AN ABSTRACT OF THE THESIS OF

Sandra J. Mitchell for the degree of Doctor of Philosophy in Foods and Nutrition presented on December 9, 1983.

Title: Protein and Carbohydrate Intake, Plasma Neutral Amino Acid Levels, and Hunger Ratings of Young Men with Changes in Breakfast Protein Content.

Abstract approved: _____
James E. Leklem

The work of others supports a dietary intake feed-back mechanism in the rat whereby dietary protein and carbohydrate contents affect the plasma ratio of tryptophan to the sum of valine, isoleucine, leucine, tyrosine, and phenylalanine (TRP/ΣNAA). This ratio in turn affects central serotonin metabolism which affects the freely-selected intake of dietary protein and/or carbohydrate. The present study tested the hypothesis that when young men consumed breakfasts of differing protein content (4 g and 18 g) and carbohydrate "to appetite," differences would be observed in plasma neutral amino acid levels and subsequent freely-selected intake at meals with regard to protein and carbohydrate. Thirteen, young (ages 18-22), normal weight men participated in this study and consumed breakfasts with each level of protein for one week. Although plasma TRP/ΣNAA was significantly ($p < .01$) higher

two and four hours after the 4 g protein breakfast than after the 18 g breakfast, subsequent intake of calories, protein, and carbohydrate did not differ. Fasting values of plasma neutral amino acids did not correlate with caloric, protein, or carbohydrate intakes of the previous two days. Food intakes were measured by weighing in a university cafeteria. This method of food intake measurement was found to be similar in time costs to that of a laboratory metabolic study but had some advantages in monetary savings and presenting subjects with a familiar variety of foods. Subjective hunger ratings, as measured on a Visual Analog Scale, decreased with meal ingestion but were neither predictive of meal caloric, protein, or carbohydrate intakes nor related to plasma neutral amino acid levels or ratios. Two and four hours after breakfast, subjective hunger ratings did not differ with the different breakfast contents. Variability in pupil responses to pictures of food, when measured two hours after breakfast, made the pupillometric results inadequate as measures of interest in food. It was concluded that although breakfast contents of protein and carbohydrate affected plasma neutral amino acid ratios two and four hours after the breakfast, alterations in subsequent dietary intakes, as observed in rats by other researchers, and in post-breakfast hunger ratings were not found in the human subjects in the present study.

Protein and Carbohydrate Intake, Plasma Neutral Amino Acid Levels, and Hunger Ratings of Young Men with Changes in Breakfast Protein Content

by

Sandra J. Mitchell

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed December 9, 1983

Commencement June 1984

APPROVED:

Associate Professor of Foods and Nutrition in charge of major

Head of Department of Foods and Nutrition

Dean of Graduate School

Date Thesis is presented December 9, 1983

Typed by Jean Degnan for Sandra J Mitchell

ACKNOWLEDGEMENT

The past nine years have been spent in pursuit of the Ph.D. There have been several steps and interruptions along the way, but pursuit of this goal has had a central position in my life during this period. There is no way to acknowledge all those who have helped me in various ways. There are, however, two very special people who have provided leadership and encouragement throughout the entire graduate school experience. The first of these is Jim Leklem who has been my major advisor for both the M.S. and Ph.D. degrees. Dr. Leklem has shown me how to continue problem solving when no solutions were in sight, and he has demanded excellence when it would be easier to be sloppy. His excellence as a scientist, a teacher, and a human being has provided an example to emulate. I am proud to be associated with him. The second person who has profoundly influenced my pursuits is Margy Woodburn. Dr. Woodburn was my first contact at Oregon State. Her understanding of where I was and where I wanted to be helped me in my initial decision to start my graduate studies. Through the years I have continued to appreciate her insights and knowledge. Her counsel and her example as a professional woman have elicited my affection and admiration. I have been very fortunate to have studied with the guidance of these two people.

Financial sources of assistance have been several. The funds for research came from an American Home Economics Association Foundation grant, a Sigma Xi research fellowship, and the Dean's Research Fund. Personal assistance was received from the General Foods Fund and a Tartar award.

My family and my friends have been supportive, and their faith has helped me over the hard times. My employers have been understanding, and my students have been appropriately empathetic. So many people helped in the research. Subjects, technicians, faculty, and fellow graduate students made long hours bearable and frequently enjoyable.

This thesis is dedicated as a memorial to my father, Woodrow Mitchell. Without his values of perseverance and dedication, this thesis would never have been written.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Appendix Tables

PROTEIN AND CARBOHYDRATE INTAKE, PLASMA NEUTRAL AMINO ACID LEVELS, AND HUNGER RATINGS OF YOUNG MEN WITH CHANGES IN BREAKFAST PROTEIN CONTENT

CHAPTER I

INTRODUCTION

The study of human behavior has long been of interest, and throughout human existence various causes of behavior have received attention. Early in man's history, that which was not understood was attributed to uncontrollable "forces." The forces which caused rains and droughts were placated or encouraged by rituals. Antisocial behavior was the result of demons which could be exorcised by ritual.

Departure from traditional views of behavior was initiated by the controversial writings of Sigmund Freud early in this century. His view point of the biological underpinnings of behavior and the focus on cause and effect in behavior did not elicit universal acceptance, but the idea of systematic explanation for components of human behavior stimulated others to construct their own theories, and the search was underway to identify and control the forces behind social and emotional dysfunction with the ultimate goal of influencing behavior.

The growth of science and the development of understanding of scientific methodology caused many to turn to

behaviorism as the key to understanding behavior. Stimulus-response experimentation was a vehicle to understanding, and the scientist, who must rely on tangible causes and results, could operate well in the realm of conditioning studies. Erikson's concept of critical stages of development (1963, p. 271) and Maslow's differentiation of basic and higher motives (Maslow, 1971, p. 300) have influenced systematic studies of human behavior by forcing consideration of the interaction of innate developmental schemes with the environmental influences.

As knowledge of human behavior has grown, exceptional growth has also occurred in the fields of human physiology and medicine. The human body has been a fascinating and accessible subject for the scientific method. The spiral of history has brought us quickly back to parallel the era of Freud, a doctor of medicine who stepped outside the boundaries of his profession's rigidity to examine the psychological bases of organic disorders. Modern science has a knowledge base to explore not only the psychological components in organic dysfunction but the organic bases of behavior.

