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High-fat Diet Enhances Mammary Tumorigenesis and Pulmonary Metastasis and Alters Inflammatory and Angiogenic Profiles in MMTV-PyMT Mice

SNEHA SUNDARAM and LIN YAN

U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND, U.S.A.

Abstract. *The MMTV-PyMT transgenic mouse model is commonly used to study luminal B subtype, which has a lower prevalence but a worse prognosis than luminal A subtype among patients with breast cancer. The objective of the present study was to determine whether an obesogenic, high-fat diet enhances primary tumorigenesis and pulmonary metastasis in female MMTV-PyMT mice. The high-fat diet slightly but significantly increased caloric intake and body fat mass compared to the AIN93G diet. The high-fat diet significantly increased primary mammary tumor progression by 59%, primary tumor weight by 60%, and the number of lung metastases by 147%. Compared to the AIN93G diet, the high-fat diet significantly increased the abundance of proinflammatory cytokines (e.g. leptin, monocyte chemotactic protein-1, plasminogen activator inhibitor-1, resistin, and tumor necrosis factor-α) and angiogenic factors (e.g. hepatocyte growth factor, tissue inhibitor of metalloproteinase inhibitor-1, and vascular endothelial growth factor) in plasma and mammary tumors. We conclude that the obesogenic high-fat diet enhances primary tumorigenesis and metastasis in MMTV-PyMT mice. This enhancement may be the result of increased proinflammation and angiogenesis signaling.*

Breast cancer is a heterogeneous disease with several intrinsic subtypes including luminal B breast cancer (1). Luminal B breast cancer is characterized by the expression of estrogen and progesterone receptors and overexpression of human epidermal growth factor receptor 2 and Ki67 (a marker of cell proliferation) (2). Luminal B breast cancer accounts for only 12.4% of all invasive breast cancers (3). However, it is more aggressive and of a higher grade with a worse prognosis than luminal A breast cancer (4).

Epidemiological studies provide strong evidence that obesity and its associated adipose inflammation are risk factors for breast cancer, including the luminal B subtype (5, 6). Being obese at the time of diagnosis of breast cancer can be predictive of poor prognosis (7, 8); luminal B breast cancer is more frequent in obese than in non-obese premenopausal women (9). Weight gain after age 18 years is also strongly associated with the luminal B subtype (10). Furthermore, obese postmenopausal women are at a greater risk of developing luminal B breast cancer (9, 11).

The MMTV-PyMT mouse model is commonly used to study luminal B breast cancer. The mouse mammary tumor virus (MMTV) long terminal repeat drives the mammary gland-specific expression of the polyoma virus middle T antigen (PyMT) and transforms the mammary epithelia, which results in mammary gland tumors (12). The mammary tumorigenesis in this model is characterized by hyperplasia, adenoma, neoplasia, and carcinoma with high incidence of lung metastasis (12, 13). Classification and hierarchical clustering analyses suggest that the MMTV-PyMT mammary tumors are similar to those of the luminal subtype (14). Further genetic and marker analyses suggest that MMTV-PyMT tumors exhibit the luminal B tumor signature, including short latency and high penetrance (14, 15).

Studies using animal models of breast cancer support findings from human studies; obesogenic high-fat diets enhance mammary tumorigenesis in animals (16, 17). Adipose tissue is considered an endocrine organ that produces adipokines (proinflammatory cytokines) which contribute to high-fat diet-enhanced malignant progression (18, 19). We hypothesized that obesity-enhanced progression of luminal B breast cancer is through the up-regulation of inflammation. The present study tested this hypothesis by assessing the effects of a high-fat diet on primary mammary

Correspondence to: Lin Yan, USDA, ARS, Grand Forks Human Nutrition Research Center, 2420 2nd Avenue North, Grand Forks, ND, 58202, U.S.A. Tel: +1 7017958499, Fax: +1 7017958220, e-mail: lin.yan@ars.usda.gov

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tumorigenesis and pulmonary metastasis, and alterations in inflammatory and angiogenic profiles in the MMTV-PyMT mouse model.

Materials and Methods

Animals and diets. Hemizygous female MMTV-PyMT mice were obtained from the in-house breeding colony at Grand Forks Human Nutrition Research Center. The colony was established with FVB/NJ females (stock no: 001800) and hemizygous FVB/N-Tg(MMTV-PyVT)634Mul/J males (stock no: 002374; The Jackson Laboratory, Bar Harbor, ME, USA). Mice were maintained in a pathogen-free room on a 12:12-hour light/dark cycle with a temperature of 22±1˚C. Two diets (prepared by the Animal Diet Kitchen at Grand Forks Human Nutrition Research Center) were compared; the AIN93G diet (20) and a modified AIN93G diet, providing 16% and 45% (high-fat diet) of energy from soybean oil, respectively (Table I). Both diets were powder diets; they were stored at −20˚C until feeding. Gross energy of each diet (Table I) was analyzed by using oxygen bomb calorimetry (Model 6200; Oxygen Bomb Calorimeter, Parr Instrument, Moline, IL, USA).

