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Modifications in Food Intake and Energy Metabolism in Rats as a Function of Chronic Naltrexone Infusions[1]

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MARKS-KAUFMAN, R., T BALMAGIYA AND E. GROSS. *Modifications in food intake and energy metabolism in rats as a function of chronic naltrexone infusions* PHARMACOL BIOCHEM BEHAV **20**(6) 911–916, 1984.—The effects of chronic naltrexone infusions on food intake and energy balance were examined in male rats. Animals were fed either Purina Chow, or chow plus a 32% sucrose solution. After one week of being maintained on these diets, animals were implanted (intrascapularly) with osmotic minipumps infusing either 200 μg/kg/hr naltrexone hydrochloride or saline. Sucrose + chow-fed animals exhibited increased O_2 consumption, increased CO_2 production and an elevation in the respiratory quotient (RQ) relative to chow-fed controls When infused with naltrexone, sucrose + chow-fed animals decreased food intake and body weight gain. While chow-fed animals also suppressed food intake and body weight gain, these decreases were not as great as those observed in sucrose + chow-fed animals. As a function of naltrexone administration, both chow-fed and sucrose + chow-fed animals altered their metabolism as reflected by decreased RQ and adiposity as determined by skinfold measurements In addition, sucrose feeding led to a hyperthermia which was reversed by naltrexone infusions Thus, chronic naltrexone administration depressed appetite, reduced energy production and induced hypothermia in rats As naltrexone is thought to block the endogenous opioid system, this suggests that the endorphins are involved in the regulation of food intake and thermogenesis

Naltrexone Endorphins Energy metabolism Obesity Gas exchange Rat

ACUTE administration of opioid antagonists has been shown to decrease feeding behavior under a variety of experimental conditions (for review [16,22]). Both free-feeding and food-deprived rats suppress food intake following the administration of naloxone [4, 8, 14, 19]. In addition to acute injections of opioid antagonists modifying energy intake, chronic administration of these substances has also been found to alter feeding behavior In general, chronic injections of opiate antagonists have been found to decrease food intake [2, 9, 12, 18]. For example, infusing animals with naloxone over a one-week period led to a sustained suppression in food intake [9]. Both decreases in food intake and body weight gain following chronic opiate antagonist administration have been reported in genetically obese animals [18] and animals made obese by being maintained on a "cafeteria-style" feeding regime [13].

While there has been much work on the role of the opioid peptides in feeding behavior, there is limited information on the role of these substances in energy metabolism. It has been suggested [13] that the endogenous opiates may play a role in conserving energy. Chronic administration of naloxone in "cafeteria-fed" rats resulted in a suppression in the weight gain typically seen in these animals. This decrease in weight gain was thought to be a function of both a decrease in food intake and an increase in energy expenditure, as measured indirectly by oxygen consumption at thermoneutrality.

The present experiment further explored the role of chronic opiate antagonist administration on food intake, obesity and energy metabolism. Food intakes and body weight gains were monitored following chronic naltrexone infusions in both animals maintained on a standard laboratory diet and animals given access to this diet plus a 32% sucrose solution (a diet resulting in hyperphagia and increased adiposity) [10]. In order to determine the effects of chronic naltrexone infusions on overall energy balance, alterations in O_2 consumption, CO_2 production, and core and shell temperatures were also measured.

METHOD

Animals

Twenty-eight male Sprague-Dawley rats (CD outbred, Charles River Breeding Laboratories, Wilmington, MA), weighing an average of 330 g at the beginning of the study, were used. Animals were housed individually in standard laboratory cages in a temperature-controlled room (23–25°C) maintained on a 12:12 hour light-dark cycle (lights on 0800 hr).

