

Colon cancer in rats and diet in the Sonoran desert region of Mexico

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SUMMARY. The role of dietary fiber in colon cancer is still not clear. Epidemiological studies suggest a protective role for high fiber intake. However the data suggest that the effects of fiber must be considered in the context of the total diet and its interactions with other dietary components. The typical Sonora diet is high in dietary fiber (7.8%) and the majority of this fiber (71%) is insoluble. Comparing the incidence of colon cancer in Sonora with its neighboring state of Arizona, in 1991, Sonora reported 40 cases of colon cancer, a population of 1,823,606, compared to 1432 for Arizona (3,763,322 inhabitants). The typical regional diet was evaluated for its protective effect in the development of colon cancer in rats injected with azoxymethane, AOM. The regional diet showed a tendency to protect against colon cancer, an incidence of 45% compared to 66% for the control diet, although this effect was not significant ($p=0.15$). Further studies are needed to fully evaluate the diet and certain interactive dietary factors such as fat, quantity as well as type, protein, calcium and antioxidant nutrients.

RESUMEN. El cáncer de colon en ratas y la dieta regional en Sonora, México. Existen numerosos artículos científicos que han sugerido efecto protector de la fibra dietética en contra del cáncer de colon sin conocer exactamente el mecanismo. Se sabe por diversos estudios, que la fibra tiene que ser evaluada en la dieta total ya que existen interacciones con diferentes componentes. La dieta sonorense es alta en fibra (7.8 %) siendo en su mayoría insoluble (71 %). De acuerdo a los registros sobre la incidencia de cáncer de colon en el estado de Sonora en 1991 fue de 40 (1,823,606 habitantes) casos comparados con 1432 casos para el vecino estado de Arizona (E.U) (3,763,322 habitantes). Se evaluó el desarrollo de cáncer de colon en ratas alimentadas con la dieta regional y tratadas con un agente carcinogénico, azoximetano (AOM). Se encontró una incidencia de 45% de tumores en la dieta regional comparada a una incidencia de 66 % en la dieta control aunque no fue significativa. Los resultados obtenidos en esta investigación permiten sugerir la posibilidad de un efecto protector de la dieta regional en contra del cáncer de colon. Lo cual, puede ser debido al aporte de fibra dietética de algunos de sus componentes (principalmente frijol, tortillas de maíz y de harina). Por lo que se requiere más estudios que evalúen la dieta total y que tomen en cuenta ciertos factores que influyen como son la grasa, proteína, calcio y nutrientes antioxidantes.

INTRODUCTION

The diet in Sonora is changing and is influenced to a certain extent by the changes occurring in the socioeconomic development of the area. As the area becomes more industrialized and the economy improves, the people of Sonora have begun to change their dietary habits, influenced by neighboring regions. In the last ten years, nutrition surveys have shown low rates of protein-energy malnutrition. Ballesteros et al (1) evaluated the protein quality of the typical Sonoran diet, 45% animal protein and 55% vegetable protein, and showed it to have a high apparent digestibility (90%) and a corrected amino acid score of 90 for adults. No third degree malnutrition has been identified in pre-school children for both rural and urban sectors. Only 6-8% of the pre-school children studied showed signs of moderate malnutrition, and 25-28% were in the mild category; however, the adult population shows a considerable degree of overweight and obesity. In a region of fifteen rural municipalities,

40% of the adult men and 55% of the adult women had some degree of obesity when evaluated by the Quetelet's Index (2).

Vitamin deficiencies have been identified through dietary, clinical and biochemical studies (2,3,4). Vitamin A and ascorbic acid seem to be some of the most limiting nutrients due to the low fruit and vegetable consumption in these sectors of the population.

More recently, a dietary survey of the state of Sonora has been completed. The survey included individuals from all age and socioeconomic groups. This study showed that animal products, mainly beef, eggs and milk are prevalent in the diet; their frequencies are 83, 62 and 60% respectively (1). Nevertheless the main providers of energy and protein in the diet are beans, corn and flour tortillas (88, 53 and 61%, respectively). Basically, 20 different foods represent the most commonly consumed items in Sonora and the order of frequency is the only thing that will vary by regions. Table 1 presents the basic food items and their per capita consumption in the regional diet. Fig. 1 presents an inverse relationship of the preponderance of the 3 basic food items, beans, corn and flour tortillas. In practically all regions, beans occupy first place in frequency of consumption to be followed by coffee, sodas, corn and flour tortillas, and milk. What dictates the frequency of consumption more than anything is availability and price, that is to say in some of the small towns corn tortillas are not readily consumed because no commercial source exists and the families depend upon tortillas made in the home, which are usually of flour. Flour tortillas commercially made are much more expensive than corn tortillas and therefore are not purchased by the lower income people (5).