Experimental design. This study (Y27) was approved by the Institutional Animal Care and Use Committee of the U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center. The procedures followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (21).

Three-week-old female MMTV-PyMT mice were randomly assigned and weaned onto their respective diets (n=33 per group). Mice had free access to their diets and deionized water and were housed two per cage to avoid stress related to single housing. Food intake was recorded daily (5 days per week) for 3 consecutive weeks starting 1 week after the initiation of experimental feeding, and mice were weighed weekly. The duration of experimental feeding was 8 weeks. Body composition of conscious, immobilized mice was performed 1 week before termination by using an EchoMRI whole-body composition analyzer (Model 100; Echo Medical Systems, Houston, TX, USA).

Primary mammary tumorigenesis. Mice were palpated for mammary tumors twice weekly. Tumor latency was defined as the age at which the first palpable mammary tumor was detected (22). Palpable tumors were measured weekly by using a digital caliper (Fred V Fowler Company, Newton, MA, USA). Tumor volume was calculated using the formula: length × width^2 × 0.5. Tumor progression as the percentage change in volume over time was calculated using the formula: (end volume – start volume)/start volume ×100 (22).

Lung metastasis. Four weeks after detection of the first palpable mammary tumor, mice were euthanized by an intraperitoneal injection of a mixture of ketamine and xylazine. Lungs were harvested and fixed with Bouin's solution. The number of pulmonary metastases was counted (23) and the cross-sectional area and the volume of each metastasis were analyzed (24) by using ImagePro-Plus software- (Media Cybernetics, Silver Spring, MD, USA) and camera-equipped stereomicroscope. The cross-sectional area was defined as the surface area of each lung metastasis. The volume was estimated using the average diameter of the metastasis with the assumption that metastases were spherical (24). Plasma and mammary tumors were collected and stored at −80˚C for further analyses.

Adipokine and angiogenesis profiles. The abundance of adipokines and angiogenic factors in plasma and primary mammary tumors were analyzed by using Proteome Profiler™ Mouse Adipokine Array (ARY013) and Mouse Angiogenesis Array (ARY015; R&D Systems, Minneapolis, MN, USA) following the manufacturer's protocols. Primary tumors frozen in liquid nitrogen were pulverized and extracted in radio-immunoprecipitation assay buffer with protease and phosphatase inhibitors (25). Each array was repeated three times with plasma or mammary tumors pooled from four different mice per repetition. Fourteen molecules were included in both arrays; results were reported accordingly in their respective profiles in Table II and Table III. These molecules were dipeptidyl peptidase-4 (DPPIV), fibroblast growth factor-acidic (FGF-acidic), FGF-basic, hepatocyte growth factor (HGF), insulin-like growth factor-binding protein-1 (IGFBP1), IGFBP2, IGFBP3, interleukin-10 (IL10), leptin, monocyte chemotactic protein-1 (MCP1), pentraxin, plasminogen activator inhibitor-1 (PAI1), tissue inhibitor of metalloproteinase-1 (TIMP1), and vascular endothelial growth factor (VEGF). The array blots were analyzed by using BioSpectrum 500 Imaging System with LM-26 and BioChemi 500 camera (UVP, Upland, CA, USA), and area densities were measured using VisionWorks®LS Image Acquisition and Analysis Software (Version 8.13; UVP). Results are presented as fold changes in densitometry relative to array background.

Statistical analyses. Student *t*-test was used to compare differences between the groups. Kaplan–Meier analyses were conducted to estimate tumor latency; log-rank and Chi-square tests were used to compare differences between the groups. All data are presented as means±standard error of the mean (SEM). Differences with a *p*-value of 0.05 or less were considered significant. All analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC, USA).

Results

Body weight, body composition and caloric intake. The high-fat diet increased body weight compared to the AIN93G diet (Figure 1A). The difference was significant starting 3 weeks after initiation of the high-fat diet feeding ($p<0.05$); the increase continued throughout the experiment (Figure 1A). The high-fat diet increased the percentage body fat mass by 17% compared to the AIN93G diet (Figure 1B). There was no difference in the percentage body lean mass between groups (Figure 1C). The absolute lean mass of mice fed the high-fat diet was 16% higher than that of mice fed the AIN93G diet (Figure 1D). Caloric intake of mice fed the high-fat diet was 5% higher than that of mice fed the AIN93G diet (Figure 1E).

