

GHR Technical and Scientific Monograph for BIE

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August 12, 2005

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A. THE THEORY AND SCIENCE BEHIND GHR

Most people know that the female hormone estrogen declines with age in women. A similar decrease occurs with testosterone, progesterone, melatonin, DHEA, and human growth hormone (HGH). The standard medical treatment for hormone deficiency is replacement of the deficient hormones with synthetic hormones. However, GHR is a natural "hormone releaser", not a synthetic hormone. It stimulates natural human growth hormone (HGH) production and is free of side effects.

The science behind GHR involves the use of HGH releasers, also called agonists, which are ingredients that bring about the release of human growth hormone from the pituitary gland.

The body and its tissues and functions are only as good as the raw materials we give them for production and repair. We think it makes more sense to use quality building blocks to allow the body to make its

own HGH rather than imposing a synthetic hormone product that may or may not be accepted by the body. Using HGH agonists induces the pituitary to secrete extra HGH. You can also accentuate that effect to full potential with a proper diet and HGH-releasing exercises.

The level of growth hormones in men and women start to decline around age 25. By age 35, that level is decreasing at a rapid pace and by the late 50's a minor trace of HGH is being released. Young and old alike can testify to increased energy, muscle mass (body builders), weight loss, and looking and feeling better than they have in a long time.

The ingredients in GHR include pituitary and hypothalamus glandular extracts; an amino acid blend; a phytosterol complex; phosphatide complex; and panax ginseng.

Glandular Extracts

Oral and injectable glandular products have been used safely in Europe for decades as an antiaging therapy. These products are from animal sources -lamb, bovine or porcine. GHR extracts are pharmaceutical grade bovine and specially prepared from Argentinean stock that has never been implicated in human disease.

None of the common hormones, estrogen, testosterone, progesterone, melatonin, or DHEA is associated with antiaging. Only HGH has been shown to prevent biological aging and reverse a wide range of the signs and symptoms of aging. In fact, HGH therapy has been scientifically shown to turn back the biological clock as much as 20 years. (1) (LINK TO STUDY ON BIE WEBSITE <http://biehealth.ca/Research6.asp>)

HGH, or somatotropin, is the most abundant hormone secreted by the pituitary gland, a process that peaks during adolescence. Gradually this hormone secretion diminishes with age. By the time you reach the age of 60, you may only secrete 25% as much as the average 20 year old. This greatly contributes to the acceleration of the aging process.

HGH is primarily released during the beginning phases of sleep. HGH is quickly converted by the liver into the important growth-promoting metabolite somatomedin C, which then circulates throughout the body. Most of the beneficial effects of GHR are directly associated with somatomedin C. Somatomedin C is vital in instructing cells to produce protein and repair themselves and low levels have been clearly linked to reversing the aging process.

The decline of growth hormone with age is directly associated with many of the symptoms of aging. These include wrinkling, gray hair, decreased energy, and diminished sexual function. Lack of growth hormone contributes to increasing body fat, cardiovascular disease, osteoporosis, and an inclination toward other aging-related diseases.

Amino Acids

Twenty amino acids form the building blocks of all proteins and are needed for the body to make the proteins of enzymes, many hormones, muscle, bone, skin, organs, etc. Eight of them are essential and cannot be made in the body; they have to be ingested in the diet or by supplements. A number of these amino acids have been shown to induce growth hormone secretion — and GHR compiles them in such a way that maximizes their benefits.

Because of the poor nutritional state of the current Western diet, there is a high probability that people are not getting sufficient quality protein in their diet to perform basic life enhancing functions.

We also know that with age, most hormone levels diminish. The standard medical approach to treating symptoms of hormone deficiency, i.e., thyroid deficiency and menopause, is to use hormone replacement therapy. Unfortunately, in the case of synthetic estrogen and progestin replacement therapy –the side effects can include cancer.

Our approach to anti-aging and the benefits of growth hormone in the body is to enhance the body's ability to make it's own growth hormone using the body's own DNA and RNA blueprints, rather than introducing a synthetic form that could prove harmful over time.

B. THE INGREDIENTS IN GHR

1. Anterior Pituitary and Hypothalamus

In order to understand how the anterior pituitary and hypothalamus extracts work in GHR we need to explore live cell therapy, which is the use of animal organ extracts to promote human health. Thousands of years ago East Indian authors wrote about the use of deer testicles to promote strength. In current times, Swiss born, Dr. Paul Niehans, beginning in the early 1930's, popularized the use of animal organs to heal diseased organs in humans. His first treatment was serendipitous. He injected parathyroid glands into a patient suffering life-threatening seizures after having had her parathyroid glands accidentally removed during thyroid surgery. To everyone's astonishment, the patient survived and cell therapy was born.

Since that time German researchers proved that radioactive tagged animal organ cells find their way to the corresponding organ in humans. For example young liver cells of animal origin end up in the human liver and either provide fresh genetic material to the organ or stimulate secretions that restore healthy function. Dr. Niehans, himself, is reputed to have given about 45,000 live cell injections with no serious side effects in his 42-year career. Niehans is quoted as saying "cellular therapy is a method of treating the whole organism on a biological basis, capable of revitalizing the human organism with its trillions of cells by bringing to it those embryonic or young cells which it needs . . . selective cellular therapy offers new life to the ailing or diseased organism". Live Cell Tissue Extracts: Little Known Therapy With Great Promise By James L. Wilson, PhD. Townsend Newsletter. #205, p.73-77, 2001.)

This quote comes from Dr. James Wilson's 2001 review of live cell therapy in the Townsend Letter. He contends, "Live cell therapy is the only therapy that can actually regenerate cells and tissues". He also notes that in the years 1954 to 1993, more than 1,500 experiments involving whole cells, cell extracts and cell fractions were published in scientific journals. He cites Chase and Landsteiner, two of the founding fathers of Cellular Immunology, who conducted animal experiments proving that injections of whole spleen or thymus cells into live animals such as mice or guinea pigs could enhance immunity. As early as 1933, "tens of thousands of physicians in the U.S. had treated many thousands of patients suffering from various ailments with oral or injected live cells or dried or desiccated glandular tissue in order to restore or regenerate cells and boost function".

It is little known that "Substances such as pituitary, pancreas, thyroid, adrenal, ovary and testes extracts

were available commercially and used by some of the most eminent physicians”. Why then and not now is the obvious question. Dr. Wilson says that with “the advent of synthetic hormones and their almost immediate and seemingly miraculous effect led to a decline in the availability and the use of these whole gland concentrates”. He notes, however, that as doctors encounter the limitations and dangers of using synthetic hormones, they are turning back to oral or injected live cell or dried or desiccated glandular tissue. BIE product GHR uses dried oral glandular tissue to restore and regenerate cells and boost function.

Anterior Pituitary

The pituitary is called the master gland because it directly controls all the other endocrine glands. The pituitary is a small oval gland, the size of a pea, located at the base of the brain just behind the nose. The anterior lobe, simply called the anterior pituitary, is by far the larger of the two lobes. It makes up 80 percent of the pituitary. It produces hormones that control the thyroid, adrenals, ovaries and growth hormone.

Anterior Pituitary

Hormone	Tissue Affected	Effects Produced
Growth hormone	Liver, adipose tissue	Protein, lipid and carbohydrate metabolism
Thyroid-stimulating hormone	Thyroid gland	Stimulates secretion of thyroid hormones T2, T3, T4
Adrenocorticotrophic hormone	Adrenal gland (cortex)	Stimulates secretion of glucocorticoids
Prolactin	Breast tissue	Milk production
Luteinizing hormone	Ovary and testis	Control of reproductive function
Follicle-stimulating hormone	Ovary and testis	Control of reproductive function

Posterior Pituitary

Hormone	Tissue Affected	Effects Produced
Antidiuretic hormone	Kidney	Conservation of body water
Oxytocin	Ovary and testis	Induces milk ejection and uterine contractions

The anterior pituitary produces Growth Hormone Releasing Factor GHRH, which stimulates the production of human growth hormone (HGH). HGH stimulates cellular growth and regulation of metabolism. Growth in the body is mediated mostly by somatomedin-C, a growth factor whose synthesis is controlled by HGH. HGH works in two ways. In order to obtain building blocks for growth it acts in

part like insulin to increase glucose uptake in muscle and fat; stimulates amino acid uptake and protein synthesis in liver and muscle; and inhibiting break down of fat in adipose tissue. Once that has been established the effects on growth begin.

HGH stimulates somatomedin-C, which in turn stimulates the proliferation of cartilage cells, which results in bone growth. Somatomedin-C also encourages the growth and proliferation of muscle cells. Both HGH and somatomedin-C stimulate amino acid uptake and protein synthesis in muscle and many other tissues keeping them strong and healthy. HGH helps break down triglycerides and assists in carbohydrate metabolism helping to keep blood sugar in the normal range.

Hypothalamus:

The pituitary may be the master gland but it is the hypothalamus that directs the activity of the pituitary. The hypothalamus is an area of tissue in the brain located under the thalamus, to which it is heavily connected with nerves. It is located behind the eyes at the base of the optic nerves. It is securely attached to the pituitary via a stalk-like structure. It listens to feedback from body organs and uses this information to release or inhibit the secretion of hormones produced by the pituitary.

The hormones released by the hypothalamus are called releasing hormones and inhibiting hormones, and as mentioned above they directly influence the pituitary-specifically, the anterior pituitary. In other words, a hypothalamic hormone will be secreted into veins that communicate between the hypothalamus and anterior pituitary and attach to the appropriate hormone receptor sites. The message to the anterior pituitary is what hormone and how much of it must be released or inhibited to create a certain necessary action in the body.

Hypothalamus

Hypothalamic Hormones	Anterior Pituitary Hormones	Tissue Affected
Thyrotropin-releasing hormone (TRH) (TSH)	Thyroid-stimulating hormone (TSH)	Thyroid
Thyrotropin-releasing hormone (TRH)	Prolactin (PRL)	Breast
Gonadotropin-releasing hormone (GnRH)	Follicular-stimulating hormone (FSH) Luteinizing hormone (LH)	Ovary
Growth hormone-releasing hormone (GHRH)	Growth Hormone (GH)	All
Corticotrophin-releasing hormone (CRH)	Adrenocorticotropic hormone (ACTH)	Adrenal
Somatostatin	Inhibit the release of growth hormone (GH) Inhibit the release of thyroid-stimulating hormone (TSH)	
Dopamine	Inhibit the release of prolactin (PRL)	

Hypothalamic Hormones	Posterior Pituitary Hormones	Tissue Affected
Antidiuretic hormone (ADH)	ADH is released	Kidney
Oxytocin	Oxytocin is released	Ovary

2. Amino Acid Blend:

Amino acids are the basic structural building units of proteins. They create proteins by first forming short chains called peptides and polypeptides, which join together to form proteins. There are only 20 standard amino acids created by the human genetic code. However other amino acids can be incorporated into proteins and over 100 have been found in nature. In addition to protein synthesis, amino acids have a long list of other biologically important roles.

Isomerism

All amino acids, except glycine, occur in two optical isomer forms, called D and L. The L-amino acids represent the vast majority of amino acids found in proteins and represent the amino acids found in GHR. D-amino acids are found naturally in some proteins produced by exotic sea-dwelling organisms, such as cone snails. They are also abundant components of the cell walls of bacteria. D-amino acids are also made synthetically and should not be confused with the natural form.

The essential amino acids are: Tryptophan, Methionine, Phenylalanine, Threonine, Valine, Lysine, Leucine and Isoleucine. Histidine and Arginine are essential amino acids in children. Essential amino acids are not manufactured by the body and must be taken in the diet or in supplement form. The non-essential amino acids are Arginine, Tyrosine, Glycine, Serine, Glutamic acid, Aspartic acid, Taurine, Cystine, Histidine, Proline, and Alanine.

Effects on hGH: In 2003 a study using a standardized solution of essential amino acids found “an increase in hGH secretion with maximum concentrations being 2100+/-1013% higher than the basal values (P<0.0001). In contrast, no changes in hGH concentrations were observed in the iso-caloric controls; in the fasting controls only a slight increase in hGH was found towards the end of the fasting period.” (2,3) (LINK ABSTRACT # 1, 2)

The GHR amino acid capsules includes nearly all the essential and non-essential amino acids: Lysine, Histidine, Arginine, Aspartic acid, Threonine, Serine, Glutamic acid, Leucine, Phenylalanine, Proline, Glycine, Alanine, Cystine, Valine, Methionine, Isoleucine, Tyrosine.

Amino Acid Overview: (4)

We have included current scientific information on the amino acids in GHR. However, we uncovered a trend in the research away from the use of single amino acids to a focus the hundreds of amino acid analogues for each amino acid that are being developed by the food and pharmaceutical industries. In part the reason for this is to create patentable products for economic reasons.

L Lysine: Lysine is one of the 20 amino acids normally found in proteins. It is an essential amino acid. A deficiency in lysine can create a deficiency of niacin (vitamin B 3), and the niacin deficiency disease, pellagra.

Hydroxylysine, which is derived from lysine, is plentiful in collagen. Hydroxylysine is necessary for the formation of bones and ligaments. It is also an important component of elastin, which maintains the integrity of blood vessel walls.

Lysine is metabolized to obtain the important molecule acetyl-CoA. Acetyl-CoA is the precursor molecule to HMG CoA, which is a vital component in cholesterol and ketone synthesis. It also contributes the acetyl group for the formation of acetylcholine an important neurotransmitter.

Lysine also participates in the formation of glycogen (sugar stores), glucose, lipids (and therefore hormones), and energy.

In spite of the incredible role of lysine in the product of collagen, cholesterol, acetylcholine, glycogen, glucose and energy most of its popularity as a nutritional supplement is due to the treatment of herpes simplex virus. Studies show that lysine can decrease the recurrence rate of this common virus. It should be noted that when the immune system is forced to maintain vigilance to prevent viral infections it is distracted from its duties of regeneration and repair.

There is also preliminary research suggesting that lysine may be a treatment for osteoporosis because of its role in collagen synthesis. Studies show that lysine also facilitates the absorption of calcium from the small intestine.

In one study in Syria, lysine fortification of the food supply resulted in reduced anxiety and stress in the study population. (5)

Lysine deficiency may result in tiredness, inability to concentrate, irritability, bloodshot eyes, retarded growth, hair loss, anemia, and reproductive problems.

Effects on HGH: Many of the above effects of lysine deficiency could also be called effects of aging. The specific effects on HGH include a 1981 study by Italian researcher A. Isidori, M.D., and his associates at the University of Rome. (6) They found that a combination of 1,200 milligrams of l-lysine and 1,200 milligrams of l-arginine in fifteen male volunteers between the ages of fifteen and twenty was ten times more effective than taking arginine alone. According to the researchers, "we could demonstrate that the association of the two amino acids does result in the release of biologically active hormone able to affect peripheral cellular receptors and thus cell growth in general." The fact that lysine and arginine together were active in oral form, say the researchers, "is clearly of considerable importance in clinical and diagnostic practice, where it offers a more practical and physiological approach.

According to Roy Walford, there is evidence that a combination of lysine and arginine may increase thymus hormone secretion in older animals and humans, partially reversing the immunodeficiency of aging.

Research Profile: A MeSH search of PubMed found a total of 1265 studies on the therapeutic use of lysine. The main focus of the most recent studies was the fortification of human and animal food with lysine because of its effects on growth.

