Elevated GH/IGF-I promotes mammmary tumors in high-fat, but not low-fat, fed mice

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Introduction

Growth hormone (GH) is produced in the anterior pituitary gland and is released into the general circulation, serving to stimulate the production of insulin-like growth factor I (IGF-I) from a variety of tissues. Although many tissues, including the mammary gland, produce IGF-I, the liver is the primary source of IGF-I found in the circulation (1). It is clear that GH and IGF-I are required for normal mammary gland development (2). More controversial is the hypothesis that over-production of GH and/or IGF-I promotes mammmary gland tumor formation/progression (3). This hypothesis is based on some, but not all, epidemiologic studies showing circulating IGF-I and GH are positively associated with breast cancer risk (2,4–8). Also, more recent studies show that GH can induce chemoresistance and protect against apoptosis in estrogen-dependent and -independent neoplastic breast cancer cells lines (9,10). Animal studies support a role of GH and IGF-I in mammary tumorigenesis, as rodent models with development defects in GH and/or IGF-I production or signaling are resistant to genetic- and chemical-induced mammmary tumors (11,16).

However, the relative contribution of GH and/or IGF-I in promoting mammary tumorigenesis remains a subject of debate and is confounded by the fact that in many of the rodent models used to date, defects in GH/IGF-I signaling negatively impact mammmary gland development, which could in turn alter genetic- and chemical-induced mammmary tumor development and progression (2,11,15). Also, to our knowledge, no animal studies have been conducted to determine if elevations in endogenous GH promotes the formation of mammmary tumors. It is clinically relevant to understand the contribution of GH to mammmary tumor formation since GH levels decline with age and weight gain and medical strategies have been considered to raise GH to improve body composition and metabolic function. However, in elderly and obese individuals metabolic syndrome is prevalent, where hyperlipidemia, inflammation, insulin resistance and obesity increase breast cancer risk, elevating GH may serve to exacerbate cancer progression. To better understand the role GH/IGF-I plays in tumor formation, this study used unique mouse models to determine if reducing GH/IGF-I in adults protects against 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammmary tumor development, and if moderate elevations in endogenous GH/IGF-I alter DMBA-induced tumorigenesis in mice fed a standard-chow diet or in mice with altered metabolic function. We observed that adult-onset isolated GH-deficient mice, which also have reduced IGF-I levels, were less susceptible to DMBA-treatment. Specifically, fewer adult-onset isolated GH-deficient mice developed mammmary tumors compared with GH-replete controls. In contrast, chow-fed mice with elevated endogenous GH/IGF-I (HiGH mice) were not more susceptible to DMBA-treatment. However, high-fat-fed, HiGH mice showed reduced tumor latency and increased tumor incidence compared with diet-matched controls. These results further support a role of GH/IGF-I in regulating mammmary tumorigenesis but suggest the ultimate consequences of GH/IGF-I on breast tumor development are dependent on the diet and/or metabolic status.

