CITRIN® A VERSATILE NUTRACEUTICAL

By
Vladimir Badmaev, MD, PhD

Overview and application in weight loss

One of the key nutraceutical products developed by Sabinsa is a brand of natural hydroxycitric (HCA) acid, Citrin ®, present on the nutritional market in the US, Europe, Japan and Australia for more than a decade now.

Natural HCA has been derived from the rind of a fruit of Garcinia Gambogia, a tree indigenous to the southern part of India. Gracinia fruits, containing approximately 30% of HCA, have a long standing history of culinary use in India as a food acidifier and also as an addition to curry together with turmeric root. This fruit has also been eaten in its raw form.

During the 1970's, scientists at Brandeis University and at Hoffman LaRoche demonstrated that HCA, when blended with the diet, had a marked suppressive effect on appetite and weight gain in rats. The mechanism behind that weight-loss effect was discovered, i.e. inhibition by HCA of a specific enzyme, citrate lyase, which is responsible for the synthesis and storage of fat in our body.

The LD50 value established by Hoffman LaRoche scientists for HCA is 4000 mg/kg (comparable to the value for a known GRAS [Generally Recommended As Safe] item, citric acid). One may compare this value with a daily recommended dose of HCA established through our clinical studies to be 750 mg/person/day.

Our company sponsored, between 1993 and 1997, six clinical studies of our brand of HCA Citrin ®, i.e. three open field, and three double-blind designs. A chronological list of this research is provided for your convenience:

1. Conte AA (1993 Summer) A Non-Prescription Alternative in Weight Reduction Therapy. The Bariatrician: 17-19.
2. Conte AA (October 1994) The effects of (-) - Hydroxycitrate And Chromium (GTF) On Obesity. J Amer Coll Nutr. 13 (5): 535 [Abstract 60].
3. Katts GR, Pullin D, Parker LK, Keith PL, Keith S (March 1995) Reduction Of Body Fat As A Function Of Taking A Dietary Supplement Containing Garcinia Cambogia Extract, Chromium Picolinate And L-Carnitine - A Double Blind Placebo Controlled Study. Abstract/Poster presented at a symposium on obesity organized by the Mexican Sociedad Medical del Sureste para el Estudio de la Obesidad, March 4, 1995, Merida, Yucatan, Mexico.

4. Conte AA (June/July 1995) Effective Natural Weight Loss Techniques. Alternative Complementary Therapies. I (4): 212-215.
5. Badmaev V, Majeed M (July 1995) Open Field, Physician Controlled, Clinical Evaluation Of Botanical Weight Loss Formula Citrin ®. Nutracon 95: Nutraceuticals, Dietary Supplements And Functional Foods. Day One (Sponsored by Global Business Research LTD). Published in the symposium book.
6. Thom E (May 1996) Hydroxycitrate (HCA) In The Treatment Of Obesity. Int J Obesity. 20 (4): 75 [Abstract /Poster 08-193-WP1 at 7th European Congress on Obesity in Barcelona, Spain 14-17 May, 1996].

Based on our 8 weeks and up to 36 months clinical study of Citrin ® in overweight patients, some of which had poor glucose tolerance, we can report that Citrin ® in a dose of 1500 mg [calculated as 750 mg of pure HCA] did not affect the overall clinical status of the patients and did not alter blood biochemistry in a detrimental way.

The blood levels of triglycerides, often elevated in patients with poor glucose tolerance, assessed for the entire population studied with Citrin ® decreased significantly ($p<0.05$). The mean value of triglyceride levels before Citrin ® intake was 166.5 mg/dl and after the 8 weeks was 154.8 mg/dl. On the other hand the HDL ("good cholesterol") levels were significantly ($p<0.01$) increased after 8 weeks of Citrin ® from a mean value of 47.4 mg/dl to a mean value of 50.4 mg/dl.

The 8 week Citrin ® intake lowered the risk of coronary heart disease [CHD] (as assessed from the blood lipid profile) significantly ($p<0.01$) for the entire population studied. The risk index decreased from a mean value of 0.998 to a mean value of 0.90. This finding is particularly important in view of increased risk of CHD in patients with poor glucose tolerance.

In addition, the blood chemistry parameters like blood electrolytes, in particular sodium, potassium, chloride, calcium and phosphorus, blood urea nitrogen [BUN], creatinine, plasma proteins and liver enzymes were not altered even after several months administration of Citrin ®.

