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Modulating role of dietary fat, energy restriction, and the effect of age on the expression of proliferating cell nuclear antigen and protein kinase C activity in prostate glands of rats

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The present study investigated the role of dietary fat and energy restriction (ER) on the proliferative status and the protein kinase c (PKC) activity in the prostate glands in young (3-week-old, n = 40) and adult rats (10-week-old, n = 40). F344 male rats, young and adult, were allocated to four dietary groups (n = 10/group): high fat (23% wt/wt) or low fat (5% w/w) ad libitum (HFAL or LFAL) and high- or low-fat energy restriction (HFER or LFER). Energy-restricted rats were fed 80% of the energy intake of the ad libitum fed rats. After 12 weeks of feeding, the proliferating cell nuclear antigen (PCNA) labeling index in prostate glands was higher in young rats than in adult rats. Both the HFAL and LFAL groups had higher PCNA labeling index than both the HFER and LFER groups in each age group. In addition, the HFAL group had higher PCNA labeling index than the LFAL group. Dietary fat and ER markedly affected the PKC activity, which decreased in the order HFAL > HFER = LFAL > LFER for both young and adult animals. The effect of high-fat diet and ER were more evident in the younger animals than in the adult animals. Among the young animals, the absolute total PKC activity was higher ($P \leq 0.05$) in the high-fat groups than in the low-fat groups and a higher proportion of the total PKC was associated with the membrane fraction. In both young and adult rats, ER decreased the total PKC activity and the ratio of PKC activity in the cytosol to the membrane fractions compared with the ad lib counterparts. We conclude that age, a high-fat diet, and energy restriction modulate PCNA expression and the PKC activity of prostatic tissue. (J. Nutr. Biochem. 9:236–241, 1998) © *Elsevier Science Inc. 1998*

Keywords: prostate glands; protein kinase C; proliferating cell nuclear antigen; energy restriction; dietary fat

Introduction

Dietary lipids and energy are well established modulators of cell growth and differentiation including signal transduction and gene expression.[1–5] Evidence supports the role of dietary lipids and/or energy as a modulator of carcinogenesis in several organs.[4–12] Growth of the prostate glands is dependent on the hormonal state of the animals which in turn depends on the nutritional status and the age of the animals. Epidemiologic and animal studies have provided evidence in support of the conjecture that dietary fat and energy exert a profound effect on the carcinogenic process in the prostate glands.[4–13] The exact mechanism by which the high-fat diet exerts these effects is poorly understood. Postulations are that dietary factors may affect the promotional steps of prostate cancer development.

Animal models provide the opportunity to test and refine hypotheses linking diet in the etiology and prevention of different types of cancer. Unfortunately, attempts to develop animal models to study prostate cancer have met with only limited success. Considering that the susceptibility of an organ to carcinogenesis may depend on the growth and hormonal status of a particular organ, it is important to determine the effect of dietary lipid and energy restriction on the growth of normal prostate glands.

The main objective of the present investigation was to assess the effect of dietary fat and energy restriction on the

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Table 1 Nutrient composition (g/100g) of experimental diets[1]

Ingredient	HFAL[2]	HFER	LFAL	LFER
Casein	23.0	23.0	20.0	20.0
D-L Methionine	0.3	0.3	0.3	0.3
Corn starch	33.8	31.5	52.0	50.1
Dextrose	8.5	8.5	13.0	13.0
Corn oil	23.0	23.0	5.0	5.0
Cellufil	5.9	7.1	5.0	6.0
AIN-76 minerals mix	4.1	4.9	3.5	4.2
AIN-76 vitamins mix	1.2	1.4	1.0	1.2
Choline bitatrate	0.2	0.3	0.2	0.2
Energy density (kcal/g)	4.7	4.6	4.1	4.0
% Energy from fat	50.3	50.3	11.5	11.5

[1]AIN-76 Semisynthetic diet.
[2]HFAL, High-fat ad libitum; HFER, High-fat energy restriction; LFAL, Low-fat ad libitum; LFER, Low-fat energy restriction.

proliferation state of the prostrate glands. This was determined by enumerating the cells exhibiting proliferating cell nuclear antigens (PCNA) and protein kinase C (PKC) activity in rats of two different age groups. Proliferative status and PKC activity are two parameters that are known to be affected by the tumor modulating environment. PCNA are auxiliary proteins present during DNA synthesis and are expressed in cycling cells.[14,15] The enzyme PKC is a family of isozymes that are known to be involved in cell growth and differentiation.[16–19] In this manuscript, we document that a high-fat diet and ER affected the PCNA labeling index and PKC activity in prostate glands and that the effect of diet was more pronounced in younger animals than in their older counterparts.

