

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

Research Article

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Abbreviations: 2'- deoxynucleotide 5'-triphosphates, (dNTPs); body mass index, (BMI); Chromium Picolinate, (CrP); Chromium, (Cr); confidence interval, (CI); D2 receptor, (DRD2); estimated safe and adequate daily dietary intake, (ESADDI); Free Fat Mass, (FFM); Generally Recognized as Safe, (GRAS); high-density lipoprotein, (HDL); lean body mass, (LBM); Leptin OB, (LEPT); obesity, (OB); polymerase chain reaction, (PCR); recommended dietary allowance, (RDA); reference dose, (RfD)

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Summary

There is controversy regarding the effects and safety of chromium salts (picolinate and nicotinate) on body composition and weight loss in humans. Thus, we decided to test the hypothesis that typing the obese patients by genotyping the dopamine D2 receptor (DRD2) gene prior to treatment with Chromium Picolinate (CrP) would result in a differential treatment outcome. We genotyped obese subjects for the DRD2 gene utilizing standard PCR techniques. The subjects were assessed for scale weight and for percent body fat using dual energy X-ray absorptiometry (DEXAR). The subjects were divided into matched placebo and CrP groups (400 µg. per day) accordingly. 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 of these groups were tested separately for differences between placebo and treatment means for a variety of measures of weight change. 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. These results suggest that the dopaminergic system, specifically the density of the D2 receptors, confers a significant differential therapeutic effect of CrP in terms of weight loss and change in body fat, thereby strengthening the need for DNA testing.

I. Introduction

Chromium (Cr) is an essential nutrient involved in the regulation of carbohydrates and lipid metabolism (Anderson, 1998). Normal dietary intake of chromium in humans is often sub-optimal (Anderson, 1998; Diez et al, 2007). In addition to its effects on glucose, insulin, and lipid metabolism, chromium has been reported to increase lean body mass (LBM) and decrease percentage body fat, which may lead to weight loss in humans. The effects of chromium on body composition are controversial but are supported by animal studies, (Page et al, 1993; Lindermann et al, 1995; Kornegay et al, 1997; Min et al, 1997; Mooney et al, 1997; Ward et al, 1997, Fekete et al, 2001) which increase their validity. Negative reports of the effects of CrP on muscle mass and weight loss have also been published in rats (Gonzalez Munoz et al, 2006). Moreover in humans, most recently the effects of CrP on weight loss was not achieved in a recent randomized double-blinded study (Lukaski et al, 2007). A subject's response to chromium depends on his or her chromium status, the salt of chromium consumed, amount of supplemental chromium, study duration and possibly an individual's genome. Since we have reported on the involvement of the Taq1 Dopamine D2 receptor gene polymorphisms and percent body fat (Chen et al, 2007). We decided to test the potential nutrigenetic response of CrP as a function of D2 polymorphisms.

A. Safety issues

According to a review by Anderson in 1998 trivalent Cr found in foods and nutrient supplements, is one of the least toxic nutrients (Mertz et al, 1994). The reference dose (RfD) established by the U.S. Environmental Protection Agency for Cr is 350 times the upper limit of the estimated safe and adequate daily dietary intake (ESADDI) of 200mcg /day (3.85 micro mols.). The RfD is defined as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population, including sensitive subgroups that is likely to be without an appreciable risk of deleterious effects over a lifetime (Mertz et al, 1994). The ratio of the RfD to the ESADDI or recommended dietary allowance (RDA) is 350 for Cr ESADDI, compared with less than 2 for zinc RDA, roughly 2 for manganese (ESADDI), and 5-7 for selenium ESADDI (Mertz et al, 1994). Moreover, Anderson (1998) demonstrated a lack of toxicity of Cr chloride (CrCl) and CrP in rats at levels several thousand times the upper limit of the ESADDI for humans

