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The impact of biomics technology and DNA directed anti-obesity targeting of the brain reward circuitry

Review Article

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Abbreviations: Basal Metabolic Rate, (BMR); Body Mass Index, (BMI); Cardiovascular Disease, (CVD); Catechol-o-methyl-transferase, (COMT); chromium picolinate, (CRP); D_4 dopamine receptor gene, (D4DR); dopamine D_2 receptor gene, (DRD2); dopamine, (DA); Dutch Investigation to Evaluate Treatments, (D.I.E.T.); E2 p45 -related factor 2, (Nrf2); Garcinia cambogia extract, (GcE); Gas Chromatography/Mass Spectrometry Method, (GC/MS); hypothalamus arcuate, (ARC); Non-insulin Dependent Diabetes Mellitus, (NIDDM); norepinephrine, (NE); Obsessive Compulsive Disorder, (OCD); paraventricular, (PVN); Reward Deficiency Syndrome, (RDS); Substance Use Disorder, (SUD); tropomyosin-related kinase B, (TrkB)

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Summary

Obesity-related medical conditions are the second leading cause of death in the U.S. Classified as a chronic disease in 1985, the understanding of obesity and its causes and effects has been further elucidated through additional research into the evolution of genetic and biologic influencing factors of this deadly disease. What used to be understood as primarily a behavioral problem of overeating and under-exercising, has only contributed to continued increases in the rates of obesity despite increases in dieting and exercise. Successful strategies to effectively induce sustainable fat loss and manage obesity have been elusive. For the most part, the tactics employed

have not been multi-faceted, multi-system approaches, but have been characterized by one-dimensional metabolic approaches targeted at achieving weight loss as measured by linear criteria (i.e. scale weight, Body Mass Index (BMI), percent body fat, etc). Recent evidence indicates a much more complex and multidimensional syndrome, characterized by the simultaneous breakdown of many facets of metabolism exacerbated or limited by the predispositions of inherited genetic traits. There is significant evidence to substantiate the existence of Reward Deficiency Syndrome (RDS) as a new paradigm shift in the understanding of Obesity. Specifically, there are genetic links to the role of catecholaminergic pathways in aberrant substance seeking behavior, in particular cravings for carbohydrates. These neurological factors, regulated by genetic predisposition, are a subtype of RDS that we classify as Neurobesigenics. In the treatment of this neurogenetic mechanism, there are leading prescription pharmaceuticals as well as nutritional therapies. There is growing evidence to support the augmentation of precursor amino acid therapy and enkephalinase and Catechol-o-methyl-transferase (COMT) inhibition leading to enhanced levels of neurotransmitters: serotonin, enkephalins, GABA and dopamine/norepinephrine. Utilization of combining substances/nutrients directed at replenishing the nutrigenomic needs of multiple pathways includes brain reward targets mechanistically mimicking the brain reward cascade. The effects of a novel nutraceutical supplement contained in a DNA-customized nutraceutical denoted as LG839, including Synaptamine, Super CitriMax and Passion flower, have been evaluated in an open label trial. Based on a number of case reports, it appears that this unique combination of ingredients may have a generalized anti-stress and anti-craving effect resulting in an inhibition of carbohydrate appetite, inducing energy increase, positive focus, improved performance, a sense of well-being, fat loss and enhanced body image/composition. Published research from our laboratory on an earlier complex denoted as SG8839 provides a classical example of a Structural Equation Modeling Methodology (SEM), a statistical regression model that is used to evaluate the effectual relationships between various systems in a multi-system matrix to measure the contributory roles of those PATHs, enabling targeted and effective nutraceutical interventions. We review the potential mechanisms and rationale for the utilization of this multifaceted approach to attenuate the pleiotropic defaults in obesity stemming from the evolution of the thrifty gene concept to modern times. This is the first time the components of this formula have been combined at the clinically tested dosage levels indicated, to promote successful and sustainable results in improved body recomposition. In summary, the impact of biomics technology and the DNA directed nutraceutical (LG839™) targeting of the brain reward circuitry may provide a customized approach to prevent and treat high risk individuals who are carriers of a genetic predisposition to obesity. We are cognizant that the importance of this research is not focused on any particular product but to encourage the scientific community to incorporate pharmacogenetic/nutrigenetic testing prior to administration of any anti-obesity agent. Thus, in this review we present the necessity of exploiting systems biology and "omics" and have coined the term "Neurobesigenics".

I. Introduction

An Evolutionary Genomic Trieste

Obesity used to be understood in fairly elementary behavioral terms: eating too much and exercising too little results in excess body weight, due in large part to a lack of willpower or self-restraint. But as people have increased their dieting and exercise, the rates of obesity continue to rise as the combined prevalence of overweight and obese persons in the US have increased from 46% of the adult population (NHANES II, 1976 to 1980) to over 60% of the adult population in NHANES III (1988-1994). In 1985, obesity was recognized as a chronic medical disease with serious health implications caused by a complex set of factors. Obesity-related medical conditions contribute to 300,000 deaths each year, second only to smoking as a cause of preventable death (Hill, 2006). Obesity has been established as a major risk factor for hypertension, cardiovascular disease, Type 2 diabetes, and some cancers in both men and women. Obesity affects 58 million people across the nation and its prevalence is increasing (source: U.S. Census Bureau). Approximately one-third of American adults are estimated to be obese, and 60% are overweight.

In response to this rising epidemic, the medical, food and fitness communities have consistently told Americans to just make behavioral modifications, such as diet and exercise. As scientific advancements have demonstrated in other neurological healthcare conditions such as alcoholism, there are important biological and genetic components that limit the efficacy of behavioral adjustments alone.

Eighteen years ago, Blum and colleagues published in 1990 landmark research suggesting that another prevalent healthcare condition which had been traditionally characterized in behavioral terms like obesity, namely alcoholism, also had a hereditary or genetic component and that genetic information could explain why such a condition could be found to "run in the family". Blum's research (Blum et al, 2007) continued to explain how knowing this important genetic information could then caution certain genotypes to adjust their dietary intake and environments to overcome this genetic predisposition. In a recent study of 11,000 Americans, results suggested that more than 75% of obese Americans (n=3,100) say they have healthy eating habits. According to this survey, 40 percent of obese people also said they do "vigorous" exercise at least three times per week. In this survey by Thomson Medstat, a Michigan-based healthcare research firm, obese people reported similar behaviors in snacking, reading nutritional labels, and eating out when compared to normal weight people. Weight loss alone is difficult, but sustainable weight loss is exceedingly difficult. Most people regain as much as two-thirds of

weight lost within one year and regain all of it within five years (Stobbe, 2006).

To better understand obesity, one must recognize that it is the result of interdependent metabolic dysfunctions that are also influenced by various genetic mutations. This interdependence of neurological genetic factors relates to a condition which we classified as Reward Deficiency Syndrome (RDS) (Blum et al, 1996a). Further work from our laboratory and others have embraced this classification (Blum et al, 1995, 1996b, 2006; Goldman et al, 1997; Simonin et al, 1998; Comings and Blum, 2000; Ponce et al, 2003; Werme et al, 2003; Cheng et al, 2004; Linazaroso et al, 2004; Salmon et al, 2004; Bowirrat and Oscar-Berman 2005; Brandacher et al, 2006; Kim et al, 2006; Manzardo and Penick, 2006) while others have argued its validity (Goldman et al, 1998). This syndrome is more accurately defined as: "A multifactorial condition in which, among other things, stress, environmental influences and poor lifestyle activities can affect and/or exacerbate in-born genetic predispositions that can lead to imbalances and deficiencies retarding the brain's competent management of the multitude of functions necessary to maintain optimal neurometabolic efficiency" (Comings and Blum, 2000). This impaired or suboptimal metabolic state contributes to neurochemical imbalances that manifest through such conditions as addictive behaviors, Substance Use Disorder (SUD), a lack of well-being, increased cravings, depression, inability to cope with stress, Obsessive Compulsive Disorder (OCD), sleep disorders and ADHD among others, including a subtype of schizophrenia. Metabolic abnormalities, such as Metabolic Syndrome, also result. The systemic RDS dysfunctions induce metabolic "survival" behaviors characterized by such things as lowered resting (or basal) metabolic rate, increased fat storage, retarded fat metabolism, fatigue, inflammation, water retention, reduced muscle health (up to muscle atrophy in advanced stages), decreased bone density, distressed organ function, and diminished homeostatic capacity.

In this review, we will describe the genetic factors influencing obesity, specifically those related to neurological factors influencing eating behavior and metabolism. We propose the term Neurobesigenics to classify the sub-segment of Reward Deficiency Syndrome that governs the genetic implications of various neurological factors contributing to the obesity epidemic. It is important to realize that carriers of the DRD2 A1 allele have behaviors similar to the early Homo *sapiens* as aggressive behaviors, fearless, high risk takers, hunter gatherers, sexual predators, high cravings, and basically not very noble. In fact these behaviors fit well with the dawning of the Age of Genetics, Charles Darwin's view of survival of the fittest and what he has termed the "*Sympathin gene*". It is quite possible the DRD2 A1 allele is the older and more common allele in the 10,000 years B.C. However, even Darwin conceded near the end of his life (Darwin F. 1888), that his evolutionary theory had shortchanged the role of the environment. In an 1876 letter, to Moritz Wagner he wrote: *"In my opinion, the greatest error which I have committed has been not allowing sufficient weight to the direct action of the environment, i.e. food, climate, etc., independently of natural selection"*.

It is conceivable that as mankind became civilized throughout the explosion of the agricultural era, the DRD2 A2 allele evolved and has now become the major rather than the minor allele. There are some that believe that we are now facing a situation whereby the DRD2 A1 allele is on the rise again as suggested by David E. Comings in his book the "Gene Bomb" (Hope Press. 2000). If this turns out to be true, we could expect that mankind may revert back to cave-like behaviors. Thus the importance of this review is to provide a scientific exploration of at least one phenomena, RDS, which may have its beginnings thousands of years ago, having a negative impact on current lives and human interaction. This genomic notion in America alone may affect as much as 100 million people since the percent prevalence of the DRD2 A1 allele is over 30 %. This is also true for another dopaminergic control gene, Catechol-o-methyl-transferase (COMT) (one third of Americans carry a polymorphism that associates with addictive behaviors) (Boettiger et al, 2007). With this in mind it may be the etiological cause in-part for aberrant carbohydrate craving behavior leading to our pandemic obesity problem. It is well known that 90% of individuals that are obese have severe carbohydrate craving behaviors. Being cognizant of these genetic concepts leads us to an attempt at understanding the relationship of genes and environmental interactions and systems biology.

II. Exploiting "systems biology" in nutritional approaches to obesity

The human genome is estimated to encode over 30,000 genes, and to be responsible for generating over 100,000 functional proteins. It is our contention that unraveling; the multitude of nutrigenomic (nutritional genomics), transcriptomic (gene expression), epigenomic (organization of the chromatin) proteomics (post-translational modifications) and metabolomic (metabolic profiles) patterns that arise from nutrient-induced bioactive cellular mechanisms is likely to provide insights into a tailored approach to adhere to a diet and induce health. With regard to eating behaviors through the principles of nutrigenomics, scientists could ultimately identify molecular targets for nutritional preemption. Understanding the nature of systems biology will hold the key to a personalized approach to nutrition (Trujillo et al, 2005). The concept of nutrigenomics builds on the premises that:

- Diet and dietary components can alter the risk of disease developments modulating multiple processes involved with onset, incidence, progression, and/or severity;
- Nutrients can act on the human genome, either directly or indirectly, to alter the expression of genes and gene products;
- Diet could compensate for or accentuate effects of genetic polymorphisms; and
- The consequences of a diet are dependent on the balance of health and disease states and on an individual's genetic background.

Finally, the success of nutritional preemption approaches will depend on the ability to identify and validate nutrigenetic, nutritional epigenetic, proteomic and metabolomic biomarkers to determine cause, effect, and predisposition/susceptibility to disease. The systems biology field is of great interest but has been rather slow in progress. However, it must be pursued rigorously if we are to achieve evidence-based nutritional intervention strategies (van Omen et al, 2002, 2004; Corthesy-Theulaz et al, 2005; Spielbauer and Stahl 2005; Afman and Muller 2006; Trujillo et al, 2006).

We believe that evidence is mounting whereby gene-nutrient interactions become important targets for a novel, personalized approach to weight management and related disease. A number of examples highlight this conceptual framework:

1. a polymorphism in the angiotensin gene may determine how an individual's blood pressure responds to dietary fiber (Hegle et al, 1997).

2. a polymorphism at codon 198 of human glutathione peroxidase results in a substitution of leucine for proline, and has been associated with increased risk of lung cancer. This may be related to the amount of selenium needed to optimize enzyme activity (Hu and Diamond, 2004).

3. A study investigating the role of caffeine as a risk factor for bone loss in elderly women found that those with a variant of the vitamin D receptor (tt genotype) and who had caffeine intakes greater than 300 mg/day had significantly higher rates of bone loss than did women with TT genotype (Rapuri et al, 2001).

4. In individuals with a specific polymorphism in the PPARγ (Pro12Ala), a low-polyunsaturated-to-saturated fat ratio is associated with an increase in body mass index and fasting insulin concentrations (Luan et al, 2001).

5. DNA methylation is dependent on bioactive food components ranging from alcohol to zinc. Supplementation of choline, folic acid, vitamin B-12, methionine, and zinc to the maternal diet led to an increase in the level of DNA methylation in the agouti gene and change in the color pattern of the hair coat. This phenotypic change has recently been shown to coincide with a lower susceptibility to obesity, diabetes, and cancer (Cooney et al, 2002).

6. In term of how food components regulate genes, in addition to an inability to up-regulate glutathione s-transferase, nicotinamide adenine dinucleotide phosphate, (NADPH), quinine reductase, γ-glutamylcysteine synthetase, and epoxide hydrolase, a block in E2 p45-related factor 2 (Nrf2) in mice is also involved with regulation of xenobiotic metabolizing enzymes, anti-oxidants, and biosynthetic enzymes of the glutathione and glucoronidation conjugation pathways. Similar studies with PPARα-null mice have shown its role in regulating various sites of lipid metabolism (Pan et al, 2000).

7. By using RNA interference, investigators systematically disrupted expression of all genes in the worm model system *Coenorhabditis elegans* to determine which gene inactivation decreased body fat and increased fat storage. This allowed for the identification of a core set of fat regulatory genes and pathway-specific fat regulators (Ashrafi et al, 2003).

In the interest of brevity for this review, we will focus on certain neurobiological aspects related to the problem of obesity rather than a review on the confirmation of genetic factors or physio-pathological features at neurological and peripheral target sites (Blum et al, 1996a).

