Cancer
Research

A Low Carbohydrate, High Protein Diet Slows Tumor Growth and Prevents Cancer Initiation

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Abstract

Since cancer cells depend on glucose more than normal cells, we compared the effects of low carbohydrate (CHO) diets to a Western diet on the growth rate of tumors in mice. To avoid caloric restriction–induced effects, we designed the low CHO diets isocaloric with the Western diet by increasing protein rather than fat levels because of the reported tumor-promoting effects of high fat and the immune-stimulating effects of high protein. We found that both murine and human carcinomas grew slower in mice on diets containing low amylose CHO and high protein compared with a Western diet characterized by relatively high CHO and low protein. There was no weight difference between the tumor-bearing mice on the low CHO or Western diets.

Additionally, the low CHO-fed mice exhibited lower blood glucose, insulin, and lactate levels. Additive antitumor effects with the low CHO diets were observed with the mTOR inhibitor CCI-779 and especially with the COX-2 inhibitor Celebrex, a potent anti-inflammatory drug. Strikingly, in a genetically engineered mouse model of HER-2/neu–induced mammary cancer, tumor penetrance in mice on a Western diet was nearly 50% by the age of 1 year whereas no tumors were detected in mice on the low CHO diet. This difference was associated with weight gains in mice on the Western diet not observed in mice on the low CHO diet. Moreover, whereas only 1 mouse on the Western diet achieved a normal life span, due to cancer-associated deaths, more than 50% of the mice on the low CHO diet reached or exceeded the normal life span. Taken together, our findings offer a compelling preclinical illustration of the ability of a low CHO diet in not only restricting weight gain but also cancer development and progression. *Cancer Res; 71(13); 4484–93. ©2011 AACR.*

Introduction

More than 80 years ago, Otto Warburg found that most cancer cells, unlike normal cells, rely more on glycolysis than oxidative phosphorylation (OXPHOS) to meet their energy needs, even under normoxic conditions (1). He postulated that this "aerobic glycolysis" was due to irreversible defects in mitochondrial respiration (2). However, whereas some studies have linked mitochondrial mutations to cancer (3), a causal role for these mutations seems to be relatively rare (4), and, in most cases, glycolysis in tumors seems reversible (5). Importantly, because glycolysis is far less efficient at generating ATP, most cancer cells require higher levels of glucose than normal cells to proliferate and survive, and this is why the glucose analog, ^{18}fluorodeoxyglucose, is capable of detecting the majority of human tumors via positron emission tomography (PET; ref. 6).

The current consensus to explain why tumor cells prefer aerobic glycolysis is that even though it is far less efficient than OXPHOS at generating ATP, yielding only 2 ATPs/glucose rather than 34 ATPs/glucose, it does not catabolize glucose completely to CO_2 for ATP but instead uses the carbon chains as building blocks for nucleic acid (i.e., ribose), protein (i.e., alanine, etc.), and lipid (i.e., citrate) syntheses, all of which are essential for cell proliferation (7). This likely explains why increased glycolysis is not exclusive to solid tumors, but also occurs in leukemias (8) and some rapidly growing normal cells, such as clonally expanding T cells (9). Also, glycolysis, via its pentose phosphate pathway offshoot, provides NADPH, which generates glutathione, an important intracellular reducing agent that prevents intracellular reactive oxygen species–induced death in cancer cells (10). In addition, glycolysis leads to the secretion of lactic acid, which can decrease the extracellular pH from 7.4 to 6.0 within a poorly perfused tumor, and

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this promotes metastasis by inducing normal cell death, angiogenesis, extracellular matrix degradation, and the inhibition of tumor antigen-specific immune responses (11). So, as long as cancer cells can obtain high levels of glucose, a high glycolytic rate provides sufficient ATP, even under hypoxic conditions, for tumor cell survival and proliferation (12).

Thus, we investigated whether low carbohydrate (CHO), high protein diets could sufficiently decrease blood glucose (BG) in mice to slow tumor growth. To prevent caloric restriction (CR)-induced effects, we had isocaloric diets prepared, in which we compensated for the low CHO content by raising protein levels. We chose high protein rather than high fat because of the reported tumor-promoting effects of high fat (13, 14) and the established benefits of amino acid supplementation (15, 16).

Materials and Methods

Mice and tumor cell injections

Five- to 8-week-old C3H/HeN and Rag2M mice, from Simonsen Laboratories or bred in-house, were housed 2 to 4 mice/cage in high-top Allentown cages on static racks, with 2 times per week bedding changes. Unless otherwise stated, 2 $\times$ 10^5 murine squamous cell carcinoma VII (SCCVII) cells (from James W. Evans, Threshold Pharmaceuticals, South San Francisco, CA in 2005), cultured *in vitro* in RPMI + 10% fetal calf serum + 50 U/mL penicillin and 50 mg/mL streptomycin, were injected subcutaneously (s.c.) into the backs of shaved C3H/HeN mice. Similarly, 8 $\times$ 10^6 human colorectal carcinoma (HCT-116) cells (from ATCC in 2002 and not passaged for more than 6 months before use) were injected into Rag2M mice. Tumors were measured 2 to 3 times per week by using manual calipers, and their volumes were determined by the formula—(Length $\times$ Width $\times$ Height) $\times$ $\pi/6$—except for the Rag2M study, where the following formula was used—(Length $\times$ Width $\times$ Width)/2.

Female NOP mice, which express a HER2/Neu-Ovalbumin fusion protein under the mouse mammary tumor virus (MMTV) promoter and expressing the *Trp53* minigene (17), were put on Western (5058) or 15% CHO diets at 8 weeks of age and monitored for tumor development. They were sacrificed when tumors were palpable (with subsequent confirmation by necropsy) or when age-associated idiopathic dermatitis developed.

Measurement of blood glucose, insulin, and lactate

BG was measured via tail vein by using a OneTouch Ultra-glucose meter and LifeScan test strips. Insulin and lactate levels were determined by ELISA (Mercodia; #10-1247-01) and lactate assay kits (BioVision; #K607-100), respectively, using plasma from CO_2-euthanized mice.

