Cancer Progression in the Transgenic Adenocarcinoma of Mouse Prostate Mouse Is Related to Energy Balance, Body Mass, and Body Composition, but not Food Intake

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Abstract
Calorie restriction can inhibit or delay carcinogenesis, reportedly due to a reduction in calorie intake rather than by concurrent changes in body mass and/or composition. Our objective was to test the hypothesis that body mass and/or composition have an important effect, independent of energy intake, on the benefits or hazards associated with calorie restriction or overeating, respectively. In the first experiment, transgenic mice that spontaneously develop prostate cancer [transgenic adenocarcinoma of mouse prostate (TRAMP)] were housed at 27°C or 22°C and pair fed the same diet for 21 weeks (95% of ad libitum intake at 27°C). In the second experiment, TRAMP mice were housed at 27°C or 22°C and fed the same diet ad libitum for 21 weeks. Despite a similar calorie intake, pair-fed mice at 27°C (PF27) were heavier (28.3 ± 3.3 versus 17.6 ± 1.6 g at 21 weeks; P < 0.001; mean ± SD) and had greater fat (6.4 ± 2.1 versus 1.9 ± 0.3 g; P < 0.001) and lean mass (P < 0.001) than pair-fed mice at 22°C. Furthermore, PF27 mice had greater levels of serum leptin (P < 0.001), lower levels of adiponectin (P < 0.05), and a greater frequency of prostatic adenocarcinoma (P < 0.05). In contrast, ad libitum–fed mice housed at 22°C consumed ~30% more calories than ad libitum–fed mice at 27°C, but there was no difference between groups in body composition or cancer progression. These results imply that the ability of calorie restriction to inhibit or delay cancer incidence and progression is mediated in part by changes in energy balance, body mass, and/or body composition rather than calorie intake per se, suggesting that excess calorie retention, rather than consumption, confers cancer risk. [Cancer Res 2007;67(1):417–24]

Introduction
The prevalence of obesity has markedly increased over the past 2 decades in American adults, and excess adiposity has become more and more recognized as a risk factor for cancer incidence and mortality (1, 2). The fundamental cause of obesity is a long-term imbalance in energy intake and expenditure (i.e., positive energy balance). Calorie restriction provides an effective strategy to combat this imbalance by limiting energy intake and, hence, reducing fat stores. In animal models, a 20% to 40% reduction in calorie intake has consistently shown an ability of calorie restriction to reduce body weight and adiposity (3) and delay several age-related diseases, including cancer (4, 5).

Despite the fact that the main phenotypic change observed in calorie-restricted animals is a reduction in body and fat mass, most investigations have concluded that the benefits of calorie restriction are mediated by a reduction in food intake per se rather than changes in body mass and/or composition (6–9). However, many of the effects of calorie restriction can be alternatively explained by changes in body composition rather than a decrease in food intake (10). For example, a reduction in body weight, and specifically body fat, can lead to improvements in insulin sensitivity (11), reductions in levels of proinflammatory cytokines (12, 13), and oxidative stress (14), all of which are related to cancer incidence and mortality (15–17).

Because restricting calories in mammals typically results in a reduction in body and fat mass, attempting to differentiate the benefits attributed to a reduction in food intake from concurrent changes in body mass and composition is extremely difficult. To date, the cancer-obesity link is based on mounting epidemiologic evidence (1, 18, 19), in vitro studies (20–22), and the assumption that obesity-related risk factors implicated in the etiology of cardiovascular disease and diabetes also raise cancer risk (10). However, there remains a lack of in vivo evidence that confirms that excess body weight, and specifically body fat, is a direct cause of cancer incidence and/or progression.

In an attempt to test the role of body mass and/or body composition versus food intake on cancer progression, we used small differences in ambient temperature (5°C) as a tool to manipulate energy expenditure in a mouse model of prostate cancer [transgenic adenocarcinoma of mouse prostate (TRAMP)]. Specifically, when ambient temperature is raised closer to the thermoneutral zone for mice (approximately 30–35°C), thermoregulatory demands, and hence, energy expenditure can be significantly reduced (23). Therefore, we hypothesized that under fixed food intake conditions, mice maintained slightly below thermoneutrality would expend less energy in thermoregulation, have greater gains in body and fat mass, and have more advanced prostate cancer than mice maintained at a lower ambient temperature (27°C versus 22°C). Thus, this experiment would test the hypothesis that body mass and/or composition, but not food intake, are the links between calorie restriction or obesity and cancer.

