



**ATLAS
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The Effect of Organic Green Cell Therapy on Systemic pH

Clinical Studies By

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Abstract

Chronic, low-grade metabolic acidosis has been implicated as an underlying cause of a variety of conditions with adverse effects on health, in particular the catabolic effects on skeletal and muscle tissue. Metabolic acidosis is a state in which the blood pH is low (under 7.35) due to increased production of H⁺ by the body or the inability of the body to form bicarbonate (HCO₃⁻). It is well documented that the composition of the diet can affect the body's acid-base balance due to the ability of specific foods to produce acid or base as a result of their digestion and metabolism; and by manipulating diet this balance can be tilted in favor of net acid accumulation (lower pH) or net base, primarily bicarbonate, production (higher pH). Foods high in mineral salts and organic acids, namely most fruits and vegetables, tend to increase pH. This study examined the effects of a nutritional supplement based on concentrated "green" (vegetable) extracts, digestive enzymes, probiotics, and anti-oxidants on acid-base status by observing changes in urinary and salivary pH. Twelve healthy subjects between the ages of 23 and 44 were recruited to participate in the study. All participants consumed a protein supplement daily during the course of the study. Six subjects consumed the Green Cell product for 10 days followed by 10 days not taking the product and six subjects served as controls not taking product for the 20 day duration of the study. Both groups measured their urine and saliva pH 3 times per day and the average pH were recorded for each day. There was a significant group by time interaction ($p < .05$) between the first 10 days and second 10 days and a significant difference in pH between the groups during the first 10 days ($p < .01$). These results suggest that the Green Cell Therapy product appears to be effective in maintaining increased urinary and salivary pH.

The Effect of Organic Green Cell Therapy on Urinary and Salivary pH

Normal pH is held between a narrow range of 7.35 and 7.45 in arterial blood and slightly lower, 7.31-7.41 in venous blood, however it is likely that when pH is close to the low end of this range, even though still considered to be "normal" the body is attempting to increase pH and correct a "low-grade" metabolic acidosis (4). Metabolic acidosis can be generated by increased acid (hydrogen ion) production, extra-renal loss of base (bicarbonate ion), impaired renal acid excretion, or a combination of any of these factors. Metabolic acidosis, in a clinical sense, can be serious and life threatening subsequent to conditions such as diabetes, kidney failure, shock, and ingesting certain toxins (13). However metabolic acidosis can also be a mild, chronic condition. Under normal physiologic circumstances, the net endogenous acid production rate (NEAP) and the degree of the attendant low-grade metabolic acidosis are determined primarily by the composition of the diet (4, 7, 15). And there is also a link between low-grade chronic metabolic acidosis, diet and clinical disorders, including nephrolithiasis, osteoporosis, sarcopenia, epilepsy, and endocrine disturbances (1, 4, 8, 11, 13, 15, 16, 17, 19, 26). Chronic low grade metabolic acidosis can occur even in healthy individuals if consumption of foods high in acid precursors is disproportionate to that of foods rich in base precursors (vegetable foods) (4, 8).

As stated above the cause of diet induced metabolic acidosis is an excess consumption of acid forming foods compared with base forming foods. Protein metabolism yields non-carbonic acids due largely to oxidation of cationic and sulfur-containing amino acids (8, 9, 14). In addition high sodium chloride intake can also induce mild metabolic acidosis (8, 12). Consumption of most vegetable foods on the other hand increases bicarbonate production due to the metabolism of dietary organic acids/alkali salts (e.g. sodium citrate, potassium citrate, potassium gluconate, etc.)(8, 9, 15).The carbon dioxide produced by oxidation of organic acids from fruits and vegetables is exhaled, which also helps reduce the acid load on the body. Ingested bicarbonate as mineral salts (e.g. sodium bicarbonate, potassium bicarbonate, etc.) can also increase the plasma bicarbonate concentration (1, 6, 8, 9, 15, 17).

