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Mealtime food intake and behavior of normal weight adult males: Effects of phenylalanine and aspartame

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The University of North Carolina at Greensboro, 1987

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MEALTIME FOOD INTAKE AND BEHAVIOR OF NORMAL WEIGHT ADULT MALES: EFFECTS OF PHENYLALANINE AND ASPARTAME

by

Milly Ryan Harshman

A Dissertation Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
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Approved by

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APPROVAL PAGE

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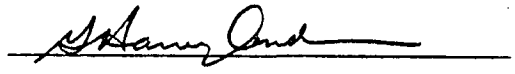
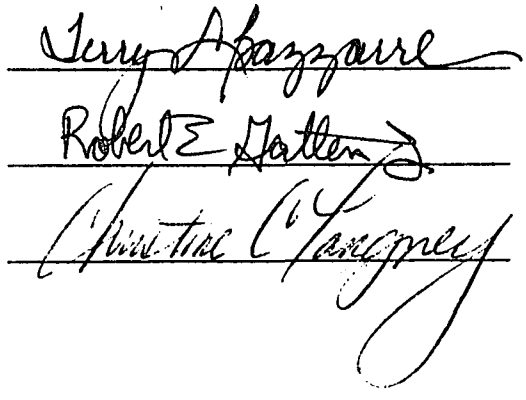
Research Supervisor 

Committee Members 

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RYAN-HARSHMAN, MILLY, Ph.D. Mealtime Food Intake and Behavior of Normal Weight Adult Males: Effects of Phenylalanine and Aspartame. (1987) Directed by Dr. Lucille M. Wakefield and Dr. G. Harvey Anderson. 107 pp.

Two experiments were conducted to investigate the neurobehavioral effects of phenylalanine (PHE; 0.84, 2.52, 5.04, and 10.08 g) and aspartame (APM; 5.04 and 10.08 g) on energy and macronutrient selection and on subjective feelings of hunger, mood and arousal in normal weight adult males. Neither PHE nor APM altered mean energy intakes or macronutrient selection during lunch which began 60 or 105 min after the amino acids were consumed. During this time, increased ($p < .05$) visual analog scale scores for emptiness, rumbling, weakness, degree of hunger and urge to eat were found in both experiments, but no treatment effects or interactions were seen for any variable in either experiment. Plasma amino acid levels were measured after capsule administration at 45 min in experiment 1 and at 90 min in experiment 2 and were compared to baseline samples. Plasma PHE levels and ratios to other large neutral amino acids (NAA) rose significantly ($p < .05$) after all treatments except 0.84 g PHE; plasma tyrosine (TYR) levels increased ($p < .05$) only when greater than 2.52 g PHE was given. TYR/NAA ratios were higher ($p < .05$) after 2.52 and 5.04 g PHE, and 10.08 g APM. No relationships were found between food intake and plasma amino acid levels.

A third experiment was performed to further test the neurobehavioral effects of PHE, as APM, on subjective feelings of hunger, mood and arousal and on plasma amino acid levels. APM or placebo (10.08 g) capsules were administered concurrently with a

high carbohydrate (118 g) breakfast at 8 a.m. Visual analog scales (administered at 8 a.m., 10 a.m., 12 noon and 2 p.m.) showed no treatment effects or interactions for any variable in this experiment, but time affected scores for emptiness, hunger, and urge to eat. When absolute and relative plasma amino acid levels at 90 min following the high carbohydrate breakfast were compared to baseline values, plasma PHE, TYR, and tryptophan (TRP) levels decreased ($p < .05$). The PHE/NAA ratio increased ($p < .05$), but TYR/NAA and TRP/NAA ratios were unchanged. When APM was given with the high carbohydrate breakfast, plasma PHE and its ratio to the other large NAA increased ($p < .05$), but the increase in TYR and the TYR/NAA ratio was not significant. Plasma TRP decreased ($p < .05$), but the TRP/NAA ratio was not significantly decreased. The author concludes that PHE, when given as the free amino acid or as APM in doses up to 10 g, does not affect feeding behavior in normal weight adult males, even when administered concurrently with carbohydrate.

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CHAPTER I

INTRODUCTION

Although the brain comprises only 2% of adult body weight, this organ receives 15% of cardiac output and accounts for 20-30% of the resting metabolic rate (Solokoff, Fitzgerald, & Kauffman, 1977). Logically then, because the central nervous system (CNS) demands a steady influx of nutrients, it could be involved in regulating energy and macronutrient (protein, carbohydrate and fat) intakes. Much research has been focused on the regulation of energy intake, but only recently have the putative roles of the macronutrients in regulating feeding behavior been explored. Brain neurotransmitters, such as the catecholamines norepinephrine and dopamine, may play a key role in appetite regulation through their influence on energy intake and macronutrient selection in the diet. Amino acids serve as precursors in the synthesis of brain neurotransmitters, but the effect of bolus doses of single amino acids on food intake has not been investigated thoroughly. The importance of understanding mechanisms which control appetite is apparent when studying eating disorders such as obesity. Differences in the regulation of feeding behavior may exist between normal and obese individuals. Whether these possible differences exist prior to onset of weight gain or as a result of obesity is unknown. However, obese people often attempt to reduce caloric intake by substituting artificial sweeteners for

sucrose; therefore, understanding the effect of artificial sweeteners on food intake regulation is an important research goal. Aspartame (APM), a relatively new non-nutritive sweetener which is widely used in foods in many countries, contains approximately 50% by weight of phenylalanine (PHE), the amino acid precursor to the catecholamines.

Purpose and Objectives

The purpose of this project was to test the effect of premeal consumption of PHE, as the free amino acid or as APM, on short-term energy intake (kcal), macronutrient selection (percent protein, carbohydrate, fat), and subjective feelings of hunger, mood and arousal during lunch time in normal weight adult males.

The specific objectives were the following:

1) to examine the effect of large intakes (up to 10 g) of PHE on energy intake and macronutrient selection in a meal 2) to examine the effect of large intakes (up to 10 g) of APM on energy intake and macronutrient selection in a meal 3) to measure the effects of premeal consumption of PHE and/or APM on subjective feelings of hunger, mood and arousal as assessed by Visual Analogue Scales (VAS; Maxwell, 1978) 4) to determine the effect of administration of PHE or APM on plasma levels of the large neutral amino acids (NAA) PHE, tyrosine (TYR), tryptophan (TRP), leucine, isoleucine and valine, and on the relative concentrations of PHE, TYR and TRP to the other NAAs (e.g., PHE/NAA) 5) to determine whether the concurrent consumption of a high carbohydrate breakfast (118 g) with APM (10 g)

will enhance the effect of PHE on subjective feelings of hunger, mood and arousal and 6) to investigate whether the concurrent consumption of a high carbohydrate (118 g) breakfast with APM (10 g) will alter the absolute and relative plasma concentrations of PHE, TYR and TRP.

Hypotheses

The following null hypotheses were tested:

1) premeal consumption of PHE and APM will have no effect on energy intake and macronutrient selection in a meal consumed by normal weight adult males 2) premeal consumption of PHE and APM will have no effect on plasma NAAs in normal weight adult males 3) premeal consumption of PHE and APM will have no effect on subjective feelings of hunger, mood and arousal in normal weight adult males 4) concurrent consumption of a high carbohydrate breakfast with APM will have no effect on plasma NAA levels and 5) concurrent consumption of a high carbohydrate breakfast with APM will have no effect on subjective feelings of hunger, mood and arousal in normal weight adult males.

CHAPTER II

REVIEW OF THE LITERATURE

Overview of Appetitive Mechanisms

Mechanisms by which the CNS may regulate feeding behavior must be reviewed before discussing the possible effects of PHE and APM on control of appetite. Of primary interest is that the hypothalamus was identified as the portion of the brain involved in the feeding mechanism. The ventromedial hypothalamus (VMH) is associated with satiety while the lateral hypothalamus (LH) is described as the feeding center (Anderson, Li, & Glanville, 1984). However, subsequent to hypothalamic experiments, evidence was presented which indicated that catecholaminergic and serotoninergic fiber tracts cross the hypothalamus; the introduction of brain lesions in the hypothalamic area to study feeding behavior may actually damage these important neuronal systems (Grossman, 1972; Mogenson, 1974). In experimental animals, the manipulation of brain neurotransmitter (serotonin, norepinephrine, dopamine) systems may alter feeding responses.

The classic glucostatic and aminostatic theories of appetite control cannot be overlooked in a discussion of feeding behavior. Louis-Sylvestre and Le Magnen (1980) observed a 7% decrease in blood glucose concentration prior to the onset of eating in the rat. This observation supports the glucostatic theory of appetite control, i.e., the brain responds directly to alterations in blood glucose

concentration. However, the glucostatic theory of appetite control can be challenged by the observation that hypoglycemia (Stricker, Rowland, & Saller, 1977) and hyperglycemia (Rezek, Havlicek, & Novin, 1975) do not always influence feeding. Because blood glucose concentration does not necessarily indicate the rate of glucose utilization by tissues, brain cellular glucose utilization may be more closely related to feeding behavior (Anderson et al., 1984). Satiation in animals corresponds with a high rate of cellular glucose utilization (Glick & Mayer, 1968) while a low rate of cellular glucose utilization by tissues is associated with eating (Muller, Paneri, Cocchi, Frohman, & Mantegazza, 1973).

The aminostatic theory of appetite control originated from the observation that an inverse relationship existed between serum amino acid concentration and appetite in man (Mellinkoff, Franklin, Boyle, & Geipell, 1956). From this research, Mellinkoff et al. (1956) concluded that, to control appetite, the brain monitored shifts in the plasma amino acid pattern. The protein specific appetite of rats was first described by Musten, Peace, and Anderson (1974). Young rats were given two diets which differed in protein content; both diets contained identical nutrient to energy ratios and were nutritionally complete. Yet, the rats were able to regulate their protein intake from the two diets at a constant proportion of total dietary energy. Cold exposure (Leshner, Collier, & Squibb, 1971) or increased activity (Collier, Leshner, & Squibb, 1969) caused rats to adjust food intake to meet the increased energy requirement, but the

proportion of protein consumed remained unchanged. Overall, the rats chose a diet which resulted in normal growth.

Diet and Brain Neurochemistry

Currently, research is being conducted to determine if neurotransmitters play a role in appetite regulation. The feeding of dietary protein elicits a shift in both plasma and brain amino acid patterns in rats; this shift may influence subsequent feeding behavior through neurotransmitter synthesis (Tews, Good, & Harper, 1978). Until recently, diet was thought to influence brain biochemistry and function only when biochemical and clinical evidence of nutrient deficiency was present. The provision of amino acids, monoamines and peptides for brain neurotransmitter synthesis was thought to be independent of diet for two reasons. Scientists believed the brain could produce sufficient quantities of substrates by utilizing its own resources. In addition, they believed that neurotransmitter synthesis was regulated by metabolic feedback where fluctuations in synthesis are linked to utilization, not precursor availability (Anderson & Johnston, 1983). However, feeding behavior may be directly influenced by the elevation in brain free amino acid pools, or may be indirectly influenced by a block in the brain's uptake of the NAAs (Tews et al., 1978).

Dietary components may affect neurotransmitter synthesis by enhancing the transmission of neural impulses across the synapse; therefore, dietary alterations may result in increased neurotransmitter synthesis. Certain biochemical processes must

follow in sequence if the consumption of a meal rich in a particular nutrient will increase the brain's synthesis of the neurotransmitter for which the nutrient is the precursor. No feedback mechanism can exist which maintains a relatively constant plasma level of the nutrient, and there must be no impenetrable blood-brain-barrier for the precursor nutrient. Both the transport mechanism that mediates the passage of the nutrient from blood to brain and the neuronal enzyme that catalyzes the conversion of the precursor nutrient into the neurotransmitter must be of the low affinity type, i.e., they must become more completely saturated when the nutrient level rises. Finally, the neuronal enzyme must not be susceptible to feedback inhibition when the intracellular level of the neurotransmitter rises. All of these conditions have been met for the synthesis of serotonin, the catecholamines dopamine and norepinephrine, and acetylcholine (Wurtman, 1982).

Anderson and Johnston (1983) noted that the availability of dietary precursors influences the synthesis of at least five neurotransmitters: serotonin (TRP), catecholamines (TYR), acetylcholine (lecithin and choline), histamine (histidine), and glycine (threonine). The relationship between dietary intake of TRP and serotonin synthesis has been established in many studies (Ashley & Anderson, 1975; Fernstrom, Larin, & Wurtman, 1973; Fernstrom & Wurtman, 1971a; Fernstrom & Wurtman, 1971b; Fernstrom & Wurtman, 1972; Li & Anderson, 1984; Wurtman & Fernstrom, 1975). Consumption of a meal high in carbohydrate will increase plasma TRP levels

relative to other large NAAs because of the effect of insulin on plasma amino acids. Insulin secretion following ingestion of carbohydrate will enhance the uptake of large NAAs by peripheral tissues; but, TRP is normally bound to albumin, which prevents its uptake by peripheral tissues. At the level of the blood-brain-barrier, TRP is released from albumin because TRP has a greater affinity for the brain's large NAA transport system carrier. A reduction in plasma concentrations of the competitive amino acids allows more TRP to cross the blood-brain-barrier. Hence, brain TRP levels rise and brain serotonin synthesis is enhanced. In short, carbohydrate consumption increases brain serotonin while protein consumption decreases brain serotonin (Anderson et al., 1984); consequently, rats regulate their macronutrient (carbohydrate and protein) intakes if appropriate dietary choices (Li & Anderson, 1983) are available on both a day-to-day (Musten et al., 1974) and a meal-to-meal basis. If the previous meal consumed was high in carbohydrate, then the rat will choose a meal higher in protein for its next meal (Li & Anderson, 1982).

Unfortunately, the relationship between dietary TYR and brain catecholamine synthesis is not yet clear. Elevations in plasma TYR may influence catecholamine synthesis; in turn, catecholamines may regulate energy balance (Anderson, 1979) and food choice (Wurtman & Wurtman, 1979). In animals, brain catecholamine synthesis seems to respond to food consumption and composition (Landsberg & Young, 1978) as well as to the availability of the precursor, TYR (Gibson &

Wurtman, 1978; Sved & Fernstrom, 1981). Shimazu and Takahashi (1980) stated that norepinephrine and dopamine are both involved in energy expenditure; furthermore, Wurtman, Hefti, and Melamed (1981) reported that normal animals subjected to cold stress exhibited enhanced brain synthesis and release of norepinephrine upon administration of TYR. Leibowitz (1976) studied the roles of norepinephrine and dopamine in mechanisms of appetite regulation. Leibowitz (1976) observed that central injections of norepinephrine in rats caused an increase in meal size and rate of eating; the rats also showed a greater preference for carbohydrate. Similar injections of dopamine increased rate of eating and decreased preference for protein. Central injections of norepinephrine and dopamine may, however, elicit different appetite effects than those observed with dietary manipulation because of the various brain regions and receptor sites affected (Anderson et al., 1984; Leibowitz, 1986).

Dopamine and norepinephrine are formed from TYR by the enzymatic action of TYR hydroxylase (Fernstrom & Wurtman, 1974). TYR is abundant in dietary proteins and, in rats, approximately half of the PHE in protein is converted to TYR during each passage through the hepatic circulation; however, PHE is an essential amino acid and its conversion to TYR is probably not sufficient to meet the body's requirement for TYR (Wurtman et al., 1981). A recent study (Clarke & Bier, 1982) indicates that the rate of conversion of PHE to TYR in man may be somewhat lower than the rate obtained from

PHE loading studies. Yet, the enzyme PHE hydroxylase, which converts PHE to TYR, is also present in catecholamine synthesizing neurons (Bagchi & Zarycki, 1973).

Some evidence exists which supports the concept that the rate-limiting factor in brain catecholamine synthesis is TYR hydroxylase activity, but data presented by Wurtman, Larin, Mostafapour, and Fernstrom (1974) suggest that brain TYR concentration may be an additional factor. The administration of 50 mg/kg of TYR caused, after 45 min, an 81% increase in brain TYR and a 13% increase in the accumulation of dopa ($p < .05$) in rats. A 50 mg/kg dose of PHE did not significantly enhance dopa synthesis, although brain TYR levels were elevated (Wurtman et al., 1974). Agharanya, Alonso, and Wurtman (1981) reported that accelerated catecholamine synthesis can be achieved in the human sympathoadrenal system by TYR administration, most likely by enhancing saturation of TYR hydroxylase. However, Fernstrom (1984) noted that brain TYR levels must increase twofold before TYR hydroxylase activity is enhanced. A short-term increase in the physiological activity of catecholaminergic neurons, such as occurs during stress, can accelerate the conversion of TYR to a catecholamine, perhaps by decreasing the end-product inhibition of TYR hydroxylase by norepinephrine. Also, TYR hydroxylase activity is increased with long-term enhancement of the physiological activity of catecholaminergic neurons (Wurtman & Fernstrom, 1975). Wurtman (1983a) stated that the biochemical mechanism which links the firing

frequency of a neuron to its responsiveness to TYR apparently involves the activation of TYR hydroxylase by phosphorylation. Phosphorylation of TYR hydroxylase increases the enzyme's affinity for TYR and diminishes the enzyme's sensitivity to feedback inhibition by catecholamines. Therefore, the brain selects which catecholaminergic neurons will be allowed to respond to increased precursor levels by modulating the firing frequencies of the neurons; this event is not observed in serotoninergic neurons (Wurtman, 1983a).

Because a single mechanism mediates the uptake of all the large NAAs by the brain, the passage of TYR through the blood-brain-barrier can be accelerated either by raising plasma TYR levels or by lowering plasma levels of other NAAs. Although insulin facilitates the uptake of TYR into skeletal muscle, plasma TYR levels are not lowered to as great an extent as are the branched-chain NAAs (leucine, isoleucine, and valine) which are metabolized primarily in muscle. Therefore, a protein-free meal will cause no change or only a small increase in the plasma ratio of TYR/NAA, but a meal containing protein will increase the plasma TYR/NAA ratio and possibly accelerate the synthesis of catecholamines (Wurtman et al., 1981).