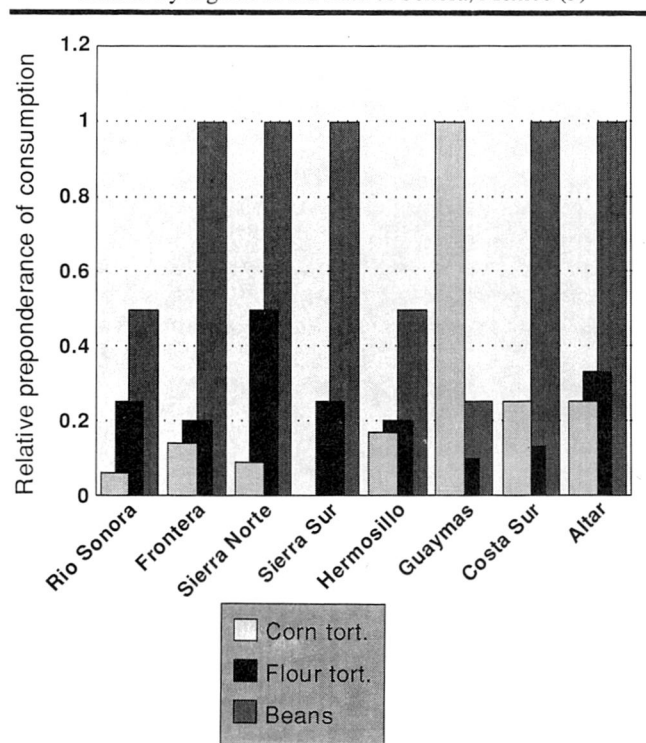
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TABLE 1
Basic food items and percapita consumption (g/d)
for the regional diet (5)

| Basic food item | |
|------------------|-----|
| Beans | 211 |
| Milk | 223 |
| Soda | 192 |
| Coffee | 156 |
| Corn tortilla | 121 |
| Meat | 67 |
| Flour tortilla | 88 |
| Eggs | 52 |
| Potatoes | 42 |
| Tomato | 32 |
| White bread | 27 |
| Pasta soup | 26 |
| White mex cheese | 13 |
| Rice | 20 |
| Beer | 35 |
| Oranges | 42 |
| Bananas | 15 |
| Fish | 10 |
| Sugar | 13 |
| Lettuce | 10 |

FIGURE 1

Relative preponderance of consumption of beans, corn and flour tortillas by regions in the state of Sonora, Mexico (5)



The distribution of energy is 50% from carbohydrates, 14% from protein and 36% from fat. Although some studies in the United States reported 40% of the energy from fat (6), the Sonoran diet shows a tendency similar to the US and other industrially developed countries to have a high fat content. This similarity is due to the close proximity

of Sonora to the United States, which greatly influences diets as well as life-style.

The main difference in the Sonoran diet from westernized diets is the high dietary fiber content, which can be attributed principally to the consumption of beans, corn and flour tortillas, with a daily per capita consumption of 211, 121 and 88 g respectively. These three items constitute 68% of the total dietary fiber intake of the Sonoran diet which provides approximately 50 g of fiber daily (5).

Many studies have described the relationship of dietary factors with colon cancer mostly based upon epidemiology studies (7). Fiber has received a great deal of attention as well as fat in the diet, type of fat, calories and certain antioxidant compounds such as ascorbic acid, vitamin E, selenium (Se) and carotenoids. Type of fiber, soluble or insoluble, is also very important. Experimental diets rich insoluble dietary fiber have been shown to be the most successful at reducing colon cancer (7). Insoluble dietary fiber passes through the digestive tract largely unmetabolized and is excreted in the feces.

Health statistics for the state of Sonora present an interesting panorama in regards to the incidence of colon cancer in comparison to our neighboring state and other more industrialized countries. In 1991, Sonora with a population of 1,823,606, report 40 cases of colon cancer compared to 1432 for Arizona, with a population of 3,763,322 (8,9).

The incidence of colon cancer remains low in Sonora regardless of what is happening in other parts of Mexico. However it is believed that this situation will change quickly in the near future. As a result of the North American Free Trade Agreement (NAFTA), the food scene is changing rapidly with an influx of fast food chain restaurants and introduction into the supermarkets of a wide variety of imported foods, including prepared and frozen foods.

