

Effect of Morphine and Nalmefene on Energy Balance in Diabetic and Non-Diabetic Rats

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LEVINE, A S, M GRACE, C J BILLINGTON, B A GOSNELL, D D KRAHN, D M BROWN AND J E MORLEY *Effect of morphine and nalmefene on energy balance in diabetic and non-diabetic rats* PHARMACOL BIOCHEM BEHAV 29(3)495-500, 1988 —Male rats made diabetic by intravenous injection of streptozotocin were used to evaluate the effect of the diabetic state on morphine- and nalmefene-induced changes in food intake and body weight Morphine increased 4 hour food intake in non-diabetic rats after an initial injection, but increased intake in diabetic rats only after repeated injections Unlike short term measurements, morphine decreased food intake when measured over 24 or more hours in both groups Chronic injection of morphine decreased body weight only in non-diabetic rats Feed efficiency data suggest that morphine had a more potent effect on energy balance in the non-diabetic rats The opioid antagonist, nalmefene, did not alter body weight in either group and only altered food intake in the diabetic animals These data are in concert with other reports indicating that the diabetic state renders animals less responsive to the effects of morphine on nociception and smooth muscle contraction

Opioids Diabetes Morphine Nalmefene

GLUCOSE has been shown to modulate the responsiveness of laboratory animals to opioid effects In 1956 Davis *et al* [3] found that hypoglycemia potentiated the antinociceptive action of morphine in rats as quantified by the tail flick method Simon *et al* [29,30] demonstrated that the analgesic effects of morphine, phenazocine and levorphanol were decreased in rats made diabetic by injection of streptozotocin Pretreatment with hypertonic dextrose or fructose produced the same effect [29,30] Shook *et al* [28] also reported that increasing the concentration of glucose in the media reduced the responsiveness of the electrically stimulated ileum to normorphine which suggests that glucose concentration, rather than insulin alteration, is the mediator of their *in vivo* findings Recently, Shook and Dewey [27] found that diabetic mice were less physically dependent upon morphine than non-diabetic controls Genetically diabetic mice display increased tail flick latencies to radiant heat relative to their littermate controls [9] Both diabetic patients and subjects infused with glucose had decreased pain thresholds and pain tolerance [22]

Since it is known that glucose levels or glucose utilization can modulate feeding behavior, that opioids can stimulate food intake [7,21] and that glucose modulates opioid nociceptive effects, it seems reasonable to believe that glucose might modulate opioid effects on food intake Our laboratory found that both genetically diabetic mice (C57 BL/Ks-db+/db+) and streptozotocin diabetic mice show enhanced sensitivity to naloxone-induced suppression of food intake [6] Rats made hypoglycemic by injection of insulin, a procedure that stimulates food intake, are relatively insensitive to naloxone diminution of food intake [5,10], however, this may depend upon the environment in which the study is conducted [25] In general, then, it appears that elevated glucose levels potentiate the naloxone effect, although we have found this to be markedly influenced by the animal's environment [8]

Data concerning long term administration of opioid agonists and antagonists on food intake and body weight are few and confusing Chronic naltrexone infusions decreased food intake in rats fed chow plus a 32% sucrose solution

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better than in those ingesting only chow [16] Repeated injections of a zinc tannate preparation of naloxone decreased food intake only in rats receiving a high fat cafeteria diet [15] In a group of lean and obese Zucker rats, the long-acting opioid antagonist, nalmefene (6-desoxy-6 methylene naltrexone), decreased food intake but only slightly altered body weight [11,12] On the other hand it has been reported that mice gain weight when given naloxone and decrease their food intake after chronic morphine administration [26] In long term studies in humans naltrexone has been shown to slightly decrease or have no effect on body weight [1,17]

The effect of chronic administration of opioids and their antagonists on food intake and body weight in diabetic vs non-diabetic rats is not known In the present study we addressed this issue by injecting the opioid agonist, morphine, and the opioid antagonist, nalmefene, in a group of streptozotocin-induced diabetic rats and non-diabetic rats of the same strain for a period of 24 days while measuring food intake and body weight Nalmefene is potent and long lived relative to naloxone and does not possess as much opioid agonist activity as naloxone [4]