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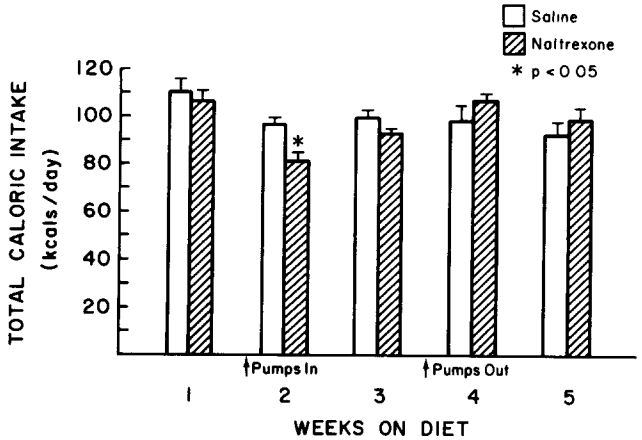

FIG. 1 Mean caloric intakes ±S.E M., averaged over one week periods, of Chow-fed animals implanted with osmotic minipumps infusing either saline or naltrexone. Minipumps were implanted in animals at the end of week 1 on the diet, and removed at the end of week 3 on the diet.*Significantly different from saline

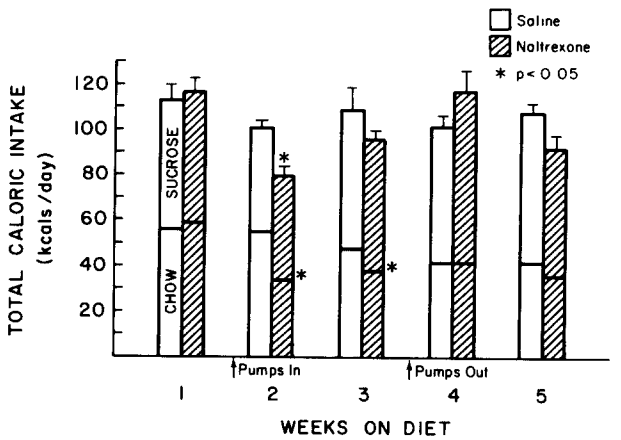

FIG 2 Mean total caloric intakes ±S E M (sum of calories from Purina Chow plus the 32% sucrose solution), averaged over one week periods, of Sucrose + Chow-fed animals implanted with osmotic minipumps infusing either saline or naltrexone Minipumps were implanted in animals at the end of week 1 on the diet, and removed at the end of week 3 on the diet *Significantly different from saline

Procedure

Animals were divided into two groups matched on the basis of body weight. The sucrose + chow-fed group (n=14) was given ad lib access to ground Purina Rodent Chow No. 5001 (caloric density=3.6 kcal/g), water and a 32% sucrose solution (1.28 kcal/ml). The sucrose solution was prepared from commercial-grade sugar and tap water on a weight per volume basis. The chow-fed group (n=14) received only Purina Chow and water. Food was provided in Wahmann (Timonium, MD) LC-306A spill-proof stainless steel food cups. Both the sucrose solution and water were provided in inverted 250 ml glass bottles with rubber stoppers and non-leaking metal drinking spouts. Body weights, food, sucrose and water intakes were measured daily throughout the experiment.

After one week of being maintained on their respective diets, animals in each diet condition were again divided into two groups. Half the sucrose + chow-fed and half the chow-fed animals were implanted subcutaneously (intrascapular region) with osmotic minipumps (Alzet No. 2002) filled with naltrexone hydrochloride (160 mg naltrexone/ml in 0.9% saline). Naltrexone hydrochloride was generously provided by Endo Laboratories (Garden City, NY). Delivery rate of the drug was approximately 200 μg/kg/hr for a period of 14 days. The remaining animals in each group were implanted with minipumps filled only with the saline solution. After a two-week drug period the pumps were removed and data collected for another two weeks. Both implantation and removal of the pumps were done under ketamine anesthesia (40 mg/kg, IP).

CO_2 production, O_2 consumption, skinfold thickness, and rectal, paw and tail temperatures were measured both during the baseline (pre-implantation) and drug (post-implantation) periods.

Resting metabolic rate was calculated using indirect calorimetry by measuring gas exchange in an open circuit system [15]. Prior to the initial measurements, rats were acclimated to the experimental chambers. The air in the chambers was maintained at 23–25°C, equal to the air temperature in the animal room. The metabolic chamber (glass dessicator) was equipped with inlet and outlet ports of 1/4 inch in diameter The expired air from the animals was mixed with room air flowing through the metabolic chamber This mixture of gases was drawn from the chamber by a gas sampling pump (AS-300, Spectrex) equipped with rheostatic rough and fine adjusters for maintaining constant air flow. Air was then passed successively through a drying cylinder (4 mesh anhydrous $CaSO_4$, drierite, Hammond), a 7 micron brass filter and a fine metering valve (Nupro Co.) to a paramagnetic oxygen analyzer (Beckman, model 755) and to a non-dispersive Infrared CO_2 Analyzer (Beckman, model 864) at flow rates of 800 ml/min for each analysis. The flow rate was constantly monitored by an electronic mass flowmeter with a digital scale (Matheson, model 8160) The output of both the O_2 analyzer and CO_2 analyzer were recorded on a dual chart recorder (Beckman, model 8720A) as percent gas concentrations with a charge speed of 38 mm/min