III. Evolutionary basis of neurobesigenics

The human ability to gain weight today has been influenced by the course of human history. At the risk of sounding colloquial, we wish to review the historical context and implications of early human eating behavior on our modern day weight challenges. Whereas today the food supply is plentiful in the Industrial World, in the time of prehistoric human civilization, the "hunter gatherers" did not have a plentiful food supply (Groop and Tuomi, 1997). For example, when berries and roots were in season and when wild animals were not hibernating, prehistoric humans ate well and "they fattened up". However, when these foods were not available, they relied on the stored fat to see them through the lean times.

To better understand the importance of weight gain, two biological functions assisted prehistoric humans in their struggle to survive this perpetual cycle of "feast" and "famine". Some scientists suggest that an abundant supply of food induced efficient fat storage in early humans. When there was less fat, their metabolism slowed to adjust to the smaller quantities programmed to adapt their metabolic rates to food intake (Dulloo and Jacquet, 1999). Thus, survivability resulted for those humans with "fat storage genes" while those who lacked these genes perished. This suggests that the survivors passed their "fat storage" genes on to future generations, or what has been called "thrifty genes". With today's advent of conglomerated agribusiness, mass food processing and distribution, this genetic evolution may guide our bodies to increase fat stores as a result of the consumption of high-calorie nutrient-deficient foods, like concentrated sugar, processed carbohydrates, and adulterated fats that contribute to insulin resistant-based conditions (a.k.a. Metabolic Syndrome) like Non-insulin Dependent Diabetes Mellitus (NIDDM), Cardiovascular Disease (CVD), hypertension and obesity.

This inherited genetic predisposition presents a challenge to the Industrialized World where humans live in a perpetual feast with food available in their homes, grocery stores, and at restaurants on every corner. Unfortunately, many of the foods humans now consume are deficient in essential nutrients. By being overfed yet undernourished, humans generally respond as they did prehistorically, storing calories and fat during the perpetual "feast" season, waiting for the "famine" season that never appears. The exception to this response is when humans participate in "low-fat diets". Like prehistoric humans, today's human brain and body respond to this reduction in fat by wanting to eat more, and reacts by quickly regaining the lost weight in preparation for the next food shortage, just as it did in ancient times.

In this regard it has been suggested that the modern lifestyle in the Industrialized World appears to provide the social and environmental conditions that favor maximum expression of underlying individual genetic differences in susceptibility to becoming overweight.

This is an important view because we now know that in today's society, with its highly processed foods, chemicals, and pollution, with regard to metabolic effects, the body's instinct is to prepare for and defend against famine, but there is even a more important facet to the genetic propensity to gain excess weight and it does not reside in genes which control fat storage, and/or resting metabolic rates. Instead it is in the genes that control, for the most part, human cravings. These genes are termed "reward genes".

In this regard, we must be reminded of some earlier work involving the concept of a "thrift genotype" which was originally discussed in reference to Diabetes Mellitus in 1962 by Neel. This earlier work has been discussed most recently by Prentice and colleagues in 2005. These newer authors suggest that the pandemic of obesity is caused by a profound mismatch between humanity's present environmental circumstances and those that have molded evolutionary selection. This concept was first articulated when gestational diabetes was described as being the result of a "thrifty genotype rendered detrimental by progress". More recently, this hypothesis has been extended to the concept of a "thrifty phenotype" to describe the metabolic adaptations adopted as a survival strategy. To date thrifty genes remain little more than a nebulous concept propagated by the intuitive logic that the human has been selected to survive episodic famine and seasonal hungry periods. In this regard, the search for candidate "thrifty genes" needs to cover every aspect of energy balance and food seeking behavior and even oxidative metabolism. In the paper by Prentice and colleagues in 2005, a number of candidate genes have been proposed and include: maternally-transmitted mitochondrial genes; the uncoupling proteins; apoE4, whose geographical distribution has been linked to a possible thrifty role in lipoprotein and cholesterol metabolism.

Furthermore, prolonged practice of unhealthier tactics can lead to malnutrition, disrupt the body's homeostatic regulatory capabilities and in more extreme cases lead to such disorders as anorexia and bulimia. Many of these tactics are used individually or conjunctively to achieve rapid "weight loss" results. As previously stated, the primary goal of these tactics is "weight loss" and/or image enhancement. These objectives are often pursued without considering the potential impact on health, the body's natural genetically mandated and homeostatic response to such tactics, or the fact that depriving the body of resources essential to maintain health is counterproductive. Essentially, such tactics artificially mimic (and eventually induce) a state of famine in proportion to the degree or magnitude of nutrient and energy deprivation (a.k.a. deprivation syndrome). This in turn, induces the inevitable, genetically mandated energy-conserving survival responses. After some time and at some point in the energy conservation sequela, appetite naturally and automatically increases. Moreover, binge eating, in response to deprivation, has been associated with altered energy metabolism, suppression of fat oxidation and increased fat storage (Gniuli et al, 2005). Alarmingly, many of these one-dimensional tactics are approved, administered and/or supervised by medical or health professionals. While initially appearing to promote "weight loss" (phase 1), such tactics are destined to fail as gene-induced recalibration of energy management and storage instructions homeostatically adjusts to the imposition and influence of these unnatural, genetically defiant tactics, generally by lowering the basal metabolic rate, increasing energy storage requirements and promoting increased fat retention (phase 2) (Dulloo and Jacquet, 1998). The consequences of chronic and repeated attempts to lose weight with such tactics are referred to as the "yo-yo" rebound weight gain effect. This phenomenon is responsible for ever-increasing frustration, anxiety and a sense of helplessness caused by the out-of-control "weight loss"/gain juggernaut.

Finally, the overwhelming research now directed to finding genomic solutions have only further frustrated the field and has resulted in the discovery of over 600 candidate genes and genetic based networks have shown that over 50% of 17,000 genes are either up or down regulated associated with metabolic syndrome. The good news is however, newer methods will allow scientists to discover a few key drivers of the genome to target and develop new pharmaceuticals with a certain degree of specificity. Three genes in this network, lipoprotein lipase (Lpl), lactamase beta (Lactb), and protein phosphatase1-like (Ppm11), are genes that until now were not known to be related to obesity (Schadt et al, 2008).

IV. RDS - a new perspective in the treatment and prevention of obesity

Reward Deficiency Syndrome (RDS), first coined by 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, 2007). Dopamine influences appetite and growth hormone through receptor-mediated activity. Dopaminergic agonists are known to suppress appetite and dopamine D2 receptor antagonists enhance it. Research has shown that DRD2 polymorphisms are significantly associated with BMI as well as with height (Comings et al, 1993). In another study, it was found that striatal dopamine D2 receptor availability was significantly lower in ten obese individuals than in lean controls (Wang et al, 2001). The availability of the D2 receptors was decreased in obese individuals in proportion to their BMI. Dopamine modulates motivation and reward circuits and hence dopamine deficiency in obese subjects may perpetuate pathological eating as a means to compensate for decreased activation of these circuits. Hence, strategies aimed at improving dopamine function may be beneficial in the treatment of obese individuals.

RDS offers an important paradigm shift in the understanding of obesity. Genetics provide a dominant

control in manifesting the body's biological "direction" and performance. However, the multidimensional aspects of lifestyle exert a leveraging influence on gene variations. This area of science is called Nutrigenomics. In recent years there is an ever-increasing abundance of research being conducted to determine the effects that nutrients and/or botanicals exert on various types of genetic variations. This type of information is invaluable in developing therapeutic dose-dependent nutraceutical targets. In addition, nutraceutical ingredients must be qualified and validated based on their ability to positively influence genetic variations.

Like others, we have established a role of catecholaminergic pathways in aberrant substance seeking behavior, in particular cravings for carbohydrates. The genetic basis for generalized craving behavior is the subject of intense investigation.

A. The neuronal inter-relationship between glucose and dopamine release mechanisms

In light of data suggesting frequent co-morbidities to obesity, including diabetes mellitus, atherosclerosis, osteoporosis, and potentially others, Blum and colleagues in 2006 hypothesized that the biologic and genetic factors, synergistically with behavioral modifications, must be addressed to adequately treat this disease. They hypothesized that one such genetic factor that influences behavior and thus obesity is a predisposition to glucose craving and the overall effect of dopaminergic activity in the reward center of the brain. To understand the important relationship between dopamine and glucose, it is of utmost importance to realize that in the mesolimbic system, the glucose receptor is in proximity with the enkephalinergic neurons. There are also other important connections in the substantia nigra, tuberoinfundibular neurons, globus pallidus, and other important brain regions.

For brevity purposes and to eliminate repetition in the literature, the salient points were presented earlier (Blum et al, 2006). It is well known that glucose modulates substantia nigra (SN), dopamine neuronal activity and GABA terminal transmitter release by actions of an ATP-sensitive potassium channel. A study on the effect of altering SN glucose levels on striatal dopamine release was assessed by placing microdialysis probes into both the SN and striatum of male rats. During 50 mM glucose infusion, striatal DA efflux increased transiently by 50% and returned to baseline after 60 minutes. Moreover, when biculline, a GABA (A) antagonist, was added, DA efflux increased by a further 30%. Furthermore, at basal glucose levels, nigral biculline alone raised striatal dopamine efflux by 31%, which suggests the well-known tonic GABA inhibits input to the DA neurons. Thus striatal dopamine release is affected by changing SN glucose levels. This response may reflect the known effect of glucose on K (ATP) channel activity both on SN Dopamine neurons and on GABA axon terminals in the SN. These interactions could provide a mechanism whereby glucose modulates motor activity involved in food intake. Long-term incubation with a high concentration of glucose increased the capacity of calcium uptake to enhance depolarization-induced dopamine release from Pheochromocytoma-12 cells. These data, taken together, suggest that a high concentration of glucose-induced activation of the calcium channel stimulates dopamine release from P12 cells. It was found that restricted feeding with scheduled sucrose access results in an upregulation of the rat dopamine transporter in the nucleus accumbens and ventral tegmental area of the brain. It was found that dopamine can activate B_3 adrenoreceptors to lower glucose uptake into rat white adipocytes which lack dopaminergic receptors. It is of interest that intrastriatal injection of D_1 and D_2 dopamine agonists affects glucose utilization in both the direct and indirect pathways of the rat basal ganglia. Dopamine receptor antagonism influences fat intake in rats depending upon dosage and timing. Both $D_1$and D_2 receptor co-activation may reduce body weight, body fat, food consumption and serum concentrations of glucose, triglycerides, free fatty acids and insulin while increasing protein mass. Blood glucose concentrations seem to be significantly correlated with cerebrospinal fluid concentrations of homovanillic acid, a dopamine metabolite.

Dopamine has been known to induce hyperglycemia, in both animals and man. Along these lines, a direct effect of dopamine on glucose release from primary cultured rat hepatocytes was demonstrated by Japanese researchers. Dopamine was shown not only to have a direct effect on glucose release from hepatocytes, most likely through the glycogenolytic and/or gluconeogenic pathways, but also at the same time, to determine the main type of adrenergic receptor involved in glucose release. This suggests that increasing glucose release from tissue would reduce cravings for glucose and carbohydrates. Glycogen-rich and gluconeogenic-depleted hepatocytes were prepared in order to study glycogenolytic and gluconeogenic-depleted glucose release, respectively. Dopamine caused release of glucose which was inhibited by the β blocker propranolol. Hence, dopamine has a direct effect on hepatocytes, increasing glucose release via both glycogenolytic and gluconeogenic pathways and mediated by β adrenergic receptors (Trugman and James, 1993; Shiroyama et al, 1998; Adler et al, 2000; Bina et al, 2000; Koshimura et al, 2000; Levin 2000; Conti et al, 2001; Colantuoni et al, 2002; Kogan et al, 2002; Bello et al, 2003; Umhau et al, 2003).

B. Other dopaminergic reward genes, risk taking behavior and overeating

Although we believe the gene for the D_2 receptor plays a critical role in RDS behaviors, other dopaminergic receptor genes (such as the dopamine transporter genes, dopamine-β-hydroxylase genes and the D_3 and D_4 dopaminergic recep tor genes) undoubtedly are involved in the different manifestations of the syndrome. But, for example, both the dopaminergic transporter and the dopamine-β-hydroxylase genes are not associated with obesity. It may turn out that one type of overeating is exhibited by high-risk takers whose initial dopamine-linked addictive risk-taking behavior evolved into

carbohydrate bingeing. This is not surprising in light of recent molecular genetic findings (Benjamin et al, 1993, 1996; Caine and Koob, 1993; Noble et al, 1993; Volkow et al, 1993; Poston et al, 1998).

Many genes have been identified that may play a role in increasing susceptibility to obesity. Reduced dopamine function appears to play a role in dysfunctional eating patterns and may predispose some individuals to obesity. The long version of the D_4 dopamine receptor gene (D4DR) has been shown to alter receptor function and reduce intracellular response to dopamine. It also has been associated with novelty-seeking-related personality traits that are found with greater frequency in obese individuals. The association between the D4DR and obesity was shown in a study of 115 obese patients participating in a weight management program. This study constructed four models of increased obesity that included combinations of traditional risk factors (i.e. history of obesity, parental obesity, a body mass index > 40) and elevations on the novelty scales of the Karolinska Scales of Personality. There was a significant increase in the frequency of the D4DR long alleles in individuals defined as high risk using the combination of novelty-seeking-related personality traits, severe obesity (i.e. BMI > 40), and any other traditional risk factor, but not with the traditional risk factors alone. These preliminary data suggest a potential role for the D4DR gene in increasing obesity susceptibility.

C. Other obesity genes

Currently there are over 600 genes that have been identified with one or more sequela associated with obesity. Moreover, twin and family studies suggest that genetic factors potentially influence energy and nutrient intake. In this regard, the HERITAGE group, utilizing a genome-wide scan for dietary energy and nutrient intakes, has determined that in whites, the strongest evidence of linkage appeared for dietary energy and nutrient intakes on chromosomes Ip21.2 and 20q14.1. The linkage evidence on chromosomes 1-20 is related to total energy intake rather than to the intake of specific macronutrients. In blacks, promising linkages for macronutrient intakes occurred on chromosomes 12q23-q24.21, Oq32.1, and 7q11.1. Several potential candidate genes are encoded in and around the linkage regions on chromosomes Op21.2, 12q14.1 and 20q13.13 (Collaku et al, 2004; Rankinen et al, 2005).

There is growing evidence that one gene in particular, the POMC gene, and subsequent mutations in the melanocortin peptides might predispose to obesity. In this regard, Farooqi and O'Rahilly identified in 2006 five unrelated probands who were heterozygous for a rare misense variant in the region encoding B-MSH, Tyr221Cys. This frequency was significantly increased ($p < 0.001$) compared with the general UK Caucasian population and the variant co-segregated with obesity/overweight in affected family members. In other studies, obese children carrying the Tyr221Cys variant were hyperphagic and showed increased linear growth both of which are features of melanocortin deficiency (MC4R) (Biebermann et al, 2006). These studies support a role for B-MSH in the control of human energy homeostasis. Although, at present there is no specific treatment for MC4R deficiency, it is highly likely that these subjects would respond well to pharmacotherapy that overcame the reduction in the hypothalamic, melanocortinergic tone that exists in these patients. Because most patients are heterozygotes with one functional allele intact, it is possible that small molecule MC4R agonists might in the future be worthwhile treatments for obesity (Nargund et al, 2006).

