Excess weight gain accelerates 1-methyl-1-nitrosourea-induced mammary carcinogenesis in a rat model of premenopausal breast cancer

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Abstract

In contrast to the null effects generally reported, high-risk premenopausal women (Gail score ≥1.66) enrolled in the Breast Cancer Prevention P-1 Trial were recently reported to be at increased risk for breast cancer when overweight (HR, 1.59) or obese (HR, 1.70). To investigate this clinical observation in a preclinical setting, ovary-intact female rats were intraperitoneally injected with 50 mg/kg 1-methyl-1-nitrosourea at 21 days of age to simulate premenopausal women with increased risk. Two commercially available strains of Sprague Dawley rat (Taconic Farms) were used which are dietary resistant (DR) or dietary susceptible (DS) to excess weight gain when fed a purified diet containing 32% kcal from fat, similar to levels consumed by the typical American woman. DS rats were approximately 15.5% heavier than DR rats at study termination and plasma leptin indicated a marked difference in adiposity. DS rats had higher incidence (26% increase), multiplicity (2.5-fold increase), and burden (5.4-fold increase) of mammary carcinomas with a concomitant reduction in cancer latency (16% earlier detection) compared to DR rats (P <.001 for all analyses), and displayed a higher proportion of hormone receptor negative tumors compared to DR rats (OR=1.78, 95% CI 0.83-3.81). Circulating levels of several breast cancer risk factors including leptin, adiponectin:leptin ratio, insulin, IGF-1, IGF-1:IGFBP3 ratio, and calculated insulin resistance (HOMA-IR) were negatively impacted in DS rats (P <.05 for all analyses). These findings support further investigation of the effects of excess weight in high-risk premenopausal women and demonstrate a useful preclinical model for rapid evaluation of mechanistic hypotheses.
Introduction

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer death in US women (1). Current estimates indicate that 17% of breast cancer cases in the US are preventable by maintaining a healthy weight (2). This relationship is of considerable public health importance given the ongoing obesity epidemic, in which two out of three women are reportedly overweight or obese as defined by body mass index (BMI) ≥25 kg/m² (3, 4). Obese women with breast cancer typically have larger tumors, higher rates of metastasis, higher rates of recurrence, and increased all-cause and breast cancer-related mortality at any age compared to normal weight women with breast cancer (5, 6).

The nature of the association between obesity and breast cancer has been reported to hinge on menopausal status. In postmenopausal women, a positive association between excess body weight and breast cancer risk has been consistently reported (2). Conversely, most studies report a null or inverse association of excess weight with breast cancer risk in premenopausal women though this topic remains controversial (2, 7-9). Recently, in a study based on the National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention (P-1) Trial, Cecchini et al. provided evidence contrary to this mainstream of thinking. In the Cecchini study, a large cohort of premenopausal women aged ≥35 at high risk for breast cancer (Gail score ≥1.66) was evaluated at three tiers of BMI: <25.0 (normal weight), 25.0-29.9 (overweight), and ≥30.0 kg/m² (obese). Risk of invasive breast cancer was increased in overweight and obese women (HR, 1.59 and 1.70, respectively) compared to women of BMI <25 (10), suggesting that in women with multiple breast cancer risk factors, which comprise a subpopulation of premenopausal women, excess weight gain may further increase risk.

Despite the abundance of preclinical studies evaluating the relationship between dietary fat, adiposity, and breast cancer risk, several gaps remain in our understanding of the effect of
overweight and obesity on breast carcinogenesis as well as in available preclinical tools to fill these gaps. Though most strains are resistant to diet-induced obesity (DIO), MacLean et al. have characterized subpopulations of Wistar rats with sensitivity or resistance to diet-induced excess weight gain; however these studies have only been performed in a postmenopausal model for breast cancer (11-14). Studies in mice, while mechanistically informative and likewise trending towards identification of obesity-resistant and sensitive subpopulations in reports from Cleary et al. (15-17), are limited by two factors. First, in most mouse models for breast cancer, predominantly estrogen and progesterone receptor-negative tumors are induced, a molecular subtype of the disease which is less common in premenopausal women (reviewed in (18, 19)). Second, preclinical models of diet-induced obesity in susceptible mouse strains generally utilize high fat diets containing 45-60% kcal from fat, levels of fat above relevance to human populations. Moreover, non-obese control mice are frequently fed a low fat diet thus adding differences in dietary composition as potentially confounding variables in those investigations.