Nutritionists are constantly confronting the interaction of the physical and psychological aspects of the human organism. The effects of nutrition on behavior and emotional well being have bases in both science and mysticism. Eating is the means of acquiring nutrients and it is a behavior which must be understood if the control or

alteration of food selection is to be realized.

Overeating is very common in our society with the abundance of food that has been provided by our agricultural and economic advances (Hirsch, 1978). Because obesity threatens our life span and our quality of life, many methods have been devised to alter eating behavior. Behavior modification can be effective for those who merely have lapsed into poor dietary habits, will power is the putative cure for overeaters with inner "demons," and psychotherapy is useful for those who are eating to kill themselves or who became fixated in the oral stage. But the continuing difficulty so many people have in controlling food intake is sufficient reason to examine the physiological bases to desire for food in excess or for specific foods that threaten the nutritional balance in our diets.

Cravings for certain foods may be explained by conditioning or social pressures, but physiological causes are suspected with reports of specific cravings during the premenstrual period (Smith & Sauder, 1969) and as a result of drug therapy (Paykel et al., 1973).

Clara Davis, in her classic study of infants' self-selected feeding, reported that children will select a well-balanced diet when they are presented with a variety of "wholesome foods." Also revealed in this 1928 study, however, was that the children would go on "jags" during which one particular food would be eaten in large quantities for several days.

Rats which are not thought to share our spiritual weaknesses and oral fixations have been studied as models for biologically based appetites. Many of the studies on the presence of instinctive discrimination in the rat which allows for selection of an appropriate and nutritionally balanced diet have been designed around omission of a nutrient in a formulated food. The apparent presence of instinctive discrimination has usually been discounted by the confounding effect of aversive conditioning (Rozin, 1976). Rozin believes that the desire for a varied diet is genetically based and is the end product of the evolutionary pressures which produced omnivores such as the rat and man. With the variety of edible foods available in nature, the desire for variety will ensure nutritional balance. Thus, examining instincts with formulated foods is relying on the presence of sensors that were never required in nature.

Anderson and coworkers, however, observed that when weanling rats were offered choices of diets differing in protein content they exhibited a steady intake of protein as a percentage of dietary calories. This percentage varied with the type of protein in the diet but was not related to amino acid adequacy (Musten, Peace, & Anderson, 1974). Judith and Richard Wurtman demonstrated in a series of experiments that rats had an apparent appetite mechanism for controlling the intake of carbohydrates (1977b). The

results of these studies could not be explained by simple aversive conditioning. Although conditioning may play a role in development of discrimination between foods, the explanation of the underlying cause lies in a feed-back mechanism which appears to involve the central neurotransmitter, serotonin.

R. Wurtman and Fernstrom (1972) found that plasma neutral amino acid levels can affect the quantity of tryptophan that is transported into the brain, and these levels are influenced by the dietary components of protein and carbohydrate. Because the neutral amino acids compete for the same transport system into the brain, the ratio of tryptophan to the sum of the other neutral amino acids (valine + isoleucine + leucine + tyrosine + phenylalanine) (TRP/ΣNAA) in the plasma determines the rate of tryptophan entry into the brain (Pardridge, 1977). Tryptophan availability is a limiting factor in brain serotonin metabolism (Ashley & Curzon, 1981). Drugs which alter serotonin metabolism have been found to affect the ingestion of calories (Panksepp & Nance, 1974), protein (Ashley & Anderson, 1977b), and carbohydrate (Hirsch & Wurtman, 1981) in rats. Such drugs have also been observed to alter calorie (Shoulson & Chase, 1975) and carbohydrate (Wurtman, Wurtman, Growdon, Henry, Lipscomb, & Zeisel, 1981) intake in some humans.

The experiment conducted for the present study was designed to examine the possibility that the protein and

carbohydrate contents of one meal (breakfast) can affect the desire for protein or carbohydrate containing foods during the remainder of the day. In addition, plasma neutral amino acids were measured to determine whether changes in the TRP/ΣNAA were reflected in subsequent food interest or intake. Food interest was measured with subjective hunger ratings and pupil responses to pictures of food. Food intake was determined by weighing the food actually consumed by subjects in a free-selection situation.

Development of the theoretical basis for this study is discussed in a review of the literature in Chapter II. Chapter III contains the overall experimental design and the resulting plasma amino acids and food intake. The fourth chapter describes the procedure developed for measuring food intake, and Chapter V presents an analysis of the limitations of subjective hunger ratings as measured in this study. The sixth chapter reports the pupillometric analysis which was included in the experimental design.

Tables of data which were summarized in the text are presented in the appendices. It is hoped that these data will be useful to other researchers who may want to consider the data in a different way.

CHAPTER II

REVIEW OF LITERATURE

Understanding why people eat what they do has been a subject of speculation for a period that is probably longer than recorded history. The question is not merely of philosophical interest but of practical economic and medical interest as well. Recent observations of an apparent appetite in rats for protein (Musten et al., 1974) or carbohydrate (Wurtman & Wurtman, 1977b) have stimulated studies designed to examine the physiological responses to intake of these nutrients. A mechanism relying on plasma neutral amino acid responses to nutrient intake and consequent alteration of brain neurotransmission has been proposed (Anderson, 1977) as part of a physiological system in the rat in which the protein consumed affects the future ingestion of protein.

Because the components of this regulatory feed-back mechanism are apparently present in humans, this study was undertaken to determine whether human plasma amino acid responses to ingestion of protein and carbohydrate are similar to those of the rat. In addition, this study examined the food intake patterns of young men to determine whether the relationship to plasma amino acids of macronutrient (protein and carbohydrate) intake, as observed by

other investigators in the rat, could also be demonstrated in humans.

After examination of the evidence for a macronutrient appetite in human, theoretical and experimental evidence for macronutrient appetite regulation in the rat will be presented, and the components of the proposed physiological system related to the appetite mechanism will be discussed. Experimental evidence for physiological parallels in the human will be considered. Finally, the theoretical need for a macronutrient appetite will be compared to experimental evidence for food intake regulation in humans.