METHOD

Male Sprague-Dawley rats were used for all studies and were housed under standard lighting and temperature conditions (12 hour/day artificial light, 7 a.m. to 7 p.m., 25°C) Animals were given free access to Purina laboratory chow and to water At about 7 weeks of age rats were given an intravenous injection (tail vein) of streptozotocin (55 mg/kg in 1 3% citric acid buffer, pH 4.5) followed by 2 ml of 30% dextrose Three weeks later they were bled via the tail vein and glucose was measured using a Beckman Glucose Analyzer (Fullerton, CA) to assure a diabetic state (glucose=632±22 mg/dl). Age-matched rats were used as non-diabetic controls Food intake and body weight were quantified for 3 days before the start of the experimentation to acclimate the animals to handling and daily interruptions The rats were divided into five groups of ten and 1 group of eleven which allowed for three groups of diabetic and three groups of non-diabetic rats. To enable a balanced design for statistical analysis, one rat was omitted at random from the group containing eleven rats

On day one of the study a single injection of either morphine sulfate (10 mg/kg, Eli Lilly and Co., Indianapolis, IN), nalmefene (10 mg/kg, kindly provided by Key Pharmaceuticals, Inc., Miami, FL) or vehicle was injected subcutaneously Food intake was then measured at hours 1, 2, 4, 6 and 24. During the ensuing 21 days, rats were injected twice per day at about 0900 and 1600 hr On days 2 and 7 food intake was again quantified at hours 1, 2, 4 and 6 (only 4 hour data are presented) Food intake and body weight were measured on days 3, 5, 10, 12, 15, 17, 19, and 22 Food intake was corrected for spillage at each measurement and average daily intake was calculated by dividing the total food intake by the representative time period (eg grams eaten between (day 3/day 5)/3) Animals were sacrificed on day 24 following one injection of the appropriate drug and trunk blood was collected for analysis of glucose with a glucose oxidase procedure (Sigma Chemical Co., St Louis, MO)

All data are shown as mean±SEM Data were analyzed by analysis of variance (ANOVA) (repeated measures when appropriate). Post-hoc tests (least significant difference procedure-LSD) were only conducted if the overall ANOVA demonstrated an interaction The error term for each post-hoc test was calculated by a one factor ANOVA

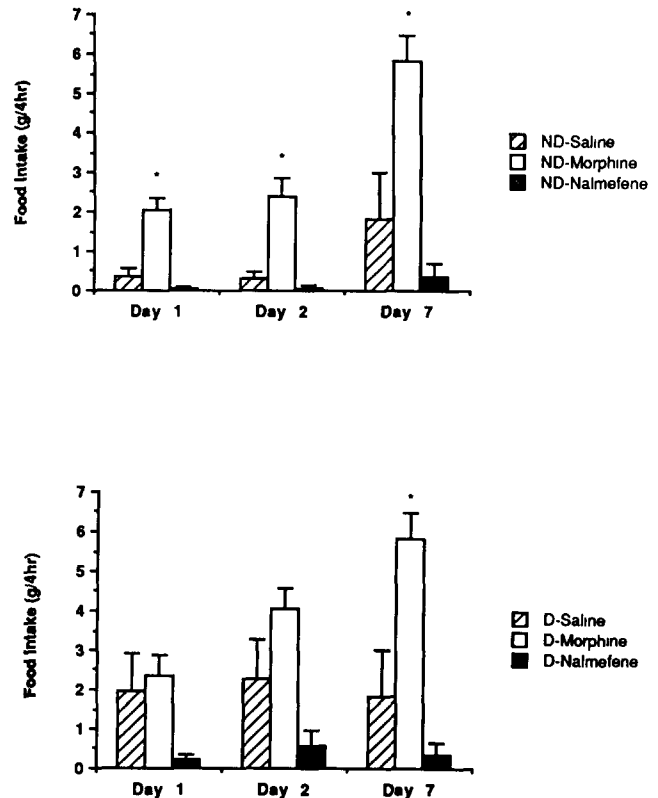


FIG 1 The effect of morphine (10 mg/kg bid) and nalmefene (10 mg/kg bid) on 4 hour food intake in diabetic (D) and non-diabetic (ND) rats on days 1, 2 and 7 of the study * $p < 0.05$ compared to saline controls for rats of same day and diabetic status