Materials and methods

Animal models and DMBA treatment

This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Jesse Brown VA Medical Center. All mouse strains were in a C57Bl/6 background. All mice were housed under a 12-h light, 12-h dark cycle (6:00 a.m. lights on–6:00 p.m. lights off) at 22–24°C and provided standard rodent chow [CHOW, 3.1 kcal/g; fat, 17% kcal (from unspecified amounts of corn, soybean, wheat, fish and porcine fat); carbohydrate, 56% kcal; protein 27% kcal] Formulab Diet 5008, Purina Mills, Richmond, IN] unless otherwise indicated. The AOiGHD mouse model was developed and validated by our laboratory, as reported previously (22). Briefly, 8-week-old female mice heterozygous for the inducible diphertheria toxin receptor (iDTIR), with or without somatotrop-specified expression of the Cre-recombinase transgene (cCHoPGcre) Tg were infected with DT via osetostatic murine (6 mg/ for 7 days, sc). DT treatment selectively destroys GH-producing cells in iDTIR/Cre+ mice (AOiGHD), but not iDTIR-Cre- mice (controls). At 10 weeks of age AOiGHD (n = 25) and control (n = 25) mice were treated with DMBA in olive oil [500 mg/10 g body weight; DMBA, 7.12-dimethylbenz[a]anthracene; EB, end bud; ER, estrogen receptor; GH, growth hormone; GHR, GH receptor; HF, high-fat; iDTIR, inducible diphertheria toxin receptor; IGF-I, insulin-like growth factor I. 4These authors contributed equally to this work. 5AOiGHD, adult-onset isolated GH deficiency; BW, body weight; DMBA, 7.12-dimethylbenz[a]anthracene; EB, end bud; ER, estrogen receptor; GH, growth hormone; GHR, GH receptor; HF, high-fat; iDTIR, inducible diphertheria toxin receptor; IGF-I, insulin-like growth factor I. 6AOiGHD, adult-onset isolated GH deficiency; BW, body weight; DMBA, 7.12-dimethylbenz[a]anthracene; EB, end bud; ER, estrogen receptor; GH, growth hormone; GHR, GH receptor; HF, high-fat; iDTIR, inducible diphertheria toxin receptor; IGF-I, insulin-like growth factor I. 7AOiGHD, adult-onset isolated GH deficiency; BW, body weight; DMBA, 7.12-dimethylbenz[a]anthracene; EB, end bud; ER, estrogen receptor; GH, growth hormone; GHR, GH receptor; HF, high-fat; iDTIR, inducible diphertheria toxin receptor; IGF-I, insulin-like growth factor I. A1AOiGHD, adult-onset isolated GH deficiency; BW, body weight; DMBA, 7.12-dimethylbenz[a]anthracene; EB, end bud; ER, estrogen receptor; GH, growth hormone; GHR, GH receptor; HF, high-fat; iDTIR, inducible diphertheria toxin receptor; IGF-I, insulin-like growth factor I. 8AOiGHD, adult-onset isolated GH deficiency; BW, body weight; DMBA, 7.12-dimethylbenz[a]anthracene; EB, end bud; ER, estrogen receptor; GH, growth hormone; GHR, GH receptor; HF, high-fat; iDTIR, inducible diphertheria toxin receptor; IGF-I, insulin-like growth factor I. 9AOiGHD, adult-onset isolated GH deficiency; BW, body weight; DMBA, 7.12-dimethylbenz[a]anthracene; EB, end bud; ER, estrogen receptor; GH, growth hormone; GHR, GH receptor; HF, high-fat; iDTIR, inducible diphertheria toxin receptor; IGF-I, insulin-like growth factor I.
The HiGH mouse model was developed and validated by our laboratory, as reported previously (23). Briefly, this mouse model has elevated GH levels due to Cre-mediated somatotrope-specific inactivation of the IGF-I receptor (IgfIr) and the insulin receptor (Insr). All experimental mice were obtained by crossbreeding IgfIr,Insr<sup>−/−</sup> female (control) mice with IgfIr,Insr<sup>+/−</sup> and IgfIr,Insr<sup>−/−</sup> (AOiGHD) male mice, in order to avoid the confounding effects of elevated maternal GH/IGF-I levels on pup development. In the first study, female mice were maintained on CHOW diet (control n = 24 and HiGH n = 25) whereas the second group of mice were fed a high-fat diet (HF; controls n = 17, HiGH n = 34; 5:24 kcal fat, 66 kcal protein, 54 kcal; carbohydrate, 20% kcal; protein 20% kcal; Research Diets), starting at 4 weeks of age and maintained for the remainder of the study. In both studies, at 8 weeks of age, mice were treated with DMBA in olive oil (500 mg/10 g BW, by oral gavage) once a week for 5 consecutive weeks. The development and progression of mammary gland tumors was monitored for 24 additional weeks in CHOW-fed and 28 weeks in HF-fed mice. Based on IACUC standards requiring euthanasia of mice with tumors >1 cm³, the HF-fed study had to be terminated 4 weeks earlier (20 weeks after DMBA treatment) than the CHOW-fed study (24 weeks after DMBA treatment). Whole body composition (lean, fat and extracellular water content) was assessed by NMR (MiniSpec LF50, Bruker Optics, Manning Park, Billerica, MA) every two weeks in HF-fed mice starting at 8 weeks of age whereas, in CHOW-fed mice, whole body composition was measured composition every two weeks starting at 20 weeks of age.

