Abstract:

Title: UTRAGENOMICS

Compositions and methods are provided using GENOPROFILE to measure and direct the customization of a subsequent nutraceutical to act as a therapeutic modality wherein said GENOPROFILE is based on the analysis of certain known polymorphic genes associated with Substance Use Disorder (SUD). Nutraceutical composition comprising at least one herbal component, at least one vitamin component, at least one mineral component, at least one opiate destruction-inhibiting substance, at least one neurotransmitter precursor, at least one tryptophan concentration enhancing substance, at least one catecholamine catalytic inhibitor and at least one homeopathic component is useful in treating disease affected by genetic and neuro-metabolomic factors.
BACKGROUND OF INVENTION

NUTRAGENOMICS

[0001] In this provisional patent application, we are suggesting that in this era, genes and nutrition will be the target of ongoing research (see drawings 1-13). Currently, the nutraceutical world has seen only limited research in this field of nutrigenomics (NGx). However, the concept of gene-based response, especially in the pharmaceutical world is growing, and billions of research dollars are being poured into the field known as pharmacogenomics (PGx). In this application, our purpose is to show how one’s genome is ever important in a response to any biologically-active substance such as drugs and more importantly nutrients. As our knowledge of genomics continues to grow so will nutrigenomics in all of its facets, especially to help us understand the basis of individual differences in response to dietary patterns and targeted supplementation.

[0002] The recent completion of the draft sequence of the human genome and related developments has increased interest in genetics, but confusion remains among health professionals and the public at large. Inaccurate beliefs about genetics persist, including the view that in the past it had no effect on the practice of medicine and that its influence today is pervasive. We have recently entered a transition period in which specific genetic knowledge is becoming critical to the delivery of effective health care for everyone. While we do not know precisely how many genes the human genome contains, current data indicate that the human genome includes approximately 30,000 to 35,000 genes - a number that is substantially smaller than was previously thought.

[0003] If genetics has been misunderstood, genomics is even more mysterious-what exactly is the difference? Genetics is the study of single genes and their effects. "Genomics", a term coined only 17 years ago, is the study not just of single genes, but of the functions and interactions of all the genes in the genome. Genomics has a broader and more ambitious reach than does genetics. The science of genomics rests on direct experimental access to the entire genome and applies to common conditions, such as breast cancer, colorectal cancer, human immunodeficiency, cardiovascular, Parkinson's disease and certain brain and neurological disorders such as Alzheimer's, bipolar disorder, Neurogenobolic Deficiency Syndrome (NGDS), Reward Deficiency Syndrome (RDS), and even Attention Deficit Hyperactivity Disorder (ADHD) and related behaviors. These common disorders are also all due to the interactions of multiple genes and environmental factors.

[0004] Only about half these genes have recognizable DNA sequence patterns that suggest possible functions. Mutations known to cause disease have been identified in approximately 1000 genes. However, it is likely that nearly all genes are capable of causing disease if they are altered substantially. Whereas it was dogma that one gene makes one protein, it now appears that, through the mechanism of alternative splicing, more than 100,000 proteins can be derived from these 30,000 to 35,000 genes. Rather than DNA expression being fixed in stone, new evidence now suggests that DNA expression is a dynamic process. In addition to alternative splicing, a number of "epigenetic" phenomena, such as methylation and histone modification, can alter the effect of a gene. Furthermore, a complex array of molecular mandates allows specific genes to be "turned on" (expressed) or "turned off" in specific
tissues and at specific times. Genes are distributed unevenly across the human genome. Certain chromosomes particularly 17, 19, and 22 are relatively gene dense as compared with others, such as 4, 8, 13, 18, and Y.

[0005] Interestingly, gene density varies within each chromosome, being highest in areas rich in the bases cytosine and guanine, rather than adenine and thymine. Moreover, not all genes reside on nuclear chromosomes; several dozen involved with energy metabolism are on the mitochondrial chromosome. Since ova are rich in mitochondria and sperm are not, mitochondrial DNA is usually inherited from the mother. Therefore, mitochondrial genes, and diseases due to DNA sequence variants in them, are transmitted in a matrilineal pattern that is distinctly different from the pattern of inheritance of nuclear genes.

[0006] One characteristic of the human genome with medical and social relevance is that, on average, two unrelated persons share over 99.9 percent of their DNA sequences. However, given the more than 3 billion base pairs that constitute the human genome, this also means that the DNA sequences of two unrelated humans vary at millions of bases. Since a person's genotype represents the blending of parental genotypes, we are each thus heterozygous at about 3 million bases. Many efforts are currently under way, in both the academic and commercial sectors, to catalogue these variants, commonly referred to as "single-nucleotide polymorphisms" (SNPs), and to correlate these specific genotype variations with specific genotypic variations relevant to health. Some SNP-phenotype correlations occur as a direct result of the influence of the SNP on health. More commonly, however, the SNP is merely a marker of biologic diversity that happens to correlate with health because of its proximity to the genetic factor that is actually the cause. In the case of mood there are multiple genes (polygenic inheritance) involved and thus potentially hundreds of SNPs. In general terms, the SNP and the actual genetic factor are said to be in linkage disequilibrium.

[0007] The convergence of Pharmacogenetics and rapid advances in human genomics has resulted in pharmacogenomics and/or nutrigenomics, terms used here to mean influence of DNA-sequence variation on the effect of a drug and/or a natural substance or nutrient. With the completion of the Human Genome Project, and the ongoing annotation of its data, the time is rapidly approaching when the sequences of virtually all genes encoding enzymes that catalyze phase 1 and phase 11 drug metabolism will be known including genes that encode drug (nutrient)-transporters, drug (nutrient) receptors, and other drug (nutrient) targets.

[0008] It is well known that individuals respond differently to medications and certain nutraceuticals in terms of both toxicity and treatment efficacy. Potential causes for such variability in drug (nutrient) effects include the pathogenesis and severity of the disease being treated: drug (nutrient) interactions; the individual's age, nutritional status; kidney and liver function; and concomitant illnesses. Despite the potential importance of these clinical variables in determining drug/nutrient effects, it is now recognized that inherited differences in the metabolism and disposition of drugs/nutrients, and genetic variants (polymorphisms) in the targets of drug/nutrient therapy (such as receptors like the dopamine D2 receptor [DRD2]), can have even greater influence on the efficacy and toxicity of either medications or nutraceuticals.
Clinical observations of such inherited differences in drug effects were first documented in the 1950’s, exemplified by the prolonged muscle relaxation after the drug known as suxamethonium (an inhibitor of the breakdown of acetylcholine) and an inherited deficiency in the genes that encode the enzyme responsible for the breakdown of this drug as marked by plasma cholinesterase (the enzyme which breaks down acetylcholine). The second gene-based drug response was observed when researchers found that certain patients bled to death after they were treated with an anti-malarial therapy because they carried a gene variant which lowered their blood cell glucose 6-phosphate dehydrogenase activity. Such observations gave rise to the field of “pharmacogenetics” the antecedent to pharmacogenomics, the current topic. However, we now know that individual differences in response to drugs and or nutrients are not due to single gene variants but rather they are determined by the interplay of several genes encoding proteins (enzymes, receptors, transporters) involved in multiple pathways of drug/nutrient metabolism, disposition and effects. We are embarking on new era where efficacy of any substance is governed by an individual’s inherited genotype to a greater degree than even other non-genetic factors. Understanding structure/function normal physiology and certain observable dysfunctions may indeed lead to promising nutrient based targets, but without the knowledge afforded by accurate DNA based prescreening (genotyping) subsequent supplementation becomes nothing more than a crap shoot. Similar to the pharmaceutical industry the nutraceutical industry can become an equal opportunity player and begin to initiate ongoing research and development by incorporating these genomic-based doctrines as described herein.

Out of the 3 million unshared DNA bases, individuals could carry gene variants (polymorphisms) that might lead to either an increase or a decrease of a certain important drug/nutrient response related proteins such as receptors, enzymes, cell cycle control, chemical messenger synthesis or catabolism (breakdown) or many other cellular events. As stared earlier, while there is a paucity of molecular studies involving genome-based response in the nutrition field (see below), a plethora of molecular studies have revealed that many genes encoding drug targets exhibit genetic polymorphism (variants), which in many cases alters their sensitivity to specific medications and/or offer specific targeted therapy.

Such examples include the following:

- Asthma- Polymorphisms in Beta-adrenergic receptors (adrenalin -like) impart differential sensitivity to substances that stimulate these receptors (beta-agonists) in asthmatics.
- Renal function and Blood pressure -angiotensin converting enzyme (ACE) gene polymorphisms impart differential sensitivity to inhibitors of ACE.
- Cardiovascular - angiotensin II T1 receptor gene polymorphisms impart differential sensitivity to the substance phenylalanine and subsequent vascular reactivity.
- Diabetes- polymorphisms in the sulfonyurea receptor gene imparts differential responsiveness to sulfonyurea hypoglycemic agents.
- Coronary atherosclerosis - polymorphisms in the gene that controls the enzyme cholesteryl ester transfer protein impart differential efficacy of the drug pravastatin in patients with coronary disease.
- Dysrhythmias- Potassium channel mutations predict drug-induced dysrhythmias as an adverse effect.
• Drug Metabolism- Polymorphisms in the P-450 enzymes responsible for metabolizing drugs such as caffeine and codeine impart differential clearance of these and other substances. One such enzyme is the CYP2D6.
• Breast Cancer- Trasruzumab is a drug known to target a certain genetic mutation in a protein product of the HER2/neu oncogene (which is overexpressed in breast cancers) and has been found compared to standard therapy to be superior in preventing metastatic breast cancer.
• Diuretic therapy- There is a gene known as C825T involved with a second messenger G-protein (beta)3 whereas polymorphisms in this gene predict responsiveness to the anti-diuretic drug (used to treat hypertension ), hydrochlorothiazide.
• Lipid response- Genetic variation of the apolipoprotein constituents of the lipoprotein molecules (APOE gene locus) predicts plasma low-density lipoprotein cholesterol (LDL-C) concentrations. Interesting carrying one form of the APOE (E4) seems to be more responsive to dietary modification than carriers of E3 and or E2 forms of the same gene.
• Nicotine patch - Variation of the CT and TT allele of the dopamine D2 receptor gene confirms a differential response to the nicotine patch. At the eight-year mark, 12% of women with the CT or TT allele of the dopamine D2 receptor gene who had received the patch had remained abstinent. Only 5% of women with the CC allele had maintained their non-smoking status. No difference based on genetics was noted in men.
• The polymorphic CYP2D6 regulates the O-demethylation of codeine and other weak opioids to more potent metabolites with poor metabolizers having reduced antinociception in some cases.

[00012] Certainly we have come full circle from the "Naturalistic Era" (400B.C. -1750 AD), to the "Chemical Analytical Era" (1750-1900) to the "Biological Era" (1900-present), to the "Cellular Era" (post 1955) and the current era of the 21st century where "genomics" is the new buzz word. Utilizing tools derived from this new science will allow us to identify and understand molecular-level interaction between nutrients and other dietary bioactives with the human genome during transcription, translation and expression, the process during which proteins encoded by the genome are synthesized and expressed. There is growing evidence that certain gene polymorphisms predict response to nutrients.

[00013] Pharmacogenetics is the study of the role of genetics in inter-individual variability to drug response and therapy. In this regard there are 232 PUBMED reports concerning pharmacogenetic studies for opioid drugs. Opioid analgesics are widely used clinically for pain management, and inter-patient variability with opioid therapy is often reported. Information on genetic polymorphisms in enzymes, receptors and transporters related to opioid disposition (pharmacokinetics) and pharmacology (pharmacodynamics) is documented. Pharmacogenetics of enzymes, including the cytochrome P450s and uridine diphosphoglucuronosyltransferases, opioid receptors and the ABC family of transporters are a few examples.

[00014] In the broadest terms, the interface between the nutritional environment and cellular/genetic processes is being referred to as "nutrigenomics". While nutrigenomics in this sense seeks to provide a molecular genetic understanding for how common dietary chemicals ( i.e. nutrition) influences health by altering the expression and/or structure of an individual's genetic makeup, the more restricted view is governed by the same principles as seen with advent of pharmacogenomics in clinical medicine which involves DNA based - targeted response to biologically active compounds.
The tenants for nutritional genomics include in the broadest sense the following:

- Common dietary substances act on the human genome
- Diet, specifically the consequences of poor diet (especially for certain genotypes), can be a risk factor that potentiates certain gene expressions and promotes a number of genetic diseases or behavioral disorders.
- Diet-regulated genes are likely to play a role in the onset, incidence, progression and/or severity of chronic diseases.
- Diet affects the balance between healthy and disease states and this interaction depends on an individual's genetic makeup
- Dietary intervention based on knowledge of nutritional requirement, nutritional status, and genotype (i.e. "individualized nutrition") can be used to prevent, mitigate, or cure chronic disease or behavioral disorders.

While there is plethora of scientific information concerned with four of the five tenets, there is paucity with regard to "individualized nutrition".

In terms of dietary intervention based in individualized nutrition such examples of a number of gene-disease association studies have shown promise of this approach as follows:

- Hypertension- The amount of circulating angiotensinogen (ANG) is associated with increased blood pressure. A SNP (polymorphism) designated AA, at nucleotide position -6 of the ANG gene, is linked with the level of blood ANG protein. Individuals with the AA genotype who eat the Dietary Approaches To Stop Hypertension (DASH) diet show reduced blood pressure, but this diet was less effective for carriers of the GG genotype.
- Cardiovascular Apo-Al gene plays a role in lipid metabolism and coronary heart disease. The A allele (variant) was associated with decreased serum HDL levels. The variant was coupled with consumption of type of fat and subsequent effect on HDL levels in both males and females carrying different genotypes.
- Cancer- Methylene Tetrahydrofolate Reductase (MTHFR) is a key gene in one-carbon metabolism and, indirectly, in all methylation reactions. The C677T polymorphism of this gene, which reduces enzymatic activity, is inversely associated with occurrence of colorectal cancer and acute lymphocyte leukemia. Low intake of folate, B12, B6 and methionine was associated with increased for cancer among those with the MTHFR TT genotype.
- Rheumatoid arthritis - Polymorphisms in the proinflammatory cytokine tumor necrosis factor (TNF) impart a differential response to fish oil supplementation to treat rheumatoid arthritis.
- Oxidant stress and inflammation- Polymorphisms in the TNF gene impart a differential response to vitamin E to promote anti-oxidant activity and reduce inflammatory processes.
- Carbohydrate metabolism-Based on polymorphisms in the gene called carbohydrate responsive element-binding protein (ChREBP), a key regulator of glucose metabolism and fat storage, Cyclic AMP and a high fat diet inhibit ChREBP and slow down glucose utilization.
- Obesity- In overweight women carriers of the C polymorphisms of the Leptin receptor gene lost more weight in response to low calorie diet than the non carriers.
- Central Nervous System - Extracts of Ginkgo biloba induce differential expressions of 43 cortex genes, 13 hippocampus genes, and four other genes common to both brain regions.
[00017] A Case Study: Chromium and Dopamine Genes. While there is still controversy regarding the effects of chromium salts (picolinate and nicotinate) on body composition and weight loss in general, recent work seems to support the positive change in body composition in humans. The inventors embarked on a study with chromium picolinate to test out the principles of nutrigenomics. In this study they genotyped obese subjects for the dopamine D2 receptors gene (DRD2). The subjects were assessed for scale weight and for percent body fat. The subjects were divided into matched placebo and chromium picolinate (CrP) groups. The sample was separated into two independent groups; those with either an Al/Al or A1/A2 allele and those with only the A2/A2 allelic pattern. The measures of the change in fat weight, change in body weight, the percent change in weight, and the body weight change in kilograms were all significant, whereas no significance was found for any parameter for those subjects possessing a DRD2 A1 allele. These results suggest that the dopaminergic system, specifically the density of the D2 receptors, confers a significant differential therapeutic effect of CrP in terms of weight loss and change in body fat. Moreover, the inventors propose for the first time that mixed effects now observed with CrP administration in terms of body composition, may be resolved by typing the patient via DRD2 genotyping prior to treatment with chromium salts.

[00008] There is a current interest in the relationship between toxins, diet and the role of our genes and biological response. There is emerging data showing differential response to heart disease and other medical conditions based on levels of specific toxins as well as genetics. There is some interesting data on excitotoxins and their widespread use in foods (especially inartificial sweeteners). Blaylock has reviewed the effects of such toxins like lead, aluminum, cadmium, mercury, manganese etc and biological response and the role of genes. To give just one example of an interaction between race, diet and a toxin, American Blacks tend to have a genetic vulnerability to lead due to lactose intolerance, which results in low levels of calcium in their diet. Since lead is, like calcium, a divalent cation, exposure to lead by individuals with very low calcium in their circulating blood or body stores are more likely to absorb lead. And insofar as both genetics and poverty have reinforcing effects in this vulnerability, this may have important ramifications. After all, the prevailing cultural stereotypes of black inferiority just have to coincide with the effect of lead neurotoxicity.

[00019] In terms of obesity research it is noteworthy that genetic manipulation in nutrition metabolism may involve current standard methods for overexpressing, inactivating, or manipulating genes. These molecular biology procedures can be carried out with the maintenance of the genetic information to subsequent generations (transgenic technology) or devised to exclusively transfer the genetic material to a given target organism, which cannot be transmitted to the future progeny (gene therapy). Moreover, the novel technique of RNA interference (RNAi) approach allows for the creation of new experimental models by transient ablation of gene expression by degrading specific mRNA, which can be applied to assess different biological functions and mechanisms.

[00020] DNA-Based Individualized Nutrition - Certainly, if we could get the cost of identifying a person's SNPs down to pennies rather than hundreds of dollars, we will be on the correct path to realizing nutrigenomics. Current costs of genetic tests range from $250 for prenatal tests assessing 76 diseases to $1,595 for Alzheimer's. While there are a number of companies involved in genotyping an individual's DNA, there are few that couple DNA with individualized nutrition, but no other company utilizes genetic
and/or metabolomic testing to customize formulations. Other companies will recommend a host of different supplement pills, but only LifeGen changes the contents of the pill.

[00021] On the other hand, tools are now available and new ones are in progress which will have relevance to the arising field of nutrigenomics. One company already involved in "individualized nutrition", developed a computerized program called which catalogues health priorities and screens out drug - nutrient interactions using approximately 5000 evidence -based rules which will identify individualized nutritional needs. In one scenario a person can swab their mouth for cheek cells and submit the swab to a central DNA laboratory and determine brain related neurotransmitter gene (serotonin, endorphins, GABA, dopamine, acetylcholine, etc.) polymorphisms. If a person carries a gene variant in the serotonin receptor (deficient) then it quite plausible to induce receptor proliferation by providing that individual a tryptophan enhancing substance like chromium and or 5-hydroxytryptophan.

This may be important for adjunctive supplementation to offset some of the symptoms related to a "sweet tooth" which could ultimately result in a reduction of weight. This can then be incorporated into a program on a genome based individualized basis using the Baxter customized packeting system already utilized commercially by a number of companies. We believe that nutrigenomics is closer than ever before and will indeed be the wave of the future. We propose in this provisional that LifeGen has a unique and proprietary process of analyzing this genetic information to deliver customized nutraceutical formulations by using a polymorphic, multi-variant analysis of DNA.

[00022] LifeGen intends on pursuing additional DNA tests, algorithms, and nutraceutical formulations as product lines and indications related all common healthcare concerns, including but not limited to:

- Alcoholism affecting 12,264,000 American
- Drug Addiction affecting 12,500,000 Americans
- Smoking Addiction affecting 46,000,000 Americans
- Obesity affecting 60,000,000 Americans
- Attention Deficit Hyperactivity Disorder affecting 11,200,000
- Pre-Menstrual Dysphoric Disorder affecting 4,000,000 Americans
- Pain sensitivity intolerance

[00023] As scientists engaged in understanding the potential of drug/nutrient responses as a function of our genome and all of its ramifications including academic and commercial aspects, our future looks bright. While, however, being cautious about our future especially in terms regulatory issues, gene-nutrition interactions especially related to genome based response will indeed be the next cornerstone of solid scientific approaches to assist individuals in choosing dietary supplements, functional foods, and even nutritional beverages on an individualized basis. Nutrigenomics is the key to what we have termed "nutritional gene therapy" and from its origin will spring gene mapping as the wave of the future in nutrition. The information provided in this provisional application will serve as evidence of our conviction of this scientific opportunity.
Reward Deficiency Syndrome (RDS) - In order to understand the potential role of RDS as a link to inflammation, pain, and other conditions, we provide important information as a way of background in support of the novel formulae so proposed in this application. Since dopamine is a major component in mechanisms involving RDS and brain function and certain polymorphisms of the dopamine D3 receptor gene plays a role in the function of prostaglandin induced transcription activity, RDS seems to be linked. The Reward Deficiency Syndrome (RDS) results from a dysfunction in the Brain Reward Cascade which directly links abnormal craving behavior with a defect in the DRD2 Dopamine Receptor Gene as well as other dopaminergic genes (D1, D3, D4, and D5). Dopamine is a very powerful neurotransmitter in the brain, which controls feelings of well being. This sense of well-being is produced through the interaction of dopamine and neurotransmitters such as serotonin, the opioids, and other powerful brain chemicals. Low serotonin levels are associated with depression. High levels of the opioids (the brain's opium) are associated with a sense of well-being. Kenneth Blum has termed the complex interactions of these powerful neurotransmitters ultimately regulating the Dopaminergic Activity in the Reward Center of the Brain as "The Brain Reward Cascade".

In individuals possessing an abnormality in the DRD2 Dopamine Receptor Gene, the brain lacks enough Dopamine receptor sites to use the normal amount of Dopamine in the Reward Center of the brain and thus reduces the function of Dopamine in this area of the brain. In individuals possessing the variant in the Dopamine Receptor Gene tend to be serious cocaine abusers, may have unhealthy appetites which lead to obesity or overeating or on the other extreme be anorexic with extremely low caloric intake, have levels of stress over an extended time period time period and their addictive brains lead to high generalized craving behavior. In essence they seek substances including alcohol, cocaine, nicotine, and/or glucose (substances known to cause preferential release of dopamine at the Nucleus Accumbens [NAcc]) to activate dopaminergic pathways as a self-healing process to offset their low D2 receptors caused by genetic antecedents known as the dopamine D2 receptor gene Taql A1 allele.

The overall effect is inadequate Dopaminergic Activity in the Reward Center of the Brain. This defect drives individuals to engage in activities, which will increase brain Dopamine function. Consuming large quantities of alcohol or carbohydrates (carbohydrate bingeing) stimulate the brain's production and utilization of Dopamine. So too does the intake of crack/cocaine and the abuse of nicotine. Also, it has been found that the genetic abnormality is associated with aggressive behavior, which also stimulates the brain's use of Dopamine.

Reward Deficiency Syndrome involves a form of sensory deprivation of the brain's reward or pleasure mechanisms. Reward Deficiency Syndrome can be manifested in relatively mild or severe forms that follow as a consequence of an individual's biochemical inability to derive reward from ordinary, everyday activities. We believe that we have discovered at least one genetic aberration that leads to an alteration in the reward pathways of the brain. It is a variant form of the gene for the dopamine D2 receptor, called the A1 allele. This genetic variant also is associated with a spectrum of impulsive, compulsive, and addictive behaviors. The concept of the Reward Deficiency Syndrome unites those disorders and may explain how simple genetic anomalies give rise to complex aberrant behavior.
Evidence for the existence of RDS in Substance Use Disorder. In 1990, Blum and colleagues, using the TaqI polymorphism of the dopamine D2 receptor gene locus (DRD2), for the first time reported a strong association between a virulent form of alcoholism and the minor allele (A1) of the Drd2 gene in this population. Other more recent studies further support an association of the A1 allelic form of the DRD2 gene with substance abuse vulnerability and other compulsive behaviors. This association serves as the cornerstone of the biogenetic disease model and could ultimately lead us to better diagnosis and targeted treatment. A complete review of this work can be found in the Journal of Psychoactive Drugs.

This provisional patent application will highlight the importance of a new concept, which provides a clearer understanding of impulsive, addictive, and compulsive behaviors. It is our notion that the real genesis of all behavior, whether so-called normal (socially acceptable) or abnormal (socially unacceptable) behavior, derives from an individual’s genetic makeup at birth. This predisposition, due to multiple gene combinations and polymorphisms, is expressed differently based on numerous environmental elements including family, friends, educational status, economical position, environmental pollutants, and availability of psychoactive drugs including food. We believe the core of predisposition to these behaviors is a set of genes which promote a feeling of well-being via neurotransmitter interaction at the “reward site” of the brain (located in the meso-limbic system), leading to normal dopamine release. We also subscribe to the notion that at least one major gene, the dopamine D2 receptor gene, is responsible for the synthesis of dopamine D2 receptors. And further depending on the genotype (allelic form A1 versus A2), the dopamine D2 receptor gene dictates the number of these receptors at post-junctional sites.

A low number of dopamine D2 receptors suggests a hypodopaminergic function, as described by Eliot Gardner in a series of published works. When there is a paucity of dopamine receptors the person will be more prone to seek any substance (including glucose) or behavior that stimulates the dopaminergic system as a form of self-healing. In this regard we know that substances such as alcohol, cocaine, heroin, nicotine and glucose, as well as a number of behaviors like gambling and sex, preferentially release dopamine at the n. accumbens (the reward site). Understanding this preamble allows us to introduce the concept of reward deficiency syndrome into the field of addictive behavior, which will serve as a model to explain the commonality of a number of seemingly diverse addictions based on shared genetics and neurochemistry. In this regard, most recently, Qing-Shan Yan reported that ethanol, at a peak concentration within five to 10 minutes after interparenteral administration, significantly increased both extracellular dopamine and serotonin in the n. accumbens, supporting the role of these two neurotransmitters in the reinforcing properties of ethanol. Moreover, Honkanen and associates also found low basal dopamine release in alcohol accepting (AA) compared to alcohol non-accepting (ANA) rats, showing that dopamine plays a role in high alcohol preference of AA rats. One important study from Nora Volkow’s group further provides support for the role of the dopamine D2 receptor gene in alcohol intake in rats. Utilizing a cDNA construct of the dopamine D2 receptor gene implanted into the n. accumbens of rats, they found that following a four-day treatment, the dopamine D2 receptors increased to 150% above pretreatment level and alcohol drinking was reduced by 50%.

After a period of 8 eight days, the D2 receptor density returned to pretreatment level and so did alcohol
drinking. Twenty-four days later, second injections of the same construct caused a similar increase in
density with a two-fold decrease in drinking. The same group has confirmed this work in mice.

[00031] Reward Genes and The Addictive Brain - In 1990 Kenneth Blum in conjunction with Ernest P.
Noble from UCLA and our colleagues, published a paper suggesting that a specific genetic anomaly was
linked to alcoholism. Unfortunately it often was reported erroneously that we had found the
"alcoholism gene. "Such misinterpretations are common-readers may recall accounts of an "obesity
gene" or a "crime gene." These reports imply that there is a one-to-one relationship between a gene and
a specific behavior. Needless to say, there is no such thing as a specific gene for alcoholism, obesity, or
criminal behavior. However, it would be naive to assert the opposite, that these complex problems of
human behavior are not associated with any particular genes. Rather the issue at hand is to understand
how certain genes and behaviors are connected.

[00032] In the past nine years scientists have pursued the association between certain genes and
various behavioral disorders. In molecular genetics, an association refers to a statistically significant
incidence of a genetic variant (an allele) among genetically unrelated individuals with a particular
disease or condition compared to a control population. In the course of our work Blum and others
discovered that the genetic anomaly previously found to be associated with alcoholism also is found
among people with other addictive, compulsive, or impulsive disorders. The list is long and remarkable-it
comprises overeating and obesity, Tourette Syndrome, attention deficit and hyperactivity disorder (as
well as just ADD) and pathological gambling. We believe these disorders are linked by a common
biological substrate, a "hard-wired" system in the brain (consisting of cells and signaling molecules) that
provides pleasure in the process of rewarding certain behavior. Consider how people respond positively
to safety, warmth and a full stomach. If these needs are threatened or are not being met, we experience
discomfort and anxiety. An inborn chemical imbalance that alters the intercellular signaling in the brain's
reward process could supplant an individual's feeling of well-being with anxiety, anger or a craving for a
substance that can alleviate the negative emotions. This chemical imbalance manifests itself as one or
more behavioral disorders termed "Reward Deficiency Syndrome."

[00033] This syndrome involves a form of sensory deprivation of the brain's pleasure mechanisms. It can
be manifested in relatively mild or severe forms that follow as a consequence of an individual's
biochemical inability to derive reward from ordinary, everyday activities. The inventors believe that we
have discovered at least one genetic aberration that leads to an alteration in the reward pathways of
the brain. It is a variant form of the gene for the dopamine D2 receptor, called the A2 allele (low D2
receptors), which may have been the natural prehistoric trait. This is the same genetic variant that was
previously found to be associated with alcoholism as well as obesity (see below).

[00034] We look at evidence suggesting the A2 allele also is associated with a spectrum of impulsive,
compulsive, and addictive behaviors, including a predisposition to overeating. The concept of the
Reward Deficiency Syndrome unites these behaviors (impulsive/addictive/compulsive) and may explain
how simple genetic anomalies give rise to complex aberrant behavior. Oddly enough, compared to the
so called "normal" variant the A2, which occurs in approximately two-thirds of Americans having a
normal compliment of D2 receptors, the A1 carriers may be predisposed to overeating, have a higher percent body fat, and have innate craving for carbohydrates.

[00035] The Biology of Reward - The pleasure and reward system in the brain was discovered by accident in 1954. The American psychologist James Olds was studying the rat brain’s alerting process, when he mistakenly placed the electrodes in a part of the limbic system, a group of structures deep within the brain that generally are believed to play a role in emotions. When the brain was wired so the animal could stimulate this area by pressing a lever, Olds found that the rats would press the lever almost nonstop, as much as 5,000 times an hour. The animals would stimulate themselves to the exclusion of everything else except sleep. They would even endure tremendous pain and hardship for an opportunity to press the lever. Olds clearly had found an area in the limbic system that provided a powerful reward for these animals. Olds’ research on human subjects revealed the electrical stimulation of some areas of the brain (medial hypothalamus, which is in the limbic system) produced a feeling of quasi-orgasmic sexual arousal. If certain other areas of the brain were stimulated, an individual experienced a type of light-headedness that banished negative thoughts. These discoveries demonstrated pleasure is a distinct neurological function that is linked to a complex reward and reinforcement system.

[00036] It is useful to think of the brain’s reward system as a cascade in which one reaction triggers another. At the level of individual neurons, the reward cascade is catalyzed by a number of neurotransmitters. Each neurotransmitter binds to certain types of receptors and serves a specific function. The binding of the neurotransmitter to a receptor on a neuron, like a key in a lock, triggers a reaction that is part of the cascade. Disruption of these intercellular cascades results in one form or another of the Reward Deficiency Syndrome.

[00037] The Cascade Theory of Reward - During the past four decades, considerable attention has been devoted to the investigation of neurochemical and neuroanatomical systems underlying chemical dependency. The research on the neuropharmacological basis of dependence on alcohol, opiates, cocaine and glucose points to the involvement of common biochemical mechanisms. It appears as if a limbic-accumbens-pallidal circuit is the critical substrate for the expression of drug reward. However, while each substance of abuse appears to act on this circuit at a different step, the end result is the same, the release of dopamine the primary chemical messenger of reward at such reinforcement sites as the NAcc and the hippocampus. In a normal person, neurotransmitters (the messengers of the brain) work together in a pattern of stimulation or inhibition, the effects spreading downward from complex stimuli to complex patterns of response like a cascade, leading to feelings of well-being: the ultimate reward (Cascade Theory of Reward). Although the neurotransmitter system is too complex and still not completely understood, the main central reward areas in the human brain’s meso-limbic system are summarized in Drawings 3a &3b.
In the reward areas the following interactions take place:

- serotonin (1) in the hypothalamus (I) indirectly activates opiate receptors (2) and causes a release of enkephalins in the ventral tegmental region A10 (II). The enkephalins inhibit the firing of GABA (3), which originates in the substantia nigra A9 region (III);
- GABA’s normal role, acting through GABA B receptors (4), is to inhibit and control the amount of dopamine (5) released at the ventral tegmental regions (II) for action at the nucleus accumbens (IV). When the dopamine is released in the nucleus accumbens it activates dopamine D2 receptors (6), a key reward site [there are at least five dopamine receptors, including D2]. This release also is regulated by enkephalins (7) acting through GABA (8). The supply of enkephalins is controlled by the amount of the neuropeptidases (9), which destroy them;
- dopamine also may be released into the amygdala (V). From the amygdala, dopamine (10) reaches the hippocampus (IV) and the CA, cluster cells (VII) stimulates dopamine D2 receptors (11), another reward site.
- an alternate pathway involves norepinephrine (12) in the locus of ceruleus A6 (VIII) whose fibers project into the hippocampus at a reward area centering around cluster cells which have not been precisely identified, but which have been designed a CAx (IX). When GABA A receptors (13) in the hippocampus are stimulated, they cause the release of norepinephrine (14) at the CAx site (See Figure 3b).

It is to be noted that the glucose receptor (GR) in the hypothalamus is intricately involved and "links" the serotonergic system with opioid peptides leading to the ultimate release of dopamine at the n. accumbens. In the "cascade theory of reward" as defined by Blum and Kozlowski, these interactions may be viewed as activities of subsystems of a larger system, taking place simultaneously or in sequence, merging in cascade fashion toward anxiety, anger, low self-esteem, or other "bad feelings" or toward craving for a substance that will make these bad feelings go away, for example sugar. Certainly, many overweight individuals also cross abuse other psychoactive substances (e.g. alcohol, cocaine, and nicotine). Alcohol activates the norepinephrine fibers of the mesolimbic circuitry through a cascade of events, including the interaction of serotonin, opioid peptides, and dopamine. In a more direct fashion, through the subsequent formation of the neuroamine condensation products TlQs, alcohol may either interact with opioid receptors or directly with dopaminergic systems.

In the cascade theory of carbohydrate bingeing, genetic anomalies, long-continued stress, or long-term abuse of sugar can lead to a self-sustaining pattern of abnormal craving behavior in both animals and humans. Animal model support for the cascade theory can be derived from a series of experiments carried out by T.K. Li et al. upon their substance-prefering (P) [seek carbohydrates, alcohol, opiates, etc.] and nonpreference (NP) rat lines. They found that P rats have the following neurochemical profile:

- lower serotonin neurons in the hypothalamus;
- higher levels of enkephalin in the hypothalamus (due to a lower release);
• more GABA neurons in the nucleus accumbens;
• reduced dopamine supply at the nucleus accumbens;
• reduced densities of dopamine D2 receptors in the meso-limbic areas.

[00041] This suggests a four-part cascade sequence leading to a reduction of net dopamine release in a key reward area. This was further confirmed when McBride et al. found that administering substances which increase the serotonin supply at the synapse, or by stimulating dopamine D2 receptors directly, craving behavior could be reduced. Specifically, D2 receptor agonists reduce alcohol intake in high alcohol preferring rats whereas D2 dopamine receptor antagonists increase alcohol drinking in these inbred animals.