Sabinsa enjoys now several years of success in providing Citrin® to the nutritional market world-wide, with a solid safety record of using this product in supplements, foods and beverages. Based on our extensive experience with HCA we recommend use of this product in the USA as a category of Food for Special Dietary Use (FSDU), or as a Dietary Supplement under the new Dietary Supplement Health and Education Act of 1994 (DSHEA).

Inhibition of citrate lyase by Citrin® contained hydroxycitric acid may increase physical endurance by increasing glycogen levels in the body.

What Glucose means to the body

A steady availability of blood glucose is a basic requirement for body functions. Glucose is the fuel of choice for the brain, and also an essential energy source for muscles. This valuable
energy source is provided to the body in the form of different dietary sources, such as starch, disaccharides lactose, maltose, and sucrose; from monosaccharides fructose, galactose or
mannose; from glucose yelding (glucogenic) amino acids and small carbohydrates, e.g. glycerol and glyceraldehyde. These compounds can eventually be converted into glucose by the pathway of gluconeogenesis, neogenesis meaning new production, in this particular case of the glucose.

The many ways of securing an uninterrupted glucose supply to the body shows how important the steady availability of this nutrient is for proper functioning of the body. To sum-up, there are three master plans for glucose supply to the body: supply with food (particularly as a component of starches), readily disposable storage in the form of glycogen (a security reserve often saving us from collapse in the course of hectic pace of life and poor nutrition) and finally when food supply is inadequate and reserves of glycogen have been used up, the body resorts to sacrificing other nutrients (e.g. amino acids) just to manufacture glucose. Obviously the glucose supply is one of the most elemental requirements for our daily performance, if not for our existence.

In addition to the discussed mechanisms, glucose per se can yield the molecule, pyruvic acid, which increases glucose extraction from the blood to the tissues, like muscles. Pyruvic acid or its salt form pyruvate yield up to a 50% increase in muscle glycogen, thus contributing to greater capacity of the muscles for performance and endurance.

Because blood glucose levels are so critical, the stores of readily available glucose are found in organs which have particularly high energy requirements. Approximately 400 gm of glycogen
makes up 1% to 2% of the fresh weight of resting muscle, and approximately 100 gm of glycogen makes up 6% to 10% of the fresh weight of a healthy liver.

Nutritional regulation of glycogen

Nutrition is the key in supplying carbohydrates and other nutrients from which glucose can be manufactured. Nutrition also supplies natural compounds which can improve glucose metabolism by decreasing the amount of body lipids while increasing the glycogen contents in the liver.

The well researched natural compound that is known to improve glucose metabolism is (-) hydroxycitric acid, derived from the rind of *Garcinia cambogia* (Fam. Clusiaceae) fruit. From literature data it is known that (-)hydroxycitric acid [HCA] can inhibit citrate lyase, a key enzyme required for production of fatty acids, or building blocks of body fat. As a result, less dietary glucose is utilized for synthesis of fatty acids, and the dietary

glucose which could be converted to fatty acids is instead converted into its storage form - glycogen.

In experiments carried out with laboratory animals at the Department of Food Sciences and Technology, Kyoto University, Faculty of Agriculture, Japan, rodents were fed with 5 mg of HCA for 3 days. The results suggested that mice fed with HCA as compared to the control animals benefited with higher content of glycogen in the muscles. In the endurance test those mice which were fed with HCA could swim significantly longer than the control group.

In a clinical weight-loss study involving Citrin®, a brand form of HCA, the 52 participating patients were also asked to report in a questionnaire on their energy levels in the course of their eight week treatment. Each patient was taking 500 mg of calcium salt of HCA tid (corresponding to 750 mg of pure HCA daily). The questionnaire was filled out before the study was started and after four and eight weeks of the treatment. Analysis of the subjective perception of energy levels showed a significant ($p<0.01$) increase in energy after four weeks (score 2.27 ± 0.7), and eight weeks (score 2.29 ± 0.69) as compared to the energy levels at the onset of the study - score 1.7 ± 0.98. These subjective observations of increased energy levels should be further studied since they may reflect, or be a result of, increased levels of glycogen stores in the body (Badmaev, V., Majeed, M [July 1995] Open Field, Physician Controlled, Clinical Evaluation Of Botanical Weight Loss Formula Citrin®. Nutracon 95: Nutraceuticals, Dietary Supplements And Functional Foods. Day One (Sponsored by Global Business Research LTD).

