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Buffer Therapy for Cancer

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Abstract

Oral administration of pH buffers can reduce the development of spontaneous and experimental metastases in mice, and has been proposed in clinical trials. Effectiveness of buffer therapy is likely to be affected by diet, which could contribute or interfere with the therapeutic alkalinizing effect. Little data on food pH buffering capacity was available. This study evaluated the pH and buffering capacity of different foods to guide prospective trials and test the effect of the same buffer (lysine) at two different ionization states. Food groups were derived from the Harvard Food Frequency Questionnaire. Foods were blended and pH titrated with acid from initial pH values until 4.0 to determine "buffering score", in mmol H^+/pH unit. A "buffering score" was derived as the mEq H^+ consumed per serving size to lower from initial to a pH 4.0, the postprandial pH of the distal duodenum. To differentiate buffering effect from any metabolic byproduct effects, we compared the effects of oral lysine buffers prepared at either pH 10.0 or 8.4, which contain 2 and 1 free base amines, respectively. The effect of these on experimental metastases formation in mice following tail vein injection of PC-3M prostate cancer cells were monitored with *in vivo* bioluminescence. Carbohydrates and dairy products' buffering score varied between 0.5 and 19. Fruits and vegetables showed a low to zero buffering score. The score of meats varied between 6 and 22. Wine and juices had negative scores. Among supplements, sodium bicarbonate and Tums® had the highest buffering capacities, with scores of 11 and 20 per serving size, respectively. The "de-buffered" lysine had a less pronounced effect of prevention of metastases compared to lysine at pH 10. This study has demonstrated the anti-cancer effects of buffer therapy and suggests foods that can contribute to or compete with this approach to manage cancer.

Keywords: Food buffering capacity; Acid-base; pH; metastasis; Sodium bicarbonate

Introduction

Solid tumors exhibit a higher rate of glucose uptake and metabolism compared to normal surrounding tissues, which is a strong negative prognostic factor for disease outcome [1]. It is notable that cancer cells maintain a high level of glucose metabolism even in the presence of oxygen, which was first documented by Warburg more than 80 years ago [2,3]. This is a consistent finding across a variety of cancers, and has been recognized as a "hallmark" of cancer [4].

A significant consequence of increased glucose metabolism is the production of acids, such as lactic acid, which can be an independent negative prognostic factor for cancer outcome [5]. Prior mathematical models and empirical studies have shown that solid tumors export acid to the surrounding parenchyma [6,7]. This is consistent with measurements of tumor pH in mouse models, which have shown that the extracellular pH of solid tumors is acidic [8,9]. Combined, these observations have led to the generation of the "Acid Mediated Tumor Invasion" hypothesis, which proposes that fast-growing tumors export acid to surrounding stroma, and that reduced pH contributes to the tissue remodeling required for tumor invasion [6]. Furthermore, the acid produced by hyperglycolytic cancer cells selects for increased acid resistance in the tumor population, while the normal stromal cells are relatively more sensitive to acid-induced cell death [10-12].

These observations suggest that interfering with the intra-tumoral acidification could preserve the extracellular matrix and the stromal cell population surrounding the tumor, and hence retard invasion. To this end, we have shown that oral administration of alkalinizing pH buffers (sodium bicarbonate, imidazoles, and lysine) significantly reduced the development of spontaneous and experimental metastases in animal models [13-15], even though the growth of the primary tumors was not affected. In these experiments the blood pH remained unchanged due to compensation, but there was an increase in its pH buffering capacity by an increase in the concentration of bicarbonate anions. These initial preclinical observations have led to two early clinical trials targeting patients with pancreatic cancer and bone metastases to evaluate the feasibility and effectiveness of administering sodium bicarbonate diluted in water at a dose of ca. 0.6 g/kg of body weight per day [16,17] to increase buffering capacity in the gastrointestinal (GI) tract. Although previous case studies demonstrated promising results [14] using bicarbonate supplementation, the unpleasant taste of sodium bicarbonate, the volume required and other grade I/II gastrointestinal symptoms experienced by patients in these trials, has resulted in poor compliance. Based on these initial observations, future studies to test effectiveness of pH buffering on tumor progression should consider dietary augmentation as an adjuvant therapy.