Villalobos et al (10), reported an increase in the incidence of cancer of the gastrointestinal tract from 24% to 32% during the period of 1978 to 1981. These authors attributed this increase in a large part to the decrease in consumption of the foods indicated as basics such as beans and tortillas to the inclusion of more processed foods, refined sugar and thus a resultant decrease of dietary fiber.

To evaluate the effect of the typical Sonoran diet, high in insoluble fiber, in the development of colon cancer a model diet was prepared and this diet was evaluated in rats. Male rats (F344) were injected with the carcinogen axoxymethane (AOM) and control animals were injected with saline solution.

Experimental Procedures: Preparation of diets: The composition of this diet was based upon the results of a study published by Wyatt et al (11) on the chemical composition of diets for low income adults 25 years or older in Sonora, Mexico. The food items and the g/d that constitute this model diet are shown in Table 2 and the chemical composition is shown in Table 3. As can be seen in the Table the insoluble dietary fraction represents 71% of the total dietary fiber. All of the individual food items were prepared according to Wyatt et al (11), weighed and mixed together using a food blender (Leland Mixer, Model 100D, Leland Detroit Manufacturing Co, Detroit MI). Because of the high moisture content of the diet, it was pre-dried at 70 °C for 10-12 hrs (Vorton Oven Model 380, Beloit, WI). The pellets were prepared in a Hobart Mill, (Model 4152 Hobart Co, Ann Arbor, MI) using a 3/8 inch screen. A final drying to a moisture content of less than 10% was obtained by drying the pellets at 62 °C for an additional 6-8 hrs. The diet was stored at 5 °C until fed to the animals. Semipurified rat diet AIN 76 (American Institute of Nutrition, United States Biochemical Corp. Cleveland OH) was used as the control diet.

TABLE 2
Composite diet for low income 25 years or older in Sonora
Mexico (g/d, wt/wt) (11)

| Food | Intake (g) |
|--|------------|
| Refried beans (<i>Phaseolus vulgaris</i> Var. Pinto) | 329 |
| Corn tortilla | 253 |
| Flour Tortilla | 105 |
| Patatoes fried | 110 |
| Cheese, white | 40 |
| Chile serrano | 10 |
| Tomatoes | 41 |
| Onion, white | 25 |
| Soda | 380 |
| Meat, fried | 60 |
| Milk | 228 |
| Coffee | 435 |
| Sugar | 24 |
| Eggs, fried | 70 |
| Vegetable oil | 10 |
| Rice, cooked | 200 |

TABLE 3
Chemical composition of the experimental diet

| | Dry wt g/100g |
|-------------------------------|---------------|
| Fat | 14.62 ± 0.66 |
| Protein | 12.62 ± 0.42 |
| Ash | 3.55 ± 0.08 |
| Carbohydrates (by difference) | 61.31 |
| Dietary Fiber | |
| Soluble | 1.33 ± 0.28 |
| Insoluble | 5.61 ± 0.23 |
| Total | 7.86 ± 0.14 |

n=5

Animals: Fifty males Fisher 344 (Sprague Dawley, Indianapolis, IN), 5-6 weeks old, were placed in quarantine for 1 week fed the control diet then weighed and randomly placed in 4 treatment groups. Each rat was injected once a week for 3 weeks with 15 mg/kg body weight of azoxymethane dissolved in saline solution (Sigma Chemical Co., St. Louis MO) or saline solution (Table 4). Body weights were obtained weekly (Ohaus Model 01010-00, Cole Parmer Instrument Co., Chicago, IL) and at the end of 35 weeks after the last injection, the animals were sacrificed by asphyxiation with ether. All organs were dissected and weighed and the location and number of tumors identified.

TABLE 4
Experiment Design

| Diet | Treatment | |
|-------------------------|-----------|---------|
| | AOM | Saline |
| Control (AIN76) | 13 rats | 12 rats |
| Experimental (Regional) | 13 rats | 12 rats |

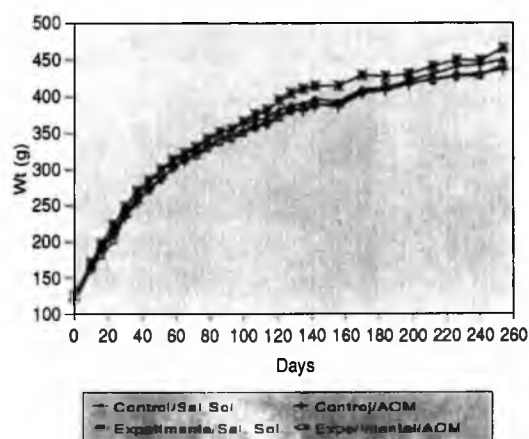
Tissue analysis: Both normal and tumorous tissues were fixed in 10% formalin (Merck of Mexico), imbedded in paraffin and tissue analysis conducted by the method of Rodríguez and Rosai, (12).