Macronutrient appetite in humans

There is a long history of the study of appetite and hunger in humans. Much of the interest in appetite and hunger arises from concerns about obesity, but there has also been an interest in decreased body weight from loss of appetite in disease states such as cancer. The more prominant interest in controlling excessive caloric intake has resulted in basic and applied research on the hunger mechanisms and means of controlling them. A complete understanding of the means of altering food intake is still elusive.

Recently, interest has focused on the appetite for specific macronutrients (i.e., protein and

carbohydrate). Ashley and Anderson (1975b, 1977a) observed that rats, when given dietary options, consumed a constant percentage of their caloric intake as protein. Judith and Richard Wurtman have studied carbohydrate intake regulation in rats (1977b) and in obese humans (Wurtman, Wurtman, Growdon, Henry, Lipscomb, & Zeisel, 1981). The underlying mechanism appears to involve serotonin in the brain, and the model for this mechanism has been well developed by many laboratories from research with rats.

Dietary sources of energy include protein, carbohydrate, fat, and alcohol. Most of the studies on caloric appetite have not given the subjects (humans or rats) dietary options, and thus this bulk of research does not provide information on specific macronutrient appetites. Alcohol has not been considered as a natural energy source, and an appetite for fat does not seem to occur in the human or the laboratory rat. Many of the studies which have been made on protein and carbohydrate appetite have altered the ratio of these two nutrients in the diet. The caloric density of these nutrients is very similar, and adjustments to the diet are simplified by altering only these components. In many cases, although results are given in terms of protein or carbohydrate intake, intakes of both of these macronutrients have been variables.

Evidence of macronutrient appetite in humans

The only evidence for a protein appetite in humans is the result of a study by Anderson et al. (1979) in which eleven male subjects were allowed to choose from a variety of foods at breakfast and lunch. A significant negative correlation was seen between the percentage of calories ingested as protein (% protein) in the breakfast and the lunch.

Cravings for carbohydrate foods, particularly sweets, have been reported by many people who are attempting weight reduction. In a survey of 300 female members of the nursing staff of a general hospital, two-thirds of the respondents reported desires to eat compulsively, and a similar number reported cravings for sweets (Smith & Sauder, 1969). Report of cravings for carbohydrates was significantly correlated with weight gain in a group of women who were being treated for depression with amitriptyline (Paykel et al., 1973). There was no evidence of hypoglycemia or hyperinsulinemia from the women's fasting blood samples, and the cravings were not related to their depression ratings. Although 87% of those women being treated with amitriptyline reported carbohydrate cravings, only 29% of the women treated with placebo or with no drugs reported such cravings. No differences were seen in the overall appetite. Paykel et al. (1973) found that

elimination of the amitriptyline therapy reversed the weight gain and the carbohydrate cravings.

In a study by Judith Wurtman and her colleagues (1981), obese subjects were given three meals a day and allowed to choose snacks which were rich in protein or carbohydrate. Subjects had been chosen because of their reported tendency to snack on carbohydrate rich foods, and this tendency was confirmed in the study. Fenfluramine and tryptophan (TRP) administration reduced the carbohydrate snacking in several of the subjects, but placebo administration did not affect it.

Study of appetite in man is difficult because there are so many factors affecting food choice. Pilgrim (1957) categorized the factors into three major groups. These groups are physiology, sensation, and attitudes. Each of these contribute to the perception and acceptance of a particular food at a particular time. The three groups each contain several components which are not necessarily independent, and mutual interaction among components within the same group and between components in different groups have been found. In Pilgrim's categorization, appetite is a component of the physiological group, and she points out that measures of food acceptance or consumption are measuring not only appetite but the momentary interaction of all the components in all the groups.

Humans have some complications in feeding behavior

that may not be seen in other animals. We often eat when we are not hungry, and as Booth, Toates, and Platt stated:

> Anticipatory control of feeding offset may be particulary important for man in societies like ours, because other behavioral mechanisms for regulating energy balance have little chance to operate--social factors largely determine feeding onset and the size of a meal is often fixed at its beginning when the plate or tray is filled. (1976, p. 141)

Seigel (1957) found evidence for a "completion compulsion" in an experiment with college students. The food items were served individually and in particular quantities, but the subjects were allowed to take as many servings as they liked. The exact number of servings was eaten with much greater frequency than partial servings.

Evidence for macronutrient appetite in rats

Rats and humans have two eating behavior traits in common; both species are omniverous and neophobic (Rozin, 1976). But because the rat's environment can be better controlled for experimentation, it is presumed that internal factors such as appetite will contribute the greatest share of components influencing food consumption. Observation of factors influencing food choice and consumption by the rat may reveal physiological components that are also present in human food choice and consumption.

Energy consumption has been thought to be the dominant factor influencing the food consumption of rats

(Rozin, 1976), but Kanarek, Marks-Kaufman, and Lipeles (1980) found that rats which were allowed to self select from separate sources of macronutrients chose fewer (10-15%) calories than when given only commercial lab chow. This indicated that some regulation of macronutrient intake could override the caloric regulation.

When offered a choice of diets containing either 3, 10, 25, or 50% casein with a methionine supplement, both young and old rats exhibited a preference for the 10% diet. When the only choices were 25 or 50% protein, the preference was for the 25% diet (Peng et al., 1975). Schoenfeld and Hamilton (1976) also observed a constant percent protein consumption in rats, and compensation for dietary changes occurred within the first day during experiments by Booth (1974b).

Li and Anderson (1982a) observed that rats which had been fed a 2 g "pre-meal" of a 45% casein diet consumed less protein in the meal selected 30 minutes later than was consumed by rats fed a protein-free premeal of the same size. A pre-meal containing 70% casein resulted in a still lower amount of protein consumption in the meal, and it also reduced the total food intake during the entire 12 hour feeding period. The 0 and 45% casein pre-meals did not result in different 12 hour intakes (Li & Anderson, 1982a).

Rats which were given a cafeteria choice of liquid carbohydrate, protein, and fat compensated for dilution of the protein source by drinking more of the diluted solution. Dilution of the carbohydrate source resulted in no compensation. When protein or carbohydrate was omitted from the choices, no specific compensation was observed (Rozin, 1968). These results were different from those seen by Kratz (1977) using solid foods. Dilution of the protein source with Alphacel resulted in a decreased protein intake, and compensation for the caloric dilution was made by increasing the consumption of carbohydrates.