RESULTS

At sacrifice, trunk blood was sampled and serum was analyzed for glucose concentration Serum glucose levels in diabetic and non-diabetic animals were not altered following chronic injections of morphine or nalmefene (Diabetic saline=530±14, morphine=496±2, nalmefene=511±8 mg/dl) (Non-Diabetic saline=142±3, morphine=138±2, nalmefene=148±5 mg/dl)

Short-Term Feeding Studies

The effect of morphine and nalmefene on food intake in diabetic and non-diabetic control rats on day 1, day 2 and day 7 was analyzed by a 3-factor repeated measures ANOVA There were main effects of diabetic state, $F(1,54)=11.72$, of drug, $F(2,54)=22.53$, and of day of the study, $F(2,108)=5.25$ (all p 's<0.01) Also, there was a significant diabetic state × drug × day interaction Individual group comparisons (LSD procedure) indicated that morphine stimulated 4 hour food intake in the non-diabetic rats on days 1, 2, and 7, whereas morphine only stimulated food intake in the diabetic rats on day 7 (Fig 1) Nalmefene had no significant effect on 4 hour food intake, although the mean intakes after nalmefene were consistently less than control means Twenty-four hour food intake was significantly decreased in both diabetic and non-diabetic animals following one injection (day 1) and following three injections (day 1 + day 2) of morphine and nalmefene (Fig 2)

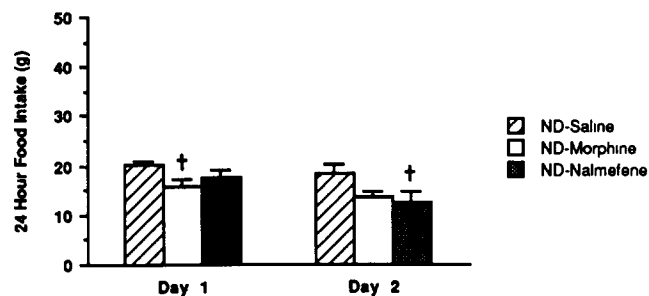
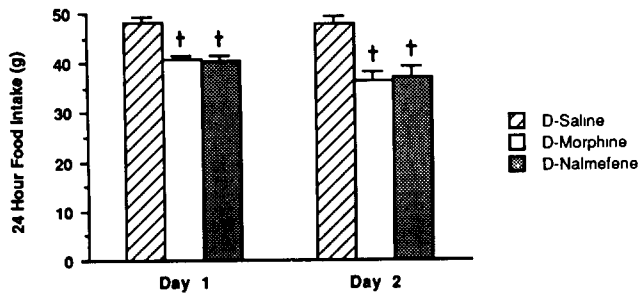


FIG 2 Twenty-four hour food intake in diabetic and non-diabetic rats following morphine (10 mg/kg bid) and nalmefene (10 mg/kg bid) injections on days 1 and 2 of the study † $p < 0.05$ compared to saline controls for rats of same day and diabetic status

Chronic Drug Studies

The effect of morphine and nalmefene on average food intake and on body weight change over 22 days was analyzed by a 3-factor repeated measures ANOVA. There were main effects of diabetic state, $F(1,54)=846.93$, of drug, $F(2,54)=9.34$, and of day, $F(7,378)=39.16$ (all p 's < 0.001). There was a significant diabetic \times drug interaction, $F(2,54)=5.22$, $p < 0.05$, and a significant diabetic \times drug \times day interaction, $F(14,378)=2.86$, $p < 0.001$. One factor ANOVA followed by LSD tests indicated that food intake was decreased by nalmefene and morphine in the diabetic rats, whereas only morphine decreased feeding at some time points in the non-diabetic control rats (Fig. 3).