Statistical analysis: Animal weights were analyzed by analysis of variance utilizing the Duncan's multiple ranges for averages and the incidence of tumors was analyzed by calculation the Z value (13).

RESULTS AND DISCUSSION

Body weights: The average body weight gain of the animals in the study according to the treatment is presented in Fig. 2. No significant differences were noted in weight gains in the animals treated with AOM and those injected with saline solution either for the regional diet or the control AIN 76 diet. Both diets were equal in calories/g. Lower body weights for AOM treated animals were expected. In a study by Nigro et al (14), they showed lower body weights for treated animals on diets with different types of fibers.

FIGURE 2
Average weight (g) of F 344 rats fed a control
and experimental diet



Tumor incidence: Table 5 presents the tumor incidence and location according to treatment. In total, 13 tumors were examined histologically. Eight animals in the control diet treated with AOM had tumors, predominantly located in the colon, however, 1 tumor each was located in the duodenum and jejunum. Five animals fed the regional diet treated with AOM had well differentiated adenocarcinomas, again predominantly located in the colon, however, 1 was located in the ileum. An incidence of 66% tumors was observed in the control diet compared to 45% for the regional diet ($p=0.15$). No tumors were found in the animals not treated with AOM. In a study conducted by Nigro et al (14) the addition of 20-30% fiber to the experimental diet reduced the average number of tumors. They also found a positive correlation between tumor promoting fecal bile acid concentration and tumor frequency.

Location of the tumors: The tumors were polypodes and ranged in diameter from 0.4 to 3.5 cm. No apparent differences were noted in regards to treatment (control vs. regional diets) for tumor size in the colon, however, we found larger tumors located in other parts of the intestinal tract, in the rats fed the control diet. In Fig. 3, the largest tumor found is shown, which was located in the jejunum of a AOM

treated rat fed the control diet. Fig. 4 shows a segment of the colon of a rat with a malignant mucosa polypoid tumor. This animal was AOM treated and fed the control diet.

TABLE 5

Effect of experimental diets in the development of cancer in F344 rats treated with AOM

| Diet | No of animals at end of experiment | Carcinomas | Incidence % | Area of tumor |
|------------------------|------------------------------------|------------|-------------|--|
| Control (sal sol) | 12 | 0 | 0.00 | ————— |
| Control (AOM) | 12 | 8 | 66.66 | colon (6), jejunum (1), duodenum (1) |
| Experimental (sal sol) | 12 | 0 | 0.00 | ————— |
| Experimental (AOM) | 11 | 5 | 45.45 | colon (4), ileum (1) |

FIGURE 3

Stomach, duodenum and a segment of the jejunum with a 3.5 cm in diameter adenocarcinoma AOM treated, control diet



FIGURE 4

Segment of a rat colon with a polipoide mucosa tumor, AOM treated, control diet



Histological examination: Fig. 5 presents the examination fo a section of an adenocarcinoma located in the colonic mucosa of a rat treated with AOM, fed the regional diet, showing a well differentiated dysplatic adenoma. Fig. 6 presents normal tissue and a section of the colonic mucosa from a rat treated with AOM on the control diet, whereby one can observe the differences in the cellular structure.

FIGURE 5

Malignant colonic mucosa tissue from a rat treated with AOM and fed the regional diet (10X)