The extent to which diet contributes to acidosis can quantitatively determined by measuring the potential renal acid load (PRAL) of individual foods (22, 24). On average this is estimated to be 1-2 mEq of acid per kg body weight of acid per day or 70-150 mEq/d on the diet typical of industrialized societies, which are high in processed foods based on processed cereal grains, animal protein, and a high salt content. This is believed to result in a low-grade CMA with secondary bone and muscle catabolism due to increased NEAP and loss of base/bicarbonate (6, 7). It

has been estimated that pre-agricultural dietary PRAL was negative 88 mEq total per day, indicating the retention of base (8, 22, 23, 24). The PRAL (calculated for a 24-hour period), together with a relatively constant daily amount of urinary excreted organic acids (in healthy subjects proportional to body surface area or body weight), yields the daily net acid excretion (7, 9, 22, 23, 24).

The body attempts to normalize pH by two primary mechanisms: 1) plasma bicarbonate to buffer the increase in endogenous acid production, and 2) urinary excretion of hydrogen ions. As plasma bicarbonate concentration decreases progressively when endogenous acid production is increased by dietary changes to acid producing foods in normal subjects, the skeletal stores of bicarbonate are tapped into, thereby releasing bicarbonate ions that can buffer increases in hydrogen ions ultimately forming carbon dioxide and water by the reaction: $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+$ (17). In addition, acidosis-induced proteolysis appears to be an acid-base homeostatic mechanism. Amino acids released from skeletal muscle, in particular glutamine, which is used by the kidney for synthesis of ammonia; the kidney can increase the excretion of acid (as ammonium) in the urine, thereby mitigating the severity of the acidosis (4, 13). It has been reported that chronic metabolic acidosis induces muscle protein breakdown, and that correction of acidosis reverses the effect (6, 7, 26). Indeed, because of the attendant low grade chronic acidosis, habitual ingestion of typical net acid-producing diets might chronically sustain a slightly increased state of protein breakdown and consequent nitrogen wasting that can be considered a tonic "normal" state in adult humans, accounting for the normal progressive decrease in muscle mass (sarcopenia) as adults get older. Normal age-related decline in kidney function can increase the severity of diet-dependent acidosis thus amplifying acid induced muscle wasting in older individuals (8, 13).

It is also possible that skeletal muscle hypertrophy induced by resistance training may be attenuated since a positive nitrogen balance is necessary for muscle hypertrophy may be more difficult to achieve. The increased protein intake required for optimal muscle adaptation could be negated by the acidifying effect of dietary protein unless countermeasures are taken to ensure a net dietary alkaline load instead of an acid load.

In order to correct this low grade CMA and bring acid-base balance towards a more alkaline state consuming a diet high in alkaline/base producing foods compared to acid producing foods is necessary. When dietary protein intake and consumption of processed foods high in sodium chloride is low, the usual content of the diet or moderate increases in base producing vegetables and fruits can usually suffice (5, 16). However as protein intake increases it is necessary to consume increasing amounts of these foods to offset endogenous acid production,

which can be difficult to achieve because it is often inconvenient to consume large amounts of vegetables and fruit. This consideration prompted us to investigate, in healthy adult subjects eating a normal net acid-producing diet, whether a dietary supplement containing a blend of vegetable powder extracts, minerals, digestive enzymes, and probiotics is effective in maintaining alkaline conditions within the body.

