Enterostatin decreases postprandial pancreatic UCP2 mRNA levels and increases plasma insulin and amylin

Denis Arsenijevic,1 Eva Gallmann,1 William Moses,1 Thomas Lutz,2 Charlotte Erlanson-Albertsson,3 and Wolfgang Langhans1
1Institute of Animal Sciences, Eidgenössische Technische Hochschule Zurich, Schwerzenbach; 2Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland; and 3Department of Cell and Molecular Biology, Biomedical Center, Lund University, Lund, Sweden

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Arsenijevic, Denis, Eva Gallmann, William Moses, Thomas Lutz, Charlotte Erlanson-Albertsson, and Wolfgang Langhans. Enterostatin decreases postprandial pancreatic UCP2 mRNA levels and increases plasma insulin and amylin. Am J Physiol Endocrinol Metab 289: E40–E45, 2005. First published February 15, 2005; doi:10.1152/ajpendo.00367.2004.—This study investigated the chronic effect of enterostatin on body weight and some of the associated changes in postprandial metabolism. Rats were adapted to 6 h of food access/day and a choice of low-fat and high-fat (HF) food and then given enterostatin or vehicle by an intraperitoneally implanted minipump delivering 160 nmol enterostatin/h continuously over a 5-day infusion period. Enterostatin resulted in a slight but significant reduction of HF intake and body weight. After the last 6-h food access period, enterostatin-treated animals had lower plasma triglyceride and free fatty acid but higher plasma glucose and lactate levels than control animals. Enterostatin infusion resulted in increased uncoupling protein-2 (UCP2) expression in various tissues, including epididymal fat and liver. UCP2 was reduced in the pancreas of enterostatin-treated animals, and this was associated with increased plasma levels of insulin and amylin. Whether these two hormones are involved in the observed decreased food intake due to enterostatin remains to be determined. As lipid metabolism appeared to be altered by enterostatin, we measured peroxisome proliferator-activated receptor (PPAR) expression in tissues and observed that PPARα, -β, -γ1, and -γ2 expression were modified by enterostatin in epididymal fat, pancreas, and liver. This further links altered lipid metabolism with body weight loss. Our data suggest that alterations in UCP2 and PPARγ2 play a role in the control of insulin and amylin release from the pancreas. This implies that enterostatin changes lipid and carbohydrate metabolic pathways in addition to its effects on food intake and energy expenditure.

body weight; appetite; glutathione; peroxisome proliferator-activated receptors; uncoupling protein-2

THE PANCREATIC PENTAPEPTIDE ENTEROSTATIN, which is cleaved from the pancreatic procolipase, has been implicated in control of body weight and food intake (21). Enterostatin appears to affect fat intake more than carbohydrate or protein intake (21). The exact mechanism of enterostatin’s effect on feeding is still unknown, but some evidence suggests that it modifies neurotransmitters such as dopamine and serotonin (13). Enterostatin has also been shown to modify the uncoupling protein 2 (UCP2), a mitochondrial transport protein found in various tissues (21). Several structurally related UCPs exist, but their precise functions are unknown, except for UCP1, which is involved in thermogenesis. UCP2 is found in tissues and cells that are important in metabolic regulation (3), such as white adipose tissue (WAT) and liver. UCP2 is also believed to be involved in regulating mitochondrial reactive oxygen species (ROS) (3). ROS can react with lipids resulting in the formation of malondialdehyde (MDA). Increased MDA levels suggest increased oxidative stress. The levels of ROS are determined by mitochondrial antioxidant levels, in particular manganese superoxide dismutase and glutathione. We have recently shown a close association between UCP2 regulation of ROS by mitochondrial glutathione (GSH) (7). Changes in antioxidant state have been associated with increased UCP2, in particular with the elevated ROS and reduced levels of the antioxidant glutathione found in diabetics (1). UCP2 is found in the pancreas, and it has recently been implicated in regulation of insulin secretion (27).

One aim of this study was to expand on previous investigations showing that enterostatin resulted in a decrease in body weight associated with a decreased intake of a high-fat (HF) diet (21). Using ad libitum-fed rats that had a choice of HF and low-fat (LF) diets, we studied the effect of chronic administration of enterostatin through intraperitoneally implanted osmotic minipumps on food intake and body weight. We also examined whether enterostatin changed certain metabolic parameters in the postprandial phase, including the relationship between expression of the peroxisome proliferator-activated receptors (PPARs) (10), which can regulate UCP2 expression and metabolism in various tissues. More specifically, we chose epididymal fat pad, liver, and pancreas. Finally, because enterostatin has been shown to influence insulin secretion, we measured plasma concentrations of insulin and amylin, two hormones that are cosynthesized in and cosecreted by pancreatic β-cells and are involved in energy balance.