[00042] Inhibitors of Enkephalinase(s) and Craving Behavior - As stated earlier, although it is known that opiates and/or opioids reportedly increase food intake in animals and humans, some papers suggest the opposite-suppression of food intake, especially when one considers macro selection of food sources (i.e., sugar/ carbohydrates). Moreover, Broekkamp et al. reported that infusion of enkephalin into the ventral tegmental AIO area of the brain induces a short-term latency behavioral stimulant effect reminiscent of effects produced by stimulation of the meso-limbic dopamine pathway; this effect is blocked by pretreatment of the opiate receptor antagonist naloxone. This takes on importance in terms of feeding behavior, as feeding has been shown to increase dopamine levels in various brain structures such as the posterior hypothalamus, the nucleus accumbens, and the amygdala.

[00043] It is well known that dopamine in sufficient concentration can inhibit food intake. Gilman and üchttingfeld proposed as an appropriate therapeutic for carbohydrate binging (i.e., bulimia) a selective D2 agonist such as bromocriptine [or natural released dopamine], providing D2 occupancy. In this regard, using a push-pull cannula technique, Chesseelet et al. were able to induce dopamine release in the "brain reward center" after local application of enkephalin, which suggests regulation by delta receptor stimulation. Indeed Kelotphan (an inhibitor of the opioid peptide degrading enzyme) may protect against possible cholecystokinin-8 (CCK-8) degradation by brain peptidases. This important satiety neuropeptide is co-localized with dopamine in the nucleus accumbens, and there is a close interaction between CCK-8, dopamine, and endogenous opioid peptides (like enkephalins). The opioid peptides are involved not only in macro-nutrient intake, but have been implicated in substance seeking, as well as brain self-stimulation behavior. In essence, there are a substantial number of animal experiments which support not only the "Brain Reward Cascade" but the subsequent sequela induced by a defected reward cascade leading to a number of addictive, compulsive and impulsive behaviors-defined as the "Reward Deficiency Syndrome".

[00044] In this regard, Blum et al. reversed alcohol-seeking behavior in genetically preferring C57Bl/6J mice with the chronic administration of an enkephalinase inhibitor. In other work by George et al., they concluded that a relative lack of enkephalin peptides trans-synaptically, possibly resulting from enhanced enkephalin degradation, might contribute to increased alcohol consumption in C57Bl/6J mice. Moreover, others showed that intracranial self-stimulation by rats was reduced by nucleus accumbens microinjections of ketorphan, a potent enkephalinase inhibitor.
Brain Hypodopaminergic Function and The Self-Healing Process - Since deficits have been found in neurotransmitter functions underlying craving behavior, and since these deficits may be alleviated by facilitated dopamine release consequent to the use of drugs, nicotine, alcohol, and food, the studies mentioned above indicate enkephalinase inhibition may similarly compensate for neurotransmitter imbalance (i.e., opioids, thereby attenuating craving behavior). In an attempt to understand that carbohydrate craving is a subset of generalized craving behavior ("Reward Deficiency Syndrome"), due to hypodopaminergic function (an impaired "reward cascade"), scientists believe individuals self-heal through biochemical (licit or non-illicit) attempts to alleviate the low dopaminergic brain activity via drug-receptor activation (alcohol, heroin, cocaine, and glucose). It is conjectured this will substitute for the lack of reward and yield a temporary sense of well-being. In order to help explain this so called self-healing process, it is germane that the reinforcing properties of many drugs of abuse may be mediated through activation of common neurochemical pathways, particularly with regard to the meso-limbic dopamine system. In this regard, glucose, opiates, nicotine, cocaine, tetrahydrocannabinol (THC), and ethanol have been shown to directly or indirectly enhance release or block re-uptake of dopamine in at least one of the primary terminal sites for the limbic dopamine neurons, the nucleus accumbens.

A number of studies of genetically bred animal models support the D2 dopamine receptor involvement in substance-seeking behavior due to lower D2 receptor sites in preferring compared to non-prefering animals. One inference from these observations is that ethanol intake, as well as the self-administration of other substances (i.e., glucose), might be altered by manipulation of dopamine receptors. Of interest, Gardener observed further confirmation of the "Reward Deficiency Syndrome" in generalized substance-behavior involving slow dopamine release in the nucleus accumbens in polysubstance seeking Lewis animals.

Reward Deficiency Syndrome: Human Studies - Human support for the Reward Deficiency Syndrome can be derived from a series of clinical trials with neuronutrients (precursor amino acid loading technique and enkephalinase inhibition) indicating:

- Reduced alcohol and cocaine craving
- Reduced stress rates
- Reduction of leaving treatment against medical advice (AMA)
- Facilitated recovery
- Reduced relapse rates
- Reduction in carbohydrate bingeing
- Loss of body weight
- Prevention of weight regain
- Reduction of glucose craving
- Enhancement of insulin sensitivity
- Reduction of cholesterol
- Enhancement of memory and focus
- Enhanced compliance with narcotic antagonists.
There are a number of studies using precursor amino-acids and enkephalinase inhibition which have been shown to affect various aspects of RDS (see below).

### [00048] Summary of Completed Clinical Studies with Nutraceutical Supplementation (A Literature Review)

<table>
<thead>
<tr>
<th>Drug Abused or Dysfunction</th>
<th>Supplement Used</th>
<th>No. of Patients</th>
<th>No. of Days</th>
<th>Study Type</th>
<th>Significant Results</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>Tropamine</td>
<td>54</td>
<td>30</td>
<td>TO IP</td>
<td>Drug hunger significantly reduced in patients taking SAAVE as compared to controls; 4.2 percent AMA rate for patients</td>
<td>Blum et al. Enkephalinase inhibition and precursor amino acid loading improves inpatient treatment of alcoholics and polydrug abusers: a double-blind placebo-controlled study of the neuronutrient intervention adjunct SAAVE. <em>Alcohol</em>. 1989; 5:481.</td>
</tr>
<tr>
<td>Condition</td>
<td>Treatment</td>
<td>Number</td>
<td>Patient</td>
<td>TO/OP</td>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------------</td>
<td>--------</td>
<td>---------</td>
<td>-------</td>
<td>--------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Alcohol and Cocaine</td>
<td>SAAVE and Tropamine</td>
<td>60</td>
<td>379</td>
<td>TO CP</td>
<td>At end of one year over 50 percent of the alcoholic DUI offenders not using SAAVE dropped out of the program while less than 15 percent of those using SAAVE dropped out. For the cocaine abusers over 90 percent of the Non-Tropamine group dropped out, but less than 25 percent of the patients in the control group.</td>
<td>Brown et al. Neurodynamics of relapse prevention: a neuronutrient approach to outpatient DUI offenders. <em>J. Psychiatric Drugs.</em> 1990; 22:173.</td>
</tr>
<tr>
<td>Over-Eating</td>
<td>PCAL 103</td>
<td>27</td>
<td>90</td>
<td>TO OP</td>
<td>The PCAL 103 group lost an average of 27 pounds in 90 days compared with an average loss of 10 pounds for the control group. Only 18.2 percent of the PCAL 103 patient group relapsed compared to 82 percent of the patients in the control group.</td>
<td>Blum et al. Neuronutrient effects on weight loss on carbohydrate bingeing in a bariatric setting. <em>Curr Ther Res.</em> 1990; 48:2a17.</td>
</tr>
<tr>
<td>Over-Eating</td>
<td>PCAL 103</td>
<td>247</td>
<td>730</td>
<td>PCOT OP</td>
<td>After two years, craving and binge eating were reduced one-third in group of patients on PCAL 103, as compared to the control patients. PCAL 103 group regained 14.7 pounds of their lost weight compared with 41.7 percent weight regained in control patients.</td>
<td>Blum K, Cull JG, Chen JHT, Garcia-Swan S, Holder JM, Wood R, et al. Clinical relevance of PhenCal in maintaining weight loss in an open-label, controlled 2-year study. <em>Curr Ther Res.</em> 1997; 58:745-63.</td>
</tr>
<tr>
<td>Over-Eating</td>
<td>Chromium Picolinate (CP) and L-Carnitine</td>
<td>40</td>
<td>112</td>
<td>RDBPC CP</td>
<td>21 percent increase (p&lt;0.001) in resting metabolic rate (RMR), no change in lean body</td>
<td>Kaats FE et al. The short-term therapeutic effect of treating obesity with a</td>
</tr>
<tr>
<td>Over-Eating</td>
<td>Chromium Picolinate</td>
<td>32</td>
<td>180</td>
<td>DBPC OP</td>
<td>After six months the CrP group had an increase in lean body mass and avoided non-fat related weight loss. Difference between groups was significant at p&lt;0.001.</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>---------------------</td>
<td>-----</td>
<td>------</td>
<td>---------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>154</td>
<td>72</td>
<td>RDBPC OP</td>
<td>200 and 400 mcg of CrP brought about significant changes in Body Mass composition indices when compared with placebo.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>122</td>
<td>90</td>
<td>RDBPC OP</td>
<td>After controlling for differences in caloric expenditure and caloric intake as compared with the placebo group, 400 mcg CrP group lost significantly more weight (p&lt;0.001) and body fat (p&lt;0.004), had a greater reduction in body fat (p&lt;0.001), significantly improve body composition (p&lt;0.004).</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>122</td>
<td>90</td>
<td>RDBPC OP</td>
<td>Measures of changes in fat weight, change in body composition.</td>
<td></td>
</tr>
</tbody>
</table>


Blum K, Kaats G, Eisenbery A, Sherman
<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Study Design</th>
<th>Duration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over-Eating</td>
<td>Chromium Picolinate and Chromium Picolinate comparison</td>
<td>43 63 ROTPC OP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

  CrP supplementation resulted in significant weight gain, while exercise training combined with CrP supplementation resulted in significant weight loss and lowered insulin response to an oral glucose load. Concluded high levels of CrP supplementation are contraindicated for weight loss, in young obese women. Moreover, results suggested that exercise combined with CrP supplementation may be more beneficial than exercise training alone for modification of certain CAD or NIDDM risk factors.


<table>
<thead>
<tr>
<th>Healthy Volunteers</th>
<th>Tropagen</th>
<th>15 30 DBPC OP</th>
<th>Non-drug subjects with Tropagen performed better on computer memory and performance tasks as measured with P300 wave evoked potential. Changes in P300 wave evoked potential result in better focusing ADHD patients</th>
</tr>
</thead>
</table>
Most recently, research by Ortiz and associates at Yale University School of Medicine and the University of Connecticut Health Services Center supported the notion of dopamine as the "final common pathway" for a number of diverse drugs of abuse such as cocaine, morphine, and alcohol, as well as glucose. This support demonstrates that chronic treatment with cocaine, morphine, or alcohol similarly results in several biochemical adaptations in the meso-limbic dopamine system, which may "underlie prominent changes in the structural and functional properties of the neuronal pathway" related to the above. The brain reward cascade schematic (DRAWING 3B), since then, became the blueprint for the search for "reward genes". We propose that the Reward Deficiency Syndrome gives rise to a wide range of disorders that can be classified as impulsive-addictive-compulsive diseases. Impulsive diseases include attention deficit disorder and Tourette's Disorder. Addictive diseases include substance-seeking behavior involving alcohol, drugs, nicotine, and most importantly food. Compulsive diseases include pathological gambling and excessive sexual activity. In terms of personality disorders it includes conduct disorder, oppositional defiant disorder, antisocial personality disorder, schizoid/avoidant behavior, violent aggressive behaviors (See DRAWING 1).

**Reward Genes - Historical background** - In the late 1980's Blum was inspired by a Jane England (1987) paper reporting the association of a variant found on chromosome 11 at the tyrosine hydroxylase loci in bi-polar affective disorder among the Amish. This molecular genetic observation coupled with the then current research on the inheritance of alcoholism provided the impetus for Blum, and associates to investigate potential genetic differences between alcoholics and nonalcoholics. They suspected that one of the differences was the activity of chemical signaling molecules in the brain. Over the course of two years they compared eight genetic markers associated with various neurotransmitters and metabolic enzymes (including serotonin, endogenous opioids, GABA, transferrin, acetylcholine, and alcohol and aldehyde dehydrogenases). In each instance they failed to find a direct association between the genetic markers and alcoholism. Finally, as we stated above, Blum and Noble and others began to study the gene which controls the laying down of dopamine D2 receptors-dopamine D2 receptor gene. They found a very significant association between the Dopamine D2 receptor gene and severe alcoholism. In their original study, over 70 percent of the alcoholics had cirrhosis of the liver, a disease suggestive of severe and chronic alcoholism. Quickly following this first study published in the Journal of the American Medical Association (JAMA), a number of other flawed studies were negative. The negative studies failed to adequately assess controls to eliminate alcoholism, drug abuse, and other related "reward behaviors" including carbohydrate binging and used less severe alcoholics. In this regard, Drs. Katherine Neiswanger and Shirley Hill of the University of Pittsburgh (funded by the National Institutes of Alcoholism and Alcohol Abuse) found a strong association of the D2 A1 allele and alcoholism. Hill suggested failures reported in the literature were due to poor assessment of controls. Their suggestion significantly bolsters the appropriate use of "super"; controls to more accurately assess a true phenotype. This is especially important when studying complex behavioral diseases. The same
researchers found evidence for linkage between the dopamine D2 receptor gene and severe alcoholism, early onset, physical dependence symptoms, and Antisocial Personality Disorder.

**Scientific Support**

*Alcohol-opiates common mechanisms*

[00051] In 1970's, some postulated that brain chemistry involving craving behavior for alcohol was similar to that of opiates like heroin and morphine. In fact it was shown that you could block the central nervous responses of both alcohol and opiates with the narcotic antagonist naloxone. During this time, multiple studies were conducted that built upon this body of science.

In an attempt to find a common agent to reduce craving for alcohol, especially in genetically bred alcohol accepting mice (with low endorphin levels), it was found that the substance D-phenylalanine (a substance known to inhibit the breakdown of peptides) shown earlier as an antidepressant, significantly reduced alcohol acceptance in these drinking mice while increasing brain levels of endorphins. Then it was found that stress induced a reduction of endorphins in the brain with concomitant reduction of alcohol seeking behavior. It was also found that brain injections of endorphins into the reward center significantly reduced alcohol intake in alcohol preferring rats and mice.

[00052] Later, an analysis of the brains (limbic system) of Golden hamsters (love alcohol) that drank alcohol for one year (equivalent to 20 years in the human) resulted in a significant reduction of brain endorphins compared to non-drinking Golden hamsters (no alcohol). This was also found to be true for morphine in terms of brain endorphin reduction.

[00053] Shortly after these two published reports it was found that there was a one-third reduction of brain reward endorphins in humans drinking alcohol for over 20 years compared to non-drinking humans. It was also shown in mice that low levels of brain endorphins resulted in high alcohol intake; middle levels of brain endorphins resulted in moderate alcohol intake and high brain levels of endorphins resulted in mice hating alcohol. Based on these experiments three types of alcoholics or drug seeking behavior individuals were categorized. Type 1 = genetically prone with low levels of endorphins; Type 2 = stress induced low levels of endorphins; Type 3 = alcohol/opiates induce low levels of endorphins. Then a number of studies showed that narcotic antagonists like Naltrexone could significantly reduce human alcohol and morphine consumption.

**Brain-Reward Cascade**

[00055] The interactions of separate subsystems below merge together into the much larger global system of "brain reward". These activities take place simultaneously and in a specific sequence, merging like a cascade. The end result is a sense of peace, pleasure, and well-being when these systems work normally. If there is a deficiency or imbalance, the system will work abnormally, causing the sense of well-being to be displaced by feelings of anxiety, anger, low self-esteem, or other "bad feelings". This can lead to the craving for a substance that masks or relieves those bad feelings such as carbohydrate...
binge drinking, alcohol, or cocaine; or to other addictive behaviors such as compulsive gambling, compulsive sex, workaholism, or engaging in high risk activities.

[00056] We are cognizant that both the Glutamate and endocannabinol pathways are involved in the inhibitory process which impinges on GABA neurons to cause a preferential release of Dopamine in the n accumbens. Of cause there is also interaction with NMDA and CBI receptors.

Serotonergic system

[0057] Many studies in the late 60's began to show the relationship between serotonin and alcohol intake. This was coupled with the early work showing a significant relationship between low brain levels of serotonin and stress. Then it was shown that stress induced aberrant alcohol drinking behavior. Later, it was shown that stress and low brain serotonin levels linked to high alcohol intake. In another study, it was shown that if you place certain rats (alcohol non-prefering rats) in a dark closet you induce high alcohol intake. Moreover, if you inject these rats with a substance melatonin (synthesized in the pineal gland-seat of the soul-regulator of circadian rhythm) in the daytime the non-prefering rats drink as if they were in the dark closet. Additionally, if you sever the nerve controlling the pineal gland this effect is eliminated. It is well known that melatonin is involved in the synthesis of serotonin. In the night serotonin is low thus drinking behavior goes up. Injections of melatonin increase brain serotonin so reduced drinking even in the day. Then, based on this and other pharmacological evidence, large pharmaceutical companies targeted this pathway to reduce aberrant drinking behavior with serotonergic uptake inhibitors like Zoloft. However, there was more to the story.

Endorphinergic system

[0058] Between the 70's and 80's there were a series of papers that suggested an induction of a brain chemical called TIQ's that linked alcohol with opiates. TIQ's were found in the brain of alcoholic rodents and monkeys and had analgesic activity like opiates. Their pharmacological effects were blocked by naloxone and the TIQ's induced a significant reduction of alcohol intake. Like endorphins they activated mu opiate receptors having a similar profile of the endorphins. Thus many studies even to date continue to show not only this link but support the important role of endorphins in alcohol and other craving behaviors.

[0059] A recent study emphasizes the endorphinergic connection to alcoholism. Single injections of alcohol to high-alcohol preferring rats results in an increase in blood endorphin levels. The new drug to treat alcoholism approved by the FDA is called Acamprosate. This drug can also increase blood levels of endorphins. However, what seems to be important is that acamprosate prevents the depleting effects of alcohol induce loss of brain endorphins. Since endorphins are important and necessary as a brain component to provide a balanced brain and normal physiological craving desires for food, thirst and sex, this work provides a potential mechanism of understanding. It may be concluded that acamprosate modulates the endogenous opioid system and that is one mechanism for its anti-alcohol effects.
Gabergic system

[00060]GABA is the most ubiquitous inhibitory compound in the brain. This substance, works as an anti-anxiety natural brain chemical in the reward system of the brain through a well known brain receptor system benzodiazepines (a calming chemical-tranquilizer). Many studies show the unique relationship between alcohol actions and GABA.

[00061]One of the important findings related to GABA is that it is involved in regulating another important chemical brain reward messenger Dopamine (DA). In fact, if you activate GABA you tend to inhibit the neuronal release of DA at the brain reward site. Thus if you inhibit GABA you will increase DA release in the brain reward site. In fact, this is the second mechanism involved in the action of acamprosate. It was designed to do just that. Moreover, you could also accomplish this by stimulating the cannabinoid receptors (i.e. marijuana) as well. Since it is well know that chronic alcohol reduces the action of GABA, it helps explain why under high alcohol consumption there is a poor DA release and thus a powerful craving response. DA is indeed the brain "pleasure molecule" and anti-stress substance. When it is low craving for the normal physiological drives of food, thirst and sex go up. While the blocking of this GABA-DA connection may reduce immediate drug induced euphoria (a short term fix proposed by many in the addiction field) in the long term may prevent normal physiological drives or reward (knocking out the DA response) and cause mood disorders (depression, more craving behaviors). This has been now seen with the FDA rejected drug Accomplia, a cannabinoid receptor blocker.

Dopaminergic System

[00062] In the early 70's scientists working in the addiction field began to show the potential connection of DA and various forms of mental states (e.g. depression, schizophrenia and craving behavior). Many studies then and now have connected DA and alcohol pharmacologic effects. One of the first studies to illustrate this interaction showed that alcohol induced withdrawal symptoms in rodents can be blocked by injections of L-Dopa (needed for the production of DA) and even DA. This pleasure inducing substance being found at the nucleus accumbens (brain reward site) is released by alcohol and its release is controlled by GABA. It is well known that substances that stimulate DA receptor sites in the brain can reduce craving behavior in all its forms (i.e. drugs, sugar, sex, etc). The key here is that therapeutic targets that involve DA activation may indeed be the best approach to substance seeking behaviors.

[00063] Thus, with this background we have proposed something called the brain-reward cascade. In this cascade stimulation of the serotonergic system in the hypothalamus leads to the stimulation of delta /mu receptors by serotonin to cause a release of enkephalins. Activation of the enkephalinergic system induces an inhibition of GABA transmission at the substantia nigra by enkephalin stimulation of mu receptors at GABA neurons. This inhibitory effect allows for the fine tuning of GABA activity. This provides the normal release of dopamine at the projected area of the nucleus accumbens (reward site of the brain). Thus the release of DA induces a feeling of well-being, "reward" and "happiness". Without being too bold, any company holding the IP around DA activation by both pharmaceutical and
nutraceutical intervention may hold an important key to one of the world’s most devastating dilemmas’
- reward deficiency.

Reward Deficiency Syndrome Concept

[00064] By the early 80’s there were many suggestions that alcoholism had genetic antecedents and a
very high hereditability rate. The world of genetics as it related to finding gene associations was just
beginning. In fact, in the late 70’s and early 80’s even in single gene disorders like Huntington’s scientists
did not find gene associations. Harvard scientists utilizing a new technique called Restriction Fragment
Length Polymorphisms (RFLP) just found the gene for this devastating disease. Then in 1987, scientist
using this technique found the first gene connection to depression in an Amish population. This was the
gene involved in the conversion of the amino-acid tyrosine to DA. This prompted other scientist to also
utilize this methodology to explore candidate genes for another complex disorder called alcoholism. The
result was an association of the dopamine D2 receptor gene (DRD2) and severe alcoholism (see below
for more detail).

[00065] Following great controversy many studies around the globe found this same association for
alcoholism. While the press erroneously coined the finding which was published in JAMA as “experts
found the alcoholic gene” the scientists never stated that they found an alcoholic gene. Instead they
clearly stated that they have found a “reward gene”. This was borne out by many subsequent papers
that also found association with the DRD2 gene variant called the A1 allele with not only alcoholism,
children of alcoholics, but cocaine dependence, heroin addiction, smoking behavior, marijuana
dependence, ADHD, Tourette Syndrome, autism, tics, early onset sexual intercourse, high defense style
(lying), pathological gambling, obesity, BMI, overeating, percentage body fat, energy expenditure,
pathological aggression, inability to cope with stress, schizoid avoidance behavior, glucose metabolism,
memory, shopping addiction, among other addictive behaviors. It was also calculated utilizing a Bayes
theorem (named after a 16th century monk) that carrying the DRD2 A1 allele at birth should provide a
74% chance that these positive carriers would engage in one or more of these behaviors. These
behaviors were grouped into a classification of an umbrella termed “Reward Deficiency Syndrome (RDS).

[00066] This concept was first published in the scientific journal of the Royal Society of Medicine in 1996.
Since this time a number of reports supported his novel concept and the number of supportive increases
yearly. In this regard, a number of other genes and their variants (called polymorphisms) have emerged
in the scientific literature. These genes seem to include the same systems involved in the proposed
brain-reward cascade (genes involved synthesis, release mechanism(s), second messenger responses,
 synaptic activity genes [transporter], catabolism and metabolism etc). The systems involve serotonergic,
opioidergic, cannabinoidergic, gabaergic, dopaminergic, etc.

[00067] The brain reward circuitry, in particular, the dopaminergic system and the dopamine D2
receptor, has been implicated in reward mechanisms (Blum et al. 1991). The net effect of neurotransmitter
interaction at the mesolimbic brain region induces “reward” when dopamine (DA) is released from the
neuron at the nucleus accumbens and interacts with a dopamine D2 receptor (Blum et al. 1996, Blum
The "reward cascade" (Blum and Kozlowski, 1990) involves the release of serotonin, which in turn at the
hypothalamus stimulates enkephalin, which in turn inhibits GABA at the substantia nigra, which in turn
regulates the amount of DA released at the nucleus accumbens or "reward site" (Gessa et al.1985,Yadid, 1994, Parsons et al.1996, Halibus et al.1997, Dick et al.2004). It is well known that under
normal conditions in the nucleus accumbens DA works to maintain our normal drives (Adler et al.2000,

[00068] In fact, DA has come to be known as the "pleasure molecule" (Hall and Bloom,1977, Blum, 1991,
Comings et al. 1991, Koob,1992, Nakajima, 1989, Blum et al. 1996c, Miller et al. 1999) and/or the "anti-
stress molecule" (Comings et al. 1996, Kreek and Koob, 1998, Pani et al., 2000). When DA is released into
the synapse, it stimulates a number of DA receptors (D1-D5), which results in increased feelings of well-
being and stress reduction (Robinson and Berridge, 1989, Blum and Braverman, 2000).

[00069] A consensus of the literature suggests that when there is a dysfunction in the brain reward
network or cascade, which could be caused by certain genetic variants (polygenic), especially in the DA
system causing a hypodopaminergic trait as suggested by Gardner (1997), the brain of that person
requires dopaminergic activation. This trait leads to multiple drug seeking behaviors (Blum et al. 1996a,b, Comings et al. 1994, Comings and Blum, 2000, Volkow et al. 2001). This holds true as alcohol,
psychostimulants like cocaine, heroin, marijuana, nicotine, and glucose all result in activation and
neural release of brain DA which in turn could help attenuate craving behavior (Di Chiara, and
Imperato, 1988).

[00070] Further support of this notion is derived from the first report by Blum et al (1990) and other
subsequent work (Blum et. al. 1991, 1993) showing an association of the dopamine D2 TaqAl allele with
severe alcoholism and other work which found decreased D2 receptors in carriers of the A1 allele (Noble
et al. 1991, Hietata, 1994). A recent multiple population study (Xu et al. 2004) from the National
Institute on Alcohol Abuse and Alcoholism, supported a role of the D2 Dopamine Receptor gene (a
haplotype block at 25.8kb region) in Substance Use Disorder (SUD) [alcohol and heroin]. Utilizing
positron emission tomography (PET) others have found substantial lower levels of D2 receptors in
alcohol and drug dependent subjects compared to non-dependent individuals (Volkow et al. 1996).
Moreover, Volkow's group found that subjects with high levels of D2 receptors did not like the effects of
psychostimulants, while individuals with low D2 receptors enjoyed the effects of psychostimulants
(Volkow et al. 2001). In animals, overexpression of the D2 receptor via vector delivery of the D2 gene
directly into the nucleus accumbens resulted in significant reduction of alcohol consumption (Myers and

[00071] Reward Deficiency Syndrome (RDS), first coined by Dr. Kenneth Blum in 1995 and published in
1996, links genetic polymorphisms to a common thread of dopaminergic dysfunction leading to
addictive, compulsive and impulsive aberrant behavior (Blum et al. 1996b). Many natural rewards
increase dopamine neurotransmission.
For example, eating something that you enjoy or engaging in sexual behavior can cause dopamine levels to increase. In these graphs, dopamine is being measured inside the brains of animals, its increase shown in response to food or sex cues. This basic mechanism has been carefully shaped and calibrated by evolution to reward normal activities critical for survival. However, there are certain genes, including the DRD2 gene Taq 1 allele that make someone genetically predisposed to a deficiency in this reward circuitry. This deficiency results in cravings of substances or activities to compensate. Drugs of abuse increase dopamine neurotransmission. Because drugs activate these brain regions (usually more effectively than natural rewards) they have an uninhinherent risk of being abused.

Drug-induced repeated disturbances in dopamine cell activity can lead to long-term and deleterious effects in the brain. These effects can be detected using brain imaging technologies. Positron emission tomography (PET), for example, is a powerful technique that can demonstrate functional changes in the brain. The images depicted in the image below using PET show that similar brain changes result from addiction to different substances, particularly in the structures containing dopamine. Dopamine D2 receptors are one of five receptors that bind dopamine in the brain. In this image below, the brains on the left are those of normal controls, while the brains on the right are from individuals addicted to cocaine, methamphetamine, alcohol, or heroin. The striatum (which contains the reward and motor circuitry) shows up as bright red and yellow in the normal controls, indicating numerous D2 receptors. Conversely, the brains of addicted individuals (on the right row) show a less intense signal, indicating lower levels of D2 receptors. This reduction likely stems from a chronic over-stimulation of the second (post-synaptic) neuron (schematically illustrated in the right hand column), a drug-induced alteration that feeds the addict's compulsion to abuse drugs.

Gene directed therapeutic targets

Gene therapy for many diseases seems to be the wave of the future. While we are still in its infancy some exciting research has emerged in many disciplines. Studies on rodents revealed the first successful gene therapeutic model for RDS behaviors. Nucleus accumbens injection of a viral vector carrying the cDNA (compliment DNA) of the DRD2gene resulted in an increase of D2 receptors with a concomitant reduction of alcohol seeking behavior. In terms of treatment outcomes compliance is an important issue. For most therapeutics even in the pharmaceutical field less than half of patients receiving medication actually comply. As early as 1995, it was found that certain genotypes might hold the clue to poor compliance. One example is the finding that carriers of the DRD2 A2 variant (allele) [the normal gene variant] had a higher attrition rate compared to the carriers of the DRD2 A1 variant [the RDS variant] with regard to alcoholism treatment using a DA D2 receptor activator (agonist), known as bromocriptine. Most recently this effect was confirmed in a study utilizing an experimental DNA customized nutraceutical called Genotrim. Carriers of the DRD2 A2 variant had a higher attrition rate (50.1 days on treatment), compared to the DRD2 A1 variant (110 days on treatment.). This tends to suggest that possibly the DRD2 A1 variant may be a persistency genotype that may have utility for a wide array pharmaceutical and nutraceutical modalities (see Figure 2).

Certainly many (100's) other genes are involved. A short list includes: DRD1, DRD2, DRD3, DRD4, DRD5, DAT1, HTT, HTRIA, TD02, DBH, ADRA2A, ADRA2C, NET, MAOA, COMT, GABRA3, GABRB3, CNRI,
CNRA4, NMDARI, PENK, AR, CRF, HTR1D, HTR2A, HTR2C, interferon-CD8A, or PSI, ANKKl, TD02, SREBP-lc, PPAR-gamma-2, MGPAT, NYP, AgRP, POMC, CART, OBR, Mc3R, Mc4R, UCP-I, GLUT4, C-FOS, C-JUN, C-MYC, Interleukin 1-alpha, interleukin-1 beta, interleukin-8, tumor necrosis factor-alpha, intracellular adhesion molecule, and interleukin-10, CYP2D6, P-glycoprotein, ABCBl, mu opioid receptor, delta opioid receptor, kappa opioid receptor, sigma opioid receptor, gamma opioid receptor, among other genes (see below).

Description of Clinical Trials

[00076] There are a number of studies using precursor amino-acids and enkephalinase inhibition that have been shown to affect various aspects of RDS [see Table 1 below]. TABLE 2 Summary of Completed Clinical Studies with Nutraceutical Supplementation (A Literature Review) Detoxification measures: Blum K. Trachtenberg MC, Ramsey IP reduction in benzodiazepine requirement, reduction in J. Improvement of inpatient treatment withdrawal tremors after 72 hours, reduction in of the alcoholic as a function of depression neuronutrient restoration: a pilot study, Int J Addiction. 1988; 23: 991-98. Blum K. Trachtenberg MC. Neurogenic deficits caused by alcoholism: restoration by SAAVE. Journal of Psychoactive Drugs. 1988; 20: 297. Alcohol SAAVE 62 21 DBPC Reduction in psychosocial stress reduction as measured Blum et al. Enkephalinase inhibition plus IP by SCL, reduced BESS score, improved physical score, and precursor amino acid loading Poly- six-fold decrease in likelihood of leaving AMA after five improves inpatient treatment of drugs days, alcoholics and poly-drug abusers: a double-blind placebo-controlled study of the neuronutrient intervention adjunct SAAVE. Alcohol. 1989; 5: 481. Cocaine Tropamine 54 30 TO Drug hunger significantly reduced in patients taking Blum et al. Reduction of both drug IP SAAVE as compared to controls: 4.2 percent AMA rate hunger and withdrawal against advice for patients on Tropamine versus 28 percent for patients rate of cocaine abusers in a 30 day on SAAVE and 37 percent for controls, inpatient treatment program with the neuronutrient tropamine. Curr Ther Res. 1988; 43: 1204. Alcohol SAAVE and 60 379 TO At end of one year over 50 percent of the alcoholic DUI Brown et al. Neurodynamics of and Tropamine CP offenders not using SAAVE dropped out of the program relapse prevention: a neuronutrient Cocaine while less than 15 percent of those using SAAVE approach to outpatient DUI offenders, dropped out. For the cocaine abusers over 90 percent J. Psychiatric Drugs. 1990; 22: 173. of the Non-Tropamine group dropped out, but less than 25 percent of the patients in the control group. Over- PCAL 103 27 90 TO The PCAL 103 group lost an average of 27 pounds in 90 Blum et al. Neuronutrient effects on Eating OP days compared with an average loss of 10 pounds for weight loss on carbohydrate bingeing the control group. Only 18.2 percent of the PCAL 103 in a bariatric setting. Curr Ther Res. patient group relapsed compared to 82 percent of the 1990; 48: 2a7. patients in the control group. Over- PCAL 103 247 730 PCOT After two years, craving and binge eating were reduced Blum K. Cull JG, Chen JHT, Eating OP one-third in group of patients on PCAL 103, as Garcia-Swan S, Holder JM, Wood R. compared to the control patients. PCAL 103 group et al. Clinical relevance of PhenCal regained 14.7 pounds of their lost weight compared with in maintaining weight loss in an 41.7 percent weight regained in control patients, open-label, controlled 2-year study. Curr Ther Res. 1997; 58: 745-63. Over- Chromium 40 112 RDBPC 21 percent increase (p < 0.001) in resting metabolic rate Kaats FE et al. The short-term Eating Picolinate CP (RMR), no change in lean body mass (LBM), RMR: LBM therapeutic effect of treating obesity (CP) and L increased 25 percent (p < 0.001).
Body fat decreased with a plan of improved nutrition and Carnitine approximately 1.5 lbs./week, and
reduction in serum moderate caloric restriction. Cholesterol while increasing RMR with no loss of LBM
Curr Ther Res. 1992; 51: 261. Over- Chromium 32 180 DBPC After six months the CrP group had an
increase in lean Bahadori B, Habersack S, Schneider Eating Picolinate OP body mass and avoided non-fat
related weight loss. H, Wascher TC, Topiak H. Treatment Difference between groups was significant at p
< 0.001. with chromium picolinate improves lean body mass in patients following weight reduction.
significant Kaats FE, Blum K, Fisher JA, Eating Picolinate OP changes in Body Mass composition indicies
when Aldeman JA. Effects of chromium compared with placebo picolinate supplementation on body
mass composition: a randomized, double-blind, placebo-controlled study. Curr Ther Res. 1996; 57: 747-
56 Over- Chromium 122 90 RDBPC After controlling for differences in caloric expenditure Kaats FE, Blum
K, PulMn D, Keith Eating Picolinate OP and caloric intake as compared with the placebo group, Sc. Wood
R. A randomized 400 meg CrP group lost significantly more weight double-masked placebo-controlled (p
< 0.001) and body fat (p < 0.004), had a greater study of the effects of chromium reduction in body fat (p
< 0.001), significantly improve picolinate supplementation on body composition (p < 0.004).
RDBPC Measures of changes in fat weight, change in body Blum K, Kaats G, Eisenbery A, Eating
Picolinate OP weight, percent change in weight, and body weight Sherman M, Davis K, Comings DE.
changes in kgms were all significant in A2/A2 group, Cull JG, Chen THJ, Wood R, and non-significant in
A1/A2 and Al/Al carriers. Bucci L, Wise JA, Braverman ER, and PulMn D. Chromium Picolinate Induces
Changes in Body Composition as a Function of the TaqI Dopamine D2 Receptor Al Alleles ( in press)
Gene Therapy & Molecular Biology. Chromium 43 63 ROTPC CrP supplementation resulted in significant
weight gain, Grant KE, Chandler RM, Castle AL, Eating Picolinate OP while exercise training combined
with CrP Ivy JL. Chromium and exercise and supplementation resulted in significant weight loss and
training; effect on obese women. Chromium lowered insulin response to an oral glucose load. J Am
Sports Med 1997; 29(8): Picolinate Concluded high levels of CrP supplementation are 992-8. comparison
contraindicated for weight loss, in young obese women. Moreover, results suggested that exercise
combined with CrP supplementation may be more beneficial than exercise training alone for
modification of certain CAD or NIDDM risk factors Healthy Tropagen 15 30 DBPC Non-drug subjects with
Tropagen performed better on Defrance JJ, Hymel C, Trachtenberg Volun- OP computer memory and
performance tasks as measured MC et al. Enhancement of attentionwith P300 wave evoked potential.
Changes in P300 processing by KANTROL in healthy wave evoked potential result in better focusing ADHD
Solution

It is our belief that if there is a genetic tendency to abuse alcohol, opiates, stimulants,
carbohydrates, nicotine, especially in individuals carrying the DRD2A1 allele, which causes a one-third
decrease of D2 receptors in the reward system of the brain, nutraceutical manipulation of the brain
reward circuitry will be beneficial. High craving behavior may indeed be tied to low D2 receptors. Low
D2 receptors are tied to DRD2A1 allele. Slow D2 agonistic action of any D2 agonist including natural
dopamine, causes a slow but steady proliferation of D2 receptors even against one's genetic make up. It
is also our belief that the Synaptamine Complex will cause a preferential DA relapse at the NAC which
will ultimately increase D2 receptors and reduce craving behavior.

