LOCAL REGULATION OF INTESTINAL BLOOD FLOW DURING DIGESTION

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ABSTRACT

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In order to examine the relationship between post-prandial intestinal hyperemia and the increase in local metabolism, various constituents of chyme were placed in the canine small intestine and their effects on venous outflow and oxygen consumption were monitored.

The results indicate that in the jejunum, digested food plus bile has a greater hyperemic and metabolic effect than digested food alone or undigested food plus bile. While both digested food and undigested food plus bile increased flow, only the former increased oxygen consumption. Bile in the jejunum did not have any effect on blood flow or oxidative metabolism. Bile in the ileum increased flow but not oxygen consumption. There was a significant correlation between the changes in blood flow and oxygen consumption during the placement of digested food. Although digested food plus bile produced a greater increase in both blood flow and oxygen consumption, there was no significant correlation between the changes in these

two variables. These findings indicate that postprandial intestinal hyperemia may involve both metabolically and nonmetabolically linked factors. The food solutions used in the above study contained many nutrients and each of them may have different mechanisms in causing an increase in flow. Thus, in subsequent experiments, the mechanisms by which a major nutrient, glucose, causes an increase in blood flow in the intestine were examined.

In the first two series of experiments, the vascular and metabolic effects as well as absorption rates of glucose, 3-0-methyl glucose and 2-deoxy glucose were compared because they have different absorptive and metabolic characteristics in the small intestine. The results show that glucose and 3-0-methyl glucose are absorbed and increase blood flow. 2-deoxy glucose was not absorbed and did not increase flow. Thus, absorption of glucose may play a role in the glucose-induced hyperemia. Of the three glucoses, only glucose increased oxygen consumption and its hyperemic effect was significantly greater than 3-0-methyl glucose. This suggests that an increase in oxidative metabolism may play a role in the hyperemia. Bile did not alter jejunal blood flow when placed into the lumen but it markedly enhanced the hyperemic effect of glucose and not 3-0-methyl glucose or 2-deoxy glucose. This indicates that the influence of bile on the glucose-induced hyperemia has chemical structural specificity.

In the third series of experiments, the effects of luminal placement of glucose and glucose plus bile on blood flow, glucose absorption and oxygen consumption were compared in the proximal jejunum.

During the placement of glucose, there was a significant correlation between the changes in blood flow and oxygen consumption as well as blood flow and glucose absorption. However, the addition of bile into the glucose solution altered the relationship between the changes in blood flow and oxygen consumption. The changes in these variables were no longer significantly correlated during the placement of glucose plus bile. In contrast, the relationship between the changes in blood flow and glucose absorption remained the same and was significantly correlated with or without bile.

It is concluded that the local regulation of blood flow during postprandial intestinal hyperemia may involve metabolic and nonmetabolic factors. Various constituents of chyme can elicit different responses in blood flow and oxygen consumption in the small intestine. The hyperemic effect of glucose in the jejunal lumen is, at least in part, related to glucose absorption and an increase in oxidative metabolism. Bile enhances both the hyperemic as well as the metabolic effects of glucose and digested food. Bile also alters the positive relationship between the changes in blood flow and oxygen consumption produced by glucose and digested food.

To Jean, Ling Ling, Kristofer

and

my parents

Whose unwavering support and

sacrifices made this possible

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CHAPTER I

INTRODUCTION

Postprandial intestinal hyperemia is characterized by an increase in blood flow through the digestive organs with little or no change in flow to other vascular beds (25, 120-122). Recently it has been demonstrated that the intestinal hyperemia is a local phenomenon and is confined only to that region of the small intestine which is exposed to food (25). In addition, it was observed that the constituents of chyme responsible for the hyperemia were the digested products of food (26). The mechanisms by which these digested products cause an increase in local blood flow however, are still not clear.

Many studies have shown that the hemodynamic changes after a meal are associated with an enhancement of splanchnic oxidative metabolism (29, 30). In addition, the presence of various digested products in the gut lumen have been shown to increase both blood flow and oxygen consumption of the small intestine (118, 119). The results from these studies have led to a hypothesis suggesting that the local regulation of postprandial intestinal hyperemia is linked to the heightened metabolic activities during

digestion and absorption (34, 118). However, unlike the heart or the skeletal muscle where many classical studies have been performed to evaluate the relationship between the rate of oxidative metabolism and functional hyperemia (69), there is virtually no similar study performed on the small intestine during digestion and absorption. Thus the purpose of this study was to examine the relationship between the intestinal hyperemia and the increase in oxidative metabolism during digestion. In addition, the study also examined the relationships between blood flow, oxidative metabolism and glucose absorption during glucose induced intestinal hyperemia.

CHAPTER II

LITERATURE REVIEW

Splanchnic Circulation

The splanchnic viscera receives up to 28% of the total cardiac output. This constitutes a greater share than any other organ in the body. The stomach, pancreas, duodenum, and gallbladder receive a supply of blood from the celiac artery; while the inferior mesenteric artery supplies blood to the transverse, descending, and pelvic colon as well as the rectum. In addition, the superior mesenteric artery supplies blood to the pancreas, duodenum, small intestine, cecum, ascending colon, and transverse colon. In contrast to the rest of the organs in the body, venous outflow from the stomach, small and large intestine, pancreas, and spleen is not drained by the central venous system. Rather it is drained by the portal vein which, together with the hepatic artery, supplies blood to the liver. This review will focus on the mesenteric circulation and its relationship with some of the functions of the intestine that are pertinent to this study.

Gastrointestinal Circulation

Blood supply to the gastrointestinal tract is best characterized by its complex system of vascular arcades. These extend even into the intramural layers of the intestine as well as the stomach (82, 113, 115). In 1958, Grim and Lindseth (51) using ^{24}Na-labeled glass microspheres first described the flow distribution in the wall of the small intestine. They suggested that the supply of blood to the three layers (mucosa, submucosa and muscularis) of the intestine may be separate entities in parallel to each other. However, it has become increasingly clear that the mucosa-submucosa layers are coupled in series and, as a whole, are in parallel with the muscularis layer (82, 113). The bulk of evidence (9, 25, 47, 51, 73, 82, 113, 126) thus far indicates that the mucosa-submucosa receives a major portion (82 to 88%) of total wall flow as compared with the muscularis layer (12 to 18%). In addition, blood flow to these two layers as expressed by volume per unit weight of tissue, is much greater than the muscularis (9, 25, 47, 51, 73, 82, 113, 126). Svanvik and Lundgren (113) attributed this phenomenon to the higher metabolic demands of these two layers due to active transport as well as secretion.

While the distribution of flow to the intramural layers has been well recognized, the blood supply to the intestinal villus has, until recently, been controversial (6, 8, 12, 64, 65, 82, 86, 87, 111, 113). Levitt and Levitt

(86) first proposed that there are two sets of blood vessels within the villus possessing distinct anatomical and functional characteristics. One set of these blood supplies runs close to the tip of the villus while the other is at the crypt or the submucosa. They cited as evidence that there seemed to be two distinct components in the absorptive patterns of various substances, one being flow dependent while the other diffusion dependent. As such, materials entering the villus could be readily carried away by the circulation close to the tip and thus the rate was directly proportional to blood flow. Substances that entered the crypt or submucosa circulation were limited by the longer distance between the lumen and the vessels and thus were diffusion dependent. This is in contrast to the hypothesis of Lundgren et al. (64, 65, 87, 111) and Jacobson and Noer (62). They suggested that each villus is supplied by a single arterial vessel (around 20 μm in diameter) emerging from the submucosa. It runs "in the central core without branching" and the direction of flow is opposite to the venous vessel draining the villus (64, 65, 87, 111). These vessels are about 15 to 20 μ apart and appear to be permeable to a variety of substances, especially those that are lipid-soluble.

An obvious implication of this anatomical arrangement is the possibility of a countercurrent exchanger mechanism analogous to the one observed in the kidney. Thus, materials (especially those that are lipid-soluble)

absorbed from the lumen of the gut should be trapped at the tip of villus since what entered the capillaries and vein could diffuse back into the arterial vessel. On the other hand, blood borne, diffusable materials could be accumulated at the base of the villus due to shunting of the substances among the two vessels. This has been proven to be the case (6, 8, 64, 65). For example, Jodal and Lundgren (64, 65) observed that during absorption there was a tip to base concentration gradient of 5:1 for the lipid soluble palmitic acid and only a 2:1 concentration gradient for the water soluble short chain butyric acid. Jodal and Lundgren (63) have also demonstrated that during intestinal absorption of sodium chloride, there was a four-fold tip to base gradient in the villus with the interstitial fluid osmolality reaching 1,200 mOsm/kg at the tip. Regarding the second possibility, Kemp et al. (74) has shown that there was indeed extravascular shunting of oxygen from the arterial to the venous vessel, resulting in a gradient of the gas from the base to the tip of the villus. It has also been observed (1, 74) that this shunting of oxygen was more pronounced during hypovolumic shock, due to a prolonged transit time of blood in the villus as would have been predicted by the countercurrent exchanger model. It is apparent that the dual circulation hypothesis proposed by Levitt and Levitt (86) can not explain the osmotic gradient as observed by so many investigators (6, 8, 63, 64, 74). However, the countercurrent exchanger mechanism theory

is not without its share of criticisms (11, 85). As suggested by Levitt et al. (85), diffusion of lipid-soluble materials from the lumen to the artery in the villus would effectively destroy the concentration gradient between the two vessels and render the exchanger theory inoperative. In addition, these investigators (11, 85) failed to find any evidence that may indicate there was a delay in the luminal absorption of diffusable gases in the rabbit small intestine as observed earlier in cats (85). However, in a recent finding this same group (12) did find a significant delay in the absorption of these gases in dogs and they conceded that there may be a species difference in the efficiency of the countercurrent exchanger mechanism. Although it now appears that the countercurrent exchanger theory of the villus is gaining acceptance, definite proof of its existence is still lacking and further testing is necessary (113).

Capillary perfusion in the intestinal vascular bed is mainly determined by the arterioles and precapillary sphincters (69, 82, 113). It has been observed that at rest, only about 20 to 40% of the capillaries in the villi are opened for perfusion (69, 113). The arterioles and precapillary sphincters are arranged in series and controlled by both extrinsic and intrinsic mechanisms (113). Since there is no known parasympathetic vasodilator outflow to the mesenteric bed, it is believed that the sympathetic vasoconstrictor fibers, circulating hormones and gases

constitute the major extrinsic controlling mechanisms of blood flow in this area (82). In contrast, the local controlling factors of blood flow to the intestine have not been identified (69, 82, 113). There is no doubt that the intestine is capable of autoregulation and autoregulatory escape as demonstrated so convincingly by P. C. Johnson et al. (67, 68, 69) as well as Folkow et al. (38, 39). The precise mechanism(s) for this phenomenon however, remains elusive. Two major functions of the intestine are the digestion and absorption of nutrients (30). Since food intake is known to increase gut blood flow, postprandial intestinal hyperemia has been "loosely" referred to as a functional hyperemia of the mesenteric bed (46). The rest of this review will be devoted to examining the control of blood flow during this mesenteric hyperemia.

Circulatory Adjustments to Food Intake

The cardiovascular responses of the body to food intake have long been a subject of interest to many investigators. Grollman (52) as well as Gladstone (44) studied the effect of eating on cardiac output of human subjects and found it increased after food ingestion. Herrick et al. (56) found in dogs that blood flow through the femoral, carotid and superior mesenteric arteries increased following a meal. Dagenais et al. (29) studied the demodynamic effects of carbohydrate and protein meals in man. A protein rich meal brought a greater increment of cardiac output

than did a carbohydrate rich diet. Brandt and associates (14) found in humans that protein feeding induced a 35% increase in splanchnic blood flow while glucose feeding did not elicit any response.

With the advent of research in chronic conscious animals, the ability to study the hemodynamic responses of the body to food intake in intact, awake animals became possible. Fronek and Stahlgren (43) performed feeding experiments on conscious dogs with implanted electromagnetic flow transducers around the ascending aorta, brachiocephalic, superior mesenteric and iliac arteries. During the anticipation and ingestion periods, cardiac output was observed to increase by 142% with a concomitant rise in both blood pressure and heart rate. The changes in these parameters reached a maximum in the first minute after the beginning of ingestion and gradually declined back to control level 1 hour after feeding. Total peripheral resistance tended to drop during anticipation of food but increased modestly (5%) during ingestion. This slight increase persisted even after the completion of food intake. Brachiocephalic flow increased during ingestion by 196% while the iliac flow dropped to 75.4% of control level. Although there was a slight increase in superior mesenteric blood flow at this time, mesenteric regional resistance was found to increase by 28%. The authors suggested that there was a general sympathetic response to anticipation and actual ingestion of food. During

digestion (1 and 3 hours after ingestion), blood pressure, cardiac output, heart rate and total peripheral resistance were not significantly different from respective control values. Superior mesenteric blood flow increased by as much as 133% of control while the brachiocephalic and iliac arterial flows decreased to 86.5% and 74% of control, respectively. The authors concluded that there seemed to be a redistribution of cardiac output to the splanchnic bed during digestion. Indeed the ratio of flow in the superior mesenteric artery to cardiac output was found to have increased from 9% at control to 13% three hours after food intake.

Since the introduction of the redistribution hypothesis by Fronek and Stahlgren (43), many new studies have been performed to investigate this phenomenon. Burns and Schenk (16) in 1969 measured cardiac output and blood flow through the superior mesenteric artery of conscious dogs following a meal. Electromagnetic flow meters were planted on the ascending aorta and the superior mesenteric artery to monitor blood flow. After consuming a standard meal of 15 oz. of horsemeat, mesenteric blood flow began to rise within 5 minutes and reached a plateau approximately 50 minutes later. The mean flow was still 50% above the control level 3 hours after feeding. Although there were occasional changes in cardiac output during ingestion, no detectable increment was observed during digestion. Vatner et al. (121) found in conscious dogs that anticipation and

ingestion of a meal caused increases in cardiac output, heart rate and aortic blood pressure. These parameters returned to the control level within 10 to 30 minutes after food presentation and remained stable throughout the digestive period. Although mesenteric blood flow decreased (10%) transiently during anticipation, it began to increase during actual ingestion and reached maximal values (115% to 300% of control) within an hour. The increase in blood flow to the viscera has been reported to last up to 7 hours postprandially. The same group of investigators (120) found in a subsequent study that in addition to the above mentioned vascular changes, renal resistance increased by 24%, iliac and coronary resistances decreased by 33% and 62% respectively during the anticipation and ingestion period. During digestion, the increase in mesenteric flow was accompanied by a slight decrease (10%) in iliac flow while cardiac output, heart rate, renal and coronary vascular resistances returned to their control levels. This suggested that there was vasoconstriction in the skin and skeletal muscle to compensate for the increase in flow to the mesenteric vascular bed during digestion.

In 1974, Vatner et al. (122) studied the regional circulatory adjustments to eating and digestion in conscious primates. During ingestion of a meal consisting of different fruits, baboons experienced an increase in both heart rate (82%) and atrial pressure (25%). Likewise, iliac and coronary flows were observed to increase by 84%

and 152% respectively. There was a transient but significant decrease in both mesenteric (12%) and renal (4%) blood flow during this time. During digestion, mesenteric blood flow rose and reached a maximum in an hour. This increase in flow was reported to last up to 4 hours and was accompanied by a significant decrease in iliac flow (31%). Heart rate, arterial pressure, coronary and renal blood flow remained at control levels throughout digestion.

These recent studies have therefore shown that although cardiac output, heart rate and systemic pressure increase during the anticipation and ingestion of food, the changes are transient and decline to control levels 30 minutes after ingestion (16, 43, 120, 121, 122). Mesenteric blood flow begins to increase from 5 to 30 minutes after completion of food intake and the hyperemia may last up to 7 hours. This increase in mesenteric blood flow during digestion is accompanied by increased resistance in the brachiocephalic and iliac arteries (43, 120, 122). There is a postprandial redistribution of cardiac output favoring the mesenteric vascular bed at the expense of the muscle and skin.

These studies, however, have not determined the specific localization of the hyperemia within the mesenteric circulation.

Postprandial Intestinal Hyperemia

Fara et al. (34) observed that intraduodenal instillation of corn oil in cats caused increases of blood flow through the superior mesenteric, pancreatic and jejunal arteries while flow through the stomach and colon were not altered. Chou et al. (25) found in anesthetized dogs that the hyperemia through the various organs within the gastrointestinal tract seemed to follow the pattern of chyme movement. The flow through the celiac artery was observed to increase when food was introduced into the stomach but did not change when instilled into the duodenum. Superior mesenteric blood flow on the other hand, fell transiently but not significantly during intragastric placement of food; started to rise 30 minutes after the placement and remained increased for 3 hours. This was significant since the celiac artery supplies the stomach whereas the superior mesenteric artery supplies the small intestine. These findings were interpreted to indicate that blood flow may increase only in that region exposed to food and not indiscriminantly throughout the gastrointestinal tract.

This possibility was further explored by these investigators when they infused food intraduodenally. In addition to monitoring the superior mesenteric arterial flow, they measured venous outflow from an isolated *in situ* jejunal segment. As before, superior mesenteric blood flow was found to increase during the intraduodenal food

infusion but venous outflow from the isolated jejunal segment which had no contact with food was not altered. When the same food was placed only into the isolated jejunal segment, the blood flow through the segment increased but that through the superior mesenteric artery did not. Subsequently venous outflows from two isolated adjacent in situ jejunal segments were measured simultaneously; one segment contained 10 ml of food and the other, the same amount of saline. The segment with food increased its flow while no change was observed in the control segment with saline. These findings suggested that the intestinal hyperemia that occurs during digestion was a local phenomenon. Radioactive microspheres were also used by these authors (25) to study the compartmental blood flow in the jejunum. Three isolated in situ jejunal segments were used, one was left empty and the other two contained either 10 ml of non-absorbable polyethylene glycol solution or the same amount of food. It was found that only the segment containing food showed an increase in blood flow. The increase in the total wall flow was the result of an increase in mucosal-submucosal flow; while flow to the muscularis was not altered. Yu et al. (126) in a similar study using radioactive microspheres observed that placement of 50% glucose solution in an isolated jejunal segment in dogs induced an increase in blood flow in the mucosa-submucosa but did not affect flow to the muscularis. Thus the increase in the superior mesenteric blood flow during digestion may

well be due to an increased flow to that segment of the small intestine that contains chyme. Furthermore, the increased flow appears to be localized to the mucosal-submucosal layer of the intestinal wall (25, 126).

Although some information has surfaced in recent years concerning the mechanisms involving the cardiovascular responses to food intake, the overall picture is not entirely clear. Opinions are not always unanimous as to what mediates the local intestinal hyperemia during digestion. Nor is there any concensus as to what initiates the increase in flow to the part of the intestine that is in contact with food. Nonetheless, investigations have been done to elucidate the mechanisms of the postprandial responses. Among the mediators that have been implicated are: (1) local neural pathways and (2) various humoral substances that are released into the blood stream during digestion.

Neural Mechanisms

There are two major intrinsic nerve plexuses within the gastrointestinal tract: the submucosal plexus of Meissner and the myenteric plexus of Auerbach. The former is situated between the muscularis mucosa and the circular muscles of the intestinal wall. The latter plexus is located between the longitudinal and circular muscle coats. The sensory endings of the visceral afferent fibers are dendrites from the submucosal plexus that may be situated

in the mucosal epithelium, in the muscle layers or in the plexuses. They are chemical and mechanical receptors that are activated by changes in the intestinal contents (peptides, amino acids, fats, pH and osmolality) and by stretch and distention of the gut wall (30, 97, 115). The afferent impulses may be conducted centrally via the vagal and sympathetic fibers or be transmitted locally via the submucosal plexus to the myenteric plexus (30, 97, 115).

Zamiatina (127) found that the presence of food or its digestive products in the lumen of the gut can stimulate the receptors in the intestinal wall. During digestion in cats that were fed meat, especially high neural activity was seen from the afferent nerves of the jejunum, mesentery and pancreas but not of the colon. This is also true with glucose and amino acid solutions infused into the gut. Glucose (4 to 10%) solutions have a stimulating effect on the upper part of the jejunum whereas amino acid mixtures intensify the neural activities in the jejunum, mesentery and pancreas. In a similar experiment Sharma and Nasset (102) have also been able to demonstrate that when glucose and amino acid solutions were perfused through the lumen of the small intestine, the frequency of firing from mesenteric nerves increased by as much as 400% from control.

Fronek and Stahlgren (43) characterized the increases in heart rate, blood pressure and cardiac output during anticipation and ingestion of food as a generalized

sympathetic response. They cited as evidence the findings of Ehrlich et al. (32) that there was no increase in mean arterial blood pressure during ingestion in catecholamine-depleted dogs.