The gas analyzers were calibrated with room air (20.93% O_2 and 0.03% CO_2) and span gas mixture (16% O_2 and 2% CO_2, Matheson). Each animal was placed in the chamber for a 30 minute acclimation period followed by a 30 minute continuous monitoring test period. All data were converted to standard temperature and pressure, dry.

Body temperatures were monitored with a Telethermometer (model 73TA, Yellow Springs Instr. Co., Yellow Springs, OH). Rectal temperature measurements were taken with a thermistor probe (No 701) inserted 6 cm into the rectum. A small surface, teflon-covered stainless disc temperature probe (No. 727) was used to monitor paw and tail temperatures

Skinfold thickness was measured with a Adipometer Skinfold Caliper for rats (Ross Laboratories, OH). Skinfold thickness was taken as the sum of skinfolds from three sites (neck, subscapular and back).

Statistical Analysis

All data were analyzed using two-way analysis of vari-

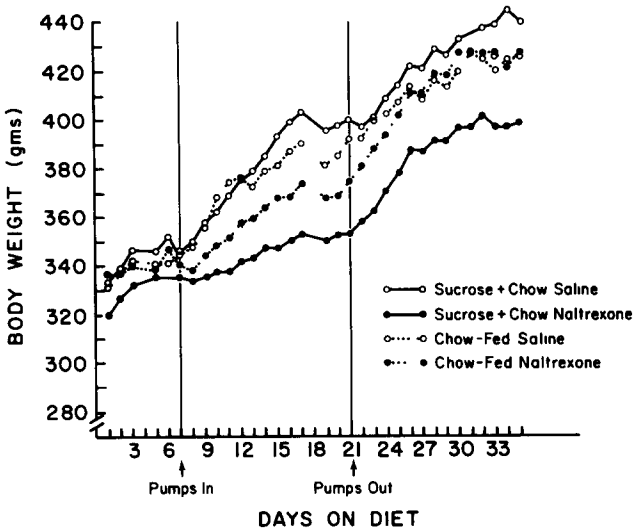

FIG. 3. Mean daily body weights (gms) of Chow-fed and Sucrose + Chow-fed animals implanted with minipumps infusing either saline or naltrexone.

TABLE 1

	Skinfold Thickness (mm) ± S E M	
	Pre	Post
Sucrose + Chow-fed		
Saline-infused	8.14 ± 0 30	10 43 ± 0 58
Naltrexone-infused	8 86 ± 0 36	8.11 ± 0 37*
Chow-fed		
Saline-infused	8.91 ± 0 21	9.93 ± 0.49
Naltrexone-infused	8 64 ± 0 32	7 97 ± 0 35*

Mean skinfold thickness (mm) ± S.E M of Sucrose + chow-fed and Chow-fed animals infused with either saline or naltrexone, before minipump implantation (PRE) and after minipump implantation (POST)

Skinfold thickness was taken as the sum of skinfolds from 3 sites (neck, subscapular and back).

*Significantly different from saline

ance with diet and drug conditions as between group factors. Caloric intakes and body weights were analyzed across 7-day periods. All analyses were followed by a posteriori multiple comparison tests. Data reported as significant have a p-value of 0.05 or less.

RESULTS

Food Intake

Following the first week of drug administration, chow-fed animals receiving naltrexone exhibited a significant suppression in food intake relative to saline-infused animals (Fig. 1). This difference was no longer significant by the second week of the drug period. Feeding behavior was not modified as a function of withdrawal of the drug in chow-fed animals.