Whole mount mammary gland analysis

The right inguinal mammary glands were excised from either young untreated mice (8 weeks of age) or DMBA-treated mice at sacrifice. The glands were spread onto a glass slide, fixed in 10% neutral buffered formalin overnight, defatted in aceton and rehydrated in graded ethanol ending in water. The whole mounts were then stained with carmine (0.2% carmine, 0.5% aluminum potassium sulfate) (Sigma, St Louis, MO), washed in water and dehydrated in ascending graded ethanol solutions. Stained whole mounts were stored and photographed in methyl salicylate (Sigma) as described previously (14,16). In whole mounts prepared from inguinal glands of young mice, photographs were used to assess the number of terminal end buds (TEBs) and the extent of ductal branching, by counting the number of intersecting branches along a line drawn between the leading edge of the ducts and the lymph node. The number of branches per unit length was used as an indicator of gland complexity (2,24,25). In whole mounts obtained from DMBA-treated mice, tumors and hyperplastic lesions were scored by two blinded observers (14,26).

Circulating hormones

Commercial ELISA kits were used to assess circulating GH (Millipore, MA), IGF-I (Immunodiagnostic Systems, AZ) and insulin (Merckia, Uppsala, Sweden) levels. Triglycerides (TG), cholesterol and ketones were determined using reagents and microtiter plate procedures from Wako Diagnostics (Richmond, VA). Details on the performance parameters of the assays employed have been reported previously (23).

Tumor histopathology

A subset of tumors from CHOW- and HF-fed HiGH and control mice were dissected, formalin-fixed and sectioned in 4-μm paraflin sections for hematoxylin-eosin staining. Estrogen receptor (ER) presence was evaluated using the anti-estrogen receptor α (Cat. Number: 06-935; Millipore) following standard protocols. Two independent pathologists performed the histopathological analysis of the tumors following a blinded protocol.

Data analysis

Tumor latency was calculated as the time between the last DMBA dose and the date the tumor was first detected by palpation of the mammary glands. Tumor multiplicity indicates the average number of palpable tumors found per animal at the termination of the study (including those animals that did not develop tumors). Tumor burden indicates the average number of palpable tumors found per animal at the termination of the study (including those animals that did not develop tumors). Tumor burden indicates the average number of palpable tumors found per animal at the termination of the study (including those animals that did not develop tumors).

Results and discussion

Adult-onset isolated GH deficiency reduces DMBA-induced mammary gland tumor development