To circumvent these issues, this study was conceived as a means to rapidly evaluate Cecchini et al.’s findings in a reverse translational setting, and to our knowledge has not been previously attempted. We describe the integration of a novel model for the study of diet-induced obesity introduced in 1997 by BE Levin of Veterans Affairs Medical Center in East Orange, NJ, with a well-characterized approach to cancer induction that was developed by our laboratory in 1995. The model for polygenic obesity developed by Levin utilizes two strains of Sprague Dawley rats selectively bred for >20 generations for resistance (DR) or susceptibility (DIO, or DS, as used herein) to diet-induced excess weight gain when fed diet containing ~32% kcal as fat. This model has been extensively characterized by Levin’s group (e.g. (20-22)). When fed the 32% fat diet, DS rats rapidly gain excess weight and have expanded peripheral and visceral fat depots by 3 months of age (21), display hyperlipidemia (total cholesterol and triglycerides) by 2 months, hyperleptinemia by 3 months, and pronounced fat infiltration of the liver by 6 months of age (21).
DS rats display pre-diabetic measures of glucose homeostasis including hyperinsulinemia by 2 months, insulin resistance by 3 months, worsened oral glucose tolerance by 2 months, and eventual reduced pancreatic insulin secretion by 9 months of age, though rats do not fully progress to diabetes up to 2 years of age (21). Our model for breast cancer induction involves injecting weanling Sprague Dawley rats, which are highly sensitive to mammary cancer induction, with a non-toxic dose of a chemical carcinogen (23). Tumors begin to emerge after sexual maturity and incidence, multiplicity and latency of mammary tumors are dependent on carcinogen dose (23-25). The histogenesis, morphological stages of disease development, and the types of carcinomas induced recapitulate the process of breast carcinogenesis in women (18, 26). In ovary-intact animals, the disease process provides a biologically relevant model for premenopausal breast cancer. Using this integrated preclinical model, we evaluated Cecchini et al.’s hypothesis that excess weight gain resulting in overweight or obesity increases incident cancer in premenopausal women with increased risk for breast cancer.
Materials and Methods

Animal Breeding and Husbandry

Breeder pairs (approximately 30 pairs each Levin DR and DS) were obtained from Taconic (Taconic, Hudson, NY) at 5-7 weeks of age. These outbred strains of Sprague Dawley rats were originally obtained by Taconic from BE Levin after 20 generations of selective breeding for rapid weight gain on sucrose and moderate fat (32%) (SUMO32) diet and were subsequently outbred using a rotational breeding scheme for an additional 30+ generations, and are commercially available from the Taconic repository (strain: TacLevin:CD(SD)DIO, stock #DS; TacLevin:CD(SD)DR, stock #DR). In-house breeding was conducted using a Poiley rotational breeding scheme, in which breeder pairs are systematically rotated in each breeding cycle (27).

Pups were weaned at 3 weeks of age and were immediately switched to SUMO32 diets. Post-weaning, rats were housed 3 per cage, maintained on 12 hour light:dark cycle at 24 ± 2°C with 30% relative humidity, and given ad libitum access to SUMO32 diet and distilled water. Animals were weighed weekly. To initiate mammary carcinogenesis according to the rapid emergence model first developed by our laboratory (23), female ovary intact DR (n = 103) and DS (n = 101) rats were injected intraperitoneally (50 mg/kg) with 1-methyl-1-nitrosourea (MNU) (Ash Stevens, Detroit, MI-prepared fresh in acidified saline) at 21 days of age as previously described (24). Bi-weekly palpations for detection of mammary tumors began 24 days post-carcinogen and continued until study termination. All animal studies were performed in accordance with the Colorado State University Institutional Animal Care and Use Committee.