Collier, Leshner, and Squibb (1969) introduced an activity wheel into the cages of some rats which were allowed to select from separate carbohydrate and protein sources or were given a complete diet. Although growth rates were similar with both diets, active rats consumed less food than the inactive ones, and in this case, the inactive rats consumed more food when presented with a selection. Active rats consumed a lower percentage of calories as protein than the inactive ones, but activity did not correlate with protein intake. This study indicates that freely-selected intake of protein and carbohydrate may respond to internal states which result from having some activity. (Although the response is not understood, the activity factor may be important in considering applications of studies on hospital patients to ambulatory

populations.)

Changes in percentage of calories as protein have been observed in rats made diabetic by streptozotocin (Booth, 1974a) and alloxan (Vartiainen & Bastman-Heiskanen, 1950). In both studies the percentage of calories as protein increased over that of control rats. This increase occurred whether total calorie intake was greater or less than the controls and was not altered by insulin treatments (Vartianin & Bastman-Heiskanen, 1950).

Insulin administration to normal rats resulted in increased carbohydrate consumption in a study by Kanarek et al. (1980). Carbohydrate and calorie consumption were

decreased on the second day after cessation of insulin administration. These self-selecting animals also avoided hypoglycemic shock which was the result in 37% of insulin-treated animals which were given access to a standard chow diet only.

Feed-back regulatory mechanism for macronutrient appetite: a rat model

A mechanism for regulation of macronutrient intake would be one which could respond rapidly to changes in diet (Booth, 1974b) and to the internal state (Vartianen & Bastman-Heiskanen, 1950). Peripheral changes which would be reflected in the blood and alter the functioning of the brain seem to be a logical basic mechanism. The development of a specific theory for a mechanism involving plasma neutral amino acids, availability to the brain of TRP, and altered synthesis of the central neurotransmitter, serotonin (5-HT), was developed from the results of experiments in several different laboratories.

Musten, Peace, and Anderson (1974) found that when rats were given a choice between diets differing in protein concentration, they maintained a constant percentage of their calories as protein. This percentage changed with different sources of protein. To determine what peripheral changes occurred to permit such control, plasma amino acids were measured in weanling rats which had been

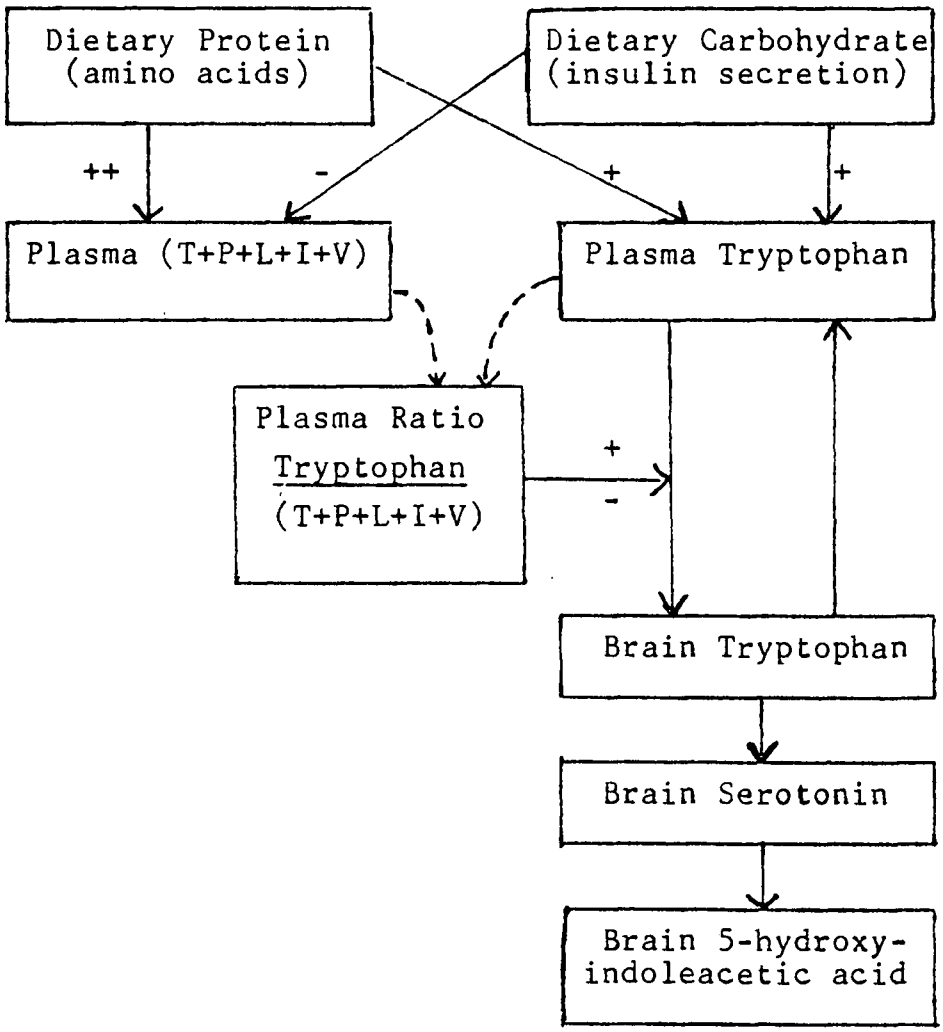
allowed to self-select from two diets differing in protein concentration. Ashley and Anderson (1975a) fed the animals for four weeks and pooled the blood of the six rats in each group after they had been killed between 900 and 1100 h. Lights in the animal room were off between 2000 and 800 h, and the animals had been fasted overnight. The protein used varied, and additions of the limiting essential amino acid or groups of amino acids were made to gluten, casein, and zein. Comparison of the food intake and the plasma amino acids showed a consistent negative correlation between the percentage of calories as protein and the plasma ratio of TRP to the sum of the other large neutral amino acids (TRP/ΣNAA). The "other large neutral amino acids" are tyrosine (TYR), phenylalanine (PHE), leucine (LEU), isoleucine (ILE), and valine (VAL). Total and individual plasma amino acids did not correlate with either the energy or the protein intake.

