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Reviewing the role of putative candidate genes in "Neurobesigenics," a clinical subtype of Reward Deficiency Syndrome (RDS)

Research Article

Thomas J.H. Chen[1], Kenneth Blum[2,4,9,*], Gilbert Kaats[3], Eric Braverman[4], Dennis Pullin[5], Bernard W. Downs[6], Manuel Martinez-Pons[7], Seth H. Blum[8], Julie Mengucci[8], Debasis Bagchi[10,11], Manashi Bagchi[11], Ariel Robarge[6], Brian Meshkin[9], Vanessa Arcuri[4], Michael Varshavskiy[4], Allison Notaro[4], David E. Comings[12], Lisa White[13]

[1]Chang Jung Christian University, Tainan, Taiwan and Changhua Christian Hospital, Changhua, Taiwan
[2]Department o Physiology & Pharmacology, University of Wake Forest School of Medicine, Medical Center Boulevard Winston-Salem, North Carolina
[3]Health and Medical Research Foundation, San Antonio, Texas
[4]Path Medical Foundation, Park Ave., New York, New York
[5]Sports Medicine Institute, Baylor College of Medicine, Houston, Texas
[6]Allied Nutraceutical Research, Lederach, Pennsylvania
[7]Department of Education, Brooklyn College CUNY, New York, New York
[8]Synaptamine, Inc., San Antonio, Texas
[9]Salugen, Inc. San Diego, California
[10]Department of Pharmacy Sciences, Creighton University Medical Center, Omaha, Nebraska
[11]InterHealth Research Center, Benicia, California
[12]Carlsbad Science Foundation, Monrovia, California and City of Hope National Medical Center, Duarte, California
[13]Baylor College of Medicine, Department of Molecular Gentics Houston TX and DNA Services of America, Lafayette, LA.

***Correspondence**: Kenneth Blum, Ph. D. Department of Physiology and Pharmacology, University of Wake Forest School of Medicine, Medical Center Boulevard, North Carolina, 27157, USA; TEL-1-210-823-1504; FAX- 1-210-694-2680; e-mail: DRD2gene@aol.com

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Abbreviations: 2'- deoxynucleotide 5'- triphosphates (dNTPs); 2-deoxyglucose (2DG); acetylcholine (ACh); Attention-deficit –disorder with or without hyperactivity (ADHD); body mass index (BMI); dopamine D_2 receptor (D2R); dopamineD_2 receptor gene (DRD2); dual energy X-ray absorptiometry (DEXA); hydroxycitric acid (HCA-SX); Minnesota Twin and Family Study (MTFS); mitochondrial DNA (mtDNA); neuropeptide Y (NPY); nonpreferring (NP); polymerase chain reaction (PCR); Reward Deficiency Syndrome (RDS); single nucleotide polymorphism (SNP); substance use disorder (SUD)

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Summary

While there is a considerable body of literature correlating the role of dopaminergic genes and obesity, body mass index, body type, overeating, carbohydrate binging, energy expenditure and low dopamine D_2 receptor (D2R) density, there is a paucity of research concerning the dopamine D_2 receptor gene (DRD2) variants and percent body fat. We propose that the degree of obesity involving the interactive relationship of the brain's reward circuitry and the body's response to stress and caloric deprivation is represented by a new term Reward Deficiency Syndrome (RDS), which is subject to strong genetic influence. We report here the first potential association of DRD2 genotypes and the percent fat phenotype. We genotyped, at the DRD2 Taq1 A1 polymorphism, 122 obese Caucasian subjects and 135 non-obese controls. 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 consisted of 105 non-obese Caucasians

screened to exclude substance abuse and psychiatric disorders (Controls B). Controls A were assessed for weight, body mass index (kg/m^2) [BMI] and percent body fat using dual energy X-ray absorptiometry (DEXA). The controls B were assessed for weight and BMI. The sample was separated into two groups, those with the Taq1 A1 allele (A1/A1 or A1/A2) and those without the A1 allele (A2/A2). The controls A had a normal range of body fat (25-31% for females and 18-25% for males), a mean % body fat of 28.4±3.4 % and a mean BMI of 22.4 ± 2.9 kg/m^2. The controls B had a mean BMI of 21.9 ± 2.9 kg/m^2 The obese subjects had a percent body fat value of over 32 % for females and over 25% for males, a mean % body fat of 42.1± 7.5%, and mean BMI of 29.3 ± 6.25 kg/m^2. 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: c^2 = 39.6 d.f. =1, p<0.0001, and Controls B vs. Obese subjects c^2 = 25.9 d.f. 1, p <.0001. These results are consistent with a role of the DRD2 gene in obesity as measured by percent body fat as well as by BMI. Additionally, it is proposed that since fat distribution is under extensive genetic control (h^2<0.80) one putative gene may be the DRD2.

I. Introduction

Overeating is a biogenetic condition that comes in many forms (Wang et al, 2001). Large family studies in different populations have consistently demonstrated a familial correlation in adult body mass index (BMI) at about 0.2 between parents and offspring and about 0.3 between siblings. Moreover, according to many twin and adoption studies, these correlations are attributable mainly to genetic influences rather than to effects of the shared environment (Males et al, 1997). Fewer studies have addressed the genetic and environmental influences on body shape, assessed by body circumferences, skinfold measurements, and on body composition of fat and lean mass (Carey et al, 1996; Rice et al, 1997; Rose et al, 1998). Most recently, Schousboe and colleagues in 2004 using a more stringent design, found in both Caucasian women and men, heritability for BMI was 0.58 and 0.63, for body fat %, 0.59, and 0.63, and for lean body mass, 0.61 and 0.56 respectively. The same authors found no strong evidence of common environmental effects but did find a significant decrease in heritability with advancing age (Schousboe et al. 2004). Similar findings were found among African- American twins (Nelson et al, 2002), however, there are differences between Blacks and Caucasian probands where certain gene polymorphisms are concerned such as leptin expression and genes associated with BMI (Chagnon et al, 2000). There are certain environmental factors which may reduce body fat, body mass and fat-free mass in both adults and children. These include but are not limited to low-intensity, long-duration exercise, aerobic exercise combined with high-repetition resistance training and exercise programs combined with a behavioral-modification component. Moreover, genetics may even play a more significant role (LeMura and Maziekas, 2002).

