

Nutrients regulate diamine oxidase release from intestinal mucosa

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Wollin, Armin, Xiaolin Wang, and Patrick Tso. Nutrients regulate diamine oxidase release from intestinal mucosa. *Am. J. Physiol.* 275 (Regulatory Integrative Comp. Physiol. 44): R969–R975, 1998.—Diamine oxidase is continuously released from the intestinal mucosa and carried to the circulation by the lymphatics. The effect of nutrients on this release was examined. Rats were prepared with duodenal and intestinal lymph cannulas. Test mixtures of lipid emulsions containing triolein, oleic acid, or tricaprylin and solutions of carbohydrate and protein were infused into the duodenum. The enzyme release and triglyceride transport were determined and in some experiments were done in the presence and absence of Pluronic L-81, an inhibitor of chylomicron formation, and aminoguanidine, an inhibitor of diamine oxidase activity. The data indicate that nonlipid nutrients did not increase diamine oxidase activity in the intestinal lymph, but the mucosal tissue content was significantly reduced in the distal small intestine, particularly after protein infusion. Triglycerides and fatty acids increased diamine oxidase in the intestinal lymph, and the longer-chain triglyceride was more effective. Inhibition of triglyceride transport did not interfere with the enzyme release, and the inhibition of diamine oxidase activity had no significant effect on lipid absorption. According to our observations, only lipids increase intestinal lymph diamine oxidase. Nonfat nutrients appear to increase diamine oxidase in the intestinal lumen. Diamine oxidase is not directly required for lipid absorption.

triglycerides; dietary lipids; Pluronic L-81; aminoguanidine; heparin

THE APICAL CELLS of the intestinal mucosa contain a wide range of enzymes that participate in the process of digestion and absorption and the maintenance of the mucosal barrier. One of these enzymes is diamine oxidase. It is primarily associated with mature enterocytes in the villus tips (21), and the tissue content of the enzyme increases along the small intestines, being lower in the duodenum and higher in the distal ileum (15). Diamine oxidase is continuously released from the intestinal epithelia into the lamina propria (20, 27). There it binds to the basolateral membranes (6), and the remainder is transported into the bloodstream where it binds to endothelial cells (2, 11, 13, 14). Diamine oxidase metabolizes polyamines and histamine (2, 18), and, on the basis of this action and the extracellular location, it is postulated that it prevents the absorption of polyamines and histamine originating from ingested food and intestinal bacteria (17, 24). The protective function is not restricted to the intestinal tissue but extends to all tissues because of its transport from the intestinal mucosa to the circulation

(13, 15). Several factors increase and possibly regulate the release of diamine oxidase from the intestinal tissue. For example, heparin greatly increases diamine oxidase activity in the lymph fluid and plasma. Its action is thought to be the result of displacement of the enzyme from extracellular binding sites (11, 12, 15). Histamine, one of the substrates, when given intravenously or intraduodenally, increases the enzyme activity in the intestinal lymph as well (24). The mechanism responsible for this release is not known. Intestinal ischemia also releases diamine oxidase from the intestinal mucosa by villus epithelial exfoliation and by its transportation into the bloodstream via an unknown mechanism (25). Ingestion of olive oil was also observed to increase intestinal lymph diamine oxidase activity (26, 27), but how the long chain triglycerides release the enzyme from its source is still unclear. It is also unclear whether nutrients other than fat have an effect on intestinal diamine oxidase mobilization. Overall, there is little information available regarding the regulation of diamine oxidase secretion, particularly in response to food ingestion.

The present study examined the action of all major food constituents on the release of intestinal diamine oxidase and compared their effects on enzyme transport into the intestinal lymph and on tissue content. The stimulatory action and specificity of triglycerides were examined, and the relationship of the enzyme activity and fat absorption was assessed.

MATERIALS AND METHODS

Materials

Triolein, tricaprylin, oleic acid, phosphatidylcholine (egg), sodium taurocholate, aminoguanidine bicarbonate, putrescine, heparin, bovine albumin fraction V, sucrose, diamine oxidase, and a triglyceride-ultraviolet kit were purchased from Sigma (St. Louis, MO); Pluronic L-81 was supplied by BASF (Parsippany, NJ); and radiolabeled putrescine, [2,3-³H(N)]putrescine dihydrochloride (38 Ci/mmol), was purchased from NEN (Boston, MA).