(Anderson, 1998). In 1995, there was concern over the demonstration by Stearns and colleagues in 1995 that, at concentrations thousands of times higher than physiological levels, trivalent chromium (CrP and picolinic acid *per se*) can break chromosomes in cell culture. Others (WHO, 1973; Evans, 1993; Lukaski, 1999) suggest that this finding may not be relevant to nutritional supplementation. In this regard, a prediction that CrP will accumulate in tissues to dangerous levels during long-term supplementation is based on an inappropriate pharmacokinetic model and is at odds with data from long-term rat feeding studies (Anderson, 1979; Evens, 1994). Furthermore, clastogenicity (breakage of chromosomes leads to the production of micronucleus formation of acentric fragments called clastogens) is not equivalent to either mutagenicity or carcinogenicity and studies in animals reveal that any effects observed with regard to clastogenicity of trivalent chromium is only relevant to cell culture studies, not to living animals or humans (Stoecker, 1990; Loveday, 1996; McCarty, 1996). Moreover, the therapeutic-toxic-dose ratio for trivalent chromium is 1:10,000. Thus, clarifying the safety issues concerning Cr⁺³ in particular the picolinate salt, we believe is quite relevant to the use of this substance as an important dietary supplement, which is utilized on a large scale worldwide to assist in reducing the obesity epidemic (Anderson, 1995). Furthermore, cats tolerate 1000 mg of Cr⁺³ daily and rodents have no adverse effects with 1000mg of Cr per kg of diet (Lukaski, 1999). It may be noteworthy that hexavalent Cr, according to Lukaski in 1999 is much more toxic than the trivalent form.

However, most recently Stallings and colleagues in 2006 in a three-part study examined the effects of several chromium-containing supplements and their components on hatching and eclosion rates and success of development of first generation progeny of adult *Drosophila* fed food containing these compounds. It further examined the effects of the compounds on longevity of virgin male and female adults. Finally, the chromosomes in the salivary glands of *Drosophila* late in the third instar larval stage, which were the progeny of *Drosophila* whose diets were supplemented with nutritional levels of [Cr(pic)(3)], are shown to contain on average over one chromosomal aberration per two identifiable chromosomal arms. No aberrations were observed in chromosomes of progeny of untreated flies. The results suggest that human consumption of the supplement should be a matter of concern and continued investigation to provide insight into the requirements of

chromium-containing supplements to give rise to genotoxic effects. There have been both positive and negative cellular findings regarding this potential danger of CrP in humans (Bagchi et al, 2002; Gudi et al, 2005).

A review of recent studies on the safety of chromium picolinate was presented by Ronald Slesinski, the president-elect of the regulatory and safety specialty section of the society, at a Centers for Disease Control and Prevention (CDC) conference on metal toxicity and carcinogenesis in 2004:

"The safety research overwhelmingly confirms that Chromax chromium picolinate is a safe nutritional supplement," said Dr. Slesinski. *"Research studies conducted by the United States Department of Agriculture (USDA), National Toxicology Program (NTP) and at independent testing laboratories, show no evidence of genetic toxicity."*

As of April 2003, a panel of experts concluded that the weight of the evidence clearly demonstrates the safety of CrP is Generally Recognized as Safe (GRAS) for addition to a number of food categories for human consumption after reviewing the available data. However, other forms of chromium including the polynicotinate form should be considered as an alternative Cr salt (Bagchi et al, 2002).

B. Weight related measurements

Signs of Cr deficiency in humans consuming parenteral diets that have been documented on numerous occasions by Anderson in 1995, 1998 and Brown and colleagues in 1986, include elevated blood glucose, insulin, cholesterol, and triglycerides and decreased high-density lipoprotein (HDL) cholesterol (Mertz, 1993).

Although numerous reports document the role of Cr in glucose and insulin metabolism (Mertz 1993; Anderson, 1995, 1998b) the role of Cr in the regulation of LBM, percentage body fat, and weight reduction is still highly controversial because a significant number of studies (Clancy et al, 1994; Trent and Thieding-Cancel, 1995; Lukaski et al, 1996; Hallmark et al, 1996; Passman et al, 1997; Walker et al, 1998; Campbell et al, 1999; Lukaski, 2000; Luvolsi et al, 2000; Volpe et al, 2001) do not support an effect of Cr on body composition. Other studies (Evans, 1989; Grant et al, 1992; Hasten et al, 1992; Kaats et al, 1992, 1996, 1998; Bulbulian et al, 1996; Bahadori et al, 1997; Crawford et al, 1999) however, do report an effect of Cr on body composition in humans. Additional support is derived from a study by Rubin and colleagues in 1998 showing both acute and chronic resistive exercise increased Cr losses. These data demonstrate that the improvements in body composition due to resistive exercise are associated with increased urinary ⁵³Cr isotope losses that are consistent with increased absorption.