The way that HCA can improve aerobic exercise

These preliminary findings on the role of HCA in glycogen storage and its ready availability to increase levels of physical energy leads to an interesting application of HCA in sports nutrition. While exercise training increases the capacity of skeletal muscle to oxidize free fatty acids as a source of energy, it nevertheless remains true that glucose must be metabolically available to support continuos exercise. It is also true that stores of glycogen are depleted in muscles during exercise, and this is accompanied by a depletion of glycogen in the liver with substantially lowered blood glucose levels in the blood. This chain of events in the course of exercise shows how important to physical performance and endurance is the relationship between glycogen and glucose. Especially since maintaining adequate blood glucose levels during exercise is crucial to the central nervous system. The well-known practice of carbohydrate loading to promote aerobic endurance reflects the metabolic importance of glycogen stores during exercise.

During exercise the caloric needs of muscles are initially met by glycogenolysis (glucose release from glycogen) in muscle along with increased uptake of glucose by the muscles. Blood glucose initially rises with increased hepatic glycogenolysis but may fall with prolonged strenuous exercise. There is an increase in gluconeogenesis. Plasma insulin falls, and plasma glucagon (a hormonal substance opposing insulin action) rises. After

exercise, liver glycogen is replenished by additional gluconeogenesis and a decrease in hepatic glucose output. Therefore replenishment of glycogen, and any nutritional intervention to increase stores of glycogen is particularly desired in the training.

When hepatic glycogen level are high, the rate of deamination of amino acids is depressed, and the amino acids are thus preserved for other uses. Importantly, for body builders protein catabolism (proteins are utilized for the process of glucose production in the gluconeogenesis process) is decreased as a result of high liver glycogen levels - the result is a protein-sparing effect. Also an added benefit of high glycogen in the liver is an enhancement of the detox processes, e.g. acetylation and glucuronide conjugation. Properly functioning detox mechanisms in the liver are particularly important in handling metabolic demands during training and the heavy nutritional supplementation that is usually recommended in training.

CITRIN IN URINARY STONE PREVENTION

Urinary citrate plays an important role in preventing formation of calcium-containing kidney stones by chelating calcium, preventing crystallization and precipitation of calcium and calcium oxalate complex formation. Low urinary citrate occurs in approximately half of patients with kidney stones. One of the possible mechanisms leading to low levels of urinary citrate is due chronic metabolic acidosis and hypokalemia (low levels of potassium) associated with adaptive increases in ATP citrate lyase. ATP citrate lyase is an intracellular enzyme which cleaves citrate to oxaloacetate and acetyl CoA, thus its excess activity would lead to low levels of biologically available citrate. In recent study at Northwestern University Medical School, Chicago, Illinois, a potential role of Citrin Mg/K (potssium magnesium salt of hydroxycitric acid) in inhibiting ATP citrate lyase and alleviation of hypocitraturia in rats was studied. Using the animal model it was found that CitrinMg/K administered by the oral route increased urinary citrate excretion by 5 fold as compared to the untreated animals. This preliminary work indicates that Citrin may provide an important therapeutic benefit to those patients with hypocitraturia and kidney stones.

A POSSIBLE NEW USE OF HYDROXYCITRIC ACID (Citrin®) AS A FOOD SUPPLEMENT IN ANTI-CANCER THERAPY

Role of lipids and lipid metabolism in the development of cancer

One of the important directions of research into the mechanisms of cancer development is the link between nutrition and the cancer; in particular the association between dietary fat and the origins of human colorectal, breast, prostatic, ovarian and endometrial cancers.

Many animal studies have shown a definite positive correlation between dietary fat and the rate of tumor growth and the severity of metastases. It is a recognized fact that the rate

of lipid synthesis in tumor cells is quite rapid. This phenomenon can be understood, because rapidly dividing cells not only need fresh copies of DNA and proteins, but they also require the security of new biomembranes composed of phospholipids and cholesterol.

The cholesterol synthesizing pathway has been implicated as a promoter of tumor cell growth since the 1980s (Maltese, WA et al. J.Clin. Invest 76:1748-1754, 1985 & Buchwald, H. Lancet 339:1154-1156, 1992). The cholesterol intermediate, Farnesyl-PP, has been implicated as a factor promoting cell proliferation (Goldstein, JL and Brown, MS. Nature. 343: 425-430, 1990). A covalently attached farnesyl group is essential for the normal functioning of the Ras protein, a key regulator of cell division in normal cells.

Excessive activity of Ras produces uncontrolled cell growth, and activated Ras genes are the most frequently identified oncogenes in human tumors. Farnesyl-PP is an intermediate in the cholesterol synthesis pathway and must be synthesized de novo within the cell where it is to be used.