Primary mammary tumorigenesis. All mice, regardless of dietary treatments, developed mammary tumors. Feeding mice the high-fat diet increased the latency of mammary tumor; the median latency was 6.0 weeks and 6.1 weeks for

Table I. *Composition of experimental diets.*

Ingredient	AIN93G g/kg	High-fat g/kg
Corn starch	397.5	42.5
Casein	200	239.4
Dextrin	132	239.4
Sucrose	100	119.7
Soybean oil	70	239.4
Cellulose	50	59.8
AIN93 mineral mix	35	41.9
AIN93 vitamin mix	10	12
L-Cystine	3	3.6
Choline bitartrate	2.5	3
t-Butylhydroquinone	0.014	0.017
Total	1,000	1,000
Energy	%	%
Protein	20	20
Fat	16	45
Carbohydrate	64	35
Analyzed gross energy kcal/g[a]	4.3±0.1	5.2±0.1

[a]Values are means±SEM of three samples analyzed from each diet.

the AIN93G and the high-fat groups, respectively (hazard ratio=1.88, 95% confidence interval=1.40-3.85, $p<0.01$; Figure 2A). Tumor progression was defined as the change in tumor volume from detection of the first palpable mammary tumor to termination 4 weeks later. The high-fat diet increased tumor progression by 59% (Figure 2B) and primary tumor weight by 60% (Figure 2C) compared to the AIN93G diet.

Lung metastasis. There was no significant difference in the incidence of lung metastasis between mice fed the AIN93G diet (72.7%) and those fed the high-fat diet (75.7%). The high-fat diet significantly increased the number of metastases by 150% compared to the AIN93G diet (Figure 2D). There were no differences in metastatic cross-sectional area (data not shown) and volume between the groups (Figure 2E). Gross necropsy at termination found no metastatic lesions in other organs.

Adipokine and angiogenesis profiles. Consumption of the high-fat diet altered adipokine and angiogenesis profiles in plasma and primary mammary tumors. The adipokine arrays showed that the high-fat diet compared to the AIN93G diet significantly increased expression of 9 out of the 38 adipokine-related proteins analyzed in plasma and mammary tumors, respectively. These included IGFBP6 in plasma and C-C motif chemokine ligand 5 in tumors, and HGF, leptin,

Table II. *Adipokine profile (fold-change in densitometry relative to array background) in plasma and mammary tumor of MMTV-PyMT mice fed the AIN93G or the high-fat diet. Mice were weaned onto diets at 3 weeks old and continued on diets until termination 4 weeks following tumor detection (the duration of experimental feeding was 8 weeks). Values are means±SEM (n=3).*

	Plasma		Mammary tumor	
Adipokine	AIN93G	High-fat	AIN93G	High-fat
Adiponectin	9.7±1.0	6.1±0.6*	40.1±2.5	25.7±2.1*
AgRP	1.3±0.01	1.1±0.01*	1.5±0.1	1.6±0.2
ANGPTL3	5.2±0.7	5.1±1.0	1.6±0.04	1.7±0.1
C-Reactive protein	6.8±0.5	8.5±1.0	21.5±0.5	13.5±0.5*
DPPIV	9.2±1.3	12.7±1.4	8.4±1.0	5.4±0.5
Endocan	2.9±1.0	4.3±1.5	3.5±0.6	5.2±0.3
FetuinA	1.6±0.1	1.9±0.2	5.5±0.6	3.5±0.6
FGF-acidic	1.0±0.02	1.0±0.01	5.5±0.5	5.3±0.9
FGF21	5.6±3.0	5.6±2.8	1.2±0.1	1.4±0.1
HGF	1.0±0.01	1.8±0.1*	1.3±0.1	1.6±0.1*
ICAM1	2.6±0.5	3.7±0.7	7.3±0.4	4.7±0.4*
IGF1	1.6±0.2	2.5±0.2	1.5±0.01	1.3±0.1
IGF2	1.2±0.01	1.3±0.1	2.3±0.04	1.7±0.1*
IGFBP1	12.8±3.6	19.5±5.6	4.8±1.1	7.8±0.9
IGFBP2	9.3±1.0	10.2±1.2	9.6±1.6	9.6±0.9
IGFBP3	11.8±1.6	14.7±1.6	17.2±1.0	12.4±1.1*
IGFBP5	5.4±0.4	6.3±0.7	7.0±0.4	5.0±0.4*
IGFBP6	14.4±0.8	18.8±1.1*	10.5±1.1	6.2±0.6*
IL6	1.0±0.02	1.0±0.02	1.2±0.1	1.4±0.1
IL10	1.0±0.03	1.0±0.01	1.2±0.1	1.4±0.1
IL11	1.1±0.10	1.1±0.1	1.4±0.1	1.4±0.1
Leptin	1.2±0.04	1.9±0.1*	1.5±0.04	2.9±0.1*
LIF	1.0±0.06	1.0±0.04	1.9±0.2	2.5±0.1
Lipcalin2	2.9±0.53	4.0±0.5	2.2±0.2	1.5±0.1
MCP1	1.1±0.04	2.4±0.2*	2.8±0.6	5.4±0.3*
M-CSF	4.3±0.5	5.6±0.8	8.7±0.5	5.5±0.3
OncostatinM	1.1±0.02	1.1±0.02	1.4±0.1	1.5±0.1
Pentraxin 2	2.3±0.1	2.5±0.1	2.7±0.2	2.4±0.2
Pentraxin 3	2.2±0.7	3.0±1.3	5.4±0.5	3.3±0.2*
PREF1	1.4±0.2	1.7±0.4	1.5±0.03	1.3±0.1
RAGE	1.2±0.1	1.1±0.1	1.2±0.05	1.3±0.1
RANTES	1.0±0.04	1.1±0.1	1.5±0.1	2.2±0.1*
RBP4	1.3±0.10	1.5±0.2	3.9±0.2	3.1±0.2
Resistin	25.2±2.8	41.3±4.0*	22.6±3.7	35.5±1.2*
PAI1	2.1±0.10	4.3±0.7*	11.1±2.2	23.4±1.0*
TIMP1	1.4±0.3	3.2±0.5*	4.2±1.2	10.5±0.5*
TNFα	1.0±0.1	1.4±0.02*	1.1±0.03	1.5±0.1*
VEGF	1.2±0.1	1.6±0.1*	4.2±1.3	9.2±0.9*