In another laboratory, Fernstrom and his colleagues (1973) studied the short term effects of diet on plasma and brain concentrations of amino acids of rats. The animals were deprived of food for more than 12 hours. In the afternoon, one group of rats was killed (controls), and the other rats were given one of three diets: no-protein, the no-protein diet with the addition of 18% casein by weight, and lab chow with 24% protein. The rats were then killed two hours after they had been given free access to

their food. Plasma TRP was higher after consumption of lab chow than after the no-protein diet, but the brain TRP was no higher than the controls. Brain TRP was 50% higher than the controls' after the animals were fed the no-protein diet. No correlation was seen between plasma and brain TRP, but there was a significant positive correlation in each case between the concentration of brain TRP and both the plasma TRP/ΣNAA and the ratio of TRP to each of the neutral amino acids. To further consider the effect of the neutral amino acids, Fernstrom et al. (1973) fed rats a diet in which the neutral amino acids other than TRP were eliminated. Plasma concentrations of the other neutral amino acids did not rise after ingestion of this diet. The concentrations of plasma and brain TRP now were correlated, and the correlation between brain TRP and plasma TRP/ΣNAA still existed. In all of these comparisons, the consideration of aromatic or branched chain amino acids (BCAA) alone did not improve any correlations.

The presence of TRP in the brain is important in the consideration of a feed-back mechanism because TRP is the essential precursor of 5-HT, a neurotransmitter which has been implicated in the control of macronutrient intake. A diagram of proposed influences of dietary components on brain 5-HT synthesis is presented in Fig. II-1 (p. 19).

Because of the limitations in studying the central nervous system of humans, all direct evidence of the role



Note: (T+P+L+I+V) indicates the sum of the molar concentrations of tyrosine, phenylalanine, leucine, isoleucine, and valine.

\+ indicates positive effector

\- indicates negative effector

Figure II-1. Proposed sequence describing diet-induced changes in brain serotonin concentration in the rat. (Fernstrom & Wurtman, 1972)

of 5-HT in the brain has been found in rat studies. Indirect evidence in human studies does not indicate that any contradiction exists with the rat model (Chase & Shoulson, 1975; Shoulson & Chase, 1975; Noble, 1969; Wurtman & Wurtman, 1981).

Neurotransmitters

The nerve cell, or neuron, is the basic structural and functional unit of the central nervous system, and behavior is the net result of the complex interaction of neurons. An electrochemical impulse, which is conveyed along the fiber of a neuron, brings information to the synapse, or gap, between the neuron terminal and the receiving neuron. Bridging the synapse and reinitiating electrochemical transmission requires the release of a neurotransmitter which travels across the synapse. The interaction of the neurotransmitter with receptors on the outside of the receiving neuron's membrane causes a change in the electric potential across the membrane (Nathanson & Greengard, 1977).

Interference with the binding of a neurotransmitter to its receptor will disrupt the normal neuronal communication and thereby alter behavior. A receptor agonist is a molecule with a configuration similar enough to the neurotransmitter that it can bind to the membrane receptor and mimic the neurotransmitter's action. A receptor

antagonist will also bind to the receptor, but it will not activate it. While the antagonist is bound to the receptor the neurotransmitter cannot act. Some drugs have a mixed action in that they bind to the receptor and cause partial activation. Other drugs have the effect of altered transmission because of the changes they make in neurotransmitter availability (Nathanson & Greengard, 1977).

Use of drugs with known action can elucidate the factors in neurotransmission and the resulting responses by the animal. The action of drugs used to study 5-HT and its effects are primarily ones which would be categorized as drugs which alter neurotransmitter availability. Some of these are:

1. Para-chlorophenylalanine (PCPA) - inhibits tryptophan hydroxylase (Wurtman et al., 1974).
2. Fenfluramine [(m-trifluoromethyl)-N-ethyl-amphetamine] - inhibits release and synthesis of 5-HT (Fuller et al., 1978).
3. Fluoxetine - inhibits re-uptake of 5-HT from brain synapses (Reinhard & Wurtman, 1977). And
4. Lilly 110140 - inhibits re-uptake of 5-HT from brain synapses (Goudie et al., 1976).

In addition, quipazine has been used in studies, and its mode of action is as a receptor agonist (Samanin et al.,

1977).

Fernstrom and Wurtman (1974) named six well-established neurotransmitters which are found in the brains of mammals: 5-HT, acetylcholine, gamma-amino butyric acid, epinephrine (adrenalin), norepinephrine (NE), and dopamine (DA). The last three of these are the catecholamines (Fernstrom & Wurtman, 1974). Some neurotransmitters (e.g., acetylcholine) are excitatory (i.e., changes in ionic flux increase the chance of depolarization). Others (e.g., 5-HT) are inhibitory (Wurtman, 1979).

Some of the neurotransmitters' synthesis is dependent on changes in plasma precursors. Wurtman, Hefti, and Melamud (1981) observed that all the neurotransmitters which have their synthesis influenced by precursor availability receive these precursors in whole or in part from the diet. Storage of the precursors in tissue proteins and membrane lecithin means that there is little decrease in plasma levels of the precursors beyond that from an overnight fast if they are not included in the diet. It would seem logical that there must be a mechanism to maintain a narrow range of the levels of these precursors after meal consumption or neurologic alterations would occur. Also needed are mechanisms in the blood-brain barrier that allow precursor molecules in the circulation to equilibrate with those in the brain extracellular fluid.

The transport system of the blood-brain barrier must be the low-affinity type and not saturated at physiological plasma concentrations. The absence of a feed-back mechanism to regulate firing is essential if availability of the substrate is to have an effect on transmission. The synthesis and release of 5-HT seems to vary directly with TRP availability from the plasma throughout the physiological range, and the changes in release may give information to the rest of the brain (Wurtman, Hefti, & Melamud, 1981).

Each meal's composition can alter brain choline and TYR levels as well as the TRP level (Wurtman, 1979). The alterations in choline and TYR can modify the synthesis of acetylcholine and the catecholamines DA and NE, respectively. Brain TRP and TYR levels are, in part, dependent on the proportions of carbohydrate and protein in the meal while the choline level is dependent on the lecithin content of the diet.