The other major lipid class, fatty acids, are also involved in tumor cell growth. In fact many human and experimental cancers express elevated levels of fatty acid synthase (FAS), the major enzyme required for endogenous fatty acid biosynthesis. For example a prognostic molecule isolated from a number of breast cancer patients was identified as FAS (Kuhajda, FP et al. Proc.Natl.Acad.Sci. USA 91:6379-6383, 1994). Inhibition of FAS leads to loss of clonogenic capacity and induction of programmed cell death in breast cancer cells (Pizer, ES et al. Cancer Res 56:2745-2747, 1996).

The potential anti-cancer mechanism of Citrin®

The effect of the CitrinK® brand of the potassium salt of (-)hydroxycitric acid, isolated from the fruit of the Garcinia cambogia tree on lipid synthesis and cancer cell growth was evaluated (George Washington University, Department of Physiology, Washington D.C.). The tumor cells were incubated in a growth phase for eight days in the presence and/or absence of various levels of CitrinK. Subsequently the amount of lipid in the form of triglyceride palmitate was measured in the cultures. The results clearly showed that the lipid increased in the untreated cultures as the cells grew in the untreated cultures. However, increasing concentrations of CitrinK lead to decreases in the lipid content and also inhibited the cell growth.

The inhibitory effect of CitrinK on lipid production can be prevented by the prior adding 10mM acetate, which initiates the lipid synthesis pathways independent of citrate lyase. This last finding clearly implicates the ATP citrate lyase inhibition as the mechanism of action of CitrinK, and that this mechanism operates to prevent tumor cell growth. It is proposed therefore that because CitrinK blocks de novo lipogenesis of tumor cells, it can be used in preventing cancer growth.

In another study, the effect of CitrinK on lipid synthesis derived from glutamine has been evaluated in cancer cells. Recent experimental findings show that glutamine can contribute 20 to 40% of the acetyl CoA utilized for lipid synthesis in the cancer cells. CitrinK increased catabolism of glutamine in the cancer cells making it less available for lipid synthesis. This experiment shows that CitrinK can block lipid synthesis independently of its other mechanism of inhibiting the enzyme citrate lyase.

The following mechanism of CitrinK is proposed:

1. Blocking de novo synthesis of all cholesterol intermediates including acetyl CoA, glutamine and Farnesyl-PP leading to an inability of Ras to stimulate cell growth and division.

2. Reducing the precursor supply for fatty acid synthase and thus blocking tumor cell proliferation.

Since Citrin-K is already used as a food supplement, it is proposed to investigate its potential as a cancer fighting functional food.

General Considerations on Citrin®

We feel that certain diets, e.g. high fiber diet (although a common choice among dieters) may actually diminish bioavailability of hydroxycitrate from the gastrointestinal tract. On the other hand a diet including a moderate amount of simple carbohydrates and low in dietary fats (an average diet) may enhance the bioavailability of hydroxycitrate from the digestive tract or otherwise enhance the inhibition of ATP lyase by hydroxycitrate.

The other important consideration for bioavailability of hydroxycitrate to the body could depend on the water solubility of the compound carrying hydroxycitrate. Thus in addition to the water insoluble calcium salt of hydroxycitric acid (the originalCitrin) we have developed the water soluble versions including potassium, magnesium and magnesium-potassium salts of hydroxycitric acid.

CITRIN® PRODUCTS : STANDARDIZED EXTRACTS

Citrin®: Standardized for a minimum of 50% (-) HCA this calcium salt is a pale brown powder slightly soluble in hot water, soluble in dilute acids and insoluble in alcohol. It contains about 19-20% calcium. The product provides supplemental calcium in addition to facilitating weight loss. The recommended dose is 500 mg three times a day. The

product finds versatile applications in nutritional supplements and functional food products where water solubility is not critical.

Citrin®DC : Standardized for a minimum of 50% (-) HCA, this calcium salt is a pale brown powder, slightly soluble in hot water, soluble in dilute acids and insoluble in alcohol. In addition to the advantages listed for Citrin®, this product is in granular form, "directly compressible" for tableting applications.

Citrin®WJ: Standardized for a minimum of 50% (-) HCA, this product is the calcium salt with a ligher color, suitable for applications requiring a colorless weight loss ingredient. It is a white to off-white powder slightly soluble in hot water, soluble in dilute acids and insoluble in alcohol. Except for the color, the product is similar to Citrin®.

