Effects of Castration on Insulin Levels and Glucose Tolerance in the Mouse Differ From Those in Man

Takamitsu Inoue,[1,2] Mahvash Zakikhani,[1] Stéphanie David,[1] Carolyn Algire,[1,2]
Marie-José Blouin,[1] and Michael Pollak[1,2]*

[1]Lady Davis Institute for Medical Research of the Jewish General Hospital, Montreal, Quebec, Canada
[2]Department of Oncology, McGill University, Montreal, Quebec, Canada

BACKGROUND. Plasma insulin concentration is increased in prostate cancer patients during androgen deprivation therapy (ADT) and hyperinsulinemia has been associated with aggressive prostate cancer behavior. To investigate the possible role of castration-induced hyperinsulinemia as a mechanism that may attenuate the beneficial effects of ADT in patients with prostate cancer, a murine model would be useful. We therefore investigated long-term metabolic effects of castration in several mouse models.

METHODS. We studied the long-term influence of castration on energy intake, body weight, glucose tolerance, plasma-insulin, plasma insulin-like growth factor-1 (IGF-1), plasma adiponectin, and plasma leptin in C57BL/6, Swiss nu/nu, and CB17 scid mice receiving various diets. In each case, mice were randomized to have either bilateral orchiectomy or a sham operation.

RESULTS. Energy intake, body weight, blood glucose levels in glucose tolerance test, plasma insulin, plasma IGF-1, and plasma leptin level in all had a trend to be decreased in castrated as compared to sham operated mice. Plasma adiponectin level was increased in the castrated mice.

CONCLUSIONS. The effects of castration on glucose, insulin, and related markers in several mouse models studied does not coincide with clinical observations; further studies in this area will require clinical research and/or the use of alternate models such as the dog.

KEY WORDS: prostate cancer; castration; insulin; IGF-1; metabolic syndrome; mouse

INTRODUCTION

Prostate cancer is the most frequent malignancy in men with >190,000 new cases and >27,000 deaths estimated in 2009 in the United States [1]. Androgen deprivation therapy (ADT) with a gonadotropin-releasing hormone (GnRH) agonist or bilateral orchiectomy is the standard therapy for patients with metastatic or locally advanced prostate cancer [2]. ADT is initially effective in approximately 70–80% of untreated metastatic prostate cancer patients [3]. However, virtually all of these patients eventually develop castration resistant prostate cancer, and die whether ADT is continued or not.

There is evidence that ADT leads to increased fat mass, body mass index (BMI), and other components of ''metabolic syndrome'' [4–6]. The risk of new onset type II diabetes is increased in men undergoing

ADT [7–11]. Moreover, there is evidence that plasma insulin concentration is increased in men during ADT [6,12–14].

In this context, it is important to note that a growing body of population studies suggests that obesity is associated with risk of prostate cancer progression [15–19]. Candidate mediators of the adverse effect of

obesity on prostate cancer prognosis include insulin, insulin-like growth factor 1 (IGF-1), and adipokines such as adiponectin and leptin [20]. We observed that high BMI and high plasma concentration of C-peptide, a marker of insulin secretion, at or prior to the time of prostate cancer diagnosis were each strongly associated with shorter prostate cancer-specific survival [19]. There is additional evidence that increased serum insulin level is associated with increased risk of prostate cancer diagnosis [21,22] and recurrence [23]. Furthermore, we [24] and others [25] have showed that men with high plasma IGF-1 level have an increased risk of prostate cancer and of advanced-stage prostate cancer [26].

The possibility that insulin and/or IGF-1 levels could influence prostate cancer risk and/or prognosis is plausible because the insulin as well as the IGF-1 receptor is expressed on primary human prostate cancers [27]. An in vivo study showed that a high carbohydrate diet is associated with increased tumor growth related to hyperinsulinemia and elevated IGF-1, and with activation of signaling pathways distal to the insulin receptor of the tumor cells [28]. However, no in vivo models to study the influence of castration-induced hyperinsulinemia on prostate cancer biology have been reported.

In order to investigate the possible role of androgen deprivation-induced hyperinsulinemia as a factor that might attenuate the benefits of ADT and lower the development of castration resistant prostate cancer, a murine model would be useful. We therefore investigated long-term effects of androgen deprivation on energy intake, body weight, glucose tolerance, serum insulin, IGF-1, and adipocytokine levels in several mouse models.