L-Tyrosine: Tyrosine has a phenol (carbolic acid) side chain. It plays a key role in the transmission of signals from one cell to another. Tyrosine is a vital precursor of thyroxin, the thyroid hormone; the pigment melanin; and the brain neurotransmitters dopamine, noradrenalin and adrenaline, which are involved in mood, mental function, and sex drive.

Effects on HGH: Thyroxin, the thyroid hormone that is synthesized from tyrosine, is a vital hormone involved in regulating growth, metabolism, skin health and mental state. Clinical studies indicate that Tyrosine can be helpful in reducing the irritation, fatigue and depression of PMS sufferers.

Research Profile: A MeSH PubMed search found 1,116 studies on the therapeutic use of tyrosine. Many studies focused on tyrosine analogues. The most recent studies investigated the use of tyrosine in the following: hyperthermia; increasing norepinephrine; animal growth; sleep; and cognitive function.

L-Glutamine: Glutamine is the most abundant amino acid in the body. It is a conditionally essential amino acid, meaning that the body may not be able to synthesize all it needs when under physical stress, illness, and injury.

Glutamine is the amino acid that is most used by the body, particularly during times of stress. The immune system and the gut are very dependent on glutamine. Glutamine is the energy source for rapidly dividing cells, for example immune cells and gut mucosa. If the body does not produce enough glutamine, muscle loss and immune dysfunction can occur. Without rapid cell division in the GI tract, the gut atrophies, meaning nutrients of all kinds cannot be properly absorbed.

A 1995 study by Vanier in human skeletal muscle showed that glutamine supplementation enhances the build up of glycogen after exercise thus preventing acidosis and muscle breakdown. (7) According to Tudy Shabert, M.D., author of *The Ultimate Nutrient Glutamine*, supplementation with glutamine, especially in times of stress, would prevent muscle wasting. (8) In a foreword Shabert's book, Douglas Wilmore, M.D., of Harvard Medical School, points out that glutamine is a key to the metabolism and maintenance of muscle; the primary energy source for the immune system; and essential for DNA synthesis, cell division, and cell growth -all factors that are enhanced by HGH. It also crosses the blood-brain barrier into the brain, where it increases energy and mental alertness.

Scientific reviews in 2003 and 2004 revealed some of the benefits of glutamine that have accumulated to date. (9,10) The authors reported that glutamine has a major impact on the function of the immune system. It has a protective effect on cells of the immune system and also cells in other organs such as heart cells. It is able to protect proteins against heat shock, along with taurine. It has a very important effect of being able to protect the gut barrier function from gut permeability. Glutamine also seems to be intimately related to the gut-associated lymphoid tissue called GALT having a positive effect on that tissue system. The authors of this review determined that glutamine has a beneficial effect on infectious complications and can decrease the time spent in hospital. In critically ill patients glutamine supplementation, by itself, reduces morbidity and mortality.

We know that amino acids work better together. A study by Hickson showed that a combined alanine and glutamine infusion in animals prevented muscle atrophy. (11)

Effects on HGH: Glutamine is the latest amino acid to generate excitement as a HGH-releaser thanks to a 1995 study by Thomas C. Welbourne of Louisiana State University College of Medicine in Shreveport. (12) (LINK ABSTRACT # 3) Welbourne showed that a surprisingly small oral dose of about 2 grams of glutamine raised growth hormone levels more than four times over that of a placebo. Even more exciting, age did not diminish the response at least in this small study of volunteers, who ranged from thirty-two to sixty-four years.

Research Profile: A MeSH PubMed search found 1079 studies on the therapeutic use of glutamine. The most recent studies investigated the use of glutamine in the following: lung infection; sepsis; inflammation; sports; low birth weight infants; intestinal permeability; surgical patients; colitis; HIV; intensive care; acute pancreatitis; drug side effects; trauma; and much more.

L-Alanine: Alanine is one of the 20 most common natural amino acids. It is hydrophobic and has a methyl group side chain. Alanine is second to glycine in size. It is non-essential, meaning the body can synthesize alanine. Alanine is involved with sugar and acid metabolism, increases immunity, and provides energy for muscle tissue, the central nervous system, and the brain. It is crucial to energy metabolism and is a building block of muscle protein. Alanine is synthesized in muscle from pyruvate (LINK PYRUVATE BELOW using glutamate as the nitrogen donor. In the liver, alanine can be transformed into pyruvate by the reverse reaction.

LINK Pyruvate: is the ionized form of pyruvic acid, a very important biochemical compound that is formed when glucose is broken down to ultimately create energy. When glucose goes through the process of glycolysis, pyruvic acid is formed and becomes the fuel of the citric acid cycle (also known as the Krebs cycle). The end result of the Krebs cycle is the production of ATP-the energy molecules that fuel all cells in the body. Vitamins and minerals are essential cofactors for each step of the glycolysis cycle and the Krebs cycle.

END LINK

Research Profile: A MeSH PubMed search found 494 studies on the therapeutic use of alanine. However most studies were on the use of alanine analogues with some focus on animal growth and hypoglycemia for the free amino acid.

L Arginine: Arginine is a non-essential amino acid in adults but essential in children. However, many studies have confirmed that it is conditionally essential in people under physical stress and must be obtained from the diet or supplementation. A 2005 review paper reported that arginine is used in the synthesis of body proteins; is essential for ammonia detoxification via urea synthesis, which prevents toxicity caused by elevations in tissue ammonia; and it is essential for the formation of creatine, (a major source of high energy phosphate for regeneration of ATP in muscle) as well as ornithine and is involved in the formation of active enzyme centers. (13) It is also an important component of wound healing and the treatment of renal disease. (14,15)

Arginine in therapeutic amounts stimulates the thymus and stimulates GH secretion according to a 1986 review. One study reported that in mature rats glucose tolerance, the rate of repletion from severe protein under nutrition, and recovery from trauma are significantly accelerated by dietary arginine. (16,17) LINK # 4, 5) Another study reported in this review showed that oral or intravenous administration of excessive arginine reverses nitrogen loss and immune suppression after trauma in rats. Further studies were reported in healthy human volunteers who demonstrated significantly enhanced lymphocyte immune reaction in their blood on an oral dose of 30 grams of arginine.

The authors of the 1986 review found that calculations based on creatinine excretion show that 0.8 grams of protein/kg body weight of the quality supplied by the usual American diet provides insufficient arginine for synthesizing the quantity of creatinine excreted daily in the urine of 70-kg adults. Human patients who often consume less than this amount of protein show a decline in creatinine excretion during illness; the decrease suggests that their intake of arginine is less than optimal. A 1996 paper confirmed that the demands for arginine are increased during stress and encouraged the use of arginine for injured patients. (18)

A review of arginine and its effects on heart disease show that it is closely related to an important signal molecule nitric oxide (NO). (19) In fact, L-arginine is the only substrate of NO production. It therefore has a tremendous affect on the cardiovascular system (blood vessels and heart). The majority of experimental and clinical studies clearly show a beneficial effect of l-arginine on endothelium (the lining of blood vessels) when it is hypofunctioning and therefore producing less NO. Studies involving healthy volunteers or patients suffering from hypertension and diabetes indicate that it may also regulate vascular hemostasis (a complex interplay of various cellular and molecular components within the blood vessels). Experiments performed on animals and in vitro data suggest that l-arginine may have a complex antiaggregatory, anticoagulatory and profibrinolytic effect.

Effects on HGH: Arginine triggers the secretion of growth hormone. In fact, a 15 to 30 gram intravenous infusion of arginine is used as a standard endocrine test to stimulate the pituitary into releasing growth hormone. In a 2005 review of arginine, research shows that a 5 and 9 g of oral arginine caused a significant GH response approximately 30 minutes after ingestion and peaking approximately 60 minutes post ingestion. (20) The authors acknowledge that IV arginine causes an elevation GH but wanted to confirm that oral dosing does the same. A review by Cynober and his colleagues confirms that arginine stimulates growth hormone and insulin secretion. (21)

Arginine helps to improve exercise performance, because, along with glycine, it is one of the main ingredients that the liver uses to make creatine. Supplements of creatine monohydrate are very popular in the bodybuilding community because they raise the level of high-energy creatine phosphates within the muscle and nerve cells needed for high-intensity, short-duration exercises. So with arginine you get higher growth hormone levels and the raw material for increasing your energy.

Arginine appears to stimulate HGH by blocking the secretion of the growth-hormone inhibitor somatostatin. It also greatly enhances the effect of growth hormone-releasing hormone when they are given together.

Positive claims for arginine include increasing fat burning and building muscle tissue probably through the stimulation of growth hormone, increasing the weight and activity of the thymus gland, boosting

immunity, fighting cancer, promoting healing of burns and other wounds, protecting the liver and detoxifying harmful substances, and enhancing male fertility (almost all of which are enhanced by GH). It also restores sexual function in impotent men. A 1994 study in the department of urology at New York University School of Medicine, found that six of fifteen men who took 2,800 milligrams of arginine a day for two weeks had renewed sexual performance with improved erectile function. None of the men on the placebo had any improvement. (22) The researchers believe that arginine worked because it is a precursor of nitric oxide, which plays a key role in initiating and maintaining an erection.

A study using arginine and yohimbin showed beneficial effects on erectile dysfunction; there were no side effects with this treatment. (23)

Research Profile: In a MeSH PubMed search there are 2768 studies on arginine. The most recent studies investigated the use of arginine in the following: endothelial enhancement; hemorrhagic shock; major surgery; pulmonary hypertension; fat loss; anal fissures; drug side effects; infection and sepsis; wound healing; cystic fibrosis; preeclampsia; intestinal healing; diabetic foot ulcers; prevention of surgical infection and post op infection; immune system enhancement; hypertension; and blood thinning.

L-Histidine: Histidine is one of the 10 essential amino acids for infants. It is a conditionally essential amino acid for adult, which means that even though histidine can be synthesized in adult human tissues, sufficient amounts may not be produced to meet extra requirements imposed by stress and disease. Research shows that histidine may act as an antioxidant and affect the immune system.

There are many aspects of histidine that are being scientifically researched. Patients with rheumatoid arthritis (RA) tend to have low levels of free histidine in their serum whereas other amino acid levels are normal. It transpires that histidine is an excellent chelating agent to remove excess copper, iron and zinc from the body. However in rheumatoid arthritis patients who do not have enough histidine these metals can accumulate and act as free radicals causing destruction in tissues and joints. PDRHealth.com reports that in a pilot study, RA patients received up to 6 grams of supplemental histidine daily and were said to benefit with as little as 1 gram daily.

Histidine is also an important precursor of histamine, a compound released by immune system cells during an allergic reaction. Histamine is a chemical transmitter similar to serotonin, epinephrine, and norepinephrine, involved in local immune responses, regulating stomach acid production and in allergic reactions.

Histamine possesses antioxidant activity and modulates the immune system. Suppressor T cells have a certain receptor that is activated by histamine. Promotion of suppressor T cell activity could be beneficial in rheumatoid arthritis and other autoimmune diseases. Histamine has also been shown to down-regulate the production of reactive oxygen species in phagocytic cells, such as monocytes, by binding to the H2 receptors on these cells. Decreased reactive oxygen species production by phagocytes could play antioxidant, anti-inflammatory and immune modulating roles in many diseases.

This latter mechanism is the rationale for the use of histamine itself in several clinical trials studying histamine for the treatment of certain types of cancer and viral diseases. In these trials, down-regulation

by histamine of reactive oxygen species formation appears to inhibit the suppression of natural killer (NK) cells and cytotoxic T lymphocytes, allowing these cells to be more effective in attacking cancer cells and virally infected cells. Low dose histamine injections are also a viable treatment for asthma and allergies.

As with most amino acids, histidine is needed for growth and repair of tissue. It is also important for the maintenance of the myelin sheaths that act as protector for nerve cells. It is further required for the manufacture of white blood cells and therefore supports the immune system. (24) Histidine also helps to protect the body from damage caused by radiation and helps remove heavy metals from the body. In the stomach, histidine is also produces gastric juices. People with a shortage of gastric juices or suffering from indigestion may also benefit from this nutrient.

There are some reports that an increase in the intake of histidine helps with the lengthening of orgasms and also more intense sexual enjoyment.

Research Profile: A MeSH search of PubMed for therapeutic use of histidine found 311 studies. The most recent studies included research with histidine on: diarrhea; appetite suppressant; fat loss; minimizing the side effects of shock therapy; regulation of copper levels; assimilation of zinc; and cerebral ischemia.

Aspartic acid: Aspartic acid is an excitatory transmitter amino acid shown to raise growth hormone levels in the blood, possibly by affecting neural transmission in the hypothalamus, the brain center that directly controls GH production and secretion. It aids in the expulsion of harmful ammonia from the body. When ammonia enters the circulatory system it acts as a highly toxic substance that can be harmful to the central nervous system. Recent studies have shown that aspartic acid may also increase resistance to fatigue and increase endurance.

Research Profile: A MeSH PubMed search found 1034 studies on the therapeutic use of aspartic acid. However, the majority of these studies were on NMDA, which is a water-soluble synthetic derivative of aspartic acid that is not normally found in biological tissue.

Threonine: Threonine is an important constituent of collagen, elastin, and enamel protein and helps maintain protein balance in the body. It helps the digestive and intestinal tracts function more smoothly and assists metabolism and assimilation. It is also involved in liver functioning; it helps prevent fat build-up in the liver. Threonine has enhanced lipotropic functions when combined with aspartic acid and methionine. It assists the immune system by helping the production of antibodies and promotes thymus growth and activity.

Other nutrients are also better absorbed when threonine is present, and it has also been used adjunctive treatment of mental health. This makes sense because in humans, deficiency may result in irritability and mood swings.

Research Profile: A MeSH PubMed search found 192 studies on the therapeutic use of threonine. The most recent studies investigated the use of threonine in the following: intestinal mucus production;

animal growth studies; amyotrophic lateral sclerosis; multiple sclerosis; spastic conditions.

L-Serine: Serine is a non-essential amino acid synthesized from glycine or threonine. It acts as a site of storage for glucose in the liver and muscles; helps create the fatty acid layer around nerve cells; and helps strengthen the immune system by producing antibodies. Serine and glycine are both major sources of one-carbon units necessary for the *de novo* synthesis of purine nucleotides and thymidylate. Purine nucleotides are precursors of DNA and RNA, and thymidylate is a precursor of DNA.

Research Profile: A MeSH PubMed search found 780 studies on the therapeutic use of serine. The most common uses of serine were in the treatment of schizophrenia and prevention of homocystinuria.

L-Glutamic acid (glutamate). It is one of the 20 most common natural amino acids used in protein synthesis. Glutamic acid is critical for proper cell function, but, by definition, it is not considered an essential nutrient in humans because the body can manufacture it from simpler compounds. It prevents muscle breakdown by serving as fuel for muscle energy like glucose. Glutamic acid is stored in muscle and is the most common primary amino acid found there.

Glutamic acid is converted to L-glutamine, which crosses the blood brain barrier where it functions as an excitatory amino acid. Glutamic acid is the most common neurotransmitter in the central nervous system and is a precursor for the synthesis of GABA (a growth hormone stimulant). Glutamic acid activates NMDA receptors and is involved in cognitive functions like learning and memory.