Citrin®80 mesh : Standardized for a minimum of 50% (-) HCA, this calcium salt is Citrin® ground to a particle size of 80 mesh. It is a pale brown powder slightly soluble in hot water, soluble in dilute acids and insoluble in alcohol. The smaller particle size facilitates encapsulation and the product is popularly used in soft gel formulations.

Citrin®Liquid: Standardized for a minimum of 45% (-) HCA, this brown colored mobile liquid is miscible with water and alcohol. It finds applications in functional food/beverage products and soft gel capsules.

Citrin®K: Standardized for a minimum of 48% (-) HCA, this patented potassium salt (U.S. patent # 5,783,603, 1998) containing about 30-33% potassium, is a beige to pale brown hygroscopic powder, deliquescent when exposed to moist air. It is soluble in water and in dilute acids and insoluble in alcohol. At a recommended dose of 500 mg three times a day, this product provides supplemental potassium (which regulates metabolic processes) in addition to weight loss. Popular applications include functional food products of various types, where water solubility is important, as well as tablet and capsule formulations.

Citrin®Mg: Standardized for a minimum of 55-60% (-) HCA, this Magnesium salt is a beige to brown completely soluble powder. It contains about 10% Magnesium and is particularly useful in sports nutritional food products and supplements facilitating weight management, metabolic regulation, muscle toning and energy enhancement. The recommended dose is 455 mg, three times daily.

Citrin®Mg/K: Standardized for a minimum of 55-60% (-) HCA, this magnesium/potassium salt is a pale brown completely soluble powder. It contains about

7% magnesium and about 6% potassium, providing supplemental essential minerals in weight loss formulations (functional food products, nutritional beverages, tablets and capsules). Health benefits include weight management, metabolic regulation, muscle toning and energy enhancement. The recommended dose is 455 mg, three times daily.

Citrin® Ca/K: Standardized for a minimum of 55-60% (-) HCA, this calcium/potassium salt is beige to a pale brown powder soluble in water and in dilute acids and insoluble in alcohol. It contains about 10% calcium and about 10% potassium, providing supplemental essential mineral nutrients in weight loss formulations (functional food products, nutritional beverages, tablets and capsules). Health benefits include weight management, metabolic regulation, muscle toning and dietary calcium enrichment. The recommended dose is 455 mg, three times daily.

Citrin® crystals: Standardized for a minimum of 10% (-) HCA, these bright pink obtained from *Garcinia indica* crystals, is soluble in water. In addition to weight loss effects, it functions as a natural coloring agent in nutritional beverages.

THE EFFECTS OF (-) – HYDROXICITRATE AND *CHROMIUM (GTF) ON OBESITY*

Conte A. Lifestyle Medical Center, Hilton Head, SC 29926, USA

Abstract

The search for an effective alternative to pharmaceutical weight loss enhancers has been the goal of many physicians in the field of Bariatric Medicine for several decades. Early laboratory studies of (-) – Hydroxycitrate,* an extract from Garcinia cambogia that grows in Asia, have recorded its dual action as an appetite suppressant and as a fat synthesis inhibitor. The effect of chromium, a non-toxic trace mineral, is to keep insulin levels in check by making the hormone more efficient at regulating blood sugar levels. The author wishes to report on three clinical studies he conducted on obese subjects using these non-drugs, all natural, weight-loss enhancers, as well as use of them in his private bariatric practice. In an 8-week double-blind study in 1991, the active ingredients group lost an average of 11.1 pounds per person while the placebo group lost an average of 4.2 pounds per person. The 1993 study, a consumer study with no placebos and no exclusions because of associated medical conditions and therapies, confirmed the effectiveness and safety of the previous study. The newest study, presently in progress, appears to confirm the usefulness of this alternative method of treating obesity and eating disorders with a non-drug appetite suppressant. The enhancers are used in conjunction with a carefully selected diet of everyday foods that emphasize moderation, variety and balance, plus a regular, sensible activity program. The combined method offers significant benefits to obese individuals and the physicians treating them.

* The study was done with Citrin® calcium salt (info as per Dr. V. Badmaev)
Conte AA (October 1994) The effects of (-) - Hydroxycitrate And Chromium (GTF) On Obesity. J Amer Coll Nutr. 13 (5): 535 [Abstract 60].

REDUCTION OF BODY FAT AS A FUNCTION OF TAKING A DIETARY SUPPLEMENT CONTAINING GARCINIA CAMBOGIA EXTRACT, CHROMIUM PICOLINATE AND L-CARNITINE- A DOUBLE BLIND PLACEBO CONTROLLED STUDY

*1. Gilbert R. Katts, Ph.D., 2. Dennis Pullin, MS, 3. Larry K. Parker, M.D.,
4. Patti L. Keith and 4 Samuel Keith.