Moreover, Brain-derived neurotropic factor (BDNF) regulates the development, survival and differentiation of neurons through its high-affinity receptor, tropomyosin-related kinase B (TrkB). Unlike other neurotropins, BDNF is secreted in an activity-dependent manner that allows for highly-controlled release. Recently, BDNF has been implicated in the regulation of body weight because its expression is reduced by fasting, and BDNF administration causes weight loss in wild-type mice through a reduction in food intake (Xu et al, 2003). Other supporting studies with regard to the involvement of BDNF and obesity include the work of Yen and colleagues in 2004 and Gray in 2007.

Twenty-four different Mendelian disorders have been reported exhibiting obesity as one clinical manifestation. From animal research we know of 115 genetic sites associated with obesity and related problems. Moreover, in humans, over 250 genes, markers, and chromosomal regions have been associated with obesity and related behaviors. Studies of twins provide the clearest evidence for genes and environment both exerting a significant influence on body composition. In 1997, researchers examined data from 25,000 pairs of twins and a total of 50,000 family members. On average, obesity was 67% genetic and 33% environmental. A small sampling of important candidate obesity genes presented in **Table 1** just touches the surface (Rankinen et al, 2005).

V. Pharmacologic mechanisms of Meridia® and Acomplia®

A. Common neurogenetic mechanisms as an anti-obesity target.

Many of the leading anti-obesity therapies target a neurogenetic mechanism related to obesity, or what we are terming Neurobesigenics. For the purposes of this review, we will briefly outline two prescription therapies and one novel DNA-customized nutritional therapy -- prescription pharmaceutical Meridia® (sibutramine), prescription pharmaceutical Acomplia® (rimonobant), and an LG839 DNA-customized nutraceutical. In the following sections, we will briefly review the pharmacologic mechanisms of sibutramine and rimonobant.

B. Meridia

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 Merida's effect on inhibiting 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 (Balcioglu and Wurtman, 2000).

With the most obvious outcome, nutraceutical ingredients in a patented composition proposed for anti-craving effects, mirror the Meridia mechanism and should produce similar anti-craving effects. However, the notion is that the nutraceuticals, unlike the Meridia, work by answering certain nutrient needs required to silence excessive cravings originating from those systems. In this section we will point out the potential of the ingredients in a proposed formula, based on a large body of neurochemical evidence concerning, among others, precursor amino-acids, the role of chromium as a tryptophan enhancing substance; d-amino acid inhibition of enkephalinase; Rhodiola as a suspected inhibitor of COMT; as well as l-tyrosine, a substance that can mimic some of the effects of catecholamines (Kim et al, 2001). Thus, it is anticipated that instead of pharmacological prolongation of bioactivity of the same three neurotransmitters affected by Meridia (Sibutramine), an increase in those neurotransmitters could be produced by supplying certain "precursor" nutraceutical ingredients, resulting in similar effects without the noted side effects. It could be hypothesized that by increasing precursor intake (i.e. phenylalanine, tyrosine, and chromium and or 5-hydroxytryptophan or any other neurotransmitter enhancers even via transport) and inhibiting enzymatic degradation by COMT, greater levels of 5HT and 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 vi GABA inhibition. Thus the sum total effect is very similar to Meridia. The following information will assure the scientific potential of such a novel natural formula.

The effects of intravenous sibutramine (Meridia) on brain dopamine and serotonin flux into striatal and hypothalamic dialysates of freely moving rats was investigated. 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 (Balcioglu and Wurtman, 2000). The importance here is

Table 1. Example of a number of Candidate obesity genes.

PATHWAY	**GENE POLYMORPHISMS**	**REFERENCE (S)**
CNS-Neurotransmitter Mesolimbic "reward" system and appetite regulatory pathway.	Leptin OB Gene OB1875 < 208-bp allele	Comings DE, Gade R, MacMurray JP, Muhlleman D, Johnson P, Verde R, Peters WR (**1996**) Genetic variants of the human obesity (OB) gene: association with body mass index in young women psychiatric symptoms, and interaction with the dopamine D2 receptor gene. **Molecular Psychiatry** 1, 325-335
Serotonergic pathway involving "sweet tooth" and appetite regulation. Implicated in Bulimia nervosa and anorexia nervosa Serotonin concentrating substance, Percent body fat reduction, cholesterol reduction, glucose regulation, reduction of glucose craving in atypical depression.	5HT2A -1438G/A and 102/C	Tochigi M, Umekage T, Kato C, Marui T, Otowa T, Hibino H, Otani T, Kohda K, Kato N, Sasaki T (**2005**) Serotonin 2A receptor gene polymorphisms and personality traits: no evidence for significant association. **Psychiatric Gen** 15, 67-69. Fuentes JA, Lauzurica N, Hurtado A, Escartí A, Barrios V, Morandé G, Soriano J, Jáuregui I, González-Valdemoro MI, García-Camba E (**2004**) Analysis of the -1438G/A polymorphism of the 5-HT_{2A} serotonin receptor gene in bulimia nervosa patients with or without a history of anorexia nervosa. **Psychiatric Gen** 14, 107-109. Roy S, Rink C, Khanna S, Phillips C, Bagchi D, Bagchi M, Sen CK (**2004**) Body weight and abdominal fat gene expression profile in response to a novel HCA dietary supplement. **Gene Expression** 11, 251-262.
Association with total cholesterol and triglyceride levels.	Acid phosphatase (ACPI); A^* / A^*, A/B and A/C and non-allele genotypes B/B, B/C, C/C. These genotypes have been associated with total cholesterol and triglycerides.	Bottini N, MacMurray J, Peters W, Rostamkhani M, Comings DE (**2002**) Association of the acid phosphatase (ACP1) gene with triglyceride levels in obese women. **Mol Gen Metab** 77, 226-229.

Neurotransmitter gene interaction especially serotonin precursors.	Phenylethanolamie NMethytransferase PNMT G-148A of PNMT G/G, A/A and G/A. Compared with the heterozygous PNMT variant, G/A, the presence of the homozygous PNMT variant, either G/G or A/A, was associated with a statistically significant weight loss when challenged with Sibutramine (adrenergic/serotonergic) after 6 months.	Peters WR, MacMurry JP, Walker J, Giese RJ Jr, Comings DE (**2003**) Phenylethanolamine NMethytransferase G-148A genetic variant and weight loss in obese women. **Obes Res** 11, 415-419.
receptor is involved in both Pleasure and anti-anxiety. It is involved in craving for sugar as well as other addictive substances. Dopamine is a major neurotransmitter released at the n. accumbens and acts as a reward substance in the brain.	D2 receptor gene DRD2 The Taq1A1 allele as well as the B1 allele has been associated with a number of Reward Deficiency behaviors including eating and craving for glucose. It has also been associated with Obesity, elevated BMI and increased fat storage. The Ser311Cys of the D2gene has been associated with low energy expenditure.	Tataranni PA, Baier L, Jenkinson C, Harper I, Del Parigi A, Bogardus C (**2001**) Ser311Cys mutation in the human dopamine D2 gene is associated with reduced energy expenditure. **Diabetes** 50, 901-904. Comings DE, Gade R, MacMurray JP, Muhlleman D, Johnson P, Verde R, Peters WR (**1996**) Genetic variants of the human obesity (OB) gene: association with body mass index in young women psychiatric symptoms, and interaction with the dopamine D2 receptor gene. **Molecular Psychiatry** 1,325-33. Noble EP, Noble RE, Ritchie T, Grandy DK, Sparkes RS (**1994**) D2 dopamine receptor gene and obesity. **J Eating Disorders** 15, 205-17. Blum K, Chen TJH, Kaats G, Braverman E, Pullin D, Downs BW, Wood R, Blum SH, Meshkin B, Mengucci J, Comings DE, Bagchi D, Bagchi M (**2005**) Correlation of the *Taq1* Dopamine D2 Receptor Gene and Percent Body Fat in Obese and Screened Control Subjects : A Preliminary Report (presented at the 2005 IPS conf in Venice, Italy).
Neurotransmitter synaptic clearance	Monoamine -Oxidase A MOA-A MOA-A repeat polymorphisms include 2 allele; 3 allele; 3 allele; 4 allele, 5 allele; Short (2,3) and Long (4,5). An important MAOA polymorhism is called FNU4 i.e. 941 Fnu4 exon 8 in LD with EcoV polymorphism. Absence of site assoc with low MAO activity.	Tsugeno T, Ito A (**1997**) A key amino acid responsible for substrate selectivity of monoamine -oxidase A and B. **J Biological Chem** 272, 14033-14036. Manoli I, Le H, Alesci S, McFann KK, Su YA, Kino T, Chrousos GP, Blackman MR (**2005**) Monoamine-oxidase-A is a major target gene for glucocorticoids in human skeletal muscle cells. **FASEB J** 19, 1359-61 Manor I, Tyano S, Mel E, Eisenberg J, Bachner-Melman R, Kotler M, Ebstein RP (**2002**) Family-based and association studies of monoamine oxidase A and attention deficit hyperactivity disorder (ADHD): preferential transmission of the long promoter-region repeat and its association with impaired performance on a continuous performance test (TOVA). **Mol Psychiatry** 7, 626-632. Ito H, Hamajima N, Matsuo K, Okuma K, Sato S, Ueda R, Tajima K (**2003**) Monoamine oxidase polymorphisms and smoking behavior in Japanese. **Pharmacogenetics** 13, 73-79.
DHEA a natural substance effects female belly fat and is associated with the STS gene.	Steroid sulfatase (STS) gene variation STS "G" allele (n = 36) had greater acute changes in DHEA (+4.4 (0.7) vs. +2.0 ng/ml (0.5), S1; +3.2 (0.6) vs. +1.0 ng/ml (0.4), S30; $P < 0.01$) and DHEAS:DHEA (-37 (11) vs. 5 (7), S30, $P < 0.05$) than those subjects with only an "A" allele (n = 84).	Riechman SE, Fabian TJ, Kroboth PD, Ferrell RE (**2004**) Steroid sulfatase gene variation and DHEA responsiveness to resistance exercise in MERET. **Physiol Genomics** 17, 300-6. Villareal DT, Holloszy JO (**2004**) Effect of DHEA on abdominal fat and insulin action in elderly women and men: a randomized controlled trial. **JAMA** 292, 2243-8.

Interaction of DHEA and adipose tissue as correlated with the PPARgamma gene.	Pro12Ala polymorphism of PPARgamma gene A corresponding increase in peroxisome proliferator-activated receptor gamma (PPARgamma) mRNA expression suggests that PPARgamma may be involved in the up-regulation of adiponectin gene expression after DHEA treatment. Primer sequence is 5' CTGTTATGGGTGAAACTCTGG-3' Adiponectin gene; the primer sequence is: 5' TGGCAGAGATGGCACTCC-3'	Karbowska J, Kochan Z (**2005**) Effect of DHEA on endocrine functions of adipose tissue, the involvement of PPARgamma. **Biochem Pharmacol** 70, 249-57. Dillon JS, Yaney GC, Zhou Y, Voilley N, Bowen S, Chipkin S, Bliss CR, Schultz V, Schuit FC, Prentki M, Waxman DJ, Corkey BE (**2000**) Dehydroepiandrosterone sulfate and beta-cell function: enhanced glucose-induced insulin secretion and altered gene expression in rodent pancreatic beta-cells. **Diabetes** 49, 2012-2020.
Glucose conversion to fat is controlled by the ChREBP gene. The ChREBP is a very important gene which controls glucose metabolism and may be a key in the etiology of obesity. A high fat diet inhibits glucose metabolism, and is sometimes referred to as the "fatty acid sparing effect of glucose'. It has been showed that the activity of ChREBP was inhibited by a high fat diet. Therefore glucose stimulates ChREBP activity while fat inhibits ChREBP activity which in turn reduces glucose conversion to fat. In terms of health risk DNA analysis of the ChREBP gene will provide very important information which may result in the proper adjustment of known glucose reducing substances.	Carbohydrate responsive element-binding protein (ChREBP) gene. This is an important gene which controls the end step in the conversion of glucose to fat. The mechanism of glucose activation appears to involve the import of ChREBP protein from the cytosol to the cell nucleus. This involves the dephosphorylation of the phoso-SER196 of ChREBP. A Ser568ASP mutant shows weak DNA-binding. There appears to be two levels of regulation of ChREBP by cAMP-dependent protein kinase (PKA)-mediated phosphorylation as a result of a rise in cAMP. One is the phosphorylation of Ser196, which inhibits nuclear import, and the other is Thr666 which inhibits DNA-binding activity. A Ser 626 ASP (with Ser196Ala) mutant loses transcriptional activity, as a result of DNA-binding activity. because of the presence of the similar double mutant of Thr666Ala (Ser196Ala) which inhibits DNA-binding.	Uyeda K, Yamashita H, Kawaguchi T (**2002**) Carbohydrate responsive element-binding protein (CHREBP) a key regulator of glucose metabolism and fat storage. **Biochem Pharmacol** 63, 2075-2080.

that it provides further support for a novel formula containing the aforementioned nutraceutical ingredients that promote improved body composition via both serotonergic, dopaminergic and other mechanistic anti-obesity actions based on known neuropharmacological mechanisms (Cooper et al, 1966; Koob and Bloom 1988; Blum and Payne 1991).

C. Acomplia

It is well established that *Cannabis sativa* can increase appetite, particularly for sweet and palatable foods. In laboratory animals, cannabinoid CB_1 receptor antagonism decreases motivation for palatable foods, and most recently, the CB_1 antagonist SR141716A, or rimonabant (Acomplia) was reported to produce weight loss in obese human subjects (Van Gaal et al, 2005). It is believed that the mechanism of action involves the hypothalamus arcuate (ARC) and paraventricular (PVN) nuclei, suggesting that they are interconnected with the mesolimbic DA pathways, which are implicated in the reinforcing effects of non-drug natural rewards such as food, as well as psychostimulants and other drugs of abuse (Di Chiara et al, 1993; Gardner and Vorel, 1998; Carelli, 2004). It is believed that the reduction of sweet food by Acomplia may be due in part to an effect on mesolimbic DA (Ward and Dykstra, 2005). There are other effects of Acomplia that may contribute to its anti-obesity activity including metabolic effects (Vickers and Kennett, 2005).

The major difference between Acomplia and our nutritional approach is that the former drug reduces the natural function of DA (a dopaminergic antagonist to block reinforcement) compared to the DA agonist induction of our approach. In fact, because of the mechanism of action of long term utilization of Acomplia as an anti-DA agent, mood changes, including suicide, occur and this has led to the FDA rejected clearance in America.