The specific effect of bolus doses of PHE on food intake and brain neurotransmitter synthesis has received even less attention than the effect of TYR on catecholamines and food intake. PHE administration by intragastric infusion has been shown to suppress

food intake in rats (Anika, 1982), dogs (Meyer & Grossman, 1972), and monkeys (Gibbs, Falasco, & McHugh, 1976). This appetite suppressing effect of PHE has been commonly attributed to the fact that PHE is a potent gut cholecystokinin (CCK) secretogogue (Walsh, 1981). However, the possible influence of PHE on brain catecholamine synthesis and food intake cannot be ignored.

Catecholamines and Obesity

Although obesity has been thoroughly investigated, the causes are still unidentified. Wooley (1971) studied the long-term effects of caloric density of food and self-reported hunger in obese and non-obese individuals. No significant differences were observed in energy intakes between normal and obese individuals, but obese subjects' hunger ratings increased throughout the study while normal subjects' hunger ratings remained consistently low. Wooley (1971) concluded that obese subjects' hunger was appetitive (ratings increased in response to the presence of nonexperimental food), but normal subjects' hunger was physiological. Nevertheless, studies have indicated that obesity may have a physiological nature, e.g., obesity may be associated with abnormal catecholamine metabolism. Catecholamine metabolism has been shown to be altered in obese rodents (Coscina, McArthur, Stancer, & Godse, 1978; Cruce, Thoa, & Jacobowitz, 1978; Orosco, Jacquot, & Cohen, 1981). In a study by Johnston, Warsh, and Anderson (1983), baseline urinary excretion of 3-methoxy-4-hydroxy phenylethyleneglycol (MHPG), a norepinephrine metabolite, was associated with body fat content, while excretion of

the norepinephrine metabolite vanilmandelic acid (VMA) was related to lean body mass and total energy intake in 12 normal subjects. These relationships were not observed in nine obese females. Normal subjects responded to TYR with elevations in plasma TYR/NAA, MHPG, VMA and homovanillic acid (HVA, a dopamine metabolite), but not dihydroxy phenylethyleneglycol (DHPG). In the obese, only VMA and HVA excretion increased with TYR supplementation, and these women also exhibited altered circadian rhythms. Johnston et al. (1983) concluded that, if, as believed, the majority of MHPG originates in the brain, the relationship between MHPG and body fat may indicate that central norepinephrine metabolism is linked to energy storage in normal weight subjects, but not in obese subjects. An investigation into the effects of PHE on food intake and brain catecholamine synthesis in experimental animals and to the extent possible in normal weight humans must be conducted before comparisons of PHE's effects can be made between obese and normal weight subjects.

Artificial Sweeteners and Weight Control

In view of the proposed mechanisms by which the CNS regulates appetite, is it possible to achieve weight loss by replacing sucrose in the diet with low calorie artificial sweeteners? The effects of artificial sweeteners on food consumption were investigated as early as 1933. Rats on an ad lib diet preferred glucose and saccharin solutions to water. When the rats drank sugar solutions, they kept caloric intake constant by reducing food intake (Hausmann, 1933).

Sheffield and Roby (1950) reported that hungry animals, but not satiated animals, responded appropriately to a stimulus when saccharin was available as a reward. Friedhoff, Simon, and Friedhoff (1971) reported that mice forced to ingest water containing sucrose ate less solid food than those drinking either plain water or artificially sweetened water. The value of low calorie beverages for weight control or weight reduction was questioned by Friedhoff et al. (1971) because an individual could replace calories missing in the beverages with other foods, and furthermore, ingestion of sugar-containing beverages may not increase body weight because spontaneous reduction of solid food intake may occur.

McCann, Trulson, and Stulb (1956) studied a group of 147 obese individuals. Because 43% of the subjects used non-caloric sweeteners and/or artificially sweetened products, the group was divided into users and non-users. Individuals in both groups lost weight; there was no significant difference in weight loss between the two groups. The length of time non-caloric sweeteners were used did not alter the patterns of weight loss. Also, no relationship existed between the degree of obesity and the use of non-caloric sweeteners. Farkas and Forbes (1965) reported that adherence to a carbohydrate-restricted diet was independent of the use of low-calorie sweeteners in 100 diabetics tested. Parham and Parham (1980) compared 57 women who used saccharin seven or more times weekly (mean = 11.3 times/week) with 45 women who used saccharin

less frequently (mean = 1.0 times/week). Among saccharin users, 41.5% stated that they needed to lose weight while only 17.9% of the non-users thought they needed to lose weight. Although the saccharin users consumed significantly ($p < .05$) fewer servings per week of nutritive sweeteners and foods containing them, the saccharin users still reported consuming 23 servings per week. In addition to substituting saccharin for sugar, the users consumed significantly ($p < .05$) less bread, bakery products and soft drinks than the non-users. The saccharin users also maintained lower ($p < .05$) intakes of energy, carbohydrates, sugars, protein and iron which indicated that the decreased caloric intake may have been due to more than just the replacement of sugar with saccharin. Yet, Parham and Parham (1980) concluded that saccharin is mildly beneficial to healthy individuals who are watching their weight or limiting their sugar consumption. Porikos and Van Itallie (1984) suggested that the use of a non-caloric sweetener by subjects in the Parham and Parham (1980) study facilitated adherence to a diet by satisfying some need for sweets.

Aspartame as an Artificial Sweetener

APM, more commonly known by its commercial name Nutrasweet, is a dipeptide which yields PHE, aspartic acid and methanol upon hydrolysis; APM has a sweetening potential 180-200 times greater than sucrose (Stegink, Filer, & Baker, 1977). APM was discovered accidentally in the G. D. Searle & Co. research laboratories in the early 1960's. Research scientists were conducting experiments with

the inhibition of the gastrointestinal hormone gastrin as a possible treatment for ulcers, and APM was an intermediate in the synthesis of the hormone (Mazur, 1984). The Food and Drug Administration (FDA) approved APM as a tabletop sweetener (Equal) and for certain dry food applications in 1981; approval was extended to allow APM's use in soft drinks in 1983 (The Australian Sugar Industry, 1984).

Attempts have been made to estimate the average daily consumption of APM. Roak-Foltz and Leveille (1984) estimated the potential APM intake as 867 mg daily. An APM intake of 34 mg/kg body weight represents the 99th percentile of projected daily intake if APM replaces 50% dietary sucrose. However, these calculations were carried out before approval of APM for use in carbonated beverages (Stegink, 1984). In Canada, soft drinks contain 102 to 156 mg of APM per 10 ounce can or bottle.

The importance of APM consumption is related to the artificial sweetener's possible influence, through PHE, on brain neurochemistry and behavior, and to its potential ability to control weight through weight reduction and/or maintenance.

Aspartame and Weight Control

The effectiveness of APM in weight reduction and/or weight control has only begun to be explored. An August 1984 petition by The Sugar Association, Inc. before the Federal Trade Commission (FTC) requested immediate action be taken by the FTC against the G. D. Searle Co. for misrepresentation in the advertisement of its product, APM. The association stated that advertisements suggesting

that Nutrasweet and Equal may be useful in weight control were misleading because the company failed to disclose that no long-term studies had been conducted to demonstrate the effectiveness of these sugar substitutes in either weight control or weight reduction (The Sugar Association, Inc., 1984).

Knopp, Brandt, and Arky (1976) reported that weight loss was apparent in two groups of young persons consuming APM (2.79 g/day) or a lactose placebo in the first seven weeks of study, but remained unchanged in the final six weeks, presumably because the subjects lost interest in the study. Because researchers have suggested that obesity occurs in individuals who have an elevated set point for energy storage, Porikos, Booth, and Van Itallie (1977) conducted an experiment to determine if the obese individual had the ability to defend his excess weight when challenged by a covert decrease in the caloric density of his diet. When APM was substituted for sucrose (25% reduction in caloric content of the diet), no change occurred in the quantity (weight) of sweet foods consumed, but caloric intake decreased ($p < .05$) from baseline by 23% (3274 kcal/day vs 2512 kcal/day). The data indicated that the decrease in energy intake was due to APM substitution for sucrose to reduce caloric content, rather than to a rejection of APM sweetened products. However, the increase in caloric intake (energy intake stabilized at 86% of baseline) seen in period III of APM substitution may have marked the beginning of an adaptation period in which the obese individual attempts to defend his energy stores (Porikos et al., 1977). Long

term studies of dietary manipulation are necessary to establish this theory.

Porikos and Van Itallie (1984) conducted two additional studies to determine the effectiveness of APM in weight reduction. APM substitution for sucrose caused energy intake to stabilize at 85% of baseline after 12 days in six normal weight men. In the second study, eight normal weight and five obese men (18-45% overweight) were compared. Obese subjects consumed an average of 20% more calories per day than controls (4131 kcal/day vs 3418 kcal/day), but showed the same pattern of adaptation to decreased energy intake (3464 kcal/day vs 2880 kcal/day). Substitution of APM for sucrose produced a 15% decrease in total energy intake for both groups. Porikos and Van Itallie (1984) concluded that APM may be useful in weight maintenance as well as weight loss. Yet, an undesirable shift in food selection that may occur with APM substitution must be noted. Even though APM substitution for sucrose resulted in a reduction of calorie intake, food preference shifted so that the energy deficit was partially alleviated by choosing foods high in fat. This shift to a higher fat intake must be viewed as a possible undesirable consequence of artificial sweetener consumption because high fat diets are believed to be associated with an increased prevalence of cardiovascular disease (Ball, 1980).

Aspartame and Brain Neurochemistry

Despite the fact that APM has been approved for use as a tabletop sweetener and as a food additive in certain foods and

beverages, controversy still exists concerning the effect of APM on brain neurochemistry. Since the FDA approved the use of APM in soft drinks, Wurtman (1983b) has withdrawn his support of the product. Wurtman (1983b) suggested that individuals with hypertension, Parkinson's disease, insomnia, hyperkinesia, or phenylketonuric heterozygotes and persons taking drugs which interact with plasma PHE or TYR would most likely exhibit behavioral or functional changes following APM ingestion.

Fernstrom, Fernstrom, and Gillis (1983) reported that although APM administration in rats produced substantial elevations in both blood and brain levels of TYR and PHE, its administration caused only a small change in brain TRP, and did not significantly alter the activity of either TYR or TRP hydroxylase, or affect the levels of serotonin, dopamine or norepinephrine. In addition, APM had no significant effect on brain levels of the branched-chain amino acids leucine, isoleucine and valine. The researchers suggested that the anticipated decrease in serotonin synthesis was not realized because even a 200 mg/kg dose (which produced blood levels of PHE in rats comparable to those achieved in humans receiving 34 mg/kg body weight) of APM was not sufficient to raise blood PHE and TYR levels to a point where TRP uptake into the brain was inhibited, or where high brain PHE levels inhibited TRP hydroxylase (Fernstrom et al., 1983). In later research, Fernstrom (1984) observed that a 50 mg/kg body weight dosage of APM raised blood and brain TYR and PHE concentrations in rats, but did not reduce brain TRP levels.

Fernstrom (1984) noted that TYR levels in the blood and brain may have risen because 1) PHE may have been hydroxylated to TYR in the liver, causing blood levels of TYR to rise 2) higher plasma TYR levels may have allowed TYR to more effectively compete with other NAA for transport into the brain and 3) PHE is a substrate for TYR hydroxylase in the brain. Fernstrom (1984) concluded that:

> Aspartame, even when administered to animals in amounts that cause large increments in brain tyrosine and phenylalanine concentrations, produces minimal effects on the brain levels of other [NAA], on the rates of formation of the monoamine transmitters, and on the pharmacological potency of centrally acting [NAA] drugs.

In experiments with various groups, the results have largely demonstrated that APM consumption has no adverse effects on brain neurochemistry. Visek (1984) and Frey (1976) reported that no significant differences in plasma PHE, TYR or PHE to TYR ratios were observed in healthy children and adolescents consuming APM. Plasma TYR levels were slightly, but not significantly, higher during APM administration in young people participating in a weight reduction program (Knopp et al., 1976). Stegink, Filer, Baker, and McDonnell (1980) reported that administration of 100 mg/kg APM caused PHE levels to be significantly higher ($p < .02$) in phenylketonuric (PKU) heterozygous adults than in normal adults. Metabolism of PHE was slower in heterozygous adults because of decreased levels of PHE hydroxylase in the liver, and the expected increase in plasma TYR

levels was delayed in PKU heterozygotes. Although metabolism of PHE was slower in PKU heterozygous adults, Stegink et al. (1980) concluded that the metabolism was adequate to clear an abuse dose (greater than 34 mg/kg/day) of APM. Koch, Shaw, Williamson, and Haber (1976) also reported that no significant medical or biochemical changes occurred in PKU heterozygotes receiving APM during a 28-week study. In children with PKU, elevated plasma PHE levels are associated with mental retardation; however, in PKU children plasma PHE levels range between 180 and 300 moles/100 ml. After an APM load (34 mg/kg body weight), plasma PHE levels rose significantly (12 $\pm$ 3 moles/dl, $p < .001$), but remained within normal postprandial limits (Stegink et al., 1977). Two normal adolescents and two adolescents with PKU were tested with an APM load (34 mg/kg/day) and a PHE load (19 mg/kg/day). No clinically significant alterations occurred in the subjects' excretion of PHE metabolites, although plasma PHE levels rose in the PKU subjects (Koch, Schaeffler, & Shaw, 1976).

However, Yokogoshi, Roberts, Caballero, and Wurtman (1984) suggested that alterations in brain neurochemistry may occur when APM is consumed with carbohydrate. In a preliminary experiment with humans, Yokogoshi et al. (1984) determined that plasma PHE and TYR ratios were elevated beyond the normal ranges in six healthy subjects following ingestion of APM (15 mg/kg) with a snack providing 200 g of carbohydrate. In rats, plasma PHE levels increased ($p < .01$) by 62% and TYR levels by 142% after APM

administration (200 mg/kg). Administration of glucose (3 g/kg) plus APM significantly increased ($p < .01$) plasma PHE and TYR ratios by causing a reduction in plasma branched-chain amino acid levels. Glucose administration apparently resulted in insulin secretion, which enhanced the uptake of the branched chain amino acids into muscle tissue. A 100% increase in brain PHE and a 50% increase in brain TYR was observed after administration of APM plus glucose. When APM was administered with glucose, the expected increase in the plasma TRP ratio was completely blocked. In addition, APM plus glucose resulted in a blockage of serotonin synthesis normally seen following carbohydrate ingestion. The 200 mg/kg dose of APM was given to the rats because, according to the calculations of Yokogoshi et al. (1984), the changes seen paralleled those which would be expected if 1) a 30 kg child obtained 20 mg/kg of APM by drinking a quart of diet beverage containing 500 mg of APM 2) the child consumed an additional 100 mg of APM from other dietary sources and 3) the child concurrently ate carbohydrate, but not protein. In conclusion, Yokogoshi et al. (1984) stated that "aspartame administration may, by elevating brain tyrosine levels, amplify catecholamine release from...neurons, and thus influence the physiological and behavioral mechanisms that they mediate."

Subsequently, Yokogoshi and Wurtman (1986) studied the effects of 200 or 300 mg/kg APM on catecholamine metabolism in various regions of rat brain. A strain-dependent increase in regional brain levels of TYR or MHPG sulfate was reported following oral

administration of APM. Regional increases in brain norepinephrine, dopamine and their major metabolites following oral administration of APM was also observed by Coulombe and Sharma (1986), but serotonin and its metabolite, 5-hydroxy indole acetic acid (5-HIAA), were unaffected by APM. However, neither of these studies addressed the issue of the functional significance (through behavioral testing of the rats) of these brain neurotransmitter alterations.

Much of the speculation concerning APM's effects on human brain neurochemistry has been extrapolated from studies of patients with PKU or hyperphenylalaninemia. Severe maternal hyperphenylalaninemia (plasma PHE levels greater than 0.6 to 1.0 mM), can cause mental retardation in the fetus, but what is unclear is whether the effects of hyperphenylalaninemia on brain development follow a threshold or a linear relationship. One would not expect mental retardation to occur in a fetus whose mother's plasma PHE levels were below 0.6 mM, but would subtle, undetectable changes in IQ occur (Pardridge, 1986)?

Aspartame and Behavior

Reports concerning APM's effect on behavior in humans are limited. Possible behavioral side effects noted in the literature include disorientation, depression (Monte, 1984), nervousness, loss of appetite (Stern et al., 1976) or change in appetite (Knopp et al., 1976; Wurtman, 1983b) and emotional disturbances (Knopp et al., 1976). Other potential neurochemically related behavioral effects of large doses of APM which are associated with its PHE component

that have been suggested include subtle changes in seizure thresholds in susceptible individuals, insomnia, blockade of therapeutic effects of drugs, menstrual irregularities, and cardiovascular functions such as blood pressure regulation (Pardridge, 1986).

No symptoms, such as loss of appetite or nervousness, could be attributed to the administration of either APM or placebo in non-insulin dependent diabetic subjects (Stern et al., 1976). In a chart concerning the number of side effects reported by young obese subjects consuming APM, five complaints of appetite change were reported for subjects ingesting APM, while eight complaints of appetite change were listed for subjects receiving a lactose placebo (Knopp et al., 1976).

Although studies (Lieberman, Corkin, Spring, Wurtman, & Growdon, 1985; Leathwood & Pollet, 1982/83) have clearly demonstrated an effect of TRP on behavior in humans, these same studies have failed to show any behavioral effects of TYR. The potential behavioral effects of PHE and APM have been virtually unexplored in clinical investigations. Recently, the Centers for Disease Control (CDC) in Atlanta published an evaluation of anecdotal reports of adverse reactions to APM. These complaints were divided into three general categories - those which affect the central nervous system, the gastrointestinal tract, and gynecological function. In this report, the authors emphasized the importance of developing well-defined criteria to detect and

evaluate, in focused clinical studies, unexpected adverse reactions to food additives such as APM (Bradstock et al., 1986).

In summary, two mechanisms exist by which APM may affect food consumption. Because APM is much sweeter than sucrose, less APM than sugar can be used to sweeten foods, thereby reducing the caloric content of sweets. If APM substitution for sucrose causes a reduction in caloric intake, then the success of APM, which has no bitter aftertaste, can be measured by its effectiveness in weight reduction in obese individuals. In addition, PHE, which can be readily converted to TYR, is a major constituent of APM. Appetite regulation is one behavior that is likely to be sensitive to APM ingestion because amino acids, such as PHE and TYR, may influence appetite regulatory mechanisms through precursor control of neurotransmitter secretion. The discovery of APM may lead to important benefits in man, but controlled experiments are necessary to clarify the effects of PHE and APM on food consumption.