It has been suggested that if CrP can lower insulin resistance as reported earlier (Anderson, 1998) it can improve body composition, because insulin resistance or deficiency results in impaired entry of glucose and amino acids into muscle cells, increased catabolism of muscle protein and the potential acceleration of lipid deposition (Evans et al, 1973; Anderson, 1995). In our earlier publication (Kaats et al, 1998), after controlling for differences in caloric intake and expenditure, as compared with the placebo group, subjects in the active treatment group lost significantly more weight (7.79 kg vs. 1.81 kg, respectively) and fat mass (7.71 kg vs. 1.53 kg respectively), and had a greater reduction in percent body fat (6.30% vs. 1.20%, respectively) without any loss of fat-free mass. It was concluded that this study replicated earlier findings (Kaats et al, 1992, 1996) that supplementation with CrP can lead to significant improvements in body composition.

Lukaski has argued in 1999 that data from these studies by Kaats and colleagues in 1996, 1998 have the following potential flaws; lack of control of the actual Cr

intake and failure to maintain constant energy intake and expenditure. Furthermore, the calculation of fat loss based on a 3500-kcal energy expenditure resulting from physical activity produces dubious results. Thus, according to Lukaski in 1999, our findings (Kaats et al, 1996, 1998) that CrP supplementation promotes fat loss with a preservation of Free Fat Mass (FFM) conflicts with reports of no changes in body composition in adults given CrP supplemented diets and not provided a supplemental exercise program. He further points out that CrP supplementation in conjunction with an exercise-training program also does not facilitate a preferential loss of FFM (Hasten et al, 1992; Trent and Thieding-Cancel, 1995; Lukaski et al, 1996; Hallmark et al, 1996; Grant et al, 1997; Passman et al, 1997).

On the other hand, Anderson in 1998 has counter-argued and favored the effect of Cr on lean body mass. As stated earlier, there have been at least ten studies of Cr supplementation and resistive exercise. However, the methods used to determine LBM have been questioned, but Anderson suggests in 1998 that this does not negate the actual observed statistical differences between placebo and Cr supplementation. Moreover, three studies (Clancy et al, 1994; Trent and Thieding-Cancel, 1995; Passman et al, 1997), reported no significant increases in LBM from Cr, in subjects performing resistance exercise training. However, changes caused by exercise alone also were not significant. Anderson further points out that it seems unrealistic to assume that if methods and statistics used do not detect changes caused by resistive exercise in LBM, any changes owing to Cr would be detected. Changes in LBM owing to Cr would certainly be anticipated to be much less than those achieved through strenuous resistive exercise. Moreover, explanation for a number of negative studies could be due to low dosage, small sample size and duration of the study. In a study by Bulbulian and colleagues in 1996, in a study of 20 male and 20 female swimmers who received 400 mcg Cr/day (as CrP), Cr significantly increased LBM, decreased fat mass, and decreased percentage body fat compared with the placebo group. Effects were not significant after 12 weeks but were after 24 weeks.

Weight loss is common and relatively easy for many people, but maintaining weight loss is extremely difficult. The failure rate for maintaining weight loss more than 2 years is often greater than 95%. Although there is ample evidence that Cr has an effect on body composition, decreases in body weight from supplemental Cr alone are likely to be small. In studies by Anderson and colleagues (1979, 1998a, b, 1995) during the past 20 years of daily supplementation of 200 mcg CrC and up to 1000 mcg in the form of CrP ranging from 5 weeks to 4 months of supplementation, Andersons' group have been unable to detect an effect of supplemental Cr on body weight.

C. Genetic aspects

To date there has been no resolution and explanation for the controversial findings related to the effects of Cr salts on body composition and other weight related parameters. However, none of the investigators seriously considered genetic aspects, in spite of the emerging field

of nutrigenetics (the biological response to an element is dictated by one's genotype).

The advent of molecular genetic knowledge and techniques, in the past two decades, has revolutionized our understanding of inherited disorders. Although much success has been achieved in localizing genes in Mendelian disorders, great difficulty has been experienced in identifying genes in behavioral disorders (alcoholism, drug dependence, smoking, food and bingeing behavior, etc). In this regard, however, the D2 dopamine receptor gene has been one of the most extensively investigated genes in neuropsychiatric disorders. After the first association of the Taq1 A DRD2 minor (A1) with severe alcoholism in 1990 by Blum and colleagues, a large number of international studies have followed. Variants of the DRD2 gene have also been associated with other addictive disorders including cocaine, nicotine, opioid dependence and obesity. It is hypothesized that the DRD2 gene is a reinforcement or reward gene (Noble, 2003; Blum et al, 1996a,b; Comings et al, 1996; Thanos et al, 2001).