Conventional testing of acid-base balance on a clinical basis typically involves sampling blood and/or urine and measuring the difference between major plasma cations and major plasma anions; Anion Gap (AG) = $([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-])$ or Strong Ion Difference [SID] = $[Na^+] + [K^+] + [Ca^{2+}] + [Mg^{2+}] - [Cl^-] - [other\ strong\ anions]$ (10, 13). However it is obviously unrealistic for healthy individuals to have these measurements taken on a daily basis. Long-term acid loading in humans causes an increase in renal acid excretion, normally leading to the urine becoming more acidic (22). This can also be measured clinically where net acid excretion (NAE) is $= NH_4 + \text{titratable acid} - \text{urinary } HCO_3^-$ (15, 22). The total increment in NAE by the kidneys, however, is often lower than total acid production, resulting in a positive hydrogen-ion balance. Thus urinary pH will decrease as the kidneys attempt to excrete excess acid and thus can be used as an indicator of excess acid excretion and acid-base balance (22, 25). In addition, the pH of saliva can also provide an indication of the body's acid-base status (2, 3, 14). Immediately following feeding bicarbonate is released into the blood due to hydrochloric acid production by stomach parietal cells (alkaline tide). Subsequently sodium bicarbonate is secreted from the pancreas into the small intestine to neutralize the acid chyme entering the small intestine from the stomach after feeding. The bicarbonate is then retained by absorption back into the blood plasma. As serum bicarbonate is used to help buffer the excess hydrogen ions, and remaining bicarbonate is available to be taken up by alkaline glands in the body including the salivary glands (21).

The purpose of this investigation was to evaluate the effect of an Organic Green Cell Therapy supplement on daily urine pH and saliva pH in subjects consuming a protein supplemented diet.

Method

Subjects

Subjects were divided into 2 groups of six (6) participants. Participants were given an informed consent as to the testing of the product. Subjects were chosen at random, and randomly selected for the test group and control group.

Equipment

Participants weighed on a Detecto 5000 scale for body weight. A detailed body fat analysis was then performed using the Lange Skin Fold Caliper (Model # 014921). All participants were test strips to measure urine and saliva pH (phion Nutrition Scottsdale, AZ)

Experimental Protocol

All participants were required to take in 40-70 additional grams of supplemental protein (Metabolix Nutrition Systems, Inc., Lutherville, MD) on a daily basis for 20 days. The test group was instructed to take two (2) Green Cell Therapy capsules (Atlas Operations Inc. Pompano Beach, FL) with each meal for the first ten day period, followed by no supplement for the second 10 days, while the control group was instructed to take just the supplemental protein for the entire 20 days. The components of the proprietary blend Green Cell Therapy are listed in Table 1. Each subject was instructed to take urinary and salivary pH levels using pH test strips three times per day - one hour after the first meal of the day, one hour after the second meal of the day and prior to bedtime. pH levels were recorded for a period of 20 days and mean pH level for each day was calculated using the equation provided by the pH test strip manufacturer:

$$(\text{avg urine pH} + \text{avg saliva pH} * 2) / 3$$

Taken three times per day and using the above equation provides a "moving average" proposed by Reams (3) that accounts for the relatively wide fluctuations in urinary and salivary pH throughout the day while also accounting for the relative contribution of the respective values as indicators of blood and tissue acid-base status.

Statistical Analysis

Statistical analyses were conducted using SPSS PC 13.0 version software. Means \pm SD were calculated for all variables. A One Way ANOVA was used to determine if there were any differences between groups with respect to age, weight, and percent body fat. A two-way group (green cell therapy vs. no therapy) x time (first 10 days vs. second ten days) analysis of variance (ANOVA) with repeated measures on time was used to compare the two groups over time on the daily average combined urine and saliva pH. Significant group x time interactions were followed up with pairwise comparisons based on estimated marginal means with Bonferroni adjustment for multiple comparisons. Differences were considered significant at $p < 0.05$.

Table 1: Green Cell Therapy

Brown Rice Bran
Wheat Grass Juice Powder
Barley Grass juice Powder
Chlorella
Carrot Juice Powder
Beet Juice Powder
Parsley Leaf Powder
Kale Juice Powder
Lipase
Lactase
Amylase
Protease
Bifidobacterium Longum
Lactobacillus Rhamnosus
Lactobacillus Acidophilus
Lactobacillus Casei
Grape Seed Extract
COQ10
Milk Thistle Extract
Lycopene
Alpha Lipoic Acid
Quercetin
Dandelion Powder
Spinach Powder
Blue Green Algae
Bee Pollen Powder
Aloe Vera Powder
Flax Seed Powder
Lecithin
Green Tea
Astragalus Powder
Royal Jelly
**Contains milk, soy.

