AMINO ACID LOADING AND ENKEPHALINASE INHIBITION IN FAMILIAL OVEREATING:CLINICAL EVIDENCE FOR EFFECTIVENESS IN MAINTAINING WEIGHT LOSS IN A OPEN TRIAL CONTROLLED TWO YEAR STUDY.

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ABSTRACT

A group of 247 outpatients in a low calorie,fasting program were studied for two years.At two years the group taking the amino acid regimen of *Kan-100* compared to the no *Kan-100 /* Centrum vitamin group showed:1) a twofold decrease in percent overweight for both males and females;2) a 70% decrease in craving for females and 63% decline for males;3) a 66% decrease in binge eating for females and 41% decrease for males; 4) most important, the experimental group regained only 13.5% of the weight they lost during fasting while the control group regained 51% of their lost weight;and 5) logistic regression modeling revealed that with KANTROL treatment ,female gender, morbid obesity and family history of obesity were significant predictors of weight gain after two years.

INTRODUCTION

Obesity is generally defined as 20% or more over ideal body weight.In the United States 25% of white males , 26% of white females,30% of black males, and 48% of black females are obese.Moreover,over 20million people are involved in dieting or weight control not to mention those focusing on wellness through increased physical activity.Numerous methods of weight reduction have been attempted including hypocaloric balenced diets, "fad" diets, behavior modification,drugs(i.e D-phenflouramine,phenteramine etc),surgery, total starvation,jaw wiring,and combinations of these methods.Most of these are short-term approaches to the problem and have been only transiently effective and some can even pose serious danger(1).Even

if weight loss is demonstrated in the short-term, the weight is usually regained following discontinuation of the weight-loss regiment.Despite the fact that about 28% of the American population is obese,obesity is widely percieved as a food-addiction,a self -imposed condition with cosmetic rather than health indications(2,3).

An understanding is emerging from recent studies of some of the causes of obesity and the difficulties of treating this condition.Studies of twins in Pima Indians have substaniated a strong genetic basis for obesity(4,5).Obesity is a heterogenous and prevalent disorder which has both genetic and environmental components.The relationship between macroselection of various foods and familial substance use disorder(SUD) has been documented throughout the literature and neurochemical studies have supported the commonality of reinforcement through dopaminergic systems by alcohol,nicotine,cocaine and carbohydrates.In this regard,both obesity and SUD can be condidered appetitive compulsions.Some genes such as the dopamine D2 receptor (DRD2), and dopamine transporter (DAT1) genes may be a risk factor not only for obesity(6-8) but also for SUD in general and othe psychiatric disorders (6,9-13).additionaly,the cloning and sequencing of the mouse *ob* gene and its human *OB* homologue raised hopes that defects in this gene may play a significant role in the cause of obesity in man and that Leptin,it's gene product, would be useful in treatment(14,15).While genetic effects can act alone,in most cases the genetic profile only sets the stage defining the opportunity for a genetic -environmental interaction (ie dramatic increase in weight when coupled with increased food).for persons with such a genetic risk profile obesity is a life-long condition requiring long term therapy as in other chronic diseases.

Although the causes of obesity are multifactorial, it is clear that obesity is a physiological condition involving the integration of the nervous and endocrine systems which regulate weight.Maintenance of a particular weight involves a balence of energy intake and expenditure(ie "set-point weight").Both intake and expenditure are regulated by genetic and environmental factors operating alone and in concert(16).

The specific causes of uncontrollable ingestive behavior for alcohol,drugs,and food (in particular,carbohydrates) are incompletely understood .Neverless, it is clear that these appetative compulsive behaviors are a product of genetic predisposition and environmental insult factors.Both the genetic and environmental factors may be understood as operating through particular alterations in brain neurochemical balance which may induce carbohydrate bingeing ,as well as other "Reward Deficiency Syndrome "(RDS) related behaviors(17).Previously, Blum et.al (18) proposed that a multi-neuronal cascade of events in the reward system may play a role in neuropharmacology of RDS (19).Others have hypothesized that multiple brain neurotransmitters play a significant role in the control of food intake, appatite for specific macronutrients, and patterns of meal-taking behavior.Leibowitz (20) summerized extensive evidence for the role of a number of brain monoamines and neuropeptides in the control of normal eating behavior operating in concert at the mesolimbic reward system(21).Analyses of cerebrospinal fluid in both humans and animals indicate specific disturbances in brain neurochemical function in association with abnormal eating patterns(22,23).

