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Morphofunctional Changes in Gastrointestinal Tract of Rats due to Cafeteria Diet

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Female rats fed a cafeteria diet from birth developed obesity at 60 days of age and their stomach, small intestine and caecum were enlarged when compared with controls, i.e. these regions had greater food storage capacity. In spite of the enlargement, these regions had similar or reduced weight and linear density, which is seen as proof of reduced mechanical performances. Cafeteria diet produced increased glucose duodenal absorption in older animals unlike the typical reduction known in controls. Tryptophan absorption was maintained high in adulthood, compensating for the low structural nutritive properties of the cafeteria diet. The results are interpreted as an adaptation to the cafeteria diet effects and properties: the characteristic overeating of foodstuffs with greater energy density, lower mechanical requirements and lower structural nutritive value than pelleted chow.

Key words: Cafeteria diet, Obesity, Body weight, Gastrointestinal morphometry, Glucose and Tryptophan absorption

The relation between the quality of a diet and the morphology and morphometry of the gut has been investigated in birds (2, 5). After these studies, a valid index for this relation has been established (6, 7). This index has been used as indicative of the gastrointestinal response to the seasonal dietary changes (16).

These studies were extended to wild rodents (4), considering that the gastrointestinal length is a consequence of the energetic requirements and the quality of a diet. These were considered the main factors determining the anatomic variability between species and affecting mainly to the structure and dimensions of the stomach, caecum and colon (8).

The morphometric characteristics of the gastrointestinal tube are important because they determine largely the digestive function. The enzymatic secretion and the

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intestinal absorption are surface dependent processes; they should correlate well with general morphometric parameters and specifically to surface area.

This paper deals with diet induced changes in morphometry and function of the gut, when related to the development of obesity through the consumption of a highly palatable diet, the «cafeteria diet» (13). The functional aspects of the adaptation to cafeteria diet will be studied through the absorption of glucose and tryptophan, as examples of carbohydrates and essential aminoacids.

Materials and Methods

Animals. — Wistar rats, housed in solid bottomed cages with bedding material have been used. The study has been carried out only in females due to their higher sensitivity to cafeteria diet (3). All animals were kept under controlled conditions of light (12/12 hours light/dark) and temperature (23 °C). All animals were born from multiparous females and the litter size was always normalized to 8 pups. At 21 days of age the infant rats were separated from their dams and caged in groups of four until the end of experiments.

Experimental design. — Depending on the diet supplied, two main groups have been studied: 1) controls, which had commercial chow (Panlab, Barcelona) continuously available from birth and 2) cafeteria fed, which received this diet from 7 days after birth to the end of the experiments together with the commercial chow. Although the puppies do not nibble solid foodstuffs until they attain an age of about 16-18 days, the early introduction of cafeteria diet was intended for habituation purposes; it was expected that a sudden switch in their food at weaning would produce a reduction in the amount of food consumed interfering with the typical overeating induced by the cafeteria diet. Both control and cafeteria fed groups were divided into three subgroups according to the age of sacrifice (30, 60 and 90 days of age). Each final group contained between 12 and 24 animals.

Diets. — The commercial chow supplied (Panlab, Barcelona) had the following composition (%): proteins 23.5, lipids 5.0, carbohydrates 48.9, fiber 4.0, minerals 5.7, and water 12.0. Its mean caloric value was 3800 Cal/kg (dry weight). The supplied cafeteria diet consisted in cookies with pâté and sobreasada (a typical majorcan pork sausage) croissants, sweets, bacon, biscuits, chocolate, peanuts, cheese, carrots, bananas and sugary milk (220 g sugar/l). Its percent composition was 13.6 lipids, 21.0 carbohydrates, 9.0 proteins, 51 water, and 5.1 other (14). A cocktail of vitamins and minerals (Vitachock) was also supplied. The averaged caloric value of the administered cafeteria diet was 4950 Cal/kg (dry weight).

Morphometric studies. — The animals were sacrificed when the adequate age was attained using an overdose of chloroform after 24 h of starvation. Immediately after death the animals were weighed and their gastrointestinal apparatus was extracted and dissected in the following segments: 1) esophagus, 2) stomach, 3 small intestine, 4) caecum and 5) colon and rectum. The dissection was quickly made after death to minimize loss of muscular tonus (2). The mesenterium was removed, but the gastrointestinal contents were left to minimize tissue traumatisms.