Because of the complexity of compulsive eating disorders, it is likely that more than one defective gene is involved. Indeed at least twelve genes [see below] involved in the neurochemistry of brain reward have been associated with morbidly obese people. Moreover, three metabolic type genes have been identified-one associated with cholesterol production, one with fat transport, and one related to insulin production (Perusse et al, 2001).

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 (Rose et al, 1998; Perusse et al, 2001). Studies of twins provide the clearest evidence for genes and environment both exerting a significant influence on body composition. In 1997, researches examined data from 25,000 pairs of twins and a total of 50,000 family members. On an average, obesity was 67% genetic and 33% environmental (Evans et al, 2001). A small sampling of important candidate obesity genes presented in **Table 1** just touches the surface.

Given the complex array of metabolic systems that contribute to overeating, it is not surprising that a number of neurochemical defects have been implicated. Carbohydrates cause the release of the pleasure-inducing brain chemical dopamine. There is general agreement that other pleasure-inducing substances such as alcohol and nicotine, like glucose, exert an effect on the dopaminergic pathways of the brain. This shows the common genetic thread of multiple addictions (Blum et al, 1996). There are a number of investigators that have observed a significant association between a variant of the dopamine D_2 receptor gene and time of the onset of obesity, carbohydrate preference or craving, high body mass index, co-morbid drug abuse, energy expenditure, hyperphagia (Blum et al, 2000) and low dopamine D_2 receptors (Wang et al, 2001).

In 1990, Blum and colleagues, reported a strong association between a virulent form of alcoholism and the *Taq1* A1 allele of the *DRD2* gene (Blum et al, 1990). Other more recent studies further support an association of this allele with substance abuse vulnerability and other compulsive behaviors (Xu et al, 2004). In this regard, the National Institute on Alcohol Abuse and Alcoholism recently reported data that strongly suggests that *DRD2* is a susceptibility gene for substance abuses across multiple populations. Specifically, a haplotype block of 25.8 kb region of the *DRD2* gene was highly associated with alcohol dependence and heroin addiction. A low number of dopamine D2 receptors in individuals carrying the *Taq1* A1 variant suggest a hypodopaminergic function, as described by Eliot Gardner in a series of published works (Gardner, 1997). When there is a paucity of dopamine receptors the person will be more prone to seek any substance (including glucose) or behavior that stimulates the dopaminergic system as a form of self-healing. In this regard, we know that substances such as alcohol, cocaine, heroin, nicotine and glucose, as well as a number of

behaviors like gambling and sex, preferentially release dopamine at the nucleus accumbens. In one study (Wang et al, 2001), striatal dopamine D2 receptor availability was significantly lower in ten obese subjects compared to normal non-obese controls. In these obese subjects, BMI correlated negatively with the measures of D_2 receptors. The individuals with the lowest D_2 values had the largest BMI. **Table 2** illustrates the role of dopaminergic genes in obesity and related behaviors.

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

PATHWAY	GENE	POLYMORPISM(S)	REFERENCE (S)
CNS-Neurotransmitter Mesolimbic "reward" system and appetite regulatory pathway.	Leptin (OB)	OB1875 < 208-bp allele	Comings et al, 1996
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.	Serotonin receptor (5HT2A)	-1438G/A and 102/C	Fuentes et al, 2004; Roy et al, 2004; Tochigu et al, 2005
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. The ACP1 * A allele may be partially protected against developing Reward Deficiency Syndrome (RDS) G-148A of PNMT	Bottini et al, 2002
Neurotransmitter gene interaction especially serotonin precursors	Phenylethanolamine N-Methyltransferase (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 et al, 2003
Receptor is involved in both pleasure and antianxiety. 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.	Dopamine receptor (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.	Noble et al, 1994; Tataranni et al, 1996; Blum et al, 2000
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).	Tsugeno and Ito, 1997; Manor et al, 2002; Ito et al, 2003; Manoli et al, 2005
DHEA a natural substance effects female belly fat and is associated with the	Steroid sulfatase (STS)	Absence of site assoc with low MAO activity. 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	Riechman et al, 2004; Villareal and Holloszy, 2004

STS gene.		DHEAS:DHEA [-37 (11) vs. 5 (7), S30, P < 0.05] than those subjects with only an "A" allele (n = 84).	
Interaction of DHEA and adipose tissue as correlated with the PPARγ gene.	Peroxisome Proliferator-Activated Receptor γ (PPARγ)	A corresponding increase in PPARγ mRNA expression suggests that PPARγ may be involved in the up-regulation of adiponectin gene expression after DHEA treatment. Pro12Ala polymorphism of PPARγ gene is a major variant.	Dillon et al, 2000; Karbowska and Kochan, 2005; Blum et al, 2007
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).	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 et al, 2002

Table 2. Mean (SD) baseline demographic data for subjects for non obese and obese subjects.

	AGE (Y)	**WEIGHT (kg)**	**BODY FAT (%)**	**BMI (kg/m2)**
Control Subject A (N = 30)	44.8 ± 7.1	61.3 ± 6.7	28.4 ± 3.4	22.4 ± 2.9
Control Subject B (N = 105)	36.5 ± 6.9	60.5 ± 6.1	Not tested	21.9 ± 1.7
Obese Subjects (N = 122)	42.3 ± 8.8	82.7 ± 21.7	42.1 ±7.5	29.3 ± 6.2

We are making progress in our understanding of the cerebral mechanisms underlying the behaviors that lead to pathological overeating and obesity. Dopamine, a neurotransmitter that is influenced by and in turn influences other neurotransmitter pathways, modulates rewarding properties of food and is likely to be involved. To test the hypothesis that obese individuals who have a high percent body fat compared to non-obese controls that may also have a high presence of the dopamine *DRD2* A1 allele, we measured percent body fat and genotyped

individuals for the presence or absence of the dopamine D_2 receptor A1 allele.