Analysis of body weight data demonstrated main effects of diabetic state, $F(1,54)=78.42$, $p < 0.001$, and of day $F(8,432)=63.85$, $p < 0.001$, but not of drug. There was a significant interaction of diabetic state \times drug \times day, $F(16,432)=1.814$, $p < 0.05$. One factor ANOVA followed by the least significance difference test indicated that body weight was reduced due to morphine injection only in the non-diabetic animals (Fig. 4). Nalmefene failed to alter mean body weight in either group of rats. Analysis of maximum percent body weight gain (maximum weight gain or at least a negative weight change/initial body weight $\times 100$) and maximum percent body weight loss (maximum weight loss or at least a negative weight change/initial body weight $\times 100$) substantiated the observation that morphine has a more potent effect in non-diabetic controls compared with diabetic rats (Fig. 5). In the non-diabetic rats the maximum percent body

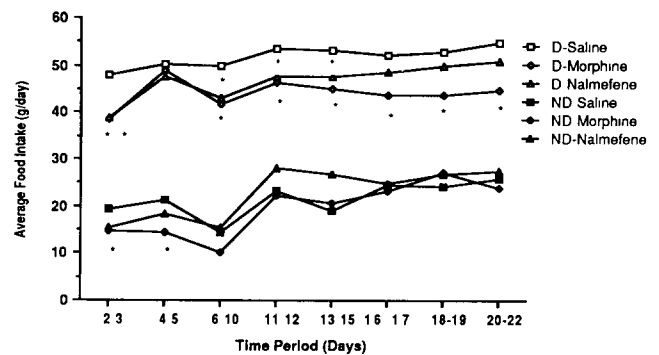


FIG 3 The effect of chronic injection of morphine (10 mg/kg bid) and nalmefene (10 mg/kg bid) on average food intake in diabetic (D) and non-diabetic (ND) rats * $p < 0.05$ compared to saline controls for rats of same day and diabetic status

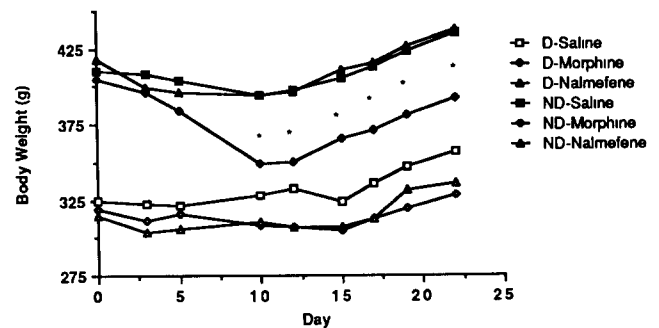


FIG 4 The effect of chronic injection of morphine (10 mg/kg) and nalmefene (10 mg/kg) on body weight in diabetic (D) and non-diabetic (ND) rats * $p < 0.05$ compared to saline controls for rats of same day and diabetic status

weight lost by the rats treated with morphine was approximately 15%, in contrast to only above a 5% maximum body weight loss in the diabetic rats.

To integrate food intake and body weight data we calculated a "feed efficiency ratio" (change in body weight [either (+) weight gain or (-) weight loss] from day 0 divided by the cumulative amount of food eaten). Morphine decreased the feed efficiency ratio in diabetics as well as non-diabetics, but had a more potent effect in the non-diabetic group (drug \times diabetic state interaction $F(2,54)=3.432$, $p < 0.05$ (Fig. 6)). In contrast, nalmefene failed to alter the feed efficiency ratio in non-diabetics, but did so in the diabetics.

DISCUSSION

This study indicates that diabetic rats respond differently than non-diabetic control rats to the acute effects of morphine on food intake as well as to the changes in food intake and body weight which occur following chronic morphine administration. Morphine failed to stimulate short term food intake in diabetic animals following an initial exposure, whereas morphine stimulated feeding in non-diabetic control rats after a single injection. This might be secondary to the increased basal intake of food in the diabetics since opioids are less potent stimulators of feeding in animals chronically food deprived [19] or during the nocturnal feeding period [22]. However, repeated exposure of diabetic

