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Effect of cafeteria diet on intestinal absorption of palmitic acid in rats

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The effect of a hypercaloric cafeteria diet on the accumulative ability of small intestine palmitic acid transport in female Wistar rats was studied using everted intestinal slices. Cafeteria diet decreased jejunal and ileal absorption of palmitic acid per intestinal mass after obesity had developed. A link between gastrointestinal functions, feeding behavior, and the development of obesity induced by cafeteria diet is suggested. (J. Nutr. Biochem. 6: 151–154, 1995.)

Keywords: obesity; cafeteria diet; palmitic acid; intestinal absorption

Introduction

The cafeteria or supermarket diet is used to approximate the varieties of highly palatable food consumed by humans and is accepted as the closest experimental analog to the majority of human cases of obesity induced by chronic voluntary hyperphagia of energy-rich food.[1–3] Offering laboratory rats ad libitum access to a source of energy-rich foods results in increased food intake and weight and body fat gain.[4–8]

However, changes in emptying rates of intraluminal contents and morphological alterations in the gastrointestinal tract may be predisposing factors in stimulating increased food intake and hence obesity. Transit delay in obese humans is explained by possible anatomical differences or by motility alterations.[9] Rapid gastric emptying and slow overall transit time were found in rats that became obese due to a cafeteria diet.[8]

In addition, dietary regulation of digestive function has also been described. Some nutrients affect their own as well as overall intestinal absorption. Dietary carbohydrate stimulates aldohexose uptake,[10] but the feeding of a diet high in a given fatty acid does not necessarily produce a change in its intestinal uptake.[11]

Finally, previous work showed that a hypercaloric cafeteria diet increased active intestinal transport of hexoses in young rats, though not when obesity was well established.[12] Hence, the aim of this paper is to show the effect of the above-mentioned cafeteria diet on the absorption of palmitic acid, a saturated fatty acid, in the small intestine of developing rats.

Methods and materials

Animals and diet

Female Wistar rats were used. The animals were housed four animals per cage at a temperature of 23 ± 1° C and relative humidity of 50% with a 12-hr on–off light cycle. They were fed ad libitum.

The rats were divided in two groups in accordance with the diet supplied: controls and cafeteria-fed. These two groups were also divided into three subgroups of 16 animals each according to age (30, 60, and 90 days old).

The control group received ad libitum tap water and rat chow pellets (Panlab, UAR A-03, Barcelona, Spain; dry wt., 26.7% proteins, 56.6% carbohydrate, 5.7% lipids, 4.5% cellulose and 6.5% ashes; energy contents, 13.37 kJ/g). The cafeteria-fed group received ad libitum tap water and rat chow together with an ad libitum cafeteria diet which was offered to the litters from day 7 of life. The latter consisted of the following foods crudely but homogenously mixed: cookies, foie-gras, croissants, sweets, bacon, biscuits, chocolate, peanuts, carrots, bananas, and cheese plus sugary milk (overall dry wt., 16.3% proteins, 45.8% carbohydrates, 34.7% lipids, and 3.2% others; energy contents, 14.72 kJ/g solid diet, 5.22 kJ/mL sugary milk). The ratio of unsaturated (poly- and monounsaturated) to saturated fatty acids was 2.68:1 for the chow diet and 1.2:1 for the cafeteria diet.

The energy content and unsaturated to saturated fatty acids ratio of the cafeteria diet were determined using food composition tables.[13] The chow diet energy content and unsaturated to saturated fatty acids ratio were supplied by the manufacturer.

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Measurement of obesity induced by cafeteria diet

To study the obesity induced by the administered cafeteria diet, the body weights (g) of the control and cafeteria rats were measured. These rats were killed by beheading and then their abdominal, ovarian, lumbar and, dermal white adipose tissue (WAT) was removed, weighed, and expressed as body WAT (g). These four regions were chosen following Vague's distribution[14] of white adipose tissue, which defined gynoid obesity.

Measurement of daily food intake

The food intakes (g/day for solid food, and mL/day for liquid food) of control and cafeteria rats were each monitored for a period of 5 days before the studied ages (30, 60, and 90 days old), during which time food consumption of all animals in the cage was recorded daily. Energy contents (kJ/day) of food intake were also calculated. These values were expressed as daily averages per animal.

Measurement of intestinal morphometry

After the animals were killed, the small intestine was removed from the ligament of Treitz to the ileocecal junction, briefly washed in saline, blotted on filter paper, and weighed (g), and the length (cm) was measured after submission to a constant tension of 10 g.