We have hypothesized that if the diet of an individual were modified in order to increase his whole body buffering capacity by the same order of magnitude as the pre-clinical sodium bicarbonate supplementation

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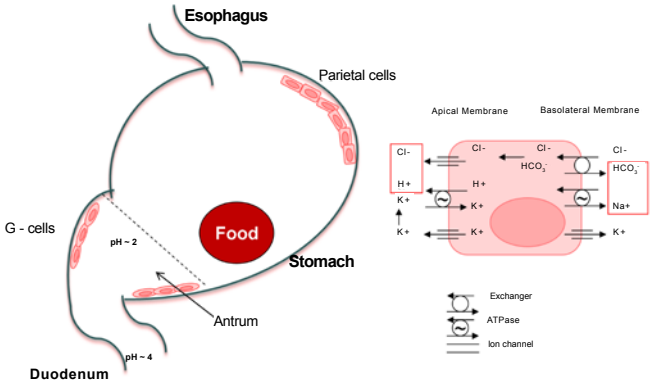

Figure 1: Acid secretion and ion transport mechanism. Luminal acidity and food contents in the stomach increase intragastric pH, which promotes gastrin secretion that increases the rate of acid secretion (HCl) by parietal cells. The concentration of bicarbonate (HCO_3^-) in the blood is increased as a response to the acid secreted in the stomach, and the extra bicarbonate is generated by the same parietal cells that produce the HCl in the lumen in order to lower the pH to digest a meal. Once the food reaches the duodenum, the cells in its lumen and pancreas secrete bicarbonate to increase the pHe of the lumen. As a consequence, while food moves from the esophagus to the stomach, and finally to the duodenum, the concentration of bicarbonate in blood increases and then decreases back to its normal levels.

(c.a. 11 mEq/kg bodyweight/day) we should observe a similar intra-tumoral pH buffering effect, and thus a significant reduction in the potential of development of metastases. There are at least two mechanisms through which dietary intake may affect the whole body pH buffering system: the first occurs through the acidification of the food in the stomach from its initial pH down to pH~2.0, where digestion by enzymes is optimized. Prior to further absorption in the GI tract, the pH of food is restored to ~4.0 at the distal part of the duodenum by secretion of bicarbonate, and the difference between the initial food pH and 4.0 would thus result in a surplus or deficit of bicarbonate anions from the body due to food digestion (Figure 1). The second mechanism consists in the production of acidic or alkaline byproducts from the metabolism of nutrients. While the second mechanism has been widely studied, especially in the literature of metabolic acidosis and alkalosis, the first has received less attention, and thus is the focus of this work. Along this manuscript, the term "dietary buffering effect" will be used to refer to the net balance of bicarbonate anions gained or lost during the passage of food through the GI tract, and not due to their metabolism.

While some types of foods may contribute to this pH buffering effect, others may actually counteract it. Specific nutritional interventions play a major role in the prevention and in the improvement of the health of individuals at pre-clinical and established stages of targeted diseases [18]. Specific diets have been used to treat patients with chronic acidosis or alkalosis, even though the rationale behind the choice of foods was focused on the byproducts of the metabolism, rather than the actual pH buffering effect of food in the GI tract [19]. The most important factor when considering food as adjuvant to pH buffering therapy is not only the initial pH of the foods, but their buffering capacities, i.e. the amount of hydrogen ions consumed to reduce the pH to physiological levels in the GI tract. In addition, subjects on buffer therapy should not consume foods that would counteract the buffer and inhibit therapy. Although the pH of some common foods was readily available in the research and food manufacturing literature, very little data on food buffering capacities were available [20-23].

The objective of the present work is to examine the acid "buffering score" of some common foods, based on the distribution of self-reported intakes on the Harvard Food Frequency Questionnaire (FFQS, https://regepi.bwh.harvard.edu/health/nutrition.html). Early clinical trials with volunteers under a controlled diet have suggested that dietary intervention is capable of interfering in the systemic acid-base balance [24]. These studies have shown that, while the blood pH in the subjects is unaffected, the bicarbonate concentration in blood and urine pH was significantly lowered in high-protein diets. Remer et al. have proposed a mathematical formula to calculate the dietary effect on acid-base balance: proteins and phosphorous would have an acidifying effect, while counter-cations such as potassium, magnesium and calcium have an alkalinizing effect [25-26]. Clinical trials examining the effect of diets on metabolic acidosis and calcium loss through urine, however, have produced conflicting results; some proposing that protein-rich diets correlated with increased fractures and lower bone density, while others could not observe correlations either way [27-30]. We propose that a contributing factor to the conflicting results in these studies may be the pH titration that occurs in the digestive system: for example, the pH buffering capacity varies significantly among different amino acids, and different protein sources have different amino acid compositions. The food preparation process also significantly changes the initial pH of meals, and consequently, their pH buffering. Consider sushi compared to ceviche. Both may contain an equivalent amount of fish, yet ceviche is prepared in acidic brine, reducing its effectiveness as a buffer. In the current study we have quantified this effect with an abundant amino acid, lysine, which has pKa values of 10.5, 9.0 and 2.2. The Henderson-Hasselbach relationship predicts that each lysine prepared at a pH of 10.5 (free base) has 1.5 non-ionized amines, whereas lysine prepared at a pH 8.4 contains approximately 0.3 non-ionized amines. Thus, the "buffer score" of high pH lysine should be ca. 5X higher than low pH lysine. We have compared the effect of these two preparations on metastasis formation.