Tryptophan metabolism in the brain

The conversion of TRP to 5-HT occurs in two steps (see Fig. II-2, p. 24). The first step is the hydroxylation of TRP in the 5-position by the enzyme tryptophan hydroxylase, and the product is called 5-hydroxytryptophan (5-HTP). 5-HTP is then decarboxylated by the aromatic L-amino acid decarboxylase to 5-HT (Lovenberg et al., 1973).

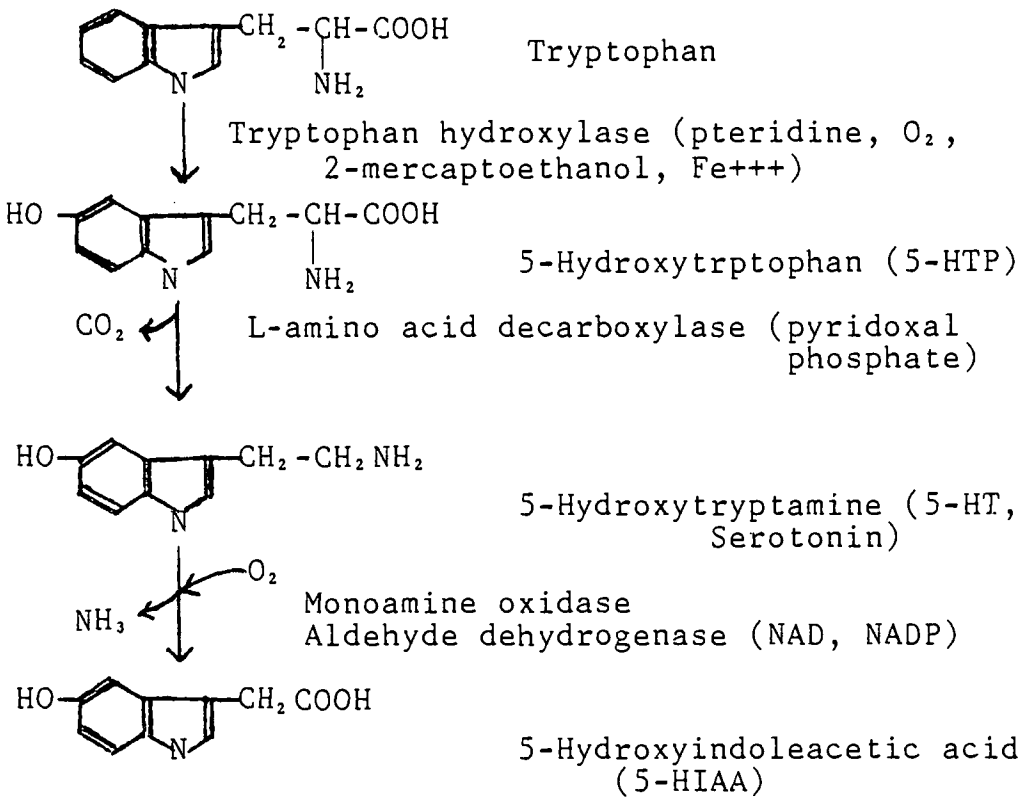


Figure II-2. Conversion of tryptophan to serotonin and oxidation to 5-hydroxyindoleacetic acid. (Lehninger, 1975; Costa & Neff, 1970; Greenberg, 1961; Harper et al., 1977)

5-HT is further metabolized by monoamine oxidase (MAO) to 5-hydroxyindoleacetic acid (5-HIAA) (Eccleston et al., 1970).

The activity of tryptophan hydroxylase was found to be unchanged when rats were given diets containing low amounts of TRP, but with supplementation of three times the normal food level of TRP, the enzyme activity was seen to vary over a four-week experimental period (Ashley & Cruzon, 1981). From these studies, Ashley and Curzon concluded that the brain level of TRP was important, but was not the sole determinant of 5-HT synthesis. Lovenberg et al. (1973) observed product inhibition at high levels of L-5-HTP, but this was much higher than physiological levels, and no inhibition was seen with addition of 5-HT or D-5-HTP.

Colmenares, Wurtman, and Fernstrom (1975) found indirect evidence that diet-induced changes in 5-HT and 5-HIAA levels reflect an acceleration of 5-hydroxyindole synthesis. They measured 5-HTP accumulation after injection of a decarboxylase inhibitor. 5-HTP accumulation was observed in those sites of the brain in which increases of TRP, 5-HT, and 5-HIAA had been observed with the experimental diet.

A system involving two pools of 5-HT in the brain was proposed by Grahme-Smith (1974). The effect of this system would be that under normal conditions, if 5-HT synthesis is

in excess of needs, the excess 5-HT would be contained by intraneuronal pools or metabolized by intraneuronal MAO. This would mean that the regulation of 5-HT metabolism could be controlled by alterations in MAO activity or by leakage from the intraneuronal pools. His conclusions were: first, brain TRP and tryptophan hydroxylase do not have to be the fine controls for prefunctional 5-HT; and second, feed-back could be exerted on (a) vesicular binding of 5-HT, (b) MAO activity, or (c) the amount of 5-HT available intraneuronally for oxidative deamination of either the prefunctional or the inactive 5-HT. From these possibilities Grahme-Smith (1974) suggested that the levels of 5-HT in the brain and of 5-HIAA in the cerebro-spinal fluid (CSF) may not always

reflect 5-HT neurotransmission in the brain.

It was suggested by Weissman (1973) that there were two main reasons that the understanding of the influence of 5-HT on behavior remained tentative and circumstantial. The first of these was that there were no known specific means for elevating or diminishing 5-HT receptor activity, and the second was that there was no unanimity on the division of behavior into unequivocal classes. Studies with various drugs and brain lesions, however, have elucidated some of the roles of 5-HT in specific eating behaviors.

Drug manipulations

Para-chlorophenylalanine (PCPA) has been observed to inhibit the synthesis of 5-HT in the brain by its inhibition of tryptophan hydroxylase (Wurtman et al., 1974). The behavioral effects of PCPA treatment were outlined by Weissman (1973). The effects of PCPA on gross behavior are to elicit mild irritability and aggressiveness, but there is little effect on motor stimulation. Rats show little effect on food consumption until the doses are high, and then anorexia occurs. PCPA alters sleep with prolonged EEG activation and sleep disturbance. The many effects were characterized as reflecting a hyperresponsiveness to the environment. Weissman (1973) suggested that functioning 5-HT systems may be necessary for inhibiting reactivity to stimuli. The observation by

Wurtman and his colleagues (1974) that injection of PCPA into rats profoundly depresses brain TYR and DA accumulation indicates that PCPA lacks specificity for inhibition of serotonin synthesis.

