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The Effect of Early Caloric Restriction on Colonic Cellular Growth in Rats

Demetrius Albanes, Arline D. Salbe, Orville A. Levander, Philip R. Taylor, Daniel W. Nixon, and Myron Winick

Abstract

Although the inhibitory effect of caloric restriction on tumorigenesis is substantial and well known, the pertinent mechanisms remain to be determined. We recently suggested that the risk of cancer may be directly related to the total number of dividing cells within an affected organ. This study evaluates the effects of early caloric restriction on the cellular growth of the colon. The experiment began one day postpartum and ended six weeks later with the killing of all animals. It consisted of two consecutive periods: a) three weeks of suckling and b) three weeks postweaning. Animals whose food was restricted only during the suckling period showed normal colons when killed at six weeks. Caloric restriction (40%) for three weeks postweaning resulted in colons of lower weight with fewer cells (less total DNA) and reduced total DNA synthesis ($[^3\text{H}]$ thymidine uptake, dpm/colon) when compared with animals fed ad libitum postweaning. Conversely, only rats fed ad libitum from birth through the first three weeks after weaning demonstrated an increase (21%) in the rate of DNA synthesis (dpm/mg DNA) compared with other animals. In addition, the colonic crypts showed no differences in the number of cells or the number of dividing cells, as determined by autoradiography. By contrast, the total number of crypts (and/or the number of mucosal cells between crypts) are reduced, and hence the total number of colonic mucosal cells dividing at any given time are similarly decreased. The reduced number of dividing cells in the colons of these animals (i.e., those restricted postweaning) could explain previous data suggesting that they are resistant to the induction of colon cancer.

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Introduction

Normal cellular growth in organs that do not continuously regenerate themselves can be divided into three stages: hyperplasia, hyperplasia with hypertrophy, and hypertrophy (1).

D. Albanes, P.R. Taylor, D.W. Nixon, and M. Winick are affiliated with the Cancer Prevention Research Program, Division of Cancer Prevention and Control, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. A.D. Salbe and O.A. Levander are affiliated with the US Department of Agriculture, Human Nutrition Research Center, Beltsville, MD 20705.

Protein-calorie undernutrition during the hyperplastic phase results in an attenuated rate of cell division and a permanent reduction in cell number in nonregenerating organs, including the heart, brain, and skeletal muscle (2). Excess food during hyperplastic growth results in an increased rate of cell division and permanently larger organs with a greater number of cells (3). For tissues that are constantly regenerating their cells (e.g., mucosal cells of the gastrointestinal tract, breast ductiles, and bronchial epithelium), there are no studies examining the effect of early protein-calorie undernutrition on either cellular growth or cell turnover rate.

Recently, we proposed a theory relating the incidence of certain tumors, particularly those in the colon and the breast, to the total number of cells within the colonic mucosa or the breast ductiles that are normally undergoing mitosis (4). The total number of dividing cells depends on the total number of cells within the mucosa or ductiles and the rate of cell division (i.e., the percentage of these cells dividing) at any given time. The theory is based on extrapolation from older as well as more recent studies. First, animals whose food is restricted for several weeks after birth not only are permanently stunted, with smaller organs, but also live longer and are more resistant to the later induction of certain tumors (5). Second, there is a direct relationship between height and the incidence of colon and breast cancer in humans (6,7). Assuming that taller people have longer and perhaps broader large intestines (8), their total number of colonic mucosal cells could be increased; at any given rate of mucosal cell turnover they would be at greater risk for developing colon cancer than shorter people would be. Our experiment was designed to examine the effects of early caloric restriction imposed at specific times during rat growth on the total number of colonic cells, the number of cells lining the colonic crypts, and the total number of dividing cells in the colon.