We have evaluated the pH buffering score of various foods to estimate their effect in the whole-body pH buffering system. For each of these food types, we performed pH titrations from the initial pH values until the pH reached 4.0. From these, we have determined a "buffer score", based on the number of mEq H^+ consumed to lower the pH to 4.0, which is the fasting (steady-state) pH of the distal duodenum (see Results). The actual contribution of foods to systemic pH buffering is a combination of the buffer score and the frequency of these foods in the diet. Future goals of this project are to test the hypothesis in observational epidemiologic studies of dietary therapy and cancer, and to ultimately develop and test adjuvant nutrition interventions with an "alkalinizing" diet that could supplement the amount of therapeutic buffer required to prevent cancer progression.

Materials and Methods

In vitro Experiments

Food source and sample preparation: A comprehensive list of foodstuffs based on the 2007 Harvard FFQS was prepared, containing different sections such as multi-vitamins and minerals; dairy food; fruits; vegetables; eggs & meat; breads, cereals & starches; beverages; sweets, baked goods, and miscellaneous. Foods were purchased from the local grocery stores (Publix Super Markets Inc., Target Corporation, and Walmart Inc.) in Tampa, Florida, in their raw, individual, or processed form, cut in small pieces, and stored in airtight polyethylene bags in a refrigerator at a temperature of 4°C until the pH measurements were performed.

pH buffer supplements: In order to compare the pH "buffering

score" of food with the one of commercial antacids, we also performed the pH titration of sodium bicarbonate from ARM & HAMMER and SIGMA-ALDRICH (1 g of powder/150 mL of water, $NaHCO_3$, molecular weight 84 g), calcium carbonate TUMS® (TUMS Ultra Strength 1000, 1 tablet [2.58 g] containing 1 g calcium carbonate, 400 mg calcium, 5 mg sodium, 1.5 g sugars), L-lysine free base SIGMA-ALDRICH (1 g of powder/150 mL of water, reagent grade ≥ 98%, catalog number L5501, linear formula H2N(CH2)4CH(NH2)CO2H, and molecular weight 146.19 g), L-lysine monohydrochloride SIGMA-ALDRICH (1 g of powder/150 mL of water, reagent grade ≥ 98%, catalog number L5626, linear formula H2N(CH2)4CH(NH2)CO2H·HCl, and molecular weight 182.65 g), L-lysine Monohydrochloride GNC (two tablets (1.52 g)/150 mL of water, 0.5 g of L-Lysine in the monohydrochloride form and 1 g of cellulose per tablet, code 010711), Spirulina GNC® (1 g/150 mL of water, 0.5 g of *Arthrospira platensis* per capsule, filled with gelatin and cellulose, code 198311), Peptone SIGMA-ALDRICH (10 g of powder/150 mL of water, microbiology grade, catalog number 70169), and "Whey Protein Gold Standard" OPTIMUM NUTRITION (10 g/150 mL of water, 90% protein weight).

pH titration: Based on water solubility of the test substances, we used a suspended mass varying between 1 g and 100 g, liquefied in a volume between 50 mL and 150 mL of de-ionized water, by using a Rival® 5 Speed Blender. The volume of juices, carbonated and non-carbonated beverages, alcoholic and non-alcoholic beverages were arbitrarily tested between 100 mL and 355 mL and normalized to 100 mL.