The primary neurotransmitters involved in eating brhavior include the monoamines dopamine (DA), norepinephrine(Ne),epinephrine(EPI),and serotonin (5-HT);the inhibitory neurotransmitter gama-aminobutyric(GABA); and a variety of neuropeptides such as the pancreatic polypeptides, opioid peptides, hormone-releasing factors, and various gut-brain peptides.(for reviews see 24.25.26.)

We believe that RDS is the response to one or more neurotransmitter deficits. Attemps to alleviate this neurotransmitter imbalence through drug-receptor activation will only substutute for lack of reward, and will yield a temporary sense of well-being.In this regard, we have shown that recovery from certain forms of uncontrollable ingestive behavior(ie,SUD) is significantly facilitated by the use of neuronutrients designed to restore brain chemical deficits through the administration of both precursor amino acids and enkephalinase inhibitors(27-29).Specificaly, in terms of overeating, a study with 27 overeaters demonstrated that experimental subjects taking avariant of *Kan-100* lost an average of 27 lbs in 90 days compared to 10 lbs lost in the control group (30).Utilizing a similar approach we decided to extend this work and evaluate the question of wheather amino acid precursor loading and enkephalinase inhibition would enhance maintenance of weight loss in an outpatient setting over a two year period.

METHODS

Subject Selection and Program

The subjects of this study were 247 outpatients in a very low calorie, supplemented fasting program at the Behavioral Medicine Group Clinic in Sacramento, California. Subjects used Optifast as a nutritional fasting product until they were within 15% of their goal weight or until the patient elected not to continue fasting.Standard Metropolitan Life Insurance height/weight tables were used to determine ideal weight.All subjects took Centrum vitamins during the entire study.Each subject gave informed consent and the protocol was approved by the Behavioral Medicine Medical Group Clinic Institutional Review Committee and the University of Texas Health Science Center at San Antonio Institutional Review Board under a protocol of obesity research with the Life at Goal Weight Center of San Antonio,Texas.

The decision of whether or not a patient would take *Kan-100* or not was made at the end of fasting.Patients that were complaining the most or were not in control of their eating were selected to take *Kan-100* (experimental group;N=130).If maintenance appeared to be effortless, then the patient was not offered *Kan-100* (control group;N=117).For the selected subjects, *Kan-100* treatment was begun at the end of fasting and continued during the maintenance period until the end of the study at two years.*Kan-100* is an amino acid and vitamin supplement and is the forerunner of *Kantroll* manufactured by Kantroll,Inc.,San Antonio ,Texas .The selected patients took six capsules of *Kan-100* per day, which consisted of 460 mg.Dl-phenylalanine,25mg. L-tryptophan.25mg. L-glutamine, and 5mg. pyridoxal -5'-phosphate.

Patients were weighed weekly before or after attending an educational class.In addition their

psychological status and medical condition was monitored on a weekly basis.Psychological status was monitored as scores on rating scales for food craving,mmdiness and binge eating.Craving and binging were recorded on a scale of 1 to 5.A score of 5 on the craving scale indicated feeling totally out of control and eating past discomfort.A score of 1 indicated a nagging desire even though the person knew he/she was satisfied and physiologically should not be craving.Moodiness was rated on a similar scale with 5 being very moody and 1 being minimally moody.Binging was recorded as number of episodes per week of eating past the point of being full.Laboratory analyses of blood alternated every other week with urinalyses provided information about medical condition.A chemistry panel and SMAC-CBC were performed on the blood, while urine was analyzed mainly for ketones and/or glucose.The educational class was one hour per week.Principles of nutrition,exercise, behavioral changes, and stess management to support weight loss and long term maintenance were emphasized.

Characteristics of the subject population

Of the 247 subjects,84% were female.All subjects were Caucasian and the mean age was 40 years.The average subject was 74% overweight upon entry to the program (see Table 1).

Table 1. Characteristics of the treatment population

	Number	Age	Female	% ideal weight	% Over-weight[a]	% OB+[b]	% CD+[c]
Total Population	247	39.8	84.2	130.2	74.3	68.0	53.0
PCAL-103 Group	130	38.9	83.8	129.1	74.4	64.6	65.4
No PCAL-103 (Control)	117	40.7	84.6	131.4	74.3	71.8	39.3

a) % overweight = (start weight - ideal weight)/ideal weight
b) % OB+ = percent of group that reported a family history of obesity
c) %CD+ = percent of group that reported a family history of chemical dependency

The 130 experimental subjects taking *Kan-100* did not differ significantly from the 117 control subjects in age , ideal weight, start weight, percent overweight , craving, mood swings, binge eating, or family history of obesity (see table 1).However, they did differ in family history of chemical dependency (CD+). Of the subjects in the experimental group, 65% had a family history of chemical dependcy (CD+) while 39% of the control subjects were CD+ ($p < .005$).Since subjects were selected to be in the experimental group if they complained more or had a loss of control- during weight maintenance, these data suggest that CD+ subjects complained more and appeared to have difficult time during weight maintenance period.