MATERIALS AND METHODS

Animals and Diets

We studied the influence of castration on body weight and selected endocrine endpoints in several groups of mice. In each case, mice were randomized to have either bilateral orchiectomy or a sham operation. The groups studied include [A] male C57BL/6 mice aged 6 weeks (Charles River, Saint-Constant QC, Canada) fed with diet 3, or [B] male C57BL/6 mice aged 6 weeks fed with diet 4, or [C] male C57BL/6 mice aged 8 months (Charles River) fed with diet 2, or [D] male Swiss nu/nu mice aged 6 weeks (Taconic Farms Inc., Hudson, NY) fed with diet 3, or [E] male Swiss nu/nu mice aged 6 weeks fed with diet 4, or [F] male CB17 scid mice aged 6 weeks (Taconic Farms Inc.) fed with diet 1. Diets compositions of diet 1 (Ren's Feed and Supplies Ltd., Oakville, ON, Canada; #5TEE), diet 2 (Harlan Teklad Ltd., Madison, WI; #2018), diet 3 and 4

TABLE I. Diet Compositions

	Diet 1	Diet 2	Diet 3	Diet 4
Energy (kcal/g)	3.9	3.4	5.1	5.1
% Weight				
Carbohydrate	69.8	50.0	47.5	11.4
Fat	5.0	6.0	23.8	22.8
Protein	15.0	18.8	17.9	51.5
% Energy contribution				
Carbohydrate	72.6	60.0	40.0	10.0
Fat	11.7	17.0	44.9	44.7
Protein	15.6	23.0	15.1	45.1

(Ren's Feed and Supplies Ltd; #5381 and #5382) were shown in Table I. Mice were allowed ad libitum access to food and water from the commencing of the diets to the time of euthanasia. Mice were placed in individual cages to avoid fighting. Diets were commenced at the same time as the mice underwent castration. Uneaten food was weighed to allow calculation of grams and kcal consumed. Mice were weighed every week and weight gain rate was calculated based on the weight before castration as a baseline. All experiments performed were approved by the McGill University Animal Care Committee.

Bilateral Orchiectomy

Mice were anesthetized using isoflurane. Abdominal wall including skin and muscle layer was cut using scissors. Testicular fat pads were grasped and pulled using fine forceps. Blood vessels proximal to the testis avoiding vas deferens were tied using 6–0 silk (Ethicon, Somerville, NJ) and testis with testicular fat pads was excised. Abdominal muscle wall was sutured using 6–0 silk and skin was closed using AutoClips (MikRon Precision, Gardena, CA). In the sham operation, only the testicular fat pads were excised avoiding the testicular vessels. AutoClips were removed 2 weeks after the operation.

Glucose Tolerance Test

Mice were starved for 16 hr before glucose tolerance test. Blood glucose concentration was measured before, 30, 60, and 90 min after the intraperitoneal (IP) injection of the 1.5 g/kg glucose using the One Touch Ultra glucometer and test strips.

Enzyme-Linked Immunosorbent Assay

Plasma for the measurement of insulin, IGF-1, adiponectin, and leptin concentration was collected from saphenous vein using heparinized micro-hematocrit capillary tubes. Heparinized microhematocrit capillary tubes were centrifuged 11,700 rpm for 3 min using

Autocrit Ultra (Becton Dickinson, Franklin Lakes, NJ) to collect the plasma and stored in $-80°C$ freezer until the measurement. Plasma testosterone, insulin, IGF-1, adiponectin, and leptin concentrations were measured using a testosterone enzyme-linked immunosorbent assay (ELISA) kit (Immuno-biological Laboratories, Inc, Minneapolis, MN; #IB79106), a rat/mouse insulin ELISA kit (Millipore, St. Charles, MO; #EZRMI-13K), a mouse/rat IGF-1 ELISA kit (Diagnostic systems laboratories Inc, Webster TX; #DSL-10-29200), a rat/mouse adiponectin ELISA kit (Millipore; #EZMADP-60K) and a rat/mouse leptin ELISA kit (Millipore; #EZML-82K).

