# APPLIED LONGEVITY, LTD.

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## Mission Statement

**Applied Longevity, Ltd.**™ is dedicated to advances in neuro-endocrine research and cosmeceutical delivery (liposomal systems) by utilizing proprietary, non-invasive testing procedures with special interests in nutritional, immunological and metabolic research.

## Introduction

Applied Longevity, Ltd.™ is the first company to combine evidence based technology (24 hour circadian hormonal screening and urinary metabolic comprehensive profile) and targeted supplementation (transdermal and oral) positively affecting the endocrine system. **Applied Longevity™** has made it possible to scientifically identify the areas of concern and categorize them as three easy steps to hormonal and metabolic balance and overall well-being. We call these steps **BASELINE – BALANCE – RESULT**. The simplicity of our system is what makes it so advanced. The guesswork is gone, now you can implement a scientific approach that helps achieve a healthier, more productive lifestyle.

**Three Easy Steps to Metabolic Balance and Overall Well-Being**

Applied Longevity™ crèmes are the first all natural endocrine support crèmes that combine all the elements necessary to support a rational endocrine program. Designed to deliver a potent, yet responsible dose of several important factors, every Applied Longevity product helps the body attain optimal balanced metabolic levels. This improves delivery payload, avoiding many of the obstacles encountered when trying to supplement orally. Studies have shown a dramatic increase in absorption when comparing transdermal vs. oral administration.

## THE BODY'S HORMONAL BALANCE CAN SIGNIFICANTLY AFFECT:

1. **Energy Production**
   Abnormal adrenal function can alter the ability of cells to produce energy for activities of daily living. People who have a hard time getting up in the morning, those who suffer from energy slumps during the day (and may even be tired all the time), often have abnormal adrenal circadian rhythms (see diagram page II-4).

2. **Muscle and Joint**
   Abnormal adrenal rhythms are known to compromise tissue healing. Reduced tissue repair and increased tissue breakdown can lead to muscle and joint injury and chronic pain.

3. **Bone Health**
   Adrenal rhythm determines how well we build bone. If night and morning cortisol levels are too high, bones do not rebuild well and are more prone to osteoporosis.

4. **Immune System Health**
   Various immune cells (white blood cells) cycle in and out of the spleen and bone marrow for special conditioning and possible nourishment and instruction. This immune system trafficking follows the cortisol cycle. If the cycle is disrupted, especially at night, then the immune system is adversely affected.

5. **Sleep Quality**
   The ability to enter REM sleep cycles, i.e. regenerative sleep, is interrupted by high cortisol values at night and in the morning. Chronic lack of REM sleep can reduce the mental vitality and vigor of a person and induce depression.

6. **Skin Regeneration**
   Human skin regenerates mostly during the night. With higher night cortisol values, less skin regeneration takes place. So, a normal cortisol rhythm is essential for optimal skin health.

7. **Thyroid Function**
   The level of cortisol at the cell level controls thyroid production. Quite often, hypothyroid symptoms such as fatigue and low body temperature are due to adrenal mal-adaptation.

# BASELINE

## ENDOCRINE SALIVARY TESTING

### Why Saliva?

Saliva collection is simple, non-invasive and can be performed in the privacy of ones home. The Saliva Test Kit is sent by mail to the laboratory. The results will be sent to the individual or the referring practitioner within 10 to 14 working days following receipt of the Test Kit. The individual analytes are stable at room temperature for 5 days and require no special handling for shipment.

Saliva testing measures the levels of circulating analytes available to body tissues in men and women. Saliva is considered to be a better indicator of biologically active hormone levels than blood – more accurately reflecting the body's functional hormone status.

Saliva testing provides a simple noninvasive means of determining whether hormone levels are within the expected normal range for one's age and gender. It is also an accurate method of evaluating how hormone replacement therapy, topical hormone creams, sublingual hormone drops, diet, herbal therapy and exercise influence these levels.

### Biomarkers and Biological Age

Biological age is measured by evaluating a number of physiological and biochemical parameters (biomarkers) that are known to change with age. One of the most common and predictable aspects of aging is the age-related shifting of hormone levels, resulting in many of the diseases and disabilities of aging. Measuring these biomarkers, and comparing them with levels of healthy young adults (age 25-30 years), provides a clear indication of the degree of age-related dysfunction of particular organs or organ systems. Restoration of these parameters to a functional balance should result in a reduction of biological age and the likelihood of a healthy extended life span.

## BALANCE

### LIPODERMAL™ CRÈME DELIVERY SYSTEM

The APPLIED LONGEVITY Crèmes are the first all natural endocrine support crèmes that combine all the elements necessary to support a rational endocrine program. Designed to deliver a potent yet responsible dose of several important factors, every APPLIED LONGEVITY product helps the body attain optimal balanced hormonal levels.

APPLIED LONGEVITY employs a very advanced delivery mechanism that uses lipid spheres. These lipid globules are filled with the active components and processed to maintain a protective shell until it reaches the bloodstream. Once there, its outer layers break down and release a chemical cargo into the bloodstream. This improves delivery payload avoiding many of the obstacles encountered when trying to supplement orally. Studies have shown a dramatic increase in absorption when comparing LipoDermal vs. oral administration.

APPLIED LONGEVITY uses two different forms of lipodermes to specifically target tissue. The primary lipoderme is multi layered, containing up to one hundred shells. This form of lipoderme is known as a multilamellar, and is capable of delivering its payload directly to the bloodstream. The secondary base (unilamellar lipoderme – single wall) releases its homeopathic and flower essence ingredients into the local tissue, where it becomes anchored. This is important when using vibrational medicines such as homeopathics. In his twenty eighth publication on the subject of flower essence, Dr. Ditmar Kramer proves that the topical use of vibrational medicine (homeopathics and flower essence) can have a more profound, and longer lasting effect, than when used sublingually. This topical application allows them to influence the surrounding tissues, while also stimulating the natural energy flow via the acupuncture meridians. It is believed that this natural stimulation of the energy flow (Chi) causes a release of neuro-chemicals. By utilizing a pulsatile delivery system (PDS™), the LipoDermal crèmes mimic the endocrine system and release payloads in the same manner.

## RESULTS & MAINTENANCE

Applied Longevity offers Testing to verify that the hormone levels are normal. Once achieved, Applied Longevity maintenance crèmes and metabolic balancing protocols can be used to maintain a younger, healthier body.

# WHY SHOULD ONE BE CONCERNED WITH HORMONE LEVELS?

Adequate levels and an appropriate balance of the steroid hormones (estradiol progesterone, testosterone, and DHEA) are necessary for maintaining optimal health and well being in both females and males. This family of steroid hormones supports a wide range of essential physiological functions including blood lipid balance, bone mineral density, fertility, sexuality, normal thyroid function, a general sense of well-being, and certain aspects of normal brain function.

**Who Should Be Tested for Hormone Levels?**

- Those concerned about decreasing hormone levels.
- Cycling women experiencing PMS symptoms that may be related to a hormonal imbalance.
- Pre and postmenopausal women concerned with their estradiol and progesterone levels for replacement considerations.
- Those wishing to monitor their hormone levels following replacement therapy (oral, sublingual or topical), and subsequently regulate their supplements levels.
- Men concerned with their sexual drive and testosterone levels.
- Those with sleep disorders that wish to know their natural melatonin levels.
- Those with stress related disorders, fatigue, depression, and immune weakness.

**Indicators for Hormonal Imbalances**

- Chronic stress and related health problems.
- Lack of vitality and energy.
- Muscle and joint pain.
- Migraine headaches.
- Osteoporosis.
- Sleep disturbances.
- Poor memory.
- Alcohol intolerance.
- Stress maladaptation.
- Low sex drive.
- Low body temperature.

## TESTING™ RESEARCH DIVISION

**Why Hormone Testing?**

A primary marker of the aging process in both men and women is a reduction in normal hormone levels which is responsible in large part for infertility, decreased energy and muscle strength, loss of libido, and an increase in the symptoms of menopause.

Hormone levels can also be related to the symptoms of PMS, sleep disorders, menopause, andropause, osteoporosis, sex hormone imbalance, and heart health.

Saliva endocrine levels are useful for:

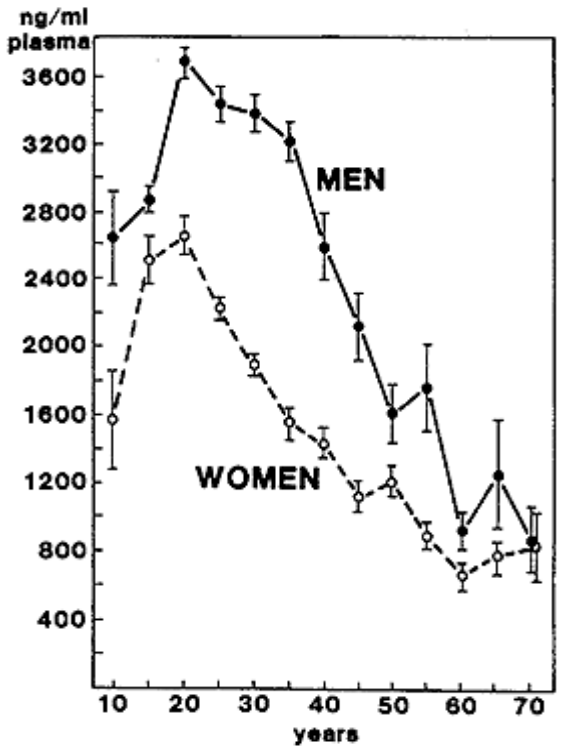
(1) Monitoring the effectiveness of products (skin creams, pills and suppositories) containing hormones or their precursors.
(2) Measuring the effect of phytoestrogens consumed in foods and herbs.
(3) Determining whether the body compensates and maintains its balance while undergoing hormone replacement therapy.

# Adrenal Glands

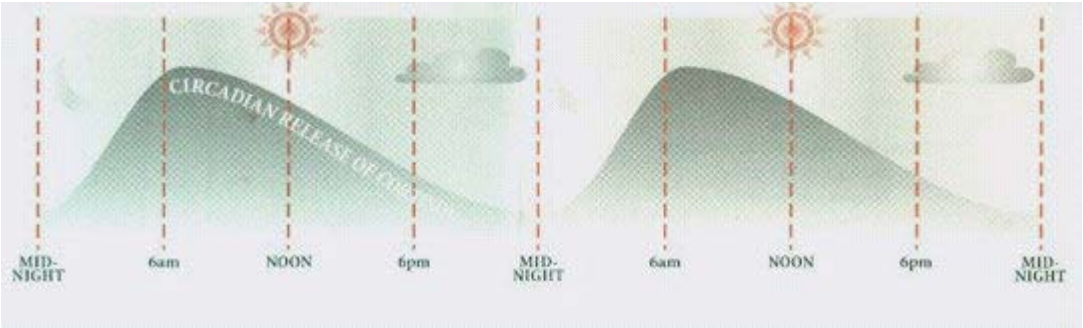
The Adrenals are two small glands; one located above each kidney, each weighing three to five grams. Each adrenal gland is composed of two separate functional entities. The outer zone, or cortex, accounts for 80% to 90% of the gland, and secretes adrenal steroids (cortisol, dehydroepiandrosterone (DHEA), and aldosterone). The inner zone, or medulla, comprises 10% to 20% of the gland, and secrete epinephrine (adrenaline), nor-epinephrine (nor-adrenaline), called catecholamines. Cortisol, DHEA and adrenaline are the three adrenal stress hormones. DHEA declines dramatically with age.

## DHEA Levels Related to Age

DHEA is the 2nd most abundant steroid in the body (Cholesterol is number 1). Decreased levels of DHEA maybe associated with diabetes, Alzheimer's disease, age associated memory impairment (AAMI), and senility.



The human adrenal gland does not secrete its steroid hormones at a constant level throughout the day. The hormones are actually released in a cycle with the highest value in the morning and lowest at night. This is easily understood by looking at the diagram below.




This 24-hour cycle is called the circadian rhythm. An abnormal adrenal rhythm can influence many functions of the body.

# THE THYROID

## Tissues

Since the response to thyroid hormone is expressed at the cell level, it is logical to assume that hormone concentration in tissues should correlate best with its action. Methods for extraction, recovery, and measurement of iodothyronines from tissues have been developed but, for obvious reasons, data from thyroid hormone measurements in human tissues are limited. Preliminary work has shown that under several circumstances, hormonal levels in tissues such as liver, kidney, and muscle usually correlate with those found in serum.[188]

Measurements of T3 in cells most accessible for sampling in humans, namely, red blood cells gave values of 20 - 45 ng/dl (0.31 - 0.69 nmol/L) or one-fourth those found in serum.[189] They are higher in thyrotoxicosis and lower in hypothyroidism.

The concentrations of all iodothyronines have been measured in thyroid gland hydrolysates.[18,133,139] In normal glands, the molar ratios relative to the concentration of T4 are on average as follows: T4/T3 = 10; T4/rT3 = 80; T4/3,5'-T2 = 1,400; T4/3,3'-T2 = 350; T4/3',5'-T2 = 1,100; and T4/3'-T1 = 4,400.

## Effects of Thyroid Hormone on Body Tissues

Thyroid hormone regulates a variety of biochemical reactions in virtually all tissues. Thus, ideally, the adequacy of hormonal supply should be assessed by the tissue responses rather than by parameters of thyroid gland activity or serum hormone concentration, which are several steps removed from the site of thyroid hormone action. Unfortunately, the tissue responses (metabolic indices) are nonspecific because they are altered by a variety of physiologic and pathologic mechanisms unrelated to thyroid hormone deprivation or excess. The following review of biochemical and physiologic changes mediated by thyroid hormone has a dual purpose: (1) to outline some of the changes that may be used for evaluation of the metabolic status, and (2) to point out the changes in various determinations commonly used in the diagnosis of a variety of non thyroidal illnesses, which may be affected by the concomitant presence of thyroid hormone deficiency or excess.

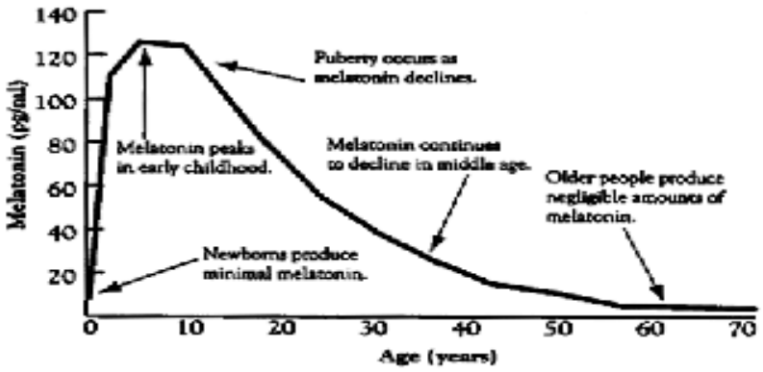
| Table 2. Biochemical and Physiologic Changes Related to Thyroid Hormone Deficiency and Excess (+ = up, - = down, N = normal) | | |
|---|---|---|
| Entity Measured | During Hypothyroidism | During Thyrotoxicosis |
| Metabolism of various substances and drugs Fractional turnover rate (antipyrine,197 dipyrone,198 PTU, and methimazole,197 albumin,199 low-density lipoproteins,200 cortisol,201,202 and Fe203,204 ) | - | + |
| Serum Amino Acids Tyrosine (fasting level and after load)205,206 | - | + |
| Glutamic acid205 | N | + |
| Proteins | | |
| Albumin207 | - | - |
| Sex hormone- binding globulin14,208,209 | - | ++ |
| Ferritin210,211 | - | + |
| Low-density lipoproteins200 | - | + |
| Fibronectin212 | | + |
| Factor VIII-related antigen212 | | + |
| Tissue-plasminogen activator212 | | + |
| TBG83 | + | - |
| TBPA213 | N | - |
| Hormones | | |
| Insulin | | |
| Response to glucose214 | - | - |
| Response to glucagon215 | + | - |
| Estradiol-17ß216 , testosterone14,208,216 and gastrin217 | - or N | + |
| Parathyroid hormone concentration218,219 | + | - |
| Response to PTH administration219 | - | + |
| Calcitonin220 | - | + |
| Calcitonin response to Ca++ infusion221 | - | |
| Renin activity and aldosterone222,223 | - | + |
| Catecholamines224 and noradrenaline225 | + | + |
| Atrial naturetic peptide226,227 | - | + |
| Erythropoietin204 | N or - | + |

# Pineal Gland

## Pineal Hormone Testing

The pineal gland is a flattened, conical organ that lies beneath the posterior border of the corpus callosum and between the superior colliculi. It receives sympathetic nervous "information" which is suppressed when the retina responds to light. In response to this input, the pineal gland secretes melatonin, an amine hormone. The synthesis and secretion of melatonin vary within a 24-hour period, thereby providing the body with a 24 hour circulating clock. As a biological timekeeper, melatonin plays a key role in hormone secretion, is critically involved in the synchronization of hormone secretion and is responsible for regulating many biorhythms, including the sleep-wake cycle. Melatonin drops precipitously with age. Melatonin is also a powerful anti-oxidant, regulator of stress, and immune modulator. Consequently, the low levels of melatonin that occur with aging, result in sleep dysfunction, increased susceptibility to free radical-related diseases, increased stress-related diseases, and reduced levels of immunity.

***Melatonin Levels Related to Age***




(R. Reiter et al.

## Steroid Sex Hormones

**Other hormones** that decrease with age include estrogen and progesterone in females, and testosterone in both males and females. This family of steroid hormones supports a variety of essential physiological functions and an appropriate balance is necessary for optimal health. Steroid hormonal changes or imbalances result in reduction of bone density and muscle mass, decreased energy levels, and reduced libido.

## Estradiol

Estradiol is the most potent estrogen of a group of endogenous estrogen steroids including estrone and estriol. Estradiol is responsible for maturation of long bones, development of breasts, reproductive organs and secondary female characteristics. Estradiol is mainly produced by the ovaries with secondary production by the adrenal glands and conversion of steroid precursors into estrogens in fatty tissue.

Upon reaching menopause, estrogen levels fall. This fall is associated with hot flashes, vaginal dryness, thin skin and an increase of bone loss.

Estradiol levels are used to evaluate amenorrhea, fertility, and monitor replacement therapy.

## Progesterone

Progesterone is a steroid hormone synthesized by the corpus luteum. Progesterone stimulates the cyclic changes in the uterine endometrium that allows the implantation of the fertilized egg. Progesterone also is responsible for maintenance of the uterus during pregnancy, suppression of uterine contractions, and preparation of the breasts for lactation.

Progesterone levels are low during the follicular phase. Levels increase sharply for a maximum of 10 days following ovulation. Levels decline rapidly at about 4 days prior to menstruation.

## Testosterone

Testosterone is a steroid hormone synthesized primarily by the testes in males, the ovaries in females, and adrenal glands of both sexes. Testosterone is synthesized from androstenedione, a metabolite of DHEA and progesterone, the precursors being pregnenolone and cholesterol.

From puberty thru the reproductive years, males synthesize 20 times more testosterone than females. In males, testosterone is utilized to develop the external genitalia and secondary hair patterns, stimulation of spermatogenesis, development of muscle mass, and behavioral patterns. In females, testosterone affects pubescent musclo-skeletal development, general anabolic activity and libido. Testosterone enhances aerobic metabolism and increases protein synthesis in males and females.

Testosterone decreases with age in both males and females.

# Electrolytes

**ELECTROLYTES** are chemical substances that help to maintain the vital electrical charge of the body necessary for all life functions. Electrical charges vital to nerve, muscle, and proper metabolic function are three examples. All the principal electrolytes are asymmetrically distributed across cell membranes. The principal electrolytes of the extracellular fluids are sodium, chloride, and bicarbonate. The major electrolytes of intracellular fluids are potassium, magnesium, calcium, and organic anions, including proteins.

Stability of electrolyte balance is dependent upon several factors. One is proper intake of water. Although the term *dehydration* is often used for the combined loss of water and salts, the usage is misleading. Dehydration should be used to describe relatively pure water depletion, *volume depletion* should be used for combined deficits (loss of fluid and electrolytes). Adrenal hormone imbalances can lead to dehydration and volume depletion, thus adding to electrolyte imbalances. Proper hydration and electrolyte intake and/or supplementation are critical for normal water metabolism. Just drinking water will not balance electrolytes.

# Electrolyte Control

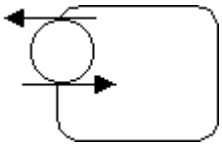
**Control of Sodium Excretion**

Sodium concentration and osmolarity (the total concentration of solutes) are controlled and maintained by thirst, antidiuretic hormone (ADH), the renin-angiotensin-aldosterone axis and the kidneys.

The hypothalmic-pituitary-adrenal axis (HPA) can also affect changes in osmolarity, causing an increase in thirst and in circulating levels of ADH, which, of course, ultimately affects sodium levels.

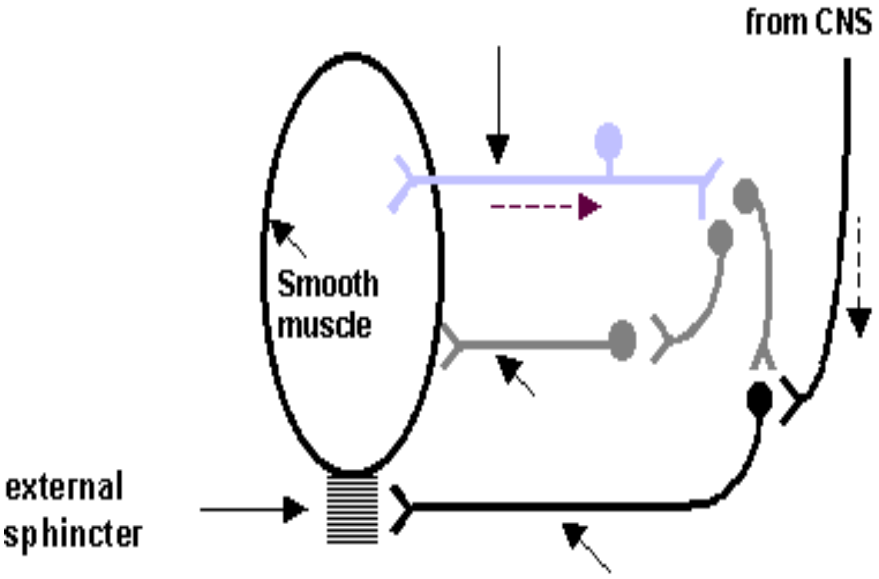
*Aldosterone*, synthesized by the adrenal cortex, is regulated primarily by serum potassium but also is released in response to sodium loss through the *renin-angiotensin-aldosterone axis*. Aldosterone causes re-absorption of sodium in the kidney (at the distal renal tubule). Sodium retention obligates free water retention. Generally, disorders of sodium balance can be traced to a disturbance in thirst or water acquisition, ADH, aldosterone, or renal sodium transport.

**Control of Potassium Excretion**



High $K^+$ stimulates aldosterone secretion

Aldosterone increases $Na^+/K^+$ ATPase pumping, so more $K^+$ exits tubule cells into filtrate




Symptoms: These symptoms, especially when coupled with a recent history of altered fluid balance, suggest the probability of electrolyte imbalance.

| | | |
|---|---|---|
| Anorexia | Agitation | Nausea and vomiting |
| Confusion | Difficulty concentrating | |
| Headache | Lethargy | |

**Causes:** Electrolyte imbalance develops as sodium, potassium and/or chloride and free water are lost and replaced inappropriately. Electrolytes can be lost through renal or non renal routes. Non renal routes include GI losses, excessive sweating, or third spacing of fluids.

External and internal potassium balances are regulated to maintain an extracellular fluid (ECF) concentration of 3.5 to 5.5 mEq/L and a total body content of about 50 mEq/Kg (40 mEq/kg in females). A simplified daily balance sheet would show the following:

| Input | Output |
|---|---|
| Dietary $K^+$ 50-125 mEq | Urine 45-112 mEq |
| | Feces 5-12 mEq |

A more detailed example that accounts for the distribution of potassium within the body is given in the following figure.

Balance usually is disrupted by increase in renal, gastrointestinal, or skin losses, which produce **negative balance**; or decreases in renal excretion, which produces a **positive balance**. **Changes in body potassium content (>95% in cells) are not reflected in plasma potassium concentration**. Therefore, it is necessary to examine factors that regulate internal balance by regulating the distribution of potassium between the intracellular and extracellular fluids.

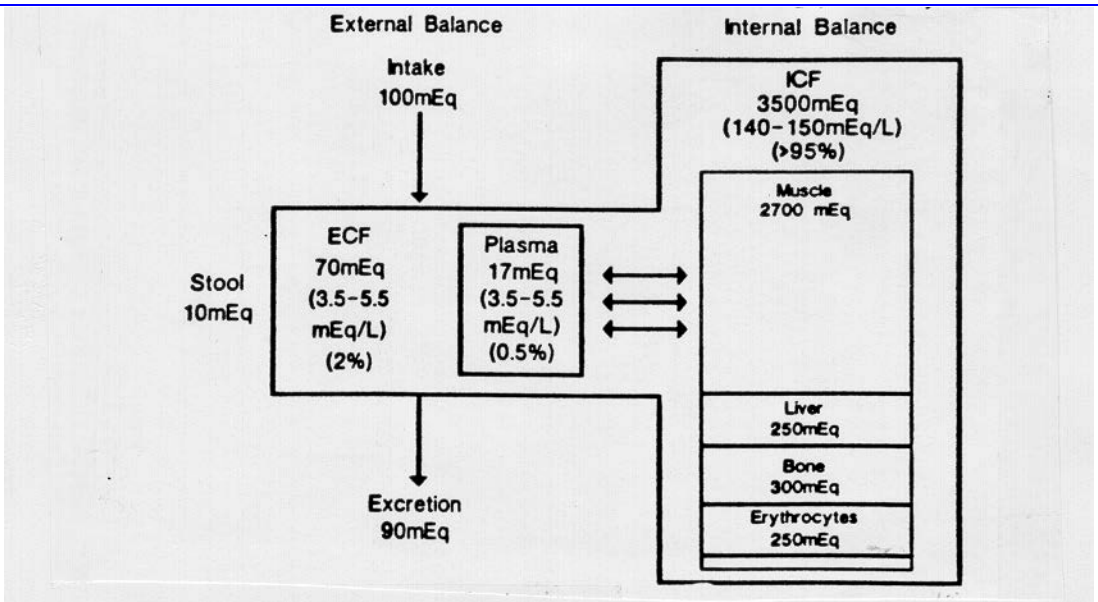



**Figure 1.** Internal potassium homeostasis is a 70 kg person. The potassium concentration in the extracellular fluid (ECF) depends on both the external balance (intake and output) and the internal balance (distribution between extracellular and intracellular fluid, (ICF)). Factors affecting internal balance are discussed below. Note the large IC pool exists at a far greater potassium concentration than the small EC pool. The EC pool will therefore change more dramatically with changes in total body potassium distribution.