Materials and Methods

Animals

Eleven timed-pregnant Sprague-Dawley dams (Charles River, Wilmington, MA) were received in the laboratory seven days before parturition. Dams were housed in plastic cages with wood shavings used as bedding and were allowed free access to Purina Rat Chow (Product 5001, Ralston Purina, St. Louis, MO) and distilled water during pregnancy and lactation. Temperature was controlled at $24 \pm 1^\circ\text{C}$, and a 12:12-hour light-dark schedule was maintained. One day postpartum, 7 dams had excess pups removed to leave litter sizes of 4 (growth-accelerated group), while 4 dams had pups added to their litters to attain a litter size of 12 pups each (growth-retarded group). No rejection of added pups was noted, and lactation proceeded without difficulty.

The experiment, which began one day postpartum and ended six weeks later with the killing of all animals, consisted of two consecutive periods: a) the three-week suckling period and b) the three-week postweaning period. At the time of weaning (21 days postpartum), the female pups of each litter were rank ordered by body weight and sex and housed individually in wire mesh cages (males were used for a separate experiment). The pups were randomly assigned to one of two dietary regimens, which were also designated as growth accelerated or growth retarded. This randomization yielded four treatment groups: growth accelerated/growth accelerated (GA/GA), growth accelerated/growth retarded (GA/GR), growth retarded/growth accelerated (GR/GA), and growth retarded/growth retarded (GR/GR). Body weight was measured at weaning, weekly until the end of the experiment, and just before killing.

Diets

The diets were based on the formulations of Ruggeri and co-workers (9) and are shown in Table 1. Animals in the postweaning growth-accelerated groups (GA/GA and GR/GA) were

Table 1. Composition of Experimental Diets		
Component	Percent in Diet	
	Control diet	Experimental diet
	(High calorie) ^a	(Low calorie) ^b
Sucrose	58.0	30.1
Casein	21.6	36.0
AIN mineral mix	3.8	6.3
AIN vitamin mix	1.0	1.7
DL-Methionine	0.3	0.5
Corn oil	5.0	8.3
Cellulose	10.1	16.8
Choline H ₂ citrate	2.70 ml/kg	4.05 ml/kg

a: Growth-accelerated group.
b: Growth-retarded group.

fed the control diet ad libitum. Rats in the postweaning growth-retarded groups (GA/GR and GR/GR) consumed the experimental diet at 40% less than the total intake, pair fed to the nearest weight-matched animal (immediately postweaning) in the ad libitum group. Whereas the diets were designed to provide equal absolute intake of protein, fat, vitamins, and minerals for the GA and GR groups postweaning, the percent of calories from protein and fat were higher in the GR groups (protein, 43% vs. 24%; fat, 22% vs. 12%). Food spillage was estimated and accounted for daily. Animals were fed daily (late in the afternoon) and were maintained on these dietary regimens for three weeks.

Tissue Preparation for Histology

One hour before killing, the animals were injected intraperitoneally with [³H]thymidine (1 μCi/g body wt, specific activity 88 Ci/mmol, diluted in 0.9% NaCl, Amersham, Arlington Heights, IL). Animals were killed by decapitation and exsanguination, and whole-animal length was measured. Colons were removed, perfused clean of intestinal contents with 1.15% KCl, patted dry, measured, and weighed. Individual colons were divided into three equal segments, and a 1-cm slice from the proximal end of each segment was fixed in 10% neutral buffered formalin. The remaining colon tissue from each colon segment was frozen at -80°C until ready for extraction of total DNA, RNA, and protein.

Histology

Colonic crypt morphology and epithelial cell proliferation were determined by autoradiography. Three 1-cm sections (ascending, transverse, and descending segments) from each colon were processed from the formalin solution to paraffin blocks. These were sectioned (5 μm), stained with hematoxylin and eosin, immersed in Kodak NTB photographic emulsion (Eastman Kodak, Rochester, NY) in darkness for 10 days, and then developed and fixed. Under light microscopy, cells in the first four longitudinally oriented crypts that spanned the muscularis mucosa and the luminal surface were counted for each animal. The total number of epithelial cells and the number (and position) of the [³H]thymidine-labeled cells (i.e., cells showing at least 5 grains/nucleus) were counted for the two columns (sides) of each crypt. The position of the highest labeled cell per crypt, absolute as well as relative to total crypt height, was determined. Average values for each of these parameters were calculated for each animal.

