

Dietary Microbes Modulate Transgenerational Cancer Risk

Theofilos Poutahidis^{1,2}, Bernard J. Varian¹, Tatiana Levkovich¹, Jessica R. Lakritz¹, Sheyla Mirabal¹, Caitlin Kwok¹, Yassin M. Ibrahim¹, Sean M. Kearney^{1,3}, Antonis Chatzigiagos², Eric J. Alm^{3,4}, and Susan E. Erdman¹

Abstract

Environmental factors are suspected in the increase of obesity and cancer in industrialized countries but are poorly understood. Here, we used animal models to test how future generations may be affected by Westernized diets. We discover long-term consequences of grandmothers' *in utero* dietary exposures, leading to high rates of obesity and frequent cancers of lung and liver in two subsequent

generations of mice. Transgenerational effects were transplantable using diet-associated bacteria communities alone. Consequently, feeding of beneficial microbes was sufficient to lower transgenerational risk for cancer and obesity regardless of diet history. Targeting microbes may be a highly effective population-based approach to lower risk for cancer. *Cancer Res*; 75(7); 1197–204. ©2015 AACR.

Introduction

Inflammation-associated, metabolic, and neoplastic diseases have increased in frequency in the industrialized world due to numerous factors, including reduced activity levels, increased total energy intake, environmental carcinogens, and immune status (1, 2). Compelling hypotheses aim to explain this phenomenon and reshape preventive medicine. According to one microbe immune-centric hypothesis, inhabitants of developed countries have immune systems of reduced lifelong regulatory capacity due to societal practices in the form of refined diets, antibiotics, and Caesarian births with insufficient beneficial microbe exposure during perinatal life (1–3). Impaired regulatory capacity leads to uncontrolled immune inflammatory responses, obesity, and cancer later in life (2, 3). Our recent studies in mice have shown that Westernized diet (NWD)-associated obesity and cancer coincide with changes in gastrointestinal tract microbial communities and immunoregulatory capacity preventable by dietary enrichment with beneficial bacteria (4–7). We test here whether these postulated effects of gut microbes transcend generations. We discover that mother mice consuming NWD during pregnancy convey detrimental long-term consequences to subsequent generations, in particular high rates of lung and liver cancer

with obesity and premature senescence. We show this transgenerational predisposition is mediated by intestinal bacteria, highlighting a key role for microbial flora in reducing the risk of cancer.

Materials and Methods

Animals

Outbred conventional or germ-free CD-1 Swiss stock mice (Charles River) were housed and handled in Association for Assessment and Accreditation of Laboratory Animal Care-accredited facilities with diets, experimental methods, and housing as approved by the Institutional Animal Care and Use Committee. A genetically outbred stock with robust breeding capacity and absent transgenic predilections to cancer was selected for these transgenerational studies. To test the transgenerational impact of dietary microbes on progeny, the experimental design was to expose F0 (grandmother) mice to diets starting at the age of 8 weeks. Special dietary treatment continued until the birth of their pups. Progeny were later examined to determine health risks to subsequent generations. Euthanasia was performed using carbon dioxide overdose at 1 year of age, unless otherwise specified. Because of early-life morbidity, F2 progeny (grandchildren) were euthanized at 6 months of age or younger according to institutional humane criteria and clinical disease. Tissues were collected upon necropsy and then examined histologically. Each experiment included 5 to 10 animals per sex per treatment group, performed in duplicate, as described in detail below.

Special diets for animals

Outbred Swiss CD-1 mice were placed on experimental diets starting at 8 weeks of age: control AIN-76A (Harlan-Teklad) and a NWD with high fat and low fiber with substandard levels of vitamins B and D (TD.96096; Harlan-Teklad; Supplementary Table S1) as previously described (7). Subsets of CD-1 mice who had been treated with NWD *in utero* subsequently received in their drinking water an anti-inflammatory strain of *Lactobacillus reuteri* ATCC-PTA-6475 cultivated as described elsewhere (8, 9), with live organisms supplied at a starting dosage of 3.5×10^5 organisms/

¹Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts. ²Laboratory of Pathology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece. ³Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts. ⁴Broad Institute of MIT and Harvard, Cambridge, Massachusetts.

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T. Poutahidis and B.J. Varian contributed equally to this article.

Corresponding Author: Susan E. Erdman, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139. Phone: 617-252-1804; Fax: 617-258-5708; E-mail: serdman@mit.edu

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mouse/day in drinking water (4). Live bacterial counts in water bottles were calculated to be 1.4×10^6 colony-forming units (CFU) per mouse on day 1, 4.1×10^5 CFU on day 2, and 1.1×10^5 CFU on day 3; and *L. reuteri* was detectable by PCR in feces and bowel of mice undergoing the dosing regimen, as described in detail in Lakritz and colleagues (4). Control mice received regular drinking water. Fresh drinking water for both groups of animals was replaced twice weekly throughout the experiments.