II. Research Methods and Procedures

This study was conducted with IRB approval of both the Path Research Foundation (registration # IRB00002334) and the City of Hope National Medical Center. All patients filled out and signed an approved consent form prior to entering this study. The genotyping was performed at the Department of Medical Genetics at the City of Hope National Medical Center, Duarte, California. Lean controls were provided by Dr. Mathew McGue of the University Of Minnesota (see control B). The recruitment and characterization of the obese subjects were accomplished at the Health and Medical Research Foundation, San Antonio, Texas and the Sports Medicine Institute, Baylor College of Medicine, Houston, Texas. The recruitment and characterization of the well-screened controls were accomplished at the PATH Medical Research foundation, New York City, New York. The statistical analysis was performed in part at the University Of Texas School Of Public Health, San Antonio, Texas and Brooklyn College, CUNY, New York.

A. Controls A

In order to perform scientifically sound genetic association studies in a complex disease such as obesity, certain exclusion/inclusion criteria must be satisfied. It is now known that polymorphisms of the *DRD2* gene (A1, B1, C1 and the haplotype In6-Ex7 and other variants) are associated with a number of impulsive-addictive-compulsive disorders. These include severe alcoholism, polysubstance dependence, crack/cocaine dependence, smoking, obesity (BMI over 25), carbohydrate binging, conduct disorder, defense style personality, schizoid/avoidant personality, violent crimes, pathological gambling, autism, Tourette Syndrome, Attention-deficit – disorder with or without hyperactivity (ADHD), severe withdrawal depression, posttraumatic stress disorder, parental history of alcoholism, drug abuse, obesity in Caucasians, and inability to cope with stress. Therefore, we decided to develop stringent non-Hispanic Caucasian "super normal" controls (Blum et al, 1996, 1990, 2000; Gardner, 1997; Noble, 2003; Xu et al, 2004). Specifically, the exclusion criteria included careful assessment of alcoholism, substance use disorder, family history of chemical dependence, obesity, nicotine dependence (smoking behavior), BMI over 25, carbohydrate binging, Autism, Tourettes, ADHD, mood disorders, personality disorder (novelty seeking), schizophrenia, movement disorders, migraine, pathological gambling and post-traumatic stress. Thus, we carefully stratified 184 individuals attending the Path Medical Clinic of New York City. These patients were stringently assessed to eliminate any impulsive, addictive (including carbohydrate bingeing behavior and obesity as defined by BMI, and scale weight), or compulsive behaviors including absence of both Axis 1 and Axis 2 diagnosis.

Computer analysis revealed a sub population that fit the above exclusion/inclusion criteria. This sub-population consisted of a total of 30 non-Hispanic Caucasians subjects (4 males and 26 females) with an average age of 44.8 (46.3 females and 43.4 males) recruited from the total patient population of the Path Medical Clinic in New York City. Both BMI and body fat of the 30 controls was within the normal range (BMI normal range below 25 kg/m^2, and had a normal range of body fat between 25-31% for females and 18-25% for males). The percent body fat as assessed by Dual Energy X-Ray Absorptiometry (DEXA) was below 31%. Over a five-year period we studied a total of 1506 subjects (91% Caucasian, 1% black, 7% Hispanic, less than 1% Asian), attending the Path Medical Clinic utilizing DEXA and found the average percent body fat to be 28.7.

B. Controls B

This sample consisted of 105 non-Hispanic Caucasian female adults from the Minnesota Twin and Family Study (MTFS) (Iacono et al, 1999). The MTFS is a large, multi-discipline, multi-year study to examine the interaction between genetic and environmental risk factors in the development of adolescent and adult alcoholism and drug abuse. The advantage of the study is that it uses a population based twin ascertainment in which all same-sex twins born in the state of Minnesota are identified by public birth records, thus providing a measure of a random ascertainment. Since most of the other individuals in this study were females (13.6% males and 86.4% females), genotyping of control B subjects was restricted to the mothers of the twins. In terms of inclusion/exclusion criteria for the control subjects they were administered the parent version of the DICA-R (Diagnostic Interview for Children and Adolescents (Welner et al, 1987)) and the Structured Clinical Interview for DSM-III-R (SCID-R) (Spitzer et al, 1987). Subjects with any substance abuse or other DSM III-R diagnosis and subjects with a BMI of greater than 24 were excluded. The range of BMI was 19 to 24.

C. Obese subjects

A total of 130 unrelated non-Hispanic Caucasian obese subjects (BMI above 25 Kg/m^2 and percent body fat above 32%.) were enrolled in the study; 122 subjects were genotyped for the DRD2 gene polymorphisms (17 men and 105 women [mean age, 42.3 years ± 8.8 S.D.]). Subjects were recruited from a variety of fitness and athletic clubs in San Antonio and Houston, Texas, by fitness instructors and sales personnel who provided information about the study to potential participants. In order to ensure compliance, the fitness instructors were paid to monitor the subjects as they progressed through the study to ensure that the subjects reported their physical activity levels and caloric intake and completed the testing. All subjects were asked to consult with their personal physician before giving written informed consent. In terms of inclusion/exclusion criteria the experimental subjects were identified as being obese and had multiple failures in their attempts at dieting. The minimum BMI was $25kg/m.^2$

D. Dual energy x-ray absorptiometry (DEXA)

A number of studies have shown that DEXA can accurately measure fat and lean content of skeletal mass with a typical precision error for total body bone mineral content <1% (Tataranni and Ravussin, 1995). DEXA has also been shown to be a precise method for assessing body composition on obese and non-obese subjects. DEXA correlates highly with underwater weighing, deuterium dilution, and total potassium. The reliability of DEXA makes it possible to monitor the effects of relatively short-term dietary restrictions and exercise on both regional and total body composition.

DEXA provides a three-compartment model of body composition: fat, lean tissue mass and bone mineral content. Measurements are made using a constant potential energy source at 78kVp and a K-edge filter (cerium) to achieve a congruent, stable, dual-energy beam with effective energies of 40 to 70 keV. The unit performs a series of transverse scans moving form head

to toe at 1-cm intervals. The area being scanned is approximately 60 x 200cm. Data is collected for 120 pixel elements per transverse, with each pixel approximately 5 x 10 mm. Total body measurements are completed in 10 to 20 minutes with a scan speed of 16 cm/s, or in 20 minutes with a scan speed of 8 cm/s. The rate value (ratio of low – to high – energy attenuation in soft tissue) ranges from 1.2 to 1.4.