Experiments

Animal preparation. Male Sprague-Dawley rats weighing 290–370 g were maintained on a 12:12-h light-dark cycle, with free access to water and Purina rat chow. After an overnight fast, the main mesenteric lymph duct was cannulated with vinyl tubing (0.8 mm OD) while rats were under halothane anesthesia, according to the procedure originally described by Bollman et al. (3) and modified by Tso et al. (22). In addition, a silicone tube (2.2 mm OD) was placed ~2 cm into the duodenum through the proximal stomach. The

incision in the fundus of the stomach wall was closed by a purse-string suture. The rats were placed into restraining cages, and the surrounding temperature was kept at 30°C during the recovery period and the experiments. Postoperatively, the animals were given a glucose-saline solution (145 mM NaCl, 4 mM KCl, and 0.28 M glucose) via the intraduodenal cannula at a rate of 3 ml/h. Fourteen to fifteen hours before the experiment, the infusate was changed to saline. The experiments were always done on the day after surgery. The rat cannulation protocol was approved by the Animal Care Committees from the Louisiana State University Medical Center and the University of Saskatchewan.

Effect of nutrients on intestinal diamine oxidase release. LYMPH DIAMINE OXIDASE ACTIVITY. A series of nutrients were tested for their action on the release of diamine oxidase. On the day of the experiment, the initial 1-h lymph sample was collected and represented the fasting sample. The saline infusion into the duodenal cannula was then replaced by a nutrient infusate. Lipid emulsion contained 40 µmol triolein or tricaprylin or 120 µmol oleic acid, plus the emulsion medium, 7.8 µmol phosphatidylcholine, and 57 µmol sodium taurocholate in 3 ml PBS at pH 6.4. The lipid concentration was equivalent to 11.8 mg/ml triolein, 6.3 mg/ml tricaprylin, and 11.3 mg/ml oleic acid. For the evaluation of the effect of the emulsion medium, lipids were replaced by PBS in the above mixture. The carbohydrate solution contained 90 mg/ml sucrose in PBS. The protein solution contained 50 mg/ml albumin in PBS. The amount of nutrient delivered to the intestinal lumen was within the range of a normal dietary load. All infusions were at a rate of 3 ml/h for 6 h. Lymph was collected in hourly intervals in graduated glass tubes surrounded by ice. The volume and enzyme activity were measured.

TISSUE DIAMINE OXIDASE ACTIVITY. The tissue enzyme determination was done on rats without the lymph cannula, but we retained the duodenal cannula for intestinal infusions. The infusion rates and length of experiments were the same as for the lymph collection experiments. In addition, the effect of heparin on tissue diamine oxidase was tested for comparison, because intraperitoneal injection of heparin releases diamine oxidase from the intestinal mucosa (20, 27). A single dose of 2,500 U/kg was used along with an intraduodenal saline infusion. At the end of the 6-h infusion, the rats were lightly anesthetized and decapitated. The blood was collected in a heparinized centrifuge tube, and the plasma was separated and stored at 4°C for the determination of diamine oxidase activity. The animals were eviscerated from the pylorus to the proximal colon, and the lumen was washed with saline. Two-centimeter segments were taken from the following regions of the intestinal tract: duodenum and jejunum, 2 and 9 cm from the pylorus, respectively; midgut, midway between the pylorus and the cecum; ileum, 14 cm from the cecum; and colon, 5 cm from the cecum. The tissue was homogenized in 4 ml of 0.01 M sodium phosphate buffer at pH 7.4 and was analyzed for diamine oxidase activity and protein content. Protein was measured by the Lowry method.

Inhibition of chylomicra transport. To determine whether diamine oxidase release is linked to chylomicra transport, we treated animals with Pluronic L-81. This nonionic hydrophobic surfactant reversibly inhibits the formation of chylomicra in the mucosal cells by blocking the trafficking of lipids from the endoplasmic reticulum to the Golgi apparatus (22). Pluronic L-81 was added to the triolein infusate at a concentration of 0.33 mg/ml. The infusion of triolein plus Pluronic L-81 was carried out for 6 h and then replaced with saline and continued for another 6 h. Diamine oxidase activity and triglyceride content were measured in the intestinal lymph.