During the first week of naltrexone infusions, sucrose + chow-fed animals exhibited a greater suppression in total caloric intake relative to saline-infused controls than chow-fed animals (Fig. 2) Sucrose + chow-fed animals infused with naltrexone consumed approximately 76% of the calories consumed by saline-infused sucrose + chow animals. In contrast, chow-fed animals receiving the drug ate approximately 83% of the calories of their controls. While the total caloric intake of the sucrose + chow-fed animals receiving naltrexone returned to saline control levels by the second week of drug administration, chow intake of these animals remained significantly suppressed during the entire drug period. In contrast to chow intake, sucrose intake was not significantly modified as a function of the drug. No alterations in feeding behavior were observed when the drug was withdrawn (Fig 2).

Body Weight and Adiposity

Although no significant modifications in body weight were observed in chow-fed animals as a function of naltrexone administration, these animals did appear to have slightly suppressed body weights across the drug period relative to animals infused with saline. Body weights in these animals returned to saline control levels when the pumps were removed (Fig. 3).

Body weights of sucrose + chow-fed animals receiving naltrexone were significantly suppressed across the entire drug period relative to saline animals. When the minipumps were removed, animals began to rapidly gain weight, though they never attained control levels (Fig. 3).

Although no significant modifications in body weight were observed in chow-fed animals as a function of drug administration, naltrexone administration resulted in a significant suppression in skinfold thickness in chow-fed animals relative to animals infused with saline. This was also observed in sucrose + chow-fed animals infused with naltrexone. These animals exhibited significantly less adiposity than saline-infused controls, as indirectly measured by skinfold thickness (Table 1).

Energy Production

While there were no significant modifications in either O_2 consumption (Table 2) or CO_2 production (Table 2) in chow-fed animals infused with naltrexone, these animals exhibited a significant suppression in their respiratory quotient (RQ) relative to saline-infused controls (Table 2).

Sucrose + chow-fed animals exhibited modifications in energy metabolism relative to chow-fed animals. Differences in O_2 consumption (Table 2) were observed by the third week of the study as a function of diet, with sucrose + chow-fed animals consuming significantly more O_2 than chow-fed controls. Significant increases in CO_2 production (Table 2) were observed in sucrose + chow-fed animals relative to chow-fed animals by the first week of access to the diets. Changes in CO_2 production were reflected in significant modifications in RQ (Table 2), with sucrose + chow-fed animals exhibiting elevations in RQ both during the first and third weeks of the experiment. Changes in energy metabolism were also observed in sucrose + chow-fed animals as a function of naltrexone administration. While there were no significant modifications in O_2 consumption, CO_2 production was significantly suppressed in sucrose + chow-fed animals receiving

TABLE 2
GAS EXCHANGE PARAMETERS

	O_2 consumption		CO_2 production		Respiratory Quotient	
	Pre	Post	Pre	Post	Pre	Post
Sucrose + chow-fed						
saline-infused	0 81 ± 0 02	0 84 ± 0 03	0 75 ± 0 03	0 77 ± 0 04	0 93 ± 0 08	0 92 ± 0 01
naltrexone-infused	0 80 ± 0 12	0 74 ± 0 05	0 71 ± 0 05	0 59 ± 0 03*	0 91 ± 0 02	0 82 ± 0 03*
Chow-fed						
saline-infused	0 81 ± 0 06	0 69 ± 0.04	0 67 ± 0 02	0 57 ± 0 03	0 82 ± 0 02	0 82 ± 0 01
naltrexone-infused	0.76 ± 0 04	0 72 ± 0 03	0 64 ± 0 02	0 54 ± 0 01	0 84 ± 0 02	0 75 ± 0 02*

Mean O_2 consumption (ml O_2/g/hr) ± S.E.M , mean CO_2 production (ml CO_2/g/hr) ± S E M , and mean respiratory quotient (RQ) of Sucrose + Chow-fed and Chow-fed animals infused with either saline or naltrexone, before minipump implantaion (PRE) and after minipump implantation (POST)
*Significantly different from saline

naltrexone. These changes were reflected in decreases in RQ in naltrexone-infused animals relative to saline-infused animals.

Core and Shell Temperature

No modifications in rectal, paw or tail temperature were observed in chow-fed animals as a function of naltrexone administration.

While there were no modifications in shell temperature as a function of diet, rectal temperature was significantly elevated by the third week of the study in sucrose + chow-fed animals relative to chow-fed animals (Fig. 4). When sucrose + chow-fed animals were infused with naltrexone, there was a significant suppression in rectal temperature, with no changes in paw or tail temperatures observed as a function of the drug.