One form of glutamic acid to be avoided is its sodium salt, monosodium glutamate (MSG), which is used as a taste enhancer for bland foods. Many people have serious reactions to MSG, which may stem from a vitamin B6 deficiency and the inability to break down MSG before it acts like an excitotoxin in the brain.

Research Profile: A MeSH PubMed search found 1497 studies on the therapeutic use of glutamic acid. However, most studies investigated derivatives of glutamic acid used in cancer chemotherapy.

L-Leucine, L-Isoleucine and L-Valine: These are essential amino acids that cannot be synthesized in the body but must be provided in the diet. These three essential amino acids are identified as branched-chain amino acids (BCAAs) that have many similar functions. They are found in the proteins of all life forms.

L-Leucine and L-Isoleucine contribute to the structure of protein by the tendency of their side chains (composed only of carbon and hydrogen) to seek an environment consisting of similar side chains, like those of valine, tryptophan, and phenylalanine, and to exclude water. This hydrophobic property is analogous to that which prevents oil from dissolving in water. The tendency for these hydrophobic residues to associate with one another is evidently quite important in determining the bending and folding (tertiary structure) of the peptide chain characteristically seen in every protein. Isoleucine was isolated from beet sugar molasses in 1904.

The chemical composition of isoleucine is identical to that of leucine, but the arrangement of its atoms is slightly different resulting in different properties. They both provide ingredients for the manufacture of other essential biochemical components in the body, some of which are utilized for the production of energy and stimulants to the brain.

L-Valine: It is one of the eight essential amino acids needed in the diet since the human body cannot synthesize it from de novo. The side chain of valine is hydrophobic and accounts for its properties. It is a growth enhancer in children and helps maintain nitrogen balance in adults. It has the properties described above under branched-chain amino acids

According to PDR Health, a pilot study indicated that amyotrophic lateral sclerosis (ALS) patients showed symptomatic improvement when given large doses of BCAAs. It was theorized that BCAAs might protect against neuronal damage from the neuroexcitatory neurotransmitter glutamate. Based on this pilot study, branched-chain amino acids received orphan drug approval for the treatment of ALS. Unfortunately, most of the follow up studies were negative. This is an indication that no one amino acid holds the key to health.

Branched-chain amino acids are sometimes used in enteral and parenteral feedings in the management of hepatic encephalopathy. They can also be used in the management of tardive dyskinesia, extensive burns and severe trauma conditions because of their anticatabolic action in these conditions. In other words, BCAAs prevent muscle catabolism and promote protein synthesis.

It has been theorized that some of the symptoms of hepatic encephalopathy are due to the accumulation toxic metabolites that act as false neurotransmitters in the brain. BCAAs may improve encephalopathy symptoms in some by decreasing the accumulation of these false neurotransmitters and other substances involved in the encephalopathy.

BCAAs serve as important fuel sources for skeletal muscle during periods of metabolic stress including heavy exercise. Under such conditions, BCAAs may promote protein synthesis, suppress protein catabolism and serve as substrates for gluconeogenesis. BCAAs are mainly catabolized in skeletal muscle, stimulating the production of, among other substances, L-alanine and L-glutamine.

The BCAAs are distributed to the various tissues of the body via the systemic circulation. The BCAAs appear to be preferentially taken up by skeletal muscle, where they undergo similar catabolic reactions to those described above. Skeletal muscle appears to be the major site of both BCAA transamination and oxidation in humans. BCAAs are also taken up by other organs, particularly the brain and kidney, where they also undergo oxidation.

Warning: Branched-chain amino acids are contraindicated in those with the rare inborn errors of metabolism maple syrup urine disease and isovaleric acidemia. BCAAs are also contraindicated in those with hypersensitivity to any component of a BCAA-containing supplement.

Research Profile: A MeSH PubMed search found 1,128 studies on the therapeutic use of valine. Much research in this category is on valine analogues. The most commonly investigated area for the use of valine is in animal growth experiments.

L-Phenylalanine: Proline is an essential amino acid, which must be obtained in the diet or by supplementation. It is one of the twenty common amino acids used to synthesize protein. Phenylalanine is a precursor of melanin, dopamine, noradrenalin, and thyroxin. Thus it is associated with mood elevation and thyroid function.

Warning: All children are tested at birth for a genetic disorder called phenylketonuria, which is an inability to metabolize phenylalanine. Those who have been diagnosed with phenylketonuria should avoid GHR.

Research Profile: A MeSH PubMed search found 14,973 studies on the therapeutic use of phenylalanine. However most of the recent studies revolve around the use of analogues of phenylalanine and not L-phenylalanine as a precursor amino acid.

L-Proline: Proline is one of the twenty amino acids used by living organisms as a building block of proteins. It is a non-essential amino acid synthesized from glutamic acid. Structurally, proline is extremely important for the proper functioning of joints and tendons because it is an essential component of collagen. Collagen also helps maintain and strengthen heart muscles.

Research Profile: A MeSH PubMed search found 5,111 studies on the therapeutic use of proline. The most recent studies investigated the use of proline in the following: wound and fracture healing; ulcers; and iron deficiency.

L-Glycine: Glycine is a non-essential amino acid, meaning that cells of the body can synthesize sufficient amounts to meet physiological requirements –given the right building blocks. It is the simplest of the 20 natural amino acids and its side chain is a hydrogen atom. Because of this small side chain it can fit into many places where no other amino acid can. For example, only glycine can be the internal amino acid of a collagen helix (LINK COLLAGEN BELOW). Most proteins contain only small quantities of glycine. However, collagen is about one-third glycine.

LINK

Collagen is the main protein of connective tissue. It has great tensile strength, and is the main component of ligaments and tendons. It is responsible for skin elasticity, and its degradation leads to wrinkles that accompany aging. Collagen also fills out the cornea where it is present in crystalline form. Collagen is the most abundant protein in mammals.

Collagen contains large amounts of glycine and proline as well as two amino acids –hydroxyproline and hydroxylysine derived from proline and lysine in an enzymatic process for which vitamin C is required. This is related to why vitamin C deficiencies can cause scurvy, a disease that leads to loss of teeth and easy bruising caused by a reduction in strength of connective tissue due to a lack of collagen or defective collagen.

The large number of glycine residues in collagen, because of its small size, allows very tight coiling of the collagen helix. There are eleven types of collagen. Type I collagen is the most abundant in the

human body. It is present in scar tissue when tissue heals by repair. It is also found in tendons and the organic part of bone. Other types of collagen are found in cartilage, granulation tissue, basal lamina (the basement membrane on which epithelium sits), and connective tissue.

END LINK

Glycine is an inhibitory neurotransmitter in the central nervous system, especially in the spinal cord. However, in the CNS (central nervous system) it is an excitatory neurotransmitter along with glutamate.

Glycine is very evolutionarily stable at certain positions of some proteins (for example, in cytochrome c (LINK CYTOCHROME C BELOW) myoglobin, (LINK MYOGLOGIN BELOW) and hemoglobin, because mutations that change it to an amino acid with a larger side chain could break the protein's structure.

LINK Cytochrome C is a small heme protein found loosely associated with the inner membrane of mitochondria. It is a soluble protein and an essential component of the electron transfer chain. Cytochrome c is a highly conserved protein across the spectrum of species, found in plants, animals, and many unicellular organisms.

END LINK

LINK Myoglobin is a single-chain protein of 153 amino acids, containing a heme (iron-containing porphyrin) group in the center. It is the primary oxygen-carrying pigment of muscle tissues. Myoglobin is the target protein that causes acute renal failure in rapid breakdown of muscle (e.g. rhabdomyolysis (from statin drugs) severe crush trauma, malignant hyperthermia, status epilepticus, and neuroleptic malignant syndrome) due to its toxicity to renal tubular epithelium. Myoglobin is a sensitive marker for muscle injury, making it a potential marker for myocardial infarction in patients with chest pain.

END LINK

Research Profile: A MeSH PubMed search found 1,720 studies on the therapeutic use of glycine. The most recent studies investigated the use of glycine in the following: periodontal disease; ulcers; hemorrhagic shock; animal growth; alcoholic liver; schizophrenia; prevention of reperfusion injury after liver transplantation; antioxidant activity; intestinal ischemia reperfusion injury; liver injury; inflammation; and insulin response.

L-Cystine: Cystine is the oxidized form of cysteine, which is two cysteine molecules joined by a disulfide bond. Cysteine is a naturally occurring hydrophobic amino acid, which has a sulfhydryl group and is found in most proteins in small quantities. The sulfur group in cysteine makes it a powerful antioxidant protecting the body against radiation and pollution. As such it can help slow down the aging process, deactivate free radicals, neutralize toxins, and prevent cellular damage. It is a necessary component of skin and hair, accounting for 10-14 percent of their content. Before cysteine was produced by fermentation, all cysteine dietary supplement products were made from hair.

Cystine is an essential precursor to N-acetyl-cysteine (NAC), a powerful antioxidant.

Research Profile: A MeSH PubMed search found 182 studies on the therapeutic use of cystine. The most recent studies investigated the use of cystine in the following and included many studies using

NAC: normal requirements; animal growth; homocystinuria; nephropathy; antioxidant potential; lung damage from smoking; lung infection; animal growth; and acetaminophen overdose.

L-Methionine: Methionine is an essential amino acid that helps break down fats. The only two amino acids that have a sulfur group are cysteine and methionine. Methionine is important in the production of cysteine, carnitine, and taurine. It also functions in lecithin production and the synthesis of Phosphatidyl choline and other phospholipids.

Methionine is a safe and effective chelating agent that binds with metals, removing them from the body. In its role as a principle supplier of sulfur it helps prevent disorders of the hair, skin and nails while promoting hair growth. Methionine also regulates the formation of ammonia and creates ammonia-free urine, which reduces bladder irritation.

Because it is lipotropic, methionine helps lower cholesterol levels and also because it increases the liver's production of lecithin. It also reduces liver fat and protects the kidneys.

Research Profile: A MeSH PubMed search found 1,935 studies on the therapeutic use of methionine. Many studies focused on methionine as a component of SAME or selenomethionine. The most recent studies investigated the use of methionine in the following: homocystinuria; animal growth; high cholesterol; and myelopathy.

3. PHYTOSTEROLS

Beta sitosterol, Campesterol, Stigmasterol,

Phytosterols are the cholesterol of the plant kingdom. All sterols have a chemical ring structure. We eat phytosterols every day in our diet. Typical daily dietary intakes of phytosterols range from 100 to 300 milligrams with the higher amount eaten by vegetarians. Since less than 3 percent of the population are vegetarians, we might assume that the majority of the population is not obtaining a sufficient quantity of phytosterols in their diet.

Beta-sitosterol is the most abundant of about 40 phytosterols; it comprises about 50 percent of dietary phytosterols. The next most abundant phytosterols are campesterol (about 33 percent) and stigmasterol (about 2 to 5 percent). Other phytosterols found in the diet include brassicasterol, delta-7-stigmasterol and delta-7-avenasterol.

Beta-sitosterol only differs from cholesterol by the presence of an ethyl group at the 24th carbon position of the side chain. In the case of campesterol, a methyl group occupies this position.

Unlike cholesterol, phytosterols do not raise cholesterol levels but can lower them. As early as 1951, it was shown that phytosterols lowered cholesterol in chickens, and subsequently they were found to lower cholesterol in humans. In one study using plant sterols, cholesterol levels dropped by 15.1 percent in nondiabetic subjects and by 26.8 percent in diabetic subjects. (25) Not only are there plant sterol supplements but functional foods containing plant sterols are being marketed in the form of margarines, spreads and salad dressings.

There is a tremendous amount of basic research on the plant sterols. A MeSH PubMed search for phytosterols produced 3,720 studies.

4. PHOSPHOLIPIDS

Phosphatidyl serine, Phosphatidyl ethanolamine, Phosphatidyl inositol

Phospholipids are formed from four components: fatty acids; a negatively charged phosphate group; an alcohol; and a backbone –such as glycerol. Phospholipids with a glycerol backbone are known as glycerophospholipids or phosphoglycerides. Phospholipids are a major component of all biological membranes, along with glycolipids and cholesterol.

Phytosterols are crucial to membrane permeability and the transport of nutrients from the blood into cells. Due to its polar nature, the head of a phospholipid is attracted to water (hydrophilic) and the tail avoids water (hydrophobic). When placed in water, phospholipids form what is called a bilayer where the hydrophobic tails line up together to avoid water and form a membrane with the hydrophilic heads extending into the water. Such a membrane can spontaneously form liposomes, which are small fat vesicles, which are used to transport materials into living organisms. This membrane is what is required to move nutrients across membranes. It is partially permeable, very flexible, and has fluid properties.

Research Profile Phosphatidyl serine: A MeSH PubMed search found 82 studies on the therapeutic use of phosphatidyl serine. The most recent studies investigated the use of phosphatidyl serine in the following: inflammation; immunity; stress; memory; behavior; cognition; mood; heart rate; ischemia; amnesia; depression; neuritis; Alzheimer's; photosensitivity; epilepsy; tumor necrosis factor.

Research Profile Phosphatidyl ethanolamine: A MeSH PubMed search found 200 studies on the therapeutic use of phosphatidyl ethanolamine. The majority of studies focus on the use of phosphatidyl ethanolamine as a carrier for chemotherapeutic agents in the treatment of cancer.

Research Profile Phosphatidyl inositol: A MeSH PubMed search found 26 studies on the therapeutic use of phosphatidyl inositol. The most recent studies investigated the use of phosphatidyl inositol in the following: colitis, lung tissue, insulin activation, immunity, and lactation.

6. Panax Ginseng: The root (panax) of Ginseng is marketed as a remedy for fatigue but it does much more. According to scientific research, it improves abstract thinking, speeds up reaction time, and boosts resistance to viral infections.

The American Botanical Council (ABC) offers a public monograph on panax ginseng that describes its beneficial effects and its 2,000 yearlong safety record. (26) This monograph identifies the pharmacological and clinical studies that have been conducted over the past 40 years as focusing on “radioprotective, antitumor, antiviral, and metabolic effects; antioxidant activities; nervous system and reproductive performance; effects on cholesterol and lipid metabolism, and endocrinological activity.”

According to ABC, early research in Bulgaria provided a “pharmacological basis for a stimulative effect of ginseng on the central nervous system, a hypotensive effect, respiratory stimulation effect, blood sugar lowering activity, an increase of reactivity of cerebrocortical cells in response to stress, increase of erythrocyte and hemoglobin counts, and blood cholesterol lowering effects.” Soviet research found that soldiers who took ginseng ran faster; made fewer errors and worked faster as radio operators; and experienced improved stamina.

The monograph confirmed that ginseng contains a number of active constituents including saponins, essential oil, phytosterol, carbohydrates and sugars, organic acids, nitrogenous substances, amino acids and peptides, plus vitamins and minerals. At least 22 saponins, known as ginsenosides (or panaxosides) have been isolated and found to be the most active constituents.

ABC reports that research on the “pharmacological actions of pure ginseng saponins indicates that ginsenoside Rb-1 has CNS-depressant activity, is anticonvulsant, analgesic, antipyretic, antipsychotic, ulcer-protective, inhibits conditioned avoidance response, has weak anti-inflammatory activity, an antihemolytic action, and increases gastrointestinal motility. In addition it accelerates glycolysis, and accelerates serum and liver cholesterol, nuclear RNA, and serum protein synthesis.”