During the pH titration, each sample was stirred continuously with a magnetic stir bar using the VWR® Standard Magnetic Stirrer. For each of these food types with pH higher than 4, we titrated by gradual addition of 1 mL of 1 N hydrochloric acid (HCl) until pH decreased to 2 (the normal stomach pH), and for food with pH lower than 4 we titrated by gradual addition of 1 mL of 1 N sodium hydroxide (NaOH) until pH increased to 4 (the normal distal duodenal pH) using a Denver Instrument model 250 pH meter adjusted to room temperature with a calibrated combination electrode (Denver Instrument Micro Glass-body pH Electrode, body diameter 0.20" (5 mm) and pH 0 to 14). Initial pH levels and all further measurements taken during titration were recorded following 30 seconds to 1 minute equilibration period after addition of acid or base, and the total volume of acid or base added to each sample was recorded separately.

Determination of "acid-buffering score"

For each of these food types, we measured pH titrations from the initial pH values until pH < 4.0. From these, we have assigned a "base excess" score, which is the number of excess H^+ consumed to lower the pH to 4.0, which is the postprandial pH of the distal duodenum. These data were normalized to 100 g dry weight or 100 g wet weight. The final "buffer score" normalized the base excess to Harvard FFQS and United States Department of Agriculture (USDA) [31] serving sizes.

In vivo experiments

Animal housing and diet: Animals were housed in the USF Vivarium located onsite at the H. Lee Moffitt Cancer Center according to IACUC protocol. Bioluminescent imaging was completed within the facility using the In Vivo Imaging System (IVIS 200). 4-6 week old SCID-beige mice (Harlan, Madison, WI) were placed in two cohorts (8 mice each) and were provided with either tap water or 200 mM Lysine pH 8.4 water six days prior to injection, and was continued for the duration of the experiment. Lysine (free base from Sigma Aldrich, St. Louis, MO) was dissolved in tap water and the pH was adjusted to pH 8.4 with 1N HCl. Water consumption and animal weights were measured biweekly.

The effect of lysine at pH 8.4 observed in this work was compared to a previous study from our group, where mice were supplemented with lysine at pH 10 [15]. Briefly, 4 days prior to injection of prostate cancer cells (PC-3M) 4-6 week old SCID-beige mice were separated in two groups with six mice each. The control group received normal tap water, while the second group received tap water with 200 mM lysine at pH ~10. Tumor burden and presence of metastases was determined through bioluminescence.

In the animal studies performed in the current work, the blood chemistry (pH, concentration of bicarbonate anion, etc.) was not analyzed. However, our previous works with SCID-beige mice under oral pH buffering supplementation [13] consistently showed that after a transient (c.a. 1 day) increase, blood pH returns to normal levels by compensation from the whole body buffering regulatory system, while a stable increase in bicarbonate anion concentration remains for the course of the experiment, and this increase is the actual responsible for the increase in intra-tumoral pH buffering.

Cell culture and preparation of cells for injection: PC-3M cells stably expressing luciferase (Luc6 clone) were obtained from Xenogen Caliber (Hopkinton, MA). PC-3M cells were cultured as detailed previously [32]. In preparation for injection, PC-3M cells were trypsinized and washed once with sterile 1X PBS before being suspended at a concentration of 2.4 x 10^6 cells in 200 µL PBS. Cells were injected via tail vein and successful injection was confirmed immediately by bioluminescent imaging.

Bioluminescent imaging: Animals were anesthetized with isoflurane and injected intra peritoneally with 10 µL per g body weight with 15 mg/mL d-luciferin. Five minutes after injection, mice were imaged with the IVIS 200, resulting in a photographic image overlayed with the corresponding bioluminescent image. Images were analyzed using LIVINGIMAGE V3.2 software (Caliper Life Sciences, Hopkinton, MA).

Results

The "buffer score"

In order to quantify the "buffering score" of foods, it was first important to establish *ab initio* a physiologically relevant metric to which values can be compared. Once foods are ingested, they are transported through the esophagus to the stomach, where they mix with gastric juices comprised of pepsin, lipase, mucin, intrinsic factor, peptides, nucleic acids and electrolytes (Figure 1). Throughout the gastrointestinal tract, a mucosa provides a dynamic barrier within the host, allowing the passage of certain ions and molecules into the body and restricting the entry of other luminal contents. This maintenance of barrier function is not so much an anatomic barrier as it is a series of consecutive defense mechanisms, each of them finely regulated [33,34]. Gastric juices are kept at low pH (~2) by the secretion of HCl by parietal cells, which are part of a control mechanism to keep the gastric pH at this optimum level, which maximizes the activation of pepsin and absorption of nutrients [35-37]. The H^+ are generated in parietal cells by the hydration of CO_2. Hence for each H^+ there is a stoichiometrically equivalent amount of HCO_3^- produced on the basolateral (blood) side of the gastric epithelium. Importantly, this HCO_3^- is re-released into the gastrointestinal lumen in the duodenum (Figure 1), in order to raise the pH back to ca. 4.0. During the gastric phase of digestion, a transient