The roles played by the autonomic nervous system during anticipation, ingestion and digestion of food were examined in detail by Vatner et al. (121). The effects of food presentation, actual ingestion and digestion on cardiac output, heart rate, aortic pressure and mesenteric blood flow before and after adrenergic and cholinergic blockade in conscious dogs were investigated. Alpha and beta adrenergic blockade by phenoxybenzamine and propranolol attenuated the increases in heart rate, blood pressure and mesenteric resistance during the anticipation and ingestion periods. No effect was observed on the mesenteric hyperemia during the digestion period. Cholinergic blockade prevented the mesenteric vasodilation during digestion but bilateral thoracic vagotomy had no effect on the response. Fara et al. (34) also observed in cats that atropine blocked the increase in superior mesenteric blood flow seen after the intraduodenal instillation of milk, corn oil (fat), L-phenylalanine or hydrochloric acid. Bilateral splanchnicectomy or combined alpha and beta adrenergic blockades have no effect on the vasodilation. Vatner et al. (122) have shown in primates that the mesenteric vasodilation could be attenuated by prior cholinergic blockage

with atropine. Again the blockade had no effect on the vascular responses seen in the early ingestion phase of a meal.

Thus, these findings seem to suggest that the sympathetic nervous system is involved in the anticipation and ingestion responses to food. The fact that bilateral thoracic vagotomy had no effect on the postprandial vascular response indicates that cholinergic influence is at the local level; most likely involving the intramural nerve plexus.

Chen et al. (20) have demonstrated that dibucaine can attenuate the vascular effects of intraluminally placed hypertonic salt solutions. Before applying the local anesthetic, 1500 mOsm of NaCl, KCl, $MgCl_2$ or $CaCl_2$ salt solutions all increased blood flow in an in situ jejunal loop. However, after the mucosa of the segment had been exposed to dibucaine, only $MgCl_2$ increased blood flow. Other solutions either lowered blood flow or had no effect on the jejunal vasculature. Chou et al. (21) showed that dibucaine can also attenuate the increase in blood flow evoked by a hypertonic glucose solution in the lumen of the gut. Yu et al. (126) observed that the inhibitory effect of the local anaesthetic on the vasocilation is limited to the mucosal layer of the luminal wall. They observed that dibucaine attenuated the mucosal hyperemic response to hypertonic glucose solution in the lumen.

Blood flow to the other two layers was not affected by the anaesthetic.

As discussed above, the cardiovascular responses seen during the anticipation and ingestion of food may in part, be mediated by the sympathetic fibers. Local cholinergic plexuses have been suggested to play a role in mediating the mesenteric hyperemia during digestion. Precisely what neural mechanisms are involved in the vascular adjustments during digestion is not clear. It is possible that receptors on the mucosa continuously monitor the content of the chyme and provide vital information for a local reflex arc during the presence of food in the lumen. In turn, the intraluminal cholinergic plexus may either act on the blood vessels or release local vasoactive agents to produce the vascular responses to food intake.

Humoral Mechanisms

Among the many substances that are released into the blood stream during digestion are the GI hormones. The specific actions of these hormones, i.e., gastrin, secretin and cholecystokinin (CCK) on secretion, absorption, motility and metabolism of the gastrointestinal tract have long been under intense scrutiny. It was not until recently that attention has been given to their possible vascular effects. All three of these hormones are released from the upper gastrointestinal tract during digestion. They have been shown to have specific vascular effects on different organs.

The antrum of the stomach is the main source of gastrin. The release of the hormone can be stimulated by vagal stimulation, gastric distention or various chemicals (e.g., meat extracts, amino acids, histamine and alcohol) in the stomach. Inhibition of the release of the hormone occurs when the antral pH drops below 3.5 (66).

Chou et al. (22) observed in dogs that infusions of gastrin extracted from porcine antrum into the superior mesenteric artery decrease the resistance of the intestine by 10% to 12%. Laureta et al. (83) found local intra-arterial infusion of the hormone to have a similar effect on the canine stomach. It was concluded that gastrin may very well be vasoactive in both the gastric and mesenteric vascular beds. Swan and Jacobson (114) on the other hand, found that gastrin had no effect on the total blood flow to a gastric pouch but did increase its mucosal blood flow. The effects of subcutaneous injection of gastrin extracted from porcine antrum were examined by Burns and Schenk in dogs (16). Injection of the extract subcutaneously increased mesenteric blood flow within 5 minutes, flow reached a peak (45% above control) in 1 to 2 hours and remained elevated for up to 3 hours. In addition to its effects on the gastric and mesenteric vasculature, gastrin has also been shown to increase pancreatic secretion and blood flow (45) as well as decrease hepatic vascular resistance (91). Whether the hormone has a direct influence on the blood vessels or acts indirectly through the release

of intermediary agents is still not clear. Fasth et al. (36) observed in cat small intestine that atropine can attenuate the increase in intestinal motility but not the increase in blood flow induced by the intra-arterial infusion of gastrin. Bowen et al. (13) recently found that cholinergic blockade by atropine abolished the vasodilating effect of pentagastrin in the canine distal ileum. Thus, depending on the species, the hormone's actions on both motility and blood flow may well be mediated through cholinergic receptors.

Secretin was first isolated by Mutt and Jorpes (89) and synthesized by Bodansky et al. (7). It is believed that secretin is produced by villus epithelial cells in the duodenum (30, 66). Hydrogen ions in the duodenum are the most effective stimulant for the release of the hormone. Intraduodenal installation of fatty acids and amino acids have also been shown to cause the release of the peptide (66). Secretin is most effective in stimulating the secretion of bicarbonate and fluid in the pancreas and pepsin in the stomach (30, 66, 95).

The vascular effects of secretin in the gastrointestinal vasculature have been studied in cats (34, 95), dogs (13, 22, 45, 83) and humans (117). Chou et al. (22) and Laureta et al. (83) did not observe any change in vascular resistance when they infused the hormone intra-arterially into the canine superior mesenteric artery and the stomach. Goodhead et al. (45) found that intravenous

infusion of secretin increases blood flow to the pancreas and duodenum but does not affect blood flow to the other parts of the alimentary tract. Recently Bowen et al. (13) observed in dogs that synthetic secretin administered intra-arterially into a loop of distal ileum did not elicit any response. Burns and Schenk (16) on the other hand, found in dogs that intravenous injection of an impure extract of secretin increased mesenteric blood flow within 5 minutes. This increment reached a maximum (38% above control) in 2 hours. Ross (95) examined the effects of a natural secretin extract on flow rates in the femoral, hepatic and superior mesenteric arteries in cats. Rapid injection decreased resistance in the superior mesenteric and femoral arteries but not the hepatic artery. These changes were accompanied by a prolonged increase in blood pressure. Fara et al. (34) observed that intravenous administration of a pure, natural secretin into cats increased the superior mesenteric, pancreatic and jejunal arterial blood flow. Uden (117) observed in humans that secretin injection increased mesenteric blood flow. Thus, substantial disagreement exists as to the vascular effects of secretin in the stomach and the small intestine. This may stem from the fact that most studies were conducted with purified secretin rather than the synthetic one. Naturally obtained extracts of the hormone are known to contain various other vasoactive substances such as CCK or plasma kinin. These contaminants may have contributed to many of the

vasodilating effects of secretin. Only Bowen and associates (13) have used synthetic secretin in their studies. Unfortunately, they did not examine the vascular effects of the hormone in the entire gastrointestinal tract.

Ivy and Oldberg's cholecystokinin (CCK) (60) and Harper and Raper's pancreozymin (55) have been proven to be one single hormone (72). CCK is a strong stimulant of gallbladder contraction and a weak stimulant of gastric secretion. Presence of amino acids, fatty acids and hydrogen ions in the duodenum stimulates the release of the hormone from the upper small intestine.

Fara et al. (35) was able to mimic the vascular effects of intraduodenal fat by intravenous infusion of CCK. In cats, infusion of the partially purified hormone increased both superior mesenteric blood flow and duodenal motility. It was postulated that CCK may be the mediator of fat-induced vasodilation in the duodenum. Fasth et al. (36) found that local intra-arterial infusion of the hormone into the cat's small intestine caused an immediate but short lasting increase of blood flow. Both the vascular and metabolic effects of CCK were examined by Fara et al. (34) in cats. CCK increased blood flow and O_2 consumption in the jejunum and pancreas. These effects were not blocked by atropine or vagotomy. Bowen et al. (13) however, observed in the canine ileum that the increases in superior mesenteric blood flow and O_2 consumption after infusion of CCK could be blocked by atropine. Route of infusion

and dosage of the hormone as well as species difference may account for the contrasting results.

Fara et al. (34) suggested that the mesenteric vasodilation caused by CCK may have been the consequence of the release of local vasoactive substances triggered by the hormone. Their in vitro studies have shown that CCK does not affect the active tension of a superior mesenteric arterial strip. Thus, it is unlikely that the hormone has any direct effect on the mesenteric vasculature. Biber et al. (7) observed in cats that blocking the vascular effects of 5-hydroxytryptamine (5-HT) by an α-receptor antagonist (dihydroergotamine) can abolish the intestinal vascular responses to CCK infusion. It was suggested that 5-HT, may be the intermediary vasoactive agent for the mesenteric vasodilation elicited by CCK. Hilton and Jones (58) perfused the cat's pancreas with CCK and found increases in pancreatic blood flow and secretion. In addition, they observed that the activity of the active kinin-forming enzyme "kallikrein" in the perfusate had increased about fourfold. This led them to speculate that plasma kinin may play a prominent role in the functional vasodilation of the pancreas during CCK infusion.

In summary, all three gastrointestinal hormones have been shown to be vasoactive in the gastrointestinal vasculature. Infusion (both i.a. and i.v.) of gastrin in dogs and cats produced vasodilation in the superior mesenteric, pancreatic and hepatic arterial blood flows (16,

22, 45, 83, 91). Whether this hormone acts directly or through other vasoactive agents is not known. Findings concerning the vascular effects of secretin are controversial. There is conflicting evidence as to whether or not the hormone is vasoactive in the small intestine (13, 16, 22, 34, 83, 95). Its vasodilating effect on the pancreas however, has been well established (41, 45, 90, 117). CCK is known to decrease vascular resistance in the small intestine (7, 13, 22, 34, 35, 116), stomach (83), pancreas (45, 58, 90) and in the portal vein (91, 116). It was suggested that the hormone's action may be mediated by 5-hydroxytryptamine (7) and kallikrein (58).

It must be emphasized that much of the vascular effects of the hormones described above were obtained from pharmacological doses. Thus, any extrapolation from these data must be treated with caution. Chou et al. (24) recently found that of the 3 hormones, only CCK was able to exert its vascular effects at physiological doses (2 to 8 μ units/ml). This was compatible with the observation by Fara et al. (34) in cats that infusion of low doses of CCK mimicked the vascular response induced by intraduodenal fat. Furthermore, atropine, which prevents the release of CCK (123), has been shown to prevent postprandial mesenteric vasodilation (34, 120, 122). What these findings seem to suggest then, is that the mesenteric vasodilation could be a consequence of the release of CCK. It is unlikely, however, that CCK was the sole vasoactive agent

involved since mesenteric blood flow has been shown to increase by as much as 300% of control postprandially (120, 122). The average increase of flow in the superior mesenteric artery after CCK infusion (even at pharmacological doses) is less than 100% (7, 13, 34).

Many other substances have been shown to be vasoactive in the mesenteric vasculature. Unfortunately, their exact role in postprandial hyperemia has not been determined. As mentioned earlier both 5-hydroxytryptamine (7) and kallikrein (58) have been suggested as possible mediators for the vascular effects of CCK in the small intestine and the pancreas (7, 58). Local hypoxia and hypercapnia (78, 107) as well as the intra-arterial infusion of magnesium, ATP, ADP, AMP, bradykinin, prostaglandins, acetylcholine or histamine in the dog cause intestinal vasodilation (30, 48, 54, 99, 115). What role these substances play in postprandial hemodynamics, however, has not been determined. Thus, besides the three gastrointestinal hormones, little is known concerning the possible role of other endogenous humoral substances in eliciting the local increase of mesenteric blood flow.

Local Regulation of Intestinal Blood Flow, Absorption and Oxidative Metabolism

Chou and co-workers (26) recently observed that the constituents of chyme responsible for the hyperemia are the digested products of food. Existing evidence seems

to agree with this view. For example, many elementary digested products of proteins, carbohydrates and lipids have been shown to decrease vascular resistance when placed inside the lumen of the intestine (21, 26, 34, 118, 119, 126). Studies by Fara et al. (34) as well as others (25) have also shown that the time of the onset of postprandial hyperemia occurs more rapidly with digested food than with undigested food. Thus, it is very likely that digestion plays a vital role in postprandial hyperemia and is probably a prerequisite to the increase in intestinal blood flow caused by the presence of food.

The question of how the processes of absorption trigger postprandial hyperemia has never been fully elucidated. It is well known however that the absorption of some nutrients (amino acids and glucose) is an energy requiring process (30) and addition of exogenous substrates into the intestinal lumen accelerates local metabolism (84). For example, glucose is absorbed by an active transport mechanism against a concentration gradient (5, 18, 28, 30, 125). The transport of glucose into the mucosal epithelial cell is made possible by a mobile sodium carrier located in the brush border plasma membrane (18, 28, 30, 125). Crane (28) postulated that there are two binding sites on the carrier molecule, one for sodium and the other for glucose. It was further suggested that the glucose molecule cannot attach to the carrier unless sodium is already occupying its site on the carrier molecule. Once

the carrier has bound sodium and glucose, the entire complex is driven (by the concentration gradient of sodium) from the luminal to the cytoplasmic side of the brush border plasma membrane, where both molecules are then discharged (5, 28, 30). Accumulation of the sugar inside the cell allows it to diffuse into the circulation. The sodium concentration gradient, i.e., 100 to 140 mEq/kg in the lumen and 50 mEq/kg or less inside the cell, is maintained by the active extrusion of the ion at the expense of ATP by a Na-pump located on the serosal side of the cell (5, 28, 30).

Thus, the absorption of glucose is an energy-requiring process and indeed, the absorption of the sugar has consistently been shown to increase intestinal oxygen consumption and blood flow (118, 119). But unlike the heart and the skeletal muscle where many classical studies have been performed to correlate the rate of metabolism and functional hyperemia (2, 4, 15, 31, 40, 75, 76, 79, 80), there is virtually no data available to clearly establish that the regulation of the glucose-induced hyperemia is related to factors that are linked to oxidative metabolism.

It is without a doubt that all cells in the body require an adequate supply of oxygen to maintain their function. Since blood is the only means of oxygen transport to the cells, it is vital that blood flow to the tissue is closely monitored and regulated by cellular oxygen

demand. This in fact is the rationale behind the metabolic hypothesis of the local regulation of blood flow (99). The hypothesis suggests that the flow adjustments of an organ in response to changes in metabolism are caused by the alterations in the concentrations of oxygen or other metabolites in the tissue fluids surrounding the arterioles and precapillary sphincters (99). For example, an increase in oxidative metabolism (as during increased work load of the heart or skeletal muscle) increases the production of metabolites and decreases PO_2 in the tissue fluids, resulting in the relaxation of the vascular smooth muscles (vasodilation) and thus, oxygen delivery is augmented in response to an elevated metabolic demand. Alternatively, a sudden increase in blood supply causes an increase in oxygen delivery above and beyond that which is needed by the tissues, resulting in an increase in the concentration of tissue oxygen which leads to an active contraction of the vascular smooth muscles (vasoconstriction) and returns oxygen delivery to normal.

The causal relationship between metabolic activity and blood flow is best illustrated by studies done on the heart and skeletal muscle (2, 4, 15, 31, 40, 75, 76, 79, 80, 105). For example, Shipley and Gregg (105) first demonstrated that the increase in myocardial contractility during stimulation of the canine stellate ganglia was accompanied by increases in both coronary blood flow and oxygen consumption. They suggested that the increase in coronary

flow was in part related to the augmented cardiac metabolism. Eckenhoff et al. (31) measured coronary blood flow and oxygen consumption at rest, during hemorrhage and after epinephrine injection in anesthetized dogs. Arterial and venous oxygen samples were taken from the carotid artery and greater cardiac vein. Oxygen content in whole blood was analyzed by the method of Van Slyke and Neil (31). Left descending coronary arterial flow was monitored by a bubble flowmeter. Oxygen consumption was calculated as the products of A-V O_2 difference and coronary blood flow. It was observed that there was a good correlation ($r = 0.85$) between coronary blood flow and cardiac oxygen consumption. Foltz et al. (40) and Alella et al. (2) also observed that there was a significant correlation between coronary flow and myocardial oxygen consumption. Braunwald et al. (15) in a subsequent study, observed that this relationship between the two variables remains unchanged over a wide range of arterial blood pressure and cardiac output. Kramer et al. (79, 80) as well as Barger et al. (4) have also demonstrated a similar relationship between flow and oxygen consumption in the canine skeletal muscle during exercise. They observed that during steady state, work done by the muscle, blood flow and oxygen consumption were linearly correlated.

Certainly, the intestine must regulate its blood flow to meet its metabolic demands. The question is whether this is the only factor that determines the control

of blood flow by the gut. The problem is complex in that unlike the heart or skeletal muscle, the intestine has to perform various functions during digestion besides muscular contraction (30). These include secretion and absorption (30). It is the last function in particular that makes the gut unique among the major organs in the body.

In addition to relying on its blood supply for chemical energy to maintain cellular integrity, the intestine must also depend on its circulation to perform absorption (30, 82). Blood vessels and lymphatics are the only means by which nutrients can be transported from the intestine to the rest of the body for use or storage. Teleologically, it is certainly most advantageous for the gut to increase its blood flow during the height of absorption (postprandial) to transport the nutrients. Thus, in addition to meeting its metabolic demands, the gut probably also regulates its blood flow to accomplish its absorptive function. The mechanisms by which the intestine regulates its blood flow to serve these two functions is not clear. Whether the functional hyperemia during digestion correlates with the increase in metabolism is not known. Since the rate of metabolism increases during intestinal absorption (30), the possibility exists that the regulation of the hyperemia is linked to factors that are closely related to oxidative metabolism. On the other hand, it is not inconceivable that different mechanisms are involved, since

the two functions (providing chemical energy and nutrient transport) do not serve the same purpose.

The purpose of this study, therefore, is to examine the relationship between the metabolic activity of the gut and its local regulation of blood flow during postprandial intestinal hyperemia.

There are two specific aims: (1) To examine the relationship between the intestinal hyperemia and the increase in oxygen consumption during digestion, and (2) To study the relationship between the increase in blood flow, oxygen consumption and glucose absorption during the luminal placement of glucose and its synthetic analogs, 2-deoxy glucose and 3-0-methyl glucose in the small intestine.

CHAPTER III

METHODS

Surgical Procedures

Mongrel dogs of either sex (15-25 kg) were fasted for 24 hours, anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and ventilated with a positive pressure Harvard respiration pump (Model #607, Dover, Mass.) to achieve normal arterial blood pH. Systemic arterial blood pressure (mm Hg) was monitored by a Statham pressure transducer (Model #P23Gb, Hato Ray, Puerto Rico) via a catheter (PE 280) inserted into the femoral artery, and continuously recorded on a four channel Hewlett Packard oscillograph (7700 series, Waltham, Mass.).

The abdominal cavity was exposed through a midline incision and a loop of the proximal jejunum or distal ileum supplied by a single artery and vein was exteriorized. After administration of heparin sodium (6 mg/kg), the vein was cannulated for measurement of venous outflow. The outflow was directed to a reservoir and was returned to the animal through a femoral vein at a rate equal to the venous outflow. A rubber tube was placed into the lumen of the segment for the introduction and withdrawal of

solutions to be tested. At all other times, the tube was connected to a Statham pressure transducer (P23Gb) to monitor intraluminal pressure. Both ends of the segment were cut to exclude collateral blood flow and were tied to prevent bleeding. The segment was covered with a plastic sheet to prevent desiccation and kept at 37°C with a heat lamp. In the double jejunal segment preparation, two segments of equal length were prepared from the jejunal loop as described above (21-26).

Preparation of the Solutions Studied

Food Solutions

The solutions studied were: (1) digested food, (2) undigested food plus bile, (3) digested food plus bile, and (4) bile alone.

The food solutions were made from a commercially available dog food (protein 12%, fat 7%, fiber 1.5%, ash 3%, linoleic acid 0.4%, moisture 78%, Alpo Beef, Allen Products Co., Allentown, Pa.). A can of this dog food was homogenized in an electric blender, then the pH of the homogenate was adjusted to 7.0 by adding sodium bicarbonate. A part of the homogenate was retained to be used in the experiments as undigested food. To the other part of the homogenate a pancreatic enzyme preparation (187.5 mg/100 cc; Viokase R, Viobin Co., Monticello, Ill.) was added and the whole was gently mixed with a magnetic stirrer at room temperature for 5 hours to permit digestion. The digested

food was then made near isotonic by adding 2 parts of distilled water to 1 part of the digested food. The mean ± SEM osmolality of the diluted digested food made from 6 cans of the dog food was 278 ± 29 mOsm/kg.

Prior to each experiment, bile was aspirated from the animal's gallbladder with a needle and syringe. The volume of bile obtained from each dog varied from 15 to 30 ml. The digested food plus bile solution was prepared by mixing equal parts of undiluted digested food, distilled water and gallbladder bile together. The whole was then gently mixed with a magnetic stirrer for approximately 15 minutes. The undigested food plus bile solution was prepared in a similar fashion. The pH and osmolality of this solution was 7.0 ± 0.47 and 317 ± 8 mOsm/kg, respectively. The bile to be placed in the lumen was diluted with 2 parts normal saline to 1 part bile. The diluted gallbladder bile had a pH of 7.0 ± 0.37 and an osmolality of 284 ± 7.4 mOsm/kg.