Each segment was measured according to the method of LEOPOLD (5), doing a stitch at both ends of each one of the lineal segments (esophagus, small intestine and colon-rectum), using suture thread to hang the segment from the oral end and supporting the opposite end a 20 g weight. After a pilot study, this standard weight was selected as producing an optimal trac-

tion to measure the length of the segment considered.

The non linear segments (stomach and caecum) were measured after tracing their shape over millimetric paper and measuring its main axis.

After these morphometric measures each segment was cleansed of its contents with saline, blotted dry over filter paper and then weighed.

Absorption studies. — Glucose absorption was studied *in vivo* after the method of PONZ *et al.* (10). The animals were anesthetized with sodium penthobarbital (45 mg/kg, i.p.) and a small incision was made in the abdominal wall. Through this incision, the duodenum (from antrum to Treitz ligament) was connected to a peristaltic pump with silicone tubing. A phosphate buffered (pH 7.4) solution (15 ml total volume) containing 8 mM glucose and ^{14}C-glucose, with a final activity of 0.02 mCi/ml, was recirculated at 1 ml/min through the intestinal segment. Samples of this solution (2 µl) were extracted after 0, 15, 30, 60 and 120 min from the beginning of perfusion. The samples were mixed with 10 ml of liquid scintillation cocktail and its activity was measured in a liquid scintillation spectrograph (Beckman, LS 1800).

A segment of approx. 10 cm long, beginning in the Treitz ligament, was used to measure tryptophan jejunal absorption using the same technique. The intestine was perfused with 15 ml of phosphate buffer solution added with tryptophan plus L-metylen-^{14}C-tryptophan with a final concentration of 10 mM and activity of 0.53 mCi/ml. Sampling and activity measuring was made in the same way as for glucose.

At the end of the experiments in both glucose and tryptophan studies, the perfused intestinal segments were extracted, weighed and measured. The results were expressed as the total amount of substrate absorbed in the studied time interval, relative to weight and length (µmol/g · cm) of intestine in agreement with the method of DEBNAM (1). As the recirculating volume during the experiment changed less than 2-4 %, no correction was made with respect to this variable.

Statistics. — Tests for significant differences between means were made using parametric statistics (Student T test) after normality of data assessment using the Kolmogorof test. For graphic and table representation, S.E.M. has been calculated.

Results

Body weight and fat. — Figure 1 shows the mean body weight and dissectable white fat weight at the considered ages. The cafeteria diet has produced a 30 % (at 60 days) and a 50 % (at 90 days) overweight. However, at 30 days of age the cafeteria fed animals had significant ($p < 0.05$) lower weight than controls.

The length of the gastrointestinal segments is shown in table I. There are no clear differences between controls and cafeteria fed in esophagus and colon-rectum, but there is a statistical difference in the remaining segments, being always longer in cafeteria fed animals. The differences in stomach and small intestine were statistically significant at all studied ages.

Table II shows the wet weight of the gut segments. Only the small intestine shows significant differences from controls, being always heavier in cafeteria fed animals.

Glucose and tryptophan absorption. — Fig. 2 shows the final amount (after 120 min of recirculation) of glucose and tryptophan absorbed at the three studied ages, with respect to linear density (g · cm) after the method of Debnam. With respect to glucose, two main points are apparent: a) In controls the amount of absorbed sub-

Table I. *Length of Intestinal Segments (cm)*
Values are mean ± S.E.M. Significance of difference from controls: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Age (days):	30		60		90	
Segment	Control (21)	Obese (15)	Control (16)	Obese (15)	Control (21)	Obese (25)
Esophagus	5.7 ± 0.08	4.9 ± 0.08***	7.4 ± 0.26	7.5 ± 0.14	8.5 ± 0.22	8.3 ± 0.16
Stomach	3.0 ± 0.08	3.6 ± 0.05***	3.2 ± 0.09	4.1 ± 0.07***	3.5 ± 0.07	4.4 ± 0.1***
Small Intestine	94.0 ± 0.66	99.7 ± 1.99***	103.1 ± 1.12	120.8 ± 2.28***	108.8 ± 1.14	123.4 ± 2.43***
Caecum	3.6 ± 0.1	3.6 ± 0.05	4.1 ± 0.16	5.1 ± 0.09***	3.9 ± 0.38	5.6 ± 0.12***
Colon-Rectum	15.5 ± 0.36	15.0 ± 0.29	21.5 ± 0.69	21.8 ± 0.33	23.1 ± 0.33	24.0 ± 0.36