*Significantly different at $p<0.05$ compared to the AIN93G diet. AgRP: Agouti-related protein; ANGPTL3: angiopoietin-like 3; DPPIV: dipeptidyl peptidase-4; FGF: fibroblast growth factor; HGF: hepatocyte growth factor; ICAM: intercellular adhesion molecule; IGF: insulin-like growth factor; IGFBP: insulin-like growth factor-binding protein; IL: interleukin; LIF: leukemia-inhibiting factor; MCP1: monocyte chemotactic protein-1; M-CSF: macrophage colony-stimulating factor; PREF1: preadipocyte factor-1; RAGE: receptor for advanced glycation end-products; RANTES: C-C motif chemokine ligand 5 or regulated on activation, normal T-cell expressed and secreted; RBP: retinol-binding protein; PAI1: plasminogen activator inhibitor-1; TIMP: tissue inhibitor of metalloproteinases; TNFα: tumor necrosis factor-α; VEGF: vascular endothelial growth factor.

Table III. *Angiogenesis profile (fold-change in densitometry relative to array background) in plasma and mammary tumor of MMTV-PyMT mice fed the AIN93G or the high-fat diet. Mice were weaned onto diets at 3 weeks old and continued on diets until termination 4 weeks following tumor detection (the duration of experimental feeding was 8 weeks). Values are means±SEM (n=3).*