Another ginsenoside fraction, known as Rg-1 has shown “weak CNS-stimulant activity, anti-fatigue action, aggravation of stress ulcer, and a slight increase in motor activity. In behavioral tests it showed an acceleration of discrimination behavior in pole-climbing tests and the Y-maze tests, a reversal learning response in the Y-maze test, and one-trial passive avoidance learning using the step-down method.”

A function of special importance to antiaging is the research on nerve tissue. Work in the 1980’s showed that ginsenosides Rb-1 and Rd potentiated nerve growth factor. ABC reports “Nerve growth factor is recognized as having an important role for the survival, regeneration, and regulation of catecholaminergic neurons of brain and ganglion in adult animals.”

At the dosage in the combined GHR formula there are no side effects or drug interactions with ginseng.

Research profile: A PubMed search for ginseng found 2,314 studies.

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D. ABSTRACT LINKS

ABSTRACT # 1 LINK

Groschl M, Knerr I, Topf HG, Schmid P, Rascher W, Rauh M. *J Endocrinol.* 2003 Nov;179(2):237-44. Endocrine responses to the oral ingestion of a physiological dose of essential amino acids in humans. Department of Paediatrics, Friedrich-Alexander-Universitat Erlangen-Nuremberg, Germany.

The response of insulin, human growth hormone (hGH), cortisol, leptin and ghrelin, in addition to various metabolic parameters, was measured at 10 minute intervals following the oral ingestion of a standardised physiological dose of essential amino acids (AA). Twenty-eight healthy male, fasted volunteers (aged 18-40 yrs, BMI 18.0-24.5 kg/m²) took part in the study; 13 volunteers in the AA group, nine subjects in an iso-caloric control group, and a further six subjects served as fasting controls. Twenty minutes after ingestion, insulin reached peak concentrations that were up to 500% higher than basal values (P<0.0001). The AA group and iso-caloric control group showed a similar insulin response. AA ingestion led to an increase in hGH secretion with maximum concentrations being 2100+/-1013% higher than the basal values (P<0.0001). In contrast, no changes in hGH concentrations were observed in the iso-caloric controls; in the fasting controls only a slight increase in hGH was found towards the end of the fasting period. While cortisol decreased significantly (P<0.01) during the study in the AA group, neither control group showed a significant change in this parameter. Changes in leptin levels remained insignificant in all three groups, whereas ghrelin showed a different profile in each of the three groups, i.e. a continuous rise towards the end of the study period (P<0.001) in the AA group, a less significant effect for the fasting group, and no effect at all in the iso-caloric control group. There was no significant correlation between the concentrations or the area under curve of the hormones measured in any of the groups. The endocrine data provided in this study indicate that a single bolus of essential AA in fasted individuals is associated with both anabolic and catabolic hormonal responses.

ABSTRACT LINK # 2

Di Luigi Luigi, Guidetti Laura, Pigozzi Fabio, Baldari arlo, Casini Alessandro, Nordio Maurizio, Romanelli Francesco. Acute amino acids supplementation enhances pituitary responsiveness in athletes. *Medicine & Science in Sports & Exercise.* Dec 1999. 31(12):1748-54.

Purpose: The purpose of this study was to determine the effect of a mixture of amino acids on pituitary responsiveness to a stimulation test (GnRH + CRH) in athletes.

Methods: In a double blinded counterbalanced experimental protocol, 10 moderately trained male athletes performed the pituitary stimulation test 60 min after a single oral administration of a placebo (Pl-AS) or an amino acid mixture solution (AS) (L-arginine hydrochloride 100 mg[middle dot]kg⁻¹ + L-ornithine hydrochloride 80 mg[middle dot]kg⁻¹ + L-branched chain amino acids 140 mg[middle dot]kg⁻¹: 50% L-leucine, 25% L-isoleucine, 25% L-valine) on two different occasions. Plasma ACTH, LH, FSH, GH, and cortisol were evaluated before (-60, -30, 0 min) and after (+15, +30, +45, +60, +90 min) the stimulation test.

Results: The ACTH, LH and FSH response to CRH + GnRH was significantly higher in AS group both as absolute values and area under curve (AUC) values than in Pl-AS group. Pre-test and post-test cortisol AUC levels were significantly higher in Pl-AS group although a higher percent increase in post-

test cortisol was found in AS group. The total GH-AUC was higher in AS group and, as expected, the absolute GH concentrations at different time points were not influenced by CRH + GnRH administration.

Conclusion: The amino acid mixture used enhanced the ACTH, LH, and FSH response to CRH + GnRH.

ABSTRACT LINK # 3

Welbourne TC. *Am J Clin Nutr.* 1995 May;61(5):1058-61. Increased plasma bicarbonate and growth hormone after an oral glutamine load.

Department of Physiology, Louisiana State University College of Medicine, Shreveport 71130, USA.

An oral glutamine load was administered to nine healthy subjects to determine the effect on plasma glutamine, bicarbonate, and circulating growth hormone concentrations. Two grams glutamine were dissolved in a cola drink and ingested over a 20-min period 45 min after a light breakfast. Forearm venous blood samples were obtained at zero time and at 30-min intervals for 90 min and compared with time controls obtained 1 wk earlier. Eight of nine subjects responded to the oral glutamine load with an increase in plasma glutamine at 30 and 60 min before returning to the control value at 90 min. Ninety minutes after the glutamine administration load both plasma bicarbonate concentration and circulating plasma growth hormone concentration were elevated. These findings demonstrate that a surprisingly small oral glutamine load is capable of elevating alkaline reserves as well as plasma growth hormone.

ABSTRACT LINK # 4

Cynober L, et al. Arginine-enriched diets: Rationale for use and experimental data. *Nutr Clin Metab.* 1996, 10/2 (89-95).

Since the pioneering work of Rose who classified arginine as a non-essential amino acid, subsequent works have revealed that arginine can become an essential amino acid in stress situations. In septic rats, arginine-enriched nutrition (either enteral or parenteral) improves nitrogen balance and total body and liver protein synthesis. In addition, arginine stimulates growth hormone and insulin secretion. The most remarkable action of arginine is certainly that exerted on cellular immunity. This action concerns thymus and extra-thymus areas. Finally, arginine favours wound healing improves host defenses in cancer and slows tumour growth. The pharmacological action of arginine probably depends upon various mechanisms: its action on immunity may be mediated by the synthesis of nitric oxide and polyamines (via ornithine synthesis). The effect on wound healing may be related to proline synthesis. The effects on nitrogen metabolism may be linked to growth hormone secretion. These observations form the rationale for the administration of arginine-enriched diets to injured patients.

ABSTRACT LINK # 5

Visek WJ. Arginine needs, physiological state and usual diets. A reevaluation. *J Nutr.* Jan 1986, 116 (1) p36-46.

Evidence is discussed that puts in question the widely held belief that adult mammals, including human beings, can meet all of their arginine needs by endogenous synthesis. Arginine, used in synthesis of

body proteins, is essential for ammonia detoxification via urea synthesis, which prevents metabolic derangements caused by elevations in tissue ammonia. It is a precursor for polyamine synthesis and is the only source of amidino groups for the formation of creatine, a major source of high energy phosphate for regeneration of ATP in muscle. Arginine at supraphysiologic doses is thymotropic and a secretagogue for hormones that control growth and metabolism. Studies in mature rats show that glucose tolerance, the rate of repletion from severe protein undernutrition and recovery from trauma are significantly accelerated by dietary arginine. Oral or intravenous administration of excessive arginine reverses nitrogen loss and immune suppression after trauma in rats, and healthy human volunteers consuming 30 g of oral supplements or arginine have shown significantly enhanced immunoreactivity of the lymphocytes of their peripheral blood. Calculations based on creatinine excretion show that 0.8 g of protein/kg body weight of the quality supplied by the usual American diet barely provides sufficient arginine for synthesizing the quantity of creatinine excreted daily in the urine of 70-kg adults. Human patients who often consume less than this amount of protein show a decline in creatinine excretion during illness; the decrease suggests that their intake of arginine is less than optimal. Recent studies of intraspecies and interspecies differences in responses to arginine reemphasize that dispensability or indispensability of arginine is a matter of definition and that growth and nitrogen balance data impose significant limitations on the drawing of far-reaching conclusions about the needs for arginine by mammalian adults including humans. Orotic acid excretion, immune responsiveness and circulating hormone levels are measures that should be evaluated for identifying when enhancement of arginine intakes might prove beneficial.

E. AMINO ACID REFERENCES

1. ARGININE REFERENCES

Cylwik D, Mogielnicki A, Buczko W. L-arginine and cardiovascular system. *Pharmacol Rep.* 2005 Jan-Feb;57(1):14-22.

Department of Pharmacodynamics, Medical University, Mickiewicza 2C, 15-089 Bialystok, Poland.

L-arginine is a basic endogenous amino acid. Its significant metabolic role as the product of ammonia detoxification, the urea cycle metabolite, the precursor of proteins, ornithine, urea and creatinine, and the amino acid involved in the formation of active enzyme centers was very well established. The current interest in this amino acid refers mainly to its close relation with an important signal molecule nitric oxide (NO). Literature review demonstrates that L-arginine, the only substrate of the NO production, affects cardiovascular system (blood vessels and heart). The majority of experimental and clinical studies clearly show a beneficial effect of L-arginine on endothelium in conditions associated with its hypofunction and thus with reduced NO synthesis. Some clinical studies involving healthy volunteers or patients suffering from hypertension and diabetes indicate that it may also regulate vascular hemostasis. Moreover, experiments performed on animals and in vitro data also suggest that L-arginine may have a complex antiaggregatory, anticoagulatory and profibrinolytic effect. Therefore, a novel therapeutic potential of L-arginine should be taken into consideration.

Collier SR, Casey DP, Kanaley JA. Growth hormone responses to varying doses of oral arginine. *Growth Horm IGF Res.* 2005 Apr;15(2):136-9. Epub 2005 Jan 26.

Department of Exercise Science, Syracuse University, 820 Comstock Avenue, Room 201, Syracuse, NY 13244, USA.

Intravenous (IV) arginine invokes an increase in growth hormone (GH) concentrations, however, little is known about the impact of oral arginine ingestion on the GH response. **OBJECTIVE:** The purpose of this study was to determine the dose of oral arginine that elicits an optimal GH response and to determine the time course of the response. **DESIGN:** Eight healthy males (18-33 years - 24.8±1.2 years) were studied on 4 separate occasions. Following an overnight fast at 0700 h, a catheter was placed in a forearm vein. Blood samples were taken every 10 min for 5 h. Thirty minutes after sampling was initiated, the subject ingested a dose of arginine (5, 9 or 13 g) or placebo (randomly assigned). **RESULTS:** Mean resting GH values for the placebo, 5, 9 and 13 g day were 0.76, 0.67, 2.0 and 0.79 microg/L (n=6), respectively. Integrated area under the curve was not different with 13 g (197.8±65.7 min microg/L), yet it increased with 5 and 9 g compared with the placebo (301.5±74.6, 524.28±82.9 and 186.04±47.8 min microg/L, respectively, P<0.05). Mean peak GH levels were 2.9±0.69, 3.9±0.85, 6.4±1.3 and 4.73±1.27 microg/L on each day for the placebo, 5, 9 and 13 g days. **CONCLUSION:** In conclusion, 5 and 9 g of oral arginine caused a significant GH response. A 13 g dose of arginine resulted in considerable gastrointestinal distress in most subjects without augmentation in the GH response. The rise in GH concentration started approximately 30 min after ingestion and peaked approximately 60 min post ingestion.

Mertz PM, Davis SC, Franzen L, Uchima FD, Pickett MP, Pierschbacher MD, Polarek JW. Effects of an arginine-glycine-aspartic acid peptide-containing artificial matrix on epithelial migration in vitro and experimental second-degree burn wound healing in vivo
Journal of Burn Care and Rehabilitation (USA), 1996, 17/3 (199-206)

Cells central to dermal tissue repair such as dermal fibroblasts and keratinocytes interact with arginine-glycine-aspartic acid (RGD)-containing proteins of the extracellular matrix such as fibronectin. It has been shown that synthetic peptides containing this RGD sequence can also support cell attachment and migration in vitro. We therefore set out to test whether the use of these peptides, when formulated as a synthetic RGD-peptide matrix consisting of peptide complexed with hyaluronic acid, would have an effect on the rate of epithelial migration and wounds. Evaluation consisted of measuring the extent of epithelial outgrowth from human dermal explants and the epithelization of experimental second-degree burn wounds in pigs. We show here that the RGD-peptide matrix supports epithelial sheet migration from explants in a dose-dependent manner. In second-degree burn wounds in pigs, wounds treated with daily applications of the RGD peptide matrix under occlusion resurfaced at a significantly faster rate (day 7 = 57% completely epithelized) than wounds treated with hyaluronic acid under occlusion (day 7 = 13% completely epithelized, p < 0.01), occlusion alone (day 7 = 13% completely epithelized, p < 0.01), or air exposed (day 7 = 0% completely epithelized, p < 0.001). Histologic examination showed that wounds treated with the RGD-peptide matrix also had thicker epithelial covering and greater granulation tissue deposition than occluded, air-exposed, and hyaluronate-treated wounds. These data therefore show that the use of RGD-peptide matrix induces faster explant epithelial migration and results in faster healing of experimental second-degree burns.

Cooper ML, Hansbrough JF, Polarek JW. **The effect of an arginine-glycine-aspartic acid peptide and hyaluronate synthetic matrix on epithelialization of meshed skin graft interstices.** J Burn Care Rehabil. 1996 Mar-Apr;17(2):108-16

Keratinocytes and fibroblasts interact with proteins of the extracellular matrix such as fibronectin and vitronectin through RGD (arginine-glycine- aspartic acid) cell-attachment sequences. This study evaluated the ability of a provisional synthetic matrix composed of an RGD peptide and hyaluronic acid to accelerate the epithelialization of the interstices of meshed, human, split-thickness skin when placed on full-thickness wounds of athymic mice. Full-thickness skin defects, sparing the panniculus carnosus, were created on athymic mice and 3:1 meshed, human skin was placed on them. The grafts had four central, isolated interstices, which epithelialized by migration of human keratinocytes. Conditions were either the addition to the wound of the synthetic matrix or a matrix of hyaluronic acid alone. The time to closure of the graft interstices was decreased ($p < 0.02$) in the wounds treated with the RGD peptide-hyaluronic acid provisional matrix. The resultant epithelium of the closed interstices was significantly thicker 8 days after surgery for the RGD-treated wounds. Basement membrane proteins (laminin and type IV collagen) were also found to be present at the dermoepidermal junction earlier in the RGD-treated wounds. These results imply that use of the RGD peptide conjugate to effect-cell-matrix interactions may have clinical significance in the field of wound healing.

Can the length of hospital stay be influenced by enteral immunonutrition?
Anesthesiologie und Intensivmedizin (Germany), 1997, 38/3 (137-147)

The balance of current clinical data suggests that early enteral nutrition may influence infectious complications in the critically ill patients. Certain nutrients may affect organ function, independent of their general nutritional effects. Four of these nutrients are arginine, nucleotides, omega-3-fatty acids and glutamine. The target cells for the action of these nutrients appear to be T-lymphocytes and macrophages. An enteral nutrition enriched with such nutrients is called 'immunonutrition'. Recent evidence has suggested that an immunonutrition can have a beneficial effect on the prevention of infectious complications and SIRS, reduction of ventilator days, ICU- and hospital stay. This seems to be translated into a reduction in hospital charges. Beside a therapeutic approach with specific inhibitors and receptor antagonists the so called 'immunonutrition' seems to have a place in the therapy of the critically ill patient.