E. Genotyping

All subjects were genotyped based on a neutral identification number and read without knowledge of the individual being typed. Total genomic DNA was extracted from each coded blood sample, and aliquots were used for polymerase chain reaction (PCR) analysis. The oligo- nucleotide primers 5'-CCGTCGACCCTTCCTGAGTGTCATCA-3' and 5'CCGTCGACGGCTGGCCAAGTTGTCTA-3'were used to amplify a 310-base pair (bp) fragment spanning the polymorphic *Taq1A1* site of the DRD2 gene. The D2A1 and D2A2 genotyping were performed by a PCR technique (Blum et al, 1990; Comings et al, 1996, 2000). PCR was performed in 30- μL reaction mixtures containing 1.5mM $MgCl_2$, 2mM 2'- deoxynucleotide 5'-triphosphates (dNTPs), 05 μM primers, 1 ug of template DNA 1, 5U of *Taq* polymerase (Boehringer Mannheim Corp., Indianapolis, IN), and PCR buffer (20 mM Tris-HCL [pH 8.4] and 50m*M* KCL. After an initial denaturation at 94°C for 4 minutes, the DNA was amplified with 35 cycles of 30 seconds at 94°C, 30 seconds at 58°C, and 30 seconds at 72°C, followed by a final extension step of 5 minutes at 72°C. The PCR product was digested with 5 U of *Taq* 1 for 22 hours at 65°C for the *Taq1A* polymorphism. Digestion products were then resolved on a 3% agarose gel (5V/cm) containing 0.65 μg/ml ethidium bromide. There were three *DRD2 Taq1A genotypes:* 1) the predominant homozygote A2/A2, which exhibits two restriction fragments of 180 and 130 bp; 2) the heterozygote A1/A2, which exhibits three restriction fragments of 310, 180, and 130bp; and 3) the rare homozygote A1/A1, which produces only the uncleaved 310-bp fragment.

F. Statistical analysis

Demographic, clinical, laboratory, interview, and questionnaire data were coded and entered into a computer database. *DRD2* allelic prevalence, obtained by personnel blinded to the aforementioned information, was also coded. The chi-square statistic with Yates' correction for continuity (Siegel, 1956), as appropriate, was used for group comparisons using SPSS statistical software (SPSS, Inc, Chicago, IL).

III. Results

Table 2 and **Figure 1** summarize the subject demographics and results. For controls A (N =30) the mean (± S.D.) age was 44.8 ± 7.1 years, weight 61.3 ± 6.7 Kg, percent body fat 28.4 ± 3.4, and BMI 22.4 ± 2.9 kg/M^2. For controls B (N = 105) the mean age was 36.5 ± 6.9, weight 60.5 ± 6.1 Kg, and BMI 21.9 ± 2.9 kg/m^2.

Table 3. The role of dopaminenergic pathways in obesity and related behaviors.

DRDS Receptors and Psychoactive Drugs
Certain ant-psychotropic drugs increase feeding behavior and cause weight gain because they block dopamine D2 receptors and dopamine response.
Drugs that stimulate either dopamine D1 and/or D2 receptors reduce overeating
Dopamine D2 Receptors and Gene
Dopamine stimulators normalize overeating, body fat and blood glucose levels by increasing serum dehydroepiandrosterone (DHEA)
There is an evidence that BMI and even personality disorders (temperament) are directly correlated with the ability of dopamine to bind to its respective brain receprots
Certain dopamine gene mutations associate with BMI
The A1 form of the dopamine D2 receptor gene is associated with an increase of fat storage
The A1 form of the dopamine D2 receptor gene is associated with an increase a high BMI
In humans using PET scan low dopamine d2 receptors have been found with obese individuals not lean controls
Glucose causes a release of dopamine at brain sites
Dopamine itself increases the release of glucose from blood cells (hepatocytes)
Other Dopamine Gene
Other genes such as the dopamine 4 receptor was also associated with obesity and a high BMI
Both dopamine D1 and d2 receptors are linked to glucose metabolism and subsequent feeding bahevior
Certain dopamine gene forms associate with low energy expenditure.

Source: Welner et al, 1987; Spitzer et al, 1987; Noble, 2003

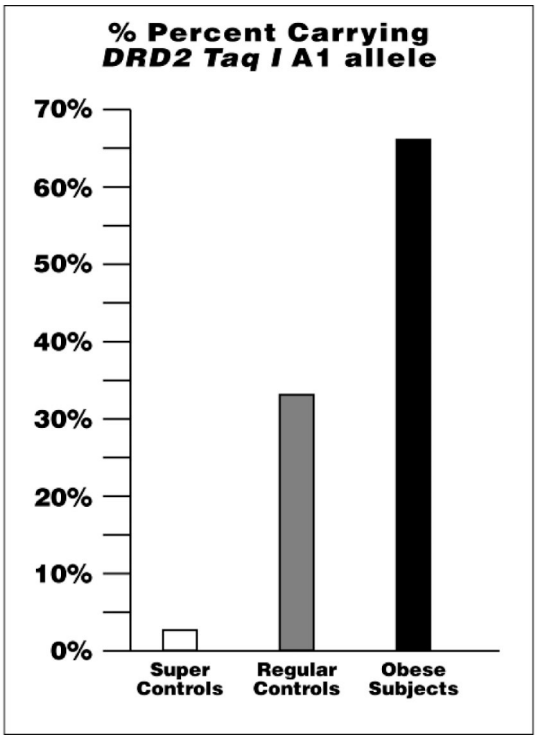

Figure 1. Percent prevalence of the DRD2 gene *Taq 1* allele in super controls (N= 30), regular controls (N = 105) and Obese subjects (N= 122l). For Controls A vs. Obese subjects $X^2 = 39.6$ d.f. = 1, p = < .0001. For Controls B vs. Obese subjects $X^2 = 25.9$ d.f. = 1, p = < .0001.

IV. Discussion

This is the first study to associate the prevalence of the DRD2 A1 allele and percent body fat. Our findings of a 67% A1 allele prevalence in obese subjects compared to 33% A1 allele prevalence in non-obese controls appears to be in agreement with the work of Evans et al, 2001.