Reagents

All diets (Table 1) were from TestDiet. Unless otherwise stated, diets were switched 7 days before tumor implantation. Celebrex (Pfizer) was formulated into the diets by TestDiet. CCI-779 (LC Labs) was diluted from a 100% ethanol stock into the vehicle (5% Tween-80 + 5% PEG400 in PBS) used for intraperitoneal injections into mice at 1.5 mg/kg on days 4 and 7 after tumor implantation. All other reagents were from Sigma Chemical Co., unless otherwise stated.

Table 1. Macronutrient breakdown of diets used

	TestDiet 5058	8% CHO	15% CHO[a]	10% CHO[a]
CHO	55.2	8.0	15.6	10.6
Protein	23.2	69.4	58.2	63.5
Fat	21.6	22.6	26.2	25.9

NOTE: Values are given in % kcal.

[a] CHO content is 70% high amylose cornstarch.

Statistical analyses

GraphPad Prism (GraphPad Software, Inc.) was used for statistical analyses. Briefly, tumor sizes and ELISA results were tested for statistical significant differences by using a 1-tailed *t* test, and regressions were tested by using the Spearman rank correlation and *F* tests. A log-rank (Mantel–Cox) test was used to determine the significance of the difference between the survival curves in the spontaneous tumor study. Numbers were considered statistically significant if *P* value was 0.05 or less, unless otherwise stated.

Results

Tumors grow slower in mice on an 8% CHO, 69% protein, 23% fat (8% CHO) diet, but the mice lose weight

As it is well established that most human and murine tumors take up more glucose than normal tissues (6), we asked if we could decrease BG levels sufficiently, by decreasing dietary CHO, to significantly reduce tumor growth rates. We considered this possible because no-CHO ketogenic diets (NCKD) have recently been shown to reduce tumor growth rates in mice and rats (18). However, as it would be extremely difficult for humans to maintain such a NCKD, we asked if a more moderate, CHO-reduced diet could decrease BG levels and reduce tumor growth rates. To test this, we first designed a mouse diet containing 8% CHO (% of total calories consumed), because this level is used in the Atkins diet (19). However, we kept fat levels in the range of a Western diet (23%) rather than the 50% used in the Atkins diet because of the tumor-promoting effects of high fat (13, 14), and raised the level of protein instead (Table 1). Comparing the effect of this diet, given *ad libitum*, with an isocaloric Western diet (TestDiet 5058; Table 1) on BG levels in nontumor bearing Rag2M mice revealed that BG, indeed, dropped significantly after 4 to 7 days on the 8% CHO diet to a new, stable plateau (Fig. 1A), in keeping with previous reports showing that BG drops within 7 days on a ketogenic diet (20). Interestingly, this drop was more pronounced in male mice, consistent with the reported BG buffering effects of estrogen (21). On the basis of these results, we carried out the majority of our studies with male mice.

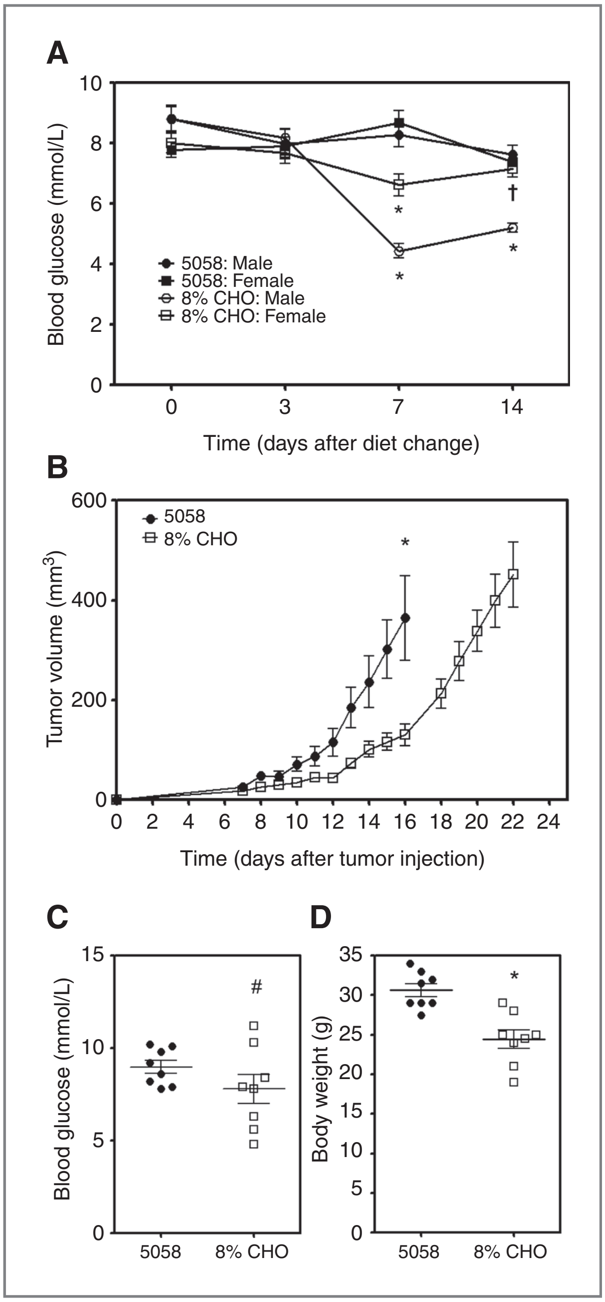

Figure 1. Tumors grow slower in mice on an 8% CHO diet than those on a Western diet, but the mice weigh less. A, BG time course of male and female Rag2M mice after switching to an 8% CHO diet. B, growth of SCCVII tumors in C3H/HeN mice on the 8% CHO versus 5058 diets ($n = 8$ for both groups). BG (C) and body weights (D) of these mice on the 8% CHO diet 6 days after diet switch. Results are given as mean ± SEM. *, $P < 0.05$ in a t test comparing the 8% CHO group to its respective 5058 group; #, $P < 0.10$ in a t test comparing the 8% CHO and 5058 groups; †, $P > 0.10$ for a t test comparing the 8% CHO group to its respective 5058 group.