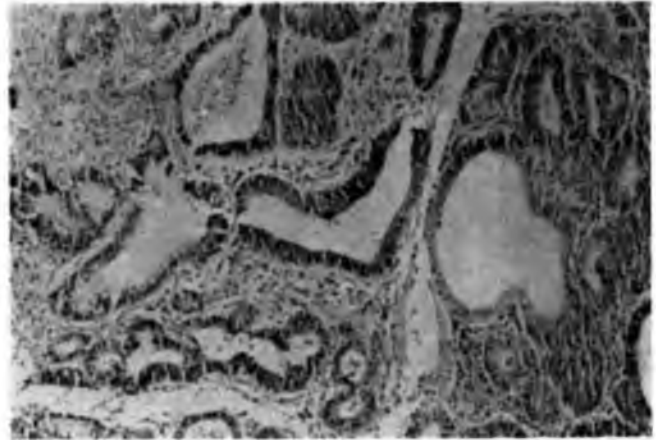
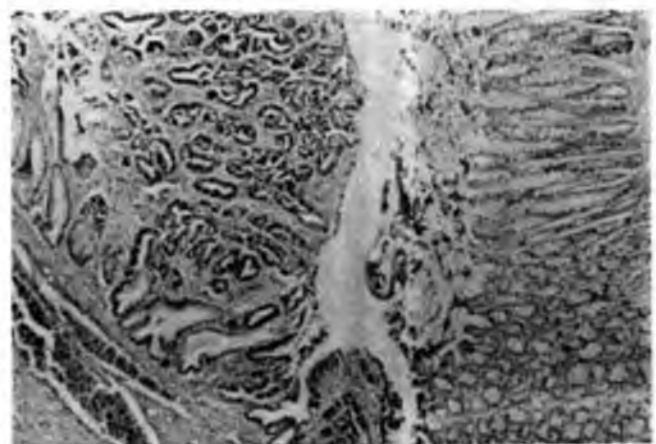


FIGURE 6

Mixed tissue, normal and malignant colonic mucosa from a rat treated with AOM fed the control diet (10X)



Unanswered questions: From the data of this study it appears that the typical regional diet offers a degree of protection against the development of colon cancer, however not completely. Of course the obvious controversy exists, that is, how valid is it to extrapolate data from animal studies to humans? This issue has long been argued, but it is generally accepted that animal data does provide insight as to what might be expected in humans. Nigro (15) states «Conclusions arrived at from animal studies certainly are speculative when applied to humans. However, evidence from human studies correlate fairly well with those from animal experiments. Therefore, such speculations are worth considering. «The majority of the studies relating fiber and colon cancer have either been epidemiological studies examining certain populations and their diet or animal studies evaluating certain

isolated fibers such as bran, cellulose, alfalfa, etc. Few studies have been conducted feeding mixed natural diets. The diet factors most often correlated with colon cancer incidence are the absence or presence of fruits and vegetables, cereals, fats (amounts and type), calcium, and red meats. The interaction among dietary components is quite complex and difficult to study in intestinal carcinogenesis. The protective effect of dietary factors such as fiber, antioxidant nutrients, seems to be well defined but many factors come to play in a mixed diet and there are certain chemical aspects of the Sonoran diet that lend itself to some very interesting dilemmas. There seems to be a general consensus that diets high in fiber and low in fat, particularly saturated fat, are inhibitory to colon cancer. The Sonoran diet is clearly high in fiber, 56% of the calories coming from carbohydrates, primarily legumes and cereals. Relatively high in fat, 36% of the calories coming from fat and probably this fat is high saturated, however specific chemical data is lacking to substantiate this, and overtly absent in fresh fruits and vegetables. The average fiber consumption of 50 g/d is as high as in the African natives diet that was first historically described by Burkitt (16) and shown to have a low colon cancer incidence.

Not all studies support this simple relationship that diets high in fat and low in fiber induce colon cancer. Some workers have shown a high Ca content (in this case the Ca coming primarily from dairy products) helps overcome the adverse effect of a high fat content (17,18). The Sonoran diet is high in Ca (1164 mg/d) (19) but how available is this Ca remains to be studied. Approximately 9% of the Ca comes from the corn tortilla. Some studies have shown the lye treating process for the corn used in making tortillas makes the Ca more soluble. In our studies conducted in this center, we found that 80% of the Ca in corn tortillas was soluble (19). However other researchers based studies have concluded that the Ca in Mexican diets is not very available (20). The 3 main providers of energy in the diet, beans, flour and corn tortillas, provide 24% of the daily intake of Ca.

Also lacking is information of the effect of the regional diet in regards to what happens with fecal bile acids. Jensen et al (21) showed that fecal bile acid concentration was correlated positively with the risk of colon cancer. A comparison of fecal bile acid concentrations in Finns and Americans showed that while the two groups excreted the same amount of bile acid (275 mg/d), the concentration of fecal bile acids was 4.6 mg/g in the Finns and 12.3 mg/g in the Americans (22). Hill et al (23) found average fecal bile acid concentrations in Uganda, Japan and India (countries with low incidence of colon cancer) to be 0.61 ± 0.13 mg/g feces, whereas the average concentration in England, Scotland and the United States was 6.15 ± 0.66 mg/g (countries with higher incidence of colon cancer). Dietary fiber binds bile acids and bile salts, the level of binding depending on both the fiber and the bile acid derivative (24,25). No data exists for Mexico.

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