It has been recently reported that humans with inactivating mutations in the GH receptor show reduced incidence of neoplasia, including breast cancer (27) and that patients with congenital IGF1 deficiency seem protected from future cancer development (28). Likewise, rodent models with developmental deficits in GH and/or IGF-1 production or signaling exhibit reduced or delayed susceptibility to chemical- or genetically-induced mammary gland tumors (1,12-14,16). However, many of these rodent models also show impaired mammalian gland development (11,15), which could indirectly alter mammary tumor formation and progression. In order to circumvent this problem and test if lowering GH/IGF-I in the adult would impact mammary tumorigenesis, we used DMBA to induce mammary tumors in AOiGHD mice and their GH-replete littermate controls (22), in which the induction of GH and tumorigenesis was performed after normal mammary gland development had occurred. We have reported previously that female AOiGHD mice have circulating GH levels that are reduced to 30–50% of control mice and this is associated with a modest (20%), but significant, decrease in circulating IGF-1 (29). It should be noted that the pituitary and the reproductive axes remain intact in AOiGHD female mice as they have normal prolactin levels and the time to conception, litter size and pup weight at weaning does not differ from control mice (22). Since it has been reported that GH can also be expressed locally in the mammary gland (2) and Cre recombinase expression is driven by the rat GH promoter in our model system, the expression of Cre recombinase and DTR was assessed by qRT-PCR in the mammary fat pads of iDTR-/Cre<sup>−/−</sup> mice with AOiGHD and controls found to be undetectable in both genotypes (data not shown). These validation studies indicate that any differences in DMBA-induced mammary tumors between AOiGHD and controls are due to the selective reduction in circulating GH and/or the concomitant, associated decline in IGF-I. Although AOiGHD mice did not significantly impact tumor latency or tumor burden (number of tumors per tumor-bearing mouse), it reduced tumor incidence and tumor multiplicity (number of tumors per mouse) 24-week post-DMBA treatment (Figure I and Table I), as compared with DMBA-treated controls. Specifically, only 14% of AOiGHD mice exhibited palpable tumors at the end of the study, compared with the 42% of the DMBA-treated controls (P < 0.01; Table I). Evaluation of whole mounts of the inguinal glands of AOiGHD mice, revealed no tumors and only a small proportion (10%) of glands displayed hyperplasia (Table I). This was in contrast to inguinal glands of control mice, where 10% had visible tumors and 35% displayed clear hyperplasia (P < 0.05 compared with AOiGHD mice). Examples of whole mounts of inguinal mammary gland from DMBA-treated, control and AOiGHD mice are provided in Supplementary Figure I, available at Carcinogenesis Online. It should be noted that although the relative impact of DMBA on mammary tumorigenesis in C57Bl/6 mice is less than that of other background strains (30), the magnitude of response in the control mice used in this study was similar to that reported previously in other studies using C57Bl/6 mice (26,31,32). Also, DMBA-induced morbidity/mortality (Table I) and body weights at sacrifice did not differ between genotype or between non-tumor-bearing and tumor-bearing mice within genotype (control mice without tumors: 23.0 ± 0.3 g, control mice with tumors 23.3 ± 0.5 g, AOiGHD without tumors 22.7 ± 0.4 g, AOiGHD with tumors 21.2 ± 0.8 g).

Taken together, these data provide evidence that selectively lowering GH/IGF-I after mammary gland development can protect against or delay the formation of chemical-induced mammary gland tumors.
Endogenous GH/IGF-I levels and mammary gland tumors

During the peripubertal period, GH is required to achieve normal body development and DMBA-induced mammary gland tumorigenesis is diet-dependent. It remains to be determined if this protection is due to a reduction in GH/IGF-I input to the mammary gland per se or indirectly through changes in metabolic function. This latter possibility is based on our previous studies showing female AOiGHD are more glucose tolerant, consistent with the antagonistic actions of GH on insulin’s actions (29). Nevertheless, our present observations support the possibility that GH receptor and/or IGF-I receptor antagonists may serve as effective adjunct therapies in treating breast cancer (33,34).

The impact of elevating endogenous GH/IGF-I on mammary gland development and DMBA-induced mammary gland tumorigenesis is diet-dependent

During the peripubertal period, GH is required to achieve normal body growth, while in adults GH plays an important role in maintaining metabolic homeostasis through its pro-anabolic, pro-lipolytic and anti-lipogenic actions (35). The metabolic actions of GH have prompted its off-label use and abuse to improve body composition and athletic performance (36,37). Since GH levels have been shown to decline with age and weight gain, it is believed that the loss of GH contributes to metabolic dysfunction. Therefore, medical strategies to raise endogenous GH levels or exogenous GH therapy are being considered to counteract the metabolic dysfunction observed with aging and obesity (38,39). Given GH/IGF-I, as well as features of the metabolic syndrome (insulin resistance, dyslipidemia, inflammation and obesity) are positively associated with breast cancer risk (2,3,18–21), in the current study, we sought to determine if DMBA-induced mammary gland tumor development and progression is altered in a unique mouse model with moderately elevated endogenous GH levels (HiGH mice) (23), when mice are maintained under normal metabolic conditions (standard CHOW diet), or under altered metabolic conditions by feeding them a HF diet.