Experimental design

Experiment 1. Six eight-week-old CD-1 female mice were fed *ad libitum* a NWD diet TD.96096 mimicking fast-food starting at 8 weeks of age (Fig. 1A). Females who ultimately served as mothers and grandmothers were immediately arranged into breeding pairs to induce pregnancy. Special diets were replaced with control AIN-76A chow upon birth of F1 progeny. To serve as controls, 6 separate 8-week-old CD-1 female mice were fed *ad libitum* a control chow AIN-76A and similarly arranged into breeding pairs to produce experimental control progeny animals. All animals had *ad libitum* access to diets with unrestricted exercise. Their progeny were also examined for obesity and cancer when they reached 1 year of age, including body weights

along with tissue collections upon necropsy for histologic examination.

Experiment 2. To test whether gut microbes were sufficient for transgenerational effects, 5 germ-free female Swiss CD-1 mice were fed by gastric gavage the feces from NWD-fed mother mice in Experiment 1 (Fig. 1B). Five germ-free mice received comparable stool collected from control diet-fed moms in Experiment 1 to serve as controls. To achieve this, the germ-free mice were dosed 3 times every other day by gastric gavage with 0.05 grams of fresh fecal slurry per mouse per dose. Subsequently, both groups of fecal transplant-recipient moms were co-housed with a male mouse in autoclaved caging and fed only control chow AIN-76A diet to produce progeny for future examination. Progeny were examined for cancer at 1 year of age when tissues were collected upon necropsy.

Experiment 3. Six F1 female progeny with *in utero* exposure to NWD from Experiment 1 (above) were randomly arranged into breeding pairs at 8 weeks of age to produce offspring (grandchildren) for further observation (Fig. 1C). Six F1 progeny from control diet-fed moms in Experiment 1 were similarly arranged

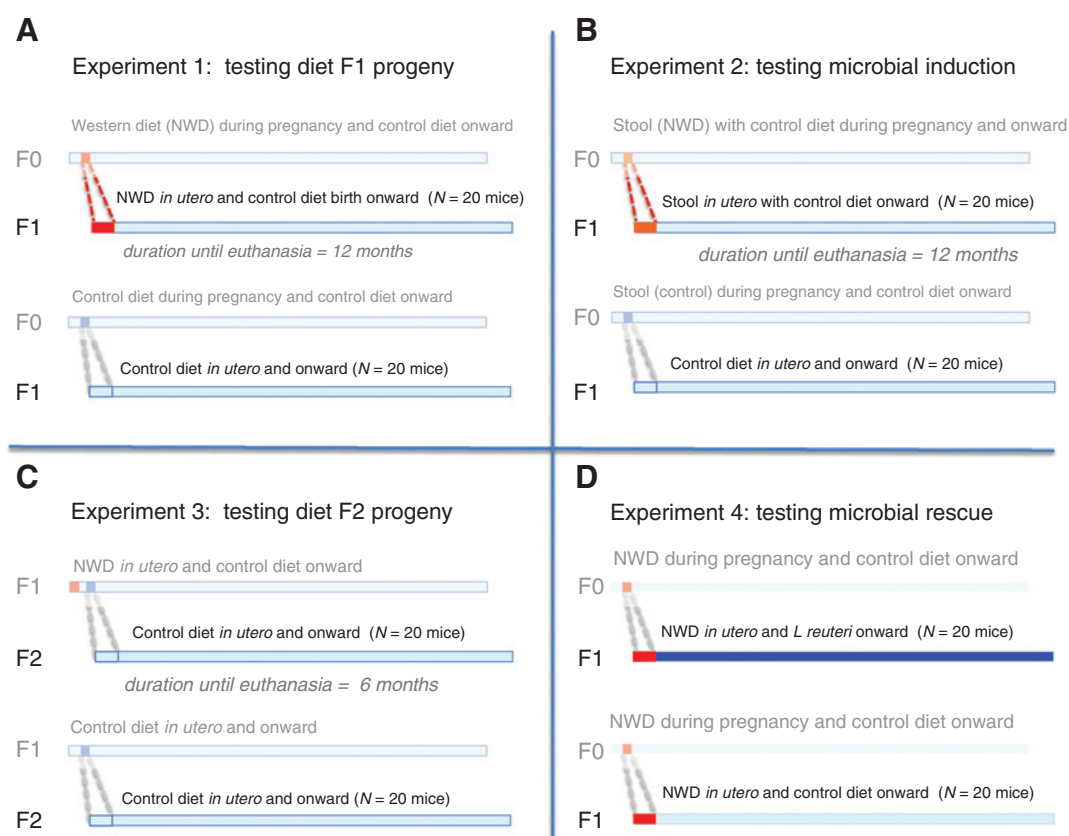


Figure 1.