In our first tumor studies, we acclimatized 5- to 6-week-old male C3H/HeN mice to either 5058 or the 8% CHO diet for 1 week, then injected s.c. SCCVII cells and monitored tumor growth. As shown in Figure 1B, tumors in the mice on the 8% CHO diet grew significantly slower, with the mean tumor size of the 8% CHO group (130.9 ± 21.76 mm^3) being less than half that of the 5058 group (364.3 ± 85.01 mm^3) at 16 days after tumor implantation. Also, BG levels in the 8% group were significantly lower (Fig. 1C). Similar results were obtained in Rag2M mice injected with human colorectal cancer cells (HCT-116 cells; data not shown).

Although mice on 8% CHO diet had slower growing tumors, they lost weight, weighing, on average, 20% less than mice on 5058 diet (Fig. 1D). This was consistent with the mice eating less than the 5058 group (data not shown), likely because the 8% CHO pellets were significantly harder to chew. This confounded our results because CR, which is known to cause cells to switch, via AMPK activation, to OXPHOS to generate more ATP for survival (22), has been shown to slow tumor growth (23). Thus, we could not rule out the possibility that the slower tumor growth rates were due to the effects of CR rather than to reduced dietary CHO.

A 15% high amylose CHO, 58% protein, 26% fat (15% CHO) diet reduces both fasting and constitutive BG

To prevent CR, we formulated a new diet consisting of 15.6% CHO, 58.2% protein, and 26.2% fat. Instead of sucrose, which was in our 8% CHO diet, this diet contained cornstarch with 70% amylose because it allowed for a pellet consistency similar to 5058, and because amylose is digested more slowly than sucrose or amylopectin (in 5058), which results in less pronounced postprandial BG and insulin spikes (24). We found that mice ate this chow at the same rate as 5058, and that, after a short fasting period, it did not increase BG to the same extent as 5058, two hours after feeding (Fig. 2A). Moreover, mice on this 15% CHO diet had lower constitutive BG levels than mice on the 5058 diet (Fig. 2B).

Tumors grow slower in mice on the 15% CHO diet without weight loss

We then compared SCCVII tumor growth in C3H/HeN mice on the 15% CHO versus 5058 diets and found that tumors grew significantly slower in the 15% CHO group, with an average volume of 321.0 ± 79.79 mm^3 versus 542.9 ± 78.80 mm^3 in the 5058 group, 16 days after implantation (Fig. 2C). Significantly, there was no difference in caloric intake (data not shown), average body weight (Fig. 2D, left), or rate of weight gain (Fig. 2D, right) between these diet groups.

We also compared the effect of this 15% CHO diet with 5058 on the growth of HCT-116 tumors in Rag2M mice and found that 15% CHO mice had significantly smaller tumors, with a mean size of 255.6 ± 10.50 mm^3 versus 401.7 ± 35.21 mm^3 in the 5058 group, 21 days after tumor implantation (Fig. 2E). Once again, there was no difference in the average body weight or rate of weight gain between the 2 groups (Fig. 2F).

A 10% CHO diet slows tumor growth more than a 15% CHO diet without significant weight loss

To see if we could further reduce tumor growth rates by decreasing dietary CHO levels even more, we tested another isocaloric diet containing 10% high amylose CHO, 64% protein, and 26% fat (Table 1). Comparing tumor growth in male C3H/