Glucose Solutions

The solutions studied were: 3-0-methyl glucose, 2-deoxy glucose and glucose (Sigma Chem. Co.). In addition, the effects of these glucoses plus bile were also studied. The solutions were made as follows: One part of normal saline or gallbladder bile was added to 2 parts of a solution containing 300 mM of each of these glucoses. These

solutions were isotonic, at pH 6.8-7.2, and were kept at 37°C.

Experimental Procedures

Food Studies

The purpose of these studies was to examine the relationship between postprandial intestinal hyperemia and local oxygen consumption. In the first series of experiments, various food solutions were placed into the lumen of a single jejunal segment and their effects on blood flow, oxygen consumption, luminal pressure, as well as systemic arterial pressure were measured. The solutions studied were digested food, digested food plus bile, undigested food plus bile, and bile alone. In addition, the effects of bile in the ileum were also examined. Each of these solutions was individually placed inside the lumen of the jejunal or ileal segment for a 30 minute period during which the changes in local blood flow, oxygen consumption, luminal pressure and systemic arterial pressure were monitored. Blood flow was determined by timed collection of venous outflow by graduated cylinders. Oxygen consumption was calculated as the product of blood flow times arteriovenous (A-V O_2) difference. Arterial O_2 samples were taken from the femoral artery and venous samples from the venous outflow of the intestinal segment under study. The O_2 content in mg% of whole blood was determined by the Lex-O_2-Con (Lexington Instrument Corp., Waltham, MA.). These variables

were measured before and at 5, 10, 15, 20, 25, and 30 minutes after placement of each solution.

In the second series of experiments, the temporal relationship between the increase in blood flow and oxygen consumption during placement of digested food and digested food and bile was studied. Blood flow was continuously monitored by an electromagnetic blood flow probe (Model BL 2024 F17, Biotronex Lab. Inc., Silver Spring, MA.) and Biotronex flow meter (BL-610). The vein draining the intestinal segment was cannulated with the in-line flow probe through which blood flowed to a reservoir. The outflow was then returned to the animal through a femoral vein at a rate equal to venous outflow. Prior to and during each experiment, the flow meter was calibrated with the timed collection of venous outflow by graduated cylinders. A-V O_2 difference was determined continuously by perfusing the femoral arterial blood and a portion of the jejunal venous outflow through an A-V O_2 difference analyzer (#1020, A-Vox Systems, San Antonio, TX.) at a constant rate (6.0 to 6.8 ml/min). These variables, along with luminal pressure and systemic arterial pressure were continuously recorded on a Hewlett-Packard oscillograph (7700 series, Waltham, MA.). The protocol for this series of experiments was the same as the first one. The percent changes from control of blood flow and oxygen consumption were then correlated using the least squares regression analysis.

Glucose Studies

Three series of experiments were performed. In the first two series, the effects of glucose, 3-0-methyl glucose and 2-deoxy glucose on blood flow, absorption and local metabolism were compared. It has been shown that glucose is both actively absorbed and metabolized, 3-0-methyl glucose is actively absorbed but not metabolized (18, 28, 125), and 2-deoxy glucose is neither absorbed nor metabolized by the small intestine (28). Thus, by comparing the vascular and metabolic effects as well as the absorption of these glucoses, one can determine whether the glucose induced hyperemia is related to its metabolism and/or absorption. In addition, the effects of bile on these parameters were also examined.

Series 1. The vascular effects of glucose and its synthetic analogs, 3-0-methyl glucose (3-MG) and 2-deoxy glucose (2-DG) with or without bile, were compared in the double jejunal segment preparation (21-26). The protocol for the experiments consisted of three 15 minute periods: control, test, and post-experimental control. In the pre- and post-experimental periods, both segments contained 10 ml normal saline while in the test period one segment contained 10 ml of glucose solution and the other contained 10 ml of either 3-MG or 2-DG solution so that their vasoactivities could be compared.

In each 15 minute period, venous outflow from both segments were simultaneously collected in graduated cylinders in 3 minute samples with 1 minute intervals in between collections. The volume of blood was determined and the blood poured into the reservoir. After each period, the luminal contents were withdrawn and the lumen was gently and thoroughly washed with normal saline.

Series 2. Only one jejunal segment was prepared for this series of experiments. In addition to venous outflow, oxygen consumption and glucose absorption were measured during luminal placement of glucose + bile, 3-MG + bile, 2-DG + bile, or bile alone. A-V O_2 difference was continuously monitored by the A-V O_2 analyzer as described above. Arterial and venous samples were obtained before and at 5, 10, and 15 minutes after the placement of test solutions for the determinations of plasma glucose and 2-DG (glucose oxidase method) or 3-MG concentration (Somogyi and Nelson method, see appendix). Oxygen consumption of the jejunal segment was calculated as the product of A-V O_2 difference and venous outflow. The protocol for the experiments in this series was similar to that of the first series.

Series 3. In this series of experiments, the temporal relationship between the increase in blood flow, oxygen consumption, and glucose absorption was examined. A single segment of the proximal jejunum was used. Blood

flow was determined by an in-line electromagnetic flow probe (Model BL 2024 F17) and a Biotronex flowmeter (BL-610). A-V O_2 difference was continuously monitored by the A-Vox analyzer as described above. Blood samples were obtained from a femoral artery and the venous outflow of the jejunal segment for the determination of the plasma glucose concentration by the oxidase method by a glucose analyzer (Model 23 A, Yellow Springs Instr. Co. Inc., Yellow Spring, OH.). Isotonic glucose (300 mM) diluted with either normal saline or gallbladder bile was placed inside the jejunal lumen for a 20 minute period. Blood flow, glucose absorption (blood flow x A-V glucose difference) and oxygen consumption (blood flow x A-V O_2 difference) were determined at control and at 15 second intervals during the first five minutes of glucose placement and at 1 minute intervals thereafter. The percent changes from control in these three variables were then correlated.

Statistical Analysis of the Results

The Student's t-test modified for paired comparisons was used to evaluate the changes from control in the variables produced by the test solutions. The Student's group t-test for equal variance with unpaired observations was used to compare the changes in the monitored variables between groups of animals. In addition, the data from the studies on glucose and its synthetic analogs were analyzed by a two-way analysis of variance. The least squares

regression analysis was used to correlate the changes in two variables. The statistical significance was set at p values less than 0.05.

CHAPTER IV

RESULTS

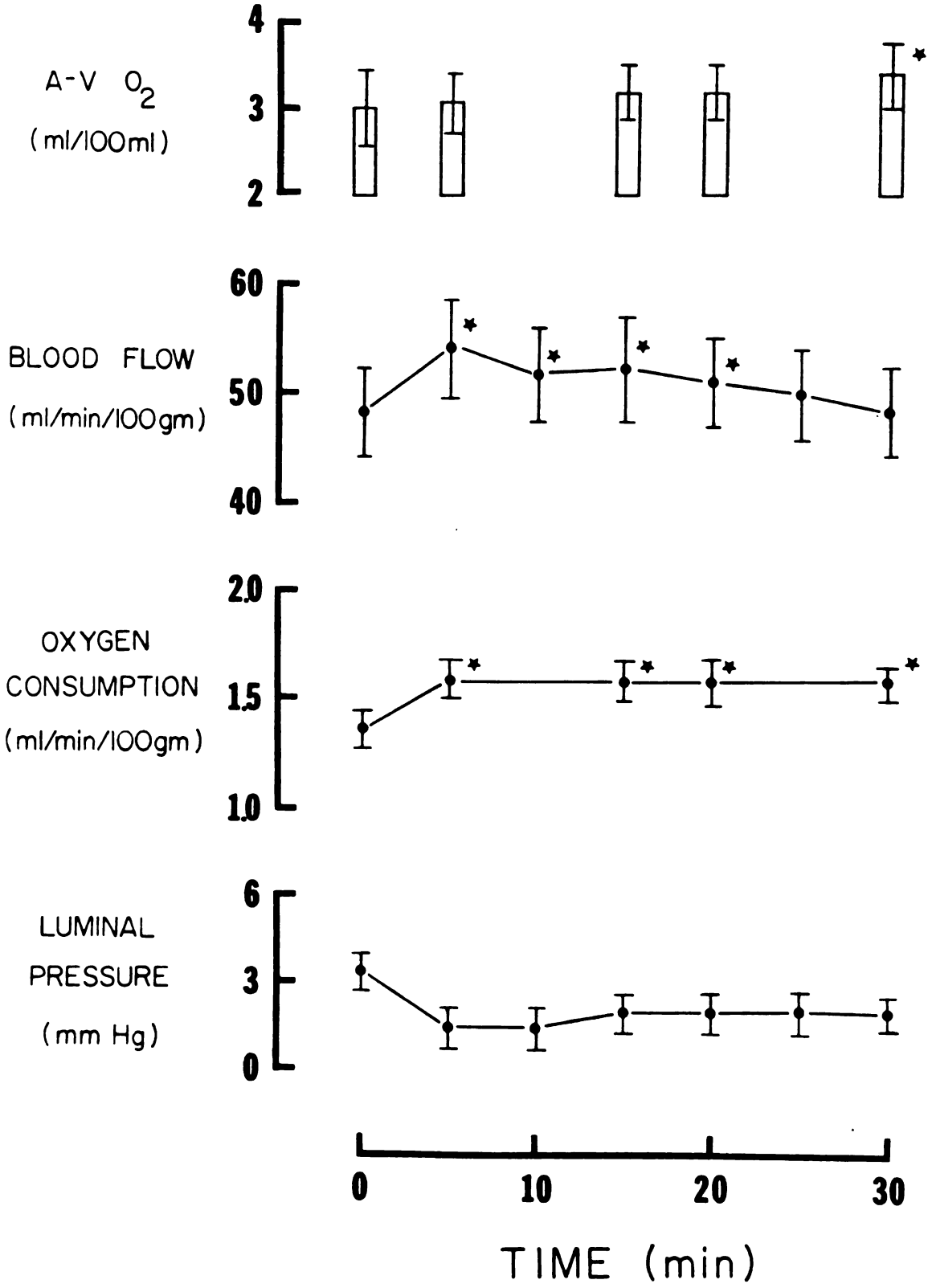
The mean ± SEM systemic arterial blood pressure was 122 ± 2 mmHg (N = 102) and was not altered by the placement of various test and control solutions into the intestinal lumen.

Food Studies

Series 1

The effects of luminal placement of digested food, undigested food plus bile, and digested food plus bile on venous outflow, oxygen consumption, and luminal pressure were examined in the single jejunal segment. In addition, the effect of bile alone on these variables in both the jejunum and ileum were also studied. The purpose was to determine whether the increase in local blood flow in response to the presence of food (or bile) in the gut lumen is accompanied by an enhancement of local oxidative metabolism. As shown in Figure 1, placement of digested food significantly increased blood flow as well as oxygen consumption. Blood flow increased from a control value of 48.1 ± 4.2 to 54.1 ± 4.4 ml/min/100 gm at 5 minutes after

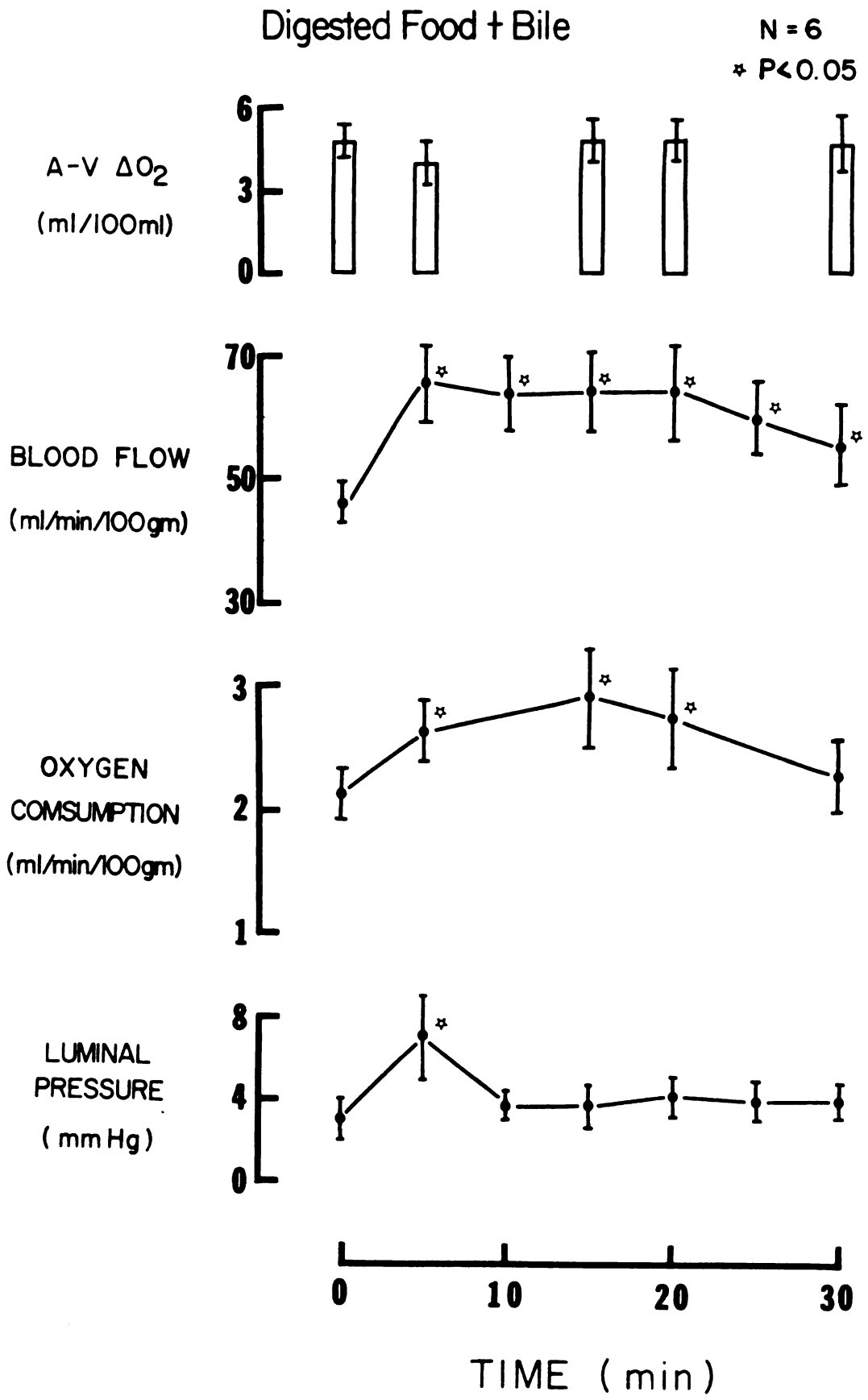
Fig. 1.--Effects of luminal placement of digested food alone on jejunal A-V O_2 difference, blood flow, oxygen consumption and luminal pressure. Asterisks indicate values significantly different from control (time = 0) at $p < 0.05$; $n = 7$.


A-V O_2
(ml/100ml)
4
3
2
BLOOD FLOW
(ml/min/100gm)
60
50
40
OXYGEN
CONSUMPTION
(ml/min/100gm)
2.0
1.5
1.0
LUMINAL
PRESSURE
(mm Hg)
6
3
0
0
10
20
30
TIME (min)

the introduction of the food solution. A steady state of blood flow was reached within 5 to 10 minutes after the food placement and this lasted for approximately 20 minutes. Blood flow at this state was 52.8 ± 4.5 ml/min/100 gm. The mean ± SEM percent increase in flow from precontrol during the steady state was approximately 10 ± 1.9%. There was a concomitant increase in oxygen consumption from 1.36 ± 0.08 ml/min/100 gm at control to 1.58 ± 0.1 ml/min/100 gm at 20 minutes after food placement. This represents a 16.4 ± 4.1% increase from precontrol. This increase in oxygen consumption was met by an increase in blood flow since A-V O_2 difference was not altered. There was no significant change in luminal pressure throughout the entire 30 minute period.

Figure 2 shows the effect of digested food plus bile on blood flow, oxygen consumption, A-V O_2 difference, and luminal pressure. Within 5 minutes after placement of the food solution, blood flow increased from 46.8 ± 4.3 ml/min/100 gm at control to 65.9 ± 6.7 ml/min/100 gm, a 37.7 ± 8.1% increase. The increase is significantly greater than the 10% increase in blood flow observed when digested food markedly enhanced its hyperemic effect. Furthermore, the hyperemia was maintained throughout the 30 minute experimental period. The blood flow remained at 55.5 ± 6.4 ml/min/100 gm at 30 minutes after food placement. Along with the increase in flow, there was also an increase in oxygen consumption, which peaked at 15 minutes and

Fig. 2.--Effects of luminal placement of digested food plus bile on jejunal A-V O_2 difference, blood flow, oxygen consumption and luminal pressure. Asterisks indicate values significantly different from control (time = 0) at $p < 0.05$, $n = 6$.


Digested Food + Bile
N = 6
* P<0.05
A-V ΔO2
(ml/100ml)
6
3
0
BLOOD FLOW
(ml/min/100gm)
70
50
30
OXYGEN
COMSUMPTION
(ml/min/100gm)
3
2
1
LUMINAL
PRESSURE
(mm Hg)
8
4
0
0
10
20
30
TIME (min)

gradually returned to control at 30 minutes. The peak increase in oxygen consumption was about 29.8 ± 7.9% above control (from the control of 2.18 ± 0.28 to 2.91 ± 0.46 ml/min/100 gm at 15 minutes after the placement) which, like the increase in flow, was greater ($p < 0.05$) than the effect of digested food alone in the lumen (+16.4%). Since A-V O_2 was not significantly altered, the increase in oxygen consumption was provided by an increase in oxygen delivery rather than by an increase in extraction by the intestine. Luminal pressure was slightly but significantly elevated only at 5 minutes after food placement.

Figure 3 shows the effect of undigested food plus bile on flow and local metabolism. There was a slight but significant increase in blood flow (12.7 ± 1.4%) beginning 5 minutes after placement of the food solution. The hyperemia persisted for almost 30 minutes. However, in contrast to the digested food and digested food plus bile solution, there were no significant changes in oxygen consumption. Also, both A-V O_2 difference and luminal pressure were unchanged. Luminal placement of bile (three fold diluted gallbladder bile with normal saline) in the jejunum had no effect on any of the variables that were monitored (Figure 4).

Since bile salts are absorbed in the ileum and bile produces an increase in local blood flow when placed into the ileal lumen (23), the effects of bile on blood flow, oxygen consumption and luminal pressure in the terminal ileum were also studied. As shown in Figure 5, blood flow

Fig. 3.--Effects of luminal placement of undigested food plus bile on jejunal A-V O_2 difference, blood flow, oxygen consumption and luminal pressure. Asterisks indicate values significantly different from control (time = 0) at $p < 0.05$; $n = 7$.

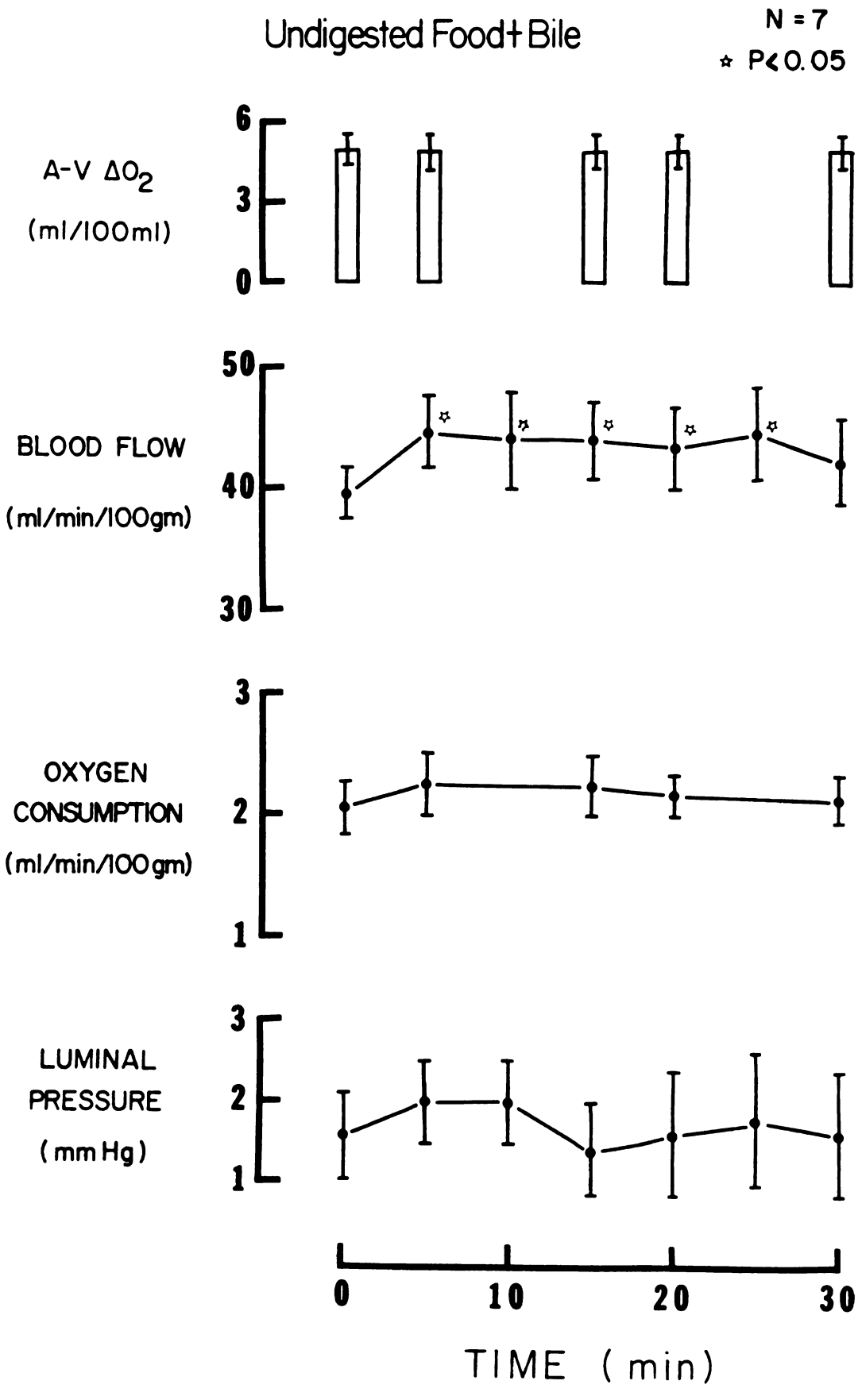

Undigested Food+Bile
N = 7
☆ P<0.05
A-V ΔO2
(ml/100ml)
6
3
0
BLOOD FLOW
(ml/min/100gm)
50
40
30
OXYGEN
CONSUMPTION
(ml/min/100gm)
3
2
1
LUMINAL
PRESSURE
(mm Hg)
3
2
1
0
10
20
30
TIME (min)

Fig. 4.--Effects of luminal placement of bile on jejunal A-V O_2 difference, blood flow, oxygen consumption and luminal pressure. N = 6.