Field Of invention

[00078] Brain Nutrition and Behavior - A detailed account of this subject is treated in the books Alcohol
and The Addictive Brain (Blum, 1991 The Free Press), and To Binge or Not to Binge? (Blum, Cull & Miller,
1998 Psychiatric Genetic Press). In short, if genetic anomalies result in neurotransmitter imbalance, then
how could we help to restore balance? At the functional level, it seems clear that neurotransmitter
imbalance may be a problem of brain nutrition: more specifically, a deficiency or excess of amino acids.

In the healthy body, amino acids are in balance; if there is an excess or shortage, distortions of brain
function can result.

[00079] As we know the brain cannot synthesize all of the amino acids involved in the formation of
neurotransmitters; some are derived from food metabolism, and come to the brain via the blood supply.

There are two categories of amino acids: essential and nonessential. There are five essential amino acids
necessary for the manufacture of neurotransmitters, thought to play a role in obesity: methionine,
leucine, phenylalanine, tyrosine, and tryptophan (see above for more detail). Among the nonessential
amino acids manufactured in the body, Glutamine probably plays a significant role, because it is involved
in the manufacture of GABA. Two forms of amino acids are found in nature. The amino acids in the brain
that make up the neurotransmitters, and the enzymes that regulate them, are all derived from the L-
form. The D-form (as in D-phenylalanine) is found in a few microorganisms and in multi-cellular
organisms like frog skin.

[00080] Single Versus Multiple Amino acid Neuronutrients

- First, although a single amino acid may be involved in the formation of a given neurotransmitter,
it does not act alone. It needs the help of co-factors such as vitamins and minerals before the
formation can take place. For example, vitamin B6 (in the alcoholic, pyridoxal -5-phosphate
form is required) is needed for the manufacture of dopamine.
- Second, obesity is the result of a complex disorder that involves processes taking place in the
neuron, at the synapse, and at receptors.
- Third, we cannot determine (until we use DNA tests) the specific defect that is producing a
particular part of the problem. Therefore, in the effort to offset neurotransmitter deficits, it is
not feasible to depend on single amino acids. This is why we include both serotoninergic and
dopaminergic precursors.
- Fourth, an odd characteristic of the blood/brain barrier actually makes treatment easier. Most
overweight individuals have compounded stress and may have comorbid addictions like alcohol,
smoking, and other drugs; it is known that all of these weaken the barrier facilitating the
passage of restorative substances such as amino acids into the brain. This is particular important
when you consider large neutral amino carrier system and competition of tryptophan,
phenylalanine and tyrosine. It is equally important when you consider, as mentioned earlier,
that the rate limiting enzyme Tyrosine Hydroxylase works best under stressful conditions and
the precursor tyrosine will indeed be converted to dopamine and will be subsequently released
into the synapse of the N. accumbens.
• Fifth, it is well known that the degradation of catecholamines by COMT plays a role, albeit only partial, in clearing these neurotransmitters from synaptic cleft. Dopamine, norepinephrine and serotonin reuptake into nerve terminals via membrane transporter is thought to play a more significant role. However, it is our position that any enhancement of the neurotransmitters in the synapse is positive. In this regard, the effects of synephrine on norepinephrine receptors and the central nervous system effects of Rhodiola rosea could contribute to a sibutramine/ d-fenfluramine-like effect. The amount of Rhodiola rosea recommended in the formula is 240 mg per day (based on an extract standardized to 3% rosavin), which is somewhat higher than the recommended dose for use of Rhodiola rosea as an antidepressant (200mg/day). Moreover, the NGI formula also contains synephrine, derived from citrus aurantium (6% synephrine) at a daily dose of 50mg. This amounts to only 6 mg per day. While this is less than what is normally recommended as a sympathomimetic agent, when combined with caffeine thermogenesis could be achieved without the stimulatory effects seen with much higher doses (104mg/day).

[00081] Studies Showing Anti-craving Efficacy of Precursor Amino-acids and Enkephalinase Inhibitor

Activity - It is our contention that with the formula as designed for anti-craving, additive or even synergistic outcomes might be observed since the ingredients are included that could act through several different mechanisms to enhance the activity of the neurotransmitters. The patented complex has been named Synaptamine™.

• In a number of experiments we have shown brain changes of the enkephalins using d-phenylalanine (500mg/kg/day for 18 days and or its metabolite hydrocinnamic acid (intracerebral ventricular injection of 25 micrograms) in mice; Using the same doses these known enkephalinase inhibitors significantly reduced alcohol preference in both acceptance and 14 day preference test.

• We have shown in healthy volunteers electrophysiological changes (enhanced memory and focus) with the combination of DL-phenylalanine (1500mg/day), L-tyrosine (900mg/day), L-glutamine (300mg/day), chromium picolinate (360 micrograms /day) and other co-factors;

• Positive effects in alcoholics in an in-patient hospital including lower building up to drink scores, required no PRN benzodiazepines, (0% vs. 94%), ceased trembling at 72 hours, had no severe depression on the MMPI, in contrast to 245 of control group (Blum et al. 1988). The ingredients included DI-phenylalanine( 2760mg/kg/day), L-tryptophan (150mg/day), L-glutamine (150mg/day), and pyridoxal-5-phosphate (30mg/day);

• In a double-blind placebo controlled study of polysubstance abusers in an in-patient hospital, the combination of DI-phenylalanine (2760mg/day), L-tryptophan (150mg/day), L-glutamine (150mg/day), and pyridoxal-5 -phosphate (30mg/day), significantly reduced stress, improved physical and emotional scores, a six -fold reduction in AMA rates, enhanced treatment recovery;

• Utilizing DL-phenylalanine (1500mg/day), L-tyrosine(900mg/day), L-glutamine (300mg/day), L-tryptophan (400mg/day) and pyridoxal -phosphate (20mg/day) in inpatient treatment of cocaine abusers over a 30 day period compared to controls significantly reduced drug hunger and withdrawal against advice rate (AMA), reduced need for benzodiazepines, and facilitated retention in the treatment program;

• In an outpatient clinic DUI offenders (alcoholics and/or cocaine addicts) were treated with a combination of dl-phenylalanine, L-tyrosine, L-glutamine, Chromium, pyidoxy-5-phosphate over a ten month period. Compared to a vitamin control (only B-complex and vitamin c), the
experimental group significantly reduced relapse rates and enhanced recovery in these DUI
outpatient offenders. The retention rates obtained for alcoholics was 87% for the experimental
group compared to only 47% of the control patients and for cocaine abusers the numbers are
80% vs. only 13%. For alcoholics: DL-phenylalanine (2760mg/day), L-Glutamine (150mg/day),
chromium picolinate (360 micrograms/day), pyridoxal -5-phosphate; For cocaine abusers: DL-
phenylalanine (1500mg/day), L-Tyrosine (900mg/day), L-glutamine (300mg/day), pyridoxal -5-
phosphate (20mg/day).

- Utilizing amino-acid and enkephalinase inhibitory therapy, J.A. Cold found significant
improvement in both cocaine craving and withdrawal symptoms in out patient cocaine addicts.
The ingredients included DL-phenylalanine (1500mg/day), L-Tyrosine (900mg/day), L-glutamine
(300mg/day), pyridoxal -5-phosphate (20mg/day).
- With only chromium picolinate it was found in two double - blind placebo controlled studies
that doses of either 00 meg or 400 meg resulted in a body composition improvement, loss of
body fat, gain in nonfat mass;
- In addition see above for similar results dependent on the DRD2 Al variant (unpublished Blum &
Kaats);
- With DL-phenylalanine (2700mg/day), L-tryptophan (150mg/day), L-glutamine (150mg/day) and
pyridoxal -5 phosphate (30mg/day) it was also found that 27 outpatients with high
carbohydrate bingeing behavior where females were assigned 800 calories total intake per day
and males were assigned 1,000 to 1,200 calories per day and all withdrew from sugar use
attending a supervised diet- controlled treatment program, the supplement group over a 90
day period lost an average of 26.96 pounds compared to the control group (no supplement) lost
only 10 pounds. In fact, only 18.2 % of the experimental group relapsed (lost less than 15
pounds over the 90 day period) compared to 8.3 % in the control group;
- In another study where the supplement contained dl-phenylalanine (2760mg/day), L-tryptophan
(150mg/day), L-glutamine (150mg/day), pyridoxal -5 phosphate (30mg/day), chromium
Picolinate (200 micrograms/day), and carnitine (60mg/day) over a 2-year period in 247 obese
patients the following results were obtained in a dual blind non-randomized open trial utilizing
Centrum vitamin as a control: compared with the Non-PhenCal / Centrum group the
experimental PhenCal/Centrum group showed a two-fold decrease in percent overweight for
both males and females; a 70 % decrease in food cravings for females and a 63% decrease for
males; and a 66% decrease in binge eating for females and a 41 / % decrease for males. Most
importantly, the experimental group regained only 14.7% of the lost weight, and multiple
regression modeling revealed that with PhenCal treatment, morbid obesity and binge eating
score were significant predictors of weight gain after 2 years. In contrast, family history of
chemical dependence was most closely associated, although not statistically significant, with
improved results with PhenCal.
- Blum decided to test the hypothesis that possibly by combining a narcotic antagonist and amino
acid therapy consisting of an enkephalinase inhibitor (D-Phenylalanine) and neurotransmitter
precursors ( L-amino -acids) to promote neuronal dopamine release might enhance compliance
in methadone patients rapidly detoxified with the narcotic antagonist TrexanR. ( Duponr,5
Delaware). In this regard, Thanos et. al. and associates found increases in the dopamine D2
receptors (DRD2 ) via adeno viral vector delivery of the DRD2 gene into the nucleus accumbens,
significantly reduced both ethanol preference (43%) and alcohol intake (64%) of ethanol
preferring rats, which recovered as the DRD2, returned to baseline levels. This DRD2
overexpression similarly produced significant reductions in ethanol non-prefering rats, in both
alcohol preference (16%) and alcohol intake (75%). This work further suggests that high levels of
DRD2 may be protective against alcohol abuse. The DRD2 Al allele has also been shown to
associate with heroin addicts in a number of studies. In addition, other dopaminergic receptor
gene polymorphisms have also associated with opioid dependence. For example, Kotler et al.
showed that the 7 repeat allele of the DRD4 receptor is significantly overrepresented in the opioid
dependent cohort and confers a relative risk of 2.46. This has been confirmed by Li et al. for
both the 5 and 7 repeat alleles in Han Chinese case control sample of heroin addicts. Similarly
Duaux et al. in French Heroin addicts, found a significant association with homozygotes alleles
of the DRD3-Bal 1. A study from NIAAA, provided evidence that strongly suggests that DRD2 is a
susceptibility gene for substance abusers across multiple populations. Moreover, there are a
number of studies utilizing amino acid and enkephalinase inhibition therapy showing reduction
of alcohol, opiate, cocaine and sugar craving behavior in human trials. Over the last decade, a
new rapid method to detoxify either methadone or heroin addicts utilizing TrexanR sparked
interest in many treatment centers throughout the United States, Canada, as well as many
countries on a worldwide basis. In using the combination of TrexanR and amino-acids, results
were dramatic in terms of significantly enhancing compliance to continue taking Trexan®. The
average number of days of compliance calculated on 1,000 patients, without amino-acid
therapy, using this rapid detoxification method is only 37 days. In contrast, the 12 subjects
tested, receiving both the Trexan® and amino-acid therapy was relapse-free or reported taking
the combination for an average of 262 days (P < 0.0001). Thus coupling amino-acid therapy and
enkephalinase inhibition while blocking the delta receptors with a pure narcotic antagonist may
be quite promising as a novel method to induce rapid detox in chronic methadone patients. This
may also have important ramifications in the treatment of both opiate and alcohol dependent
individuals, especially as a relapse prevention tool. It may also be interesting too further test
this hypothesis with the sublingual combination of the partial opiate mu receptor agonist
buprenorphine. The ingredients tested included DL-phenylalanine (2760mg/day), L-Glutamine
(150mg/day), chromium picolinate (360 micrograms/day), pyridoxal -5-phosphate (30mg/day).

Most recently a study was performed by Julia Ross best selling author of The Diet Cure ( Viking
Press USA, 1999; Penguin UK, Au, and USA, 2000), in an outpatient clinic in Mill Valley,
California involving amino-acid therapy and enkephalinase inhibition based on Blum's work. At
Recovery Systems, Ross has successfully utilized this approach to treat a number of RDS
behaviors, especially eating disorders. In a preliminary evaluation, utilizing the following
ingredients tailored made for each client, dl-phenylalanine, 5-hydroxytryptophan, L-tryptophan,
L-tyrosine, L-glutamine, chromium, vitamin B6, follow-up interviews of six randomly selected
former eating disordered female clients (three were also chemically dependent), were
contracted nine months to three years post - treatment to evaluate efficacy of combining
targeted nutritional elements (amino-acids, vitamins, digestive enzymes, a diet low in refined
carbohydrates but adequate in calories and other nutrients) with conventional counseling,
education, and peer support. Follow-up confirmed significant initial benefits in mood and
freedom from compulsive behavior and ideation in 100% tested. While one subject relapsed
within six months, the remaining five subjects all sustained, and in some cases exceeded
expectations. Following this preliminary evaluation, the authors, also evaluated an additional
100 patients and the data collected revealed 98% significant improvement in both mood and
reduced craving for not only carbohydrates but other abusable substances as well. According to
Ross this work further suggests the positive potential of adding targeted nutritional protocols to
conventional treatment elements to improve outcome in an RDS intransigent population.

A study in Las Vegas at an outpatient clinic has been completed. The following results have been
evaluated and presented herein. Relapse rates: CCD: Out of 15 patients only 2 patients dropped
out, while the other 13 patients remained in the program for 12 months. Therefore, the percent
relapse for this group is 13.33; CC - Out of 43 patients 11 patients dropped out, while the other
32 patients remained in the program for 12 months. Therefore, the percent relapse for this group is 23.2.; FCS- Out of 10 patients only 2 dropped out, while the other 8 patients remained in the program for 12 months. Therefore, the percent relapse for this group is 20.0.; SR- Out of 8 patients none dropped out, thus 8 patients remained in the program for 12 months. Therefore, the percent relapse for this group is 0.0. If we calculate the percent relapse of the entire program which included a total of 76 patients with a total of 15 patients that dropped out it is a remarkable 19.9 % relapse. The majority of drop outs (11 out of 15 or 73.3 %) were methamphetamine abusers. The ingredients include DL-phenylalanine (2700mg/day), 5 - hydroxytryptophan (20mg/day), L-Tyrosine (750mg/day), L-glutamine (350mg/day), Rhodiola rosea (3% rosavin) (66mg/day), Chromium dinicotinate glycerate 1000 micrograms/day), DMAE (40mg/day), Huperzine A (150 micrograms/day). Combination of vitamins (C,E, Niacin, Riboflavin, Thiamin, B6 [20% Pyridoxal - 5 phosphate and 80% Pyridoxine], folic acid, B12, Biotin, Pantothenic acid, Calcium, Magnesium, zinc, Manganese and a herbal calming blend, focus blend or mood enhancing blend. The ingredients and dosage was dependent on type of abusers including diagnosis of ADHD.

Fortunately, if a broad menu of amino acids is available in sufficient quantity, the brain appears to have the ability to choose from the menu the one or ones needed to manufacture more of the neurotransmitter that is deficient. Based on the patents and technology afforded to us, the following nutrients are scientifically formulated and have been clinically tested for over 20 years and have relevance to the problem defined as "Reward Deficiency Syndrome", more specifically-overeating and carbohydrate bingeing. However, the work to date supports a generalized anti-craving claim.

- D-Phenylalanine, to inhibit enkephalinase, the enzyme that metabolizes or breakdown enkephalins, thereby increasing the availability of enkephalins and, presumably, making more dopamine available at the reward sites especially under stressful conditions.
- L-Phenylalanine, to stimulate the production of dopamine, and/or increase norepinephrine levels in the reward area of the brain. The major problem with this amino acid is that it could compete with other amino acids, such as blood borne L-tryptophan and L-tyrosine at the large neutral amino acid brain carrier system (see Milner et al. 1986). However, other data demonstrates for the first time that the synthesis and release responses to some dopaminergic agents may be elicited from synaptosomal dopamine, which is formed by the hydroxylation of phenylalanine. Amphetamine and Cogentin increased the release of dopamine formed from 14C-phenylalanine in rat caudate nucleus synaptosomal preparation and concomitantly stimulated the synthesis. Amfocal acid also caused a net release of that dopamine. In conclusion, the results suggest that synaptosomal particles represent a unit capable of synthesizing dopamine from L-phenylalanine and that synthesis from this precursor may be under the regulatory control of the particles.
- L-glutamine, to increase brain GABA levels at receptors associated with anxiety. Its major use is to maintain balance in case of over inhibition by D-phenylalanine.
- L-5-hydroxytryptophan (or its natural form) - The effect of systemic administration of 5-hydroxy-L-tryptophan on the release of serotonin in the lateral hypothalamus of the rat in vivo as examined utilizing brain microdialysis. Administration of 5-HTP caused an immediate increase of the 5-HT in dialysates, which was long lasting and dose dependent. When calcium was omitted from the perfusion medium, thereby limiting exocytosis, levels of basal 5-HT were significantly decreased and the 5-HTP- induced response of 5-HT was markedly attenuated.
• Pyridoxal-5-phosphate, the active ingredient of vitamin B6 to serve as a co-factor in the production of neurotransmitters and to enhance the gastrointestinal absorption of amino acids.

• Chromium Salts (Nicotinate and Picolinate), have a number of metabolic effects including: increase of insulin sensitivity; reduction of cholesterol; reduction of percent body fat; reduction of weight loss; maintaining muscle mass promoting lean; enhancing body composition; promotes brain serotonin production (see above).

• Calcium, promotes neurotransmitter release based on many studies

• Rhodiola rosea - Several clinical trials with double-blind placebo controls in Russia provide evidence that R. rosea possess positive mood enhancing and anti-stress properties with no detectable levels of toxicity. Generally, R. rosea extract has been shown to have a positive influence on the higher nervous system, increasing attention span, memory, strength and mobility of the human body, and weight management. It is believed that R. rosea can act as a COMT inhibitor where brain levels of serotonin and dopamine has been observed. Studies by Saratikov and Marina suggest that R. rosea can increase the level of neurotransmitters by 30 percent and decrease COMT activity by 60 percent. In the weight management area there are double-blind studies with regard to weight loss and fat mobilization.


[00083] Meridia is an approved FDA drug for "weight loss" and weight management. The major effect of this drug is an anti-craving action derived from its effect to inhibit the reuptake of serotonin (5HT), dopamine (DA) and norepinephrine (NE). This inhibition of neurotransmitter reuptake results in an increase in the length of time 5HT, DA, and NE are available to act in the synaptic junction, and ultimately in an amplification of the neurotransmitter effects to reduce sugar/glucose cravings.

[00084] In its simplest form, the ingredients in the patented composition proposed for anti-craving effects mirrors the Meridia mechanism and should produce similar anti-craving effects. In this section we will point out the potential of the ingredients in the proposed formula, based on a large body of neurochemical evidence concerning precursor amino-acids; the role of chromium as a tryptophan enhancing substance; d-amino acid inhibition of enkephalinase; Rhodiola as a suspected inhibitor of catechol-O-methyl transferase (COMT) as well as Synephrine, a substance that can mimic some of the effects of catecholamines. Thus it is anticipated that since the same three neurotransmitters affected by Meridia (Sibutramine), could potentially be affected by certain ingredients, it should produce similar effects. It could be hypothesized that by increasing precursor (i.e. phenylalanine, tyrosine, and chromium and or 5-hydroxytryptophane or any other neurotransmitter enhancer even via transport) intake and inhibiting enzymatic degradation by COMT greater levels of 5HT, DA would be available at the synapse. The availability of the synapse is also increased since the D-phenylalanine causes preferential release of dopamine via opioid peptide breakdown inhibition. Thus the sum total effect is very much like Meridia and the following information will assure the scientific potential of this novel natural formula.

[00085] Most recently, Balcioglu and Wurtman, measured the effects of sibutramine (Meridia), given intravenously, on brain dopamine and serotonin flux into striatal and hypothalamic dialysates of freely
moving rats. While low doses of the drug had no effect, higher doses increased both serotonin and
dopamine concentrations in the striatal and hypothalamic brain regions. These findings further support
the neurochemical effects of sibutramine, and suggest that the drug’s anti-obesity action may result
from changes it produces in brain dopamine as well as serotonin metabolism. The importance here is
that it provides further support for the SYNAPTAMINE formula and both serotonergic and dopaminergic
anti-obesity actions.

**SUMMARY of GNAP**

[00086] In essence, formulations of this type will cause the synthesis of the brain reward
neurotransmitters like serotonin and catecholamines and through its effect on the natural opioids will
by virtue of inhibiting GABA cause a significant release of dopamine at the nucleus accumbens. This
constant release of possibly therapeutic amounts of dopamine (anti-stress substance) occupies
dopamine D2 receptors, especially in carriers of the A1 allele (low D2 receptors and high glucose
craving), and over time (possibly 6-8 weeks) effects RNA transcription leading to a proliferation of D2
receptors, thereby, reducing craving for aberrant substances, improving joint health and reducing the
signs and symptoms of arthritis, reducing fat and optimizing, and providing anxiety relief.

**Example**

**Injured Workers and High Narcotic Use**

The Problem: Preferred Embodiment

[00087] Based on consensus of the literature and past clinical treatment programs individuals that are
gene expression predisposed to Substance Use Disorder (SUD) may be more prone to work related accidents.
This high risk population will posses one or more gene variants (polymorphisms) related to the brain
reward cascade and/or brain circuitry such as:

**Table 1: Genetic Testing - Brain Reward Cascade Allele Genes**

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Genes</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopaminergic</td>
<td>DRD2 receptor genes</td>
<td>Pleasure</td>
</tr>
<tr>
<td>Serotonergic</td>
<td>5-HT2 receptor genes</td>
<td>Depression</td>
</tr>
<tr>
<td>Endorphinergic</td>
<td>Pre-Enkephalin genes</td>
<td>Pain</td>
</tr>
<tr>
<td>Gabaergic</td>
<td>GABA_A receptor genes</td>
<td>Anxiety</td>
</tr>
<tr>
<td>NT Metabolizing</td>
<td>MAO and COMT genes</td>
<td>Enzymatic Breakdown</td>
</tr>
<tr>
<td>Opiate receptor(s)</td>
<td>Delta, Mu, Kappa, Sigma</td>
<td>Pain</td>
</tr>
</tbody>
</table>

Moreover, narcotic addiction must be avoided with these individuals in order to improve their eventual
outcome. These workers typically are the revolving door patients one sees in case management. The
cycle of (injury ~ doctor visit ~ narcotic Rx ~ injury ~ etc.) must be stopped and substituted with a
healthier and more successful methodology of therapy.

[00088] The ACOEM (American College of Occupational and Environmental Medicine, pg 115), guidelines
are very concerned about the problems relating to addiction to narcotic medications. ACOEM states,
"Pain medications are typically not useful in the sub-acute and chronic phases and have been shown to
be the most important factor impeding recovery of function ... Prolonged use of narcotic medications
may cause both physiologic and psychological addiction and may reduce the body's supply of
endorphins, causing depression and delayed recovery."

2. Background:

Treatment of chronic, nonmalignant pain syndromes has been largely suboptimal and the most
debilitating conditions; such as lower back problems, arthritis, and neuropathic pain continue to pose a
significant burden to individuals and society. The answer to these pain syndromes is a double edge
sword. First, is to eliminate or significantly reduce the actual physical pain condition without addicting
pharmaceuticals and the other side of the sword is to identify, treat and follow-up on those individuals
who seem to constantly re-injure themselves. We sometimes discount and label these individuals as
accident prone or clumsy. The truth of the matter is that the majority of these individuals have a genetic
predisposition for addiction called the Reward Deficiency Syndrome (RDS). They have a lower utilization
of the pleasure chemicals in the brain called neurotransmitters (NT) than those with normal counts. This
puts them in a disadvantage and so being makes them prone for accidents, drug seeking behaviors and
placing an overburden on the health care delivery system as a whole.

The causation of drug addiction is probably one of the most complex and difficult issues to
address in the insurance industry. The primary problem is how the patient is to be treated for his/her
narcotic dependency. These individuals are being prescribed behavioral or psychiatric modification
instead of being treated for the underlying medical condition. These patients need to be categorized
into the most successful treatment groups. Unfortunately, a tremendous amount of time and money is
expended in a guessing game as to where these patients would be best suited for treatment.

Unfortunately, the drug seeking patients are the ones who are driving this care. They are very smart and
creative in finding ways to obtain their medication in order to satisfy their neurological dependency for
narcotic usage. These areas are, but are not limited to; frequent Emergency Room usage, doctor
shopping, sharing narcotic drugs, buying street narcotics and partnering with scrupulous medical
providers who will dispense any type of narcotic for a price. The answer is to genetically diagnose and
treat these individuals in clinics with highest integrity.

[00091] At issue is the Reward Deficiency Syndrome (RDS). A drug addict is fairly easy to detoxify and get
off their prescribed narcotic. However, to stop drug relapse and the intense physiological &
psychological cravings are another matter. Most all drug-seeking behaviors originate in the
dopaminergic centers of the mesolimbic brain. (See diagram 1) These centers are responsible for the
feelings of pleasure and a sense of well-being. Any decrease in the dopaminergic system will lead to a
loss of pleasure and eventually lead to drug seeking or risk behaviors. We have now documented that
there is a genetic relationship of the Reward Deficiency Syndrome to the dopaminergic system. The Reward Deficiency Syndrome is based primarily on a common genetic deficiency in the dopamine D2 receptors and other genes, (see Table 1) above. A genetically dependant decrease in the number of neurotransmitter receptors will decrease or attenuate the propagation of the neurological pleasure signal to the affected target organs, thus a lower sense of well-being. DNA gene testing can identify these individuals who carry the affected Reward Deficiency Genes.

[00092] Over 25% of the US population has some form of this genetic deficiency; it is estimated in the Workers Compensation industry that number rises to around 40%. Important to note, is that just because you have a genetic predisposition for an addictive behavior does not mean you will be an addict. Environmental triggers may expose these individuals to addiction. Some of these environmental triggers or influences are more important to some groups over others. The equation below is a prime example of the Nature vs. Nurture dilemma.

### Type I: Born Addiction - Genetic

\[ DCB = G_{\text{DNT}} + E \]

- **DCB** = Drug Craving Behavior
- **G_{\text{DNT}}** = Genetically Decreased Neurotransmitters
- **E** = Environmental influences

Type I individuals have a genetic deficiency in the dopaminergic system. Environmental issues may trigger this behavior but the genetic genotype is much stronger than the environmental influence. This group of individuals will relapse very easy and are usually accident-prone. This may explain why in the workers compensation system this group represents about 35-40% of the W/C injuries. The most successful treatment for this group is a medical adjunctive dopaminergic therapy; The Gnap Program. Psychosocial counseling has a minor influence. When this group is treated correctly, this group has the greatest chance of recovery.

### Type II: Stress Addiction

\[ DCB = G_{\text{NNT}} + E_{\text{S\_DNT}} \]

- **DCB** = Drug Craving Behavior
- **G_{\text{NNT}}** = Genetically Normal Neurotransmitters
- **E_{\text{S\_DNT}}** = Environmental (Stress) Decreased Neurotransmitters

Type II individuals have no genetic deficiency and are drawn into the addiction cycle due to environmental stressful or pain conditions. A good example of this individual would be a woman who was abused as a child. Opiates and alcohol produce a euphoric condition, which will reduce stress. The most successful treatment for this group is a combination therapy of a modified Gnap program to
attenuate the use of narcotics and psychosocial therapy. Psychosocial behavioral therapy is the primary treatment regime for these Type II individuals in order to reduce and or remove any negative Environmental stress influences.

**Type III: Drug Toxicity**

\[ ACB = G_{\text{ANT}} + E_{\text{A\_DNT}} \]

\[ DCB = \text{Drug Craving Behavior} \]

\[ Gnnt = \text{Genetically Normal Neurotransmitters} \]

\[ E_{\text{ADNT}} = \text{Environmental (Abuse) Decreased Neurotransmitters} \]

Type III individuals have no genetic deficiency and are drawn into the addiction cycle due to a long-term drug abuse history of getting high. These individuals usually started taking drugs or alcohol as a social activity and have continued well into their adult life. These individuals are very difficult to treat. They need both medical adjunctive dopaminergic therapy and prolonged psychosocial counseling. Even when this group is treated correctly, they have the lowest success rate of recovery. Luckily, there are a lower percentage of these individuals in the Workers Compensation System vs. the Criminal Justice System.

The purpose of the Gnap program is to identify and correctly treat with gene therapy those individuals who are Type I. Genetic Identification is the KEY to success to isolate and successfully treat these individuals who are Type I. These individuals are the category which will run up the financial costs faster than any of the other groups. With the addition of DNA testing, we now have the tools that will allow the physician to make clinical decisions in the formulation of treatment protocols that are specific to the individual. This program is not a "one-size-fits-all" approach. We customize their specific treatment regime to their genetic footprint. This is what is meant by the statement "gene-therapy." One of the cost effective components of the program is that we are able to treat and contain the individual with their primary treating physician or that of a specialist, there is no reason to advance this person to another level of care and cost, Detox, Rehab and Psychiatric care.

This program has worked very well with hard core narcotic addicts within the Judicial system for over six years with a very high level of patient compliance. It is due to the DNA identification that makes this program possible for the Worker Compensation system. The true estimated cost savings of this program can run between a low of $20,000 to over $150,000 depending upon the recommended treatment and awards given the injured worker. By utilizing the Gnap program a reduction in narcotic addiction would lead to improved health and quicker return to function for the injured worker and significant cost savings to the carrier and employer.

Gnap program has an innumerable amount of Evidence Based Medical studies already published in peer reviewed Pubmed journals and the cost savings that this program affords is certainly worth investigating it further. At this time of the programs implementation we are primarily interested in those individuals which are Type I as discussed above; this means they demonstrate a high narcotic use with low functionality. The duration of narcotic use is not a factor; the individual could be on narcotics for
months or years. In layman’s terms Gnap works well with the Work Comp fires or nightmare patients that everyone wants to avoid. Surprisingly, when you are able to put these fires out with this type of classification and treatment of the individual they become one of the best citizens and workers. They do not need to spend the majority of their time seeking after that which they no longer need or desire and can become more functional and industrious.

The Process

We propose that a threefold approach is needed for the successful treatment of these individuals.

The first step is very important; it is the identification of these predisposed individuals to narcotic abuse through DNA analysis. By taking a swabbing sample inside of the individual’s cheek we have enough cells to perform a DNA analysis, no blood draw is required. With this information we are able utilize empirical medical evidence to categorize these individuals into the most appropriate treatment group. The current mode of differential diagnoses is to give your best educated guess as to which group they belong to and use a trial by error methodology in order to find the most effective course of treatment. Just this one step alone will save hundreds of thousands of dollars by utilizing gene-therapy during the early stages of treatment instead of an ineffective trial by error methodology.

Unfortunately, patients are not obtaining this service at an early treatment intervention but obtaining this genetic testing later down the road of medical treatment usually at Pain Clinic’s.

This condition has been treated through behavioral modification or other non-medical therapies over the past 40 years with a low success rates due to a lack of specific identification of these individuals. DNA testing is the key to the Gnap program. With the appropriate identification of these individuals the prescribing physician can attenuate these individuals off narcotics and assist the employee to become a functional employee within an office setting environment. The cost savings for the employer is substantial. In 2005, ACOEM saw the potential cost savings industry wide and approved genetic testing within the workplace. The Gnap program adheres to all the DNA protocols established by ACOEM.

The second step is the treatment of the RDS by augmenting and balancing the pleasure chemicals in the brain called neurotransmitters (NT) without negative side effects.

Depending upon the DNA genetic results of addiction severity, the individual is placed on either a high-level or a low-level treatment regime in many administrative forms of Synaptamine™, for example in prescription compounded oral suspension or IM injections, in order to obtain the highest possible level of success.