Tissue Preparation for and Analysis of Total DNA, RNA, and Protein

The colon tissue remaining after the 1-cm segments were taken for histology was thawed, weighed, diluted in four volumes of 1.15% KCl, and homogenized with a polytron mixer (Brinkmann Instruments, Westbury, NY). DNA, RNA, and protein were extracted from colon homogenates according to the Munro and Fleck (10) modification of the Schmidt and Thannhauser procedure with 100 mg wet wt colon homogenate. DNA was quantified according to the diphenylamine assay method of Burton (11). Protein content was measured by the method of Lowry and colleagues (12). Extrapolation to total colon was based on original colon weight and weight of the colon after removal of the segments for histology. [³H]Thymidine uptake was measured by counting 200 μ l DNA extract in 3 ml Dimiscint scintillation fluid (National Diagnostics, Manville, NJ) for 10 minutes (Beckman Scintillation Counter LS3801, Beckman Instruments, Fullerton, CA) and calculating dpm [³H]thymidine per total colon.

Statistical Analysis

Data were analyzed by ANOVA and tested with Duncan's multiple-range test (13).

Results

Food intake and physical parameters of the four groups of animals are shown in Table 2. The GA/GA group demonstrated the greatest food and energy intake, weight gain, and final body size. Restriction during the earlier suckling period resulted in substantially reduced body weight at weaning. Animals restricted in their food and calorie intake during the later three-week experimental period had significantly lower weight gain, final weight, and final length than did animals fed ad libitum during the same period.

Table 3 shows that regardless of the diet during the nursing period, calorie restriction for three weeks postweaning produced lighter colons with fewer cells (less total DNA). These same animals also showed a reduced total colonic DNA synthesis (dpm/colon) when compared with animals well fed postweaning. Only for animals who were well fed from birth through the first three weeks after weaning (GA/GA) was there a suggestion of an increased rate of DNA synthesis (dpm/mg DNA), but this finding was only statistically significant when compared with all other groups combined (Student's *t* test 2.26; *p* < 0.05). Similarly, cell size, as measured by protein/DNA ratio, was significantly higher only in the GA/GA group.

Table 2. Daily Dietary Intake and Physical Characteristics of Experimental Groups

	Groups ^{a,b}			
	GA/GA	GA/GR	GR/GA	GR/GR
No. of animals	6	7	7	7
Food intake, g/day	13.4 \pm 0.6*	7.9 \pm 0.3 [†]	11.8 \pm 0.5 [‡]	7.5 \pm 0.4 [†]
Energy intake, kcal/day	49.0 \pm 2.2*	27.1 \pm 1.0 [†]	43.2 \pm 1.7 [‡]	25.7 \pm 1.2 [†]
Body weight at weaning, g	45.0 \pm 2.0*	46.0 \pm 2.0*	33.0 \pm 1.0 [†]	34.0 \pm 1.0 [†]
Body weight gain, g	106.0 \pm 6.0*	65.0 \pm 3.0 [†]	98.0 \pm 5.0*	61.0 \pm 3.0 [†]
Final body weight, g	151.0 \pm 5.0*	111.0 \pm 2.0 [†]	131.0 \pm 6.0 [‡]	95.0 \pm 3.0 [§]
Body length, cm	16.1 \pm 0.2*	14.6 \pm 0.2 [†]	15.2 \pm 0.2 [‡]	13.7 \pm 0.2 [§]

a: Values are means \pm SEM. Within rows, means not sharing superscripts are significantly different at *p* \leq 0.05. Intake and weight gain during last 3 wks of expt; final body weight and length on day of death.

b: GA, growth accelerated; GR, growth retarded.