We have reported previously that CHOW-fed adult female HiGH mice display elevated circulating GH levels that are 2–3-fold that of controls and this increase is associated with a 20% increase in total IGF-I (23). As with AOiGHD mice, HiGH mice have normal prolactin levels and show no derangement in reproductive-axis function (23). Of note, the increase in GH/IGF-I leads to a small but significant increase in body weight (1–2 g in female mice), which can only be observed after 8 weeks of age (23). In the present study, we observed similar differences in the body weights between control and HiGH mice fed either a CHOW or a HF diet from 4 to 8 weeks of age. High-fat feeding increased body weight and fat mass in control and HiGH mice, relative to their CHOW-fed counterparts (Figure 2A). As shown in Figure 2B, HF-feeding had an overall stimulatory effect on mammary gland complexity, which has been observed in other HF-fed rodent models (40,41) and this was associated with an increase in the number of TEBs (Figure 2B). Independent of diet, HiGH mice displayed enhanced mammary gland complexity and a non-significant increase in the number of TEBs (Figure 2B). These findings are consistent with previous reports showing elevation of GH/IGF-I during the peripuberal period promotes mammary gland development (2,24).

Despite enhanced mammary gland development, CHOW-fed, HiGH mice were not more susceptible to DMBA-induced tumor formation compared with controls (Figure 3), although GH and IGF-I levels remained elevated above control levels until the end of the study, at 24-week post-DMBA (Supplementary Figure 2, available at Carcinogenesis Online). In fact, there was a significant delay in tumor appearance (latency; P < 0.05) in HiGH mice compared with controls (Figure 3) and only 26% (5/19) of HiGH versus 42% (9/21) of controls developed tumors (Table I). In addition, there was a non-significant reduction in tumor multiplicity (P = 0.09) and tumor burden (P = 0.10), where the number of HiGH mice that developed large tumors (>1 cm²) was less than that of controls (5% versus 19%, respectively; Table II). Whole mount analysis of inguinal mammary glands supports these findings, as HiGH mice did not develop more tumors (10.5% versus 14.3%) and hyperplastic lesions (21.1% versus 31.7%; Table II). Whole mount analysis of inguinal mammary glands reveals these findings, as HiGH mice did not develop more tumors (10.5% versus 14.3%) and hyperplastic lesions (21.1% versus 31.7%; Table II). Whole mount analysis of inguinal mammary glands reveals these findings, as HiGH mice did not develop more tumors (10.5% versus 14.3%) and hyperplastic lesions (21.1% versus 31.7%; Table II).

In striking contrast to that observed in CHOW-fed mice, elevated levels of endogenous GH/IGF-I in combination with HF-feeding significantly exacerbated DMBA-induced mammary gland tumor formation (Figure 3 and Table I). Indeed, the tumor latency was significantly reduced in the HiGH mice (Figure 3) and the tumor incidence was dramatically increased (P = 0.003), with 85% of HiGH mice developing tumors compared with 50% of diet-matched, control mice (P < 0.05; Table II). In addition, the number of animals that developed large tumors (>1 cm²) doubled, from 14% in controls to 30% in HiGH mice (Table II). Whole mounts of inguinal mammary glands from HiGH control and HiGH mice confirmed the results observed in vivo. In control mice, only 17% of the glands showed tumors and 33% exhibited hyperplastic lesions, while in HiGH mice 24% of