Active treatment duration is 3 months. This program is meant to rebuild the dopamine receptor sites, giving the individual a greater sense of pleasure and well-being, essentially stopping the drug seeking and relapse behaviors. Thus, attenuating the individual from their Narcotic medication and increasing their functional status while at the same time drastically reducing costs. Another benefit of
increased Dopamine is a rising of the patient's pain threshold; patients are able to cope with more of their existing pain than they were before. (See drawings 2 & 3)

The individual also has overlap of true physical pain that needs to be addressed since a non-narcotic treatment intervention is being implemented. For the third step the patient is placed on a non-addictive alternative for pain control. There are a myriad of pain devices and weak acting pain medications on the market today. These will be utilized on a trial basis to see which modality or medication is best suited for the individual. When all the components of the Gnap program are utilized opiate addicts can be drug free in three months without a Psychiatric claim or the use of a Detox/Rehab facility.
## Synaptamine Formulation

### Table 1. Amino Acid Nutrition Therapy

<table>
<thead>
<tr>
<th>Supplemental Ingredient</th>
<th>Restored Brain Chemical</th>
<th>Addictive Substance Abuse</th>
<th>Amino Acid Deficiency Symptoms</th>
<th>Expected Behavior Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Phenylalanine or DL-Phenylalanine</td>
<td>Enkephalins, Endorphins</td>
<td>Heroin, Alcohol, Marijuana, Sweets, Starches, Chocolate, Tobacco</td>
<td>Most Reward Deficiency Syndrome (RDS) conditions sensitive to physical or emotional pain. Crave comfort and pleasure. Desire certain food or drugs. D-phenylalanine is a known enkephalinase inhibitor.</td>
<td>Reward stimulation. Anti-craving. Anti-depression. Mild improved energy and focus. D-Phenylalanine promotes pain relief, increases pleasure.</td>
</tr>
<tr>
<td>L-Phenylalanine or L-Tyrosine</td>
<td>Norepinephrine, Dopamine</td>
<td>Caffeine, Speed, Cocaine, Marijuana, Aspartame, Chocolate, Alcohol, Tobacco, Sweets, Starches</td>
<td>Most Reward Deficiency Syndrome (RDS) conditions. Depression, low energy. Lack of focus and concentration. Attention-deficit disorder.</td>
<td>Reward stimulation. Anti-craving. Anti-depression. Increased energy. Improved mental focus.</td>
</tr>
</tbody>
</table>
Rhodiola rosea has been added to the formula and is a known Catechoi-O-methyS transferase inhibitor (COMT). This provides more synaptic dopamine in the VTA/NAc.

Source: **Perfumi M, Mattioli L.** *Adaptogenic and central nervous system effects of single doses of 3% rosavin and 1% salidroside Rhodiola rosea L extract in mice.* *Phytother Res.*21 2007 37-43.

Chromium salts -This has been added to the formula to enhance insulin sensitivity and resultant brain concentrations of serotonin.

Note: To assist in amino-acid nutritional therapy, the use of a multi-vitamin/mineral formula is recommended. Many vitamins and minerals serve as co-factors in neurotransmitter synthesis. They also serve to restore general balance, vitality and well-being to the Reward Deficiency Syndrome (RDS) patient who typically is in a state of poor nutrition! health. The utilization of GABA is limited due to its polar nature and ability to cross the blood brain barrier. Glutamate is used in a low level only to prevent over-inhibition of enkephalin breakdown and subsequent inhibition of Gabaergic spiny neurons of the substantia nigra.
In terms of formulation we propose a number of forms for the delivery of Synaptamine. These include
but are not limited to the following:

- Oral - Pills, Capsules, tablets, Sublingual, Troche, dissolvable paper thins
- Liquid - Oral suspension, beverage
- Injectable - Intramuscular, Intravenous, intrathecal
- Intra-Rectal
- Ointments
- Patches
- Pellets
- Beverages with powder application

*Genes and Opiate Addiction: A Pharmacogenomic Trieste*

It has been appreciated for some time now that humans react differently to opioids. A specific
opioid such as morphine sulfate may have specific analgesic effects for certain patients with post
herpetic neuralgia whereas in other patients with post herpetic neuralgia, it may provide quite different
analgesic qualities. Also, in any one individual patient a particular opioid may provide better analgesia
than other opioids. Furthermore, these differences are not unique to analgesia; they can also be seen
with other opioid effects/toxicities. Though many of the differences can be classified neatly into
pharmacokinetic and pharmacodynamic differences, there are certain differences which still remain
incompletely understood. Also, clinicians are not yet able to easily predict which patients will respond
well or poorly to various opioids. As research unravels the various genetics, biochemical, and receptor
interaction differences of opioids in humans, it is hoped that easily obtainable, cost-effective testing will
become available to aid clinicians in choosing an optimal opioid analgesic for an individual patient, a
process which is currently accomplished via health care provider judgment along with trial and error. In
the future, knowledge gained from databases on knockout rodents, pharmacogenetics, and gene
polymorphisms may impact on the ability of clinicians to predict patient responses to doses of specific
opioids in efforts to individualize optimal opioid analgesic therapy. It is conceivable that eventually
information of this type may translate into improved patient care. In the future, armed with data of this
type, clinicians may become quite adept at tailoring appropriate opioid therapy as well as optimal opioid
rotation strategies. Currently it is not obvious as to what gene or genes are perfect candidates for gene
directed opioid therapy.

In terms of pain sensitivity certain candidate genes have been studies. Candidate genes such as those for
catechol-O-methyltransferase, melanocortin-1 receptor, guanosine triphosphate cyclohydrolase and
mu-opioid receptor have been intensively investigated, and associations were found with sensitivity to
pain as well as with analgesic requirements in states of acute and chronic pain. In contrast, the impact of
genetic variants of drug-metabolizing enzymes on the response to pharmacotherapy is generally well
described. Polymorphisms of the cytochrome P450 enzymes influence the analgesic efficacy of codeine,
tramadol, tricyclic antidepressants and nonsteroidal anti-inflammatory drugs. Together with further
candidate genes, they are major targets of ongoing research in order to identify associations between an
individual's genetic profile and drug response (pharmacogenetics). Moreover, sensitivity and tolerance to morphine were determined in 2 strains of mice, BALB/cBy and C57BL/6By, their reciprocal F1 hybrids and seven of their recombinant inbred strains. Sensitivity was established based on locomotor activity following the administration of saline, 10 or 20 mg/kg of morphine hydrochloride while tolerance was established according to the "hot plate" method following the single or repeated administration of saline, 5, 10, or 20 mg/kg of morphine hydrochloride. Results indicate that both sensitivity and tolerance to morphine are genotype-dependent and their inheritance is characterized by dominance or partial dominance.

[000104] The most common treatment for opioid dependence is substitution therapy with another opioid such as methadone. The methadone dosage is individualized but highly variable, and program retention rates are low due in part to non-optimal dosing resulting in withdrawal symptoms and further heroin craving and use. Methadone is a substrate for the P-glycoprotein transporter, encoded by the ABCB1 gene, which regulates central nervous system exposure. ABCB1 genetic variability influenced daily methadone dose requirements, such that subjects carrying 2 copies of the wild-type haplotype required higher doses compared with those with 1 copy and those with no copies (98.3 +/- 10.4, 58.6 +/- 20.9, and 55.4 +/- 26.1 mg/d, respectively; P = .029). In addition, carriers of the AGCTT haplotype required significantly lower doses than noncarriers (38.0 +/- 16.8 and 61.3 +/- 24.6 mg/d, respectively; P = .04). Although ABCB1 genetic variability is not related to the development of opioid dependence, identification of variant haplotypes may, after larger prospective studies have been performed, provide clinicians with a tool for methadone dosage individualization.

[000105] Studies of polymorphisms in the mu opioid receptor gene, which encodes the receptor target of some endogenous opioids, heroin, morphine, and synthetic opioids, have contributed substantially to knowledge of genetic influences on opiate and cocaine addiction. Other genes of the endogenous opioid and monoaminergic systems, particularly genes encoding dopamine beta-hydroxylase, and the dopamine, serotonin, and norepinephrine transporters have also been implicated.

[000106] Moreover, genetically caused inactivity of cytochrome P450 (CYP) 2D6 renders codeine ineffective (lack of morphine formation), slightly decreases the efficacy of tramadol (lack of formation of the active O-desmethyl-tramadol) and slightly decreases the clearance of methadone. MDRI mutations often demonstrate pharmacogenetic consequences, and since opioids are among the P-glycoprotein substrates, opioid pharmacology may be affected by MDRI mutations. The single nucleotide polymorphism A118G of the mu opioid receptor gene has been associated with decreased potency of morphine and morphine-6-glucuronide, and with decreased analgesic effects and higher alfentanil dose demands in carriers of the mutated G118 allele. Genetic causes may also trigger or modify drug interactions, which in turn can alter the clinical response to opioid therapy. For example, by inhibiting CYP2D6, paroxetine increases the steady-state plasma concentrations of (R)-methadone in extensive but not in poor metabolizers of debrisoquine/sparteine. So far, the clinical consequences of the pharmacogenetics of opioids are limited to codeine, which should not be administered to poor metabolizers of debrisoquine/sparteine. Genetically precipitated drug interactions might render a standard opioid dose toxic and should, therefore, be taken into consideration. Mutations affecting opioid receptors and pain perception/processing are of interest for the study of opioid actions, but with
modern practice of on-demand administration of opioids their utility may be limited to explaining why
some patients need higher opioid doses; however, the adverse effects profile may be modified by these
mutations. Nonetheless, at a limited level, pharmacogenetics can be expected to facilitate individualized
opioid therapy. It has been demonstrated that the muOR 304G variant significantly reduces intrathecal
fentanyl ED(50) for labor analgesia, suggesting women with the G variant may be more responsive to
opioids and require less analgesic drugs. These findings for intrathecal fentanyl pharmacogenetics may
have implications for patients receiving opioids in other settings.

The following is a sampling of genes involved in the addictive process that we propose can be
informative which relate to Opiate addiction:

mu opioid receptor, delta-opioid receptor; the metabotropic receptors mGluR6 and mGluR8, nuclear
receptor NR4A2 and cryptochrome 1 (photolyase-like), DRD gene (D1-D5), Datl, DBH, proenkephalin
(PENK) and prodynorphin (PDYN), CAMKII; GnRH, CYP2D6; BDNF; NT-3 genes; GABA receptor subunit
genes on 5q33; GABA(A)gamma2; OPRM1; G-protein alpha subunits; OPRK1; alpha2-adrenoceptor;
TTC12; ANKK1; NCAMI; ZCRB1; CYP2B6; CYP2C19; CYP2C9; interleukin-2; RGS-R7; Gbeta5; MAO-A; 287
A/G polymorphism of catechol-O-methyltransferase; serotonin transporter; Ca2+/cAMP responsive
element binding protein; CNRI; ABCB1, P-glycoprotein, UGT2B7, and CREB.

EXAMPLE 1.

Table 1. Synaptamine™ Gene Map for GnAP

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human kappa opioid receptor gene (OPRK1)</td>
<td>In humans, the 36G &gt; T single nucleotide polymorphism (SNP) on KOR gene.</td>
</tr>
<tr>
<td></td>
<td>The kappa opioid receptor (KOR) system seems to play a role in stress</td>
</tr>
<tr>
<td></td>
<td>responsivity, opiate withdrawal and responses to psycho-stimulants,</td>
</tr>
<tr>
<td></td>
<td>inhibiting mesolimbic dopamine. KOR gene polymorphisms have been reported</td>
</tr>
<tr>
<td></td>
<td>to contribute to predisposition to voluntary alcohol-drinking</td>
</tr>
<tr>
<td></td>
<td>DL-Phenylalanine L-Tyrosine Passion Flower</td>
</tr>
</tbody>
</table>

Human kappa opioid receptor gene (OPRK1) polymorphism is associated with opiate addiction.
<table>
<thead>
<tr>
<th>Mu opioid receptor</th>
<th>A118G SNP of the mu opioid receptor gene (OPRM1)</th>
<th>Mu opioid receptors are critical for heroin dependence, and A118G SNP of the mu opioid receptor gene (OPRM1) has been linked with heroin abuse. In our population of European Caucasians (n = 118), approximately 90% of 118G allelic carriers were heroin users.</th>
<th>DL-Phenylalanine L-Tyrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>D(2) dopamine receptor gene (DRD2)</td>
<td>A haplotype block of 25.8 kilobases (kb) was defined by 8 SNPs extending from SNP3 (TaqIB) at the 5' end to SNP10 site (TaqIA) located 10 kb distal to the 3' end of the gene.</td>
<td>Within this block, specific haplotype cluster A (carrying TaqIB1 allele) was associated with a high risk of heroin dependence in Chinese patients (P = 1.425 x 10(-22); odds ratio, 52.80; 95% confidence interval, 7.290-382.5 for 8-SNP analysis). A putative recombination &quot;hot spot&quot; was found near SNP6 (intron 6 ins/del G), creating 2 new daughter haplotypes that were associated</td>
<td>DL-Phenylalanine L-Tyrosine Passion Flower</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lawford BR, Young RM, Noble EP, Sargent J, Rowell J, Shadforth S, Zhang X, Ritchie T. The D(2) dopamine receptor A(1) allele and</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
with a lower risk of heroin dependence in Germans (P = 1.94 x 10^-11 for 8-SNP analysis).

Other studies show the relationship of carrying TAq1A1 vs. A2 alleles in the treatment outcomes for heroin abuse. The results indicate that DRD2 variants are predictors of heroin use and subsequent methadone treatment outcome and suggest a pharmacogenetic approach to the treatment of opioid dependence. Others found association between nasal inhalation of opiates and DRD2 promoter - 141DeltaC polymorphism. Significantly stronger cue-elicited heroin craving was found in individuals carrying D2 dopamine receptor gene (DRD2) TaqI RFLP


<table>
<thead>
<tr>
<th><strong>ANKK1 Gene</strong></th>
<th><strong>A1 allele than the non-carriers (P &lt; 0.001).</strong></th>
<th><strong>L-Tyrosine</strong></th>
</tr>
</thead>
</table>
| With a non-synonymous G to A transition, rs2734849 produces an amino-acid change (arginine to histidine) in C-terminal ankyrin repeat domain of ANKK1. | Since DRD2 expression is regulated by transcription factor NF-kappaB, we suspect that rs2734849 may indirectly affect dopamine D (2) receptor density. The rs273849 ANNK1 variant alters expression level of NF-kappaB-regulated genes. | \{ \}

**Catechol-O-methyltransferase (COMT) gene**

| Val(108/158)Met polymorphism of the catechol-O-methyltransferase (COMT) gene | Genotyping 38 Israeli heroin addicts and both parents using a robust family-based haplotype relative risk (HRR) strategy. There is an excess of the val COMT allele (likelihood ratio = 4.48, P = 0.03) and a trend for an excess of the val/val COMT genotype (likelihood ratio = 4.97, P = 0.08, 2 df) in the heroin addicts compared to the HRR control group. | **L-Tyrosine DL** |
| Phenylalanine | Rhodiola rosea | \{ \}

**Proenkephalin**

<table>
<thead>
<tr>
<th>&gt; or = 81 bp allele</th>
<th>Among the</th>
<th><strong>Comings DE, Blake H, Dietz</strong></th>
</tr>
</thead>
</table>
Gene (PENK) subjects with opioid dependence, 66% carried the > or = 81 bp allele compared with 40% of subjects with other types of substance abuse (chi2 = 11.31, p < 0.004) and 49% of controls (chi2 = 6.0, p < 0.015). These results are consistent with a role of the PENK gene in opioid dependence.

In another study, Heroin abuse was significantly associated with PENK polymorphic 3' UTR dinucleotide (CA) repeats; 79% of subjects homozygous for the 79-bp allele were heroin abusers. Such individuals tended to express higher PENK mRNA than the 81-bp homozygotes, but PENK levels within the nucleus accumbens (NAC) shell were most strongly correlated to catecholamine-O-methyltransferase

<table>
<thead>
<tr>
<th>Phenylnalanine</th>
<th>L-Tyrosine</th>
<th>Rhodiola rosea</th>
</tr>
</thead>
</table>


<table>
<thead>
<tr>
<th>Serotonin transporter (hSERT)</th>
<th>Homozygosity at hSERT (especially 10/10) was associated with early opiate addiction, while genotype 12/10 proved to be protective.</th>
<th>Reward system pathway</th>
<th>5-hydroxytryptophan</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Dopamine</th>
<th>In the case of</th>
<th>Reward System</th>
<th>DI-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Galeeva AR, Gareeva AE,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transporter (DAT1)</td>
<td>DAT1, genotype 9/9 was associated with early opiate addiction. The combination of hSERT genotype 10/10 with DAT1 genotype 10/10 was shown to be a risk factor of opiate abuse under 16 years of age.</td>
<td>Pathway</td>
<td>Phenylalanine L-Tyrosine</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Cannabinoid CB1 (brain) receptor gene (CNR1)</td>
<td>A microsatellite polymorphism (AAT)n at the cannabinoid CB1 (brain) receptor gene (CNR1) consists of 9 alleles. The number of i.v. drugs used was significantly greater for those carrying the &gt; or =/&gt; or = 5 genotype than for other genotypes (P = 0.005).</td>
<td>Cannabinoid receptors in the modulation of dopamine and cannabinoid reward pathways</td>
<td>L-Glutamine (decrease) L-Tyrosine DL-Phenylalanine</td>
</tr>
</tbody>
</table>

**[0007]**Twelve Genes For Pharmacogenomic Solution To Pain Compounds

Medical Necessity Explanation and references for support

Human kappa opioid receptor gene (OPRK1)

**Polymorphism**

In humans, the 36G > T single nucleotide polymorphism (SNP) on KOR gene.
Pathway

The kappa opioid receptor (KOR) system seems to play a role in stress responsivity, opiate withdrawal and responses to psycho-stimulants, inhibiting mesolimbic dopamine. KOR gene polymorphisms have been reported to contribute to predisposition to voluntary alcohol-drinking behavior in experimental animals. It is also associated with opiate response.

Action Required

The finding of the 36g>T single nucleotide polymorphism (SNP) on KOR gene indicates that more opiate is necessary to reduce pain and reduce stress.

Reference(s)


Human kappa opioid receptor gene (OPRK1) polymorphism is associated with opiate addiction.


Mu opioid receptor

Polymorphism

A118G SNP of the mu opioid receptor gene (OPRM1)

Pathway

Mu opioid receptors are critical for heroin dependence, and A118G SNP of the mu opioid receptor gene (OPRM1) has been linked with heroin abuse. In one population of European Caucasians (n = 118), approximately 90% of 118G allelic carriers were heroin users.

Action required

Carriers of the mu receptor polymorphism will be tagged as high risk for opiate dependence. This will necessitate that the physician should decrease oral narcotics and increase ointment compounds to avoid opiate dependence.

Reference(s)


Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers.

Proenkephalin gene (PENK)

**Polymorphism**

> or = 81 bp allele

**Pathway**

Among the subjects with opioid dependence, 66% carried the > or = 81 bp allele compared with 40% of subjects with other types of substance abuse (chi2 = 11.31, p < 0.004) and 49% of controls (chi2 = 6.0, p < 0.015). These results are consistent with a role of the PENK gene in opioid dependence.

In another study, Heroin abuse was significantly associated with PENK polymorphic 3′ UTR dinucleotide (CA) repeats; 79% of subjects homozygous for the 79-bp allele were heroin abusers. Such individuals tended to express higher PENK mRNA than the 81-bp homozygotes, but PENK levels within the nucleus accumbens (NAc) shell were most strongly correlated to catecholamine-O-methyltransferase (COMT) genotype. Altogether, the data suggest that dysfunction of the opioid reward system is significantly linked to opiate abuse vulnerability and that heroin use alters the apparent influence of heritable dopamine tone on mesolimbic PENK and tyrosine hydroxylase function.

**Action Required**

Carriers of the PENK polymorphism will be tagged as high risk for opiate dependence. This will necessitate that the physician should decrease oral narcotics and increase ointment compounds to avoid opiate dependence. Polymorphisms of this gene also associate with poor pain tolerance so there would be the requirement for potential longer term treatment with the pain compounded ointment. It is also proposed that by increasing DL-Phenylalanine we could increase the baseline enkephalin which will offset pain intolerance.

**Reference(s)**


D(2) dopamine receptor gene (DRD2)

**Polymorphism**

A haplotype block of 25.8 kilobases (kb) was defined by 8 SNPs extending from SNP3 (TaqlB) at the 5′ end to SNPIO site (TaqlA) located 10 kb distal to the 3′ end of the gene.
**Pathway**

Within this block, specific haplotype cluster A (carrying TaqIBI allele) was associated with a high risk of heroin dependence in Chinese patients ($P = 1.425 \times 10^{-22}$; odds ratio, 52.80; 95% confidence interval, 7.290-382.5 for 8-SNP analysis). A putative recombination "hot spot" was found near SNP6 (intron 6 ins/del G), creating 2 new daughter haplotypes that were associated with a lower risk of heroin dependence in Germans ($P = 1.94 \times 10^{-4}$), for 8-SNP analysis.

Other studies show the relationship of carrying TAqIBI vs. A2 alleles in the treatment outcomes for heroin abuse. The results indicate that DRD2 variants are predictors of heroin use and subsequent methadone treatment outcome and suggest a pharmacogenetic approach to the treatment of opioid dependence.

Others found association between nasal inhalation of opiates and DRD2 promoter - 141DeltaC polymorphism. Significantly stronger cue-elicited heroin craving was found in individuals carrying D2 dopamine receptor gene (DRD2) TaqRFLP A1 allele than the non-carriers ($P < 0.001$). Genotyping 38 Israeli heroin addicts and both parents using a robust family-based haplotype relative risk (HRR) strategy. There is an excess of the val COMT allele (likelihood ratio = 4.48, $P = 0.03$) and a trend for an excess of the val/val COMT genotype (likelihood ratio = 4.97, $P = 0.08$, 2 df) in the heroin addicts compared to the HRR control group. Another study showed that carriers of the Drd2 A1 allele show significantly less bind sites for Naltrexone.

**Action Required**

Carriers of the D2 receptor polymorphism will be tagged as high risk for opiate dependence. As well as poor methadone treatment outcome. This will necessitate that the physician should decrease oral narcotics and increase ointment compounds to avoid opiate dependence. Additionally, if Subloxin is utilized in treatment there will be a need to increase the dose in carriers of the Drd2 A1 allele. It is proposed that by increasing Synaptamine we could offset narcotic addiction liability.

**Reference(s)**


Catechol-O-methyltransferase (COMT) gene

**Polymorphism**

Val(108/158)Met polymorphism of the catechol-O-methyltransferase (COMT) gene

**Pathway**

Genotyping 38 Israeli heroin addicts and both parents using a robust family-based haplotype relative risk (HRR) strategy. There is an excess of the val COMT allele (likelihood ratio = 4.48, P = 0.03) and a trend for an excess of the val/val COMT genotype (likelihood ratio = 4.97, P = 0.08, 2 df) in the heroin addicts compared to the HRR control group.

**Action Required**

Carriers of the COMT polymorphism will be tagged as high risk for opiate dependence. This will necessitate that the physician should decrease oral narcotics and increase ointment compounds to avoid opiate dependence. It is proposed that by increasing Synaptamine we could offset Narcotic addiction liability.

**Reference(s)**


serotonin transporter (hSERT)

**Polymorphism**

Homozgyosity at hSERT (especially 10/10) was associated with early opiate addiction, while genotype 12/10 proved to be protective.
Pathway

VNTR polymorphisms of the serotonin transporter and dopamine transporter genes in male opiate addict.

Action Required

Carriers of the hSERT polymorphism will be tagged as high risk for opiate dependence. This will necessitate that the physician should decrease oral narcotics and increase ointment compounds to avoid opiate dependence. It is proposed by increasing Chromium and or L-tryptophan or 5-Hydroxytryptophan we could increase serotonin. This could decrease opiate dependence.

Reference(s)


Dopamine Transporter (DATI)

Polymorphism

In the case of DATI, genotype 9/9 was associated with early opiate addiction. The combination of hSERT genotype 10/10 with DATI genotype 10/10 was shown to be a risk factor of opiate abuse under 16 years of age.

Pathway

VNTR polymorphisms of the serotonin transporter and dopamine transporter genes in male opiate addicts are common genes associated with risk behaviors and potential opiate dependence.

Action Required

Carriers of the DATI polymorphism will be tagged as high risk for opiate dependence. This will necessitate that the physician should decrease oral narcotics and increase ointment compounds to avoid opiate dependence. It is proposed that by increasing Synaptamine we could stabilize the deficiency in the dopamine system thereby decreasing substance seeking behavior including narcotics.

Reference(s)

Cannabinoid CBL (brain) receptor gene (CNRI)

**Polymorphisms**

A microsatellite polymorphism (AAT)n at the cannabinoid CBL (brain) receptor gene (CNRI) consists of 9 alleles. The number of i.v. drugs used was significantly greater for those carrying the > or =/> or = 5 genotype than for other genotypes (P = 0.005).

**Pathway**

Cannabinoid receptors and dopaminergic receptors in reward system of the brain affecting IV opiate abuse

**Action Required**

Carriers of the CBL polymorphism will be tagged as high risk for opiate dependence. This will necessitate that the physician should decrease oral narcotics and increase ointment compounds to avoid opiate dependence especially in those individuals dependent on IV heroin utilization.

**Reference(s)**


P450 Liver Enzyme Gene

**Polymorphisms**

Common CYP2C8 and CYP2C9 polymorphisms and other polymorphisms (P450 GENE VARIANTS)

**Pathway**

Drug metabolism and pharmacogenomic response tied to narcotic drugs which will include any opiate used orally or in the transdermal form including Ketamine and even Gabapentin. Moreover these polymorphisms are also tied to NSAID metabolism and have been established as high risk gene polymorphisms for GI bleeds.

**Action Required**

Carriers of these polymorphisms (CYP2C8 and CYP2C9) will have a problem in metabolizing narcotics. Depending on the P450 polymorphism the physician will be required to either decrease or increase the said narcotic. Of equal importance the carriers of these polymorphisms will suggest NSAID GI risk in bleeding and thus the amount of NSAIDs used in the compounds will have to be adjusted accordingly. It is proposed that by increasing D-Phenylalanine we could have a natural anti-inflammatory response eliminating the need for high dosage NSAIDs.
There are 10 studies relating polymorphisms of this gene and opiate response and there are over 20 studies involving NSAIDs GI bleed risk and P450 gene polymorphisms.

TNF-alpha Polymorphisms

TNF-alpha (-308(G->A)), IL-10(-1082(G->A))

Pathway

High risk for development of inflammatory secondary messengers. The carrying of the TNF-alpha polymorphism provides medical evidence for proper utilization of NSAIDs in the treatment of pain inflammation. This includes any NSAID such as Ketoprofen, Baclofen, Cyclobenzapine, Diclofenac, Capsaicin, Ibuprofen. It is proposed that by increasing D-Phenylalanine we could have a natural anti-inflammatory response eliminating the need for high dosage NSAIDs.

Action Required

Carriers of the TNF-alpha polymorphism would require an increase in NSAIDs compounded in the pain ointment as prescribed the attending physician.

References

There are 2700 studies relating polymorphisms of this gene and the inflammatory response 3 studies specific to opiate response.

Nitric Oxide Gene (eNos)

Polymorphisms

-786T/C, -922A/G, 4B/4A, and 894G/T polymorphisms of eNOS

Pathway

Nitric oxide (NO) plays critical role in endothelial dysfunction and oxidative stress, pointing to the significance of endothelial nitric oxide synthase gene (eNOS) variants. Nitric Oxide deficiency leads to oxidative stress which prevents tissue healing. Furthermore, data imply that NMDA receptors and nitric oxide production in rostral ventromedial medulla modulate the transmission of opioid pain-inhibitory signals from the periaqueductal grey. It is proposed that by increasing Rhodiola rosea we could reduce oxidative stress. It is also proposed that by coupling the H-Wave device we could increase Nitric Oxide production as well.
Carriers of the eNos gene polymorphisms will have an increased risk of slow healing due to oxidative stress. The physician will be required to increase the amounts of pain medication and increase the number of prescriptions due to the reduced healing and the need to enhance the opioid pain-inhibitory responses.

There are 75 studies relating polymorphisms of this gene and oxidative stress. Additionally there are 21 papers showing the relationship of eNos polymorphisms and morphine actions related to pain inhibition.

Vascular Endothelial Growth Factor Gene (VEGF)

Polymorphisms

SNP genotypes, -160C, -152A (rs13207351), -116A (rs1570360)

Pathway

Angiogenesis Factor-required for proper tissue healing, these polymorphisms will slow the healing process. It has been demonstrated that there is a clear association between VEGF SNPs and severity of diabetic retinopathy. Furthermore, results suggested that endogenous opioid peptides (endomorphin-1 and -2 and deltorphin I) stimulated angiogenesis in the CAM assay, and these effects were modulated with the opioid receptors.

Carriers of the VGEF gene polymorphisms will have an increased risk of slow healing due to lack of angiogenesis in the healing process. The physician will be required to increase the amounts of pain medication and increase the number of prescriptions due to the reduced healing and the need to enhance the opioid pain-inhibitory responses by its induction of angiogenesis. A polymorphism in this gene will provide the medical necessity to prolong treatment past 30 days. It is also proposed that by coupling the H-Wave device we could increase angiogenesis as well.

There are 3423 studies relating polymorphisms of this gene and angiogenesis.

Example 2

[0008] Coupling RX pain compounds with Synaptamine and GeneMap

While there 626 PUBMED papers on the general topic of pain ointments, there is a paucity of studies on the following constituents of the proposed transdermal compounds:

**Gabapentin** - A search found no published PUBMED studies

**Ketamine** (C-III) - Only one published study showing positive effects in patients with complex regional pain syndrome type 1.

**Ketoprofen** - Ketoprofen (KP) is a potent nonsteroidal anti-inflammatory drug (NSAID) widely used in clinical practice for the control of acute and chronic pain of soft tissues and skeletal muscle system. The importance of KP in the therapeutic field, has stimulated the development of topical dosage forms to improve its percutaneous absorption through the application site. Moreover they could provide relatively consistent drug levels for prolonged periods and avoid gastric irritation, typical side effect of NSAID oral administration. Since the topical formulation efficiency depends on vehicle characteristics, some different ointments, at 1% and 5% concentrations of KP showed promise. There are only eight published PUBMED studies using this drug in pain ointments. None of which are double-blinded.

**Baclofen** - Baclofen is a potent nonsteroidal anti-inflammatory drug (NSAID) widely used in clinical practice for the control of acute and chronic pain of soft tissues and skeletal muscle system. While there are 5242 studies related to its oral effects there are no PUBMED studies related to Baclofen's efficacy in ointments.

**Cyclobenzaprine** - Antispasmodic agents, such as cyclobenzaprine, are primarily used to treat musculoskeletal conditions. While there are 156 PUBMED studies on the oral efficacy of this drug there are no published studies related to its use in ointments.

**Ibuprofen** - Is a well characterized NSAID with 7265 PUBMED oral studies. There are only 23 studies to date related to its efficacy in ointments.

**Diclofenac** - Is a well characterized NSAID with 6010 PUBMED oral studies. There are only 21 studies to date related to its efficacy in ointments.

**Capsaicin** - Is a well characterized NSAID with 8831 PUBMED oral studies. There are only 42 studies to date related to its efficacy in ointments.

**Lidocaine** is a topical anesthetic cream with 2243 PUBMED oral studies. There are only 344 studies to date related to its efficacy in ointments.

**Menthol** - There are 42 studies related to methanol in ointments.

**Camphor** - There are 34 studies related to methanol in ointments.
**CX-659S**, a newly discovered anti-inflammatory compound, exerts inhibitory effects on chronic contact hypersensitivity responses (CHRs) induced by repeated application with picryl chloride (PC), which is known to mimic many, if not all, events occurring within lesional skin of patients with atopic dermatitis (AD). CX-659S suppresses the expression of mRNA for interleukin (IL)-4 and IL-10 but not that for IFN-gamma, and inhibits serum IgE production in a chronic CHR model. Although topical corticosteroids have been widely utilized in steroid-responsive dermatoses such as AD, their chronic use may be associated with significant side effects. In addition, a rebound phenomenon often occurs after discontinuation of prolonged use of topical corticosteroids, with enhanced production of IgE and Th2 cell cytokines. The purpose of this study was to assess whether CX-659S inhibits the rebound phenomenon after discontinuation of chronic treatment with prednisolone in a chronic CHR model in mice. The efficacy of CX-659S as a sequential therapeutic agent after discontinuation of chronic treatment with prednisolone was tested on PC-treated ears of BALB/c mice with chronic CHR. Effects were quantified by measurements of ear thickness, serum IgE and cytokine mRNA expression. The rebound phenomenon was confirmed after discontinuation of chronic treatment with prednisolone in chronic CHR in mice, i.e. by evidence of flare thickening of the ear, enhanced expression of mRNA for IL-4 and IL-10 and increased serum IgE. Sequentially applied CX-659S suppressed these rebound phenomena with a good cosmetic result. CX-659S is the first promising compound with inhibitory activity on the rebound phenomenon following withdrawal of corticosteroid therapy without immunosuppression (Inoue et al 2003).

**Nimesulide gel**

A study was conducted to compare the analgesic efficacy of a new topical gel formulation of nimesulide (10 mg of pure drug) with that of placebo, diclofenac and piroxicam gels (10 mg of pure drug) in three parallel groups in a double-blinded, randomized fashion with vehicle placebo. The analgesic activity of nimesulide was subsequently correlated with its pharmacokinetic profile. The drugs were applied on a fixed marked area on the skin of the right forearm. Pain stimulus was administered using a modification of the Hollander method, before and at 15, 30, 60, 120 min and 240 min post-treatment. The pain experienced by the subjects was ranked separately on the visual analogue scale (VAS) and the ten-point category scale. Antinociception induced by the treatments was evaluated through the placebo-related ratings (PRR) and total pain relief (TOTPAR) analysis. The plasma concentration of nimesulide was estimated using high-performance liquid chromatography (HPLC). Nimesulide exhibited better efficacy than diclofenac, piroxicam and placebo. It demonstrated faster onset of action in concordance with earlier studies. Peak analgesic effect was observed at 120 min post-treatment, which correlated with the pharmacokinetic profile of the drug in gel formulation. In this study, diclofenac was found to be superior to piroxicam though both drugs exhibited peak analgesic effect at 60 min post-treatment. In the modified Hollander method, a good correlation was found between the ten point category scale and the VAS, indicating that it may serve as a sensitive and reliable method for the screening of analgesic drugs. The superior analgesic activity of nimesulide (as gel formulation), correlating with its pharmacokinetic profile, indicates that the topical route of administration may be a safe and effective alternative to the presently used oral and rectal routes (Sengupta et al 1998).
Novel Drug Delivery Systems

Soya-lecithin aggregates

In one study soya-lecithin aggregates, prepared by a technique using compressed gas, are used to formulate new dermal preparations. Ketoprofen (KP), a nonsteroidal anti-inflammatory drug (NSAID) is included as a model drug. The technique offers the possibility of incorporating auxiliary agents, such as penetration enhancers, anti-irritants and moisturizers together with the drug in one process. Apparent partition coefficients for n-octanol-phosphate buffer were determined for each of the lecithin aggregates. In general, soya-lecithin improves the partition of KP into n-octanol. The resulting products were included in widely used hydrophilic and hydrophobic vehicles. After 24 h, the cumulative amount of drug released through an artificial membrane was higher from the hydrophilic gels (2.6-4.3 mg) and the hydrophobic creams (0.23-0.392 mg) than from the control preparations (control hydrogel: 1.3 mg; control hydrophobic cream: 0.141 mg). However, the cumulative amount released from the hydrophobic vehicles was generally lower than from the hydrophilic matrices. Cumulative amounts such as those released from the hydrophilic preparations can also be achieved using supersaturated formulations based solely on the drug-loaded lecithin aggregates and a suitable oily component (4.07 mg). Results from the diffusion studies using artificial membranes were confirmed by permeation studies using excised rat skin. The improvement in skin permeation is related to both the solubilizing effect of the lecithin matrix and the penetration enhancing effect of lecithin itself. The novel soya-lecithin aggregates are promising candidates for new drug delivery systems in dermatology and cosmetology. Lecithin aggregates loaded with drugs are multifunctional carriers that also act as penetration enhancers.