Results

The anthropometrical data for the participants are presented in Table 2. The groups differed with respect age with the experimental group significantly younger than the control group ($31.5y \pm 6.1y$ vs. $39.8y \pm 2.4y$, $p = .012$). There were no significant differences between the groups with respect to weight ($184 \pm 58.5\text{lbs}$ vs. $181 \pm 66.1\text{lbs}$) and percent body fat ($16.3\% \pm 5.7$ vs. $21.5\% \pm 5.8$).

Table 2: Subject Data

Experimental group

Gender	F	M	M	F	F	M
Age (y)	34	28	28	23	39	37
Weight (lbs)	133	218	263	115	153	222
BF%	9.1%	16.8%	14.6%	21.2%	12.1%	24.5%
	Age	Weight	BF%			
Mean	31.5	184.0	16.3			
Std. Deviation	6.1	58.5	5.7			

Control group

Gender	F	M	M	F	F	M
Age (y)	44	39	41	38	37	40
Weight (lbs)	159	211	119	167	131	299
BF%	29.0%	11.6%	15.7%	12.9%	23.4%	36.2%
	Age	Weight	BF%			
Mean	39.8	181.0	21.5			
Std. Deviation	2.4	66.1	9.8			

The average daily pH values are shown in Table 3 and Figure 1 for the test group and the control group for the initial 10 day period where the test group was using the Green Cell Therapy product followed by the second 10 days of the 20 day experimental period where both groups were not using the product. For the first 10 days of the study the mean combined urine and saliva pH for the experimental group ingesting the Green Cell Therapy was $6.82 \pm .22$ and for the second 10 days it dropped to $6.49 \pm .39$. For the control group the mean daily combined pH was $6.46 \pm .07$ for the first 10 days and $6.51 \pm .05$ for the second 10 days. Statistical analysis revealed a significant group (experimental vs. control) by time (1st 10d vs. 2nd 10d) interaction ($p < .05$). Post hoc analysis revealed a significant difference in pH during the first 10 days between the groups ($p < .01$) while there was not a significant difference between groups for the second 10 days.

Table 3: Cumulative mean pH for the first 10 days followed by the second 10 days; for the control group and the group ingesting the Green Cell Therapy product for the first 10 days only.

Group		10 day average	10 day post average
Control	Mean	6.46	6.51
	N	6	6
	Std. Deviation	.07	.05
Green Cell	Mean	6.82	6.49
	N	6	6
	Std. Deviation	.22	.39
Total	Mean	6.64	6.50
	N	12	12
	Std. Deviation	.24	.27