Tissue uptake studies

The animals were killed at 15.00 hr and segments of small intestine were taken distally from 5 cm after the ligament of Treitz to 5 cm before the ileocecal junction. Then the small intestine was cut into two segments of similar length which were taken as the jejunum and the ileum. Once the adherent mesenteric tissue and Peyer's patches were trimmed off, both jejunum and ileum were washed with ice-cold Krebs–Henseleit buffer devoid of divalent cations (pH 7.4) (KHDD), everted, and then divided into slices of similar size (~1 cm in length and 50 to 100 mg in weight).

A micellar solution (MS) was obtained according to Borgstrom[15] using 1-monooleoyl-rac-glycerol (18:1, cis-9) taurodeoxycholic acid 0.3 mM, sodium salt monohydrate 3.4 mM, palmitic acid 0.6 mM, and ^{14}C-palmitic acid as a tracer in KHDD.

Everted slices were preincubated in KHDD for 10 min at 37° C and then incubated in 10 mL of MS containing labeled palmitic acid at 37° C and bubbled with carbogen gas (95% O_2 + 5% CO_2). The stream of gas was adjusted to allow proper stirring of the incubation medium. The samples were incubated for 1, 2, 3, 5, 10, 15, and 20 min periods. After incubation, the samples were removed from the medium, briefly washed in ice-cold saline, opened lengthwise, blotted on filter paper, and weighed. The tissue was digested in perchloric acid and hydrogen peroxide at 50° C for 24 hr, neutralized with 0.1 N NaOH, and then radioactivity counted using standard liquid scintillation procedures.

In order to correct for the amounts of palmitic acid associated with the adherent fluids, tissue slices were incubated at the above-mentioned incubation times with 0.6 mM polyethylene glycol-4000 (PEG-4000), and ^{14}C-PEG-4000 as a tracer in KHDD with the same conditions as above.

The number of slices that can be prepared from each animal is limited. This impedes determination of absorption and adherent fluid measurements simultaneously at the seven incubation times for a single animal. Thus, for each incubation time, the following procedures were carried out: five slices were taken for palmitic acid uptake studies and three slices for adherent fluids studies. These were obtained from a pool of n different rats and the standard error accounts for interanimal variability.

Materials

Taurodeoxycholic acid sodium salt monohydrate and 1-monooleoyl-rac-glycerol (18:1, cis-9) were supplied by Aldrich Chem Co. (Milwaukee, WI USA) and Sigma Chem. Co. (St. Louis, MO USA), respectively. (U-^{14}C)-palmitic acid and ^{14}C-polyethylene glycol-4000 were purchased from The Radiochemical Centre, (Amersham, UK); unlabeled palmitic acid and PEG-4000 were from Sigma Chem. Co. The specific activity and final activity of the palmitic acid were 3.22 mCi/mg and 0.04 mCi/mL, respectively; those of PEG-4000 were 15 mCi/g and 0.1 mCi/mL.

Calculations

Intracellular uptake of the substrate was expressed as nmol/100 mg wet weight after corrections were made from the amount of substrate associated with adherent fluids. In order to determine the overall ability of tissue to uptake palmitic acid, the trapezoidal method[16] was used to calculate the area under the curve (AUC) obtained by interpolation of intracellular uptake mean values of each experimental incubation time. This was expressed as nmol · min/100 mg wet wt.

Statistics

Results are expressed as a mean ± SE. Differences between means were determined by using the Student's t-test. Differences between groups were determined by using analysis of variance (ANOVA). A P value of ≤ 0.05 was taken to be statistically significant.

Results and Discussion

Table 1 shows the results of body weight, WAT, intestinal weight, and length, plus average daily intake and its energy content in rats fed with rat chow (RC) and a cafeteria diet (CD) at 30, 60, and 90 days old.

These results showed that the cafeteria diet used resulted in obesity in rats. This was demonstrated by the highest body weight and the highest WAT weight of rats fed with rat chow and the cafeteria diet (for the cafeteria diet-fed group), except in the youngest animals, which showed no differences in body weight between animal groups. These results agree with previous data which showed that the development of obesity becomes clearly established after 30 days of cafeteria diet feeding.[8]

The small intestine of the cafeteria-fed rats showed significantly higher weight and length than that of lean rats as also shown by previous work.[8]

The average values of daily intake per animal showed that the rats ate a larger quantity of the cafeteria diet than the rat chow, as demonstrated by the low consumption of commercial rat chow when cafeteria food is given.

The energy contents of the average daily intake per animal were higher in cafeteria-fed rats than in controls, except at 30 days of age when no difference between animal groups was observed. The increase of energy intake followed a pattern similar to the above observed development of obesity.