In the second experiment, we used the same housing temperatures but allowed the animals to feed ad libitum. We hypothesized that TRAMP mice maintained at 22°C would increase food intake in a manner that would offset the increased thermoregulatory demands and that both groups would then have similar body mass and composition throughout the experiment. The purpose of the second experiment was to determine whether increased food...
intake, without a difference in body mass and composition, would affect cancer progression. The results of these two studies show that a reduction in body, fat, and lean mass, without changing food intake, can significantly inhibit or delay carcinogenesis and that, in the absence of changes in body composition, increasing caloric intake by ~30% does not accelerate cancer progression. However, to what extent these results may be attributed to energy balance differences is not clear.

Materials and Methods

Transgenic animals. TRAMP mice on a pure C57BL/6 background were obtained from our breeding colony located at the University of Alabama at Birmingham (Birmingham, AL). Transgenic females were bred with nontransgenic males, and all breeders were maintained on a standard 12-h light/12-h dark photoperiod and fed standard rodent chow (Harlan Teklad, Madison, WI) and water ad libitum. After weaning, male pups were separated from females, and a tail biopsy was collected for determination of transgene incorporation by PCR as described previously (24). Subsequently, all male TRAMP mice were singly housed in cages measuring 28 × 13 × 13 cm on a standard 12-h light/12-h dark photoperiod in air- and humidity-controlled rooms. All work was approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Determination of ad libitum food intake. Initially, ad libitum food intake was determined in 7-week-old male TRAMP mice singly housed at 27°C in a temperature- and humidity-controlled incubator (model IS79SD, Powers Scientific, Inc., Pipersville, PA) for 4 weeks. Food consumption was determined by measuring the loss of food from the hopper of each animal over a 3-day period (one 3-day determination per week). The average 4-week food consumption was determined (2.32 g/d), and 95% of this amount (2.20 g/d) was used for the pair-feeding study.

Experiment 1 design: pair-feeding study. For the pair-feeding experiment, 7-week-old male TRAMP mice were randomly assigned to one of two groups, singly housed in a temperature- and humidity-controlled incubator at 27°C (PF27; n = 54) or singly housed in a temperature- and humidity-controlled room at 22°C (PF22; n = 54) for up to 21 weeks (28 weeks of age). Both groups were fed 2.20 g of a phytoestrogen-free diet (AIN-76A, Harlan Teklad) daily; food was placed in the food hopper of the animal each afternoon at ~1500 h. Any food remaining in the bedding or hopper after 24 h was removed, weighed, and recorded. In addition, body weights were recorded weekly for all animals.

Mice were removed from the experiment if one or more of the following criteria were met: (a) >50% of food (>1.10 g) remained in the hopper for 2 consecutive days coupled with signs of sickness, lethargy, or a palpable tumor; (b) weight loss exceeding 20% of the body weight from the previous week; or (c) a tumor diameter ≥20 mm in PF27 mice or ≥15 mm in PF22 mice. This smaller diameter was used for PF22 mice because they were considerably smaller than PF27 mice.

Experiment 2 design: ad libitum–feeding study. Seven-week-old male TRAMP mice were randomly assigned to one of two groups, singly housed at 27°C (AL27; n = 24) or singly housed at 22°C (AL22; n = 21) and fed the same diet ad libitum for up to 21 weeks (28 weeks of age).

Mice were removed early from this experiment if one or more of the following criteria were met: (a) weight loss exceeding 20% of the body weight from the previous week or (b) a tumor diameter ≥15 mm was detected. This diameter was chosen as the cutoff for this experiment because ad libitum–fed mice showed a propensity for their condition to deteriorate more quickly than pair-fed mice. Furthermore, a reduction in food intake was not used as an exclusion criterion because significant variability in food intake existed from week to week in these animals. Body weight was recorded weekly, and weekly measures of food intake were determined by measuring loss of food from the hopper of each animal over a 3-day period (one 3-day determination per week).