Diet Formulation and Composition

The sucrose and moderate 32% fat (SUMO32) diet is a purified formulation described in Table 1. Anhydrous milk fat and corn oil together contribute 32.1% of kcal in the SUMO32 diet, comprised of 29.2% saturated fatty acids (9.4% of total dietary kcal), 28.4% monounsaturated
fatty acids (9.1% of total dietary kcal), and 42.4% polyunsaturated fatty acids (13.6% of total dietary kcal) ((28), manufacturer’s label). The SUMO32 diet provided 16.7% protein and 51.2% carbohydrate by kcal, comparable to the macronutrient composition of the average American woman’s diet as reported in Table 1 (29). The SUMO32 rodent diet provided 4.35 kcal/g (18.2 kJ/g) and was mixed on-site at our laboratory’s diet mixing facility and stored at -20°C until used.

**Necropsy**

The study was terminated 63 days post-carcinogen when rats were 84 days of age, whereupon fasted rats were euthanized within a 4-hour window via CO₂ inhalation and cervical dislocation. Blood was collected into EDTA VacuTainers (Becton-Dickinson, Franklin Lakes, NJ) and centrifuged to separate plasma. After separation, plasma was kept on ice before freezing at -20°C until use. Rats were skinned and mammary gland chains were examined under translucent light; grossly visible tumors were excised, weighed, and processed for histopathological analysis and classification by H&E staining as previously described (30).

**Plasma Analyses**

Body weights of both DR and DS strains are normally distributed; therefore to maximize statistical power, animals chosen for plasma analysis were purposely sampled from non-overlapping areas of the distributions, e.g. lean DR and heavy DS. All systemic analytes were determined using commercial ELISA kits performed according to manufacturer’s specifications. Specifically, glucose was determined using a kit obtained from Thermo Fisher (Waltham, MA). Insulin, leptin, IL-6, IL-1β, and TNF-α were determined using a multiplex kit, while adiponectin and IGF-1 were separately quantified using commercial single-plex kits from Millipore (Billerica, MA). C-reactive protein (CRP) was determined using a commercial rat ELISA from Helica Biosystems Inc. (Fullerton, CA). Estradiol, progesterone, and sex hormone-binding globulin
(SHBG) were determined using commercial ELISAs from GenWay Biotech (San Diego, CA). IGF-1 binding protein (IGFBP)-3 kit was from BioVendor (Asheville, CA). Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated as previously described (31).

**Immunohistochemical Assessment of Hormone Receptor Status**

The estrogen and progesterone receptor status of mammary carcinomas was determined using our previously published procedure (32). Specificity of the staining is illustrated in Supplementary Figure S1.

**Statistical Analyses**

Statistical analyses were performed on data from palpable mammary adenocarcinomas and 95% CIs were constructed for each outcome. Differences were assessed for statistical significance as follows: final body weight at study termination was assessed by unpaired t test with Welch-Satterthwaite correction; cancer incidence (%) and proportion of estrogen and progesterone positive and negative tumors were assessed by chi square test of equal proportions; cancer multiplicity (# carcinomas/ rat) was assessed using Poisson regression; carcinoma burden (g/ rat) was assessed using a two-stage model combining chi-square (incidence) and log linear (burden) P-values; and cancer latency was assessed using a log-rank (Mantel-Cox) test in Kaplan-Meier survival analysis, where animals without palpated tumors were right-hand censored at 60 days post-carcinogen, the last day rats were palpated for detectable tumors before study termination (censored: DR n = 39, DS n = 9). Between-group differences in plasma analytes were assessed by unpaired t test with Welch-Satterthwaite correction and log_{10}-data transformation as needed to satisfy statistical assumptions.
Results

SUMO32 diet induces rapid excess weight gain in DS rats

All rats were fed SUMO32 diet (Table 1) ad libitum throughout the 9 week duration of the study. Rats sensitive (DS) to diet-induced excess weight gain ($n = 101$) were 3 g heavier than rats resistant (DR) to diet-induced excess weight gain ($n = 103$) at the outset of the study (body weights for DS and DR: $56.7 \pm 8.7$ vs. $53.6 \pm 6.3$ g, respectively, $P = .004$). As depicted in Figure 1A, body weights of DS and DR rats began to diverge immediately with SUMO32 feeding, and DS rats continued to gain weight at an accelerated rate for the duration of the study ($P$ for trend < .001). At study termination 63 days post carcinogen, DS rats were 15.5% heavier than DR rats ($217.0 \pm 24.5$ vs. $187.9 \pm 14.6$, respectively, $P < .001$). Plasma leptin, which is related to fat mass (33, 34), was substantially higher in DS versus DR rats at study termination ($5.6 \pm 1.4$ vs. $0.9 \pm 0.8$ for DS vs. DR, respectively) ($P < .001$).