Certainly, it appears that DA pathways seem to be a common mechanism involved in craving behavior. More intensive research is required to tease out differences between Meridia, Acomplia and our nutritional approach (Blum et al, 2006a), but certainly dopaminergic activation rather than blockade, with its potential side effects, would seem to be favored.

VI. Brain nutrition and behavior

There is growing evidence to support the use of DNA-targeted ingredients in helping to restore optimal body composition, such as augmentation with precursor amino acid therapy and enkephalinase and COMT inhibition leading to enhanced levels of neurotransmitters such as serotonin, enkephalins, GABA and dopamine/norepinephrine. This nutraceutical technology, designated as LG839 (formerly SG8839), has been evaluated in an open label trial and has been demonstrated to exert a generalized anti-craving effect in alcoholics (Blum et al, 2006b), and can inhibit carbohydrate binging, making an important contribution to inducing significant healthy fat loss and relapse prevention (Blum et al, 2006c).

Based on a number of case reports, it appears that this unique technology and combination of ingredients may have a generalized anti-stress and anti-craving effect, resulting in an inhibition of carbohydrate appetite, inducing energy increase, increasing positive focus, improving performance and a sense of well-being, promoting fat loss and enhancing body image/composition. Targeting certain pathways provided a means of combining natural ingredients at the clinically tested dosage levels indicated, to answer nutrigenomic and pleiotropic needs to promote successful and sustainable improved body recomposition results.

A detailed account of reviewing the intricate relationship of brain nutrition and behavior is treated in the book "Alcohol and The Addictive Brain" (Blum and Payne, 1991). 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 or, more specifically, a deficiency or excess of amino acids and co-factors. In the healthy body, if dietary sources are sufficient, amino acids are kept in balance. However, if there is an excess or shortage of the amino acids and/or their co-factors, distortions of brain function can result (Bongiovanni et al, 2006).

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. However, other D-amino acids like D-serine have been found to play an important role in brain development (Mustafa et al, 2004). What is even more important is the recent finding of the existence and identification of a D-amino oxidase system in neurons (Molla et al, 2006). However, to date D-serine is the only naturally occurring D-amino acid ever found in the brain of mammals (Snyder and Kim, 2000), but may be an important therapeutic neurotransmitter target. Normal amino acid metabolism is essential for many physiological and behavioral functions. In fact, a small percentage of children are born with a genetic anomaly involving neurotransmitter synthesis and metabolism. Deficiency of aromatic L-amino acid decarboxylase (AADC) is associated with severe developmental delay, oculogyric crisis (OGC), and autonomic dysfunction. Treatment with dopamine agonists and MAP inhibitors is beneficial, yet lon-term prognosis is unclear (Pons et al, 2004). Interestingly, these children cannot properly regulate sugar intake and are chubby in the first year of their life.

A. 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, the 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 serotonergic 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 co-morbid 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 particularly important when you consider the large neutral amino carrier system and competition of tryptophan, phenylalanine and tyrosine. It is equally important when you consider that the rate-limiting enzyme, Tyrosine Hydroxylase, works best under stressful conditions and the precursor tyrosine will indeed be converted to dopamine, only to be released subsequently into the synapse of the nucleus accumbens.
- Fifth, it is well known that the degradation of catecholamines by COMT plays a role, albeit only partial, in clearing these neurotransmitters from the synaptic cleft. Dopamine, norepinephrine and serotonin reuptake into nerve terminals via membrane transporter is thought to play a more significant role (Saratikov et al, 1968). 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 (Germano and Ramazanov, 1999) plus the central nervous system effects of *Rhodiola rosea* (Ramanazov and del Mar Bernard 1999; Brown et al, 2005; Panossian and Wagner

2005) 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 the extract standardized to 3% rosavin), which is somewhat higher than the recommended dose for use of Rhodiola rosea as an antidepressant (200mg/day) (Panossian and Wagner 2005). Moreover, one reiteration of the novel nutraceutical formula also contains synephrine, derived from Citrus aurantium (6% synephrine) at a daily dose of 50 mg. This amounts to only 3 mg/per day. While this is less than what is normally recommended as a sympathiomimetic agent when combined with caffeine, thermogenesis could be achieved without the stimulatory effects seen with much higher doses (104 mg/day) (Sale et al, 2006). Furthermore, our approach is to avoid excessive stimulation of the central nervous system, even as a means of inducing fat oxidation for fat loss. The phase 2 consequences of Phase 1 CNS stimulation, without the other components needed for a multidimensional nutrigenomic solution, in an obese-RDS afflicted individual, would include, among other things, a compensatory down regulation of Basal Metabolic Rate (BMR) an increase in fat storage and an increase in appetite (cravings), ultimately resulting in rebound weight gain – a well known and epidemic phenomenon.

VII. Synaptamine™complex: studies showing anti-craving efficacy.

It is our contention that with the formula as designed, anti-craving, additive or even synergistic outcomes might be observed since the ingredients are included that could act through several different mechanisms (see above) to enhance the activity of the neurotransmitters (Blum et al, 1987, 1997, 1988a, 1990; Defrance et al, 1997; Chen et al 2004). However these results must be interpreted with caution.

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, the combination might enhance compliance in methadone patients rapidly detoxified with the narcotic antagonist Trexan®· (Dupont, Delaware) (Chen et al, 2004). In this regard, other research has found that increases in the dopamine D_2 receptors (DRD2) via adenoviral 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 (Thanos et al, 2001; Volkow et al, 2006). This DRD2 overexpression similarly produced significant reductions in ethanol non-preferring rats, in both alcohol preference (16%) and alcohol intake (75%). This further suggests that high levels of DRD2 may be protective against alcohol abuse (Blum et al, 1990, 1991). The DRD2 A1 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, it has been shown 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 for both the 5 and 7 repeat alleles in Han Chinese case control sample of heroin addicts (Li et al, 1997). Similarly in French Heroin addicts, a significant association with homozygote alleles of the DRD3-Bal 1 was found. 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 (Ross 2001);

Over the last decade, a new rapid method to detoxify either methadone or heroin addicts utilizing Trexan® 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 Trexan® 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 were 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 of further interest to test this hypothesis with the sublingual combination of the partial opiate mu receptor agonist buprenorphrine. The ingredients tested included DL-phenylalanine (2760 mg/day), L-Glutamine (150 mg/day), chromium picolinate (360 micrograms/day Cr), and pyridoxal-5-phosphate (30 mg/day) (Chen et al, 2004);

A recent 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, the following ingredients were utilized and tailored made for each client: DL-phenylalanine, 5-hydroxytryptophan, L-tryptophan, L-tyrosine, L-glutamine, chromium, and vitamin B6. Follow-up interviews of six randomly selected former eating disordered female clients (three were also chemically dependent) were conducted 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, Ross and associates also evaluated, in 2001, an additional 100 patients. 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 in 2001, 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 recent study from our laboratory 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 taken include DL-phenylalanine (2700 mg/day), 5-hydroxytryptophan (20 mg/day), L-Tyrosine (750 mg/day), L-glutamine (350 mg/day), Rhodiola rosea (3% rosavin) (66 mg/day), Chromium dinicotinate glycerate (1000 micrograms/day), DMAE (40 mg/day), and Huperzine A (150 micrograms/day). The composition included a combination of vitamins (C, E, niacin, riboflavin, thiamin, B6 [20% pyridoxal-5-phosphate and 80% pyridoxine] folic acid, B12, biotin, pantothenic acid), minerals (calcium, magnesium, zinc, manganese) and an herbal calming blend, a focus blend or mood enhancing blend were also used. The ingredients and dosages were dependent on type of abusers including diagnosis of ADHD (Blum et al, 2007).

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/are deficient. Based on evidence-based medicine, we propose that DNA testing be used to tailor the inclusion and amounts of certain qualified ingredients, which have been clinically tested for over 20 years. These ingredients have relevance to RDS, more specifically, overeating and carbohydrate bingeing, and support a generalized anti-craving claim.

VIII. LG839™ - searching for a DNA-customized nutraceutical

In contrast to existing tactics that attempt to "manipulate" body composition in unnatural ways as previously explained, there is a need to achieve healthy body composition through supporting the body with essential nutrients based upon its genetic predisposition. We propose the use of an LG839 DNA-customized nutraceutical that currently involves a genetic analysis of at least five to sixteen genes involved in multiple contributing factors to weight, including the stress, inflammation, pleasure and food craving in the reward circuitry of the brain, the immune system, the neuroendocrine system, and the bodys ability to metabolize and produce energy (Blum et al, 2006d).

In an observational study, a total of 1,058 subjects were genotyped in our laboratory. Each subject self-identified themselves as obese or overweight by selecting LG839™, a DNA-customized nutritional solution for weight loss, as an adjunct to their weight loss efforts. In this case, each subject was genotyped based upon the following genetic mutations: *Sweet Tooth Gene*™ (Dopamine D2 Receptor Gene Taq 1 Alelle (DRD2 A1)), *Nervous Eating Gene*™ (5-Hydroxytrytamine 2A-1438G/A promoter polymorphism (5-HT2a-1438G/A)), *New Cell Gene*™ (Methylene Tetrahydrofolate Reducatse C677T polymorphism (MTHFR C677T)), *Obesity Risk Gene*™ (Leptin Genetic Polymorphism - OB1875 < 208-bp alleles (Leptin OB1875)), and the *Fat Regulator Gene*™ (Peroxisome Proliferator-Activated Receptor-γ Gene Pro12Ala polymorphism (PPAR-γ Pro12Ala Allele)).

The Sweet Tooth Gene was present in 38.09% of the study subjects (n=1,058) versus 29% of the literature controls (n=3,259). This difference was significant (Z = 8.393, p = 0.0001). The Nervous Eating Gene was present in 64.18% of the study subjects (n=1,058) versus 61% of the literature controls (n=284). This difference was not statistically significant (Z = 0.755, p = 0.23). The New Cell Gene was present in 69.85% of the study subjects (n=1,058) versus 54% of the literature controls (n=100). This difference was significant (Z* = 2.23, p = 0.01). The Obesity Risk Gene was present in 75.61% of the study subjects (n=1,058) versus 45.6% of the literature controls (n=206). This difference was significant (Z = 5.612, p = 0.0001). The Fat Regulator Gene was present in 25.05% of the study subjects (n=1,058) versus 14% of the literature controls (n=2,245). This difference was significant (Z=17.398, p = 0.001).

Based upon this genetic information, LG839 provides a DNA-customized nutraceutical formula provides a solution to meet the genetic predisposition of the individual (**Figure 1**). The ingredients used include a form of chromium, a proprietary raw material complex called Synaptamine, an adjunct to that raw material complex including Passiflora and several other ingredients that have demonstrated weight loss potential in clinical studies.

A. Pharmacogenomic targeting of chromium picolinate (CRP); a case study

Despite considerable evidence correlating dopaminergic genes and obesity, body mass index (BMI), body type, overeating, carbohydrate binging, and low dopamine D_2 receptor (D2R) density, there are few studies

involving the dopamine D_2 receptor gene (DRD2) variants and percent body fat (%BF). The effectiveness of chromium picolinate (CRP) in altering body composition has been controversial. Evidence from an unpublished multi-centered study may provide a functional basis linking dopaminergic gene polymorphisms first to %BF and obesity, and secondarily to the nutrigenomic response of CRP in producing body composition effects**.** In an unpublished multi centered study, a total of 901 subjects were genotyped at the DRD2 Taq A1 allele in three different institutions involving subject volunteers: Baylor University School of Medicine, University Of Texas Health Science Center, Wake Forest University School Of Medicine. A total of 257 subjects were assessed for weight, BMI (kg/m^2) and %BF using dual energy X-ray absorptiometry (DEXA). The remaining 644 subjects, part of the Dutch Investigation to Evaluate Treatments of DNA-customized nutritional solutions for weight management (D.I.E.T.) study, were assessed by a cross sectional survey known as the Blum-Meshkin OPAQuE Scale (Overweight Patient Assessment Questionnaire and Evaluation) without DEXA. For the %BF experiment, the first control group consisted of 30 non-obese Caucasians screened to exclude a wide range of addictive behaviors (Controls A). The second control group, from the City Of Hope National Medical Center, consisted of 105 non-obese Caucasians screened to exclude substance abuse and psychiatric disorders (Controls B). For the CRP experiment, subjects were divided into matched placebo (n=60) and CRP (n=62) groups (400 ug per day). The sample was separated into two independent groups: those with either an A1/A1 or A1/A2 allele or those with only the A2/A2 allelic pattern. Each group was tested separately for differences between placebo and treatment means for a variety of weight change measures. The DRD2 Taq1A1 allele was present in 67% of the obese subjects compared to 3.3 % of the well-screened controls A and 33.3 % for controls B. These differences were significant: Controls A vs. Obese subjects: r^2 = 39.6 d.f. =1, p<0.0001, and Controls B vs. Obese subjects r^2 = 25.9 d.f. 1, p <0.0001. With regard to the effects of CRP the measures of the change in fat weight (p<0.041), change in body weight (p<0.017), the percent change in weight (p<0.044), and the body weight change in kilograms (p<0.012) were all significant for carriers of the DRD2 A2 genotype, whereas no significance was found for any parameter for those subjects possessing a DRD2 A1 allele. In terms of the role of dopamine in global obesity, the DRD2 A1 allele was present in 37% of the self identified obese subjects in the D.I.E.T. study. Compared to literature controls (N=3,329), a significant association was found (X^2 14.47; df=1, P < 0.0001) (Odds Ratio = 1.407 95% conf int for OR (1.179, 1.679)) (**Figure 2**) (Chen et al, 2007).