Materials and methods

Animals

Eighty male F344 rats (3-week-old, $n = 40$; 10-week-old, $n = 40$) were obtained from Charles River Canada, Inc. (Montreal, Quebec, Canada) and were housed individually in wire cages, with a 12-hr light and 12-hr dark cycle. Temperature and humidity were controlled at 22°C and 50%, respectively. Animals were given Purina Laboratory Chow and water ad libitum until initiation of the experiment. Animal care was in accordance with the guidelines of the Canadian Council of Animal care.

Diets

The diets were high-fat ad libitum (HFAL), high-fat energy restricted (HFER), low-fat ad libitum (LFAL), and low-fat energy restricted (LFER) diets, which were formulated based on the AIN-76A diet with modification.[20–22] Additional fat was added to the high-fat diet at the expense of an isocaloric amount of corn starch. Therefore, all essential nutrients was similar to on a caloric basis in the high- and low-fat diets. The amount of essential nutrients in each of the ER diets was increased by 20% by weight at the expense of corn starch, so that the level of essential nutrients was similar to that of the corresponding AL group. This approach to diet formulation and ER has been used previously by other researchers and our group.[4,5,22] The amount of food consumed by the AL groups was monitored every 2 days. The corresponding ER group received 80% of the amount of feed consumed by the AL group. The diet composition (*Table 1*) ensured that the consumption of vitamins, minerals, and non-nutritive fibre in each ER group was equal to that of the corresponding ad libitum counterpart.

Study design

Within each age category, rats were randomly allocated to four diet groups ($n = 10$/group). The ER groups were fed 80% of the amount of food consumed by their AL counterparts. Body weights were determined weekly until the end of the study. All animals were terminated after 12 weeks by CO_2 asphyxiation. Liver and prostate samples were obtained from animals immediately after termination, and the samples were weighed and processed for biochemical and histological procedures.

Measurement of proliferating cell nuclear antigen (PCNA) indices

The prostate gland, consisting of ventral, dorsal, and lateral lobes, was fixed in 70% ethanol, embedded in paraffin wax, sectioned (5-μm thick), and processed for immunohistochemistry using the unlabeled antibody bridge method and the Universal Peroxidase kit from Signet Laboratories (ID Labs Inc., London, ON Canada) as described previously.[14] Tissue sections were deparaffinized, flooded with normal goat serum, and incubated for 20 min to block nonspecific binding. The anti-PCNA monoclonal antibody (Dimension Laboratories Inc., Mississauga, ON, Canada) diluted with antibody diluting buffer (1:40) was applied to tissue sections, and the slides were incubated for 1 hr. Each tissue section was then sequentially incubated with anti-mouse IgG (bridging antibody) and mouse IgG peroxidase. The peroxidase reaction was initiated by immersing the slides in 0.06% 3,3′-diaminobenzidine tetrahydrochloride (Sigma) in phosphate-buffered saline (PBS) to which 0.03% H_2O_2 had been added immediately before use. Finally, the slides were lightly counterstained with haematoxylin, dehydrated, and mounted with Permount (Fisher, Scientific, Ottawa, ON, Canada). All incubations were performed in a humidified chamber at room temperature, and between incubations, slides were extensively washed with PBS. The PCNA labeling index was determined for 10 well-orientated acini in the central and the lateral lobes. The PCNA labeling index is the number of labeled cells per acinus divided by the total number of cells per acinus multiplied by 100.[23]