	Plasma		Mammary tumor	
Angiogenic molecule	AIN93G	High-fat	AIN93G	High-fat
ADAMTS1	1.2±0.2	2.3±0.4*	2.3±0.1	3.0±0.1*
Amphiregulin	1.7±0.2	1.3±0.1*	2.6±0.2	2.5±0.3
Angiogenin	5.7±1.2	4.9±1.2	3.4±0.4	3.7±0.4
Angiopoietin 1	5.7±2.0	10.9±4.1	13.4±1.3	20.0±1.7
Angiopoietin 3	1.8±0.2	1.8±0.3	1.6±0.01	1.8±0.1*
Coagulation factor III	1.6±0.2	2.4±0.3	10.6±1.6	8.0±0.6
CXCL16	10.4±2.9	12.9±3.2	5.3±0.8	7.0±0.7
Cyr61	3.8±1.0	6.4±1.1	9.4±0.8	11.6±0.8
DLL4	1.7±0.2	2.06±0.4	2.2±0.2	2.7±0.2
DPPIV	11.0±1.7	10.8±1.5	7.2±1.4	7.3±1.2
EGF	1.6±0.1	1.2±0.05*	26.0±5.0	7.3±1.9*
Endoglin	2.0±0.3	3.2±0.03*	3.6±0.5	4.0±0.3
Endostatin/collagen XVIII	12.3±0.7	13.5±0.6	10.6±1.6	7.7±0.9
Endothelin1	2.5±0.3	2.1±0.4	2.7±0.3	3.1±0.1
FGF-acidic	3.1±0.2	2.2±0.3*	4.3±0.9	4.7±0.7
FGF-basic	2.0±0.3	2.0±0.3	3.2±0.1	2.6±0.2*
KGF	2.0±0.4	1.6±0.3	1.5±0.1	1.8±0.1
Fractalkine	4.7±1.4	3.9±1.1	4.0±0.2	5.7±0.5*
GM-CSF	1.9±0.3	1.4±0.1	1.4±0.2	1.7±0.2
HB-EGF	1.5±0.2	1.3±0.1	2.3±0.4	1.9±0.2
HGF	1.8±0.3	4.1±0.3*	3.3±0.2	4.4±0.3*
IGFBP1	24.0±1.4	33.2±1.5*	4.0±0.4	8.0±1.3
IGFBP2	24.4±1.0	16.5±0.3*	13.1±2.1	15.7±2.2
IGFBP3	26.9±3.1	23.8±1.2	15.7±2.3	20.2±2.0
IL1α	1.4±0.1	1.2±0.03*	2.0±0.1	3.3±0.2*
IL1B	1.8±0.2	1.6±0.2	1.2±0.01	1.7±0.1*
IL10	1.9±0.4	1.6±0.2	1.6±0.04	1.6±0.02
IP10	1.8±0.3	1.6±0.3	1.5±0.09	1.6±0.03
KC	2.3±0.5	2.0±0.4	6.0±0.4	10.0±0.7*
Leptin	1.9±0.3	3.6±0.4*	1.6±0.1	2.4±0.2*
MCP1	1.8±0.3	3.2±0.2*	4.8±0.2	7.9±0.7*
MIP1a	2.9±0.4	2.2±0.3	1.8±0.1	2.1±0.2
MMP3	29.0±2.4	38.5±2.1*	17.2±1.5	12.5±1.0
MMP8	5.2±1.6	6.0±1.6	1.6±0.1	1.9±0.1
MMP9	12.5±2.5	16.6±3.4	8.0±1.5	11.6±1.8
NOV	28.1±1.8	34.9±2.1*	5.3±0.5	9.0±0.8*
Osteopontin	11.4±1.0	8.0±0.4*	2.9±0.5	2.6±0.2
PD-ECGF	1.5±0.2	1.5±0.2	1.5±0.1	1.7±0.2
PDGFAA	2.0±0.4	2.9±0.9	4.0±0.3	5.1±0.2*
PDGFAB/BB	2.4±0.7	2.9±0.9	2.6±0.1	3.0±0.2
Pentraxin	5.3±1.1	5.7±1.5	5.5±0.7	6.7±0.6
Platelet factor4	23.8±0.2	18.4±1.3*	12.0±1.2	14.1±1.5
PLGF2	5.2±1.4	7.2±1.5	20.5±2.7	24.9±2.6
Prolactin	5.8±1.4	5.1±1.4	1.5±0.1	1.7±0.1
Proliferin	1.9±0.3	2.3±0.4	1.5±0.1	1.8±0.1
SDF1	13.3±3.8	19.3±5.2	3.5±0.4	4.6±0.4
PAI1	13.3±3.4	26.4±4.1*	22.0±2.7	31.2±0.9*
SerpinF1	6.3±1.8	8.0±2.3	11.7±2.1	14.3±1.6
Thrombospondin 2	2.1±0.4	4.2±0.7*	4.4±0.2	6.0±0.1*
TIMP1	2.1±0.4	3.9±0.6*	2.7±0.1	3.6±0.1*
TIMP4	6.4±2.0	5.4±1.7	1.4±0.1	1.5±0.03
VEGF	1.2±0.1	2.5±0.2*	1.2±0.1	1.7±0.1*
VEGFB	1.8±0.2	1.6±0.3	1.8±0.1	2.0±0.1

*Significantly different at $p<0.05$ compared to the AIN93G diet. ADAMTS1: A disintegrin and metalloproteinase with thrombospondin motifs 1; CXCL16: C-X-C motif chemokine ligand-16; Cyr61: cysteine-rich angiogenic inducer-61; DLL4: delta-like ligand-4; DPPIV: dipeptidyl peptidase-4; EGF: epidermal growth factor; FGF: fibroblast growth factor; KGF: keratinocyte growth factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; HB-EGF: heparin-binding EGF-like growth factor; HGF: hepatocyte growth factor; IGFBP: insulin-like growth factor-binding protein; IL: interleukin; IP: interferon gamma-induced protein; KC: keratinocyte-derived CXC motif; MCP1: monocyte chemotactic protein 1; MIP: macrophage inflammatory protein; MMP: matrix metalloproteinase; NOV: nephroblastoma overexpressed; PD-ECGF: platelet-derived endothelial cell growth factor; PDGF: platelet-derived growth factor; PLGF2: placental growth factor-2; SDF1: stromal cell-derived factor-1; PAI1: plasminogen activator inhibitor-1; serpinF1: Pigment epithelium-derived factor; TIMP: tissue inhibitor of metalloproteinases; VEGF: vascular endothelial growth factor.