Statistics

Data are presented as means $\pm$ SEM. The distribution of variables was tested for normality. Significant difference between the sham operation group (non-castrated mice) and the bilateral orchiectomy group (castrated mice) were evaluated using General Linear Model or Mixed Procedure. A one-way repeated measures analysis of variance-covariance model (ANOVA-ANCoVA) was used to determine between-group differences and within-group changes over time.

Statistical analyses were performed using Statistical Analysis System software, version 9.2 (SAS Institute, Cary, NC), with P-values <0.05 considered significant.

RESULTS

Energy Intakes

Energy intake of the castrated mice was significantly less than that of non-castrated in experiments [E] and [F] ($P = 0.039$ and $P = 0.0001$, respectively). Castrated mice consumed 9–32% less energy than non-castrated mice in these significant groups. There was no significant difference in energy intake in experiments [A]–[D] (Fig. 1).

Weight Gain

Body weight gain rate of the castrated mice were significantly less than that of non-castrated in experiments [C], [D], [E], and [F] ($P = 0.024$, $P = 0.034$, $P = 0.023$, and $P < 0.0001$, respectively). Castrated mice had 9–20% less weight than non-castrated mice in these significant groups. There was no significant difference in body weight gain in experiments [A] and [B] (Fig. 2).
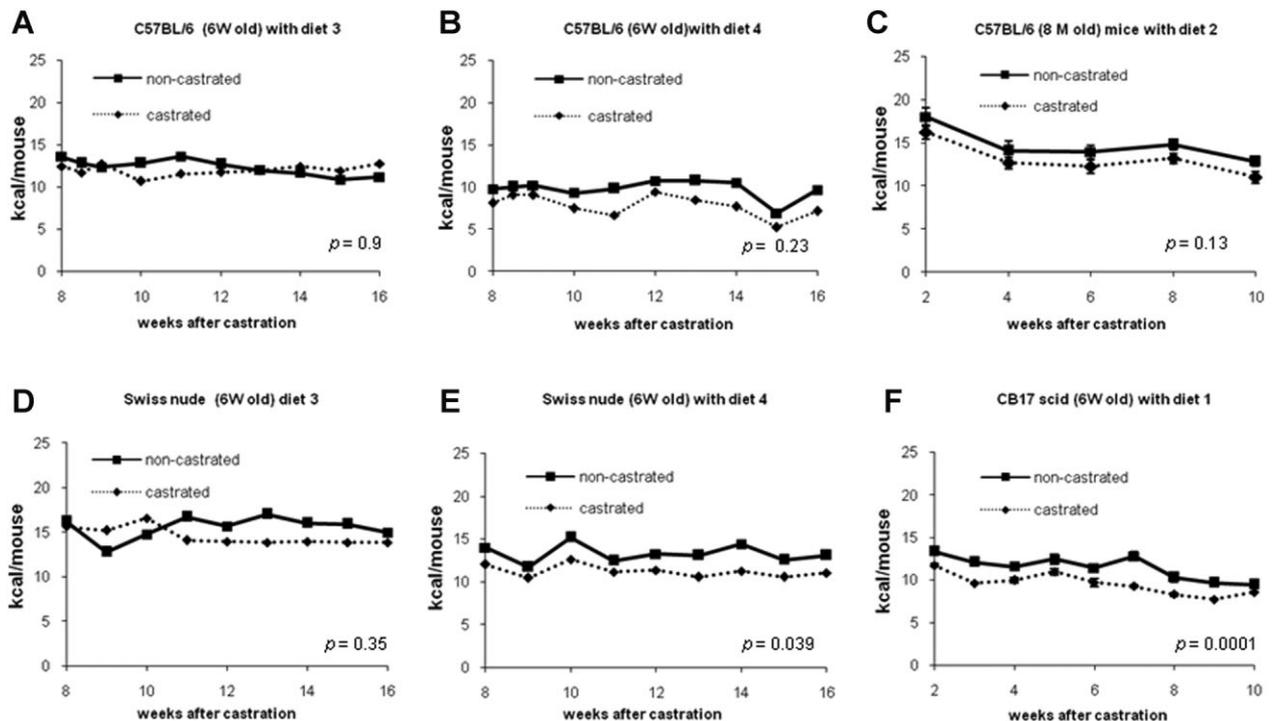
Fig. 1. Comparison of energy consumption per mouse between castrated and non-castrated. **A**: C57BL/6 aged 6 weeks with diet 3. No difference was observed. **B**: C57BL/6 aged 6 weeks with diet 4. No difference was observed. **C**: C57BL/6 aged 8 months with diet 2. No difference was observed. **D**: Swiss nude aged 6 weeks with diet 3. No difference was observed. **E**: Swiss nude aged 6 weeks with diet 4. Non-castrated consumed more than castrated ($P = 0.039$). **F**: CB17 scid aged 6 weeks with diet 1. Non-castrated consumed more than castrated ($P < 0.0001$).
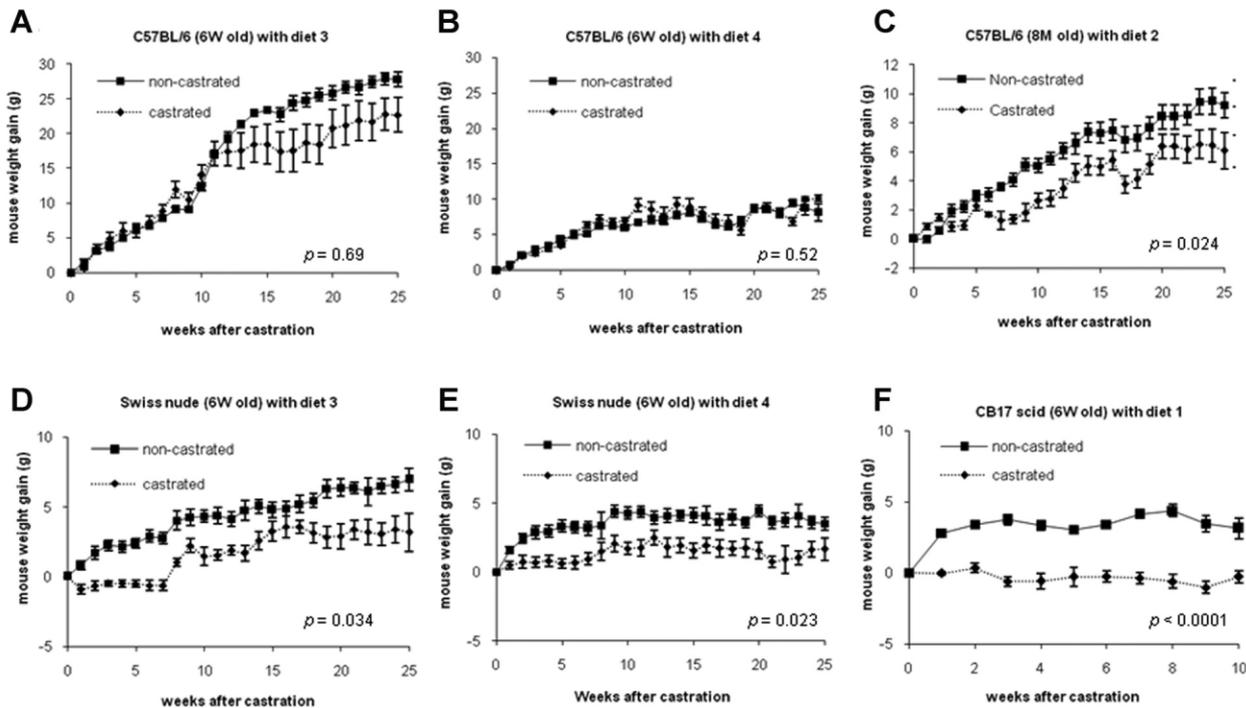
Fig. 2. Comparison of mouse body weight between castrated and non-castrated. **A**: C57BL/6 aged 6 weeks with diet 3. No difference was observed. **B**: C57BL/6 aged 6 weeks with diet 4. No difference was observed. **C**: C57BL/6 aged 8 months with diet 2. Non-castrated were heavier than castrated ($P = 0.024$). **D**: Swiss nude aged 6 weeks with diet 3. Non-castrated were heavier than castrated ($P = 0.034$). **E**: Swiss nude aged 6 weeks with diet 4. Non-castrated were heavier than castrated ($P = 0.023$). **F**: CBI7 scid aged 6 weeks with diet I. Non-castrated were heavier than castrated ($P < 0.000I$).