Inhibition of diamine oxidase activity. Triolein absorption stimulated the release of diamine oxidase from the intestinal mucosa. The question arose whether diamine oxidase activity was required for triolein absorption and transport. This point was evaluated by measuring triglyceride absorption in the presence of aminoguanidine, an inhibitor of diamine oxidase. During the initial 1-h fasting collection, the animals treated with the inhibitor received 50 mg·kg⁻¹·h⁻¹ of aminoguanidine as a pretreatment and during the triolein infusion received 10 mg·kg⁻¹·h⁻¹ as a maintenance dose. The control animals were the same as in the preceding experiments. Triglyceride content was measured in the hourly collections of intestinal lymph using a triglyceride-ultraviolet kit (Sigma). The lymph flow rate was measured for each collection, and diamine oxidase activity was determined in one animal to verify the effectiveness of aminoguanidine.

Measurement of Diamine Oxidase

The lymph collections for diamine oxidase activity were stored at 4–5°C until the time of assay, which was restricted to within 1 wk. Short-term storage at 5°C caused very little loss of enzyme activity. Before taking aliquots for measurements, we dispersed clotted lymph samples to release any trapped diamine oxidase. The enzyme activity was estimated by a radiometric assay described by Forget et al. (7). Briefly, the reaction mixture consisted of a 50-µl enzyme sample and a 50-µl substrate mixture. The substrate mixture was a combination of cold and labeled putrescine prepared in 0.1 M sodium phosphate buffer at pH 7.4 at a ratio of 10 µl of [³H]putrescine per 1,000 µl of 0.3 mM unlabeled putrescine. The final concentration of putrescine in the reaction mixture was 0.15 mM, which is slightly below the substrate concentration for the maximum velocity of the enzyme reaction. The total count of 50 µl of substrate was ~0.8 million dpm. A standard curve was prepared with a sample of known enzyme activity of 0.05 U/mg (Sigma). One unit was defined as the enzyme activity that oxidized 1 µmol of putrescine per hour at 37°C. The range of the standard curve was 0–4 mU/test. The determinations were done in duplicate, and the enzyme activity was expressed in milliunits.

Statistical Analysis

The values were expressed as means ± SE. Statistical significance was calculated by ANOVA. Differences between treatments were considered significant when the probability that they occurred by chance was <0.05.

RESULTS

Relationship Between Nutrients and Diamine Oxidase Release

The initial experiments examined the effects of three types of nutrients on the diamine oxidase release from the intestinal mucosa. Infusion of 270 mg/h sucrose or 150 mg/h albumin into the duodenal lumen caused no significant change in the diamine oxidase output into the intestinal lymph over the 6-h test period (Fig. 1). In contrast, infusion of 35 mg/h of triolein gradually increased the rate of diamine oxidase release, which reached a near plateau by 5 h. The rise in the hourly output of enzyme was linear with time over the 6-h test period ($r = 0.98$). At the highest level of enzyme secretion, it was five times higher than the fasting level. Oleic acid, the fatty acid of triolein, increased

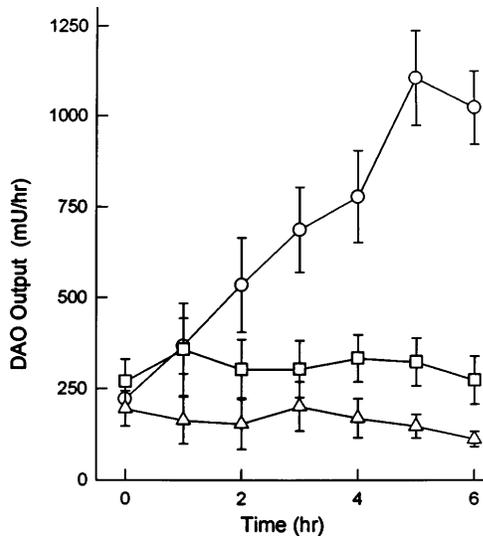


Fig. 1. Diamine oxidase (DAO) release into intestinal lymph during continuous infusion of triolein (○, 35 mg/h, n = 8), sucrose (□, 270 mg/h, n = 4), and albumin (△, 150 mg/h, n = 4). DAO output is product of DAO activity (mU/ml) and volume of lymph collected per hour (ml/h). Values are means ± SE.

diamine oxidase output into the intestinal lymph to the same amount as the equimolar triolein infusion. The emulsion medium, consisting of 7.8 μmol phosphatidylcholine and 57 μmol sodium taurocholate in 3 ml of PBS, had a very weak response at hours 5 and 6 (Fig. 2). However, when the variations in the response were taken into consideration, the increase was not statistically significant ($P > 0.05$).