D. DRD2 gene and pharmacogenetics

In terms of pharmacogenomic and pharmacogenetic studies involving the D2 receptor gene, Lawford and colleagues showed in 1995 a selective positive effect of bromocriptine, a D2 agonist, in reducing relapse rates in alcoholics as a function of dopamine D2 receptor genotype (in 3,329 unscreened controls the A1 allele is present in 29.4% of the general population, whereas the A2 allele is present in 70.6 %) (2003). Other studies involving the D2 receptor gene and drug response includes the work of Nobles' group showing a differential response of the serotonergic re-uptake inhibitor paroxetine and response of patients with posttraumatic stress disorder. Individuals with the D2 A1 allele were more responsive than those with the D2 A2 allele (Lawford et al, 2003).

E. DRD2 genes and obesity

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 was 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), prevalence similar to that found in alcoholics, nicotine and other drug-dependent subjects. In addition, the A1 allele was significantly associated with carbohydrate craving. Variants of the human obesity (OB) and the DRD2 genes have been examined in relationship to obesity (Comings et al, 1996). Polymorphisms of the Leptin OB gene and the DRD2 A1 allele each associated significantly with obesity. These two polymorphisms together accounted for about 20% of the variance in body mass index (BMI), particularly in younger women. Another study has ascertained the relationship of the DRD2 A1 allele in obese subjects with and without comorbid substance use disorders (Blum et al, 1996c). In obese subjects, the A1 allelic prevalence was significantly higher than controls ($P < 10^{-4}$). Moreover, the progressive increase in comorbid substance use disorders

in these obese subjects was positively related to increased A1 allelic prevalence ($P < 10^{-6}$). Furthermore, another case control study (Spitz et al, 2000) compared variants of the DRD2 gene in obese (BMI > 30) and non-obese control subjects. 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 risk of obesity associated with the DRD2 A1 genotype was 3.48 compared to 4.55 for the DRD2 B1 genotype. Moreover, Thomas and colleagues assessed in 2001 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.

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 Ser311-encoding allele (Jenkinson et al, 2000). Further, total energy expenditure and 24-hour resting energy expenditure were lower in homozygotes for the Cys311-encoding allele when compared to heterozygotes and homozygotes for the Ser-311-encoding allele (Tataranni et al, 2001). Finally, another study (Rosemond et al, 2001) determined the association of a Nco1 Polymorphism (C-T transition) in exon 6 of the DRD2 gene with hypertension. Subjects with the TT genotype had significantly higher systolic blood pressure than subjects with the CT genotype ($P=0.049$). Moreover, subjects with TT genotype had significantly higher diastolic blood pressure than either subjects with the CYT or CC genotype ($P=0.011$). The importance of the latter finding relates to the fact that, there is strong evidence from epidemiological studies of a positive relationship between increased body weight and hypertension (Thomas et al, 2000).

In order to resolve the issue of non-responders, we decided to test the hypothesis that typing the obese patients by genotyping the DRD2 gene prior to treatment with CrP would result in a differential treatment outcome. This notion was based on previous research, as discussed above, which in summary, indicated that the DRD2 TaqA1 allele was associated with obesity in general (Comings et al, 1993; Spitz et al, 2000), BMI (Noble et al, 1994), carbohydrate bingeing (Comings et al, 1996), co-morbid substance use disorder (Blum et al, 1996c), reduced receptor density (Wang et al, 2001) in very obese people, energy expenditure (Jenkinson, et al, 2000; Tataranni et al, 2001), hypertension (Thomas et al, 2000), and contributed to the overall variance of percent body fat in the present population at a significant rate (Chen et al, 2007). We predicted that carriers of the DRD2 A2 allele, would retain the positive metabolic effects of CrP. In contrast the DRD2 A1, carriers because of a proclivity to increase carbohydrate bingeing, would possibly mask the metabolic effects of CrP on weight loss and change in body fat.