Body composition. All body composition scans for animals were done early in the morning. The first 60 animals included in the pair-feeding experiment [PF27 (n = 29) and PF22 (n = 31)] were anesthetized with isoflurane (2%), and body composition was assessed in vivo by dual-energy X-ray absorptiometry (DXA; GE Lunar PIXImus software version 1.45, Lunar, Madison, WI) at baseline and at 6, 12, 18, and 21 weeks in study. The remaining 48 pair-fed animals [PF27 (n = 25) and PF22 (n = 23)] and all animals in the ad libitum–feeding experiment were anesthetized i.p. with Nembutal (0.693 mg/g) for the collection of blood and assessment of body composition. For PF27, fat mass, soft lean tissue mass, bone mineral content (BMC), and bone mineral density (BMD) were determined as described previously (25). Briefly, mice were anesthetized and placed in a prostrate position on the imaging plate, and a total body scan was done in ~5 min. All analyses were done excluding the head.

Indirect calorimetry. Energy expenditure was assessed by indirect calorimetry at 12 weeks in study as reported by our laboratory previously (26). Energy expenditure was determined from O2 consumption and CO2 production using the equation of Weir (27). Total energy expenditure (TEE) was calculated as the sum of the energy expended over the measurement period, extrapolated to 24 h. Resting energy expenditure (REE) was estimated from the average energy expenditure over three nonconsecutive 10-min intervals during which energy expenditure was minimal. Non-REE (NREE; i.e., activity-related energy expenditure) was calculated as the difference between TEE and REE.

Data were collected for 22 h from a subset of PF27 (n = 5) and PF22 (n = 5) mice, respectively, from the pair-feeding study. A 22-h measurement period was used to allow time for the chambers to be cleaned and for the animals to be calibrated between experiments on consecutive days. Mice were placed in the chamber at ~1600 h, provided a weighed food pellet (2.20 g), and given ad libitum access to water during the measurement period. The mice were measured at the temperature at which they were housed (27°C or 22°C), and the photoperiod was set at 12 h light/12 h dark.

Blood and tissue collection. For mice anesthetized with Nembutal, immediately following the DXA scan, ~200 μL of blood were collected from the tail under anesthesia at 6 and 12 weeks in study and by cardiac puncture at termination for assessment of hormone and cytokine levels. Serum was separated from blood samples by centrifugation and stored at ~70°C until further analysis. In addition, tissue weights were obtained at necropsy for the urogenital system (prostate, bladder, and seminal vesicles) and testes.

Serum measures. Total testosterone was measured in duplicate from serum collected at 6 and 21 weeks in study using a RIA kit (Diagnostic Systems Laboratories, Webster, TX). Serum at 12 weeks in study was assessed in duplicate for adiponectin, interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and leptin. Adiponectin was measured by RIA (LINCO Research, St. Louis, MO); IL-6, TNF-α, and leptin were measured by the Luminex(®) instrument (Luminex Corp., Austin, TX) using a LINCOplex immunoassay panel (LINCO Research). Serum concentrations of IL-6 and TNF-α are not presented because the values obtained were below the lower detection limit of the assay (12.2 pg/mL). The intra-assay coefficient of variation for reported assays was 7.4% for testosterone, 2.3% for adiponectin, and 4.2% for leptin.

Histopathology. To assess cancer progression and metastasis, a portion of the dorsolateral prostate (DLP) and lymph nodes were collected for histologic analysis. The DLP was placed in an acid-alcohol fixative containing 96% ethanol, 1% glacial acetic acid, and 3% distilled water at 4°C overnight as described by Folkvord et al. (28), and the lymph nodes were placed in phosphate-buffered formalin overnight. The fixed tissue was then embedded in paraffin, and 5 μm sections were mounted onto slides. All DLP sections were stained with Gomori trichrome staining, and lymph node sections were stained with H&E.

Pathologic changes in the DLP were scored as described previously (29, 30) on a 0 to 6 scale by a pathologist who was blinded to the experiment (L.A.E.). Scores 1, 2, and 3 indicated normal tissue, low-grade prostatic intraepithelial neoplasia (PIN), and high-grade PIN, respectively; scores 4, 5, and 6 indicated well-differentiated, moderately-differentiated, and poorly-differentiated prostatic adenocarcinoma, respectively. The presence or absence of cancerous cells in periaortic lymph nodes was used to determine if metastasis of the primary tumor had occurred.
**Statistical analyses.** All statistical analyses were carried out separately for the pair-feeding study and *ad libitum*—feeding study because they were not run concurrently and protocols differed slightly. In both experiments, food intake, body weight, and body composition were analyzed by repeated measures ANOVA and followed up with planned contrasts when appropriate. Mice that were removed early from the experiment were not included in the repeated measures analyses. Serum concentrations, energy expenditure, and tissue weights between groups were compared with Student’s *t* test, and χ² analysis was used to determine if a significant difference existed among groups in the distribution of pathologic scores of the DLP and for the presence or absence of metastasis to lymph nodes. For the χ² analysis of pathologic score distribution, animals were assigned to one of three groups: normal prostate (score, 1), PIN (score, 2–3), and adenocarcinoma (score, 4–6). All analyses were done using Statistical Analysis System version 8.0 (SAS Institute, Inc., Cary, NC) and considered statistically significant when *P* < 0.05.