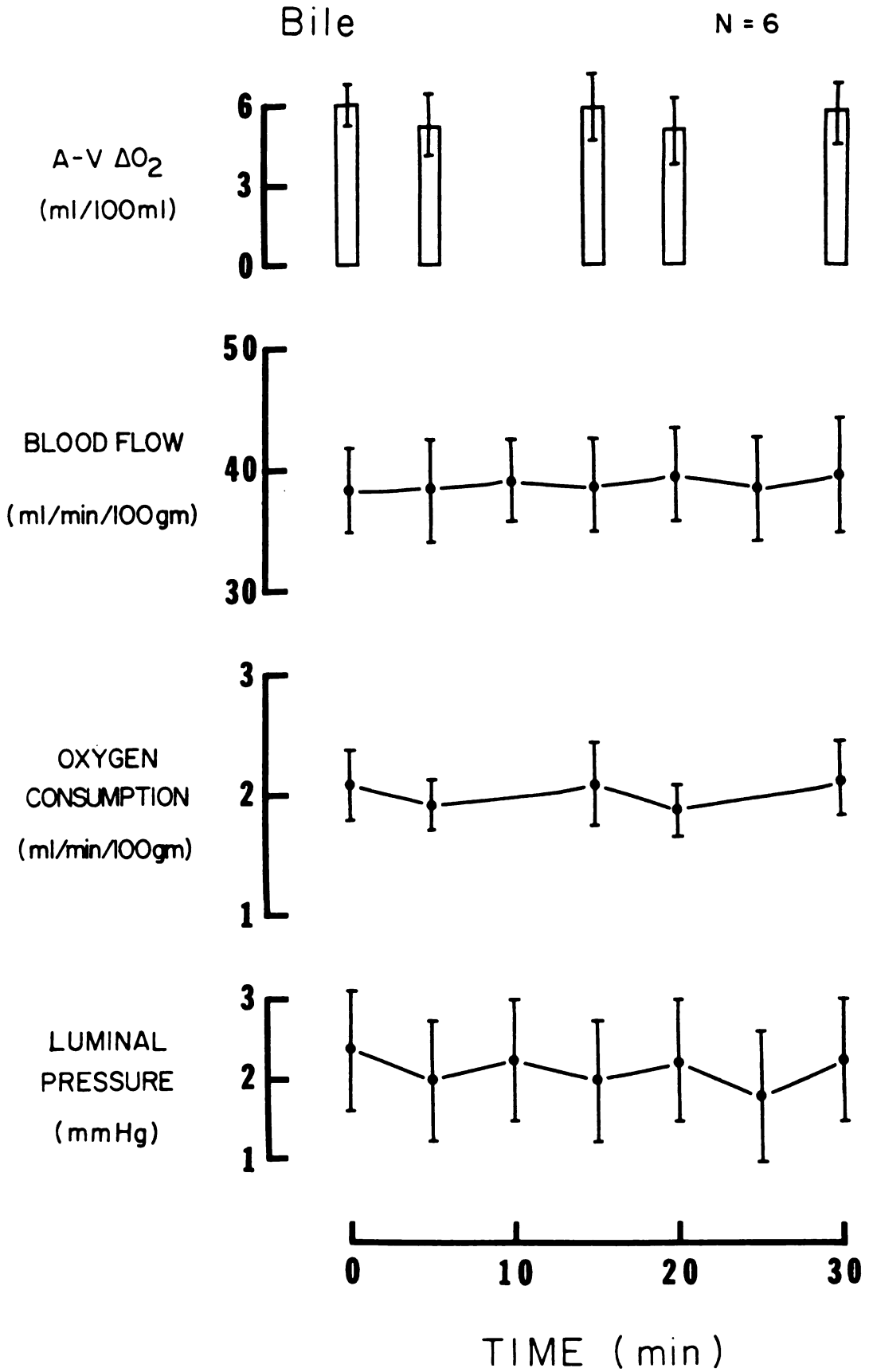
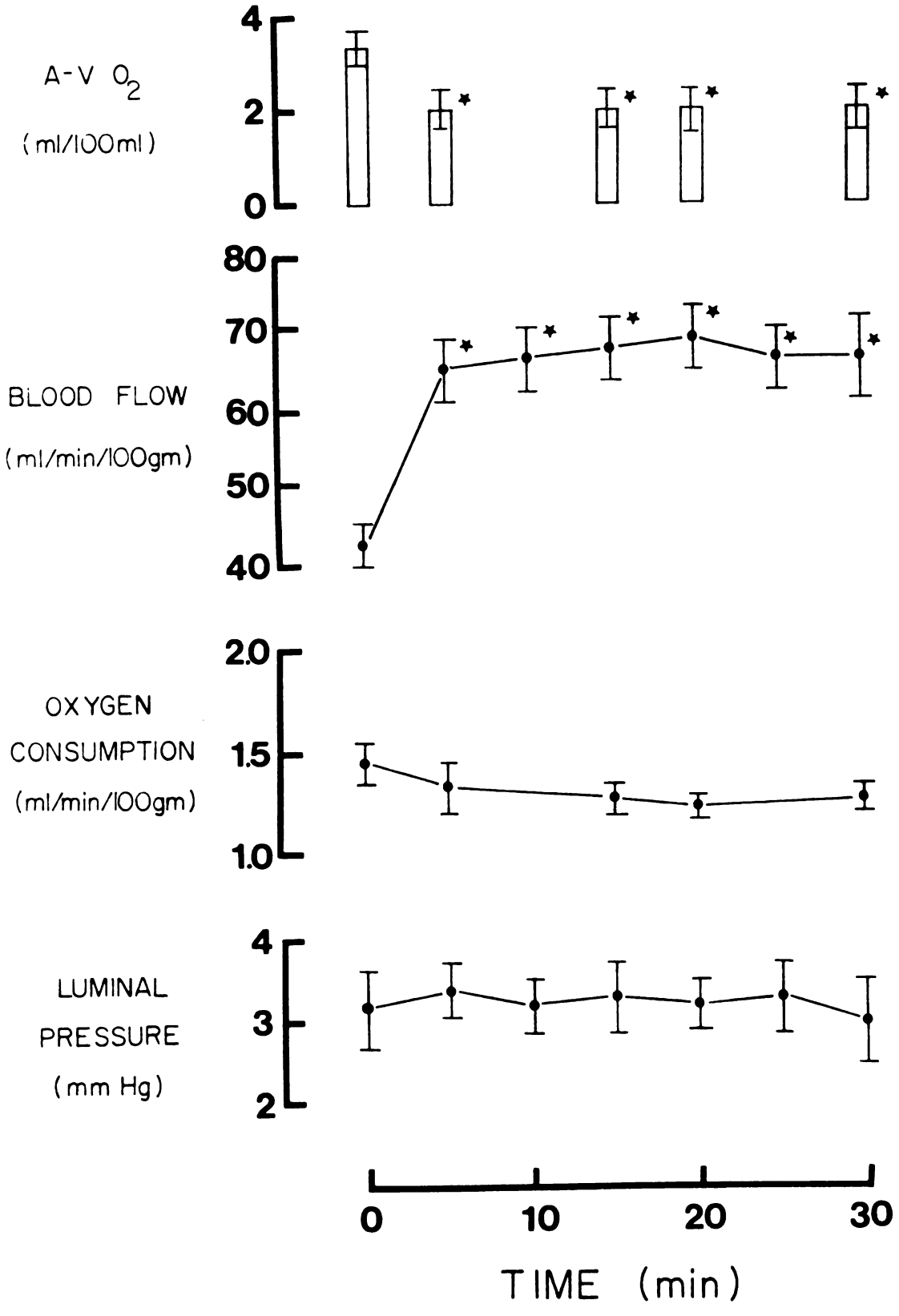

Bile
N = 6
A-V ΔO_2
(ml/100ml)
6
3
0
BLOOD FLOW
(ml/min/100gm)
50
40
30
OXYGEN
CONSUMPTION
(ml/min/100gm)
3
2
1
LUMINAL
PRESSURE
(mmHg)
3
2
1
0
10
20
30
TIME (min)

Fig. 5.--Effects of luminal placement of bile on ileal A-V O_2 difference, blood flow, oxygen consumption and luminal pressure. Asterisks indicate values significantly different from control (time = 0) at $p < 0.05$; $n = 6$.


A-V O_2
(ml/100ml)
4
2
0
BLOOD FLOW
(ml/min/100gm)
80
70
60
50
40
OXYGEN
CONSUMPTION
(ml/min/100gm)
2.0
1.5
1.0
LUMINAL
PRESSURE
(mm Hg)
4
3
2
0
10
20
30
TIME (min)

increased within 5 minutes after the placement and remained at a steady level for 30 minutes. The control flow was 42.1 ± 3 ml/min/100 gm which rose to 68.0 ± 8.2 ml/min/100 gm 20 minutes after the placement, a 61.5% increase in local blood flow. Oxygen consumption decreased slightly but not significantly throughout the 30 minute placement period.

The results from this series of experiments indicate that digested food plus bile, has a greater hyperemic as well as metabolic effect than digested food alone and undigested food plus bile. Addition of bile to digested food, which contained pancreatic enzymes could promote digestion and absorption of lipids in the food thereby enhancing the hyperemia. While both digested food alone and undigested food plus bile increased flow by approximately 10%, only the former increased oxygen consumption. Bile in the jejunal lumen did not have any effect on blood flow or oxygen consumption, but bile in the ileum increased flow by 60% and did not alter oxygen consumption (Figure 5). These results thus indicate that postprandial intestinal hyperemia is a complex phenomenon and different constituents of chyme can elicit different responses in blood flow and oxygen consumption. In the case of digested food and digested food plus bile, the increase in blood flow was accompanied by an increase in local metabolism (Figures 1 and 2). The possibility thus exists that the heightened oxidative metabolism may contribute to the hyperemia induced

by these food solutions. Accordingly, the temporal relationship between these two variables during food placement was examined more closely in the next series of experiments.

Series 2

In this series of experiments, digested food and digested food plus bile were individually placed into the lumen of the proximal jejunum for 30 minutes during which blood flow and oxygen consumption as well as luminal pressure were monitored continuously. Figure 6 shows the hyperemic and metabolic responses of the jejunal segment to the placement of digested food. Flow increased within 15 seconds (8.7 ± 1.7%) after the introduction of this food solution and was accompanied by an increase in oxygen consumption (12.5 ± 2.0%). Flow remained significantly elevated until 20 minutes after placement and then declined back to control level. In contrast, oxygen consumption remained significantly increased throughout the entire 30 minutes placement. These changes were not accompanied by any alterations in blood pressure, luminal pressure or A-V O_2 difference.

Figure 7 shows the relationship between the percent changes from controls of blood flow and oxygen consumption. These data were taken at 15 second intervals for the first 5 minutes after food placement and at 1 minute intervals thereafter. There was a significant

Fig. 6.--The temporal relationships between blood flow, A-V O_2 difference, oxygen consumption, aortic pressure and luminal pressure during placement of digested food alone in the jejunum. The food solution was placed into the lumen at time 0. Both blood flow and oxygen consumption increased significantly ($p < 0.05$) 15 seconds after the placement. Flow remained significantly elevated until 20 minutes after placement and declined gradually back to control level ($p > 0.05$ as compared to 0 time 20 minutes after the placement). In contrast, oxygen consumption remained significantly ($p < 0.05$) increased throughout the entire 30 minute placement period. Aortic pressure, luminal pressure and A-V O_2 difference were not significantly altered ($n = 7$).

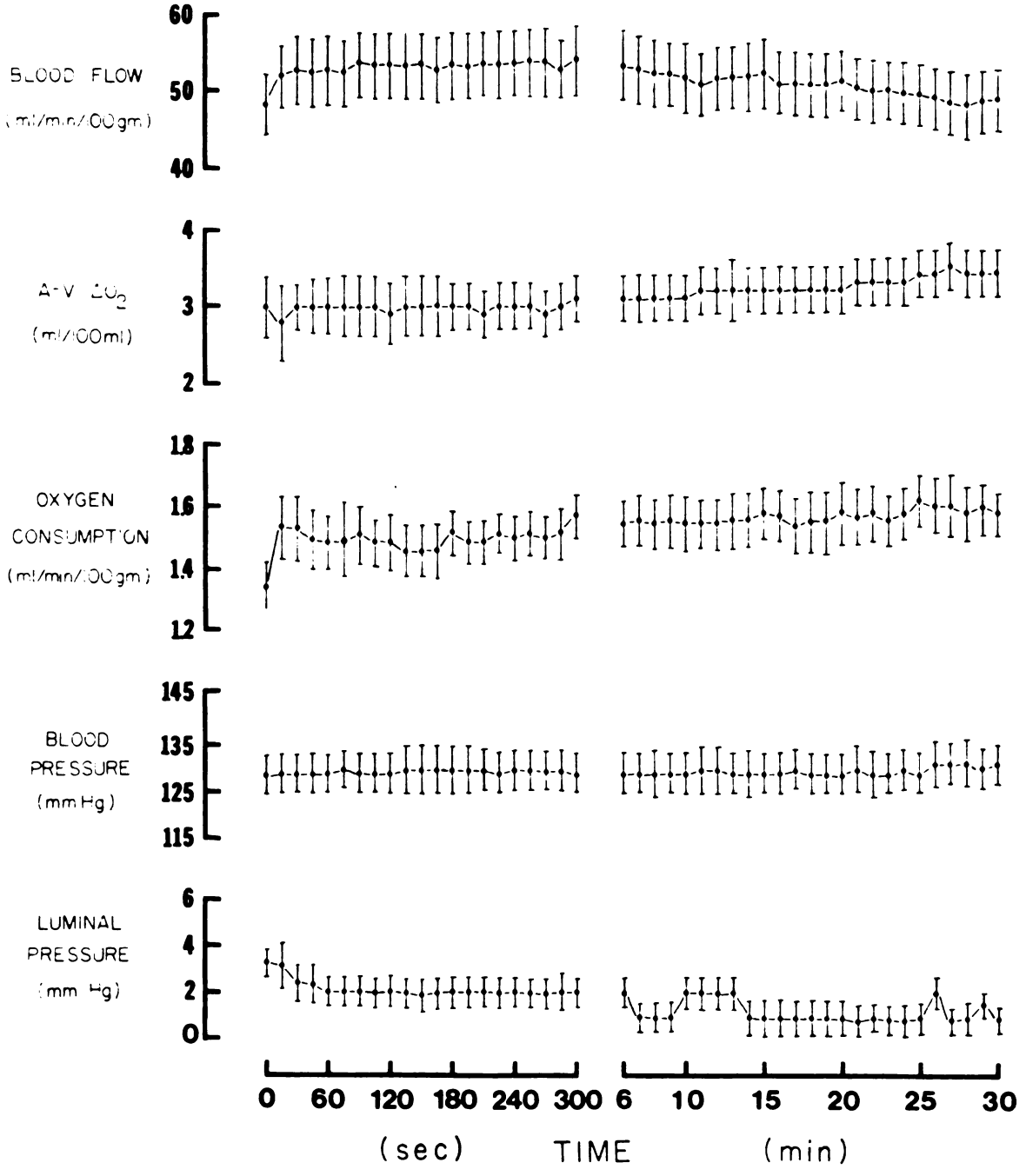
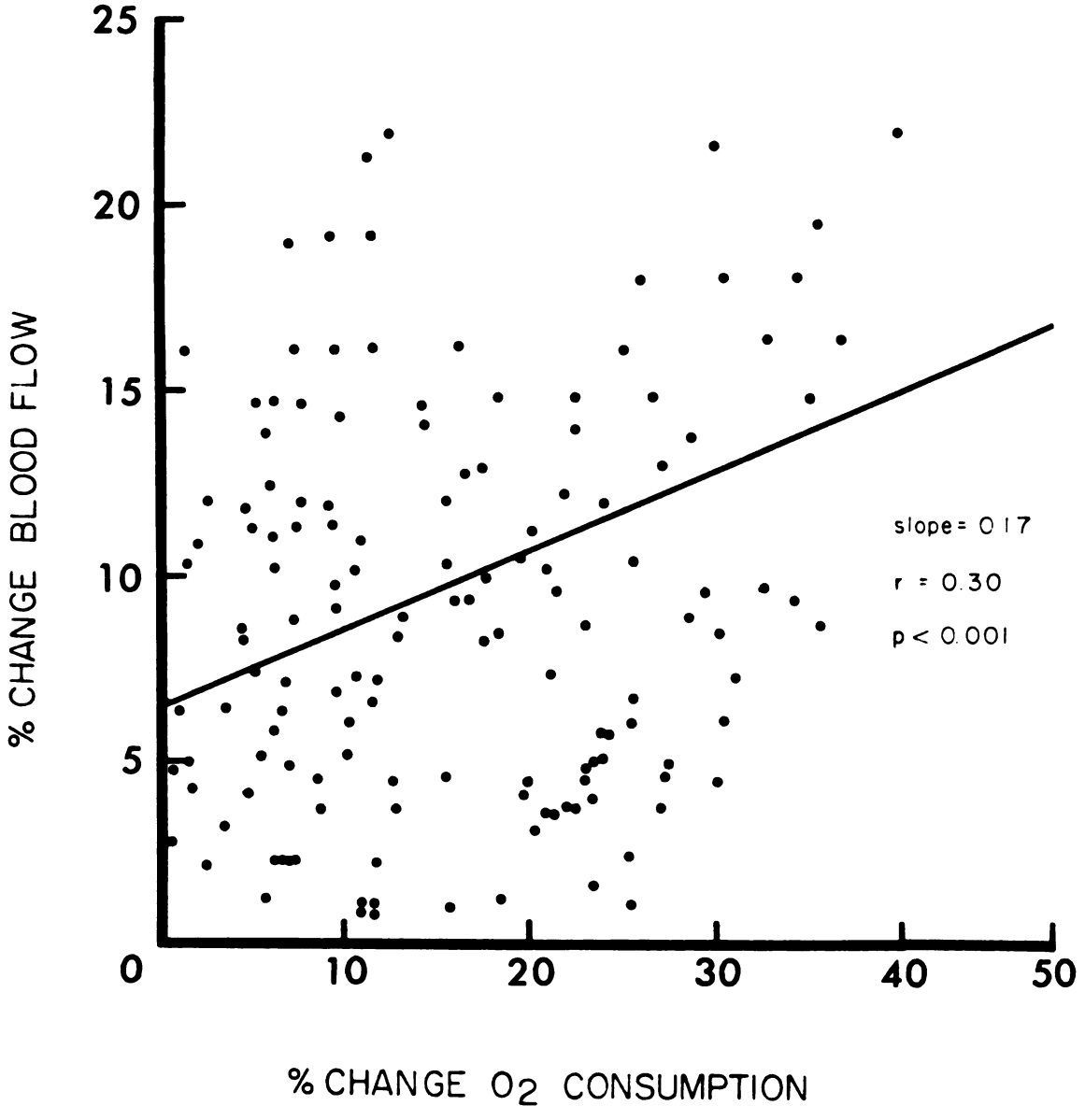

BLOOD FLOW
(ml/min/100gm)
60
50
40
A-V CO_2
(ml/100ml)
4
3
2
OXYGEN
CONSUMPTION
(ml/min/100gm)
1.8
1.6
1.4
1.2
BLOOD
PRESSURE
(mm Hg)
145
135
125
115
LUMINAL
PRESSURE
(mm Hg)
6
4
2
0
0 60 120 180 240 300 6 10 15 20 25 30
(sec) TIME (min)

Fig. 7.--Relationship between the percent changes in blood flow and oxygen consumption during placement of digested food in the jejunum. The variables were recorded at 15 second intervals for the first 5 minutes after food placement and at 1 minute intervals thereafter.