Figure 1 shows the results of jejunal and ileal in vitro absorption of palmitic acid per intestinal mass (100 mg of wet weight) after 1, 2, 3, 5, 10, 15, and 20 min of incubation, respectively, for a 90-day-old control or cafeteria-fed rats. Similar results were also obtained when animals

Table 1 Body weight, WAT, intestinal weight and length, food and energy intake in rats (n = 8 per group) fed with rat chow (RC) and cafeteria diet (CD) at 30, 60, and 90 days old

Age (days)	30		60		90	
Group	RC	CD	RC	CD	RC	CD
Body weight (g)	89 ± 3	89 ± 3	193 ± 3	233 ± 6*	211 ± 4	308 ± 6*
White adipose tissue (g)	2.4 ± 0.1	4.8 ± 0.1*	7.2 ± 0.1	27.2 ± 0.5*	12.2 ± 0.3	50.8 ± 1.0*
Intestinal weight (g)	2.3 ± 0.1	3.4 ± 0.2*	3.0 ± 0.1	3.8 ± 0.1*	3.1 ± 0.2	4.4 ± 0.2*
Intestinal length (cm)	94.0 ± 0.7	104.0 ± 0.7*	103.1 ± 1.2	120.2 ± 2.7*	110.2 ± 2.5	125.0 ± 3.2*
Average daily intake†						
Rat chow (g/day)	18.5 ± 1.2	3.5 ± 0.6*	18.9 ± 0.8	4.8 ± 0.6*	19.5 ± 0.7	5.9 ± 0.9*
Cafeteria diet (g/day)	—	11.3 ± 1.4	—	14.7 ± 4.2	—	14.8 ± 2.2
Sugared milk (mL/day)	—	12.6 ± 1.3	—	19.1 ± 2.7	—	22.5 ± 2.1
Energy (kJ/day)	247 ± 16	279 ± 36	253 ± 11	381 ± 84*	261 ± 9	415 ± 56*

*$P \leq 0.05$ as compared with RC at the same age using Student's t-test.
†Mean values (±SE) of daily intake of eight animals each calculated 5 days before the experiment.

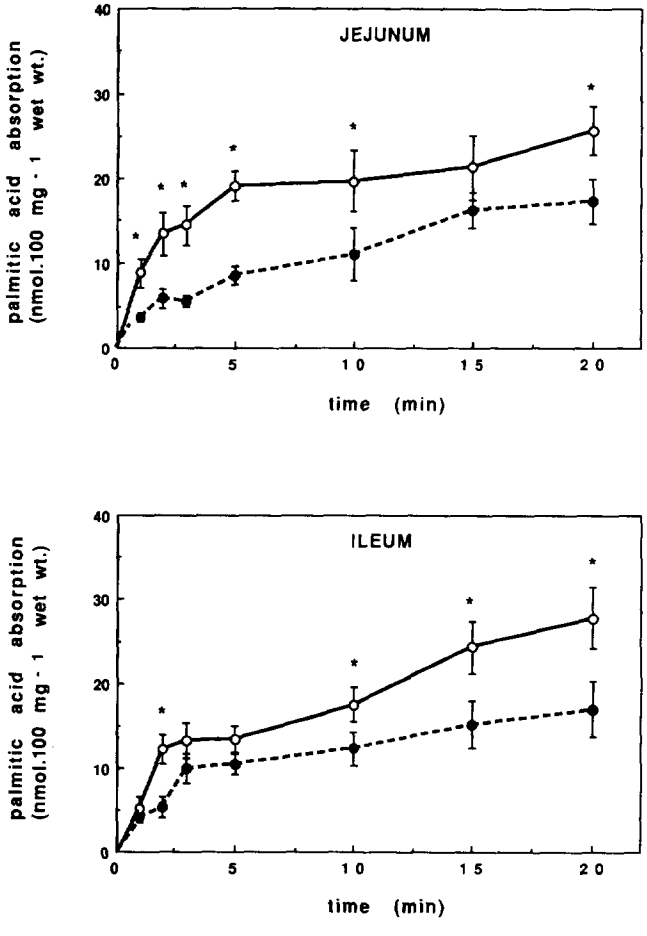

Figure 1 Jejunal and ileal palmitic acid absorption (nmol/100 mg wet wt.) in vitro in 90-day-old controls (open symbols) and cafeteria-fed (full symbols) rats, depending on time of incubation. Similar results were obtained when animals were 30 and 60 days old. Data are given as mean ± SE for n = 8 rats (*$P \leq 0.05$, using Student's t-test).

were 30 and 60 days old. These results show that the cafeteria diet decreased intestinal absorption of palmitic acid per intestinal mass ($P \leq 0.05$).