Abstract

(-) Hydroxycitrate (HCA), an analog of citric acid found naturally in Garcinia cambogia, inhibits citrate lyase. This enzyme influences both the synthesis and oxidation of fat in the liver through its role in synthesis of malonyl-coA. In animal studies, HCA administration reduced deposition of body fat, and in one previous double-blind study, HCA promoted increased weight loss in human subjects. Our study was designed to examine changes in body fat in 200 subjects who were assigned either to a "placebo" or "active" group and were asked to follow a low-fat, high-fiber eating plan with increased physical activity for four weeks. The "active" gorup consumed a dietary supplement ***Brindall Trim*** containing a daily total of 1,500 mg of HCA (as Citrin®), 1,200 mg of L-carnitine and 600 mcg of chromium (as picolinate). Body fat measurements were taken using underwater tests, displacement method, before and after the test period and all subjects reported to a research center each of the four weeks obtaining a scale weight and briefly reviewing their progress with a research technician. A total of 194 (97%) of the subjects completed the four-week test period and the pre and post-underwater tests. An additional eight subjects where excluded from the data analysis since, based on interviews and counting the remaining capsules, they consumed less than half the number of supplements required in the study. For the remaining 186 subjects (93%), comparisons were made between the changes in body fat levels between the placebo and the active group. The average loss of body fat for the placebo group during the four week test period was –1.40 lb. compared to –2.84 lb. in the active group, a statistically significant difference ($p<.01$). Two subjects reported gastrointestinal distress, both of whom were consuming the placebo supplement. No adverse effects were reported for subjects consuming the ***Brindall Trim***. It is concluded that the garcinia, L-carnitine, chromium picolinate supplement can facilitate the loss of body fat when combined with a low-fat, high fiber diet and increased physical activity.

*1. Principle Investigator, Health & Medical Research Foundation, 1840 Lockhill Selma Road, San Antonio, Texas 78213.

2. Baylor Sports Medicine Institute, Baylor College of Medicine, Houston, Texas.

3. Women's Total Health Care, Enegton, Texas.

4. The Center for Applied Research, San Antonio, Texas.

Katts GR, Pullin D, Parker LK, Keith PL, Keith S (March 1995) Reduction Of Body Fat As A Function Of Taking A Dietary Supplement Containing Garcinia Cambogia Extract, Chromium Picolinate And L-Carnitine - A Double Blind Placebo Controlled Study. Abstract/Poster presented at a symposium on obesity organized by the Mexican Sociedad Medical del Sureste para el Estudio de la Obesidad, March 4, 1995, Merida, Yucatan, Mexico.

The American Pediatric Society and The Society for Pediatric Research 1999 Abstract Form

Renal ATP Citrate Lyase (ATP CL) Protein Localizes Throughout the Nephron and Increases Only in the Proximal Tubule with Chronic Metabolic Acidosis (CMA) Krishna Puttaparthi[1], Thomas Rogers[1], Nabil A Eishourbagy[2], Moshe Levi[1], and Joel Z. Melnick[3], [1]UT Southwestern, Dallas, TX; [2]SmithKline Beecham, King of Prussia, PA and [3]Northwestern University Medical School, Chicago, IL. (Sponsored By: Craig B. Langman)

Decreased urinary citrate excretion, an important cause of kidney stones, occurs in CMA or hypokalemia (lowK). Microperfusion studies have shown that proximal tubular citrate reabsorption is the major regulator of urinary citrate. ATP CL, a cystosolic enzyme responsible for cleaving citrate to oxaloacetate and acetyl-CoA, plays an important role in the development of hypocitraturia (***J Clin Invest*** 98:238). CMA and lowK increase ATP CL activity and protein in renal cortex. However, the specific tubular segment where this increase occurs in unknown. Furthermore, administration of the specific inhibitor of ATP CL, 4S-hydroxycitrate, has been shown to increase urinary citrate. The purpose of these studies was to identify the portion of the kidney responsible for the observed increase in renal cortical ATP CL protein and to examine the effect of 4S-hydroxycitrate on ATP CL activity and abundance.

Male rats, 180-220g, were placed in individual metabolic cages. Control animals received ***ad lib*** water and were pair-fed rat chow to experimental animals that received 0.28M NH_4CL water, ***ad lib***. After 7 days, animals were perfused at 300 Torr with 3% paraformaldehyde and 0.05% picric acid in a 0.1M cacodylate buffer with 10% Pentaspan® (DuPont). Kidney slices were fixed in 10% formalin and sliced into 3µ sections. Immunoperoxidase labelling was performed using the Vectastain® ABC kit (Vector Laboratories, California). The primary rabbit anti-rat antibody that had been used previously for immunoblotting was used at a 1:200 dilution.