METHODS AND MATERIALS

Animals and Experimental Procedures

Male Sprague-Dawley rats (200–225 g body wt at the start of the experiment, n = 5) from our own breeding facility (Schwerzenbach, Zurich, Switzerland) were caged individually in a temperature-controlled room with a 12:12-h dark-light cycle with the lights off at 0700. All procedures were approved by the Canton of Zurich’s Animal Use and Care Committee. The rats had access to both HF and LF diets every day for 6 h (0930 to 1530) (15). Diets were obtained from Kliba (Kaiseraugst, Switzerland). The LF diet (Kliba...
Continuous intraperitoneal enterostatin infusion reduced body weight \((P < 0.01)\) over the 5-day period (Fig. 1A). Enterostatin decreased HF diet intake \((P < 0.02)\) over the 5-day period but had no effect on LF diet intake (Fig. 1B). The number of meals during the feeding period was the same in both groups, with the last meal eaten between 1400 and 1520. Animals from both groups ate on average two HF and two LF meals during the feeding period (data not shown).

**Tissue UCP2, MDA, and GSH**

Continuous intraperitoneal enterostatin infusion resulted in elevated UCP2 expression in liver and epididymal fat pad (WAT), whereas UCP2 expression in the pancreas was decreased by enterostatin \((P < 0.001); \text{Fig. 2A}). Also, enterostatin increased MDA in liver and WAT \((P < 0.001)\) but not in the pancreas (Fig. 2B).
In comparison, enterostatin reduced GSH in liver and WAT substantially (P < 0.001) but to a much smaller extent in the pancreas (P < 0.01) and, to a lesser extent, in WAT (P < 0.05) but decreased in liver (P < 0.01; Fig. 4D).

Enterostatin increased PPARα expression in liver (P < 0.01) and pancreas (P < 0.01) but not in WAT (Fig. 4A). PPARβ was markedly elevated by enterostatin in WAT and pancreas and to a lesser extent in the liver (P < 0.01 for all tissues; Fig. 4B). PPARγ1 was elevated by enterostatin in all tissues examined (P < 0.01 for all tissues; Fig. 4C). PPARγ2 was increased in pancreas (P < 0.01) and, to a lesser extent, in WAT (P < 0.05) but decreased in liver (P < 0.01; Fig. 4D).

**Plasma Insulin, Amylin, FFA, Triglycerides, Glucose, And Lactate**

Enterostatin increased circulating levels of insulin (P < 0.02), amylin (P < 0.002), glucose (P < 0.005), and lactate (P < 0.002), whereas plasma FFA (P < 0.01) and triglycerides (P < 0.006) were reduced by enterostatin (Fig. 3, A–D).

**Tissue PPARs**

Enterostatin increased PPARα expression in liver (P < 0.01) and pancreas (P < 0.01) but not in WAT (Fig. 4A). PPARβ was markedly elevated by enterostatin in WAT and pancreas and to a

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**Fig. 2.** Five-day IP enterostatin infusion changed uncoupling protein-2 (UCP2) expression (A) as well as malondialdehyde (MDA; B) and glutathione (GSH; C) levels in white adipose tissue (WAT, epididymal fat pad), pancreas, and liver. A: UCP2 expression was decreased in pancreas but increased in WAT and liver (P < 0.001). B: MDA was increased in WAT and liver (P < 0.001) but not in pancreas. C: GSH was decreased in WAT and liver (P < 0.001) and less so in the pancreas. All values are given as % changes compared with saline (control) group; n = 5 for each group. ***P < 0.001.

**Fig. 3.** Five-day ip enterostatin infusion increased plasma insulin and amylin (P < 0.02 and P < 0.002, respectively; A and B). Plasma lactate and glucose were increased (P < 0.002 and P < 0.005, respectively) by enterostatin (C), whereas plasma triglycerides and free fatty acids (FFA) were decreased (P < 0.006 and P < 0.01, respectively; D); n = 5 for each group. *P < 0.05, **P < 0.01.
The present findings confirm previous reports of a reduction in body weight following acute enterostatin administration (21) and extend these results by demonstrating that this effect of enterostatin is maintained for several days with continuous administration. We also demonstrate for the first time that enterostatin affected HF but not LF diet intake over the 5-day administration period starting on the day after minipump implantation. Enterostatin did not affect the frequency of HF or LF meals. Consistent with previous observations (21), we also found an increased level of UCP2 in various tissues, such as stomach, intestine (data not shown), liver, and WAT. This increase in UCP2 was associated with increased MDA and decreased GSH. Our novel finding in this context is that enterostatin specifically decreased UCP2 expression in the pancreas after the feeding period, and this was associated with lower levels of MDA and higher levels of GSH. The observed enterostatin-induced changes in MDA (increase) and GSH (decrease) associated with an increase in UCP2 suggest that increased oxidative stress leads to an increase in UCP2 and, hence, support an antioxidant role for UCP2. These findings also suggest that enterostatin can alter tissue oxidative stress directly or indirectly, possibly due in part to the alteration in circulating glucose. Yet, if glucose were the only factor affecting tissue oxidative stress, one would expect the pancreas to be affected in the same manner as other tissues.