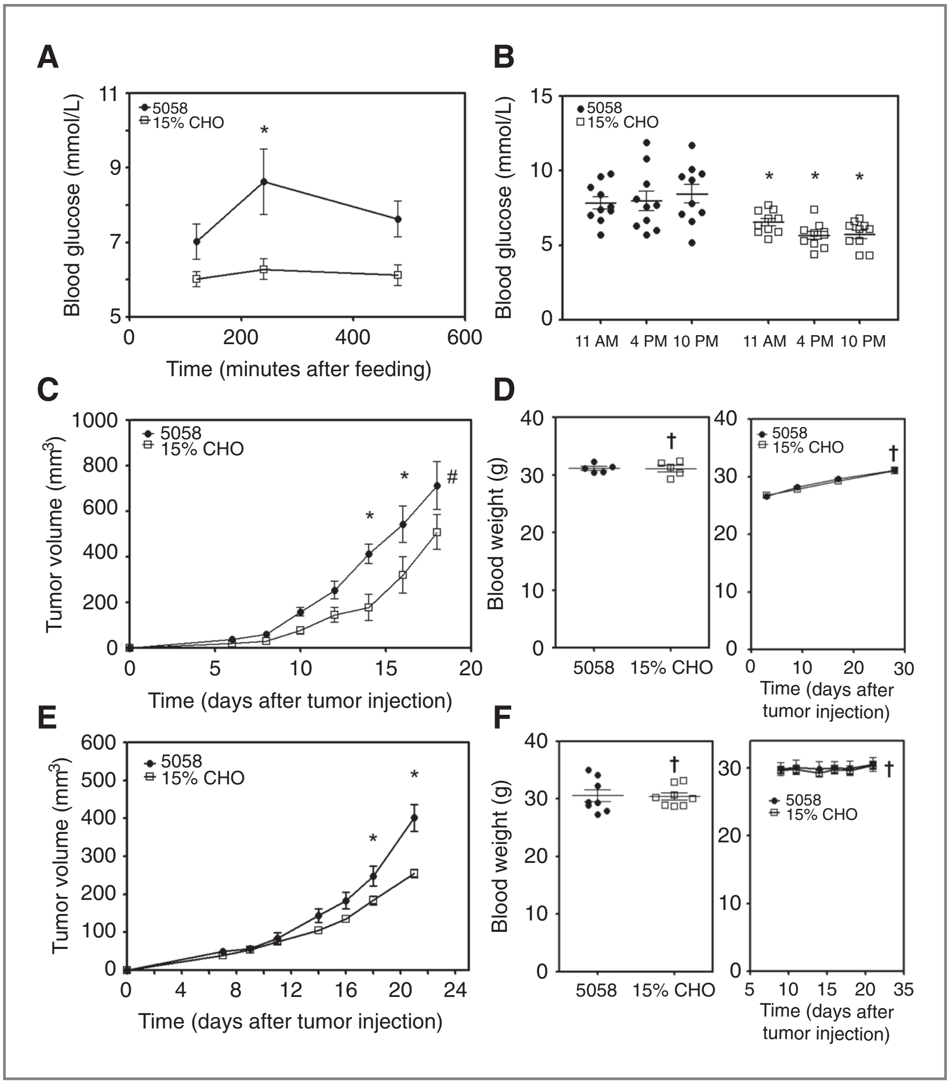

Figure 2. Tumors grow slower in mice on a 15% CHO diet than a Western diet, and the mice weigh the same. A, postprandial BG in C3H/HeN mice fed 5058 or 15% CHO diets after a 6-hour fast ($n = 5$ for 5058, $n = 4$ for 15% CHO). B, constitutive BG of mice at 3 different times of day. C, SCCVII tumor growth in mice fed the 15% CHO versus 5058 diets ($n = 5$ for both groups); D, their body weights on final measurement (left) and weight change (right). E, HCT-116 tumor growth in Rag2M mice on the 15% CHO versus 5058 diet ($n = 8$ for both groups). F, weights of mice on final measurement (left) and weight change (right) of these Rag2M mice. Results are given as mean ± SEM. *, $P < 0.05$ for a *t* test comparing the low CHO group to its respective 5058 group; #, $P < 0.10$ for a *t* test comparing the low CHO and 5058 groups; †, $P > 0.10$ for a *t* test comparing the low CHO and 5058 groups.

HeN mice, we found that tumors in mice fed this 10% CHO diet were significantly smaller (572.3 ± 215.7 mm^3) than those on 5058 (1153.0 ± 108.0 mm^3), 22 days postimplantation (Fig. 3A). This difference was more pronounced than with the 15% CHO diet and on par with the 8% CHO diet. As expected, the BG of mice on the 10% CHO was lower than those on 5058 (Fig. 3B). Even though the mice on the 10% CHO diet gained weight throughout the study and ate the same amount of food (data not shown), their average body weight at the end was slightly (~7%) lower (Fig. 3C), raising the concern that the smaller tumors in the 10% CHO group might be because of a smaller body size. To investigate this, we carried out a meta-analysis of pooled data from 3 independent experiments and found no significant positive correlation between body weight and tumor size for either the 5058 (Fig. 3F, left) or 10% CHO groups (Fig. 3F, right). This indicated that, within the range of mouse weights tested, smaller body sizes were not related to smaller tumors. Nonetheless, we cannot say with absolute certainty that the slightly lower weights of the 10% CHO-fed mice had no impact on tumor size.

Low CHO diets cause a drop in plasma insulin and lactate

To gain some insight into how the low CHO diets were reducing tumor growth rates, we measured plasma insulin levels and found that all the low CHO diets reduced plasma insulin, with the 8% and 10% CHO having a more marked effect than the 15% CHO diet (Fig. 4A). As high BG triggers insulin release from pancreatic β-cells, and the released insulin then enhances cellular uptake of BG via insulin receptor-mediated upregulation and activation of glucose transporters (25), these insulin results suggest that low CHO diets can reduce insulin-mediated glucose uptake into tumor cells. Consistent with this and our hypothesis that glucose supply is related to tumor growth, we found a positive correlation between plasma insulin levels and tumor size (Fig. 4B). We also compared plasma lactate levels in 5058 versus 10% CHO