Water Intake

Animals given access to sucrose + chow drank significantly less water than chow-fed animals over the entire course of the study. Naltrexone infusions led to a significant decrease in water intake in chow-fed animals over the two-week drug period, with water intake returning to control levels when the minipumps were removed (Table 3). While sucrose + chow-fed animals infused with naltrexone decreased water intake during the drug period relative to saline-infused animals, this decrease was not significant. This was probably due to the low baseline levels of water being consumed by these animals (Table 3).

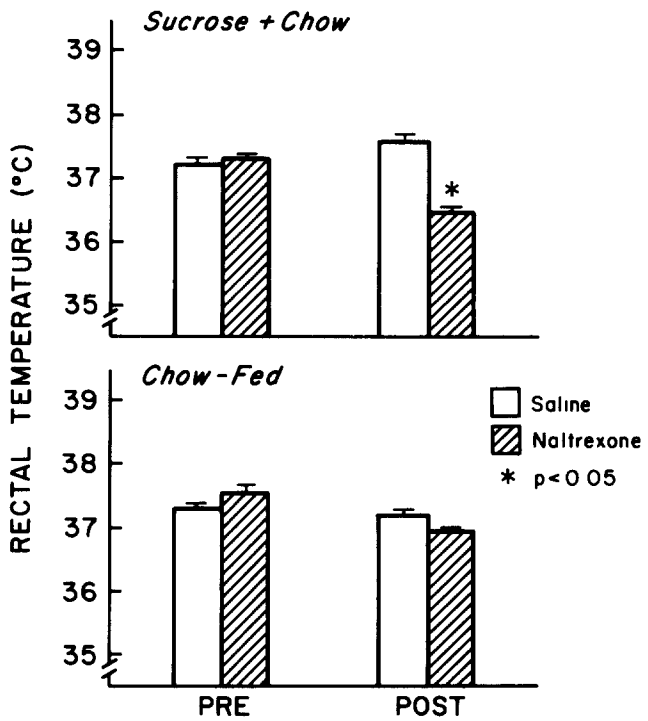

FIG 4 Mean rectal temperature (°C) ±S.E M of Sucrose + Chow-fed and Chow-fed animals infused with either saline or naltrexone, before minipump implantation (PRE) and after minipump implantation (POST) *Significantly different from saline

DISCUSSION

Chronic naltrexone infusions resulted in decreased energy intake in both animals maintained on a standard laboratory diet and animals given access to a 32% sucrose solution in addition to this diet. Food intakes were suppressed for the first week of the naltrexone infusions, but returned to baseline levels by the second week of the drug period. These data are consistent with previous studies which have reported early suppressions in feeding behavior following chronic opiate antagonist administration [2, 9, 23] For example, rats injected with naloxone in a slow-release vehicle decreased food intake for a 2 to 4 day period. This was followed by a return of feeding towards control levels [2] Similarly, it was reported that some tolerance to the feeding effects of naloxone may develop following one week of minipump infusions [9]. In contrast, it was noted that when rats were maintained on a cafeteria-style feeding regime, decreased feeding still occurred after 30 days when naloxone was administered in a long-lasting vehicle [13]

Interestingly, in the present study, even though total en-

TABLE 3

WATER INTAKE (ml) ± S E M

	Baseline	Pumps In		Pumps Out	
	Week 1	Week 2	Week 3	Week 4	Week 5
Sucrose + Chow-fed					
saline-infused	12 21 ± 2 58	11.36 ± 2 48	10 43 ± 2 03	8.26 ± 1 57	13 10 ± 2 45
naltrexone-infused	11.86 ± 1 99	6.97 ± 1 12	7.74 ± 1 80	6.17 ± 1 11	15 21 ± 2 65
Chow-fed					
saline-infused	46.59 ± 1 46	49.64 ± 2 43	48 77 ± 1 49	46 09 ± 1 75	49 77 ± 3 74
naltrexone-induced	43 56 ± 1 86	36 76 ± 2 04*	38 30 ± 1.77*	49 44 ± 1 96	44.93 ± 2 68