Overview of experimental design. A, in experiment 1, to determine the risk of cancer in progeny animals, 8-week-old CD-1 female mice were fed *ad libitum* a NWD chow mimicking fast food consumed by humans, and then arranged into breeding pairs to induce pregnancy. Their progeny were examined for obesity and cancer when they reached 1 year of age. B, in experiment 2, germ-free female CD-1 mice were fed by gastric gavage the feces from NWD-fed mice, paired with males, and then fed only control chow. Their progeny were examined for cancer at 1 year of age. C, in experiment 3, F1 progeny from NWD-fed mother mice were arranged into breeding pairs to produce offspring (grandchildren) for further observation. Progeny were examined for cancer starting at 6 months of age. D, three-week-old progeny (F1) from NWD-fed mother mice in experiment 1 were subdivided and provided in their drinking water *L. reuteri* ATCC-PTA-6475 to test effects of beneficial microbes. Mice receiving NWD *in utero* from experiment 1 served as regular drinking water controls.

into breeding pairs to serve as controls. Both groups were fed control AIN-76A diet throughout Experiment 3. Unanticipated fertility problems arose in half (3 of 6) of the matings involving NWD-fed mother mice, whereas control mice had no such issues. Two litters with ancestral exposure to NWD also exhibited a scurfy-like thymic atrophy and failure-to-thrive in 9 of 20 (45%) infant mice and were euthanized at 8 weeks of age. Surviving F2 progeny ($n = 10$ males and $n = 10$ females) were examined for cancer starting at 6 months of age (earlier than their parent's cohort) due to unexpected morbidity requiring euthanasia. Tissues were collected upon necropsy and then examined histologically to confirm diagnoses.

Experiment 4. To further test the microbe-centric hypothesis, 3-week-old progeny (F1) from NWD-fed CD-1 mother mice in Experiment 1 were randomly subdivided into groups of 20 mice (10 males and 10 females per treatment) and received in their drinking water *L. reuteri* ATCC-PTA-6475 as described elsewhere (8, 9) continuously until 1 year of age (Fig. 1D). Mice from Experiment 1 that received NWD while *in utero* that then got regular drinking water served as controls. Tissues were collected upon necropsy at 1 year of age.

Stool microbiome analyses

Genomic DNA was extracted from stool samples using the Qiagen QIAamp DNA Stool Mini Kit. Samples for paired-end Illumina sequencing were constructed using a 2-step PCR amplicon approach targeting the V4 region of the 16S rRNA gene (U515F and E786R) and reads were quality filtered and clustered into operational taxonomic units (OTU) at 97% nucleotide identity as previously described (7).

Histopathology and immunohistochemistry

For histologic evaluation, formalin-fixed tissues were embedded in paraffin, cut at 5 μm , and stained with hematoxylin or immunohistochemistry (IHC) as previously described (3, 10). Primary antibodies for IHC included rabbit antibodies against myeloperoxidase, p53 (ThermoFisher Scientific/Lab Vision), IL17 (Santa Cruz Biotechnology, Inc.), Thyroid transcription factor 1 and Ki-67 (TTF1, Abcam). Heat-induced antigen retrieval was performed with citrate buffer, pH 6, for myeloperoxidase, with EDTA buffer, pH 8, for TTF1 and p53 detection or with CC1 epitope retrieval solution (Ventana Medical Systems, Inc.) for Ki-67 and IL17. Primary antibody binding was detected with goat anti-rabbit polymer HRP (ZytoChem Plus). Color was developed with DAB substrate-chromogen system (ThermoFisher Scientific/Lab Vision) and tissues were counterstained with hematoxylin.

Statistical analyses

The Mann-Whitney *U* test was used for whole body and thymic weight analyses. Tumor incidence in experimental groups was compared with the Fisher exact test. All analyses were performed with the GraphPad Prism version 5.0 for windows, GraphPad software. $P < 0.05$ was statistically significant.

Results

Offspring of mice consuming Western diet have high risk for cancer

Lifestyle changes, including Westernized diet, appear to underlie many of the chronic inflammatory diseases, including

obesity, diabetes, heart disease, autoimmune diseases, and cancer (11). To test whether NWD diet-induced obesity (7) and neoplasia (4) may be passed to subsequent generations, we fed NWD to mother mice (F0) during pregnancy and then examined health outcomes in their children reared eating a regular control chow diet (Fig. 1a). For these studies, we used outbred white Swiss CD-1 stock without additional exposure to carcinogens. Mother mice of generation F0 consuming NWD for several weeks during pregnancy were not themselves affected by cancer and obesity in this study. However, we found that F1 progeny of moms consuming NWD during pregnancy had more frequent cancers when compared with offspring of mother mice eating control diet during pregnancy (Table 1).