In the experiments reported herein, it is proposed that the PHE component of APM may, by enhancing the synthesis of central catecholamines, cause subjects to alter energy intake and macronutrient selection (increased preference for carbohydrate) during lunch time. In addition, differences may be observed in the subjects' perception of hunger, mood and arousal. Plasma amino acid levels will reflect alterations in absolute and relative concentrations of PHE, TYR and TRP.

CHAPTER III

METHOD

Design

Initially, two independent experiments were conducted with healthy males between the ages of 20 to 35 years, and within 90 and 110% of ideal body weight according to the Metropolitan Life Insurance Co. (1983) tables. All subjects were recruited from the University of Toronto campus through posted notices and advertisements. The subjects gave informed consent to participate in the experiments, which were approved by the University of Toronto Human Subjects Review Committee. Individuals who were identified as possible restrained eaters because scores were higher than 40 on an eating habits questionnaire (Herman & Polivy, 1980) or those individuals who practiced unusual eating habits (e.g., vegetarianism) or those following restrictive therapeutic diets were excluded.

General demographic, health, dietary and anthropometric information was collected for all subjects prior to each experiment. Habitual food selection patterns, reflecting food preferences, were assessed using a food frequency instrument. Height and weight were recorded, and triceps, biceps, subscapular and suprailiac skinfold thicknesses were measured with Harpenden skinfold calipers. Lean body mass and percent body fat were approximated from the sum of four skinfolds (Durnin & Womersley, 1974).

In experiment 1 (n = 13), four feeding trials were conducted following administration of capsules containing PHE in doses of 0 g (5.04 g alanine (ALA) placebo), 0.84 g, 2.52 g and 5.04 g. Four to five subjects were tested concurrently in sessions held at one week intervals. The order of treatments was double blind and randomized according to a randomized complete block design.

After an overnight fast (which began at 8 p.m.), the subjects were given a standardized breakfast (600 kcal) which consisted of cereal (35 g), milk (225 ml), whole wheat bread (60 g), butter (10 g), jam (15 g), orange juice (169 ml) and coffee or tea if requested. Following breakfast, the subjects were free to leave the test facilities, but were instructed not to consume any foods or beverages, except tap water, during the morning period. To disguise the true purpose of the experiments, subjects were advised that they were participating in a study examining the effect of a nutrient on taste perception; accordingly, taste tests were included in the protocol. Subjects tasted 2 g portions of four foods (ham, cheddar cheese, bread, chocolate cookie) without swallowing, and the perceived sweetness, sourness, saltiness, bitterness and overall liking of each food item was rated on scales which were later discarded.

Visual analog scales (VAS; Maxwell, 1978) were administered at 11 a.m. (capsules taken at 11:15 a.m.), 12 noon (buffet style luncheon served at 12:15 p.m.), and 12:45 p.m. to assess 17 subjective feelings of hunger, mood and arousal. Subjects were asked to record their feelings regarding stomach sensations

(emptiness, rumbling, ache, nausea), head sensations (headache, dizziness, faintness), general sensations (drowsy, weak, nervous, tense, drugged, depressed, alert, mentally slow), degree of hunger and urge to eat on 100 mm unnumbered scales. The time interval (45 min) between administration of the first and second VAS was selected because of its reported sufficiency in producing a maximum rise in plasma PHE after an oral dose (Stegink, Filer, & Baker, 1981).

Twelve capsules identical in appearance were administered with 300 to 500 ml of tap water; the appropriate dose was achieved by mixing placebo and PHE capsules. The buffet style luncheon consisted of dinner rolls, butter, cold meats, cheeses and cookies; water was given as a beverage. All foods were pre-weighed and served in excess on separate trays for each subject (Table 1). Subjects were left alone and unobserved during lunch, but were instructed not to exchange foods because their food intake would be compared to taste test results. Following the experimental sessions, all leftover foods were re-weighed and meal size (kcal) and macronutrient (percent protein, fat, carbohydrate) selection were determined (from table 1) in all trials for each of the 13 subjects. All food preparation and testing was carried out in the experimental kitchen/dining facility located in the Fitzgerald Building at the University of Toronto.

In experiment 2 (n = 13), conditions were similar to experiment 1 except that doses were different and additional time (a duration of 90 min) was allowed between administration of PHE and APM by capsule and the second VAS test. The length of time between capsule

Table 1

Nutrient Content of Foods Served

Food	Amount Served[a] (g)	Energy Density (kcal/g)	Nutrients (g/g food) Protein	CHO[b]	Fat
Dinner Rolls	250	2.90	0.09	0.56	0.03
Butter[c]	30	7.20	0	0	0.80
Ham	250	1.40	0.20	0.01	0.06
Salami	250	2.16	0.16	0.01	0.17
Cheddar Cheese	200	3.72	0.25	0.02	0.30
Havarti Cheese	200	4.29	0.21	0.01	0.38
Chocolate Cookies	80	4.92	0.04	0.70	0.22
Chocolate Chip Cookies	110	4.83	0.05	0.65	0.23
Fig Newtons[c]	110	3.58	0.04	0.75	0.06

[a] Average amount served. [b] CHO = carbohydrate. [c] Composition obtained from food composition tables; other foods by proximate analysis.

administration and behavioral or biochemical testing was increased on the chance that insufficient time was allowed for neurobehavioral effects of phenylalanine to occur. Doses administered were ALA placebo (10.08 g), 5.04 g APM plus 5.04 g ALA, 10.08 g APM and 10.08 g PHE, and VAS tests were administered at 10 a.m. (24 capsules taken at 10:15 a.m.), 11:45 a.m. (buffet lunch served at noon) and 12:30 p.m.

Some subjects (n=5, experiment 1; n=7, experiment 2) volunteered to provide blood samples for plasma amino acid analysis. Venous blood was drawn on days separate from the feeding experiments. After an overnight fast, the subjects were given the same breakfast as before. A baseline blood sample was obtained 2 1/2 hr later, immediately before capsule administration. A second sample was drawn 45 min (90 min in experiment 2) after the capsules were ingested.

A third experiment was designed to test further the behavioral effects of a large dose of APM (10 g) ingested concurrently with a high carbohydrate (118 g) breakfast, and to determine the effects of this treatment on plasma amino acid levels.

Six male subjects, between the ages of 20 to 35 whose weights were between 90 and 110% ideal body weight, were recruited from the Department of Nutritional Sciences at the University of Toronto. The experiment was approved by the University of Toronto Human Subjects Review Committee, and subjects gave their informed consent to participate on two separate occasions (APM vs. ALA placebo).

On experimental days, baseline blood samples were drawn at 7:30 a.m. following an overnight fast; a high carbohydrate breakfast (orange juice, 500 ml; whole wheat toast, 90 g; butter, 10 g; jam, 20 g) was served at 7:45 a.m. At 8 a.m. the subjects ingested (with their juice) 24 capsules containing either 10.08 g APM or ALA. The subjects were also asked to complete the first of four VAS assessing subjective feelings of hunger, mood and arousal. At 9:30 a.m. (90 min after capsule administration) a second blood sample was obtained. The remaining sets of VAS were completed at 10 a.m., 12 noon and 2 p.m.

To standardize protein intake during the trials, subjects were given portions of vanilla flavored Ensure (1.4 g protein/kg lean body mass; Ensure contains 14% protein, 31% fat, 54% carbohydrate, 1.06 kcal/ml). Ensure allotments for each individual were divided into three equal meals at 12 noon, 4 p.m. and 8 p.m. Subjects had been instructed not to eat or drink anything, except tap water, between breakfast and the second blood sample. Individual energy requirements were met by consuming additional calories from apple juice, whole wheat bread and plain cake type doughnuts.

Analytical Methods

Plasma amino acid levels were determined fluorometrically by high pressure liquid chromatography (HPLC) following the method described by Fernstrom and Fernstrom (1981).

Statistical Analyses

A one-way analysis of variance (ANOVA) with repeated measures was performed on food intake data from experiments 1 and 2 (Winer,

1971). Marks on the VAS were converted to numerical scores on which a two-way (treatment vs. time period) ANOVA with repeated measures was performed (Winer, 1971). Plasma amino acid levels were analyzed by paired t-tests (Steel & Torrie, 1980).

CHAPTER IV

RESULTS

In experiment 1, the 13 subjects were (mean ± S.D.) 24.2 ± 4.4 years of age, 177.7 ± 4.1 cm in height, 68.7 ± 5.9 kg in weight and 11.0 ± 2.6 % body fat. Mean energy intakes did not differ significantly (Table 2). Macronutrient selection was not significantly different between feeding trials, but remained relatively constant at about 16% protein, 36% carbohydrate and 48% fat. Analysis of the VAS indicated that there were no treatment effects on stomach, head or general sensations or on degree of hunger or urge to eat (data not shown).

Incremental changes from baseline plasma levels of PHE, TYR and the ratios of PHE, TYR and TRP to the other large neutral amino acids (NAA) are listed in Table 3. Plasma PHE levels were significantly increased ($p < .05$) at 5.04 g PHE (at 2.52 g PHE, the standard deviation was large and obscured statistical significance). Plasma TYR rose significantly ($p < .05$) at doses of 2.52 g PHE and 5.04 g PHE. Plasma PHE/NAA was significantly higher ($p < .05$) after all PHE doses, but plasma TYR/NAA was significantly increased ($p < .05$) only after doses of 2.52 g PHE and 5.04 g PHE. Plasma TRP/NAA was significantly lower ($p < .05$) following the 5.04 g PHE dose.

Table 2

Food Intake Following Phenylalanine (PHE) Administration in Adult Males

	Treatment			
Food Intake	Placebo 5.04 g ALA[a]	PHE 0.84 g	PHE 2.52 g	PHE 5.04 g
Grams Consumed (dry wt.)	244.0 ± 61.0	283.0 ± 91.0	248.0 ± 90.0	226.0 ± 72.0
Energy (kcal)	1330.0 ± 311.0	1543.0 ± 471.0	1334.0 ± 479.0	1215.0 ± 366.0
Protein (%)	17.4 ± 3.2	15.6 ± 3.5	16.2 ± 2.5	17.0 ± 3.2
Carbohydrate (%)	34.1 ± 4.9	35.5 ± 8.0	37.0 ± 6.6	36.5 ± 7.0
FAT (%)	48.8 ± 4.6	49.2 ± 5.2	47.1 ± 5.7	47.0 ± 5.1
CHO[c]/Protein (g/g)	2.1 ± 0.7	2.5 ± 1.1	2.4 ± 0.6	2.3 ± 0.9

[a]ALA = alanine. [b]N = 13, $\bar{x} \pm$ SD. [c]CHO = carbohydrate.

Table 3

Changes in Plasma Amino Acid Concentrations and Ratios Following Phenylalanine (PHE) Administration in Adult Males

Amino Acid	Baseline	Treatment			
		Placebo 5.04 g ALA[a]	PHE 0.84 g	PHE 2.52 g	PHE 5.04 g
PHE (μmoles/dl)	5.4 ± 0.7[b]	-0.36 ± 0.58	3.2 ± 2.4	10.1 ± 9.4	22.4 ± 7.2*
TYR[c] (μmoles/dl)	6.7 ± 0.9	-1.2 ± 1.1	1.5 ± 3.0	2.7 ± 2.2*	3.3 ± 1.5*
PHE/NAA[d]	0.097 ± 0.010	0.005 ± 0.01	0.065 ± 0.04*	0.174 ± 0.13*	0.419 ± 0.14*
TYR/NAA	0.123 ± 0.012	-0.004 ± 0.02	-0.026 ± 0.05	0.029 ± 0.02*	0.016 ± 0.02*
TRP[e]/NAA	0.123 ± 0.005	0.009 ± 0.01	-0.008 ± 0.02	-0.019 ± 0.01	-0.037 ± 0.02*

Note. The baseline shown is the average of the mean baselines for each individual which were calculated from the plasma values obtained prior to the four treatments. The treatment effects are shown as the differences between plasma values 45 min after treatment minus baseline values for that day's trial for each individual.

[a]ALA = alanine. [b]N = 5, $\bar{x} \pm$ SD. [c]TYR = tyrosine. [d]NAA = neutral amino acids. [e]TRP = tryptophan.

*Different ($p < 0.05$) from baseline, paired t-tests.

For the five individuals who participated in the biochemical study, no consistent relationship existed between food intake and plasma amino acid levels. At the highest dose of PHE (5.04 g), plasma PHE increased more than 400% from baseline, and plasma TYR increased 60% from baseline.

In experiment 2, the 13 subjects were (mean $\pm$ S.D.) 23.1 $\pm$ 3.8 years of age, 176.0 $\pm$ 7.2 cm in height, 66.5 $\pm$ 6.7 kg in weight, and 10.9 $\pm$ 2.7 % body fat. Neither APM nor PHE affected mean energy intakes and macronutrient selection was not significantly different, but was similar to experiment 1 (approximately 16% protein, 37% carbohydrate, 47% fat; Table 4). In spite of an absence of effect of PHE and APM on VAS scores in both these experiments, VAS scores were significantly changed ($p < .05$) over time (Figures 1 and 2). Before lunch, the subjects reported higher values for emptiness, stomach rumbling, weakness, degree of hunger and urge to eat; these effects of time were eliminated by eating lunch. No interactions were seen for any variable in either experiment.

Once again, no consistent relationships were found between food intake and plasma amino acid concentrations or ratios in those individuals from whom blood was drawn. Values for plasma PHE, TYR, PHE/NAA, TYR/NAA and TRP/NAA are listed in Table 5. Plasma PHE concentration and its ratio to the other NAA rose significantly ($p < .05$) at all doses given (placebo excepted), but plasma TYR only rose ($p < .05$) at the highest dose of PHE (10.08

Table 4

Food Intake Following Phenylalanine (PHE)

and Aspartame (APM) Administration in Adult Males

	Treatment			
Food Intake	Placebo 10.08 g ALA[a]	APM 5.04 g	APM 10.08 g	PHE 10.08 g
Grams Consumed (dry wt.)	226.0 ± 63.0[b]	203.0 ± 57.0	207.0 ± 66.0	197.0 ± 69.0
Energy (kcal)	1230.0 ± 358.0	1103.0 ± 355.0	1124.0 ± 387.0	1070.0 ± 385.0
Protein (%)	16.7 ± 3.2	16.5 ± 3.6	15.6 ± 3.3	16.1 ± 4.0
Carbohydrate (%)	35.8 ± 5.4	37.5 ± 7.2	38.1 ± 7.0	36.7 ± 5.5
Fat (%)	47.7 ± 4.2	46.1 ± 6.1	46.6 ± 6.2	47.3 ± 3.5
CHO[c]/Protein (g/g)	2.3 ± 0.9	2.5 ± 1.0	2.6 ± 1.0	2.5 ± 1.0

[a]ALA = alanine. [b]N = 13, $\bar{x}$ ± SD. [c]CHO = carbohydrate.

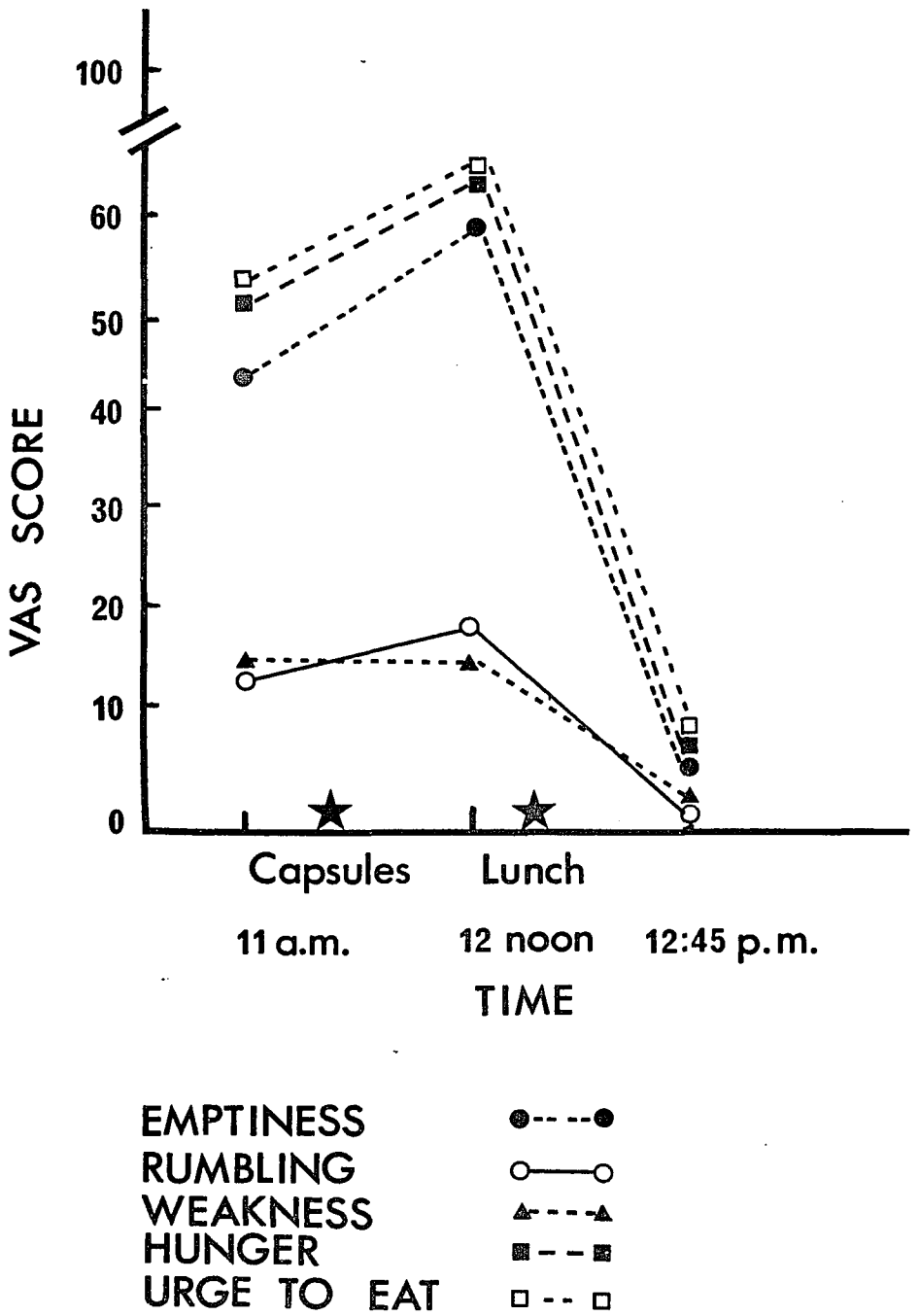

Figure 1. Mean VAS scores for three time periods in experiment 1 illustrate the effect of lunch time on perceived feelings of hunger, mood and arousal.