Mean water intake (ml) ± S E.M , averaged over one-week periods, of Sucrose + Chow-fed and Chow-fed animals implanted with osmotic minipumps infusing either saline or naltrexone

Minipumps were implanted at the end of week 1 on the diet, and removed at the end of week 3

*Significantly different from saline

ergy intake was no longer decreased in sucrose + chow-fed animals by the second week of naltrexone infusions, body weights were still suppressed. Brands *et al* [2] reported that while food intake was reduced for only a 2 to 4 day period following the injection of naloxone in a slow-release vehicle, body weights were significantly suppressed for up to eleven days. Decreases in body weight gain with chronic opioid antagonist administration have also been reported in both cafeteria-fed rats [13] and genetically obese mice [18].

In addition to changes in energy intake, modifications in energy production were also observed in the present study. Animals given access to sucrose + chow exhibited elevations in O_2 consumption, CO_2 production and RQ. The high CO_2 production observed in the present study, reflects the fact that the main source of fuel for the sucrose + chow-fed animals was carbohydrates, with 50 to 60% of their calories alone coming from sucrose. In addition, changes in O_2 consumption observed in these animals are consistent with the well-described phenomenon of so called longstanding "diet-induced thermogenesis" (DIT) [20, 24, 26] DIT allows animals consuming excess calories to try to maintain energy balance by increasing heat production. This increase in heat production is thought to occur mainly by non-shivering thermogenesis (NST) via sympathetic stimulation of brown adipose tissue [1,7]. Interestingly, sucrose feeding has been shown to induce increased peripheral sympathetic nervous system activity, with both increased catecholamine content and turnover found in tissues [11].

Naltrexone-treated sucrose + chow-fed animals showed a decrease in both CO_2 production and RQ. While these animals showed no suppression in sucrose intake by the second week of the drug period, it appeared as if they were no longer using carbohydrate as their main source of fuel. Changes in RQ suggest that animals were now burning calories from either fat or protein stores as a source of energy In addition to changes in RQ, decreases in both body weight and skinfold thickness support this suggestion. In contrast to the present study, Mandenoff *et al.* [13] found that chronic naloxone injections resulted in significant elevations in resting O_2 consumption in both cafeteria fed rats and rats maintained on a standard laboratory diet. One possible explanation for these different findings may be that Mandenoff *et al.* tested animals at thermoneutrality (29°C), while in the present study animals were monitored at ambient temperature (23–25°C) At thermoneutrality, the basal metabolic rate would be monitored as the main source of heat production. This would not be the case at ambient temperature where both the basal metabolic rate and NST would contribute to changes in oxygen consumption. Differences between the two studies may then be a function of monitoring changes in NST, particularly since NST is greatly influenced by both nutritional factors and drug challenges [21,25].

There are a number of possible explanations for the observed changes in energy metabolism following opioid antagonist administration. First, naltrexone may be affecting the gastrointestinal tract resulting in malabsorption of nutrients. If nutrients were malabsorbed in the gut, animals might start to utilize their own fat and protein stores as a source of energy. This possibility is presently being investigated.

Second, sucrose overfeeding is reported to lead to increases in lipoprotein lipase (LPL) activity and elevations in circulating triglyceride (TG) levels. Both increases in LPL and TG are thought to enhance fat deposition and contribute to obesity by stimulating the uptake of free fatty acids into white adipocytes [5,17]. In addition to increasing both LPL activity and plasma TGs, overfeeding with carbohydrates has been found to elevate insulin levels [6]. Naltrexone may be suppressing these carbohydrate-induced changes, resulting in decreased fat deposition

Finally, chronic naltrexone infusions resulted in an impairment in thermoregulation in animals given access to sucrose + chow. While sucrose feeding itself led to a hyperthermia, naltrexone infusions resulted in a significant suppression in core temperature in these animals. In general, at ambient temperature, naloxone and naltrexone are reported to have little effect on body temperature regulation when injected alone [3]. However, in the present study, when core temperature had been elevated following sucrose feeding, naltrexone suppressed this increase.

While similar changes in food intake and energy metabolism were observed in animals on both dietary regimes, these effects were more pronounced in the sucrose + chow-fed animals. As naltrexone is thought to block the endogenous opioid system, these data suggest that there is an interaction between diet and the opioid peptides. Further research is necessary to help delineate this complex interaction.

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