Glucose Tolerance

Blood glucose levels in glucose tolerance test in castrated mice were significantly lower than those of non-castrated mice in experiment [C] and [D] ($P = 0.003$ and $P = 0.014$). Although blood glucose levels tended to be higher in non-castrated mice, there was no significant difference in blood glucose levels between castrated and non-castrated mice in experiment [A], [B], [E], and [F] (Fig. 3).

Hormonal Measurements

The efficacy of castration was confirmed by testosterone measurements, which were 0.968 ± 0.285 ng/ml in the non-castrated mice, and 0.143 ± 0.015 ng/ml in the castrated mice, as expected (n = 33 vs. 34, $P = 0.006$). The remaining data are summarized in Table II. There were no consistent changes in insulin levels associated with castration across the experimental groups. In the cases where castration influenced insulin levels, it was associated with lower rather than the hypothesized higher levels; also, where castration had an influence on IGF-1 levels, it was associated with lower IGF-1 level. There was a consistent increase in adiponectin levels, which was significant in the experiments with immunocompetent mice, but the effect of castration on leptin levels varied with mouse strain and diet.

DISCUSSION

In humans, ADT leads to body composition and hormonal changes with similarities of metabolic syndrome and type II diabetes [29]. Smith et al. [30] have reported that mean body weight increased by 1.8% and fat mass by 11.0% after 12 months of ADT for the patients with non-metastatic prostate cancer. They have shown also that mean serum insulin concentration increased by 25.9% after 12 weeks of ADT [6,12]. Another report revealed that fasting serum glucose and insulin level in the patients who were treated with ADT for at least 12 months were 35.1% and 104% higher than that of age-matched men [13]. ADT for men with prostate cancer were associated with an increased risk of type II diabetes [7,10,11] using both GnRH agonist treatment and bilateral orchiectomy [8,9].

Androgen deprivation-induced features of metabolic syndrome have been observed in both cat and dog models. In cats, neutering was observed to be a factor associated with obesity [31]. Neutered male cats gained
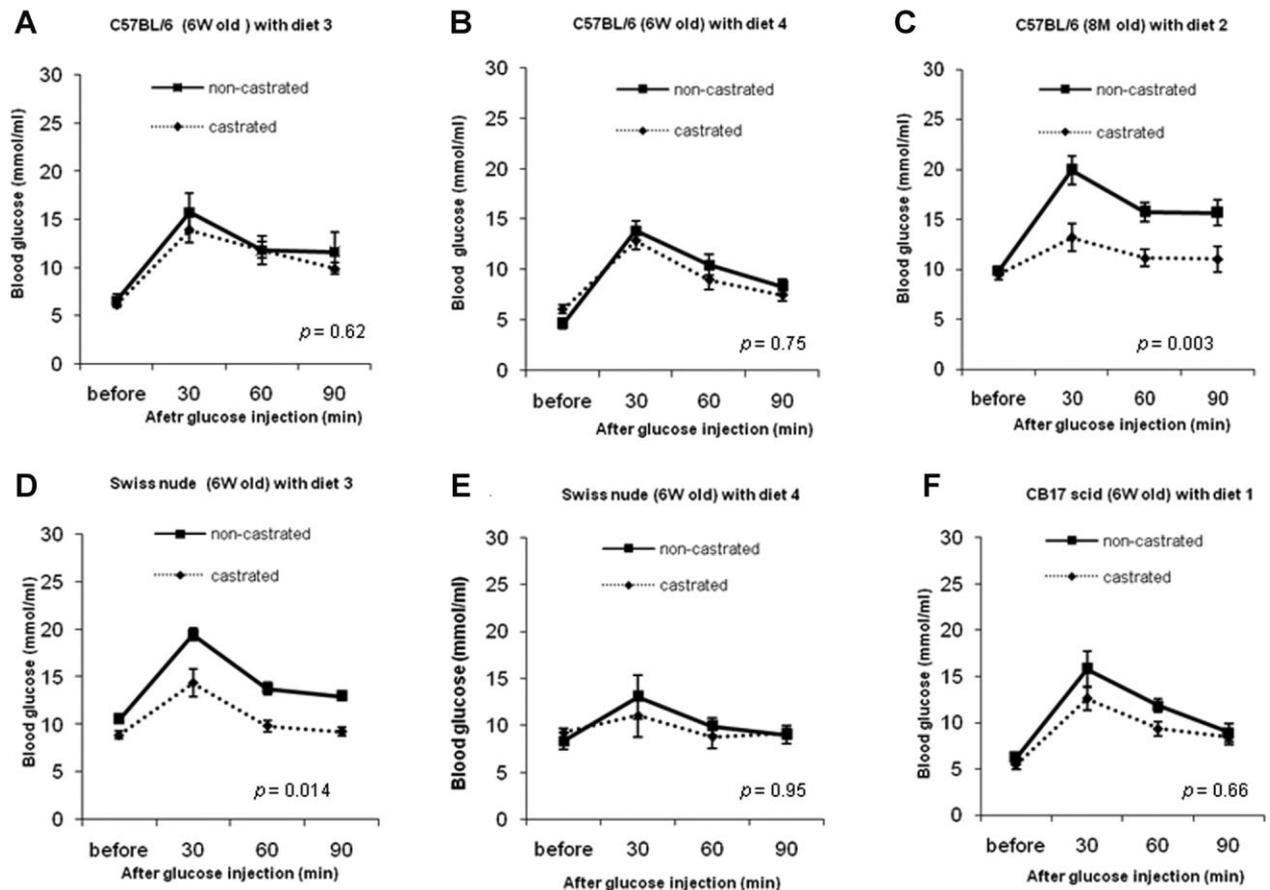
Fig. 3. Glucose tolerance test between castrated and non-castrated mice. Blood glucose concentration was measured before, 30, 60, and 90 min after the IP injection of the 1.5 g/kg glucose. **A**: C57BL/6 aged 6 weeks with diet 3. No difference was observed. **B**: C57BL/6 aged 6 weeks with diet 4. No difference was observed. **C**: C57BL/6 aged 8 months with diet 2. Non-castrated had higher blood glucose level than castrated ($P = 0.003$). **D**: Swiss nude aged 6 weeks with diet 3. Non-castrated had higher blood glucose level than castrated ($P = 0.014$). **E**: Swiss nude aged 6 weeks with diet 4. No difference was observed. **F**: CB17 scid aged 6 weeks with diet 1. No difference was observed.