Fenfluramine alters brain 5-HT metabolism in several ways which include the release of serotonin, inhibition of serotonin synthesis, and depletion of brain serotonin content (Fuller et al., 1978). Studies by Knapp and Mandell (1976) determined that after treatment with fenfluramine there is a close temporal relationship between the duration of the block of ^{3}H-5-HT reuptake in the nerve endings and an adaptive decrease in the cell body tryptophan hyroxylase. The effects of fenfluramine occur over a 10-14 day period. The early effect is to increase the nerve-ending conversion of TRP to 5-HT and to block the reuptake of 5-HT. Later there is an adaptive decrease in the hydroxylase which normalizes when the reuptake block is released.

Free-feeding rats which were injected with fenfluramine exhibited anorexia in the first hour and then decreased their % protein intake. Leathwood and Ashley (1981) observed that changes in 5-HT metabolism paralleled these feeding alterations. Feeding behavior of rats with and without lesions of the hypothalamus was studied by Blundell and Leshem (1975) after they treated the rats with amphetamine, 5-HTP, and fenfluramine. During the

first hour, 5-HTP treatment resulted in a reduction of food intake which was more severe in the lesioned than in the control animals. The anorectic effect lasted only eight hours. Amphetamine reduced the food intake more in the controls, and fenfluramine caused more reduction in the lesioned rats. 5-HTP enhanced the effects of each of the other drugs (with more enhancement of amphetamine) in the lesioned animals, but in the controls, only the amphetamine was enhanced. Blundell and Leshem (1975) concluded that 5-HTP and fenfluramine may cause anorexia by the same mechanisms.

The degeneration of central serotonergic neurons is a selective effect of the administration of 5,6-dihydroxytryptamine (5,6-DHT). This agent was used by Clineschmidt (1973) to determine whether the anorexegenic action of fenfluramine was dependent on release of endogenous 5-HT from central monoamine-containing neurons. Ten or 13 days after pretreatment with 5,6-DHT, rats were injected with fenfluramine 30 minutes before their regular feeding period (900 - 1100 h). Food consumption was compared to that of the previous day. After fenfluramine injection there was more consumption of food by the 5,6-DHT treated rats than by the untreated controls. Fenfluramine was also more effective in depressing motor activity in the controls. Injections of PCPA for three days prior to fenfluramine administration resulted in reduced food intake, but

there was no effect on the fenfluramine action. The antagonistic effect of 5,6-DHT on fenfluramine was concluded to be caused by its destruction of 5-HT containing neurons. The effect of 5,6-DHT on 5-HT itself does not seem to be a factor because PCPA was not antagonistic to the fenfluramine anorectic effect (Clineschmidt, 1973).

Fluoxetine (Reinhard & Wurtman, 1977) and Lilly 110140 (Goudie et al., 1976) have been used to potentiate 5-HT action by inhibiting the reuptake of 5-HT from the brain synapses. Experiments with fluoxetine determined that the reuptake precedes the transformation of 5-HT to 5-HIAA (Reinhard & Wurtman, 1977). Quipazine was found by Samanin and his colleagues (1977) to decrease food intake by direct stimulation of central 5-HT receptors, and thus it could mimic the effects of 5-HT. Many other drugs have also been found to affect feeding behavior, but some such as codeine and morphine have no significant effects on the synthesis of 5-HT (Lovenberg et al., 1973).

Neurological lesions

Saavedra (1977) used a microdissection technique to study the localization of enzymes in specific areas and nuclei of the brain. The highest brain concentrations of 5-HT and the related enzymes were found in the raphé nuclei. 5-HT concentrations were also relatively high in areas and nuclei of the hypothalamus, limbic system, and

brain stem; and the synthesizing enzymes were also found in these areas. Catecholamines were also present in the raphé nuclei, and changes in catecholamine metabolism resulted in changes in 5-HT levels in specific brain areas. These observations led Saavedra to suggest that 5-HT may be involved in the regulation of several autonomic functions as well as in neuroendocrine control mechanisms.

Methods which use histochemical fluorescence were employed by Kuhar et al. (1972) to visualize various monoamine-containing neurons. They found axons and nerve terminals in the forebrain with increased intensity of fluorescence after pre-treatment with L-TRP and the MAO inhibitor, pargyline. Chemical methods were used to examine regional tryptophan hydroxylase activity and synaptosomal uptake activity before and after midbrain raphé lesions. Decreases of 5-HT, tryptophan hydroxylase, and synaptosomal uptake activity were observed in the lesioned animals' hypothalamus-thalamus (70-90%), cortical and hippocampal regions (80-90%), and the striatum (45-65%). In the cerebellum these parameters were 50% lower in the controls than in the lesioned animals. Application of the histochemical fluorescence methods indicated that the lesions had destroyed cells in the raphé nuclei and decreased the number of neurons in the forebrain which had previously been seen after pargyline and TRP treatment.

Coscina and Stancer (1977) presented evidence that midbrain raphé lesions prevent the overeating and obesity characteristic of medial hypothalamus (MH) lesions. The results of their experiments indicate that 5-HT plays a secondary rather than a primary role in MH hyperphagia. Female adult rats were given all possible combinations of lesions of the medial hypothalamus and raphé nuclei and sham lesions. The only combination that resulted in hyperphagia was a sham lesion in the raphé and a lesion of the MH. The combination of raphé and MH lesions resulted in more weight gain than with only a raphé lesion, but it was not as great as in the group with only MH lesions. Altered sucrose and quinine acceptance was seen in the MH lesioned group and was independent of the raphé lesioning. The MH lesioned group also had an increased weight gain on high fat diets, and this effect was independent of raphé lesioning. It appeared that although there was an interaction of effects on weight regulation, the acceptance behaviors were dependent on MH lesioning.

Extracellular recording of the firing of neurons in the midbrain raphé nucleus of the rat indicated that systemic and local injections of TRP and 5-HTP diminished the firing rate (Gallager & Aghajanian, 1976). These effects were inhibited by agents which prevent 5-HT synthesis. From these results, Gallager and Aghajanian concluded that the enzymatic conversion of TRP and 5-HTP to 5-HT

diminishes raphé firing.