25
20
15
10
5
0
10
20
30
40
50
% CHANGE BLOOD FLOW
% CHANGE O_2 CONSUMPTION
slope = 0.17
r = 0.30
p < 0.001

correlation between the percent change in blood flow and oxygen consumption ($r = 0.3$, $p < 0.001$). The equation for the regression line is: $y = 6.7 + 0.17\ x$, where y = % changes in flow and x = % changes in oxygen consumption.

Figure 8 shows the temporal relationship between blood flow, oxygen consumption, and luminal pressure when digested food plus bile was placed into the jejunal lumen.

Blood flow increased almost immediately and peaked at 5 minutes (51.6 ± 15.5%) after the introduction of the food solution. It remained elevated throughout the entire 30 minute placement period. A-V O_2 difference, on the other hand, fell transiently for 10 minutes ($p < 0.05$) but returned back to control level during the rest of the experimental period. Oxygen consumption increased significantly 15 seconds after the placement of food. This increase reached a peak 10 minutes after placement (+33.3 ± 8.5%) and remained elevated throughout the entire 30 minutes of food placement. Motility was elevated during the initial 10 minutes after food placement but returned to control level during the final 15 minutes. As shown in Figure 9, there was no significant correlation between the percent change in flow and oxygen consumption during the presence of digested food plus bile in the jejunum ($r = 0.03$, $p > 0.5$). The equation for the line is: $y = 44.7 - 0.66\ x$.

This series of experiments shows that both digested food alone and digested food plus bile produce

Fig. 8.--The temporal relationships between blood flow, A-V O_2 difference, oxygen consumption, aortic pressure and luminal pressure during placement of digested food plus bile in the jejunum. The food solution was placed into the lumen at time 0. Both blood flow and oxygen consumption increased significantly ($p < 0.05$) 15 seconds after the placement. They remain elevated throughout the entire 30 minute placement period. There was a significant decrease ($p < 0.05$) in A-V O_2 difference during the initial 5 minutes after food placement. Luminal pressure was elevated significantly ($p < 0.05$) during the first 10 minutes of placement. Aortic pressure was not significantly altered ($n = 6$).

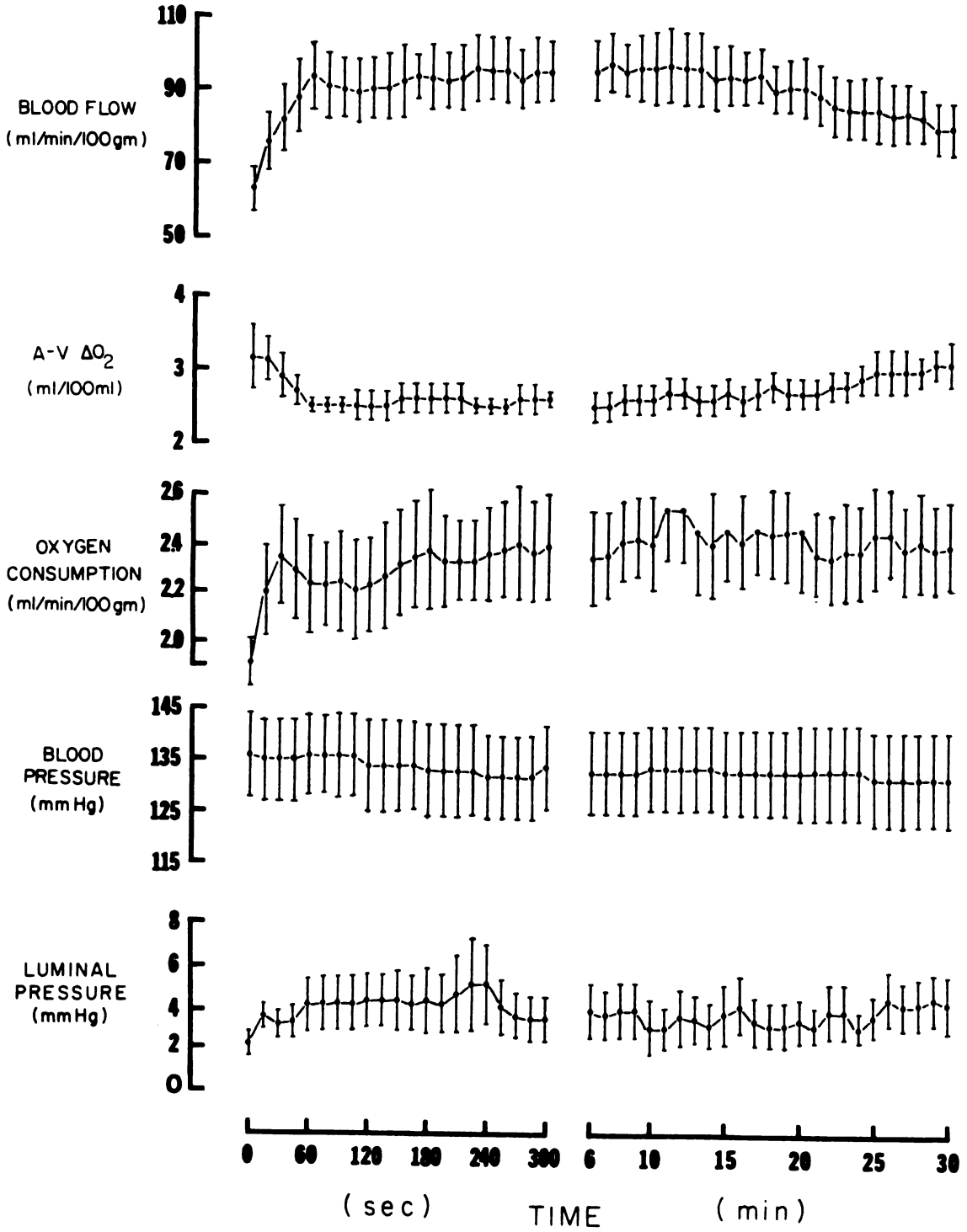
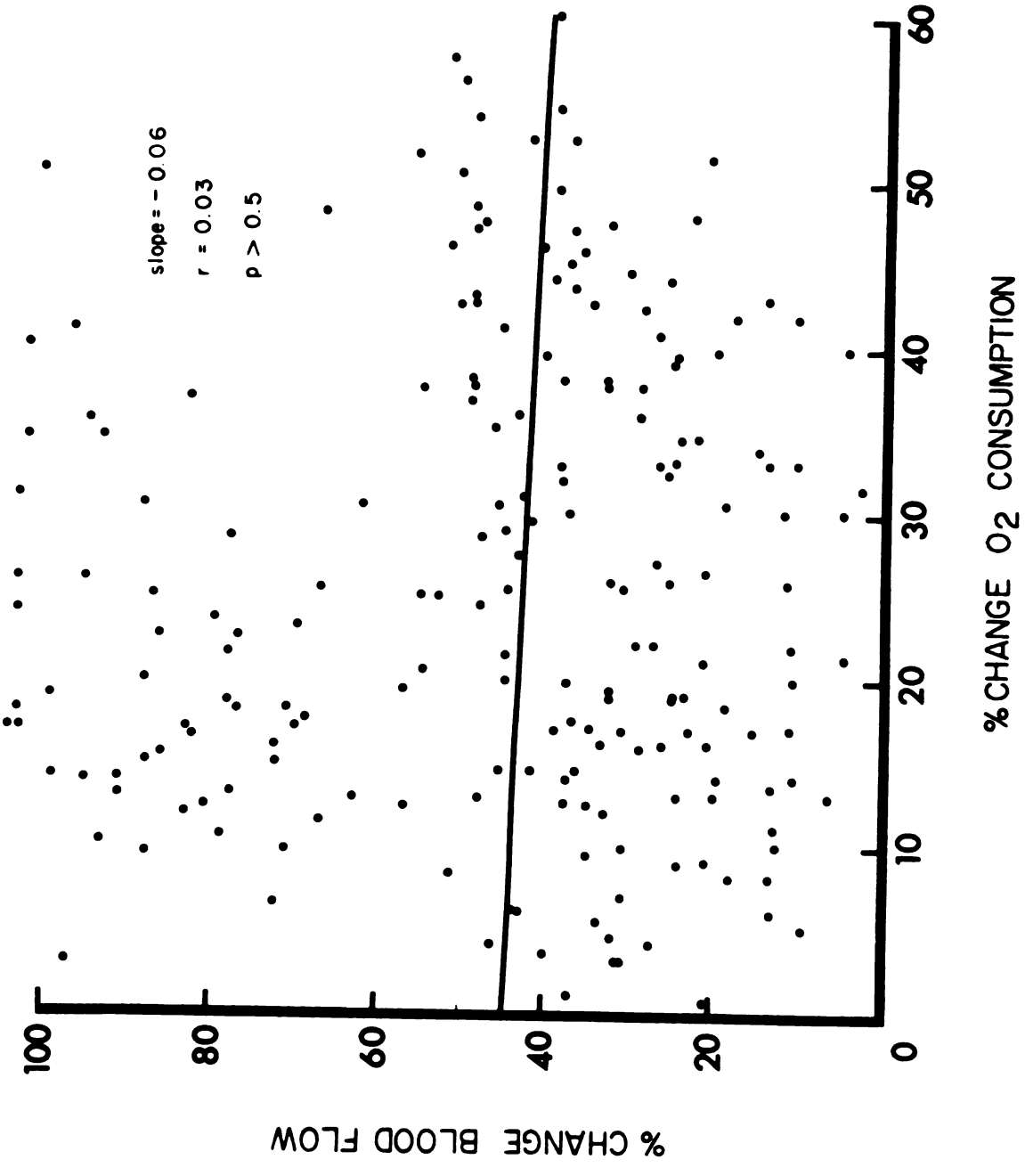

BLOOD FLOW
(ml/min/100gm)
110
90
70
50
A-V ΔO_2
(ml/100ml)
4
3
2
OXYGEN
CONSUMPTION
(ml/min/100gm)
26
24
22
20
BLOOD
PRESSURE
(mmHg)
145
135
125
115
LUMINAL
PRESSURE
(mmHg)
8
6
4
2
0
0
60
120
180
240
300
6
10
15
20
25
30
(sec)
TIME
(min)

Fig. 9.--Relationship between the percent changes in blood flow and oxygen consumption during the placement of digested food plus bile in the jejunum. The variables were recorded at 15 second intervals for the first 5 minutes after food placement and at 1 minute intervals thereafter.


slope = - 0.06
r = 0.03
p > 0.5
% CHANGE BLOOD FLOW
100
80
60
40
20
0
10
20
30
40
50
60
% CHANGE O2 CONSUMPTION

a prompt and simultaneous increase in blood flow and oxygen consumption. In both cases, the increases in flow were maintained at a steady level for 20 minutes and then gradually returned toward control level but the increase in oxygen consumption was maintained at a steady level throughout the 30 minute placement period (Figures 6 and 8). The A-V O_2 difference (oxygen extraction) was either decreased or unchanged during the first 5 minutes and tended to increase during the last 10 minute placement period. Thus, it appears that during the early part the increase in oxygen consumption is primarily met by an increase in blood flow but during the last 10 minutes, an increase in tissue oxygen extraction plays a role in the increase in oxygen consumption since blood flow returned toward control level.

There was a significant correlation between the changes in oxygen consumption and blood flow when digested food alone was in the lumen ($r = 0.30$, $p < 0.001$). Although digested food plus bile produced a much greater increase in both blood flow and oxygen consumption, the correlation between the changes in these variables were not significant. Furthermore, while the y intercept of the regression line of digested food was 6.7% and was not significantly different from the origin (Figure 7), that of digested food plus bile was 44.7% and was significantly different from the origin (Figure 9). This seems to indicate that the presence of bile in the digested food

produces a 44.7% increase in flow even in the absence of any apparent change in oxygen consumption. Moreover, the slope of the line (-0.06, Figure 9) indicates that this increase in flow remained virtually constant over a wide range of increases in oxygen consumption.

The food solutions in the above study contain many nutrients and each of them may have different mechanisms in causing an increase in flow. Thus, in the second part of this study, the mechanisms by which a major nutrient, glucose, causes an increase in local blood flow in the jejunum was examined.

Glucose Studies

Series 1

In this series of experiments, the vascular effects of glucose and its synthetic analogs, 3-0-methyl glucose and 2-deoxy glucose, with or without bile, were compared in a double jejunal segment preparation. During the test period, one segment contained 10 ml of glucose solution and the other contained 10 ml of either 3-0-methyl glucose or 2-deoxy glucose solution so that their vasoactivities could be compared.

After the introduction of the test solutions, blood flow often reaches a steady state within 5 to 10 minutes which lasts for about 20 minutes. Thus, the flow values taken at the 12 to 15 minute period were used for expressing and analyzing the data.

As shown in Figure 10, glucose and 3-0-methyl glucose in the absence of bile significantly increased venous outflow by 8.9% and 4% of precontrol respectively; the difference in their hyperemic effect of 5.0% was statistically significant ($p < 0.05$) the hyperemic effect of glucose (17.2% above control) but not 3-0-methyl glucose (4.3%). The difference in their hyperemic effect thus increased to 13.3%. Figure 11 shows that while glucose significantly increased blood flow, 2-deoxy glucose did not alter flow either with or without bile. Thus, of the three glucoses, only glucose and 3-0-methyl glucose were capable of increasing blood flow with the hyperemic effect of glucose being greater than that of 3-0-methyl glucose. The addition of bile enhanced the hyperemic effect of glucose and not 3-0-methyl glucose or 2-deoxy glucose.

Series 2

The purpose of this series of experiments was to determine whether the differences in the vascular effect of the three glucoses were related to its absorption and/or an increase in local metabolism. A single segment of the proximal jejunum was used in these experiments. Each glucose plus bile was individually placed into the jejunal lumen for 20 minutes. As shown in Figure 12, in the presence of bile, both glucose and 3-0-methyl glucose increased blood flow but only glucose significantly increased oxygen consumption. The placement of glucose

Fig. 10.--Comparison of vascular effects of glucose (G) and 3-0-methyl glucose (3-MG) with or without bile, in double jejunal segments (n = 6). Values above bars indicate percent changes from precontrol. A - B = Difference in changes produced by the two paired solutions. Asterisks indicate that the values are significant at $p < 0.05$. Addition of bile markedly enhanced the hyperemic effect of glucose ($p < 0.05$, two-way analysis of variance) but not 3-0-methyl glucose.

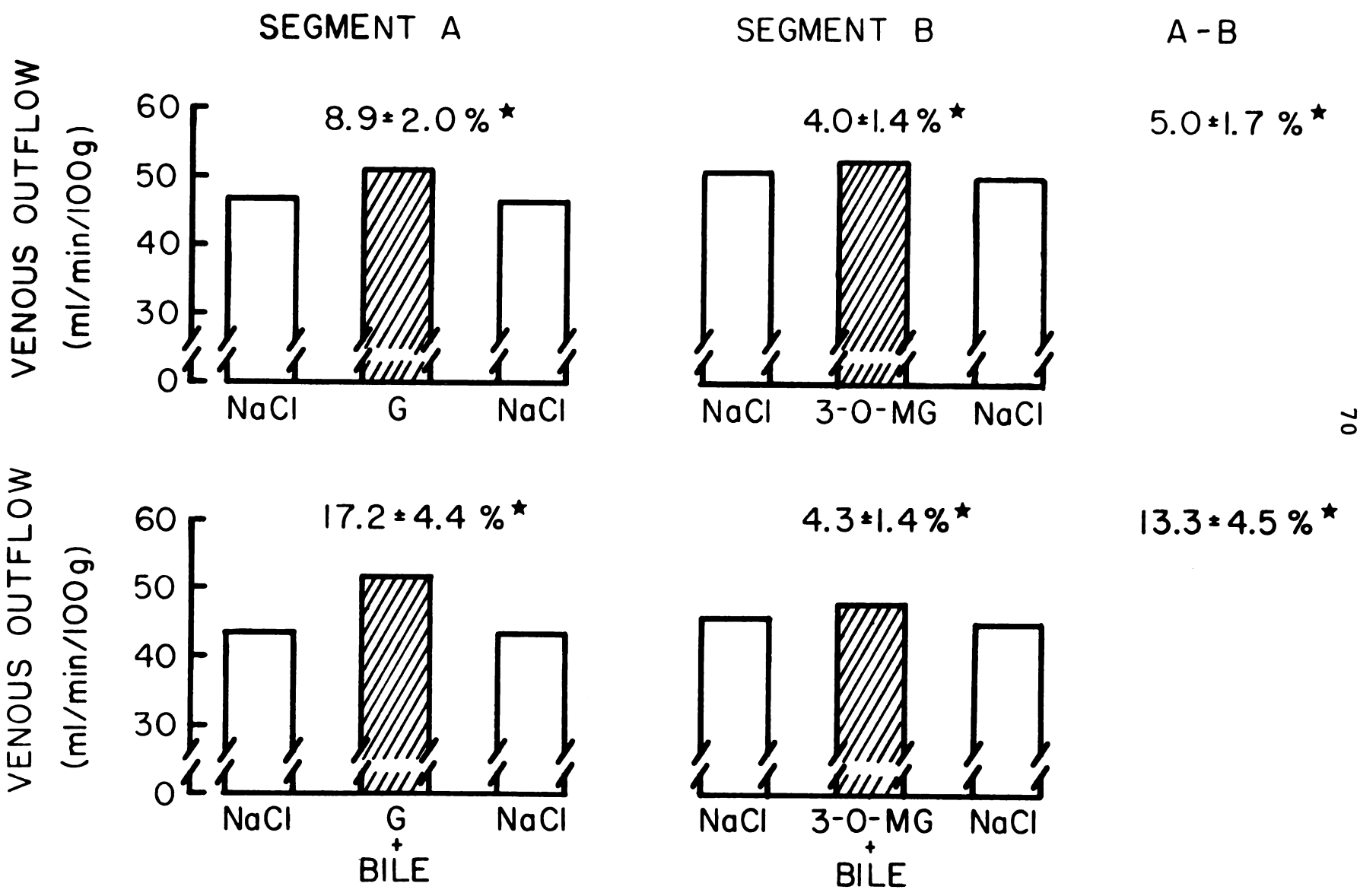

SEGMENT A
SEGMENT B
A - B
VENOUS OUTFLOW (ml/min/100g)
60
50
40
30
0
8.9±2.0%*
4.0±1.4%*
5.0±1.7%*
NaCl
G
NaCl
NaCl
3-O-MG
NaCl
VENOUS OUTFLOW (ml/min/100g)
17.2±4.4%*
4.3±1.4%*
13.3±4.5%*
NaCl
G + BILE
NaCl
NaCl
3-O-MG + BILE
NaCl

Fig. 11.--Comparison of the vascular effects of glucose (G) and 2-deoxy glucose (2-DG) with or without bile, in the double jejunal segments (n = 7). Values above bars indicate percent change from precontrol. A - B = Difference in changes produced by two paired solutions. Asterisks indicate that the values are significant at $p < 0.05$. Addition of bile markedly enhanced the hyperemic effect of glucose ($p < 0.05$, two-way analysis of variance) but not 2-deoxy glucose.