Cumulative mean pH values

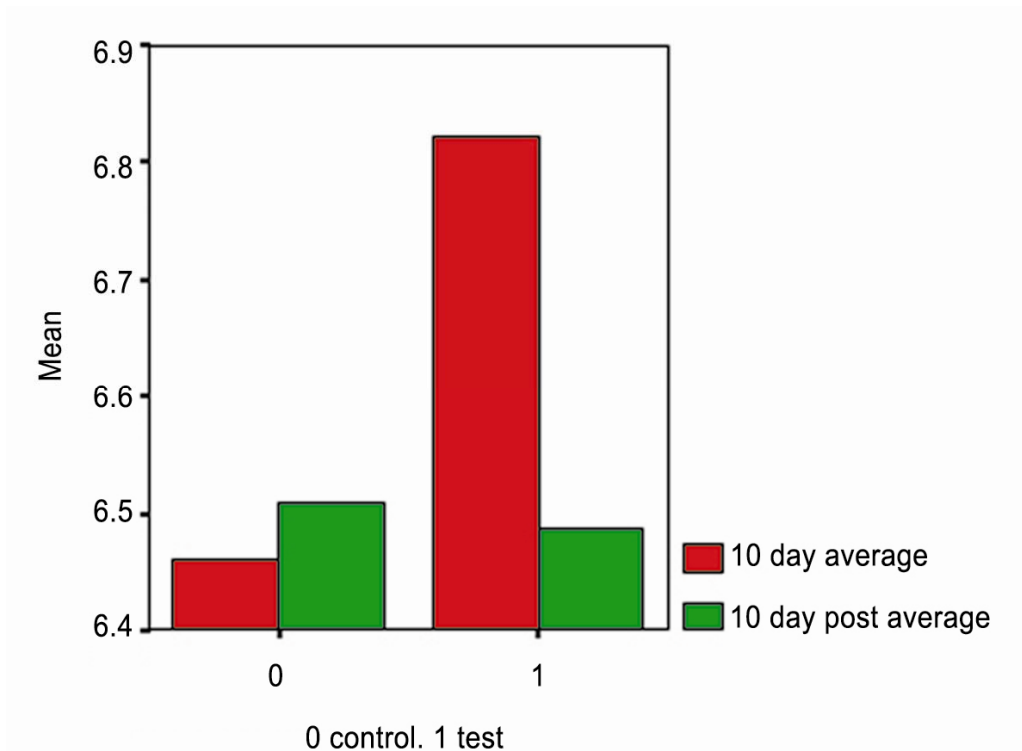


Figure 1: Cumulative mean pH values. First 10 days (red bar) followed by the second 10 days (green bar) for the control group and the group ingesting the Green Cell Therapy product for the first 10 days only.

Discussion

The results of this study demonstrated that consuming the Green Cell Therapy product maintained a significantly higher combined urinary and salivary pH. This result is not surprising since the test product contains a blend of agents individually known to have an alkalizing effect on the body; these include the vegetable extracts, algae, and digestive enzymes. A previous study by Burns examined the alkalizing effect of a mineral based supplement (pH Control, pH Sciences, Inc.) on urine pH. In this study 23 subjects (no control group) ingested 4 x 1000 mg tablets for five days. Urine was collected only on waking up in the morning on the first and on the 5th day. A mean significant increase of 0.32 pH units was observed which they calculated to be a 53% reduction in urine acidity. This value is in

agreement with the increased pH in the current study of 0.33 pH units for the treatment group.

Allie and Rogers (1) examined the effects of mineral (calcium, magnesium) and citrate supplementation for 7 days on mean urinary pH in an attempt to evaluate urinary stone risk. In this study all 3 supplements increased urinary pH with the citrate having the largest effect increasing pH from 6.83 to 7.40. An interesting finding from this study was that citrate supplementation resulted in reduced excretion of calcium and magnesium but increased sodium excretion whereas the supplements containing the minerals also increased excretion of those minerals. Citrate is a major component of diets that result in a reduced PRAL, NEAP, and acid excretion (8, 24).

Although only urine and saliva pH was recorded in the current study, this data can be compared to measured urinary net acid excretion in previous studies. In a review on bone buffering of acid and base in humans, subjects eating a control diet had a mean urinary pH = 6.05 ± 0.44 and mean urinary net acid excretion = 49 ± 28 meq/day (17). Subjects that received NH_4Cl had a mean urinary pH = 5.40 ± 0.13 and mean urine net acid excretion = 272 ± 61 meq/day while the subjects that received KHCO_3 or Na HCO_3 had a mean urinary pH = 6.67 ± 0.18 ; mean urinary net acid excretion = 6 ± 13 meq/day. Therefore it is probable that a more detailed urine analysis would have shown such an increase in net acid excretion after the participants ceased taking the supplement.