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	Groups ^{a,b}			
	GA/GA	GA/GR	GR/GA	GR/GR
Colon weight, g	0.78 ± 0.05*	0.64 ± 0.03 [†]	0.77 ± 0.04*	0.62 ± 0.02 [†]
Colon length, cm	17.40 ± 0.70*	16.80 ± 0.50*	17.20 ± 0.70*	15.50 ± 0.50*
Total colon DNA, mg/colon	4.74 ± 0.27* [†]	3.74 ± 0.39 [‡]	5.46 ± 0.26*	3.90 ± 0.33 ^{†,‡}
Total colon DNA synthesis, dpm/colon × 10 ⁻⁵	12.40 ± 1.40*	7.80 ± 1.20 [†]	12.20 ± 1.10*	8.70 ± 0.90 [†]
Rate of DNA synthesis, dpm/mg DNA × 10 ⁻⁵	2.66 ± 0.32*	2.09 ± 0.31*	2.27 ± 0.28*	2.21 ± 0.13*
Protein/DNA ratio	14.40 ± 0.70*	13.20 ± 1.00 [†]	12.60 ± 0.30 [†]	11.70 ± 0.60 [†]

a: Values are means ± SEM. Within rows, means not sharing superscripts are significantly different at $p \leq 0.05$. All factors are those from last day of expt.
 b: GA, growth accelerated; GR, growth retarded.

Results of the autoradiographic analysis appear in Table 4. Regardless of the diet, by six weeks of age the adult pattern was attained and showed that the number of cells from the base of the crypt to the luminal surface was smallest in the ascending colon. There were no significant differences in crypt height or number of labeled cells in any region of the colon as a result of dietary treatment, nor was the percent of cells labeled consistently different in any dietary group across regions. Finally, although the highest labeled cell usually appeared in the transverse colon, the position of the highest labeled cell was significantly different only between rats in the GA/GR and GR/GA groups in the transverse colon and between rats in the GA/GA and GA/GR groups in the descending colon.

	Groups ^{a,b}			
	GA/GA	GA/GR	GR/GA	GR/GR
Crypt height (cells)				
Ascending	32.3 ± 1.6*	31.8 ± 0.8*	30.6 ± 0.5*	30.2 ± 0.7*
Transverse	40.7 ± 0.9*	39.5 ± 0.9*	40.4 ± 0.3*	38.9 ± 1.0*
Descending	37.1 ± 1.4*	35.6 ± 1.7*	37.1 ± 1.0*	37.5 ± 0.8*
No. of labeled cells per crypt column				
Ascending	2.5 ± 0.3*	2.9 ± 0.3*	3.3 ± 0.3*	2.9 ± 0.2*
Transverse	2.4 ± 0.3*	2.7 ± 0.6*	2.2 ± 0.3*	2.2 ± 0.3*
Descending	2.8 ± 0.4*	3.2 ± 0.4*	2.6 ± 0.3*	2.5 ± 0.5*
% Labeled cells ^c				
Ascending	7.8 ± 0.6*	9.0 ± 0.8* [†]	10.8 ± 0.8 ^{†,‡}	9.4 ± 0.7* [‡]
Transverse	6.0 ± 0.7*	6.7 ± 1.4*	5.6 ± 0.7*	5.6 ± 0.6*
Descending	7.8 ± 1.3*	9.0 ± 0.9*	7.0 ± 0.8*	6.8 ± 1.3*
Highest labeled cell ^d				
Ascending	17.0 ± 1.3*	15.6 ± 1.0*	16.7 ± 1.4*	17.3 ± 2.1*
Transverse	25.2 ± 2.4* [†]	21.1 ± 1.3*	26.6 ± 1.1 [†]	22.9 ± 1.1* [†]
Descending	16.7 ± 1.1*	21.1 ± 2.2 [†]	19.1 ± 1.3* [†]	18.6 ± 1.6* [†]

a: Values are means ± SEM. Within rows, means not sharing superscripts are significantly different at $p \leq 0.05$.
 b: GA, growth accelerated; GR, growth retarded.
 c: (No. of labeled cells/total no. of cells in each crypt) × 100.
 d: Cell location from base of crypt.