Table 2 shows the area under the curve (AUC) obtained by interpolation of jejunal and ileal uptake mean values of 30-, 60-, and 90-day-old control and cafeteria-diet rats. The cafeteria diet decreased the ability of the jejunum and the ileum to accumulate palmitic acid per intestinal mass (100 mg of wet weight) at all the ages studied ($P \leq 0.05$).

Table 2 Area under the curve (AUC) (expressed as nmol · min/100 mg wet wt.) of jejunal and ileal uptake of palmitic acid in rats fed with rat chow (RC) and cafeteria diet (CD)

	Diet	AUC jejunum	AUC ileum
30 days old	RC	353.4 ± 24.6	350.9 ± 17.5
	CD	258.4 ± 10.2*	262.0 ± 5.9*
60 days old	RC	301.1 ± 19.8	286.9 ± 16.3
	CD	195.0 ± 7.9*	233.5 ± 11.2*
90 days old	RC	335.9 ± 17.8	322.5 ± 14.9
	CD	216.3 ± 9.9*	215.5 ± 14.1*

Data are given as mean ± SE for n = 8 rats (*$P \leq 0.05$ CD versus RC for jejunum and ileum, using Student's t-test).

The observed effect of the cafeteria diet on the intestinal absorption of palmitic acid could be explained as due to changes of palmitic acid uptake and/or the structure of the intestinal wall, both induced by cafeteria diet components. This effect is directly related to food transit time through the intestinal lumen and is also related to gastrointestinal motility characteristics.

The fastest gastric emptying and the slowest overall transit time of luminal contents have previously been observed[8] after 30-day-old female Wistar rats were fed with the cafeteria diet used in the present work from day 7 of life, results which concur with the findings of the present work showing an increased intestinal weight and length following the above-mentioned cafeteria diet.

In addition, dietary fat proved to have a trophic action on the small intestinal mucosa, increasing mucosal weight, protein, and DNA contents of each of the small intestinal regions: proximal, medial, and distal.[17] According to these findings, it may be concluded that the hyperlipidic cafeteria diet used in the present work also had a direct effect on the small intestinal mucosa.

It is also well known that voluntary hyperphagia is due to the higher palatability of the diet, i.e., the variety of food

increases the average meal size and frequency.[7] From this and previous findings,[18,19] certain relationships were established between anatomical alterations, gastrointestinal motility, food intake, food energy density, and the time that food is in contact with the intestinal wall. Thus, people who choose energy-dense meals may tend to show increased rates of gastric emptying, resulting in shorter satiety periods, increased eating at the next meal and increased net intestinal absorption. Such implications correlate with previous results which have shown increased jejunal absorption per intestinal mass (100 mg of wet weight) of α-MG (an aldohexose) in young cafeteria-fed rats.[12]

The increased intestinal uptake of this glucose analog could also be explained according to the properties of each dietary nutrient. It is well know that the intestinal uptake of glucose in enhanced in proportion to the dietary level of glucose or sucrose,[10] and the cafeteria diet employed[12] had high contents of refined sugars, especially sucrose.

The results from this work showed that rats fed a cafeteria diet exhibited decreased intestinal absorption per intestinal mass (100 mg of wet weight) of palmitic acid, a saturated fatty acid. It is well known that feeding diets high in a given fatty acid does not change jejunal or ileal uptake.[11] The cafeteria diet used in the present work had a high lipid content, and the ratio of unsaturated (poly- and monounsaturated) to saturated fatty acids was 2.68:1 for the chow diet and 1.2:1 for the cafeteria diet. Hence, the uptake pattern of palmitic acid observed in the present work could be explained by the effect of nutrients on their own absorption.

Additionally, the lipid energy equivalent is more than twice that of carbohydrate per weight, and this may also help explain the different uptake pattern of α-MG versus palmitic acid despite the rats being fed the same cafeteria diet. However, the observed increase of intestinal weight and length in cafeteria-fed rats showed that similar overall intestinal absorption of palmitic acid between control and cafeteria-fed rats could also be expected.

Finally, this work supports the implied link[12] between gastrointestinal structure, motility and absorption, food intake, feeding behavior, and the development of obesity induced by a cafeteria diet. Thus obesity developed after the first month of cafeteria diet feeding, and this obesity was demonstrated by a high body and white adipose tissue weight.[8] It is also noted that there is a relationship between meal frequency and body fat; a high level of body fat suppresses the tendency for rats to eat, inducing a decrease in meal frequency proportional to obesity development.[7] This suggests a relationship between fat metabolism and feeding behavior that illustrates how feeding patterns of obese subjects may be, at least partly, a consequence rather than a cause of their obesity.