Gender Differences

The 208 females in the study were an average of 76% overweight at the start, while the 39 males were 66% overweight.Almost three-quarters (73%) of the females were morbidly obese , i.e. 50% or more overweight, compared with about half (49%) of the males.Females outnumbered males by more than five to one in this program.During the intake interview over 90% of the females in the study reported craving food, while slightly less than 80% of the males reported craving..Occurrence of binge eating was also significantly different between males and females.Eighty percent of females reported binge eating, versus only 64% of the males.

Familial Aspects of Patients

Upon entry to the program, each subject was asked about family history of obesity and family history of chemical dependency.Slightly more than 70% of the females, and 56% of the males reported a family history of obesity.Of those with a family history of obesity, about twice as many subjects reported having an obese mother (73%) compared to an obese father (38%). Eleven percent of those with a family history of obesity reported both parents obese.

Almost half of both females and males reported a family history of chemical dependency.In this case, however , it was the father that carried the trait.Of the subjects with a family history of chemical dependency, a full 86% reported their fathers were chemically dependent, compared with only 31% of the mothers.

About two thirds (63%) of the morbidly obese (50% or more overweight) subjects reported having a mother that was obese and 60% reported having a father with some form of chemical dependency.Inasmuch as males represent only 16% of the total population, any statements with regard to family history subgroups are, at best, preliminary.

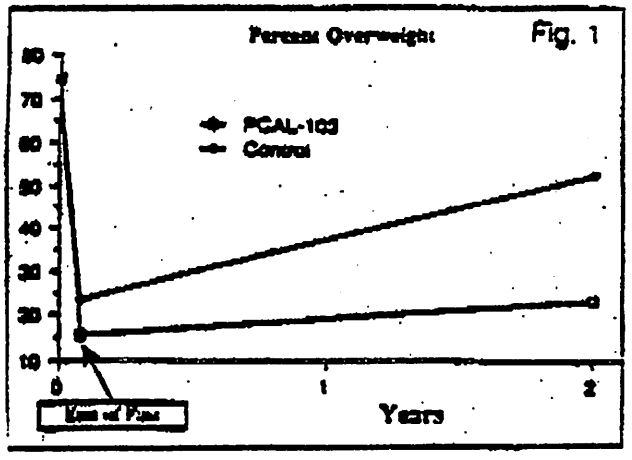
RESULTS

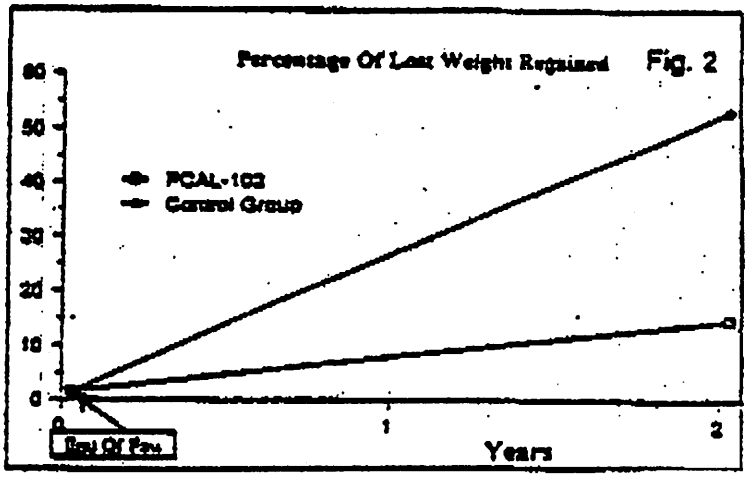
Mainentenance of weight loss

The 247 subjects lost an average of 68.4 lbs over an average of 20.0 weeks of fasting.
The experimental group differed from the control group at the end of fasting and before begining th*Kan-100* but this difference was non-significant.The experimental group was 22% overweight at the end of fasting compared to 32% overweight for the control group.Despite this difference, statistical analysses demonstrated that the weight-at the end of fasting did not affect the weight reduction at two years.At the end of the two year study, subjects taking *Kan-100* were a mean 23.5% overweight compared with 52.8% for the control group not taking *Kan-100* ($p< 0.0$)[figure 1].At two years subjects in the experimental group regained only 13.5% of their percent lost weight, compared to 51% in control subjects ($p<0.0$)[figure 2].