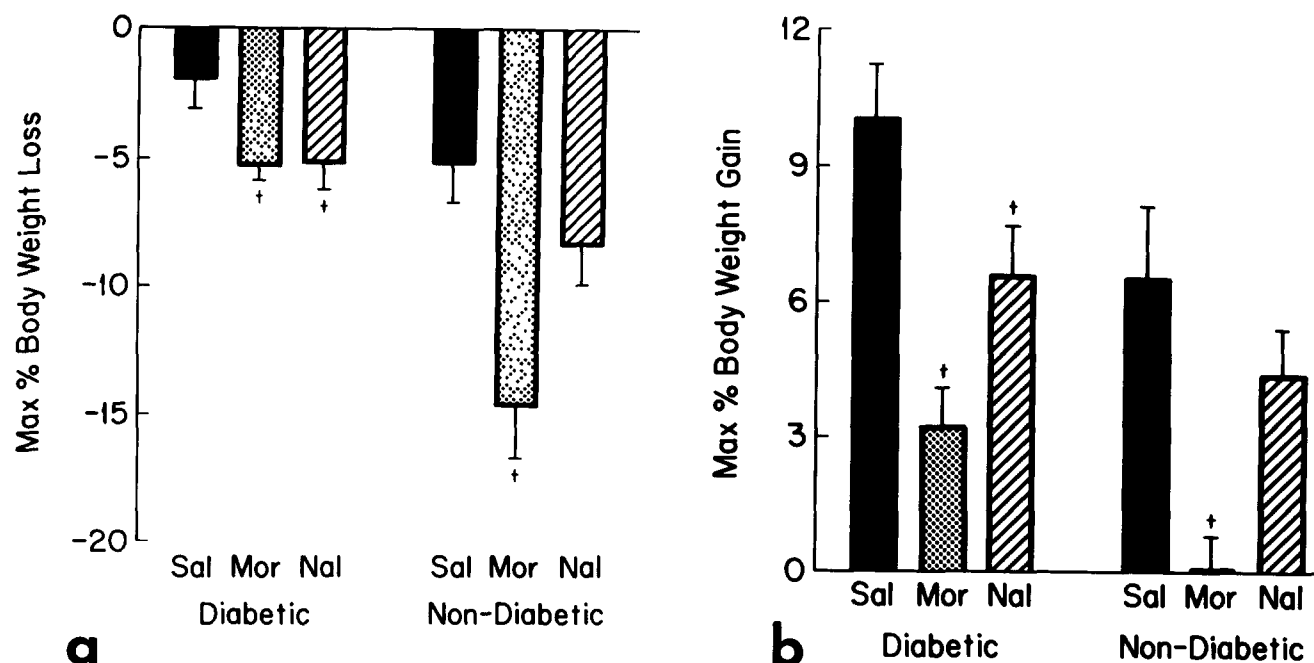


FIG 5 Maximum percent body weight loss (a) and percent body weight gain (b) in diabetic and non-diabetic rats following chronic injection of morphine (10 mg/kg bid) and nalmefene (10 mg/kg bid) [†] $p < 0.05$ compared to saline controls for rats of same day and diabetic status

animals to morphine ultimately resulted in enhanced food intake. We have previously reported this "reverse-tolerance" effect in normal animals [22]. Nalmefene failed to alter short term food consumption in either group of rats, probably due to a floor effect. In contrast to the short term studies, chronic administration of morphine to both diabetic and non-diabetic rats resulted in decreased cumulative food intake. Also, morphine resulted in a more marked decrease in body weight in the non-diabetic animals. The feed efficiency ratios also substantiated the latter finding, suggesting that diabetic rats were somewhat resistant to morphine's effect on energy balance.

The above data are in concordance with those reported by Simon and his colleagues [29,30]. They found that streptozotocin-induced diabetic rats were less sensitive to the antinociceptive effects of morphine. Recent data from the same laboratory indicates that hyperglycemia modifies the responses of guinea pig ileum and mouse vas deferens responses to normorphine [4,5]. Preliminary studies [31] indicate that glucose can enhance the binding of naloxone to brain membranes. It has also been shown that diabetic humans display decreased pain thresholds and pain tolerance [22]. In general, it appears that diabetic animals respond differently to the effects of morphine on both pain and food intake when compared to non-diabetic control animals.

Relatively few studies have examined the effect of chronic administration of opioid agonists or antagonists on food intake and body weight. In our study the long-acting opioid antagonist, nalmefene, failed to alter body weight in either diabetic or non-diabetic rats. Nalmefene did not decrease food intake in the non-diabetic rats, but did decrease food intake slightly in the diabetic rats. McLaughlin and Baile [11] found that nalmefene decreased meal size and daily food intake, but increased meal frequency in Zucker rats and their lean littermates. Body weight decreased during the first week of the study in the nalmefene treated animals compared with the saline treated controls, however, subse-