increase in blood pH can be measured, which is known as the "Alkaline Tide". This phenomenon consists of a temporary increase in the pH and bicarbonate levels of blood after a meal, as a consequence of the delay between the acid secretion in the stomach and later secretion of bicarbonate in the duodenum [36,38,39] (Figure 1).

Despite the low pH of the contents released by the stomach, the epithelial cells of the duodenum are maintained in a virtually neutral pH due to a layer of mucus and the secreted bicarbonate, which plays an important component of a larger system of acid-base balance [40,41]. Between the proximal and distal sections of the duodenum, a gradient of 2 pH units is established. Thus, we considered that the number of protons consumed to reduce the pH of foods to a pH of 4.0 would contribute to a net alkaline tide, and reflect an increase in systemic buffering.

It is important to clarify that the term "buffering score" of food in this study refers to the number of protons absorbed by the food from the body during its passage through the GI tract. This should not be confused with the general term buffering capacity of a solution (β), which is the instant derivative of a pH titration curve of a solution at a given pH value. The "buffering score" of a food, as described in this work, would thus consist in the integration over pH, of the buffering capacity from the initial food pH until pH~4.0.

Buffering score of pH buffer supplements

All preliminary *in vivo* work has been performed using sodium bicarbonate as the paradigmatic buffer. As expected, bicarbonate had a high buffering score, requiring 11 mEq HCl to reduce the pH of 1g to a pH of 4.0 (equivalent to 0.93 mEq HCl per mEq bicarbonate).

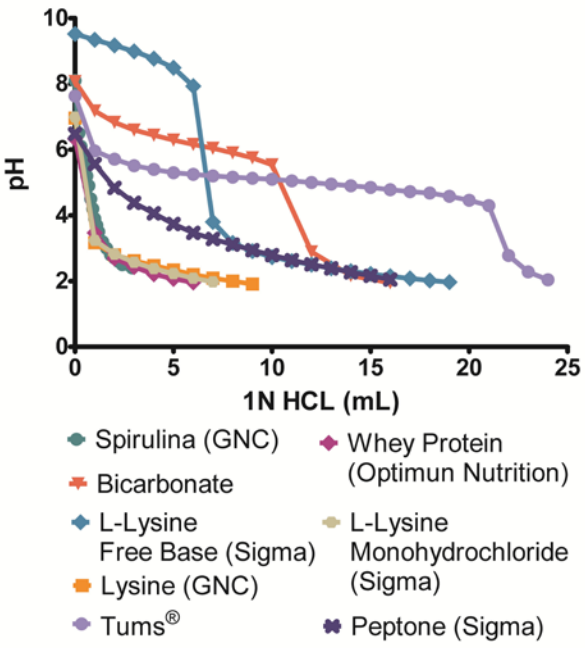

Figure 2 Titration curves of pH buffer supplements. Different supplements commercially available were tested as candidates for pH buffering therapy. Each supplement was re-suspended in distilled water and titrated using HCl (1N) from its original pH down to pH 2.0. The amounts of supplement and volumes of distilled water were 1g/150mL for Spirulina, 1g/150mL for sodium bicarbonate, 1g/150mL for lysine (free base), 1.52g/150mL for lysine (GNC), 50g/150mL whey protein, 1g/150mL for lysine monohydrochloride, 2.58g/150mL for Tums, and 4g/50mL for peptone. L-lysine free base and calcium carbonate are the best substitutes for sodium bicarbonate.