For studies using the ATP CL inhibitor, rats were given normal rat chow and NH_4CL water, ***ad lib***. After 7 days, experimental animals received CitrinK-MG® (Sabinsa, New Jersey), 2 mmole/kg/day, by gavage. After 2 days, animals were sacrificed and perfusion-fixed as above. One kidney was clamped and removed prior to the perfusion for determination of ATP CL activity and protein abundance as we have done previously.

Results of our immunohistochemistry revealed that ATP CL protein localized throughout the nephron. However, in animals with CMA, a 3-fold increase in staining occurred only in the proximal tubule segment, particularly in the brush border. For studies in animals that had received the ATP CL inhibitor, urinary citrate increased by 5-fold. However, there was no difference in ATP CL activity (in nmole hydroxamate produced/mg protein/30 min;
132 ± 16 vs. 134 ± 14) nor ATP CL protein by immunoblotting.

In conclusion, the inhibition of ATP CL by 4S-hydroxycitrte observed ***in vivo*** does not persist ***in vitro***. By in conclusion, the inhibition of ATP CL by 4S-hydroxycitrate observed ***in vivo*** does not persist ***in vitro***. By immunohistochemisty, ATP CL protein is found throughout the nephron. The increase in proximal tubular ATP CL immunostaining seen in rats with CMA provides further evidence of the importance of this enzyme and of this tubular segment in the development of hypocitraturia.

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HYDROXYCITRATE (HCA) IN THE TREATMENT OF OBESITY

Erling Thom; Medstat Research, Brotergt. 2 Norway – 2001 Lilestrom.

ABSTRACT

Sixty patients, 44 females and 16 males, with obesity were included in a randomized placebo-controlled double-blind study to investigate the efficacy and tolerability of hydroxycitrate (HCA) (Citrisan®) in weight reduction. The double-blind study had a duration of 8 weeks.

HCA, or an identical placebo capsule, was taken t.i.d., 30 minutes before breakfast, lunch and dinner. All patients were on a low fat diet of 1200 kcal/d and were instructed to exercise 3 times/week. The daily dose of HCA was 1320 mg/day.

The mean weight reduction in the HCA group (30 patients) was 14.11 lbs., while the patients in the placebo group reduced their weight by 8.37 lbs. The difference in weight reductions is highly significant ($p<0.001$). The composition of the weight loss, determined with NIR technique, shows that 87% of the weight loss in the HCA group is due to fat loss, while the corresponding figure in the placebo group is 80%. Blood pressure, total cholesterol and hip and waist circumferences were significantly reduced in

both groups. A statistically significant difference between the groups in favor of the HCA group was seen in all these parameters ($p<0.001$). Appetite scores during the study, using visual analog scales, were significantly reduced in the HCA group, but not in the placebo group ($p<0.001$).

The tolerability of the treatments was excellent. Two patients stopped the treatment due to stomach pain, one in the HCA group and one in the placebo group.

The patients will be followed regularly for 12 months in an open follow-up study where all patients will receive HCA.

Conclusion: HCA (Citrisan®) is an effective and well tolerated short-term treatment of overweight and obesity, when combined with a sensible low fat diet and exercise. Long-term data are needed for efficacy and tolerability assessments.

The exclusive source of HCA in Citrisan® is Citrin® .

Citrin® is a registered trademark of Sabinsa Corporation.

Thom E (May 1996) Hydroxycitrate (HCA) In The Treatment Of Obesity. Int J Obesity. 20 (4): 75 [Abstract /Poster 08-193-WP1 at 7th European Congress on Obesity in Barcelona, Spain 14-17 May, 1996].

OPEN FIELD, PHYSICIAN CONTROLLED CLINICAL EVALUATION OF A BOTANICAL WEIGHT LOSS FORMULA BASED ON GARCINIA CAMBOGIA DERIVED (-) HYDROXYCITRIC ACID

Vladimir Badmaev, M.D., Ph.D., Muhammed Majeed, Ph.D., and Anthony A. Conte, M.D.* Sabinsa Corporation Piscataway, NJ, * Life Style Medical Center, Hilton Head, N.C.