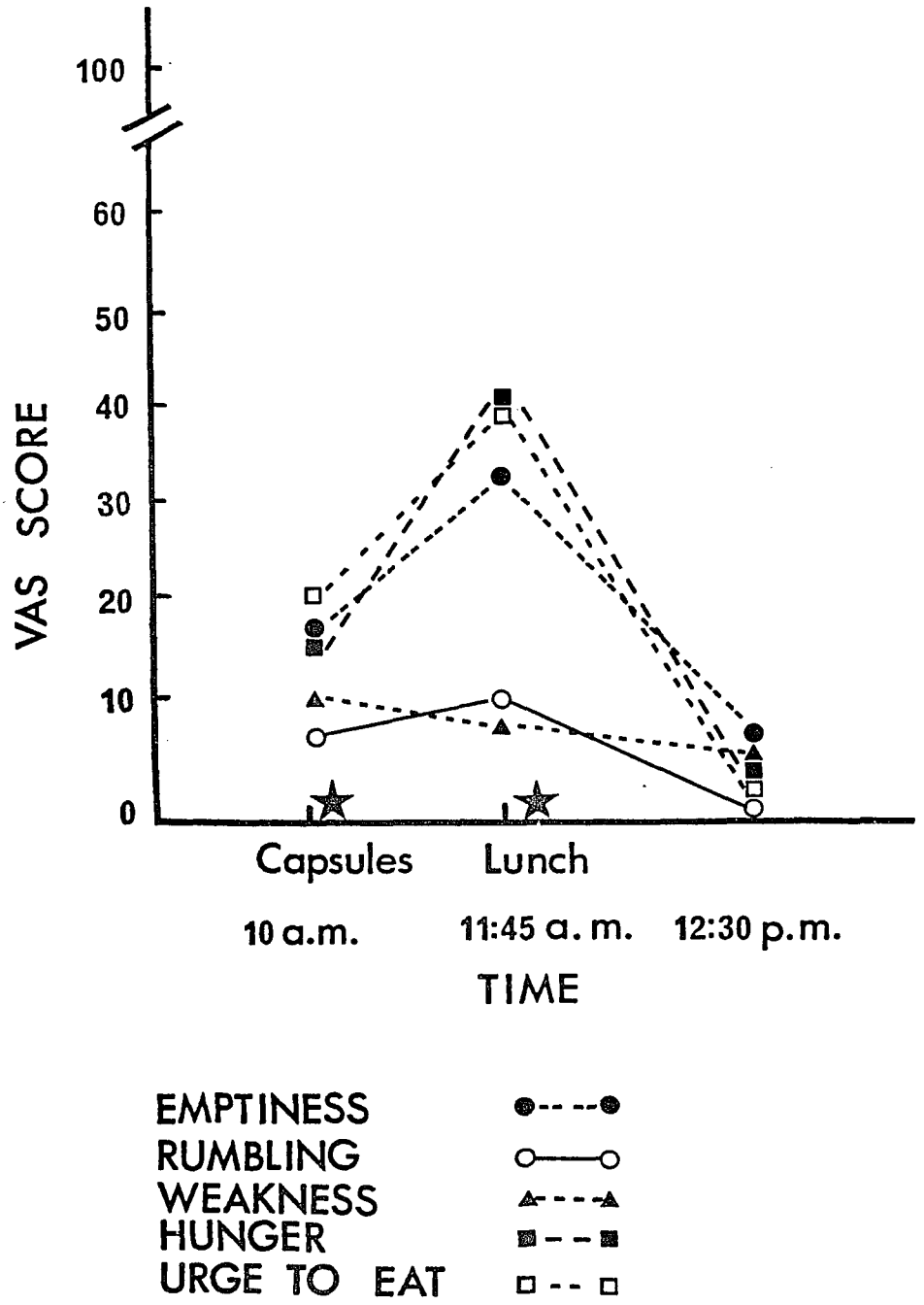

Figure 2. Mean VAS scores for three time periods in experiment 2 illustrate the effect of lunch time on perceived feelings of hunger, mood and arousal.

Table 5

Changes in Plasma Amino Acid Concentrations and Ratios Following Phenylalanine (PHE) and Aspartame (APM) Administration in Adult Males

		Treatment			
Amino Acid	Baseline	Placebo 10.08 g ALA[a]	APM 5.04 g	APM 10.08 g	PHE 10.08 g
PHE (μmoles/dl)	7.1 ± 0.7[b]	-1.0 ± 0.6	5.5 ± 3.9*	19.3 ± 7.8*	76.0 ± 33.1*
TYR[c] (μmoles/dl)	7.7 ± 1.2	0.1 ± 2.5	2.5 ± 1.5	4.7 ± 2.9	8.2 ± 5.4*
PHE/NAA[d]	0.116 ± 0.013	0.017 ± 0.014	0.083 ± 0.066*	0.397 ± 0.204*	1.261 ± 0.827*
TYR/NAA	0.129 ± 0.015	0.012 ± 0.044	0.032 ± 0.030	0.065 ± 0.039*	0.0 ± 0.051
TRP[e]/NAA	0.136 ± 0.028	-0.014 ± 0.036	-0.028 ± 0.020*	-0.046 ± 0.018*	-0.99 ± 0.017*

Note. The baseline shown is the average of the mean baselines for each individual which were calculated from the plasma values obtained prior to the four treatments. The treatment effects are shown as the differences between plasma values 90 min after treatment minus baseline values for that day's trial for each individual.

[a]ALA = alanine. [b]N = 7, $\bar{x} \pm SD$. [c]TYR = tyrosine. [d]NAA = neutral amino acids. [e]TRP = tryptophan.

*Different ($p < 0.05$) from baseline, paired t-tests.

g). The ratio of TYR to the other NAA increased significantly ($p < .05$) at a dose of 10.08 g APM, but the high plasma PHE levels at 10.08 g PHE caused the plasma TYR/NAA to remain unchanged. TRP/NAA was significantly decreased ($p < .05$) at all doses except placebo.

In experiment 3, the six subjects were (mean $\pm$ S.D.) 26.7 $\pm$ 3.1 years of age, 173.6 $\pm$ 4.8 cm in height, 68.3 $\pm$ 7.0 kg in weight and 12.7 $\pm$ 3.6 % body fat. Analysis of the VAS indicated that there were no treatment effects on stomach, head or general sensations or on degree of hunger or urge to eat (data not shown). However, the effects of time were observed for the variables emptiness, hunger and urge to eat. In the hours before lunch (10 a.m. and 12 noon), the subjects rated these sensations higher than after lunch (Figure 3). No interactions were seen for any variable in this experiment.

Plasma values for PHE, TYR, TRP, PHE/NAA, TYR/NAA and TRP/NAA are listed in Table 6. Comparisons are shown between the effect of a 10.08 g APM dose administered concurrently with a high carbohydrate (118 g) breakfast and the high carbohydrate breakfast alone (with placebo). The high carbohydrate breakfast caused a reduction ($p < .05$) in the plasma concentrations of PHE, TYR and TRP. Because the branched-chain amino acid levels were also reduced (Table 7), the ratio of PHE to the other NAA was increased ($p < .05$). However, the increases in TYR/NAA and TRP/NAA ratios did not reach significance. When APM was given with the high

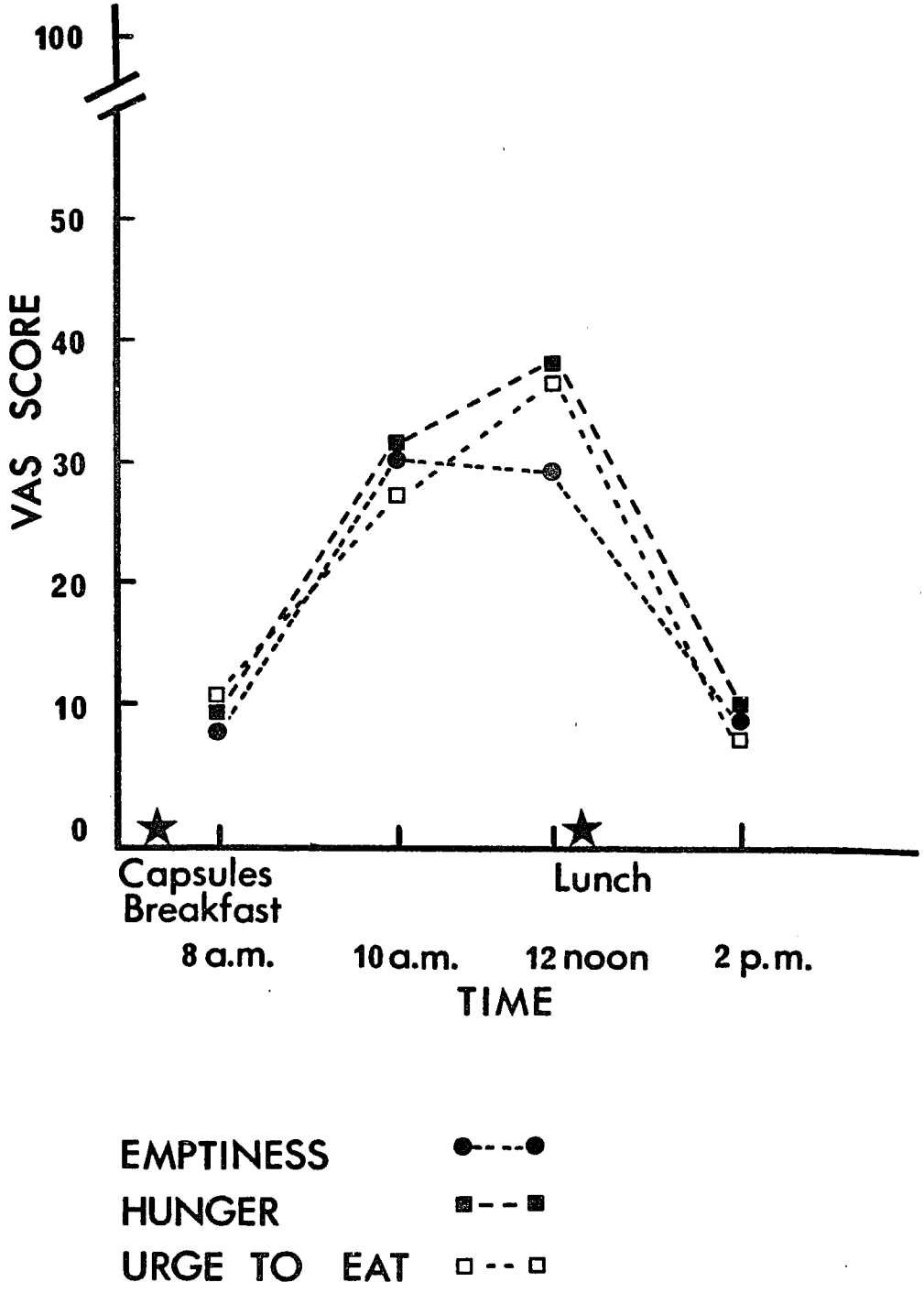

Figure 3. Mean VAS scores for four time periods in experiment 3 illustrate the effect of lunch time on perceived feelings of hunger, mood and arousal.

Table 6

Plasma Amino Acid Concentrations and Ratios Following Administration of Aspartame (APM) or Alanine (ALA) Placebo Concurrently With a High Carbohydrate Breakfast

Amino Acid	Experimental Condition			
	Baseline	APM (10.08 g) + Breakfast	Baseline	ALA (10.08 g) + Breakfast
PHE (μmoles/dl)	6.4 ± 1.1[a]	15.3 ± 5.3*	6.8 ± 0.6	5.9 ± 0.9*
TYR (μmoles/dl)	8.7 ± 0.9	10.7 ± 3.2	8.6 ± 1.6	6.5 ± 1.3*
TRP (μmoles/dl)	7.2 ± 0.9	6.7 ± 1.0*	8.0 ± 1.4	6.3 ± 1.5*
PHE/NAA	0.114 ± 0.018	0.303 ± 0.095*	0.117 ± 0.026	0.131 ± 0.032*
TYR/NAA	0.169 ± 0.050	0.190 ± 0.032	0.150 ± 0.036	0.153 ± 0.059
TRP/NAA	0.132 ± 0.019	0.116 ± 0.021	0.137 ± 0.022	0.139 ± 0.023

[a] N = 6, $\bar{x} \pm$ SD

*Different ($p < .05$) from baseline, paired t-tests.

Table 7

Pre- and Posttreatment Branched-Chain Amino Acid Levels Following a High Carbohydrate Breakfast and Aspartame (APM) Administration in Adult Males

	Treatment			
	ALA[a] Placebo Plus Breakfast		APM[b] Plus Breakfast	
Amino Acid	Baseline	Experimental	Baseline	Experimental
Valine (μmoles/dl)	20.9 ± 8.0[c]	17.3 ± 7.5*	18.8 ± 7.9	16.6 ± 7.0
Leucine (μmoles/dl)	15.8 ± 2.1	11.3 ± 2.0*	14.6 ± 2.2	11.4 ± 1.4*
Isoleucine (μmoles/dl)	7.7 ± 0.8	5.6 ± 1.0*	7.4 ± 1.0	5.9 ± 0.7*

[a]ALA = Alanine

[b]APM = Aspartame

[c]N = 6, $\bar{x}$ ± S.D.

*Significantly ($p < 0.05$) different from baseline.

carbohydrate breakfast, the plasma concentration of PHE increased ($p < .05$) significantly. However, the plasma TYR level did not significantly increase, primarily because of the large variation among subjects. The plasma level of TRP was significantly decreased ($p < .05$) following this treatment. The ratio of plasma PHE/NAA was increased ($p < .05$), but the increase in the TYR/NAA ratio did not reach significance. The TRP/NAA ratio was not significantly decreased because of the large variation among the six subjects.

CHAPTER V

DISCUSSION

Our results show that lunchtime energy intake and macronutrient selection were not affected by PHE or APM in doses up to 10 g. In addition, subjective feelings of hunger, mood and arousal were unaffected by these doses.

We chose the lunchtime feeding paradigm to assess the affect of PHE on food intake because it allows subjects to freely select from a variety of appealing foods, and provides a situation which allows a quantitative assessment of food consumption. Foods offered to the subjects were chosen for their lunch time appeal (i.e. sandwiches, cookies), but the high fat content (Table 1) of the cheeses, butter, salami and cookies with chocolate may have confounded preferences for protein or carbohydrate. Although no effects of PHE and APM were observed, this same feeding paradigm has been demonstrated to be appropriate in detecting effects of amino acids. TRP, the amino acid precursor for the brain neurotransmitter serotonin (Anderson et al., 1984), caused a significant ($p < .001$) reduction in energy consumption (250-400 kcal) when two or 3 g TRP was given to lean, healthy adult males (Hrboticky, Leiter, & Anderson, 1985). Also, a greater suppression ($p < .05$) of carbohydrate food choices following TRP was observed in normal weight women, particularly women who were

unrestrained eaters (Hrboticky, 1986). TRP has been shown to modulate behaviors such as sleep and mood (Leathwood & Pollet, 1983; Lieberman et al., 1985). To provide subjective evaluation of hunger, mood and arousal, VAS are frequently used measures which correlate reasonably well with expressed behavior (Maxwell, 1978). The scales appear to be sensitive to the effects of amino acids, if they occur, because administration of two or 3 g TRP produced mild psychotropic effects (increased drowsiness, increased dizziness and/or decreased mental alertness; $p < .05$) in normal weight men (Hrboticky et al., 1985). PHE and APM did not affect the VAS, suggesting that if they did have mild psychotropic effects, these were much less robust than those of TRP and were undetectable by an assessment which has a large within subject variability.

The lack of effect on VAS of large doses of APM are of particular interest because of the Centers for Disease Control (CDC) report evaluating consumer complaints regarding APM (Bradstock et al., 1986). The VAS used in our experiments contained descriptive words such as headache, dizziness, stomach ache, nausea, drowsiness, alertness and depression which are similar to those symptoms reported by complainants after APM consumption in the CDC report. That none of our subjects reported any unusual reactions to APM consumption (in very high doses) may indicate that the symptoms noted by complainants were likely idiosyncratic and not representative of the general population.

Alternatively, these responses may be normal responses (see Figure 1) to increasing energy (caloric) deprivation, which is self-inflicted and the reason many consumers choose APM containing beverages or foods.

PHE administration by intragastric infusion has been shown to suppress food intake in rats (Anika, 1982), dogs (Meyer & Grossman, 1972), and monkeys (Gibbs et al., 1976). This appetite suppressing effect of PHE has been commonly attributed to the fact that PHE is a potent gut CCK secretogogue (Walsh, 1981). Our data on PHE do not contradict the literature because the dose of PHE required to suppress food intake in Rhesus monkeys was 500 mg/kg body weight (Gibbs et al., 1976) and in rats, 1 g/kg body weight (Anika, 1982). In our experiments, the highest average PHE dose was approximately 150 mg/kg body weight. Perhaps a much smaller endogenous CCK response than those used exogenously (Nicholl, Polak, & Bloom, 1985) accounts for the lack of effect of PHE on food consumption in men in the present study. In another human study, an amino acid mixture (PHE, 3 g; valine, 2 g; methionine, 2 g; TRP, 1 g) preload produced short term satiety in four overweight subjects. The appetite suppression was attributed to a CCK mediated satiety by the authors (Butler, Davies, Gehling, & Grant, 1981). It seems more likely; however, that appetite suppression was mediated by TRP and valine which have been shown to suppress food intake in humans and rats, respectively (Hrboticky et al., 1985; Li et al., 1986).