While there is increasing evidence that genetic factors can influence differences in vulnerability to obesity, there still is controversy as to the exact genetic mechanism. Dopamine, acting through many receptors, can modulate the activity of neuronal reward pathways and thus affect a number of impulsive, addictive and compulsive behaviors, including overeating and carbohydrate bingeing. While obesity is a heterogeneous and prevalent disorder with both genetic and environmental components, the causes of this disease are still unknown. Over the last decade, a number of genetic variants have been associated with obesity and related substrates. Included in this list is CNS regulatory genes such as the OB (LEP) gene and the dopamine D_2 receptor gene. In this regard a number of studies (Comings et al, 1993, 1996; Noble et al, 1994; Blum et al, 1996; Levitan et al, 2000; Wang et al, 2001) (**Table 1**) have reported a positive association of the Taq1 dopamine D_2 receptor A1 allele and low density of D_2 receptors in obesity, body mass index, carbohydrate binging, parental history of obesity, co-morbid substance use disorder (SUD) and reduced energy expenditure.

In this study we found a significant association of the DRD2 A1 allele and percent body fat. In terms of the phenomena observed herein, there are a number of plausible mechanisms that may contribute. They include: (1) role of the DRD2 gene polymorphisms in obesity and related behaviors; (2) role of the DRD2 mutations and energy expenditure; (3) the interaction of glucose and dopamine release and hyperphagia; (4) role of dopamine and hyperglycemia; and (5) the importance of using "super" normal controls in association studies.

A. Role of the DRD2 gene polymorphisms in obesity and related behaviors

Specifically, the reinforcing properties of food have also led to an examination of the involvement of *DRD2* polymorphisms in obesity. Haplotype 4 (GT) of intron 6 and exon 7 of the *DRD2* gene were found to be associated with increasing risk for obesity (Comings et al, 1993). In another study, the *DRD2* A1 allele was present in 45.2% of obese subjects (Noble et al, 1994), a prevalence similar to that found in alcoholics, nicotine and other drug dependent subjects. In addition, the A1 allele was significantly associated with carbohydrate cravings. Variants of the human leptin *(LEPT, OB)* and the *DRD2* genes have been examined in relationship to obesity. Polymorphisms of the OB gene and the *DRD2* A1 allele each associated significantly with obesity (Comings et al, 1996). These two polymorphisms together accounted for about 20% of the variance in BMI, particularly in younger women. Another study has ascertained the relationship of the DRD2 A1 allele in obese subjects with and without co-morbid substance use disorders (Blum et al, 1996). In obese subjects, A1 allelic prevalence was significantly higher than controls ($P< 10^{-4}$). Moreover, the progressive

increase in co-morbid substance use disorders in these obese subjects was positively related to increased A1 allelic prevalence ($P < 10^{-6}$). Furthermore, another case control study (Blum et al. 1996) compared variants of the *DRD2* gene in obese (BMI > 30) and non-obese control subjects. The prevalence of the *DRD2* A1 allele was significantly higher in obese subjects compared to controls ($P = 2 \times 10^{-3}$) as was the *DRD2* B1 allele ($P = 3 \times 10^{-3}$). The odds ratio for obesity associated with the *DRD2* A1 genotype was 3.48 compared to 4.55 for the *DRD2* B1 genotype. Moreover, Thomas et al, (2000) and Rosmond et al, (2001), assessed *Taq1* A *DRD2* alleles in 484 obese and 506 non-obese Chinese subjects. Obese subjects, using either BMI or waist-to-hip ratio criteria, had a significantly higher prevalence of the A1 allele (P=0.02) and A1 allelic frequency (P= 0.03) than non-obese subjects.

B. Role of the DRD2 mutations and energy expenditure

Two studies (Jenkinson et al, 2000; Tataranni et al, 2001), assessed the role of other *DRD2* mutations on weight and energy expenditure in Pima Indians. Individuals with a Cys-encoding allele had a higher BMI than those homozygous for the Ser-311 allele (Jenkinson et al, 2000). Further, total energy expenditure and 24 hour resting energy expenditure were lower in homozygotes for the Cys311 allele when compared to heterozygotes and homozygotes for the Ser-311-encoding allele (Tataranni et al, 2001).

C. The interaction of glucose and dopamine release and hyperphagia

Moreover, it is well known that pharmacologic doses of the glucose analogue, 2-deoxyglucose (2DG), cause acute glucoprivation and are associated with enhanced dopamine turnover in pre-clinical studies. In fact, lines of evidence indicate that a variety of metabolic stressors, including acute glucose deprivation are associated with dopamine release. Using PET, Adler and colleagues (2000) found that 2DG administration enhanced synaptic dopamine concentrations. The administration of 2DG is associated with significant striatal dopamine release even in healthy volunteers. These studies are important because they further closely tie glucose levels to dopaminergic activity and strengthen our understanding of the interactive symbiotic relationship between insulin, serotonin and dopamine. As such, there is a relationship between insulin levels and dopamine release in the tuberonfundibular neurons. The insulin effect is dependent on CA^{++} ions, protein kinase C, and the Na^{+}-H^{+} exchange system. Additionally, when there is lower glucose in the brain leading to cerebral global transient ischemia, monoamine release, especially dopamine, is inhibited. In this regard, Trugman and James (1993) showed D1 antagonists lowered glucose utilization by 24 %-28 % in the globus pallidus, entopeduncular nucleus, subthalmic nucleus, substantia nigra, and even the motor cortex, suggesting that stimulation of the D1 receptor by endogenous dopamine contributes to basal metabolism in these regions. In contrast both D1 and D2 agonists increase glucose utilization. These results suggest that feeding behavior is tied into the stimulation of both D1 and D2 receptors and provides metabolic evidence for the importance of D1 and D2 functional linkage in the brain, which relates to hyperphagia or overeating**.**

D. Role of dopamine and hyperglycemia

The direct effect of dopamine on glucose release from primary cultured rat hepatocytes was studied in Japan by Shiroyama and colleagues in 1998. In this regard, dopamine is known to induce hyperglycemia in both animals and man. Their study investigated whether dopamine has any direct effect on glucose release from hepatocytes through the glycogenolytic and/or gluconeogenic pathways, and at the same time determined the main type of adrenergic receptor involved in glucose release. 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 beta blocker propranolol. Their study demonstrates that dopamine has a direct effect on hepatocytes, increasing glucose release via both glycogenolytic and gluconeogenic pathways and mediated by beta adrenergic receptors.