Specifically, the most profoundly increased cancer types arising in mice with *in utero* exposure to NWD included pulmonary adenoma (3 of 14) and adenocarcinoma (11 of 14; 14 of 20; $P < 0.0001$), liver hepatocellular carcinoma (12 of 20; $P < 0.0001$), and spleen or mesenteric lymph node lymphoma (11 of 20; $P < 0.003$; Table 1, Fig. 2A–C). Other types of neoplasia were increased, although to a lesser extent, including mammary carcinoma (Fig. 2D), reproductive organ malignancies such as ovarian theca and granulosa cell tumors and cystadenoma, and primary hepatic hemangiosarcoma, small intestine adenoma, and islet cell adenoma of pancreas (Supplementary Fig. S1). In addition, progeny of NWD-fed moms were more likely to suffer obesity than their counterparts whose mothers had eaten a control diet (Fig. 3A), earlier linked with high levels of cytokine IL17 (7). Using IL17A-specific IHC, we discovered extensive fat pyogranulomas displaying high levels of IL17 protein *in situ* in offspring when their mothers had eaten the fast-food-style NWD chow (Fig. 3A). We also found higher abundance of *Firmicutes* and lower abundance of *Bacteroidetes* in NWD-treated mice consistent with our previous findings and also those of other groups who have studied obesity and metabolic syndrome (Supplementary Fig. S2; refs. 7, 12). Knowing that gastrointestinal tract microbial communities change within days after eating NWD (7), we hypothesized that microbial dysbiosis in pregnant mother mice placed their infants at increased risk for cancer.

Fecal transplant from NWD-fed donors is sufficient for cancer in progeny

To test our microbe-centric hypothesis, we used fresh fecal slurry containing gut bacteria from NWD-fed mother mice delivered by gastric gavage to recipient 8-week-old germ-free Swiss female mice subsequently arranged in matings (Fig. 1B). Control germ-free mice received comparable stool collected from control diet-fed moms. Subsequently both groups of stool-recipient moms ate only the standard control chow diet. When progeny were examined 1 year later, we found that mice receiving NWD-donor microbiota had higher rates of lung ($P < 0.0001$) and liver ($P < 0.0001$) cancers, at levels similar to those in offspring from NWD-consuming moms (Table 1). Conversely, offspring of germ-free mothers receiving control stool gavage rarely exhibited neoplasms in the target organs. It remains to be determined whether *in utero* activities of specific microorganisms result entirely from increased inflammatory tone or other mechanisms (13). Nonetheless, these findings demonstrated that transplantable fecal microbes were sufficient for increased risk of cancer in progeny mice.

Table 1. Frequency of cancers diagnosed in vital organs of progeny animals

Generation	Treatment	Age	At necropsy	Group ID	Organ type examined					
					Lung	Liver	Lymph	Intestine	Pancreas	Mammary
Experiment 1: Testing whether <i>in utero</i> exposure to NWD increases risk of cancer in first generation progeny										
F1	Control	12 mo	All	0/20	0/20	5% (1/20)	0/20	0/20	0/10	0/10
			Females	0/10	0/10	1/10	0/10	0/10	0/10	0/10
			Males	0/10	0/10	0/10	0/10	0/10	nd	nd
F1	NWD <i>in utero</i>	12 mo	All	70% (14/20)	66% (13/20)	55% (11/20)	5% (1/20)	0/20	20% (2/10)	30% (3/10)
			Females	7/10	6/10	7/10	1/10	0/10	2/10	3/10
			Males	7/10	7/10	4/10	0/10	0/10	nd	nd
Experiment 1.			All	$P < 0.0001$	$P < 0.0001$	$P < 0.003$	ns	ns	ns	ns
Fisher exact P										
Experiment 2: Testing whether transplantable microbes are sufficient for increased cancer risk in first generation progeny										
F1	control feces	12 mo	All	1/20	1/20	3/20	0/20	0/20	0/10	0/10
			Females	1/10	0/10	2/10	0/10	0/10	0/10	0/10
			Males	0/10	1/10	1/10	0/10	0/10	nd	nd
F1	NWD feces	12 mo	All	70% (14/20)	70% (14/20)	66% (13/20)	5% (1/20)	5% (1/20)	20% (2/10)	20% (2/10)
			Females	8/10	7/10	8/10	1/10	1/10	2/10	2/10
			Males	6/10	7/10	5/10	0/10	0/10	nd	nd
Experiment 2.			All	$P < 0.0001$	$P < 0.0001$	$P < 0.003$	ns	ns	ns	ns
Fisher exact P										
Experiment 3: Testing whether <i>in utero</i> exposure to NWD increases risk of cancer in second-generation (grandchildren) progeny										
F2	control <i>in utero</i>	6 mo	All	0/20	0/20	1/20	0/20	0/20	0/10	0/10
			Females	0/10	0/10	1/10	0/10	0/10	0/10	0/10
			Males	0/10	0/10	0/10	0/10	0/10	nd	nd
F2	NWD <i>in utero</i>	6 mo	All	75% (15/20)	80% (16/20)	50% (10/20)	5% (1/20)	5% (1/20)	20% (2/10)	30% (3/10)
			Females	8/10	8/10	7/10	0/10	0/10	2/10	3/10
			Males	7/10	8/10	3/10	1/10	1/10	nd	nd
Experiment 3.			All	$P < 0.0001$	$P < 0.0001$	$P < 0.003$	ns	ns	ns	ns
Fisher exact P										
Experiment 4: Testing whether treatment of first-generation progeny with beneficial bacteria inhibits cancer risk after <i>in utero</i> NWD exposure										
F1	Oral LR tx of NWD <i>in utero</i>	12 mo	All	5% (1/20)	5% (1/20)	(20%) 4/20	0/20	0/20	0/10	0/10
			Females	1/10	0/10	3/10	0/10	0/10	0/10	0/10
			Males	0/10	1/10	1/10	0/10	0/10	nd	nd
F1	NWD <i>in utero</i>	12 mo	All	70% (14/20)	66% (13/20)	50% (10/20)	10% (2/20)	5% (1/20)	20% (2/10)	30% (3/10)
			Females	7/10	6/10	6/10	1/10	1/10	2/10	3/10
			Males	7/10	7/10	4/10	1/10	0/10	nd	nd
Experiment 4.			All	$P < 0.0001$	$P < 0.0001$	$P < 0.04$	ns	ns	ns	ns
Fisher exact P										