The observed changes in circulating pancreatic hormone levels support the idea that enterostatin-induced changes in UCP2 in the pancreas may contribute to metabolic regulation. Results from UCP2 knockout mice suggest that UCP2 is a negative regulator of insulin (27). Thus, with a reduction of UCP2 in the pancreas, one might expect an increase in circulating insulin, and this is what we actually found in the rats treated with enterostatin. The enterostatin effect did not seem to be specific for insulin, as amylin was also increased, consistent with the fact that amylin is always cosecreted with insulin (16). Previous studies have shown that enterostatin acutely decreases rather than increases the plasma insulin level (18). Yet insulin was not measured postprandially in that acute study, whereas we looked at the effect of a continuous enterostatin administration at 1–2 h after the 6-h feeding period. The two apparently contradictory results may in fact be compatible, because enterostatin has been shown to initially decrease and later increase intracellular ATP in insulin-secreting INS-1 cells in vitro (4). A chronic increase in ATP would favor insulin release (4). Whether the observed increase in circulating insulin and amylin in response to enterostatin is due to increased transcription, translation, or secretion of the two proteins remains to be determined.

Although the increase in circulating insulin and amylin levels may be mainly due to increased secretion, a reduced degradation rate of the two proteins might contribute, based on the fact that hepatic GSH expression was reduced by enterostatin. Hepatic GSH is required for insulin degradation (6). Whether the reducing effect of enterostatin on food intake is mediated by the elevated concentrations of circulating amylin and insulin remains to be determined. Pancreatic GSH levels could also influence insulin secretion by a direct effect (5, 19). Finally, the increase in circulating insulin and amylin levels may, in part, reflect a response to the hyperglycemia induced by chronic enterostatin.

We found that one class of transcription factors, the PPARs, was altered by chronic enterostatin in pancreas, liver and WAT, as would be expected from a substance that alters lipid levels. An increase in PPARα expression in the pancreas may...
also contribute to an increase in insulin release (11), as could increased expression of PPAR\(\gamma\)1 and -\(\gamma\)2 (12). PPAR expression was also altered in other tissue, suggesting that both carbohydrate and lipid metabolism were altered by chronic enterostatin administration. Increased PPAR\(\alpha\) expression in the liver could implicate augmented lipid catabolism, as would an increased expression of PPAR\(\gamma\)1 and PPAR\(\gamma\)2 in the liver. Alteration of PPAR\(\gamma\)1 and PPAR\(\gamma\)2 expression in the WAT tissue may also indicate changes in fat deposition, i.e., adipogenesis (17, 25).

Enterostatin has been shown to increase sympathetic nervous system activity (19) and UCP1 expression in brown adipose tissue (BAT) (21). This suggests that enterostatin increases metabolic rate and enhances lipolysis, which could contribute to the decrease in body weight. It has previously been shown that epididymal fat mass is reduced by enterostatin (21). An increase in adipocyte conversion of glucose to lactate could also be a way to diminish fat stores, and this is consistent with our findings with an increased plasma lactate level in response to enterostatin. An enhanced white adipocyte anaerobic metabolism has been shown to occur with an increase in circulating insulin (8, 23). The increase in lactate could, in turn, fuel gluconeogenesis and, hence, contribute to the observed increase in plasma glucose levels (8). Yet, whether the lactate in this model is really derived from adipose tissue remains to be demonstrated.

The finding that enterostatin increased circulating levels of insulin, amylin, glucose, and lactate, whereas it reduced FFA and triglycerides, could reflect a shift from oxidative to anaerobic metabolism. This switch may favor the reduction of mitochondrial oxidative metabolism and therefore decrease mitochondrial ROS, particularly in tissues where UCP2 expression is increased, such as adipose tissue. Enterostatin could decrease body fat by increasing BAT UCP1 activity, and it may also shift adipose tissue metabolism from fat storage to release, as is suggested by altered PPAR expression in tissues involved in lipid metabolism.

Overall, our studies suggest that chronic enterostatin can reduce body fat by attenuating high-fat intake and by altering metabolism, in particular by favoring anaerobic metabolism as seen by the increase in lactate levels and the increase in glucose levels possibly due to an increased gluconeogenesis. The circulating levels of glucose, lactate, and FFA after enterostatin treatment are more consistent with an action of amylin than of insulin (9). Whether amylin, alone or together with nonhypo-glycemic circulating insulin, contributes to the observed food intake reduction needs to be examined. Furthermore, enterostatin can differentially modify tissue and mitochondria oxidative state, as seen by changes in UCP2. Whether enterostatin can modify postprandial insulin and amylin levels directly merits further investigation, as does the role of enterostatin in PPAR expression and the way lipid metabolism is modified. In conclusion, we have shown that enterostatin alters energy balance and may play a role in energy partitioning between glucose and fat metabolism.

**REFERENCES**