Micronized

The bioavailability of S(+) and R(-) ketoprofen (KTP) in six horses was investigated after oral administration of the racemic (rac) mixture. Two oral formulations were studied, an oil-based paste containing micronized rac-KTP and powder from the same source in hard gelatin capsules, each at a dose rate of 2.2 mg/kg. For the oil-based paste two feeding schedules were used: horses were either allowed free access to food or access to food was restricted for 4 h before and 5 h after dosing. The drug in hard gelatin capsules was administered to horses with restricted access to food. After intravenous administration of rac-KTP, S(+) enantiomer concentrations exceeded those of the R(-) enantiomer. For S(+) and R(-)KTP, respectively, pharmacokinetic parameters were, t1/2 beta 0.99 +/- 0.14 h, 0.70 +/- 0.13 h; ClB 0.56 +/- 0.09, 0.92 +/- 0.20 L/h/kg; Vd(ss) 0.53 +/- 0.11, 0.61 +/- 0.10 L/kg. Following oral administration of rac-KTP as the oil-based paste to horses with free access to food, there were no detectable concentrations in plasma in three animals at any sampling time, while a fourth animal showed very low concentrations at two sampling times only. In the two remaining horses very low but detectable concentrations were present for 5 h. In the horses with restricted access to food, rac-KTP paste administration produced higher concentrations in plasma. However, bioavailability was very low, 2.67 +/- 0.43 and 5.75 +/- 1.48% for R(-) and S(+)KTP, respectively. When administered as pure drug substance in hard gelatin capsules, absorption of KTP was fairly rapid, but incomplete. Bioavailability was 50.55 +/- 10.95 and 54.17 +/- 9.9% for R(-) and S(+)KTP, respectively. This study demonstrates that rac-KTP had a modest bioavailability when administered as a micronized powder in hard gelatin capsules.
to horses with restricted access to food. When powder from the same source was administered as an oil-based paste, it was for practical purposes not bioavailable, regardless on the feeding schedule.

**Cyclic monoterpenes**

The percutaneous absorption promoting effect and skin irritancy of cyclic monoterpenes were investigated in rats and with rabbits, respectively. Ketoprofen (KPF) was applied to rat skin in gel ointments containing various cyclic monoterpenes. Plasma concentrations of KPF markedly increased with the addition of the hydrocarbons of cyclic monoterpenes such as trans-p-menthan e and d-limonene, whereas no significant enhancing effect was observed in the cases of other terpenes such as l-menthol, l-menthone and 1,8-cineole. The lipophilicity of the enhancers seems the important factor in promoting penetration of KPF through the skin. The enhancing activity of d-limonene was found to be much higher than that of Azone. Irritancy of the hydrocarbons of cyclic monoterpenes and Azone to the skin was evaluated using a Draize scoring method with rabbits. No change was observed on the skin surface when ethanol containing 2% of the hydrocarbons was applied to the dorsal skin, though a slight edema and erythema were observed in the case of Azone. In particular, an obvious difference was observed in the erythema formation between Azone and the hydrocarbons of cyclic monoterpenes.

**Cyclohexanone derivatives**

The promoting effect of cyclohexanone derivatives on the percutaneous absorption of ketoprofen and indomethacin from gel ointments was investigated in rats. Drug absorption was markedly enhanced by the addition of 2-tert-butylcyclohexanone. Promoting activities of 2,6-dimethyl and 4-tert-butylcyclohexanone were also observed, but their effects were significantly lower than that of the 2-tert-butyl derivative. The effect of side chain length at the 2-position of the cyclohexanone ring on the percutaneous absorption of these drugs was determined similarly using a series of 2-n-alkylcyclohexanones. Pronounced effects were observed in the case of 2-n-octylcyclohexanone, suggesting that a chain length of eight carbons is an important factor for absorption enhancement in this series. The extent of absorption enhancement was found to be an almost linear function of 2-n-octycyclohexanone concentrations in the range from 0 to 10%.

Generally, a procedure which can serve as a possible basis for the laboratory study of the topical effect of NSAID was investigated in rats or guinea pigs. The effect of NSAID was greatly influenced by physical characteristics of the preparation such as drug particle size, solubility, ointment base and concentration of drug. Moreover, it was also found to be affected by many technical factors such as animal fixation, drug application times and methods (rubbing times or occlusive dressing technique) and amounts applied which play an important role in topical preparation. The topical application of NSAID ointment (1% of indomethacin, ketoprofen or diclofenac sodium) markedly inhibited the paw edema by carrageenin in rats. The inhibitory activity was the same as that of steroidal ointment (0.12% betamethasone 17-valerate or 0.05% fluocinonide), but was less than that by oral administration of these NSAID. Also, the NSAID ointment obviously inhibited the ultraviolet erythema in guinea pigs and the swelling in the hind feet of adjuvant arthritic rats. The inhibitory activities of NSAID ointments on these inflammatory responses were almost the same as those obtained by oral administration of such NSAID and more potent than those of steroidal ointments. Furthermore, NSAID ointments increased the pain threshold in the inflamed foot as determined by the method of Randall and Seltitto. The analgesic activity of NSAID ointment was more potent than that of steroidal ointment, but less than that of NSAID administered orally. On the other hand, neither the systemic effects such as decrease in weight of the adrenals and thymus which were noted when steroidal ointment was used, nor the gastrointestinal
lesions which were found by oral administration of NSAID, were recognized in rats in which NSAID ointment was applied topically. The anti-inflammatory effects of NSAID ointment correlated well with the drug concentration at the site of inflammation. These findings suggest that NSAID ointment has a clinical use in the treatment of inflammatory diseases.

**isosorbide dinitrate ointment**

In complex regional pain syndrome type 1 (CRPSI) vascular changes occur from the initial, inflammatory event onto the trophic signs during chronicity of the disease, resulting in blood flow disturbances and marked temperature changes. Pharmacotherapeutic treatment is generally inadequate. To determine whether local application of the nitric oxide donor isosorbide dinitrate (ISDN) could cause vasodilation and thereby improve tissue blood distribution in the affected extremity a pilot study was performed by Groeneweg et al (2008). In a pilot study, 5 female patients with CRPSI in one hand were treated with ISDN ointment 4 times daily during 10 weeks. As a primary objective video thermography was used to monitor changes in blood distribution in both the involved and contralateral extremities. Patients treated with ISDN showed an increase of 4 degrees C to 6 degrees C in mean skin temperature of the cold CRPSI hands, reaching values similar to that of the contralateral extremities within 2 to 4 weeks time, suggesting normalization of blood distribution. This was confirmed by an improvement in skin color. In 3 patients the Visual Analog Scale pain declined, whereas in the other 2 patients the Visual Analog Scale pain was unchanged over time. In the pilot study, topical application of ISDN seems to be beneficial to improve symptoms for patients with cold type CRPSI, but further study is needed.

**Liopoderm**. This substance increases absorption but there are no PUBMED published reports.

To the inventors knowledge this is the first unobvious proposed invention to couple the polymorphic genes with specific customized pain ointment compounds (described below). These genes will be explored in terms of their relationship to nutrients.

**Synaptamine™**

The combining of the Synaptamine complex protected by US patent # 724 with any compounded pain ointment would have a number of important benefits.

The minimum ingredient complex comprising of:

- **Rhodiola rosea**
- **DL-Phenylalanine**
- **Chromium salts / l-tryptophan**

However and advanced formula includes Passion flower and a source of vitamin B12 and calcium, magnesium and potassium.
The inventors are providing specific studies published to validate efficacy of individual ingredients utilized in the patented complex Synapatamine™. When combined with Passion Flower and AlgaeCal as proposed in the advanced formula it is noteworthy that since the combination of subsequent ingredients have not been reported to date the combination cannot be considered obvious.

**Rhodiola rosea**


**Rhodiola: a promising anti-aging Chinese herb.**

**Jafari M, Feigner JS, Bussel II, Hutchili T, Khodavari B, Rose MR, Vince-Cruz C, Mueller LD.**

Using the fruit fly, Drosophila melanogaster, we investigated the effects of Rhodiola on life-span. Rhodiola is a plant root used in traditional Chinese medicine that may increase an organism’s resistance to stress. It has been proposed that Rhodiola can extend longevity and improve health span by alleviating oxidative stress. Rhodiola supplied every other day at 30 mg/mL significantly increased the lifespan of Drosophila melanogaster. When comparing the distribution of deaths between Rhodiola-supplemented and control flies, Rhodiola-fed flies exhibited decelerated aging. Although the observed extension in lifespan was associated with statistically insignificant reductions in fecundity, correcting for a possible dietary restriction effect still did not eliminate the difference between supplemented and control flies, nor does the effect of Rhodiola depend on dietary manipulation, strongly suggesting that Rhodiola is not a mere dietary restriction mimetic. Although this study does not reveal the causal mechanism behind the effect of Rhodiola, it does suggest that the supplement is worthy of continued investigation, unlike the other Chinese herbals, Lu Duo Wei (LDW), Bu Zhong Yi Qi Tang (BZYQT), San Zhi Pian (SZP, Three Imperial Mushrooms), Hong Jing Tian (Rhodiola) that were evaluated in this study.


**Protective effects of salidroside on hydrogen peroxide-induced apoptosis in SH-SY5Y human neuroblastoma cells.**


Oxidative stress plays an important role in Alzheimer’s disease and other neurodegenerative disorders. Salidroside, a phenylpropanoid glycoside isolated from Rhodiola rosea L. shows potent antioxidant property. In this paper, the neuroprotective effects of salidroside on hydrogen peroxide (H2O2)-induced apoptosis in SH-SY5Y cells were investigated. Pretreatment with salidroside markedly attenuated H2O2-induced cell viability loss and apoptotic cell death in a dose-dependent manner. The mechanisms by which salidroside protected neuron cells from oxidative stress included the induction of several antioxidant enzymes, thioredoxin, heme oxygenase-1, and peroxiredoxin-1; the down regulation of pro-apoptotic gene Bax and the up regulation of anti-apoptotic genes Bcl-2 and Bcl-X(L). Furthermore, salidroside dose-dependently restored H2O2-induced loss of mitochondrial membrane potential as well
as the elevation of intracellular calcium level. These results suggest that salidroside has protective
effects against oxidative stress-induced cell apoptosis, which might be a potential therapeutic agent for
treating or preventing neurodegenerative diseases implicated with oxidative stress.

Biofactors. 2006;26(3):209-19

Antioxidative effects of Cinnamomi cassiae and Rhodiola rosea extracts in liver of diabetic mice.

Kim SH, Hvun SH, Choung SY.

Both Cinnamomi cassiae and Rhodiola rosea extracts are used as anti-diabetic folk medicines. Recently,
increased oxidative stress was shown to play an important role in the etiology and pathogenesis of
diabetes mellitus and its complications. This study was designed to examine the effects of Cinnamomi
cassiae and Rhodiola rosea extracts on blood glucose, lipid peroxidation, the level of reduced
glutathione and its related enzymes (glutathione reductase, glutathione S-transferase), and the activity
of the antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) in the liver of
db/db mice. Diabetic C57BL/Ks db/db mice were used as experimental models. Mice were divided into
control (n=10), Cinnamomi cassiae (200 mg/kg/day, n=10), and Rhodiola rosea (200 mg/kg/day, n=10)
treated groups for 12 weeks of treatment. These type II diabetic mice were used to investigate the
effects of Cinnamomi cassiae and Rhodiola rosea on blood glucose, reduced glutathione, glutathione
reductase, glutathione S-transferase, glutathione peroxidase, lipid peroxidation, catalase and superoxide
dismutase. Cinnamomi cassiae and Rhodiola rosea extracts significantly decreased on blood glucose,
increased levels of reduced glutathione and the activities of glutathione reductase, glutathione S-
transferase, glutathione peroxidase, catalase and superoxide dismutase in the liver. Extract treatment
also significantly decreased lipid peroxidation. Cinnamomi cassiae and Rhodiola rosea extracts may be
effective for correcting hyperglycemia and preventing diabetic complications.


Cytoprotective and antioxidant activity of Rhodiola imbricata against tert-butyl hydroperoxide
induced oxidative injury in U-937 human macrophages.

Kanupriva , Prasad D, Sai Ram M, Kumar R, Sawhney RC, Sharma SK, Ilavazhagan G, Kumar D, Banerjee PK.

The present study reports cytoprotective and antioxidant activity of aqueous and alcoholic extracts of
Rhodiola imbricata rhizome on tert-butyl hydroperoxide (tert-BHP) induced cytotoxicity in U-937 human
macrophages. There was an increase in cytotoxicity and apoptosis significantly in the presence of tert-
BHP over control cells. The tert-BHP induced cytotoxicity can be attributed to enhanced reactive oxygen
species (ROS) production which in turn is responsible for fall in reduced glutathione (GSH) levels; further
there was a significant decrease in mitochondrial potential and increase in apoptosis and DNA
fragmentation. Both aqueous and alcoholic extracts of Rhodiola rhizome at a concentration of 250
microg/ml were found to inhibit tert-BHP induced free radical production, apoptosis and to restore the
anti-oxidant levels to that of the control cells. The alcoholic extract of Rhodiola showed higher
cytoprotective activities than aqueous extract. These observations suggest that the alcoholic and
aqueous extracts of Rhodiola have marked cytoprotective and antioxidant activities.
Rhodiola rosea as antioxidant in red blood cells: ultrastructural and hemolytic behaviour.

Rhodiola rosea L. (Crassulaceae) is a plant that lives at high altitude in Europe and Asia, widely used for its high capacity to increase the organism resistance to different stress conditions. Although a few international literature supports these effects, today R. rosea has become a common component of many dietary supplements also in the Western world. The aim of the present study was to investigate the effect of the R. rosea roots aqueous extract on in vitro human erythrocytes exposed to hypochlorous acid (HOCl)-oxidative stress. Several damages occur in human erythrocytes exposed in vitro to HOCl, among these membrane protein and lipid modifications, shifting from the discocyte shape to the echinocyte one, and determining lysis ultimately. Therefore, in the present work, the evaluation of the antioxidant capacity of the Rhodiola extract has been carried out by means of scanning electron microscopy and of hemolytic behaviour on human erythrocytes exposed to HOCl in the presence of increasing doses of the aqueous extract in different experimental environments (co-incubation and subsequent incubations). The results obtained are consistent with a significant protection of the extract in presence of the oxidative agent, but a cautionary note emerges from the analysis of the data related to the cell exposition to the plant extract in the absence of any induced oxidative stress. In fact, the addition to erythrocyte of high doses of R. rosea extract always determines severe alterations of the cell shape.

The present study reports the radioprotective properties of a hydro-alcoholic rhizome extract of Rhodiola imbricata (code named REC-7004), a plant native to the high-altitude Himalayas. The radioprotective effect, along with its relevant superoxide ion scavenging, metal chelation, antioxidant, anti-lipid peroxidation and anti-hemolytic activities was evaluated under both in vitro and in vivo conditions. Chemical analysis showed the presence of high content of polyphenols (0.971 +/- 0.01 mg% of quercetin). Absorption spectra analysis revealed constituents that absorb in the range of 220-290 nm, while high-performance liquid chromatography (HPLC) analysis confirmed the presence of four major peaks with retention times of 4.780, 5.767, 6.397 and 7.577 min. REC-7004 was found to lower lipid oxidation significantly (p < 0.05) at concentrations viz., 8 and 80 microg/ml respectively as compared to reduced glutathione, although the optimally protective dose was 80 microg/ml, which showed 59.5% inhibition of induction of linoleic acid degradation within first 24 h. The metal chelation activity of REC-7004 was found to increase concomitantly from 1 to 50 microg/ml. REC-7004 (10-50 microg/ml) exhibited significant metal chelation activity (p < 0.05), as compared to control, and maximum percentage inhibition (30%) of formation of iron-2,2'-bi-pyridyl complex was observed at 50 microg/ml, which correlated well with quercetin (34.9%), taken as standard. The reducing power of REC-7004 increased in a dose-dependent manner. The absorption unit value of REC-7004 was significantly lower (0.0183 +/- 0.0033) as compared to butylated hydroxy toluene, a standard antioxidant (0.230 +/- 0.091), confirming its high reducing ability. Superoxide ion scavenging ability of REC-7004 exhibited a dose-
dependent increase (1-100 microg/ml) and was significantly higher (p < 0.05) than that of quercetin at
lower concentrations (1-10 microg/ml), while at 100 microg/ml, both quercetin and REC-7004 scavenged
over 90% superoxide anions. MTT assay in U87 cell line revealed an increase in percent survival of cells
at doses between 25 and 125 microg/ml in case of drug + radiation group. In vivo evaluation of radio-
protective efficacy in mice revealed that intraperitoneal administration of REC-7004 (maximally effective
dose: 400 mg/kg b.w.) 30 min prior to lethal (10 Gy) total-body gamma-irradiation rendered 83.3%
survival. The ability of REC-7004 to inhibit lipid peroxidation induced by iron/ascorbate, radiation (250
Gy) and their combination [i.e., iron/ascorbate and radiation (250 Gy)], was also investigated and was
found to decrease in a dose-dependent manner (0.05-2 mg/ml). The maximum percent inhibition of
formation of MDA-TBA complex at 2 mg/ml in case of iron/ascorbate, radiation (250 Gy) and both i.e.,
iron/ascorbate with radiation (250 Gy) was 53.78, 63.07, and 51.76% respectively and were found to be
comparable to that of quercetin. REC-7004 (1 microg/ml) also exhibited significant anti-hemolytic
capacity by preventing radiation-induced membrane degeneration of human erythrocytes. In
conclusion, Rhodiola renders in vitro and in vivo radioprotection via multifarious mechanisms that act in
a synergistic manner.

Biofactors., 2004;20(3):147-59

In vitro protective effect of Rhodiola rosea extract against hypochlorous acid-induced oxidative
damage in human erythrocytes.


Rhodiola rosea L. (Crassulaceae) is a plant living at high altitudes in Europe and Asia. Its roots have long
been used in the traditional medical system of these geographical areas to increase the organism
resistance to physical stress; today, it has become an important component of many dietary
supplements. In this study we investigate the antioxidant capacity of the R. rosea aqueous extract
evaluating its ability to counteract some of the main damages induced by hypochlorous acid (HOCI), a
powerful oxidant generated by activated phagocytes, to human erythrocytes. Ascorbic acid was used as
a reference substance because of its physiological HOCl-scavenging ability. Our study demonstrates that
R. rosea is able to significantly protect, in a dose-dependent manner, human RBC from glutathione (GSH)
depletion, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inactivation and hemolysis induced by
the oxidant. Furthermore, we demonstrate that R. rosea aqueous extract acts from the inside of the
erthrocyte suggesting a probable involving of cell components. The protection on GSH afforded by the
R. rosea extract with respect to ascorbic acid, occurred also if added 2 or 5 min. later than the oxidant,
suggesting a more rapid or powerful effect.


Lack of effect of Rhodiola or oxygenated water supplementation on hypoxemia and oxidative stress.

Wing SL, Askew EW, Luetkemeier MJ, Rvuin DT, Kamimori GH, Grissom CK.

This study investigated the effects of 2 potentially "oxygen promoting" dietary supplements on hypoxia
and oxidative stress at a simulated altitude of 4600 m. Fifteen volunteers (ages 20-33) received 3
separate 60-minute hypoxic exposures by breathing 13.6% oxygen at an ambient barometric pressure of
633 mm Hg (simulating the partial pressure of oxygen at 4600 m elevation). Each subject received, in
random order, treatments of a 7-day supply of placebo, Rhodiola rosea, and an acute dose of stabilized oxygen dissolved in water. Arterialized capillary blood oxygen samples (PcO2) were measured at baseline and at 30 and 60 minutes of exposure. Pulse oximeter oxyhemoglobin saturation (SaO2) was measured at baseline and at every 10 minutes of hypoxic exposure. Oxidative stress markers measured included baseline and 60-minute exposure serum lipid peroxides (LPO) and urine malondialdehyde (MDA). For each treatment group, PcO2 decreased by approximately 38% from baseline to 60-minute hypoxic exposure. Similarly, SaO2 also decreased among groups from approximately 97 to 81%. Serum lipid peroxides increased significantly in the placebo group and decreased significantly from baseline in response to the stabilized oxygen treatment (P = .02); there was a trend for decreased LPO with the Rhodiola treatment (P = .10). There were no significant changes for MDA among groups. The 2 dietary supplements investigated did not have a significant effect on blood oxygenation after 60 minutes of sedentary hypoxic exposure. Hypoxia-induced oxidative stress was observed in the control group only. Both supplements appeared not to increase oxidative stress and may decrease free radical formation after hypoxic exposure compared with the control.

**Biol Pharm Bull.** 2002 Aug;25(8):1101-4

**Neuroprotective effects of constituents of the oriental crude drugs, Rhodiola sacra, R. sachalinensis and Tokaku-joki-to, against beta-amyloid toxicity, oxidative stress and apoptosis.**


We tested the constituents of two Rhodiola plants, Rhodiola sacra s. H. Fu and R. sachalinensis A. BOR, and an Oriental crude drug, Tokaku-joki-to, for their neuroprotective effects. Of the 58 compounds tested, six had considerable protective effects against beta-amyloid-induced death of B103 neuronal cells in vitro. These six compounds also showed protective effects against staurosporine-induced cell death, and two of the six compounds protected neurons from H2O2-induced cell death. These results suggest that some of the tested compounds protect neurons from beta-amyloid toxicity based on antiapoptotic and antioxidative activity.


**Phyto-adaptogens protect against environmental stress-induced death of embryos from the freshwater snail Lymnaea stagnalis.**

**Boon-Niermeijer EK, van den Berg A, Wikman G, Wiegant FA.**

The main purpose of the studies presented in this paper is twofold: 1) to evaluate whether phyto-adaptogens (Acanthopanax senticosus and Rhodiola rosea) are able to exert a protective action against stress-induced death of embryos of the pond snail Lymnaea stagnalis; and 2) whether a possible protective action by phyto-adaptogens can be explained by the induction of heat shock proteins. Enhancement in resistance by phyto-adaptogens was studied by applying plant extracts for a period of 20 hours to 3-day-old larvae of the pond snail Lymnaea stagnalis. Subsequently they were exposed to a high and toxic dose of different environmental stressors. The following stress conditions were selected: a physical stress condition (heat shock: 43 degrees C for 4 minutes), an oxidative stress condition (superoxide radicals induced by menadione [600 microM for 2 hours]) and heavy metal-induced stress (copper [150 microM for 1 hour] or cadmium [20 microM during 1 hour]). Both Acanthopanax and
Rhodiola exert a strong protective action against a lethal heat shock. These adaptogens also significantly protect against the negative effect of superoxide radicals as induced by menadione. With respect to the protective action against exposure to heavy metals a small but significant protection was observed against intoxication with copper or cadmium by the phyto-adaptogens. In summary, there appears to be a difference in efficiency in enhancing resistance to the various stress conditions used (heat shock>menadione>copper>cadmium). Based on the results presented in this paper, we can conclude that phyto-adaptogens are able to enhance the resistance against the different stress conditions tested in developing individuals of Lymnaea. Although the degree to which resistance is enhanced appears to depend on the type of stressor applied, our results confirm the definition of phyto-adaptogens as being universal enhancers of non-specific resistance against different kinds of stress conditions. With respect to the mechanism of enhanced resistance, the question was asked whether this protective action is caused by an induction of heat shock proteins (hsps), which are known to be involved in tolerance and adaptation. The phyto-adaptogens did not induce the synthesis of any of the hsps, nor did they modulate the normal heat shock induced synthesis of these stress proteins. We conclude that it is unlikely that hsps play a major role in obtaining an enhanced state of resistance provided by phyto-adaptogens.

**D-Phenylalanine**


D-phenylalanine markedly potentiates opiate analgesia - an example of nutrient/pharmaceutical up-regulation of the endogenous analgesia system.

**Russell AL, McCarty M F.**

In the author's clinical experience, concurrent treatment with DL-phenylalanine (DLPA) often appears to potentiate pain relief and also ease depression in patients receiving opioids for chronic non-malignant pain. An analysis of this phenomenon suggests that it may be mediated, at least in part, by up-regulation of the 'endogenous analgesia system' (EAS), a neural pathway that projects caudally from medullary nuclei to the dorsal horn of the spinal column; when stimulated by chronic pain or therapeutic measures such as opiates or acupuncture, the EAS suppresses activation of second-order pain-receptive neurons in the dorsal horn, and thereby alleviates pain. Since serotonin and enkephalins are key neurotransmitters in the EAS, it is reasonable to predict that measures which promote serotonin activity (such as 5-hydroxytryptophan and serotonin-reuptake inhibitors) as well as enkephalin activity (such as D-phenylalanine, an enkephalinase inhibitor) should potentiate EAS-mediated analgesia - a view consistent with much previous medical research. Comprehensive support of the EAS with well-tolerated nutrients and pharmaceuticals may amplify the analgesic efficacy of chronic opiate therapy, while enabling dosage reductions that minimize opiate side-effects. Analogously, this approach may complement the efficacy of acupuncture and other analgesic measures that activate the EAS.


[A comprehensive study of the neurochemical and immune mechanisms of morphine tolerance: the effects of nalozone]
Litvinova SV, Shul'govskii VV, Gruden' MA, Panchenko LF, Terebilina NN, Aristova VV, Kaliuzhnyi AL

To test the authors' hypothesis about the role of endopeptidase (enkephalinase A, in particular) in mechanisms of morphine tolerance and blocking action of small doses of naloxone, they studied nociception reactions, morphine antibodies titres and enkephalinase A activity after morphine, d-phenylalanine and naloxone injection in brain structures. It is shown that activity of enkephalinase A in structures of endogenous antinociceptive system increased simultaneously with morphine antibodies titres in tolerance condition. Injection of small dose naloxone inhibited enkephalinase activity in brain structures and decreased morphine antibodies titres to these in control morphine-sensitive rats and therefore suppressed morphine tolerance. Prolonged naloxone injection decreased morphine antibodies titres to the levels of intact animals and highly increased titres of antidiotopic morphine antibodies. Thus, these results confirm the role of enkephalinase as a neuromodulator. A strong relationship exists between enkephalinase and immune mechanisms of development of morphine tolerance which can be blocked by small naloxone doses. It is concluded that naloxone in small doses can be used in patients to suppress morphine tolerance.


The analgesic action of new enkephalin analogs

Solov'eva EV, Kulikov SV, Khar’kovskii AO, Bogdanov EG.

The enkephalin analogue peptide IKB-901 containing epsilon-ACA and cysteine with the modified S-end shows an analgetic activity in rats (1 micron, intrathecally and 5 mg/kg intravenously) and in cats (0.35 and 0.7 mg/kg intravenously). Naloxone (0.1 mg/kg) prevents the analgetic effect of peptide. The coadministration of the peptide and the enkephalinase inhibitor D-phenylalanine (0.35 and 10 mg/kg, respectively) enhances analgesia and displays an antihypertensive effect in nociceptive stimulation.


The enkephalinase mechanisms of the resistance and tolerance to the analgesic effect of morphine in rats. Differences in the effects of the action of D-phenylalanine in morphine-sensitive, morphine-tolerant and morphine-resistant rats

Litvinova SV, Kozlov Alu, Kaliuzhniy LV.

In morphine-sensitive (s.c. 1.5 mg/kg) Wistar rats (60%) i.p. inoculation of 300-600 mg/kg d-Phenylalanine (d-Pha) did not change the nociception (tail-flick test), but in morphine-resistant rats (40%) evoked a dose-dependent analgetic effect. In morphine-sensitive rats (40%) chronic morphine administration induced the tolerance and d-Pha injection evoked analgetic effect. Morphine injection just after d-Pha analgesia was over evoked analgetic effect in morphine-resistant and -tolerant rats. It is suggested that morphine-resistant rats have a congenital and morphine-tolerant rats an acquired high level of enkephalinase activity which blocked the morphine analgetic action.


The analgesic action of d-phenylalanine in combination with morphine or methadone
The analgesic action of D-phenylalanine (D-Phe) is well known. It has been demonstrated in hot-plate tests on mice that combining D-Phe with narcotic analgesics already with doses inactive on separate application. In combination with D-Phe, a dose of morphine less by half compared to its unique use does not reduce analgesic activity in rats, but after six weeks of treatment some undesirable side effects like dependence, behaviour disorders and growth retardation are markedly lowered. These results suggest the possibility to design a combined drug similarly effective as well-introduced narcotic analgesics, but better tolerated.


Action of an enkephalinase blocker on the effect of acupuncture in acupuncture sensitive and resistant rabbits

Kaliuzhnii LV, Kozlov ALu.

In unanesthetized acupuncture-sensitive rabbit d-phenylalanine injection didn't change the EP in response to tooth pulp electrostimulation, but prolonged the analgetic effect of auriculo-acupuncture stimulation 15 Hz expressed by decreasing of the amplitude of N1P2 component EP in acupuncture-resistant rabbit d-phenylalanine injection induced analgetic effect which was enhanced and prolonged by auriculo-acupuncture stimulation. It's suggested that the recovery of pain sensibility after acupuncture analgesia is determined by enkephalinase's mechanism activation which is activated permanently in acupuncture-resistant rabbits.


Morphine analgesia mediated by activation of the acupuncture-analgesia-producing system.

Sato T, Takeshige C, Shimizu S.

Analgesia caused by intraperitoneal 0.5 mg/kg morphine (MA) in rats is equivalent to acupuncture analgesia (AA) caused by low frequency stimulation of the tibial muscle (Tsusanli acupuncture point). Analgesia equivalent to both AA and MA was produced by intrathecal application of 0.05 microgram morphine. This analgesia exhibits individual variation in effectiveness which is parallel to those of both AA and MA, and disappears after 250 mg/kg intraperitoneal D-phenylalanine. Analgesia that persisted after termination of acupuncture stimulation was not affected, maximally developed MA and AA were both partially antagonized, and the initial development of AA and MA were completely antagonized by intrathecal application of 0.2 microgram naloxyone. Analgesia caused by intrathecal 0.05 microgram morphine was abolished by bilateral lesion of the anterolateral tract (ALT) of the spinal cord and that caused by acupuncture stimulation was abolished by contra-lateral lesion. Analgesia caused by larger doses (0.1-0.2 microgram) of intrathecal morphine was not abolished, but persisted after ALT lesion, unilateral lesion of the dorsal periaqueductal central gray (D-PAG), or hypophysectomy. Potentials were evoked by acupuncture stimulation in the bilateral D-PAG. Analgesia produced by D-PAG stimulation was not affected by ALT lesion nor by intrathecal naloxyone, but was abolished by lesion of the dorsolateral funiculus. These results imply two types of morphine action in the spinal cord to produce analgesia: activation of the ascending AA pathway; and direct inhibition of pain message in the spinal
cord. They also show that the AA producing pathway ascends contralateral in the ALT and then bilaterally in the D-PAG.


Potentiation of swim analgesia by D-amino acids in mice is genotype dependent.

Panocka I, Sadowski B.

The effect of combined treatment with 125 mg/kg of D-phenylalanine plus 125 mg/kg of D-leucine (IP) on magnitude and duration of analgesia caused by 3 min swim at 20 degrees C was studied in mouse lines selectively bred for 20 generations toward high and low level of stress-induced analgesia. The D-amino acids administered 30 min prior to swimming increased post swim tail-flick latencies and prolonged antinociception more in the high analgesia line (HA) than in concomitantly bred unselected controls, but were not effective in the low analgesia line (LA). The potentiation of swim analgesia by D-amino acids was prevented by simultaneous administration of 1 mg/kg of naloxone hydrochloride which, given alone, antagonized the analgesia more in the HA line than in controls, but not in the LA line. The results are interpreted in terms of genetic differentiation of opioidergic transmission in the selectively bred mouse lines.


Analgesic effects of D-amino acids in four inbred strains of mice.

Ninomiya Y, Kawamura H, Nomura T, Uebavashi H, Sabashi K, Funakoshi M.

1. Prominent strain differences of mice were found in analgesic effects of D-amino acids. 2. In C57BL/6CrSlc and C3H/HeSlc mice, pain threshold, which was determined by using a hot-plate method, increased to 140-175% of the control after the systemic treatment of all three D-amino acids employed, such as D-phenylalanine, -leucine and -methionine, whereas in DBA/2CrSlc or BALB/cCrSlc mice, out of three only one D-amino acid, D-phenylalanine or -leucine, produced significant increase of pain threshold. 3. This lack of ability to perceive analgesic effects of specific amino acids observed in the latter two strains suggests that there probably exist different analgesia-inducing mechanisms for each of three D-amino acids in mice and the latter two strains lack two of them.


Studies on the enhanced effect of acupuncture analgesia and acupuncture anesthesia by D-phenylalanine (2nd report)-schedule of administration and clinical effects in low back pain and tooth extraction.


D-phenylalanine (DPA) is known to block the activity of carboxypeptidase, an enzyme which degrades enkephalins, endogenous morphine-like substances. Therefore, it is considered that DPA administered as an inhibiting drug of this degrading enzyme might prolong analgesia induced by acupuncture. 1)
Thirty patients suffering from chronic low back pain were treated with acupuncture 30 minutes after the oral administration of 4.0 grams of DPA. The results were: excellent in 7 cases, good in 11, fair in 6 and poor in 6. Cases graded excellent and good were then compared with a placebo group. The effect was increased 26% in the DPA-acupuncture group, which shows no statistically significant difference (P less than 0.1). 2) In 56 patients, tooth extraction was performed under acupuncture anesthesia: 18 had received 4.0 gram of DPA (P.O.) 30 minutes earlier. The results were excellent in 8, good in 6, fair in 3, and poor in 1. The excellent and good cases were compared with 38 placebo group cases. The effect in the DPA-acupuncture anesthesia group was significantly increased by 35% (P less than 0.01). 3) In order to determine the optimum time for the administration of DPA, two schedules of administration were compared. [1] DPA was given on the previous day in three 0.5 gram doses (26 cases). [2] A single 4 gram dose was administered 30 minutes before treatment (30 cases). The results from the "excellent", "good" and "fair" cases showed a 16% increase in effectiveness when DPA was administered the day before, not a statistically significant difference (P less than 0.1), but a clear tendency to increase was observed. The above findings show that DPA has an enhancing effect on acupuncture analgesia and anesthesia in clinical practice.


Studies on the enhanced effect of acupuncture analgesia and acupuncture anesthesia by D-phenylalanine (first report)-effect on pain threshold and inhibition by naloxone.