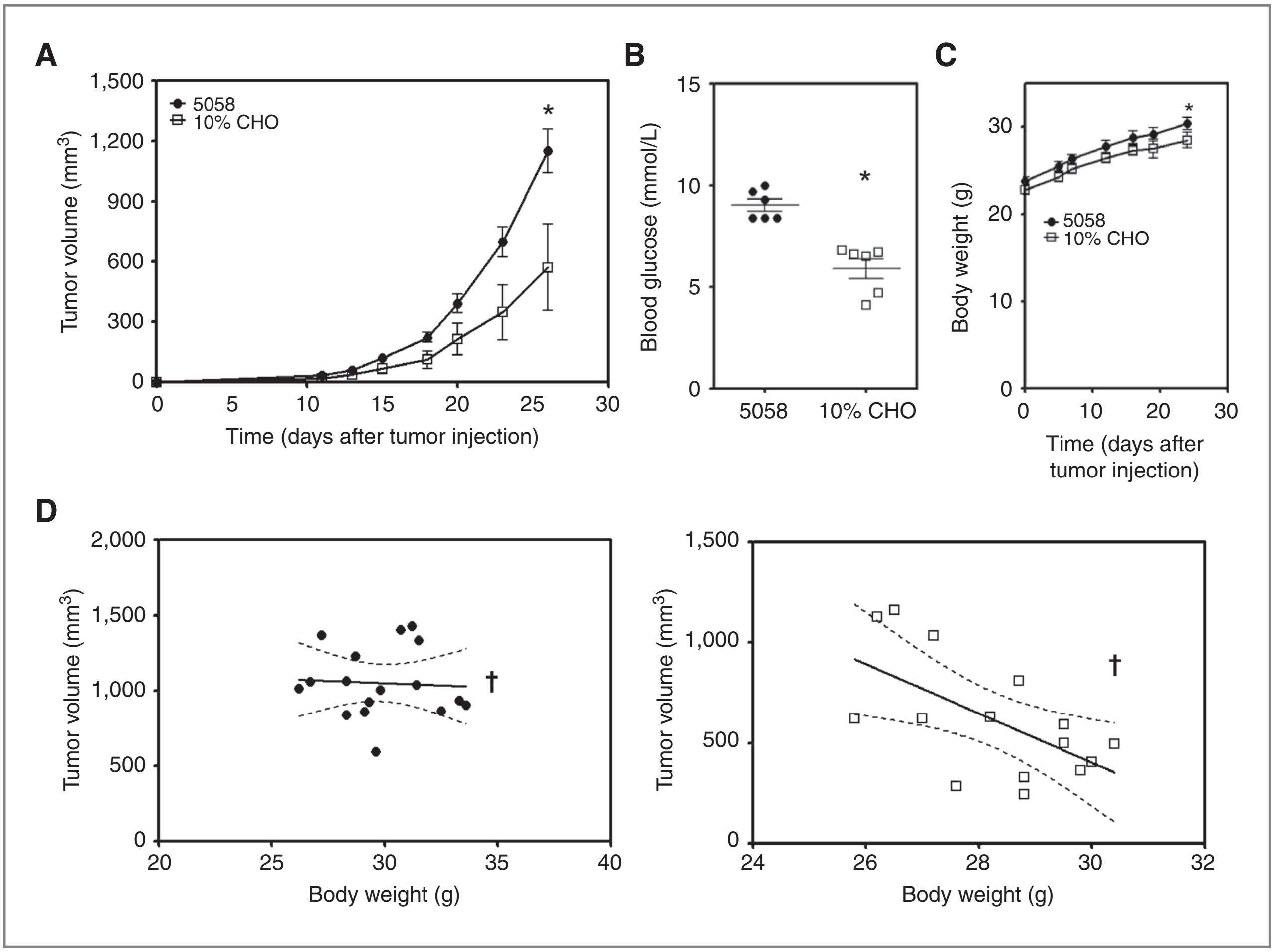

Figure 3. A 10% CHO diet is more effective than the 15% CHO diet at slowing tumor growth with only a slight effect on mouse weight. A, tumor growth in male C3H/HeN mice receiving 1 × 10^5 SCCVII cells on the 10% CHO versus 5058 diet ($n = 5$ for both groups). B, BG measurements of 10% CHO and 5058 groups at sacrifice. C, changes in body weight. D, linear regression of tumor size versus body weight for the 5058 (left) and the 10% CHO groups (right). Except for the linear regression, all results are given as mean ± SEM. *, $P < 0.05$ in a *t* test comparing the low CHO and 5058 groups; †, $P > 0.10$ for both an *F* test for a >0 slope and a positive correlation in a Spearman rank test.

mice and found the 5058-fed mice had significantly higher lactate levels (0.713 ± 0.03 mmol/L versus 0.572 ± 0.03 mmol/L; Fig. 4C), consistent with reduced glycolysis in the low CHO-fed mice. Once again, we found a positive correlation between plasma lactate levels and tumor size (Fig. 4D).

Low CHO diets act additively with known cancer therapeutic agents to reduce tumor growth

Having shown that the 10% and 15% CHO diets slowed tumor growth without significant weight loss, we asked if they might be additive with known cancer therapeutic agents. To test this, we first compared the growth of SCCVII (Fig. 5A, left) and Lewis lung carcinoma (data not shown) tumors in mice on the 10% CHO or 5058 diets ± the mTOR inhibitor, CCI-779, and found that, in both, combining the 10% CHO diet with CCI-779 resulted in an additive effect, with a negligible effect on mouse weights (Fig. 5A, right). Most exciting, however, were the results obtained with the 15% CHO diet containing the COX-2 inhibitor, Celebrex. Not only was tumor growth significantly reduced with the 15% CHO diet containing 1 g/kg Celebrex, but the overall slope of the tumor growth was lower (Fig. 5B, left). Once again, there were negligible effects on mouse weights (Fig. 5B, right), and although the Celebrex-treated mice weighed slightly less than the mice not treated with Celebrex, they did not fall outside the range tested in the meta-analysis, suggesting that the effect of Celebrex was not related to lower body weights.

The 15% CHO diet reduces the incidence of tumors in a spontaneous mouse model of breast cancer

We then asked if our low CHO diets could reduce cancer incidence in a spontaneous cancer model by using female NOP mice, which express a dominant-negative allele of p53 and the HER2/Neu oncogene under the control of the MMTV promoter, thus mimicking human breast cancers (17). These mice have a 70% to 80% chance of developing mammary tumors over their lifetime (17). Mice were switched onto the 15% CHO or 5058 diets when they reached adulthood (8 weeks), and,