Chemically-induced mammary carcinogenesis is markedly accelerated in DS rats

To determine the effect of excess weight gain on the development of chemically induced mammary carcinogenesis, female ovary-intact rats (DR $n = 103$, DS $n = 101$) were injected intraperitoneally with 50 mg/kg 1-methyl-1-nitrosurea (MNU) at 21 days of age as delineated in the rapid emergence model of mammary carcinogenesis (23). Latency to the first palpated mammary carcinoma per rat was evaluated by to time-to-event analysis with tumor-free rats right-hand censored at 60 days post-carcinogen, the last day rats were palpated before study termination (censored: DS $n = 9$, DR $n = 39$). Cancer outcomes are reported in Table 2 and are based on data for palpable histopathologically confirmed mammary adenocarcinomas. As shown in Figure 1B, cancer-free survivor functions for DR and DS rats were significantly different ($P < .001$) and 50% incidence of mammary adenocarcinoma in the DS group was achieved by 39 days post-carcinogen, compared 49 days post-carcinogen in the DR group. Final cancer incidence was 91% in DS rats versus 65% in DR rats. Cancer multiplicity and
cancer burden were also markedly higher in the DS versus DR group (Figure 1C and Table 2; 
$P < .001$ for all analyses). The carcinomas used to construct Figure 1B were assessed for 
estrogen and progesterone receptor status. The percent of carcinomas that were 
estrogen/progesterone receptor negative was increased in the DS group (34.9%) vs. DR group 
(22.8%) though this difference did not achieve statistical significance ($P = 0.134$; odds ratio 
=1.78 (95% CI, 0.83 to 3.81)).

**DS rats display widespread alterations in circulating analytes**

Given previous reports describing metabolic alterations of DS rats, we evaluated circulating 
levels of a broad panel of analytes in plasma from DR ($n = 10$) and DS ($n = 10$) rats, with values 
reported in Table 3. DS rats displayed statistically significant elevations in fasted plasma insulin, 
IGF-1, IGF-1:IGFBP-3 ratio, leptin, and adiponectin, with a concomitant reduction in 
adiponectin:leptin ratio versus DR rats. Calculated insulin resistance, estimated through 
homeostasis model assessment-estimated insulin resistance (HOMA-IR), was elevated in DS 
versus DR rats. A trend towards elevated TNF-$\alpha$ and estradiol in DS rats was observed but 
differences between these and other evaluated analytes did not reach statistical significance.
**Discussion**

This study characterizes a preclinical rat model designed to interrogate the effects of excess weight and fat mass accumulation on breast cancer risk. This preclinical model was developed in response to a recent report by Cecchini *et al.* in which high-risk women aged ≥ 35 enrolled in the Breast Cancer Prevention P-1 Trial with BMI ≥ 25 had substantially elevated risk of invasive breast cancer compared to women with BMI < 25. Cecchini *et al.* concluded that overweight and obesity are not protective among premenopausal women in a high-risk population, and that the relationship between BMI and breast cancer may not be the same for all women (10). The Cecchini study challenges currently held conventions in the field of premenopausal breast cancer research (reviewed in (9)), and has paradigm-shifting potential as it provokes a shift in thinking away from considering individuals with elevated BMI as a homogeneous population, and towards viewing this population as highly diverse with wide variance in diet, lifestyle, genetics, and biology, and the accompanying risk tiers that these factors, in addition to BMI, confer.