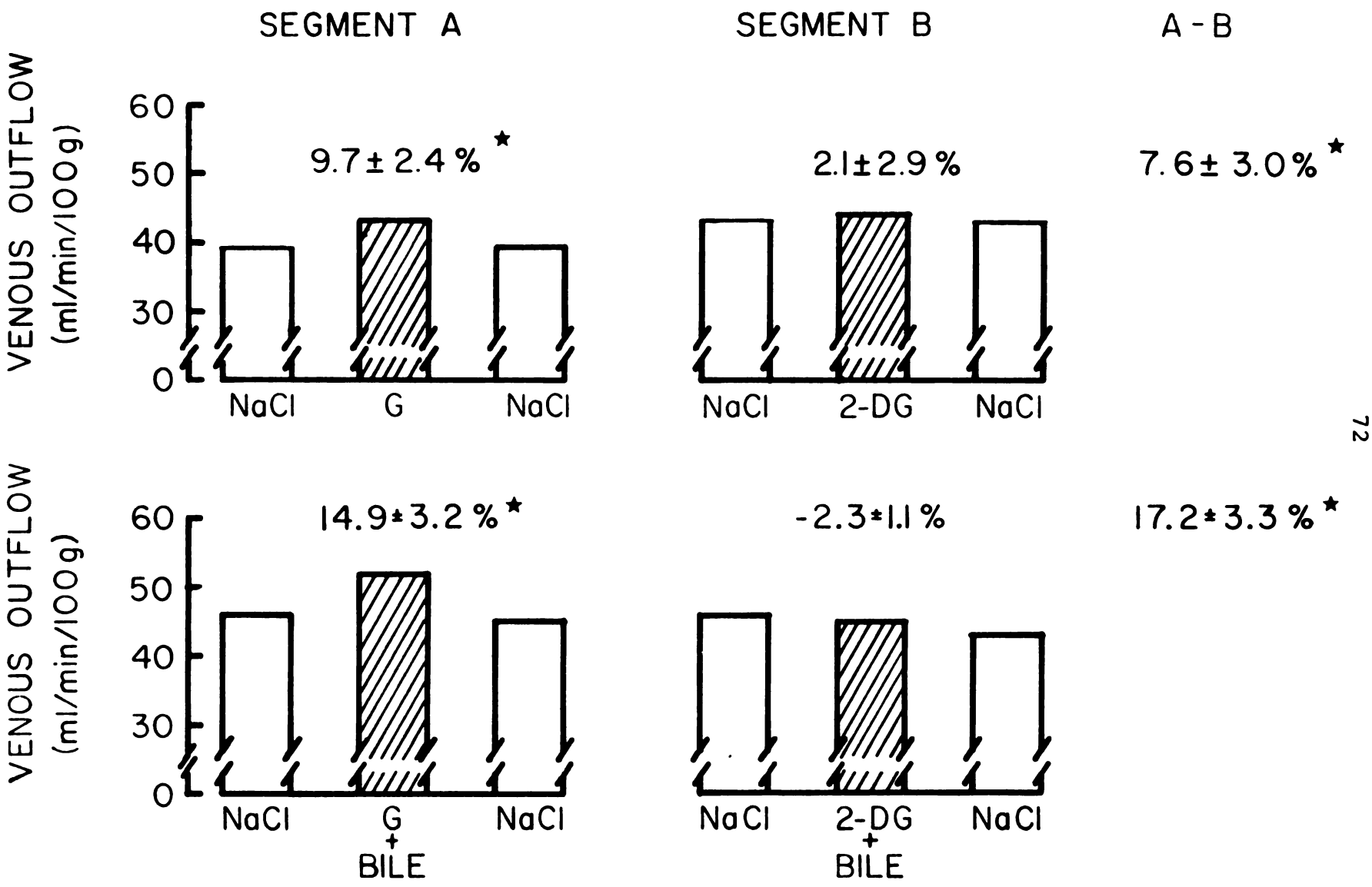
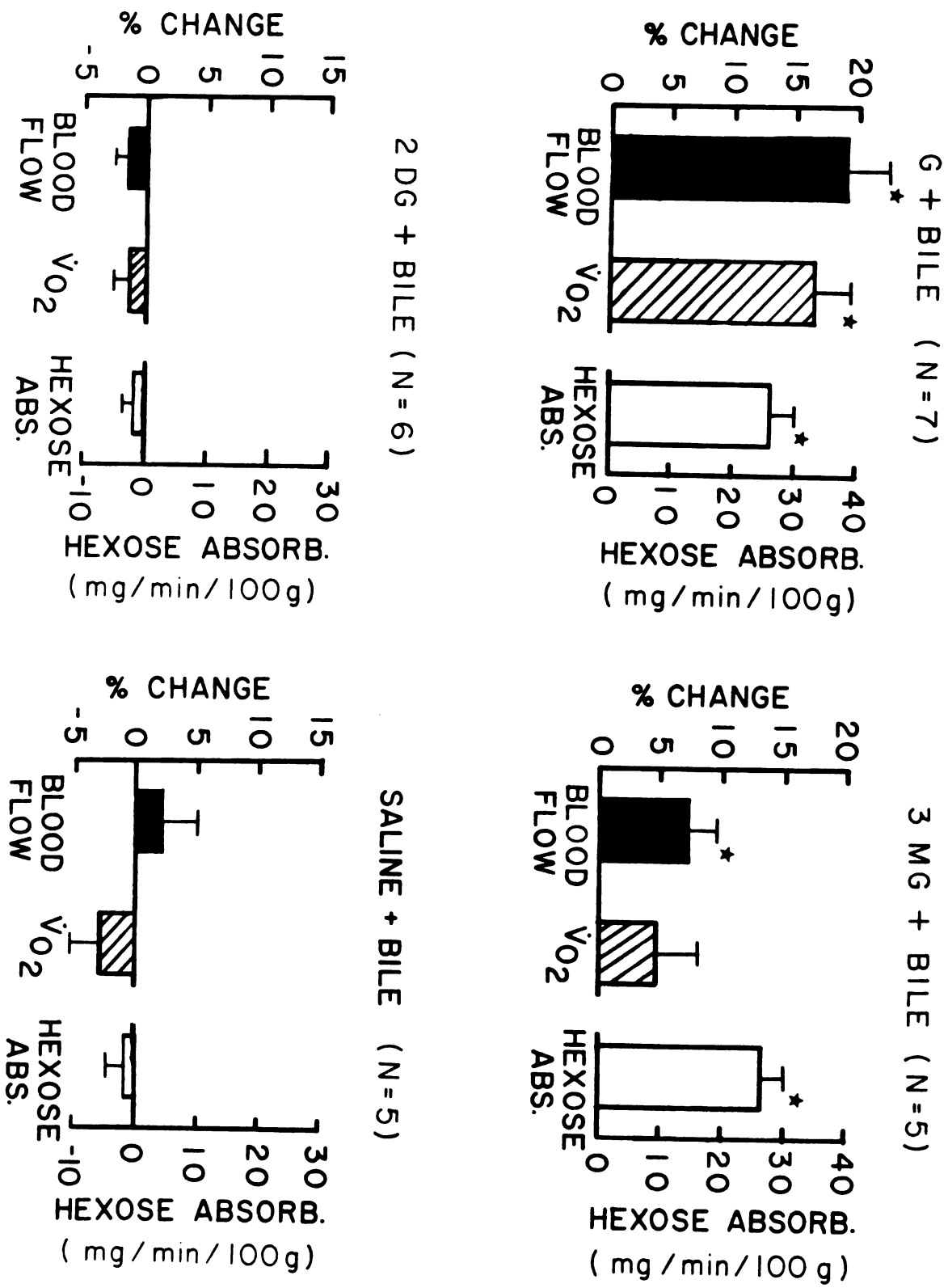

SEGMENT A
SEGMENT B
A - B
VENOUS OUTFLOW (ml/min/100g)
60
50
40
30
0
9.7 ± 2.4 % ★
2.1 ± 2.9 %
7.6 ± 3.0 % ★
NaCl
G
NaCl
NaCl
2-DG
NaCl
14.9 ± 3.2 % ★
-2.3 ± 1.1 %
17.2 ± 3.3 % ★
NaCl
G + BILE
NaCl
NaCl
2-DG + BILE
NaCl

Fig. 12.--Percent changes in blood flow and oxygen consumption from precontrol after luminal placement of glucose (G), 3-0-methyl glucose (3-MG), 2-deoxy glucose (2-DG), with bile, and bile alone. Control blood flow was 42.5 ± 2.1 ml/min/100 g and VO_2 was 1.50 ± 0.05 ml/min/100 g. HEXOSE ABS. denotes the rate of absorption of the hexose placed into the lumen. During the placement of Saline + bile, the rate of glucose absorption was determined. Asterisks indicate that the values are significant at $p < 0.05$.


% CHANGE
BLOOD FLOW
$\dot{V}O_2$
HEXOSE ABS.
HEXOSE ABSORB. (mg/min/100g)
G + BILE (N=7)
2 DG + BILE (N=6)
3 MG + BILE (N=5)
SALINE + BILE (N=5)

plus bile increased local oxygen consumption by 17.8 ± 5.9%. The hyperemic effect of glucose (18.2 ± 4.8%) was significantly greater than that of the 3-0-methyl glucose (7.4 ± 2.3%). There was however no significant difference between the absorption rates of glucose and 3-0-methyl glucose ($p > 0.05$, unpaired t-test). Bile alone or 2-deoxy glucose plus bile did not alter blood flow or oxygen consumption. There was no hexose absorbed when saline (during control periods), bile, or 2-deoxy glucose plus bile were in the lumen.

The results from these two series of experiments show that of the three glucoses, only those that are absorbed (glucose and 3-0-methyl glucose) caused an increase in blood flow and that glucose, which increased oxygen consumption, caused a greater hyperemic effect than 3-0-methyl glucose, which did not increase oxygen consumption. This seems to indicate that both the absorption of glucose and the increase in oxidative metabolism may contribute to the glucose induced hyperemia. The results also indicate that bile only enhanced the hyperemic effect of glucose but not that of 3-0-methyl glucose or 2-deoxy glucose. This enhancing effect of bile does not appear to be due to the vascular effect of bile since bile alone in the jejunal lumen does not increase local blood flow. The enhancing effect of bile may be related to glucose absorption and/or oxygen consumption. Thus, in the next series of experiments, the effects on blood flow, glucose

absorption and oxygen consumption of luminal placement of glucose alone and glucose plus bile were compared in the proximal jejunum.

Series 3

Glucose alone or glucose plus bile was placed in the lumen of a jejunal segment for 20 minutes during which blood flow, oxygen consumption and luminal pressure were continuously monitored.

Figure 13 shows the effect of luminal placement of 200 mM glucose in saline. Blood flow increased immediately after glucose was introduced into the lumen. Flow increased from a control value of 49.3 ± 3.6 to 52.6 ± 4.1 ml/min/100 gm at 15 minutes. This represented a 6.1 ± 1.4% increase from control. Oxygen consumption increased gradually from 1.60 ± 0.16 at control to 1.72 ± 0.17 ml/min/100 gm at 15 minutes. This was a 9.1% increase from control. The increase in oxygen consumption was not through an enhanced extraction of oxygen since A-V O_2 difference was not significantly altered. Thus the increase in oxygen consumption was met by an increase in blood flow. Glucose absorption increased from 0 at control to 24.0 ± 3.2 mg/min/100 gm at 15 minutes after placement of the solution. Luminal pressure on the other hand, was not significantly altered. Thus, all three monitored variables (blood flow, oxygen consumption and glucose absorption) increased during the 20 minute period when glucose was present in the lumen.

Fig. 13.--Effects of luminal placement of 200 mM glucose in saline on jejunal blood flow, A-V O_2 difference, oxygen consumption, glucose absorption, and luminal pressure. Asterisks indicate values significantly different from control (time = 0) at $p < 0.05$, $n = 6$.

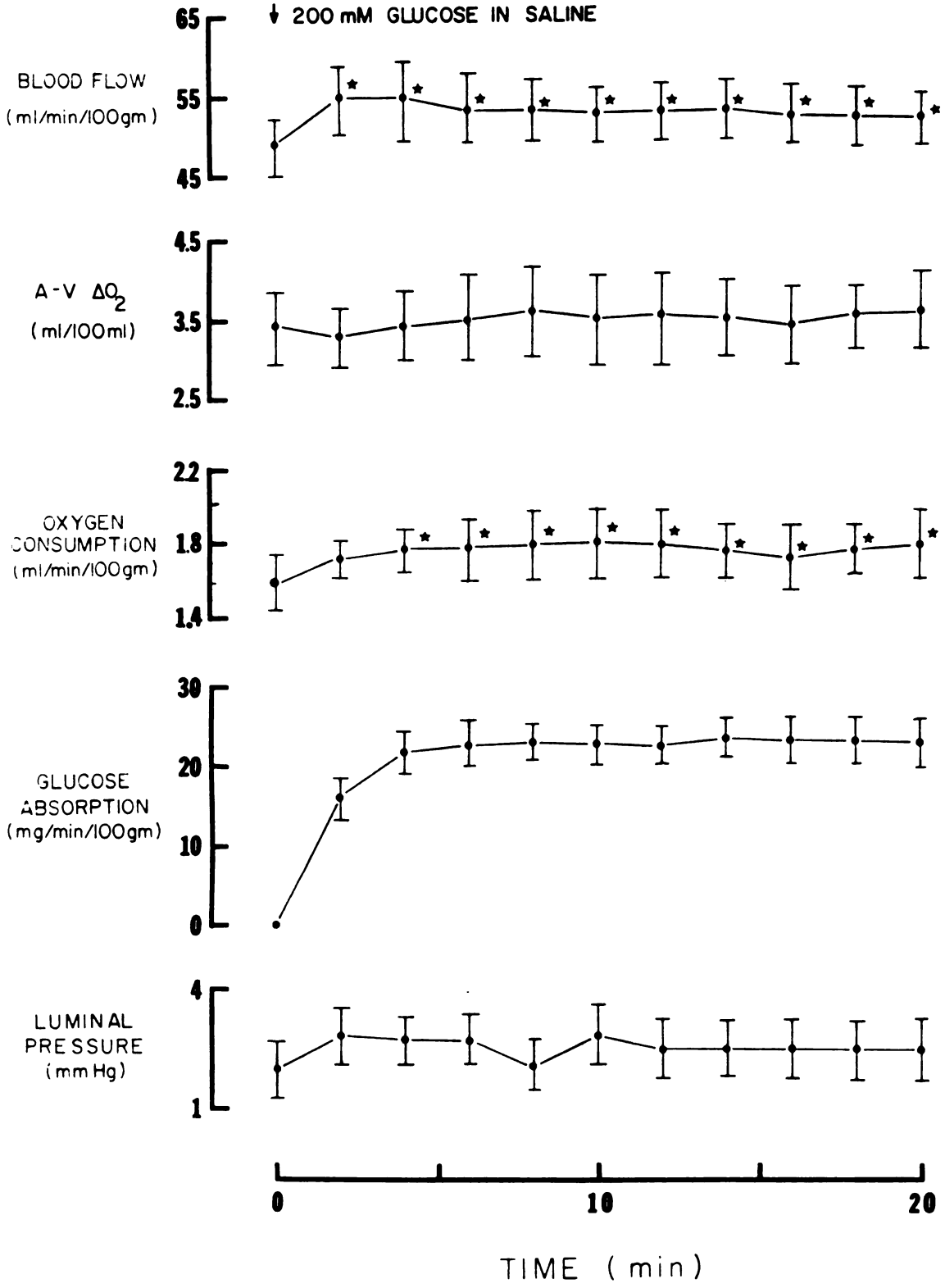

200 mM GLUCOSE IN SALINE
BLOOD FLOW
(ml/min/100gm)
65
55
45
A-V ΔO_2
(ml/100ml)
4.5
3.5
2.5
OXYGEN
CONSUMPTION
(ml/min/100gm)
2.2
1.8
1.4
GLUCOSE
ABSORPTION
(mg/min/100gm)
30
20
10
0
LUMINAL
PRESSURE
(mm Hg)
4
1
0
10
20
TIME (min)

Figure 14 shows the relationship between the percent changes in blood flow and oxygen consumption. There was a significant correlation between the changes in the two variables ($r = 0.25$, $p < 0.01$). The equation for the line is: $y = 5.9 + 0.19\ x$. There was also a significant correlation between the change in blood flow and glucose absorption as shown in Figure 15 ($r = 0.27$, $p < 0.001$). The equation for the regression line is $y = 4.9 + 0.12\ x$.

Figure 16 shows the effects of luminal placement of glucose plus bile on local blood flow, A-V O_2 difference, oxygen consumption, glucose absorption and luminal pressure. Blood flow increased immediately following introduction of glucose plus bile and the hyperemia (25.0 ± 4.3% at 10 minutes) persisted for the entire 20 minute placement period. Oxygen consumption increased gradually from 1.58 ± 0.14 at control to 2.00 ± 0.19 ml/min/100 gm at 15 minutes. This represented a 21.9 ± 4.5% increase from control. The increase in oxygen consumption was not through an enhanced extraction of oxygen since A-V O_2 difference was not significantly altered. Glucose absorption increased from 0 at control to 26.7 ± 4.7 mg/min/100 gm at 15 minutes after placement of the solution. Luminal pressure was not significantly altered.

Figure 17 shows the relationship between the percent changes in blood flow and oxygen consumption. There was no significant correlation between the changes in these variables ($r = 0.05$, $p > 0.5$). The equation for the line

Fig. 14.--Relationship between the percent changes in blood flow and oxygen consumption during the placement of 200 mM glucose in saline in the jejunum. The data included were the values recorded at 15 second intervals for the first 5 minutes after glucose placement and at 1 minute intervals thereafter. The data was linearly correlated using the least squares regression analysis.

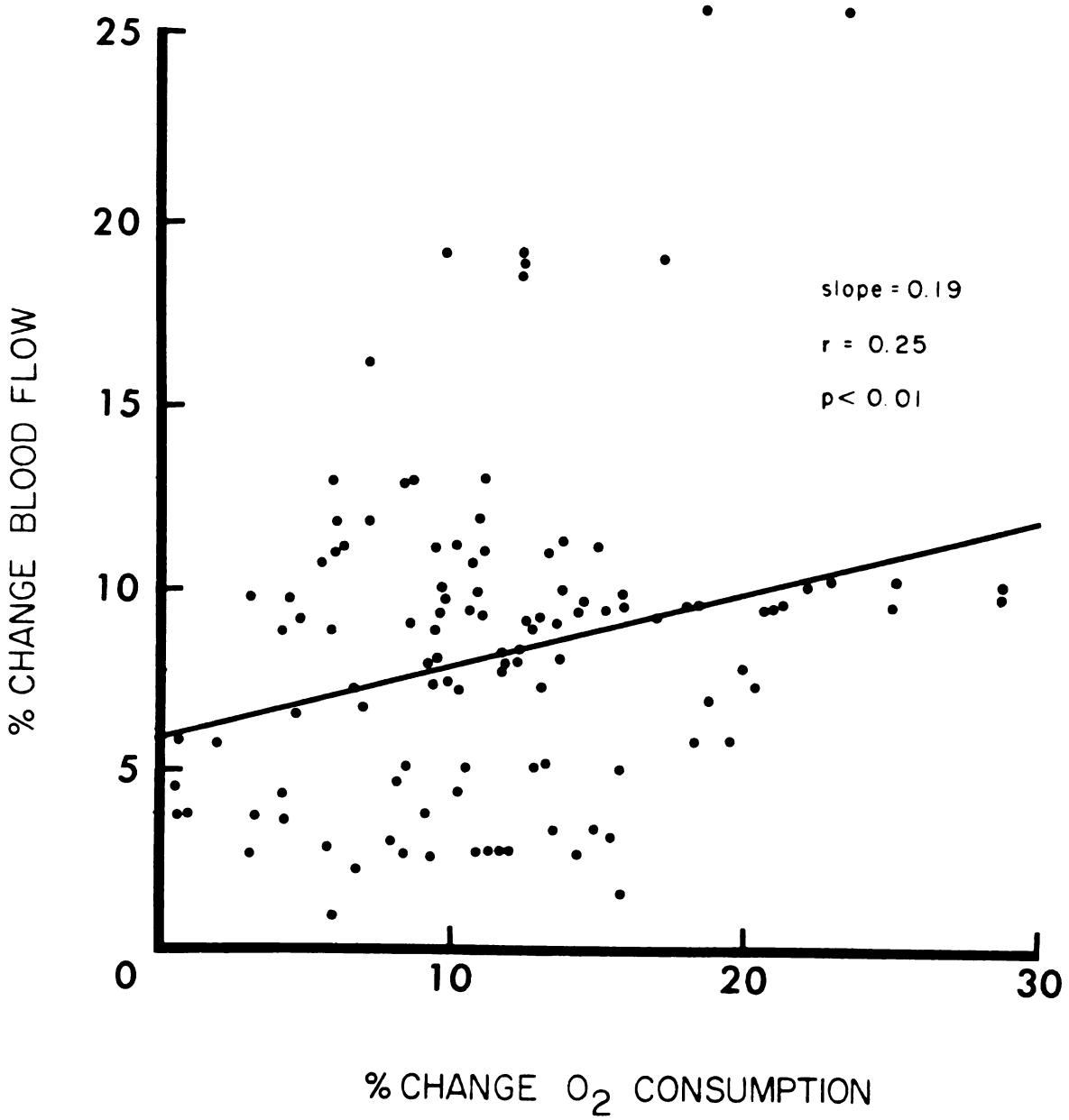

25
20
15
10
5
0
10
20
30
slope = 0.19
r = 0.25
p< 0.01
% CHANGE BLOOD FLOW
% CHANGE O_2 CONSUMPTION

Fig. 15.--Relationship between the percent changes in blood flow and the rates of glucose absorption during the placement of 200 mM glucose in saline in the jejunum. The data included the values recorded at 15 second intervals for the first 3 minutes after glucose placement and at 1 minute intervals thereafter. The data was linearly correlated using the least squares regression analysis.