This is a baseline score, to which all other supplements and foods can be compared. Previous studies [42] have suggested that diets rich in protein would consume more gastric acids. Consistent with this, Peptone (a tryptic digest of casein) buffers over a large pH range and consumes 4 mEq H^+/gram dry weight to reach a pH of 4. Similarly, lysine base is a dibasic amino acid that should have a high base excess. Titration results (Figure 2) showed that L-lysine free base had a pH buffering score comparable to sodium bicarbonate on per gram (7 vs. 11 mEq HCl per gram) and per mEq (~1 mEq HCl per mEq buffer) basis. Notably, neither of the "protein-rich" common supplement preparations (spirulina and whey protein) exhibited significant buffering, suggesting that their protein contents were less than 100%. A common antacid whose active ingredient is calcium carbonate, Tums®, showed approximately the same buffering score as sodium bicarbonate on a per gram basis (7.7 vs. 11 mEq HCl per gram). On a per gram active ingredient basis, $CaCO_3$ was much more effective than $NaHCO_3$, as expected (20 vs. 11 mEq HCl per gram). On a molar basis, $CaCO_3$ was twice as effective, consuming 2 mEq H^+ per mEq $CaCO_3$, and as such is a suitable candidate for a systemic pH buffer.

Supplementary tables 1 and 2 lists the titration results for foods and supplements in this study.

Buffering score of food from different groups

Table 1 summarizes the buffering scores, in mEq H^+, for the foods in this study. Notably, foods showed similar buffering score within the same group.

The groups of carbohydrates and dairy products showed a buffering capacities varying from 5 to 25 mEq of H^+/serving size, which is equivalent to approximately 0.45-2.3 g of sodium bicarbonate, respectively. The fruits and vegetables group showed the lowest pH buffering score, while the meats group showed the highest buffering score.

Our results show that the order of magnitude of the pH buffering score of a diet containing protein-rich foodstuff such as meats and dairy products is the same as that of the amount of sodium bicarbonate given to cancer patients in the two clinical trials (0.9 g/kg of body mass). For instance, the replacement of 3 servings of carbonated cola by 3 glasses of milk, and an additional 3 servings of meat would increase a person's pH buffering score by $3\times(20+3)+3\times(50-5)=204$ mEq of H^+, which is equivalent to approximately 20 g of sodium bicarbonate.

Lysine free base reduces metastases through pH buffering

Previously we have shown that an amino acid, lysine, reduces metastasis in a prostate cancer model, which we hypothesized occurs through buffering of peri-tumoral pH [32]. Lysine has three pKas and can be protonated at the side chain amino group, backbone amino group and carboxyl group. To test the effect of lowering the buffering capacity of free base lysine, we lowered the pH of a 200 mM lysine solution to pH 8.4, below the middle pKa. Titration analyses of 200 mM Lysine showed that lysine pH 8.4 has about one-fifth the buffering capacity of Lysine pH 10.0 (Figure 3b). In this experiment, mice were injected intravenously with luciferase-expressing PC-3M prostate cancer cells and allowed to drink either tap water or water with 200 mM lysine pH 8.4. Weekly monitoring of metastasis formation with bioluminescence imaging revealed that 200 mM lysine pH 8.4 had a less significant effect on the development of metastatic burden compared to the fully buffered (pH 10.0) lysine supplementation (figures 3c and 3d). These results confirm that the pH buffering of lysine, and not its metabolism, was the main factor in prevention of metastases in animal models.

Food / supplement	Serving size	Points per serving size
Dairy Products		
Sour cream	1tbsp	+0.5
Peach yogurt	1 cup (8 fl oz)	+8.0
Swiss cheese	1 slice or 1oz serving	+9.5
Whole milk	8 fl oz	+13.0
Low fat milk	8 fl oz	+19.0
Fruits		
Orange	1 fruit (2-5/8" diameter)	+0.0
Apple	1 large (3-1/4" diameter)	+0.0
Peach	1 medium (2-1/2" diameter)	+0.0
Black Grapes	1oz serving	+0.0
Banana	1 extra small (less than 6" long)	+3.0
Watermelon	1 slice	+4.5
Cantaloupe	1 cup, cubes	+5.0
Vegetables		
Lettuce	1 leaf outer	+1.0
Eggplant	1/2 cup	+1.0
Tomatoes	1 Italian tomato	+1.5
Green beans	1/2 cup	+2.0
Kidney beans	1/2 cup	+3.0
Garbanzo beans	1/2 cup	+2.0
Carrots	1/2 cup cooked	+2.5
Brussels' sprouts	1/2 cup	+3.0
Beets	1/2 cup	+3.0
Rice (white)	1/2 cup	+4.0
Broccoli	1/2 cup	+3.0
Asparagus	1/2 cup	+5.5
Eggs, Meats, Etc.		
Eggs (whole)	1 unit	+6.0
Chicken breast	1 can (5 oz)	+15.0
Tuna	1 can	+24.0
Hamburger (fat)	1 unit (patty)	+52.0
Hamburger (lean)	1 unit (patty)	+22.0
Breads, cereals, etc.		
White bread	1 slice (medium)	+2.5
Oatmeal	1 cup	+5.5
Cheese pizza	1 slice	+14.0
Beverages		
White wine	1 serving (5 fl oz)	-4.5
Red wine	1 serving (5 fl oz)	-1.5
Caffeine-free cola	1 can (12 fl oz)	-3.0
Apple juice	1 cup	-2.5
Regular cola	1 can (12 fl oz)	-2.0
Regular coffee	1 cup (8 fl oz)	+2.4
Beer	1 can or bottle (12 fl oz)	+2.0
Arizona fruit punch	1 can (12 fl oz)	-2.0
Hawaiian punch	1 can (12 fl oz)	-2.5
Diet cola	1 can (12 fl oz)	-1.5
Caffeine-free and sugar-free cola	1 can (12 fl oz)	-1.5
V8 100% vegetable juice		+3.5
Supplement		
Spirulina	1g	+1.0
NaHCO3	1g	+11.0
L-Lysine Free Base (Sigma-Aldrich)	1g	+1.0
L-Lysine (GNC)	1 pill (1.52g)	+1.0
Whey protein (O.N.)	10g	+0.5
L-Lysine monohydrochloride (Sigma-Aldrich)	1g	+1.0
CaCO3 (TUMS)	1 tablet (2.58g)	+20.0
Peptone (Sigma-Aldrich)	10g	+11.0