F2 progeny display increased cancer and accelerated aging

Recognizing extensive pathology existing in F1 mice, we next tested whether subsequent generations are also at increased risk for cancer arising from ancestral *in utero* dietary indiscretions. Thus, we performed mating experiments among the F1 children of mother mice who had consumed NWD or control chow during pregnancy (Fig. 1C). Initially, infertile mating of Swiss F1 progeny [3 of 6 (50%) vs. 0 of 6; not significant] made it difficult to produce the necessary F2 grandchildren from NWD-fed grandmother mice. Upon further examination of this generation, F1 female nulliparous littermate progeny exhibited not only obesity but also polycystic ovaries ($P < 0.001$) and endometrial disease (Fig. 3B). In these same female mice, elongated anogenital distances (data not shown) and urogenital atypia suggestive of masculinization were not extensively characterized. Another feature in daughters of NWD-fed mothers, hepatocellular carcinoma, is more typical of male animals with high levels of IL6 (14). Specific causes of impaired fertility in these mice remain to be investigated.

Grandchildren (F2 progeny) emerging from fertile F1 mating ultimately exhibited lung ($P < 0.0001$) and liver ($P < 0.0001$)

cancers with neoplasms arising at younger ages than those seen in their parents (Table 1). Similar early-onset transgenerational phenomena have been described in F2 progeny of rats undergoing experimental stressors while *in utero* (15), raising the possibility that *in utero* microbial "stress" is linked with underlying epigenetic mechanisms. Ancestral exposures to NWD were also manifested as failure-to-thrive (9 of 20 vs. 0 of 20; $P < 0.05$) in 8-week-old F2 progeny (Fig. 3C), affecting both male [50% (5 of 10)] and female [40% (4 of 10)] animals. The syndrome included thymic involution and inflammatory infiltrates throughout vital organs resembling "scuffy" mice with immunoregulatory deficits (16).

Recognizing that pathogenic gut bacteria trigger accumulations of neutrophils (17), we examined non-neoplastic tissues from descendants with gut dysbiosis and found increased numbers of intravascular and extravasated neutrophils, an observation supported by myeloperoxidase (MPO)-specific IHC in liver, adipose tissue pyogranulomas, and mesenteric arteries (Fig. 3C). In contrast, the presence of neutrophils in control diet- and LR-treated mouse tissues was minimal. It was previously shown that reciprocal relationships exist between neutrophils and host regulatory