Dietary fatty acids with long and short chain lengths are transported across the mucosa by separate pathways. To test the specificity of the lipid effect on the liberation of diamine oxidase, we substituted a medium-chain triglyceride for the C₁₈ chain triolein. Tricaprylin, with a C₈ fatty acid chain length, was infused into the duodenum under the same conditions and the same molar concentration as triolein. Tricaprylin produced a

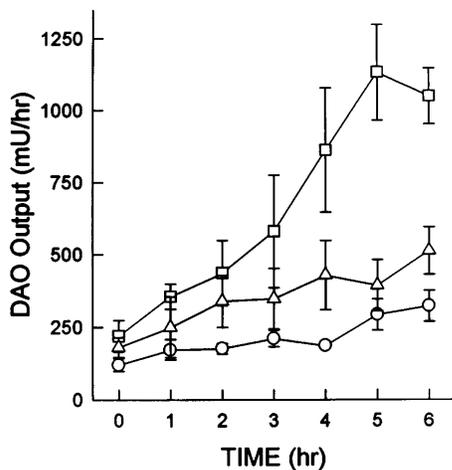


Fig. 2. DAO release into intestinal lymph during infusion of emulsion medium (○, 3 ml/h, n = 3) and lipids tricaprylin (△, 19 mg/h, n = 3) and oleic acid (□, 35 mg/h, n = 3). Values are means ± SE. Hourly infusion quantities of the 2 lipids were equimolar.

Table 1. Intestinal lymph flow after infusion of nutrients and nutrients plus inhibitors of chylomicra formation and diamine oxidase activity

Nutrients	n	Lymph Flow Rates, ml/h		
		Fasted	3 h after infusion	6 h after infusion
Sucrose	4	2.5 ± 0.2	3.8 ± 0.3*	3.6 ± 0.2*
Albumin	4	2.8 ± 0.2	3.4 ± 0.2	3.4 ± 0.1*
Triolein	4	3.0 ± 0.2	3.8 ± 0.2*	3.8 ± 0.3*
Triolein and Pluronic L-81	5	2.9 ± 0.1	2.1 ± 0.2*	3.8 ± 0.3*
Triolein and amino-guanidine	5	2.2 ± 0.2	4.0 ± 0.1*	4.3 ± 0.2*

Results are means ± SE. *Significant ($P < 0.05$) change relative to fasted value.

much smaller response (Fig. 2). The increase in diamine oxidase output was linear, but the slope was three times smaller than the triolein response. The lymph flow increased with all nutrient infusions, ranging from 21 to 52% above the fasting rate (Table 1).

Effect of Nutrients on Diamine Oxidase Content in Intestinal Tissue

In the preceding experiments, the release of diamine oxidase into the intercellular space was measured by the changes in enzyme content in the intestinal lymph, the fluid draining from the intestinal intercellular space. In the following experiments, the effect of nutrients on the enzyme content in the intestinal tissue was assessed 6 h after a continuous exposure to either saline, triolein, albumin, or sucrose. In fasted rats (saline infusion), the tissue diamine oxidase content increased from the proximal to the distal region of the small intestine and dropped to the duodenal level in the proximal colon. All nutrients decreased the enzyme content in the small intestine, but the reduction reached statistical significance only in the ileal tissue when it was exposed to albumin and triolein (Table 2). The colon was unaffected. Heparin, which displaces diamine oxidase from specific binding sites, caused a widespread reduction in diamine oxidase content in samples from the jejunum to the ileum, including the colon (Table 2). Because heparin releases diamine oxidase into the lamina propria from all intestinal