Quantification of PKC activity in rat prostate glands

Each prostate gland was fractionated into cytosolic and membrane fractions by the previously described method with minor modifi-

cations.[17] Briefly, the prostate glands were removed and homogenized in cytosolic buffer, buffer A (25-mM Tris buffer that contains 3.305 g/L Trizma HCl, 0.485 g/L Trizma base and pH adjusted to 7.5, 0.25 mM phenylmethyl sulfonyl fluoride, 15 mM mercaptoethanol and aprotinin, 0.007 g pepstatin, and 0.007 g trypsin inhibitor). The homogenized mixture was centrifuged at 100,000 × g for 60 min. The supernatant represented the cytosolic fraction. The pellet was resuspended and homogenized in the membranous buffer containing 0.8 vol/vol Triton-X 100 and cytosolic buffer (pH = 7.5), and then centrifuged at 100,000 × g for 30 min. The supernatant represented the membrane fraction. All procedures were performed at 4°C unless stated otherwise.

The measurement of PKC activity in the cytosolic and membrane fraction was determined by quantifying the transfer of ^{32}P from γ-[^{32}P]adenosine triphosphate (ATP) using a commercially available assay system (Amersham International plc, Amersham, UK). This system quantifies calcium and lipid-dependent protein kinase C, which includes the three classical isoforms α, β, and γ, and uses a peptide substrate whose amino acid sequence (RKRTLRRL) is based on the sequence of epidermal growth factor receptor. A brief description of the procedure is as follows. All assay components were brought to 25°C before beginning the assay. A 25-μL aliquot of the reaction mixture was added to 25-μL of sample; the reaction was then initiated by addition of 25-μL of magnesium [32P] ATP buffer (10 μCi/ml) and allowed to incubate at 25°C for 15 min. Another 100-μL stop reagent was added to terminate the reaction; then 125 μL of the terminated reaction mixture was transferred onto small squares of P81 phosphocellulose paper. The paper was immersed in 75 mM acetic acid (5% vol/vol) and the dried squares were counted for radioactivity on a Beckman LS 6000 TA Liquid Scintillation Counter (Beckman Instruments, Fullerton, CA USA). Protein content was analysed by using the Coomasie Blue protein assay (Sigma).

Statistical analysis

SAS statistical software for microcomputers was used for all statistical analyses. Statistical analyses of energy intake, organ and body weights, total and subcellular PKC activities were performed by analysis of variance (ANOVA) and Duncan's multiple range test. A P value of ≤ 0.05 was considered significant.

Results

Body weights and organs weights

Body weights of young animals increased with time (*Figure 1*) From Week 3 and thereafter average body weight in the ER groups was less ($P < 0.05$) than the AL groups (*Figure 1*). Differences in the body weight of the adult animals were evident at the second week of feeding. After 12 weeks of feeding, significant differences were noted among all groups (HFAL>LFAL>HFER>LFER). At Week 12, weights of the liver and prostate glands of ER groups were generally less than those of their respective AL groups for both ages (*Table 2*). Note that despite a lower average body weight in the LFAL group compared to the HFAL group, the weight of the liver and the prostate were similar in both groups.

PCNA labeling index of the prostate glands as affected by dietary treatments and age

The PCNA labeling indices in the ER groups were significantly different ($P \leq 0.05$) from the indices in the AL groups in both the ventral and lateral lobes of the young and adult rats (*Figure 2*). Between the HF and LF groups, a significant difference in PCNA labeling indices was observed for the AL groups for both ages. For the ER groups the difference was significant only in adult rats. In each diet group, the number of PCNA labeled cells was higher in the ventral lobe than in the lateral lobe and was higher among young rats than adult rats.