Abstract

(-) Hydroxycitric acid derived from the rind of the Garcinia cambogia fruit competitively inhibits the cytosolic enzyme adenosine triphosphate (ATP) citrate lyase in vitro and in vivo. This mechanism seems to operate in experimental animals fed with (-)HCA, and is likely responsible for the noticeable decreased feeding frequency, decrease in total body weight and body fat, as well as increase in energy expenditure. The current open field, physician controlled eight week clinical study was designed to evaluate the efficacy of (-)HCA for body weight loss in overweight human subjects. Of the 77 ambulatory patients selected for the study, a total of 22 patients dropped out due to non-side effects related

reasons, with 42 women and 13 men (20-64 years old) completing the eight-week long trial. Subjects received a HCA formula consisting of: 1500 mg of calcium salt of HCA (750 mg of pure HCA) and 300 mcg of chromium picolinate per day. The body weight, appetite and energy self-assessment, and vital signs were evaluated on zero, four and eight weeks. In addition, blood biochemistry was performed on zero and eight weeks. Patients lost on average <5 lbs. after four and <10 lbs. after eight weeks of regimen on the HCA formula (significance $p<0.001$). This weight loss was independent of gender or age of the population studied. The blood levels of triglycerides (TG), VLDL in the entire population and LDL in men over 60 years of age were significantly ($p<0.05$) lowered in the course of treatment (TG mean value before treatment 167mg/dl vs. 155mg/dl after eight weeks; VLDL 34 mg/dl before vs. 29 mg/dl after; LDL 124 mg/dl before vs. 116 mg/dl after), with the cholesterol levels unchanged. Blood levels of HDL were significantly ($p<0.01$) increased for the entire population studied (mean value before treatment 47.4 mg/dl vs. 50.4 mg/dl after). Cardiovascular risk or Coronary Heart Disease (CHD) risk based on the total cholesterol/HDL ratio was evaluated before and after the eight-week treatment. The eight week intake of the formula lowered CHD risk significantly ($p<0.01$) for the entire sample studied, decreasing the risk index from a mean value of 0.99(CI: 0.87-1.13) to a mean of 0.90 (CI: 0.76-1.04). Blood sugar homeostasis was not affected by the treatment as assessed by unaltered blood glucose levels before and after eight weeks of treatment. No side effects subjective or objective were reported. The vital signs including pulse rate and blood pressure remained stable in course of the treatment.

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Evaluation of water soluble Citrins

METHOD OF ANALYSIS

WATER SOLUBLE CITRINS – SUITABILITY TEST FOR THEIR USAGE IN DRINKS

A. <u>Scope of Experiment:</u>

To find out the suitability of our new water soluble Citrin® products like Citrin® K, Citrin® Mg, Citrin® - Ca & K and Citrin® Mg & K for their usage in drinks. Since most of the drinks invariably contain citric acid which may interact with these salts and cause precipitation, this study becomes essential.

B. <u>Design of the Study:</u>

500 mg each of Citrin® salt is dissolved separately in 350 ml of water in which 50 mg of citric acid is added and thoroughly mixed. This solution, after ensuring its clarity, is incubated for 7 days at 35 deg. C. The water used for dissolution is the mineral water (Bisleri) A blank, which contains only water and citric acid is also incubated.

C. Observation:

S1. Product	**Clarity on Storage**						
No.	Initial	Day-1	Day 2	Day 3	Day 4	Day 5	Day 6 Day 7
1. Blank	Clear	Clear	Clear	Clear	Clear	Clear	Clear Clear
2. Citrin®-Mg	Clear	Clear	Clear	Clear	Slightly hazy	Slightly hazy	Slightly hazy Slightly hazy
3. Citrin®-K	Clear	Clear	Clear	Clear	Slightly hazy	Slightly hazy	Slightly hazy Hazy
4. Citrin®-Mg and K	Clear	Clear	Clear	Slightly hazy	Slightly hazy	Slightly hazy	Hazy
5. Citrin®-Ca and K	Clear	Clear	Clear	Slightly hazy	Slightly hazy	Hazy	Hazy

D. Conclusion:

1. All the Citrin® salts are not precipitating on incubation at 35 deg. C for 7 days.

2. Citrin® Mg is better in clarity when compared with other salts. It is followed by Citrin® K, Citrin® Mg & K and Citrin® Ca & K.

BASED ON THE ABOVE EXPERIMENTS, IT CAN BE CONCLUDED THAT ALL OUR WATER SOLUBLE CITRIN® SALTS ARE SUITABLE FOR DRINK USE.

Evaluation conducted by R&D Center Sami Chemicals, Bangalore, India.