Another neurobesigenic ingredient in the LG839 nutraceutical is the Synaptamine Complex that provides anti-craving efficacy through precursor amino-acids and enkephalinase inhibitor activity. Synaptamine includes:

- 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;

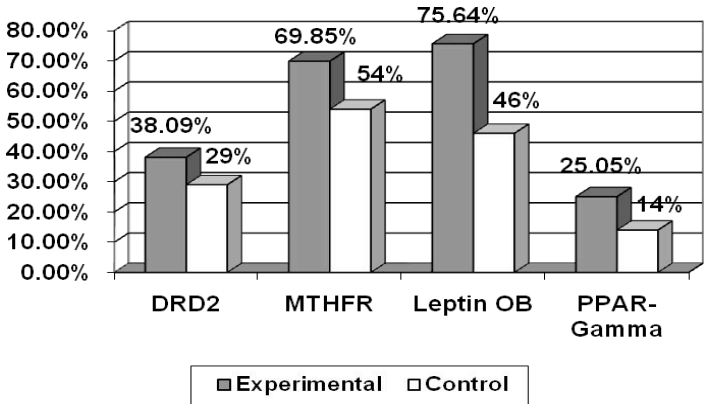
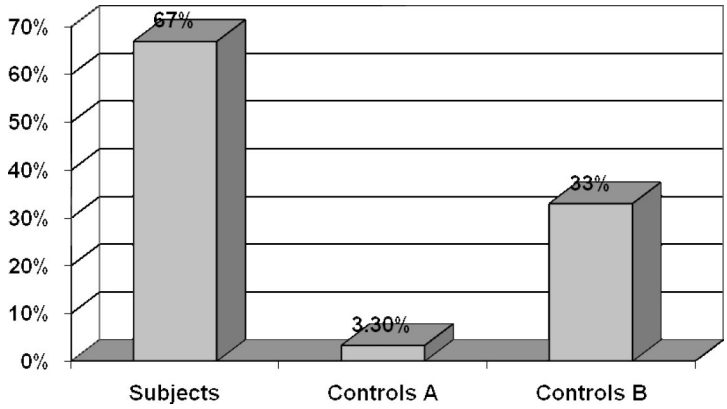


Figure 1. Prevalence of LG839 Genes in Self-Identified Obese Persons

Gene	Experimental	Control	Significance
DRD2	38.09% (n=1,058)	29% (n=3,259)	Z=8.393, p=0.0001
MTHFR	69.85% (n=1,058)	54% (n=100)	Z=2.23, p=0.01
Leptin OB	75.61% (n=1,058)	45.6% (n=206)	Z=5.612, p=0.0001
PPAR-γ	25.05% (n=1,058)	14% (n=2,245)	Z=17.398, p=0.001



Controls A vs. Obese subjects: $r^2 = 39.6$, d.f. =1, $p<0.0001$
Controls B vs. Obese subjects $r^2 = 25.9$, d.f. =1, $p <0.0001$
PPAR data reproduced from Blum et al, 2007 with kind permission from Gene Therapy Press.

Figure 2. Prevalence of DRD2 Taq 1 Allele in Obese Subjects versus Non-Obese Subjects.

- 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 born L-tryptophan and L-tyrosine at the large neutral amino-acid brain carrier system (Milner et al, 1986). However, other data demonstrate 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 ^{14}C-phenylalanine in a rat caudate nucleus synaptosomal preparation and concomitantly stimulated the synthesis of dopamine. Furthermore, Amfoelic acid also caused a net release of 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 synaptosomal particles (Bagchi et al, 1979).
- 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-Tyrosine, to increase brain dopamine levels as a direct precursor and amino-acid synthesis promoter. Its major use is for the repletion of utilized and thereby metabolized neurotransmitter (i.e. dopamine).
- L-5-hydroxytryptophan (optional) - The effect of systemic administration of 5-hydroxy-l-tryptophan (5-HTP) on the release of serotonin in the lateral hypothalamus of the rat *in vivo* was 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 (Gartside et al, 1992).
- Pyridoxal-5-phosphate, the active ingredient of vitamin B_6 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). A novel oxygen-coordinated chromium nicotinate has been shown to improve insulin sensitivity, promote lean muscle-sparing benefits (enhancing energy metabolism) and enhance fat loss in overweight subjects. Chromium salts also have a number of other metabolic effects including; reduction of cholesterol; reduction of percent body fat; induction of weight loss; maintaining and promoting lean muscle mass; enhancing body composition; promoting brain serotonin production (see above). Administration of 600 mcg of elemental chromium as NBC (ChromeMate®) in three divided doses daily over a period of 2 months to African-American women with a moderate diet and exercise regimen influenced weight and fat loss and sparing muscle, improving body composition. Another study at the University of Texas found that young obese women consuming 400 mcg of elemental chromium as NBC (niacin bound chromium or chromium nicotinate) per day with exercise experienced significant weight loss over an eight week period. Other studies in Fisher F344/BN rats using a chronic dose of 400 mcg of elemental chromium per day showed no adverse effects in body and organ weights, and blood chemistries.
- Calcium, promotes neurotransmitter release based on many studies (Kasim et al, 2006).
- Rhodiola rosea - Several clinical trials with double-blind placebo controls in Russia provide evidence that R. rosea possesses 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 lower brain levels of serotonin and dopamine have been observed. It was shown that R. rosea can increase neurotransmitter levels by 30 percent and decrease COMT activity by 60 percent. Furthermore, several double-blind

studies have demonstrated the efficacy of R. rosea extract in the treatment of obesity (Panossian and Wagner, 2005). Most recently, the positive effects of Rhodiola were confirmed in studies on mice. The extract was tested on antidepressant, adaptogenic, anxiolytic, nociceptive and locomotor activities at doses of 10, 15 and 20 mg/kg, using predictive behavioral tests and animal models. The results show that 3% R. rosea extract significantly, but not dose-dependently, induced antidepressant-like, adaptogenic, anxiolytic-like and stimulating effects in mice. This study thus provides evidence of the efficacy of R. rosea extracts after a single administration, and confirms many preclinical and clinical studies indicating the adaptogenic and stimulating effects of such R. rosea extracts. Moreover, antidepressant-like and anxiolytic-like activities of R. rosea were shown in mice for the first time (Perfumi and Mattioli, 2007).

It is well known that stress induces the preferential release of the circulatory hormone cortisol in humans (Rivier et al, 1986). It is also well know that lipolysis is the major activity that is involved in the burning of fat in adipose tissue. It was shown that cortisol significantly reduced the basal rate of lipolysis ($p < 0.01$) and specifically cortisol reduced the catecholamine lipolysis stimulators isoprenaline and noradrenalin *in vitro* (Ottosson et al, 2000). Thus, cortisol decreases lipolysis. In addition, the pathogenesis of obesity has been suggested to include and be intimately linked to the catecholaminergic regulation of lipolysis and the function of the sympathetic nervous system. Norepinephrine and epinephrine activate lipolysis via B_1, B_2 and B_3 - adrenoreceptors and inhibit it via α_2 – adrenoreceptors. These neurotransmitters are the most important lipolytic substances *in vivo*. Defects of catecholamine-induced lipolysis have been observed in a number of obese subjects associated with polymorphisms of the B_2- and B_3 receptors (Clement et al, 1995; Large et al, 1997).

In a recent publication (Blum et al, 2006a), our laboratory used S.E.M. analysis to evaluate the effectual relationships between various systems vulnerable to dysfunction from RDS. This analysis produced a multi-system matrix to measure the contributory roles of those various dysfunctions or PATHs, enabling targeted and effective interventions. S.E.M. analysis is a type of statistical regression analysis that evaluates the contributory effect (in a "cause and effect sequence") of at least 2 or more causal models (factoring their relative variables) in a multi-dimensional matrix, to develop a statistical means of defining the importance (weight), impact and relational contributions of each interactive path toward the total outcomes.

In this regard, we found a number of very interesting correlations (negative and positive): Stress is negatively correlated with sleep (r=-0.49), whereby when stress is high sleep is low; stress is positively correlated with appetite (r=0.42), whereby when stress is high appetite is high; stress is negatively correlated with energy (r=-0.24) whereby when stress is high energy is low; sleep is positively correlated with energy (r=0.44), whereby when sleep is high energy is high; stress is negatively correlated with well-being (r= -0.36), whereby when stress is high well-being is low; sleep is positively correlated with performance (r= 0.40), whereby when sleep is high performance is high; appetite is negatively correlated with focus (r= - 0. 30), whereby when appetite is high focus is low; appetite is positively correlated with weight (r= 0.21), whereby when appetite is high weight is high; and finally weight is positively correlated with inches (r = .63), whereby when weight is high so are inches (**Figure 3**).

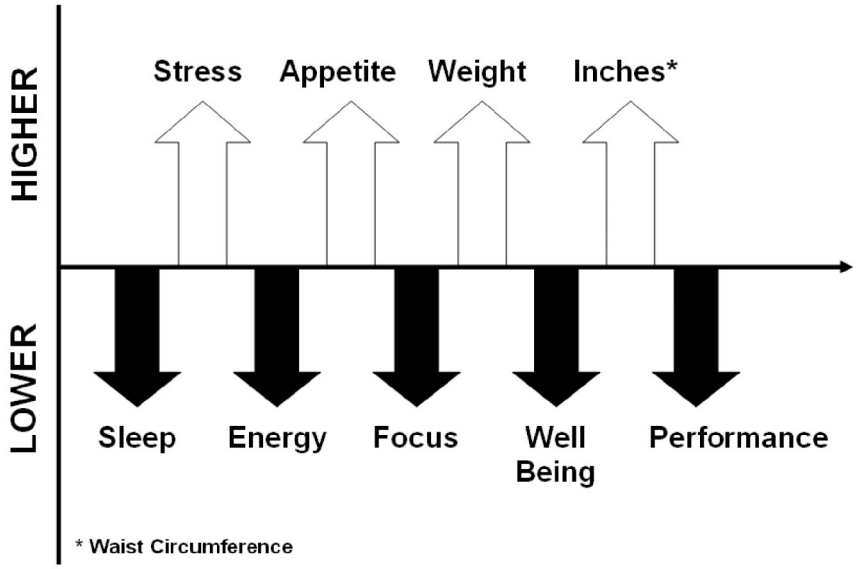


Figure 3. Modified representation of a Structural Equation Modeling Methodology (SEM) to evaluate the clinical effects of LG839 in obesity. Reproduced from Blum et al, 2006a with kind permission from Advances in Therapy.

The findings obtained in this open-label observation study revealed a number of beneficial effects of the Synaptamine™ complex. The duration ranged from 8 days to 12 months. Interestingly even after only 8 days of usage the subject experienced a reduction of stress, increased well-being, a reduction of sugar cravings and a reduction of appetite. While one individual experienced an increase in body weight (3lbs over 5 month duration, (however, changes in lean to fat mass ratios were not evaluated), 83% achieved weight reduction over the duration of the study. The survey evaluation was not able to reveal whether that one individual's increase in weight was due to an increase in heavier lean muscle development or fat tissue. Out of the 24 people responding, 96% obtained a greater feeling of well-being; 83% achieved weight reduction, 79% reported a significant suppression of appetite; craving for sugar was reduced in 75% of the respondents; 75% reported a reduction of stress; importantly 70% achieved loss of inches; increased energy was found in 54%; positive sleep was observed in 42% of the subjects; enhanced focus was reported in 25% of the respondents; and enhanced performance was observed in 21% of the subjects. Out of 24 respondents, 13 (54%) measured the pounds actually lost. Not withstanding other factors, exercise, better eating habits etc., the average loss of scale weight was 17.15 lbs. Out of 24 respondents 5 (20%) measured inches lost and found that on the average the subjects lost 10.7 inches. Only one respondent measured actual percent body fat and lost 3 % over a month period following utilization of Synaptamine an LG839, complex. In a very small number of cases other reporting features included sugar level reduction (0.42%); blood pressure reduction (0.83%), and cholesterol reduction (0.83%) (Blum et al, 2006a).

B. Salts of HCA

Garcinia cambogia extract (GcE) containing HCA, has been purported to reduce appetite and promote fat loss, without stimulating the central nervous system, by competitively inhibiting the enzyme, ATP Citrate Lyase that converts excess citrate (a glucose metabolite) into fat (Lowenstein, 1971; Wheatley, et al, 1972; Greenwood, et al, 1981; Sakariah and Nageswara, 1988; Lowenstein and Brunengraber, 1993; Schwarz, et al, 2001).

However, controversy abounds over its effectiveness, with many factors contributing to the controversy. Differences in the composition and potency of GcEs can affect pH, solubility, bioavailability, and efficacy; and would account for profound differences in results between different complexes used in various studies. Varying the number and kinds of co-ingredients is also a confounding factor. Further, omitting details about product specifications, product analysis, other product components and characteristics (aside from HCA) make it virtually impossible to accurately evaluate, compare and explain results from one study to the next. Without such information, given a valid study method, the level of effectiveness of *Garcinia cambogia* extract, by default, has been solely attributed to or refuted based on the "HCA value", when in fact other components by their presence or absence can significantly contribute to or detract from the therapeutic effect. In order to be effective, orally ingested HCA must reach optimum plasma levels and cellular concentrations. The clinical significance of HCA for weight management cannot be ascertained without establishing bioavailability. The omission of accurate bioavailability analysis, at least in blood, indicates that HCA, *per se*, is responsible for the lack of beneficial weight loss evidence. This omission relinquishes the opportunity to explain the significant differences between studies.

A Gas Chromatography/Mass Spectrometry Method (GC/MS) to accurately identify and quantify plasma HCA levels in humans was developed in 2001 (Preuss, et al, 2004a). Plasma HCA concentrations were measured over 3.5 hour period in fasting and fed subjects ingesting 2 g of HCA. While HCA levels varied between subjects, peak plasma levels were reached in approximately 2 hours. The source of HCA used in this study was a novel stabilized Ca/K salt of a 60% HCA GcE (CitriMax HCA-600-SXG-P capsules ~500 mg each, supplied by InterHealth Nutraceuticals). This source is currently patent pending and is the same source used in the formula that is the subject of the present discussion**.** The Ca/K moiety of HCA and its effects on the extract's physical and chemical properties appear crucial. This research demonstrated that HCA's polar functional groups render it susceptible to poor absorption due to the possibility of interactions with other compounds in foods. Bonding of HCA with other salts, individually or in combination could significantly alter physical and chemical properties and the therapeutic effects accordingly.

Furthermore, the bioavailability and efficacy of HCA (and calcium and potassium) from this material has been demonstrated by its ability to influence leptin metabolism and various metabolic parameters crucial for healthy fat loss (Downs et al, 2005). In a randomized, double-blind and placebo-controlled study, the effects of a novel Ca/K 60% HCA, alone (Group A) and in combination with a patented niacin bound chromium complex (4mg/d providing 400mcg chromium) and *Gymnema sylvestre* (400mg/d) (Group B) were investigated (Filozof et al, 2000). In both groups, (-) HCA intake was 2800 mg/day, given to moderately obese humans consuming a 2000 kcal diet/day and engaging in a walking exercise regimen. Among other factors, body weight, BMI, lipid profile, and serum leptin levels (a marker of obesity gene) were assessed at 4 and 8 weeks. At the end of 8 weeks, supplementation of HCA-SX in Group A decreased serum leptin levels by 36.6%. Body weight and BMI were decreased by 6.3% respectively and food intake decreased by 4%. Total cholesterol LDL and triglycerides were decreased 6.3%, 12.3%, and 8.6% respectively with no significant adverse effects observed. HDL and serotonin levels increased by 10.7% and 40% respectively. In Group B, serum leptin levels decreased 40.5%, body weight and BMI reduced by 7.8% and 7.9% respectively. Total cholesterol, LDL and triglycerides were reduced by 9.1%, 17.9% and 18.1% respectively, while HDL and serotonin levels increased by 20.7% and 50% respectively. Food intake was reduced by 14.1%. This research demonstrates bioavailability and efficacy of this novel preparation of

Ca/K 60% HCA (commercially available as Super CitriMax) on various metabolic parameters integral to safely achieving and maintaining healthy body composition.