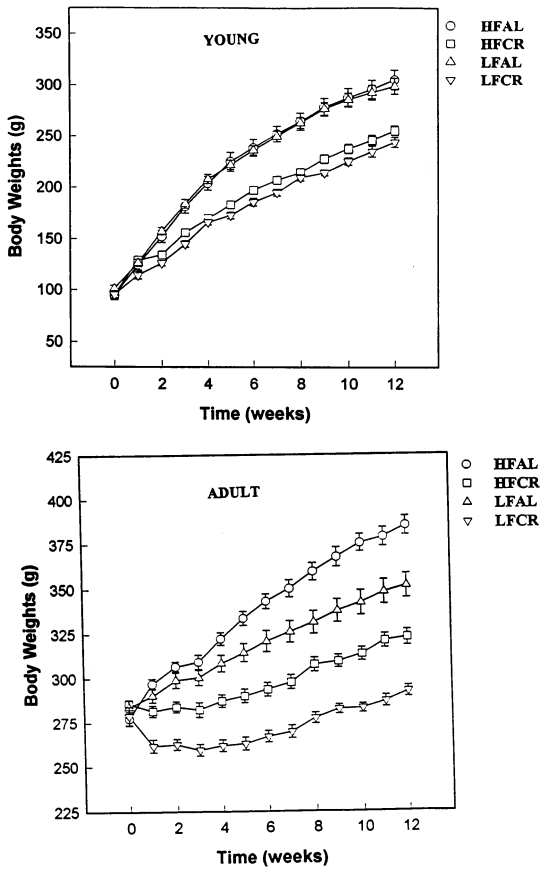

Figure 1 Body weights of young and adult rats fed different diets (n = 8) during the experimental period. Values are the mean ± standard error (SE).

Table 2 Organs weights of young and adult rats fed different diets

Group	Diet	Liver (g)	Prostate glands (g)
Young	HFAL	13.08 ± 0.67—a	0.62 ± 0.05—a
	HFER	9.64 ± 0.21—b	0.42 ± 0.05—b
	LFAL	14.36 ± 0.85—a	0.66 ± 0.05—a
	LFER	9.75 ± 0.60—b	0.41 ± 0.04—b
Adult	HFAL	13.90 ± 0.51—a	0.80 ± 0.04—a
	HFER	12.00 ± 0.50—b	0.58 ± 0.01—b
	LFAL	15.90 ± 0.82—c	0.74 ± 0.04—a
	LFER	11.80 ± 0.53—b	0.52 ± 0.04—b

Values shown are the means ± SE. HFAL, High-fat ad libitum; HFER, High-fat energy libitum; LFAL, Low-fat ad libitum; LFER, Low-fat energy libitum.
Means with different letters attached are significantly different from each other ($P \leq 0.05$).

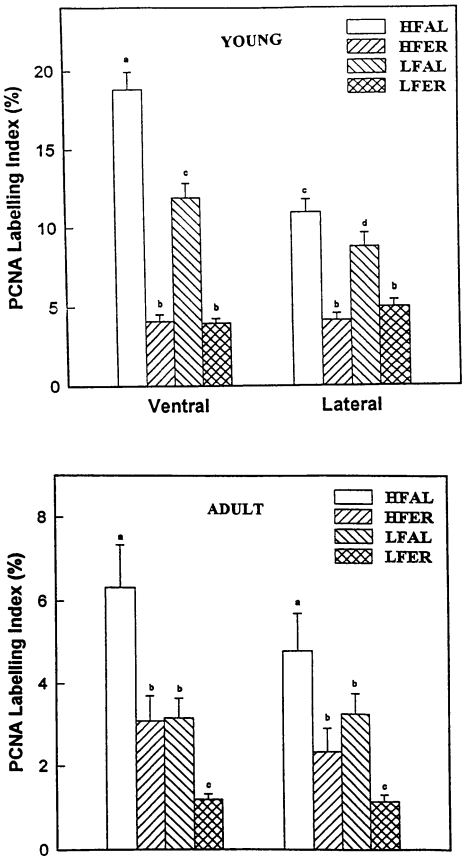

Figure 2 PCNA labeling index in the ventral and lateral lobes of prostate glands in young and adult rats ($n = 8$). Values are the mean ± SE. Columns not sharing a letter are significantly different from each other ($P \leq 0.05$).

Effect of dietary treatments and age on PKC activity in the prostate glands

Prostatic PKC activities in the cytosolic and membranous fractions are reported in Table 3. Generally, PKC activities were higher in the young rats than their adult counterparts in each diet category and were higher in the membranous fractions than in the cytosolic fractions. The prostatic tissues of the ER groups exhibited lower membrane associated PKC activity than their ad lib counterparts in each diet group for both ages.