ALA was selected as a placebo in our experiments for two reasons. First, it does not stimulate the release of CCK (Walsh, 1981), and second, it provides an equivalent source of nitrogen which, through the production of ammonia and urea, may influence food intake regulation centers (Ashley, 1974). To rule out this non-specific effect of amino acids and thus identify a unique role for an amino acid in influencing feeding behavior, ALA is a more appropriate control than a carbohydrate. By increasing the ALA placebo from 5.04 g in experiment 1 to 10.08 g in experiment 2, energy intake was not suppressed to any greater degree (1330 kcal vs. 1230 kcal); therefore our results also suggest that ALA, and the nitrogen it contains does not affect food intake.

Biochemical measures such as plasma amino acid levels lend strong support to the interpretation of data arising from experiments in which behavioral testing is employed. Because the large neutral amino acids (NAA), primarily PHE, TYR, TRP, leucine, isoleucine, and valine, share a common carrier mechanism for uptake across the blood-brain-barrier (Pardridge, 1977), changes in plasma amino acid concentrations is an important factor in predicting precursor availability for brain neurotransmitter synthesis. Clearly, the doses of PHE and APM led to changes in plasma PHE, TYR and amino acid ratios which would lead to increased brain uptake of PHE and TYR. In our experiments, administration of 5.04 g PHE resulted in a plasma PHE concentration of 27.7 μmoles/dl after 45 min, whereas 10.08 g

APM, which contains roughly 5 g PHE, resulted in a plasma PHE concentration of 26.2 μmoles/dl after 90 min. This suggests that plasma PHE levels do reach a peak by 45 min (e.g. as found in experiment 1) and are maintained for up to 90 min after capsule administration. Current views on precursor control of neurotransmitter synthesis suggest that alterations in brain neurotransmitter synthesis would be expected from the increased brain PHE and TYR (Sved, 1983). Clearly, if this occurred, no behavioral consequences were detected.

The results of the VAS in experiment 3 were consistent with those of both experiments 1 and 2; only time had a significant effect on emptiness, hunger and urge to eat. The effects of time were expected and even the large within subject variability which is associated with VAS probably cannot obscure these effects. However, this large within subject variability may hinder the detection of more subtle effects of amino acids such as PHE, even when hunger, mood and arousal are assessed by VAS over a longer duration as conducted in experiment 3. However, the existence of any behavioral effects of PHE remains to be demonstrated. To this date, studies (Lieberman et al., 1985; Leathwood & Pollet, 1983) have been unable to demonstrate any behavioral effects of TYR in humans, and PHE would be expected to function in a similar fashion as TYR.

Blundell and Hill (1986) reported that although consumption of an APM sweetened solution (162 mg in 200 ml) resulted in a

depression of the perceived pleasantness of 10 samples of 20% sucrose. Appetite motivation (hunger, desire to eat) was not suppressed and ratings of fullness were decreased compared to a glucose load (50 g in 200 ml) of equivalent sweetness. These results are not unexpected; the energy deficit created by the ingestion of APM sweetened beverages may indeed increase hunger and urge to eat when compared with calorie containing sugar solutions. The popularly held view that artificial sweeteners provide the sensory pleasure and satisfaction of hunger that sugar provides, but without calories, was first demonstrated to be untrue by Hausmann (1933). Rats preferred glucose and saccharin solutions to water, but only when the rats drank sugar solutions did they reduce their solid food intake. That significant differences in appetite motivation were observed between APM and water loaded volunteers, except when testing was done on volunteers who fasted for 4 hr in the Blundell and Hill experiment has not been observed before; therefore, this aspect should be explored more thoroughly. Certainly, in our experiment when APM was uncoupled from its sweet taste by administering it in capsule form and the subjects were allowed to eat (there were no differences in energy consumption between the two trials), there were no differences in hunger or urge to eat between APM and the ALA placebo (20 $\pm$ 19 APM vs 23 $\pm$ 22 AIA, hunger; 17 $\pm$ 20 APM vs 23 $\pm$ AIA, urge to eat).

Comparing the effects on plasma amino acids of a high carbohydrate breakfast alone vs. APM plus a high carbohydrate breakfast provides useful data because of the suggestion that APM plus carbohydrate will influence behavioral mechanisms mediated by catecholamines (Coulombe & Sharma, 1986; Yokogoshi et al., 1984). In a preliminary experiment with humans, Yokogoshi et al. (1984) reported that plasma PHE and TYR ratios were elevated beyond the normal ranges in six healthy subjects following ingestion of APM (15 mg/kg) with a snack providing 200 g of carbohydrate. For a 70 kg male, the APM dose is roughly equivalent to 1 g; in our experiment, we gave a dose of approximately 10 g.

The reduction in branched-chain amino acids (Table 7) following the administration of carbohydrate is related to the secretion of insulin which enhances the uptake of branched-chain amino acids by muscle. PHE and TYR are also taken up by peripheral tissues for the same reason, but TRP is generally protected from peripheral uptake because it is bound to albumin (Anderson et al., 1984). Plasma PHE and TYR may be reduced in experiment 3 after a high carbohydrate meal because of peripheral or central uptake, but it is likely that, after 90 min, plasma TRP is reduced because of enhanced brain uptake. When APM was administered with the high carbohydrate breakfast, the absolute and relative concentrations of PHE increase as expected. However, of special significance is that the administration of 10.08 g APM caused a .19 millimolar rise in plasma PHE after 90 min (6.9 $\pm$ 1.2

μmoles/dl to 26.2 ± 7.8 μmoles/dl) in experiment 2, but when 10.08 g APM was administered concurrently with carbohydrate, a rise of only .09 millimoles in plasma PHE (6.4 ± 1.1 μmoles/dl to 15.3 ± 5.3 μmoles/dl) was observed after 90 min. Although some PHE may have been taken up by the brain, it is also likely that the rise in insulin due to carbohydrate ingestion may have resulted in peripheral uptake and oxidation of PHE.

Notably, the absolute and relative concentrations of TYR did not increase when APM was given with the high carbohydrate breakfast. The conversion of PHE to TYR varies considerably among subjects and may be influenced by the individual's needs for PHE and TYR, both essential amino acids.

The reduction in plasma TRP may still be caused by brain uptake, but it must be noted that the TRP/NAA ratio is not necessarily favorable (mean = 0.132 baseline vs. mean = 0.116 at 90 min) to brain uptake because of the high plasma PHE levels (6.4 ± 1.1 μmoles/dl baseline vs. 15.3 ± 5.3 μmoles/dl at 90 min). In addition, if both PHE and TRP are taken up by the brain, then synthesis of both catecholamines and serotonin might be expected. These neurotransmitters have been demonstrated to have conflicting influences on feeding behavior in animals (Liebowitz, 1984). The changes in plasma PHE, TYR, TRP and their ratios to the other large NAA are dependent on two factors: 1) the decline of branched-chain amino acids due to insulin secretion following carbohydrate consumption and 2) the appearance of PHE and TYR in

plasma due to APM consumption. Overall, individual differences among subjects' responses to both APM consumption and/or ingestion of carbohydrate may be related to gastrointestinal transit time, insulin release and the speed at which enzymatic conversion of PHE to TYR takes place.

CHAPTER VI

SUMMARY

In conclusion, both PHE and APM in doses up to 10 g failed to alter food intake or hunger, mood and arousal in normal weight adult males, even though significant changes in the absolute and relative concentrations of plasma PHE and TYR were observed. The highest PHE load was greater than two times the usual daily intake (National Research Council, 1974), and the highest APM load approximates that present in 70-100 cans of soft drink containing this sweetener.

Further research is necessary to determine if the failure of these amounts to alter behavior was due to an inability of PHE and APM to alter neurotransmitter synthesis, or if neurochemical effects were counteracted by the multiplicity of control systems which regulate behaviors in humans. Two recommendations for further research are to measure urinary catecholamine metabolites as an indicator of neurotransmitter synthesis and to conduct long-term studies with PHE and APM.

The measurement of urinary catecholamine metabolites, which partially reflect CNS activity (Johnston et al., 1983), should be included in future experiments. Because PHE had no effect on behavior in the current experiments, the possibility that urinary metabolites were unchanged exists. However, even if urinary catecholamine metabolites are found to respond to these large

doses, one should remember that feeding behavior may be controlled by several brain monoamine and neuropeptide systems whose actions vary depending on what brain regions and/or receptor sites are affected (Leibowitz, 1986; Anderson et al., 1984).

The approaches used in the experiments reported herein may not qualitatively assess the interactions among neurotransmitter precursors and the multiple neurochemical systems regulating food intake, hunger, mood and arousal under usual conditions. Detection of subtle responses to PHE and APM, if they occur, will be difficult. Because short-term studies may be unable to detect subtle changes, the need for long-term studies is apparent.

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APPENDIX A

CONSENT FORMS

University of Toronto

Department of Nutritional Sciences

University of Toronto

Informed Consent

A research study related to the role of dietary supplements in taste perception, has been explained to me and I have been asked to participate. I understand that I will be required to participate in up to five experimental sessions. These sessions will consist of:

1. an orientation session during which dietary, demographic, health and anthropometric (height, weight, triceps, biceps, subscapular and suprailiac skinfolds) data will be obtained.

2. four sessions which will involve the following protocol

   (i) eating a standardized breakfast, after an overnight fast, provided by the research team (arrival at 8:30 a.m.)

   (ii) taste evaluation of several food samples, before and after administration of a dietary supplement obtained from normal food sources (10 a.m.) (Sweeteners, Amino Acids).

   (iii) a buffet style luncheon, followed by a final taste evaluation (12 noon).

Each test session will be approximately 2 hours in duration, excluding breakfast.

In addition, I agree, if selected, to provide a blood sample during each of the 5 sessions. The blood (20 ml, equivalent to 4 teaspoons) will be drawn from a vein in my arm. Blood drawing procedures may result in possible slight discomfort and bruising from the venepuncture, but I am aware that the risk involved is equivalent to that of giving a routine blood sample.

Informed Consent (cont'd)

I am aware that the following conditions will prevail at all times.

1. My participation will not involve any health risk or discomfort.

2. All personal information given by me will remain confidential and my name will not appear in any publication.

3. I may withdraw from the study at any time without prejudice.

I also understand that it is hoped that I shall take part in the full series of sessions, and that unless I complete all sessions, my efforts will be of little use to the researchers.

Upon completion of the 5 sessions, I will receive financial compensation of $50.

Results of the study will be made available to me upon completion of the experiment; if I have any questions I should contact Milly Ryan-Harshman in the Department of Nutritional Sciences (978-6894).

I hereby agree to participate in the study.

Date _____ Participant's signature _____

University of Toronto

Department of Nutritional Sciences

Informed Consent

A research study related to phenylalanine and its effect on behaviour has been explained to me and I have been asked to participate. I understand that I will be required to participate in two experimental sessions.

The protocol for the two sessions is as follows:

1) consumption of either phenylalanine, as aspartame, or placebo (10 g) in capsules at 8 a.m. (breakfast served at 7:45 a.m.).

2) completion of visual analog scales which assess subjective mood, hunger and arousal at 8 & 10 a.m., noon and 2 p.m..

3) participate in five consecutive urine collection periods (8 a.m. to 12 p.m., 12 p.m. to 4 p.m., 4 p.m. to 8 p.m., 8 p.m. to 12 a.m., and 12 a.m. to 8 a.m.).

4) meals will be provided by the researcher in the form of Ensure.

In addition, I agree to provide a blood sample at 7:30 a.m. and 9:30 a.m. during each of the sessions. The blood (20 ml, equivalent to 4 teaspoons) will be drawn from a vein in my arm. Blood drawing procedures may result in possible slight discomfort and bruising from venepuncture, but I am aware that the risk involved is equivalent to that of giving a routine blood sample.

I am aware that the following conditions will prevail at all times.

1) My participation will not involve any health risk or discomfort.

2) All personal information given by me will remain confidential and my name will not appear in any publications.

3) I may withdraw from the study at any time without prejudice.

INFORMED CONSENT (cont'd)

Results of the study will be made available to me upon completion of the experiment; if I have any questions I should contact Milly Ryan-Harshman in the Department of Nutritional Sciences (978-6894).

I hereby agree to participate in this study.

Date _____ Participant's Signature _____

APPENDIX B

DEMOGRAPHIC AND HEALTH INFORMATION QUESTIONNAIRE

Date: _____ Subject ID _____

Demographic and Health Information

Name:

Address: Telephone:

Date of Birth:

Place of Birth:

Sex: Height: Weight:

Occupation:

If student, field of study:

Highest education level reached:

Marital Status:

.....

1. How would you describe your present weight?

- ◯ underweight
- ◯ normal weight
- ◯ moderately overweight
- ◯ very overweight

2. What was your weight at these periods in your life?

Age	Normal Weight	Moderately Overweight	Very Overweight
0-6 preschool	◯	◯	◯
7-12 childhood	◯	◯	◯

Demographic and Health Information (cont'd)

2.(cont'd)

Age	Normal Weight	Moderately Overweight	Very Overweight
13-15 early teens	○	○	○
16-19 late teens	○	○	○
20-29	○	○	○
30-39	○	○	○

3. Has your weight varied from your present weight in the last 5 years?

If yes, when _____

If yes, number of pounds _____

4. Have you ever attempted to lose weight?

YES

NO

gain weight? YES

NO

5. Are you presently attempting to lose weight?

YES NO

How: _____

gain weight: YES NO

How: _____

Demographic and Health Information (cont'd)

6. How would you describe your father's weight?

- ◇ underweight
- ◇ normal weight
- ◇ moderately overweight
- ◇ very overweight

7. How would you describe your mother's weight?

- ◇ underweight
- ◇ normal weight
- ◇ moderately overweight
- ◇ very overweight

8. Any of your siblings (brothers, sisters) overweight?

YES NO NO SIBLINGS

Specify: _____

9. Do you have any of the following health problems?

diabetes _____

high blood pressure _____

thyroid disorder _____

loss of appetite _____

poor sense of smell _____

Others? _____

Demographic and Health Information (cont'd)

10. Are you presently taking any medications on a daily or weekly basis?

YES NO

Type: _____

Dose: _____ Frequency: _____

11. Are you presently taking vitamin and/or mineral supplements?

YES NO

Type: _____

Dose: _____ Frequency: _____

12. Do you consider yourself to be:

- ◯ extremely athletic
- ◯ moderately athletic
- ◯ slightly athletic
- ◯ not athletic

13. Which of the following health problems has your father had?

Check the appropriate boxes.

- ☐ diabetes
- ☐ high blood pressure
- ☐ stroke
- ☐ angina (chest pain)
- ☐ heart attack before age 50
- ☐ other heart or artery problems

describe _____

Demographic and Health Information (cont'd)

13. (cont'd)

- ☐ phlebitis
- ☐ other vein problems

  describe _____

- ☐ gout
- ☐ gall bladder disease
- ☐ goitre
- ☐ other thyroid problems

  describe _____

- ☐ arthritis

14. Which of the following health problems has your mother had? Please check the appropriate boxes.

- ☐ diabetes
- ☐ high blood pressure
- ☐ stroke
- ☐ angina (chest pain)
- ☐ heart attack before age 50
- ☐ other heart or artery problems

  describe _____

- ☐ phlebitis
- ☐ other vein problems

  describe _____

Demographic and Health Information (cont'd)

14.(cont'd) ☐ gout

☐ gall bladder disease

☐ goitre

☐ other thyroid problems

describe _____

☐ arthritis

15. Are you presently following any dietary restriction for medical, religious or personal reasons? (e.g., vegetarianism).

YES NO

Specify: _____

16. Do you suffer from any food allergies?

YES NO

Specify: _____

17. Do you suffer from any food intolerances?

YES NO

Specify: _____

18. How many times a week do you eat?

	0	1	2	3	4	5	6	7
breakfast	—	—	—	—	—	—	—	—
mid-morning snack	—	—	—	—	—	—	—	—
lunch	—	—	—	—	—	—	—	—
afternoon snack	—	—	—	—	—	—	—	—
dinner	—	—	—	—	—	—	—	—
evening snack	—	—	—	—	—	—	—	—

Demographic and Health Information (cont'd)

19. Describe your typical

Breakfast:

Lunch:

Dinner:

20. Do you smoke?

YES NO

APPENDIX C

EATING HABITS QUESTIONNAIRE

Eating Habits Questionnaire

Date:

Name:

I.D.:

The following questions refer to your normal eating pattern and weight fluctuations. Please answer accordingly.

1. How often are you dieting? (Circle one)

   Never Rarely Sometimes Usually Always

2. What is the maximum amount of weight (in pounds) you have ever lost within one month? (Circle one)

   0-4 5-9 10-14 15-19 20+

3. What is your maximum weight gain within a week? (Circle one)

   0-1 1.1-2 2.1-3 3.1-5 5.1+

4. In a typical week, how much does your weight fluctuate? (Circle one)

   0-1 1.1-2 2.1-3 3.1-5 5.1+

5. Would a weight fluctuation of 5 lbs. affect the way you live your life? (Circle one)

   Not at all Slightly Moderately Very much

6. Do you eat sensibly in front of others and splurge alone? (Circle one)

   Never Rarely Often Always

7. Do you give too much time and thought to food? (Circle one)

   Never Rarely Often Always

Eating Habits Questionnaire (cont'd)

8. Do you have feelings of guilt after overeating? (Circle one)

Never Rarely Often Always

9. How conscious are you of what you're eating? (Circle one)

Not at all Slightly Moderately Extremely

10. What was your maximum weight ever? _____

11. How many pounds over your desired weight were you at your maximum weight?

0-1 1-5 6-10 11-20 21+

APPENDIX D

FOOD USE FREQUENCY FORM

Name: _____

Subject ID: _____

HOW OFTEN DO YOU USE THESE FOODS?	Milk, homogenized	Milk, 2%	Milk, skim	White Bread	Whole Wheat bread	Ham	Cold cuts, salami etc.	Cottage cheese	Hard cheese, sharp	Hard cheese, mild	Butter	Margarine	Soup, vegetable	Cucumber, raw	Celery, raw
FREQUENCY	> 3	> 3	> 3	> 3	> 3	> 3	> 3	> 3	> 3	> 3	> 3	> 3	> 3	> 3	> 3
1. DAILY (no. of times per day)	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1
2. WEEKLY (no. of times per week)	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1
3. MONTHLY (no. of times per month)	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1
4. LESS THAN ONCE A MONTH	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
5. TRIED BUT DO NOT EAT OR DRINK	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
6. NEVER TRIED	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7. DON'T KNOW FOOD ITEM	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D

Name: _____

Subject ID: _____

HOW OFTEN DO YOU USE THESE FOODS?