L-arginine restores dilator responses of the basilar artery to acetylcholine during chronic hypertension.
Hypertension (UNITED STATES) Apr 1996, 27 (4) p893-6

The objective of this study was to test the hypothesis that administration of L-arginine, a substrate for nitric oxide synthase, restores acetylcholine-induced dilatation of the basilar artery in chronically hypertensive rats. Basilar artery diameter was measured through a cranial window in anesthetized stroke-prone spontaneously hypertensive rats (SHRSP) and normotensive Wistar-Kyoto rats (WKY) aged 6 to 7 months (adult) and 12 months (older adult). Under control conditions, baseline basilar artery diameter was smaller in SHRSP (adult, 239 +/- 30 micron; older adult, 198 +/- 13 micron) (mean +/- SE) than in WKY (adult, 261 +/- 10 micron; older adult, 259 +/- 7 micron) ($P < .05$ versus SHRSP). Topical application of acetylcholine ($10(-5)$ mol/L) produced dilatation of the basilar artery in WKY, which was impaired in both adult and older SHRSP ($P < .05$). Topical L-arginine ($10(-3)$ mol/L for 30

minutes) did not affect responses to acetylcholine in adult SHRSP but enhanced vasodilatation in response to acetylcholine (10^{-5} mol/L) in older SHRSP without affecting responses to sodium nitroprusside. In contrast, D-arginine did not affect acetylcholine-induced vasodilatation in older SHRSP. These results suggest that impaired dilatation of the basilar artery in response to acetylcholine in older SHRSP is restored toward normal by L-arginine, a substrate for nitric oxide synthase.

Yukihito Higashi; Tetsuya Oshima; Mitsuaki Watanabe; Hideo Matsuura; Goro Kajiyama. Renal response to L-arginine in salt-sensitive patients with essential hypertension. *Hypertension*.1996;27:643-648.)

This study examined whether disturbances in nitric oxide formation contribute to renal dysfunction in salt-sensitive essential hypertensive patients. We evaluated the effects of intravenous administration of L-arginine (500 mg/kg given over 30 minutes) on systemic and renal hemodynamics in 23 patients with mild essential hypertension during 1 week of a low NaCl diet (50 mmol/d) followed by 1 week of a high NaCl diet (340 mmol/d). Patients were classified as salt sensitive (n=10) or salt resistant (n=13) based on salt-induced changes in their blood pressures. Salt loading increased renal vascular resistance but not renal plasma flow in salt-sensitive patients. The L-arginine-induced renovascular relaxation was significantly reduced by a high NaCl diet (renal vascular resistance: low NaCl $-12.4 \pm 2.3\%$ versus high NaCl $-7.1 \pm 1.8\%$, $P < .001$) in salt-sensitive patients, whereas it was unchanged in salt-resistant patients. The increase in plasma cGMP in response to L-arginine was also reduced by a high NaCl diet in the salt-sensitive patients (low NaCl $49 \pm 7\%$ versus high NaCl $36 \pm 8\%$, $P < .05$) but not in the salt-resistant patients (low NaCl $51 \pm 6\%$ versus high NaCl $58 \pm 6\%$). These findings suggest that NaCl loading in salt-sensitive patients with mild essential hypertension reduces the ability of L-arginine to produce nitric oxide in the endothelium of the renal vasculature.

Albert Mimran; Jean Ribstein; Guilhem DuCaila. Contrasting effect of antihypertensive treatment on the renal response to L-arginine. *Hypertension*. 1995;26:937-941.

We assessed the renal hemodynamic response to L-arginine infusion (30 g within 60 minutes) in normotensive subjects, patients with never-treated essential hypertension, and hypertensive patients controlled by long-term (more than 2 years) treatment with or without an angiotensin-converting enzyme inhibitor. The renal vasodilator response to L-arginine observed in normotensive subjects ($15 \pm 4\%$ increase in effective renal plasma flow) was abolished in untreated hypertensive patients and restored only in the group treated by angiotensin-converting enzyme inhibition. In the whole population a positive correlation between the change in effective renal plasma flow and the change in urinary cGMP was obtained. It is suggested that abnormalities of the renal nitric oxide pathway not corrected by increased availability of L-arginine and reversible only on long-term treatment by angiotensin-converting enzyme inhibition may underlie the abnormal renal resistance observed in essential hypertension.

Drexler H. **Endothelial dysfunction: Clinical implications.** Germany *Progress in Cardiovascular Diseases* 1997, 39/4 (287-324)

The endothelium is involved in the control of vascular tone and homeostasis. Risk factors for arteriosclerosis, as well as other conditions have been shown to be associated with a dysfunctional endothelium. Clinically, endothelial function and dysfunction have been mostly evaluated by the assessment of endothelial dependent relaxation, for example in response to acetylcholine or increase inflow. The functional implications of endothelial dysfunction in cardiovascular disease are not well defined, but recent clinical trials have suggested that endothelial dysfunction may affect vascular tone and organ perfusion particularly during stress situations such as exercise. Moreover, endothelial dysfunction may represent an early event in the development of arteriosclerosis. Therefore, recent clinical studies have been performed to restore normal endothelial function in patients, using interventions such as L-arginine, lipid lowering drugs, vitamin C, other antioxidants, or exercise.

L-Arginine reduces lipid peroxidation in patients with diabetes mellitus. Free Radic Biol Medicine.1997, 22 (1-2) p355-7

A current concept for the development of diabetic long-term complications is the involvement of oxidative stress, as, e.g., lipid peroxidation, in the diabetic state. Data published recently show also oxidative damage to DNA, which might be one factor for accelerated aging and diabetic microangiopathy. In our study we tested the hypothesis that L-arginine can reduce lipid peroxidation in patients with diabetes. We performed a blind placebo controlled study with crossing over two treatment periods for 3 months. Thirty patients with diabetes mellitus were randomly assigned to treatment group A (first treatment then placebo) and B (first placebo then treatment). Treatment consisted of two daily dosages of 1 g L-arginine free base. Lipid peroxidation as reflected by malondialdehyde was evaluated in urine using a standard HPLC assay. After 3 months of treatment there was a significant reduction in malondialdehyde levels in group A ($p < .0032$), whereas there was no difference compared to the baseline values after three months of placebo treatment in group B ($p < .97$). After crossing over, there was a significant reduction in malondialdehyde levels in group B ($p < .0002$). Group A showed a significant increase in malondialdehyde levels ($p < .0063$) returning to baseline values. L-Arginine treatment was able to reduce the lipid peroxidation product malondialdehyde. This provides evidence that treatment with L-arginine may counteract lipid peroxidation and thus reduce microangiopathic long-term complications in diabetes mellitus.

Short-term oral administration of L-arginine reverses defective endothelium-dependent relaxation and cGMP generation in diabetes. Eur J Pharmacol (NETHERLANDS) Dec 19 1996, 317 (2-3) p317-20

In the present study, we evaluated whether acute dietary supplementation with L-arginine in vivo could reverse the defective endothelium-dependent relaxation in diabetic blood vessels assessed ex vivo. At 8 weeks of diabetes, streptozotocin-induced diabetic rats were given 1.25% L-arginine in drinking water 3 days prior to isolation of aortic rings for evaluation ex vivo. Plasma arginine concentration was reduced by diabetes but restored to normal in diabetic rats receiving dietary L-arginine. In norepinephrine-contracted rings, relaxation to acetylcholine but not to nitroglycerin was reduced by diabetes. Dietary treatment with L-arginine restored relaxation to acetylcholine without altering relaxation to nitroglycerin and restored the defect in acetylcholine-stimulated cGMP generation. These data suggest that the substrate for nitric oxide synthesis by the endothelium is likely to be limited in diabetes but can be overcome by dietary supplementation with L-arginine.

Metformin improves hemodynamic and rheological responses to L-arginine in NIDDM patients. *Diabetes Care*. Sep 1996, 19 (9) p934-9

OBJECTIVE: The endothelium plays a pivotal role in the regulation of vascular tone by releasing nitric oxide (NO). Increased availability of L-arginine, the natural precursor of NO, induces vasodilatation and inhibits platelet activity. We studied the effect of metformin on hemodynamic and rheological responses to L-arginine in patients with NIDDM. **RESEARCH DESIGN AND METHODS:** Ten newly diagnosed NIDDM patients with mild fasting hyperglycemia (7.5 ± 0.3 mmol/l) and without evidence of both micro- and macrovascular complications were investigated. They received an intravenous infusion of L-arginine (1 g/min for 30 min) with evaluation of plasma glucose and insulin, systolic (sBP) and diastolic (dBP) blood pressure, heart rate and plasma catecholamines, platelet aggregation, and blood viscosity and filterability. The L-arginine test was repeated after an 8-week treatment with metformin (850 mg b.i.d.). **RESULTS:** Metformin treatment significantly reduced basal fasting plasma glucose, HbA1c, and platelet aggregation to ADP ($P < 0.05$); the other parameters did not change. During pretreatment test, L-arginine infusion decreased sBP (from 137 ± 4.1 to 129 ± 4.5 mmHg, $P < 0.01$) and dBP (from 79 ± 1.9 to 75 ± 1.2 mmHg, $P < 0.01$) without affecting heart rate or plasma catecholamines. Both platelet aggregation and blood viscosity showed significant decrements after L-arginine, while blood filterability did not change. After metformin treatment, the decrease in blood pressure after L-arginine infusion was significantly enhanced, with a maximal decrease of sBP of 12 ± 3.4 mmHg (8 ± 2.5 mmHg pretreatment, $P < 0.05$) and dBP of 9.5 ± 2.4 mmHg (4.5 ± 1.9 mmHg pretreatment, $P < 0.01$). Heart rate, plasma norepinephrine levels, and blood filterability also rose significantly ($P < 0.05$ - 0.01). The decrease in both platelet aggregation and blood viscosity after L-arginine was significantly amplified after metformin. **CONCLUSIONS:** We conclude that L-arginine infusion in newly diagnosed NIDDM patients without vascular complications produces relevant hemodynamic and rheological changes, which are amplified by an 8-week treatment with metformin. Whether these vascular effects of metformin will improve the poor cardiovascular outlook of the diabetic patient is still unknown.

ARGININE + LYSINE

Role of lactose, arginine and lysine combination in fracture healing (an experimental study). *Ann Ital Chir*. Jan-Feb 1996, 67 (1) p77-82; discussion 82-3

L-arginine and L-lysine are essential amino acids which seem to possess some properties able to influence bone fractures healing. In fact, the increase of intestinal calcium adsorption but also in collagen synthesis, in insulin and growth hormone secretion and in osteoblastic activation. So, an experimental in vivo model was carried out by using 50 adult rabbits which, under general anaesthesia, were submitted to an osteotomy of the left fibula. Animals were divided into 5 groups and were daily treated with a mixture of lactose, L-arginine and L-lysine or with the only lactose (control group) at the same dosage as recommended for humans. They were sacrificed after 15, 30, 40, 50 and 60 days for radiological and histological studies. The results of the study showed that the pharmacological mixture containing L-arginine and L-lysine accelerates and ameliorates the healing processes and this positive effect was particularly evident from the 30th day after the osteotomy. We think that these results are linked not only to calcium metabolism but also to different biological properties which positively contribute to good healing of bone fractures.

2. TAURINE REFERENCES

Taurine deficiency after intensive chemotherapy and/or radiation. *Am J Clin Nutr*; 55(3):708-11 1992

Taurine, a nonessential amino acid (AA), is the most abundant free AA in the intracellular space. We measured plasma AA concentrations in 36 patients 7-28 d after intensive chemotherapy and/or radiation. Plasma taurine concentrations were uniformly low in all patients (20.0 +/- 6.4 $\mu\text{mol/L}$, mean +/- SD). Plasma taurine in 11 healthy volunteer control subjects was 45.0 +/- 20.3 $\mu\text{mol/L}$ (P less than 0.001). Other AA concentrations, specifically those of precursor AAs methionine and cystine, were normal. We prospectively measured plasma AA concentrations in 12 patients before starting and 6-10 d after completing intensive cytotoxic treatment. Values before treatment were 37.2 +/- 11.6, 109.6 +/- 30.7, and 18.5 +/- 4.8 for taurine, cystine, and methionine, respectively, and were 24.3 +/- 6.0, 111.2 +/- 23.8, and 24.0 +/- 14.5 after treatment. Pretreatment plasma taurine correlated directly with the magnitude of decrease in plasma taurine during cytotoxic treatment (n = 12, r = 0.85, P less than 0.01). Intensive cytotoxic chemotherapy and/or radiation leads to a reduction in plasma taurine concentrations without any change in its precursor AAs, methionine and cystine. The clinical relevance of plasma taurine depletion will need further study.

Usefulness of taurine in chronic congestive heart failure and its prospective application. *Jpn Circ J (JAPAN)* Jan 1992, 56 (1) p95-9

We compared the effect of oral administration of TAURINE (3 g/day) and coenzyme Q10 (CoQ10) (30 mg/day) in 17 patients with congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy, whose ejection fraction assessed by echocardiography was less than 50%. The changes in echocardiographic parameters produced by 6 weeks of treatment were evaluated in a double-blind fashion. In the TAURINE-treated group significant treatment effect was observed on systolic left ventricular function after 6 weeks. Such an effect was not observed in the CoQ10-treated group.

Platelet taurine in patients with arterial hypertension, myocardial failure or infarction. *Acta Med Scand Suppl (SWEDEN)* 1980, 642 p79-84

The content of TAURINE in the hypertrophied left ventricle is increased in congestive heart failure and in spontaneously hypertensive (SH) rats. In SH rats the TAURINE content of and TAURINE uptake by the platelets are also increased. The present results indicate that, as in the heart, the TAURINE content may also increase in the platelets of those patients with congestive heart failure. The TAURINE content and uptake are not increased in the platelets of hypertensive patients as they are in the platelets of SH rats. It is likely that in acute myocardial infarction, a considerable amount of TAURINE is released from the heart into the plasma. However, there is no simultaneous increase in the platelet TAURINE content. From this work one can only conclude that platelets may reflect TAURINE changes in the heart in some pathological states, e.g. congestive heart failure.

Physiological and experimental regulation of TAURINE content in the heart. *Fed Proc (UNITED STATES)* Jul 1980, 39 (9) p2685-90

High concentrations of TAURINE are found in the heart and these are increased still further in congestive heart failure. It appears that TAURINE is largely derived by influx from the circulation, and this influx is stimulated by cyclic AMP, whereas influx of alpha-amino acids is unaffected. Influx occurs via a saturable transport system that has strict requirements for ligands. Other substances are transported by this system, including beta-alanine, hypoTAURINE, guanidoethyl sulfonate, and, to a lesser extent, guanidinopropionate; and these are competitive antagonists for TAURINE transport. Guanidinoethyl sulfonate, in vivo, markedly lowers TAURINE concentrations over the course of a few days in all tissues examined in the rat and mouse (but not in the guinea pig). The concentrations of other amino acids are unaffected. Guanidinoethyl sulfonate may prove to be a useful substance in the study of the biological role of TAURINE, in view of its ability to regulate TAURINE content in a number of species. Despite the numerous pharmacological actions of TAURINE, its physiological function in the heart remains problematic. One function appears to be the modulation of calcium movements. The inotropic actions of TAURINE and beta-adrenergic activation may be linked via the cyclic AMP-dependent regulation of TAURINE influx.