Thus, it could be inferred that the gastrointestinal tract showed an adaptation to the relationship between fat metabolism and feeding behavior: decreasing differences of gastrointestinal motility between lean and obese rats,[8] increasing jejunal absorption of α-MG in young cafeteria-fed rats,[12] but decreasing palmitic acid jejunal and ileal absorption in cafeteria-fed rats although the obese rats had similar[8] or longer[20,21] small intestinal transit times than the lean rats.

References

1 Sclafani, A. and Springer, D. (1976). Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. *Physiol. Behav.* **17,** 461–471

2 Kanarek, R.B. and Hirsch, E. (1977). Dietary-induced overeating in experimental animals. *Fed. Proc.* **36,** 154–158

3 Rothwell, N.J. and Stock, M.J. (1979). Regulation of energy balance of two models of reversible obesity in the rat. *J. Comp. Physiol. Psychol.* **93,** 1024–1034

4 Rolls, B.J. and Rowe, E.A. (1979). Exercise and the development and persistence of dietary obesity in male and female rats. *Physiol. Behav.* **23,** 241–247

5 Rolls, B.J., Rowe, E.A., and Turner, R.C. (1980). Persistent obesity in rats following a period of consumption of a mixed, high energy diet. *J. Physiol.* **298,** 415–427

6 Sclafani, A. (1980). Dietary obesity. In *Obesity* (A.J. Stunkard, ed.), p. 166, Saunders, Philadelphia, PA USA

7 Rogers, P.J. and Blundell, J.E. (1984). Meal patterns and food selection during the development of obesity in rats fed a cafeteria diet. *Neurosci. Biobehav. Rev.* **8,** 441–453

8 Dameto, M.C., Rayó, J.M., Esteban, S., Planas, B., and Tur, J.A. (1991). Effect of cafeteria diet on the gastrointestinal transit and emptying in the rat. *Comp. Biochem. Physiol.* **99A,** 651–655

9 Basilisco, G., Camboni, G., Bozzani, A., Vita, P., Doldi, S., and Bianchi, P.A. (1989). Orocecal transit delay in obese patients. *Dig. Dis. Sci.* **34,** 509–512

10 Ferraris, R.P. and Diamond, J.M. (1989). Specific regulation of intestinal nutrient transporters by their dietary substrates. *Ann. Rev. Physiol.* **51,** 125–141

11 Thomson, A.B.R., Keelan, M., Garg, M., and Clandinin, M.T. (1987). Spectrum of effects of dietary long-chain fatty acids on rat intestinal glucose and lipid uptake. *Can. J. Physiol. Pharmacol.* **65,** 2459–2465

12 Dameto, M.C., Rayó, J.M., Esteban, S., Prieto, R.M., and Tur, J.A. (1994). Effect of cafeteria diet on α-MG intestinal absorption in rats. *Comp. Biochem. Physiol.* **108A,** 467–470

13 Jiménez A., Cervera P., and Bacardi M. (1990). Tabla de composición de alimentos. Ed Wander-Sandoz/Nutrición, Barcelona, Spain

14 Vague J. (1947). La différenciation sexuelle-facteur déterminant des formes de l'obesité. *Presse Méd.* **39,** 452–458

15 Borgstrom, B. (1965). The dimensions of bile salt micelle measurements by gel filtration. *Biochim. Biophys. Acta* **106,** 171–183

16 Gibaldi, M. and Perrier, D. (1982). *Farmacocinética,* p. 299–302, Reverté, Barcelona, Spain

17 Maxton, D.G., Cynk, E.U., Jenkins, A.P., and Thomson, R.P.H. (1980). Effect of dietary fat on small intestinal mucosa. *Gut* **30,** 1252–1255

18 Hunt, J.N., Cash, R., and Newland, P. (1975). Energy density of food, gastric emptying and obesity. *Lancet* **2,** 905–906

19 Vela, J.S., Yerra, N., Pitchumoni, C.S., and Jain, N.K. (1988). Carbohydrate (CHO) absorption in obesity. *Clin. Res.* **36,** 773A

20 Hunt, J.N. (1980). A possible relation between the regulation of gastric emptying and food intake. *Am. J. Physiol.* **239,** G1–G4

21 Billich, C., Bray, G.A., Gallagher, T.F., Jr., Hoffbrand, A.V., and Levitan, R. (1972). Absorptive capacity of the jejunum of obese and lean subjects. *Arch. Intern. Med.* **130,** 377–380