In preclinical studies, use of a carcinogen to induce cancer simulates a population of individuals at increased risk in that while carcinogen treatment establishes risk, the biology of each animal determines the response. Not all animals treated with a chemical carcinogen develop cancer; rather the process is stochastic in nature. Using the selectively outbred Levin strains of diet-induced excess weight gain susceptible (DS) or resistant (DR) rats, we evaluated the effects of excess weight gain on development of mammary cancer. DS rats gained excess weight despite both strains consuming identical SUMO32 diet; thus these strains allow for investigation of excess weight gain independent of dietary macronutrient composition, unlike models of diet-induced obesity which utilize high-fat formulations for the obese group and low-fat formulations for the referent control.
In the current study, ad libitum feeding of SUMO32 diet resulted in a 15.5% difference in body weights between DS and DR rats, a relevant comparison to the clinical population as stratified by BMI tiers for normal weight (BMI <25 kg/m²), overweight (25.0-29.9 kg/m²), or obese (≥30.0 kg/m²) women. For example, a 5’4” adult woman weighing 140 lbs with BMI 24.0 kg/m² is considered normal weight, while a 5’4” woman weighing 161.7 lbs (15.5% increase) with BMI 27.8 kg/m² is considered overweight and, if preexisting risk factors are present, may be at increased risk of breast cancer compared to the normal weight woman (HR, 1.59), based on Cecchini’s findings (10). This elevated risk conferred by excess weight was recapitulated in the current study, in which moderate excess weight gain in DS rats (15.5%) was accompanied by elevated risk of mammary adenocarcinoma (relative risk, 1.40), a level of risk strikingly similar to the value reported in the clinical study. DS rats displayed marked acceleration of mammary carcinogenesis compared to DR rats, with statistically significant increases in cancer incidence (26% increase), multiplicity (2.5-fold increase), and tumor burden (5.4-fold increase) with a concomitant reduction (16% earlier detection) in cancer latency in DS rats. In addition, a trend towards an increased proportion of estrogen/progesterone receptor negative mammary carcinomas was observed in the DS group (11.7% increase compared to DR rats), a finding that parallels the 14% increase in estrogen/progesterone receptor negative breast cancer reported in the clinical study (10). Also of interest was the dramatic increase in tumor burden in DS compared to DR rats, as multiple reports describe a trend towards larger breast tumors in obese women compared to normal weight women (35, 36). As the carcinogen responsiveness of the Levin rat strains was unknown, these studies utilized very large sample sizes; however future studies seeking to declare differences in tumor incidence between DR and DS groups are estimated to be well-powered (>80.8% power) with \( n = 40 \) rats per group. Studies in which multiplicity is the primary endpoint are estimated to be well-powered (>95% power) with \( n = 20 \) rats per group, due to the magnitude of the difference in tumor multiplicity between groups.
While the current study identified alterations in a broad panel of circulating analytes in DS compared to DR rats (Table 3), a great deal of further investigation is required in order to establish the mechanisms driving this phenomenon. However, we would be remiss if we failed to briefly examine results of the initial assessment of plasma biomarkers in the context of multiple mechanisms that have been proposed to explain the relationship between obesity and breast cancer, including altered adipokine and growth factor signaling, chronic inflammation, and increased production of sex hormones (reviewed in (37-40)).

Statistically significant elevations in circulating insulin, IGF-1, and IGF-1:IGFBP-3 ratio were observed in plasma from DS compared to DR rats. Moreover, as an indirect assessment of insulin resistance, HOMA-IR was elevated in DS rats, suggesting that insulin and insulin-related signaling may be deregulated in DS rats. Several studies report that insulin resistance is an independent risk factor for breast cancer in clinical populations and is positively correlated with tumor angiogenesis and metastasis (reviewed in (40)). Furthermore, Calori et al. reported that obese insulin-resistant individuals had elevated risk of cancer mortality (HR, 1.52) compared to obese insulin-sensitive individuals (HR 1.04) (41), again suggesting that a population of individuals with elevated BMI is heterogeneous with an array of associated risk. In the Cecchini study, a greater percentage of obese (5.9%) and overweight (2.3%) women were diabetic than lean women (1.4%); however, full multivariable adjustment for several variables including history of diabetes only minimally reduced hazard ratios from the final multivariable assessment of 1.59 and 1.70 for overweight and obesity to 1.55 and 1.66, respectively (10), suggesting that other factors or interaction of factors are influencing these risk ratios.