**Results**

**Experiment 1: Pair-Feeding Study**

PF27 mice consumed less food but had greater body mass. Despite our intention for pair-fed mice to have the same food intake (2.20 g/d), PF27 mice consumed less food (Fig. 1A). Although this difference was very small (≤0.1 g/d), it was nevertheless significant (*P* < 0.05). Regardless of this small reduction in food intake, PF27 mice were significantly heavier than PF22 animals, differing by ~11 g at the end of the study (*P* < 0.001; Fig. 1B).

PF27 mice had greater fat mass, lean mass, and bone content. In addition to weighing less than PF27 mice, PF22 animals had less fat mass (Fig. 1C), lean mass (Fig. 1D), and percentage fat (Fig. 1E) as measured by DXA (*P* < 0.001). Specifically, PF22 mice lost a small but significant amount of lean mass after 6 weeks of treatment but fat mass was unchanged. However, subsequent measures of body composition with DXA indicated that PF22 mice began to gain lean mass whereas fat mass decreased (*P* < 0.001; Fig. 1C and E).

With regard to bone, despite no differences in measures of BMD and BMC between groups at the beginning of the study, PF27 mice had a more substantial increase in these measures throughout the remainder of the experiment. Specifically, compared with their leaner counterparts, BMD and BMC in PF27 were significantly greater at 6, 12, 18, and 21 weeks in study for BMD (0.044 ± 0.001 versus 0.042 ± 0.002 g/cm² at 21 weeks; *P* < 0.001; mean ± SD) and BMC (0.341 ± 0.100 versus 0.320 ± 0.030 g at 21 weeks; *P* < 0.001), respectively.

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**Figure 1.** Phenotypic characteristics for pair-fed mice housed at 27°C (PF27; *n* = 44) or 22°C (PF22; *n* = 49). Measurements were taken either weekly or at predetermined intervals for (A) food intake, (B) body mass, (C) fat mass, (D) lean mass, and (E) percentage fat. F. TEE was measured in a subset of PF27 (*n* = 5) and PF22 (*n* = 5) mice at 12 wks of treatment. Columns, mean; bars, SE. *, *P* < 0.05, significantly different from PF22 mice; †, *P* < 0.001, significantly different from PF22 mice.
Pair-fed mice had similar resting, nonresting, and TEE. At 12 weeks of treatment, PF27 and PF22 mice had similar absolute TEE (P = 0.62; Fig. 1F), REE (3.99 ± 1.65 versus 3.29 ± 1.00 Kcal/d; P = 0.44; mean ± SD), and NREE (3.00 ± 0.23 versus 3.25 ± 0.58 Kcal/d; P = 0.40). Furthermore, after adjusting for lean mass, TEE remained similar between PF27 and PF22 mice (6.84 ± 1.44 versus 6.69 ± 1.44 Kcal/d; P = 0.89) as did REE (4.37 ± 1.46 versus 2.91 ± 1.46 Kcal/d; P = 0.75) and NREE (2.47 ± 0.45 versus 3.77 ± 0.45 Kcal/d; P = 0.48).

PF27 mice had greater levels of serum leptin and lower levels of adiponectin. At 12 weeks of treatment, serum leptin was greater in PF27 mice, which had more body fat (P < 0.001; Fig. 2A), and leptin was significantly related to fat mass in the PF27 group (r = 0.52; P < 0.01) but not in the PF22 group (r = −0.11; P = 0.65). Adiponectin was significantly greater in PF22 mice (P < 0.05; Fig. 2B), but adiponectin was not associated with fat mass in the PF27 group (r = 0.05; P = 0.83) or in the PF22 group (r = −0.07; P = 0.77).