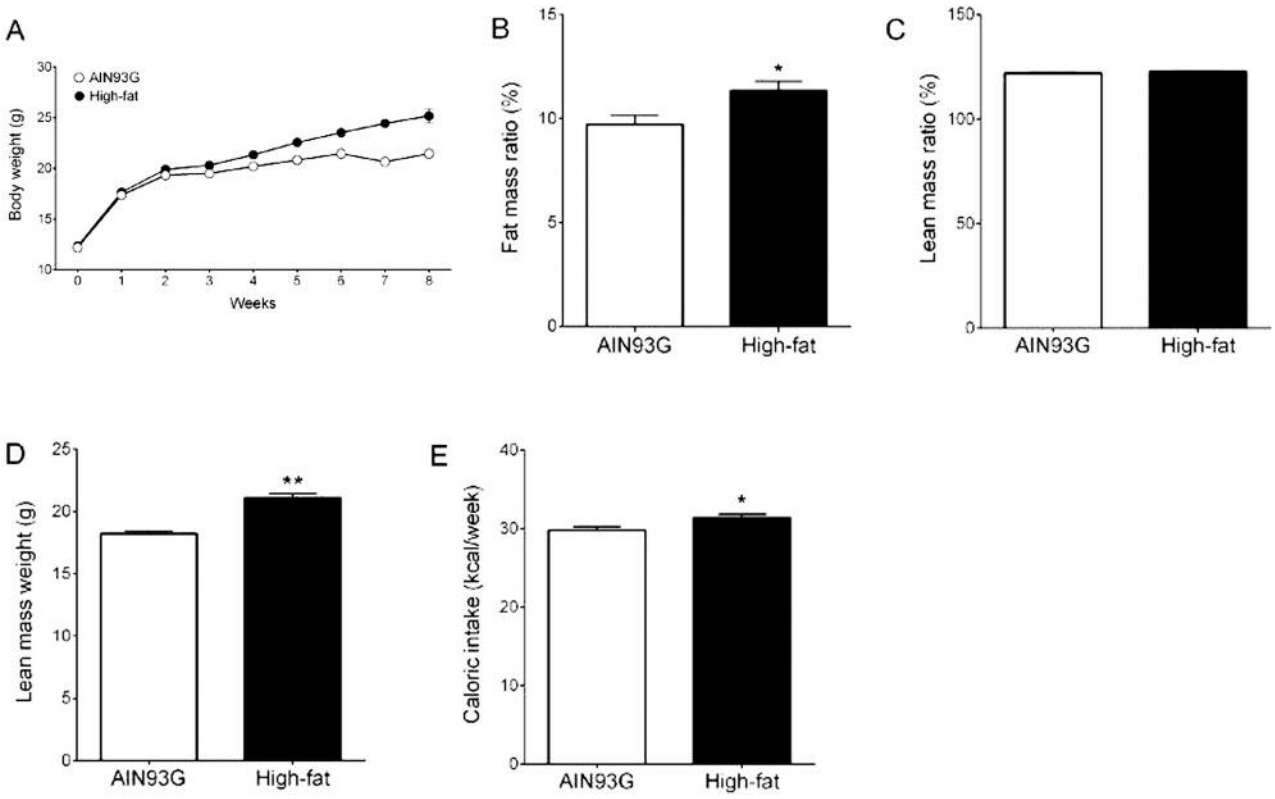

Figure 1. *Body weight (A), body fat mass ratio (B), lean mass ratio (C), lean mass weight (D), and caloric intake (E) of MMTV-PyMT mice fed the AIN93G or the high-fat diet. Mice fed the high-fat diet were heavier than those fed the AIN93G diet, the difference being significant from 3 weeks after the initiation of experimental feeding ($p<0.05$); the significant increase continued throughout the experiment. The measurement unit of caloric intake was from two mice per cage. Values are means±SEM (n=33 per group for A, B, C, D; n=6 per group for E). Significantly different compared to the AIN93G diet at *$p<0.05$, and **$p<0.01$.*

MCP1, resistin, PAI1, TIMP1, tumor necrosis factor-α (TNFα), and VEGF in both plasma and tumors (Table II). The high-fat diet significantly reduced adiponectin and agouti-related protein in plasma and 8 out of the 38 adipokine-related proteins assayed in tumors [adiponectin, C-reactive protein, intercellular adhesion molecule-1, insulin-like growth factor-II (IGF-II), IGFBP3, IGFBP5, IGFBP6, and pentraxin-3] (Table II). The angiogenesis arrays showed that out of the 53 angiogenesis-related proteins analyzed the high-fat diet significantly increased expression of 12 and 15 proteins in plasma and mammary tumors, respectively. These included endoglin, IGFBP1, and matrix metalloproteinase-3 in plasma and angiopoietin-3, fractalkine, IL1α, IL1β, keratinocyte-derived chemokine, and platelet derived growth factor-AA in tumors, and a disintegrin and metallopeptidase with thrombospondin type 1 motif 1, HGF, leptin, MCP1, nephroblastoma overexpressed, PAI1, thorombospondin-2, TIMP1, and VEGF in both plasma and tumors (Table III). The high-fat diet significantly reduced seven out of the 53 angiogenesis-related proteins assayed in plasma [amphiregulin, epidermal growth factor (EGF), FGF-acidic, IGFBP2, IL1α, osteopontin, and platelet factor-4] and two in tumors (EGF and FGF-basic) (Table III).