It has been claimed that the mechanism of acupuncture analgesia can be explained in part by endogenous opioids. If so, it might be possible to enhance the analgesic effect of acupuncture by the administration of endorphins. If D-phenylalanine (DPA), an inhibitor of the endorphin degrading enzyme, is administered, the analgesic effect of acupuncture should be prolonged due to the increased level of endorphins. From the changes of the pain threshold (PT), we investigated whether or not the pre-administration of DPA can enhance the analgesic effect of acupuncture in humans. In addition, we examined the inhibitory effect of naloxone. 1) In all five subjects whose PT was raised after acupuncture anesthesia (respondents), the rise in PT was significantly prolonged by DPA. 2) Out of 10 subjects whose PT remained almost unchanged after acupuncture anesthesia (non-respondents), the PT was increased by DPA in 5 cases. 3) The rise in PT was most prominent when DPA was administered 30 minutes before the start of acupuncture anesthesia. 4) In all 4 respondents in whom the rise in PT persisted after DPA and acupuncture anesthesia, their raised PT dropped after the intravenous injection of naloxone (10 mg). 5) These findings show that DPA enhances the analgesic effect of acupuncture by the "endorphin mechanism."


Comparative characteristics of the functioning of brain structures exposed to morphine and D-phenylalanine
larosh AK, Goruk PS, Luk'ianov EA.

In experiments on rats it was shown that morphine and D-phenylalanine in doses of 5 and 100 mg/kg, respectively, produce a similar by the degree increase of pain reaction thresholds at stimulation of paws through the electrified floor of the chamber. Experiments on rabbits demonstrated that the main factor in morphine action is a decrease of excitability and blood filling of the reticular formation of the midbrain and the central gray matter and an increase of excitability of the dorsal hippocampus without significant changes in the frontal cortex excitability. D-phenylalanine also caused a decrease of excitability of the reticular formation but in contrast to morphine failed to change excitability of the dorsal hippocampus and enhanced excitability of the central gray matter.


Attenuation of tourniquet-induced pain in man by D-phenylalanine, a putative inhibitor of enkephalin degradation.

Nurmikko T, Pertovaara A, Pöntinen PJ.

The effect D-phenylalanine (DPA), a putative inhibitor of enkephalin degradation, on the two separate pain components produced by the submaximal effort tourniquet test was evaluated in healthy human volunteers (N = 8). DPA attenuated the increase of the intensity of the ischemic and pressure pain components with increasing ischemia duration, but only the effect on the pressure pain component was significant. The results support some earlier reports suggesting that DPA has analgetic properties.


Studies on the mesolimbic loop of antinociception-II. A serotonin-enkephalin interaction in the nucleus accumbens.

Xuan YT, Shi YS, Zhou ZF, Han JS.

In a previous report we have shown that the antinociceptive effect elicited by microinjection of morphine into the periaqueductal gray is due, at least in part, to the activation of an ascending serotonergic pathway which releases 5-hydroxytryptamine in the nucleus accumbens. We now report that antinociception induced by intra-periaqueductal gray injection of morphine can be attenuated also by the narcotic antagonist naloxone or the enkephalin antibodies administered into the nucleus accumbens, and potentiated by D-phenylalanine, a putative inhibitor of the degradation of enkephalins. Moreover, the antinociceptive effect induced by 5-hydroxytryptamine administered into nucleus accumbens could be blocked by naloxone injected into the same site, whereas the antinociception elicited by intra-accumbens injection of [D-Ala2,D-Leu5]enkephalin was not affected by cinanserin, a 5-hydroxytryptamine blocking agent. It is concluded that morphine administered to the periaqueductal gray is capable of activating an ascending serotonergic pathway to release 5-hydroxytryptamine in the nucleus accumbens, which in turn activates an enkephalinergetic mechanism within the same nucleus, resulting in an antinociceptive effect.
Pharmacological "enkephalinase" inhibition in man.

Marcello F, Grazia SM, Sergio M, Federigo S.

"Enkephalinase", a peptidase capable of degrading enkephalins, has been recently characterized in man, in both plasma and cerebro-spinal fluid (CSF). This study was designed to evaluate the ability of putative "enkephalinase" inhibitors, D-phenylalanine, captopril and thiorphan to decrease "enkephalinase" activity (EKA) in plasma and CSF in human sufferers. All drugs studied decreased plasma EKA. Captopril and thiorphan also decreased CSF EKA. Of the three drugs tested thiorphan proved to be the most potent "enkephalinase" inhibitor in both plasma and CSF. These results show the usefulness of EKA assessment as a procedure for evaluating the potency and specificity of putative "enkephalinase" inhibitors in man.

Prog Clin Biol Res. 1985;192:363-70.

Analgesic properties of enkephalinase inhibitors: animal and human studies.

Ehrenpreis S.

D-phenylalanine, bacitracin and puromycin produce long-lasting, naloxone-reversible analgesia in mice. Analgesic potency parallels potency of these compounds as inhibitors of met-enkephalin degradation by mouse brain enzymes. D-phenylalanine potentiates acupuncture analgesia in mice and humans and has been used to ameliorate a variety of human chronic pain conditions.


Pharmacology of enkephalinase inhibitors: animal and human studies.

Ehrenpreis S.

We have shown that a number of compounds which inhibit the degradation of met-enkephalin can produce naloxone-reversible analgesia in mice. These compounds also potentiate the analgesia produced by acupuncture, foot shock, and transcutaneous nerve stimulation in animals and humans. The potency of their effectiveness as analgesics or potentiators parallels their potency as inhibitors of mouse brain enkephalinase. D-Phenylalanine (DPA), one of these enkephalinase inhibitors, has been used successfully for the management of chronic intractable pain in humans and to potentiate the treatment of many painful conditions by acupuncture. Other aspects of pharmacology of DPA will be discussed, including its effects on the cardio-vascular system, behavior, and lack of development of tolerance and dependence when used chronically in animals and humans.


Differentiation between acupuncture and non-acupuncture points by association with analgesia inhibitory system.
Acupuncture and non-acupuncture points were differentiated by their connection to different pathways in the central nervous system. We have found that the pathway connected to the acupuncture point is different from the pathway connected to the non-acupuncture point. In addition, pathway connected to the non-acupuncture point is inhibited within the lateral periaqueductal gray when the analgesia inhibitory system (AIS) is activated. We have explored these pathways by means of selective lesioning of discrete brain regions, selective stimulation of brain regions, as well as by recording evoked potentials arising from stimulation of acupuncture and non-acupuncture points. It was found that the lateral centromedian nucleus of the thalamus and the posterior hypothalamus are parts of the AIS. The acupuncture (tibialis muscle) and non-acupuncture (abdominal muscle) points are both connected to the AIS. Analgesia caused by stimulation of the acupuncture point is naloxone reversible, while that caused by stimulation of the non-acupuncture point after lesion of AIS is dexamethasone reversible. Stress-induced analgesia caused by low frequency electrical shock is naloxone as well as dexamethasone reversible. All three kinds of analgesia were abolished by hypophysectomy. The features and the degree of analgesia caused by intraperitoneal 0.5 mg/kg morphine were similar to analgesia caused by acupuncture point stimulation. D-phenylalanine acts like a lesion of AIS in analgesia caused by stimulation of acupuncture and non-acupuncture points, and enhances naloxone reversible analgesia. The descending pain inhibitory system plays a role as the common pathway to produce these three kinds of analgesia. This pathway is found in the arcuate nucleus (dopaminergic), ventromedial nucleus of the hypothalamus, raphe nucleus (serotonergic), reticular gigantocellular nucleus (noradrenergic) and reticular paragigantocellular nucleus.

Modulation of deprivation-induced food intake by D-phenylalanine.

D-phenylalanine has been shown to possess opiate-like effects upon pain perception. The present study examined whether it would have similar opiate-like effects upon food intake in deprived rats. The first experiment demonstrated that food intake of rats deprived for 24 h prior to injection was significantly reduced for 2 h following a 250 mg/kg dose of D-phenylalanine. However, intake over a 24 h period following injection was significantly increased following a 125 mg/kg dose of D-phenylalanine. The second experiment revealed that 0.3, 1.0, 3.0 and 10.0 mg/kg doses of naloxone dose-dependently reduced intake for 2 h in deprived rats when paired with a vehicle injection. However, the inhibitory actions of the two lower naloxone doses were significantly attenuated when paired with an injection of a 250 mg/kg dose of D-phenylalanine. These results are discussed in terms of whether D-phenylalanine possesses direct or indirect opiate-like effects upon ingestion.
Naloxazone and pain-inhibitory systems: evidence for a collateral inhibition model.

Kirchgessner A L, Bodnar R J, Pasternak GW.

The analgesic responses following morphine and cold-water swims (CWS) can be dissociated from each other. Indeed, certain manipulations in rats such as hypophysectomy or D-phenylalanine injections decrease CWS analgesia while increasing morphine analgesia. The present study examined the reciprocal notion, namely whether a manipulation that decreases morphine analgesia would increase CWS analgesia. Naloxazone, an opiate antagonist which selectively inhibits the high affinity binding site in a long-acting manner, was administered intracerebroventricularly and assessed for its effects upon morphine analgesia and CWS analgesia as measured by the jump test. While intracerebroventricular injections of naloxazole reduced morphine analgesia at 0.5 and 24 hr following microinjection, the same 50 micrograms dose significantly increased CWS analgesia at 0.5 hr after injection, suggesting a mechanism of collateral inhibition between opioid and non-opioid pain-inhibitory systems.

Systemic D-Phenylalanine and D-Leucine for Effective Treatment of Pain in the Horse.

McKibbin LS, Cheng RS.

This study showed that subcutaneous injection of a solution of D-amino acids produced effective analgesia in horses. It is postulated that systemic D-phenylalanine and D-leucine may become one of the safe, effective and nonaddictive drugs for acute and chronic pain treatment. These D-amino acids cause analgesia by presumably preserving brain endorphins. They may bind reversibly to enkephalinases and prevent enzymatic degradation of enkephalins.

D-phenylalanine and other enkephalinase inhibitors as pharmacological agents: implications for some important therapeutic application.

Ehrenpreis S.


A number of compounds have been shown to inhibit the degradation of enkephalins. As expected, these compounds produce naloxone reversible analgesia and potentiate the analgesia produced by enkephalins and by acupuncture. One of these, D-phenylalanine, is also anti-inflammatory. D-phenylalanine has proven to be beneficial in many human patients with chronic, intractable pain. It is proposed the enkephalinase inhibitors may be effective in a number of human "endorphin deficiency
diseases" such as depression, schizophrenia, convulsive disorders and arthritis. Such compounds may alleviate other conditions associated with decreased endorphin levels such as opiate withdrawal symptoms.


Curing trial of complicated oncologic pain by D-phenylalanine


Aim of investigations: Very often, chronic pain treatments used for the management of terminal ill cancer patients do not prevent acute or incident pain from coming up. For twenty months D-phenylalanine (DPA), an enkephalinase inhibitor, has been investigated in order to forestall this pain.

Methods: Nine Caucasian patients, three males and six females, between forty-nine and seventy-eight, were selected for this trial after informed consent. They were all undergoing severe incident pains related to complications (scabies, osteoporosis, painful cough or colic, Charley-Horse, RX-necrosis of skin or mucous membranes, etc) in spite of having their chronic pain component cured: phenol-rhizotomy: two cases, neuro-adrenolysis by alcohol: four cases, Brampton mixture: three cases. They were administered DPA, 250 mg three times a day for fifteen days, followed by a ten days pause, resumption and so on. Results: Seven patients out of nine were alleviated and they never claimed for more or other analgesics until they died. Four of them got very good ataraxia during the same time (survival mean x = 99,33 days). No side effect was reported, even in patients taking Brampton mixture. Conclusions: DPA seems a useful drug to prevent acute or incident pain in malignant diseases. Our data point out the consequences the enkephalinases inhibitors will take up for the cure of intractable cancer pain.


Antagonism of stress-induced analgesia by D-phenylalanine, an anti-enkephalinase.

Bodnar RJ, Lattner M, Wallace MM.

Methionine- and leucine-enkephalin produce mild and transient analgesic effects, presumably because of enzymatic degradation. Administration of high (250 mg/kg) doses of D-phenylalanine retards the degradation process and elicits analgesia which is reversed by naloxone and which summates with electroacupuncture analgesia. The present study evaluated D-phenylalanine's dose-dependent effects upon a non-opioid analgesic treatment, cold-water swims (CWS), and compared this with morphine.

following determination of flinch-jump baselines, three groups of rats received respectively either 25, 50 or 100 mg/kg of D-phenylalanine intraperitoneal in three conditions: alone, with CWS (2 degrees C for 3.5 min), and with morphine (5 mg/kg, SC). Parallel controls with saline were also tested. Simultaneous exposure with each minimally analgesic dose of D-phenylalanine reduced significantly the analgesic, but not hypothermic effects of CWS. By contrast, morphine analgesia was unaffected by D-phenylalanine.

These data provide further support that different pain-inhibitory systems mediate CWS and morphine analgesia and suggest that activation of one system is capable of exerting collateral inhibition upon the other.
A combined treatment with D-amino acids and electroacupuncture produces a greater analgesia than either treatment alone; naloxone reverses these effects.

Cheng RS, Pomeranz B.

The D-amino acids (DAA), D-phenylalanine and D-leucine, produce naloxone reversible analgesia; electroacupuncture (EA) also produces analgesia which is blocked by naloxone. Combining the two treatments produces an additive effect with a larger analgesia than that produced by either treatment given alone; this combined effect is also blocked by naloxone. Moreover only 62% of the mice show EA analgesia and 53% show D-amino acid (DAA) analgesia; 80% of the animals show marked analgesia with both EA plus DAA treatment. Perhaps the combination of EA with DAA will provide a potent method for the treatment of clinical pain.

Chromium Salts

Chromium salts are known enhancers of serotonin synthesis. This fact provides important inference that serotonergic activity being enhanced will influence pain mechanisms both peripheral and central. In this regard a PUBMED search resulted in 857 studies that coupled serotonin function and pain mechanisms.


Central serotonergic neurons are differentially required for opioid analgesia but not for morphine tolerance or morphine reward.

Zhao ZQ, Gao YJ, Sun YG, Zhao CS, Gereau RW 4th, Chen ZF.

Opioids remain the most effective analgesics despite their potential adverse effects such as tolerance and addiction. Mechanisms underlying these opiate-mediated processes remain the subject of much debate. Here we describe opioid-induced behaviors of Lmx1b conditional knockout mice (Lmx1bf/f/p), which lack central serotonergic neurons, and we report that opioid analgesia is differentially dependent on the central serotonergic system. Analgesia induced by a kappa opioid receptor agonist administered at the supraspinal level was abolished in Lmx1bf/f/p mice compared with their wild-type littermates. Furthermore, compared with their wild-type littermates Lmx1bf/f/p mice exhibited significantly reduced analgesic effects of mu and delta opioid receptor agonists at both spinal and supraspinal sites. In contrast to the attenuation in opioid analgesia, Lmx1bf/f/p mice developed tolerance to morphine analgesia and displayed normal morphine reward behavior as measured by conditioned place preference. Our results provide genetic evidence supporting the view that the central serotonergic system is a key component of supraspinal pain modulatory circuitry mediating opioid analgesia. Furthermore, our data suggest that the mechanisms of morphine tolerance and morphine reward are independent of the central serotonergic system.

The relationship between chromium and wound healing is direct but not necessarily as obvious as that of, say, zinc to wound healing. However, the 'secret' to the 'Cr to wound healing relationship' can be revealed by just understanding one simple fact. Cr improves insulin sensitivity AND insulin has a profound relationship to wound healing. Insulin resistance is directly related to wound (and diseased
tissue) promoting disorders. There are many debilitating physical and mental maladies associated with advanced insulin-resistant (Met Synd X) disorders, like diabetes, chronic inflammation, increased infections, etc. Below is just one citation that references some mechanisms associated with insulin-resistance. So the Cr/wound healing relationship is irrefutable.


Insulin Signaling, GSK-3, Heat Shock Proteins and the Natural History of Type 2 Diabetes Mellitus: A Hypothesis.

Hooper PL

Metabolic syndrome and type 2 diabetes are progressive, indolent, multi-organ diseases. Understanding the abnormalities of heat shock proteins (HSPs) in these diseases is paramount to understanding their pathogenesis. In insulin resistant states and diabetes, heat shock factor 1 (HSF-1) is low in insulin sensitive tissues, resulting in low Hsp 60, 70, and 90 levels. We propose that low Hsps levels are the result of decreased insulin action leading to less phosphorylation of PI3K, PKB, and glycogen synthase kinase-3 (GSK-3). Importantly, less GSK-3 phosphorylation (and thus more GSK-3 activity) will lower HSF-1. Low Hsps make organs vulnerable to injury, impair the stress response, accelerate systemic inflammation, raise islet amyloid polypeptide, and increase insulin resistance. Feeding this cycle is excess saturated fat and calorie consumption, hypertension, inactivity, aging, and genetic predisposition- all of which are a associated with high GSK-3 activity and low Hsps. Support for the proposed "vicious" cycle is based on the observation that GSK-3 inhibition and Hsp stimulation result in increased insulin sensitivity, reduced accumulation of degenerative proteins with in the cell, improved wound healing, decreased organ damage and improved recovery from vascular ischemia. Recognizing GSK-3 and Hsps in the pathogenesis of insulin resistance, the central common feature of the metabolic syndrome, and type 2 diabetes will expand our understanding of the disease, offering new therapeutic options.

L-Phenylalanine

L-Phenylalanine is the precursor of dopamine in the ventral tegmental area of the brain.


Morphine reward in dopamine-deficient mice.

Hnasko TS, Sotak BN, Palmiter RD.

Dopamine has been widely implicated as a mediator of many of the behavioural responses to drugs of abuse. To test the hypothesis that dopamine is an essential mediator of various opiate-induced responses, we administered morphine to mice unable to synthesize dopamine. We found that dopamine-deficient mice are unable to mount a normal locomotor response to morphine, but a small dopamine-independent increase in locomotion remains. Dopamine-deficient mice have a rightward shift in the dose-response curve to morphine on the tail-flick test (a pain sensitivity assay), suggesting either a decreased sensitivity to the analgesic effects of morphine and/or basal hyperalgesia. In contrast,
dopamine-deficient mice display a robust conditioned place preference for morphine when given either caffeine or l-dihydroxyphenylalanine (a dopamine precursor that restores dopamine throughout the brain) during the testing phases. Together, these data demonstrate that dopamine is a crucial component of morphine-induced locomotion, dopamine may contribute to morphine analgesia, but that dopamine is not required for morphine-induced reward as measured by conditioned place preference.


A common neurobiology for pain and pleasure.

**Leknes S, Tracey I.**

Pain and pleasure are powerful motivators of behaviour and have historically been considered opposites. Emerging evidence from the pain and reward research fields points to extensive similarities in the anatomical substrates of painful and pleasant sensations. Recent molecular-imaging and animal studies have demonstrated the important role of the opioid and dopamine systems in modulating both pain and pleasure. Understanding the mutually inhibitory effects that pain and reward processing have on each other, and the neural mechanisms that underpin such modulation, is important for alleviating unnecessary suffering and improving well-being.

**Arch Gen Psychiatry.** 2008 Feb;65(2):220-31.

Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses.

**Scott DJ, Stohler CS, Egnatuk CM, Wang H, Koepp RA, Zubieta JK.**

Placebo and nocebo effects, the therapeutic and adverse effects, respectively, of inert substances or sham procedures, represent serious confounds in the evaluation of therapeutic interventions. They are also an example of cognitive processes, particularly expectations, capable of influencing physiology. To examine the contribution of 2 different neurotransmitters, the endogenous opioid and the dopaminergic (DA) systems, to the development of placebo and nocebo effects. Using a within-subject design, subjects twice underwent a 20-minute standardized pain challenge, in the absence and presence of a placebo with expected analgesic properties. Studies were conducted in a university hospital setting. Twenty healthy men and women aged 20 to 30 years recruited by advertisement. Activation of DA and opioid neurotransmission by a pain stressor with and without placebo (changes in the binding potential of carbon 11 [HC]-labeled raclopride and [HC] carfentanil with positron emission tomography) and ratings of pain, affective state, and anticipation and perception of analgesia. RESULTS: Placebo-induced activation of opioid neurotransmission was detected in the anterior cingulate, orbitofrontal and insular cortices, nucleus accumbens, amygdala, and periaqueductal gray matter. Dopaminergic activation was observed in the ventral basal ganglia, including the nucleus accumbens. Regional DA and opioid activity were associated with the anticipated and subjectively perceived effectiveness of the placebo and reductions in continuous pain ratings. High placebo responses were associated with greater DA and opioid activity in the nucleus accumbens. Nocebo responses were associated with a deactivation of DA and opioid release. Nucleus accumbens DA release accounted for 25% of the variance in placebo analgesic effects. Placebo and nocebo effects are associated with opposite responses of DA and endogenous opioid neurotransmission in a distributed network of regions. The brain areas involved in...
these phenomena form part of the circuit typically implicated in reward responses and motivated behavior.

Neurosci Bull. 2007 May;23(3):185-8.

Differential effects of dopamine on pain-related electric activities in normal rats and morphinistic rats.

Zhang Y, Xu MY, Su J.

To investigate the influence of dopamine (DA) and DA receptor's antagonist on the transmission of noxious information in the central nervous system of normal rats or morphinistic rats. The influence of DA on the electric activity of the pain-excited neuron (PEN) in the caudate nucleus (Cd) of normal rats or morphinistic rats was recorded after the sciatic nerve was noxiously stimulated. DA shortened the average latency of the evoked discharge of PEN in the Cd of normal rats, indicating that DA could increase the activity of PEN and pain sensitivity in normal rats. This effect could be inhibited by Droperidol. DA increased the average latency of the evoked discharge of PEN in the Cd of morphinistic rats, indicating that DA could inhibit the activity of PEN and pain sensitivity in morphinistic rats. The responses to painful stimulation were completely opposite between normal rats and morphinistic rats after the intracerebroventricular injection of DA.

Passion Flower

Addict Biol. 2003 Dec;8(4):379-86

Drug/substance reversal effects of a novel tri-substituted benzoflavone moiety (BZF) isolated from Passiflora incarnata Linn.- a brief perspective.

Dhawan K.

The present work is a mini-review of the author's original work on the plant Passiflora incarnata Linn., which is used in several parts of the world as a traditional medicine for the management of anxiety, insomnia, epilepsy and morphine addiction. A tri-substituted benzoflavone moiety (BZF) has been isolated from the bioactive methanol extract of this plant, which has been proposed in the author's earlier work to be responsible for the biological activities of this plant. The BZF moiety has exhibited significantly encouraging results in the reversal of tolerance and dependence of several addiction-prone psychotropic drugs, including morphine, nicotine, ethanol, diazepam and delta-9-tetrahydrocannabinol, during earlier pharmacological studies conducted by the author. In addition to this, the BZF moiety has exhibited aphrodisiac, libido-enhancing and virility-enhancing properties in 2-year-old male rats. When administered concomitantly with nicotine, ethanol and delta-9-tetrahydrocannabinol for 30 days in male rats, the BZF also prevented the drug-induced decline in sexuality in male rats. Because the BZF moiety isolated from P. incarnata is a tri-substituted derivative of alpha-naphthoflavone (7,8-benzoflavone), a well-known aromatase-enzyme inhibitor, the mode of action of BZF has been postulated to be a neurosteroidal mechanism in which the BZF moiety prevents the metabolic degradation of testosterone and upregulates blood - testosterone levels in the body. As several flavonoids (e.g. chrysin, apigenin) and other phytoconstituents also possess aromatase-inhibiting properties, and the IC50 value of such phytoconstituents is the main factor determining their biochemical efficacy, by altering their
chemical structures to attain a desirable IC50 value new insights in medical therapeutics can be attained,
keeping in view the menace of drug abuse worldwide

AlgaeCal

Unpublished data

Joel E. Michalek et al (2009)

In this Bone Health Report to the Nation, the US Surgeon General (SG) concluded that America’s bone health is in jeopardy and issued a call to action for the development of bone health programs designed to increase health literacy, physical activity, and nutrition. To examine the safety and efficacy of a bone health plan that incorporated the three components recommended by the SG with two versions of a bone health supplement and examine the effects of compliance. Two groups of subjects who expressed an interest in improving their bone health were tested with Dual-energy X-ray Absorptiometry (DXA) and reviewed the AlgaeCal Bone Health Plan (the Plan), an original version of the bone health supplement, and the requirements of a 6-month open-labeled protocol. In the first group (Group 1), 274 potential subjects aged 18-85 expressed an interest in improving their bone health, 158 agreed to participate, and 125 completed the study per protocol (PP) completing DXA, blood chemistry and quality of life tests at baseline and 6 months later. Two weeks after the last subject in Group 1 completed the study, the same procedure was followed with a second group of 80 potential subjects (Group 2), 58 of whom volunteered and 51 completed PP following the same plan, but taking an revised version of the bone health supplement. The two supplements contained different amounts of a sea-algae calcium with multiple naturally-occurring magnesium and trace minerals, and supplemental magnesium, boron, and vitamins D-3, K-2, and C. There were no significant differences in mean baseline bone mineral density (BMD) between the two groups or in variables related to BMD (age, sex, height, weight, percent fat, fat mass, or lean mass). For both groups, no significant differences were found between volunteers and non-volunteers and those who completed PP and those who were lost to attrition with regard to variables related to BMD. As compared to the expected mean annualized percent change (MAPC), both groups experienced significant increases in MAPC above expected [Group 1: 1.2%, p=0.001; Group 2: 2.8%, p=0.001]. The MAPC from baseline in Group 1 (0.48%) was not significant (p=0.14), but the MAPC was significant in Group 2 (p<0.001) and the MAPC in Group 2 was significantly greater than that in Group 1 (p=0.005). The MAPC contrast between compliant and non-compliant subjects was significant in both Groups (p=0.001 and p=0.003 respectively) with compliant subjects increasing their MAPC more than non-compliant subjects. No clinically significant changes in blood chemistries or self-reported quality of life were found in either group. Following the Plan as recommended for six months with either version of the bone health supplement was associated with improvements in mean annualized percentage change in BMD. Increased compliance facilitated greater increases as did modifying the bone health supplement with different amounts and types of nutrients, while holding all other components of the Plan constant.
Preferred Embodiments

Sample Formulas for Pain Ointments

Each formulation consists of a base ointment cream containing a solubilizer (e.g. Soya-lecithin aggregates, Micronized, Cyclic monoterpenes, Cyclohexanone derivatives, isosorbide dinitrate and Lipoderm etc.). The ingredient percentages will vary dependent on genotype results. Base ointment (BO) constitutes just the base cream with the solubilizer. The range of dosing for each cream could be between 10 and 160 grams. The directions as per prescription would be to apply a thin layer to affected area 2-3 times a day. The table provides a matrix whereby each ingredient can either be compounded alone (just Bo) or with any of the listed ingredients as depicted in the matrix. Any and all combinations are applicable.

<table>
<thead>
<tr>
<th>D-Phenylalanine</th>
<th>BO</th>
<th>LID</th>
<th>MT</th>
<th>CAMP</th>
<th>GBP</th>
<th>KET</th>
<th>KEPF</th>
<th>CAP</th>
<th>DICLO</th>
<th>IBUF</th>
<th>BAC</th>
<th>AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Phenylalanine</td>
<td>BO</td>
<td>LID</td>
<td>MT</td>
<td>CAMP</td>
<td>GBP</td>
<td>KET</td>
<td>KEPF</td>
<td>CAP</td>
<td>DICLO</td>
<td>IBUF</td>
<td>BAC</td>
<td>AM</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>BO</td>
<td>LID</td>
<td>MT</td>
<td>CAMP</td>
<td>GBP</td>
<td>KET</td>
<td>KEPF</td>
<td>CAP</td>
<td>DICLO</td>
<td>IBUF</td>
<td>BAC</td>
<td>AM</td>
</tr>
<tr>
<td>L-5-Hydroxytryptophan</td>
<td>BO</td>
<td>LID</td>
<td>MT</td>
<td>CAMP</td>
<td>GBP</td>
<td>KET</td>
<td>KEPF</td>
<td>CAP</td>
<td>DICLO</td>
<td>IBUF</td>
<td>BAC</td>
<td>AM</td>
</tr>
<tr>
<td>Rhodiola rosea</td>
<td>BO</td>
<td>LID</td>
<td>MT</td>
<td>CAMP</td>
<td>GBP</td>
<td>KET</td>
<td>KEPF</td>
<td>CAP</td>
<td>DICLO</td>
<td>IBUF</td>
<td>BAC</td>
<td>AM</td>
</tr>
<tr>
<td>Chromium salt</td>
<td>BO</td>
<td>LID</td>
<td>MT</td>
<td>CAMP</td>
<td>GBP</td>
<td>KET</td>
<td>KEPF</td>
<td>CAP</td>
<td>DICLO</td>
<td>IBUF</td>
<td>BAC</td>
<td>AM</td>
</tr>
<tr>
<td>Pyridoxal phosphate</td>
<td>BO</td>
<td>LID</td>
<td>MT</td>
<td>CAMP</td>
<td>GBP</td>
<td>KET</td>
<td>KEPF</td>
<td>CAP</td>
<td>DICLO</td>
<td>IBUF</td>
<td>BAC</td>
<td>AM</td>
</tr>
<tr>
<td>L-tyrosine</td>
<td>BO</td>
<td>LID</td>
<td>MT</td>
<td>CAMP</td>
<td>GBP</td>
<td>KET</td>
<td>KEPF</td>
<td>CAP</td>
<td>DICLO</td>
<td>IBUF</td>
<td>BAC</td>
<td>AM</td>
</tr>
<tr>
<td>Synaptamine complex</td>
<td>BO</td>
<td>LID</td>
<td>MT</td>
<td>CAMP</td>
<td>GBP</td>
<td>KET</td>
<td>KEPF</td>
<td>CAP</td>
<td>DICLO</td>
<td>IBUF</td>
<td>BAC</td>
<td>AM</td>
</tr>
<tr>
<td>Kyotorphin</td>
<td>BO</td>
<td>LID</td>
<td>MT</td>
<td>CAMP</td>
<td>GBP</td>
<td>KET</td>
<td>KEPF</td>
<td>CAP</td>
<td>DICLO</td>
<td>IBUF</td>
<td>BAC</td>
<td>AM</td>
</tr>
</tbody>
</table>

Lidocaine (LID); Menthol (MT); Camphor (CAMP); Gabapentin (GBP); Ketamine (KET); Ketoprofen (KEPF); Capsaicin (CAP); Diclofenac (DICLO); Ibuprofen (IBUF); Baclofen (BAC); Amitriptyline (AM); Cyclobenzapine (CLB). "" combinations. Chromium salts include but limited to Picolinate, polynicotinate etc.

Sample additional combinations:

Example 1.

D-phenylalanine, LID, GBP, KET, KEPF (10/5/10/10/10%); D-Phenylalanine, GBP, KET, BAC (10/10/10/4%); D-Phenylalanine, GBP, KET, LID (10/6/10/10%); D-Phenylalanine, GBP, KET, AM, BAC (10/6/4/4%); D-Phenylalanine, KEPF (10/10%); D-Phenylalanine, KEPF (10/20%); D-Phenylalanine, KEPF, LID (10/10/5%); D-Phenylalanine, KEPF, CLB (10/20/2%); D-Phenylalanine, KEPF, LID, CLB (10/20/5/2%); D-Phenylalanine, IBUF, KEPF, CLB (10/10/1%); D-Phenylalanine, LID (10/10%); D-Phenylalanine, DICLO (10/10%); D-phenylalanine, CAP, MT, CAMP (10/0.0375%); D-phenylalanine, CAP, MT, CAMP (10/0.05%); D-phenylalanine, KEPF, KET, CAP (10/10/6/0.075%).

Example 2.

L-phenylalanine, LID, GBP, KET, KEPF (10/5/10/10/10%); L-Phenylalanine, GBP, KET, BAC (10/10/10/4%); L-Phenylalanine, GBP, KET, LID (10/6/10/10%); L-Phenylalanine, GBP, KET, AM, BAC (10/6/4/4%); L-Phenylalanine, KEPF (10/10%); L-Phenylalanine, KEPF (10/20%); L-Phenylalanine, KEPF, LID (10/10/5%); L-Phenylalanine, KEPF, CLB (10/20/2%); L-Phenylalanine, KEPF, LID, CLB (10/20/5/2%); L-
Phenylalanine, IBUF, KEPF, CLB (10/10/10/1%); L-Phenylalanine, LiD (10/10%); L-Phenylalanine, DICLO (10/10%); L-phenylalanine, CAP, MT, CAMP (1/0.0375%); L-phenylalanine, KEPF, KET, CAP (10/10/6/0.075%).

Example 3.

Example 4.

Example 5.

Example 6.
Example 7

Pyridoxal -phosphate, LID, GBP, KET, KEPF (0.05/5/10/10/10%); Pyridoxal -phosphate, GBP, KET, BAC (0.05/10/4%); Pyridoxal -phosphate, GBP,KET, LID (0.01/6/10/10%); Pyridoxal -phosphate, GBP,KET, AM, BAC (0.05/6/4/4%); Pyridoxal -phosphate, KEPF(0.05/10 %); Pyridoxal -phosphate, KEPF (0.05/20%); Pyridoxal -phosphate, KEPF, LID (0.05/10/5%); Pyridoxal -phosphate, KEPF, LID, CLB (0.05/20/2%); Pyridoxal -phosphate, KEPF,LID,CLB (0.01/20/5/2%); Pyridoxal -phosphate, IBUF,KEPF,CLB (0.01/10/10/1%); Rhodiola rosea, LID (0.01/10%); Pyridoxal -phosphate, DICLO (0.05/10%); Pyridoxal -phosphate, CAP, MT, CAMP (0.05/0.0375%); Pyridoxal -phosphate, CAP, MT, CAMP (10/05%); L-Tyrosine, KEPF, KET, CAP (10/10/6/0.075%).

Example 8.

L-Tyrosine, LID, GBP, KET, KEPF (10/5/10/10/10%); L-Tyrosine, GBP, KET, BAC (10/10/4%); L-Tyrosine, GBP,KET, LID (10/6/10/10%); L-Tyrosine, GBP,KET, AM, BAC (10/6/4/4%); L-Tyrosine, KEPF (10/10 %); L-Tyrosine, KEPF (10/20%); L-Tyrosine, KEPF, LID (10/10/5%); L-Tyrosine, KEPF, CLB (10/20/5/2%); L-Tyrosine, KEPF, LID, CLB (10/10/1%); L-Tyrosine, LID (10/10%); L-Tyrosine, DICLO (10/10%); L-Tyrosine, CAP, MT, CAMP (0.0375%); L-Tyrosine, CAP, MT, CAMP (10/05%); L-Tyrosine, KEPF, KET, CAP (10/10/6/0.075%).

Example 9

Synaptamine, LID, GBP, KET, KEPF (10/5/10/10/10%); Synaptamine, GBP, KET, BAC (10/10/4%); Synaptamine, GBP,KET, LID (10/6/10/10%); Synaptamine, GBP,KET, AM, BAC (10/6/4/4%); Synaptamine, KEPF (10/20%); Synaptamine, KEPF, LID (10/10/5%); Synaptamine, KEPF, CLB (10/20/2%); Synaptamine, KEPF, LID, CLB (10/20/5/2%); Synaptamine, IBUF, KEPF, CLB (10/10/1%); Synaptamine, LID (10/10%); Synaptamine, DICLO (10/10%); Synaptamine, CAP, MT, CAMP (0.0375%); Synaptamine, CAP, MT, CAMP (10/05%); Synaptamine, KEPF, KET, CAP (10/10/6/0.075%).