18.4% more weight than entire males in 3 months [32]. Plasma insulin and leptin concentration of neutered cats were also significantly increased [33,34]. Neutered dogs are more likely to be overweight [35,36].

Murine models are convenient for allografts, xenografts, and transgenic studies of prostate cancer, but metabolic effects of castration in mice have not been well defined. Two studies reported that castration causes loss of body weight and muscle weight in mice, but did not include hormone measurements [37,38]. In rats, it has been reported that castration leads to loss of body and muscle weight [39] or to have no effect on body weight and on total amount of fat [40].

Our results indicate that androgen deprivation does not induce features of metabolic syndrome in any of the mouse models studied. The reasons for the inter species differences are unclear, but may relate to various effects of castration on physical activity and energy expenditure, as well as appetite and food intake. Our data are compatible with the possibility that androgen defi-

ciency reduces pituitary growth hormone output, leading to lower IGF-1 levels, as well as to reduced energy intake and insulin levels [41].

Adiponectin is produced predominantly by white adipose tissue, and circulating levels are inversely correlated with obesity and insulin resistance in both humans and rodents [42,43]. Low-adiponectin level has been shown to be a risk factor for prostate cancer [44,45]. In humans, increased serum adiponectin levels have been reported both in patients with prostate cancer treated by ADT and in patients with primary hypogonadism [6,46]. In rodents, castration increased plasma adiponectin [47,48]. In vitro studies with adipocytes demonstrated that testosterone reduced adiponectin secretion into the culture media [47,48]. Our current results showing increased adiponectin associated with castration are consistent with these previous human, rodent and in vitro reports. As the effect of castration on insulin differs between human and mouse, we speculate that the adiponectin changes

TABLE II. Plasma Concentration of Insulin, IGF-I, Adiponection and Leptin on Castrated and Non-Castrated Mice