Influence of Genetics

In comparison of experimental to control subjects by family history (OB+/CD+,OB+/CD-,OB-?CD+, aand OB-?CD- groups) allgroups taking *Kan-100* were significantly less overwweight at 2 years than any control group ($p<0.001$).All groups with a history of OB+ were more overweight after two years than the comparable OB- groups ($p<0.0$).Subjects with a family history of chemical dependency responded well to *Kan-100*.



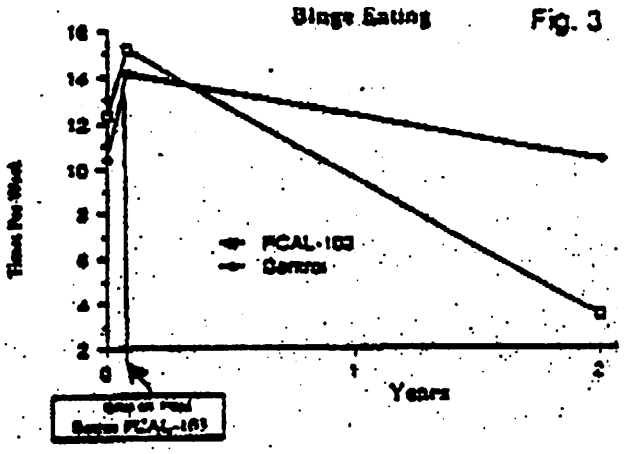


Genetics and Gender

As a whole , every family history group of males regained dramatically less weight than the comparable group of females($p < 0.0002$). In the best of cases (OB-/CD+) at the end of two years the males regained virtually none of the weight lost during the fast.Because females outnumbered males in this study by more than five to one, and since females differed in several characteristics from males, we decided to further characterize females.Seventy percent of the females reported a family history of OB+ while 54% reported a family history of CD+.Only 12% reported neither a family history of CD- nor OB-.The females that were OB+ and CD+ were on the average 58% heavier than OB-/CD- females.Moreover, OB+/CD+ females were the most overweight; followed by OB+/CD- then OB-/CD+, with OB-/CD- females the least overweight.A similar progression emerged for food craving, binging, eating and moodiness scores, with OB+/CD+ females reporting the most craving, binging and moodiness.

Food craving and binge eating

At the end of the study craving was reduced three-fold in the subjects taking *Kan-100* compared to the control group (at least $p < 0.05$) ; it was not reduced at all in the controls.The number of episodes of binge eating was also significantly reduced in the *Kan-100* subjects compared to controls. Upon entry into the study the experimental subjects reported bingeing episodes 10.9 times per week.At the end of the study the experimental subjects reported binging behavior only 2.9 times per week. In contrast upon entry of the study the non-*Kan-100* control group reported episodes of 8.3 times per week.At the end of the study the control group reported bingeing episodes of 8.3 times per week indicating no significant change (see figure 3).



At two years craving for food and binge eating were both reduced three-fold in the group taking *Kan-100* compared to the control group (subjects in the experimental group regained only 13.5% compared to 51% regained by the control group).

Multiple Regression and Analysis of Variance

A stepwise multiple regression was used to test the significance of predictors of percent weight gained back two years after the start of the treatment program, The predictors were categorized as absent (0) or present (1) and represented if the patient was morbidly obese or not, suffered from bingeing, suffered from craving, had a family history of chemical dependency, had a family history of obesity, female gender, and were administered *Kan-100* .The stepwise selection procedure (SPSS Version 6.13.SPSS INC of Chicago) selected *Kan-100* treatment, female ghender, morbid obesity and family history of obesity as significant predictors of weight gain after two years.Bingeing behavior , craving behavior and family history of chemical dependency were not statistically significant as predictors.The overall model of selected predictors was significant (p,.0001) with 39% of the variability in two year weight gain explained by the predictors.the most influential predictor in the predictor set was *Kan-100* , followed by morbid obesity, female gender and family history of obesity (see table 2.)

---------- Variables in the Equation ----------

Variable	B	SE B	Beta	T	Sig T
MORBID OBESE	20.235268	3.490780	.305151	5.797	.0000
OBESITY FH	10.056926	3.449490	.152177	2.915	.0039
FEMALE	10.315273	4.295429	.122028	2.401	.0171
SAAVE (YES)	-28.085989	3.087353	-.454957	-9.097	.0000
(Constant)	22.815756	4.804960		4.748	.0000

A second analysis compared the bingeing scores before and after (2 years) between the *Kan-100* and the control Centrum groups.a two factor Analysis of Variance with a between group factor for *Kan-100* treatment and a repeated measures factor for before and after two-years bingeing scores was found to have a significant interaction ($p < 0.001$).Paired t-tests were employed to test for changes in the bingeing scores seperately for the *Kan-100* and control groups.Statistically the control group had no detectable change while the change in bingeing was dramaticaly reduced for the *Kan-100* group.