To answer these questions, we used DEXA testing to determine body composition and genotyped each subject for polymorphisms of the DRD2 gene and carried out a randomized, double-masked, placebo-controlled study of

the effects of CrP on body composition.

II. Research methods and procedures

A. Subjects

A total of 130 Caucasian subjects were enrolled in the study, 122 (17 men and 105 women; men age, 42.3 years) of which completed the testing (93.8%). Fitness instructors and sales personnel who provided information about the study to potential participants recruited subjects from a variety of fitness and athletic clubs in San Antonio and Houston, Texas. 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. All patients signed an approved IRB consent form for both treatment and genotyping by the University of Texas Health Science Center, University Of North Texas, Baylor College of Medicine and the City of Hope National Medical Center and the PATH Medical Foundation.

B. Dual Energy X-Ray absorptiometry

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% (Fredi et al, 1991).

DEXA has also been shown to be a precise method for assessing body composition on obese and non-obese subjects (Tataranni et al, 1995; Wang et al, 1995). DEXA correlates highly with underwater weighing (Nord and Payne, 1995), Deuterium dilution (Jensen et al, 1993), and total potassium (Beshya et al, 1995). 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 from head to toe at 1-cm intervals; the area being scanned is approximately 60 x 200cm. Data are collected for about 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.

C. Procedure

Kaats and colleagues have previously published in 1998 the procedure utilized in this study. After completing an initial DEXA test, subjects were provided with a report of their test results and randomly assigned a number from 1 to 130, which corresponded to a bottle containing capsules with 400 microgram of CrP or placebo. None of the investigators, research technicians dispensing the product, or participants, knew which subject number corresponded to the placebo or active product. An independent local pharmacist acted as trustee for the study and randomly assigned subject numbers to bottles that had been pre-labeled with either an "X" or "Y" to correspond with either active or placebo.

Subjects recorded the total number of steps taken each day in the same daily log used to record their caloric intake, which was subsequently used to adjust the subject's net change in body fat by using the following formula: 3500 calories = a change of 1

pound of body fat. Subjects checked in at the research center on a weekly basis to obtain a scale weight and to report their weekly physical activity levels, estimated caloric intake and any adverse side effects (none reported).

To monitor and adjust for differences in energy expenditure through physical activity throughout their waking hours, all subjects wore a pedometer (same method as used in previous studies (Kaats et al, 1998b) that reflected the number of steps they took during each day or the step equivalents for activities in which it was impractical to wear the unit. In terms of compliance, each participant was required to provide a \$100 deposit by check or credit card, which would not be processed unless the subject failed to complete the last DEXA test and end-of-study questionnaire. Participants were advised that return of their deposit was based solely on their completing the last tests no matter how well or poorly they adhered to the research protocol, as long as they reported candidly on how much or how little they complied.

On completion of the study and when all data were gathered and entered in the computer system, the trustee opened an envelope supplied by the manufacturer indicating which product was active and subsequently notified the co-senior investigator (GRK). The Department of Computing Resources at the University of Texas Health Sciences Center at San Antonio, Texas, analyzed all information under the supervision of the corresponding author.

D. Genotyping

A total of 130 subjects were genotyped for the dopamine D2 receptor gene. 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' CCGTCGACGGCTGG CCAAGTTGTCTA-3' were used to amplify a 310-base pair(bp) fragment spanning the polymorphic *TaqI* site of the DRD2 gene. The D2A1 and D2A2 genotyping was performed by a PCR technique (Blum et al, 1990; Comings et al, 1996). PCR was performed in 30- μ L reaction mixtures containing 1.5mM MgCl₂, 2mM 2'- deoxynucleotide 5' – triphosphates (dNTPs), 0.5 μ M primers, 1 μ g of template DNA 1.5U of *Taq* polymerase (Boehringer Mannheim Corp., Indianapolis, IN), and PCR buffer (20 mM Tris-HCL [pH 8.4] and 50mM 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* I for 22 hours at 65°C for the *TaqI*A polymorphism. Digestion products were then resolved on a 3% agarose gel (5V/cm) containing 0.65- μ g/ml ethidium bromide. There were three DRD2 *TaqI*A genotypes: 1) the predominant homozygote A2/A2, which exhibits three 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.

We controlled for any false positives by having more than one person extract the genotyping data and the principle investigators (KB and GK) were blinded.