E. "Super" versus normal controls in association studies.

This is the first report that provides direct genetic evidence that the dopamine D_2 receptor A1 allele, which has been associated with lower D_2 receptors in humans (Noble et al, 1991; Wang et al, 2001), is positively associated with increased percent body fat. The process of fat storage and the role of genes are poorly understood. However, with confirming data, our findings point to a significant involvement of human percent body fat and dopamine functionality. In regards to using highly screened, "super" controls, using similarly well screened controls, Neiswanger and colleagues found a strong association of the D_2 A_1 allele and alcoholism (Neiswanger et al, 1995). Hill suggested failures reported in the literature were due to poor assessment of controls. Their suggestion significantly bolsters the appropriate use of "super" controls to more accurately assess a true phenotype. This is especially important when studying complex behavioral diseases (Hill, 1998). The same researchers found evidence for linkage between the dopamine D_2 receptor gene and severe alcoholism, early onset, physical dependence symptoms, and Antisocial Personality Disorder (Hill et al, 1999). However, the use of "super" controls may not be appropriate because these individuals do not mirror the general population, and similar comorbid disorders were not eliminated from the obese group. Thus these results should be regarded as preliminary. Moreover, we also examined a non-super control group, screened only to exclude obesity and DSM-IIIR axis I disorders. There the prevalence of the *DRD2* A1 allele was still significantly lower than for the obese group.

F. The ANNKI gene: A potential candidate dopaminergic linkage site

It is noteworthy, that regarding the observed polymorphic association in this paper, a major difficulty with an association of the DRD2 ***Taq1 A1*** allele with other complex behaviors such as alcoholism, is that the Taq1 A polymorphism is located more than 10kb downstream from the coding region of the DRD2 gene (Johnson, 1996) and a mutation at this site would not be expected to lead to any structural change in the dopamine receptor. The most likely explanation for an association is that *Taq1* A polymorphism is in linkage disequilibrium with an upstream regulatory element, or a 3' flanking element, or another gene which confers susceptibility to RDS behaviors. Several linkage disequilibrium studies have found strong linkage disequilibrium between *Taq1* A1 allele and the Taq1 B allele and the SSCP 1 allele (Blum et al, 1991; Hauge et al, 1991; Goldman et al, 1993; O'Hara et al, 1993; Johnson, 1996). As we have pointed out, the dopamine D2 receptor has been implicated extensively in relation to alcoholism, substance use disorder, nicotine dependence, anxiety, memory, glucose control, pathological aggression, pathological gambling, and certain sexual behaviors; all RDS behaviors. The most frequently examined polymorphism linked to this gene is the *Taq1 A* restriction fragment length polymorphism which has been associated with a reduction in D2 receptor density. In a recent study, within the 10kb downstream region of the *Taq1 A1 RFLP*, Neville and associates identified a novel kinase gene, named ankyrin repeat and kinase domain containing 1 (*ANKK1*), which contains a single serine/threonine kinase domain and is expressed at low levels in placenta and whole spinal cord RNA but its presence has not localized to brain tissue as yet. This gene is a member of an extensive family of proteins involved in signal transduction pathways. The DRD2 Taq1A allele is a single nucleotide polymorphism (SNP) that causes an amino acid substitution within the 11th ankyrin repeat of ANKK1 (p. Glu713lYs), which, while unlikely to affect structural integrity, may affect substrate-binding specificity. If this is the case, then changes in ANKK1 activity may provide an alternative explanation for previously described associations between the DRD2 gene and RDS behaviors (Neville et al, 2004). Most recently additional studies of this gene and nicotine dependence suggests that the ANNK1 flanking gene is closely tied to the DRD2 gene and it is strongly associated with smoking behavior (Gelenter et al, 2006).

G. Reexamining the consequences of calorie deprivation on obesity and related RDS characteristics

Some of the earliest research providing data still relevant today assessed the effects of starvation and subsequent refeeding in healthy volunteers (Keys et al, 1950). Keys and colleagues reported in 1950 that semi-starvation dieting resulted in a down regulation of metabolic rate, reduced thermogenesis and subsequent phase 2 accelerated fat deposition. Thirty two men volunteered to participate in an experiment in which their average daily food intake was cut in half. This was to be carried out for six months so the researchers could study an extended physiological and biochemical response to semi-starvation. The men were provided with 1600 calories a day including the vitamins, minerals and protein calculated to meet needs. Over the course of the six months all the subjects lost on average a quarter of their initial body weight with over half of the weight being from lean tissue. They also experienced a 40% decrease in basal metabolic rate and a significant increase in food and weight obsession. All of the men began to sneak food, binge eat, obsessively collect recipes and food became the sole topic of conversation. They also experienced a loss of interest in sex and daily activities. By the end of the study all of the men were extremely apathetic. Interestingly, this "old" research still provides insight into the repercussions of low calorie dieting as a method of weight loss.

More recently, Dulloo and Jacquet reexamined in 1998 data from this study and confirmed that semi-starvation lowers BMR and thermogenesis. However, they noted that upon refeeding it was observed that fat deposition was accelerated in a preferential fashion over lean tissue synthesis. They further noted that the rate and amount of fat deposition, termed catch-up fat gain, was proportional to the degree of initial fat storage. In other words, the greater the pre-starvation BMI, the greater the suppression of thermogenesis, and the greater the rate and quantity of fat deposition upon refeeding. In addition, they found that the lower protein ratio during refeeding was not due to a shift in the individual's energy partitioning characteristic, but was attributed to other mechanisms operating via the suppression of thermogenesis in response to severe fat depletion, with the energy thus conserved being directed specifically towards accelerating fat (and not protein) recovery. Under conditions whereby the refed animals were pair fed with weight-matched controls, the rate of protein deposition was the same as in the controls but that of fat deposition was increased by 3-fold as a result of energy spared from a 10–15% lower energy expenditure during the early phase of weight recovery (Dulloo and Girardier, 1990; Dulloo and Jacquet, 1998). A number of animal studies and human research by some of our own authors (already cited elsewhere) demonstrates that calorie reductions result in an attempt to increase dopamine, and/or dopamine stimuli, i.e. hyperphagia, sugar, alcohol, nicotine, gambling, etc (Hao et al, 2000; Johansen et al, 2001). We have already shown that calorie deprivation or restriction promotes abnormal dopamine activity that, especially in individuals carrying the DRD2 Taq 1 A1 allele, leads to a number of excessive RDS behaviors like hyperphagia, sugar cravings, binge eating, etc.