**1. Factors Regulating Plasma Potassium (Internal Balance)**

a. Blood pH

Acidosis causes a shift of $K^+$ from the intracellular space of cells into the plasma; whereas, alkalosis causes a shift of $K^+$ from the plasma into cells. These shifts result in a change in internal potassium balance. A guide of the magnitude of this redistribution of potassium is that plasma $K^+$ may rise about 0.6 mEq/L for each decrease in pH of 0.1 units.

This redistribution of potassium is not solely dependent on the acidity since different types of acids result in different magnitudes of potassium shifts. Mineral acidosis (where the anion associated with the acidosis is chloride, sulfate or phosphates) is usually associated with the degree of shift described above. On the other hand, shifts in the distribution of potassium usually do not result from acidosis caused by nonmineral or organic acidosis (where the accompanying anion is lactate, acetate, beta-hydroxybutyrate etc.).

The plasma bicarbonate concentration seems to have an effect on the uptake of potassium by cells that is independent of the pH of the blood. Under conditions of constant blood pH, infusion of sodium bicarbonate leads to a decrease in plasma potassium concentration.

b. Insulin

Insulin is the first-line defense against hyperkalemia. A rise in plasma $K^+$ stimulates insulin release by the pancreatic beta cell. Insulin, in turn, enhances cellular potassium uptake, returning plasma $K^+$ towards normal. The enhanced cellular uptake of $K^+$ that results from increased insulin levels is thought to be largely due to the ability of insulin to stimulate activity of the sodium potassium ATPase located in cell plasma membranes. The insulin induced cellular uptake of potassium is not dependent on the uptake of glucose caused by insulin. Insulin deficiency allows a mild rise in plasma $K^+$ chronically and makes the subject liable to severe hyperkalemia if a potassium load is given. Conversely, potassium deficiency may cause decreased insulin release. Thus plasma potassium and insulin participate in a feedback control mechanism.

c. Catecholamines

Catecholamines are also involved in the regulation of the distribution of potassium. Beta-2-agonists lower plasma potassium (by causing a cellular uptake of potassium) and alpha agonists increase plasma potassium concentration.

d. Physical Conditioning and Exercise

Strenuous exertion may injure muscle cells and allow leakage of $K^+$ into the ECF.

e. Activity of Cell Membrane Na-K ATPase

These ion pumps use ATP to fuel transport of sodium from the cell to the ECF in exchange for the uptake of potassium by the cell, and thus are an important means of regulating the distribution of potassium between intra and extracellular compartments. Some of the other regulators of internal potassium balance such as insulin or catecholamines, may exert their effect by altering the activity of the sodium potassium ATPase in cell membranes.

**2. Factors Regulating Body Potassium Content (External Balance)**

a. Renal Excretion of $K^+$

The Kidney can rapidly excrete large loads of potassium, 200-300 mEq/day, with out a change in plasma $K^+$ or body $K^+$ content. Potassium is filtered freely at the glomerulosa but 90-95% is reabsorbed in the proximal tubule. The major site of renal regulation of potassium excretion occurs in the distal tubules and collecting ducts where variations in the amount of potassium absorbed from or secreted into the urine regulates potassium balance. In contrast to the ability to increase excretion rapidly to meet increased input, the ability to reduce excretion to zero or very low levels is slow, taking perhaps 2 to 4 weeks. Thus, negative external balance due to sudden decrease in $K^+$ intake or increase in gastrointestinal or skin losses will be furthered by a continued leak of $K^+$ into the urine until the condition is chronic. The major determinants of urinary potassium excretion include the following:

1) Aldosterone

Aldosterone stimulates distal nephron secretion of potassium. The stimulation of secretion is related to the ability of aldosterone to stimulate sodium potassium ATPase activity in cells of the distal tubule as well as its ability to alter the urinary membrane conductance of potassium in these cells. In the absence of aldosterone, body potassium content and plasma $K^+$ are increased due to a decrease in renal excretion of potassium. In the presence of excess aldosterone both total body $K^+$ and plasma $K^+$ are decreased. An increased plasma $K^+$ stimulates aldosterone secretion and decreased plasma $K^+$ suppressed it.

2) Urine Flow Rate

Increase urinary flow rate increases urinary potassium excretion, presumable by maintaining chemical gradient for potassium that favor the passive secretion of potassium from the cell into the urinary filtration cells of the distal tubule.

3) Urinary Sodium Concentration

Sodium delivery to distal nephron may promote $K^+$ excretion (Na-K exchange), but it is not certain if this is independent of flow rate since in most instances increased urinary sodium is accompanied by increased urinary flow rate.

4) Non Re-absorbable Anions (Urinary Cl- Concentration)

Sulfates and others create favorable electrical (lumen negative) gradients for passive secretion of potassium into the urine. Additionally, the decreased urinary chloride concentrations present under these circumstances contribute to potassium excretion by inhibiting the re-absorption of potassium by the K-H ATPase present in intercalated cells of the distal tubule.

5) Plasma $K^+$ Concentration

Changes in the peritubular plasma $K^+$ concentration lead to changes in the rate of secretion of potassium by distal tubular cells. Increased plasma $K^+$ leads to an increased rate of secretion presumably due to an increased cellular $K^+$ concentration that creates a more favorable gradient for the passive secretion of $K^+$ into the urine. Decreased plasma $K^+$ has the opposite effect.

6) pH of the Blood

Acute alkalosis leads to an increase in the $K^+$ concentration of the cells of the distal tubule, this leads to a more favorable gradient that is associated with increased urinary secretion of potassium. Acidosis has the opposite effect. With

chronic changes in acid-base status, the relationship between changes in pH of the blood and potassium excretion are more complex and the relationship found in the acute state may be altered.

b. Gastrointestinal Potassium Excretion

Normally 10 – 15% of $K^+$ intake is excreted by the gut. Aldosterone is one of the regulators of secretion of potassium by the gastrointestinal tract. Diarrhea increases fecal $K^+$ loss, particularly laxative-related diarrhea. Diarrhea may contain 100 mEq/L of $K^+$.

c. Skin Potassium Excretion

Normally only a trivial amount of $K^+$ is excreted in perspiration. However, working in hot temperatures may produce up to 10-12 liters of sweat per day containing 10 mEq/L of $K^+$. Thus, major $K^+$ losses may occur via this route. Sweat $K^+$ is also under control of the hormone aldosterone.

Consequences of Potassium Deficiency

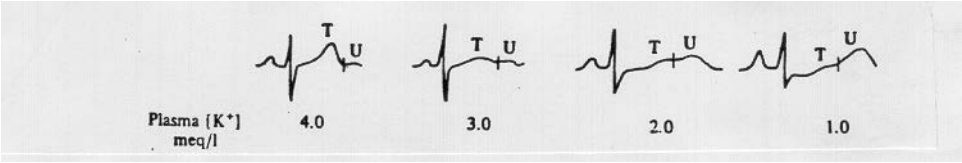
a. Metabolic Effects

Hypokalemia suppresses insulin release leading to glucose intolerance. Potassium deficiency in children retards growth.

Hypokalemia causes intracellular acidosis and increased renal ammonia production.

b. Cardiovascular Effects

Hypokalemia causes electrophysiologic abnormalities that result in changes in the EKG depicted in the follow figure.




3. Preventative strategy for Hypokalemia/Potassium Deficiency

The goal of the preventative strategy is to restore plasma and total body $K^+$ to normal by supplementing with potassium.

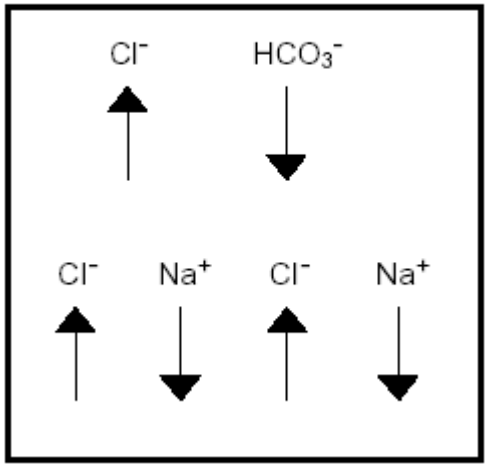
**Control of Chloride**

Chloride has several vital functions:

1) It works with sodium to maintain serum osmolarity and fluid balance. A shift in sodium and chloride concentration will trigger a fluid volume change to restore normal solute and water ratios.
2) It helps maintain acid base balance. Chloride has an inverse relationship with bicarbonate which is part of the major chemical buffering system responsible for maintaining a normal pH
3) It helps maintain the balance of extracellular cations/anions and therefore, electrical neutrality.

It does this through its relationship with both bicarbonate and sodium. In order to understand chloride's role, it is important to understand its interaction with both sodium and bicarbonate. Any alteration
in one of the three may affect the other two. The number of anions (negatively charged ions) and cations
(positively charged ions) in body fluid must always be equal. If excessive amounts of bicarbonate ions (anions) accumulate, the number of chloride ions (anions) will decrease. If sodium ions (cations) increase, the number of chloride ions (anions) will also increase. **(Figure 1)** Chloride and sodium work together, whereas chloride and bicarbonate have an inverse relationship. Chloride losses will usually follow sodium losses and chloride gains will usually follow sodium gains. In the presence of an acid-base imbalance, chloride levels will change independently of sodium.

**Figure 1: Chloride/Bicarbonate/ Sodium Relationships**




Chloride is the most abundant negatively charged ion (anion) in extracellular fluid. In the plasma it combines with sodium as sodium chloride and with potassium as potassium chloride. Chloride is also found with hydrogen as hydrochloric acid in the stomach and with sodium in cerebro-spinal fluid and sweat. Normal serum chloride values in children and adults are 97-109 mEq/L.

Although chloride was the first electrolyte to be easily measured, it has often been considered the least important of the major electrolytes because of its attachment to both sodium and potassium. The importance of chloride was discovered in the early 1980's. At that time, some infant formulas were being made without chloride. One hundred forty one cases of hypochloremic metabolic alkalosis with symptoms of lethargy, vomiting and failure to grow were reported in infants who were given chloride-free formula. The administration of chloride reversed the symptoms.

Chloride intake is through the diet as salt and sodium containing foods such as cereals, fruit and vegetable juices, soups, canned vegetables, and processed meats.

**In order to understand chloride's role, it is important to understand its interaction with both bicarbonate and sodium.**

Because of its inverse relationship with bicarbonate, chloride losses will result in an increase in bicarbonate and chloride gains will result in a decrease in bicarbonate. Chloride concentration is regulated both through the kidneys and the gastrointestinal tract. In the kidneys, chloride re-absorption depends on sodium re-absorption, which is regulated by aldosterone in the renal tubules. Therefore, any alteration in sodium re-absorption will affect the chloride level, as chloride passively follows sodium. The amount of chloride excreted in the urine is related to the amount taken in by diet, infusion, or the amount required by the body.

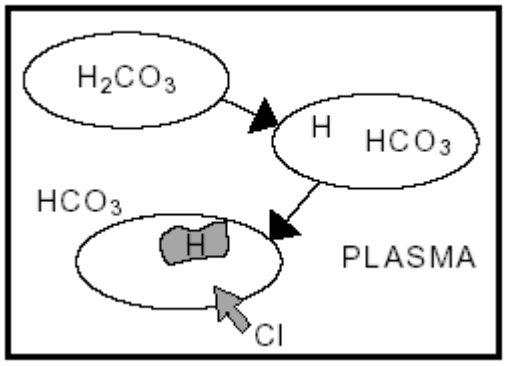
In the gastrointestinal tract, chloride is absorbed both actively and passively. Passive absorption occurs when chloride follows sodium across the bowel wall. Active absorption occurs when bicarbonate moves from the blood into the bowel and is exchanged for chloride ions to maintain electrical neutrality.

Because chloride is produced in the stomach as hydrochloric acid, gastrointestinal disorders can produce alterations in chloride levels.

**Role in Acid-Base Balance:**

One of the most important functions of chloride is its role in maintaining acid-base balance. It does this through an exchange process known as the "Chloride Shift." (**Figure 2**) Chloride and bicarbonate shift into and out of red blood cells to maintain acid-base balance. When carbon dioxide (CO2), which is the primary by-product of oxygenation is dissolved in tissue fluid and Cl- HCO3 -CI- Na+ CI- Na+

**Figure 2: Chloride Shift**




excessive fluid buildup in the body and subsequent dilution of chloride levels. This is usually associated with hyponatremia. Patients with chloridorrhea, a congenital disorder that presents with frequent loose stools resulting in a loss of hydrochloric acid can develop hypochloremia. Certain drugs such as bicarbonate, loop diuretics, thiazide diuretics, or infusions of 5% DW can also lead to hypochloremia.

Physical signs and symptoms of hypochloremia are usually those of metabolic alkalosis. Alkalosis will cause more calcium than normal to bind to albumin resulting in a decrease in serum calcium. Because calcium helps regulate neuromuscular function, signs and symptoms often include muscle excitability, muscle cramps, tremors and tetany. Respirations will be shallow and slow due to the body's efforts to compensate for the alkalosis by conserving carbon dioxide, which combines with water to form carbonic
acid. Increased levels of carbonic acid will lead to a decrease in pH. Hypotension may be present in cases where fluid losses are excessive. Severe hypochloremia can cause seizures, severe respiratory depression, cardiac arrhythmias, and coma.

**CONTROL OF CALCIUM AND PHOSPHATE**

## I. INTRODUCTION

A. Roles of Ca and $PO_4$ in body

*1. Soft tissues*

**Calcium**:

Muscle contraction, stimulus-secretion coupling in nerves and endocrine tissues

Maintain membrane stability

Blood clotting

**Phosphate**:

Part of organic molecules (ATP, DNA, RNA, Phospholipid)

Buffer in blood

*2. Bone*

Primarily in form of Ca- $PO_4$ -$H_2O$ crystals (hydroxyapetite)

Small amount in solution in ICF of bone

Provide tensile strength of bone and

Large reservoir of calcium (99% of total) and phosphate (85% of total)

B. Regulation of Ca and $PO_4$

*1. Hormones involved*

Parathyroid hormone (PTH)

Calcitonin (CT)

Vitamin D

*2. Organ systems involved*

GI tract: regulation of calcium/phosphate entry into body

Kidney: regulation of calcium/phosphate exit from body

Bone: reservoir of calcium/phosphate used to maintain homeostasis

## II. ENDOCRINE REGULATION OF CALCIUM AND PHOSPHATE METABOLISM

A. Hormones involved

**PTH**: from parathyroid glands
**CT**: from C-cells of thyroid
**Vitamin D** - originally thought to be a vitamin, now known to be hormone

B. Calcium homeostasis vs calcium balance

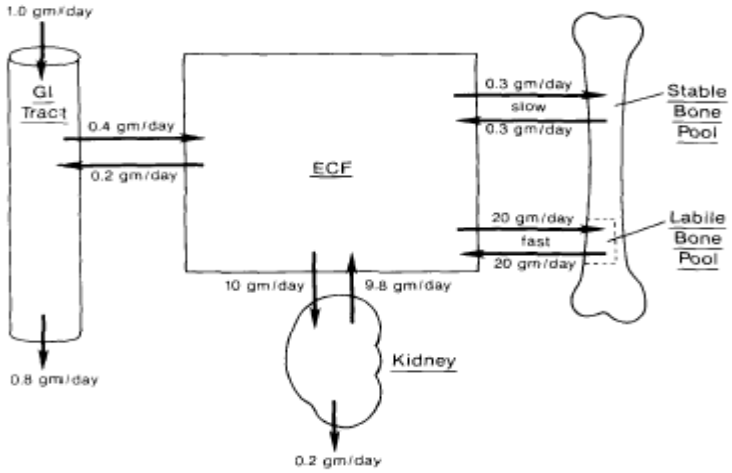
*1. Homeostasis*
Maintains constant blood calcium concentrations
Very rapid - operates on a minute-to-minute basis so any change in calcium is compensated for within 1 hr

*2. Balance*
Ensures that over the long-term calcium intake=calcium excretion
Maintains total body calcium levels; operates over weeks & months

## III. CALCIUM EXCHANGES

A. Extracellular fluid (ECF) calcium

**Plasma calcium**: 50% free and 50% bound (primarily to proteins); free is active
**Free calcium** in plasma is in equilibrium with calcium in interstitial fluid (ICF)- considered single calcium pool = extracellular fluid calcium
**ECF calcium** concentrations is maintained at constant levels




B. Exchanges between ECF and GI tract

**Calcium absorption**: active process that can become saturated; vitamin D essential for adequate calcium absorption in GI tract
**Calcium secretion**: with digestive enzymes; not under hormonal control

C. Exchanges between ECF and kidney

Large amount of calcium filtered, 98-99% reabsorbed. Rate of resorption controlled by PTH. Can go to 100% (0 excretion) if one is calcium deficient

D. Exchanges between ECF and bone: Two exchanges- slow and fast

*1.Slow exchange*
Involves most of calcium in bone reflects bone remodeling
Not important for calcium homeostasis

*2.Fast exchange*
very rapid turn over, involves small pool of calcium (~4 gm)in solution in bone ICF. Calcium movement across osteocyte-osteoblast membranes controlled by hormones. Critical for calcium homeostasis.

## IV. PARATHYROID HORMONE

A. Synthesis and secretion of PTH
Prepro PTH to pro PTH to PTH

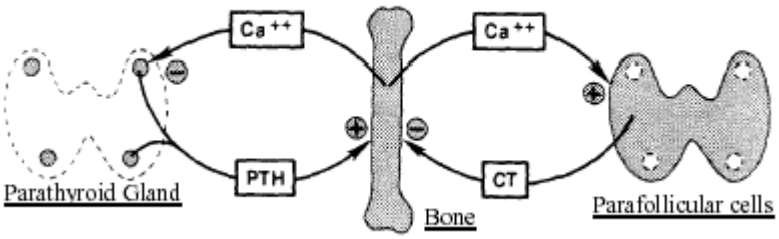
B. Actions on bone and kidney to increase ECF calcium

**Kidney**: increases calcium re-absorption and decreases phosphate re-absorption
Decreases Ca and increases $PO_4$ excretion; rapid effects (~15-30 min)

**Bone**: increases calcium efflux from bone ICF- rapid (~20-30 min), no $PO_4$
Stimulates bone resorption; slow (8-10 hrs), get Ca and $PO_4$
**GI tract**: increase calcium absorption (indirect effect via vitamin D)

C. Metabolism of PTH and circulating forms
First step is cleavage into active and inactive - latter hangs around for long time

D. Control of PTH secretion by calcium
Only important control is inhibition by calcium: classic negative feedback loop




## V. CALCITONIN

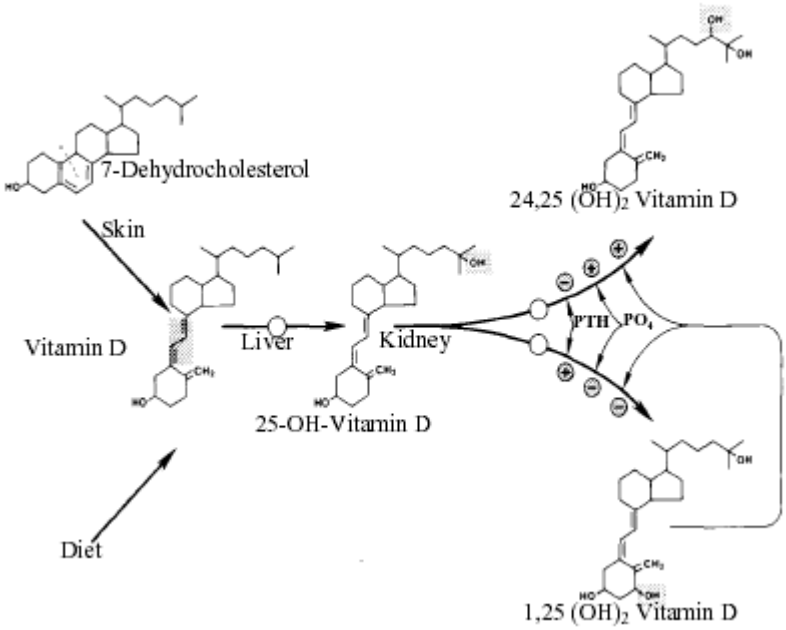
A. Action on bone to decrease ECF calcium
Deceases calcium efflux and bone resorption

B. Control of calcitonin secretion by calcium
Secretion stimulated by ECF calcium: negative feedback loop

C. Physiological role of calcitonin
NONE (e.g., can remove thyroid and have no effect on calcium levels)

## VI. VITAMIN D (CHOLECALCIFEROL)

A. Synthesis and activation of vitamin D




Can come from diet or be formed in skin if exposed to UV light. Must undergo two additional hydroxylations at 25 position (liver) and at 1 position (kidney) to be active

B. Transport in blood: bound to plasma proteins (transcalciferin)

C. Actions on GI tract and bone to increase ECF calcium
*1. GI*: increases calcium and phosphate absorption; acts via nuclear receptors
slow effect (takes 18-24 hrs)

*2. Bone*: increases response to PTH and calcium efflux

D. Control of 1,25 $(OH)_2$ vitamin D synthesis

1. *PTH*: stimulates 1-hydroxylase enzyme

**Note: decrease in ECF calcium will increase PTH so calcium indirectly controls formation of 1,25 $(OH)_2$ vitamin D**

*2. $PO_4$* : Inhibits 1-hydroxylase

Important for regulation of phosphate metabolism

## VII. OVERVIEW OF REGULATION OF CALCIUM AND PHOSPHATE

A. Physiological importance of PTH, vitamin D, and calcitonin

Calcitonin not important; PTH important for both homeostasis and balance

Vitamin D important for balance; actions too slow to play role in homeostasis

B. Control of calcium homeostasis

Decrease in ECF calcium leads to increase in PTH

PTH stimulates calcium re-absorption in kidney and calcium efflux from bone

These actions return ECF calcium back to normal. PTH also increases 1,25 $(OH)_2$ vitamin D, but this is too slow to be important

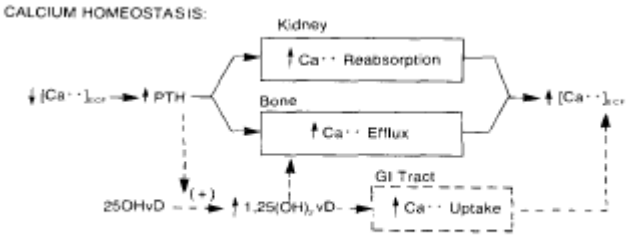
C. Control of calcium balance

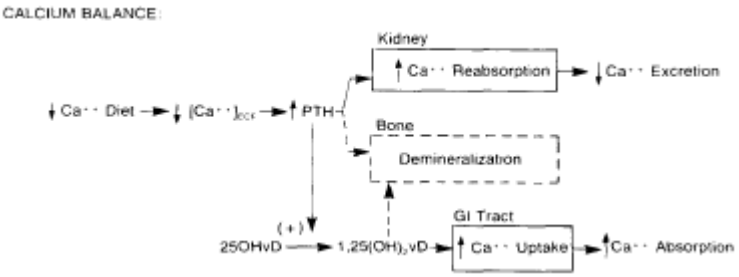
Decrease in calcium in diet leads to fall in ECF calcium, which stimulates PTH

PTH increases calcium re-absorption in kidney, decreasing calcium excretion

PTH increases 1,25 $(OH)_2$ vitamin D, which stimulates calcium absorption in GI

PTH will also stimulate bone resorption, but this does not affect calcium balance because it shifts calcium from one pool to another within the body




D. Control of phosphate metabolism

*1. Effects of PTH*: Inhibits phosphate re-absorption in kidney. Thus, decrease in PTH will increase calcium excretion by kidney.

*2. Effects of 1,25* $(OH)_2$–vitamin D: Stimulates phosphate absorption in GI

*3. Effects of $PO_4$ on synthesis of 1,25 $(OH)_2$–vitamin D*: Inhibits 1-hydroxylase

*4. Effects of $PO_4$ of PTH secretion*: Stimulates PTH secretion

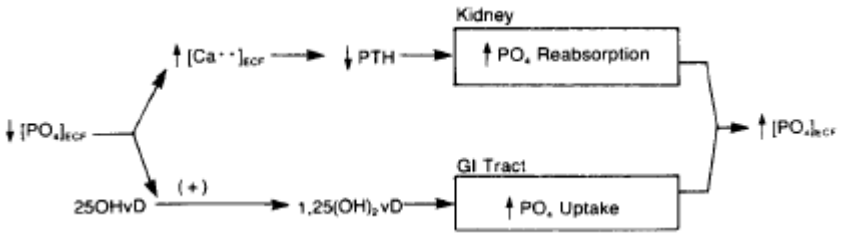
Increase in phosphate decreases Ca by mass action; fall in Ca stimulates PTH

*Overall response of this system to fall in phosphate*:

Increases ECF calcium, which inhibits PTH. Fall in PTH increases phosphate Re-absorption in kidney, decreasing loss of phosphate from body.

Stimulates 1-hydroxylase so 1,25 $(OH)_2$–vitamin D increases, which stimulates

phosphate absorption in GI tract.
Both these responses compensate for the decreases in phosphate.




## VIII. PHARMACOLOGY

A. Hormones

*1. PTH*: not used clinically

*2. Calcitonin*:

a. Can be used to acutely lower plasma calcium, but effectiveness decreases with continued use

b. Used to treat Paget's disease (disordered bone remodeling)

*3. Vitamin D and analogs*: most widely used of three hormones; used to treat;

a. Vitamin D deficiency (rickets in children; osteomalacia in adults)

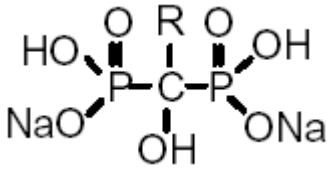
b. Renal failure

c. Hypoparathyroidism

B. Other agents

*1. Bisphosphophonates*: slow formation and dissolution of hydroxyapeptite

a. general structure




b. Incorporated into bone matrix, taken up into osteoclasts and inhibit their activity

c. Used clinically primarily to inhibit bone resorption in Paget's disease and post-menopausal women.

**Synergestic Effects of Combining Magnesium, Calcium, Vitamin D and Creatine**

Several investigators have reported that creatine supplementation enhances ergogenesis during repeated bouts of maximum intensity exercise. Other research suggests, however, that absorption and retention of dietary creatine may be more a function of physical activity than quantity ingested.

Recent research shows that ergogenic improvements are possible when laboratory rat diets were supplemented concurrently with creatine and magnesium. It also showed that *the source* of magnesium may affect ergogenic activity.[1]

Although most metabolic investigations have focused on creatine phosphate or ATP when considering muscle energy, it is important to not overlook the potential significance of magnesium. Stending-Lindberg et al reported that high muscle magnesium significantly ($P < 0.001$) improved endurance during strenuous exercise[2].

## Magnesium and Type 2 Diabetics

Mg++ replacement therapy (100mg/K body weight) improved insulin sensitivity in diabetic patients with poor glycemic control and low magnesium levels. Mg++ replacement resulted in a slight increase in basal insulin levels after 8 weeks; there also was a significant decrease in Hgb-A1c levels ($p<0.01$) in mg++ replacement group. Results show that in diabetics with

[1] jrnlappliedresearch.com/articles/Vol3Iss1/ASHMEAD

[2] Stendig-Lindberg G, Bergstrom J, Hultrman E: Hypomagnesemia and muscle electrolytes and metabolites. Acta Med Scand 201:273–80, 1977.

poor glycemic control, insulin sensitivity might be improved with mg++ replacement. Mg++ replacement, could improve insulin sensitivity, and in turn glycemic control, without increasing insulin secretion[3].