quent weight gain was greater in the nalmefene treated rats. The weight loss was more marked in the obese Zucker rats. Thus, McLaughlin and Baile [11] found only slight effects of nalmefene on body weight. Naltrexone, when given twice a day at 10 mg/kg to genetically obese (ob/ob) mice decreased the rate of weight gain in these obese mice but had no effect on their lean littermates [23]. From the latter studies it seems that opioid antagonism seems to affect energy balance more readily in obese animals. Similarly, it appears that opioid antagonism is more potent in animals fed palatable foods. Repeated injections of a zinc tannate preparation of naloxone abolishes diet-induced obesity in rats fed a high fat cafeteria diet, whereas no effect of opioid blockade was noted in rats fed a low fat diet [15]. Chronic naltrexone infusions (200 μ g/kg/hr) decreased appetite more effectively in rats fed chow plus 32% sucrose compared to those fed chow alone [16]. Brands *et al* [2] also found that a zinc tannate salt of naloxone only decreased food intake during the first few days of the study and body weight during the first ten days of the study.

Shimomura *et al* [26] reported that lean mice given daily morphine injections decreased food intake. McLaughlin and Baile [12] reported that rats autoimmunized against β -endorphin increase both their food intake and body weight. This suggests that chronically available endogenous β -endorphin might result in a decrease in food intake and body weight. This finding is in keeping with our previous study showing that peripherally infused beta-endorphin decreased food intake [18]. The present study indicates that normal rats chronically treated with morphine lose weight and ingest somewhat less food suggesting that morphine's effect on body weight could be due to increased energy expenditure, rather than secondary to its effects on feeding. Also, nalmefene failed to decrease body weight in diabetic animals, although food intake was decreased. However, it is difficult to generalize these effects of morphine and/or β -endorphin to all opioids.

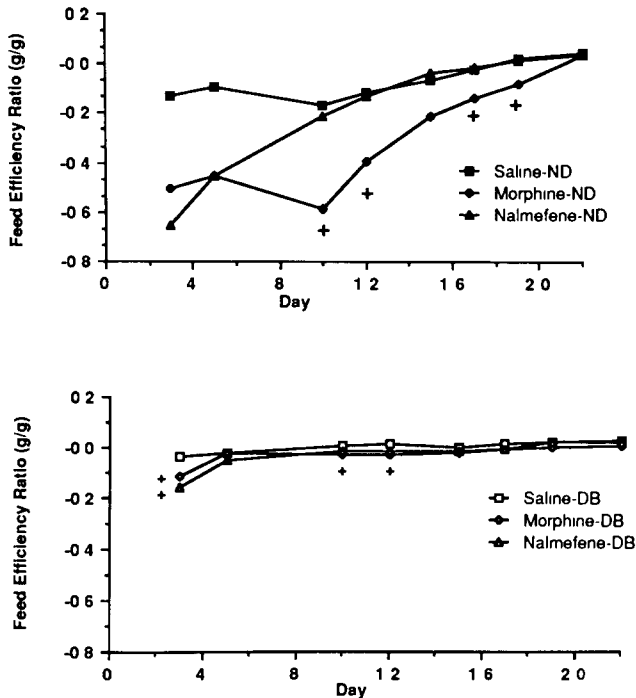


FIG 6 The effect of chronic administration of morphine (10 mg/kg bid) and nalmefene (10 mg/kg bid) on feed efficiency in diabetic and non-diabetic rats + $p < 0.05$ for rats of same day and diabetic status

The decreased feed efficiency noted with chronic injection of morphine suggests an effect on energy conservation. Morphine seems to have decreased body weight to a greater extent than can be accounted for by food intake alone, particularly in the non-diabetic animals. Chronic naloxone injection has been reported by several investigators to increase oxygen consumption [14,15]. However, Marks-Kaufman *et al* [16] found that although naltrexone treatment altered the respiratory quotient, it had no effect on total oxygen consumption. Opioids are also known to affect lipid metabolism. For example, Richter *et al* [24] demonstrated that β -lipotropin stimulated lipolysis in rabbits.

Thus, the present data indicate that morphine, when injected chronically, can alter body weight. The effect of morphine on body weight is different in diabetic animals when compared to non-diabetic controls. In general, diabetic animals appear to be less sensitive to the acute effects of morphine on food intake and the effects of chronic administration of morphine on body weight.

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