H. Metabolic and neurogenomic integration: future application and perspective

The body's number one "Prime Directive" is survival. There are many aspects of gene induced behavior, from normal metabolism to emergency failsafe protective metabolism that can be and are induced to ensure survival. As previously shown, there is a clear connection between

dopamine D2 Receptor gene polymorphisms and excessive craving-induced aberrant behaviors. In addition, as stated earlier, chronic stress causes a phase 2 reduction in dopamine concentration and the number of active dopamine cells (Gambarana, 2001; Moore et al, 2001; Miyasaka et al, 2005). As such, in a population carrying a particular polymorphism (i.e. *DRD2 Taq1 A1* allele), our notion is that chronic stress (or stressors) causes reduced dopamine secretion/receptivity, thereby increasing the craving or need for reward compensation.

Evidence has been demonstrated that persistent stress can lead to a self-sustaining pattern of abnormal craving behavior in both animals and humans, such as carbohydrate bingeing or long-term abuse of sugar. Animal model support for this premise can be derived from a series of experiments carried out by Li and associates (Mcbride et al 1990, 1997; Hwang et al, 1990; Volkow and Li 2005, 1990, Russell et al, 2004) upon substance-preferring (P) [seek carbohydrates, alcohol, opiates, *etc*.] and nonpreferring (NP) rat lines. Among a number of other factors, they found that P rats had a reduced dopamine supply at the *nucleus accumbens and* reduced densities of dopamine D_2 receptors in the *meso-limbic* areas, accompanied by lower serotonin neurons in the hypothalamus, higher levels of enkephalin in the hypothalamus (due to a lower release) and more GABA neurons in the nucleus accumbens.

Crossing a certain stress threshold not only affects dopamine, but can induce survival gene expressions that affect neurogenobolic signaling, which can retard phase 2 energy, fat and endocrine metabolism (the aftermath of initial stress response).

Work by Kogan et al. confirms that the drug DR4004, a putative 5-HT_7 receptor antagonist, also has functional activity at the dopamine D2 receptor (Kogan, et al, 2002). It is of interest that neuroanatomical data suggest a potentially interactive role between accumbens acetylcholine (ACh) and dopamine. There is evidence that Nacc ACh is apparently related to neural processes underlying not only psychostimulant reward but also natural consumption behavior (i.e. feeding). In this regard, Hajnal and colleagues found in 2000 that accumbens cholinergic interneurons play a role in the regulation of body weight and metabolism. In this context both stress and the role of dopamine play an important part in the Ach response (Hajnal et al, 2000).

It has also been known for some time that stress induces the preferential release of the circulatory hormone cortisol in humans (Reddy, 2006). Additionally, lipolysis is the major activity involved in the burning of fat in adipose tissue. Ottosson and colleagues clearly showed in 2000 that cortisol significantly reduced the basal rate of lipolysis ($p<0.01$) and the catecholamine lipolysis stimulators isoprenaline and noradrenalin *in vitro* (Ottosson et al, 2000). Furthermore, stress induction studies in animals showed a significantly higher food intake than controls within a few days following the stress-induced events (Miyasaka et al, 2005). These findings are consistent with those in humans demonstrating that stress alters dopamine metabolism, contributing to overeating and increases in other aberrant craving behaviors. In contrast, a reduction in stress, via massage therapy, was shown to reduce cortisol and increase levels of serotonin and dopamine (Field et al, 2005). In addition, dopamine has been associated with pleasure, and has been called the "anti-stress molecule" and/or the "pleasure molecule" (Blum et al, 2000). When dopamine is released into the synapse, it stimulates a number of receptors (D_1-D_5) which results in increased feelings of well-being and stress reduction.

Sufficient stress also increases cell turnover, induces apoptosis and causes a cascade of survival events. Gene-mediated apoptosis (programmed cell suicide) is an attempt to "get rid of the bad" so as not to encumber or interfere with and/or essentially "make room" for the good. Recent research in mice corroborates that stress-induced mutations in mitochondrial DNA (mtDNA) accumulate in tissues of mammalian species and are believed to be a significant contributor to aging. Accumulation of mtDNA mutations was not associated with increased markers of oxidative stress defects in cellular proliferation, but was correlated with the induction of apoptosis, particularly in tissues characterized by rapid cellular turnover. The levels of apoptotic markers were also found to increase during aging in normal mice (Dulloo and Jacquet 1998). The more severe and stressful the adverse environment in the body/tissues, the more aggressive is apoptosis, as has been shown by research in which apoptosis is curtailed (with appropriate genes being "silenced") as a result of exposure to certain nutraceutical substances (i.e. botanicals/nutrients) (Chanvitayapongs et al, 1997; Jang et al, 1997; Joshi et al, 1999; Ray et al, 1999; Criswell et al, 2005; Kujoth et al, 2005).

While in the past it was thought that DNA was a static non-changeable constituent of the organism, today it well established that environmental, pharmaceutical and nutraceutical elements can indeed have profound effects on altering polymorphic gene expressions. One example of these interesting phenomena is the influence of folic acid upon the methylation of certain DNA. Other examples include predisposition to aberrant sugar craving behavior, such as with excessive carbohydrate/alcohol binging and development of the polymorphism for the Dopamine Receptor D2 A1 Taq1 allele, which increases pleasure cravings in the reward circuitry of the brain (the dopamine system). The actual gene expression depends on an individual's life style and nutritional status rather than the DNA per se. As way of an example, this known and established polymorphism requires an almost excessive need for certain precursor amino acids to assist in the synthesis of certain brain neurotransmitters and minerals to "over nourish" and answer the excessive needs of this pathway and reduce, eliminate and/or normalize the excessive behavior that results from those demands (Blum et al, 2000).

We propose that a complete understanding of neurometabolic systems, as related to obesity and all of its behavioral subsets when coupled with genomic principles will provide novel targets to combat genetic, physiological, neurological, nutritional and peripheral metabolic dependent deficiencies.