Discussion

Our findings demonstrate that the colon, like nonregenerating tissues formerly studied, is susceptible to nutritional manipulation during the hyperplastic period of growth. Specifically, continuous food restriction imposed either at birth or at weaning (3 wks old) resulted in reduced body weight and length, as well as smaller colons composed of fewer cells, by six weeks of age. The data also suggest that the most rapid hyperplasia occurs after weaning, because no greater reduction in cell number was attained by undernourishing the animals additionally during the earlier suckling period. It is possible, however, that the degree of restriction during suckling (12 animals nursing from a single mother) was not great enough to seriously retard cellular growth of the colon during that period. In contrast, animals whose food intake was restricted only during the suckling period and then fed ad libitum had only partially caught up in body weight and length by six weeks, whereas colon weight and length were equivalent to those of animals well fed from birth. The "catch-up" growth exhibited in the colons of these animals (compared with animals food restricted for the entire experiment) was accompanied by a concomitant increase in cell number (total colon DNA), presumably because of an earlier increase in the rate of cell division. This finding is consistent with previous studies that show a reversal of starvation-induced colon hypoplasia when animals are refed (14-16).

In contrast to these findings for total cell number, undernutrition during the immediate postsuckling period does not affect the number of cells extending from the base of the crypt to the luminal surface in the ascending, transverse, or descending colon. Thus, the reduced total number of colonic cells is not due to a reduction in cell number within colonic crypts. We therefore conclude that the observed reduction in the total number of colonic cells is primarily due to a reduction in the number of crypts, the number of mucosal cells between the crypts, or both. Also, whereas the total number of dividing cells within the colon (dpm/colon) is significantly reduced in both groups of animals undernourished during the postsuckling period, the number of cells dividing within each crypt (number of labeled cells) is not affected by restriction at this time. These data suggest that the cells involved in the formation of new crypts and mucosal epithelium are different from the cells normally dividing in the lower portion of the crypts as observed by autoradiography. The latter appear to be concerned with maintaining the cellular integrity of the crypts and mucosa and are not affected by the degree of undernutrition imposed during these experiments.

While the present study is unique in that it assessed both the number of cells in the colon and their rate of division, changes in colonic mucosal cell proliferation in response to dietary alterations (including caloric restriction) were previously observed (17-19), and some of these studies demonstrate associations with tumor incidence (19,20). In one experiment, a "low-risk" diet (including decreased calories and fat and increased fiber compared with the "high-risk" diet) resulted in decreased colonic crypt cell division (18) and reduced colon tumor incidence (20). In contrast, Klurfeld and colleagues (19) observed both increased colonic crypt cellular proliferation and decreased colon tumorigenesis in calorie-restricted rats compared with animals fed ad libitum. The authors attributed these "paradoxical" findings to the stimulation of mucosal proliferation by bile acids resulting from increased fat consumption in the calorie-restricted group. In our study, only one group of animals (GA/GA) demonstrated an increase in the rate of DNA synthesis (dpm/mg DNA). While this increase was modest (21%), it is consistent with one of the previous studies (20) and suggests that prolonged overfeeding beginning at birth not only increases total colon cell number but may also increase the rate of cell division within the colon. Although we certainly cannot extrapolate these findings to humans, the implications for childhood obesity are intriguing.

The data from this study are consistent with our hypothesis. The food-restricted animals are stunted (shorter), have smaller colons with fewer cells, and, although the rate of cell division is not greatly affected, the total number of dividing cells is significantly reduced. These animals should therefore be more resistant to the induction of colon cancer. While these experiments need to be done, animals raised in a similar manner showed a lower incidence of induced cancers of a variety of types (5). If our theory is correct, then animals showing both an increased total number of cells and an increased rate of cell division may be the most susceptible to the induction of colon cancer. These data also hold out the possibility that experimental diets which protect against cancer of the colon can be devised in rats by observing their effects on total colonic cell number and the rate of colonic cell division. Such diets might give us some clues as to the makeup of protective diets for humans during both early and adult life.