Table 2. Intestinal tissue diamine oxidase activity after duodenal infusion of nutrients

Nutrients and Treatment	Tissue Diamine Oxidase Activity, mU/mg protein				
	Duodenum	Jejunum	Midgut	Ileum	Colon
Saline control	14 ± 5	34 ± 2	52 ± 4	78 ± 5	9 ± 3
Sucrose	11 ± 2	28 ± 10	43 ± 18	53 ± 16	10 ± 4
Albumin	9 ± 2	30 ± 9	32 ± 11	37 ± 12*	8 ± 3
Triolein	9 ± 1	30 ± 7	34 ± 7	44 ± 9*	14 ± 4
Heparin	8 ± 2	12 ± 4*	13 ± 4*	25 ± 1*	3 ± 1*

Values are means ± SE; n = 4. Duodenal infusion rate was 3 ml/h for 6 h. Infusates: NaCl (control), 9 mg/ml; sucrose, 90 mg/ml; albumin, 50 mg/ml; triolein, 11.8 mg/ml. Drug treatment: heparin, 2,500 U/kg ip. *Statistical significance ($P < 0.05$) compared with control in each tissue.

tissues (6), it was not surprising that the plasma levels in the heparin-treated animals increased from 0 to 4.4 ± 1.0 mU/ml. The method for diamine oxidase used in this study was not sufficiently sensitive to detect diamine oxidase activity in the fasted saline-infused controls and sucrose- or albumin-infused animals. The triolein-infused animals showed a small inconsistent increase of 0.7 ± 0.7 mU/ml.

Effect of Pluronic L-81 on Diamine Oxidase Release

The relationship between diamine oxidase release and triglyceride transport across the mucosal cells was evaluated with the help of Pluronic L-81. This compound inhibits the formation of chylomicrons in the mucosal epithelial cells by blocking the trafficking of lipids from the endoplasmic reticulum to the Golgi apparatus and thereby preventing the transport of triglycerides into the lymphatics. Infusion of triolein plus Pluronic L-81 prevented the appearance of triglycerides in the intestinal lymph (Fig. 3B). The diamine oxidase output was the same whether or not triglyceride transport was blocked (Fig. 3A). Because the Pluronic L-81 effect is known to be readily reversible, replacing the triolein-Pluronic L-81 infusate with saline resulted in a washout of the blocking agent. This in turn allowed the absorbed triglycerides to exit the enterocytes and appear in the intestinal lymph (Fig. 3B). The increase in triglyceride transport after saline infusion had no effect on the gradual decline of diamine oxidase release associated with the withdrawal of luminal triolein. The diamine oxidase output continued as long as lipids were infused into the intestinal lumen. When the infusion of triolein was replaced by saline, the rate of enzyme secretion into the intestinal lymph immediately decreased and gradually returned to the fasting level. The decline lasted 5–6 h (Fig. 3). The inhibition of triglyceride transport by Pluronic L-81 was initially accompanied by a reduction in lymph flow.

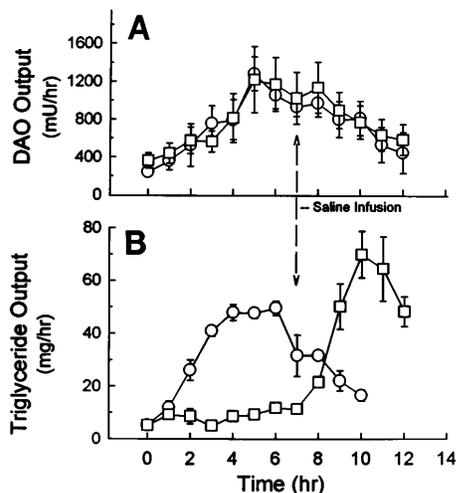


Fig. 3. A: DAO output in intestinal lymph during infusion of triolein (\circ , 35 mg/h) and triolein (35 mg/h) + Pluronic L-81 (1 mg/h) (\square). B: triglyceride content in intestinal lymph in same lymph samples collected in A. Values are means \pm SE; $n = 5$.