Table 1: Dietary pH buffering capacity point system. The results from the animal experiments[13] suggested a sodium bicarbonate dose of 1g/kg/day, which was suggested for clinical trials[16,17]. The table below contains a sampling of different foods, and their respective buffering "points" per serving size. In other words, an adult who is undergoing a pH buffering treatment may reduce the daily intake of sodium bicarbonate by 1g at every 11-point increase in his/her diet. This can be achieved by adding positive point foods such as dairy products, or by removing negative ones, such as wine and carbonated colas.

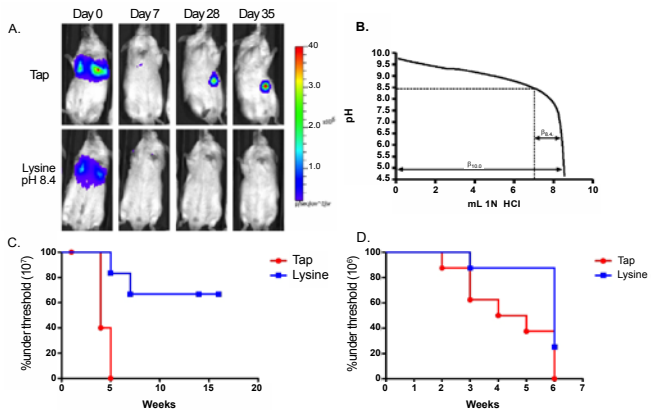

Figure 3: Lysine free base reduces metastases through pH buffering. SCID mice were divided in two groups: the "control" group and a "lysine" group, which received supplementation of lysine free base in drinking water at pH 8.4. Both groups were injected with the bioluminescent PC-3M prostate cancer cell line (A). In order to determine if the metastatic inhibition was due to the pH buffering properties of lysine, or due to its metabolism, we titrated lysine down to a pH of 8.4, using HCl (1N), a pH at which the pH buffering capacity is significantly reduced ($\beta_{10.0}$=8.5 mEq vs. $\beta_{8.4}$=1.5 mEq). (B) Tumor burden was quantified through *in vivo* bioluminescence along 6 weeks, and the effect of "de-buffered" lysine in survival was compared with the previously published results of lysine at pH 10 [15]. The lethal burden threshold for the survival curves was set at 3-fold the background signal (Rose criterion), which was considered as being the bioluminescent signal one week post injection. According to the Log-rank (Mantel-Cox) test, there was a significant increase in overall survival in the group orally supplemented with lysine pH 10 (P=0.0038, median survival control=4 weeks, median survival lysine pH10.0=undefined) (C). In this work, mice in the group supplemented with "de-buffered" lysine (pH8.4) showed a less noticeable difference in survival compared to the control group (P=0.0417, median survival for control group=4.5 weeks, median survival for lysine pH8.4=6), which reinforces the idea that the anti-metastatic properties of lysine are linked to its pH buffering capacities, rather than its metabolic properties (D).