Strain (age), diet	Weeks	Total mice number	Plasma insulin (ng/ml)			Plasma IGF-1 (ng/ml)			Plasma adiponectin (µg/ml)			Plasma leptin (ng/ml)		
			Castrated	Non-castrated	P-value	Castrated	Non-castrated	P-value	Castrated	Non-castrated	P-value	Castrated	Non-castrated	P-value
Regular mice														
C57BL/6 (6 weeks old)														
[A] Diet 3	Baseline	12	0.441 ± 0.067	0.368 ± 0.065	0.454	774 ± 60.7	624 ± 46.3	0.086						
	8W	11	1.151 ± 0.143	0.989 ± 0.152	0.484	539 ± 40.1	663 ± 32.4	0.039						
	28W	10	4.361 ± 1.289	8.085 ± 2.564	0.242	275 ± 69.0	407 ± 59.7	0.189	10.172 ± 0.915	5.68 ± 1.547	0.043	48.335 ± 3.715	39.225 ± 2.510	0.081
[B] Diet 4	Baseline	12	0.417 ± 0.051	0.360 ± 0.064	0.504	775 ± 39.9	768 ± 97.1	0.949						
	8W	12	0.794 ± 0.095	0.665 ± 0.075	0.312	751 ± 23.0	648 ± 41.6	0.063						
	28W	12	0.462 ± 0.212	1.221 ± 0.246	0.773	374 ± 46.2	488 ± 37.7	0.087	16.070 ± 2.077	9.266 ± 0.806	0.020	9.596 ± 1.660	5.374 ± 1.990	0.135
C57BL/6 (8 months old)														
[C] Diet 2	Baseline	20	0.813 ± 0.088	0.747 ± 0.069	0.559	533 ± 25.9	521 ± 21.5	0.746						
	8W	19	0.917 ± 0.086	1.732 ± 0.188	<0.001	506 ± 17.5	580 ± 20.7	0.014						
	16W	19	0.949 ± 0.131	1.674 ± 0.091	<0.001									
	32W	16	0.735 ± 0.110	1.093 ± 0.229	0.191	689 ± 41.9	697 ± 32.5	0.889	25.08 ± 2.185	15.02 ± 1.472	0.002	18.41 ± 3.560	28.557 ± 2.287	0.033
Immunodificient mice														
Swiss nude (6 weeks old)														
[D] Diet 3	Baseline	12	0.261 ± 0.014	0.213 ± 0.009	0.483	714 ± 116	503 ± 169	0.271						
	8W	11	1.376 ± 0.188	1.581 ± 0.444	0.718	604 ± 18.5	618 ± 63.9	0.130						
	25W	10	0.502 ± 0.120	1.023 ± 0.320	0.204	367 ± 27.7	385 ± 22.7	0.625	4.868 ± 0.183	3.522 ± 0.674	0.151	6.779 ± 1.546	9.540 ± 2.623	0.432
[E] Diet 4	Baseline	12	0.225 ± 0.010	0.217 ± 0.010	0.582	635 ± 74.2	506 ± 102	0.349						
	8W	11	0.763 ± 0.123	1.233 ± 0.290	0.909	621 ± 19.6	534 ± 17.4	0.522						
	25W	9	0.370 ± 0.120	0.566 ± 0.192	0.387	357 ± 13.8	463 ± 29.4	0.026	4.979 ± 1.072	3.971 ± 0.348	0.341	1.635 ± 0.377	3.791 ± 0.347	0.003
CB17 scid (6 weeks old)														
[F] Diet 1	Baseline	16	0.938 ± 0.142	0.974 ± 0.250	0.904	1120 ± 39.1	1188 ± 57.2	0.363						
	8W	10	0.789 ± 0.072	2.792 ± 0.565	0.023	955 ± 83.5	838 ± 40.5	0.254	19.251 ± 1865	15.455 ± 0.647	0.113	6.153 ± 1.477	10.866 ± 2.040	0.113

reflect direct effects of androgen, rather than indirect regulation of adiponectin by insulin.

Castration therapy of prostate cancer patients leads to a variety of hormonal changes including hyperinsulinemia. This, together with epidemiologic [19] and experimental [28] evidence that high levels of insulin are associated with aggressive prostate cancer behavior, raises the possibility that long-term benefits of ADT may be attenuated by insulin stimulation of insulin-receptor positive prostate cancer cells. However, the results presented here suggest that experimental work to investigate this hypothesis cannot be carried out in the mouse models studied. It has been suggested that the risk of prostate cancer progression is increased in neutered dogs compared with intact dogs [49]. Thus, dog models of prostate cancer, while less convenient than murine models, may be more appropriate for experimental investigations of the relationship between castration, hyperinsulemia, and prostate cancer. The absence of a convenient mouse model also increases justification to expand clinical research in this area.

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