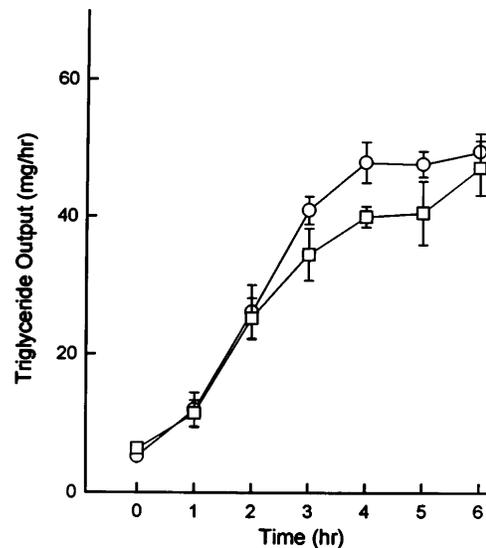


Fig. 4. Triglyceride output in intestinal lymph during infusion of triolein (\circ) and triolein + aminoguanidine (\square). Values are means \pm SE; $n = 5$.

However, it recovered fully before Pluronic L-81 was withdrawn (Table 1).

Effect of Diamine Oxidase Activity Inhibition on Triolein Absorption

The relationship between triglyceride absorption and diamine oxidase activity was further evaluated by treating animals with aminoguanidine, an inhibitor of diamine oxidase. During the infusion of triolein, triglyceride output into the intestinal lymph of control animals increased with time. After 4 h of infusion, it reached a maximum, which was maintained to the end of the experiment (Fig. 4). Animals treated with aminoguanidine 1 h before and during triolein infusion showed a small but nonsignificant decrease in the triglyceride output from that of the control during hours 3–5. Aminoguanidine treatment increased lymph flow slightly compared with animals receiving triolein alone (Table 1). Diamine oxidase activity in the lymph from aminoguanidine-treated rats was not detectable, confirming the effective inhibition.

DISCUSSION

It was observed in earlier studies that feeding olive oil as a single intragastric bolus temporarily increased diamine oxidase activity in the intestinal lymph (27). In the present study, we further examined the enzyme release from the intestinal mucosa using a continuous duodenal infusion. The intention was to bypass the gastric-retention process, allowing the comparison of the effects of several nutrients with different emptying rates. Intraduodenal infusion of nonlipid nutrients such as sucrose and albumin did not change the basal level of diamine oxidase activity in the intestinal lymph. Despite an increased lymph flow generated by the absorption of the nonlipid nutrients, no washout of enzyme was evident. A significant increase in enzyme release was found in response to the triglyceride infu-

sion. The free fatty acids of the same triglyceride in an equivalent molecular amount gave the same response as the triglyceride infusion. It is of interest that the triglyceride with a shorter chain length gave a weaker response. The medium-chain triglyceride, tricaprylin (C_8), when given in equimolar amounts, was less effective than the 18-carbon chain triolein. Whether fatty acid chains greater than C_{18} cause a still greater response or whether the degree of saturation is a contributing factor needs to be evaluated. The relationship between the magnitude of response and carbon chain length is similar to that known to exist for the release of cholecystokinin (CCK) (23) and gastric inhibitory peptide (GIP) (16). The similarity in response raises the question whether the released hormones may be the mediators that stimulate the diamine oxidase release rather than a direct action of the fatty acids on the enterocytes. But such a mechanism appears unlikely, at least for the lymph-transported diamine oxidase, because, in the rat, proteins are a strong stimulus of CCK release (23) and glucose releases GIP (16). In our studies, albumin and sucrose did not increase lymph diamine oxidase, therefore eliminating the possibility of CCK or GIP mediating the mucosal enzyme release into the lamina propria. However, other intestinal hormones may mediate this enzyme release. The present observations suggest that a mechanism that interlinks various pathways and thus integrates the digestion of the more lipophilic nutrients may be initiated via a possible receptor to fatty acids with longer chain lengths.