More importantly, results of the adipokine arrays showed that the high-fat diet compared to the AIN93G diet increased plasma abundance of HGF by 83%, leptin by 61%, MCP1 by 115%, resistin by 64%, PAI1 by 107%, TIMP1 by 138%, TNFα by 31%, and VEGF by 31%, and reduced plasma adiponectin by 37% (Table II). The mammary tumor analysis exhibited similar findings (Table II). Similar results were obtained from the angiogenesis arrays (Table III).

Discussion

The present study showed that an obesogenic high-fat diet increases the growth of primary mammary tumors and the number of metastases formed in the lungs. This indicates

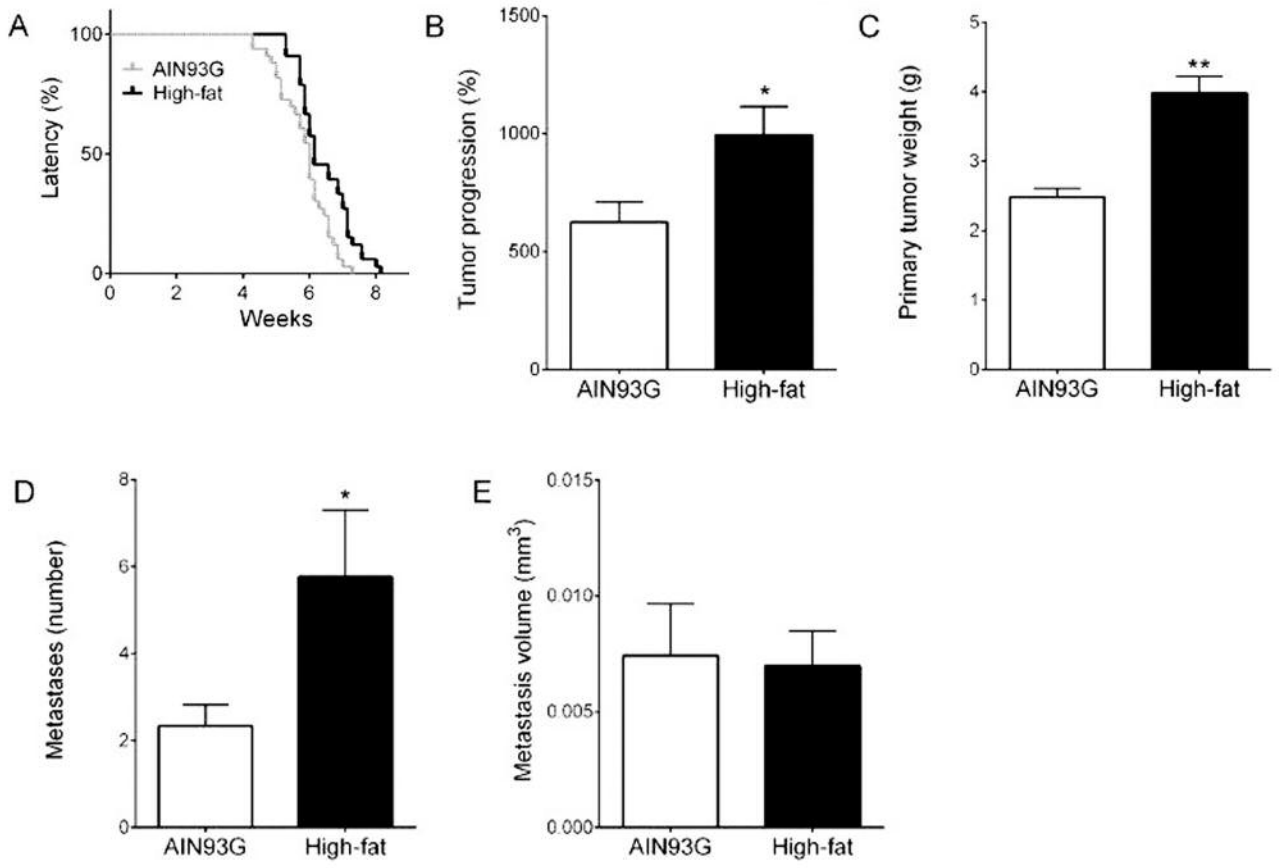

Figure 2. *Tumor latency (A), tumor progression (B), and weight (C) of primary mammary tumors, and number (D) and volume (E) of lung metastases in MMTV-PyMT mice fed the AIN93G or the high-fat diet. The median latency was 6.0 and 6.1 weeks for the AIN93G and the high-fat diet-fed groups, respectively (hazard ratio=1.88, 95% confidence interval=1.40-3.85, p<0.01) (A). Values are means±SEM (n=33 per group). Significantly different compared to the AIN93G diet at *p<0.05, and **p<0.01.*

that an obesogenic diet enhances malignant progression in this MMTV-PyMT mouse model of luminal B breast cancer.