Another key finding of the present study was the nearly 6-fold elevation of leptin in DS compared to DR rats. Leptin is strongly correlated with adiposity (33, 34); while we did not measure adiposity per se, Levin has previously reported elevations in circulating leptin in 12
week old male DS animals of a similar magnitude as found in our 12 week old females (male rats: DS, 5.1 ng/ml, DR: 1.8 ng/ml), which was associated with a 2 to 4 fold increase in individual fat pad mass and a cumulative doubling of total adiposity compared to DR rats (21). Breast tumor expression of leptin receptor in conjunction with elevated circulating leptin has been associated with poor prognosis and tumor metastasis (42, 43); conversely, studies examining the relationship between circulating adiponectin and breast cancer risk in both premenopausal and postmenopausal women report mixed outcomes (44-46). In the current study, we observed increased levels of circulating adiponectin with a concomitant reduction in the ratio of adiponectin:leptin in DS compared to DR rats. Though the elevated adiponectin in DS rats was unexpected, previous studies utilizing obese (fa/fa) Zucker rats have reported elevated levels of circulating adiponectin with reduced tissue expression of the adiponectin receptor 1 in obese vs. lean animals, whereby adiponectin signaling may be impaired despite elevated circulating levels (47). Furthermore, it has recently been suggested that the adiponectin:leptin ratio, rather individual adipokine levels, may be more informative for evaluating breast cancer risk profile (37, 48).

Both chronic low-grade inflammation and increased conversion of androgen to estrone by the cytochrome P450 aromatase in peripheral fat tissues have been implicated in mammary carcinogenesis, the latter particularly in the development of hormone-responsive tumors (49, 50); interestingly, Cecchini et al. report that BMI was significantly associated with elevated risk of estrogen receptor-positive tumors in premenopausal women. Though inflammatory cytokines and sex hormones were not significantly altered at the systemic level in the current study, effects on the carcinogenic process were observed at an early stage of body fat accumulation. The published work of Levin indicates that DS rats continue to accumulate both central and peripheral adipose tissue as well as in tissues such as the liver (21). Trends in both preclinical and population data suggest that inflammation and sex hormones may play a role in tumor
promotion and progression. An advantage of our carcinogenesis model is the relative ease with which carcinogen dose is modified and hence rate of tumor development, and consequently study duration and fat accumulation, is regulated, enabling future investigation of the effects of early, intermediate, and late phase adiposity-associated metabolic sequelae on the carcinogenic process.

In conclusion, the current study describes a model with potential value to a wide range of researchers, from basic scientists to clinicians, in which to study the impact of excess weight and adiposity on mammary carcinogenesis. Furthermore the findings highlight the potential importance of identifying and characterizing at-risk groups which stratify a population with elevated BMI. The Levin strains of selectively outbred DR and DS rats in conjunction with the rapid emergence model of mammary carcinogenesis provide a platform on which to conduct preclinical research into the effects of excess weight gain on a population at high risk of malignancies of the breast as recently reported by Cecchini, and represent rapid and cost-effective preclinical tools with high relevance to clinical populations.
Acknowledgments

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References


**Figure 1 Title:** Excess weight gain accelerates mammary carcinogenesis.

**Figure 1 Legend:** Diet-induced excess weight gain susceptible (DS) \((n = 101)\) and resistant (DR) \((n = 103)\) rats were switched from chow to sucrose and moderate fat (32% dietary fat by kcal) (SUMO32) diet ad libitum at weaning. Mammary carcinogenesis was initiated by injecting rats intraperitoneally with 50 mg/kg 1-methyl-1-nitrosourea (MNU) at 21 days of age. Study was terminated at 63 days (9 weeks) post-carcinogen; only palpable confirmed mammary adenocarcinomas were included for analysis. **A,** Rats were weighed weekly while on study; data is expressed as mean ± SD. Weight gain trend was analyzed with non-linear regression. DS display significantly more weight gain compared to DR rats, \(P\) for trend <.001. **B,** Rats were palpated twice weekly for the detection of mammary tumors. Data was subjected to Kaplan Meier survival analysis with log-rank (Mantel-Cox) test, and cancer-free survival (survivor function) for DS rats was markedly reduced compared to DR rats (censored: DR \(n = 39\), DS \(n = 9\)). Mammary adenocarcinoma incidence was increased in DS compared to DR rats, \(P <.001\). **C,** Cancer multiplicity (#/rat) was analyzed by Poisson regression; data is expressed as mean ± 95% CI. Multiplicity was significantly elevated in DS compared to DR rats, \(P <.001\).
Table 1. Sucrose and moderate fat (32% kcal) (SUMO32) diet formulations.