One limitation of the current study was that detailed dietary records for the 20 day period were not kept nor were detailed exercise records. This would have enabled us to examine other factors that may affect urinary and salivary pH and determine how the treatment affected acid-base balance. It is possible to estimate the net rate of endogenous noncarbonic acid production (NEAP) by recording the precise amounts of all foods eaten based on the tables provided by Remer and Manz (22). Frassetto, et al. was able to estimate net endogenous noncarbonic acid production in humans from diet potassium and protein contents exclusively (7). Exercise can also affect urinary pH. Moriguchi measured circadian changes in urinary pH as well as bicarbonate in a group of female volleyball players during an intense 3 day training regimen. They found that urine pH dropped by approximately one full pH unit during exercise and that recovery back to pre-exercise levels took as much as 7 hours (20). Another limitation was that the pH measurements were taken using litmus paper test strips which are not as precise as more sophisticated (and expensive) equipment and also have some degree of subjectivity. However use of the test strips enables individuals to monitor their urine and saliva pH conveniently and consistently and this feedback can be important in enabling them to make necessary dietary adjustments to correct acid-base status.

Conclusion

To maintain optimal acid-base balance the body is constantly seeking to get rid of the excess acids that accumulate as a result of cellular metabolism and diet. These acids negatively affect the tissues and can deplete the body of minerals. One of the principal systems it uses for this purpose is the renal system (kidneys). The normal rate of acid excretion through the kidneys gives urine a pH that falls between 6.75 and 7.25 (22, 25). By testing the degree of acidity of the urine, you can determine whether your body is eliminating a normal quantity of acids. If the acid excretion rate is higher than normal, the urinary pH will be more acidic (lower). This low urinary pH is a potential indication that the body is in an acid state. Another indicator of the overall pH balance in your body is the pH of your saliva. When the body has adequate mineral reserves, saliva pH is 7.0 to 7.4. A low saliva pH reading indicates that the mineral reserves are low, and are being used to buffer acids elsewhere in the body (3). The current study showed that using the Green Cell Therapy product is associated with increased pH levels up to these values.

References:

1. Allie, S. and Rogers, A. Effects of calcium carbonate, magnesium oxide, and sodium citrate bicarbonate on the urinary risk factors for kidney stone formation. *Clin Chem Lab Med* 41(1): 39-45, 2003.
2. Bardow A, Madsen J, Nauntofte B The bicarbonate concentration in human saliva does not exceed the plasma level under normal physiological conditions. *Clin Oral Investig* . 2000 Dec;4(4):245-53.
3. Beddoe AF (1984). *Biological Ionization as Applied to Human Nutrition, Principles and Techniques*. Agro-Bio Systems, Fort Bragg, Ca
4. Brown, SE, Jaffe, R. Acid-alkaline balance and its effect on bone health. *Int. J. Ingerative Med.* 2(6), 2000
5. Celia J Prynne, Gita D Mishra, Maria A O'Connell, Graciela Muniz, M Ann Laskey, Liya Yan, Ann Prentice and Fiona Ginty Fruit and vegetable intakes and bone mineral status: a cross-sectional study in 5 age and sex cohorts^{1,2,3} *American Journal of Clinical Nutrition*, Vol. 83, No. 6, 1420-1428, June 2006
6. Frassetto LA, Morris RC Jr, A. Potassium Bicarbonate Reduces Urinary Nitrogen Excretion in Postmenopausal Women *Journal of Clinical Endocrinology and Metabolism* Vol. 82, No. 1, 1997
7. Frassetto LA, Todd KM, Morris RC, and Anthony Sebastian. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents¹⁻³ *Am J Clin Nutr* 1998;68:576-83.
8. Frassetto L, Morris RC Jr, Sellmeyer DE, Todd K, Sebastian A. Diet, evolution and aging--the pathophysiologic effects of the post-agricultural inversion of the potassium-to-sodium and base-to-chloride ratios in the human diet. *Eur J Nutr.* 2001 Oct;40(5):200-13.
9. Frassetto LA, Morris RC Jr, Sebastian A. A practical approach to the balance between acid production and renal acid excretion in humans. *J Nephrol.* 2006 Mar-Apr;19 Suppl 9:S33-40.
10. Gattinoni L, Carlesso E, Cadringer P, Caironi P. Strong ion difference in urine: new perspectives in acid-base assessment. *Crit Care.* 2006 Apr 7;10(2):137
11. Gibney MJ, Walsh M, Brennan L, Roche HM, German B, van Ommen B. Metabolomics in human nutrition: opportunities and challenges. *Am J Clin Nutr.* 2005 Sep;82(3):497-503.
12. Heer, M., Frings, P., Baecker, N (Cologne, D) High sodium chloride intake causes mild metabolic acidosis. Is this the cause for bone resorption? 6th International Symposium on Nutritional Aspects of Osteoporosis Lausanne, Switzerland 04.05.2006 - 06.05.2006
13. Johnson, LR. *Essential Medical Physiology*, 3rd Ed. Elsevier Academic Press, San Diego, CA. 454-461. 2003
14. Kamisawa T, Y, Egawa N, Sakaki N, Inokuma S, Kamata N. Salivary gland involvement in chronic pancreatitis of various etiologies. *Am J Gastroenterol.* 2003 Feb;98(2):323-6.
15. Kurtz I, Maher T, Hulter HN, Schambelan M, Sebastian A. Effect of diet on plasma acid-base composition in normal humans. *Kidney Int* 1983;24:670-80. Estimation of net endogenous