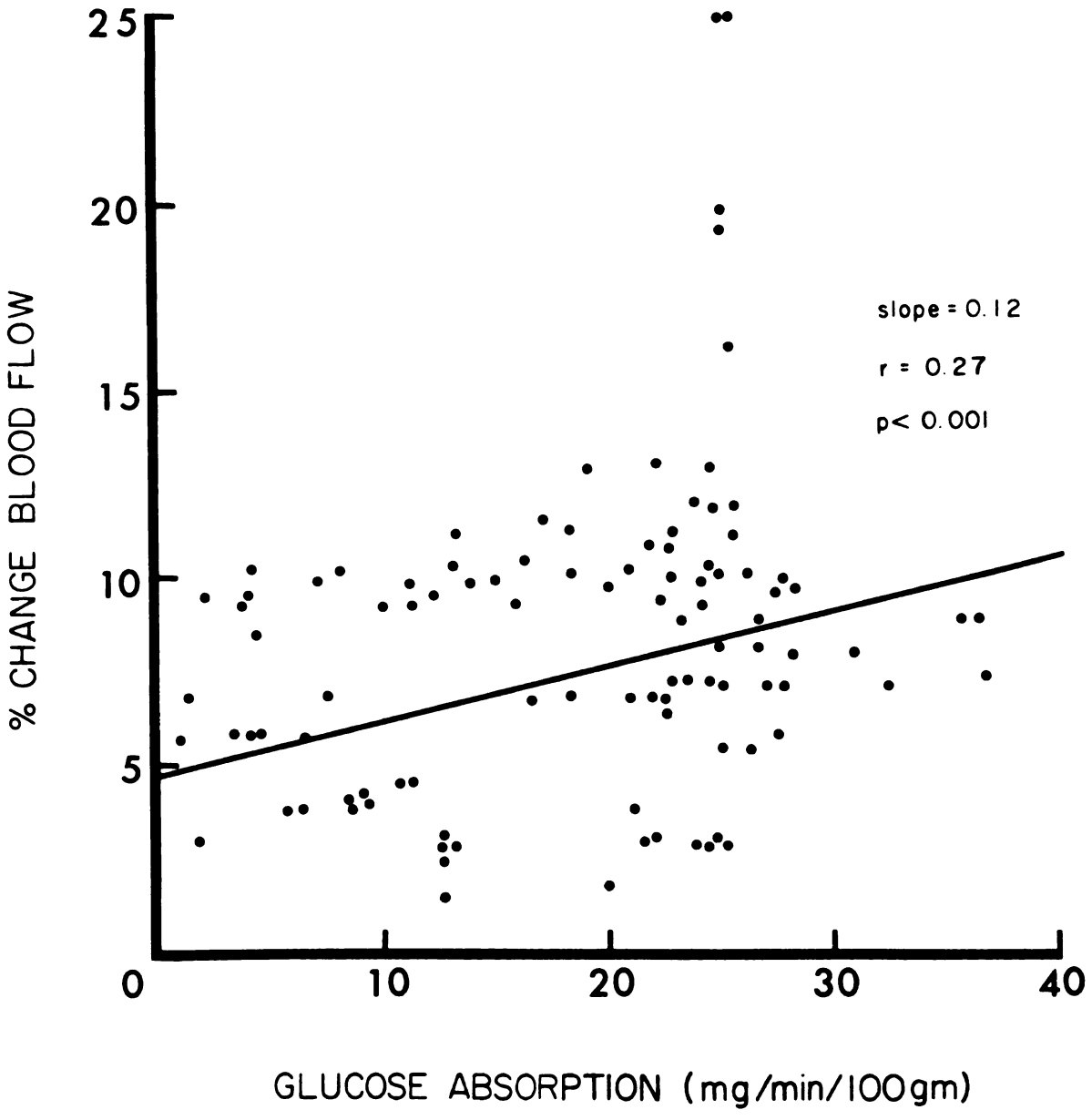

25
20
15
10
5
0
10
20
30
40
% CHANGE BLOOD FLOW
GLUCOSE ABSORPTION (mg/min/100gm)
slope = 0.12
r = 0.27
p< 0.001

Fig. 16.--Effects of 200 mM glucose plus bile on jejunal blood flow, A-V O_2 difference, oxygen consumption, glucose absorption, and luminal pressure. Asterisks indicate values significantly different from control (time = 0) at $p < 0.05$; $n = 6$.

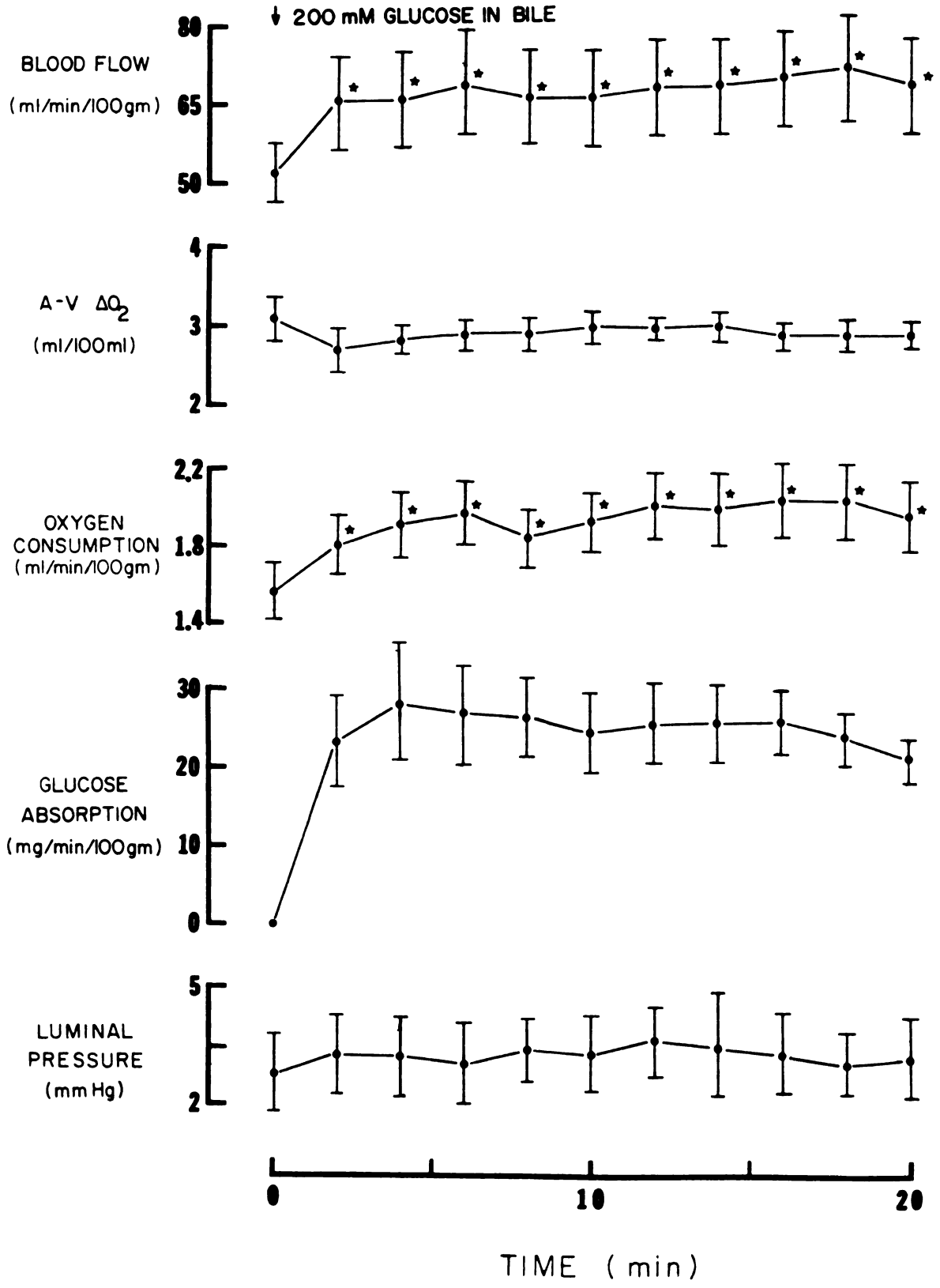
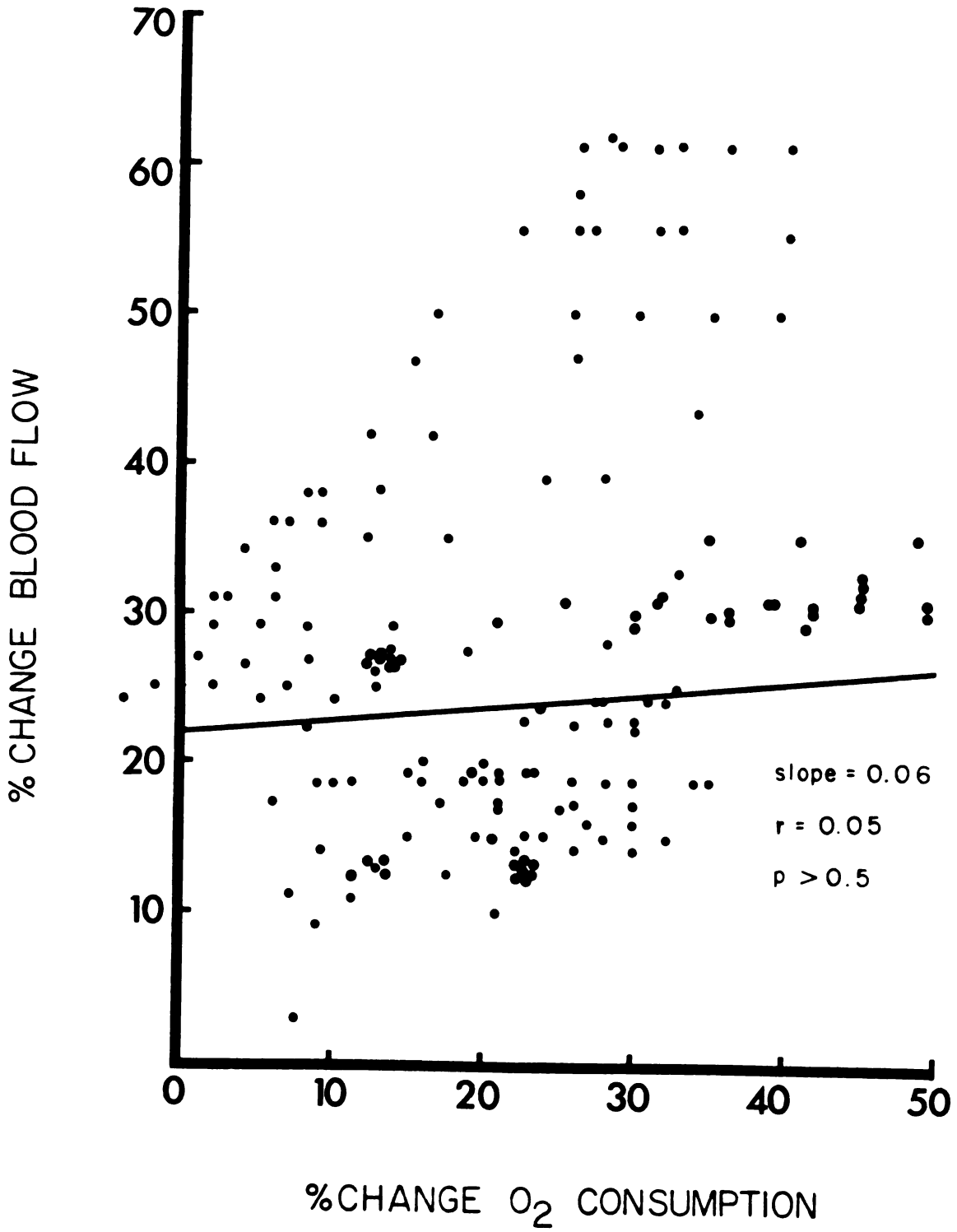

↓ 200 mM GLUCOSE IN BILE
BLOOD FLOW
(ml/min/100gm)
80
65
50
A-V ΔO2
(ml/100ml)
4
3
2
OXYGEN
CONSUMPTION
(ml/min/100gm)
2.2
1.8
1.4
GLUCOSE
ABSORPTION
(mg/min/100gm)
30
20
10
0
LUMINAL
PRESSURE
(mm Hg)
5
2
0
10
20
TIME (min)

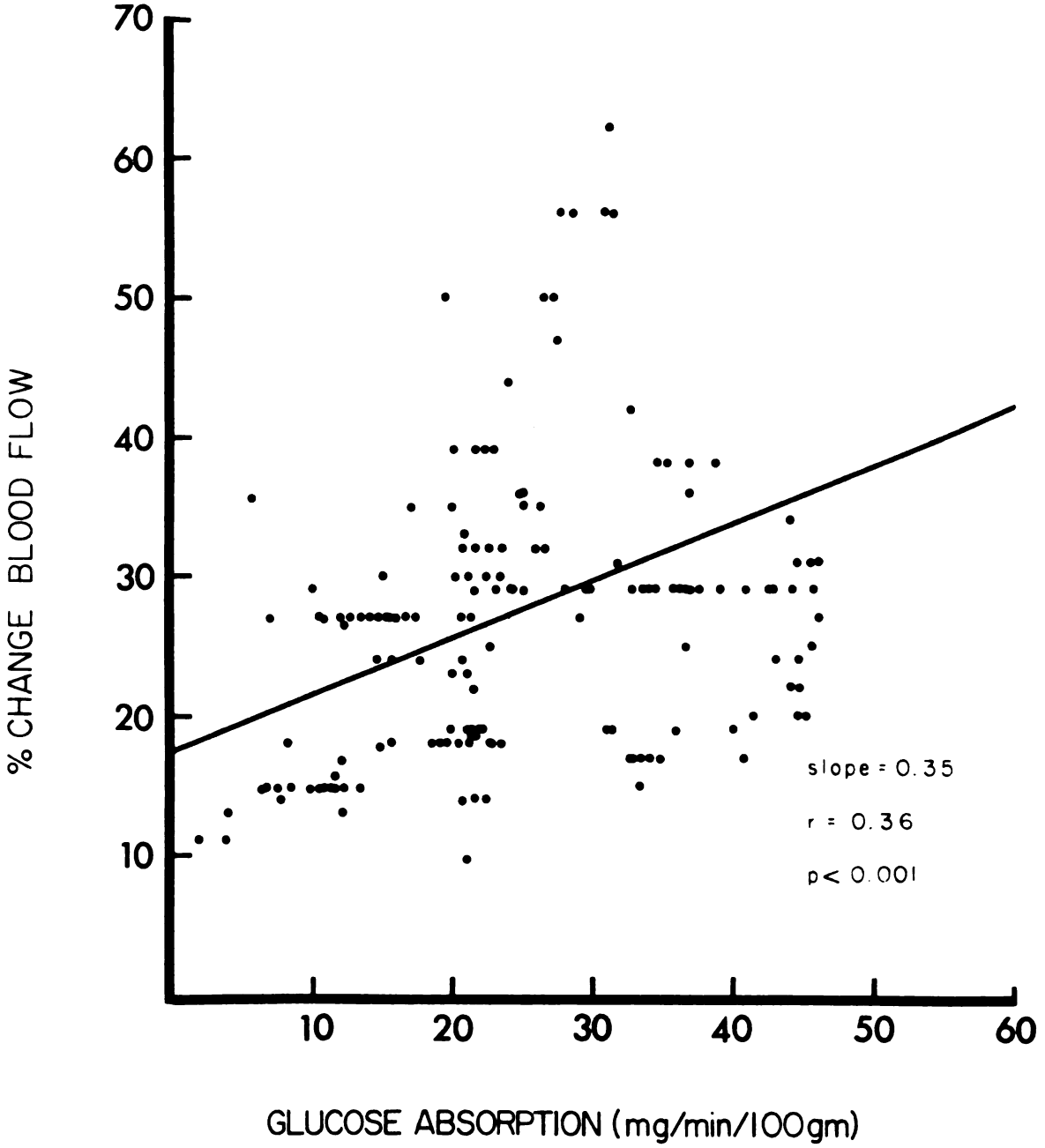
Fig. 17.--Relationship between the percent changes in blood flow and oxygen consumption during the placement of 200 mM glucose plus bile in the jejunum. The variables were recorded at 15 second intervals for the first three minutes after glucose placement and at 1 minute intervals thereafter. The data was linearly correlated using the least squares regression analysis.


70
60
50
40
30
20
10
0
10
20
30
40
50
% CHANGE BLOOD FLOW
%CHANGE O_2 CONSUMPTION
slope = 0.06
r = 0.05
p > 0.5

is $y = 23.6 + 0.06\ x$. In contrast, there was a significant correlation between the percent change in blood flow and glucose absorption ($r = 0.36$, $p < 0.001$; Figure 18). The equation for the line is: $y = 17.9 + 0.35\ x$.

Thus the results from this series of experiments indicate that the hyperemic effect of glucose plus bile was significantly greater than that of the glucose plus saline solution. This greater increase in blood flow was accompanied by a greater increase in oxygen consumption ($p < 0.05$) but not glucose absorption. The increase in oxygen consumption during the placement of glucose plus saline was 14.0 ± 3.4% above control and during glucose plus bile placement was 22.7 ± 5.6% above control. Although the changes in blood flow and oxygen consumption produced by glucose plus saline were significantly correlated (Figure 14), changes in these two variables caused by glucose plus bile were not significantly correlated (Figure 17). Also, while the y intercept of the regression line of glucose plus saline was 5.9% which was not significantly different from the origin (Figure 14), that of glucose plus bile was 23.6% which was significantly different from the origin (Figure 17). This indicates that glucose plus bile could produce a 23.6% increase in flow even in the absence of any apparent change in oxygen consumption. Furthermore, the slope of the line, i.e., 0.06, seems to suggest that the hyperemia could occur over a wide range of increases in oxygen consumption. Thus, the influence

Fig. 18.--Relationship between the percent changes in blood flow and glucose absorption during the placement of 200 mM glucose plus bile in the jejunum. The variables were recorded at 15 second intervals for the first three minutes after glucose placement and at 1 minute intervals thereafter. The data was linearly correlated using the least squares regression analysis.


70
60
50
40
30
20
10
% CHANGE BLOOD FLOW
slope = 0.35
r = 0.36
p< 0.001
10
20
30
40
50
60
GLUCOSE ABSORPTION (mg/min/100gm)

of bile on the relationship between glucose induced increases in flow and oxygen consumption is essentially similar to its influence on digested food induced increases in flow and oxygen consumption, i.e., addition of bile to digested food altered the significant relationship between the two variables and the slope of the regression line (Figure 9).

The addition of bile into the glucose solution however did not change the rate of glucose absorption (Figures 13 and 16). Moreover, the relationship between the changes in blood flow and glucose absorption remained the same, i.e., significantly correlated, with or without bile (Figures 15 and 18). Thus, while bile abolished the significant correlation between glucose induced increases in blood flow and oxygen consumption, it did not alter the relationship between changes in blood flow and glucose absorption.

CHAPTER V

DISCUSSION

Postprandial intestinal hyperemia is characterized by an increase in blood flow through the digestive organs with little or no changes in cardiac output, systemic pressure, or flows to other peripheral vascular beds (16, 25, 43, 120-122). In an extensive series of studies, Chou and co-workers (7, 13) demonstrated that this mesenteric hyperemia is a local phenomenon and is confined only to that region of the small intestine which is exposed to food. Brandt et al. (14) and Dagenais et al. (29) observed that the hemodynamic changes after a meal in humans are associated with an enhancement of splanchnic oxidative metabolism. In addition, the presence of glucose and amino acids in the gut lumen has consistently been shown to increase both blood flow and oxygen consumption of the small intestine (118, 119).

The results from these studies have led to a hypothesis suggesting that the postprandial increase in gut blood flow is linked to the heightened metabolic activities during digestion and absorption (34, 118). However, unlike the heart or the skeletal muscle where many classical

studies have been performed to correlate the rate of metabolism and functional hyperemia (69), there are virtually no data available to clearly establish that local regulation of intestinal blood flow during digestion is related to factors that are linked to oxidative metabolism.

The purpose of this study was to examine the relationship between the intestinal hyperemia and the increase in oxidative metabolism during digestion. In the first series of experiments, various food solutions were placed in the small intestine and their effects on blood flow and oxygen consumption were studied. The food solutions used were: digested food plus bile, which is a completely digested food solution that has been shown to cause a maximal hyperemic response; digested food alone and undigested food plus bile, which are partially digested food solutions that cause a slight increase in flow (26); and bile alone, which has no vascular effects in the jejunum but increases flow in the ileum (26). The aim was to examine the relationship between the changes in blood flow and oxygen consumption at different levels of the intestinal hyperemic response to food. Each of these food solutions was individually placed inside the lumen of the intestinal segment for a 30 minute period during which the changes in blood flow, oxygen consumption, luminal pressure and systemic pressure were monitored.

The results show that completely digested food solution, i.e., digested food plus bile, has a greater hyperemic (+50%) as well as metabolic (+30%) effect than the other two solutions--digested food and undigested food plus bile (Figures 1, 2 and 3). While digested food alone and undigested food plus bile increased flow by approximately 10%, only the former increased oxygen consumption (+16%). Luminal placement of bile in the jejunum did not have any effect on blood flow or oxygen consumption. In contrast, bile in the ileum increased flow by 60% but did not alter oxygen consumption (Figure 5). These results indicate that the local regulation of blood flow during postprandial intestinal hyperemia is a complex phenomenon and different constituents of chyme can elicit different responses in blood flow as well as oxygen consumption. Indeed, as illustrated by the effects of undigested food plus bile in the jejunum and bile in the ileum (Figures 3 and 5), the hyperemic response of the small intestine to the postprandial lumen contents is not always accompanied by an enhancement of oxygen consumption.

Thus, the overall local control of intestinal blood flow after a meal may involve both metabolically and nonmetabolically linked factors. This conclusion is supported by the results from the series of experiments in which the temporal relationship between the increases in blood flow and oxygen consumption during the placement of digested food and digested food plus bile was examined

in detail. As shown in Figure 7, when digested food alone was in the jejunal lumen, there was a significant correlation between the changes in blood flow and oxygen consumption. On the other hand, the changes in these two variables were not significantly correlated when digested food plus bile was placed in the jejunum (Figure 9). What this data suggests is that the hyperemic effect of digested food alone but not digested food plus bile may in part be related to factors that are linked to oxidative metabolism. As shown in Figure 7, the y intercept during digested food placement was 6.7% and was not different from the origin. However, the addition of bile into the food solution increased the y intercept to 44.7% (Figure 9). Thus the presence of bile in the digested food produced a 44.7% increase in flow even in the absence of any apparent change in oxygen consumption. Moreover, the slope of the line (-0.06, Figure 9) indicates that this increase in flow remained virtually constant over a wide range of increases in oxygen consumption. Thus, although bile enhanced both the hyperemic and metabolic effect of digested food, the greater increases in these two variables were not significantly correlated. That is, the increase in flow was not accompanied by a proportional increase in oxygen consumption.