DISCUSSION

the data presented in this two year open trial study suggest that the neuronutrient *Kan-100* suppreses abarant eating - behavior in known carbohydrate bingers while preventing regained weight loss.

Numerous studies have implicated the interaction of opiates,opioid peptides, CCK_8, glucagon, DA, and insulin in glucose utilization and selective intake of carbohydrates(31-34).

We believe that the apparent beneficial effects of *Kan-100* may be explained by the action of both the precursor amino acids and enkephalinase inhibition operating on mesoloimbic reward circuitry.We cannot at this time provide an exact mechanism of action for this neuronutrient mixture, nor can we pinpoint which ingedient or combination of ingredients best suppresses carbohydrate binging in our study.

However, an underlying presumption in the field is that a derangement or imbalance of the actions of some or all of the reward neurochemistry is responsible for eating disorders.Further, the pricipal candidate region for such imbalance is in the meso-limbic area.Similar data and logic underlies thinking about drug-dependent disorders.Thus alcohol, opiates, cocaine, and glucose induce reward by activating the mesolimbic reward multineuronal circuitry.

Blum and associates (35-37),have developed a neurotransmitter reward-cascader model that may play a role in the neuropharmacology of RDS (17,19).In this cascade, the hypothalamic serotonergic neurons innervate met-enkephalinergic neurons that, in turn, inhibit GABA neurons, which then activate DA neurons of the ventral tegmentum,.These DA neurons then project to the nucleus accumbens and to Ca1 cluster cells in the hippocampus, where the neurotransmitter DA acts as the primary reward substrates (38).

The importance of both the nucleus accumbens and enkephalins in this complex circuit is attested to by the report of Heidreder et.al. (39) as noted above.Additionally, using a push-pull cannula technique,Chesselet et.al. (40) were able to induce DA release in the striatum after loacal application of enkephakin, which suggests regulation by delta receptor stimulation.Indeed, Kelatorphan may also protect against possible CCK-8 degradation by brain peptidases.this important satiety neuropeptide is colocalized with DA in the nucleus accumbens,. And there is a close interaction between CCK-8, DA , and endogenous opioid peptides (41).

The neurotransmitters 5-HT , DA, NE, and enkephalins have been shown to reduce intake of sweet foods.Thus *Kan-100* was especially designed to enhance these food inhibitory neurotransmitters through precursor amino acid loading, including l-tryptophan (5-HT precursor) ,l-phenylalanine(DA and NE precursor), as well the enkephalinase inhibitor d-phenylalanine ( ).[to raise enkephalins].

A plausable positive mechanism for the obseved effects of *Kan-100* in these studies includes restoration of deficient monoamines such as 5-HT, NE, EPI , as well as the neuropeptides met-enkephaline and CCK-8. All of which are considered to be eating (carbohydrate) substances influenced by either glucose or genetics ( ).

It is noteworthy that *Kan-100* induced it's greatest effect on females with OB+ and CD+ compared to males with the same family history.This difference may be related to the recent findings of Comings et.al. who observed that for females alone both genetic variants of the human obesity (OB) and the human dopamine dopamine D2 (DRD2) genes accounted for up to 22.8% of the variance of the BMI.In terms of the interaction between the OB and DRD2 genes in obesity the binding of leptin to the OB receptor may be involved.This binding activates an intermediate neurotransmitter or neuropeptide that has an effect on behavior as well as on appetite and metabolism.In this regard it is known that Ob/ob mice also show significant decreased levels of dopamine in the arcuate-infundibullum ( ).

Based on this work , we believe that glucose binding is, as previously proposed, similar to other chemical dependencies (ie,alcohol, cocaine, heroin).Finding that *Kan-100* or other similar neuronutrients, as previously observed ( ) with alcoholics, polydrug abusers, heroin abusers, and cocaine - dependent individuals, facilitates recovery, further supports a common mode of treatment for these diverse substances as we proposed earlier (17,19).

This work certainly warrents more extensive research in a double-blind fashion, and should stimulate our colleaques to perform similar trials with other potent enkephalinase inhibitors with their eating- disorder patients,Moreover,this research should provide both impetus and hope for the future development of novel therapeutic measures.

Acknowledgements:we are grateful to the PATH Foundation for the generous contributions.we also acknowledge the financial support of 1899 L.L.C.