![Fig. 1. DMBA-induced mammary gland tumor formation in AOiGHD mice. 10-week-old, CHOW-fed AOiGHD and control mice were treated with DMBA (500mg/10 gBW) for 5 consecutive weeks and the development and progression of mammary gland tumors was monitored for 24 weeks. Mice (n = 25/group) were palpated weekly to follow tumor appearance. The graph indicates the moment when tumors were first detected.](https://academic.oup.com/carcin/article-abstract/35/11/2467/417324/fig1)
the glands showed tumors and 59% exhibited hyperplastic lesions. Therefore, the percentage of mammary glands affected with tumors or hyperplastic lesions was significantly elevated in HF-fed HiGH mice (83%) compared with HF-fed controls (50%; \( P = 0.05 \)). Examples of DMBA-induced hyperplasia and tumors, appearing in whole mounts of control and HiGH, HF-fed mice, are provided in Supplementary Figure 4, available at Carcinogenesis Online. Based on IACUC standards requiring euthanasia of mice with tumors \( >1 \text{ cm}^3 \), the HF-fed

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 2.** Impact of diet on control and HiGH mice, prior to DMBA treatment. (A) Body weight and fat mass and (B) Mammary gland complexity and number of TEBs of mice fed a standard chow (CHOW) or HF diet from 4 to 8 weeks of age. Values represent average ± SEM (\( n = 3–8 \) mice). Asterisks (**\( P < 0.01 \); **\( P < 0.001 \); *\( P < 0.05 \)) indicate values that significantly differ from controls.
Endogenous GH/IGF-I levels and mammary gland tumors

However, these results are consistent with a recent report and rodent models (including the data generated in the AOiGHD mice in this study) suggest a positive correlation with GH/IGF-I and mammary tumorigenesis (1,11–16). However, these results are consistent with a recent report showing mice with elevated circulating IGF-I levels, due to liver-specific expression of an IGF-I transgene (TTR-IGF-I mice), did not alter their susceptibility to ErbB2-induced mammary tumors (25). It should be noted that in HiGH mice, as well as in TTR-IGF-I mice, circulating IGF-I levels were only modestly elevated (20% and 30%, respectively). Interestingly, this elevation is comparable to the 31% increase in circulating IGF-I shown to be associated with increased breast cancer risk in premenopausal women. In contrast to HiGH mice, the TTR-IGF-I mice do not show altered mammary gland development. These differences may be due to the fact that in HiGH mice both GH and IGF-I are elevated due to loss of IGF-I and insulin negative feedback to the GH-producing cell, while in TTR-IGF-I mice elevated IGF-I probably serves to suppress GH secretion, although this was not reported (25).

In opposition to that observed in CHOW-fed HiGH mice, HF-fed HiGH mice exhibited a pronounced increase in the incidence of mammary gland tumors compared with diet-matched controls. These differences may be related to specific alterations in metabolic function mediated by elevated GH. We have reported previously that HiGH mice have higher insulin levels, compared with controls, which is associated with systemic insulin resistance, as assessed by insulin tolerance tests (23). Insulin resistance, hyperinsulinemia and associated inflammation are all considered risk factors in mammary cancers (3,33,34). We have also observed that HF feeding dramatically suppresses IGFBP1 and increases IGFBP3 to similar levels in both control and HiGH female mice (23). However, given total IGF-I levels are elevated in HF-fed HiGH mice (Supplementary Figure 2, available at Carcinogenesis Online), with low IGFBP1 and high IGFBP3, this would suggest that HF-fed HiGH mice would have more bioavailable IGF-I compared

Available at Carcinogenesis Online). In addition, as GH could regulate 17β-estradiol-dependent breast cancer cell proliferation (45), we determined ER presence in this subset of tumors and found no impact of GH/IGF-I status on tumor histotype was found (Supplementary Figure 5, available at Carcinogenesis Online).