Taurine deficiency after intensive chemotherapy and/or radiation. *Am J Clin Nutr*; 55(3):708-11 1992

Taurine, a nonessential amino acid (AA), is the most abundant free AA in the intracellular space. We measured plasma AA concentrations in 36 patients 7-28 d after intensive chemotherapy and/or radiation. Plasma taurine concentrations were uniformly low in all patients (20.0 +/- 6.4 mumol/L, mean +/- SD). Plasma taurine in 11 healthy volunteer control subjects was 45.0 +/- 20.3 mumol/L (P less than 0.001). Other AA concentrations, specifically those of precursor AAs methionine and cystine, were normal. We prospectively measured plasma AA concentrations in 12 patients before starting and 6-10 d after completing intensive cytotoxic treatment. Values before treatment were 37.2 +/- 11.6, 109.6 +/- 30.7, and 18.5 +/- 4.8 for taurine, cystine, and methionine, respectively, and were 24.3 +/- 6.0, 111.2 +/- 23.8, and 24.0 +/- 14.5 after treatment. Pretreatment plasma taurine correlated directly with the magnitude of decrease in plasma taurine during cytotoxic treatment (n = 12, r = 0.85, P less than 0.01). Intensive cytotoxic chemotherapy and/or radiation leads to a reduction in plasma taurine concentrations without any change in its precursor AAs, methionine and cystine. The clinical relevance of plasma taurine depletion will need further study.

The antiarrhythmic effects of taurine alone and in combination with magnesium sulfate on ischemia/reperfusion arrhythmia. *Chinese Pharmacological Bulletin (China)*, 1994, 10/5 (358-362)

The effect of taurine (Taur) alone and in combination with magnesium sulfate (MgSO₄) on ischemia/reperfusion arrhythmia was investigated. The arrhythmia as produced by coronary artery occlusion for 10 min followed by reperfusion. In addition, the present study also observed the effect of MgSO₄ alone and in combination with Taur on hemodynamics. The results showed that Taur (50 mg . kg⁻¹) and MgSO₄ (25 mg . kg⁻¹) had partly antiarrhythmic effect. Taur (100, 150mg. kg⁻¹) MgSO₄ (50, 100mg. kg⁻¹) had significantly antiarrhythmic effect. Taur (50 mg. kg⁻¹) combined with MgSO₄ (25 mg. kg⁻¹) shortened the duration of ventricular tachycardia (VT) more than that either drug did alone. The hypotensive effect of MgSO₄ (25 mg. kg⁻¹) was not increased by coadministration of Taur, but the myocardial oxygen consumption was reduced. These findings indicate that Taur in combination with MgSO₄ is more effect on reperfusion arrhythmia, and that the mechanism of antiarrhythmic effect of Taur and MgSO₄ may be involved in the effect of defence on myocardium.

METHIONINE

Dietary methionine imbalance, endothelial cell dysfunction and atherosclerosis. Nutrition Research (USA), 1996, 16/7 (1251-1266)

Dietary factors can play a crucial role in the development of atherosclerosis. High fat, high calorie diets are well known risk factors for this disease. In addition, there is strong evidence that dietary animal proteins also can contribute to the development of atherosclerosis. Atherogenic effects of animal proteins are related, at least in part, to high levels of methionine in these proteins. An excess of dietary methionine may induce atherosclerosis by increasing plasma lipid levels and/or by contributing to endothelial cell injury or dysfunction. In addition, methionine imbalance elevates plasma/tissue homocysteine which may induce oxidative stress and injury to endothelial cells. Methionine and homocysteine metabolism is regulated by the cellular content of vitamins B6, B12, riboflavin and folic acid. Therefore, deficiencies of these vitamins may significantly influence methionine and homocysteine levels and their effects on the development of atherosclerosis.

Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA (UNITED STATES) Jun 11 1997, 277 (22) p1775-81

CONTEXT: Elevated plasma homocysteine is a known risk factor for atherosclerotic vascular disease, but the strength of the relationship and the interaction of plasma homocysteine with other risk factors are unclear. **OBJECTIVE:** To establish the magnitude of the vascular disease risk associated with an increased plasma homocysteine level and to examine interaction effects between elevated plasma homocysteine level and conventional risk factors. **DESIGN:** Case-control study. **SETTING:** Nineteen centers in 9 European countries. **PATIENTS:** A total of 750 cases of atherosclerotic vascular disease (cardiac, cerebral, and peripheral) and 800 controls of both sexes younger than 60 years. **MEASUREMENTS:** Plasma total homocysteine was measured while subjects were fasting and after a standardized methionine-loading test, which involves the administration of 100 mg of methionine per kilogram and stresses the metabolic pathway responsible for the irreversible degradation of homocysteine. Plasma cobalamin, pyridoxal 5'-phosphate, red blood cell folate, serum cholesterol, smoking, and blood pressure were also measured. **RESULTS:** The relative risk for vascular disease in the top fifth compared with the bottom four fifths of the control fasting total homocysteine distribution was 2.2 (95% confidence interval, 1.6-2.9). Methionine loading identified an additional 27% of at-risk cases. A dose-response effect was noted between total homocysteine level and risk. The risk was similar to and independent of that of other risk factors, but interaction effects were noted between homocysteine and these risk factors; for both sexes combined, an increased fasting homocysteine level showed a more than multiplicative effect on risk in smokers and in hypertensive subjects. Red blood cell folate, cobalamin, and pyridoxal phosphate, all of which modulate homocysteine metabolism, were inversely related to total homocysteine levels. Compared with nonusers of vitamin supplements, the small number of subjects taking such vitamins appeared to have a substantially lower risk of vascular disease, a proportion of which was attributable to lower plasma homocysteine levels. **CONCLUSIONS:** An increased plasma total homocysteine level confers an independent risk of vascular disease similar to that of smoking or hyperlipidemia. It powerfully increases the risk associated with smoking and hypertension. It is time to undertake randomized controlled trials of the effect of vitamins that reduce plasma homocysteine levels on vascular disease risk.

Hyperhomocysteinemia induced by folic acid deficiency and methionine load--applications of a modified HPLC method. *Clin Chim Acta (NETHERLANDS)* Aug 15 1996, 252 (1) p83-93

The increasing possibility that homocysteine might be involved in atherosclerosis in non-homocysteinuric subjects has required the measurement of low concentrations of this aminothiols in biological samples. The procedure described here represents an improvement of different HPLC methods. We utilized an isocratic HPLC system with fluorescence detection of plasma total homocysteine derivatized after reaction with ammonium 7-fluoro-benzo-2-oxa-1,3-diazole-4-sulphonate. With the help of the rapidly eluting internal standard N-acetyl-cysteine, the method ensures very good recovery (approximately 100%), reproducibility and precision (within-assay 2.31%; day-to-day: 2.8%) in the physiological concentration range. This procedure allowed us to validate various animal models of hyperhomocysteinemia such as dietary folic acid deficiency in rat and acute methionine loads in rat and hamster. Using this method, we also confirmed that men have higher plasma total homocysteine levels than women. Due to its simplicity and reliability, our procedure is suitable for routine analysis of total homocysteine and other aminothiols (cysteine, cysteinyl-glycine and glutathione) in biological samples, as required in clinical and research laboratories.

Male rats fed methyl- and folate-deficient diets with or without niacin develop hepatic carcinomas associated with decreased tissue NAD concentrations and altered poly(ADP-ribose) polymerase activity. *Journal of Nutrition (USA)*, 1997, 127/1 (30-36)

Folate is an essential cofactor in the generation of endogenous methionine, and there is evidence that folate deficiency exacerbates the effects of a diet low in choline and methionine, including alterations in poly(ADP-ribose) polymerase (PARP) activity, an enzyme associated with DNA replication and repair. Because PARP requires NAD as its substrate, we postulated that a deficiency of both folate and niacin would enhance the development of liver cancer in rats fed a diet deficient in methionine and choline. In two experiments, rats were fed choline- and folate-deficient, low methionine diets containing either 12 or 8% casein (12% MCFD, 8% MCFD) or 6% casein and 6% gelatin with niacin (MCFD) or without niacin (MCFND) and were compared with folate-supplemented controls. Liver NAD concentrations were lower in all methyl-deficient rats after 2-17 mo. At 17 mo, NAD concentrations in other tissues of rats fed these diets were also lower than in controls. Compared with control values, liver PARP activity was enhanced in rats fed the 12% MCFD diet but was lower in MCFND-fed rats following a further reduction in liver NAD concentration. These changes in PARP activity associated with lower NAD concentrations may slow DNA repair and enhance DNA damage. Only rats fed the MCFD and MCFND diets developed hepatocarcinomas after 12-17 mo. In Experiment 2, hepatocarcinomas were found in 100% of rats fed the MCFD and MCFND diets. These preliminary results indicate that folic acid deficiency enhances tumor development. Because levels of NAD in these animals were also low, further studies are needed to clearly define the role of niacin in methyldeficient rats.

Azuma J, Sawamura A, Awata N. Usefulness of taurine in chronic congestive heart failure and its prospective application. *Jpn Circ J.* 1992 Jan;56(1):95-9.
Third Department of Internal Medicine, Osaka University Medical School, Japan.

We compared the effect of oral administration of taurine (3 g/day) and coenzyme Q10 (CoQ10) (30 mg/day) in 17 patients with congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy, whose ejection fraction assessed by echocardiography was less than 50%. The changes in echocardiographic parameters produced by 6 weeks of treatment were evaluated in a double-blind fashion. In the taurine-treated group significant treatment effect was observed on systolic left ventricular function after 6 weeks. Such an effect was not observed in the CoQ10-treated group.

Azuma J, Sawamura A, Awata N, Ohta H, Hamaguchi T, Harada H, Takihara K, Hasegawa H, Yamagami T, Ishiyama T, et al. Therapeutic effect of taurine in congestive heart failure: a double-blind crossover trial. *Clin Cardiol.* 1985 May;8(5):276-82.

In a double-blind, randomized, crossover, placebo-controlled study, we investigated the effects of adding taurine to the conventional treatment in 14 patients with congestive heart failure for a 4-week period. Compared with placebo, taurine significantly improved the New York Heart Association functional class (p less than 0.02), pulmonary crackles (p less than 0.02), and chest film abnormalities (p less than 0.01). A benefit of taurine over placebo was demonstrated when an overall treatment response for each patient was evaluated on the basis of clinical examination (p less than 0.05). No patient worsened during taurine administration, but four patients did during placebo. Pre-ejection period (corrected for heart rate) decreased from 148 +/- 14 ms before taurine treatment to 137 +/- 12 ms after taurine (p less than 0.001), and the quotient pre-ejection period/left ventricular ejection time decreased from 47 +/- 9 to 42 +/- 8% (p less than 0.001). Side effects did not occur in the patients during taurine. The results indicate that addition of taurine to conventional therapy is safe and effective for the treatment of patients with congestive heart failure.

Azuma J, Hasegawa H, Sawamura A, Awata N, Ogura K, Harada H, Yamamura Y, Kishimoto S. Therapy of congestive heart failure with orally administered taurine. *Clin Ther.* 1983;5(4):398-408.

The clinical efficacy of 2 gm BID of oral taurine (2-aminoethane sulfonic acid) was studied in 24 patients with congestive heart failure (CHF). We expressed the severity of CHF by a score based on clinical signs and symptoms and on roentgenographic data. The maximum possible score, corresponding to the worst CHF, was 23 points. How much the 24 patients improved after receiving taurine for four or eight weeks was estimated by the difference between their pretreatment and posttreatment scores. In 19 of the 24 patients, taurine was effective. In the group as a whole, mean (+/- SEM) scores fell significantly, from 7.3 +/- 0.6 before treatment to 4.4 +/- 0.5 after treatment. Thirteen of the 15 patients who were designated as New York Heart Association (NYHA) functional class III or IV before receiving taurine could be designated as class II after they completed the study. This pilot study should prompt further investigation into the possible use of taurine in the treatment of patients with CHF.

Kohashi N, Katori R. Decrease of urinary taurine in essential hypertension. *Jpn Heart J.* 1983 Jan;24(1):91-102.

In order to evaluate how taurine relates to the pathogenesis of essential hypertension, the taurine content of plasma, whole blood and urine was measured in 18 normals and in 79 hypertensive patients. The patients included 32 untreated cases of essential hypertension, 32 treated cases and 15 cases with labile hypertension. There were no statistically significant differences between normals and essential hypertensives in either plasma or whole blood taurine content. However, in comparison to urinary taurine excretion in normals, 1594.0 +/- 143.7 mumol/day (mean +/- SE), that for untreated essential hypertensives, 708.1 +/- 57.1 mumol/day (p less than 0.001), and for treated essential hypertensives, 953.6 +/- 94.3 mumol/day (p less than 0.001), were significantly lower. Those with labile hypertension showed almost the same value, 1478.3 +/- 134.3 mumol/day, as normals. Taurine clearance and the taurine/creatinine ratio were also markedly decreased in essential hypertensives without treatment. For all subjects, taurine clearance had a positive correlation ($r = 0.327$, p less than 0.01) with creatinine clearance, but there were significant negative correlations between systolic blood pressure and daily urinary taurine excretion ($r = -0.472$, p less than 0.01) and between diastolic blood pressure and daily urinary taurine excretion ($r = -0.382$, p less than 0.01). There were also significant positive correlations between daily urinary taurine excretion and serum high-density lipoprotein cholesterol ($r = 0.559$, p less than 0.01) and between the former and cardiac index ($r = 0.547$, p less than 0.01). These results suggest that a deficiency of taurine plays an important role not only in elevating blood pressure in essential hypertension but also in atherogenesis as well.

Hansen SH. The role of taurine in diabetes and the development of diabetic complications. *Diabetes Metab Res Rev.* 2001 Sep-Oct;17(5):330-46.

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The ubiquitously found beta-amino acid taurine has several physiological functions, e.g. in bile acid formation, as an osmolyte by cell volume regulation, in the heart, in the retina, in the formation of N-chlorotaurine by reaction with hypochlorous acid in leucocytes, and possibly for intracellular scavenging of carbonyl groups. Some animals, such as the cat and the C57BL/6 mouse, have disturbances in taurine homeostasis. The C57BL/6 mouse strain is widely used in diabetic and atherosclerotic animal models. In diabetes, the high extracellular levels of glucose disturb the cellular osmoregulation and sorbitol is formed intracellularly due to the intracellular polyol pathway, which is suspected to be one of the key processes in the development of diabetic late complications and associated cellular dysfunctions. Intracellular accumulation of sorbitol is most likely to cause depletion of other intracellular compounds including osmolytes such as myo-inositol and taurine. When considering the clinical complications in diabetes, several links can be established between altered taurine metabolism and the development of cellular dysfunctions in diabetes which cause the clinical complications observed in diabetes, e.g. retinopathy, neuropathy, nephropathy, cardiomyopathy, platelet aggregation, endothelial dysfunction and atherosclerosis. Possible therapeutic perspectives could be a supplementation with taurine and other osmolytes and low-molecular compounds, perhaps in a combinational therapy with aldose reductase inhibitors. Copyright 2001 John Wiley & Sons, Ltd.