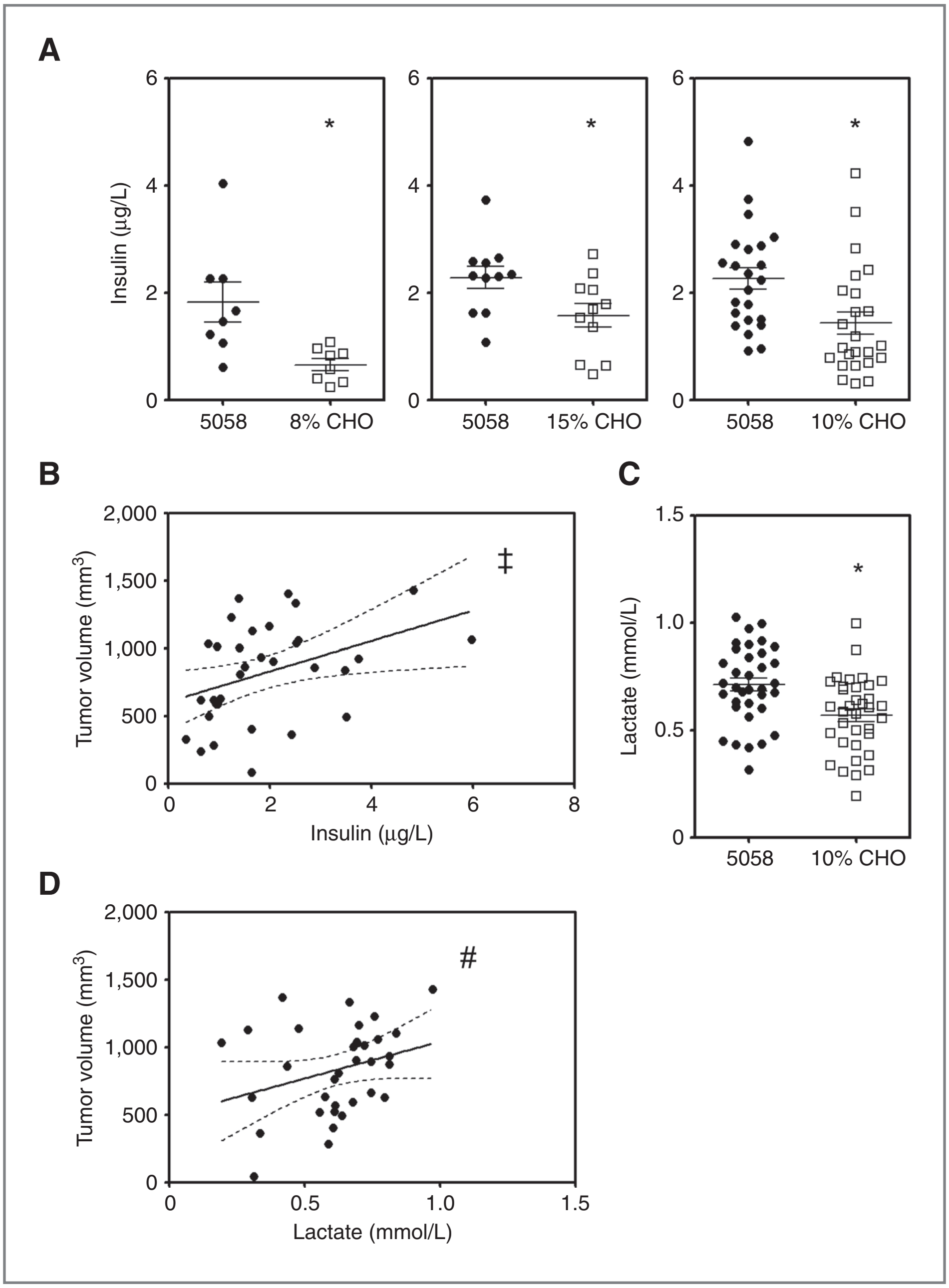

Figure 4. Low CHO diets reduce plasma insulin and lactate levels. A, plasma insulin levels of SCCVII tumor-bearing male C3H/HeN mice on the low CHO (8%, 15%, 10%) versus their respective 5058 experimental controls. Results of the 10% CHO diet are from data pooled from 3 experiments. B, linear regression of final plasma insulin levels versus final tumor volumes on all mice; data pooled from 3 experiments by using the 10% CHO and 5058 diets. C, lactate levels in the plasma of these mice on the 10% CHO and 5058 diets. D, linear regression of final plasma lactate levels versus final tumor volumes on all mice; data pooled from 3 experiments by using the 10% CHO and 5058 diets. Except for the linear regression, all results are given as mean ± SEM. *, $P < 0.05$ in a *t* test comparing the low CHO and 5058 groups; ‡, $P < 0.05$ for both an *F* test for a >0 slope and a positive correlation in a Spearman rank test; #, $P < 0.10$ for an *F* test for a >0 slope and $P < 0.05$ for a positive correlation in a Spearman rank test.

9 weeks later, we found that BGs were significantly low in the 15% CHO group (Fig. 6A, left). Interestingly, whereas the weights were stable in both groups after 8 to 9 weeks on the diets, they were consistently low in the 15% CHO group (Fig. 6B), which is not unexpected, given that long-term low CHO diets reduce body mass (26). Also, plasma insulin levels, taken at death, were significantly low in the 15% CHO group (Fig. 6C). Importantly, as shown in Figure 6D, at 1 year of age almost half the mice on 5058 had developed tumors compared with none in the mice on the 15% CHO diet. Furthermore, 70% (7 of 10) of mice on 5058 developed tumors during their life span, with only 1 reaching normal life expectancy, whereas less than 30% (3 of 11) of the mice on the 15% CHO diet developed tumors, with more than half reaching or exceeding normal life expectancy. Of note, in the 5 mice on the 15% CHO diet that exceeded normal life spans, only 1 had kidneys that showed above-normal levels of protein in the urine (data not shown). These long-term mouse studies suggest that this 15% high amylose CHO, 58% protein, 26% fat diet is both safe and efficacious.

Discussion

To exploit the fact that cancer cells rely more heavily on glycolysis than normal cells, we designed low CHO, high protein diets to see if we could limit BG and tumor growth.