## Magnesium Linked to Aging & Calcification

Normal development apparently depends on the presence of magnesium. Approximately 70 percent of the magnesium in the body is found in the skeletal system. At least half of the magnesium in the body is combined with calcium and phosphorus in the bones. The remainder is in the muscles, red blood cells and the other tissues of the body.

Magnesium ensures the strength and firmness of the bones, and it makes the teeth harder. Adequate intake of magnesium counteracts acidity, poor circulation and glandular disorders. Children with magnesium deficiency are very often mentally backward.

**Influences on Absorption**

The absorption of magnesium from the intestines may be influenced by (1) the parathyroid hormone, (2) the condition of the intestines, (3) the rate of water absorption, and (4) the amounts of calcium, phosphate and lactose (milk sugar) in the body.

Recent studies have shown that magnesium deficiency is found in 25 percent of eating disorders, such as obesity and anorexia nervosa. Symptoms such as weakness, leg cramps, anxiety and confusion will often clear up with magnesium therapy. A magnesium deficiency in humans can occur in patients with diabetes, chronic diarrhea or vomiting.

Heart palpitations, "flutters" or racing heart, otherwise called arrhythmias, usually clear up quite dramatically on 500 to 1000 milligrams of magnesium gluconate (or aspartate) once daily.

Without sufficient magnesium, one cannot control the adrenals, and this lack of control can result in diabetes, hyper excitability, nervousness, mental confusion and difficulty coping with simple day-to-day problems. Depressed and suicidal people often display inadequate levels of magnesium.

**Human Cell's Power Plant**

The power plant of human cell is called the mitochondrion. The mitochondrion is what generates energy for the cell to use. What everyone refers to as "energy" is derived from the oxidative reduction of the cellular respiration. This is done through the mitochondria.
But the problem arises when the cell is low in magnesium, relative to calcium. Adenosine triphosphate, the energy source of the cell, is magnesium dependent. This means it is obvious that the calcium pump at the cell membrane is also magnesium dependent.
Without enough biologically available magnesium, the cellular calcium pump slows down and a vicious cycle is established. The low levels of available magnesium inhibit the generation of energy, and the low levels of energy inhibit the calcium pump.

The mitochondrion, the powerhouse of the cell and the entire body, becomes calcified. This is the beginning of aging. It all starts in the cell. First the cells age, leading to organ aging. Once the organs age, individual aging occurs. Since calcium is readily accumulated by mitochondria, this ion is potentially capable of antagonizing the activating influence of magnesium on many intra mitochondrial enzyme reactions.

This means that every function of your body can be inhibited when the mitochondria calcify. It's like going through life with the emergency brakes on. Calcium is the brake. Magnesium is the accelerator. To be in optimal health, there must be a balance between the two.

**Chronological age and biological age**

The ratio of calcium to magnesium within your cells affects your “biochemical age.” Although both minerals are vitally important, Applied Longevity has found that cellular efficiency and “biochemical age” involves the body's ability to **utilize the supplements** you so meticulously take.

**All of this fits so well with Applied Longevity’s basic belief, which rests upon the word *balance*.**

[3] *Archives of Internal Medicine*, 1999

**Statlyte™,** produced by Applied Longevity, is a balanced transdermal crème delivering all the necessary ingredients necessary for effective electrolyte supplementation and balancing.

**IsoBlast**™ , produced by Applied Longevity is the first oral liposomal concentrate to address mitochondrial inefficiency. Vital minerals, amino acids, co-factors, vitamins, electrolytes, are efficiently delivered for intracellular utilization.

# Salivary Electrolyte Levels

# Magnesium

*Magnesium, the second most abundant intracellular cation, has several critically important roles in the body. In addition to energy production and maintaining electrolyte balance, magnesium is essential for normal neuromuscular function. Intracellular magnesium affects all normal tissue and organ functions by restricting the loss of mg-ATP (the essential substrate for many cellular reactions) and has been shown to be a physiological ca++ blocker. While low serum levels of magnesium may be indicative of tissue deficiency, low tissue levels may exist although serum levels may be normal. Bone contains 50% to 60% of the body's magnesium while tissue contains about 98% of the body load. Serum contains 1% of the total. Evidence suggests that a deficit of magnesium is closely interrelated to potassium depletion and refractory potassium repletion. The primary organs involved in magnesium metabolism are the intestinal tract and the kidneys.*

## I. Decreased Levels (Magnesium)

***A. Causative factors.***
**- Low Levels –**Low salivary magnesium levels may be caused by physical or emotional stress, low adrenal function, endocrine dysregulation, dietary deficiency, as well as excessive use of diuretics, alcoholism, diabetes mellitus or heavy metal poisoning.

***B. Associated conditions***
Low salivary magnesium levels have been associated with osteoporosis, hypertension, muscle spasms, heart disease and periodontal disease.

***C. Therapeutic Nutritional Considerations:***
*Magnesium supplementation in general*: use highly bioavailable forms such as fine mesh (less than 5 micron) above the ground ***Coral* CalMag Plus™**, or a chelated magnesium (magnesium glycinate, magnesium lysinate, magnesium aspartate). These forms of magnesium are especially well tolerated by patients who experience the laxative effect of magnesium supplementation.

*Muscle Spasm*: use magnesium in combination with calcium.

*Cardiovascular Disease:* use magnesium in combination with potassium. Also consider using taurine, 1-carnitine, chondroitin sulfate, coenzyme Q-10, inositol hexaniacinate (no "flush" niacin source), vitamins B-6, B-12, C, folic acid, and the botanicals Crataegus oxycantha (hawthorn berry), Ginkgo biloba, Zingiber officinale (ginger) and Allium sativum (garlic).

*Hypertension*: use magnesium in combination with potassium.

*Premenstrual Syndrome*: use magnesium alone or in combination with calcium. Also consider using vitamins B-6, B-1, B-2, B-5, B-12, C, E, folic acid, beta-carotene, and **BioFèmme IC™** - natural progesterone and adrenal support transdermal crème [extract from the botanicals Dioscorea villosa (wild yam)], Vitex Agnus Castus (chaste tree), Angelica Sinensis Dangsinensis (dang kwai), Cinnamomum zeylanicum (cinnamon), and Taraxacum Officinale (dandelion).

*Hypoadrenal*: use **Endosis IC™** transdermal crème, magnesium in combination with zinc, pantothenic acid (vitamin B-5), vitamin C, beta-carotene and the botanicals Glycyrrhiza glabra (Licorice), and Eleutherococus senticosus (Siberian Ginseng).

*Diabetes Mellitus*: use magnesium, glucose tolerance factor (GTF), and chromium. Consider vitamins B-1, B-2, B-3, B-6, folic acid, and B-12. Also consider the botanicals Syzgium jambolanum (jambul), Taraxacum Officinale (dandelion root), and Glycyrrhiza glabra (licorice).

*Maldigestion/malabsorption*: consider the possibility of hypo- or achlorhydria, parasites, intestinal permeability defects, dysbiosis, celiac disease, and food allergies. Treat the underlying condition and supplement with magnesium if indicated. Use digestive enzymes (**Digest*ALL*™**) to aid digestion and absorption. Consider L-glutamine, N-acetyl-D-glucosamine, gamma oryzanol, and gamma linolenic acid for a preventative strategy of intestinal permeability defects. Use ***Ultra D*™** for intestinal cleansing/detoxing.

*Comments: increase dietary intake, reduce stress, and treat the underlying condition. Reduce meats and carbonated beverage. Physical exercise enhances magnesium transport into the cell.*

## II. Increased Levels (Magnesium)

***A. Causative factors***.
**- High Levels -** High salivary magnesium levels may be caused by hyperparathyroidism, dietary intake or by depressed excretion.

***B. Associated conditions***.
High intracellular magnesium can affect kidney function and has been associated with renal failure, hypotension, reduced mental activity, muscle weakness, poor reflexes, or lethargy.
***C. Therapeutic Nutritional Considerations:***
*Reduce magnesium intake if excessive (e.g. milk of magnesia, antacids, mineral supplementation).*
*Kidney disease:* treat the underlying condition. Consider supplementation with antioxidants including quercetin, N-acetyl-L-cysteine, vitamins C and E, beta-carotene, zinc, copper, selenium, and Ginkgo biloba. Also consider proteolytic enzymes (such as those derived from Aspergillus oryzae) for functional kidney disorders.
*Comments: in case of significant renal disease, avoid magnesium-containing medications.*

## III. The DV for magnesium is 400 mg per day.

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# Calcium

Ca++ levels are regulated by endocrine events. Ca++ is involved in most secretory function of tissues at the cellular level. Neurotransmission and neuro-muscular transmission require regulated ca++ movements. Structure of the supportive tissues, bone, cartilage, and membrane stability require normal calcium metabolism. Early ca++ loss increases intracellular levels. With continued loss, the level eventually drops.

## I. Decreased Levels (Calcium)

***A. Causative factors.***
**- Low levels -** low ca++ levels may be caused by low adrenal function, malabsorption, excessive sweating, or magnesium deficiency.

***B. Associated conditions.***
Low salivary ca++ may be associated with hypoparathyroidism, hypertension, heart disease, osteoporosis, and muscle cramps.
***C. Therapeutic Nutritional Considerations***:

*Calcium supplementation in general:* use highly bioavailable forms such as fine mesh (less than 5 micron) above the ground ***Coral* CalMag Plus™**, or a chelated calcium (e.g. calcium malate, calcium aspartate, calcium lysinate).
*Osteoporosis:* combine calcium with magnesium (a 1:1 ratio of magnesium to calcium) ***Coral* CalMag Plus™**. Consider supplementing with the trace minerals boron, manganese, zinc, copper, and silicia. Increase weight-bearing exercises, exposure to sunlight, reduce meats and carbonated beverages. Consider extra vitamin D supplementation in deficient. Phytase enzyme enhances mineral availability.
*Muscle spasm:* use calcium in combination with magnesium (***Coral* CalMag Plus™)**.
*Cardiovascular disease:* use calcium in combination with magnesium and potassium. Also consider using taurine, L-carnitine, chondroitin sulfate, coenzyme Q-10, inositol hexaniacinate (no "flush" niacin source), vitamins B-6, B-12, C, folic acid, and the botanicals Crataegus oxycantha (hawthorn berry), Ginkgo biloba, Zingiber Officinale (ginger) and Allium sativum (garlic).
*Hypertension*: use magnesium in combination with potassium.
*Kidney disease:* treat the underlying condition. Consider supplementation with antioxidants including quercetin, N-acetyl-L-cysteine, vitamins C and E, beta-carotene, zinc, copper, selenium, and Ginkgo biloba. Also consider proteolytic enzymes (such as those derived from Aspergillus oryzae) for functional kidney disorders.
*Agitation/Insomnia:* use calcium in combination with magnesium. Consider the use of the following botanicals: Valeriana Officinalis (valerian root), Scutellaria Lateriflora (skullcap), Passiflora Incarnate (passionflower), Humulus Lupulus (hops), Piper Methysticum (kava-kava) and Lactuca Virosa (wild lettuce).
*Maldigestion/malabsorption:* consider the possibility of hypo- or achlorhydria, parasites, intestinal permeability defects, dysbiosis, celiac disease, and food allergies. Treat the underlying condition and supplement with calcium if indicated. Use digestive enzymes (**Digest*ALL*™**) to aid digestion and absorption, and gamma linolenic acid for the preventative strategy of intestinal permeability defects.
*Comment: Increase weight-bearing exercises, exposure to sunlight, reduce meats and carbonated beverages. Consider extra vitamin D supplementation if deficient.*

## II. Increased Levels (Calcium)

Cellular metabolism and efficiency is affected by intracellular ca++ and mg++ levels. Increased intracellular calcium interferes with bioenergetic functions acting as a metabolic poison for ATP production. Muscle contraction and relaxation, enzymatic actions and neurotransmitter functions are suppressed by excess intracellular calcium. Aging to tissue and cell death could occur when intracellular ca++ and mg++ is suppressed.

***A. Causative factors.***
**- High Levels -** high salivary ca++ levels may be caused by endocrine dysregulation, magnesium deficiency, physical inactivity, vitamin D deficiency, and calcium channel blocking medications or high intake of dietary phosphates.
***B. Associated conditions.***
High salivary calcium levels may be a sign of the mobilization of bone calcium into soft tissues signaling the early onset of osteoporosis, and can also be associated with hyperparathyroidism, hypertension, heart disease, or muscle spasms. Muscle contraction and relaxation, enzyme actions, neurotransmitter functions are suppressed by excess intracellular calcium.
***C. Therapeutic Nutritional Considerations:***
*Magnesium supplementation:* by modulating the movement of calcium into cells, magnesium acts as the body's natural calcium channel blocker. Consider supplementing with magnesium if salivary levels of calcium are high and magnesium is low. Use highly bioavailable forms such as chelated magnesium

(magnesium glycinate, lysinate, magnesium aspartate). These forms of magnesium are especially well tolerated by patients who experience the laxative effect of magnesium supplementation.
*Osteoporosis:* use calcium with magnesium (a 1:1 ratio of magnesium to calcium), use highly bioavailable forms such as fine mesh (less than 5 micron) above the ground ***Coral* CalMag Plus™**. Consider supplementing with the trace minerals boron, magnesium, zinc, copper, and silica. Increase weight-bearing exercises, exposure to the sunlight, reduce meats and carbonated beverages. Consider extra vitamin D supplementation if deficient. Phytase enzyme enhances mineral availability.
*Cardiovascular disease/Atherosclerosis:* use magnesium in combination with potassium. Also consider using taurine, L-carnitine, chondroitin sulfate, coenzyme Q-10, inositol hexaniacinate, vitamins B-6, B-12, C, folic acid and the botanicals Crataegus oxycantha (hawthorn berry), Ginkgo biloba, Zingiber Officinale (ginger) and Allium sativum (garlic).
*Hypertension:* use magnesium in combination with potassium.
*Muscle spasm:* use magnesium (glycinate).
*Hypoadrenal:* use magnesium in combination with zinc, pantothenic acid (vitamin B-5), vitamin C, beta carotene and the botanicals Glycyrrhiza glabra (licorice), and Eleutherococcus senticosus (Siberian ginseng). Consider using **Endosis IC™** for additional adrenal support.
*Comment: increase weight-bearing exercises, reduce meat and carbonated beverages.*

**III. The DV for calcium is 1000 mg per day.**

# Potassium

## I. Decreased levels (Potassium)

***A. Causative factors.***
**- Low Levels -** Low salivary potassium levels may be caused by low adrenal function, hormonal dysregulation, excessive sweating, magnesium deficiency, salt retention, diuretic or steroid medications, renal disease, chronic stress, malabsorption, or diarrhea.
***B. Associated conditions.***
Low salivary potassium levels are due to excessive potassium loss in stool or urine, not dietary deficiency, and are associated with fatigue, heart disease, muscle weakness, hypercholesterolemia, glucose intolerance, and high intracellular calcium levels. Potassium is a major intracellular cation acting with sodium and calcium causing profound effects on membrane potentials. In some patients even a moderate fall may have serious consequences on neuromuscular and cardiac functions. The extent of potassium loss in tissue cannot be accurately assessed by serum measurements.
***C. Therapeutic Nutritional Considerations***:
*Potassium supplementation in general*: most cases of low intracellular potassium are due to excessive loss of potassium in the urine or stool, not dietary deficiency. Find and treat the cause of the potassium loss. Consider supplementation with potassium if salivary levels are very low (**Stat*Lyte*™ -** a proprietary transdermal electrolyte crème).
*Magnesium supplementation:* if magnesium levels are low, use highly bioavailable forms such as fine mesh (less than 5 micron) above the ground ***Coral* CalMag Plus™**, chelated magnesium (magnesium glycinate, magnesium lysinate, magnesium aspartate). These forms of magnesium are especially well tolerated by patients who experience the laxative effect of magnesium supplementation.
*Hypoadrenal:* use magnesium in combination with zinc, pantothenic acid (vitamin B-5), vitamin C, beta carotene and the botanicals Glycyrrhiza glabra (licorice), and Eleutherococcus senticosus (Siberian ginseng). Consider using **Endosis IC™** for additional adrenal support.

*Cardiovascular disease:* use potassium in combination with magnesium. Also consider using taurine, L-carnitine, chondroitin sulfate, coenzyme Q-10, inositol hexaniacinate (no "flush" niacin source), vitamins B-6, B-12, C, folic acid and the bioanicals Crataegus oxycantha (hawthorn berry), Ginkgo biloba, Zingiber Officinale (ginger) and Allium sativum (garlic).
*Hypertension:* use potassium in combination with magnesium.
*Kidney disease:* treat the underlying condition. Consider supplementation with antioxidants including quercetin, N-acetyl-L-cysteine, vitamins C and E, beta-carotene, zinc, copper, selenium, and Ginkgo biloba. Also consider proteolytic enzymes (such as those derived from Aspergillus oryzae) for function kidney disorders.
*Diarrhea:* find and treat the cause. Consider supplementing with beneficial Pro- Biotics (PharmX), and dietary fiber (e.g. psyllium hull powder) with bentonite. Use **Ultra D™** for intestinal cleansing/detoxing. Consider the botanicals Hydrastis canadensis (goldenseal), Geranium maculatum (cranesbill), Zingiber Officinale (gingerroot), Echinacea spp. Also consider digestive enzymes (**Digest*ALL*™**). Replace electrolytes in case of excessive fluid loss (**Stat*Lyte*™**), especially if chloride and potassium are low.
*Comment: supplement with potassium. Increase dietary intake. Reduce sodium if excessive.*

## II. Increased levels (Potassium)

***A. Causative factors.***
**- High Levels -** High salivary potassium levels may be elevated despite normal serum levels. High salivary potassium levels may be caused by high dietary intake, excessive use of insulin by diabetics, physical inactivity, dehydration, medications or renal failure.
***B. Associated conditions.***
High salivary potassium levels affect cellular electrolyte balance. Cellular potassium is up to 40 times higher than serum levels. Elevated levels may be associated with EKG abnormalities, cognitive impairment, speech and memory disorders. Maintenance of cardiac, skeletal, and smooth muscle tissue depends on adequate potassium gradients between intra and extracellular spaces. All neuromuscular activity is dependent on both sodium and potassium for maintaining the electro-potential in both nerves and muscle.
***C. Therapeutic Nutritional Considerations:***
*Kidney disease:* treat the underlying condition. Consider supplementation with antioxidants including quercetin, N-acetyl-L-cysteine, vitamins C and E, beta-carotene, zinc, copper, selenium, and Ginkgo biloba. Also consider proteolytic enzymes (such as those derived from Aspergillus oryzae) for functional kidney disorders.
*Hypoadrenal:* use magnesium in combination with zinc, pantothenic acid (vitamin B-5), vitamin C, beta carotene and the botanicals Glycyrrhiza glabra (licorice), and Eleutherococus senticosus (Siberian ginseng). Consider using **Endosis IC™** for additional adrenal support.
*Comment:* increase fluids if dehydrated (usually will see high sodium and chloride). Reduce potassium intake if excessive. Increase physical activity, especially if phosphorus and calcium are high. Reduce magnesium intake (if high magnesium), as excess magnesium can cause potassium to accumulate in the tissue.

## III. A DV of 2000 mg per day is considered adequate.

# Sodium

## I. Decreased levels (Sodium)

***A. Causative factors.***

**- Low levels -** Excessive water intake, vomiting, diarrhea, excessive sweating, medications, colitis, edema, or renal failure may cause low salivary sodium levels.

***B. Associated conditions***.

Sodium is the principal cation of serum. Changes in levels affect other electrolytes. Renal sodium excretion can be affected by cardiac output. Low salivary sodium levels may be associated with dizziness, low blood pressure, abdominal cramps, muscle weakness, or cognitive impairment.

***C. Therapeutic Nutritional Considerations:***

*Diarrhea*: find and treat the cause. Consider supplementing with beneficial Pro- Biotics (PharmX), and dietary fiber (e.g. psyllium hull powder) with bentonite. Use ***Ultra D™*** for intestinal cleansing/detoxing. Consider the botanicals Hydrastis canadensis (goldenseal), Geranium maculatum (cranesbill), Zingiber Officinale (gingerroot), Echinacea spp. Also consider digestive enzymes (**Digest*ALL*™**). Replace electrolytes in case of excessive fluid loss, especially if chloride and potassium are low.

*Maldigestion/malabsorption:* consider the possibility of hypo- or achlorhydria, parasites, intestinal permeability defects, dysbiosis, celiac disease and food allergies. Treat the underlying condition. Use digestive enzymes to aid digestion and absorption (such as plant enzymes derived from Aspergillus Oryzae). Consider L-glutamine, N-acetyl-D-glucosamine, gamma oryzanol, and gamma linolenic acid for a preventative strategy of intestinal permeability defects.

*Edema*: consider proteolytic enzymes (such as those derived from Aspergillus oryzae), and Taraxacum Officinale (dandelion root). Rule out cardiovascular and kidney disease.

*Liver dysfunction:* to support and protect the liver consider supplementing with choline, L-methionine, L-glutamine, L-glutamic acid, L-glutathione, N-acetyl-L-cysteine, and the botanicals Silybum Marianum (milk thistle seed), Taraxacum Officinale (dandelion root), Chelidonium majus (celadine), Chionanthus virginicus (fringe tree bark), Beta vulgaris (beet leaf), Raphanus niger (russian black radish).

*Hypoadrenal:* use magnesium in combination with zinc, pantothenic acid (vitamin B-5), vitamin C, beta carotene and the botanicals Glycyrrhiza glabra (licorice), and Eleutherococus senticosus (Siberian ginseng). Consider using **Endosis IC™** for additional adrenal support.

*Kidney disease*: treat the underlying condition. Consider supplementation with antioxidants including quercetin, N-acetyl-L-cysteine, vitamins C and E, beta-carotene, zinc, copper, selenium, and Ginkgo biloba. Also consider proteolytic enzymes (such as those derived from Aspergillus oryzae) for functional kidney disorders).

*Cardiovascular disease:* use potassium in combination with magnesium. Also consider using taurine, L-carnitine, chondroitin sulfate, coenzyme Q-10, inositol hexaniacinate (no "flush" niacin source), vitamins B-6, B-12, C, folic acid, and the botanicals Crataegus oxycantha (hawthorn berry), Ginkgo biloba, Zingiber Officinale (ginger) and Allium sativum (garlic).

## II. Increased levels (Sodium)

***A. Causative factors.***

**- High Levels -** High dietary intake, potassium deficiency, dehydration, Cushing's syndrome may cause high salivary sodium levels.

***B. Associated conditions.***

High salivary levels of sodium may be associated with hypertension, heart disease, diarrhea, edema, hyperactivity and irritability.

***C. Therapeutic Nutritional Considerations:***

*Potassium supplementation:* low potassium leads to sodium retention, supplement with potassium if it is found to be low.

*Magnesium supplementation:* high salivary sodium has been associated with magnesium deficiency. Supplement with magnesium if it is found to be low. Use highly bioavailable forms such as fine mesh (less than 5 micron) above the ground ***Coral*** **CalMag Plus™**, chelated magnesium (magnesium glycinate,

magnesium lysinate, magnesium aspartate). These forms of magnesium are especially well tolerated by patients who experience the laxative effect of magnesium supplementation.
*Diarrhea:* find and treat the cause. Consider supplementing with beneficial Pro- Biotics (PharmX), and dietary fiber (e.g. psyllium hull powder) with bentonite. Use ***Ultra D™*** for intestinal cleansing/detoxing. Consider the botanicals Hydrastis canadensis (goldenseal), Geranium maculatum (cranesbill), Zingiber Officinale (gingerroot), Echinacea spp. Also consider digestive enzymes (**Digest*All*™**). Replace electrolytes in case of excessive fluid loss, especially if chloride and potassium are low.
*Cardiovascular disease:* use potassium in combination with magnesium if salivary magnesium and potassium levels are low. Also consider taurine, L-carnitine, chondroitin sulfate, coenzyme Q-10, inositol hexaniacinate (no "flush" niacin source), vitamins B-6, B-12, C, folic acid and the botanicals Crataegus oxycantha (hawthorn berry), Ginkgo biloba, Zingiber Officinale (ginger) and Allium sativum (garlic).
*Comment: reduce sodium intake, increase fluids if dehydrated, and treat underlying condition.*

**III. A DV of 500 mg, a level substantially exceeded by usual diets in the U.S., even in the absence of added sodium chloride, is considered adequate.**

# Chloride

## I. Decreased levels (Chloride)

***A. Causative factors.***

**- Low Levels -** Low salivary chloride levels may be caused by excessive water intake, excessive sweating without replacement, diarrhea, and vomiting or chronic respiratory illness.

***B. Associated conditions.***

Low intracellular chloride levels may be associated with muscle cramps, listlessness, heart disease, renal failure or metabolic alkalosis. All of the ions that are depleted should be repleted - magnesium, potassium and chloride to prevent or correct metabolic alkalosis.

***C. Therapeutic Nutritional Considerations:***

*Diarrhea:* find and treat the cause. Consider supplementing with beneficial bacteria (e.g. Lactobacillus acidophilus, L casei subsp. Rhamnosus, L plantarum Bifidobacterium bifidum, B. longum, B. infantis, B. adolescentis, Streptococcus faecium), and dietary fiber (e.g. psyllium hull powder) with bentonite. Consider the botanicals Hydrastis canadensis (goldenseal), Geranium maculatum (cranesbill), Zingiber officinale (gingerroot), and Echinacea spp. Also consider digestive enzymes (such as those derived from Aspergillus oryzae). Replace electrolytes in case of excessive fluid loss, especially if chloride and potassium are low.

*Hypoadrenal:* use magnesium in combination with zinc, pantothenic acid (vitamin B-5), vitamin C, beta carotene and the botanicals Glycyrrhiza glabra (licorice), and Eleutherococus senticosus (Siberian ginseng). Consider using **Endosis IC™** for additional adrenal support.

*Kidney disease:* treat the underlying condition. Consider supplementation with antioxidants including quercetin, N-acetyl-L-cysteine, vitamins C and E, beta-carotene, zinc, copper, selenium, and Ginkgo biloba. Also consider proteolytic enzymes (such as those derived from Aspergillus oryzae) for functional kidney disorders.

## II. Increased levels (Chloride)

***A. Causative factors.***

**- High Levels -** Low magnesium and/or potassium levels, excess intake of salt, dehydration, diabetes mellitus, or renal failure may cause high salivary chloride levels.

***B. Associated conditions.***

Chloride ions are present in higher concentrations in serum and interstitial fluids than inside cells. They tend to diffuse into the cell and achieve an electrolyte balance that increases peripheral resistance. If this balance is not working (possible magnesium depletion), the chloride electrolyte tends to enter the cell causing swelling, thus contributing to hypertension. High intracellular chloride levels have been associated with hypertension and imbalances of sodium and potassium electrolyte levels caused by this swelling.