Discussion

The pH regulation of food along the digestive tract is not yet fully understood. Measurements of duodenal pH have been accomplished via insertion of electrodes in different regions, and measurements have been performed for several hours to account for periods of fasting, feeding and digestion. These have been difficult measurements to obtain. Although there is a considerable amount of divergence among the reported values [43-45], most studies agree that the fasting pH in the duodenum is ca. 4.0, and that this reaches higher values (4.7-6.8) post-prandially, and returns to 4 with the influx of gastric HCl [43].

Hence, we propose that the number of protons required to lower the food pH from its original value to a final pH of 4.0 represents a base excess, which is the net balance of the effect of food on systemic buffering capacity. Diets composed of food which consume large

amounts of HCl to reach the pH 4 (alkalinizing; high base excess), will contribute to an increase in systemic buffering capacity, while diets that require consumption of bicarbonate to reach 4.0 (acidifying, negative base excess) will reduce systemic buffering capacity.

In this work, we studied the pH buffering score of different foods and supplements as a prerequisite for analyzing cohort studies of cancer in which participants reported usual diet using a food frequency questionnaire and as a potential dietary adjuvant for patients undergoing buffer therapy. In agreement with prior publications, these results showed that the initial pH of food varied widely among the different food groups [46-48] in the few extant references to food buffering score, and so does the amount of acid or base required to reach the pH of the distal duodenum. Milk and other dairy products that originated from cow's milk had the highest buffering score among the foods in our study. However, as previously observed, dairy products from other animals such as goat may have a lower initial pH [49]. A recent review noted that the association of milk and dairy product intake has been examined in only a small number of cohort studies, and data are inconsistent or lacking [24]. Meta-analyses of cohort data available to date support an inverse association between milk intake and risk of colorectal and bladder cancer [24], but more research on other cancer sites is warranted.

A review on studies of buffering score of meat [20] showed discordance with our study, with a buffering score of approximately half of what we observed. This difference may be due to the composition of the "meat" used in both studies (pork and beef muscle) [20] compared to ground beef used in our study, in addition to the titration direction. While our study focused on the interval between the initial pH and pH 4.0, these previous studies focused on the titration towards pH 9.0.

Among vegetables and grains, kidney beans showed thrice as much buffering score as white rice. This difference has been previously documented and reported to be in part due to the polishing process of the white rice [22]. Although most fruits and vegetables have been associated with lower rates of cancers in epidemiological studies, we observed that several vegetables and fruits tested in this study had a relatively low buffering score compared to protein-rich foods, including beans. The notable exceptions were the melons, which had high buffering score. Recent findings of the presence of bioactive phytochemicals in fruits and vegetables, which target various signal transduction pathways that modulate carcinogenesis, may contribute to their cancer prevention effects despite the low buffering score measured for this food group [50].

By using a buffering score system which attributes one point per mEq of HCl necessary to bring the pH of a serving size of food stuff to pH 4.0 (and conversely deduces one point per mEq NaOH needed to reach the same pH), we selected the best candidates to be included or avoided in the nutritional intervention (pH buffering) to be examined in a clinical setting. Our evaluation of an initial group of common foods indicates that meats and dairy foods are those with highest pH buffering score, while the carbonated sodas and citric juices have the lowest scores. This study shows that the substitution of low buffering score by high buffering score foods can increase the blood pH buffering score by the same order of magnitude as antacids, and thus is an alternative to reduce the amount of antacids required for patients in pH buffering clinical trials.

It is also notable that Remer [25] has shown that protein consumption per se is a strong metric for increased renal acid secretion. This underscores the difference between raw buffering power and eventual metabolism. In the current study, we were uniquely focused on the buffering score of foods as an adjuvant to buffer therapy. However, as macronutrients are metabolized, they are oxidized to volatile (carbonic) and non-volatile (sulfuric) acids, or converted to ammoniac bases (urea) that are exhaled or excreted. This metabolic acid load must also be considered when calculating the effect of diet on systemic buffering.

The animal experiment showed that the efficacy of dietary buffers (e.g. lysine) in preventing metastasis was dependent on its titration state, and is thus independent of metabolism. Therefore, we propose that dietary macronutrients can have a significant systemic pH buffering effect, as long as they are prepared and ingested in a form where they retain their pH buffering (food pH > pKa > pH 4.0). These results suggest that a highly buffered, high-protein diet prepared at a high pH can increase the systemic buffering system and potentially delay metastases.

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