The changes in the PKC activity of the cytosolic fractions attributable to ER were variable among young and adult rats. ER reduced the cytosolic PKC activity only in the young rats fed high-fat compared with the HFAL group. Among the adult rats, the HFAL group had the lowest cytosolic PKC activity in the prostate, while the LFER had the highest. These effects were reflected in the ratio between the PKC activity associated with the cytosolic and membranous fractions. The ratio was higher in the HFER or LFER group compared with their ad lib counterparts.

Discussion

There is limited information as to how the normal prostatic tissue responds to dietary factors in young and adult animals. The physiologic responses were measured by assessing the weight of the glands, number of cycling cells in the glands and the activity of PKC. The weight of the prostate gland provides a direct measure of growth whereas the number of cycling cells provides an estimate of the fraction of total cells that are cycling and the PKC activity provides a potential measure of tissue's ability to perform complex sequence of events leading to growth and differentiation. In the present study, we explored the possibility that dietary fat and energy restriction would have a marked effect on these measures. The main findings of the present study were: (a) the prostate glands of young rats had a higher number of PCNA labeled cells and higher PKC activity than those of the older rats, and (b) that the level of fat and ER both profoundly affected these parameters. The weight of the animals and prostate glands, which is a direct measure of growth, revealed that the effect of the high-fat diet on the body weight of the animals compared with that of the low-fat diet was only evident in the adult animals. This finding suggest that a high-fat diet was used by young rats differently than by the adult rats. The latter group tended to gain more weight than its low-fat counterpart. Energy restriction retarded the growth of the prostate glands in both ages but the effect was more pronounced in the young rats than in the adult rats. This could be attributable to the fact that prostate glands are rapidly developing in the

Table 3 Protein kinase C activity (pmol/min/mg tissue protein) in the prostate glands of young and adult rats fed different diets

Group	Diet	Total PKC	Cytosolic PKC (A)	Membrane PKC (B)	Ratio Between A and B
Young	HFAL	609.37 ± 80.88—a	172.10 ± 24.09—a	437.26 ± 57.28—a	0.39
	HFER	367.42 ± 3.13—b	75.24 ± 26.05—b	292.18 ± 27.30—b	0.26
	LFAL	294.14 ± 62.65—bc	19.56 ± 2.90—c	274.55 ± 64.36—b	0.07
	LFER	169.19 ± 55.13—cd	25.34 ± 6.34—c	143.85 ± 51.10—cd	0.18
Adult	HFAL	154.59 ± 58.50—cd	31.64 ± 3.52—c	122.95 ± 8.92—cd	0.26
	HFER	117.39 ± 7.59—d	39.07 ± 7.59—d	78.32 ± 5.40—f	0.50
	LFAL	143.09 ± 24.12—d	51.03 ± 7.90—b	92.07 ± 16.70—cf	0.55
	LFER	119.02 ± 22.31—d	63.29 ± 12.14—b	55.73 ± 8.26—g	1.13

All values shown are the mean ± SEM. HFAL, High-fat ad libitum; HFER, High-fat energy restriction; LFAL, Low-fat ad libitum; LFER, Low-fat energy restriction.
Means in column with different letters attached are significantly different ($P < 0.05$) from each other.

young rats and are therefore more sensitive to the growth-retarding effect of ER than in the adult rats.

Cell replication is a complex multistep process that can be regulated by a variety of hormones and growth factors. The entire cell cycle can be interrupted or stagnated at several stages by endogenous and exogenous factors.[14] Depending on the method of assessment, different stages of the cell cycle are enumerated. Proliferating cell nuclear antigens are cell-cycle-specific proteins that serve as auxiliary proteins to DNA polymerase, and participate in the control of cell proliferation in mammalian cells during normal replication or DNA repair replication.[14,24,25] The assessment of the amount of cell cycle related proteins in normal tissue is proposed to quantify the number of cycling cells.[14,15,24–25] Several studies have suggested that PCNA immunohistochemistry may be superior to thymidine labeling or bromodexyuridine immunohistochemistry as a technique for determining proliferative status.[14,24–26] The advantage of this technique is that PCNA is expressed in cycling cells, therefore, animals do not require treatment with exogenous labeling or blocking agents to identify cycling cells. The determination of PCNA is ideal if the enzymatic status of the tissues is to be performed, as colchicine, which blocks cells in metaphase, and bromodeoxyuridine, a compound used to label cells engaged in DNA synthesis, are also cytotoxic and may affect enzymatic status of an organ.