C. Passiflora incarnata (Passion Flower)

There are more than 40 species in the genus whose origins are in both the tropical and subtropical regions of the western hemisphere (Dhawan et al, 2004). Passion flower's long history in herbal medicine includes its use as a treatment for colic, diarrhea, dysentery, menstrual pain, skin eruptions, conjunctivitis, hemorrhoids, and muscle spasms. However, the inclusion in any anti-obesity formula also involves its central nervous system effects. Passion Flower has demonstrated stress reduction effects. Reducing stress is expected to result in a default lowering of cortisol, reducing the accumulation of excess abdominal fat.

One of the problems with this subtropical plant is its identity. While there are a number of alkaloids which have been sold under the rubric of Passionflower, the most important and consistently effective candidate is Passiflora incarnata. The ethnobotanical database on the U.S. Agricultural Research Service's Web site lists the total alkaloid content of P. incarnata as 100 to 900 ppm and the total flavonoid content as 1.2 – 3.9 percent. Twenty six components fall into two categories: 20 flavonoids (including a cyanogenic glycoside and gynocardine) and 6 alkaloids. Some researchers have ascribed the sedative effects of P. incarnata ip indole alkaloids such as Harmane and its relatives, harmaline and harmol. However, others have suggested that P. incarnata's alkaloid content is too small to cause this and other CNS effects and that flavonoids, such as apigenin, luteolin, or their glycosides, are more likely to account for CNS bioactivity.

Most recently, scientists have isolated a highly anxiolytic, trisubstituted benzoflavone moiety from a P. incarnata extract. In one study, P. incarnata extract restored the libido of aging male rats (Dhawan et al, 2002a). In other studies, P. incarnata extract restored fertility and libido that had been compromised by addiction to tetrahydrocannabinol (Dhawan and Sharma, 2003), alcohol (Dhawan and Sharma, 2002), and nicotine (Dhawan and Sharma, 2002). Further research suggests the effectiveness of P. incarnata extract on anxiety related to alcohol withdrawal Dhawan et al, 2002b). There are also double-blind randomized studies indicating that Passiflora extract is relatively effective compared to oxazepam for the management of generalized anxiety disorder (Akhondzadeh et al, 2001a). There is even evidence, from a double-blind randomized controlled trial, that Passiflora may be an effective adjuvant in the management of opiate withdrawal (Akhondzadeh et al, 2001b). In addition, Passiflora was shown to reduce benzodiazepine dependence in mice (Dhawan et al, 2003). In fact, many pharmacological investigations have confirmed the sedative effects of Passiflora, especially in the P. incarnata form (Krenn, 2002). We have formulated a proprietary nutraceutical using a fragmented or cut, dried aerial parts of P. incarnata supplied by Euromed. It has been demonstrated that the separated leaves afford the best possible CNS results, and in fact, selection of the entire aerial parts excluding the flowers may prove to be the optimum approach for pocking up the bioactive plant parts of P. incarnata (Dhawan et al, 2001). The importance of standardization of preparations of Passiflora has been actively studied especially as it relates to the anxiolytic activity (Dhawan et al, 2002c).

In the early '70s', it was first suggested that serotonin, a brain neurotransmitter, functions as a biological substrate of stress (Geller and Blum, 1970). In fact, induction of stress in rodents was attenuated by injections of the serotonin chemical synthesis depletor Para-Chlora-Phenylalanine (PCA). Others have also shown the involvement of serotonin and dopamine in stress production in both animals and humans. Moreover, amino-acid and enkephalinase inhibition therapy was shown to reduce stress in polysubstance abusers in a double-blind randomized controlled trial in humans using skin conductance levels (Blum et al, 1988a,b). These findings provide further support for the use of Passiflora extracts in the treatment of anxiety disorders, especially those mediated by serotonin, which, interestingly enough, is homologous to an indole phytoconstituent of Passiflora extracts. Furthermore, the DRD2 A1 allele has been associated with stress disorders (Comings and Blum, 2000).

XI. Future perspective

In the future, our goal as scientists should be to analyze before we act, test before we recommend usage and/or prescribe, so that we can better understand the genetic factors influencing the obesity confronted by an individual. By understanding the evolutionary contributing factors to their outward presentation, we can better provide the obese individual with a customized program to bring their body composition into balance involving behavioral and biological changes and treatments. This simple doctrine relates back to the understanding of the "thrifty gene" concept. In this regard, we hypothesize that using a multi-variant nutrigenomic index for the purposes of customizing or adjusting the formulation of nutritional supplements will result in an improved and novel approach to the diagnosis, stratification, prognosis, and treatment of RDS induced obesity and related behaviors. This multi-variant genetic index, or Geneprofile™ (a published and patented process, LifeGen, Inc. La Jolla, California) (Blum et al, 2006c) is derived by analyzing genotype and/or phenotype through measuring multiple genetic mutations of single nucleotide polymorphisms, gene expression, or other forms of genetic and phenotypic measurements. This process will provide the opportunity for DNA-customized nutrition covering multiple genes involved in RDS, as well as the metabolism, efficacy and/or toxicity associated with specific vitamins, minerals, herbal supplements, homeopathic ingredients and other ingredients in the nutritional and/or dietary supplement regimen. We strongly believe that utilization of this genomic approach, independent of whether we are administering a pharmaceutical and/or a nutraceutical

genomic resolution, will result in enhanced positive clinical outcomes.

X. Conclusion

Just as obesity affects multiple systems of the body resulting in a plethora of co-morbidities, its treatment must consider the contributing factors and health of multiple systems. Existing weight loss tactics for the most part have failed to provide successful means to achieve sustainable healthy body composition and weight loss accordingly due to a myopic view of the cause of obesity. Emerging technologies that customize nutritional/nutraceutical needs of an individual based on genetic traits, merit further investigation, as preliminary data appear very promising. It is common knowledge that factors like exercise or extreme exertion, disease, drug intake, poor diet, etc., alter nutritional needs. Along this line, research has shown that genetic polymorphisms also alter nutritional needs to maintain healthy homeostasis. This is particularly true for phenotypes exhibiting polymorphisms predisposing aberrant pleasure seeking (and addictive) behavior, excessive cravings and obesity, and related Metabolic Syndrome X disorders. There is significant evidence to substantiate the existence of RDS as a new paradigm shift in the understanding of obesity. Moreover, we and others have established a role of catecholaminergic pathways in aberrant substance seeking behavior, in particular cravings for carbohydrates. The genetic basis for generalized craving behavior is the subject of intense investigation. There is growing evidence to support the augmentation of precursor amino acid therapy and enkephalinase and COMT inhibition leading to enhanced levels of neurotransmitters: serotonin, enkephalins, GABA and dopamine/norepinephrine (Blum et al, 2007). Utilization of this combination in LG839 nutraceutical complex, including Synaptamine, Super CitriMax, Passion flower and a proprietary oxygen coordinated chromium nicotinate have been evaluated in an open label trial. Based on a number of case reports, it seems possible that this unique combination of ingredients may have a generalized anti-stress and anti-craving effect resulting in an inhibition of carbohydrate bingeing, inducing fat loss and enhanced body image/composition. This is the first time the components of this formula have been combined at the clinically tested dosage levels indicated, to promote successful and sustainable loss of fat and improvement in body composition. While there are a number of important pathways identified on the road toward unwarranted weight gain, weight and obesity can be independent of each other. And, it is clear from the path analysis concerning the effects of Synaptamine™ complex that these same pathways are interactive and multi-directional, resulting in a net enhancement of body composition. The utilization of Synaptamine™ is a classical example of a path analysis and is statistically borne out herein and warrants further investigation.

Additionally, there are a number of studies cited above that relate not only to the serotonergic and leptinergic pathways, weight loss and weight regain, but the dopaminergic and other endorphinergic genes (POMC) are associated with obesity in both animals and humans. There is also the work of Nora Volkow and others on D2 receptor density utilizing PET to determine low D2 receptors in obese humans. There is evidence as cited above showing the relationship between body type and the D2 receptors. There are studies to show the relationship of dopamine receptors and hyperphagia, body mass index and carbohydrate bingeing. There is also evidence showing associations with the D2 A1 allele as well as the leptin receptor gene (which has links to dopaminergic fibers in the mesolimbic system) and carbohydrate bingeing, obesity, and body mass index as well as energy expenditure (Noble, 2003).

In essence, similar to pharmaceutical counter parts such as Meridia and Acomplia, neuronutrient amino-acid based compositions 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 D_2 receptors, especially in carriers of the A_1 allele (low D_2 receptors and high glucose craving), and over time (possibly 6-8 weeks) effects RNA transcription leading to a proliferation of D_2 receptors, thereby, reducing craving for carbohydrates. Interestingly, among many other genes the dopamine D2 receptor gene is now part of the Human Obesity Gene Map (Rankinen et al, 2005). In essence understanding the evolutionary concepts related to eating behavior and genetic antecedents such as the “thrifty gene” (inter-relationships of fasting/ starvation and fat production) enabled us to develop these newer genomic concepts as they relate to obesity and eating behavior. We contend that if as scientists we test these theories proposed and obtain positive results the potential of stopping the obesity epidemic as a genetic pleiotropic disease we call “Neurobseigenics” may be achievable someday.

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References

Adler CM, Elman I, Weisenfeld N, Kestler L, Pickar D, Breier A (**2000**) Effects of acute metabolic stress on striatal dopamine release in healthy volunteers. **Neuropsychopharma** 2, 545-550.

Afman L, Muller M (**2006**) Nutrigenomics: From Molecular Nutrition to Prevention of Disease. **J Am Diet Assoc** 106, 569-576.

Akhondzadeh S, Kashani L, Mobaseri M, Hosseini SH, Nikzad S, Khani M (**2001a**) Passionflower in the treatment of opiates withdrawal: a double-blind randomized controlled trial. **J Clin Pharm Ther** 26, 369-273.

Akhondzadeh S, Naghavi HR, Vazirian M, Shayeganpour A, Rashidi H, Khani M (**2001b**) Passionflower in the treatment

of generalized anxiety: a pilot double-blind randomized controlled trial with oxazepam. **J Clin Pharm Ther** 26, 63-67.

Ashrafi K, Chang FY, Watts JL, Fraser Ag, Kamath RS, Ahringer J, Ruvkun G (**2003**) Genome-wide RNAi analysis of Caenarhaditis elegans fat regulatory genes. **Nature** 421, 268-272.

Bagchi SP, Smith TM (**1979**) Synaptosomal dopamine from phenylalanine in brain: effects of some dopaminergic agents. **Res Comm Chem Pathol Pharmacol** 26, 447-458.

Balcioglu A, Wurtman RJ (**2000**) Sibutramine, a serotonin uptake inhibitor, increases dopamine concentrations in rat striatal and hypothalamic extracellular fluid. **Neuropharmacology** 39, 2352-2359.

Basile AS, Fedorova I, Zapata A, Liu X, Shippenberg T, Duttaroy A, Yamada M, Wess J (**2002**) Deletion of the M5 muscarinic acetylcholine receptor attenuates morphine reinforcement and withdrawal but not morphine analgesia. **Proc Natl Acad Sci** 99, 11452-11457.

Bello NT, Sweigart KL, Lakoski JM, Norgren R, Hajnal A (**2003**) Restricted feeding with scheduled sucrose access results in an upregulation of the rat dopamine transporter. **Am J Physiol Regul Integr Comp Physiol** 284, R1260-1268.

Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH (**1996**) Population and familial association between the D4 dopamine receptor gene and measures of novelty seeking. **Nat Genet** 12, 81-84.

Berrendero F, Mendizabal V, Robledo P, Galeote L, Bilkei-Gorzo A, Zimmer A, Maldonado R (**2005**) Nicotine-induced antinociception, rewarding effects, and physical dependence are decreased in mice lacking the preproenkephalin gene. **J Neurosci** 25, 1103-1112.

Biebermann H, Castaneda TR, van Landeghem F, von Deimling A, Escher F, Brabant G, Hebebrand J, Hinney A, Tschop MH, Gruters A, Krude H (**2006**) A role for β-melanocyte-stimulating hormone in human body-weight regulation. **Cell Metab** 3, 141-146.

Bina KG, Cincotta AH (**2000**) Dopaminergic agonists normalize elevated hypothalamic neuropeptide Y and corticotropin-releasing hormone, body weight gain, and hyperglycemia in ob/ob mice. **Neuroendocrinology** 71, 68-78.

Blum K, Allison D, Trachtenberg MC, Williams RW, Loeblich LA (**1988c**) Reduction of both drug hunger and withdrawal against advice rate of cocaine abusers in a 30-day inpatient treatment program by the neuronutrient Tropamine. **Curr Ther Res** 43, 1204-1214.

Blum K, Braverman ER, Holder JM, Lubar JF, Monastra VJ, Miller D, Lubar JO, Chen TJ, Comings DE (**2000**) Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. **J Psychoactive Drugs** 32(Suppl:i-iv), 1-112.

Blum K, Braverman ER, Wood RC, Gill J, Li C, Chen TJ, Taub M, Montgomery AR, Sheridan PJ, Cull JG (**1996b ken**) Increased prevalence of the Taq I A1 allele of the dopamine receptor gene (DRD2) in obesity with comorbid substance use disorder: a preliminary report. **Pharmacogenetics** 6, 297-305.

Blum K, Chen TJ, Meshkin B, Downs BW, Gordon CA, Blum S, Mangucci JF, Braverman ER, Arcuri V, Deutsch R, Pons MM (**2006c**) Genotrim™ a DNA-customized nutrigenomic product, targets genetic factors of obesity: Hypothesizing a dopamine-glucose correlation demonstrating reward deficiency syndrome (RDS). **Med Hypotheses** 68, 844-52.

Blum K, Chen TJH, Downs WB, Gordon CA, Blum S, Mengucci JF, Braverman ER, Arcuri V, Varshavskiy, Deutch R, Pons, MM, Meshkin B (**2006a**) Reward deficiency syndrome in obesity: a preliminary cross-sectional trial with a Genotrim variant. **Adv Ther** 23, 1040-51.

Blum K, Chen TJH, Downs,WB, Meshkin B, Blum SH, Pons MM, Mengucci JF, Waite RL, Arcuri V, Varshofsiky M, Braverman ER (**2006b**) Synaptamine (SG8839) ™ an amino-acid enkephalinase inhibitor nutraceutical Improves recovery of alcoholics, a subtype of Reward deficiency syndrome(RDS). **Trends in Applied Sciences Research** 2, 132-138.

Blum K, Chen TJH, Meshkin B, Waite RL, Downs WB, Blum SH, Mengucci JF, Arcuri V, Braverman ER, Paloma T (**2007**) 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 Hyp** 69, 1054-60.

Blum K, Cull JG, Chen TJH, Garcia-Swan S, Holder JM, Wood R, Braverman ER, Buci LR, Trachtenberg MC (**1997**) Clinical evidence For Effectiveness of Phencal TM in maintaining weight loss in an open-label, controlled, 2-year study. **Curr Ther Res** 58, 745-763.