This study represents the first to examine the impact of elevated endogenous GH/IGF-I levels on carcinogen-induced mammary tumor formation and progression. It was somewhat of a surprise that elevations in GH/IGF-I did not augment tumor induction or progression in CHOW-fed, HiGH mice since both epidemiologic (2,4–8) and rodent models (including the data generated in the AOiGHD mice in this study) suggest a positive correlation with GH/IGF-I and mammary tumorigenesis (1,11–16). However, these results are consistent with a recent report showing mice with elevated circulating IGF-I levels, due to liver-specific expression of an IGF-I transgene (TTR-IGF-I mice), did not alter their susceptibility to ErbB2-induced mammary tumors (25). It should be noted that in HiGH mice, as well as in TTR-IGF-I mice, circulating IGF-I levels were only modestly elevated (20% and 30%, respectively). Interestingly, this elevation is comparable to the 31% increase in circulating IGF-I shown to be associated with increased breast cancer risk in premenopausal women. In contrast to HiGH mice, the TTR-IGF-I mice do not show altered mammary gland development. These differences may be due to the fact that in HiGH mice both GH and IGF-I are elevated due to loss of IGF-I and insulin negative feedback to the GH-producing cell, while in TTR-IGF-I mice elevated IGF-I probably serves to suppress GH secretion, although this was not reported (25).

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### Table II. DMBA-induced mammary gland tumor formation in CHOW- and HF-fed HiGH mice

<table>
<thead>
<tr>
<th></th>
<th>CHOW-fed&lt;sup&gt;a&lt;/sup&gt;</th>
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<th>HFD-fed&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>Control (n = 24)</td>
<td>HiGH (n = 20)</td>
<td>Control (n = 17)</td>
<td>HiGH (n = 34)</td>
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<tr>
<td>Palpable mammary gland tumors&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.1 ± 5.0</td>
<td>90.4 ± 10.8*</td>
<td>72.0 ± 6.1</td>
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<tr>
<td>Tumor latency (days)</td>
<td>42%</td>
<td>26%</td>
<td>50%</td>
<td>85%*</td>
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<tr>
<td>Mice with tumors</td>
<td>50%</td>
<td>30%</td>
<td>14%</td>
<td>30%</td>
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<tr>
<td>Mice with tumors &gt;1 cm&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.8 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>1.3 ± 0.2*</td>
</tr>
<tr>
<td>Tumor burden&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.0 ± 0.3</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.4 ± 0.1</td>
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<tr>
<td>Whole mount analysis&lt;sup&gt;f&lt;/sup&gt;</td>
<td>14.3%</td>
<td>10.5%</td>
<td>17.0%</td>
<td>23.0%</td>
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<tr>
<td>Tumor</td>
<td>14.3%</td>
<td>10.5%</td>
<td>17.0%</td>
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<td>Hyperplasia</td>
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<td>21.1%</td>
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<td>Normal</td>
<td>38.1%</td>
<td>68.4%</td>
<td>50.0%</td>
<td>18.0%</td>
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<tr>
<td>Percentage of mice that did not complete the study</td>
<td>12.5%</td>
<td>5.0%</td>
<td>17.7%</td>
<td>20.6%</td>
</tr>
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</table>

<sup>a</sup>Data obtained after 24 weeks post DMBA treatment.

<sup>b</sup>Data obtained after 20 weeks post DMBA treatment.

<sup>c</sup>Excluding those mice that did not complete the study.

<sup>d</sup>Average number of tumors per mouse.

<sup>e</sup>Average number of tumors per tumor-bearing mouse.

<sup>P</sup> < 0.05.