In the present study, prostate glands in young rats had a higher number of cycling cells than in adult rats, suggesting a higher proliferative status. Among the three lobes in the prostate gland of rats, the ventral lobe seems to be more sensitive than the other lobes to carcinogenesis.[26] Interestingly, the ventral lobes exhibited higher proliferative status than the lateral lobes. The higher sensitivity of the ventral lobe to carcinogenesis compared with the lateral lobes could be related to their proliferative status. The higher proliferative state and PKC activity in the prostatic tissue of young rats suggest that prostate glands are more vulnerable to carcinogenesis and environmental factors including diet early in the life cycle than later. Recent studies in humans also allude to this possibility.[10,28]

In the present study, dietary fat and ER significantly altered the proliferative status of both the ventral and lateral lobes of prostate glands in young and adult animals. However, the overall number of PCNA labeled cells was greater in young animals than in adult animals. Testosterone exerts a proliferative stimulus on the prostate glands.[13] The young rats are expected to have a higher level of testosterone than the adult rats. Whether an energy-rich, high-fat diet accentuated the level of testosterone remains to be determined.

Protein kinase c is a family of isozymes, which exhibit differences in co-factor dependence and activator specificity.[16,17] In the present study, calcium and phospholipid-dependent PKC were measured that include the three classical isoforms α, β, and γ. PKC activity is altered by tumor promoters, dietary lipids, and disease state in a number of tissues.[16] Information on the role of PKC in normal rat prostate glands is limited. However, a number of reports have appeared investigating the presence and expression of various isoforms in normal rat prostatic tissues and in prostatic cancer cells. Of the three classical isoforms that depend on calcium and lipids, only α and β have been identified in normal prostate glands of rats.[29,30] Therefore, one would speculate that the enzyme activity reported in the present study represented only the PKCα and β isoforms. Higher membrane associated PKC activity in the prostate glands of animals fed the high-fat diet or caloric unrestricted diet in our study suggests that these diets may have increased endogenous activators involved in translocating PKC from cytosolic to the membrane fraction. The endogenous activators could be lipid metabolites that are known modulators of PKC activity. This observation is consistent with the observation that tumor promoters seem to increase the translocation of cytosolic PKC to the membrane fraction and that the high-fat diet used in the present study has a tumor promoting activity in the colon.[31] It appears that in adult rats, an increase or decrease in the membrane associated PKC activity was accompanied by a corresponding decrease or increase in cytosolic PKC activity. In a study investigating the role of dietary fat and PKC in skin carcinogenesis, activity in the particulate fraction increased two-fold, whereas the cytosolic PKC activity decreased dramatically in animals consuming a high-fat diet.[32] A limited number of studies have investigated the effect of age on the organs' metabolism and response to a growth modulating environment. The findings of the present study suggest that age and diet are both important factors affecting PKC activity and its location in prostate glands.

The reason(s) for the noticeably higher total PKC activity associated with high-fat and energy intake than their low-fat or ER counterparts is not known. One plausible explanation is that these dietary factors affected the synthesis of the enzyme because of an increase in the proliferative status of the organ and/or that they increased the binding sites for PKC on plasma membrane. Despite the fact that we cannot explain the mechanism(s) by which fat and energy affect PKC activity, the findings of the present study have demonstrated that PKC was regulated quite differently in the young versus old rats. It is important to note that the PKC activity assessed in one gland corroborated the changes occurring in the PCNA labeling indices of the other gland in each group.

The most salient findings of the present research are that age, dietary fat, and energy intake profoundly affected the proliferative status, as measured by PCNA labeling index and PKC activity of normal rat prostate glands. The mechanism(s) by which age of animals, dietary fat, and ER affect the growth and PKC activity of prostate glands and its sensitivity to carcinogenesis remains to be investigated.

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