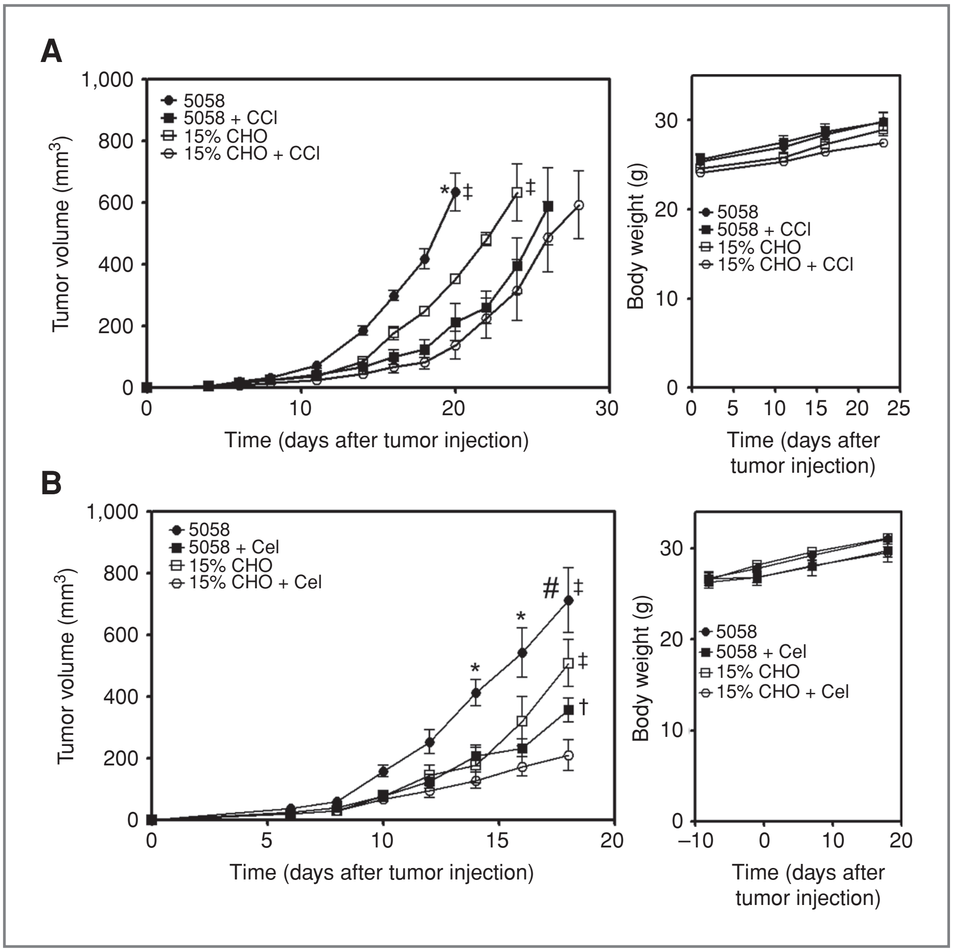

Figure 5. Low CHO diets act additively with current treatments for cancer. A, growth of SCCVII tumors in male C3H/HeN mice on the 10% CHO ($n = 3$) or 5058 diets ($n = 6$) ± CCI-779 ($n = 3$ for both CCI groups; left) and body weight versus days after tumor injection (right). B, growth of SCCVII tumors in male C3H/HeN mice on the 15% CHO ($n = 5$) or 5058 ($n = 5$) diet ± 0.1% w/w Celebrex (Cel; 5058 + Cel $n = 10$; 15% CHO + Cel $n = 6$; left) and body weight versus days after tumor injection (right). All results are given as mean ± SEM. *, $P < 0.05$ in a t test comparing the 5058 to any other diet group; ‡, $P < 0.05$ in a t test comparing drug treated (5058 or low CHO) with their respective untreated control group (same diet); #, $P < 0.10$ for a t test comparing the untreated low CHO and 5058 groups; †, $P < 0.10$ for a t test comparing drug treated groups (5058 versus low CHO).

In designing our diets, we wanted to avoid NCKDs because of the difficulty in achieving long-term compliance with no CHO diets in potential future human studies (27) and because Masko and colleagues recently reported that a 10% or 20% CHO diet slows tumor growth as effectively as NCKDs (27). Following early studies with 8% CHO diets, using 10% and 15% CHO, high protein diets in which 70% of the CHO was in the form of amylose, we found that, compared with a Western diet, they were indeed capable of reducing BG, insulin, and lactate levels and, importantly, in slowing the growth of implanted murine and human tumors, with little or no effects on mouse weight.

We assessed the effects of our low CHO diets in both murine tumor-bearing immunocompetent mice and human tumor-bearing immunocompromised mice, because immune status has been shown to influence tumor growth (7), but found that low CHO diets slowed tumor growth to a similar extent without any difference in tumor-associated immune cell composition between the low CHO and 5058 groups (data not shown). Of note, Venkateswaran and colleagues recently found that CHO reduction (from 45% to 10%) slowed the growth of LNCaP xenografts and attributed this to reduced insulin like growth factor I (IGF-I) levels (28). Interestingly, we detected no changes in IGF-I levels in mice on our low CHO diets, unless there was CR (e.g., with our 8% CHO diet; data not shown). Our findings suggest that although IGF-I reduction may be a relevant mechanism in some models, low CHO diets may also slow tumor growth in an IGF-I–independent manner.

Given the antitumor effects of ketones and ketosis (29), we measured plasma β-hydroxybutyrate and found that tumor-bearing mice on our 10% diet as well as NOP mice on our 15% CHO diet for many months had β-hydroxybutyrate levels similar to mice fed with Western diet (<5 mg/dL; Supplementary Fig. S1), and substantially less than those reported for mice on NCKDs (~15 mg/dL; refs. 27, 30). This is consistent with very recent studies showing that ketosis requires high dietary fat (31) and suggests that ketosis does not contribute to the slower tumor growth we observe with our low CHO diets.

We also found that our low CHO diets were additive with the tumor suppressive effects of CCI-779 and Celebrex. Related to this, while it has been shown that COX-2 is overexpressed in many human cancers, and that Celebrex may be beneficial in preventing/slowing colon, breast (32), and prostate cancers (33, 34) by blocking both omega-6 fatty acid-induced inflammation(33) and tumor-induced angiogenesis (35), high-dose Celebrex has cardiovascular side effects (36). As our low CHO