E. Statistical analysis

Comparisons were made between body composition variables for the two groups at baseline using a two-tailed student's t test and between baseline and post test for both groups (n=120) using paired t-test analysis. Finally, comparisons were

made between the changes occurring in the two groups without making any adjustments for caloric intake (kilocalorie) or expenditure. All data analysis was conducted at the University of Texas Health Sciences Center's Department of Computing Resources.

III. Results

Of the 130 subjects who were recruited for this study, only 8 failed to complete the final test; 1 subject became pregnant and was asked to withdraw from the study, 3 moved from the area, 1 was ill during the post testing period and 3 was lost to the follow-up. A comparison of the 122 subjects who completed the study with the 8 subjects who did not revealed no significant differences in any of the body composition variables. Baseline characteristics for the 122 subjects who completed the study are provided in **Table 1**.

No statistically significant differences in baseline characteristics were observed between the active treatment and placebo groups, independent of the *DRD2* genotyping, suggesting that the randomization process was successful in providing two equivalent groups of subjects.

Figure 1 presents a comparison between group changes that occurred in body composition variables in both active treatment and placebo groups as a function of *DRD2* genotyping over the test period. Adjusting for caloric intake and energy expenditure of the data, we then

utilized Student's t test to examine the differences between the four groups. In agreement with our *a priori* prediction t test analysis revealed that carriers of the *DRD2* A2 allele were more responsive to the effects of CrP than were the *DRD2* A1 allele carriers. In the *DRD2* A2 carriers the measures of the change of fat weight (-0.59 vs. -5.9 p<0.041), change in body weight (-1.5 vs. -8.5 p<0.017), the percent change in weight (0.98 vs. 4.1 p<0.44), and the body weight change in kilograms (-0.7 vs. -3.8 p<0.018) all parameters showing significance. In contrast, in the *DRD2* A1 carriers, the change of fat weight (-4.7 vs. -6.7; p = 0.231), change in body weight (-4.7 vs. -5.4; p = 0.7), the percent change in weight (2.8 vs. 2.8 p = 0.97) and the body weight change in kilograms (-2.12 vs. -2.5; p = 0.7) no significance was found in any parameter tested (see **Figures 1 and 2**). It is noteworthy that a subsequent analysis of these data revealed that among participants receiving CrP, the average amount consumed was 357 µg/d.

In terms of distribution of the *DRD2* genotypes, we found that carriers of the *Taq1 A1* allele constituted 68% of the 122 subjects tested, whereby the remaining 32% possessed the *Taq1 A2* allele. We only found three individuals to carry the homozygous A1/A1 allele. We believe that this high allelic presence of the *DRD2* A1 allele is because these individuals tested were obese.

Table 1. Mean (SD) baseline demographic data for 122 subjects randomized to receive either chromium picolinate (CrP) [n=62} or placebo (n=60).

	Age (Y)	Weight (kg)	Body-Fat (%)	Body-Mass Index (kg/m ²)
CrP (400 µg/d)[n=62]	41.1±10.5	85.5 ± 23.0	42.4 ± 8.3	30.2 ±7.1
Placebo [n=60]	43.5± 7.5	79.9 ± 20.4	41.8 ±6.7	28.4 ±5.4

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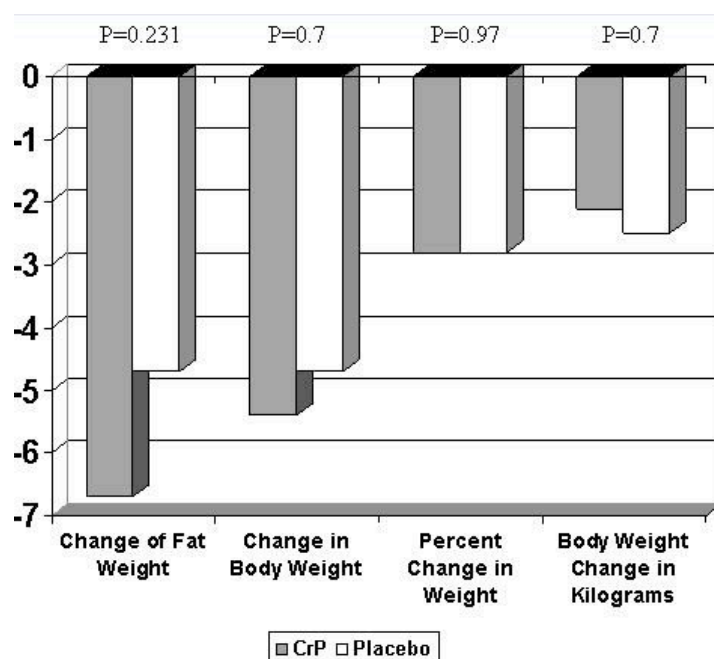