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<tr>
<th>Ingredient</th>
<th>Source</th>
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<th>%kcal/100g</th>
<th>SUMO32 diet (% kcal)</th>
<th>US woman’s diet (% kcal)</th>
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**Table 1 Legend:** Table 1 has no legend.
Table 2. Excess weight accelerates mammary carcinogenesis in rats resistant (DR) or susceptible (DS) to diet-induced weight gain.

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<th>N</th>
<th>Body Weight&lt;sup&gt;a&lt;/sup&gt; (g)</th>
<th>Incidence&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>Relative Risk&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Multiplicity&lt;sup&gt;d&lt;/sup&gt; (#/rat)</th>
<th>Burden&lt;sup&gt;e&lt;/sup&gt; (g/rat)</th>
<th>Latency&lt;sup&gt;f&lt;/sup&gt; (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>101</td>
<td>217.0 (215.9, 225.2)</td>
<td>91.1 (83.8, 94.8)</td>
<td>1.40 (1.20, 1.63)</td>
<td>4.3 (3.8, 5.0)</td>
<td>7.15 (5.99, 8.31)</td>
<td>39 (35, 41)</td>
</tr>
<tr>
<td>DR</td>
<td>103</td>
<td>187.9 (185.1, 190.8)</td>
<td>65.1 (55.0, 74.2)</td>
<td>--</td>
<td>1.7 (1.4, 2.1)</td>
<td>1.33 (0.91, 1.75)</td>
<td>49 (48, 56)</td>
</tr>
</tbody>
</table>

Table 2 Legend: Data based on palpable adenocarcinomas.

<sup>a</sup>Values are means (95% CI) at study termination. Groups differ ($P < .001$) by unpaired t test with Welch-Satterthwaite correction.

<sup>b</sup>Values are percentages (95% CI). Groups differ ($P < .001$) by chi square test of equal proportions.

<sup>c</sup>Values are point estimates of incidence risk in DS compared to DR (95% CI), from chi square test of equal proportions.

<sup>d</sup>Values are means (95% CI). Groups differ ($P < .001$) by Poisson regression.

<sup>e</sup>Values are medians (95% CI). Groups differ ($P < .001$) by two-stage model combining chi-square (incidence) and log linear (burden) $P$ values.

<sup>f</sup>Values are point estimates at 50% quartile (95% CI of linear-transformed survivor functions). Survivor functions differ ($P < .001$) by log-rank (Mantel-Cox) test. Censored: DR $n = 39$, DS $n = 9$. 
Table 3. Rats susceptible to diet-induced excess weight gain (DS) display alterations in circulating factors compared to dietary resistant (DR) rats.