Testes from PF27 mice were heavier, but no difference in serum testosterone. Testes weights were significantly heavier in PF27 mice than in PF22 mice at 21 weeks of treatment (P < 0.05; Fig. 3A). In contrast, the concentration of serum total testosterone was not significantly different between groups at 6 weeks (P = 0.89; Fig. 3B) or 21 weeks of treatment (P = 0.57; Fig. 3B).

PF27 mice had more advanced prostate cancer, but no difference in metastasis. Based on the exclusion criterion described in Materials and Methods, 10 of 54 (18.5%) PF27 mice were removed early from the study: 7 due to a tumor diameter ≥20 mm and 3 due to a weight loss exceeding 20% of the weight from the previous week. Five of 54 (9.3%) PF22 animals were removed early from the study: all due to a tumor diameter ≥15 mm. Despite the fact that 2-fold more PF27 mice had to be removed early from the study than PF22 mice, there was no significant difference in number of animals removed by χ² analysis (P = 0.16).

The urogenital system (prostate, bladder, and seminal vesicles) from PF27 mice was significantly heavier than PF22 animals (2.4 ± 2.0 versus 1.0 ± 1.7 g; P < 0.001; Fig. 4A). Furthermore, PF27 mice had a significant shift in the distribution of pathologic scores toward prostatic adenocarcinoma, whereas PF22 mice displayed a higher frequency of normal prostate (score = 1) and lower incidence of prostatic adenocarcinoma (score = 4–6; P < 0.05; Fig. 4B). However, there was no difference in metastasis to lymph nodes between PF27 (92%) and PF22 (90%) mice with poorly differentiated adenocarcinoma (score = 6; P = 0.85).

Experiment 2: Ad libitum–Feeding Study
AL22 mice consumed more food but weighed less than AL27 mice. AL22 mice consumed ~30% more food than AL27 mice (P < 0.05; Fig. 5A). Despite this large difference in food intake, AL22 mice weighed 2 to 3 g less than AL27 mice during the latter half of the study, and this difference was significant at most time points (P < 0.05; Fig. 5B).
No difference in fat or lean mass, but AL27 mice had greater BMC. There was no significant difference in fat mass (P = 0.06; Fig. 5C), lean mass (P = 0.47; Fig. 5D), or percentage fat (P = 0.10; Fig. 5E) between ad libitum–fed mice. BMD was similar between ad libitum–fed mice throughout the experiment (0.045 ± 0.001 versus 0.045 ± 0.001 g/cm² at 21 weeks; P = 0.30; mean ± SD), but AL27 mice had significantly greater BMC after 12, 18, and 21 weeks of treatment (0.387 ± 0.034 versus 0.355 ± 0.024 g at 21 weeks; P < 0.01).

No difference in testes weight or serum testosterone between ad libitum–fed mice. There was no significant difference for mean testes weights between AL27 and AL22 mice at 21 weeks of treatment (143.0 ± 23.9 versus 143.3 ± 12.5 mg; P = 0.97). Furthermore, the concentration of total testosterone was not significantly different between AL27 and AL22 mice at 6 weeks of treatment (0.347 ± 0.16 versus 0.355 ± 0.20 ng/mL; P = 0.91)

Discussion

The dogma for years has held that a reduction in body weight and body fat from calorie restriction is merely a by-product of consuming fewer calories, with no real impact on outcomes of interest, such as cancer and longevity (6, 7, 9, 10, 31, 32). Indeed, the importance of body fat has been widely discounted due to the classic viewpoint of adipose tissue as only an inert storage depot for triglycerides, and historical studies which suggested that the beneficial component of calorie restriction was the result of a reduction in food intake rather than body fat (6,7,9). However, the present study has shown that the retardation of cancer initiation and progression in the mouse prostate is not attributable to the amount of calories consumed and may instead be mediated by changes in energy balance, body weight, and adiposity.

To our knowledge, these data are the first to show that a reduction in food intake is not required for the beneficial effects of calorie restriction with regard to cancer prevention. In agreement with these findings, a previous study by Bluher et al. (33) reported that fat-specific insulin receptor knockout (FIRKO) mice had similar food intake as control animals but were leaner and longer lived, suggesting that body fat may play an important role in longevity. In contrast, Bertrand et al. (6) reported that fat mass was not related to lifespan in ad libitum–fed rats but was positively correlated with lifespan in calorie-restricted rats. A later study by Harrison et al. (7) found that calorie-restricted ob/ob mice were longer lived than ad libitum–fed wild-types despite having nearly twice as much body fat. Indeed, these studies seem to provide strong evidence against a role for body fat in the determination of lifespan, but the validity of the conclusions reached by these studies is one of continual debate (10,32).