***C. Therapeutic Nutritional Considerations:***

*Potassium supplementation:* low potassium leads to chloride retention, supplement with potassium if it is found to be low.

*Magnesium supplementation:* high intracellular chloride has been associated with magnesium deficiency. Supplementation with magnesium if it is found to be low. Use highly bioavailable forms such as chelated magnesium (magnesium glycinate, magnesium lysinate, magnesium aspartate). These forms of magnesium are especially well tolerated by patients who experience the laxative effect of magnesium supplementation.

*Kidney disease:* treat the underlying condition. Consider supplementation with antioxidants including quercetin, N-acetyl-L-cysteine, vitamins C and E, beta-carotene, zinc, copper, selenium, and Ginkgo biloba. Also consider proteolytic enzymes (such as those derived from Aspergillus oryzae) for functional kidney disease.
*Comments:* increase fluids if dehydrated. Increased Chloride may be more associated with hypertension than is sodium. Reduce intake of table salt if excessive, especially if high sodium (high intracellular sodium will cause chloride to follow). Reduce other forms of chloride. Increase dietary supplemental potassium, if low. Treat the underlying condition.

### III. A DV of 750 mg is considered adequate.

# Phosphorus

## I. Decreased Levels (Phosphorus)

***A. Causative Factors.***
**- Low Levels -** Low dietary intake, mal-absorption, endocrine dysfunction, hyperparathyroidism or liver cirrhosis may cause low salivary phosphorus levels.
***B. Associated conditions.***
Low intracellular phosphorus conditions may be associated with bone disorders, fatigue, numbness and other sensation disorders.
***C. Therapeutic Nutritional Considerations:***
*Kidney disease:* treat the underlying condition. Consider supplementation with antioxidants including quercetin, N-acetyl-L-cysteine, vitamins C and E, beta-carotene, zinc, copper, selenium, and Ginkgo biloba. Also consider proteolytic enzymes (such as those derived from Aspergillus oryzae) for functional kidney disorders.
*Liver dysfunction:* to support and protect the liver consider supplementing with **AminoStat™** (proprietary balanced formula transdermal crème containing the 9 essential amino acids), **Detoxinol™** (proprietary transdermal crème containing silymarin, DIM, and folic acid), choline, L-methionine, L-glutamine, L-glutamic acid, L-glutathione, N-acetyl-L-cysteine, and the botanicals Silybum Marianum (milk thistle seed), Taraxacum Officinale (dandelion root), Chelidonium majus (celadine), Chionanthus virginicus (fringe tree bark), Beta vulgaris (beet leaf), Raphanus niger (russian black radish).
*Maldigestion/malabsorption:* consider the possibility of hypo- or achlorhydria, parasites, intestinal permeability defects, dysbiosis, celiac disease, and food allergies. Treat the underlying condition. Use digestive enzymes to aid digestion (**Digest*All*™**) and absorption (such as plant enzymes derived from Aspergillus oryzae). Consider L-glutamine, N-acetyl-D-glucosamine, gamma oryzanol, and gamma linolenic acid for a preventative strategy of intestinal permeability defects, Pro-biotics (PharmX) to re-colonize with appropriate normal flora.
*Comments: increase dietary phosphorus, if low. Treat the underlying condition.*

## II. Increased levels (Phosphorus)

***A. Causative factors.***
High salivary phosphorus levels may be caused by high dietary intake of such sources as carbonated beverages, water high in phosphate content and red meats, hypoparathyroidism, diabetes mellitus, renal disease, vitamin D deficiency or physical inactivity.
***B. Associated conditions.***
Excessive intracellular phosphorus tends to increase the need for magnesium and other trace metals. High intracellular phosphorus may affect mineral transport at the cell membrane level and prevent entry of magnesium and the exit of calcium in cells.

***C. Therapeutic Nutritional Considerations:***

*Magnesium Supplementation:* high intracellular phosphorus has been associated with magnesium deficiency. Supplement with magnesium if it is found to be low. Use highly bioavailable forms such as chelated magnesium (magnesium glycinate, magnesium lysinate, magnesium aspartate). These forms of magnesium are especially well tolerated by patients who experience the laxative effect of magnesium supplementation.

*Kidney disease:* treat the underlying condition. Consider supplementation with antioxidants including quercetin, N-acetyl-L-cysteine, vitamins C and E, beta-carotene, zinc, copper, selenium, and Ginkgo biloba. Also consider proteolytic enzymes (such as those derived from Aspergillus oryzae) for functional kidney disorders).

*Cardiovascular disease:* use potassium in combination with magnesium if salivary magnesium and potassium levels are low. Also consider using taurine, L-carnitine, chondroitin sulfate, coenzyme Q-10, inositol hexaniacinate (no "flush" niacin source), vitamins B-6, B-12, C, folic acid, and the botanicals Crataegus oxycantha (hawthorn berry), Ginkgo biloba, Zingiber Officinale (ginger) and Allium sativum (garlic).

*Hypertension:* use potassium in combination with magnesium.

*Diabetes mellitus*: use magnesium (if low), glucose tolerance factor (GTF), and chromium. Consider vitamins B-1 (thiamine), B-2 (riboflavin), B-3 (niacin), B-5 (pantothenic acid), B-6 pyridoxine), folic acid, and B-12 (cyanocobalamin). Also consider the botanicals Syzgium jambolanum (jambul), Taraxacum Officinale (dandelion root), and Glycyrrhiza glabra (licorice).

*Osteoporosis:* combine calcium with magnesium [(a 1:1 ratio of magnesium to calcium) ***Coral*** **CalMag Plus™**]. Consider supplementing with trace minerals boron, manganese, zinc, copper, and silica. Increase weight-bearing exercises, exposure to sunlight, reduce meats and carbonated beverages. Consider extra vitamin D supplementation if deficient. Phytase enzyme enhances mineral availability.

*Comment: increase weight-bearing exercise, reduce meats and carbonated beverages. Increase sun exposure and vitamin D intake if deficient. Treat the underlying condition.*

**III. A DV of 1200 mg is considered adequate.**

# Ratio Interpretation Guide

***Phosphorus/Calcium Ratio***

Tendon ligament and bone structures are related to ratios of phosphorus and calcium. When Phosphorus is low, Ca is low. As this ratio, lowers look for protein dietary deficiency.

***Magnesium/Calcium Ratio***

As this ratio decreases, cardiac risk factors increase. Serious pathology such as calcinosis, atherosclerosis, vascular occlusion, acute myocardial vasospasm or infarction with related arrhythmias may arise.

***Magnesium/Phosphorus Ratio***

Depression of Mg/P ratio in tissue tends to block entry of magnesium into cells that may result in lowered intracellular enzyme activity. Excessive dietary phosphorus can cause physiological cellular changes particularly when the Mg/Ca ratio is also low.

***Potassium/Calcium Ratio***

Potassium lowers the tendency towards increased peripheral arterial resistance and spasm in renal and coronary circulation. This ratio can be an important early biomarker for cardiovascular or renal disease.

***Potassium/Magnesium Ratio***

Magnesium has a Potassium sparing effect and is the rate-limiting ion for Potassium transport. In most cases, when Magnesium is low, Potassium is low.

***Potassium/Sodium Ratio***

Active transport of Potassium and Sodium produces major energy processes, normal cell volume and is vital to ion transport as well as producing the membrane potentials for all secretory functions, neurotransmission and neuromuscular activity. Serum Potassium levels are not good indicators of tissue potassium levels. This ratio is vital to establishment of homeostasis for normal function of intracellular biochemical events.

# AMINO ACIDS

Amino acids are the "building-blocks" of proteins, however, it is not dietary proteins that make up the human body. Dietary proteins are broken down into amino acids, then 'reconstructed' into the specific proteins necessary for body structure, function or biochemistry. Therefore, it is amino acids, not dietary proteins that are essential nutrients.

## Introduction

An adequate supply of dietary protein is required for survival, growth and development, reproduction and lactation, and maintaining health throughout life. The amino acids released during the digestion of food proteins are essential for the synthesis of tissue proteins, which comprise ~16% of the human body.

Table I. Nutritional Classification of Amino Acids

| Indispensable (Essential) | Dispensable (Nonessential) |
|---|---|
| Histidine | Alanine |
| Isoleucine | Arginine |
| Leucine | Aspartic acid |
| Lysine | Asparagine |
| Methionine | Cystein |
| Phenylalanine | Glutamic acid |
| Threonine | Glutamine |
| Tryptophan | Glycine |
| Valine | Proline |
| | Serine |
| | Tyrosine |

Some proteins are structural components of cells and tissues; others function as enzymes (biological catalysts), hormones (chemical messengers), antibodies, carriers for the transport of lipids in the blood, and components of systems for transporting small molecules across cell membranes. The structural proteins of muscles make up the largest proportion of total-body proteins. Also, many small nitrogen-containing molecules needed for normal body functions are synthesized from amino acids. Some of the individual amino acids are precursors of the purines and pyrimidines needed for the synthesis of nucleic acids, the hereditary units that carry information from one generation to the next. Other amino acids are precursors of small biologically important molecules such as heme as well small hormones such as thyroxine and epinephrine, creatine, neurotransmitters, skin pigments, and nitrogenous constituents of phospholipids.

Besides being building blocks for tissue proteins and precursors of many biologically important molecules, some amino acids also have specific regulatory functions. The rates of protein synthesis and degradation in liver, for example, are influenced by the influx of amino acids after a meal, some of which affect those processes more than others. (1,2) Some amino acids serve as stimuli for the release of hormones from endocrine organs and the gastrointestinal tract. (3) Several of these amino acids, particularly leucine, glutamine, and arginine, have been used as therapeutic agents in a preventative strategy of patients in catabolic states (4 - 6) or with hepatic encephalopathy. (7) Because these amino acids are also components of the proteins or amino acid mixtures that serve as sources of nutrients in diets, enteral-nutrition formulations, or solutions for parenteral nutrition, their nitrogen cannot be distinguished from that of the amino acids that are provided only for nutrition purposes. A question that arises is how should amino acids used in large amounts as therapeutic agents be dealt with in assessing nitrogen utilization by such patients?

3. Nutritional Essentiality of Amino Acids

Food and tissue proteins contain 20 amino acids of nutritional importance (Table I). Nine of these -- histidine, isoleucine, leucine, lysine, methionine, phenylalanine, theronine, tryptophan, and valine -- cannot be synthesized by the body; they are therefore essential or indispensable nutrients that must be obtained from the diet. The other 11 -- alanine, arginine, aspartic acid, asparagine, cystine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine -- are also ordinarily obtained from the diet, but the body can synthesize them. They are therefore not essential nutrients; they are nutritionally dispensable or nonessential. They are, nevertheless equally as important as the indispensable amino acids for the nutrition of cells and for normal cell and organ function.

Methionine and phenylalanine are required as specific precursors for the synthesis of the dispensable amino acids cysteine and tyrosine, respectively but the other dispensable (nonessential) amino acids can be synthesized from organic acids that are intermediates in the metabolism of carbohydrates and nitrogen from surpluses of other amino acids or even from ammonium sales.

Some amino acids that are ordinarily nutritionally dispensable may not be synthesized in large enough amounts to meet the body's needs if the metabolic pathways for their synthesis are immature or impaired. This appears to be the case with cystine and tyrosine in premature infants and possibly with taurine (8) there is also evidence that, after severe trauma, glutamine may not be synthesized in adequate amounts (6) Thus, under some conditions, certain dispensable amino acids may become conditionally indispensable. (8) The requirement for protein is thus a dual requirement -- for nine amino acids that the body cannot synthesize and some that may not be synthesized in adequate amounts and for nitrogen needed for the various nitrogenous compounds that are synthesized continuously. (9)

The carbon skeleton of amino acids can be oxidized by the body, so surpluses of protein and individual amino acids can also serve as sources of energy. Organs and tissues differ greatly in their ability to use amino acids as energy sources. The liver has the capacity to oxidize most amino acids and, if they are in surplus, will oxidize them in preference to other energy-yielding molecules. (10) Most of the indispensable amino acids are not oxidized in other tissues, but the branched-chain amino acids -- leucine, isoleucine, and valine -- like many of the dispensable amino acids, can be oxidized by most tissues and organs. (11) Glutamine and glutamic acid are preferential energy sources for the intestine and lymphocytes. (12)

4. Human Requirements for Protein and Amino Acids

Body proteins are in a dynamic state. They undergo continuous breakdown and resynthesis. A large proportion of the amino acids released during the breakdown of tissue proteins are reutilized for the synthesis of new proteins. Only in pathological conditions are proteins and amino acids excreted from the body unaltered in significant amounts. Amino acids that are not reutilized and those that are consumed in excess of the amounts needed for tissue synthesis are degraded completely, and their nitrogen, after being incorporated into urea, is excreted quantitatively in the urine.

5. Nitrogen Balance

The nitrogen in foods is almost entirely in proteins, and proteins contain on the average 16% of nitrogen; hence, the amount of food nitrogen consumed multiplied by 6.25 gives a measure of the amount of protein consumed. Measurement of the amount of nitrogen excreted from the body provides an estimate of the amount of body protein that has been completely degraded and cannot be reutilized and of the amount of dietary protein consumed in excess of that needed for tissue protein synthesis. The difference between the amount of nitrogen consumed and the amount lost from the body provides a measure of nitrogen balance.

As protein intake declines, the efficiency with which amino acids are reutilized increases, but small amounts of amino acids are continuously degraded, and their nitrogen is lost from the body even when no protein is being consumed. These losses are termed obligatory nitrogen losses. If protein intake declines below the amount of these obligatory losses, the amount of nitrogen excreted by the body will exceed the amount consumed, and the body will be in a state of negative nitrogen balance. As protein intake is increased, nitrogen balance will become less negative until, at some point, a steady state will be reached such that nitrogen intake will equal nitrogen loss; i.e., nitrogen balance will be zero. An estimate of adult requirements for protein or amino acids can therefore be obtained from measurement of the minimum amount of protein or amino acid nitrogen that must be consumed to just balance body nitrogen losses. (9 13)

To determine protein requirements, human subjects are initially fed a diet containing insufficient protein to maintain nitrogen balance and then, in sequence, a series of diets in which the protein content is increased incrementally every few days. Throughout this time, the amount of nitrogen in the food consumed and the amounts excreted in the urine and feces each day are measured. As protein intake is increased, a point is reached at which nitrogen intake just balances nitrogen loss. Some nitrogen is also lost in sweat, in sloughed skin and hair, and by other minor routes. These losses are not ordinarily measured, so the amounts estimated in the few studies in which they have been determined must be added to the urinary and fecal losses to obtain the true value for nitrogen losses. (9 13) Nitrogen intake required to maintain zero balance after this adjustment multiplied by 6.25 is then taken as the estimate of the adult protein requirement. If protein consumed exceeds the amount required to maintain zero nitrogen balance, the extra amino acids will be degraded, their carbon skeletons will be oxidized for energy, and the

nitrogen released will be converted to urea and excreted in the urine. Thus, when nitrogen intake exceeds the amount needed for zero nitrogen balance, nitrogen excretion increases, but nitrogen balance is maintained at zero with a greater inflow and outflow of nitrogen and with a greater proportion of the dietary protein being used as a source of energy.

6. Protein Requirements

The amount of protein needed to meet the requirements for indispensable amino acids differs with the source of protein in the diet. Protein needs are minimal when the dietary protein is of the highest quality, as in it is highly digestible and provides indispensable amino acids in the proportions in which they are required for the synthesis of body constituents. Requirements for protein are therefore established from the results of nitrogen balance studies in which human subjects have been fed the highest-quality proteins. The results of such studies have been reviewed regularly (9 13); the most recent comprehensive reassessment of protein requirements was done by an international committee in 1985. (14) This committee concluded that the average adult requirement for high-quality protein was 0.6 g kg -1 body wt day -1.

Requirements of individuals for nutrients differ depending on their genetic makeup. It is not possible to distinguish between individuals having low or high requirements without elaborate metabolic studies, but the range of protein requirements expected in a large population can be estimated from statistical analysis of the individual values obtained in experimental studies.

From such information, the coefficient of variation of adult protein requirements is estimated to be 12.5%; a value 25% above the average is considered high enough to cover the needs of most individuals in the population. (14) The "safe intake" of high-quality proteins such as those of milk, eggs, or meat, was set on the basis of these considerations at 0.75 g kg -1 day -1. In the United States the Recommended Dietary Allowance (RDA) for protein for adults, based on similar considerations, was set in 1974 at 0.8 g kg -1 day -1. (15) It has recently been reduced to conform to the international safe intake of 0.75 g kg -1 day -1, (16) but the protein allowances for adults in the RDA table are still based on the value of 0.8 g kg -1 day -1.

Table II. Estimated Safe Intakes or Recommended Dietary Allowances for Protein

| Age | g/ kg Body Wt /Day |
|---|---|
| 1-3 mo | 2.00 |
| 6 mo | 1.50 |
| 1 yr | 1.20 |
| 6 yr | 1.00 |
| Adult | 0.75 |

Protein requirements are highest during the period of rapid growth after birth. Protein requirements of infants have been estimated from measurements of the amounts of protein consumed by infants growing satisfactorily on breast milk or formulas of comparable quality. Requirements of older children are estimated by a factorial method. The maintenance requirement for protein has been estimated in short-term nitrogen balance studies on children consuming high-quality proteins; then, with knowledge of growth rates and the protein content of the tissue being deposited, the average amount of protein needed for growth can be calculated. From the sum of the maintenance and growth needs, and estimate of the average amount of protein required at various ages is obtained. These values are increased, in the same

way as for adults, to allow for individual variability in requirements and permit estimation of safe intakes or RDA. (14) For infants, safe intakes or protein from breast milk or from formulas of comparable quality are estimated to be -2 g kg -1 day -1 shortly after birth and decline to 1.5 g Kg -1 day -1 by 6 mo of age, 1.2 g kg -1 day -1 by 1 yr, 1 g kg -1 day -1 by 6 yr, and gradually thereafter to the adult RDA value of 0.75 g kg -1 day -1 (Table II).

7. Amino Acid Requirements

Adult requirements for indispensable amino acids have been estimated with the nitrogen balance procedure in the same way as for establishing protein requirements, except that a mixture of amino acids replaces the protein in the basic diet so the quantity of each amino acid can be adjusted separately. Diets containing adequate quantities of all but one indispensable amino acid and increasing increments of the missing one are then fed in sequence to experimental subjects, and the intake at which zero nitrogen balance is achieved, as in estimating the protein requirement, is taken as the requirement for the indispensable amino acid. This process was used to determine the adult requirements for eight of the indispensable amino acids. (17 18)

The indispensable amino acid requirements of infants and young children have been estimated by observing changes in weight after the amount of one amino acid in an otherwise fully adequate amino acid diet is reduced
incrementally. If intake of one amino acid falls below the requirements, weight gain will decline. To ensure that growth will not be impaired by this procedure, the amount of the amino acid that is limiting growth is increased in the diet as soon as the first evidence of reduced weight gain is detected. Indispensable amino acid requirements of infants have also been estimated from amino acid intakes of infants growing satisfactorily, calculated from knowledge of the amounts of breast milk or formula they have consumed and the amino acid composition of these foods. Requirements determined via this procedure, except that for tryptophan, were lower than those obtained with amino acid diets. (9 14)

The most recent estimates of amino acid requirements of infants, children, and adults are shown in Table III. (14) Requirement values for infants reported by a National Research Council committee (9) using essentially the same information ranged from 20% lower to 20% higher than the Food and Agriculture Organization (FAO)/World Health Organization (WHO) values. The values for each age grouping were, nonetheless, in the same range, and differences between the age groups were similar.

Table III. FAO/WHO/UNO Amino Acid Requirements (mg/kg Body Wt/day)

| | Infants (4-6 Mo) | Children (2 Yr-9) | Children (10-12 Yr) | Adults |
|---|---|---|---|---|
| Histidine | 28 | ? | ? | ? |
| Isoleucine | 70 | 31 | 30 | 10 |
| Leucine | 161 | 73 | 45 | 14 |
| Lysine | 103 | 64 | 60 | 12 |
| Methionine + Cysteine | 58 | 27 | 27 | 13 |
| Phenylalanine + Tyrosine | 125 | 69 | 27 | 14 |
| Therenine | 87 | 37 | 35 | 7 |
| Tryptophan | 17 | 12.5 | 4 | 3.5 |
| Valine | 93 | 38 | 33 | 10 |
| Total amino acids | 742 | 352 | 261 | 84 |

| | | | | |
|---|---|---|---|---|
| Proteine | 1650 | 1200 | 1000 | 750 |
| E/T | 45 | 29 | 26 | 11.2 |

Amino acid requirements decline much more rapidly with increasing age than protein requirements. Snyderman, (19) in a carefully controlled study of phenylketonuric infants, observed that, during the first 2 yr of life, the phenylalanine requirement fell by 75%, whereas the protein requirement declined by only ~50% over this period. For most amino acids, the difference between the amino acid and protein requirements becomes greater as maturity is approached. Thus, the proportion of protein or total amino acids required as indispensable amino acids is much lower for the adult than for the infant (Table III). In other words, a protein that may not meet the indispensable amino acid requirements of the child when it is consumed in an amount that meets the total nitrogen requirement may provide amounts of amino acids in excess of the requirements for adults consuming enough protein to meet the nitrogen requirement. (9 14)

Requirements of adults for several indispensable amino acids have been reinvestigated with an isotopic procedure. (20 21) Adult human subjects were fed diets containing a mixture of amino acids instead of protein, with one carbon-labeled indispensable amino acid being included at a time in graded amounts in a series of diets. Expired air was collected for several hours after feeding each diet, and the amount of labeled amino acid oxidized was estimated from the amount of isotopic carbon in the expired carbon dioxide. From the values obtained for the amounts of several amino acids oxidized, the investigators concluded that adult requirements for indispensable amino acids had been underestimated by 50-75% by the nitrogen balance procedure. Other investigators questioned this conclusion, largely on the basis of theoretical considerations, but did acknowledge that amino acid requirements of adults are probably underestimated by the nitrogen balance procedure. (15 22)

Largely in response to criticisms of the nitrogen balance procedure, adult protein requirements have been extensively reinvestigated and reassessed, leading to the conclusion that they had earlier been underestimated by ~20%. (14) It should not be surprising, therefore, if adult amino acid requirements have also been underestimated. The question is, by how much? Although the results of oxidation studies indicate that, with the exception of the methionine requirement, amino acid requirements of adult humans have been underestimated by a factor of 2-3, (20 21) values for amino acid requirements of young rats (23) and piglets (24) estimated by the oxidation procedure and growth assays have been in good agreement. Also, young adults have been maintained in nitrogen balance and good health for 50 days while consuming cereal grain diets that provided lysine at ~50% above the current requirement. (25) These observations raise several questions. Is the nitrogen balance procedure so much less reliable for estimating adult requirements for amino acids than for protein? If it is, why? Why are methionine requirements of adults estimated by the nitrogen balance and oxidation procedures similar but values for most other amino acid requirements so widely divergent? Is the oxidation procedure for estimating amino acid requirements for maintenance subject to sources of error that are not obvious? Unfortunately, both the nitrogen balance and oxidation procedures for estimating amino acid requirements for maintenance are indirect methods, and there is no direct method of validating them in human subjects. Thus, the question of amino acid requirements for adults remains unresolved.

From a practical viewpoint, even if amino acid requirements for adults have been underestimated by 50-75%, the proportion of total amino acids needed as indispensable amino acids would increase only from ~10 to 20 or 30%. Therefore, any protein that meets the indispensable amino acid needs of young children would be more than adequate for adults, provided that the amount consumed was sufficient to meet the nitrogen requirement.

8. The Concept Of Amino Acid Balance

Indispensable amino acids are required in specific proportions, as seen from examination of the amino acid requirement values listed in Table III. Proteins that provide amino acids in the proportions in which they are required have well-balanced amino acid patterns. Provided such proteins are readily digested, their amino acids will be used highly efficiently for the synthesis of tissue proteins. If a protein contains a disproportionately low amount of one or more amino acids, i.e., has a poorly balanced or unbalanced amino acid pattern, it will be used inefficiently for tissue protein synthesis. The greater the deviation in the amino acid pattern of the dietary protein from the pattern of amino acid requirements, the less efficiently it will be used. This occurs because, if one amino acid is provided in less than the amount required, its concentration in tissues will fall, and it will become limiting for protein synthesis. Other amino acids can then be used for tissue protein synthesis only in amounts equivalent to the proportion of the requirement of the limiting amino acid that has been met, e.g., if a diet provides only 50% of the amount of the limiting amino acid required, then only amounts of the other amino acids equivalent to 50% of the amount of the requirements can be used. Quantities in excess of this will be degraded. If a protein with a well-balanced pattern of amino acids is consumed in an amount in excess of that needed to meet amino acid and nitrogen requirements, it will also be used inefficiently.

Proteins of most cereal grains have unbalanced amino acid patterns; they are disproportionately low in lysine. Such proteins are sometimes referred to as incomplete proteins; this is a misnomer. Although they are used inefficiently, they do contain all amino acids. In fact, requirements for indispensable amino acids are readily met with such proteins if the amount consumed is sufficiently in excess of the amount of high-quality protein needed. Nitrogen balance can be maintained in individuals consuming cereal grains as the sole source of protein, but efficiency of utilization of such proteins will be low (9) because, when the requirement for lysine is met, most other amino acids will be consumed in amounts well in excess of the amounts needed, and the excesses will be oxidized for energy. (10)

Although requirements for all indispensable amino acids, including the limiting one, can be met from proteins with unbalanced amino acid patterns if the total amount of protein consumed is sufficiently high, requirements for some amino acids will not be met if the protein content of the diet is low. When such conditions were produced experimentally by including a mixture of amino acids devoid of one indispensable amino acid in a low-protein diet fed to experimental animals, not only was the mixture of amino acids and protein used inefficiently, but also food intake and growth rate were depressed. These effects have been attributed to amino acid imbalances. (26) They have been observed especially with young, growing animals fed low-protein diets in which there is a severe disproportion of indispensable amino acids, with one or more of them resent in the diet in an amount well below the requirement. Under these conditions, the concentration of the limiting amino acid in the blood and brain falls sharply in association with the depressed food intake. Lesions in the prepyiform cortex of the brain prevent this response, (27) suggesting that amino acid concentrations in this area of the brain are monitored and that depletion of the brain pool of the limiting amino acid serves as a signal for depression of food intake, thereby limiting intake of the imbalanced diet and preventing more severe distortion of blood or brain amino acid patterns. If the diet provides amounts of all indispensable amino acids that meet or exceed amino acid requirements, or if the requirements are met by tube feeding (force feeding), even young animals will tolerate severely unbalanced dietary amino acid patterns. (26)

9. Evaluation of Protein Quality

Values for the nutritional quality of proteins are relative measures of the efficiency with which proteins are used to meet requirements for amino acids and nitrogen. The value for a protein depends on its amino acid composition and digestibility. If a protein contains a disproportionately low amount of one or more amino acids or is not completely digested, the amount needed to meet protein requirements will be greater than for a protein that has a well-balanced pattern of amino acids and is highly digestible. (28) Examples of high-, intermediate-, and low-quality proteins are listed in Table IV (based on total essential/total proteins x 100 values and utilization (29).