FREQUENCY	Carrots, raw	Tomato, raw	Cabbage, raw	Cabbage, cooked	Orange juice, unsweetened	Apple juice, unsweetened	Coffee	Tea	Apple	Orange	Potato chips	Cookies	Yoghurt, flavoured		
	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
1. DAILY (no. of times per day)	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1
2. WEEKLY (no. of times per week)	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1	5-6 2-4 1
3. MONTHLY (no. of times per month)	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1	2-3 1
4. LESS THAN ONCE A MONTH	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
5. TRIED BUT DO NOT EAT OR DRINK	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
6. NEVER TRIED	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7. DON'T KNOW FOOD ITEM	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D

APPENDIX E

HUNGER QUESTIONNAIRE

Hunger Questionnaire

Date: _____ Time: _____ Subject ID: _____

This questionnaire contains several sensations related to hunger. Please evaluate your present feelings on the horizontal scales below:

I. Stomach sensations

1. emptiness
none at all ——— extreme

2. rumbling
none at all ——— extreme

3. ache
none at all ——— extreme

4. nausea
none at all ——— extreme

II. Head sensations

5. headache
none at all ——— extreme

6. dizziness
none at all ——— extreme

7. faintness
none at all ——— extreme

III General sensations

8. drowsy
not at all ——— extremely

9. weak
not at all ——— extremely

10. nervous
not at all ——— extremely

Hunger Questionnaire (continued)

11. tense
not at all _____ extremely

12. drugged
not at all _____ extremely

13. depressed
not at all _____ extremely

14. alert
not at all _____ extremely

15. mentally slow
not at all _____ extremely

IV. Are you experiencing any other sensations not described by the scales? If yes, please describe:

_____

_____

V. How hungry are you?

not at all _____ as hungry as you have ever felt

Describe your urge to eat:

no urge to eat _____ very strong urge to eat

APPENDIX F

RAW DATA

Energy Intakes (kcal)
Experiment 1

ID	Placebo 5.04 g ALA	0.84 g PHE	2.52 g PHE	5.04 g PHE
101	1420	1353	1786	1242
102	1036	1245	1274	1043
103	782	898	904	538
104	1422	1371	1360	1266
105	1078	1499	1449	985
106	1744	2735	2123	1662
107	1385	1949	1707	936
108	1115	1160	823	1152
109	2081	1912	1912	2036
110	1295	1307	1427	1016
111	1327	1316	671	1391
112	1334	1266	1551	972
113	1272	2050	484	1549

Protein Intakes (%)
Experiment 1

101	14.7	10.0	14.1	15.7
102	15.3	15.0	13.5	15.0
103	20.0	15.0	20.2	19.6
104	16.0	17.0	17.1	19.9
105	20.5	20.2	17.4	18.5
106	19.0	18.5	18.4	17.8
107	18.3	17.4	14.3	16.2
108	23.5	21.4	16.6	23.6
109	11.5	12.2	13.1	11.3
110	15.3	14.1	13.0	15.6
111	14.4	13.4	15.9	17.0
112	16.4	9.9	15.9	12.0
113	21.0	18.0	20.7	19.1

Carbohydrate Intakes (%)
Experiment 1

101	36.4	43.7	37.3	36.4
102	39.5	39.0	41.5	41.9
103	28.6	34.1	22.3	31.3
104	33.8	30.3	34.0	36.5
105	27.6	28.1	27.0	31.8
106	37.0	32.1	38.7	36.5
107	34.3	33.4	40.4	40.5
108	33.2	19.3	44.5	30.3
109	46.6	48.8	44.8	49.0
110	28.8	29.5	36.1	30.2
111	31.0	35.6	43.3	26.5
112	32.0	48.1	30.3	50.7
113	34.5	39.1	39.8	36.4

Fat Intakes (%)
Experiment 1

ID	Placebo 5.04 g ALA	0.84 g PHE	2.52 g PHE	5.04 g PHE
101	49.7	46.5	49.5	49.1
102	45.2	46.3	45.1	43.0
103	51.5	51.2	57.7	50.0
104	50.6	53.0	49.3	47.3
105	52.3	52.3	56.1	50.1
106	44.4	50.2	43.6	46.3
107	48.2	50.1	46.6	44.7
108	43.5	59.4	39.0	46.6
109	40.7	40.0	42.9	40.5
110	56.1	54.0	50.2	54.2
111	55.3	51.7	41.8	57.0
112	52.4	42.1	51.5	37.6
113	44.4	42.9	39.1	44.3

Carbohydrate/Protein Intakes (%)
Experiment 1

101	2.5	4.4	2.7	2.3
102	2.6	2.6	3.1	2.8
103	1.4	2.3	1.1	1.6
104	2.1	1.8	2.0	1.7
105	1.4	1.4	1.6	1.7
106	1.9	1.7	2.1	2.1
107	1.9	1.9	2.8	2.5
108	1.4	0.9	2.7	1.3
109	4.0	4.0	3.4	4.3
110	1.9	2.1	2.8	1.9
111	2.1	2.7	2.7	1.6
112	2.0	4.9	1.9	4.2
113	1.7	2.2	1.9	1.9

Total Food Consumed (dry wt., g)
Experiment 1

101	260	252	327	229
102	194	232	239	198
103	140	161	154	98
104	257	243	248	234
105	192	268	251	179
106	331	498	406	311
107	256	356	322	179
108	212	195	161	215
109	396	376	368	399
110	224	221	255	178
111	231	237	130	239
112	239	243	268	193
113	239	390	94	291

Plasma Amino Acid Levels (μmoles/dl)
Experiment 1

ID	ALA	TYR	VAL	ILE	TRP	LEU	PHE
Placebo (5.04 g ALA)							
Baseline							
101	57.5	10.7	34.6	10.8	14.5	16.8	9.6
105	36.6	6.9	22.2	7.8	11.5	12.3	7.2
109	37.7	7.0	22.4	6.3	10.2	12.1	7.9
111	40.0	10.3	21.9	6.6	7.9	12.4	6.4
113	37.6	6.3	23.9	5.3	9.2	9.7	7.0
Experimental							
101	61.3	7.9	29.8	7.9	10.0	14.1	6.2
105	58.4	5.7	23.4	6.4	10.6	11.7	6.1
109	48.4	7.1	22.6	5.8	9.5	9.8	6.7
111	65.7	8.6	18.4	5.3	7.4	10.5	5.8
113	59.7	7.1	23.1	5.6	10.9	10.7	6.9
0.84 g PHE							
Baseline							
101	79.0	9.7	27.5	9.5	8.5	20.5	7.8
105	39.9	5.3	25.5	6.6	6.9	14.8	6.4
109	42.4	6.4	22.6	5.9	8.6	13.3	6.0
111	39.2	8.6	20.4	5.9	7.0	11.7	5.8
113	41.1	6.5	19.4	5.9	7.1	12.9	6.1
Experimental							
101	102.7	9.5	25.0	8.3	9.1	17.6	9.7
105	54.8	6.7	25.6	6.1	6.9	16.0	7.3
109	58.9	9.2	20.3	7.1	7.7	14.2	9.8
111	63.4	10.3	20.4	5.3	6.6	11.1	10.9
113	50.0	6.5	17.1	4.8	6.2	8.9	7.1
2.52 g PHE							
Baseline							
101	69.0	8.5	27.1	8.6	9.6	18.6	7.6
105	52.2	7.0	24.5	7.2	7.7	15.9	6.3
109	27.5	6.8	18.9	5.8	6.2	13.9	6.8
111	45.8	8.5	18.7	5.6	7.4	9.9	6.1
113	45.1	7.3	21.6	6.5	8.2	14.1	7.7

ID	ALA	TYR	VAL	ILE	TRP	LEU	PHE
Experimental							
101	82.7	13.0	29.0	8.3	9.3	18.4	37.5
105	63.6	8.2	23.6	6.7	6.7	15.5	11.7
109	30.1	9.2	19.0	5.8	7.2	14.1	15.2
111	56.7	10.4	21.4	5.2	7.7	10.2	15.9
113	43.1	7.9	18.7	4.0	5.7	11.6	8.0
5.04 g PHE							
Baseline							
101	64.5	10.0	26.5	9.2	9.7	17.8	7.8
105	59.2	8.9	25.7	6.4	8.0	15.8	5.9
109	27.6	5.8	17.4	4.5	7.3	11.1	5.9
111	34.2	7.6	19.9	5.9	6.8	12.4	6.8
113	35.8	7.1	17.6	4.5	7.0	11.3	5.5
Experimental							
101	53.5	12.9	24.5	7.5	9.1	14.8	30.7
105	57.4	13.3	23.1	6.8	7.7	14.5	28.5
109	28.2	12.9	17.6	5.0	7.5	11.5	38.3
111	39.3	9.2	21.4	5.9	7.6	12.0	22.8
113	33.7	10.3	18.7	4.7	6.8	11.1	14.3

Visual Analog Scale Scores
Experiment 1

ID	Dose	Time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
101	1	1	41	16	00	00	01	19	01	02	12	01	01	25	02	45	18	41	26
	1	2	72	45	01	01	01	24	01	01	15	27	26	01	01	24	01	58	45
	1	3	01	01	01	01	01	01	01	29	01	01	14	01	01	59	19	01	14
	2	1	42	30	01	01	44	01	01	50	18	01	01	01	21	34	21	44	39
	2	2	28	16	06	01	18	22	00	59	16	00	00	24	00	31	60	41	36
	2	3	01	00	00	00	00	12	13	33	00	00	00	00	00	46	27	01	01
	3	1	35	20	12	01	01	01	01	16	01	01	01	01	01	32	35	51	56
	3	2	60	14	28	00	00	00	01	01	16	01	01	01	01	57	01	55	38
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	4	2	52	00	11	00	01	22	10	08	00	00	00	00	00	48	14	42	43
	4	3	00	00	00	00	00	11	00	00	00	00	00	00	00	43	06	05	00
102	1	1	29	00	00	00	00	05	00	00	12	00	00	00	00	48	00	59	59
	1	2	53	16	00	00	00	09	00	08	09	00	00	00	00	49	00	61	65
	1	3	00	00	00	00	00	00	00	10	00	00	00	00	00	45	00	00	00
	2	1	60	37	00	00	16	17	38	39	29	19	20	00	00	53	11	58	62
	2	2	56	10	10	01	23	23	23	22	15	00	00	00	00	39	09	62	65
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	3	2	65	00	00	00	15	15	00	00	13	00	00	00	00	50	50	66	68
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	4	1	35	00	00	00	00	00	10	00	00	00	00	00	00	50	00	46	48
	4	2	53	00	05	00	08	00	00	11	12	00	00	00	00	51	00	69	70
	4	3	00	00	00	00	00	00	00	05	00	00	00	00	00	52	00	00	00
103	1	1	37	18	19	05	12	10	09	61	13	12	13	12	30	55	34	26	22
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	2	1	39	04	04	01	02	02	35	65	04	06	67	04	51	51	09	62	51
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	3	2	35	26	05	04	06	14	11	39	16	05	07	07	28	47	33	57	48
	3	3	08	08	08	08	12	34	05	32	07	08	10	10	17	58	49	16	08
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	4	2	55	41	36	15	16	14	11	53	08	08	08	09	10	51	50	60	62
	4	3	12	11	11	10	19	19	18	39	13	15	11	09	10	74	12	09	13
104	1	1	62	37	07	18	00	04	12	45	06	00	00	00	00	50	25	76	82
	1	2	73	10	00	00	00	00	05	55	07	00	00	00	00	60	12	81	87
	1	3	00	00	00	00	00	00	00	36	19	00	00	00	00	64	17	00	08
	2	1	26	09	00	00	00	00	00	42	00	00	00	00	33	63	12	33	45
	2	2	38	19	00	00	16	00	00	39	13	00	00	00	05	64	51	52	63
	2	3	00	00	00	00	00	00	00	37	00	00	00	00	00	69	17	00	05
	3	1	36	06	00	00	10	00	00	26	00	13	13	00	00	69	00	55	43
	3	2	42	16	00	00	09	00	00	48	09	02	00	00	00	59	08	56	38
	3	3	00	00	00	05	04	00	00	07	12	00	00	00	00	63	04	00	10

ID	Dose	Time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
104	4	1	50	21	00	00	45	39	05	79	29	00	00	00	00	44	61	66	45
	4	2	52	11	00	00	21	19	00	40	27	00	00	00	00	41	55	55	70
	4	3	00	00	00	00	24	00	00	19	15	00	00	00	00	66	30	00	05
105	1	1	62	01	01	01	01	01	01	10	04	00	00	00	04	81	03	62	64
	1	2	76	72	02	02	02	02	02	03	03	03	03	03	03	83	04	71	74
	1	3	08	01	01	01	01	01	01	01	01	01	01	01	01	88	05	02	02
	2	1	29	01	02	02	01	03	02	02	02	02	02	02	02	83	02	65	67
	2	2	82	01	08	01	04	04	03	02	09	02	03	04	02	76	04	82	83
	2	3	03	00	00	00	00	00	00	01	00	00	00	00	00	91	01	02	02
	3	1	26	00	00	00	00	00	00	04	00	00	00	00	00	79	16	52	54
	3	2	71	01	01	01	00	00	00	01	02	01	02	01	01	83	13	76	75
	3	3	05	01	01	01	00	00	01	00	00	00	00	00	00	88	07	02	03
	4	1	68	13	01	01	01	01	01	08	13	02	03	01	02	71	10	68	71
	4	2	76	17	18	20	27	36	26	61	11	02	02	58	02	73	06	76	79
	4	3	08	01	01	01	01	01	01	01	01	04	01	01	01	90	03	06	09
106	1	1	33	04	02	01	35	02	05	76	64	22	45	19	35	25	64	34	43
	1	2	69	25	16	13	51	30	14	54	44	40	52	23	34	42	48	67	73
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	2	1	33	04	04	03	06	08	02	33	09	01	08	02	03	49	22	49	37
	2	2	52	20	10	05	14	08	07	29	27	10	02	02	02	48	31	57	62
	2	3	00	00	16	00	00	02	04	12	00	00	00	00	00	60	29	02	02
	3	1	52	09	08	01	25	29	08	67	56	11	16	09	12	45	45	59	47
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	3	3	03	00	00	00	13	00	00	05	02	00	00	00	03	75	06	33	53
	4	1	29	02	02	01	02	24	18	28	11	05	06	02	20	41	39	41	30
	4	2	46	06	08	00	38	20	16	15	20	08	03	02	22	47	31	38	32
	4	3	07	00	00	00	00	01	01	47	15	01	01	01	06	69	19	04	03
107	1	1	68	41	38	32	06	05	06	06	08	07	07	06	06	94	02	47	53
	1	2	79	67	53	34	04	03	02	22	18	16	20	24	06	31	50	93	83
	1	3	02	13	13	09	06	26	24	35	33	15	15	18	13	75	07	02	01
	2	1	65	08	09	10	11	17	11	11	10	10	10	09	08	07	06	30	35
	2	2	68	40	21	15	11	10	07	11	15	11	09	24	07	87	06	75	73
	2	3	02	02	02	03	03	03	03	05	05	05	07	03	05	93	02	02	03
	3	1	77	19	17	08	04	04	03	05	05	04	06	06	05	87	03	69	63
	3	2	97	21	30	05	02	00	01	02	05	02	02	03	04	96	02	80	82
	3	3	03	04	02	03	04	04	08	06	05	04	04	03	04	00	00	03	04
	4	1	54	59	53	04	03	00	04	09	05	05	04	05	04	87	05	38	49
	4	2	79	41	45	43	04	03	03	07	07	06	06	05	05	90	04	75	84
	4	3	02	02	03	03	03	02	01	05	05	04	04	04	04	97	03	03	03
108	1	1	52	22	22	26	10	34	33	17	24	30	40	21	20	62	22	29	28
	1	2	55	26	21	22	19	19	33	20	21	22	32	32	22	63	16	55	34
	1	3	13	13	15	18	12	11	09	09	09	09	15	12	10	41	31	27	09
	2	1	73	56	15	19	20	23	58	12	56	14	14	17	15	79	12	59	50
	2	2	80	66	11	11	13	27	52	15	29	13	12	11	10	85	10	72	65
	2	3	40	24	06	05	07	06	08	10	21	11	09	09	08	71	18	38	08