Fig. 3. DMBA-induced mammary gland tumor formation in CHOW- and HF-fed HiGH mice. 8-week-old, CHOW- or HF-fed HiGH and control mice were treated with DMBA (500 mg/10 g BW) for 5 consecutive weeks and the development and progression of mammary gland tumors was monitored for 24 weeks or 20 weeks, respectively. Mice were palpated weekly to follow tumor appearance. The graph indicates the moment when tumors were first detected. Tumors latency indicates the mean number of days that passed from the last DMBA dose until the tumor was detected. Values represent average ± SEM (n = 17–24 mice). Asterisks (*P < 0.05) indicate values that significantly differ from controls.

study was terminated 4 weeks earlier (20 weeks after DMBA treatment) than the CHOW-fed study (24 weeks after DMBA treatment). Histopathological analysis of a subset of these tumors showed all were adenocarcinomas with squamous, epithelial or tubular differentiation (Supplementary Figure 5, available at Carcinogenesis Online), consistent with previous reports showing DMBA predominantly induces adenocarcinomas and adenosquamous carcinomas in several mouse strains (31,42–44). Importantly, no apparent effect of GH/IGF-I status on tumor histotype was found (Supplementary Figure 5, available at Carcinogenesis Online). In addition, as GH could regulate 17β-estradiol-dependent breast cancer cell proliferation (45), we determined ER presence in this subset of tumors and found no impact of GH/IGF-I status on tumor histotype was found (Supplementary Figure 5, available at Carcinogenesis Online).
with CHOW-fed HiGH mice, which could contribute to enhanced tumor formation. Other factors may also interact with elevated GH/IGF-I to promote mammary tumors in HiGH mice. For example, the micronutrient composition of CHOW and HF diets differed, where it has been shown that specific micronutrients have both positive and negative effects on tumor initiation/formation (46–48). Indeed, the HF diet used was rich in saturated fats, and it has been shown previously in rodent models and in human studies that the polyunsaturated/saturated ratio negatively associates with mammary tumor formation (47,49,50). Nonetheless, it is clear from this study that different diets can dramatically alter the impact of elevated GH/IGF-I on DMBA-induced mammary gland tumorogenesis. Since obesity has been shown to be positively associated with breast cancer risk (17,18), it is possible that the early increases in fat mass observed at the start of DMBA treatment (8 weeks of age) could have modified the effects of elevated GH/IGF-I on DMBA-induced tumor initiation. However, after DMBA treatment fat mass did not differ between CHOW-fed and HF-fed HiGH mice within genotype, from 8 to 20 weeks post DMBA treatment (Supplementary Figure 2, available at Carcinogenesis Online). Indeed, it has been reported previously that DMBA-treated mice are resistant to fat accumulation when fed a HF diet (51). Therefore, fat mass per se is unlikely a major contributor to the enhanced tumor progression observed in HF-fed, HiGH mice. In fact, the tumor formation did not appear to be associated with body weight and relative fat mass at 16 weeks after DMBA treatment (Supplementary Figure 6, available at Carcinogenesis Online). Despite the lack of elevated weight gain, HF-fed control and HiGH mice did show dyslipidemia (Supplementary Figure 2, available at Carcinogenesis Online), indicating HF diet impaired metabolic function in the context of DMBA treatment. Therefore, there may be a deleterious interaction between elevated GH/IGF-I levels and diet-induced metabolic dysfunction in terms of mammary tumorogenesis. Nevertheless, the exact mechanism(s) mediating the tumorigenic effects of elevated GH/IGF-I in the context of HF diet remains to be determined, and is probably multifactorial.

Conclusions

Taken together, our data demonstrate, under normal metabolic conditions (CHOW-diet), a reduction in endogenous GH/IGF-I levels in adults protects against mammary gland tumor formation while elevated endogenous GH/IGF-I levels do not hasten tumor formation. However, when mice are fed a HF diet rich in saturated fats, elevated endogenous GH/IGF-I levels accelerates mammary gland tumor formation and progression, suggesting the ultimate consequences of GH/IGF-I on breast tumor development is dependent on the diet and the metabolic status.

Supplementary material

Supplementary Figures 1–6 can be found at http://carcin.oxfordjournals.org/

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