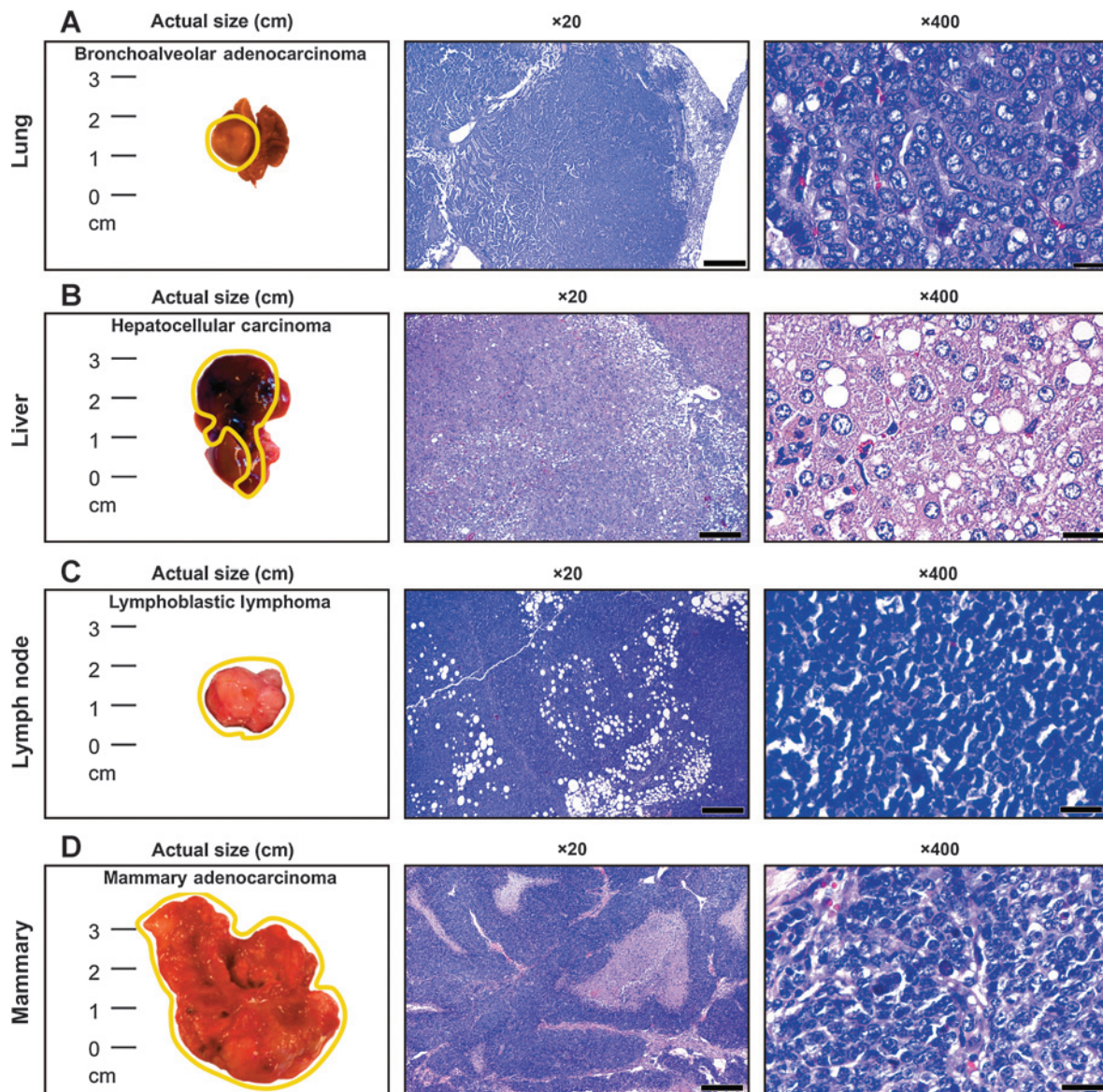


Figure 2.

Neoplastic phenotypes encountered in progeny of Swiss CD-1 mice eating NWD chow during pregnancy. A, pulmonary adenocarcinoma. A large raised mass occupies the major part of the left lobe. The expansile, unencapsulated mass shows a mixed acinar and papillary histologic growth pattern with closely packed lobules and cords supported by sparse stromal elements. Alveolar septa-like structures are lined by atypical cuboidal cells with eosinophilic cytoplasm and large, round euchromatic nuclei. B, hepatocellular carcinoma. A large irregular mass with superficial focal necroses obliterates normal liver shape and occupies approximately two thirds of the organ. The large well-circumscribed liver tumor compresses the normal liver parenchyma on the right and displays a solid growth pattern. The diagnosis is moderately well-differentiated hepatocellular carcinoma with large neoplastic hepatocytes and a high degree of nuclear pleomorphism. C, lymphoma. White lobulated mesenteric mass composed of solid sheets of neoplastic cells that infiltrate the mesenteric fat. The uniform, medium-sized lymphocytes have a scant cytoplasm and round nuclei with fine chromatin. Their histomorphology is consistent with that of lymphoblastic lymphoma. D, large, irregular, lobulated mammary tumor. Solid prominent cords and large nests of neoplastic cells are separated by moderate amounts of fibrovascular stroma. Occasional nests show central area necrosis (comedo-like pattern). Neoplastic mammary epithelial cells show marked atypia and pleomorphism and abundant mitotic figures, including abnormal ones. Hematoxylin and eosin. Scale bars, 500 μ m (middle column); 25 μ m (right column).

capacity (10, 18). Additional studies are needed to ascertain whether perinatal changes in microbe communities serve to modulate immunoregulatory capacity later in life. Taken together, these findings led us to conclude that microbial dysbiosis leads to transgenerational effects, including cancer, obesity, and accelerated aging. Knowing NWD-associated obesity and cancer are preventable by dietary enrichment with beneficial bacteria

(4, 7), we hypothesized that oral microbe therapy may similarly reduce transgenerational cancer burdens.