Table II. *Weight of Intestinal Segments (mg)*
Values are mean ± S.E.M. Significance of difference from controls: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Age (days):	30		60		90	
Segment	Control	Obese	Control	Obese	Control	Obese
Esophagus	69.2 ± 4.64	60.4 ± 4.74	128.4 ± 11.29	96.5 ± 9.55*	125.4 ± 8.5	142.6 ± 10.6
Stomach	577.1 ± 13.48	640.4 ± 30.85	998.3 ± 73.89	1129.7 ± 30.88	1303.9 ± 61.40	1243.6 ± 50.67
Small Intestine	2259.1 ± 61.88	2860.1 ± 138.55***	3111.9 ± 162.54	3405.8 ± 144.33*	3037.8 ± 104.84	3347.0 ± 86.61*
Caecum	347.6 ± 13.05	384.2 ± 22.98	567.9 ± 26.45	555.8 ± 28.42	622.2 ± 26.64	620.6 ± 28.32
Colon-Rectum	662.4 ± 19.19	674.8 ± 28.01	997.0 ± 48.13	960.2 ± 29.56	1140.3 ± 31.85	1097.0 ± 46.56

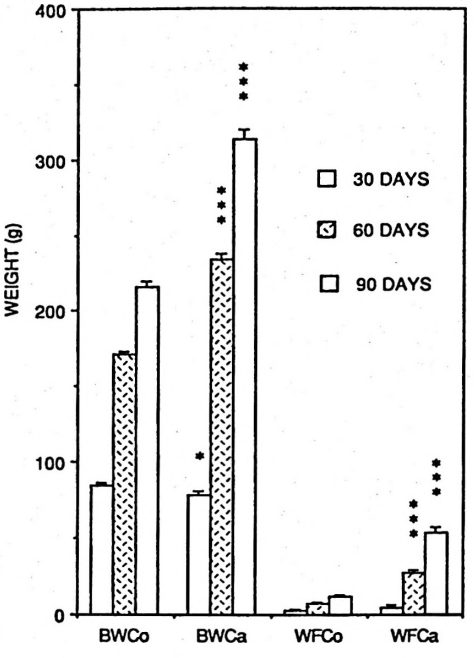

Fig. 1. *Body weight and white fat weight (mean ± SEM) for cafeteria fed and controls at the three considered age groups.*
Abbreviations: BWCo and BWCa: Body Weight for Control and cafeteria fed animals; WFCo and WFCa: White fat weight for control and cafeteria fed animals. Significance of differences between controls and cafeteria fed: * $p < 0.05$, *** $p < 0.001$.

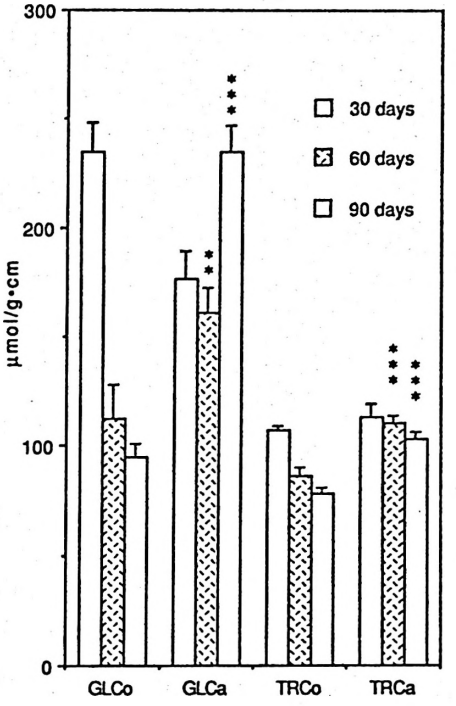

Fig. 2. *Glucose duodenal and tryptophan jejunal absorption expressed in µmol/g·cm in the three age groups.*
Abbreviations: GLCo and GLCa: Glucose absorption in Control and cafeteria fed animals; TRCo and TRCa: Tryptophan absorption for Control and cafeteria fed animals. Significance of differences between controls and cafeteria fed: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

strate is reduced when age increases, whereas in cafeteria fed animals the opposite effect is clear and b), in young animals, controls absorbed more glucose than cafeteria fed animals. On the contrary, in 90 day old animals there is more than a twofold advantage in cafeteria fed animals.

With respect to tryptophan jejunal absorption, at 30 days of age there is no difference between controls and cafeteria fed animals, but at 60 and 90 days the cafeteria fed groups absorbed more than controls. There is a significant ($p < 0.01$) decrease of absorption with age in controls and a sustained level in cafeteria fed animals (there are no significant differences between ages).