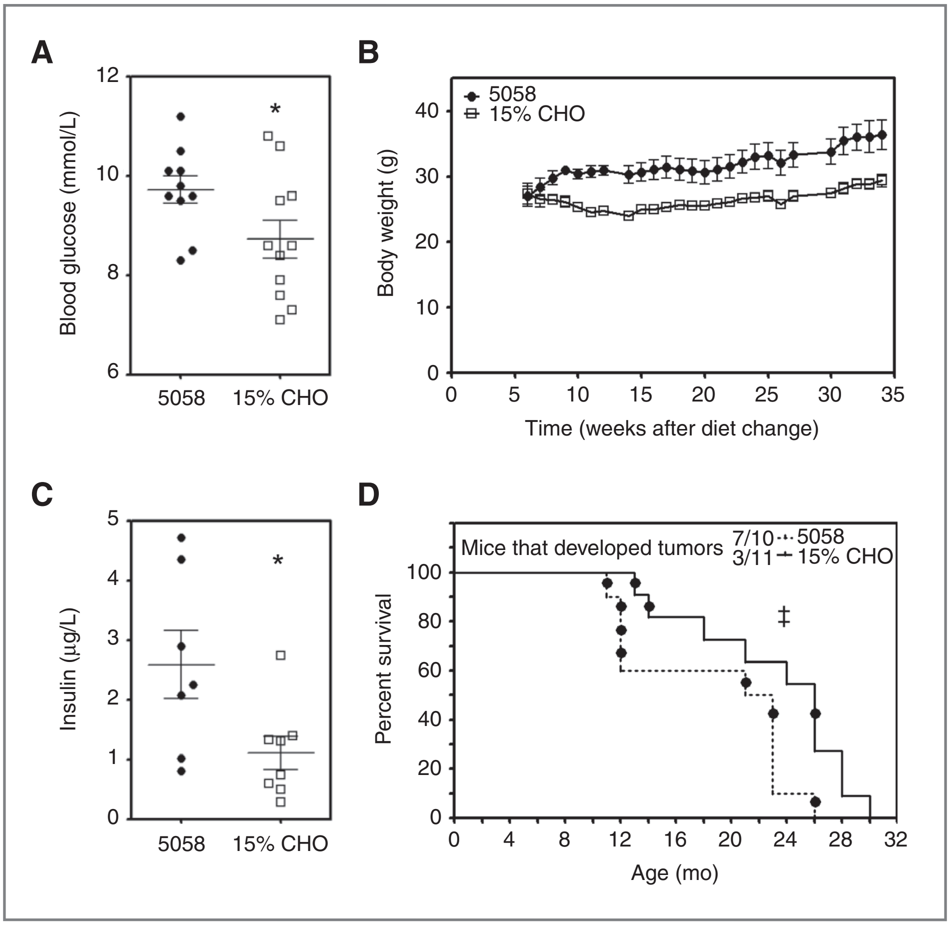

Figure 6. The 15% CHO diet reduces the incidence of tumors in a spontaneous mouse model of breast cancer. A, BG measurements at 9 weeks after diet switch of 10 (5058) and 11 (15% CHO) female NOP mice. B, body weight of these same mice over time. C, plasma insulin of NOP mice at death. D, survival curve and tumor incidence versus time (months) of NOP mice on 5058 versus 15% CHO diet. Dots indicate tumor events. Except for the survival curve, all results are given as mean ± SEM. *, $P < 0.05$ in a *t* test comparing the low CHO and 5058 groups; ‡, $P < 0.05$ in a log-rank test for significant differences between the survival curves.

diets show additive effects with Celebrex, it might allow for a lower, safer dose of Celebrex, without loss of therapeutic efficacy (37).

Although our work strongly suggests that cancer can be treated and/or prevented by limiting BG, some caution must be exercised in extrapolating our results to humans. This is because, while fasting BG levels have been shown to be significantly reduced in cancer patients on a low CHO diet (38), they may not be reduced as much as in mice (39). On the contrary, substantial postprandial reductions in BG have been reported in humans on low CHO diets (24, 38, 40, 41). Given that postprandial BG in humans is elevated for up to 2 hours after a meal and we typically eat 3 or more meals a day, it is very likely that a low CHO diet will significantly reduce the daily area-under-the-curve BG exposure. In keeping with this, it has been reported that low GI meals greatly reduce the BG area-under-the-curve compared with high GI meals in humans (40) and that reducing the CHO content of meals in mild diabetics from 55% to 20% reduces the BG area-under-the-curve by 36%, which is similar to what we see with our mice (41). Also, our low CHO studies with human HCT-116 cells in Rag2M mice and low CHO studies with other human tumors (42) suggest that there are no inherent differences between human and mouse cancer cells in their response to BG levels. Consistent with the notion that reducing BG in humans can be beneficial, there is a wealth of epidemiologic evidence showing a clear association between BG and/or insulin levels (which are determined by BG levels) and the incidence of human cancers (43–49). Thus, although our studies were conducted, out of necessity, with mice, the fact that human BG can be significantly reduced with low CHO diets and the association of many cancers with high BG levels suggest that our findings are very likely relevant to human cancers as well, particularly in cancers that have been associated with higher baseline BG and/or insulin levels, such as pancreatic (43, 44), breast (45), colorectal (46), endometrial (47, 48), and esophageal cancers (49).

In addition to these cancers, a low CHO diet may also be beneficial in early-stage prostate cancer, even though it is not typically detectable by PET (50). This is because the metastases of these tumors kill the patients and, given the pivotal role of lactate in promoting metastasis (11), our low CHO diets could significantly reduce metastasis by reducing tumor-associated lactate levels. In fact, we have preliminary data suggesting that a low CHO diet plus low dose Celebrex profoundly reduces the lung metastasis of orthotopically implanted 4T1 tumor cells (manuscript in preparation).

In terms of macronutrient composition, even though high protein has been shown to promote satiety (19)—thus reducing obesity, BG, and insulin levels—and enhance both antitumor immunity, through amino acid supplementation, and life span (15, 16, 51), we were concerned, based on the literature (52–54), that high protein levels might cause kidney damage. More recent data, however, suggest that this may only occur in individuals with existing chronic kidney disease (52, 55) and that in normal people, the increase in glomerular filtration rate and kidney cellularity that occur with long-term high protein consumption may be a normal response (52). Consistent with this, we found that while the 5 long-lived NOP mice on our 15% CHO diet had larger than normal kidneys (data not shown), only 1 had elevated urinary albumin. Moreover, because they lived beyond the normal life span of C57BL/6 mice on a Western diet, we can infer that the overall health of the mice was not adversely affected. In humans, most epidemiologic studies examining high protein diets and cancer progression have been confounded by not taking into account protein source, fat content, and red meat consumption. This is important because high fat increases cancer risk (56) and plant protein seems to decrease whereas animal protein increases cancer mortality (57). Interestingly, colonic cancer-inducing damage caused by red meats may be avoided with high amylose, low CHO diets (58). These studies suggest that macronutrient sources and combinations are very important and that testing them through highly controlled studies, such as those achieved with mice, represents a powerful approach to this question.

Our study, herein, shows that a high amylose containing low CHO, high protein diet reduces BG, insulin, and glycolysis, slows tumor growth, reduces tumor incidence, and works additively with existing therapies without weight loss or kidney failure. Such a diet, therefore, has the potential of being both a novel cancer prophylactic and treatment, warranting further investigation of its applicability in the clinic, especially in combination with existing therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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