Acknowledgments and Notes

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References

1. Winick, M, and Noble, A: "Quantitative Changes in DNA, RNA and Protein During Prenatal and Postnatal Growth in the Rat." *Dev Biol* 12, 451-466, 1965.
2. Winick, M, and Noble, A: "Cellular Response in Rats During Malnutrition at Various Ages." *J Nutr* 89, 300-306, 1966.
3. Winick, M, and Noble, A: "Cellular Response With Increased Feeding in Neonatal Rats." *J Nutr* 91, 179-187, 1967.
4. Albanes, D, and Winick, M: "Are Cell Number and Cell Proliferation Risk Factors for Cancer?" *JNCI* 80, 772-775, 1988.
5. Ross, MH, and Bras, G: "Lasting Influence of Early Caloric Restriction on Prevalence of Neoplasms in the Rat." *JNCI* 47, 1095-1113, 1971.
6. Albanes, D, Jones, DY, Schatzkin, A, Micozzi, MS, and Taylor, PR: "Adult Stature and Risk of Cancer." *Cancer Res* 48, 1658-1662, 1988.
7. Swanson, CA, Jones, DY, Schatzkin, A, Brinton, LA, and Ziegler, RG: "Breast Cancer Risk Assessed by Anthropometry in the NHANES I Epidemiological Follow-Up Study." *Cancer Res* 48, 5363-5367, 1988.
8. Hirsch, J, Ahrens, EH, and Blankenhorn, DH: "Measurement of the Human Intestinal Length *in vivo* and Some Causes of Variation." *Gastroenterology* 31, 274-284, 1956.
9. Ruggeri, BA, Klurfeld, DM, and Kritchevsky, D: "Biochemical Alterations in 7,12-Dimethylbenz[a]anthracene-Induced Mammary Tumors From Rats Subjected to Caloric Restriction." *Biochim Biophys Acta* 929, 239-246, 1987.
10. Munro, HN, and Fleck, A: "Recent Developments in the Measurement of Nucleic Acids in Biological Materials." *Analyst* 91, 78-88, 1966.
11. Burton, K: "A Study of the Conditions and Mechanisms of the Diphenylamine Reaction for the Colorimetric Estimation of Deoxyribonucleic Acid." *Biochem J* 62, 315-322, 1956.
12. Lowry, OH, Rosebrough, NJ, Farr, AL, and Randall, RJ: "Protein Determination With the Folin Phenol Reagent." *J Biol Chem* 193, 265-275, 1951.
13. SAS Institute: *SAS User's Guide. Statistics*. Cary, NC: SAS Institute, 1985.
14. Holt, PR, and Yeh, K: "Colonic Proliferation is Increased in Senescent Rats." *Gastroenterology* 95, 1556-1563, 1988.
15. Goodlad, RA, and Wright, NA: "The Effects of Starvation and Refeeding on Intestinal Cell Proliferation in the Mouse." *Virchows Arch* 45, 63-73, 1984.
16. Hagemann, RF, and Stragand, JJ: "Fasting and Refeeding: Cell Kinetic Response of Jejunum Ileum and Colon." *Cell Tissue Kinet* 10, 3-14, 1977.
17. Caderni, G, Stuart, EW, and Bruce, WR: "Dietary Factors Affecting the Proliferation of Epithelial Cells in the Same Colon." *Nutr Cancer* 11, 147-153, 1988.

18. Goettler, D, Rao, AV, and Bird, RP: "The Effects of a Low Risk Diet on Cell Proliferation and Enzymatic Parameters of Preneoplastic Rat Colon." *Nutr Cancer* **10**, 149-162, 1987.
19. Klurfeld, DM, Weber, MM, and Kritchevsky, D: "Inhibition of Chemically Induced Mammary and Colon Tumor Promotion by Caloric Restriction in Rats Fed Increased Dietary Fat." *Cancer Res* **47**, 2759-2762, 1987.
20. Rao, AV, Goettler, DM, and Bird, RP: "The Effects of Low-Risk Diet on Tumor Incidence in Chemically Induced Colon Cancer in Rats." *Nutr Cancer* **11**, 11-20, 1988.