<table>
<thead>
<tr>
<th>Analyte/ Analyte Ratio</th>
<th>DS</th>
<th>DR</th>
<th>Fold change&lt;sup&gt;a&lt;/sup&gt;</th>
<th>t statistic&lt;sup&gt;b&lt;/sup&gt;</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>INSULIN SIGNALING</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (pg/mL)</td>
<td>1402.4 ± 851.9</td>
<td>398.9 ± 577.5</td>
<td>3.5</td>
<td>4.12</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>136.4 ± 78.7</td>
<td>119.1 ± 63.5</td>
<td>1.1</td>
<td>0.64</td>
<td>.53</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>12.0 ± 6.6</td>
<td>4.6 ± 7.9</td>
<td>2.6</td>
<td>3.64</td>
<td>.003</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>285.7 ± 28.1</td>
<td>235.3 ± 39.5</td>
<td>1.2</td>
<td>3.24</td>
<td>.005</td>
</tr>
<tr>
<td>IGFBP-3 (ng/mL)</td>
<td>82.7 ± 14.0</td>
<td>80.6 ± 12.9</td>
<td>1.0</td>
<td>0.36</td>
<td>.73</td>
</tr>
<tr>
<td>IGF-1:IGFBP-3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.6 ± 2.0</td>
<td>12.3 ± 2.3</td>
<td>1.2</td>
<td>2.36</td>
<td>.03</td>
</tr>
<tr>
<td>ADIPOKINES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>5.6 ± 1.4</td>
<td>0.9 ± 0.8</td>
<td>5.9</td>
<td>7.15</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Adiponectin (μg/mL)</td>
<td>31.8 ± 13.3</td>
<td>17.1 ± 4.7</td>
<td>1.9</td>
<td>3.63</td>
<td>.002</td>
</tr>
<tr>
<td>Adiponectin:leptin&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5 ± 0.3</td>
<td>3.3 ± 2.8</td>
<td>0.2</td>
<td>4.21</td>
<td>.001</td>
</tr>
<tr>
<td>INFLAMMATORY CYTOKINES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (μg/mL)</td>
<td>695.3 ± 315.0</td>
<td>617.1 ± 200.5</td>
<td>1.1</td>
<td>0.65</td>
<td>.52</td>
</tr>
<tr>
<td>IL-1β (pg/mL)</td>
<td>17.3 ± 25.7</td>
<td>7.8 ± 9.6</td>
<td>2.2</td>
<td>1.01</td>
<td>.33</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>46.3 ± 43.44</td>
<td>111.2 ± 235.6</td>
<td>0.4</td>
<td>0.31</td>
<td>.76</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Fold Change</td>
<td>p-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------</td>
<td>-------------</td>
<td>---------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TNF-α (pg/mL)</strong></td>
<td>3.3 ± 1.0</td>
<td>2.6 ± 0.9</td>
<td>1.3</td>
<td>1.65</td>
<td>.12</td>
</tr>
<tr>
<td><strong>SEX STEROID HORMONES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>46.1 ± 11.7</td>
<td>39.8 ± 8.6</td>
<td>1.2</td>
<td>1.37</td>
<td>.19</td>
</tr>
<tr>
<td>Progesterone (ng/mL)</td>
<td>6.3 ± 4.1</td>
<td>5.2 ± 2.4</td>
<td>1.2</td>
<td>0.48</td>
<td>.64</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>12.8 ± 4.7</td>
<td>14.5 ± 7.7</td>
<td>0.9</td>
<td>0.25</td>
<td>.81</td>
</tr>
<tr>
<td>SHBG:estradiol&lt;sup&gt;c&lt;/sup&gt;</td>
<td>84.2 ± 47.8</td>
<td>99.3 ± 49.9</td>
<td>1.1</td>
<td>0.86</td>
<td>.40</td>
</tr>
<tr>
<td>Progesterone:estradiol&lt;sup&gt;c&lt;/sup&gt;</td>
<td>126.7 ± 91.5</td>
<td>113.1 ± 41.2</td>
<td>1.1</td>
<td>0.13</td>
<td>.90</td>
</tr>
<tr>
<td>Progesterone:estradiol:SHBG&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.8 ± 8.9</td>
<td>9.5 ± 5.3</td>
<td>1.1</td>
<td>0.06</td>
<td>.95</td>
</tr>
</tbody>
</table>

**Table 3 Legend:** Circulating analytes in plasma from DS (n = 10) and DR (n = 10) rats, presented as means ± SD. Analytes were log-transformed prior to analysis and Welch Satterthwaite method was used as necessary to satisfy statistical assumptions.

<sup>a</sup>Fold change is mean DS value in relation to mean DR value.

<sup>b</sup><i>t</i> statistics are absolute values.

<sup>c</sup>Analyte concentrations were converted to molarity prior to ratio determination.

HOMA-IR, Homeostasis model assessment-estimated insulin resistance.

IGF-1, insulin-like growth factor-1; IGFBP-3, IGF binding protein-3; IGF-1:IGFBP-3, ratio of IGF-1 to IGFBP-3.

CRP, C-reactive protein.

SHBG, sex hormone binding globulin; estradiol:SHBG, ratio of estradiol to SHBG; estradiol:progesterone, ratio of estradiol to progesterone; estradiol:progesterone:SHBG, ratio of estradiol to progesterone to sex hormone binding globulin.
Figure 1

A

Body Weight (g)

Days Post-Carcinogen

B

Tumor-free (%)

Days Post-Carcinogen

C

Multiplicity (#/rat)

Days Post-Carcinogen
Excess weight gain accelerates 1-methyl-1-nitrosourea-induced mammary carcinogenesis in a rat model of premenopausal breast cancer


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