Blum K, Meshkin B, Downs BW (**2006d**) DNA based customized nutraceutical "gene therapy" utilizing a genoscore: A hypothesized paradigm shift of a novel approach to the diagnosis, stratification, prognosis and treatment of inflammatory process in the human. **Med Hypoth** 66, 1008-1018.

Blum K, Noble EP, Sheridan PJ, Finley O, Montgomery A, Ritchie T, Ozkaragoz T, Fitch RJ, Sadlack F, Sheffield, D, Dahlmann T, Halbardier S, Nogami H (**1991**) Association of the A1 allele of the D2 dopamine receptor gene with severe alcoholism. **Alcohol** 8, 409-416.

Blum K, Noble EP, Sheridan PJ, Montgomery A, Ritchie T, Jagadeeswaran P, Nogami H, Briggs AH, Cohn JB (**1990**) Allelic association of human dopamine D2 receptor gene in alcoholism. **JAMA** 263, 2055-2060.

Blum K, Noble, EP, Volkow ND, Url G, Comings DE (**2001**) 1st Conference on Reward Deficiency Syndrome: Genetic Antecedents and Clinical Pathways. San Francisco, California, USA. **Mol Psychiatry** 6 Suppl S1-8.

Blum K, Payne J (**1991**) Alcohol and The Addictive Brain" The Free Press (Simon and Schuster), New York, NY.

Blum K, Sheridan PJ, Wood RC, Braverman ER, Chen TJ, Comings DE) (**1995**) Dopamine D2 receptor gene variants: association and linkage studies in impulsive-addictive-compulsive behaviour. **Pharmacogenetics** 5, 121-41.

Blum K, Sheridan PJ, Wood RC, Braverman ER, Chen TJ, Cull JG, Comings DE (**1996a**) The D2 dopamine receptor gene as a determinant of reward deficiency syndrome. **J R Soc Med** 89, 396-400.

Blum K, Trachtenberg MC, Cook DW (**1990**) Neuronutrient effects on weight loss in carbohydrate bingers: An open clinical trial. **Curr Ther Res** 48, 217-233.

Blum K, Trachtenberg MC, Elliott CE, Dingler ML, Sexton RL, Samuels AI, Cataldie L (**1988b**) Enkephalinase inhibition and precursor amino acid loading improves Inpatient treatment of alcohol and polydrug abusers: Double-blind placebo-controlled study of the nutritional adjunct SAAVE. **Alcohol** 5481493

Blum K, Trachtenberg MC, Ramsey J (**1988a**) Improvement of In Patient treatment of the alcoholic as a function of neuronutrient restoration: A pilot study. **Int J Addiction** 23, 991-998.

Blum K, Wallace JE, Trachtenberg MC (**1987**) Enkephalinase inhibition: regulation of ethanol intake in genetically predisposed mice. **Alcohol** 4, 449-456.

Blum K. Sheridan PJ, Wood RC, Braverman ER, Chen TJH, Cull JG, Comings DE (**1996b**) The D2 dopamine receptor gene as

a determinant of reward deficiency syndrome. **J R Soc Med** 89, 396-400.

Boettiger CA, Mitchell JM, Tavares VC, Robertson M, Joslyn G, D'Esposito M, Fields HL (**2007**) Immediate reward bias in humans: fronto-parietal networks and a role for the catechol-O-methyltransferase 158(Val/Val) genotype. **J Neurosci** 27, 14383-14391.

Bongiovanni R, Young D, Newbould E, Jaskiw GE (**2006**) Increased striatal dopamine synthesis is associated with decreased tissue levels of tyrosine. **Brain Res** 1115, 26-36.

Bowirrat A, Oscar-Berman M (**2005**) Relationship between dopaminergic neurotransmission, alcoholism, and Reward Deficiency syndrome. **Am J Med Genet B Neuropsychiatr Genet** 132, 29-37.

Brandacher G, Winkler C, Aigner F, Schwelberger H, Schroecksnadel K, Margreiter R, Fuchs D, Weiss HG (**2006**) Bariatric surgery cannot prevent tryptophan depletion due to chronic immune activation in morbidly obese patients. **Obes Surg** 16, , 541-548.

Brown RJ, Blum K, Trachtenberg MC (**1990**) Neurodynamics of relapse prevention: a neuronutrient approach to outpatient DUI offenders. **J Psychoact Drugs** 22, 173-187.

Brown RP, Gerbarg PL, Graham B (**2005**) The Rhodiola Revolution: Transform Your Health With the Herbal Breakthrough of the 21st Century. New York, St Martins Press.

Caine SB, Koob GF (**1993**) Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. **Science** 260, 1814-1816.

Carelli RM (**2004**) Nucleus accumbens cell firing and rapid dopamine signaling during goal-directed behaviors in rats. **Neuropharmacology** 47(Suppl 1), 180-189.

Chen TJH, Blum K, Kaats G, Braverman ER, Eisenberg A, Sherman M, Davis K, Comings DE, Wood R, Pullin D, Arcuri V, Varshavski M, Mengucci JE, Blum SH, Downs BW, Meshkin B, Waite RL, Williams L, Schoolfield J, White L (**2007**) Chromium Picolinate (CrP) a putative anti-obesity nutrient induces changes in body composition as a function of the *Taq1* dopamine D2 receptor polymorphisms in a randomized double-blind placebo controlled study. **Gene Ther Mol Biol** 11, 1-10.

Chen TJH, Blum, K, Payte JT, Schoolfield J, Hopper D, Stanford M, Braverman ER (**2004**) Narcotic antagonists in drug dependence: pilot study showing enhancement of compliance with SYN-10, amino-acid precursors and enkephalinase inhibition therapy. **Med Hypoth** 63, 538-548.

Cheng HY, Laviolette SR, van der Kooy D, Penninger JM (**2004**) DREAM ablation selectively alters THC place aversion and analgesia but leaves intact the motivational and analgesic effects of morphine. **Eur J Neurosci** 19, 3033-3041.

Clement K, Vaisse C, Manning BS, Basdevant A, Guy-Grand B, Ruiz J, Silver KD, Shuldiner AR, Froguel P, Strosberg AD (**1995**) Genetic variation in the β 3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. **N Engl J Med** 333, 352-354.

Colantuoni C, Rada P, McCarthy J, Patten C, Avena NM, Chadeayne A, Hoebel BG (**2002**) Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. **Obes Res** 10, 478-488.

Cold JA (**1996**) NeuRecover-SA in the treatment of cocaine withdrawal and craving: a pilot study. **Clin Drug Invest** 12, 1-4.

Collaku A, Rankinen T, Rice T, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C (**2004**) A genome-wide linkage scan for dietary energy and nutrient intakes: the health, risk factors, exercise training, and genetics (HERITAGE) family study. **Am J Clin Nutr** 79, 881-886.

Comings DE, Blum K (**2000**) Reward deficiency syndrome: genetic aspects of behavioral disorders. **Prog Brain Res** 126, 325-41.

Comings DE, Flanagan SD, Dietz G, Muhleman D, Knell E, Gysin R (**1993**) The dopamine D2 receptor (DRD2) as a major gene in obesity and height. **Biochem Med Metab Biol** 50, 176-185.

Conti G, Blandini F, Tassorelli C, Giubilei F, Fornai F, Zocchi A, Orzi F (**2001**) Intrastriatal injection of D1 or D2 dopamine agonists affects glucose utilization in both the direct and indirect pathways of the rat basal ganglia. **Neurosci Let** 309, 161-164.

Cooney CA, Dave AA, Wolff GL (**2002**) Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. **J Nutr** 132(supplement 8), S2393-S2400.

Cooper JR, Bloom FR, Roth RH (**1966**) The Biochemical Basis of Neuropharmacology. New York, Oxford University Press, pg 226.

Corthesy-Theulaz I, den Dunnen Jt, Fere P, Geurts JMW, Muller M, Belzen NV, van Ommen B (**2005**) Nutrigenomics: The impact of Biomics Technology on Nutrition Research. **Ann Nutr Metab** 49, 355-365.

Darwin F, Ed (**1888**) (Charles Darwin: Life and letters. London, Murray.) .

Davies KM, Heaney RP, Recker RR, Lappe JM, Barger-Lux MJ, Rafferty K, Hinders S (**2000**) Calcium Intake and Body Weight. **J Clin Endocrinol Metab** 85, 4635-4638.

DeFrance JF, Hymel C, Trachtenberg MC, Ginsberg LD, Schweitzer FC, Estes S, Chen TJH, Braverman ER, Cull JG, Blum K (**1997**) Enhancement of attention processing by Kantrol in healthy humans: A pilot study. **Clin Electroenceph** 28, 68-75.

Deriaz O, Theriault G, Lavallee N, Fournier G, Nadeau A, Bouchard C (**1991**) Human resting energy expenditure in relation to dietary potassium. **Am J Clin Nutr** 54, 628-634.

Dhawan K, Dhawan S, Chhabra S (**2003**) Attenuation of benzodiazepine dependence in mice by a tri-substituted benzoflavone moiety of Passiflora incarnata Linneaus: a non-habit forming anxiolytic. **J Pharm Sci** 6, 215-222.

Dhawan K, Dhawan S, Sharma A (**2004**) Passiflora: a review update. **J Ethnopharmacol** 94, 1-23.

Dhawan K, Kumar R, Kumar S, Sharma A (**2001**) Correct Identification of Passiflora incarnata Linn a Promising Herbal Anxiolytic and Sedative. **J Med Food** 4, 137-144.

Dhawan K, Kumar S, Sharma A (**2002a**) Beneficial effects of chrysin and benzoflavone on virility in 2-year-old male rats. **J Med Food** 5, 43-48.

Dhawan K, Kumar S, Sharma A (**2002b**) Suppression of alcohol-cessation-oriented hyper-anxiety by the benzoflavone moiety of Passiflora incarnata Linneaus in mice. **J Ethnopharmacol** 81, 239-244.

Dhawan K, Kumar S, Sharma A (**2002c**) Comparative anxiolytic activity profile of various preparations of Passiflora incarnata linneaus: a comment on medicinal plants' standardization. **J Altern Complement Med** 8, 283-291.

Dhawan K, Sharma A (**2002**) Prevention of chronic alcohol and nicotine-induced azospermia, sterility and decreased libido, by a novel tri-substituted benzoflavone moiety from Passiflora incarnata Linneaus in healthy male rats. **Life Sci** 71, 3059-3069.

Dhawan K, Sharma A (**2003**) Restoration of chronic-Delta 9-THC-induced decline in sexuality in male rats by a novel benzoflavone moiety from Passiflora incarnata Linn. **Br J Pharmacol** 138, 117-120.

Di Chiara G, Acquas E, Tanda G, Cadoni C (**1993**) Drugs of abuse: biochemical surrogates of specific aspects of natural reward? **Biochem Soc Symp** 59, 65-81.

Downs BW, Bagchi M, Subbaraju GV, Shara MA, Preuss HG, Bagchi D (**2005**) Bioefficacy of a novel calcium-potassium salt of (-)-hydroxycitric acid. **Mutat Res** 579, 149-162.

Dulloo AG, Jacquet J (**1998**) Adaptive reduction in basal metabolic rate in response to food deprivation in humans: a role for feedback signal from the fat stores. **Am J Clin Nutr** 68, 599-606.

Dulloo AG, Jacquet J (**1999**) The control of partitioning between protein and fat during human starvation: its internal determinants and biological significance. **Brit J Nutr** 82, 339-356.

Ebstein RP, Novick O, Umansky R, Priel B, Osher Y, Blaine D, Bennett ER, Nemanov L, Katz M, Belmaker RH (**1996**) Dopamine D4 receptor exon 111 polymorphism associated with the human personality trait of novelty seeking. **Nat Genet** 12, 78-80.

Farooqi S, Rau H, Whitehead J, O'Rahilly S (**2006**) ob gene mutations and human obesity. **Proc Nutr Soc** 57, 471-475.

Filozof CM, Murua C, Sanchez MP, Brailovsky C, Perman M, Gonzalez CD, Ravussin E (**2000**) Low plasma leptin concentration and low rates of fat oxidation in weight-stable post-obese subjects. **Obes Res** 8, 205-210.

Gardner EL, Vorel SR (**1998**) Cannabinoid transmission and reward -related events. **Neurobiol Dis** 5, 502-533.

Gartside SE, Cowan PJ, Sharp T (**1992**) Effect of 5-hydroxy-l-tryptophan on the release of 5-HT in rat hypothalamus *in vivo* as measured by microdialysis. **Neuropharm** 31, 9-14.

Geller I, Blum K (**1970**) The effects of 5 HTP on para chlorphenylalanine (p CPA) attenuation of "conflict" behavior. **Eur J Pharmacol** 9, 319-324.

George SR, Cheng R, Nguyen T, Israel Y, O'Dowd BF (**1993**) Polymorphisms of the D4 dopamine receptor alleles in chronic alcoholism. **Biochem Biophys Res Commun** 196, 107-114.

Germano C, Ramazanov Z (**1999**) Artic Root (Rhodiola rosea). New York, Kensington Publishing Corp pg 45.

Ghozland S, Matthes HW, Simonin F, Filliol D, Kieffer BL, Maldonado R (**2002**) Motivational effects of cannabinoids are mediated by mu-opioid and kappa-opioid receptors. **J Neurosci** 22, 1146-1154.

Gniuli D, Capristo E, Liverani E, Greco AV, Calvani M, Mingrone G (**2005**) Effects of binge eating behavior on fuel oxidation and body composition. **J Am Coll Nutr** 24, 244-249.

Goldman D, Urbanek M, Guenther D, Robin R, Long JC (**1997**) Linkage and association of a functional DRD2 variant (Ser311Cys) and DRD2 markers to alcoholism, substance abuse and schizophrenia in Southwestern American Indians. **Am J Med Genet** 74, 386-394.

Goldman D, Urbanek M, Guenther D, Robin R, Long JC (**1998**) A functionally deficient DRD2 variant (Ser311Cys) is not linked to alcoholism and substance abuse. **Alcohol** 16, 47-52.

Gray J, Yeo GS, Cox JJ, Morton J, Adlam AL, Keogh JM, Yanovski JA, El Gharbawy A, Han JC, Tung YC, Hodges JR, Raymond FL, O'rahilly S, Farooqi IS (**2006**) Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. **Diabetes** 55, 3366-71.

Green AI, Zimmet SV, Strous RD, Schildkraut JJ (**1999**) 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** 6, 287-96.

Greenwood MR, Cleary MP, Gruen R, Blase D, Stern JS, Triscari J, Sullivan AC (**1981**) Effect of (-)-hydroxycitrate on development of obesity in Zucker obese rat. **Am J Physiol** 240, E72-8.

Groop LC, Tuomi T (**1997**) Non-insulin-dependent diabetes mellitus--a collision between thrifty genes and an affluent society. **Ann Med** 29, 37-53.

Heaney RP (**2003**) Normalizing calcium intake: projected population effects for body weight. **J Nutr** 133, 268S-270S.