Proinflammatory cytokines, which can be produced in increased amounts under pathophysiological conditions, participate in cancer development and progression. Our findings of a significant increase in the abundance of proinflammatory cytokines, including MCP1, PAI1, TNFα, and resistin, in plasma and mammary tumors by the high-fat diet indicate that increased proinflammatory cytokines exacerbate mammary tumorigenesis and pulmonary metastasis in MMTV-PyMT mice. Similarly, previous studies showed that proinflammatory cytokines, *e.g.* MCP1 and PAI1, are found in plasma and mammary tumors from MMTV-PyMT mice (26-28). Expression of proinflammatory cytokines MCP1 (26), PAI1 (29, 30), TNFα (31), and resistin (32) are elevated in invasive breast cancer. In luminal B breast cancer, increased the expression of these cytokines is associated with poor prognosis and lower overall survival (26, 33, 34). Findings from the present study are further supported by our previous reports that MCP1 (18) and PAI1 (19) contribute to enhanced spontaneous metastasis of Lewis lung carcinoma in mice fed a high-fat diet.

Angiogenesis plays important roles in tumorigenesis and in transporting malignant cells to distant organs to form metastases. The high-fat diet significantly increased the content of potent angiogenic factors including HGF, VEGF, and TIMP1 in plasma and primary tumors, indicating an up-regulation of angiogenesis. Overexpression of HGF in mammary glands resulted in highly aggressive tumors with lung metastases (35). Elevated expressions of HGF (36), VEGF (37), and TIMP1 (38) in invasive breast cancer contribute to poor survival outcomes in breast cancer patients. In MMTV-PyMT mice, overexpression of VEGF leads to increased metastasis (39), the increase being through VEGF-

enhanced angiogenesis and sustained tumor proliferation and survival (39). The expression of *TIMP1* mRNA is significantly increased in malignant breast tissues (40), and the expression increases gradually with the invasive potential of carcinoma cells (38). Thus, our findings are consistent with existing knowledge of the roles of angiogenic factors in mammary tumorigenesis. This suggests that the up-regulation of angiogenesis by the high-fat diet may contribute to exacerbated tumorigenesis and metastasis in the MMTV-PyMT model.

Increased leptin and lower adiponectin concentration in plasma and mammary tumors of mice fed the high-fat diet were likely the result of increased adiposity. Physiological functions of leptin and adiponectin are to regulate energy metabolism (41, 42). However, studies also show that they can participate in carcinogenesis. An elevated plasma concentration of leptin is associated with an increased risk of breast cancer with advanced lesions (43). Adiponectin is anti-inflammatory; low circulating adiponectin levels are associated with increased breast cancer risk (44, 45). In MMTV-PyMT mice, inhibition of leptin signaling by depletion of leptin receptors inhibits tumor progression and metastasis (46), and adiponectin insufficiency facilitates mammary tumorigenesis (47). The dichotomy of leptin and adiponectin effects contribute to the pathophysiology of breast cancer (48). Thus, our results indicate that a high-fat diet alters energy metabolism such that it contributes to the exacerbated tumorigenesis and metastasis exhibited by MMTV-PyMT mice.

Our results that the high-fat diet prolonged primary tumor latency do not agree with reports that a high-fat diet shortens or does not affect primary tumor latency (28, 49). This disagreement may be caused by experimental differences, *e.g.* animal models used (49), or methods to assess the latency (28). Nevertheless, the prolonged latency did not attenuate the tumorigenic enhancement by the high-fat diet, evidenced by the increased primary tumor burden and lung metastases.

In summary, our findings show that an obesogenic diet increases primary mammary tumorigenesis and lung metastasis in MMTV-PyMT mice. The increased aggressiveness of mammary carcinogenesis by the high-fat diet may be the result of up-regulation of proinflammatory and angiogenic signaling. Lifestyle changes such as reducing excessive caloric intake or increasing energy expenditure reduce body adiposity and associated inflammation (50). The fact that these lifestyle changes may attenuate obesity-mediated breast cancer risk, including luminal B breast cancer, warrants further investigation.

Conflicts of Interest

The Authors declare that they have no conflict of interest in regards to this study.

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