Table IV. Examples of High-, Intermediate-, and Low-Quality Proteins

| | |
|---|---|
| High Quality | |
| | Cow's milk |
| | Chicken Egg |
| | Human milk |
| | Beef muscle |
| | Fish |
| Intermediate Quality | |
| | Soy flour |
| | Sunflower seed |
| | Rice |
| | Potato |
| | Oats |
| Low Quality | |
| | Peas |
| | Cornmeal |
| | White flour |
| | Cassava |
| | Gelatin |

10. Methods for Evaluating Protein Quality

Animal assays are usually used to estimate protein quality. The amount of weight gained by laboratory rats per unit of protein consumed provides a measure of the protein efficiency ratio. The proportion of dietary nitrogen retained in the body measured directly by carcass analysis or indirectly by the nitrogen balance procedure provides a measure of net protein utilization. These methods take into account both the digestibility of the protein and effects of deficits of indispensable amino acids. They provide information about differences in the quality of different food proteins but, because the assays must be done with diets low in protein, the values obtained are relative; they usually exceed the efficiency or utilization observed for proteins being consumed at the requirement level in human diets. (28)

Efficiency of nitrogen utilization or protein quality can be determined from nitrogen balance measurements made on human subjects consuming their usual diets or diets containing only specified protein sources. Considerable information about the biological value (the percentage of absorbed nitrogen retained) and the net protein utilization (the percentage of ingested nitrogen retained) of various proteins and mixtures of proteins has been obtained from such measurements made on human subjects, usually in experiments designed to investigate protein requirements. (9 30 31) The nitrogen balance procedure is expensive, time consuming, and impractical for routine determinations of protein quality. Estimating the effectiveness of proteins in meeting human amino acid and nitrogen requirements is greatly simplified, however, by use of an amino acid scoring procedure, (13) a modified chemical score method, (32) and an estimate of digestibility.

11. Amino Acid Score

The amino acid scoring procedure provides a method of predicting how efficiently a food protein will be used in meeting human amino acid needs from knowledge of its amino acid composition. The assumptions underlying the procedure are those of the amino acid balance concept: 1) tissue protein synthesis will be limited unless all amino acids are present together in appropriate amounts at sites of tissue protein synthesis, and 2) the proportion of amino acids from the dietary protein that will be used for tissue synthesis will be limited by the amount of the indispensable amino acid present in the dietary protein in least amount in relation to the amount required, i.e., by the proportion of the requirements for the limiting amino acid that is met from the dietary protein.

The initial step in the amino acid scoring procedure is to identify the limiting amino acid. To do this requires a reference amino acid scoring pattern in which amino acid requirements are expressed as milligrams needed per gram of protein, or as percentages needed in a theoretically ideal dietary protein, to meet the requirements of all of the indispensable amino acids when the amount consumed meets the nitrogen requirement. Amino acid scores are then calculated by expressing the amount of each amino acid in the dietary protein as a percentage of the amount in the scoring pattern. Values > 100% are considered to be 100; then, from among those >100, the limiting amino acid is identified as the one having the lowest score. To illustrate, if the lysine content of whole-wheat flour is 2.6% and the value for lysine in the scoring pattern based on the amino acid needs of the young child is 5.1%, the amino acid score for lysine in wheat proteins is $2.6/5.1 \times 100 = 51$. The scores for all other amino acids are higher, so lysine is the limiting amino acid, and the amino acid score for wheat proteins is 51. The score for whole egg proteins is 100; therefore, to meet the requirement for lysine, a young child would have to consume twice as much protein from whole wheat as from whole egg. (9, 14, 28-32)

A limitation of the amino acid scoring procedure is that it does not take into account protein digestibility. (9 13 28) It can be used directly to compare the nutritional quality of food or dietary proteins that are highly digestible, e.g., most animal products and refined foods that have not been heated excessively.

Many foods of plant origin, however, are not completely digested, so a correction must be made for this in assessing the nutritional quality of their proteins. Digestibility of food proteins by humans can be determined from measurements of only nitrogen intake and fecal nitrogen excretion corrected for the amount of nitrogen in the feces when the diet contains no protein. The procedure has been used extensively and remains the standard method for obtaining information about digestibility. (14) Recently, a group of consultants convened by FAO/WHO to reassess current knowledge of evaluation of protein quality concluded that digestibility measurements made with rats as the experimental subjects are as satisfactory as those obtained from measurements on human subjects. (33) If, in the example cited above, the proteins of wheat product are only 90% digestible, protein quality based on the amino acid score must be adjusted for this in estimating the quantity of protein needed to meet requirements.

The question of the appropriate amino acid scoring pattern to use for evaluating the quality of dietary proteins has been the subject of much debate. If the amino acid scoring pattern based on the amino acid requirements of the youngest age groups is used to evaluate the quality of proteins, nutritional quality of proteins with unbalanced amino acid patterns will be underestimated for adults because the amino acid requirements of adults are so much lower than those of young children. The international committee that recently reassessed protein requirements proposed that, to avoid this problem, separate patterns based on the amino acid requirements shown in Table III should be used. (14) After assessing the amino acid scores of many diets via these scoring patterns for infants, preschool children, and adults, the committee concluded that there was no need to make corrections for differences in protein quality for older children (age > 12yr) or adults consuming mixed diets. Thus, digestibility becomes the major factor in determining how much protein from mixed diets is needed to meet the protein requirements of these groups.

This subject has also been reconsidered by the recent FAO/WHO group of consultants on evaluation of protein quality. (33) The consultation concluded that, in view of questions about the validity of amino acid requirement values for school age children and adults, the amino acid scoring patterns for these groups should no longer be recommended. Instead, it proposed that the FAO/WHO amino acid scoring pattern for children of preschool age be used to evaluate dietary protein quality for all age groups except infants.

The scoring patterns (milligrams of amino acid per gram of protein) recommended for infants and preschool children (Table V) are identical to those proposed previously. (14) The pattern for infants is based on the amino acid composition of human milk, but in the 1985 FAO/WHO/UNO report, it is acknowledged that "infants consuming cow's milk proteins at the same level as breast milk show satisfactory growth and nitrogen balance." (14) Use of a single amino acid scoring pattern for all ages except infants is logical; however, the pattern proposed, although it has been accepted as satisfactory by two international expert groups, (14 35) is based on a single set of requirement values from a conference report (30) that was not subjected to peer review. The appropriateness of this scoring pattern will undoubtedly be examined further. For the populations of industrialized nations generally, whose average protein intake exceeds the requirement by ~>50%, there is little likelihood of healthy adults or older children not meeting their amino acid and protein nitrogen needs, even from diets with unbalanced amino acid patterns, unless food intake is low.

| Table V. Suggested Amino Acid Scoring Patterns (mg/g Protein) | | |
|---|---|---|
| | Infant | Preschool Child |
| Histidine | 26 | (19) |
| Isoleucine | 46 | 28 |

| | | |
|---|---|---|
| Leucine | 93 | 66 |
| Lycine | 66 | 58 |
| Methionine + Cystine | 42 | 25 |
| Phenylalanine + Tyrosine | 72 | 63 |
| Therenine | 43 | 34 |
| Tryptophan | 17 | 11 |
| Valine | 55 | 35 |
| Total | 460 | 339 |

From FAO/WHO/UNO (14) and Food and Agriculture Organization. (33) Histidine value in parenthesis obtained from interpolation from smooth curve of requirement vs. ag. See Ref. 33

**1**2. Protein Intake, Protein Quality, and Utilizable Protein

The various methods for determining protein quality are, in essence, methods for determining the proportion of the protein in a food or diet that is "utilizable" or, more specifically, the proportion of the indispensable amino acids in the protein that can be used for tissue protein synthesis. (34) The remainder, in contrast is the proportion likely to be used only as a source of energy. The amino acid score, with an adjustment for digestibility, provides a direct measure of the proportion of utilizable protein. Although measures of protein quality or utilizable protein, e.g., amino acid scores, are nutritional characteristics of food proteins, they are not absolute characteristics; protein quality varies with the amount of protein consumed.

The concept of protein quality applies only under conditions in which the amount of protein consumed is equal to or less than the amount needed to meet the requirement for the limiting amino acid. When protein intake exceeds this amount, efficiency of protein utilization, or protein quality, will decline regardless of the balance of the amino acid pattern. This will occur even with the highest-quality proteins because, after the requirement for the limiting amino acid has been exceeded, all indispensable amino acids will be present in tissues in excess of the amounts needed to saturate protein-synthesizing system, and because amino acids cannot be stored, the extra amounts of all of them will be degraded and used only as sources of energy. (28)

This relationship between protein intake and protein quality has implications for interpretation of measurements not only of the nutritional quality of individual proteins or mixtures of protein but also of diets containing proteins with unbalanced patterns of amino acids to improve the quality of the protein and reduce the quantity needed to meet amino acid requirements. Effects of amino acid supplements other than the limiting one have been examined in numerous studies of young, growing animals fed low-protein diets. The quality of the total dietary protein is lowered by such additions because, without an increase in the amount of the limiting amino acid, the other ammo acids added can be used only as a source of energy. In some of these studies, amino acid imbalances occurred, food intake and growth of the animals were depressed by the additions, and these responses were associated with a sharp decline in the concentration of the limiting amino acid in blood and tissue free pools. (26)

These effects were overcome by increasing the amount of protein in the diet or providing a supplement of the limiting amino acid, both of which improve the quality of the total dietary protein. They were also overcome, without any improvement in the amino acid balance or quality of the dietary protein, if the animals were exposed to a cold environment, which greatly increased their energy expenditure. (35) Under these conditions, they consumed much more of the low-protein diet with the unbalanced amino acid pattern and therefore obtained a much larger amount of usable protein and were able to meet

their requirement for the limiting amino acid. Adult animals, which have much lower requirements for indispensable amino acids, and younger animals receiving enough utilizable protein to meet their requirements for all of the indispensable amino acids can consume diets in which the amino acid pattern is sufficiently unbalanced to result in low overall efficiency of protein utilization without evidence of ill effects. These observations illustrate that, although the quality of the protein in a diet is critical when protein intake is low, especially for young, growing subjects, the total amount of utilizable protein is critical for meeting indispensable amino acid requirements and ensuring nutritional adequacy. The proportion of well-balanced protein needed by human adults to meet the indispensable amino acid requirements is assumed to be ~>15% of the total protein requirement. This is the basis for the conclusion of the FAO/WHO/UNO committee that only digestibility, not protein quality, need be considered in estimating protein needs of adults. (14) This does not apply to young children, whose indispensable amino acid requirements are several times those of adults. (9 13)

13. Evaluation Of Diets Containing Amino Acids as Therapeutic or Pharmacological Agents

Besides fulfilling specific nutritional or physiological roles, e.g., serving as components of body structures or metabolic systems, some nutrients may also have therapeutic or pharmacological actions. The amounts required for pharmacological effects are usually much greater than those required for nutritional function, and such effects are generally observed in individuals with some degree of metabolic or physiological impairment. This can be illustrated with tryptophan as an example. To maintain protein synthesis, the synthesis of molecules derived from tryptophan, and for regulation of the release of gastrointestinal hormones, the human adult requires ~250 mg tryptophan/day (14); however, in adults who have mild insomnia, tryptophan administered in a dose of > 1 g/day (~> 4 times the daily requirement) will induce sleep. (36) This effect is assumed to result from the increase in the concentration of the neurotransmitter serotonin (5-hydroxytrptamine) in the brain, which has been observed in animals administered a load of tryptophan. Use of tryptophan as a pharmacological agent has been associated with toxic effects, but these appear to be attributable to contaminants in some preparations.

When amino acids are used in this way as therapeutic agents, a question arises as to whether the nutritional and therapeutic functions of an amino acid should be assessed independently or whether the consequences of administering a large amount of an amino acid as a therapeutic agent should be taken into consideration in assessing nitrogen utilization and the nutritional quality of the protein component of the diet as is done with amino acid supplements. When tryptophan is administered as a pharmacological agent separately from the diet at a level of only 1 g/day, the nutritional and pharmacological effects can readily be considered independently. When other amino acids are utilized in much higher doses as therapeutic agents, the situation is more complex. The quantities used may range from 10 to 30 g of each of several amino acids per day; also, they are usually administered as part of the total diet in enteral or parental products rather than separately from the diet.

A comprehensive review of the use of amino acids and their derivatives as therapeutic agents is beyond the scope of this review.

A survey of the literature for the past 10 yr, however, reveals scattered reports of amino acids being tested as therapeutic agents, but such effects have been investigated in detail in controlled studies for only a few amino acids. A few examples from among those illustrate the nature of the problems encountered in evaluating protein or nitrogen utilization by subjects consuming diets in which large quantities of amino acids have been included for specific therapeutic purposes.

Parenteral solutions enriched with branched-chain amino acids leucine, isoleucine, and valine, have been used to improve nitrogen retention in septic and uncomplicated postoperative patients. (4 38) Formulations enriched with branched-chain amino acids along with lowered amounts of the aromatic amino acids have been used to improve plasma amino acid patterns in hepatic encephalopathy patients. (7) Leucine is the effective component in improving nitrogen retention in patients in catabolic states, but to maintain appropriate balance among the three branched-chain amino acids, they are included together in most parenteral and enteral preparations. The branched-chain amino acids may comprise ~<50% of the total amino acids in such preparations.

Arginine stimulates the release of several hormones including growth hormone and insulin, in human subjects. Arginine has been shown to reduce nitrogen loss in surgical patients with moderate trauma and improve lymphocyte function in healthy human volunteers. (5) It has been proposed that arginine is a conditionally indispensable amino acid in individuals who have been subjected to trauma. The quantities of arginine with which these therapeutic effects have been demonstrated are in the pharmacological range and may be as high as 30 g/day.

Glutamine is also considered by some investigators to be conditionally indispensable in critically ill patients. (6) Its concentration declines sharply in muscle of human subjects or animals in a catabolic state. (39) It is a preferential energy source for the rapidly proliferating cells of intestinal mucosa and is used extensively for energy by lymphocytes when they are stimulated to proliferate. In view of its therapeutic potential in the preventative strategy of human subjects in a stressed state, it has been tested for safety in healthy subjects in doses in the range considered appropriate for therapeutic effects, (~> 40g/day i.v. for 5 days). No untoward physiological effects were observed with these doses. (40)

The quantities of these amino acids that may be administered individually as therapeutic agents can greatly exceed the amounts ingested daily by healthy individuals consuming their usual diets. In fact, administration of several of these amino acids together for therapeutic purposes would result in ingestion of a total quantity of amino acids as therapeutic agents in the range of 50-100 g/day, equal to the usual daily intake of protein. Obviously, it would be inappropriate to substitute a therapeutic mixture of these amino acids for part of the dietary protein because this would reduce the quantities of indispensable amino acids in the diet, lower the proportion of balanced (utilizable) protein, and result in modification of the diet so it resembled those used experimentally in investigations of amino acid imbalances. Assuming that the amount of balanced protein in the diet is not reduced when amino acids functioning as therapeutic agents are included, the diet would provide indispensable amino acids in amounts that would meet the requirements, but as much as half of the total nitrogen intake would be from an incomplete mixture of amino acids that could not contribute to synthesis of tissue proteins.

A question that must be considered, then, is what is the appropriate way to evaluate nitrogen utilization by an individual consuming a high proportion of total dietary nitrogen from an incomplete mixture of amino acids, which has been included only to fulfill a pharmacological or therapeutic function? If the therapeutic agent were unrelated to the dietary protein, this problem would not arise.

If the incomplete amino acid mixture is considered to be strictly therapeutic, not nutritional, it would seem appropriate to evaluate protein nutriture only in relation to the amount of well-balanced or utilizable protein in the diet and ignore the nitrogenous contribution of the therapeutic components. On the other hand, if nitrogen utilization by the patient is to be evaluated via measurements of nitrogen retention or nitrogen balance, nitrogen contributed by the therapeutic agents cannot be distinguished from that contributed for nutritional purposes by the dietary protein. With equal quantities of high quality protein and an incomplete amino acid mixture, the value for the quality of the dietary nitrogen source would be low, and its efficiency of utilization would probably be ~<50%. If the therapeutic component were disregarded, efficiency of protein utilization based only on utilization of the high-quality protein component of the diet would be high and might approach 80%, close to the maximum observed for high-quality proteins consumed at the requirement level. (14)

The situation can be viewed in two ways. It might be considered analogous to a situation in which subjects are receiving a diet in which the protein component consists of a mixture of the highest-quality proteins consumed at a level that will ensure achievement of zero nitrogen balance. Viewed in this way, the therapeutic component would not be taken into account because it cannot contribute to nitrogen retention; the amount of nitrogen it provided would be subtracted from both the amounts of nitrogen consumed and excreted. Nitrogen utilization would then be estimated solely on the basis of the quantity of high-quality protein consumed. A second approach might be to view the situation as analogous to one in which subjects are receiving a diet containing an unbalanced protein that can be used only inefficiently for protein synthesis and achievement of nitrogen balance. The value of the quality of the mixture of nitrogenous constituents making up the dietary "protein" would thus be only ~50%, but the amount of nitrogen provided would be about double that needed if the protein were of high quality.

Viewed either way, the amount of utilizable protein (34) consumed would be the same. Needs for indispensable amino acids should be met equally well, as should needs for total nitrogen. The state of protein nutriture should also be the same; the extra amino acid would serve as a source of energy to substitute for carbohydrate that might have been displaced form the diet. Whichever approach is seed in assessing protein nutriture of subjects or patients consuming such diets or products, it is important that comparisons be made on the basis of utilizable protein and not total nitrogen or total protein.

Problems should arise only if extra amino acids are provided in amounts exceeding their tolerance in individuals. High intake of the amino acids used in various therapeutic mixtures has been tested individually; they should be tested together. The very high nitrogen intake associated with such therapeutic regimens may result in falsely high nitrogen retention or excretion in short-term studies.

It may require consumption of the unbalanced mixture for a considerable period until a new steady state is established with the higher nitrogen intake (41) before full nutritional and pharmacological consequences can be established.

These observations on problems in evaluating the efficiency of utilization of diets that serve as vehicles for providing amino acids as therapeutic agents represent only one part of a much larger canvas. Several nutrients, their precursors or derivatives, are being considered as therapeutic, prophylactic, and pharmacological agents. Use of large doses of nutrients in these ways has implications not only for evaluating the efficiency of nutrient utilization but also for classification of nutrients, recommended intakes of nutrients, and assessment of nutrition status. As such uses increase, to avoid confusion in the field of nutrition, it will be important to establish criteria for distinguishing clearly among physiological,

pharmacological, and medicinal effects of nutrients and to investigate interactions between physiological and therapeutic effects of nutrients.

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## Therapeutic Applications of Taurine

Taurine is a conditionally-essential amino acid, which is not utilized in protein synthesis, but rather is found free or in simple peptides. Taurine has been shown to be essential in certain aspects of mammalian development, and in vitro studies in various species have demonstrated that low levels of taurine are associated with various pathological lesions, including cardiomyopathy, retinal degeneration, and growth retardation, especially if deficiency occurs during development. Metabolic actions of taurine include: bile acid conjugation, detoxification, membrane stabilization, osmoregulation, and modulation of cellular calcium levels. Clinically, taurine has been used with varying degrees of success for the resolution of a wide variety of conditions, including: cardiovascular diseases, hypercholesterolemia, epilepsy and other seizure disorders, macular degeneration, Alzheimer's disease, hepatic disorders, alcoholism, and cystic fibrosis. (Alt Med Rev 1998;3(2):128-136)

### Introduction

Taurine (2-aminoethanesulfonic acid, see Figure 1, page 50) is a conditionally-essential amino acid, which is not utilized in protein synthesis, but rather is found free or in simple peptides. First discovered as a component of ox bile in 1827, it was not until 1975 that the significance of taurine in human nutrition was identified, when it was discovered that formula-fed, pre-term infants were not able to sustain normal plasma or urinary taurine levels.[1] Signs of taurine deficiency have also been detected in children on long-term, total parenteral nutrition,[2] and in patients with "blind-loop" syndrome.[3] In vivo studies in various species have shown taurine to be essential in certain aspects of mammalian development, and have demonstrated that low levels of taurine are associated with various pathological lesions, including cardiomyopathy, retinal degeneration, and growth retardation, especially if deficiency occurs during development.[4]

Derived from methionine and cysteine metabolism, taurine is known to play an important role in numerous physiological functions. While conjugation of bile acids is perhaps its best-known function, this accounts for only a small proportion of the total body pool of taurine in humans. Other metabolic actions of taurine include: detoxification, membrane stabilization, osmoregulation, and modulation of cellular calcium levels. Clinically, taurine has been used for the preventative strategy of a wide variety of conditions, including: cardiovascular diseases, epilepsy and other seizure disorders, macular degeneration, Alzheimer's disease, hepatic disorders, and cystic fibrosis. An analog of taurine, acamprosate, has been used as a preventative strategy for alcoholism.

### Biochemistry and Metabolism

Although frequently referred to as an amino acid, it should be noted that the taurine molecule contains a sulfonic acid group, rather than the carboxylic acid moiety found in other amino acids. Unlike true amino acids, taurine is not incorporated into proteins, and is one of the most abundant free amino acids in many tissues, including skeletal and cardiac muscle, and the brain.[5]

In the body, taurine is synthesized from the essential amino acid methionine and its related non-essential amino acid cysteine (see Figure 2, page 50). There are three known pathways for the synthesis of taurine from cysteine. All three pathways require pyridoxal-5'-phosphate (P5P), the active coenzyme form of vitamin B6, as a cofactor. A vitamin B6 deficiency has been shown to impair taurine synthesis.[6]

The activity of cysteine sulfinic acid decarboxylase (CSAD), the enzyme which converts both cysteine sulfinic acid into hypotaurine, and cysteic acid into taurine, is thought to reflect the capacity for taurine synthesis.[7] Compared to other mammals, humans have relatively low CSAD activity, and therefore possibly lower capacity for taurine synthesis.[8] Much of the published research on taurine has involved studies done on cats, which do not synthesize taurine, but must consume it in their diet.[5] Therefore, since humans have the capacity to synthesize at least some taurine, it is unclear to what extent feline studies can be extrapolated to humans.

### Cardiovascular Effects

Taurine comprises over 50 percent of the total free amino acid pool of the heart.[9] It has a positive inotropic action on cardiac tissue,[10] and has been shown in some studies to lower blood pressure.[11,12] In part, the cardiac effects of taurine are probably due to its ability to protect the heart from the adverse effects of either excessive or inadequate calcium ion (Ca2+) levels.[13] The consequence of Ca2+ excess is the accumulation of intracellular calcium, ultimately leading to cellular death. Taurine may both directly and indirectly help regulate intracellular Ca2+ ion levels by modulating the activity of the voltage-dependent Ca2+ channels, and by regulation of Na+ channels. Taurine also acts on many other ion channels and transporters. Therefore, its action can be quite non-specific.[14] When an adequate amount of taurine is present, calcium-induced myocardial damage is significantly reduced, perhaps by interaction between taurine and membrane proteins.[15] At least one study has suggested taurine's ability to function as a membrane stabilizer is related to its capacity to prevent suppression of membrane-bound NaK ATPase.[16]

Other research demonstrates taurine can protect the heart from neutrophil-induced reperfusion injury and oxidative stress. Because the respiratory burst activity of neutrophils is also significantly reduced in the presence of taurine, perhaps taurine's protective effect is mediated by its antioxidative properties.[17]

Azuma and associates have observed that taurine alleviates physical signs and symptoms of congestive heart failure (CHF).[18-20] Chazov et al were able to demonstrate that taurine could reverse EKG abnormalities such as S-T segment changes, T-wave inversions, and extra systoles in animals with chemically-induced arrhythmias.[21]

A double-blind, placebo-controlled crossover study suggested, "taurine is an effective agent for the preventative strategy of heart failure without any adverse effects." [22] Fourteen patients (9 men and 5 women) with CHF were evaluated initially and baseline data were obtained. Patients were assigned a "heart-failure score" based on the degree of dyspnea, pulmonary sounds, signs of right-heart failure, and chest film abnormalities. All patients were continued on digitalis with diuretics and/or vasodilators throughout the study period. Patients received 6 grams per day in divided doses of either taurine or placebo for four weeks, followed by a 2-week "wash-out" period. Prior to the cross-over period, baseline data were obtained for the following study period, in which patients received placebo or taurine, whichever was not taken during the first study period. Heart-failure scores fell from 5.8 ± 0.7 before taurine administration to 3.7 ± 0.5 after taurine ($p < 0.001$); the score did not change significantly during the placebo period. A "favorable response was observed in 79 percent (11/14 patients) during the taurine-treated period and in 21 percent (3/14 patients) during the placebo-treated period; 4 patients worsened during the placebo period, whereas none did during the taurine period (p less than 0.05)."[22]

Research has also been conducted in animals to determine whether oral taurine increased survivability in CHF, which resulted from surgically-induced aortic regurgitation. Albino rabbits received either taurine (100 mg/kg) or placebo after surgical damage to the aortic cusps, which produced aortic regurgitation. "Cumulative mortality at 8 weeks of non-treated rabbits following aortic regurgitation was 52% (12/23 animals) compared with 11% (1/9 animals) in taurine-treated group (p less than 0.05)... Taurine prevented the rapid progress of congestive heart failure induced artificially by aortic regurgitation, and consequently prolonged the life expectancy." [23]

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Bile Acid Conjugation and Cholesterol Excretion

The liver forms a 2-4 gram bile acid pool that has approximately ten enterohepatic cycles per day, with the terminal ileum serving as the main absorption site for the enterohepatic recycling of approximately 80 percent of these acids. Bile acids function as a detergent for emulsification and absorption of lipids and fat-soluble vitamins. Critical to this function of bile are the bile salts, which, because of their lipophilic and hydrophilic components, can lower surface tension and form micelles. Two major bile acids are derived from hepatic cholesterol metabolism: cholic acid and chenodeoxycholic acid. From these primary bile acids, intestinal bacteria form the secondary bile acids deoxycholic acid and lithocholic acid, respectively. For these bile acids to be solubilized at physiological pH, it is essential they be conjugated through peptide linkages with either glycine or taurine; these amino acid conjugates are referred to as bile salts.