Infusion of triolein or the corresponding fatty acid resulted in identical responses, which is compatible with the notion that it is the fatty acids of the triglyceride that are responsible for stimulating the release of diamine oxidase (Figs. 1 and 2). The hourly output of lymph diamine oxidase stimulated by the luminal triglycerides or fatty acids increased linearly with time ($r = 0.98$) for at least the first 5 h. Replacing the luminal lipids with saline decreased the rate of the enzyme release immediately. The enzyme release gradually returned to the baseline level. The relatively slow response of increasing and decreasing diamine oxidase secretion rates is compatible with an induction of increased enzyme synthesis and its immediate release without storage. A somewhat similar response has been observed with intestinal alkaline phosphatase, another mucosal enzyme. Its release was linked to an increase in synthesis (1, 8), but the mechanism of release appears to be different. Alkaline phosphatase is closely linked to chylomicron release, whereas diamine oxidase is not. This is based on the observation that Pluronic L-81 blocks chylomicron and alkaline phosphatase transport but does not interfere with diamine oxidase release (Ref. 28 and Fig. 3).

The diamine oxidase transport process becomes more complex when the tissue enzyme content is taken into consideration. For example, duodenal albumin infusion did not increase the release of diamine oxidase into the intestinal lymph but reduced the enzyme content in the distal intestinal (ileal) tissue by 52%. The proximal

region of the intestines where most of the digestion and absorption occurs and where nutrients come in contact with the mucosa was only slightly reduced. However, this reduction was not statistically significant ($P > 0.05$). Because the luminal presence of albumin did not cause any transport of diamine oxidase into the lamina propria, i.e., into the intestinal lymph, the enzyme release must have occurred into the lumen. How it was released is not presently known. With the assumption that the absorption of albumin was complete in the jejunum, the signal to release diamine oxidase in the ileum where the major reduction in enzyme activity was observed must have required a messenger, unless albumin did reach the ileum because of the continuous infusion and caused the enzyme release. However, the latter reason could not explain the different regional responses. Triolein infusion also lowered the diamine oxidase content in the ileal tissue, but whether the lymphatic transport accounts for the entire reduction or whether fat also caused an apical release needs further investigation. Lipids and proteins, therefore, are capable of initiating different releasing pathways. Similar to protein, the presence of carbohydrates caused no change in the lymph diamine oxidase activity, and the action on the mucosal tissue was only minor. The comparatively smaller and variable decrease in tissue diamine oxidase may be the result of nonspecific epithelial cell shedding, increased by digestive motility and the increased cell migration up the villus tip. These effects are probably common to all nutrients that come in contact with the intestinal mucosa.

Heparin injections, which increase lymph and plasma diamine oxidase activity, greatly reduced the diamine oxidase content in all mucosal tissue tested, including the colon, which was unaffected by the nutrients. According to earlier reports, heparin releases diamine oxidase by displacing it from binding sites along the basolateral plasma membrane of enterocytes and the luminal surfaces of blood vessels (6, 13, 14, 21). Therefore, on the basis of the surface binding, the extracellular location of the enzyme could be considered as belonging to an extracellular pool (5). The existence of an intracellular pool is not unreasonable. This pool would be associated with enzyme synthesis and a transient storage (5, 6, 20). It may be the source of the nutrient-stimulated release, because it is restricted to the nutrient-absorbing mucosa, excluding the colon. In addition, the gradual increase in lymph diamine oxidase release to fat infusion, reaching a maximum after 5–6 h, is compatible with enzyme induction. In contrast, the response to intraperitoneal heparin treatment was much more rapid (6, 20).

On the basis of these observations, it is conceivable that nutrients can selectively influence diamine oxidase transport into specific compartments. It is known from time course studies that diamine oxidase is synthesized continuously by mature enterocytes in the mucosal tissue (20). Some of it remains in the cells for release into the intestinal lumen, and some is constitutively released into the lamina propria where it binds to villus membrane binding sites. Here the enzyme forms a

barrier, limiting the absorption of polyamine and histamine. A portion of the released but unbound enzyme is transported via the lymphatics to binding sites in the circulation, extending the barrier over a wider range in the body. Diet can therefore regulate the location of diamine oxidase and the degree of activity.