Early life exposure to beneficial microbes inhibits transgenerational cancer

Finally, to further test our microbe-centric hypothesis involving detrimental NWD-induced changes in gastrointestinal tract

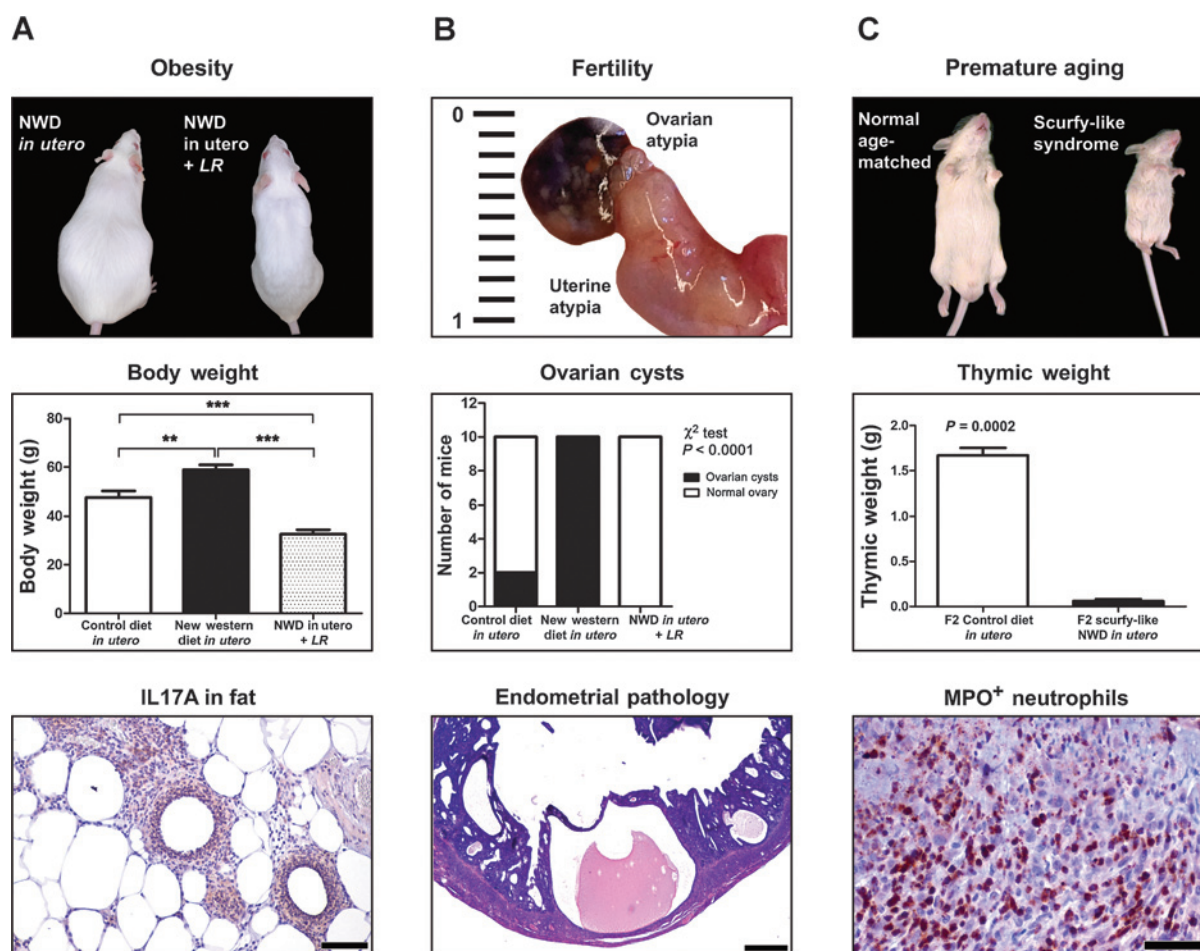


Figure 3.

Nonneoplastic pathologies observed in the offspring of CD-1 mice consuming NWD chow during pregnancy. A, side-by-side comparison of F1 mice derived from mothers consuming NWD during pregnancy after enrichment of their gut flora with *L. reuteri* (LR-treated lean mouse on right) or not (obese mouse on the left). Edible *L. reuteri* bacteria negate the transgenerational NWD-induced effect, otherwise leading to significantly increased body weight. Transgenerational obesity is characterized by typical adipose tissue pathology, including IL17-rich focal pyogranulomatous inflammatory lesions in the ependymal fat. B, markedly enlarged uterine horn with a cystically dilated ovary. The presence of ovarian cysts was a consistent finding in mice exposed to NWD *in utero* but not in those offspring of control diet-fed mice. Enlarged uteri showed cystic endometrial hyperplasia. Note markedly dilated endometrial glands. C, eight-week-old F2 progeny of NWD-diet fed grandmother mice exhibited a scurvy-like syndrome with smaller stature, sparse dull fur, and thymic atrophy. Thymic weight was significantly reduced when compared with age-matched controls. Arteritis in a mesenteric vessel involved high numbers of MPO-positive neutrophils infiltrating the vessel wall and local tissues. Hematoxylin and eosin (B, bottom). IHC; diaminobenzidine chromogen, hematoxylin counterstain (A and C, bottom). Scale bars, 500 μ m (B, bottom); 100 μ m (A, bottom); 50 μ m (C, bottom). Numbers on the y axis of bar graphs correspond to the mean \pm SEM of the parameters assessed. *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$.