Since the discovery of leptin in 1994 (34), the perception of adipose tissue as an inert storage depot has quickly evolved to that of a highly active endocrine organ, capable of secreting several proinflammatory cytokines, also referred to as “adipokines” (35). Many of these factors secreted from adipocytes and/or associated macrophages have provided viable mechanisms that may directly link obesity to increased cancer incidence and progression and not merely coincidental with the disease (10). Specifically, leptin, TNF-α, IL-6, heparin-binding epidermal growth factor, vascular endothelial growth factor, etc., may play a role in the pathology of cancer by promoting angiogenesis, inflammation, cell proliferation, and insulin resistance (21).

Leptin seems to be the most well studied adipokine due to its established role in the regulation of food intake and energy expenditure (36). More recently, leptin has garnered attention as a...
potent growth factor involved in the progression of breast (20) and prostate cancer (21, 22). In addition, leptin has been implicated in inducing oxidative stress (37) and aromatase expression (38), which converts androgens to estrogens. It has been clearly shown that the concentration of circulating leptin is positively related to the amount and location of adipose tissue (39), making leptin an attractive target for studying the obesity-cancer link. With regard to this study, leptin was profoundly elevated and significantly associated with fat mass in PF27 mice, whose fat content \(>3\)-fold that of their lean counterparts at 22°C, and may have contributed to the increased cancer progression in this group. Additionally, low concentrations of leptin in PF22 mice may have limited cancer progression.

Adiponectin, which is an adipokine secreted from adipose tissue, is paradoxically decreased in obesity (40, 41). Adiponectin may be unique in that it seems to protect from cancer by opposing proinflammatory mediators and cancer cell growth (42) while enhancing insulin function (40). Indeed, excess total and visceral fat are known to diminish circulating adiponectin and insulin sensitivity, which can lead to a compensatory increase in insulin secretion (43) and the overstimulation of inflammatory and growth-promoting pathways (44). Recent epidemiologic data support a possible inhibitory role of adiponectin on carcinogenesis by showing an inverse association between circulating adiponectin and cancer risk and progression (45–47). For example, Goktas et al. (46) found that serum adiponectin levels were lower in patients with prostate cancer and were negatively associated with the histologic grade of the disease. Because serum adiponectin was significantly greater in PF22 mice, which were leaner than PF27 mice, a reduction in body fat may have conferred additional protection from prostate cancer in these animals by diminishing serum leptin and augmenting serum adiponectin levels.

The initiation and progression of prostate cancer in the TRAMP mouse at the outset is an androgen-dependent process (48). Because calorie restriction and fasting have been shown to reduce testosterone levels (49), one possible confounder to our results may have been due to changes in circulating testosterone. Therefore, we measured testes weights and serum testosterone levels to determine if these measures were lower in PF22 mice. We found that the testes from PF22 animals weighed significantly less at 21 weeks of treatment, but this difference was small in relation to PF27 animals \((\pm 7\%)\). However, there was no difference between groups in serum testosterone levels measured at 6 weeks in study \((\sim 13\) weeks old), a time when testosterone levels would be
expected to play a role in initiation, or at 21 weeks of treatment. Thus, the difference in cancer progression observed in experiment 1 was not likely due to a deficiency in circulating testosterone (i.e., castration effect; ref. 48) to stimulate transgene expression in PF22 mice, although a difference in testosterone before 6 weeks of treatment cannot be ruled out. Instead, these data suggest that the retardation of cancer incidence and progression in PF22 mice was related to changes in energy balance, body mass, and/or composition.

The results of this study strongly suggest that the beneficial effects of calorie restriction are mediated by mechanisms other than a reduction in food intake, but to what extent these effects are the direct result of changes in body mass and/or composition versus changes in energy balance remains unclear. In the pair-feeding paradigm, food intake was clamped at 2.2 g/d, an amount that is only 5% less than what TRAMP mice consume at ad libitum at 27°C, but is ~27% less than what they consume ad libitum at 22°C. The disparate amount of voluntary food intake consumed is due to the decreased thermoregulatory demands on mice when housing temperature is raised closer to thermoneutrality (approximately 30–35°C).