It is not clear how the presence of bile can alter the relationship between blood flow and oxygen consumption. One possibility is that bile causes a redistribution of

blood flow as well as oxygen consumption within the three layers of the intestinal wall and total wall measurement is not representative of these changes. Chou et al. (25) as well as others (126), using the radioactive microsphere technique, observed that blood flow increased preferentially to the mucosa-submucosa layer of the intestine when food or hypertonic glucose was present in the gut. There is no evidence that this increase in the mucosa-submucosa flow is accompanied by a decrease in muscularis flow. Ideally, mucosal flow and oxygen consumption should be correlated rather than total wall flow and total wall oxygen consumption as was done in this present study. However, as of this writing, there is no method available to simultaneously monitor the rate of changes in these two variables in the different layers of the intestinal wall. The mucosa-submucosa layer receives a major portion of total wall flow (up to 88%) and the flow per unit tissue weight in this layer is much greater than the muscularis layer (25, 113, 126). The total wall flow as measured in this present study is, therefore, a close approximation (albeit not exact) of the changes in mucosa-submucosa flow in response to the placement of food.

In contrast to blood flow, the rates of oxygen consumption in the different layers of the intestinal wall at rest and during the presence of nutrients is largely unknown. However, because of the fact that major functions of the intestine such as active transport and

secretion are performed by the mucosa and submucosa layers, it is expected that these layers have a much greater metabolic demand than the muscularis (113), and the increase in oxygen consumption during luminal placement of food is mainly due to an increase in mucosal-submucosal oxidative metabolism. If these assumptions are correct, the total wall oxygen consumption as measured in this present study is a close approximation of the mucosal-submucosal oxygen consumption. Thus, the conclusion that the hyperemic effect of digested food plus bile is not related to local metabolism is a valid one. Conversely, if these assumptions are incorrect, this would indicate that: (1) At rest, there is a disparity between the metabolic demands of the intestinal intramural layers and their distribution of blood flow. An unlikely event that is in sharp disaccord with most experimental evidence (46, 67-71, 82, 103, 104, 113, 115, 118). (2) The increase in oxidative metabolism during digested food plus bile placement occurs in all compartments of the intestinal wall, or the mucosa-submucosa layer actually has decreased oxygen consumption; the fact that the hyperemic response of the whole wall to the presence of food is a result of the increase in mucosa-submucosa flow (25, 126) would certainly indicate that blood flow is not exclusively controlled by metabolic factors.

A second possibility of how the addition of bile to digested food alters the relationship between the

increases in blood flow and oxygen consumption is related to bile's effect on motility. In addition to absorption and secretion, another major function of the intestine is motor activity. The motility is often stimulated by the presence of foodstuffs inside the lumen of the gut (30). It has been shown in experimental settings that the motility of the intestine can affect its blood flow (61, 101, 106). For example, Sidky and Bean (106), Semba et al. (101) and Chou and Grassmick (23) have shown that rhythmic contraction may cause rhythmic changes in flow with an increase in mean flow while tonic contraction is accompanied by a decrease in flow. Thus, depending on the type of motility, intestinal blood flow can be altered in different directions. In the present study, placement of digested food plus bile often caused an initial increase in motility of a rhythmic nature (Figure 2). Could this transient increase in motility affect blood flow enough to render a poor correlation between flow and consumption? Kewenter (77) found that when graded doses of acetylcholine were given intra-arterially into the intestine, blood flow was affected only at extremely high doses, that produced an intense motor activity. This led Svanvik and Lundgren (113) to conclude that gut motility does not affect intestinal blood flow to any great extent under physiological conditions. In this present study, the increase in rhythmic contraction was small and usually stopped after the first 5 to 10 minutes during placement (Figure 2). Thus

it is not likely that flow was affected to an extent that would alter the temporal relationship between the changes in blood flow and oxygen consumption. This conclusion is supported by a recent finding from our laboratory (Sit and Chou, unpublished observation) that total abolishment of intestinal motility during digested food plus bile placement in the jejunum and bile in the ileum still resulted in an increase in local blood flow which is poorly correlated with the increase in oxygen consumption. Thus the fact that during digested food plus bile placement there is no significant correlation between the increase in flow and oxygen consumption cannot be attributed to the increase in motility.

A third possibility of how bile alters the relationship between the changes in the two variables monitored may be related to the effect of bile on food digestion. Chou and co-workers (21, 25, 26, 81, 126) have demonstrated that the constituents of chyme responsible for postprandial intestinal hyperemia are the digested products of food. Of the three major sources of nutrients, both carbohydrates and proteins can be digested to their final digestive products (glucose and amino acids) by the pancreatic enzymes used in this study (30). On the other hand, complete digestion of lipids requires the presence of both the pancreatic enzymes and gallbladder bile (30). Thus, the digested food used in this study probably contained less fatty acids than the digested food plus bile

solution. A recent study performed in our laboratory (Nyhof and Chou, unpublished observation) has indicated that the increase in flow related well with the increase in oxygen consumption during the placement of a long chain fatty acid (oleic) with bile. Thus, the difference in the correlation between the hyperemia and oxygen consumption during the placement of the two food solutions was not due to their difference in fatty acids concentration, i.e., the action of bile in facilitating the digestion of lipids. The two solutions also contained amino acids and glucose. The amino acids have been shown to have no effect on blood flow when placed into the jejunal lumen at physiological concentrations (26). On the other hand, glucose at physiological concentrations increases both flow and oxygen consumption upon placement in the lumen of the small intestine (21, 26, 118, 119).

The mechanism by which glucose induces an increase in flow in the intestine has never been elucidated. Moreover, what effect bile has on the glucose induced hyperemia is largely unknown. This possibility must be explored because in addition to its action on lipid digestion and absorption bile may alter the hyperemic effect of glucose and may cause an alteration in the relationship between the hyperemia and the increase in oxygen consumption. Thus, in the second part of this study, three series of experiments were performed to examine the possible mechanisms by which glucose increases local blood flow in

the intestine. In addition, the effect of bile on the glucose induced hyperemia was also studied.

In the first two series of experiments, glucose and its synthetic analogs, 3-0-methyl glucose and 2-deoxy glucose were used for the study; the rationale being that these glucoses have different absorption and metabolic characteristics. There are structural requirements for the active absorption and metabolism of glucose by the small intestine (18, 28, 125). Eliminating the hydroxyl group at the C-2 position of the molecule (2-deoxy glucose) can prevent its absorption by the intestine, while substituting a methyl group at the C-3 position (3-0-methyl glucose) inhibits its metabolism but not absorption (18, 28, 125). Thus, by comparing the vascular and metabolic effects as well as the absorption of these glucoses, one can identify some of the mechanisms involved in the hyperemia. In the third series of experiments, the temporal relationship between the increase in blood flow, oxygen consumption and glucose absorption during placement of a 200 mM glucose solution, with or without bile, were also examined.

The results indicate that both glucose and 3-0-methyl glucose, which were absorbed from the lumen, significantly increased local blood flow but the non-absorbable glucose, 2-deoxy glucose, did not (Figure 12). This indicates that the glucose induced hyperemia is at least in part, related to its absorption. Other studies also support this hypothesis (21, 25, 126). It has been

shown that a 20% glucose solution (1,200 mOsm/liter) in the jejunum increases local blood flow but a solution of a nonabsorbable substance (polyethylene glycol) with the same osmolality, does not (21). Furthermore, the increased jejunal blood flow during the presence of food and hypertonic glucose in the lumen is confined to the mucosal layer (25, 126). The mucosa is the site for transmembrane transport of water, electrolytes and other nutrients. Thus, the postprandial intestinal hyperemia seems to be a functional hyperemia related to the absorption of nutrients. Indeed, in the present study, there was a significant correlation between the increase in blood flow and glucose absorption during the luminal placement of glucose (Figure 15). Although the absorption rate of 3-0-methyl glucose is similar to that of glucose, the hyperemic effect of 3-0-methyl glucose is less than that of glucose (Figures 10 and 11). This appears to indicate that absorption may not be the only contributing factor of the hyperemia. This present study shows that glucose increases local oxygen consumption while 3-0-methyl glucose does not cause a significant increase (Figure 12). An increase in local metabolism therefore, may be another contributing factor for the glucose induced hyperemia. As shown in Figure 13, the increase in blood flow during the placement of the glucose solution was accompanied by an increase in oxygen consumption. The relationship between the changes in these two variables was

significantly correlated (Figure 14). A recent study has also suggested that the increased local metabolism is a factor modulating local blood flow during absorption of fluid from the ileum containing a glucose and Tyrode solution (118).

As discussed earlier, luminal placement of digested food increased blood flow and oxygen consumption (Figure 6). These increases are significantly correlated (Figure 7). Similarly, luminal placement of glucose increased both blood flow and oxygen consumption and these increases are also correlated (Figures 13 and 14). In addition, both glucose and digested food have quantitatively similar hyperemic as well as metabolic effects upon placement in the intestine (Figures 6 and 13) and , as shown in Figures 7 and 14, the relationship between the changes in blood flow and oxygen consumption during the placement of digested food is almost identical to the placement of glucose. The slope of the line for glucose was 0.19; and for digested food alone was 0.17. The digested food used in this study contained predominantly amino acids and glucose and only the latter is believed to be vasoactive at physiological concentrations in the lumen of the small intestine (26). The effect of luminal placement of digested food therefore, may well be due to its glucose content.

As mentioned earlier, the addition of bile to the digested food altered the relationship between the changes in blood flow and oxygen consumption (Figure 9). The

mechanism by which the presence of bile alters this relationship is not clear. This effect of bile is not due to its digestive action which increases the concentration of fatty acids in the solution containing both digested food and bile (Nyhof and Chou, unpublished observation). Since the effect of bile on digested food may be mediated by its action on glucose in the digested food, its effect on the glucose induced hyperemia was then determined.

As shown in Figures 10 and 11, the addition of bile to the glucose solution markedly enhanced the hyperemic effect of glucose. More importantly, while the increase in blood flow and oxygen consumption were significantly correlated during the placement of glucose alone (Figure 14), the addition of bile altered this relationship and rendered it insignificant (Figure 18). What this finding seems to suggest is that the effect of bile in altering the relationship between the changes in blood flow and oxygen consumption during digested food placement may be due to its action on glucose contained in the food solution. This conclusion is supported by the correlation analyses shown in Figures 9 and 18. Comparison of these figures indicate that the relationship between the changes in blood flow and oxygen consumption during digested food plus bile placement is virtually identical to that of glucose plus bile. The slope for digested food plus bile is -0.06 and the slope for glucose plus bile is 0.06. The increases in flow and oxygen consumption were not

significantly correlated during the placement of either of these two solutions.

Bile is an important constituent of chyme and is secreted into the small intestine after every meal. While the important role of bile in facilitating the digestion and absorption of lipids is well established (19, 30, 66, 108, 109) its contribution in postprandial intestinal hyperemia has until recently been largely unknown (26). The fact that it can enhance the hyperemic effect of digested food has been assumed to be due to its action on lipid digestion and absorption (26). The finding from this present study that bile enhances the hyperemic effect of glucose (Figures 10 and 11) further underscores the importance of bile in the overall regulation of blood flow during the mesenteric hyperemic response to food. It points out that the influence of bile goes beyond its role in the lipid induced hyperemia and that it plays a significant part in the hyperemic effect of at least one other nutrient as well. Another contribution of bile to postprandial intestinal hyperemia is likely its hyperemic effect in the ileum (Figure 5). Luminal placement of bile in the ileum increased flow markedly but the hyperemia was not accompanied by an enhancement of oxygen consumption.

The fact that the bile enhanced hyperemic effect of glucose and digested food in the jejunum as well as bile's own hyperemic effect in the ileum are not correlated with an increase in oxygen consumption strongly suggests

that the local regulation of intestinal blood flow during postprandial hyperemia is not exclusively linked to factors that are related to oxidative metabolism. This is in contrast to what was observed in the myocardium and skeletal muscle. The preponderance of evidence indicates that there is a close correlation between the functional hyperemia in these organs and their metabolic rate (2, 4, 15, 31, 40, 75, 76, 79, 80, 105). In the heart, oxygen consumption is considered a major determinant of blood flow under a wide range of conditions (99). This includes enhancement in work load due to either positive inotropism or increases in blood pressure. In the skeletal muscle increases in oxidative metabolism have been identified as a causal factor in the active hyperemia during exercise (99). In this study, there is little evidence that such a close relationship exists between the two variables during postprandial intestinal hyperemia.

It is not clear why the regulatory mechanism during the functional hyperemia in the gut is different from the heart and skeletal muscle. One possibility may be that during digestion, the functions performed by the intestinal circulare are more complex than the other two organs and thus require flow regulatory mechanisms that are not necessarily linked to oxidative metabolism. For example, whereas the basic function of the heart and skeletal muscle is to perform muscular contraction, the functions of the intestine include secretion, absorption in addition to

chyme propulsion by the visceral smooth muscles (30). While it is true that active absorption by the gut accelerates cellular oxygen consumption, it also entails the washout of nutrients from the gut lumen to other parts of the body for use or storage. Thus, the hyperemia in the intestine during digestion serves at least two distinct purposes: (1) To maintain adequate delivery of oxygen in response to the heightened demand, and (2) To transport the absorbed nutrients elsewhere. Teleologically, it is conceivable that the intestine employs different mechanisms to control its blood supply to serve these functions. Indeed, as mentioned in the literature review of this study, both local nerves and GI hormones have been proposed as mediators of postprandial intestinal hyperemia and may constitute the nonmetabolically linked controlling factors. The data from this study support this thesis and the precise role of these factors in the local regulation of intestinal blood flow during digestion warrants further investigation.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Postprandial intestinal hyperemia is defined as an increase in blood flow through the gastrointestinal tract after a meal. Associated with this phenomenon is the observation that there is also an enhancement in splanchnic oxidative metabolism. This has led to a hypothesis suggesting that the cause of the postprandial increase in mesenteric blood flow is probably linked to the heightened metabolic activities during digestion and absorption. However, there is virtually no data available to clearly establish that these two events are related to each other. The purpose of this study was to examine the relationship between the increase in blood flow and oxygen consumption during postprandial intestinal hyperemia. In addition, some mechanisms by which a major nutrient, glucose, may induce an increase in local blood flow in the jejunum were also studied. The results indicate that:

(1) Luminal placement of digested food and digested food plus bile in the jejunum increased local blood flow as well as oxygen consumption. The latter produced a greater increase in flow and

oxygen consumption than the former. Undigested food plus bile increased jejunal flow but did not have any effect on oxidative metabolism. Bile in the jejunum did not have any effect on blood flow or oxygen consumption. In contrast, bile in the ileum increased flow but did not alter oxygen consumption.

(2) There was a significant correlation between the increase in blood flow and oxygen consumption during the luminal placement of digested food. Digested food plus bile increased blood flow and oxygen consumption more than digested food alone did. But the greater increases in these two variables by digested food plus bile were not significantly correlated. Thus bile enhanced the digested food-induced hyperemia and the increase in oxygen consumption but altered the positive relationship between these two variables.

(3) Both glucose and 3-0-methyl glucose were absorbed and increased local blood flow when placed into the jejunum. Glucose increased, but 3-0-methyl glucose did not alter, oxygen consumption and the former produced a greater hyperemia than the latter. 2-deoxy glucose was not absorbed and did not alter local blood flow or oxygen consumption. These findings indicate that the glucose induced hyperemia is related to absorption and an increase

in oxidative metabolism. Of the three glucoses, only the vascular effect of glucose was enhanced by bile. Thus, the potentiating effect of bile on the glucose-induced hyperemia seems to have chemical structural specificity.

(4) During the luminal placement of glucose, there was a significant correlation between the changes in blood flow and oxygen consumption as well as blood flow and glucose absorption. Glucose plus bile increased blood flow and oxygen consumption more than glucose alone did. But the greater increases in these two variables were not significantly correlated. Thus, the influence of bile on the vascular and metabolic effect of luminal placement of glucose is similar to that of digested food. The relationship between blood flow and glucose absorption remained the same and was significantly correlated with or without bile.

In conclusion, the local regulation of blood flow during postprandial intestinal hyperemia is a complex phenomenon that may involve both metabolically and non-metabolically linked factors. Various constituents of chyme can elicit different responses in blood flow and oxygen consumption in the small intestine. The hyperemic effect of glucose in the jejunum is, at least in part, related to glucose absorption and an increase in oxidative metabolism. Bile enhances both the hyperemic as well as

the metabolic effects of glucose and digested food. Bile also alters the positive correlation between the changes in blood flow and oxygen consumption produced by glucose as well as digested food. These findings thus indicate that the contribution of bile to postprandial intestinal hyperemia goes beyond its actions in facilitating the digestion and absorption of lipids.

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APPENDIX

APPENDIX

Method of Somogyi-Nelson

Reagents:

1. Alkaline copper tartrate. 24 gm anhydrous sodium carbonate and 12 gm sodium potassium tartrate were dissolved in 200 ml distilled water. 40 ml of a 10% copper sulfate solution was then added to the solution. When dissolved, 16 gm sodium bicarbonate was added and stirred. 180 gm anhydrous sodium sulfate was dissolved in 600 ml water. The solution was heated to boil, cooled and added to the copper solution. The mixture was then transferred to a 1000 ml volumetric flask and diluted to volume.
2. Acid arsenomolybdate solution. 50 gm ammonium molybdate was dissolved in 900 ml distilled water. 42 ml concentrated sulfuric acid was added to the solution and the whole was stirred gently. 6 gm disodium O-arsenate dissolved in 50 ml water was then added to the mixture and placed in an incubator at 37°C for 48 hours.
3. Standards.
   A. Stock standard. 1 gm glucose was dissolved in a 0.2% benzoic acid solution and diluted to 100 ml

in a volumetric flask. This solution contained 10 mg glucose/ml.

B. Working standard. The stock standard solution was diluted 1:100 and 2:100 with 0.2% benzoic acid. The standard solutions thus contained 0.1 and 0.2 mg glucose/ml.

4. Zinc sulfate solution, 5.0%, 0.175M. 50 gm zinc sulfate was dissolved in distilled water and diluted to 1 L.
5. Barium hydroxide, 0.3 N. 95 gm barium hydroxide was dissolved in distilled water and diluted to 2 L.

Procedure: 1 volume of plasma sample was diluted with 5 volume water, 2 volume barium hydroxide and 2 volume zinc sulfate. This produced a 1:10 dilution of the plasma sample. It was added to a Folin-Wu sugar tube. To three other tubes, 1 ml of water was added (blank) to the first, 1 ml standard containing 0.1 mg glucose to the second, and 1 ml standard containing 0.2 mg glucose to the third. 1 ml alkaline copper tartrate was added to each tube. These tubes were then heated in a boiling water bath for 15 minutes. After cooling, 1 ml of the arsenomolybdate solution was added to each tube. The tubes were diluted to 25 ml each with distilled water and mixed by inversion. The samples were read against the blank in a photometer at 500 nm.

Calculation: Since 1 ml of a 1:10 plasma sample was treated the same as 1 ml of standard containing 0.1 mg/ml (10 mg/100 ml) of glucose or 012 mg/ml (20 mg/100 ml), the calculation was:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 10 \times 10 \text{ (or 20)} =$$

mg of glucose/100 ml sample

Glucose Oxidase Method

Reagents:

1. Phosphate buffer, 0.1 M, pH 7.0. 8.7 gm Na_2HPO_4 and 5.3 gm KH_2PO_4 were dissolved in 950 ml water. pH of the solution was adjusted to 7.0 and then diluted to 1 L.
2. Buffered peroxidase solution. 125 ml phosphate buffer, 175 ml water and 200 ml glycerin were added to a volumetric flask and mixed. 10 mg peroxidase and 100 mg 0-dianisidine dissolved in 10 ml methanol were than added to the mixutre.
3. Glucose oxidase solution. 500 mg glucose oxidase was dissolved in 50 ml of 40% glycerin.
4. Zinc sulfate and barium hydroxide as used for the preparation of the Somogyi reagents.
5. Standards.
   A. Stock standard. 10 mg/ml. Same as given for the Somogyi-Nelson method.
   B. Working standards. 1, 2 and 3 ml of the stock standard were diluted to 10 ml with 0.1% benzoic acid. These were equivalent to 100, 200, and 300 mg glucose/100 ml of the solution.

Procedure: 4.5 ml buffered peroxidase was pipetted to a test tube. After warming to 37°C, 0.02 ml of the plasma

sample was added to the solution. 0.5 ml glucose oxidase solution was added to the mixture and the whole was incubated at 37°C for 30 minutes. After incubation, 3 ml of 30% sulfuric acid was added to the solution and mixed. The standards and samples were read against the blank at 530 nm.

Calculation: Since the standards and samples were treated similarly:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Conc. of standard} = \text{Conc. of sample}$$