microbe communities, we applied a dietary enrichment strategy using beneficial bacteria originally isolated from human breast milk. Experiment 4 used F1 progeny of NWD-fed mothers (Fig. 1D), such that upon weaning at 3 weeks of age, experimental mice consumed a model microbial organism *L. reuteri* continuously in their drinking water. Control mice consumed regular water throughout the study. Upon later examination at 1 year of age, F1 children consuming *L. reuteri* had significantly lower risk for obesity (Fig. 3A) and cancer than did their untreated control counterparts (Table 1; Supplementary Fig. S3). It is promising but unknown to what extent other microbe cocktails or life stage interventions may be effective, although oral administration of purified *L. rhamnosus* was previously shown to suppress obesity in human adults (19). Mouse models and diets do not precisely mimic human conditions; however, these findings

further supported a gut microbe immune-centric hypothesis and the possibility that diet-associated bacteria may be engineered to mitigate emerging inflammatory disease epidemic in Westernized societies.

Discussion

In summary, we show that *in utero* effects of gut microbes may transcend generations. We discover that mother mice consuming a Westernized diet during pregnancy convey to their children and grandchildren detrimental long-term consequences, including high risk of obesity and cancer. In humans, there is precedent for maternal dietary folate (vitamin B9) deficiency leading to leukemia in offspring (20); although, the cancer outcome in the present study was reproducible using

microbes alone, and mothers of the most severely affected F2 progeny were actually eating a nutritionally balanced control AIN76A diet during pregnancy. Finding polycystic ovaries, endometrial disease, and infertility in F1 female mice implicates sex hormones together with immune dysregulation in transgenerational effects; however, this remains to be explicitly tested. Importantly, fecal microbiome transplant was sufficient for the transgenerational cancer effect. Grandchildren were most severely affected, exhibiting a premature aging scurfy-like syndrome and high rates of cancer at a young age, when their grandmother had consumed Western diet during her pregnancy. The scurfy-like syndrome suggests immune T regulatory (Treg) cell insufficiency (16) previously implicated in obesity and cancer (4–7). Taken together, our data suggest that dietary stress leads to gut microbe dysbiosis that elevates the risk of heritable cancers. Intestinal bacteria were sufficient to remedy the transgenerational effect, pointing to novel microbe-based personal or public health preventative medicine strategies. Intestinal bacteria were sufficient to remedy the transgenerational effect observed in mice, and further studies of novel microbe-based mechanisms in the role of human cancer and obesity prevention are warranted.

In conclusion, it is now clear that profound changes in our environment have contributed to a societal health crisis. Our previous data have built upon the perinatal microbe-driven concept to include neoplastic diseases (3, 5). Our current data expand this microbe-centric view further to include mammalian progeny and suggest that the microbial flora of a mother animal affects the predisposition of her progeny to cancer. We have shown here that a key mediator of diet-induced transgenerational disease predisposition is the gut microbial flora. Consequently, the effect was reversible by enriching the progeny gut flora with beneficial bacteria. Another well-substantiated transgenerational hypothesis suggests that westernized dietary habits during pregnancy shape disease susceptibility profiles of her descendants via epigenomics (21–23). It remains unproven that maternal microbes contribute to epigenetic changes upon their unborn progeny, and how results from an animal model in a highly controlled environment

translate into free-living human populations. Our data also suggest that the dietary indiscretions of earlier generations may be reversible by targeting more healthful gut microbes early in life. This suggests new possibilities for decreasing the risk of cancer, obesity, and other pathologies that appear more prevalent in modern societies, using targeted gut flora enrichment strategies. This suggests new possibilities for decreasing the risk of cancer, obesity, and other pathologies, using targeted gut flora enrichment strategies that remain to be validated in humans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Poutahidis, B.J. Varian, S. Mirabal, S.M. Kearney, E.J. Alm, S.E. Erdman

Development of methodology: B.J. Varian, S. Mirabal, S.E. Erdman
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Poutahidis, T. Levkovich, S. Mirabal, Y.M. Ibrahim, S.E. Erdman

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Poutahidis, C. Kwok, S.E. Erdman

Writing, review, and/or revision of the manuscript: T. Poutahidis, B.J. Varian, S.M. Kearney, S.E. Erdman

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Poutahidis, T. Levkovich, J.R. Lakritz, S.M. Kearney, A. Chatzigiagos, S.E. Erdman

Study supervision: S.E. Erdman

Other (performed the experiments): C. Kwok

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