ID	Dose	Time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
108	3	1	21	18	19	16	07	06	07	33	14	10	09	08	10	55	20	17	21
	3	2	28	09	08	09	12	10	08	24	20	07	06	06	06	49	17	22	28
	3	3	17	13	12	14	14	18	08	11	09	10	13	10	10	65	15	17	14
	4	1	73	16	18	22	13	33	31	26	32	09	44	10	38	59	16	67	65
	4	2	74	14	16	19	11	37	36	24	29	11	29	20	35	70	24	76	63
	4	3	34	06	06	07	15	14	14	21	15	16	25	17	28	66	23	34	37
109	1	1	65	01	13	01	00	00	00	00	00	00	00	00	00	83	19	55	68
	1	2	71	00	14	00	00	00	00	00	00	00	00	00	00	80	19	78	79
	1	3	00	00	00	00	00	00	00	00	00	00	00	00	00	83	14	00	00
	2	1	68	00	50	00	13	00	00	00	00	00	00	00	00	84	13	74	78
	2	2	83	00	16	00	01	01	01	00	00	00	00	00	00	00	00	76	75
	2	3	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
	3	1	64	00	00	00	00	00	00	29	00	00	00	00	00	62	07	62	68
	3	2	73	00	00	00	00	00	00	00	00	00	00	00	00	00	00	69	72
	3	3	00	00	02	02	00	00	00	00	00	00	00	00	00	85	13	11	00
	4	1	60	00	00	00	00	00	00	00	00	00	00	00	00	74	21	65	70
	4	2	68	01	01	01	00	00	00	00	00	00	00	00	00	00	00	67	63
	4	3	00	00	00	00	00	00	00	00	00	00	00	00	00	88	14	00	00
110	1	1	50	10	10	10	00	00	00	00	00	00	00	00	00	00	00	54	48
	1	2	19	18	36	18	10	00	10	00	00	00	00	00	00	00	51	69	69
	1	3	11	00	00	00	00	00	00	00	00	00	00	00	00	00	00	07	00
	2	1	64	27	48	03	00	00	00	00	00	00	00	00	00	00	20	65	50
	2	2	72	13	13	05	07	08	08	00	00	00	00	00	00	00	64	72	63
	2	3	87	07	00	00	00	00	00	00	00	00	00	00	00	00	64	22	08
	3	1	44	15	02	16	02	03	05	00	00	00	00	00	00	00	00	57	58
	3	2	85	32	83	09	00	00	21	00	00	00	00	00	00	00	55	75	82
	3	3	00	00	00	00	00	00	00	00	00	00	00	00	00	00	79	00	00
	4	1	38	24	08	15	00	12	10	00	00	00	00	00	00	00	00	37	13
	4	2	48	27	12	27	00	15	14	00	00	00	00	00	00	00	00	70	50
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111	1	1	18	02	01	02	02	15	16	24	13	08	08	03	03	59	11	41	67
	1	2	67	04	03	05	07	06	11	21	16	03	04	03	03	48	21	60	72
	1	3	17	03	03	02	04	03	04	11	06	09	10	09	09	60	28	17	54
	2	1	17	05	04	04	06	11	13	18	09	09	10	03	03	36	48	70	78
	2	2	49	09	04	03	05	12	11	17	09	08	08	02	01	33	36	67	83
	2	3	15	03	02	02	03	03	03	04	03	04	04	04	03	54	22	12	17
	3	1	29	03	05	05	08	07	06	07	08	04	10	02	01	47	46	26	50
	3	2	45	13	02	02	04	05	22	23	14	12	04	03	04	36	27	36	60
	3	3	13	04	02	04	06	05	05	16	03	08	09	02	03	49	47	14	39
	4	1	66	07	03	02	04	03	14	52	31	07	08	09	10	56	20	42	71
	4	2	25	05	05	05	07	06	06	17	15	05	04	04	04	42	44	50	73
	4	3	05	05	05	05	05	06	06	14	03	03	06	04	05	59	31	12	17
112	1	1	42	06	06	04	00	14	51	00	51	15	05	00	00	22	06	46	73
	1	2	51	03	07	00	00	06	33	54	58	19	32	09	03	33	29	55	79
	1	3	00	00	00	00	00	00	00	10	02	15	18	00	00	74	11	07	02

ID	Dose	Time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
112	2	1	68	05	12	00	00	05	16	31	30	38	50	00	08	22	69	78	88
	2	2	77	42	09	00	00	04	22	29	21	42	50	00	06	30	79	91	87
	2	3	10	04	00	00	00	03	00	00	03	30	34	00	03	78	19	46	24
	3	1	22	03	27	33	04	42	52	52	52	25	41	02	11	24	42	53	72
	3	2	56	06	30	12	21	07	26	44	48	27	26	03	06	24	57	72	71
	3	3	16	01	01	01	10	01	01	15	03	17	17	01	07	21	71	06	05
	4	1	71	25	20	04	03	12	42	41	41	03	03	00	34	21	80	75	83
	4	2	66	47	46	00	00	28	40	44	45	03	07	00	06	12	86	83	87
	4	3	09	00	00	00	00	05	00	03	05	00	03	00	03	43	52	05	02
113	1	1	19	00	00	00	00	00	00	00	02	00	00	00	00	60	00	39	51
	1	2	32	00	00	00	00	00	00	04	00	00	00	02	00	49	00	40	52
	1	3	15	00	00	00	00	00	00	00	00	00	00	00	00	65	01	10	14
	2	1	23	00	00	00	00	00	00	00	00	00	00	00	00	60	00	59	63
	2	2	55	00	00	00	00	00	00	00	00	00	05	00	00	47	00	67	69
	2	3	00	00	00	00	00	00	00	00	00	00	00	00	00	62	00	15	09
	3	1	00	00	00	00	00	00	00	00	00	00	00	00	00	63	06	10	10
	3	2	04	01	00	00	00	00	00	00	00	00	00	00	00	41	00	05	12
	3	3	00	00	00	00	00	00	00	00	00	00	00	00	00	65	00	04	02
	4	1	12	01	01	01	00	00	01	00	00	00	00	00	00	59	01	60	66
	4	2	28	01	01	01	00	00	00	00	01	00	00	00	00	64	00	62	62
	4	3	04	00	00	00	00	00	00	01	01	00	00	00	00	69	00	03	05

Variables	Doses	Times
01 Emptiness	1 5.04 g ALA	1 11:00 am
02 Rumbling	2 0.84 g PHE	2 11:45 am
03 Stomach Ache	3 2.52 g PHE	3 12:30 pm
04 Nausea	4 5.04 g PHE	
05 Headache		
06 Dizziness		
07 Faintness		
08 Drowsy		
09 Weak		
10 Nervous		
11 Tense		
12 Drugged		
13 Depressed		
14 Alert		
15 Mentally slow		
16 Hunger		
17 Urge to eat		

Energy Intakes (kcal)
Experiment 2

ID	Placebo	10.08 g ALA	5.04 g AIM	10.08 g APM	10.08 g PHE
201		644.0	598.7	628.7	623.9
202		1215.9	1021.9	1019.4	997.4
203		1539.1	1379.6	1601.4	1345.3
204		1026.3	971.9	849.1	988.9
205		1386.9	1297.3	1905.0	1573.9
206		832.4	798.6	1086.3	764.2
207		1350.9	1678.3	1532.4	947.7
208		1002.1	1223.0	858.5	566.4
209		1498.5	1029.6	785.7	1155.5
210		1839.9	1424.9	1404.3	1643.1
211		729.4	499.9	542.5	433.5
212		1720.3	941.5	1199.8	1520.5
213		1202.0	1468.3	1192.9	1349.3

Protein Intakes (%)
Experiment 2

201	14.2	13.2	15.1	12.5
202	18.2	16.9	17.1	17.6
203	15.0	21.3	14.9	12.3
204	15.4	14.3	12.4	12.1
205	16.7	15.2	15.0	17.3
206	9.6	9.8	8.9	9.6
207	15.5	16.8	16.8	18.1
208	20.5	18.6	17.1	23.2
209	18.8	15.4	16.5	18.3
210	22.5	22.4	22.1	20.7
211	20.5	21.1	19.8	15.9
212	15.3	17.4	15.2	20.3
213	15.0	11.6	11.8	11.6

Carbohydrate Intakes (%)
Experiment 2

201	42.5	44.6	51.0	43.5
202	40.0	49.4	42.0	41.2
203	29.7	30.3	31.8	39.8
204	34.8	35.4	40.4	38.8
205	34.1	33.7	33.1	32.1
206	46.6	48.5	49.4	43.8
207	37.4	32.0	30.7	29.2
208	37.1	30.6	29.0	30.6
209	31.0	35.0	36.5	31.0
210	25.2	25.0	28.1	31.1
211	34.0	39.6	40.9	42.9
212	39.8	43.2	42.1	31.8
213	33.3	39.7	39.8	41.6

Fat Intake (%)
Experiment 2

ID	Placebo 10.08 g ALA	5.04 g APM	10.08 g APM	10.08 g PHE
201	43.0	41.8	33.8	43.9
202	41.7	33.4	40.9	41.1
203	55.7	48.5	53.8	48.3
204	50.2	50.8	47.8	49.5
205	49.6	51.8	52.9	50.9
206	44.0	42.3	42.4	47.0
207	47.8	51.8	52.9	53.0
208	42.7	51.4	54.5	46.1
209	50.0	49.8	46.7	50.5
210	52.5	52.9	49.8	48.5
211	45.3	38.3	39.0	40.7
212	45.1	39.3	42.8	48.5
213	52.1	47.8	49.0	47.4

Carbohydrate/Protein
Experiment 2

201	3.0	3.4	3.4	3.5
202	2.2	2.9	2.5	2.3
203	2.0	1.4	2.1	3.2
204	2.3	2.5	3.3	3.2
205	2.0	2.2	2.2	1.9
206	4.8	5.0	5.5	4.6
207	2.4	1.9	1.8	1.6
208	1.8	1.6	1.7	1.3
209	1.6	2.3	2.2	1.7
210	1.1	1.1	1.3	1.5
211	1.7	1.9	2.0	2.7
212	2.6	2.5	2.8	1.6
213	2.2	3.4	3.5	3.6

Total Food Consumed (dry wt., g)
Experiment 2

201	122.0	114.2	127.6	147.7
202	233.3	207.1	197.1	192.1
203	267.3	252.2	282.5	247.6
204	185.9	175.5	157.3	180.3
205	252.7	233.5	340.8	283.3
206	157.6	153.8	209.7	141.9
207	250.3	301.2	272.2	167.9
208	191.8	220.1	150.9	105.2
209	270.0	186.8	144.9	207.2
210	326.6	252.8	254.1	301.3
211	136.0	97.2	105.8	83.3
212	322.9	183.6	228.9	279.8
213	214.7	266.3	219.0	250.3

Plasma Amino Acid Levels (μmoles/dl)
Experiment 2

ID	ALA	TYR	VAL	ILE	TRP	LEU	PHE
Placebo (10.08 g ALA)							
Baseline							
202	41.7	7.9	25.9	8.7	7.7	16.4	6.4
203	49.0	7.4	5.2	5.7	9.0	13.1	6.2
204	63.7	8.9	26.1	9.8	6.3	17.0	6.5
205	25.5	3.2	13.8	5.0	5.2	10.4	4.1
207	129.7	8.7	25.3	7.7	8.3	16.3	6.3
209	57.7	9.6	25.9	10.0	9.0	20.0	6.7
210	54.0	9.4	8.7	9.0	7.6	18.8	8.1
Experimental							
202	69.5	10.5	26.0	7.3	6.7	15.8	5.2
203	120.0	8.7	21.1	4.6	7.9	9.3	4.4
204	88.9	3.7	19.8	6.0	4.1	14.0	4.6
205	82.7	6.1	22.1	6.3	5.9	16.6	5.6
207	47.7	7.0	18.8	7.2	6.9	14.4	6.0
209	129.4	11.1	25.9	6.8	7.2	14.6	5.4
210	85.4	9.4	11.0	6.9	5.2	16.4	5.9
5.04 g APM							
Baseline							
202	74.3	6.5	28.4	7.4	8.2	17.3	10.9
203	61.6	10.6	29.8	9.1	10.9	19.2	9.1
204	50.6	7.6	24.0	8.9	5.3	15.1	5.5
205	57.5	4.4	15.3	10.0	6.6	11.1	8.5
207	48.0	5.9	26.5	9.0	9.8	19.1	6.8
209	41.9	13.1	25.4	9.3	8.8	18.2	6.4
210	42.2	5.2	4.7	6.6	5.0	13.2	5.8
Experimental							
202	55.9	10.9	28.0	9.4	9.3	18.2	7.1
203	94.1	8.3	27.3	7.6	9.4	16.0	20.8
204	109.1	12.3	24.2	10.0	5.7	19.9	11.7
205	63.7	8.8	15.1	7.4	5.0	9.6	11.4
207	71.4	11.1	18.6	5.4	6.1	12.4	14.7
209	62.7	8.3	26.5	6.6	7.2	14.9	11.5
210	82.7	11.2	25.2	7.8	5.2	15.7	14.3

ID	ALA	TYR	VAL	ILE	TRP	LEU	PHE
10.08 g APM							
Baseline							
202	31.3	4.4	22.0	6.6	7.1	12.3	5.5
203	54.1	8.4	20.8	6.6	10.6	12.9	6.3
204	72.8	3.2	24.5	10.2	5.0	19.2	6.6
205	47.3	9.8	25.3	8.8	10.4	15.9	6.8
207	65.5	8.9	31.1	11.6	9.8	20.1	7.0
209	48.6	7.2	33.1	12.5	10.3	22.2	9.5
210	45.5	6.9	29.1	8.4	7.2	19.3	7.0
Experimental							
202	79.9	13.0	28.7	7.1	7.8	15.2	12.2
203	39.8	10.4	14.0	3.4	5.7	7.5	36.6
204	89.3	16.5	25.2	9.1	4.9	17.8	31.7
205	30.4	10.1	13.2	4.8	5.9	9.2	17.8
207	58.9	6.3	20.7	7.8	6.5	15.5	28.8
209	62.8	12.9	22.0	6.3	6.5	14.4	28.6
210	48.8	12.4	5.2	6.7	6.2	15.1	28.1
10.08 g PHE							
Baseline							
202	49.2	8.7	29.8	9.5	10.7	18.8	12.2
203	61.7	7.9	25.2	7.9	10.8	16.3	6.7
204	94.2	12.1	32.2	10.2	6.8	20.7	6.6
205	48.3	9.0	24.9	8.8	9.0	17.4	9.1
207	35.0	3.8	2.9	5.8	6.6	12.7	5.7
209	56.9	9.5	7.8	10.1	9.1	18.0	6.7
210	55.9	8.7	28.8	9.3	9.4	18.5	7.1
Experimental							
202	39.9	14.8	24.1	6.5	7.0	15.0	24.1
203	45.9	22.1	22.2	7.0	8.1	14.7	59.2
204	51.9	13.7	31.4	10.1	7.0	12.1	80.5
205	56.7	26.9	21.1	10.1	7.5	23.0	97.1
207	43.3	9.8	5.2	6.9	6.9	15.6	139.8
209	50.3	15.3	17.9	5.2	4.9	10.2	92.7
210	42.7	14.4	24.2	6.1	5.3	13.8	92.9

Visual Analog Scale Scores
Experiment 2

ID	Dose	Time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
201	1	1	00	00	00	00	03	04	00	00	00	00	00	00	00	97	00	00	26
	1	2	30	00	00	00	00	00	00	00	00	00	00	00	00	99	00	49	67
	1	3	00	00	00	00	00	00	00	00	00	00	00	00	00	99	00	00	00
	2	1	00	00	00	00	00	00	00	00	00	00	00	00	00	99	00	00	00
	2	2	26	00	00	00	00	00	00	00	00	00	00	00	00	99	00	04	19
	2	3	00	00	00	00	00	00	00	00	00	00	00	00	00	99	00	00	00
	3	1	03	00	00	00	00	00	00	00	00	00	00	00	00	83	00	00	10
	3	2	43	00	00	00	00	00	00	00	00	00	00	00	00	76	00	52	45
	3	3	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
	4	1	00	00	00	00	00	00	00	00	00	00	00	00	00	99	00	00	12
	4	2	34	00	00	00	00	00	00	00	00	00	00	00	00	99	00	28	50
	4	3	00	00	00	00	00	00	00	00	00	00	00	00	00	99	00	00	00
202	1	1	00	00	00	00	00	00	00	08	00	00	00	00	00	50	00	00	00
	1	2	02	00	00	00	00	00	00	00	00	00	00	00	00	51	00	08	09
	1	3	00	00	00	00	00	00	00	00	00	00	00	00	00	49	00	00	00
	2	1	09	00	00	00	00	00	00	00	00	00	00	00	00	45	00	06	05
	2	2	22	00	00	00	00	00	00	00	00	00	00	00	00	58	00	24	20
	2	3	00	00	00	00	00	00	00	02	00	00	00	00	00	56	00	00	00
	3	1	00	00	00	00	00	00	00	02	00	00	00	00	00	49	48	02	02
	3	2	01	00	00	00	00	00	00	00	00	00	00	00	00	50	51	04	04
	3	3	00	00	00	00	00	00	00	14	00	00	00	00	00	49	48	00	00
	4	1	05	00	00	02	00	00	00	02	00	00	00	00	00	47	00	06	08
	4	2	02	00	00	00	00	00	00	00	00	00	00	00	00	51	00	17	27
	4	3	00	00	00	00	00	00	00	00	00	00	00	00	00	46	00	00	00
203	1	1	03	00	00	00	00	00	00	00	03	04	00	00	00	68	06	03	00
	1	2	42	00	00	00	00	00	00	15	02	00	00	00	00	72	04	41	27
	1	3	00	00	00	00	00	00	00	04	00	00	00	00	00	90	08	00	04
	2	1	33	00	08	00	00	00	04	05	36	00	00	00	14	33	33	21	47
	2	2	28	00	00	00	00	00	00	05	11	00	00	00	00	59	07	24	35
	2	3	00	00	00	00	00	00	00	08	08	00	00	00	00	60	01	00	05
	3	1	00	00	00	00	00	00	00	02	01	00	00	00	00	75	05	03	00
	3	2	22	00	00	00	00	00	00	00	04	00	00	00	00	92	06	35	17
	3	3	00	00	00	00	00	00	00	00	01	00	00	03	00	94	00	00	00
	4	1	00	00	00	00	00	00	00	04	00	00	00	00	00	87	00	00	03
	4	2	10	00	00	00	50	00	00	12	00	00	00	00	00	81	08	34	31
	4	3	00	00	00	00	04	00	00	31	00	00	00	00	00	92	05	00	00
204	1	1	20	06	18	05	48	20	17	47	46	26	17	18	08	90	12	11	04
	1	2	12	08	07	10	41	43	58	61	18	15	14	65	12	53	13	52	60
	1	3	14	11	12	02	05	05	06	09	09	09	03	52	09	73	79	15	25
	2	1	12	02	03	05	03	04	06	12	14	06	10	11	11	85	16	22	82
	2	2	11	11	75	55	50	59	64	57	61	45	42	72	65	85	55	27	85
	3	2	20	06	03	03	21	29	07	23	10	10	11	28	08	50	51	47	32
	3	3	11	04	03	03	40	45	44	15	14	13	11	09	09	72	54	22	20
	4	1	54	05	04	04	04	04	03	02	04	04	02	03	04	86	04	34	60
	4	2	21	01	02	02	17	22	07	22	15	01	00	69	03	77	01	45	39