Taurine conjugation of bile acids has a significant effect on the solubility of cholesterol, increasing its excretion, and administration of taurine has been shown to reduce serum cholesterol levels in human subjects. In a single-blind, placebo-controlled study, 22 healthy male volunteers, aged 18-29 years, were randomly placed in one of two groups and fed a high fat/high cholesterol diet, designed to raise serum cholesterol levels, for three weeks. The experimental group received 6 grams of taurine daily. At the end of the test period, the control group had significantly higher total cholesterol and LDL-cholesterol levels than the group receiving taurine.[24]

Cystic Fibrosis

Most cystic fibrosis (CF) patients suffer from nutrient malabsorption, where much of the insult is in the ileum. Since the terminal ileum serves as the main absorption site for the enterohepatic recycling of approximately 80 percent of bile acids, they are malabsorbed as well. Taurine supplementation has been shown to decrease the severity of steatorrhea associated with many CF cases.[25,26] In one double-blind crossover study, 13 CF children with steatorrhea of at least 13 grams per day were treated with a taurine dose of 30 mg/kg/day. The study continued for two consecutive 4-month durations and involved both placebo and treatment periods. Ninety-two percent of the CF children showed decreased fecal fatty acid and sterol excretion while taking taurine.[25] In CF patients with a high degree of steatorrhea, bile acid absorption was increased with taurine supplementation, suggesting a possible role for taurine in treating malabsorption.[26]

Detoxification

Due to its ability to neutralize hypochlorous acid, a potent oxidizing substance, taurine is able to attenuate DNA damage caused by aromatic amine compounds in vitro.[27] Because of taurine's unique structure, containing a sulfonic acid moiety rather than carboxylic acid, it does not form an aldehyde from hypochlorous acid, forming instead a relatively stable chloroamine compound. Hence, taurine is an antioxidant that specifically mediates the chloride ion and hypochlorous acid concentration, and protects the body from potentially toxic effects of aldehyde release.

Taurine has also been reported to protect against carbon tetrachloride-induced toxicity.[28-31] In rats exposed to carbon tetrachloride ($CCl_4$), hepatic taurine content decreased significantly 12 and 24 hours after $CCl_4$ administration. However, oral administration of taurine to $CCl_4$-exposed rats was able to protect these animals from hepatic taurine depletion, suggesting that hepatic taurine may play a critical role in the protection of hepatocytes against hepatotoxins such as $CCl_4$.[28]

Exposure to bacterial endotoxins has been suggested as one factor which can augment the magnitude of individual responses to xenobiotics.[32] Circulating endotoxins of intestinal origin have been found to create a positive feedback on endotoxin translocation from the gut, stimulating increases in serum endotoxin levels. In experimental animals, taurine was found to significantly inhibit intestinal translocation and to protect the animals from endotoxemic injury.[33] Therefore, it is possible taurine might be able to modify factors underlying susceptibility to toxic chemicals.

Hepatic Disorders

Two groups of patients with acute hepatitis, all with serum bilirubin levels above 3 mg/dl, were studied in a double-blind, randomized protocol. Subjects in the preventative strategy group received 4 grams of taurine three times daily. Bilirubin, total bile acids, and biliary glycine:taurine ratio all decreased significantly in the taurine group within one week as compared to controls.[34]

Alcoholism

Twenty-two patients undergoing preventative strategy for alcohol withdrawal were given 1 gram of taurine three times per day orally for seven days. When compared to retrospective controls, significantly fewer of the taurine-treated patients had psychotic episodes (14% vs. 45%, $p < 0.05$). The number of psychotic cases after admission who had also been psychotic before admission was 1/16 for the taurine group and 11/17 for the controls ($p < 0.001$).[35]

Recently, acamprosate, a synthetic taurine analog, has been shown to be clinically useful for the preventative strategy of alcohol dependence.[36-41] Currently available only in Europe, acamprosate (calcium acetylhomotaurinate) has a chemical structure similar to that of gamma-aminobutyric acid, and is thought to act via several mechanisms

affecting multiple neurotransmitter systems, and by modulation of calcium ion fluxes. About 50 percent of alcoholic patients relapse within three months of preventative strategy. In a pooled analysis of data from 11 randomized, placebo-controlled trials involving a total of 3,338 patients with alcohol dependence, those treated with acamprosate showed higher abstinence rates and durations of abstinence during 6- to 12-month post-preventative strategy follow-up periods, when compared to those receiving placebo.[36]

In a two-year, randomized, double-blind, placebo-controlled study, 272 patients initially were given short-term detoxification preventative strategy, and then received routine counseling and either acamprosate or placebo for 48 weeks, after which they were followed for another 48 weeks without medication. Subjects who received acamprosate showed a significantly higher continuous abstinence rate at the end of the preventative strategy period compared to those who were assigned to the placebo group (43% vs 21%, $p = .005$), and they had a significantly longer mean abstinence duration of 224 vs 163 days, or 62 percent vs 45 percent days abstinent ($p < .001$). However, there was no difference in psychiatric symptoms. At the end of a further 48 weeks without receiving study medication, 39 percent and 17 percent of the acamprosate- and placebo-treated patients, respectively, had remained abstinent ($p = .003$).[37]

Two in vitro studies have been published comparing the effects of acamprosate and calcium acetyltaurinate on ionic membrane transfer.[40,41] Ethanol has been shown to reduce ionic transfer through alterations in the cationic paracellular pathway, the coupling between two adjacent epithelial cells, the monovalent cation pump, and the antiport system. In both of these studies, the results indicate two closely related compounds have different effects on ionic membrane transfer. Therefore, caution should be used in extrapolating the effects of acamprosate to taurine or other taurine analogs.

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Ocular Disorders

The retina contains one of the highest concentrations of taurine in the body. In cats, when the retina has been depleted to about one-half its normal taurine content, changes in the photoreceptor cells begin to appear, and further depletion can result in permanent retinal degeneration.[42] In some respects, the retinal degeneration seen in the human disease retinitis pigmentosa (RP) is similar to that observed in taurine-deficient cats. However, studies of plasma and platelet taurine levels in patients with RP have yielded very inconsistent results.[43-45] A clinical trial of taurine (1-2 g/day) for one year in patients with RP did not result in any laboratory or clinical evidence of improvement, although some subjective benefits were reported.[46]

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Epilepsy

Although several clinical trials involving taurine supplementation in epileptic patients have been reported, most have major methodological flaws.[47] Depending on the criteria used, the degree of success reported in various trials using taurine for the preventative strategy of epilepsy has been between 16 and 90 percent.[48-56] In these trials, dosages ranged from 375 to 8,000 mg/day. The precise role of taurine in synaptic transmission is uncertain, and its antiepileptic action, confirmed in several models of experimental epilepsy and in short-term clinical studies, does not seem to possess major clinical relevance since trials with a longer follow-up period have generally produced less satisfactory results. Taurine's limited diffusibility across the blood-brain barrier may be the main factor restricting the antiepileptic effect of this compound.

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Alzheimer's Disease

Levels of the neurotransmitter acetylcholine have been described as abnormally low in patients with Alzheimer's disease. These insufficient levels are presumed to be related to the memory loss which characterizes the condition, and preventative strategy of Alzheimer's disease based on this premise has been proposed.[57] Taurine administered to experimental animals has been able to increase the level of acetylcholine in the brain,[58] and researchers have demonstrated that decreased concentrations of taurine are present in the cerebral spinal fluid of patients with advanced symptoms of Alzheimer's disease when compared to age-matched controls.[59] To date, no clinical trials on the use of taurine for the treatment of Alzheimer's disease have been reported in the medical literature.

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Diabetes

Both plasma and platelet taurine levels have been found to be depressed in insulin-dependent diabetic patients; however, these levels were raised to normal with oral taurine supplementation. In addition, the amount of arachidonic acid needed to induce platelet aggregation was lower in these patients than in healthy subjects. Taurine supplementation reversed this effect as well, reducing platelet aggregation. In vitro experiments demonstrated that taurine reduced platelet aggregation in diabetic patients in a dose-dependent manner, while having no effect on the aggregation of platelets from healthy subjects.

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Conclusion

Although it is readily apparent that taurine is important in conjugating bile acids to form water-soluble bile salts, only a fraction of available taurine is used for this function. Taurine is also involved in a number of other crucially important processes, including calcium ion flux, membrane stabilization, and detoxification. Some areas of investigation into the clinical uses of taurine have revealed significant applications for this amino acid: congestive heart failure, cystic fibrosis, toxic exposure, and hepatic disorders. Other conditions such as epilepsy and diabetes will require further research before a clear rationale for the use of taurine can be developed.

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**Figure 1.** Structure of Taurine

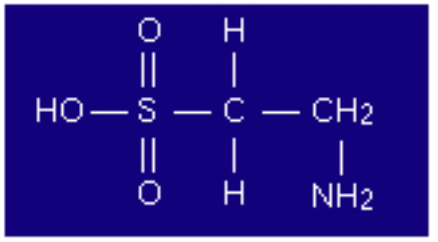
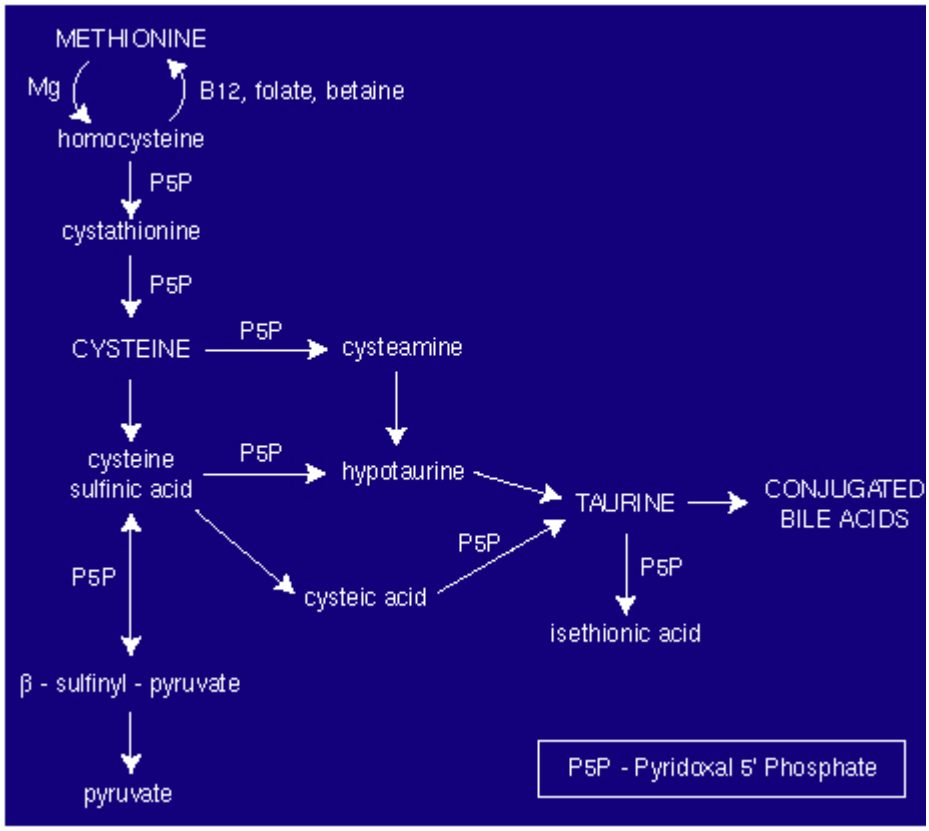



**Figure 2.** Synthesis of Taurine



# Hormone Panels

*It is Applied Longevity'ss goal to provide current and reliable hormonal information that is easily interpreted. That is why we have trade marked and included the phrase Timed Hormone Capture™ in our name. Our test results not only reflect the endogenous and supplemented ranges based on age, gender, menses status, phase cycle; but also, the time of collection- which has circadian implications.*

## ***Child Comprehensive Panel***

The *Child Comprehensive Panel* provides a reliable stress marker, revealing adrenal, sex hormone, and electrolyte imbalances. Changes in intra cellular levels of electrolytes, cortisol and DHEA-SO4 indicate shifts in adrenal function, catecholamine and aldosterone production that can affect the child's energy, emotional state, disease resistance. The 6 Electrolytes include Na, K, Cl, P, Ca, Mg.

**Sample Collection Times**
**Cortisol** is collected at 8AM, Noon, 4PM, 8PM, Midnight, and 4AM.
**DHEA-SO4** is collected at 8AM, 8PM, and Midnight.
**Electrolytes (Na, K, Cl, Ca, P, Mg)** are included in the 4PM collection tubes.

The *Child Comprehensive Panel* examines 6 samples for cortisol, 3 samples for DHEA-SO4, 1 sample for sodium, potassium, chloride, phosphate, magnesium, calcium, and 1 sample for estradiol, progesterone and testosterone.

## ***Male or Female Hormone Panel***

### Adrenal Stress Panel

The *Adrenal Stress Panel* provides a reliable stress marker, revealing adrenal imbalances. Changes in circulating levels of cortisol and DHEA-SO4 indicate shifts in adrenal function that can affect an individual's energy, disease resistance, and emotional state.

The adrenal hormones, cortisol and DHEA-SO4, are directly involved in the body's growth, immune response, and cardiovascular function. Cardiovascular disease, chronic fatigue, depression, and osteoporosis are some of the conditions that result from adrenal hormone imbalance. They affect carbohydrate, protein and lipid metabolism, act as anti-inflammatory agents, modulate thyroid function, and help in stress related conditions.

The adult *Adrenal Stress Panel* examines 6 samples for cortisol and 3 samples for DHEA-SO4. A pre-paid collection kit may be obtained from the laboratory. The adolescent (ages 8-14) *Adrenal Stress Panel* examines 6 samples for cortisol and 3 samples for DHEA-SO4..
The pediatric (ages 0-7) *Adrenal Stress Panel* examines 5 samples for cortisol. The 4AM cortisol and all DHEA-SO4 samples are not required.
**Sample Collection Times**
**Cortisol** is collected at 8AM, Noon, 4PM, 8PM, Midnight, and 4AM.
**DHEA-SO4** is collected at 8AM, 8PM, and Midnight.

## ***Female Hormone Panels***

### Female Circadian Panel

The *Female Circadian Panel* is designed to give the physician a snapshot of the current hormonal status over a 24hour period. This panel should be used as a screening resource when the physician needs to have immediate information. Cycling women need to include the calendar menses day along with time of day the specimen is taken for proper laboratory reference range matching.

The *Female Circadian Panel* examines 6 samples for cortisol, 3 samples for DHEA-SO4, 1 sample for sodium, potassium, chloride and 1 sample for estradiol, progesterone and testosterone.

A prepaid collection kit is available from the laboratory.

**Sample Collection Times**
**Estradiol, progesterone** and **testosterone** are collected at 8AM.
**Cortisol** is collected at 8AM, Noon, 4PM, 8PM, Midnight, and 4AM.
**DHEA S** is collected at 8AM, 8PM, and Midnight.
**Electrolyte Panel (Na, K, Cl)** is collected at 4PM.

### Female Hormone Panel

Both PMS sufferers and postmenopausal women usually experience significant health changes. Many of the conditions associated with PMS and menopause can be corrected with natural replacement and/or nutritional therapies. The key to successful supplementation is assessing the individual by performing baseline and follow up evaluation of their hormonal status. Only by pre and post replacement evaluation can one accurately bring the body into "hormonal balance".

The *Female Hormone Panel* examines 7 samples for estradiol and progesterone, 1 sample for sodium, potassium, chloride and testosterone, 6 samples for cortisol, 3 samples for DHEA-SO4, collected at designated days and times. This allows adrenal stress, circadian and cyclic evaluation since PMS and menopause can affect cortisol, DHEA, and melatonin levels.
A prepaid collection kit is available from the laboratory.

New cycle definition
For cycling (menstruating) women your new cycle begins the day your bleeding starts.
For menopausal women day 2 is the day you start collecting your samples.

**Sample Collection Times**

**Estradiol** and **progesterone** are collected at 8AM on days 2,10,12,14,16,18 and 24 of the cycle.
**Cortisol** is collected on day 2 at 8AM, Noon, 4PM, 8PM, Midnight, and 4AM.
**DHEA S** is collected on day 2 at 8AM, 8PM, and Midnight.
**Electrolyte Panel (Na, K, Cl)** is on day 2 at 4PM.
**Testosterone** is collected on day 2 at 8AM.

# ***Male Hormone Panel***

**Male Hormone Panel**

The *Male Hormone Panel* examines 1 sample for sodium, potassium, chloride, progesterone and estradiol, 6 samples for cortisol, 4 samples for testosterone, and 3 samples for DHEA-SO4. All samples are collected throughout the same day and at designated times. This allows for adrenal stress and melatonin circadian evaluation.
A prepaid collection kit is available from the laboratory.

**Sample Collection Times**
**Testosterone, Progesterone** and **estradiol** are collected at 8AM.
**Cortisol** is collected at 8AM, Noon, 4PM, 8PM, Midnight, and 4AM.
**DHEA S** is collected at 8AM, 8PM, and Midnight.
**Electrolyte Panel (Na, K, Cl)** is collected at 4PM.

## Timed Saliva Hormone Ranges

**(Revised 4/2014)**

**Cortisol New ranges effective 4/2014**

| | | |
|---|---|---|
| 8AM (LC/MS/MS) | 5.5 – 24.8 | nmol/L |
| Noon | 3.8 – 13.2 | nmol/L |
| 4PM | 2.2 – 9.4 | nmol/L |
| 8PM | 1.6 – 1.6 | nmol/L |
| Midnight | 0.8 – 3.3 | nmol/L |
| 4AM | 1.1 – 9.4 | nmol/L |

**DHEA-SO4**

| | | |
|---|---|---|
| 8AM | 1.6 – 18.5 | nmol/L |
| 8PM | 1.0 – 10.5 | nmol/L |
| Midnight | 0.8 – 8.3 | nmol/L |

**Estradiol** (8 AM levels)

Female

Endogenous

| | | |
|---|---|---|
| Premenopausal : | | |
| follicular | 3.50 – 8.00 | pg/ml |
| midcycle | 2.50 – 5.50 | pg/ml |
| luteal | 2.50 – 5.00 | pg/ml |
| Postmenopausal: | 0.50 – 1.50 | pg/ml |

Male

| | | |
|---|---|---|
| 20 – 49 yrs | 0.5 0 – 3.00 | pg/ml |
| 50 – 85 yrs | 0.50 – 5.00 | pg/ml |

**Estradiol (Supplemented)**

| | | |
|---|---|---|
| Oral Replacement | 2.0 – 20 | pg/ml |
| LipoDermal Cream* | 2.5 – 10 | pg/ml |
| Pharmaceutical Patch | 0.5 – 3.0 | pg/ml0 |

*Ranges represent estradiol trough levels

**Progesterone / Estradiol Ratios**

Day 2 - 30:1
Day 10 - 30:1
Day 12 - 40:1
Day 14 - 100:1
Day 16 - 100:1
Day 18 - 70:1
Day 24 - 40:1
**Menopausal (supplemented)** 100-300:1

**Estriol** (8 AM levels)

Endogenous

| | | |
|---|---|---|
| Target Range | 4 – 12 | pg/ml |
| Cycling | <3 | pg/ml |
| Menopausal | <3 | pg/ml |

**Electrolytes** (4PM Adult Ranges)

| | | | | | |
|---|---|---|---|---|---|
| Salivary Sodium | 3.1 – 39.8 | mEq/L | Salivary Calcium | 3.70 - 10.9 | mEq/L |
| Salivary Potassium | 3.7 – 31.0 | mEq/L | Salivary Phosphorous | 4.58 - 27.1 | mEq/L |
| Salivary Chloride | 20.0 – 49.6 | mEq/L | Salivary Magnesium | 0.46 - 1.28 | mEq/L |

**Progesterone** LC/MS/MS (8 AM levels) **New ranges effective 4/2014**

Female

Endogenous

| | | |
|---|---|---|
| Premenopausal | | |
| follicular | 50 – 130 | pg/ml |
| midluteal | 75 – 500 | pg/ml |
| luteal | 75 – 300 | pg/ml |
| Postmenopausal | 40 – 95 | pg/ml |
| Male | 29 – 110 | pg/ml |

**Progesterone (Supplemented)**

| | | |
|---|---|---|
| Oral Replacement | 100 – 500 | pg/ml |
| Applied Longevity Creams | 200 – 600 | pg/ml |

| Sex | **Testosterone** Age | New Ranges 9/26/2014 | Sex | Age | New Ranges 9/26/2014 |
|---|---|---|---|---|---|
| *Female* | 0 - 7 | 2.2-3.8 | Male | 0 - 7 | 2.4 - 21.6 |
| | 8 - 12 | 3.5-10.5 | | 8 - 12 | 5.7 - 47.6 |
| | 13 - 17 | 9.5-31.5 | | 13 - 17 | 10.7-127.8 |
| | 18 - 39 | 10.0-38.7 | | 18 - 39 | 10.7-148.3 |
| | 40 - 70 | 7.0-31.6 | | 40 - 70 | 8.6- 90.0 |
| | 71 - 99 | 5.0—22.1 | | 71 - 99 | 5.0- 67.7 |

# Urinary Metabolite Ranges 8/2014

| Metabolite | Age | Ranges | Units of Measure |
|---|---|---|---|
| Serotonin (LC/MS/MS) | All | 55.6 - 155.4 | ug/ g cr |
| Dopamine (LC/MS/MS) | 0 - 4 | 104.0 - 430.0 | ug/ g cr |
| | 5 - 10 | 94.0 - 420.0 | ug/ g cr |
| | 11 - 17 | 99.0 - 276.0 | ug/ g cr |
| | 18 & up | 92.7 - 212.0 | ug/ g cr |
| Serotonin/Dopamine Ratio | All | 0.49 - 1.67 | |
| Nor Epinephrine (LC/MS/MS) | 0 - 4 | 10.1 - 134.7 | ug/ g cr |
| | 5 - 10 | 11.4 - 90.0 | ug/ g cr |
| | 11 - 17 | 14.5 - 82.0 | ug/ g cr |
| | 18 & up | 15.7 - 44.9 | ug/ g cr |
| Epinephrine (LC/MS/MS) | 0 - 4 | 2.4 - 29.4 | ug/ g cr |
| | 5 - 10 | 4.6 - 30.6 | ug/ g cr |
| | 11 - 17 | 3.3 - 30.1 | ug/ g cr |
| | 18 & up | 3.8 - 9.8 | ug/ g cr |
| Norepi/Epi Ratio | 0 -10 | 4.39 - 9.12 | |
| | 11 -17 | 3.64 - 12.40 | |
| | 18 & up | 2.69 - 9.12 | |
| GABA (LC/MS/MS) | 0 - 2 | 0 - 3.10 | umol/ g cr |
| | 3 - 17 | 0 - 6.22 | umol/ g cr |
| | 18 & up | 0.12 - 4.12 | umol/ g cr |
| Glutamate (LC/MS/MS) | 0 - 2 | 0.3 - 169.2 | umol/ g cr |
| | 3 - 17 | 0.6 - 66.0 | umol/ g cr |
| | 18 & up | 0.8 - 19.8 | umol/ g cr |
| Free Histamine (LC/MS/MS) | All | 10.5 - 32.0 | ug/ g cr |
| Creatinine (10AM Collection) | All | 10.0 - 250 | mg/ dl |

Please Note: Out of Range Creatinine levels indicate collection errors and can compromise the interpretation of results. Assays will not be run and a new sample will be requested at the expense (shipping) of the patient.

## THE APPLIED LONGEVITY SYSYTEM FOR ACHIEVING HORMONAL BALANCE

The following program is designed to help you to establish a program that is individual according to your panel. By using the Applied Longevity system of hormonal balance you can, within a few months, achieve optimum hormonal function. The system has been developed taking into account the associated problems that usually help perpetuate the hormonal imbalance. This approach systematically re-establishes optimal function to all the contributing systems as well. This has a powerful impact on the entire body and allows for the regeneration of the stress handling system.

### THE TESTING™ ANALYSIS (Saliva Hormone Test): WHAT DOES IT MEAN?

It's impossible to give a simple explanation as to what your results mean. The Testing Analysis™ is a very powerful research tool and will help you to achieve whatever health goal you wish, as long as it's used properly. Unlike most traditional blood tests, it is not a matter of interpreting the result as to whether it's within normal ranges. ***Hormones work in equilibrium with one another.*** Understanding salivary hormones requires an understanding of the inter-relationships between the stress and sex hormones. The Testing Analysis™ must be reviewed by a health care professional that has been trained specifically in the interpretation of salivary hormones. Most physicians do not have this training and will want to contact the technical services department for assistance. We encourage you to work with a healthcare professional. It is our goal to develop a network of practitioners that have been trained in evaluating our results and, assist you with a structured supplementation protocol.

### READING A REPORT

Each report contains your identification information at the top of the page.
Basic interpretation: How do I determine if a result is abnormal?

On each report the numbered results appear in a specific order divided between five columns. Each column represents a different value. The first column on the left that falls over the left margin is the "abnormal" section. A result will only appear in this column if it is abnormal. The next set of numbers to the right represent the time the specimen was collected. Example 8am would mean the specimen was collected at eight in the morning. The next column contains the reported values of each test. The first result on all tests is the adrenal hormone "cortisol." The first reported value is the 8AM. This appears just right of the time indicating 8AM. The last column of numbers represents "Normal Ranges." These are the expected values for each time period.

*Applied Longevity, Testing, and DermaTrans protocols are for research and information purposes only. We make no claims or recommendations for diagnosis, preventative strategy or cure of any disease.*

## FEMALE PROTOCOL

**PRACTITIONER NOTE:**

The following protocols are intended for professional information purposes, to assist practitioners in choosing appropriate preventative strategies and is not intended to recommend preventative strategy or to make specific diagnosis. Decisions on patient care should be based on all laboratory tests, health histories and clinical presentation.

## THE BASELINE

The comprehensive Testing™ analysis has provided a baseline evaluation and starting point in your program. It is important that you review the following suggestions so that you may institute a program that will yield the desired results.

## THE BALANCE

The Applied Longevity System recognizes the importance of baseline testing, evaluation and implementing a protocol that addresses the steroid sex hormones, the steroid adrenal hormones, electrolyte and amino acid (9 essential) balancing, and digestive transformation.

**The female hormonal balance program is divided into 3 different stages**

*Stage 1* will focus on the systems involved in the fight/flight mechanism (allostatic load), affecting the adrenal stress hormones. It is important to understand that hormonal balance starts with adrenal support. In order to achieve hormonal balance we have to support the adrenal response, i.e. if the stress hormones (cortisol, DHEA) are elevated or diminished we need to help bring them into range by proper supplementation.

Stage 1 is designed to:
A. Normalize adrenal function.
B. Eliminate hyper-vigilance (reduce tendency to enter fight/flight mode).
C. Help stabilize blood sugar.
D. Help normalize digestive function and improve absorption of nutrients.
E. Length of Stage 1: 90 days.

*Stage 2* Once the body turns off the adrenal response, direct building material and the vital energy needed to repair our system can be utilized.

Stage 2 is designed to:
A. Help normalize sex hormone levels by proper supplementation.
B. Re-establish healthy dynamics of estrogen and progesterone (by adjusting the ratio of progesterone to estradiol).
C. Length of Stage 2: 120 days

Stage 3 Our records show we can optimize metabolic balance and improve hormonal utilization and clearance by electrolyte and amino acid supplementation.

Stage 3 is designed to:

A. Maintain the hormonal progress and stabilize hormonal dynamics.
B. Maintain digestive improvements (once dietary changes are implemented).
C. Utilize **AminoStat™**, **7-KetoGenic™** DHEA and **Stat*Lyte*™** combination. This will be used as a 2nd crème, applied in the early afternoon.
D. Help liver synthesis and metabolism.
E. Increase energy and stamina.
E. Length of Stage 3: 6-12 months.