The functional relationship between lipid absorption and diamine oxidase release is not obvious because dietary lipids do not contain substrates needing inactivation by diamine oxidase. It is evident from our study that the action of diamine oxidase is not directly required for lipid absorption or transport. The association of triglyceride absorption and diamine oxidase holds only for the initial interaction of the lipids with the enterocytes. Lipids have to be in the lumen for the stimulation of diamine oxidase release to take place, but chylomicron formation and transport, associated with long-chain triglyceride transport, are not required for the enzyme release. This conclusion is based on the action of Pluronic L-81, which blocks the formation of chylomicra and prevents the transport of triglycerides out of the cell (22). Under these conditions, the release of diamine oxidase was not altered. These findings also suggest that the chylomicron packaging, which is susceptible to the action of Pluronic L-81, follows a separate secretory pathway from that of diamine oxidase because diamine oxidase secretion was not disturbed in the presence of this surfactant. Furthermore, the transport of triglycerides into the lymph after the removal of Pluronic L-81 did not change the rate of diamine oxidase release. The stimulus for secretion must therefore occur at the enterocyte surface or before the lipids are packaged into the chylomicron. Neither did inhibition of the diamine oxidase activity significantly change the efficiency of lipid absorption, suggesting that diamine oxidase action was not directly required.

It is of interest that the increase in lymph flow caused by fat was somewhat greater in the aminoguanidine-pretreated group (Table 1). We know that from the substrates of diamine oxidase, only histamine has vasoactive properties capable of causing lymph flow changes. It is therefore conceivable that the increase in lymph volume may be the result of the enhanced action of increased availability of histamine spared by diamine oxidase inhibition. Earlier studies appear to support this speculation of histamine action during fat absorption. In these studies, an increased amount of plasma protein was observed in the intestinal lymph during fat absorption (26). The fat-induced increased lymph flow was thought to be the result of increased vascular permeability to plasma protein because plasma protein leakage into the lamina propria was greatly reduced by histamine antagonists (H_1 and H_2). In addition, heparin, which releases diamine oxidase capable of metabolizing free tissue histamine, also reduced protein leakage (26). On the basis of these observations, it was postulated that perhaps histamine is released during fat absorption, increasing villi vascular permeability to plasma proteins and drawing fluid into the interstitial space. The fluid then collects into the lymph capillaries, carrying the released chylomicra

into the lymphatics. The role of histamine in this phenomenon, however, was not substantiated by others in a cat model (9). In light of the present observations, the role of histamine in chylomicra transport perhaps warrants a reexamination. Besides inhibiting diamine oxidase, aminoguanidine also inhibits NO synthetase (4), but a reduction in NO would potentially cause an opposite effect on lymph flow from that observed.

The present study clearly demonstrated the specific actions of nutrients on mucosal diamine oxidase release. Lipids stimulate the release of diamine oxidase into lamina propria for transport by the lymphatic to the blood. Some diamine oxidase may be directed to the intestinal lumen, particularly into the distal ileum. Proteins stimulate the release of diamine oxidase into the lumen only, and carbohydrates do not appear to have a significant effect on the enzyme release. The functional significance of the two secretory paths for intestinal diamine oxidase is open for speculation. One could argue that the increase in enzyme transport to the extracellular location increases the degradative capacity in the lamina propria and reduces circulating polyamines and histamine, whereas the release of the enzyme into the intestinal lumen, which is more pronounced in the distal small gut, may deal with the bacterial polyamines and histamine (19). Therefore, diamine oxidase appears to prevent the buildup of free polyamines, histamine, and possibly other biologically active substrates (10), creating a barrier between the lumen and tissue interface in the mucosal and vascular regions. The intensity of the protective actions of diamine oxidase appears to be regulated by dietary components.

Perspectives

Amine oxidases are a group of enzymes regulating biogenic amine levels by catalyzing the oxidative removal of the amine group and generating hydrogen peroxide, aldehyde, and ammonia as by-products. Diamine oxidase belongs to this group of enzymes and is found in a wide range of living organisms from plants to animals. In most mammals, diamine oxidase is found in the highest concentration in the small intestines and in placental tissue, tissues with rapid cell division. The exact physiological function of diamine oxidase is not clear, but its action in a process common to plants and animals, such as cell division and maturation, has generally been accepted. The observations that dietary components release the enzyme from the intestinal mucosa to other parts of the body may indicate a possible nutritional connection with the regulation of tissue growth. The role of fat in enhancing general tissue remodeling has potentially very important physiological implications and needs to be evaluated, specifically if long-chain fatty acids have a more pronounced effect.

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