Example 10

Kyotorphin, Synaptamine, LID, GBP, KET, KEPF (10/5/10/10/10%); Kyotorphin, Synaptamine, GBP, KET, BAC (10/10/4%); Kyotorphin, Synaptamine, GBP,KET, LID (10/6/10/10%); Kyotorphin, Synaptamine, GBP,KET, AM, BAC (10/6/4/4%); Kyotorphin, Synaptamine, KEPF (10/10%); Kyotorphin, Synaptamine, KEPF, LID (10/10/5%); Kyotorphin, Synaptamine, KEPF, CLB (10/20/2%); Kyotorphin, Synaptamine, KEPF, LID, CLB (10/20/5/2%); Kyotorphin, Synaptamine, IBUF, KEPF, CLB (10/10/1%); Kyotorphin, Synaptamine, LID (10/10%); Kyotorphin, Synaptamine, DICLO (10/10%); Kyotorphin, Synaptamine, CAP, MT, CAMP (0.0375%); Kyotorphin, Synaptamine, CAP, MT, CAMP (10/05%); Kyotorphin, Synaptamine, KEPF, KET, CAP (10/10/6/0.075%).
**Example 10**

Kyotorphin, LID, GBP, KET, KEPF (10/5/10/10/10%); Kyotorphin, GBP, KET, BAC (10/10/10/4%); Kyotorphin, GBP, KET, LID (10/6/10/10%); Kyotorphin, GBP, KET, AM, BAC (10/6/6/4/4%); Kyotorphin, KEPF (10/10 %); Kyotorphin. KEPF (10/20%); Kyotorphin, KEPF, LID (10/10/5%); Kyotorphin. KEPF, CLB (10/20/2%); Kyotorphin. KEPF, LID, CLB (10/20/5/2%); Kyotorphin. IBUF, KEPF, CLB (10/10/10/1%); Kyotorphin, LID (10/10%); Kyotorphin, DICLO (10/10%); Kyotorphin, CAP, MT, CAMP (10/05%); Kyotorphin, KEPF, KET, CAP (10/10/6/0.075%).

**Refereed Gene Map for Pain Ointments:**

<table>
<thead>
<tr>
<th>Human kappa opioid receptor gene (OPRK1)</th>
<th>In humans, the 36G &gt; T single nucleotide polymorphism (SNP) on KOR gene.</th>
<th>The kappa opioid receptor (KOR) system seems to play a role in stress responsivity, opiate withdrawal and responses to psycho-stimulants, inhibiting mesolimbic dopamine. KOR gene polymorphisms have been reported to contribute to predisposition to voluntary alcohol-drinking behavior in experimental animals.</th>
<th>DL-Phenylalanine L-Tyrosine Passion Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Description</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D(2) dopamine receptor gene (DRD2)</td>
<td>A haplotype block of 25.8 kilobases (kb) was defined by 8 SNPs extending from SNP3 (TaqIB) at the 5' end to SNP10 site (TaqIA) located 10 kb distal to the 3' end of the gene. Within this block, specific haplotype cluster A (carrying TaqIB1 allele) was associated with a high risk of heroin dependence in Chinese patients (P = 1.425 x 10(-22); odds ratio, 52.80; 95% confidence interval, 7.290-382.5 for 8-SNP analysis). A putative recombination &quot;hot spot&quot; was found near SNP6 (intron 6 ins/del G), creating 2 new daughter haplotypes that were associated with a lower risk of heroin dependence in Germans (P = 1.94 x 10(-11) for 8-SNP analysis).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lawford BR</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Other studies show the relationship of carrying Taq1A1 vs. A2 alleles in the treatment outcomes for heroin abuse. The results indicate that DRD2 variants are predictors of heroin use and subsequent methadone treatment outcome and suggest a pharmacogenetic approach to the treatment of opioid dependence. Others found association between nasal inhalation of opiates and DRD2 promoter -141DeltaC polymorphism. Significantly stronger cue-elicited heroin craving was found in individuals carrying D2 dopamine receptor gene (DRD2) Taq1 RFLP A1 allele than the non-carriers (P < 0.001).

<table>
<thead>
<tr>
<th>Catechol-O-methyltransferase (COMT) gene</th>
<th>Val(108/158)Met polymorphism of the catechol-O-methyltransferase (COMT) gene</th>
<th>Genotyping 38 Israeli heroin addicts and both parents using a robust family-</th>
<th>L-Tyrosine DL Phenyllalanine Rhodiola rosea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-Tyrosine DL Phenyllalanine Rhodiola rosea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Official process to complete the treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Horowitz R, Kotler M, Shufman E.</td>
<td></td>
</tr>
</tbody>
</table>


based haplotype relative risk (HRR) strategy. There is an excess of the val COMT allele (likelihood ratio = 4.48, P = 0.03) and a trend for an excess of the val/val COMT genotype (likelihood ratio = 4.97, P = 0.08, 2 df) in the heroin addicts compared to the HRR control group.


<table>
<thead>
<tr>
<th>Proenkephalin gene (PENK)</th>
<th>&gt; or = 81 bp allele</th>
<th>Among the subjects with opioid dependence, 66% carried the &gt; or = 81 bp allele compared with 40% of subjects with other types of substance abuse (chi2 = 11.31, p &lt; 0.004) and 49% of controls (chi2 =</th>
<th>DLPhenylalanine L-Tyrosine Rhodiola rosea</th>
</tr>
</thead>
</table>
6.0, p < 0.015). These results are consistent with a role of the PENK gene in opioid dependence.

In another study, Heroin abuse was significantly associated with PENK polymorphic 3' UTR dinucleotide (CA) repeats; 79% of subjects homozygous for the 79-bp allele were heroin abusers. Such individuals tended to express higher PENK mRNA than the 81-bp homozygotes, but PENK levels within the nucleus accumbens (NAc) shell were most strongly correlated to catecholamine-O-methyltransferase (COMT) genotype. Altogether, the data suggest that dysfunction of the opioid reward system is significantly linked to opiate abuse vulnerability and that heroin use alters the

<table>
<thead>
<tr>
<th>Serotonin Transporter (hSERT)</th>
<th>Homozygosity at hSERT (especially 10/10) was associated with early opiate addiction, while genotype 12/10 proved to be protective.</th>
<th>Reward system pathway</th>
<th>5-hydroxytryptophan</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Dopamine Transporter (DAT1)</th>
<th>In the case of DAT1, genotype 9/9 was</th>
<th>Reward System Pathway</th>
<th>Dl-Phenylalanine L-Tyrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Galeeva AR, Gareeva AE, Iur’ev EB,</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Added to the above genes the inventors propose that the following genes be added to the panel because of the potential involvement in tissue healing and inflammation: eNOS, TNF-alpha, VGF.

**Dopamine and pain: A preferred embodiment**

**Background**

It is well known that individuals respond differently to medications and certain nutraceuticals, in terms of both toxicity and treatment efficacy. Potential causes for such variability in drug (nutrient) effects include the pathogenesis and severity of the disease being treated: drug (nutrient) interactions; the individual's age, nutritional status; kidney and liver function; and concomitant illnesses. Despite the potential importance of these clinical variables in determining drug/nutrient effects, it is now recognized that inherited differences in the metabolism and disposition of drugs/nutrients, and genetic variants...
(polymorphisms) in the targets of drug/nutrient therapy (such as receptors like the dopamine D2 receptor), can have even greater influence on the efficacy and toxicity of either medications or nutraceuticals.

Clinical observations of such inherited differences in drug effects were first documented in the 1950's, exemplified by the prolonged muscle relaxation after the drug known as suxamethonium (an inhibitor of the breakdown of acetylcholine) and an inherited deficiency in the genes that encode the enzyme responsible for the breakdown of this drug as marked by plasma cholinesterase (the enzyme which breaks down acetylcholine). The second gene -based drug response was observed when researchers found that certain patients bled to death after they were treated with an anti-malarial therapy because they carried a gene variant which lowered their blood cell glucose 6-phosphate dehydrogenase activity. Such observations gave rise to the field of "pharmacogenetics" the antecedent to pharmacogenomics, the current topic. However, we now know that individual differences in response to drugs or nutrients are not due to single gene variants but rather they are determined by the interplay of several genes encoding proteins (enzymes, receptors, transporters) involved in multiple pathways of drug/nutrient metabolism, disposition and effects. We are embarking on new era where efficacy of any substance is governed by an individual's inherited genotype to a greater degree than even other non-genetic factors. Understanding structure/function normal physiology and certain observable dysfunctions may indeed lead to promising nutrient based targets, but without the knowledge afforded by accurate DNA based prescreening (genotyping) subsequent supplementation becomes nothing more than a crap shoot. Similar to the pharmaceutical industry the nutraceutical industry can become an equal opportunity player and begin to initiate ongoing research and development by incorporating these genomic-based doctrines as described herein.

Out of the 3 million unshared DNA bases, individuals could carry gene variants (polymorphisms) that might lead to either an increase or a decrease of a certain important drug/nutrient response related proteins such as receptors, enzymes, cell cycle control, chemical messenger synthesis or catabolism (breakdown) or many other cellular events. As stared earlier, while there is a paucity of molecular studies involving genome-based response in the nutrition field (see below), a plethora of molecular studies have revealed that many genes encoding drug targets exhibit genetic polymorphism (variants), which in many cases alters their sensitivity to specific medications and/or offer specific targeted therapy.

Pharmacogenetics is the study of the role of genetics in inter-individual variability to drug response and therapy. In this regard there are 232 PUBMED reports concerning pharmacogenetic studies for opioid drugs. Opioid analgesics are widely used clinically for pain management, and inter-patient variability with opioid therapy is often reported. Information on genetic polymorphisms in enzymes, receptors and transporters related to opioid disposition (pharmacokinetics) and pharmacology (pharmacodynamics) is documented. Pharmacogenetics of enzymes, including the cytochrome P450s and uridine diphosphoglucuronosyltransferases, opioid receptors and the ABC family of transporters are a few examples.
Dopamine and Pain: Brain Reward Cascade

Pain System

[000115] The principle ascending pathways for pain (e.g. spinothalamic tract) originate mainly in the
dorsal horn of the spinal cord and medulla wherein second order neurons receive synaptic input from
primary afferent neurons that supply nociceptors in tissue. The second order neurons of origin are
within layer I as well as deep layers (IV-VI) of the dorsal horn (Willis, 1985). Second order neurons of
origin of pain-related pathways are mainly wide dynamic range (WDR) neurons or nociceptive-specific
(NS) neurons and these two types of neurons process both exteroceptive and interoceptive information
associated with pain. Our cutaneous nociceptive system clearly serves as an exteroceptive role in
signaling potentially dangerous stimuli impinging upon our bodies, so that we can respond
appropriately, depending upon the situational context. Our interoceptive nociceptive system signals
tissues disorders (e.g. rheumatoid ) that are essentially inescapable, and calls for responses more
obviously in the homeostatic domain.

Pharmacological aspects of pain control

[000116] Opioids such as morphine and heroin and psychostimulant drugs such as amphetamine and
cocaine are effective pharmacological tools against chronic pain. Interestingly, amphetamine and
related drugs relieve cancer pain and sometimes administered as an adjuvant analgesic in the clinical
situation because they potentiate opioid analgesia and counter opioid-related sedation and cognitive
disturbances. In support of these clinical findings, studies have shown that, in rats, psychostimulants
potentiate the analgesic effect of morphine in an animal model of persistent pain (Dhal and
Melzack,1998). There is increasing evidence that sites rostral to the brainstem play a critical role in the
analgesic effects of opioid and psychostimulant drugs.

[000117] It is well known that opioids can inhibit pain by acting at spinal sites and at sites in the
brainstem where they modulate activity in descending brain stem pathways projecting to the spinal
cord. A primary site of action in the periaqueductal gray of the brain stem where stimulation of opioid
receptors activates, through direct projections, serotonin-containing cells in the nucleus raphe magnus.
In turn, the latter cells activate neurons that project, via the dorsolateral funiculus, to the dorsal horns
of the spinal cord where they inhibit cells that transmit information about noxious painful stimulation
from the periphery to supraspinal sites. The brainstem descending pain-suppression system, however,
plays a more important role in the suppression of brief, rapidly rising, transient, and well-localized (i.e.
phasic) pain than it does in the suppression of injury-produced persistent (i.e. tonic) and inescapable
pain. However, several lines of evidence suggest that the inhibition of the tonic pain requires the
activation of neural systems in addition to those required the activation of neural systems in addition to
those required to inhibit phasic pain (Altier and Stewart 1999).

Mesolimbic dopamine in the suppression of tonic pain

[000118] There is little information to date concerning the identity of the endogenous pain systems that
serve to inhibit tonic pain. The suppression of tonic pain involves systems in addition to those known to
suppress phasic pain, and that these systems appear to involve forebrain sites, rostral to the brainstem. A clue to this problem is that both opioids and psychostimulants reduce tonic pain and increase transmission in mesocorticolimbic dopamine neurons known to be activated by natural rewards such as food and sex. These neurons arise from dopamine cell bodies that lie in the ventral tegmental area (VTA) and project to various forebrain sites such as the nucleus accumbens (NAcc), amygdala, and prefrontal cortex. Opioids cause the release of dopamine from these neurons through their indirect activation (see reward cascade Drawing), whereas psychostimulant drugs such as amphetamine and cocaine increase dopamine extracellularly by decreasing reuptake and/or inducing release. Moreover, opioids and psychostimulants have both rewarding effects and analgesic effects in the clinical setting, suggesting that reward and analgesia might share common neural substrates (Franklin, 1998). Morgan and Franklin (1990), found that dopamine-depleting 6-hydroxydopamine lesions of the ventral midbrain, which contains the cell bodies of the neurons that give rise to ascending forebrain projections, block the analgesic effects of systemic morphine and amphetamine in the formalin, but not the tail-flick test. Their findings provided the first evidence that mesolimbic dopamine neurons play a role in the suppression of tonic, but not phasic pain. In recent studies, Taylor et. al.6 (2003), found that while the D1 - selective agonist SKF 38393 was without effect at a dose of 0.5nmol/side, the D2-selective agonist quinpirole dose dependency (0.05-5.nmol/side, bilateral) inhibited the persistent phase of formalin-induced nociception. This was blocked by pre-administration of a selective S2-dopaminergic antagonist raclopride. These results indicate dopamine agonists that activate D2 receptors in the NAcc, inhibit inflammatory pain.

**Dopamine D2 receptors and chronic pain**

[000118] Plastic changes in synaptic neurotransmission in the brain are thought to play a role in chronic pain. Animal studies suggest that striatal and cortical dopaminergic systems participate in pain transmission or modulation. Dopamine D2 receptors have been reported to mediate the inhibitory role of dopamine in animal models for persistent pain (Magnusson and Fisher, 2000). Hagelberg et. al. (2002), shown in healthy volunteers that high D2 receptor availability in the putamen is associated with low cold pain threshold and a high pain modulation capacity induced by conditioning stimulation. Furthermore, decreased [18F] FDOPA uptake and increased D2 receptor availability have been demonstrated in the putamen in a chronic orofacial pain state, the burning mouth syndrome (Hagelberg et. al.,2003).

[000119] Moreover, it was found that the increase in D2 receptor availability in the left putamen and the decrease in D1/D2 ratio imply that alterations in the striatal dopaminergic system as evaluated by PET may be involved in chronic orofacial pain conditions. In essence, we hypothesize that low or hypodopaminergic function in the brain may predispose individuals to low pain tolerance. Current research would support this concept and thus carriers of the D2 Taq A1 allele as observed in Reward Deficiency Syndrome(RDS) behaviors may be good candidates for nutrients or bioactive substances designed to enhance dopamine release in the brain.
Stress and Pain

[000120] The effects of excessive stress in modern life leads to chronic states of fatigue -related depression. This is an unfortunate fact yet true that about 80% of all illness can be traced back to stress and depression. According to the American Academy of Family Physicians about 2/3 of all office visits.

[000121] The importance here is to understand that it is our position that indeed in an individual with chronic pain the subject is definitely in a stressful condition and therefore there is increased neuronal firing. There are numerous examples in the literature to support this contention. Furthermore, if a individual has the DRD2A1 variant, numerous studies have shown that resultant low dopamine D2 receptors caused an inability to cope with stress in the family and as an individual 11-13( See Blum & Braverman 2001, Noble et. al, and Comings et. al ). In this regard, it is known that stress could even reduce D2 receptor mRNA message in the substantia nigra, the lateral part of the VTA, basal ganglia especially in the "reward site" the nucleus accumbens 14( Dziedzicka -Wasylewska,1997). This work supports the concept that forebrain dopamine systems are involved in mediating the behavioral effects of chronic mild stress. It further supports the view that in obese subjects ( with chronic mild to moderate stress) with a compromised number of D2 receptor sites and reduced mRNA message, the firing frequency of a catecholaminergic neuron is enhanced and would be quite receptive to L-tyrosine supplementation as proposed in the formula. Moreover, it is also known that neuronal depletion of dopamine could also induce an independent end-product inhibitory state for TOH, which will also respond to L-tyrosine supplementation. With a slow release formula, there is constant dopamine release because of the effect of enhanced opioidergic activity via d-phenylalanine on substantia nigra GABA neurons.

Stress and dopamine: Implications for the pathophysiology of chronic widespread pain

[000122] The relationship between stress, endorphins and hypothalamic-pituitary -adrenal (HPA) axis is well known ( Kreek and Koob,1998). Certainly in the world of addiction stress plays a critical role in both the acquisition and relapse. It is known that certain genetic and environmental elements play significant roles in drug dependency and dysregulation of brain reward pathways. In fact, dopamine D2 receptor polymorphisms have been associated with stress coping mechanisms and posttraumatic stress disorder ( Comings 1996). Interestingly, either stress can induce a painful condition or it can exacerbate the pain.

Exposure to stress also activates dopamine transmission in mesocorticolimbic dopamine neurons (Deutch and Roth,1990) and this effect appear to involve opioid mechanisms in the VTA. More specifically, intra-VTA infusions of the opioid receptor antagonist, Naltrexone, prevents the stress-induced activation of dopamine metabolism in the NAcc and prefrontal cortex, and exposure to stress causes the release of met-enkephalin into the VTA (Kalivas and Abhold, 1987). These findings, combined with those indicating that exposure to stress can inhibit tonic pain and that intra-VTA morphine induces analgesia in theformalin test, suggest that the endogenous release of opioids in the VTA might be a mechanism underlying the stress-induced inhibition of tonic pain. This has been supported by the finding that intra-VTA infusions of the opioid receptor antagonist, naltrexone, block stress-induced analgesia in the formalin test (Altier and Stewart, 1999). In addition, it has been proposed that release of the tachykinin neuropeptide, substance P (SP), un the VTA might play a similar role in the stress -
induced suppression of tonic pain. In this regard, it has been found that activation of midbrain dopamine neurons by SP did indeed inhibit tonic pain in the formalin test (Altier and Stewart, 1999). The current data suggests that exposure to stress induces analgesia by causing a release of SP in the VTA, which in turn activates mesocorticolimbic dopamine neurons. Finally, opioids, amphetamine, and SP all share the ability to increase dopamine release in the NAcc. Moreover, opioids administered systemically or into the VTA augment dopamine metabolism and extracellular levels of dopamine in the NAcc.

With that background it becomes increasingly clear that tonic pain may be attenuated by dopamine D2 activation. It follows then that in this application we embrace as one inventive embodiment a natural method to cause a preferential release of dopamine in mesocorticolimbic pathways. In this regard, support of an attenuation of stress has been found with a variant of a complex with dopaminergic activation properties shown in one double-blind placebo controlled study (Blum et al. 1989). We propose herein that unless there is a way of increasing endogenous opioids, which in turn inhibit GABA causing dopamine release in the NAcc, simple neurotransmitter precursors will not be as effective in reducing tonic pain.

Fibromyalgia

One example of how stress and dopamine may interact involves fibromyalgia (FM) which has been called a "stress-related disorder" due to the onset and exacerbation of symptoms on the context of stressful events (Wood 2004).

The cardinal feature of FM is pain, the experience of which involves both afferent and efferent processes. While exposure to acute stress is known to produce stress-induced analgesia, the induction of which depends on of dopamine containing neurons within the NAcc, rat studies have demonstrated that prolonged exposure to stress eliminates this response, resulting instead in a state of stress-induced hyperalgesia (Wu et al. 1999). Chronic stress has been shown to result in the attenuation of dopaminergic activity within the NAcc and is therefore proposed to contribute to the development of stress-related hyperalgesia.

Interestingly, in FM patients clinical studies have suggested a disruption of dopaminergic function, including but not limited to decreased dopamine metabolites in cerebrospinal fluid (Russell et al. 1992; Legangneux et al. 2001). A variety of stressors result in the release of dopamine within the NAcc, including acute psychological stress a cornerstone symptom of FM (Kallivas and Duffy, 1995). Thus a vicious cycle occurs whereby stress from the pain further exacerbates the release of dopamine which in turn results in a hyperalgesia state. Hyperalgesia to both thermal and chemical stimulants persists up to 9 days after stress exposure in rats (Quintero et al. 2000). Moreover, other neurotransmitters are also involved as well. The selective 5-HT reuptake inhibitors clomipramine and fluoxetine, as well as the 5-HT reuptake precursor tryptophan, blocks development of hyperalgesia, suggesting that repeated stress produces a long-lasting increase in pain sensitivity. In fact, whereas there is a disruption of both serotonergic and dopaminergic function that occurs within the NAcc following chronic stress, the impact on dopamine outlasts that on 5-HT. In this regard there are three possibilities which have been proposed: (1) there is regulatory interaction between 5-HT and Dopamine...
during stress-induced analgesia; (2) a disruption of this interaction contributes to the inception of stress-induced hyperalgesia; and (3) dopaminergic dysfunction, which outlasts that of 5-HT, may be responsible for the persistent expression of stress-induced hyperalgesia after serotonergic function has been normalized. This phenomena may explain why strategies aimed at boosting serotonergic function only on patients with chronic widespread pain have met with limited success insofar as analgesia is concerned. Thus since FM is a stress-related disorder, one would predict that strategies aimed at boosting dopaminergic function within the mesolimbic pathway would have superior efficacy. While no one has attempted combining therapies in term of multiple pharmacogenomic targets, and the outcome of such an attempt is unknown, on this provisional we are proposing that natural manipulation of the reward signaling and circuitry could become very commercially viable. Breaking of this cycle with a stress reducing substance, such as passion flower (see below) or the proposed Synaptamine which includes this substance.

Summary of Invention

[000127] Most recently Li and his associates developed an addiction gene network that was constructed manually based on the common pathways identified in their 2008 study and protein interaction data. Addiction-related genes were represented as white boxes while neurotransmitters and secondary massagers were highlighted in purple. The common pathways are highlighted in green boxes. Related functional modules such as "regulation of cytoskeleton", "regulation of cell cycle", "regulation of gap junction", and "gene expression and secretion of gonadotropins" were highlighted in carmine boxes. Several positive feedback loops were identified in this network. Fast positive feedback loops were highlighted in red lines and slow ones were highlighted in blue lines.

[000128] Drug addiction is a serious worldwide problem with strong genetic and environmental influences. Different technologies have revealed a variety of genes and pathways underlying addiction; however, each individual technology can be biased and incomplete. Li et al (2008) integrated 2,343 items of evidence from peer-reviewed publications between 1976 and 2006 linking genes and chromosome regions to addiction by single-gene strategies, microarray, proteomics, or genetic studies. Li et al (2008) identified 1,500 human addiction-related genes and developed KARG (http://karg.cbi.pku.edu.cn), the first molecular database for addiction-related genes with extensive annotations and a friendly Web interface. Li et al (2008) then performed a meta-analysis of 396 genes that were supported by two or more independent items of evidence to identify 18 molecular pathways that were statistically significantly enriched, covering both upstream signaling events and downstream effects. Five molecular pathways significantly enriched for all four different types of addictive drugs were identified as common pathways which may underlie shared rewarding and addictive actions, including two new ones, GnRH signaling pathway and gap junction. They connected the common pathways into a hypothetical common molecular network for addiction. They observed that fast and slow positive feedback loops were interlinked through CAMKII, which may provide clues to explain some of the irreversible features of addiction. Interestingly, the common thread involves dopaminergic genes.

[000129] The subsequent coupling of these and other genes relative to polymorphisms would allow for additional nutrient based nutrigenomic mapping. The combination will provide a map which will serve as
a platform to derive novel DNA targeted areas which will link nutrients with potential anti-craving actions. Moreover, the inventors are also proposing that coupling of the Synaptamine complex and/or kyotorphin with outlined pain compounds into an ointment base with a known solubilizer is inventive and unobvious. Furthermore the coupling of this novel compounds with genotyping as suggested in the embodiment of this provisional application is inventive and unobvious as well. Both These areas are indeed novel, inventive and have not been accomplished heretofore.
REFERENCES

LifeGen-Publication support


This paper laid out the scientific data supporting a hypothesis that nutrients could be DNA-customized to affect inflammation.


This above paper shows data from an early pilot of our addiction product and how it reduces relapse and addiction.

■ Blum K, Chen TJ, Meshkin B, Waite RL, William Downs B, Blum SH, Mengucci JF, Arcuri V, Braverman ER, Palomo T. Manipulation of catechol-O-methyl-transferase (COMT) activity to influence the attenuation of substance seeking behavior, a subtype of Reward Deficiency Syndrome (RDS), is dependent upon gene polymorphisms: A hypothesis. Med Hypotheses. 2007 Apr 27; [Epub ahead of print]

This paper lays out the third party scientific evidence and our hypothesis around the measurement of the COMT gene in our addiction product.


This above paper is a publication of data on our addiction product in intravenous (IV) form showing improvement in the recovery of alcoholics.


This paper illustrates another manifestation of RDS related to aggression among adolescents.
Sampling of Publication Support


Dyr, W., Mcbride, WJ., Lumeng, L., Li, T-K., and Murphy, JM., 1993. Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats., *Alcohol 10*: 207-212.


Pre-Clinical and Clinical Support For Gnap and Genetic Risk for Reward Deficiency Syndrome (RDS).

Pre-Clinical Support


Clinical Support


**Reward Deficiency Syndrome (RDS) Support**


Green Al, Zimmet SV, Strous RD, Schildkraut JJ. Clozapine for comorbid substance use disorder and schizophrenia: do patients with schizophrenia have a reward-deficiency syndrome that can be ameliorated by clozapine. Harv Rev Psychiatry. 1999 Mar-Apr;6(6):287-96.


**Dopaminergic Genes and Addictive Behaviors (a sampling)**


O'Hara BF, Smith SS, Bird G, Persico AM, Suarez BK, Cutting GR, Uhl GR. Dopamine D2 receptor RFLPs, haplotypes and their association with substance use in black and Caucasian research volunteers. 1993; Hum Hered, 43:209-218.


**Serotonergic Genes and Addictive Behavior**


Synaptic Neurotransmitter Metabolizing Genes

MOA


**COMT**


**Gabergic Genes and Addictive Behavior**


Young RM, Lawford BR, Feeney GF, Ritchie T, Noble EP. Alcohol-related expectancies are associated with the D2 dopamine receptor and GABAA receptor beta3 subunit genes. 2004 Psychiatry Res. 127:171-83.

Enkephalinergic Genes and addictive Behavior


Blum K. Topel H. Opioid peptides and alcoholism: genetic deficiency and chemical management. 1986; Funct Neurol. 1: 71-83


H-Wave (Non-narcotic alternative to pain relief)

Blum K, Chen THJ & Ross BD. Innate properties of H-Wave device, a small fiber stimulator provides the basis for a paradigm shift of electro-therapeutic treatment of pain with increased functional restoration associated with human neuropathies by affecting tissue circulation. Medical Hypothesis 2005 1066-1067


What is claimed:

1. A composition for treating disease states affected by genetic (DNA) and neuro-metabolomic factors comprising:

   a) at least one calming herbal component;
   
   b) at least one vitamin component;
   
   c) at least one mineral component;
   
   d) an opiate destruction-inhibiting amount of at least one substance being selected from the group consisting of D-amino-acids, peptides, and structural analogues or derivatives thereof;
   
   e) a neurotransmitter synthesis-promoting amount of at least one neurotransmitter precursor selected from the group consisting of dopamine precursors L-Tyr, L-Phe and L-Dopa; serotonin precursors L-Trp and 5-hydroxytryptophane; and gamma amino butyric acid (GABA) precursors L-glutamine, L-glutamate and L-glutamic acid;
   
   f) a tryptophan concentration enhancing amount of at least one chromium salt;
   
   g) a catecholamine catalytic inhibitor of the enzyme Catecholamine o-methyl-transferase (COMT) selected from the group consisting of Rhodiola in all its forms, and/or an inhibitor of acetylcholine catabolism by blocking the acetylcholinesterase enzyme selected from a group consisting of Huperzine; and/or
   
   h) an effective amount of the at least one homeopathic component.

2. A composition of claim 1 wherein the composition is effective in reducing RDS behaviors, reducing pain, reducing inflammation acute and chronic, correcting intolerance to pain, tissue healing, promoting enhanced nitric oxide activity, inducing enhanced microcirculation and angiogenesis.

3. The composition of claim 1, wherein an effective amount of the at least one herbal component comprises at least one of the following: passion flower or fruit, BlackCurrant Oil; Black Currant Seed Oil; Ribes nigrum; Borage Oil; Borage Seed Oil; Borago officinalis; Bovine Cartilage; Bromelain; Ananas comosus; Cat's Claw; Uncaria tomentosa; Cetyl Myristoleate; Cetyl-M; Cis-9cetylmyristoleate; Cmo; Chondroitin Sulfate; Collagen Hydrolysate; Collagen; Gelatin; Gelatine; Gelatin Hydrolysate; Hydrolyzed [Denatured] Collagen; Devil's Claw; Devil's Claw Root; Grapple Plant; Wood Spider; Harpagophyllum procumbens; Dhea- Dehydroepiandrosterone; Dms-o- Dimethyl Sulfoxide; Evening Primrose Oil; Evening Primrose; Primrose; Oenothera biennis; other Oenothera species; Feverfew; Tanacetum parthenium; Fish Oil; Flaxseed; Flaxseed Oil; Flax Oil; Linseed Oil; Linum usitatissimum; Ginger; Zingiber officinale; Gingko; Gingko biloba; Ginseng; American ginseng; panax quinquefolius; Asian ginseng; panax ginseng; Siberian ginseng; eleutherothecoccus senticosus; GLA (Gamma-Ω nolenic Acid); Glucosamine; Glucosamine sulfate; glucosamine hydrochloride; N-acetyl glucosamine; Gotu Kola; Gotu Cola; Brahmi; Brahma-Buti; Indian Pennywort; Centella asiatica; Grapeseed; Grapeseed Oil; Grapeseed Extract; Vitis vinifera; Green
Tea; Chinese Tea; Camellia sinensis; Guggul; Gugulipid; Guggal; Commiphora mukul; Indian Frankincense; Frankincense; Boswellia; Boswellin; Salai Guggal; Boswellia serrata; Kava Kava; Kava; Kava Pepper; Tonga; Kava Root; Piper methysticum; Melatonin; MsM (Methylsulfonylmethane); New Zealand Green-Lipped Mussel; Perna Canaliculus; Pheliodendron Amurense; Sam-E (S-adenosyl-L-methione); Shark Cartilage; Cartilage; St. John's Wort; Hypericum perforatum; Stinging Nettle; Urtica dioica; Thunder God Vine; Tripterygium wilfordii; Turmeric; Curcuma longa; Curcuma domestica; Type II Undenatured Chicken Collagen; Chicken Collagen; Chicken Type II Collagen; Type II Collagen; Valerian; Valeriana officianalis; White Willow; Willow Bark; SaNx Alba; White Willow Bark; Wild Yam; Discorea villosa; Ganoderma Lucidum; Mangosteen Extract; Quercetin, or combinations thereof.

4. The composition of claim 3, wherein the at least one herbal component ranges from approximately 1 mg to 100,000 mg in a daily therapeutic administration.

5. The composition of claim 1, wherein an effective amount of the at least one vitamin component comprises at least one of the following: Folic Acid, Vitamin D, Vitamin C; and Vitamin B6, or combinations thereof.

6. The composition of claim 1, wherein an effective amount of the at least one mineral component comprises at least one of the following: manganese; potassium; magnesium; calcium; coral calcium; "Sierasil "; Algae CaT and any active salt thereof.

7. The composition of claim 1, wherein an effective amount of the at least one homeopathic component comprises at least one of the following: Aceonite 12X; Belladonna 12X; Bryonia 12X; Chamomilla 6X; Ferrum Phos 12X; Gelsemium 12X; and Berberis 6X.

8. The composition of claim 1, wherein the at least one other component comprises at least one of the following: an opiate destruction-inhibiting substance; a neurotransmitter synthesis precursor; a tryptophan enhancing substance; a catecholamine-O-methyl transferase (COMT) inhibitor; and/or an acetylcholinase/cholinesterase inhibitor.

9. The composition of claim 1, wherein the opiate destruction inhibiting substance comprises at least one of the following: D-phenylalanine; D-Leucine; any D-amino acid; and hydrocinnamic acid.

10. The composition of claim 1, wherein the neurotransmitter synthesis precursor comprises at least one of the following: dopamine precursors L-Tyr, L-Phe, and L-dopa; serotonin precursors L-Trp and 5-hydroxytryptophan; gamma amino butyric acid (GABA) precursors L-glutamine, L-glutamic acid and L-glutamate; acetylcholine (ACH) and acetylcarbamine precursors L-choline and L-acetylcarnitine; L-carnitine; and acetylcarnitine.

11. The composition of claim 1, wherein the tryptophan enhancing substance comprises at least one of the following chromium salts: picolinate, polynicotinate, chloride, and any active salts thereof.

12. The composition of claim 1, wherein the at least one other component comprises at least one of the following: Rhodiola (rosea extract) and/or Huperzine (A).
13. A kit comprising an assay for allelic analysis of a subject's DNA sample: and a composition for the treatment of RDS behaviors such as excessive craving behavior, Substance Use Disorder (SUD), overt prescription pain medication (i.e. narcotics), pain associated with Fibromyalgia, acute or chronic pain, or complications thereof.

14. A kit as described in claim 13 wherein said assay comprises the steps of:

Collecting DNA;

Processing, measuring and analyzing genes of the collected DNA;

Identifying any mutations of the collected DNA;

Using a custom algorithm to obtain an index score;

Formulating a composition based upon the identified index score; and

Administering to a human the custom composition for treatment of any identified mutations or disease states.

15. The kit of claim 14, wherein the DNA is collected by at least one of the following means: using a buccal swab, obtaining a whole blood sample, and other collection method.

16. The kit of claim 14, wherein the measuring of the DNA comprises at least one of the following methods: Elisa, TaqMan, PCR, and Invader.

17. The kit of claim 14, wherein the identifying mutations comprises measuring multiple genetic mutations through single nucleotide polymorphisms, gene expression, or other forms of genetic and phenotypic measurement for the purposes of customizing or adjusting the formulation of nutritional supplements.

18. The kit of claim 14, wherein the custom algorithm comprises measuring two genes through single nucleotide polymorphisms and combining genetic mutations into index scores to represent specific pre-defined formulations.

19. The kit of claim 14, wherein the index score comprises a value related to the number of identified mutations.

20. The kit of claim 19, wherein the index score is 0 for no identified mutations, 1 for an identified mutation on a gene, 2 for an identified mutation on a second gene, and 3 for an identified mutation on two separate genes.