Therefore, PF22 mice, which were housed at a lower ambient temperature (22°C) than PF27 mice and had greater thermoregulatory demands, were forced to partition a greater proportion of energy to “defend” body temperature. Because 2.2 g/d was not a sufficient amount of energy consumed by PF22 mice to support initial body mass and thermoregulation, these animals entered a brief period (~2 weeks) of negative energy balance (energy in < energy out) during which body mass was sufficiently reduced to bring them back into energy balance (energy in = energy out). They then maintained this lower mass for the duration of the study.

In contrast, PF27 mice, who were housed in a more thermoneutral environment (27°C), required less energy to maintain body temperature due to decreased thermoregulatory demands and had greater body (~8 g) and lean mass (~5 g) than PF22 mice. In addition, PF27 mice consumed less food than PF22 mice, but energy intake for PF27 mice was still in excess of energy expenditure, which resulted in calorie retention and weight gain. Interestingly, 24-h energy expenditure was not different between groups because animals remained weight stable during the measurement period. However, if weight change over the course of the study is used as a proxy of long-term energy balance, it is evident that PF27 mice expended slightly less energy than PF22 mice because these animals consumed less food but still gained weight.

In the ad libitum–feeding experiment, AL22 animals consumed ~30% more calories than AL27 mice to compensate for the additional energy expended in thermoregulation. Despite consuming more food, AL22 mice had less body mass and BMC at some time points than AL27 mice, but fat and lean mass were not significantly different. Furthermore, cancer progression was not different between ad libitum–fed animals. Thus, both experiments imply that the prevention of excess calorie retention and obesity are important mediators of the calorie restriction pathway.

Because our results cannot “tease” apart the contribution of body mass and/or composition from energy balance on cancer prevention, more studies working toward a better understanding of the cancer-preventive mechanisms mediated by body mass and/or composition and energy balance are needed. In addition, our study suggests that increased energy expenditure, via thermoregulation, can result in beneficial effects similar to those observed with calorie restriction (i.e., reduced energy intake). Therefore, from a public health standpoint, it would be interesting to determine if a similar benefit could be achieved with exercise, which can also increase energy expenditure and limit weight gain. In support of this notion, a recent study by Colbert et al. (50) found that an exercise-induced negative energy balance reduced polyph burden in male APC<sup>Min</sup> mice. Taken together, the interaction of obesity, physical activity, and calorie restriction on cancer risk offers an exciting and important challenge for both the clinician and laboratory scientist.

In summary, these data show that despite a similar calorie intake, PF22 mice weighed significantly less, and had lower fat and lean mass than PF27 mice due to increased energy expended in thermoregulation by PF22 mice. PF27 mice, which had more body fat, also had greater levels of serum leptin and lower levels of serum adiponectin. Furthermore, histologic investigation of the DLP indicated a greater frequency of normal prostate in PF22 mice, whereas PF27 mice had a greater frequency of prostatic adenocarcinoma. In contrast, AL22 mice consumed more calories.

![Figure 6. Urogenital system weights and histologic evaluation of the DLP from ad libitum–fed mice housed at 27°C (AL27) or 22°C (AL22). A, scatter plot illustrating the urogenital system (prostate, bladder, and seminal vesicles) weight obtained from each AL27 (n = 24) and AL22 (n = 21) animal at necropsy. Line, mean weight for the group. There was no significant difference between groups (P = 0.35). B, distribution of pathologic scores for the DLP from AL27 (n = 24) and AL22 (n = 21) mice. Pathologic changes in the DLP were blindly scored using the following 1 to 6 scale: scores 1, 2, and 3 indicated normal tissue, low-grade PIN, and high-grade PIN, respectively; scores 4, 5, and 6 indicated well-differentiated, moderately-differentiated, and poorly-differentiated prostatic adenocarcinoma. There was no significant difference between groups (P = 0.75).](https://www.aacrjournals.org/doi/10.1158/0008-5472.CAN-06-1244)
than AL27 mice, but there was no significant difference in fat mass, lean mass, or cancer progression between groups. These results imply that the ability of calorie restriction to inhibit or delay cancer incidence and progression is mediated in part by changes in energy balance, body mass, and/or composition rather than calorie intake per se, suggesting that excess calorie retention, rather than consumption, confers cancer risk.

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