noncarbonic acid production in humans from diet potassium and protein contents¹⁻³ *Am J Clin Nutr* 1998;68:576-83.

16. Lanham-New Fruit SA. Fruit and vegetables: the unexpected natural answer to the question of osteoporosis prevention? *American Journal of Clinical Nutrition*, Vol. 83, No. 6, 1254-1255, June 2006

17. J. Lemann Jr., D. A. Bushinsky, and L. L. Hamm Bone buffering of acid and base in humans. *Am J Physiol Renal Physiol*, November 1, 2003; 285(5): F811 - 832.

19. Maurer, M. 1, Riesen, W.2, Muser, J.1, Hulter, HN.3, and Krapf, R.1 Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans *Am J Physiol Renal Physiol* 284: F32-F40, 2003.

20. Moriguchi, T. Circadian Changes in Urinary Bicarbonate, Nitric Oxide Metabolites and pH in Female Player during Handball Camp Involved in an Exercise, Rest and Sleep Cycle.. *The Tohoku Journal of Experimental Medicine* 196(4) 2002

21. Park K, Hurley PT, Roussa E, Cooper GJ, Smith CP, Thevenod F, Steward MC, and Case, RM. Expression of a sodium bicarbonate cotransporter in human parotid salivary glands. *Arch Oral Biol*. 2002 Jan;47(1):1-9.

22. Remer T, Manz F. Potential renal acid load of foods and its influence on urine pH. *J Am Diet Assoc*. 1995 Jul; 95(7):791-7.

23. Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. *Am J Clin Nutr*. 2003 May; 77(5):1255-60.

24. Sebastian A, Frassetto LA, Sellmeyer DE, Merriam RL, Morris RC Jr. Estimation of the net acid load of the diet of ancestral preagricultural *Homo sapiens* and their hominid ancestors. *Am J Clin Nutr*. 2002 Dec;76(6):1308-16.

25. Whiting, SJ¹, Bell, J¹, and Brown, SE². First morning urine measured with pH paper strips reflects acid excretion. ¹College of Pharmacy and Nutrition, ²Osteoporosis Education Project, East Syracuse, NY, USA

26. Wiederkehr, M., Krapf, R. Metabolic and endocrine effects of metabolic acidosis in humans. *Swiss Med Wkly*. 131:127-132. 2001.

27. Yuen AW. Low-grade chronic metabolic acidosis is a contributory mechanism in the development of chronic epilepsy. *Epilepsy Behav*. 2006 Mar;8(2):347-9. Epub 2006 Feb 3.