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A carcinogenic western diet does not induce somatic mutations in various target tissues of transgenic C56BL/6 mice

Lya G. Hernández[a,b], John A. Heddle[a,*]

[a] *Department of Biology, York University, 4700 Keele Street, Toronto, Ont., Canada M3J 1P3*
[b] *Anatomy and Cell Biology Department, Queen's University, 99 University Avenue, Kingston, Ont., Canada K7L 3N6*

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Abstract

Although the importance of diet in human cancer is clear, most dietary studies of carcinogenesis in laboratory rodents have involved the use of large doses of a carcinogen, which is not comparable to the human situation. The use of carcinogens has been necessary because laboratory rodents have extremely low spontaneous rates of colon cancer. Newmark et al. (2001) showed, however, that a radical dietary manipulation sufficed to induce high rates of colon cancer in C57BL/6 mice. Here we report an investigation into whether or not this dietary manipulation acts by altering somatic mutation rates. We used the transgenic lambda *cII* locus of F_1 pups (C57BL/6 × Big Blue®) with the same C57BL/6 genetic background. The same diet (ND), high in fat, and low in calcium, vitamin D, folic acid, choline, and fibre, that was used by Newmark et al. (2001) was fed ad libitum to dams during pregnancy and lactation in order to examine its effect on mutagenesis in development and growth. There was no significant difference in mutant frequency in the small intestine ($P = 0.82$), or bone marrow ($P = 0.95$) of pups fed a ND versus the control diet. To investigate the effect of a ND during adulthood, 6-week-old F_1 pups were fed a ND ad libitum for 6, 12 and 19 weeks. There was no significant difference in mutant frequency in the small intestine ($P = 0.66$) or colon ($P = 0.49$) at the *cII* locus with no significant difference in body weight. These results indicate that Western diet-induced carcinogenesis is not mediated by alterations in mutation rate and thus may act at the promotion rather than at the initiation stage of carcinogenesis.

Keywords: *cII*; High fat; Low calcium; Vitamin D; Folic acid; Fibre

1. Introduction

Differences in cancer rates among migrant populations suggest that lifestyle and environmental factors such as diet are crucial for human cancer risk [1–4]. Cancer is a multi-step process driven by the accumulation of somatic mutations over time [5] where the initi-

* Corresponding author. Tel.: +1 416 736 2100x33053; fax: +1 416 736 5698.
E-mail address: jheddle@yorku.ca (J.A. Heddle).

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ation stage is presumed to involve mutations and to be the rate-limiting step [6]. The dietary factors involved in carcinogenesis are not well understood; therefore, the identification of ways to delay the rate and/or prevent the accumulation of mutations [7] may provide novel clues for cancer prevention. Western diets, high in fat and low in micronutrients (i.e., folic acid, vitamins C and D), have been associated with increased colorectal cancer risk in human cohort, case-control [8–12], and epidemiological studies [13,14]. Rodent studies also reveal that mice fed a Western diet show a significant increase in cellular proliferation in epithelial cells of the pancreas [15], prostate [16], colon [17,18], and terminal ducts of the mammary glands [19]. A recent study by Newmark et al. [17] demonstrated, for the first time, the induction of colonic and intestinal tumors by a Western-style diet (hereafter, referred to as ND), high in fat, and low in calcium, vitamin D, folic acid, choline, and fibre, after eighteen months of dietary exposure in the absence of a carcinogen. Newmark et al. [17] designed this diet using nutrient density for adequate nutrient comparison between humans and rodents [20]. In order to determine whether or not this carcinogenic ND was altering the colon cancer rates by altering mutation rates, we have measured the mutant frequencies in the colon of transgenic mice.

Transgenic mice harbouring prokaryotic shuttle vectors with reporter genes such as the bacterial *lacI* gene, which is present in Big Blue® mice [21] or the bacterial *lacZ* gene, which is present in Muta™Mouse [22], permit the measurement of mutations within any tissue of the mouse. The development of the lambda (λ) *cII* positive selection system by Jakubczak et al. [23], which detects mutations in the λ *cII* transgene, is an important technical advancement providing a selection method for detecting mutants. This involves infecting an *hfl*$^{-}$ strain (*E. coli* G1250) with the recovered phage containing *cII*$^{-}$ mutations forming plaques at low temperatures (24 °C), with all viable phage forming plaques at 37 °C. This system can be used in both Big Blue® and Muta™Mouse, and is favoured over the traditional *lacI* and *lacZ* mutational assays because it is a positive selection system not a screening system, the *cII* gene is one-third the size of *lacI* and one tenth the size of the *lacZ*, and the assay itself takes less time, uses less reagents, and therefore is less expensive [23,24].

In our studies, mice were exposed to the Newmark's diet during one of two phases of their lives: early growth and development (conception to 4 weeks of age) or as young adults (6 weeks of age until 25 weeks of age). Previous work has shown that the period between conception and weaning is a time of high mutation rate in both humans [25] and mice [26,27]. Hence, the earliest phase of life may be most sensitive to dietary changes. During adult life, the accumulation of somatic mutations is much slower on a normal rodent diet. For this reason, we investigated these two periods independently. Given that initiation, and thus presumably mutation, is an early step in carcinogenesis, we limited our treatments to 19 weeks with the presumption that older mice would respond differently with respect to mutation.

2. Materials and methods

2.1. Experimental design

2.1.1. Mice

All experiments were approved in advance by the Animal Care Committee of York University. Female C57BL/6 (8 weeks of age) were purchased from Charles River Canada (Quebec, Ont.). After 1 week of acclimatization, mice were assigned to two feeding groups: a control diet (AIN-76A, Research Diets, New Brunswick, NJ, USA) or the Newmark diet (ND). Female C57BL/6 were bred with male Big®Blue C57BL/6 (Stratagene, La Jolla, CA). Conception was checked by the presence of a vaginal plug. Pregnant mice were individually caged and fed their respective diet (ND or the AIN-76A control diet). Mice were housed in stainless steel wire-top cages at 58% humidity and a temperature of 22.5 ± 1 °C with a 12 h light/dark cycle. Diet and water were fed ad libitum with fresh diet provided twice per week.

2.1.2. Development and growth

Dams from both feeding groups (ND and control diet) were weighed every day until birth and F_1 pups (C57BL/6 × Big Blue®) were weighed 2, 3, and 4 weeks after birth. F_1 pups were sacrificed at 4 weeks of age.