Discussion

The cafeteria diet induced an evident obesity in animals older than 60 days as it has been repeatedly reported. Both body and white fat weight show higher values at ages other than 30 days dold. Several causes (high diet-induced thermogenesis, undernourishment produced by an unbal-

anced diet, early lack of hyperfagia etc.) may have been responsible for the low body weight (9 % less) shown in 30 day-old animals. The present data does not allow for clear interpretation of this fact.

The cafeteria diet produced an important increase in stomach, small intestine and caecum length. This increase should be considered as demonstrative of increased storage capability. As the cafeteria diet has lower fiber and higher fat contents when compared to the commercial chow, the increased storage volume means that these segments in cafeteria fed rats may deal with a higher amount of food and a very much increased caloric intake. This seems to be well adapted to the cafeteria diet characteristic overeating, which has been attributed to central mechanisms reacting to the high hedonic value of the cafeteria diet (11).

The only difference in gut weight found in small intestine, heavier in cafeteria fed animals, may be a reflection of the increased digestive efficiency reported when the cafeteria diet is administered (11).

Considering the linear density of the digestive segments (the ratio between weight and length), the cafeteria diet produced a significant reduction of this parameter in esophagus (at 60 days), stomach, caecum and colon-rectum (at 90 days). This should be attributed mainly to a thickness decrease in the walls of these regions, lowering both the mechanic passive (elastic) and the active (motor) characteristics of the gut. Again it seems to reflect an adaptation to the diet characteristics, namely the management of a diet with low fiber and high water, protein, fat and soluble sugar contents and imposing lower mechanical demands on the gut walls.

The morphometry of the colon-rectum segment has been not changed as result of eating the cafeteria diet. This seems to be an unexpected result because this region has special sensitivity to diet changes (8). It seems likely that the bigger amount of waste per weight unit of the pelleted chow has been compensated by the increased total amount of food consumed in the cafeteria groups. Hence, both diets should have produced an amount of non digested-absorbed materials quite equivalent in volume and mechanical quality which resulted in absence of effect found in this region.

The age dependent decline in glucose absortion found in controls has been repeatedly reported (9, 15). This seems to be well adapted to the lower energy needs of older animals. On the contrary, the active transport system of youth has been maintained with the cafeteria diet through age, this being well correlated with the known increased caloric intake of these animals.

The differences between controls and cafeteria fed animals in absorption rate have been found as significant with respect to linear density. Therefore, the absorption in the whole intestine may be estimated by considering together the changes in weight and length. For instance, the small intestine at 90 days measured about a 120 % more in cafeteria fed and weighed a 110 % more. If one g · cm of intestinal tissue absorbed about 230 % more in these animals, the whole intestine had about a threefold increase in absorption ($2.3 \times 1.2 \times 1.1 = 3.04$). This enormous increase in glucose absorptive capability may be an important cause of the high resistance to weight reduction known in obese men and animals.

SALTER *et al.* (12) found the intestinal transport of aromatic aminoacids regulated through a feedback loop between liver and gut. The low proteinic values of the cafeteria diet should have enhanced the activity of the absorption mechanisms through this loop, maintaining in adulthood the high ability for tryptophan absorption typical of young animals. The increased ability to absorb essential nutrients together with the increased length and weight should have counteracted its reduced availability and even may have

permitted the increased lean weight found in the cafeteria fed animals.

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Resumen

Se ha administrado dieta de cafetería a ratas hembras con lo que desarrollaron obesidad demostrable a los 60 días de edad. Su estómago, intestino delgado y ciego alcanzaron mayor capacidad de almacenamiento al ser comparadas con los controles correspondientes. Sin embargo, estas regiones presentaron un peso similar o menor, lo que se considera demostrativo de una reducción en la capacidad de tratamiento mecánico de la ingesta. La capacidad para absorber glucosa disminuyó con la edad en los animales del grupo control. Sin embargo, en los que recibieron dieta de cafetería, esta capacidad se mantuvo elevada. De la misma forma, la capacidad para absorber triptófano se mantuvo elevada en animales adultos compensando la baja calidad estructural de la dieta de cafetería. Los resultados son interpretados como una adaptación a las propiedades y efectos de la dieta de cafetería: ingesta incrementada de alimentos de elevada densidad calórica, menores requerimientos mecánicos y bajo valor estructural en contraste con los característicos del pienso normal.

Palabras clave: Dieta de cafetería, Peso corporal, Morfometría gastrointestinal, Absorción de glucosa y de triptófano.

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