**Female Protocol (Continued)**

A. First, second, and third month utilize Formula A (adrenal formula with progesterone). Menstruating females – use limited to days 7-28. Peri-menopausal and menopausal females for the entire month (days 1-28). Fourth, fifth and sixth month utilize a bi-phase crème (progesterone and phytoestrogen). Menstruating females – use limited to days 7-28. Peri-menopausal and menopausal females for the entire month (days 1-28).
C. At the end of 150 days, re-test by ordering the 24 hour retest panel.
D. Seventh month utilizes:
    1. Custom Formula Phase I days 1-15.
    2. Custom Formula Phase II days 16-28.
E. Eight month utilizes:
    1. Custom Formula Phase I days 1-15.
    2. Custom Formula Phase II days 16-28.
F. At the end of the ninth month re-test by ordering the 24 hr retest panel.

G. Repeat the full panel on a yearly basis.

**Definitions:**

Day 1 is first day of menstrual cycle.
Day 1 for non-menstruating women is first day the cream is started.

# FEMALE FORMULA ACTIVE INGREDIENTS

**Custom Formulas**

Water, Glycerin, Glycerol Monolaurate, Glycerol Stearate, Octyl Salicylate, Cocoa Glucoside, Capryllic/Capric Triglyceride, Progesterone, Avena Sativa Extract, PEG 8 Distearate, Safflower Oil, Polysorbate, Maca Extract, Proprietary Homeopathics, Pregnenolone, Stevia Extract, DHEA, Marapuama Extract, Aloe Vera, Purified Phospholipids, Carbomer, Fragrance, Alpha Lipoic Acid, Vitamin B12, Citrus Seed Extract, Phenoxyethanol, Paraben Esters, EDTA, Propyl Gallate, and Ginkgo Biloba Extract.

Custom formulas are made according to your test results, and your needs. Specific amounts of ingredients are added appropriately.

(Phyto-Estrogen Formula)
Water, Glycerine, Glycerol Monolaurate, Octyl Salicylate, Glycerol Stearate, Cocoa Glucoside, Capryllic/Capric Triglyceride, PEG 8 Distearate, Safflower Oil, Pregnenolone, Polysorbate, Maca Extract, Proprietary Homeopathics, Chasta Tree Extract, Dong Quai Extract, Soy Isoflavone, Muira Puama Extract, DHEA, Stevia Extract, Aloe Vera, Purified Phospholipids, Carbomer, Fragrance, Alpha Lipoic Acid, Citrus Seed Extract, Phenoxyethanol, EDTA, Propyl Gallate, and Ginkgo Biloba Extract.

Phase I Custom Crème formulas include the above ingredients in higher concentration.

(Progesterone Formula)
Water, Octyl Salicylate, Glycerine, Glycerol Monolaurate, Progesterone, Glycerol Stearate, Cocoa Glucoside, Capryllic/Capric Triglyceride, Avena Sativa Extract, PEG 8 Distearate, Safflower Oil, Pregnenolone, Polysorbate, Maca Extract, Proprietary Homeopathics, Collodial Silver, DHEA, Stevia Extract, Licorice Root Extract, Aloe Vera, Purified Phospholipids, Carbomer, Fragrance, Alpha Lipoic Acid, Citrus Seed Extract, Phenoxyethanol, Paraben Esters, EDTA, Propyl Gallate, Ginkgo Biloba Extract and Vitamin B12.

Phase II Custom Crème formulas include the above ingredients in higher concentration.

Apply crème to soft thin skin, (i.e. neck, chest, upper thighs, inner arms, behind the knees). The crème is not to be applied to areas of high fat concentration.

Please note that jars are packed by weight not volume. One jar will last approximately 25 to 27 days using ¼ tsp twice a day.

# MALE PROTOCOL

## THE BASELINE

The comprehensive Testing™ analysis has provided a baseline evaluation and starting point in your program. It is important that you review the following suggestions so that you may institute a program that will yield the desired results.

## THE BALANCE

**The following protocol was specifically designed to help:**

- Normalize adrenal function.
- Improve digestive function and absorption.
- Improve sexual function.
- Balance Cortisol and DHEA levels.
- Stabilize blood sugar.

**Length of Protocol** : 6 Months.
Apply ¼ tsp. upon rising daily. ¼ tsp. 2 hours before bedtime. It is best to apply crème after you have bathed (Shower or bath) in the morning, and again a couple of hours before bed. Crème can be applied to any area of soft skin (neck, chest, abdomen, inner arms and inner thighs.) It is best to apply to areas with minimal body fat. (If prostate or sexual function is your area of primary complaint apply to the inner thighs and scrotum.)
***Please note that crème jars are packed by weight and last approximately 25 to 27 days.***

**Active Ingredients**
R. roseola / Tribulus, DHEA, saw palmetto extract, Aloe Vera, pregnenolone, progesterone, avena sativa, damiana, muira puama, royal maca, Vitamins A,D,E, colloidal silver, grape seed extract.

**Dietary Changes:**
If your analysis suggests Malabsorption Syndrome, the following information will help this common cause and result of adrenal dysfunction:

The following dietary considerations are designed to help:

- Stabilize blood sugar.
- Help improve absorption.

While on the program it is recommended that you follow the following dietary considerations. (This is only while on program. After successful completion, you may incorporate them back into your diet.)

## SPECIAL DIETARY CONSIDERATIONS:

Avoid breads, pasta, and cereal or grain (wheat, oat, rye, corn) containing foods. Avoid cow's milk and all dairy products (cheese, yogurt, iced cream). Avoid all sources of caffeine (coffee, soft drinks, tea, chocolate). Decaffeinated coffee contains 50-60% of the caffeine of regular coffee. Avoid sweets, sugar, and all sources of hydrogenated oil.

## DIETARY CHANGES

Hormonal disturbances parallel those of the digestive system. Significant stress can be the result of malabsorption syndrome. Other digestive problems can also contribute to stress hormone related problems. In almost all cases that do not respond to the 3-step protocol, hormonal dysfunctions can be traced to digestive malfunction. A major contributor to sub-clinical conditions is gluten intolerance. Access the following web site to obtain information on symptoms and dietary recommendations.

http://www.csaceliacs.org/infocenter.html

SPECIAL DIETARY CONSIDERATIONS: No gluten, No milk or milk protein (cheese). No sugar. No caffeine of any type (coffee, sodas, tea.) NO ALCOHOL.

NO RECREATIONAL DRUGS ARE ALLOWED ON THIS PROGRAM.

## NUTRITIONAL SUPPLEMENTATION

Supplementation during the first phase is crucial. It provides the necessary support for repair of vital tissues.

The following nutritional supplements have been chosen specifically because of their ability to affect the systems in question.

## *LIPO*DERMAL™ ENDOCRINE CRÈME

The foundation of the program is a custom formulated LipoDermal endocrine crème. The crème will be labeled with the following: Your name. The date it was made. When and how frequently to apply it. The reference number. A printed list of ingredients will also be packaged with the crème. When you first open the crème you'll notice a pleasant natural smell (no perfumes are used) and a color that may change depending upon modifications to your formula. The crème may feel slightly grainy. This is due to some of the precursor endocrines. This is expected in some formulas and there is no need for concern. The crème should be measured using a small ¼ tsp. culinary or cosmetic spoon. (The lab does not supply a measuring devise). Take a small dab of crème and apply to any area of soft thin skin. (i.e.: neck, chest, upper thighs, and inner arms). The crème is not to be applied to areas of high fat concentration.

Note: If you are extremely sensitive to all smells, nutrients, or those components of the crème or wish to determine sensitivity prior to applying a full dose, apply a small amount to a cotton ball and place it behind your ear and secure with a small piece of paper tape. Check for any reaction after 2 hours. If you cannot tolerate tape adhesive cut a band from cotton sock approx 2" wide, use this band to hold a cotton ball against your ankle.

It is always best to start with a smaller than recommended amount. If ¼ tsp. twice daily is recommended, start with 1/8 tsp. once on the first day and for the first three days of use only in the morning. On the fourth ($4^{th}$) day add the evening/bedtime dose.

If you have any reaction (such as an inability to get to sleep) discontinue the night dose for the first two weeks then resume the night dose. **We find this weaning approach helpful for our extra sensitive clients.**

Note: A warming effect is felt in some of the crèmes. This effect is not harmful, but can sometimes be uncomfortable. For this reason do not apply to open skin, or use in the nose, mouth, anus, or vagina. Men can use the crème on the penis or scrotum with caution. *If warming or any discomfort is beyond tolerance, immediately rinse with cool clear water. The crème washes off quickly and a few moments should suffice.

The crème will be used from 6 to 12 months. The first step (90 -120 days) will be directed toward adrenal function, and the fight/flight response. The second step (120 and up) is directed toward sex endocrine (testosterone, progesterone, estrogen) balancing.

**RESTORING NORMAL FUNCTION TO THE DIGESTIVE SYSTEM**

It is important to remember that the body is integrated. The hormonal system is dependent on all other systems, especially the stress mechanisms to function correctly.

**Re-colonization program: Human Strain Probiotics**
PHASE 1: (14 days) Use Intensive Formula (powder), divide package in two equal amounts. Add 4 ounces of water and shake vigorously. Take ½ package 2 X's daily- 5 minutes prior to the start of breakfast and dinner- for the first 2 weeks.

PHASE 2: (60 days) Use High Potency formula (capsules): Take 1 Capsule by mouth 2 X's daily prior to breakfast and dinner.

PHASE 3: (indefinite) Use Maintenance formula (capsules): Take 1 capsule by mouth 2 X's daily, prior to breakfast and dinner.

Good digestion of food is important to adrenal function, energy production, as well as overall digestive health. As we get older our ability to digest protein declines. The reason for this is that the ability to digest proteins decreases after age 22. After this time, eating the same amounts of proteins may overload the system. Digestive enzymes aid digestion.

We recommend **Digest*All*™,** a dual phase digestive enzyme. This means that it affects both the digestive process in the stomach and the small intestine.

**Digest*All*™**

It is best to take digestive enzymes throughout the meal. If you are taking one, take it mid meal. If you are taking more than one, separate the dose, taking it at the beginning, as well as, at the end of the meal.

**Weight Chart**

80-140lbs: one (1) tablet taken with each meal. One (1) tablet with a large snack.

140-200lbs: two (2) tablets taken with each meal. One (1) tablet with a large snack.

Over 200lbs: two (2) to three (3) tablets taken with every meal.

# ADDITIONAL PROTOCOLS

## I. Liver Detox Protocol™

**(A) First 1 – 2 months.**

1) Detoxinol™ **formula (Use ¼ tsp @ 8AM/6PM)**

| | |
|---|---|
| Artichoke | 30g |
| DIM | 12g |
| Tyrosine | 5g |
| Glutamine | 5g |
| Folic | 5g |
| **StatLyte™** | 5g |
| Pyridoxine (B6) | 3g |

2) NattoKinase - Use 2 tabs, 3X daily, before meals.

3) Use antiviral 1 week after starting NattoKinase.

4) Start using **Ultra D™** Intestinal Cleanse immediately.

5) Start using **Digest*All*™** immediately.

6) Start Pro-Biotics after finishing with intestinal cleanse.

(B) After detox, start on the **Applied Longevity Crème Protocol** (for men or women).

## II. Adrenal Insufficiency (Flatline Cortisols/Inverted DHEA S) Protocol™ (First 2 months)

(A) Use ¼ tsp upon arising and at 2 PM
(Can start 1x/day and wean to 2X daily for extra sensitive Clients).

1) **AM Formula™** Use ¼ tsp upon awakening and at 2 PM.

| | |
|---|---|
| **TriPlex™** | 25g |
| **7 Ketogenic™ DHEA** | 10g |
| Pregnenolone | 15g |
| R. rosea extract | 10g |

2) Use 5 sprays **BioHGH™** (Sublingually) upon awakening.

Use ¼ tsp at bedtime.

(B) 1) **PM Formula™**

| | |
|---|---|
| Artichoke | 25g |
| **Stat*Lyte*™** | 15g |
| Folic | 5g |
| DIM | 10g |
| Phosphoserine | 10g |

2) Use 10 sprays **BioHGH™** (Sublingually) at bedtime.

(C) **Coral Cal/Mag Plus™** Use ½ tsp mixed in juice @ 8AM.

(D) B5 use ½ tsp mixed in juice @ 8 AM. Can be mixed with **Coral Cal/Mag Plus™.**

(E) After 2 months, start with regular crème protocol. Continue with **Coral Cal/Mag™** Plus**.**

## III. Immune Compromised (Adrenal-malabsorption-anti oxidant Support)

(A) **Adrenal Support Formula™**

| | |
|---|---|
| Pregnenolone | 15g |
| Folic Acid | 10g |
| B12 | 5g |
| **AminoStat™** | 20g |
| **TriPlex™** | 10g |
| GABA | 5g |

Application: ¼ tsp upon arising and @ 2 PM.

(B) **Anti Oxidant Formula™**

| | |
|---|---|
| **Stat*Lyte*™** | 15g |
| Melatonin | 10g |
| Artichoke | 15g |
| DIM | 10g |
| L-Taurine | 5g |
| Phosphoserine | 10g |

Application: ¼ tsp @ Bedtime.

(C) Supplement Support

1) Pro Biotics
2) **Digest*All*™**
3) **Ultra D™**
   2 tablets 3Xdaily take with minimum 12 oz of water.
4) Multi Omega Plex
   2 scoops in apple or pear juice prior to meals.
5) **Coral Cal/Mag Plus™**
   ½ tsp in juice @ 8AM/6PM.
6) Pantethenic Acid Vitamin B5.
   ½ tsp in juice @ 8AM/6PM.
7) B Complex Multi Vitamin.
8) **BioHGH™** Spray
   1500 ng/ application @ bedtime and upon arising.

(D) Dietary Controls

1) Off gluten
2) Off dairy
3) Drink 8-10 glasses of water daily

## IV. Injury and/or Inflammatory Protocol™

(A) **Inflammatory Crème Formula™**

| | |
|---|---|
| **AminoStat™** | 20g |
| L-Arginine | 20g |
| Pregnenolone | 20g |
| Folic | 5g |

**Application**

1/2 tsp apply @ site of inflammation or pain. Can be used with hormone formulas. Apply the crème 1-3 times daily, as needed.

(B) Use with NattoKinase, 2 tabs 3X daily, before meals**.**

**This protocol should be used at least for 3 months.**

## V. Profuse Sweating Protocol™ (First 2 months)

(A) **AM formula™ Use ¼ tsp @ 8AM**

| | |
|---|---|
| Artichoke | 20g |
| DIM | 15g |
| **TriPlex™** | 10g |
| Folic | 5g |
| **Stat*Lyte*™** | 10g |

(B) Use 5 Sprays **BioHGH**™ upon awakening

(C) **PM formula™ Use ¼ tsp @ bedtime.™**

| | |
|---|---|
| Melatonin | 15g |
| Gaba | 15g |
| Folic | 5g |
| DIM | 5g |
| L-Taurine | 5g |
| Phosphoserine | 20g |

(D) Use 10 sprays **BioHGH™** @ bedtime.

(E) NattoKinase - Use 2 tabs, 3X daily, before meals.

(F) Start using **Digest*All***™ immediately.

(G) Start Pro-Biotics after using **Ultra D™** intestinal cleanse.

(H) After 2 months, start on regular **Applied Longevity Crème Protocol**, maintain electrolyte support with **Stat*Lyte*™** crème.

## VI. PROTOCOL FOR 'Memory Loss' (ALZHEIMER'S SYMPTOMS)™

(A) ¼ tsp **7-KetoGenic™ DHEA**, twice daily (upon awakening and @ 1 PM).

(B) **Custom Formula** Apply crème 3x/day Dose is per ¼ tsp applied transdermally

| | |
|---|---|
| Phosphotidylcholine | 58mg per ¼ tsp |
| Centrophenoxine | 96mg per ¼ tsp |
| Aniracetam | 38mg per ¼ tsp |
| Vitamin E | 5mg per ¼ tsp |

(C) Use 3 Nattokinase tablets, 3X daily.

(D) NADH 10 -20 mg in AM.

(E) ½ tsp 1MagT subligual 3X Day

(F) Follow Pro Biotics and **Digest*All***™ protocols.

**Collagen Disease Protocol (Lupus, RA, etc.)**

(A) **Inflammatory Crème Formula™**

AminoStat™
L-Arginine
Pregnenolone
Transfer Factor

Application:

¼ tsp apply @ site of inflammation or pain. Can be used with hormone formulas. Apply the crème 1-3 times daily, as needed.

(B) Use with NattoKinase, 2 tabs 3X daily, before meals.

(C) **PM formula™ Use ¼ tsp @ 8PM**

Benfotiamine
GABA
Phenibut
5-HTP

(D) **Anti Cortisol Formula™ Use ¼ tsp @bedtime**

Phosphoserine Concentrate

(E) Use Histamine Response antagonist Crème (EzcemCare)

(F) Start using **Ultra *D*™** intestinal cleanser 7 days after starting NattoKinase.

(G) Start using **Digest*All*™** immediately.

(H) Start Pro Biotics after finishing intestinal cleanse.

(I) ***Activ8Plus*™**

## VITAMIN AND MINERAL BALANCE

**Pantothenic Acid (B-5)** supports adrenal function, increases adrenal endocrine production and often provides increased reserves of energy. We use pure powder formulas with no additives.
500 mg twice daily. This works in conjunction with biotin and folic acid. B-5 is also known for its effect on increasing peristalsis (muscular action needed to evacuate the bowel) and can be very helpful in establishing a normal bowel movement.

**Stress Formula Spray B Complex Vitamins -** sublingual: needed for energy metabolism and proper adrenal function. 3 sprays 3 times a day. Easily absorbed.

**Ascorbate Vitamin C -** nourishes the adrenals. Highest concentrations are found in the adrenals.

**Coral Cal/MAG Plus™ -** easily absorbed. Add ½ - 1 tsp to 6 oz of fruit juice and drink twice daily.

**OxyBlast™** (stabilized oxygen in a ph balanced formula) **-** helps to increase energy. OxyBlast delivers 50,000 ppm of stabilized oxygen per ml.

**Water** - Water is used as a coolant to keep the temperature of the organs constant, as well as a solvent to clean the cells of toxins and carry them out of the body. Water is very important especially for our program. Drink the minimum required amount (see examples below). Please note that tap water is unacceptable and that for the first 3 months only distilled water should be consumed.

It is very important to drink 2 to 3 ozs. per lb of body weight.

Example: 100lbs. body weight = 66 ounces of water per day.

Example: 150lbs body weight = 99 ounces of water per day.

**HERBS**

**Licorice Extract** - We use licorice extract to help maintain proper cortisol/aldosterone balance. Licorice extract is a mild source of the endocrine estradiol. It is a very important herb for females. Licorice extracts works as a stimulant on the adrenal glands. It contains glycosides, which can chemically purge excess fluid from the lungs and body. It is helpful for coughs and chest complaints. It is an important herb when recovering from illness for it will supply necessary energy to the system. It works as a laxative and helps inflammation in the intestinal tract and relieves ulcer conditions. It has a stimulating action and helps counteract stress.
It contains vitamin E, phosphorus, B-complex, biotin, niacin, and pantothenic acid. It also contains lecithin, manganese, iodine, chromium, and zinc.

**Royal Maca Extract** - Maca extract affects the control mechanisms of the hormonal system (the hypothalamus). 15 drops in 4oz. Water after breakfast and dinner.

**Saw Palmetto** – (If you suffer from prostate problems) Applied Longevity now has concentrated saw palmetto transdermal crème (2%) that can either be added to the existing custom formula or can be utilized on its own.

**Ginseng Extract** – 10-20 drops 3 times daily.

**Phosphorylated Serine** – (Use if saliva cortisol levels are high) Applied Longevity now has concentrated PS transdermal crème (2%) that can either be added to the existing custom formula or can be utilized on its own.

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Most of the above supplements are available from Applied Longevity, Ltd.. at USA 202-657-4034. Ask for the Customer service.

**RETESTING**

Retesting is a way to monitor progress of each phase, and to determine if the desired goals have been achieved.

It is recommended that you retest as follows:

1st retest should be 120 days from the time you started or prior to advancing to the maintenance crèmes. **Do not use your crème dose the night before the retest**. Resume the crème as soon as you have completed all the tests.

2nd retest should be 180 days. You want to retest prior to running out of crème. Follow the same procedure as above.

3rd retest should be done on a yearly basis. This should be a FULL 24-hour circadian test, or comprehensive (monthly cycle) retest panel. This includes the following tests: cortisol, DHEA, progesterone, estradiol, and electrolytes.

**WHAT TO EXPECT**

Hormonal problems can take years to manifest. It is unrealistic to believe that this or any program will work immediately. It is only by combining the recommendations made in the exact way and order suggested. This will insure the highest possibility of success. The first few days can bring immediate relief or an exacerbation of your symptoms. This is acceptable. Continued compliance will achieve your desired results. In the unlikely event that you experience problems (headaches, vomiting, or other extreme symptoms), contact your doctor and/or Applied Longevity immediately.

**WHAT ABOUT SUPPORT**

Client Services at Applied Longevity will be glad to discuss the system with you. They cannot give any medical advice but will discuss what the implications of each hormonal imbalance are and direct you as to selecting the appropriate supplement.

**Applied Longevity, Ltd.. is not equipped to act as your physician. Please do not ask or expect to receive medical advice from the research laboratory. We will be glad to discuss your results with your physician or practitioner.**

NOTE**: It is always best that qualified health care providers in your area monitor your progress, and work with us in helping you to achieve your desired goal.** We will be happy to talk with your physician and explain our interpretation of your case, and any recommendations felt necessary. If you do not have a doctor or someone qualified to act in this capacity, we may be able to recommend someone in your area. We have implemented a program to offer seminars for physicians to better help them utilize our program(s).

## WHAT ABOUT EXERCISE

There is a tendency when beginning a new program to throw ourselves into it head first. It is advisable to be conservative about the type and amount of exercise per week. If you have not been active prior to this program, it is best to start with non-invasive/non-impact exercise such as swimming, walking, and stretching. After the first phase, increase your exercise to include one or two aerobic exercise days or light weight training. This should be done with the help of a qualified instructor.

## HOW DO I ORDER TEST KITS AND PRODUCTS

Applied Longevity, Ltd. has made it very easy to place order for test kits, endocrine crèmes and other nutritional products. Simply call (202-657-4034) and ask for the customer service. Please be prepared to give the lab the following information:

Name, day time phone number, evening number, address where packages would be sent, address for billing purposes if different than billing and a method of payment. Applied Longevity accepts most major credit cards: American Express cards, Visa, MasterCard and Discover. If you wish to pay by check or money order, please call the customer service for information.

## ADDITIONAL INFORMATION

**MAGAZINE AND/OR ARTICLES:**
Alternative Medicine Magazine:
Issue 31 (Sept 1999) Avoiding Problems With Progesterone *by Michael Borkin*
Issue 34 (Mar 2000) Stress 101 *by Michael Borkin and Dr. William Stuppy*
Issue 37 (Sept 2000) Women's Hormones 101 *by Michael Borkin*

**WEB SITES***: RE: Endocrine Dysfunction* http://chronicfatigue.about.com
***Salivary Testing*** **http://www.Applied Longevity.com**

**OTHER WEB SITES YOU ARE ENCOURAGED TO VISIT**
http://www.alternativemedicine.com
http://www.Applied Longevity.com

**Applied Longevity also has an audiotape available by Michael Borkin that encompasses the basics of hormonal health.**

**GLUTEN INTOLERANCE**
You may contact the Celiac Association by calling or writing the following:
Telephone: (402) 558-0600
Fax (402) 558-1347
Mail: CSA/USA, Inc.
P.O. Box 31700
Omaha, NE 68131-0700
Email: celiacs@csaceliacs.org

Please read the following articles available on the Celiac Web site.

1. Celiac Disease: Then and Now
2. Celiac Disease and Insulin-Dependent Diabetes Mellitus
3. Cooking Chicken for Celiacs
4. Coping with Chronic Illness
5. Definitions Associated with Celiac Disease
6. Definitions in Food Labeling
7. Effects of Small Amounts of Gluten in the Diet of Celiac Patients
8. Food, Drug and Cosmetic Additives
9. Generics and What's New in Research
10. Gliadin in Foods
11. Medical Foods for Celiac Patients
12. Neurological Complications of Celiac Disease
13. Notes on Hypothyroidism

You may contact us by e-mail at: mailto:info@Applied Longevity.com Our website: www.Applied Longevity.com

## ***Effective Products Available***

All the products are available now by calling Applied Longevity, Ltd..™ at 202-657-4034.

| ***Benefits*** | Product |
|---|---|
| • **Attain More Satisfaction**<br>• **Elevates pheromone levels**<br>• **Helps Balance Endocrine System**<br>• **Increases Sexual Performance**<br>• **Intensity of orgasm**<br>• **Maintain Virility**<br>• **Natural Libido Enhancer** | **BIOSIS IC™ –** The original product by DermaTrans™. A libido enhancer for both men and women. Helps to buffer stress while naturally increasing important sex hormones. When used regularly, increases libido and helps to reduce overall stress. |
| • **Promotes Cell Longevity**<br>• **Antioxidant Protector**<br>• **Prevents Sun Damage**<br>• **Enhances Immune System** | **GENESIS IC™** – Systemic rejuvenation formula. Uses ultraviolet (UV) radiation as a trigger to increase its antioxidant protection. Protects against damaging UV rays. Increases immune activity, and speeds recovery of cells. May allow blood cells to live to complete maturity, thus extending cellular life. Contains potent immune modulators and growth hormone precursors. |
| • **PMS/ Hot Flashes Relief**<br>• **Menopausal Relief**<br>• **Stress Relief**<br>• **Influences Hormonal Balance**<br>• **Improve Metabolism**<br>• **Endocrine Support** | **BioFemme IC™** – Progesterone crème with estrogen blockers (natural tomoxafin). The foundation of our *BioFemme System*™, BioFemme IC is designed to re-balance your system and prepare for the use of *Bio-Est* and *Bio-Plex* maintenance crèmes. |

| ***Benefits*** | Product |
|---|---|
| | |
| | |

| Helps the Following Symptoms | |
|---|---|
| • **Loss of Libido**<br>• **Impotence**<br>• **Bloating and fluid retention**<br>• **Mood Swings**<br>• **PMS**<br>• **Menopause**<br>• **Poor Memory**<br>• **Sleep Disturbances** | **ENDOSIS™** – Adrenal formula designed to affect cortisol/DHEA equilibrium. Normalizes cortisol and allows for adrenal recovery. |
| • **Increases energy**<br>• **Increases oxygen levels** | **OXYBLAST™ –** Purified water containing 50,000 ppm-stabilized oxygen, in a pH-balanced solution. |

| Benefits | *Product* |
|---|---|

**Helps the Following Symptoms**

- **Depression**
- **Fatigue**
- **Abdominal pains**
- **Muscle weakness and cramps**

4oz/8oz

**B5 (PANTOTHENIC ACID)** Pantothenic acid (B5), a B-complex vitamin, is essential for humans and animals for growth, reproduction, and normal physiological functions. It is a precursor of the coenzymes, CoA and acyl carrier protein of fatty acid synthase, which are involved in more than 100 different metabolic pathways including energy metabolism of carbohydrates, proteins and lipids, and the synthesis of lipids, neurotransmitters, steroid hormones, porphyrins and hemoglobin.