Heaney RP, Dowell MS, Bierman J, Hale CA, Bendich A (**2001**) Absorbability and cost effectiveness in calcium supplementation. **J Am Coll Nutr** 20, 239-246.

Hegele RA, Jugenberg M, Connelly PW, Jenkins DJA (**1997**) Evidence for gene -diet interaction in the response of blood pressure to dietary fiber. **Nutr Res** 17, 1229-1238.

Hill JO (**2006**) Understanding and Addressing the Epidemic of Obesity: An Energy Balance Perspective. Endocr Rev, in press).

Hu YJ, Diamond AM (**2003**) Role of glutathione peroxidase 1 in breast cancer: Loss of heterozygosity and allelic differences in the response to selenium. **Cancer Res** 63, 3347-3351.

Kaats GR, Blum K, Fisher JA, Adelman JA (**1996**) Effects of chromium picolinate supplementation of body composition: A randomized, double-masked, placebo-controlled study. **Curr Ther Res** 57, 747-756.

Kaats GR, Wise JA, Blum K, Morin RA, Adelman JD, Craig J, Croft HA (**1992**) The short term therapeutic efficacy of treating obesity with a plan of improved nutrition and moderate caloric restriction. **Curr Ther Res** 51, 261-274.

Kasim S, Blake BL, Fan X, Chartoff E, Egami K, Breese GR, Hess EJ, Jinnah HA (**2006**) The role of dopamine receptors in the neurobehavioral syndrome provoked by activation of L-type calcium channels in rodents. **Dev Neurosci** 28, 505-517.

Kim KS, Lee KW, Lee KW, Im JY, Yoo JY, Kim SW, Lee JK, Nestler EJ, Han PL (**2006**) Adenylyl cyclase type 5 (AC5) is an essential mediator of morphine action. **Proc Natl Acad Sci** 103, 3908-3913.

Kim KW, Kim HD, Jung JS, Woo RS, Kim HS, Suh HW, Kim YH, Song DK (**2001**) Characterization of antidepressant-like effects of p-synephrine stereoisomers. **Naunyn Schmiedebergs Arch Pharmacol** 364, 21-26.

Kogan HA, Marsden CA, Fone KC (**2002**) DR4004, a putative 5-HT (7) receptor antagonist, also has functional activity at the dopamine D2 receptor. **Eur J Pharmacol** 449, 105-111.

Koob GF, Bloom FE (**1988**) Cellular and molecular mechanism of drug dependence. **Science** 242, 715-723.

Koshimura K, Tanaka J, Murakami Y, Kato Y (**2000**) Effect of high concentration of glucose on dopamine release from Pheochromocytoma -12 cells. **Metabolism** 52, 922-926.

Large V, Hellstrom L, Reynisdottir S, Lonnqvist F, Eriksson P, Lannfelt L, Arner P (**1997**) Human β-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte β-2 adrenoceptor function. **J Clin Invest** 100, 3005-3013.

Levin BE (**2001**) Glucosensing neurons do more than just sense glucose. **Int J Obes Relat Metab Disord Suppl** 5, S68-72.

Levin BE (**2000**) Glucose-regulated dopamine release from substantia nigra neurons. **Brain Res** 874, 158-64.

Li T, Xu K, Deng H, Cai G, Liu J, Liu X, Wang R, Xiang X, Zhao J, Murray RM, Sham PC, Collier DA (**1997**) Association analysis of the dopamine D4 gene exon III VNTR and heroin abuse in Chinese subjects. **Mol Psych** 2, 413-416.

Linazaroso G, van Blercom N, Lasa A (**2004**) Hypothesis: Parkinson's disease, reward deficiency syndrome and addictive effects of levodopa. **Neurologia** 19, 117-27.

Lochner C, Hemmings SM, Kinnear CJ, Niehaus DJ, Nel DG, Corfield VA, Moolman-Smook JC, Seedat S, Stein DJ (**2005**) Cluster analysis of obsessive-compulsive spectrum disorders in patients with obsessive-compulsive disorder: clinical and genetic correlates. **Compr Psychiatry** 46, 14-19.

Loe YC, Bergeron N, Rodriguez N, Schwarz JM **(2001)** Gas chromatography/mass spectrometry method to quantify blood hydroxycitrate concentration. **Anal Biochem** 292, 148-54.

Lowenstein JM (**1971**) Effect of (-)-Hydroxycitrate on Fatty Acid Synthesis by Rat Liver *in Vivo*. **J Biol Chem** 346620

Lowenstein JM, Brunengraber H, (**1993**) Effect of (-)-Hydroxycitrate On Ethanol Metabolism. **FEBS Letters** 36130.

Luan J, Brown PO, Harding Ah, Halsall DJ, O'Rahilly S, Chatterjee VK, Wareham NJ (**2001**) Evidence for gene-nutrient interaction at the PPAR γ locus. **Diabetes** 50, 1625-1638.

Manzardo AM, Penick EC (**2006**) A theoretical argument for inherited thiamine insensitivity as one possible biological cause of familial alcoholism. **Alcohol Clin Exp Res** 30, 1545-50.

Milner JD, Irie K, Wurtman RJ (**1986**) Effect of phenylalanine on the release of endogenous dopamine from rat striatal slices. **J Neurochem** 47, 1444-1448.

Molla G, Sacchi S, Bernasconi M, Pilone MS, Fukui K, Polegioni L (**2006**) Characterization of human D-amino acid oxidase. **FEBS Lett** 580, 2358-64.

Mustafa AK, Kim PM, Snyder SH (**2004**) D-Serine as a putative glial neurotransmitter. **Neuron Glia Biol** 1, 275-281.

Nargund RP, Strack AM, Fong TM (**2006**) Melanocortin-4 receptor (MC4R) agonists for the treatment of obesity. **J Med Chem** 49, 4035-4043.

Nazar K, Kaciuba-Uscilko H, Szczepanik J, Zemba AW, Kruk B, Chwalbinska-Moneta J, Titow-Stupnicka E, Bicz B, Krotkiewski M (**1996**) Phosphate supplementation prevents a decrease of triiodothyronine and increases resting metabolic rate during low energy diet. **J Physiol Pharmacol** 47, 373-383.

Neel JV (**1962**) Diabetes mellitus: a "thrifty" genotype rendered detrimental by " progress"? **Am J Hum Genet** 14, 353-362.

Noble EP, Blum K, Khalsa ME, Ritchie T, Montgomery A, Wood RC, Fitch RJ, Ozkaragoz T, Sheridan PJ, Anglin MD, et al (**1993**) Allelic association of the D2 dopamine receptor gene with cocaine dependence. **Drug Alcohol Depend** 33, 271-285.

Ottosson M, Lonnroth P, Bjorntorp P, Eden S (**2000**) Effects of cortisol and growth hormone on lipolysis in human adipose tissue. **J Clin Endocrinol Metab** 85, 799-803.

Pan DA, Mater MK, Theen AP, Peters JM, Gonzalez FJ Jump DB (**2000**) Evidence against the peroxisome proloferator-activated receptor a (PPAR a) as the mediator fpr polyunsaturated fatty acid suppression of hepatic L-pyruvate kinase gene transcription. **J Lipid Res** 41, 742-751.

Panossian A, Wagner H (**2005**) Stimulating effect of adaptogens: an overview with particular reference to their efficacy following single dose administration. **Phytother Res** 19, 819-38.

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

Ponce G, Jimenez-Arriero MA, Rubio G, Hoenicka J, Ampuero I, Ramos JA, Palomo T (**2003**) The A1 allele of the DRD2 gene (TaqI A polymorphisms) is associated with antisocial personality in a sample of alcohol-dependent patients. **Eur Psychiatry** 18, 356-360.

Poston WS 2nd, Ericsson M, Linder J, Haddock CK, Hanis CL, Nilsson T, Astrom M, Foreyt JP (**1998**) D4 dopamine receptor gene exon III polymorphism and obesity risk. **Eat Weight Disord** 3, 71-77.

Prentice AM, Rayoco-Solon P, Moore SE (**2005**) Insights from the developing world: thrifty genotypes and thrifty phenotypes. **Proc Nutr Soc** 64, 153-161.

Preuss HG, Bagchi D, Bagchi M, Rao CV, Dey DK, Satyanarayana S (**2004**) Effects of a natural extract of (-)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and Gymnema sylvestre extract on weight loss. **Diabet Obes Metab** 6, 171-180.

Ramanazov Z, del Mar Bernard SM (**1999**) Effective natural stress and weight management using Rhodiola rosea and Rhododendron caucasicum. East Canaan, ATNS/Safe Goods Publishing.

Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Perusse L, Bouchard C (**2006**) The human obesity gene map: the 2005 update. **Obesity (Silver Spring)** 14, 529-644.

Rapuri PB, Gallagher JC, Kinyamu HK, Ryschon KL (**2001**) Caffeine intake increases the rate of bone loss in elderly women and interacts with vitamin D receptor genotypes. **Am J Clin Nutr** 74, 694-700.

Rivier C, Rivier J, Vale W (**1986**) Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor. **Science** 231, 607-609.

Ross J (**2000**) Amino-acid precursor and enkephalinase inhibition therapy: evidence for effectiveness in treatment of Reward Deficiency Syndrome (RDS), with particular emphasis on eating disorders. **Mol Psych** 6, S7.

Sakariah KK, Nageswara R (**1988**) Lipid-Lowering And Antiobesity Effect Of (-) Hydroxycitric Acid. **Nutr Res** 8, 209-212.

Sale C, Harris RC, Delves S, Corbett J (**2006**) Metabolic and physiological effects of ingesting extracts of bitter orange, green tea and guarana at rest and during treadmill walking in overweight males. **Int J Obes (Lond)** 30, 764-73.

Salmon AM, Evrard A, Damaj I, Changeux JP (**2004**) Reduction of withdrawal signs after chronic nicotine exposure of a-calcitonin gene-related peptide knock-out mice. **Neurosci Lett** 360, 73-76.

Shapses SA, Von Thun NL, Heymsfield SB, Ricci TA, Ospina M, Pierson RN Jr, Stahl T (**2001**) Bone turnover and density in obese premenopausal women during moderate weight loss and calcium supplementation. **J Bone Miner Res** 16, 1329-1336.

Shiroyama K, Moriwaki K, Yuge O (**1998**) The direct effect of dopamine on glucose release from primary cultured rat hepatocytes. **In Vivo** 12, 527-529.

Simonin F, Valverde O, Smadja C, Slowe S, Kitchen I, Dierich A, Le Meur M, Roques BP, Maldonado R, Kieffer BL (**1998**) Disruption of the kappa-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective kappa-agonist U-50, 488H and attenuates morphine withdrawal. **EMBO J** 17, 886-897.

Snyder SH, Kim PM (**2000**) D-amino acids as putative neurotransmitters: focus on D-serine. **Neurochem Res** 25, 553-60.

Spanswick D, Smith MA, Mirshamsi S, Routh VH, Ashford ML (**2000**) Insulin activates ATP-sensitive K+ channels in hypothalamic neurons of lean, but not obese rats. **Nat Neurosci** 3, 757-758.

Spielbauer B, Stahl F (**2005**) Impact of microarray technology in nutrition and food research. **Mol Nutr Food Res** 49, 908-917.

Ssaratikov AS, Krasnov EA, Chnikina LA, Duvidson LM, Sotova MI, Marina TF, Nechoda MF, Axenova RA, Tscherdinzeff SG (**1968**) Rhodiolosid, a new glycoside from Rhodiola rosea and its pharmacological properties. **Pharmazie** 23, 392-5.

Stobbe M (**2006**) "Survey: Most obese claim to eat healthy." Associated Press. Accessed on October 1, 2006 on http://www.boston.com/yourlife/health/diseases/articles/2006/08/02/survey_most_obese_claim_to_eat_healthy_1154518615/.

Sullivan AC, Hamilton JG, Miller ON, Wheatley VR (**1972**) Inhibition of lipogenesis in rat liver by (-)-hydroxycitrate. **Arch Biochem Biophys** 150, 183-90.

Teegarden D (**2003**) Calcium intake and reduction in weight or fat mass. **J Nutr** 133, 249S-251S.

Thanos PK, Volkow ND, Freimuth P, Umegaki H, Ikari H, Roth G, Ingram DK, Hitzemann R (**2001**) Overexpression of dopamine D2 receptors reduces alcohol self-administration. **J Neurochem** 78, 1094-1103.

Trijillo E, Davis C, Milner J (**2006**) Nutrigenetics, proteomics, metabolomics and the practice of dietetics. **J Am Dietetic Association** 106, 403-413.

Trugman JM, James CL (**1993**) D1 dopamine agonist and antagonist effects on regional cerebral glucose utilization in rats with intact dopaminergic innervation. **Brain Res** 607, 270-274.

Umhau JC, Petrulis SG, Diaz R, Rawlings R, George DT (**2003**) Blood glucose is correlated with cerebrospinal fluid neurotransmitter metabolites. **Neuroendocrinology** 78, 339-343.

Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S; RIO-Europe Study Group (**2005**) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. **Lancet** 365, 1389-97.

van Ommen B (**2004**) Nutrigenomics : exploiting systems biology in the nutrition and health arenas. **Nutrition** 20, 4-8.

Van Ommen B, Stierum R (**2002**) Nutrigenomics: exploiting systems biology in the nutrition and health arena. **Curr Opin Biotechnol** 13, 517-521.

Volkow ND, Fowler JS, Wang GJ, Hitzemann R, Logan J, Schlyer DJ, Dewey SL, Wolf AP (**1993**) Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. **Synapse** 14, 169-177.

Wald H, Garty H, Palmer LG, Popovtzer MM (**1998**) Differential regulation of ROMK expression in kidney cortex and medulla by aldosterone and potassium. **Am J Physiol Renal Physiol** 275, F239-F245.

Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N, Fowler JS (**2001**) Brain dopamine and obesity. **Lancet** 357, 354-357.

Ward SJ, Dykstra (**2005**) The role of CB1 receptors in sweet versus fat reinforcement: effect of CB1 receptor deletion, CB1 receptor antagonism (SR141716A) and CB1 receptor agonism (CP-55940). **Behav Pharmacol** 16, 381-388.

Wasada T (**2002**) Adenosine triphosphate-sensitive (K(ATP)) channel activity is coupled with insulin resistance in obesity and type 2 diabetes mellitus. **Intern Med** 41, 84-90.

Yeo GS, Connie Hung CC, Rochford J, Keogh J, Gray J, Sivaramakrishnan S, O'Rahilly S, Farooqi IS (**2004**) A *de novo* mutation affecting human TrkB associated with severe obesity and developmental delay. **Nat Neurosci** 7, 1187-1189.

Zemel MB (**2003**) Mechanisms of dairy modulation of adiposity. **J Nutr** 133, 252S-256S.

Zemel MB (**2003**) Role of dietary calcium and dairy products in modulating adiposity. **Lipids** 38, 139-146.