ID	Dose	Time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
204	4	3	12	01	01	02	19	29	06	04	04	02	02	53	02	75	61	14	13
205	1	1	25	00	00	06	14	14	00	74	27	00	11	43	14	13	23	39	42
	1	2	32	19	06	06	06	00	06	24	07	00	14	16	12	37	30	55	33
	1	3	00	00	06	07	14	00	00	19	12	00	22	14	00	39	27	00	00
	2	1	56	15	00	20	57	46	22	50	28	15	19	39	16	32	32	52	32
	2	2	32	11	00	00	36	17	00	57	41	00	00	00	00	53	49	55	24
	2	3	14	00	00	00	23	00	00	27	17	00	00	00	00	58	17	18	04
	3	1	25	00	00	00	15	15	00	54	16	00	00	16	00	36	33	39	52
	3	2	45	11	00	00	14	14	00	48	17	00	00	14	13	28	28	58	57
	3	3	84	00	14	00	23	00	00	33	04	00	00	00	00	60	15	00	00
	4	1	26	04	31	00	00	00	00	26	08	00	09	00	15	41	12	28	26
	4	2	57	19	06	05	41	25	00	25	05	00	09	20	04	56	13	63	69
	4	3	00	00	00	12	16	06	00	28	00	00	00	23	00	46	18	00	00
206	1	1	23	03	05	05	05	07	08	35	03	04	06	06	07	72	05	16	22
	1	2	43	03	04	04	03	03	02	12	02	02	04	04	04	69	16	56	46
	1	3	07	01	02	02	03	03	03	15	02	03	03	04	05	64	16	11	05
	2	1	32	05	16	03	03	03	03	02	03	03	03	03	04	62	18	29	50
	2	2	65	02	03	03	03	03	03	20	03	04	04	04	05	70	22	57	59
	2	3	07	02	03	03	11	02	03	03	03	03	04	03	04	55	04	12	15
	3	1	15	02	03	04	03	04	03	02	02	02	02	03	03	80	07	26	57
	3	2	49	04	05	06	06	06	05	03	03	03	03	04	05	67	04	46	48
	3	3	03	03	12	11	07	08	09	19	06	06	07	09	09	71	06	04	03
	4	1	28	03	03	03	03	03	03	47	07	01	01	01	02	50	19	14	44
	4	2	26	02	02	02	18	03	03	16	02	02	02	03	03	42	21	45	46
	4	3	03	04	04	04	21	03	04	12	04	04	04	05	05	45	24	03	04
207	1	1	00	00	00	00	00	10	00	00	00	00	00	00	00	99	00	00	00
	1	2	13	00	00	00	00	07	00	00	00	00	00	00	00	99	00	12	18
	1	3	00	00	00	00	00	00	00	00	00	00	00	00	00	99	00	00	00
	2	1	00	00	00	00	00	00	00	06	00	00	06	00	00	87	00	00	00
	2	2	08	00	10	00	00	00	00	22	00	00	04	00	00	53	23	11	07
	2	3	00	00	00	00	00	00	00	00	00	00	00	00	00	82	00	00	00
	3	1	00	00	00	00	00	05	00	22	17	00	00	00	00	66	27	00	00
	3	2	12	11	00	00	00	00	00	00	00	00	00	00	00	99	00	40	55
	3	3	00	00	00	00	00	00	00	00	00	00	00	00	00	99	00	00	00
	4	1	00	00	00	00	00	00	00	11	00	00	00	00	00	72	03	00	00
	4	2	00	00	04	00	00	00	00	36	00	00	00	00	00	63	22	00	00
	4	3	00	00	00	00	00	00	00	05	00	00	00	00	00	90	07	00	00
208	1	1	14	03	03	02	02	01	00	02	02	02	02	00	01	55	05	11	04
	1	2	40	05	03	02	00	00	00	40	00	00	16	00	00	69	05	44	35
	1	3	00	00	00	00	00	00	00	00	00	00	00	00	00	56	10	00	05
	2	1	03	04	01	02	03	02	03	52	35	34	05	02	02	22	36	00	03
	2	2	29	01	01	02	02	01	02	56	33	36	00	02	02	27	45	00	37
	2	3	00	00	00	00	00	00	00	33	22	00	00	00	00	39	29	00	07
	3	1	33	11	00	02	22	01	02	02	02	00	00	00	00	52	16	20	14
	3	2	25	04	01	16	00	00	00	38	00	00	00	00	00	32	00	22	18
	3	3	03	03	03	08	03	03	02	19	05	38	03	04	04	55	08	04	00
	4	1	04	05	03	17	56	22	02	40	39	02	02	02	03	43	05	03	00

ID	Dose	Time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
208	4	2	02	00	00	00	00	00	00	76	00	02	00	00	00	30	07	00	02
	4	3	06	03	02	00	03	03	02	17	02	02	02	02	03	75	08	02	01
209	1	1	20	00	00	00	00	00	00	00	00	00	00	00	00	99	00	27	00
	1	2	51	00	00	00	00	00	00	00	00	00	00	00	00	99	00	48	29
	1	3	00	00	00	00	00	00	00	00	00	00	00	00	00	99	00	00	00
	2	1	28	00	00	00	00	00	00	25	25	00	00	00	00	77	00	00	00
	2	2	39	00	00	00	00	00	00	00	00	00	00	00	00	94	00	49	35
	2	3	00	00	00	00	00	00	00	00	00	00	00	00	00	92	00	00	00
	3	1	18	00	00	00	00	00	00	24	23	00	00	00	00	73	18	00	00
	3	2	17	00	00	00	00	00	00	00	00	00	00	00	00	89	04	22	05
	3	3	00	00	00	00	00	00	00	00	00	00	00	00	00	89	07	00	00
	4	1	45	00	00	00	00	00	00	24	23	00	00	00	00	81	13	00	00
	4	2	68	00	00	00	00	00	00	00	00	00	00	00	00	86	06	50	46
	4	3	00	00	00	00	00	00	00	00	00	00	00	00	00	99	00	00	00
210	1	1	26	22	04	04	05	04	04	07	07	06	06	04	04	77	06	22	21
	1	2	48	29	06	04	05	04	04	07	06	06	05	05	05	83	03	46	43
	2	1	05	10	03	03	06	04	04	07	06	06	06	05	05	93	05	32	37
	2	2	42	26	05	05	05	07	06	08	07	07	06	05	06	76	04	63	66
	2	3	03	03	03	04	04	03	02	03	03	02	03	03	02	77	03	13	04
	3	1	18	18	04	03	06	05	03	05	03	03	03	03	04	73	01	02	17
	3	2	53	27	03	04	05	05	05	04	05	05	05	05	05	88	03	51	35
	3	3	03	03	03	03	04	06	04	03	03	03	05	05	08	83	04	05	04
	4	1	30	24	08	22	08	08	06	07	06	07	07	05	06	79	08	21	21
	4	2	45	26	09	11	09	07	07	11	08	07	07	09	08	79	06	46	52
	4	3	05	05	04	05	06	08	08	08	07	07	05	04	04	85	03	04	04
211	1	1	03	00	00	00	08	00	00	20	07	03	18	00	03	55	03	00	00
	1	2	53	00	00	00	02	00	00	12	03	00	04	00	00	78	03	99	53
	1	3	03	00	00	00	03	00	00	31	02	00	02	00	02	85	05	00	00
	2	1	05	00	00	00	00	00	00	07	00	00	05	00	00	88	00	51	18
	2	2	71	00	00	00	00	00	00	18	00	00	08	00	00	84	00	56	55
	2	3	07	00	00	00	04	00	00	25	00	00	00	00	00	81	00	00	00
	3	1	00	00	00	00	04	00	00	05	03	00	00	00	04	59	05	00	00
	3	2	04	00	00	00	04	00	00	00	00	00	00	00	00	87	00	00	00
	3	3	00	00	00	00	00	00	00	08	00	00	00	00	04	68	00	00	00
	4	1	04	00	00	00	00	00	00	03	00	00	21	00	00	50	00	48	49
	4	2	56	00	00	00	00	00	00	00	00	00	00	00	00	53	00	68	49
	4	3	00	00	00	00	00	00	00	20	00	00	00	00	00	35	03	00	00
212	1	1	07	05	05	05	01	01	01	12	02	02	01	02	03	65	07	13	13
	1	2	29	17	03	02	02	02	02	27	04	02	02	03	03	66	18	43	44
	1	3	01	01	01	01	01	01	01	17	02	02	02	02	02	68	22	08	07
	2	1	04	02	03	03	04	04	04	15	06	06	05	05	06	55	15	11	07
	2	2	05	04	04	05	05	05	05	20	05	06	06	06	06	56	19	22	28
	2	3	03	05	05	08	05	07	08	07	08	08	08	08	08	55	24	04	06
	3	1	13	04	06	04	02	04	04	20	04	05	05	07	07	62	26	10	10
	3	2	05	07	06	06	04	05	04	15	05	02	03	03	03	65	14	44	46
	3	3	01	03	04	03	03	03	02	06	02	04	04	05	04	65	18	01	03

ID	Dose	Time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
212	4	1	00	00	00	00	00	00	00	25	00	10	00	00	00	70	43	17	17
	4	2	29	00	00	14	00	00	00	25	00	09	09	00	00	53	49	48	47
	4	3	60	00	00	01	00	00	00	22	01	01	01	01	01	63	26	12	10
213	1	1	46	14	06	07	01	04	04	28	14	03	01	00	02	43	38	39	54
	1	2	72	34	17	02	00	00	01	02	03	01	00	02	02	83	03	71	78
	1	3	00	00	00	00	00	00	00	07	00	00	00	00	00	88	00	01	04
	2	1	68	58	34	31	62	60	75	85	38	02	00	02	03	22	85	78	81
	2	2	47	47	40	16	35	13	16	20	10	02	02	02	04	68	27	78	85
	2	3	00	00	00	00	00	00	00	09	00	00	00	00	00	87	00	04	12
	3	1	35	02	02	04	18	02	02	35	04	00	00	00	00	43	42	44	47
	3	2	74	70	64	49	42	58	54	66	60	00	02	04	03	31	31	77	82
	3	3	00	00	00	00	00	00	00	19	00	00	00	00	00	77	04	00	08
	4	1	70	49	09	00	00	02	09	94	26	03	07	00	00	35	57	45	45
	4	2	43	28	02	00	02	02	00	00	00	00	00	00	00	53	06	88	64
	4	3	00	00	00	00	00	00	00	07	00	00	00	00	00	82	00	00	02

Variables	Doses	Times
01 Emptiness	1 10.08 g ALA	1 10:00 am
02 Rumbling	2 5.04 g APM	2 11:30 am
03 Stomach Ache	3 10.08 g APM	3 12:45 pm
04 Nausea	4 10.08 g PHE	
05 Headache		
06 Dizziness		
07 Faintness		
08 Drowsy		
09 Weak		
10 Nervous		
11 Tense		
12 Drugged		
13 Depressed		
14 Alert		
15 Mentally slow		
16 Hunger		
17 Urge to eat		

Plasma Amino Acid Levels (μmoles/dl)
Experiment 3

ID	ALA	TYR	VAL	ILE	TRP	LEU	PHE
Carbohydrate + 10.08 g ALA							
Baseline							
301	21.4	7.3	3.2	6.5	6.2	12.0	5.9
302	37.6	10.6	25.6	8.2	8.5	16.1	6.7
304	39.1	7.7	21.2	8.5	7.4	15.4	7.1
305	26.8	6.8	25.4	8.6	9.8	17.0	6.6
306	42.6	10.9	25.4	7.2	9.6	18.9	6.7
307	29.3	8.0	24.5	7.3	6.8	15.1	8.0
Experimental							
301	64.1	6.4	2.4	4.4	4.5	8.2	5.0
302	101.3	5.1	18.4	6.7	6.8	13.4	5.6
304	84.1	6.5	13.9	4.6	5.4	9.4	5.3
305	126.5	4.7	24.1	6.9	9.2	13.7	6.7
306	108.3	8.7	22.8	5.4	6.1	12.0	5.0
307	69.3	7.3	22.2	5.6	6.0	11.3	7.5
Carbohydrate + 10.08 g APM							
Baseline							
301	32.3	9.3	3.5	6.4	6.2	12.8	5.4
302	35.6	9.2	19.3	6.8	8.3	15.0	6.3
304	32.2	8.2	18.4	8.6	6.7	16.5	6.5
305	27.1	10.0	28.0	9.0	8.5	18.0	7.0
306	30.8	8.2	17.3	6.4	6.5	11.4	4.8
307	29.4	7.2	26.2	7.4	7.1	13.9	8.2
Experimental							
301	36.4	7.3	3.1	5.4	5.5	10.1	10.6
302	55.5	15.4	23.3	6.2	8.1	13.5	21.4
304	54.9	8.0	16.1	5.8	6.7	11.2	9.1
305	32.9	11.8	24.0	7.2	8.0	13.1	13.4
306	63.3	13.8	14.0	5.6	5.6	9.9	23.4
307	46.6	7.8	19.4	5.1	6.3	10.7	13.9

Visual Analog Scale Scores
Experiment 3

ID	Dose	Time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
301	1	1	13	64	49	06	01	13	14	11	01	01	00	00	01	27	64	05	00
	1	2	36	07	07	01	01	13	23	39	27	06	04	00	01	33	38	29	01
	1	3	08	43	47	52	57	58	62	53	36	02	02	00	01	21	74	15	06
	1	4	01	48	49	49	48	20	13	27	27	02	02	01	01	32	54	09	02
	2	1	01	01	01	01	01	32	16	58	32	00	00	01	00	55	44	01	02
	2	2	40	20	17	25	01	28	14	27	01	00	01	01	00	59	25	53	45
	2	3	17	02	01	02	03	02	02	49	31	01	01	01	00	62	13	35	36
	2	4	00	00	00	00	00	00	00	00	00	00	00	00	04	32	41	09	10
302	1	1	04	00	00	00	04	00	00	05	00	00	00	00	00	73	00	07	05
	1	2	41	00	05	00	00	00	00	06	00	00	00	00	00	79	00	64	63
	1	3	34	00	06	00	04	00	00	04	00	00	00	00	00	60	00	47	48
	1	4	06	00	00	00	17	00	00	07	00	00	00	00	00	75	00	14	10
	2	1	18	00	00	00	00	00	00	08	00	00	00	00	00	70	00	15	24
	2	2	80	14	20	00	18	00	00	06	00	00	00	00	00	72	00	72	68
	2	3	48	07	25	00	30	06	00	32	08	00	00	00	00	65	07	62	64
	2	4	38	00	00	00	48	15	00	15	00	00	00	00	00	56	07	29	18
304	1	1	13	05	05	01	02	02	02	12	10	02	01	00	04	79	18	35	34
	1	2	35	09	06	00	00	02	02	13	13	03	01	05	05	62	34	42	40
	1	3	54	22	05	02	18	07	07	06	06	03	02	00	00	83	14	62	63
	1	4	01	00	31	32	25	21	31	37	30	15	11	07	04	56	36	05	01
	2	1	13	01	04	03	05	06	07	11	00	04	04	00	00	70	21	21	22
	2	2	24	10	09	02	02	04	03	06	03	06	07	00	00	73	19	36	34
	2	3	35	34	02	02	02	00	00	04	01	03	03	00	00	81	20	40	48
	2	4	28	12	01	00	03	00	02	03	00	07	05	00	04	84	09	30	32
305	1	1	11	00	00	00	21	03	00	47	20	02	02	00	00	43	44	07	08
	1	2	65	14	12	00	17	07	01	50	22	02	17	01	00	50	11	27	34
	1	3	38	10	01	01	26	05	02	65	19	02	02	02	01	63	23	47	45
	1	4	07	02	01	01	30	02	02	29	07	02	01	01	01	70	20	01	02
	2	1	15	01	13	03	06	02	02	25	19	05	02	01	02	52	46	10	08
	2	2	27	02	02	01	18	02	02	22	17	02	02	01	01	68	26	25	24
	2	4	14	02	02	10	10	02	02	08	02	02	02	02	01	74	13	05	02
306	1	1	03	03	03	03	03	03	04	91	04	04	04	04	04	44	44	07	08
	1	2	06	06	05	23	08	07	08	41	10	10	08	07	07	48	24	06	05
	1	3	22	04	04	05	03	04	50	05	06	06	04	05	05	68	19	23	22
	1	4	05	05	05	05	04	05	06	07	07	06	07	07	07	70	16	05	05
	2	1	00	00	00	00	00	00	00	24	00	00	00	00	00	28	27	00	00
	2	2	03	00	00	00	00	00	00	14	00	00	00	00	00	43	04	07	08
	2	3	20	00	00	00	00	00	00	03	00	00	00	00	00	77	02	35	34
	2	4	00	00	00	00	00	00	00	01	00	00	00	00	00	99	02	10	09

ID	Dose	Time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
307	1	1	03	01	01	01	01	01	01	11	01	01	01	01	01	62	02	04	03
	1	2	02	01	01	00	01	01	01	04	04	01	01	01	02	73	01	01	01
	1	3	02	02	01	02	02	01	01	03	03	01	01	01	01	89	02	03	04
	1	4	04	03	01	03	02	02	02	02	02	03	02	02	03	82	04	03	03
	2	1	00	00	00	00	00	05	00	05	00	00	00	00	00	80	00	00	00
	2	2	00	00	00	00	00	00	00	00	00	00	00	00	00	76	00	00	00
	2	3	08	00	00	00	00	00	00	02	00	00	00	00	00	84	00	10	10
	2	4	00	00	00	00	00	00	00	31	00	00	00	00	00	80	00	00	00

Variables	Doses	Times
01 Emptiness	1 10.08 g APM	1 8:00 am
02 Rumbling	2 10.08 g ALA	2 10:00 am
03 Stomach Ache		3 12:00 noon
04 Nausea		4 2:00 pm
05 Headache		
06 Dizziness		
07 Faintness		
08 Drowsy		
09 Weak		
10 Nervous		
11 Tense		
12 Drugged		
13 Depressed		
14 Alert		
15 Mentally slow		
16 Hunger		
17 Urge to eat		