Militante JD, Lombardini JB, Schaffer SW. The role of taurine in the pathogenesis of the cardiomyopathy of insulin-dependent diabetes mellitus. *Cardiovasc Res.* 2000 Jun;46(3):393-402.
Department of Pharmacology, Texas Tech University, Health Sciences Center, Lubbock 79430, USA.

The cellular and molecular physiology and pathology of insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) are mostly studied and understood through the use of animal models. Fundamental differences between the IDDM and NIDDM animal models may help to explain the etiology behind diabetic cardiomyopathy, one of the most severe complications of IDDM. Experimental rat models of IDDM exhibit a characteristic increase in tissue levels of taurine in the heart, a change that is not seen in NIDDM rats. This article deals with the causes and possible consequences of this observation which may contribute to the development of diabetic cardiomyopathy. Modulation of pyruvate dehydrogenase (lipoamide) (PDH; EC 1.2.4.1) activity was found to be a possible mode for taurine involvement. PDH is a mitochondrial protein and is the rate-limiting step in the generation of acetyl CoA from glycolysis. In IDDM, PDH activity is decreased through a mechanism that includes the stimulation of the de novo synthesis of a kinase activator protein (KAP) which phosphorylates PDH and inactivates the enzyme. This lesion does not occur in NIDDM rat hearts. Taurine is known to inhibit the phosphorylation of PDH in vitro, and in taurine-depleted rats PDH phosphorylation is known to increase. Thus, the increased levels of taurine in the diabetic heart may be inhibiting this phosphorylation which in turn may be stimulating the synthesis of KAP through a negative feedback process. The main argument for this theory would be the lack of change in both the taurine levels and the activity of PDH in the NIDDM rat model.

Smith LJ, Lacaille F, Lepage G, Ronco N, Lamarre A, Roy CC. Taurine decreases fecal fatty acid and sterol excretion in cystic fibrosis. A randomized double-blind trial. *Am J Dis Child.* 1991 Dec;145(12):1401-4.

Department of Pediatrics, Hopital Ste-Justine, Montreal, Quebec, Canada.

Patients with cystic fibrosis may still have a significant degree of steatorrhea despite adequate pancreatic enzyme supplementation. Taurine is a conditionally essential amino acid that possibly improves the micellar phase of fat digestion. Thirteen children with cystic fibrosis and a significant degree of steatorrhea (> 13 g/d) were enrolled in a randomized double-blind crossover study of taurine (30 mg/kg per day) in contrast to placebo for two successive 4-month periods. No difference was noted in height and weight velocity, lung function, vitamin A level, and essential fatty acid status. Twelve of the 13 patients showed a decrease in fecal fatty acid excretion (26.5 +/- 2.6 g/24 h vs 15.4 +/- 2.5 g/24 h), affecting mainly saturates and monounsaturates, and a decrease in total sterol excretion (1492.6 +/- 303 mg/24 h vs 1211.7 +/- 213.8 mg/24 h) while ingesting taurine. Taurine may be a useful adjunct in patients with cystic fibrosis and severe steatorrhea.

Skopnik H, Kusenbach G, Bergt U, Friedrichs F, Stuhlsatz H, Dohmen H, Heimann G. Kinderklinik, RWTH [Taurine supplementation in cystic fibrosis (CF): effect on vitamin E absorption kinetics]. *Aachen. Klin Padiatr.* 1991 Jan-Feb;203(1):28-32. [Article in German]

Oral vitamin E (Vit.E) bioavailability is reduced in CF patients especially in case of malnourishment. Both exocrine pancreatic insufficiency and an altered bile acid composition showing an elevated glycine/taurine ratio of conjugated bile acids which is due to excessive loss of bile acids in the stools may contribute to this observation. Because taurine supplementation reduces the glycine/taurine ratio of bile acids in duodenal juice of CF-patients it was the objective of this study to evaluate the effect of taurine supplementation on Vit.E absorption kinetics. Oral Vit.E tolerance tests (50 mg/kg) were performed

before and after 3 months of taurine supplementation (30 mg/kg/day) in 11 CF patients (ages 7 to 22 years) under fasting conditions. Bodyweight and or weight for height of all patients were below the 25th percentile. Doses of all medications except antibiotics were kept unchanged during the study. Any additional Vit.E supplementation was stopped 14 days prior to each test. Serum Vit.E levels were measured over a 24 hour period. Determination of serum Vit.E concentrations was performed with a HPLC fluorescence technique. The glycine/taurine ratio in serum served as compliance parameter and dropped in all but one patients. Baseline Vit.E concentrations and serum Vit.E/total lipids ratios in serum considered as parameters of the Vit.E status increased significantly. Both the maximal Vit.E concentrations in serum and the areas under the oral absorption curves showed a significant increase with taurine supplementation. This study shows that the Vit.E status of malnourished CF patients can be improved with taurine supplementation due to improved Vit.E absorption kinetics.

Carrasco S, Codoceo R, Prieto G, Lama R, Polanco I. Effect of taurine supplements on growth, fat absorption and bile acid on cystic fibrosis. *Acta Univ Carol [Med] (Praha)*. 1990;36(1-4):152-6.
Department of Pediatrics, Children's Hospital La Paz, Autonoma University, Madrid, Spain.

We have evaluated the effect of taurine supplementation nutritional status, steatorrhea and bile acid in twenty two Cystic Fibrosis patients. Weight increased in fifty per cent and height in forty eight per cent of them. Steatorrhea improved significantly in six patients of group II. Glycine/taurine ratio was reduced. Bile acid malabsorption improved only in the patients with high degree of steatorrhea. Serum bile acid was observed significantly elevated in both groups. This results suggest that taurine supplementation can be useful adjunct from of therapy in Cystic Fibrosis patients with fat malabsorption.

Thompson GN. Taurine uptake by normal and cystic fibrosis fibroblasts. *Biochem Cell Biol*. 1988 Jul;66(7):702-6.
Department of Chemical Pathology, Adelaide Children's Hospital, North Adelaide, Australia.

Taurine deficiency recently has been proposed to be clinically significant in cystic fibrosis (CF). Uptake of [¹⁴C]taurine by four cystic fibrosis (CF) and three control fibroblast lines was examined to determine whether a generalized defect in taurine transport could contribute to the deficiency. The time course of uptake was linear up to 20 h and was similar in both CF and control fibroblasts. Taurine was avidly retained after uptake, and the effect of metabolic (chlorpromazine) and competitive (hypotaurine, L-leucine) inhibitors was similar in both CF and control cells. In contrast, while taurine uptake in a calcium-free medium was impaired in both CF and control fibroblasts, the impairment was significantly less in CF cells. The findings suggest that a generalized abnormality in taurine transport is unlikely to be responsible for the taurine deficiency in CF.

Thompson GN. Excessive fecal taurine loss predisposes to taurine deficiency in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1988 Mar-Apr;7(2):214-9.
Department of Chemical Pathology, Adelaide Children's Hospital, South Australia.

Elevation of the ratio of glycine: taurine-conjugated bile acids (G/T ratio) is thought to contribute to fat malabsorption in cystic fibrosis (CF). The cause, extent, and reversibility of taurine deficiency in CF

were assessed using balance studies in 6 subjects (ages 8-14 years) who were supplemented with taurine (0.24-2.4 mmol/kg/24 h) for 1 week. Taurine reduced the G/T ratio both in serum and duodenal juice in all children. The mean fecal taurine loss in CF subjects [10.8 μ mol/kg/24 h \pm 9.9 (SD), range 0.9-27.9] was much greater than that in controls (less than 0.1 μ mol/kg/24 h, n = 4) and approximated the dietary taurine intake (mean 14.6 \pm 4.4 μ mol/kg/24 h, n = 12). Absorption of an oral taurine load appeared to be normal in CF. Excessive fecal taurine loss appears to predispose CF children to bile acid taurine deficiency, a deficiency that can be corrected by oral taurine supplements.

Colombo C, Arlati S, Curcio L, Maiavacca R, Garatti M, Ronchi M, Corbetta C, Giunta A. Effect of taurine supplementation on fat and bile acid absorption in patients with cystic fibrosis. *Scand J Gastroenterol Suppl.* 1988;143:151-6.
Dept. of Pediatrics, University of Milan, Italy.

Eleven children with cystic fibrosis (CF) and pancreatic insufficiency were given supplementation with taurine (30-40 mg/kg/day) for 2 months, while taking their usual dosage of enzymatic therapy. One patient dropped out of the study because she developed severe constipation. In the other 10 patients, urinary taurine excretion (88 \pm 30.1 mg/m²s.a./24 h) was similar to that of controls (86.2 \pm 6 mg/m²s.a./24 h) before taurine and increased markedly after supplementation (618.2 \pm 79.97 mg/m²s.a./24 h), indicating efficient intestinal absorption. Their coefficient of fat absorption was 81.2 \pm 2.3% and increased significantly after taurine (91.3 \pm 1.13%; p less than 0.01); the area under the curve of plasma triglyceride postprandial levels (1 \pm 0.1 mg X min/ml) also increased significantly after taurine (1.4 \pm 0.3 mg X min/ml; p less than 0.05), showing values very similar to those of controls. Conversely, no change was observed in the serum postprandial levels of glycocholic acid: the maximum postprandial peak before (1.2 \pm 0.3 μ mol/l) and after taurine (1 \pm 0.1 μ mol/l) remained significantly lower than in controls (2.4 \pm 0.3 μ mol/l); p less than 0.01 and p less than 0.001, respectively. Mean total fecal bile acid (BA) excretion was 10.24 \pm 2.15 mg/kg/day before taurine and 12.8 \pm 4.27 mg/kg/day after taurine (normal pediatric values, 2.91 \pm 1.1 mg/kg/day); however, in the individual patients we found a variable trend, four of them showing a net increase in fecal BA excretion.(ABSTRACT TRUNCATED AT 250 WORDS)

Thompson GN, Tomas FM. Protein metabolism in cystic fibrosis: responses to malnutrition and taurine supplementation. *Am J Clin Nutr.* 1987 Oct;46(4):606-13.
Department of Chemical Pathology, Adelaide Children's Hospital, South Australia.

Increased protein breakdown has been cited as an important cause of nutrient loss in cystic fibrosis (CF). Taurine deficiency, which is common in CF, may contribute to the increased breakdown. The occurrence of and the benefit of taurine supplementation to abnormal protein metabolism in apparently optimally treated CF were assessed using a 12-mo double-blind crossover technique in 14 well-nourished and seven mildly-moderately malnourished infection-free preadolescent CF children. Muscle protein breakdown (urinary 3-methylhistidine technique) was significantly decreased in well-nourished (1.35% degraded/24 h \pm 0.15, p less than 0.05) and malnourished (1.24 \pm 0.11, p less than 0.001) CF children compared with controls (1.50 \pm 0.17, n = 13). Whole-body protein flux, synthesis, and catabolism ([¹⁵N]-glycine technique) were similar in all groups. Net protein gain was greater in CF children, particularly those who were well-nourished (0.55 g/(kg X 10 h) \pm 0.35, p less than 0.01)

compared with controls (0.16 +/- 0.26). Taurine supplementation did not significantly affect any of the indices. In the absence of infection, protein metabolism in CF children responds appropriately to malnutrition.

Belli DC, Levy E, Darling P, Leroy C, Lepage G, Giguere R, Roy CC. Taurine improves the absorption of a fat meal in patients with cystic fibrosis. *Pediatrics*. 1987 Oct;80(4):517-23.
Department of Pediatrics, Hopital Ste-Justine, Montreal, Quebec, Canada.

The effect of taurine supplementation on the absorption of a fat meal was evaluated in patients with cystic fibrosis. In a cross-over design study, five patients with cystic fibrosis (12.1 +/- 2.6 years of age) and three control subjects received either placebo or taurine (30 mg/kg/d) for two 1-week periods, a month apart, followed by a fat meal test. Blood samples were drawn 0, 1, 2, 3, 5, 8 hours after the meal. Four patients with cystic fibrosis and severe steatorrhea despite appropriate enzyme therapy showed a significant (P less than .05) improvement in the absorption of triglycerides, total fatty acids, and linoleic acid while receiving taurine supplements. Three control subjects and one child with cystic fibrosis and mild steatorrhea receiving enzyme therapy did not experience such an effect. The difference in triglyceride absorption, when calculated as the area under the curve, receiving and not receiving taurine was significantly (P less than .05) correlated with the degree of steatorrhea. Furthermore, in contrast to control subjects, the fatty acid composition of chylomicrons in these four study patients showed important discrepancies with that of the fat meal and was corrected, in part, by taurine supplementation. These results suggest that taurine supplementation could be a useful adjunct in the management of patients with cystic fibrosis with ongoing fat malabsorption and essential fatty acid deficiency.

Darling PB, Lepage G, Leroy C, Masson P, Roy CC. Effect of taurine supplements on fat absorption in cystic fibrosis. *Pediatr Res*. 1985 Jun;19(6):578-82.

Patients with cystic fibrosis have an increased proportion of glycine conjugated bile acids with diminished tauroconjugates which could contribute to fat malabsorption. Twenty-two CF children with documented steatorrhea were supplemented with taurine capsules (30 mg/kg/day) and placebo during separate 6-month treatment periods. Alteration of the glycine/taurine conjugation pattern was verified in two patients who showed a predominance of tauroconjugates as a result of taurine supplementation. On taurine, steatorrhea was reduced (p less than 0.05) by 17.6 +/- 9.7% in 19 patients who completed the study as was the excretion of long-chain saturated fatty acids. There was no change in linoleic acid (C 18:2) excretion. In the 10 patients with a more severe degree of steatorrhea the decrease in fat loss approached 20% and a close relationship was found ($r = 0.84$, p less than 0.01) between the extent of the fatty acid loss on placebo and the decrease of this loss on taurine. A linear relationship was found between the percentage decrease of individual fatty acids and their log solubility in water. No change was found in the daily excretion of bile acids, neutral sterols, and nitrogen. Fasting plasma fatty acids, cholesterol, and triglycerides were also unchanged. Monitoring of growth over the two 6-month periods revealed a marginal (p less than 0.1) increase of weight velocity expressed as a percentage expected for age (83.4 +/- 11.3----117.1 +/- 16.5). The increase in height velocity in response to taurine showed a more modest trend (95.3 +/- 7.8----110.7 +/- 10.6).