I. Reward deficiency syndrome (RDS): A paradigm shift

Taken together, results of research indicate a profoundly integrated, interdependent and compensatory relationship between the brain's reward/pleasure circuitry, stress management and the neuroendocrine system (i.e Cortisol), and the energy management system, all of which are affected by lifestyle factors and regulated by genetic "oversight" influenced by particular gene polymorphisms. The dopamine system appears to be a pivotal factor in the sequela of response initiators that, based on specific polymorphisms, results in particular types of behavior described above. We further propose that Reward deficiency Syndrome (RDS) (Blum et al, 1996) is a subset of disorders contributing to a condition we propose be termed Neurobesigenics. The condition RDS accounts for the deficiencies in metabolic competence brought on by 1) the "excessive compensatory" expression of genetic survival mechanisms provoked by chronic and significant dietary stasis and concomitant nutrient deficiencies, 2) exacerbated by yo-yo BMI induced unhealthy deprivation/stimulation tactics, 3) processed through genetic predispositions involving the brain's reward management system, energy management system, stress and inflammation management system, immune system, and 4) the interplay of each system as they're manifested through the endocrine and metabolomic system. While the net release of dopamine is important for normal neurological and metabolic functioning, other neurotransmitters are involved in dopaminergic activation through a brain reward cascade. One of the most important is serotonin, especially as it relates to sugar cravings and the so called sweet tooth phenomena. The interplay of serotonergic and dopaminergic systems are well known and established throughout the neuropharmacological literature. While this strongly suggests that dopamine metabolism should be a primary target for therapeutic intervention, and potentially the ANNKI protein, it is also evident that therapeutic address needs to include pathways like serotonin, leptin, Neuropeptide Y, and potentially other systems that exert an influence on and/or are influenced by dopamine.

In this regard, obesity is a result of a constellation of metabolic dysfunctions influenced by variation of and/or amplified genetic expressions (polymorphisms), as well as poor lifestyle decisions. Thus, successful body recomposition requires the simultaneous address of multiple systems. A novel Ca^{2+}/K^{+} salt of (–)-hydroxycitric acid (HCA-SX) from Garcinia cambogia, has shown to be effective in obesity management. Appetite suppression of HCA is thought to result from increased glycogen production and concomitant stimulation of glucoreceptors in the liver, which sends signals of satiety to the brain. However, recent clinical and pre-clinical studies show that HCA-SX increases levels of serotonin (5-HT), a vital neurotransmitter involved in a wide range of behavioral functions in the body, including mood, sleep and appetite control. Studies show that serotonin affects eating behavior and body weight. Increased plasma levels of serotonin are associated with decreased food intake, reduced weight gain and increased energy expenditure. HCA-SX significantly inhibits serotonin re-uptake and increases serum serotonin levels in the body. Another benefit of increasing serotonin levels may be in addressing many of the emotional issues overweight people face, including binge-eating and depression. HCA-SX has also been shown to reduce levels of neuropeptide Y (NPY) in the hypothalamic tissue of animals. Earlier research has shown that NPY significantly increases appetite in animals that were pre-satiated with a meal. These new studies provide powerful new evidence on HCA-SX's ability to influence brain chemicals and neuropeptides involved in appetite control and eating behavior. Another recent finding is HCA-SX's ability to increase fat oxidation and modulate blood lipid levels. Human clinical trials have shown that HCA-SX significantly increases output of urinary fat metabolites. Following exercise or other fat oxidation (burning) processes, fat tissue breaks down into small molecular components, including malondialdehyde, formaldehyde, acetaldehyde and acetone. Increased urinary levels of these fat metabolites indicate increased fat degradation. Thus, HCA-SX is a potent modulator of various metabolomic and genomic systems involved in behavior, healthy body composition and obesity management (Chanvitayapongs et al, 1997; Jang et al, 1997; Joshi et al, 1999; Ray et al, 1999; Preuss et al, 2004a,b, 2005; Criswell et al, 2005; Downs et al, 2005; Kujoth et al, 2005).

Moreover, obesity is the result of a breakdown in the harmonious (interdependent) symbiosis of multiple systems of genetically regulated neurometabolic signaling due for the most part to deficiencies in nourishment, excesses in burdensome environmental factors and/or inadequate system responsiveness necessary for that symbiotic competence between the energy management system (with regard to energy intake, expenditure and storage), the stress and inflammation management system, the pleasure management system, the immune management system, and the neuro-endocrine system. As such, tactics that address less than all of these multiple systems simultaneously can achieve only limited or no success in sustainable healthy body recomposition. The phenomenon known as "Yo-yo rebound weight gain" causes increases in fat storage that occur as a result of "successful" early onset Phase 1 weight loss efforts (from a variety of conventional "deprivation/stimulation" tactics) followed by cessation of the tactic(s) and/or the body's genetic survival response to such tactics by lowering the neurometabolic rate, increasing fat storage and increasing appetite (Keys et al, 1950; Dulloo and Jacquet, 1998; Dulloo and Girardier, 1990). Over the last few decades this phenomenon has resulted in an obesity epidemic that is spreading worldwide. Even with the overwhelming arsenal of modalities and their endless combinations, obesity is the second leading cause of preventable death in the United States after tobacco with nearly 200 million Americans (roughly 60%) categorized as overweight or obese, according to the Centers for Disease Control and Prevention.

V. Conclusion

The old adage "as the head goes, so goes the body, is

a simple but appropriate characterization of the symbiosis that exists between the brain, the genome and the resulting metabolomic symphony. We propose that while obesity is a polygenic disorder, our findings indicate a putative important role of the dopamine D_2 receptor gene in morbid obesity, especially in high risk populations (Jenkinson et al, 2000; Noble, 2003), and that high to low quality lifestyle factors can exert a positive to negative influence respectively on gene expressions accordingly. Most recently it has been determined by Malis and colleagues (2005) that total and regional fat distribution is strongly influenced by genetic factors in young and elderly twins. Specifically, the hereditability factor h^2=0.83 (young) and 0.86 (elderly). In this regard, our results further suggest that certain polymorphisms of the DRD2 gene observed to associate with percent body fat in this study and possibly fat distribution, may provide the first evidence for involvement of this gene in neurobeisigenics (a proposed new name for Obesity and related disorders) and warrants further independent systematic investigation.

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