Figure 1. Double-blind comparison of Chromium Picolinate at 400 micrograms per day vs. Placebo on a number of weight and fat measures in subjects with the Dopamine D2 receptor A2/A2 genotype. P values indicate significance in all measures tested.

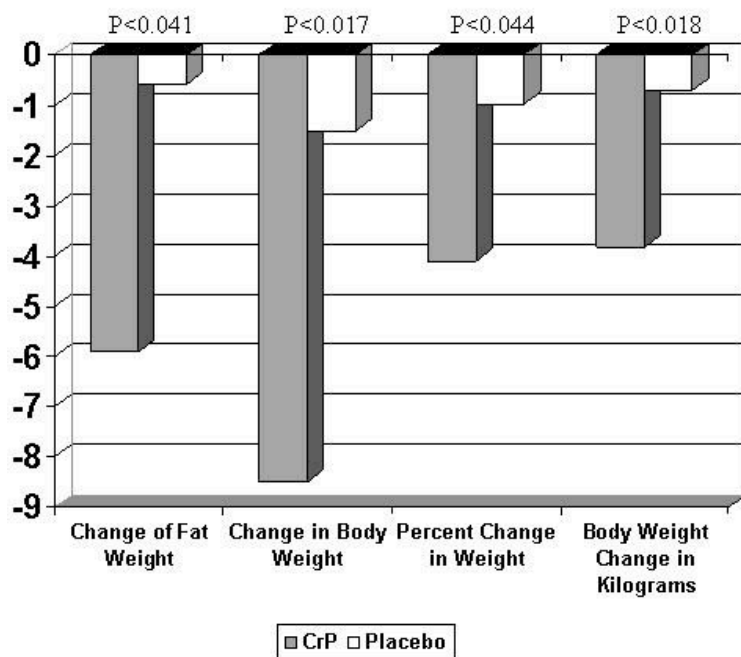


Figure 2. Double-blind comparison of Chromium Picolinate at 400 micrograms per day vs. Placebo on a number of weight and fat measures in subjects with the Dopamine D2 receptor A1/A1 and A1/A2 genotypes. P values indicate no significance in all measures tested.

IV. Discussion

In general these results confirm and expand our initial studies (Kaats et al, 1992, 1996, 1998a) showing a positive effect of CrP supplementation on body composition. We now find clear differential therapeutic effects for CrP in terms of a number of body composition variables based on genotyping for at least the DRD2 gene. This suggests that the explanation for negative findings with CrP supplementation may be due in part to a gap in information and lack of DNA testing of each patient prior to treatment. For example, as stated above in the past, such explanations discussed the possibility that strenuous physical activity increases urinary chromium loss and therefore the 200 microgram dose is too small an amount to produce positive changes in BMI when following a strenuous exercise program (Anderson, 1998) A more parsimonious explanation could simply be due to DRD2 genotype, at least in part.

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 subtypes. Included in the list are CNS regulatory genes such as the Leptin OB (LEPT) and the DRD2 genes (Comings et al, 1996). Additionally other genes have also been associated with obesity (e.g. pre-enkephalin gene, uncoupling protein, etc). With this in mind, we are proposing that since the dopaminergic pathway is involved in "reward" behaviors including substance use disorder (alcohol, cocaine, nicotine and food) (Blum et al, 1990, 1996a; Noble, 2003) and since the DRD2 A1 allele is associated with a number of body composition parameters (i.e. BMI, parental history of obesity, carbohydrate bingeing, percent body fat) carriers of the DRD2 A1 allele will be prone to excessive caloric intake. This will in turn significantly impact the positive

metabolic effects of CrP, and therefore mask its effect to lower insulin resistance, leading to negative rather than positive changes in body composition as observed in this study for only the DRD2 A2 carriers. In the present study we did find a rather high (68%) A1 allele distribution in the obese subject population, thereby confirming other studies (Noble et al, 1994; Blum et al, 1996a; Comings et al, 1996).