21. The kit of claim 14, wherein using a custom algorithm to obtain an index score comprises providing a understandable, simple report to a patient and clinician for providing insight into disease diagnosis, stratification, and prognosis.
22. The kit of claim 13, wherein the disease state comprises at least one of the following: joint health involving reducing pain, inflammation, and joint damage; stress and anxiety relief; preventing sleep loss and insomnia; lethargy or lack of energy; overall mental health and well-being; reducing the signs and symptoms of attention deficit hyperactivity disorder; reducing the signs and symptoms of depression; reducing the signs and symptoms of pre-menstrual dysphoric disorder; and, overcoming the dependence and urges of smoking, alcoholism, and drug dependence and overt prescription pain medication (non-narcotic and narcotic and pain related to Fibromyalgia).

23. The kit of claim 13, wherein the composition comprises:

a) at least one calming herbal component;

b) at least one vitamin component;

c) at least one mineral component;

d) an opiate destruction-inhibiting amount of at least one substance being selected from the group consisting of D-amino-acids, peptides, and structural analogues or derivatives thereof;

e) a neurotransmitter synthesis-promoting amount of at least one neurotransmitter precursor selected from the group consisting of dopamine precursors L-Tyr, L-Phe and L-Dopa; serotonin precursors L-Trp and 5-hydroxytryptophane; and gamma amino butyric acid (GABA) precursors L-glutamine, L-glutamate and L-glutamic acid;

f) a tryptophan concentration enhancing amount of at least one chromium salt;

g) a catecholamine catalytic inhibitor of the enzyme Catecholamine o-methyl-transferase (COMT) selected from the group consisting of Rhodiola in all its forms, and/or an inhibitor of acetylcholine catabolism by blocking the acetylcholinesterase enzyme selected from a group consisting of Huperzine; and/or

h) an effective amount of the at least one homeopathic component.

24. The kit of claim 13, wherein the composition administered comprises Synaptamine™ in a daily therapeutic administration from approximately: 32-10,000 mg of Dl-phenylalanine, 10-10,000 mg of L-tyrosine, 5-5,000 mg of L-tryptophan, 3-30,000 mg of L-glutamine, 2-30,000 mg of chromium salt, 1-300 mg of pyridoxal -5'-phosphate, and 1-10,000 mg Rhodiola rosea..

25. The composition of claim 24 further comprises 5-10,000 mg AlgaeCaf (AlgaeCal International, Las Vegas, Nevada) in a daily therapeutic administration.

26. The composition of claim 24 further comprises: 5-10,000 mg Coral Calcium (Marine Bio Tokyo, Japan) in a daily therapeutic administration.
27. The kit of claim 13, wherein the RDS behavior is selected from excessive craving, low energy, low metabolic rate, compromised immune response and anti-oxidant repair, high stress and a high Cortisol level.

28. The method of claim 27, wherein the composition administered comprises: 2-2000 mg Passion flower; 5-1500 mg Kava Kava; 5-10,000 mg Rhodiola rosea; 5-10,000 mg Rhododendron; 5-10, 000 mg diphenylalanine; 2-5000 mg L-tyrosine; 10-5,000 mg L-glutamine; 5-2000 mg 5-Hydroxytryptophane; 20-30,000 mg Chromium Picolinate or other active salt thereof; 1-1000 mg Pyridoxal phosphate; 1-1000 mg Vitamin B complex; 5-2000 mg Calcium citrate; 5-2000 mg Magnesium ascorbate; 10-20,000 mg Hydroxycitric acid (a potassium salt); and 2-2000 mg Magnolia.

29. The kit of claim 13, wherein the genes of the collected DNA processed, measured and analyzed comprise at least one of the following: polymorphisms in Beta-adrenergic receptors; angiotensin converting enzyme (ACE) gene polymorphisms; angiotensin 11 TI receptor gene polymorphisms; polymorphisms in the gene that controls the enzyme cholesteryl ester transfer protein; potassium channel mutations; polymorphisms in cytochrome P-450 enzymes including CYP2D6; genetic mutation in a protein product of the HER2/neu oncogene; polymorphisms of the C825T gene involved in second messenger G-protein {beta}3; genetic variation of the apolipoprotein constituents of the lipoprotein molecules (APOE gene locus); variation of the CT and TT allele of the dopamine D2 receptor gene; a SNP (polymorphism) designated AA, at nucleotide position -6 of the ANG gene; Apo-Al gene; Methylene Tetrahydrofolate Reductase (MTHFR) including the C677T polymorphism of this gene; polymorphisms in the proinflammatory cytokine tumor necrosis factor (TNF); polymorphisms in the carbohydrate responsive element-binding protein (ChREBP) gene; C polymorphisms of the Leptin receptor gene (Leptin Gene and Leptin Receptor Gene · R108R homozygotes, LEP A19G polymorphism and the LEPR 109R carriers); polymorphisms of the dopamine D2 receptors gene (DRD2); polymorphisms of the dopamine D1,D3, D4,D5 genes; dopamine D2 receptor polymorphisms Ser311cys and TaqIA; c-fos; c-jun and c-myc; Sterol Regulatory Element Protein-1 (SREBP-2); mitochondrial glycerol-3-phosphate acetyltransferase gene (MGPAT) and the peroxisome proliferator - activated receptor (PPAR-gamma 2); Pro2Ala polymorphism of PPARgamma gene; Tryptophan 2, 3-Dioxygenase (TDO2) gene; TCP-1, Mc4R and CART genes; interleukin -1 beta, tumor necrosis factor -alpha, intracellular adhesion molecule, and interleukin -8 and 10 genes; interferon-alpha gene; Ras-Protein and (HLA-DRBl 0404 and O1O1 olor PTPN22 R620W); Dopamine Receptor D3 Ser9Gly (-205-G/A, -7685-G/C); Glutamine:fructose-6-phosphate amidotransferase (GFPTl or GFPT 2) variant in exon 14, 1471V or 3' UTR, or glucosamine 6-P acyltransferase; Aggrecan proteoglycan allele 27; 11-beta hydroxysteroid dehydrogenase type I; FK506 binding protein 5; serum/glucoid steroid kinase; Human tryptophan 2,3 dioxygenase; Myelin and Myelin associated glycoprotein genes (myelin oligodendrocyte glycoprotein (MOG), a tetranucleotide TAAA repeat (M0G4), C10991T SNP); Edg2; Fgfr2; Decorin; Brevican; Neurotensin (NT) receptors -1; Neurotensin (NT) receptors -2; Neurotensin (NT) receptors -3; Proenkephalin; prodynorphin(946C>G); Bdnf (Neurotrophic Factor (BDNF) Val66Met and -281 C>A, T allele of the C2707T; Sgk/Serum- and glucose-regulated kinase (SGK 1) SNP Intron 6, Exon 8 (CC, CT, TT); Gabl; Id2; COMT; ANKKI; DAT1; DBH; HTT; HTRIA; HTRID; HTR2A; HTR2C (5-HT-2A, 5-HT 2B, 5-HT-4 & 5-HT-7); ADRA2A; ADRA2; NET; MAOA; GABRA3; GABRB3; CNRI; CNRA4; NMDARI; POMC; MGPAT; NYP; AgRP; OBR; Mc3R;UCP-1; GLUT4;
The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms in the proinflammatory cytokine tumor necrosis factor (TNF) for identifying a differential response to fish oil supplementation for the treatment of rheumatoid arthritis.

The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms in the TNF gene for identifying a differential response to vitamin E for promoting anti-oxidant activity and reducing inflammatory processes.

The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of the dopamine D2 receptor gene for identifying a differential response to chromium salts as well as pain intolerance and treatment compliance.

The kit of claim 13, wherein the DNA collected is analyzed for at least one of the following polymorphisms of the dopamine D2, D1, D3, D4, and D5 receptor gene and information obtained is utilized to adjust the dosage of Synaptamine complex for pain control.

The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of the human TDO2 gene and information obtained is utilized to adjust the dosage of L-tryptophan, 5-hydroxytryptophan and chromium salts.

The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of polymorphisms of the interleukin -1 alpha, interleukin 1 beta, tumor necrosis factor -alpha, intracellular adhesion molecule, interleukin -8, and interleukin -10 genes and information obtained is utilized to adjust the dosage of Echinacea.

The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of MTHFR C677T (heterozygous/homozygous mutant versus homozygous normal) gene and information obtained is utilized to adjust the dosage of Folic Acid.

The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of collected will be analyzed for polymorphisms of hippocalcin like 1 (Hpcall) gene and information obtained is utilized to adjust the dosage of calcium.

The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of proenkephalin, prodynorphin, neurotensin (1,2,3) Bdnf, TD02, Sgk, Fkbps4, Edg2,ld2, Gabl Fgfr2 genes and information obtained is utilized to adjust the dosage of Passion flower.
39. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of COMT, proenkephalin, prodynorphin, neurotensin (1,2,3) Bdnf, TD02, Sgk, Fkbp5&4, Edg2, Id2, Gabl Fgfr2 genes and information obtained is utilized to adjust the dosage of Rhodiola rosea.

40. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of COMT, proenkephalin, prodynorphin, neurotensin (1,2,3) Bdnf, TD02, Sgk, Fkbp5&4, Edg2, Id2 genes and information obtained is utilized to adjust the dosage of Rhodendron.

41. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of COMT, DRD1-5, ANKKI, DATI, DBH, TD02, HTT, HTRIA, HTRID, HTR2A, HTR2C, ADRA2A, ADRA2, NET, MAOA, GABRA3, GABRB3, CNRI, CNRA4, NMDARI, POMC genes and information obtained is utilized to adjust the dosage of di-phenylalanine.

42. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of COMT NET MAOA DRD1-5 ANKKI DATI DBH POMC proenkephalin, prodynorphin, neurotensin (1,2,3) Bdnf, TD02, Sgk, Fkbp5&4, Edg2, Id2, Gabl Fgfr2 genes and information obtained is utilized to adjust the dosage of L-Tyrosine.

43. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of COMT NET MAOA POMC Proenkephalin, prodynorphin, neurotensin (1,2,3) GABRA3 GABRB3 NMDARI genes and information obtained is utilized to adjust the dosage of L-glutamine.

44. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of COMT NET MAOA POMC proenkephalin, prodynorphin, neurotensin (1,2,3) TD02, HTT, HTRIA, HTRID, HTR2A, HTR2C genes and information obtained is utilized to adjust the dosage of 5-Hydroxytryptophane.

45. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of COMT, NET, MAOA, POMC, proenkephalin, prodynorphin, neurotensin (1,2,3), TD02, HTT, HTRIA, HTRID, HTR2A, HTR2C, DRD1-5, ANKKI HTR2A, HTR2C, DRD1-5, ANKKI, DATI, DBH genes and information obtained is utilized to adjust the dosage of Chromium (all salts).

46. The kit of claim 13 wherein the DNA collected is analyzed for polymorphisms of HTT; HTRIA; HTRID; HTR2A; HTR2C (5-HT-2A, 5-HT 2B, 5-HT-4 & 5-HT-7), COMT DRD1-5, ANKKI, DATI, DBH, TD02, ADRA2A, ADRA2 NET, MAOA, GABRA3, GABRB3, CNRI, CNRA4, NMDARI, POMC, proenkephalin, prodynorphin, neurotensin (1,2,3), Bdnf, TD02, Sgk, Fkbp5&4, Edg2, Id2, Gabl, Fgfr2 genes and information obtained is utilized to adjust the dosage of (-)-Hydroxycitric acid (HCA).

47. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of Hpcall COMT NET MAOA genes and information obtained is utilized to adjust the dosage of Pyridoxal phosphate.

48. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of Hpcall gene and all ATPase genes and information obtained is utilized to adjust the dosage of Magnesium.
49. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms of leptin receptor, dopamineD1-5, Hpcall, HTT, HTRIA, HTRID, HTR2A, HTR2C (5-HT-2A, 5-HT 2B, 5-HT-4 & 5-HT-7), ANKKI, DAT1, DBH, TD02 and information obtained is utilized to adjust the dosage of potassium.

50. The method of claim 24, wherein the composition administered further comprises at least one of the following: (-)-Hydroxycitric acid (HCA); Passion flower (Passiflora incarnata Extract; Potassium; Thiamin; Vitamin B₆; and Calcium in a daily therapeutic amount ranging from approximately 1 mg to 30,000 mg.

51. The kit of claim 13, wherein the DNA collected is analyzed for polymorphisms (Rs value of SNP of DRD2 (Rs800497, Rs6278, Rs6276, RsIO79594, Rs 6275, Rs801028, RsI076560, Rs2283265, RsI079727, RsI076562, Rs25394, Rs4648318, Rs4274224, Rs7130156, Rs4648317, Rs799732, Rs799798; 5HT2A(Rs6314, Rs3742278, Rs6561333, Rs9238866, Rs643627, Rs2770292, Rs928040, Rs2770304, Rs594242, Rs6313;ANKKI (RS2734849, RS1800497, Rs6I04671, Rs4938016); OPRKI(Rs35160174, Rs35373196, Rs34709943 R6473797) OPRMI (Rs510769, Rs553202, Rs514980, Rs561720, Rs534673, Rs524731, Rs3823010, Rs3778148, Rs7773995, Rs495491, Rs2333298, Rs461773, Rs381376, Rs3778151, Rs506247, Rs563649, Rs9479757, Rs2075572, Rs0485057, Rs540825, Rs562859, Rs548646, Rs648007, Rs9322447, Rs681243, Rs609148, Rs3798687, Rs648893); COMT (Rs737864, Rs933271, Rs5993882, Rs740603, Rs4646312, Rs65722, Rs6269, Rs7699); SLC6A3(Rs2516948, Rs042098, Rs40184, Rs564773, Rsll33767, Rs6876225, Rs3776512, Rs2270912, Rs6347, Rs27048, Rs37022, Rs2042449, Rs464069, Rs463379, Rs403636, Rs2617605, Rs1893021, Rs6350, Rs2975223, Rs2963238, Rs 11564752 Rs9752266); HTR3B(Rs3758987, Rs2276307, Rs3782025, Rs672717); NOS3 (Rs891512, Rs808593, Rs2070744, Rs3918226, Rs7830); PPPARG (Rs801282, Rs2938392, Rs75542, Rs7036314, Rs805192, Rs6484847, Rs2938392, Rs709157, Rs709158, Rsll5542); ChREBP (Rs3812316); FTO (Rs8050136, Rs421084, Rs9939609, Rs618168, Rs9337053, Rs9939973, Rs9940128, Rs558902, RsIO852521, Rs4777196, Rsll21980, Rs7193144, Rs6945088, Rs8043757, Rs3751812, Rs9923233, Rs9926289, Rs2597786, Rs7185735, Rs9931164, Rs9941349, Rs7199182, Rs9831494, Rs7817964, Rs7190492, Rs9930506, Rs9932754, Rs9922609, Rs7204609, Rs8044769, Rs2149832, Rs6499646, Rs421090, Rs2302673); TNAflpha (Rs799764, Rs800629, Rs361525, Rs800610, Rs3093662); MANEA (Rs833503); LeptinOb (Rs4728096, Rs2536535, Rs2167270, Rs2278815, Rs0244329, Rsll763517, Rs760956, RsIO954173); PEMT (Rs4244593, Rs936108); MAO-A (Rs3788662, Rs465108, Rs909525, Rs2283724, Rs2843268, Rs800659, Rs6323, Rs799835, Rs3027400, Rs979606, Rs979605 Rsll70707); CRH (Rs7209436, Rs4792887, Rsll0402, Rs242924, Rs242941, Rs242940, Rs242939, Rs242938, Rs73365, Rs876831, Rs876828, Rs397, Rs878868 Rs242948); ADIPOQ (Rs7300539, Rs2417666); STS (Rs2861247); VDR (Rs7467825, Rs731236, Rs544410, Rs229828, Rs228570, Rs2283816); DBI (Rs3091405, Rs3769664, Rs3769662, Rs956309, Rs8192506); GABRA6 (Rs8311995, Rs3219151, Rs6883829, Rs3811991); GABRB3 (Rs2912582, Rs2081648, Rs426217, Rs754185, Rs890317, Rs981778, Rs2059574); MTHFR(Rs4860488, Rs801131, Rs801133, Rs2066470); MLXIPL[ carbohydrate binding element] (Rs3812316, Rs7145738); VEGF (Rs2010963, Rs833068, Rs3025000, Rs3025010, Rs3025039, Rs3025053); DRD4 (Rs936460, Rs41298422, Rs3758653, Rs936461, Rs2720373, Rs747302, Rs800955, Rs916455, Rs916457, Rs7 124601); CLOCK (Rs801260, Rs934945, Rs3033501); Melatonin(all
polymorphisms); Orexin (all polymorphisms), PENK (RS16920581, RS1437277, RS1975285, RS260998, RS2609997) and CBl (RS1049353).

52. The kit of claim 13, wherein a pain ointment is applied and each formulation consists of a base ointment cream containing a solubilizer.

53. The kit of claim 52, wherein said solubilizer is selected from Soya-lecthin aggregates, Micronized, Cyclic monoterpenes, Cyclohexanone derivatives, isosorbide dinitrate and Lipoderm.

54. The kit of claim 13, wherein a pain ointment consists of D-phenylalanine, LID, GBP, KET, KEPF (10/5/10/10/10); D-Phenylalanine, GBP, KET, BAC (10/10/10/4%); D-Phenylalanine GBP, KET, LID (10/6/10/10); D-Phenylalanine, GBP, KET, AM, BAC(10/6/6/4/4%); D-Phenylalanine, KEPF(10/10 %); D-Phenylalanine, KEPF (10/20%); D-Phenylalanine, KEPF, LID (10/10/5%); D-Phenylalanine, KEPF, CLB(10/20/2%); D-Phenylalanine, KEPF, LID, CLB(10/20/5/2%); D-Phenylalanine, IBUF, KEPF, CLB (10/10/10/1%); D-Phenylalanine, LID (10/10/10/1%); D-Phenylalanine, D-Phenylalanine, DICLO(10/10%); D-phenylalanine, CAP, MT, CAMP(10/0.0375%); D-phenylalanine, CAP, MT, CAMP(10/0.0375%); D-phenylalanine, KEPF, KET, CAP (10/10/6/0.075%).

55. The kit of claim 13, wherein a pain ointment consists of L-phenylalanine, LID, GBP, KET, KEPF (10/5/10/10/10); L-Phenylalanine, GBP, KET, BAC (10/10/10/4%); L-Phenylalanine GBP, KET, LID (10/6/10/10); L-Phenylalanine, GBP, KET, AM, BAC(10/6/6/4/4%); L-Phenylalanine, KEPF(10/10 %); L-Phenylalanine, KEPF (10/20%); L-Phenylalanine, KEPF, LID (10/10/5%); L-Phenylalanine, KEPF, CLB(10/20/2%); L-Phenylalanine, KEPF, LID, CLB(10/20/5/2%); L-Phenylalanine, IBUF, KEPF, CLB (10/10/10/1%); L-Phenylalanine, LID (10/10/10/1%); L-Phenylalanine, DICLO(10/10%); L-phenylalanine, CAP, MT, CAMP(10/0.0375%); L-phenylalanine, KEPF, KET, CAP (10/10/6/0.075%).

56. The kit of claim 13, wherein a pain ointment consists of L-Glutamine, LID, GBP, KET, KEPF (10/5/10/10/10); L-Glutamine, GBP, KET, BAC (10/10/10/4%); L-Glutamine, GBP, KET, LID (10/6/10/10); L-Glutamine, GBP, KET, AM, BAC(10/6/6/4/4%); L-Glutamine, KEPF(10/10 %); L-Glutamine, KEPF (10/20%); L-Glutamine, KEPF, LID (10/10/5%); L-Glutamine, KEPF, CLB(10/20/2%); L-Glutamine, KEPF, LID, CLB(10/20/5/2%); L-Glutamine, IBUF, KEPF, CLB (10/10/10/1%); L-Glutamine, LID (10/10/10/1%); L-Glutamine, DICLO(10/10%); L-Glutamine, CAP, MT, CAMP(10/0.0375%); L-Glutamine, KEPF, KET, CAP (10/10/6/0.075%).

57. The kit of claim 13, wherein a pain ointment consists of 5-HTP, LID, GBP, KET, KEPF (10/5/10/10/10); 5-HTP, GBP, KET, BAC (10/10/10/4%); 5-HTP GBP, KET, LID (10/6/10/10/10); 5-HTP, GBP, KET, AM, BAC(10/6/6/4/4%); 5-HTP, KEPF(10/10 %); 5-HTP, KEPF (10/20%); 5-HTP, KEPF, LID (10/10/5%); 5-HTP, KEPF, CLB(10/20/2%); 5-HTP, KEPF, LID, CLB(10/20/5/2%); 5-HTP, IBUF, KEPF, CLB (10/10/10/1%); 5-HTP, LID (10/10/10/1%); 5-HTP, DICLO(10/10%); 5-HTP, CAP, MT, CAMP(10/0.0375%); 5-HTP, CAP, MT, CAMP(10/0.0375%); 5-HTP, KET, CAP (10/10/6/0.075%).

58. The kit of claim 13, wherein a pain ointment consists of Rhodiola rosea, LID, GBP, KET, KEPF (10/5/10/10/10); Rhodiola rosea, GBP, KET, BAC (10/10/10/4%); Rhodiola rosea GBP, KET, LID
59. The kit of claim 13, wherein a pain ointment consists of Chromium salt, LID, GBP, KET, KEFP (0.01/5/10/10/10%); Chromium salt, GBP, KET, BAC (0.01/10/10/4/4%); Chromium salt, GBP, KET, LID (0.01/6/10/10%); Chromium salt, GBP, KET, AM, BAC (0.01/6/6/4/4%); Chromium salt, KEFP (0.01/10%); Chromium salt, KEFP, LID, BAC (0.01/20/20/2%); Pyridoxal phosphate, LID, BAC (0.05/6/6/4/4%); Pyridoxal phosphate, GBP, KET, AM, BAC (0.05/6/6/4/4%); Pyridoxal phosphate, GBP, KET, LID (0.05/10/10/10%); Pyridoxal phosphate, KET, AM, BAC (0.05/6/6/4/4%); Pyridoxal phosphate, KET, LID (0.05/10/10/10%); Pyridoxal phosphate, KET, LID (0.05/10/10/5%); Pyridoxal phosphate, KET, CLB (0.05/20/20/2%); Pyridoxal phosphate, KEFP, CLB (0.01/20/5/2%); Pyridoxal phosphate, IBUF, KEFP, CLB (0.01/10/10/1%); Rhodiola rosea, LID (0.01/10%); Rhodiola rosea, LID (0.01/5/10/10%); Rhodiola rosea, LID, DICLO (0.05/10%); Rhodiola rosea, CAP, MT, CAMP (0.05/0.0375%); Rhodiola rosea, CAP, MT, CAMP (0.05/0.0375%); Chromium salt, CAP, MT, CAMP (0.05/10%); Chromium salt, CAP, MT, CAMP (0.05/10%); Chromium salt, CAP, MT, CAMP (0.05/0.0375%); Chromate, KEFP, KET, CAP (0.01/10/6/0.075%).

60. The kit of claim 13, wherein a pain ointment consists of Pyridoxal phosphate, LID, GBP, KET, KEFP (0.05/5/10/10/10%); Pyridoxal phosphate, GBP, KET, BAC (0.05/10/10/4/4%); Pyridoxal phosphate, GBP, KET, LID (0.01/6/10/10%); Pyridoxal phosphate, GBP, KET, AM, BAC (0.05/6/6/4/4%); Pyridoxal phosphate, GBP, KET, LID (0.05/10/10/10%); Pyridoxal phosphate, GBP, KET, LID (0.05/10/5/10%); Pyridoxal phosphate, KET, CLB (0.05/20/20/2%); Pyridoxal phosphate, KEFP, CLB (0.01/20/5/2%); Pyridoxal phosphate, IBUF, KEFP, CLB (0.01/10/10/1%); Rhodiola rosea, LID (0.01/10%); Rhodiola rosea, LID (0.01/5/10/10%); L-Tyrosine, GBP, KET, BAC (0.05/10/10/4/4%); L-Tyrosine, GBP, KET, LID (0.01/6/10/10%); L-Tyrosine, GBP, KET, LID (0.05/10/10/10%); L-Tyrosine, GBP, KET, LID (0.05/10/5/10%); L-Tyrosine, KEFP, LID (0.01/6/10/10%); L-Tyrosine, KEFP, LID (0.05/10/10/5%); L-Tyrosine, KEFP, CLB (0.01/20/5/2%); L-Tyrosine, KEFP, CLB (0.05/20/20/2%); L-Tyrosine, IBUF, KEFP, CLB (0.01/10/10/1%); L-Tyrosine, LID (0.01/10%); L-Tyrosine, DICLO (0.05/10%); L-Tyrosine, CAP, MT, CAMP (0.05/0.0375%); L-Tyrosine, CAP, MT, CAMP (0.05/0.0375%); L-Tyrosine, CAP, MT, CAMP (0.05/0.0375%); L-Tyrosine, CAP, MT, CAMP (0.05/0.0375%); L-Tyrosine, CAP, MT, CAMP (0.05/0.0375%); L-Tyrosine, KEFP, KET, CAP (0.05/10/6/0.075%).

61. The kit of claim 13, wherein a pain ointment consists of L-Tyrosine, LID, GBP, KET, KEFP (10/5/10/10/10%); L-Tyrosine, LID, GBP, KET, BAC (10/5/10/10/4/4%); L-Tyrosine, GBP, KET, LID (10/5/10/10/10%); L-Tyrosine, GBP, KET, BAC (10/6/6/4/4%); L-Tyrosine, GBP, KET, LID (10/6/10/10%); L-Tyrosine, GBP, KET, AM, BAC (10/6/6/4/4%); L-Tyrosine, GBP, KET, LID (10/10/5/10%); L-Tyrosine, KEFP, LID (10/10/5/10%); L-Tyrosine, KEFP, CLB (10/20/20/2%); L-Tyrosine, KEFP, UD, CLB (10/20/5/2%); L-Tyrosine, IBUF, KEFP, CLB (10/10/10/1%); L-Tyrosine, LID (10/10%); L-Tyrosine, DICLO (10/10%); L-Tyrosine, CAP, MT, CAMP (0.0375%); L-Tyrosine, CAP, MT, CAMP (10/05%); L-Tyrosine, KEFP, KET, CAP (10/10/8/0.075%).

62. The kit of claim 13, wherein a pain ointment consists of Synaptamine, LID, GBP, KET, KEFP (10/5/10/10/10%); Synaptamine, GBP, KET, BAC (10/5/10/10/4/4%); Synaptamine, GBP, KET, LID (10/6/10/10%); Synaptamine, GBP, KET, AM, BAC (10/6/6/4/4%); Synaptamine, KEFP (10/10%); Synaptamine, KEFP (10/20%); Synaptamine, KEFP, LID (10/10/5/10%); Synaptamine, KEFP, CLB (10/20/20/2%); Synaptamine, KEFP, LID, CLB (10/20/5/2%); Synaptamine, IBUF, KEFP, CLB (10/10/10/1%); Synaptamine, LID (10/10%); Synaptamine, DICLO (10/10%); Synaptamine, CAP, MT, CAMP (0.0375%); Synaptamine, CAP, MT, CAMP (10/05%); Synaptamine, KEFP, KET, CAP (10/10/6/0.075%).
63. The kit of claim 13, wherein a pain ointment consists of Kyotorphin, Synaptamine, LID, GBP, KET, KEPF (10/5/10/10/10%); Kyotorphin, Synaptamine, GBP, KET, BAC (10/10/10/4%); Kyotorphin, Synaptamine, GBP, KET, Lid (10/6/10/10%); Synaptamine, GBP, KET, AM, BAC(10/6/6/4/4%); Kyotorphin, Synaptamine, KEPF (10/10 %); Kyotorphin, Synaptamine, KEPF (10/10/20%); Kyotorphin, Synaptamine, KEPF, Lid (10/10/5%); Kyotorphin, Synaptamine, KEPF, CLB (10/10/5/5%); Kyotorphin, Synaptamine, IBUF, KEPF, CLB (10/10/10/1%); Kyotorphin, Synaptamine, Lid (10/10%); Kyotorphin Synaptamine DICLO (10/10%), Kyotorphin, Synaptamine, CAP, MT, CAMP (10/05%); Kyotorphin Synaptamine, CAP, MT, CAMP (10/05%); Kyotorphin, Synaptamine KEPF, KET, CAP (10/10/5/0.075%).

64. The kit of claim 13, wherein a pain ointment consists of Kyotorphin, LID, GBP, KET, KEPF (10/5/10/10%). Kyotorphin, GBP, KET, BAC (10/10/10/4%); Kyotorphin, GBP, KET, Lid (10/6/10/10%); Kyotorphin, GBP, KET, AM, BAC(10/6/6/4/4%); Kyotorphin, KEPF (10/10 %); Kyotorphin, KEPF (10/10/20%); Kyotorphin, KEPF, Lid (10/10/3%); Kyotorphin, KEPF, CLB (10/10/2%); Kyotorphin, KEPF, Lid, CLB (10/10/5/2%); Kyotorphin IBUF, KEPF, CLB (10/10/1%); Kyotorphin, Lid (10/10%); Kyotorphin DICLO (10/10%); Kyotorphin, CAP, MT, CAMP (10/0375%); Kyotorphin CAP, MT, CAMP (10/05%); Kyotorphin, KEPF, KET, CAP (10/10/6/0.075%).

65. The kit of claim 13 is used to improve the likelihood of reducing "dopamine resistance" and thereby improving "dopamine sensitivity" by addressing at least five pathways such as "pleasure", "stress," "energy and metabolic," "neuroendocrine" and "immunological".
[000130] DRAWING 1: The Brain Reward Cascade

Figure 1: The Brain Reward Cascade. Structures deep within the limbic system play a crucial role in the expression of emotions and the activity of the reward system of the brain. The experience of pleasure and the modulation of reward is based on a reward "cascade," a chain of events within the limbic system that interact through various signaling molecules, or neurotransmitters. The authors propose that a biochemical deficiency in one or more of these neuronal signaling molecules can explain an individual's feeling of well-being or sadness, anger or a craving for a substance that can alleviate the negative emotions.

FIGURE 1 OF 13
DRAWING 2 – Reward Deficiency Syndrome and the DRD2 Dopamine Receptor

Figure 2, Reward Deficiency Syndrome. The Reward Deficiency Syndrome comprises a spectrum of obsessive, impulsive, addictive, and personality disorders that are based on a common genetic deficiency in the Dopamine D2 receptor, according to the authors. The type of disorder that is manifested by any particular individual is determined by other genetic and environmental factors, which are not yet fully understood. A predictive model based on these factors suggests that an individual who carries the A1 allele for the dopamine D2 receptor has a 14% chance of developing one of the disorders of the reward deficiency syndromes (including a risk for substance use disorder).

FIGURE 2 OF 13
The Deep Structures Residing in the Mesolimbic System of the Brain Dissection of the Brain Showing Anatomical Sites Crucial to the Reward Circuit

FIGURE 3A OF 13

[0000] DRAWING 3B – Neurotransmitter Relations in the Reward Cascade


FIGURE 3B OF 13
Drawing 5 Brain reward chemistry is a critical treatment target.

- When a signal comes down the neuron, dopamine is released into the synapse. It then crosses to the second neuron where it binds to and stimulates dopamine receptors. It then crosses back to the first neuron where it is picked back up by dopamine transporters (reuptake molecules) for re-use.

FIGURE 5 OF 13
Drawing 6. Natural "Rewards"

FIGURE 6 OF 13

Drawing 7- DRD2 Receptor Levels in Addiction

FIGURE 7 OF 13
Drawing 8. Represents the significant differences (P < 0.045; P < 0.049) between the DRD2 A1⁻ (n = 9) and the DRD2 A1⁺ (n = 14) with regard to days on Genotrim® treatment in Dutch descent self-identified obese subjects in the D.I.E.T. pilot study. A1⁻ = (A1/A1/A1/A2) and A1⁺ = A2/A2.

FIGURE 8 OF 13

Drawing 9. Meso-limbic system

FIGURE 9 OF 13
Figure 10: Brain Transmitter Adequacy

- Transmitter/enkephalin synthesis
- Transmitter degradation enzymes
- Neuron
- Transmitter/enkephalin storage site
- Transmitter/enkephalin receptor site

**Drawing 10 Neutransmitter Adequacy**

**FIGURE 10 OF 13**
Brain Transmitter Deficiency

transmitter/enkephalin synthesis
transmitter degradation enzymes
neuron
transmitter/enkephalin storage site
transmitter/enkephalin receptor site

CRAVING AND DISTRESS

Drawing 11; Neutotransmitter Deficiency

FIGURE 11 OF 13
Drawing 12. GnAP Program outline

FIGURE 12 OF 13
Drawing 13  Hypothetical Common Molecular Network for Drug Addiction

FIGURE 13 OF 13
**INTERNATIONAL SEARCH REPORT**

**INTERNATIONAL application No.**
PCT/US 09/48074

**CLASSIFICATION OF SUBJECT MATTER**

- IPC(8)- A61K 38/00; 38/02; 31/00 (2009.01)
- USPC - 426/648; 656; 72; 74; 425/75

According to International Patent Classification (IPC) or to both national classification and IPC

**FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

USPC - 426/648; 656; 72; 74; 425/75

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC-426/648; 656; 72; 74; 424/75; 424/728; 730 (text search-see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST (USPT, PGPB, EPAB, JPAB), Google Patents/Google Scholar

Search Terms Used: dopamine receptor polymorphism, reward deficiency, pain, herbal, vitamin, mineral, opiate, gaba, tryptophan, rs627

**DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 2006/0062859 A1 (Blum et al.) 23 March 2006 (23.03.2006) para [0040], [0044], [0057], [0059], [0116], [0129], [0223], [0420], [0421], [0448], [0451], [0501], [0504], [0517], [0533], [0539], [0538], [0542], [0555], [0556], [0577], [0601], [0606], [0684], [0701], [0724], Table 5 and 11, claim 6</td>
<td>1-12</td>
</tr>
<tr>
<td>Y</td>
<td>US 2004/0254363 A1 (Bergen et al.) 16 December 2004 (16.12.2004) para [0020], [0024], [0241], Table 4</td>
<td>13, 15, 22</td>
</tr>
<tr>
<td>Y</td>
<td>US 2004/0101582 A1 (Wolicki) 27 May 2004 (27.05.2004) para [0017], [0020], [0077]</td>
<td>51</td>
</tr>
</tbody>
</table>

I J Further documents are listed in the continuation of Box C.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;T&quot;</td>
<td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td>
</tr>
<tr>
<td>&quot;X&quot;</td>
<td>document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td>
</tr>
<tr>
<td>&quot;Y&quot;</td>
<td>document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td>
</tr>
<tr>
<td>&quot;&amp;&quot;</td>
<td>document member of the same patent family</td>
</tr>
</tbody>
</table>

Date of the actual completion of the international search

02 October 2009 (02.10.2009)

Date of mailing of the international search report

22 KT 2009

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No 571-273-3201

Authorized officer

Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/2 10 (second sheet) (July 2009)