Cammarata PR, Schafer G, Chen SW, Guo Z, Reeves RE. Osmoregulatory alterations in taurine uptake by cultured human and bovine lens epithelial cells. *Invest Ophthalmol Vis Sci.* 2002 Feb;43(2):425-33. (Animal Study)

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PURPOSE: Comparative assessment of cultured human lens epithelial cells (HLECs) and bovine lens epithelial cells (BLECs) established the nature of the relationship between taurine-concentrating capability and intracellular polyol accumulation or extracellular hypertonicity. **METHODS:** The kinetic characteristics of active taurine accumulation based on the measurement of in vitro [3H]-taurine uptake were resolved by side-to-side review of cultured HLECs and BLECs pre-exposed to either galactose-supplemented medium or extracellular hypertonicity. Competitive RT-PCR was used to appraise variation in taurine transporter (TauT) mRNA abundance from cells maintained in hyperosmotic medium over a 72-hour exposure period. **RESULTS:** The capacity to accumulate [3H]-taurine was significantly lowered after prolonged (20-hour) incubation of cultured BLECs in 40 mM galactose in contrast to HLECs, the latter cells' velocity curve being indistinguishable from control cells in physiological medium. Inhibition of the intracellular taurine transport site appeared to be noncompetitive, in that there was a marked reduction in the $V(\max)$ without significant alteration in the $K(m)$ to a high-affinity transport site. Galactitol content in BLECs exceeded five times that found in HLECs. The coadministration of the aldose reductase inhibitor, sorbinil, with 40 mM galactose completely prevented the inhibitory effect of galactose on [3H]-taurine uptake. Acute exposure (3 hours) of HLECs and BLECs to a range of 10 to 40 mM galactitol or 10 to 40 mM galactose plus sorbinil-supplemented medium suggested by Dixon plot that neither galactitol nor galactose interacted with the extracellular taurine transport site. In contrast, [3H]-taurine accumulation was markedly elevated in both HLECs and BLECs after prolonged exposure to galactose-free medium made hyperosmotic by supplementation with sodium chloride. The enhanced taurine uptake capacity involved increase in peak velocity ($V(\max)$) without significant change in Michaelis-Menten constant ($K(m)$). Cultured HLECs and BLECs responded to hypertonicity with an inducible but transitory upregulation of TauT mRNA. **CONCLUSIONS:** These results demonstrate that lens epithelial cells express a high-affinity TauT protein capable of active uptake, but predisposed to inhibition by intracellular galactitol when the sugar alcohol is present in sufficiently high concentration to interfere with cell metabolism. Furthermore, lens epithelial cells respond to hypertonic stress by raising taurine transport activity. The increase in taurine uptake is due to an increase in the number of high-affinity TauTs expressed as a result of an increase in the manifestation of taurine mRNA stemming from exposure to hypertonic medium.

Obrosova IG, Stevens MJ. Effect of dietary taurine supplementation on GSH and NAD(P)-redox status, lipid peroxidation, and energy metabolism in diabetic precataractous lens. *Invest Ophthalmol Vis Sci.* 1999 Mar;40(3):680-8. (Animal Study)

Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, USA.

PURPOSE: To evaluate changes in glutathione and NAD(P)-redox status, taurine and malondialdehyde (MDA) levels, glucose utilization, and energy metabolism in diabetic precataractous lenses and to assess whether these changes can be prevented with dietary taurine supplementation. **METHODS:** The experimental groups included control and streptozotocin-diabetic rats with a 3-week duration of diabetes

fed unsupplemented or taurine (1% or 5%)-supplemented diets. The levels of glucose, sorbitol, fructose, myo-inositol, oxidized glutathione (GSSG), glycolytic intermediates, malate, alpha-glycerophosphate, and adenine nucleotides were assayed in individual lenses spectrofluorometrically by enzymatic methods, reduced glutathione (GSH) spectrofluorometrically with O-phthalaldehyde, MDA colorimetrically with N-methyl-2-phenylindole, and taurine by high-performance liquid chromatography. Free cytosolic NAD⁺/NADH and NADP⁺/NADPH ratios were calculated from the lactate dehydrogenase and malic enzyme systems. RESULTS: Sorbitol pathway metabolites and MDA were increased, and GSH and taurine levels were reduced in diabetic rats versus controls. The profile of glycolytic intermediates (an increase in glucose 6-phosphate, no change in fructose 6-phosphate and fructose 1,6-diphosphate, an increase in dihydroxyacetone phosphate, a decrease in 3-phosphoglycerate, phosphoenolpyruvate, and pyruvate, and no change in lactate), and a 9.2-fold increase in alpha-glycerophosphate suggest diabetes-induced inhibition of glycolysis. Free cytosolic NAD⁺/NADH ratios, ATP levels, ATP/ADP, and adenylate charge were reduced, whereas free cytosolic NADP⁺/NADPH ratios were elevated. Lens taurine levels in diabetic rats were not affected by supplementation with 1% taurine. With 5% taurine supplementation, they were increased approximately 2.2-fold higher than those in untreated diabetics but remained 3.4-fold lower than in controls. Lens GSH levels were similar in diabetic rats fed unsupplemented and 5% taurine-supplemented diets, whereas GSSG and MDA levels and GSSG/GSH ratios were reduced by 5% taurine supplementation. The decrease in free cytosolic NAD⁺/NADH, ATP/ADP, and adenylate energy charge were ameliorated by 5% taurine supplementation, whereas accumulation of sorbitol pathway intermediates, depletion of myoinositol, inhibition of glycolysis, a decrease in ATP and total adenine nucleotide, and an increase in free cytosolic NADP⁺/NADPH were not prevented. CONCLUSIONS: Dietary taurine supplementation ameliorates MDA levels, GSSG/GSH, and NAD⁺/NADH and fails to prevent the osmotically mediated depletion of GSH and taurine and the decrease in glucose utilization and ATP levels in diabetic precataractous lens. Dietary taurine supplementation cannot be regarded as an alternative to aldose reductase inhibition in eliminating antioxidant and metabolic deficits contributing to diabetes-associated cataractogenesis.

Gupta K, Mathur RL. Distribution of taurine in the crystalline lens of vertebrate species and in cataractogenesis. *Exp Eye Res.* 1983 Oct;37(4):379-84.

Marked heterogeneity was observed in the distribution of taurine in different regions of the lens in various species. In general low taurine pools were observed in the nucleus of all species except frog and human. The distribution of taurine in human senile cataractous lenses at different stages of maturation showed decreased contents in all the regions except capsule epithelium as compared to the normal human lenses. This decrease is progressive upto the 'mature' stage of cataract. In rat lenses with galactose cataracts taurine contents decreased by about 83-94% of the normal values in the equatorial, anterior, posterior cortical and nuclear regions.

Nakagawa K, Kuriyama K. Effect of taurine on alteration in adrenal functions induced by stress. *Jpn J Pharmacol.* 1975 Dec;25(6):737-46.

When rats were exposed to immobilized cold stress, adrenaline content in the adrenal gland as well as noradrenaline content in the brain stem were reduced drastically, while noradrenaline content in the atria was not altered by the application of stress. Oral administrations of taurine (4-7 g/kg/day, for 3 days) prevented the stress-induced decline of adrenaline in the adrenal gland and this preventive effect could

not be duplicated by the administration of L-isoleucine or DL-methionine. In hypophysectomized rats, the stress also induced a significant fall in adrenaline content of the adrenal gland, however taurine administration did not show significant preventive effects on the decline in adrenal catecholamines. The immobilized cold stress induced a significant increase in blood sugar and this increase was antagonized by pretreatment with taurine. Taurine had no significant effects on the stress-induced increase in the activity of adrenal tyrosine hydroxylase and the turnover rate of adrenaline in the adrenal gland measured by the rate of decline of this amine following alpha-methyl-tyrosine administration. The administration of taurine, in both in vivo and in vitro, inhibited the release of adrenaline from adrenal medullary granules, but that of dopamine-beta-hydroxylase was not significantly affected. The stress-induced elevation of the blood level of corticosterone was not affected by taurine administration. These findings indicate that taurine antagonizes the stress-induced elevation of blood sugar by reducing adrenaline output from the adrenal gland. The regulatory mechanism most likely involves the inhibition of adrenaline release from adrenal medullary granules, possibly by stabilizing the membrane of the granules.

Perry TL, Bratty PJ, Hansen S, Kennedy J, Urquhart N, Dolman CL. Hereditary mental depression and Parkinsonism with taurine deficiency. *Arch Neurol.* 1975 Feb;32(2):108-13.

An unusual neuropsychiatric disorder inherited in autosomal dominant fashion occurred in three successive generations of a family. Symptoms commenced late in the fifth decade in six affected patients and led to death in four to six years. The earliest and most prominent symptom was mental depression not responsive to antidepressant drugs or electroconvulsive therapy. This was accompanied by exhaustion, sleep disturbances, and marked weight loss. Later in the disease, symptoms of parkinsonism appeared, and respiratory failure occurred terminally. The most recently affected family member was investigated biochemically late in his illness. Concentrations of taurine were greatly diminished in plasma and cerebrospinal fluid, and at autopsy, all regions of brain examined had a markedly reduced taurine content. Since taurine is a putative inhibitory synaptic transmitter, deficiency of brain taurine may possibly have caused the psychiatric and neurological manifestations of this disorder.

Watt SM, Simmonds WJ. Effects of four taurine-conjugated bile acids on mucosal uptake and lymphatic absorption of cholesterol in the rat. *J Lipid Res.* 1984 May;25(5):448-55.

The importance of the bile acid structure on mucosal uptake and lymphatic absorption of cholesterol was investigated using four different taurine-conjugated bile acids. Pure synthetic conjugates of a trihydroxy bile acid, taurocholate, and three dihydroxy bile acids, tauroursodeoxycholate, taurochenodeoxycholate, and taurodeoxycholate were used to completely solubilize [¹⁴C]cholesterol and polar lipids for steady rate intraduodenal infusion for 8 hr in bile fistula rats. Lymph output and esterification of [¹⁴C]cholesterol and endogenous cholesterol were measured in hourly samples. A second group of bile fistula rats was given the same bile acids as the first group but without added cholesterol or other lipid, i.e., fasting lymph fistula group. Mucosal uptake of [¹⁴C]cholesterol was studied using recovery of [¹⁴C]cholesterol from lumen and mucosa after 1-hr infusions in conscious bile fistula rats. Lymph output of [¹⁴C]cholesterol was promoted more rapidly with taurocholate than with the dihydroxy conjugates and [¹⁴C]cholesterol output differed for the three groups given dihydroxy bile acids. The mass of cholesterol in lymph, measured chemically, varied in parallel with [¹⁴C]cholesterol absorption.

For fasting lymph, infusion of dihydroxy bile acids failed to produce a significant change in endogenous cholesterol output when compared with rats given saline only. Taurocholate infusion markedly increased endogenous cholesterol in lymph of fasted rats. Under all conditions where cholesterol output was stimulated, the increase could be accounted for mainly as esterified cholesterol. Mucosal uptake of [14C]cholesterol during 1-hr infusions in conscious bile fistula rats was slower with the dihydroxy bile acids than with taurocholate. The results indicate the marked effect of the number and configuration of the hydroxyl groups on the solubilizing bile acid for cholesterol absorption.

3. GLUTAMINE REFERENCES

M. Varnier, G. P. Leese, J. Thompson and M. J. Rennie. Stimulatory effect of glutamine on glycogen accumulation in human skeletal muscle. *Am J Physiol Endocrinol Metab.* 1995; 269: 309-315. Department of Anatomy and Physiology, University of Dundee, Scotland, United Kingdom.

To determine whether glutamine can stimulate human muscle glycogen synthesis, we studied in groups of six subjects the effect after exercise of infusion of glutamine, alanine+glycine, or saline. The subjects cycled for 90 min at 70-140% maximal oxygen consumption to deplete muscle glycogen; then primed constant infusions of glutamine (30 mg/kg; 50 mg.kg⁻¹.h⁻¹) or an isonitrogenous, isoenergetic mixture of alanine+glycine or NaCl (0.9%) were administered. Muscle glutamine remained constant during saline infusion, decreased 18% during alanine+glycine infusion (P < 0.001), but rose 16% during glutamine infusion (P < 0.001). By 2 h after exercise, muscle glycogen concentration had increased more in the glutamine-infused group than in the saline or alanine+glycine controls (+2.8 +/- 0.6, +0.8 +/- 0.4, and +0.9 +/- 0.4 mmol/g wet wt, respectively, P < 0.05, glutamine vs. saline or alanine+glycine). Labeling of glycogen by tracer [U-13C]glucose was similar in glutamine and saline groups, suggesting no effect of glutamine on the fractional rate of blood glucose incorporation into glycogen. The results suggest that, after exercise, increased availability of glutamine promotes muscle glycogen accumulation by mechanisms possibly including diversion of glutamine carbon to glycogen.

Melis GC, ter Wengel N, Boelens PG, van Leeuwen PA. Glutamine: recent developments in research on the clinical significance of glutamine. *Curr Opin Clin Nutr Metab Care.* 2004 Jan;7(1):59-70. Department of Surgery, VU University Medical Center, Amsterdam, The Netherlands.

PURPOSE OF REVIEW: The aim of this review is to describe the clinical relevance of supplementation of glutamine from the recent literature. First, new basic research is examined and subsequently recent clinical trials and a metaanalysis are illustrated. **RECENT FINDINGS:** Glutamine has a major impact on the functionality of the immune system. It has recently been established that glutamine not only has a protective effect on cells of the immune system, but also on other cells of the body, for instance cardiomyocytes. Evidence is accumulating for an effect of glutamine via glutathione, heat shock proteins as well as taurine. Another area of interest is the way glutamine enhances gut barrier function. More and more research is concentrating on the positive effect of glutamine on the gut-associated lymphoid tissue. **SUMMARY:** Based on a recent meta-analysis and up-to-date clinical trials, we may conclude that glutamine has a beneficial effect on infectious complications and reduces hospital stay. In critically ill patients glutamine supplementation may reduce morbidity and mortality. The greatest effect was observed in patients receiving high dose parenteral glutamine. A recent study with high dose enteral

glutamine demonstrated a reduced mortality in the glutamine supplemented group. In the future more trials with larger numbers of participants are needed, especially with high dose enteral glutamine in the perioperatively and the intensive care unit setting.

4. HISTIDINE REFERENCES

Cooperman JM, Lopez R. *Exp Biol Med* (Maywood). 2002 Dec;227(11):998-1000. The role of histidine in the anemia of folate deficiency.
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The amino acid histidine is metabolized to glutamic acid in mammalian tissue. Formiminoglutamic acid (FIGLU) is an intermediary in this reaction, and tetrahydrofolic acid is the coenzyme that converts it to glutamic acid. A test for folate deficiency concerns the measurement of urinary FIGLU excretion after a histidine load. It was observed that folate-deficient individuals receiving the histidine for the FIGLU test made hematological response that alleviated the anemia associated with this deficiency. This was unusual in that a biochemical test to determine the deficiency results in a beneficial effect for one aspect of the deficiency. The studies reported in this paper give a metabolic explanation for this phenomenon. Urine was collected for 24 hr from 25 folate-deficient subjects, 10 vitamin B(12)-deficient subjects, and 15 normal controls. Urinary excretion of histidine was a mean of 203 mg with a range of 130-360 mg for the folate-deficient subjects; 51.5 mg with a range of 30-76.6 mg for normal subjects; and 60.0 mg with a range of 32.3-93.0 mg for the vitamin B(12)-deficient subjects. All the folate-deficient subjects subsequently made a hematological response to the histidine administered for the FIGLU test. No hematological response was observed in the vitamin B(12)-deficient individuals. When folic acid was given to folate-deficient subjects who received no histidine, urinary histidine levels returned to normal levels rapidly and this was followed by a hematological response. Others have shown that volunteers fed a histidine-free diet developed anemia. In normal subjects, histidine is excreted much more in the urine than other essential amino acids are. Hemoglobin protein contains 10% histidine. Under normal conditions, dietary histidine can supply sufficient histidine to prevent anemia. When the dietary intake is diminished or the urinary excretion is greatly increased, anemia results. It is concluded that folate deficiency causes histidine depletion through increased urinary excretion of this amino acid. Feeding histidine replenishes tissue levels of histidine, resulting in hemoglobin regeneration. Folic acid administration results in return of histidine to normal urinary levels. Thus, a combination of folic acid histidine would be beneficial for folate deficient individuals.