In terms of therapeutic benefits, these results take on even greater significance, when one considers data which suggest that certain amino acid precursors or neurotransmitter synthesis promoters alone or in combination with CrP reduce glucose cravings as well as carbohydrate bingeing episodes (Blum et al, 1990, 1997). Therefore, we propose that future treatment of obesity should consider the importance of combining trivalent chromium salts (picolinate, nicolinate) with amino acid precursors, enkephalinase inhibitors and catecholamine o-methyl-transferase inhibitors (dl-phenylalanine, l-tryptophan, l-glutamine, rhodiola *rosea* etc.) especially in dopamine D2 receptor *Taq A1* allele obese subjects. Moreover, we propose that mixed effects now observed with CrP administration in terms of body composition, may be resolved by genotyping the prospective patient prior to administration of a trivalent chromium salt and this may be coupled with electrophysiological markers and anti-craving nutraceuticals (Blum et al, 1994; Chen et al, 2004).

It is noteworthy that CrP shows promising anti-depressant effects in atypical depression (Davidson et al, 2003). Atypical depression (American Psychiatric Association 1994) is a common form of depression (22% of all clinically diagnosed depression), characterized by mood reactivity, increased appetite, weight gain, as well as other features. Its mechanism of action may relate to 5HT_{2A} down regulation, increased insulin sensitivity, or to

other effects and other neurotransmitter receptors. While the sample size in the Davidson study in 2003 was small and the effect size was also small ($p=0.02$), based on our current data, we propose that additional work involving genotyping individuals for both serotonergic and dopaminergic gene polymorphisms linked to CrP response rate may uncover even stronger evidence for certain outcome measures related to the problem of weight gain and mood.

To shed some light on this dilemma, a study published in JAMA, by Ali H. Mokdad and colleagues in 2003 from Centers for Disease Control and Prevention, Atlanta, Georgia, using a cross-sectional random-digit telephone survey (Behavioral Risk Factor Surveillance System) of non-institutionalized adults aged 18 years or older, suggest that obesity continues to increase rapidly in the United States. In 2001 the prevalence of obesity (BMI $>$ or $=30$) was 20.9% vs. 19.8% in 2000, an increase of 5.6%. The prevalence of diabetes increased to 7.9% vs. 7.3% in 2000, an increase of 8.2%. The prevalence of BMI of 40 or higher in 2001 was 2.3%. Overweight and obesity were significantly associated with diabetes, high blood pressure, high cholesterol, asthma, arthritis, and poor health status. Compared with adults with normal weight, adults with a BMI of 40 or higher had an odds ratio (OR) of 7.37 (95% confidence interval [CI], 6.39-8.50) for diagnosed diabetes, 6.38 (95% CI, 5.67-7.17) for high blood pressure, 1.88 (95% CI, 1.67-2.13) for high cholesterol levels, 2.72 (95% CI, 2.38-3.12) for asthma, 4.41 (95% CI, 3.91-4.97) for arthritis, and 4.19 (95% CI, 3.68-4.76) for fair or poor health. Increases in obesity and diabetes among US adults continue in men and women, all ages, all races, all educational levels, and all smoking levels. Obesity is strongly associated with several major health risk factors. Since 1998, approximately one-third of the United States is overweight.

It is to be noted, Kaats and colleagues stated in 1998a that the randomization was successful in creating two equivalent groups of subjects. **Table 1**, reproduced from that earlier study shows how equivalent the groups are with respect to age, weight, body fat, and body mass index. With this mind, any adjustment for differences of groups is not expected to alter results, because the randomization utilized was so successful

Finally, because an unusually high number of subjects (93.8% completed the final testing, requiring research subjects to provide a conditionally refundable deposit (to be returned on completion of final testing) is a technique worthy of further study.

V. Conclusion

If these results could be further confirmed and extended by using PET scan to determine the pre and post density of D2 receptors in obese individuals following amino-acid precursor loading techniques and Cr, these future studies may have a powerful impact on the obesity epidemic. Cautiously we encourage additional nutrigenetic studies that will support pre-treatment DNA testing of the DRD2 gene polymorphisms and possibly other genes in obesity and other related RDS behaviors.

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