Deficiencies: Pantothenic acid deficiency has been induced in animals when fed natural feedstuffs containing low levels of pantothenic acid. Deficient animals had growth retardation with reduced food intake, functional impairments in all systems and sudden death. Pantothenic acid deficiency has also been induced in humans by use of a metabolic antagonist, w-methyl pantothenic acid along with a pantothenic acid-deficient diet. Signs and symptoms reported include depression, personality changes, cardiac instability, frequent infection, fatigue, abdominal pains, sleep disturbances and neurological disorders including numbness, paresthesia (abnormal sensation such as "burning feet" syndrome), muscle weakness and cramps. Biochemical changes include increased insulin sensitivity, lowered blood cholesterol, decreased serum potassium, and failure of adrenocorticotropin to induce eosinopenia.

Recommendations: Adults – 500mg, twice daily.

Toxicity: In humans, the only reported symptom after intakes of 10 to 20 g calcium pantothenic acid was diarrhea.

Recent research: A pantothenic acid derivative, pantethine (two molecules of pantetheine joined by a disulfide bond), has been reported to have a hypocholesterolemic effect. A metabolic antagonist of pantothenic acid, pantoyl g-amino butyric acid (called pantoyl-GABA, homopantothenate, or hopantothenate), is widely used in Japan as an antidementia drug for treating cognitive impairments in pathological states such as Alzheimer's disease, presumably through increasing cholinergic activity in vivo. Reyes-like syndrome has been reported in patients using pantoyl-GABA, presumably due to pantothenic acid deficiency. Other recent studies have shown that uptake and metabolism of pantothenic acid seem to differ among organs and tissues. Fetal growth retardation and death reported in pantothenic acid deficient animals are due to impaired placental function.

References:
Song, W.O. (1990) Pantothenic acid - How much do we know about this B-vitamin? Nutr. Today 25: 19-26 Tahiliani, A.G. & Beinlich, C.J. (1991) Pantothenic acid in health and disease. Vitamins and Hormones 46: 165-228 Annous, K.F., Song, W.O. (1995) Pantothenic acid uptake and metabolism by the red blood cell. J. Nutr. 125: 2586-2593.

| *Benefits* | Product |
| --- | --- |
| • **Strong Bones & Teeth**<br>• **Lowers Heart Disease Risk**<br>• **Lowers Liver Disease Risk**<br>• **Better Calcium Absorption**<br>• **Better Metabolism** | **Coral Cal/MAG Plus** powder is a food supplement derived from above the sea level coral reef at Yonaguni Island of Okinawa, Japan. The importance of above sea level harvest is that there is the built-in addition of solar energy in the purified aragonite (the nutritional product derived from the coral). There are two calcium mineral sources; (1) calcite (calcium derived from limestone), (2) aragonite. **Aragonite** is the preferred source since the solubility is 50 times greater than the calcite form. This solubility allows the aragonite calcium to be introduced into our body with a very effective biological reaction. There is a gradual ionization of the calcium and better PH control. Contrarily, calcite calcium reacts violently, with a sudden ionization. This increases circulating ionized free calcium, which will result in cellular damage and increase the probability of cerebral apoplexy, liver disease and heart disease.<br><br>Other distinctive differences are their oxidative characteristics. When aragonite is oxidized, a more minute product is formed, allowing for better absorption. The calcite oxide is larger and harder, affecting its absorption. Furthermore, aragonite does not affect an increase in serum calcium and does contribute to better metabolism, PH balance and the formation of bone.<br><br>The **Coral Cal/MAG Plus** includes the following – per ½ tsp. Calcium 500mg, Magnesium 500mg, Silicon 1.66mg, Sodium-130mg. |

**Helpful preventative strategy for the following:**

- **Yeast infections**
- **Herpes simplex I and II**
- **Acne**
- **Mouth Ulcers**
- **Travelers' Diarrhea**

**Pro-Biotics (Pharmax)**

The Human Lactic Commensals (HCL) range of pro-biotics have been developed within the Applied Longevity, alliance and have been proven in both clinical trials and by practitioner usage for over 11 years in Europe.

The role of Pro-Biotics in human physiology is both varied and of fundamental importance. However, part of the reason why their full benefits are not achieved in practice is the lack of delivery to the GI tract. Moreover, many bacterial isolates used as pro-biotic organisms do not reach the GI tract in either sufficient numbers or are poor colonizers and, therefore pass quickly through the GI tract, eliciting only a temporary effect. Hence for a pro-biotic to be effective, there are three primary criteria to be considered.

1. Proven product shelf stability
2. Ability of the selected cultures to survive the gastric environment.
3. Ability of cultures to attach to human epithelial tissue.

Key Features of the HLC Strain:

- Full 12-18 months shelf life at 2-8 degrees C.
- Over 3 months shelf life at room temperature
- Over 90% survival in full stomach
- High attachment efficiency

The Applied Longevity, Ltd.. Pro-Biotic system offers a concentrated form of normal flora. The Lactobacillus Acidophilus and Bifidobacteria promote intestinal and overall health. Lactobacillus acidophilus is one of the most important and well-researched microorganism that inhabits our gastro-intestinal tract. The organism makes its home in the small intestine, where nutrient breakdown and absorption take place.

Two of the many benefits of Lactobacillus Acidophilus / Bifidus Combination are its ability to manufacture B-complex vitamins, and to produce enzymes which aid in digestion of protein and lactose (milk sugar). Lactobacillus Acidophilus / BiFidus Combination also destroys problem-causing bacteria through the creation of acidophilin, a natural antibiotic. Acidophilin may help control the overgrowth of candida albicans (the culprit in yeast infections) by the production of hydrogen peroxide. Studies show acidophilus has been helpful in the preventative strategy of yeast infections, herpes simplex I and II, acne, mouth ulcers and travelers' diarrhea. Maintaining healthy gastro-intestinal levels of good bacterial organisms may also provehelpful in controlling auto-immune symptoms (rheumatoid arthritis and chronic fatigue syndrome)

| *Benefits* | Product |
|---|---|
| • **Emotional Balance**<br>• **Vaginal lubrication**<br>• **A Sharp Mind**<br>• **Better Energy**<br>• **Better Libido**<br>• **Few sugar cravings**<br>• **Youthful skin**<br>• **Better erectile function**<br>• **Better energy** | **Royal Maca** is Maca, the root of a Peruvian herb *Lepidium peruvianum chacon.*<br>Maca is an extraordinary herb; it is the highest growing food plant in the world, typically growing at altitudes of over 12,000 feet. It has rejuvenative effects on the endocrine glands. Specifically, the herb stimulates the pituitary-hypothalamus axis of the brain with four alkaloids resulting in an increase in the production of endocrine hormones secreted by the ovaries, adrenals, testes, pancreas and thyroid. The herb has no direct hormonal effects in itself. It may have an adaptogenic effect, modulating endocrine glands, down-regulating over active glands.<br><br>The herb is able to increase levels of these hormones without a negative feedback response. For example, in HRT or hormone replacement therapy, in which women take estrogen and progesterone hormones for the addition of exogenous (from out of the body) hormones cause the glands to cease their production. Sex glands atrophy and become useless. Maca, by stimulating the pituitary itself, makes the body's glands produce more natural hormone.<br><br>The effect is more energy and vitality for men and women. Post or peri-menopausal women who have been on HRT may usually utilize Maca instead. Women who take Maca report less fatigue, greater energy, less stress and relief from hot flashes and night sweats. Men who have potency problems find relief from that as well. |
| • **Promotes Weight Loss**<br>• **Increases levels of Free T3**<br>• **Increases metabolic activity**<br>• **Increases immune modulation**<br>• **Memory Enhancement** | **7 Ketogenic DHEA**<br><br>7 Ketogenic DHEA (3-acetyl-7-oxy-ehydroepiandrosterone) is a naturally occurring metabolite of the DHEA steroid hormone. DHEA is the most abundant of the adrenal hormones and serves as a precursor for all sex hormones. The 7 Ketogenic derivative was developed to allow for the most biological active form of DHEA that would not convert to the potentially harmful causing steroid sex forms (DHT, Estrogens).<br><br>A double blind study demonstrates that 7 Ketogenic DHEA promotes weight loss, increases levels of free T3, increases metabolic activity, immune modulation, memory enhancement, and thermogenesys and does not increase estrogen and/or testosterone.<br><br>Ingredients List<br>• 3-acetyl-7-(3-acetyl-7-oxy-dehydroepiandrosterone)<br>• theotic acid<br>• vitamin E |

- Essential amino acids cannot be made by the body.
- The Benefits of Direct Delivery
- Studies have shown a dramatic increase in absorption when comparing transdermal vs. oral administration.

## **AminoStat** *the transdermal answer*

Amino acids are the "building-blocks" of proteins, however, it is not dietary proteins that make up the human body. Dietary proteins are broken down into **amino acids**, then 'reconstructed' into the specific proteins necessary for body structure, function or biochemistry. *Therefore, it is amino acids, not dietary proteins that are essential nutrients. Additionally, amino acids alone cannot function properly in biochemical synthesis. They need to have the correct co-factors (including the right electrolytes and be in the right ratio)* in order to affect metabolism, and maintain cellular nutritional balance.

**Introduction:** An adequate supply of dietary protein is required for surv development, reproduction and lactation, and maintaining health throu amino acids released during the digestion of food proteins are essential of tissue proteins, which comprise ~16% of the human body. The Ap product (Amino*STAT*) is formulated to deliver a balanced core of the acids, co-factors and electrolytes.

**Table I. Nutritional Classification of Amino Acids**

| **Indispensable (Essential)** | Dispensable (Nonessential) |
|---|---|
| Histidine | Alanine |
| **Isoleucine** | Arginine |
| **Leucine** | Aspartic acid |
| **Lysine** | Asparagine |
| **Methionine** | Cystein |
| **Phenylalanine** | Glutamic acid |
| **Threonine** | Glutamine |
| **Tryptophan** | Glycine |
| **Valine** | Proline |
| | Serine |
| | Tyrosine |

1. **Nutritional Essentiality of Amino Acids**

Food and tissue proteins contain 20 amino acids of nutritional importance (Table I). **Nine** of these -- **histidine, isoleucine, leucine, lysine, methionine, phenylalanine, theronine, tryptophan, and valine** -- cannot be synthesized by the body; they are therefore essential nutrients that must be obtained from the diet or must be supplemented. The other 11 are also ordinarily obtained from the diet, but the body can synthesize them.

2. **Human Requirements for Protein and Amino Acids**

Protein requirements are highest during the period of rapid growth after birth. Requirements of older children are estimated by a factorial method. Adult requirements for indispensable amino acids have been estimated with the nitrogen balance procedure. Besides fulfilling specific nutritional or physiological roles, e.g., serving as components of body structures or metabolic systems, some nutrients may also have therapeutic or pharmacological actions.

**3. Lipodermal delivery using the WHO standards for balancing amino acids**

The Applied Longevity Amino*Stat* product for the delivery of the essential amino acids prevails since:

(a) There is no digestive influence to affect absorption.

(b) The amino acids are compounded in standardized concentrations proven to affect cellular metabolism and health.

(c) They are designed to utilize other co-factors and ingredients that are found to be defective.

**Continued use of this product is essential for the success of the Applied Longevity System**

## Libidex™ Ingredients and Indications

ACTIVE INGREDIENTS: DHEA (dehydroepiandrosterone) purified pharmaceutical grade (99.6+), , Alpha Lipoic Acid, Avena Sativa (Colloidal fluid oat bran extract) Saw Palmetto extract, Muira Puama extract, Daminana extract, Gingko Biloba extract, Colloidal Silver 40ppm, Tocopherol complex (vitamin E), Retinyl Palmitate (vitamin A) in a Lipoderme base.

HOMEOPATHICS: Agnus Castus 12x, Onosmodium 12x, Pheromone 3x, phospho. Acid 6x, Selenium 12 x. Flower Essence (Potentized Monochords) (Aloe Vera, Avena Sativa, Calendula, Crab Apple, Holly, Hornbeam, Larch, Olive, Damiana, and Ginkgo Biloba.

LIPODERME BASE INGREDIENTS: Deionized water, Distearate Ester, Glycerine, Glyceryl Stearate, Coco Caprylate / Caprate, DL- Alpha Tocopheryl Acetate, Phospholipid, 1,3-Butylene Glycol, Dimethicone, Cetearyl Alcohol, Safflower Oil, Phenonip, Aloe Vera extract, C10-30 Acrylates Crosspolymer, Sorbitan Monoleate, Cholest-5-en-3beta-ol, Tetrasodium EDTA, Wheat Germ Oil, Citrus Seed Extract.

### ***Indications for Libidex™ Crème***

- MEN AND WOMEN OVER THE AGE OF FORTY.
  As we get older sex endocrine production declines, as a result sexual dysfunction is likely to occur.

- THOSE WHO ARE HEALTHY BUT JUST DO NOT FUNCTION AS THEY USED TO.
  As early as our thirties sexual function begins to change. If proper levels of sexual factors are not replaced, function and general health can be compromised

- MEN WHO WISH TO MAINTAIN THEIR VIRIL CAPABILITIES.
  One out of five men in the United States suffers from sexual dysfunction.

- ANY WOMAN WHO WISHES TO ATTAIN A MORE SATIFYING ORGASM.
  A primary ingredient in BIOSIS IC, DHEA was recently studied by a leading research institute and was found to increase the intensity of sexual desire and orgasm.

- MEN AND WOMEN UNDER STRESS.
  Stress is a leading cause of sexual dysfunction. The ingredients in BIOSIS IC help to reduce Mental and physical stress factors.

- ANYONE USING PRESCRIPTIVE MEDICATION THAT CAUSES DYSFUNCTION.
  More than six hundred thousand medications can cause sexual dysfunction. The ingredients in BIOSIS IC may help to prevent or improve the symptoms sometimes caused by these medications.

- COUPLES WISHING TO PREVENT SEXUAL BURNOUT.
  More and more couples are experiencing a loss of interest in sex. BIOSIS IC contains ingredients to naturally stimulate the sexual appetite, also adds an element of adventure to the love making process.

- MEN AND WOMEN WISHING TO EVOLVE SEXUALLY.
  Man has evolved tremendously in many ways over the course of his existence, but sex has not evolved since the Dark Ages.

- THOSE WISHING TO USE A LUXURIOUS LUBRICANT.

- THOSE WHO ARE HEALTHY BUT JUST DON'T FUNCTION AS THEY USED TO.
  As early as our thirties sexual function begins to change. If proper levels of sexual factors are not replaced, function and general health can be compromised.

- THOSE WHO WISH TO ENHANCE THE BONDING EXPERIENCE.
  The homeopathics, and Flower essence formula in BIOSIS IC was designed to help remove physiomotional barriers that can interfere with romantic bonding.

- MEN AND WOMEN WHO SEEK A MORE COMPLETE SEXUAL EXPERIENCE.
  Many younger couples find themselves romantically incomplete. BIOSIS IC helps to focus sexual attention, and increase the pleasure associated with the act of lovemaking.

- MEN AND WOMEN WHO WISH TO USE A PREVENTATIVE APPROACH TO SEXUAL HEALTH.
  1 out of 5 men in the United States suffers from sexual dysfunction. The statistics for women are also staggering. Using BIOSIS IC daily may help prevent the progression of age related sexual dysfunction, and may also help enhance present function.

- ANY WOMAN WHO WISHES TO ATTAIN A MORE SATISFYING ORGASM
  A primary ingredient in BIOSIS IC was studied by a leading research institute and was found to increase the intensity of sexual desire and orgasm.

DESCRIPTION

**Libidex™** is the first all natural endocrine crème that combines all the elements necessary to support a rational endocrine program. Designed to deliver a potent yet responsible dose of several important sexual/hormonal factors, Libidex™ helps the body to attain higher levels of sexual awareness, satisfaction and a healthy intensification of the entire sexual experience. Researched for more than twelve years, Libidex™ is the first true mind and body supplement for proper endocrine function.

Libidex™ employs a very advanced delivery mechanism that uses lipid spheres called LIPODERMES. These lipid globules are filled with the active components and processed to maintain a protective shell until it reaches the bloodstream. Once there, its outer layers break down and release a chemical cargo into the bloodstream. This improves delivery payload avoiding many of the obstacles encountered when trying to supplement orally. Studies have Libidex™ shown a dramatic increase in absorption when comparing LipoDermal vs. oral administration.

uses two different forms of lipodermes to specifically target tissue. The primary lipoderme is multi layered, containing up to one hundred shells. This form of lipoderme is known as a multilamellar, and is capable of delivering its payload directly to the bloodstream. Libidex™ secondary base (unilamellar lipoderme – single wall) releases it's homeopathic and flower essence ingredients into the local tissue, where it becomes anchored. This is important when using vibrational medicines such as homeopathics. In his twenty eighth publication on the subject of flower essence Dr. Ditmar Kramer proves that the topical use of vibrational medicine (homeopathics, and flower essence) can have a more profound, and longer lasting effect, then when used sublingually. This topical application allows them to influence the surrounding tissues, while also stimulating the natural energy flow via the acupuncture meridians. It is believed that this natural stimulation of the energy flow (Chi) causes a release of neuro-sexual chemicals from the brain.

In addition, Libidex™ helps to elevate pheromone levels. Pheromones are produced by men and women to help make them more attractive to the opposite sex. Recent studies of the human Vamoronasal system (VMO) indicate pheromones begin to decline in production soon after the age of twenty. This decline parallels that of the other important endocrine production (DHEA) needed for proper sexual function. Libidex™ contains the basic precursors that are used by the body to increase the production of pheromones. Libidex™ helps to restore peak levels of pheromones by naturally replacing the basic components needed for pheromone production.

DOSAGE

For therapeutic purposes, it is recommended that using serum or saliva hormonal baseline studies monitor Libidex™ dosage. DHEA can easily be monitored by use of saliva testing for DHEA Sulfate. Applied Kinesiology can be used to establish exact dosage after baselines are established. The following suggestions are for dosage only, and should not be the deciding factor. Please make the appropriate decision based on the clinical data available.

Women – pre-menopause: Apply 1/8 - 1/2 tsp. to areas of soft tissue, one to three times daily, according to indications and baseline. (Breasts, inner thighs, inner arms, abdomen.)

Women – post-menopause: Apply 1/4 - 1 tsp. to areas of soft tissue, one to three times daily, according to indications and baseline.

Men under 40 years of age: Apply 1/8 - 1/4 tsp. to areas of soft skin. (Thighs, abdomen, chest, inner arms.)
Men over 40 years of age: Apply 1/2 tsp. once daily, to areas of soft skin. (Thighs, abdomen, chest, inner arms.)
It is best to maintain the initial dosage a minimum of two to four weeks before reevaluation.

FOR MAINTENANCE PURPOSES
1/4 – 1/2 the therapeutic dose for first three months, then decrease to twice weekly, and during intimacies. During maintenance phase, patient can use as much BIOSIS IC as he or she wants during lovemaking.

Libidex™ application is only limited by your imagination. Libidex™ is non-irritating and can be applied to any area of soft skin, breasts, inner thighs, inner arms, neck, and abdomen. The ingredients in Libidex™ have been formulated to be hypoallergenic, and void of irritants; therefore, it can be used anywhere on the body.

CONTRA INDICATIONS.
There are no known contraindications or drug interactions, except for the standard precautions observed with DHEA. Libidex™ should not interact or interfere with prescriptive medication. Libidex™ use is intended for healthy individuals only. DHEA (an active component) precautions should be reviewed. It is advised that men with prostate disease, or anyone with reproductive cancer should not use Libidex™ unless determined and recommended by a qualified physician.

ADMINISTRATION / APPLICATION:
Libidex™ is applied using the hands, and will absorb into the skin leaving a mild residual behind. If sexual activity follows it becomes more noticeable. As the crème becomes wet from body moisture, it takes on a slippery feel that both aids and intensifies sexual feeling. Libidex™ heats up slightly on sensitive skin. It can cause mild discomfort, but is not dangerous. Libidex™ is water-soluble, rinse skin thoroughly with clear water or water and mild soap to remove. Inform women who intend to use around the vagina, to flush with water if any discomfort occurs. Flushing removes the symptoms within moments.

IS THERE RESEARCH TO SUBSTANTIATE ITS EFFECTS?
Libidex™ is the result of over twelve years of research. The active ingredients in Libidex™ each have been tested beyond any question as to the role they play in this formula. There is extensive research (references enclosed) available that deal with the specific resources used in the development of this product.

WHAT ARE THE OTHER EFFECTS OF Libidex™?
The ingredients in Libidex™ have a multitude of long term benefits. The following is only a brief description. More specific information is available by consulting the references.

DHEA - anti aging effects, helps buffer stress, and may help to control weight.
LIPOIC ACID - Helps the body to produce more ATP our cellular fuel. This relieves stress on the system and increases the amount of available energy.

BOTANICAL EXTRACTS - these rare plant extracts help to normalize, and strengthen sexual, physical, and mental function. Included are Avena Sativa, Saw Palmetto, Damiana, Yohimbe, and Gingko Biloba.

AVENA SATIVA - this herb has been the center of much media attention lately. A study preformed at the Institute of Human Sexuality in San Francisco showed that Avena Sativa supplementation caused an increase in the intensity of orgasm in women. Over 80% of Women tested, reported a noticeable increase of climax, while also noting an increase in overall sensitivity. Researchers believe this is do to Avena Sativa's effect that frees bound hormones (the free hormone is the one utilized by the body).

SAW PALMETTO - known for its ability to possibly reduce prostate inflammation, has also been found to aid the female reproductive system.

MUIRA PUAMA - also known as Potency Wood, this rain Forrest botanical has been used for centuries to increase male potency. Recent research has shown that Muira Puama has the ability to free bound testosterone, thereby increasing plasma levels without influencing testosterone feedback mechanisms.

DAMIANA - known for centuries as an aphrodisiac, this herb is used in almost every country to treat sexual dysfunction in both men and women.

VITAMIN E - known for its ability to stimulate the reproductive glands, the tocopherol complex in BIOSIS IC also has antioxidant capabilities within the skin.
PHEROMONE PRECURSORS - help to provide the basic building blocks for the production of pheromones.
ALPHA LIPOIC ACID - this incredible nutrient holds the key to proper energy management. When supplemented, Lipoic acid helps increase production of cellular ATP (adeno-tri-phosphate). This helps to improve physical ability, stamina, and overall energy.

RETYNAL PALMATATE - Well known for its effect on the skin, vitamin A is an important factor in the normal function of the sexual organs.

HOMEOPATHIC REMEDIES - mind and body integration. These formulas are designed to help improve ones energy production, maintain proper cellular activity, and increase the flow of chi to areas of segmentation.
<u>HOMEOPATHICS</u>

PHEROMONE PRECURSORS – the following homeopathics help to stimulate the production of pheromones: Selenium 12X, Agnus Castus 12x, Onosmodium 12x, phospho acid 6x, and Calendula, affect the molecular roots most commonly associated with sexual dysfunction.

FLOWER ESSENCES - Aloe Vera, Avena Sativa, Crab Apple, Holly, Hornbeam, Larch, Olive, Damiana, and Ginkgo Biloba, when combined have the function of interfering with negative emotional feedback mechanisms. This helps restore normal function to affected areas, a result of the physio-motional manifestations.

Libidex™ is produced by Applied Longevity. It undergoes rigorous testing before, during, and after each batch production. Made of natural ingredients, Libidex™ is non-irritating, and will not stain skin or clothing. The light tropical scent of Libidex™ is derived from natural flavorings, not perfume. The supplements contained are of the highest purity. The DHEA, alpha lipoic acid, the vitamins, and botanical extract components are pure pharmaceutical grade (99.6+%).
Libidex™ is not a drug and is therefore not intended to diagnose, treat, or cure any disease. It is recommended as a supplement only, and is intended to be used only as an adjunct to established medical protocol.

**STUDIES**

Hundreds of patients over the course of fifteen years have used the crème or it's primary ingredient in one form or another. In the course of informal study over this time the response has been substantial enough to warrant continued testing and development.
Two groups of patients were included in these preliminary studies. The first group included those with normal sexual function, which desired an increase in sexual interest, and ability. The second group suffered some form of sexual dysfunction.

FIRST STUDY

| | Number of participants | Positive Response | Negative Response | No Response |
|---|---|---|---|---|
| Women | 83 | 71 | 1 | 11 |
| Pre menopause | 45 | 41 | 0 | 4 |
| Post menopause | 38 | 30 | 1 | 7 |
| Male | 121 | 94 | 3 | 24 |

RESULTS

More than 80% of the women tested reported a positive effect. Many noted a positive psychological change as well. Of the two groups of women tested, only one woman reported a]

Negative reaction involving a suspected allergy. Overall the women reported increased intensity of orgasm, heightened sexual sensation, and increased interest. Ninety five percent reported they were thinking of making love more often.

More than seventy five percent of the men tested reported a noticeable positive effect. The general comment was that sexual ability was dramatically increased, and that most had returned to their sexual peak. Three men reported irritation after using the crème. Ninety percent reported they enjoyed using the crème, and would use it again.

Due to the positive results of clinical testing, a testing protocol was initiated to isolate the effects of the crème of other possible therapeutic interrelationships. In August of 1996 a clinical study using BIOSIS IC was initiated at Michael Borkin's Holistic Health Care Center, Las Vegas, NV.

20 men 20 woman were chosen at random from a prior patient base. Subjects were divided into the following categories for test purposes:

WOMEN

| HORMONAL FACTOR | NUMBER of subjects | POSITIVE RESULTS | NEGITIVE RESULTS | NO CHANGE |
|---|---|---|---|---|
| PRE-MENOPAUSE | 12 | 10 | 0 | 2 |
| POST-MENOPAUSE | 8 | 7 | 0 | 1 |

MEN

| Sexual Health Status | Number of subjects | Positive Results | Negative Results | No Change |
|---|---|---|---|---|
| Good | 14 | 10 | 0 | 4 |
| Impotent | 6 | 4 | 0 | 2 |

Results of Study

The study was conducted between August and November of 1996. The entire time allowed for testing was ninety days. Of the forty subjects, all completed the study.

Subjects were instructed to discontinue all nutritional supplementation. This did not include medication, of which 10 men, and 12 women were prescribed.

Conclusion

Most of the men involved in the study reported an improvement in their sex drive. 90% reported a firmer erection, and 70% concluded that recovery time had decreased. 66% of the men that suffered impotency reported a positive change, and one man reported several incidence of multiple orgasm.

Of the women studied, seventeen reported a noticeable change in sexual function. All but three reported an increase in intensity of orgasm, and an increase in overall sensitivity. All reported a noticeable increase in genital sensitivity. Of the pre-menopausal woman, 90% reported better, more normalized menstruation. 80% reported a better emotional outlook. 50% of the post – menopausal woman that suffered hot flashes reported a dramatic decrease in frequency, and severity.

Ninety percent of the subjects tested enjoyed the process of applying the crème, and most looked forward to using it again. One woman reported mild vaginal burning, but remarked that it was easily controlled with water.

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