Peripheral metabolism of tryptophan

The large neutral amino acids are TRP, TYR, PHE, LEU, ILE, and VAL. The first three are considered to be aromatic amino acids, and the second three are branched chain amino acids (BCAA). It is helpful to understand at this point how TRP differs from other amino acids and how factors influencing its availability to the brain might make it the logical choice for a molecule that is central in a food intake regulating mechanism.

The peripheral metabolism of TRP has been reviewed by Young and Sourkes (1977) and is summarized below. After a meal there is an increased uptake of amino acids by the liver. Because TRP is the least available amino acid for protein synthesis it must have a regulatory effect on the protein synthesis in the liver. Increases in TRP in the liver result in increased ribosome aggregation, and dietary TRP deficiency results in depletion of free TRP stores with a marked fall in serum TRP. In TRP deficient diets there is also a decrease in brain levels of TRP. When an animal is starved, however, the levels of TRP are raised because of protein catabolism. Thus, in starvation there is an increase in brain TRP. TRP is also used in the synthesis of products other than proteins. TRP metabolism via the kynurenine pathway is regulated at

the first step via factors that influence the liver enzyme, tryptophan pyrrolase. The activity of this enzyme is subject to substrate induction, and thus excessive intake of TRP increases metabolism in the liver. This would protect the brain from high intakes of TRP. The pyrrolase activity is also induced by cortisone, and this could influence the brain levels of TRP during periods of stress. Although protein sythesis and the kynurenine pathway are probably the only routes of TRP metabolism with sufficient capacity to influence brain TRP levels, high doses of TRP may also be lowered peripherally by decarboxylation to tryptamine (Young & Sourkes, 1977).

TRP is unique among the amino acids in that a portion (74-89% in the rat, 83% in man) of it is carried bound to albumin in the plasma (Young & Sourkes, 1977). A controversy has existed over the availability of the plasma-bound TRP to the brain. As indicated below, because of the relatively higher affinity for TRP of the brain than of plasma albumin, the amount of TRP bound to albumin seems unimportant. Because the resolution of this controversy is fairly recent and not universally accepted, many studies concerned with TRP in the brain report plasma and serum values of TRP concentration in terms of free, bound, and total TRP (Madras et al., 1973; Knott & Curzon, 1972; Ashley & Curzon, 1981).

Dietary influence on availability of tryptophan to the brain

The transport of TRP into the brain appears to be influenced by dietary factors, and in this way the diet can affect the serotonergic activity of the brain. When Fernstrom and Wurtman (1971) injected male adult rats at 1200 h with a TRP dose that was less than one-twentieth of the normal daily dietary intake and decapitated the animals 10 or 60 minutes after injection, both plasma TRP and brain TRP were elevated. The concentrations were never higher than those occurring nocturnally as a result of the normal daily rhythms. The elevation of brain 5-HT 60 minutes after injection was, however, significant. Thus it seems that physiological changes in the TRP plasma levels which are less than those occurring in the diurnal rhythm can influence brain 5-HT levels.

The effects of a TRP deficient diet on plasma TRP and brain 5-HT were measured after two days, four days, and every four days thereafter during a 32 day experiment with female adult rats (Culley et al., 1962). After only four days on the deficient diet, there was a significant decrease in the concentration of plasma TRP and brain 5-HT. Further decreases were not seen until the 28th day when there was an abrupt depression of brain 5-HT. In all cases there was a highly significant correlation between

plasma TRP and brain 5-HT levels.

Another metabolite of TRP is the vitamin niacin. Zambotti, Carruba, Vicentini, and Mantegazza (1975) were concerned that the changes in brain 5-HT with TRP deficiency might be the result of a niacin deficiency because of inadequate TRP for synthesis of niacin. Because maize has low levels of both TRP and niacin, it was used as the basis of a diet fed to young adult rats for two to twenty days. As early as the fourth day there was a significant decrease in brain levels of TRP, 5-HT, and 5-HIAA. No effect was observed in the brain levels of catecholamines. With supplements of TRP, brain 5-HT returned to normal levels, but nicotinic acid (niacin) supplements had no effect. Thus Zambotti and his colleagues concluded that the availability of TRP rather than niacin was related to the levels of brain 5-HT.

Plasma albumin binds non-esterified fatty acids (NEFA) in addition to TRP. When Knott and Curzon (1972) deprived male adult rats of food for 24 hours, they found that total plasma TRP was not altered, but there was an increase in free plasma TRP and NEFA. Brain levels of TRP and 5-HIAA were increased, but there was no change in brain 5-HT levels. Curzon and Knott later (1974) experimented with various methods of altering plasma NEFA. They found that increased levels of NEFA increased the free portion but not the total plasma TRP. With this increased free

TRP there was also an increase in brain TRP and 5-HIAA but no consistent change in brain 5-HT. Decreases in plasma NEFA resulted in decreased free plasma TRP, which in one case was accompanied by an increase in total TRP. Changes in the brain were not consistent,and both positive and negative changes in brain TRP, 5-HT, and 5-HIAA were observed (Curzon & Knott, 1974).

When rats were placed on diets of skim milk, whole milk, and light cream, Fernstrom et al. (1975) observed that the plasma NEFA increased from the fasting levels in proportion to the increase in levels of dietary fat. Free plasma TRP also increased with increases in the dietary fat, but there were no significant changes in brain levels of TRP. Madras and his colleagues (1974) also fed different levels of fat to rats and observed increases in serum free TRP as dietary fat increased from 0 to 30%. Further increases in fat did not change serum free TRP. None of the fat-containing diets increased brain TRP, 5-HT, or 5-HIAA above the levels seen with the 0% fat diet.

After a series of experiments, Gessa and Tagliamonte (1974) concluded that the free serum TRP is the most important but not the only factor controlling the transport of TRP into the sites of the brain where 5-HT synthesis occurs. The results supporting their conclusion were:

1. Exogenous TRP elevated free and total serum TRP. There was a proportional increase in cerebral TRP

and 5-HT turnover which was time-related to the free but not the total serum TRP.

2. Administration of drugs which release TRP from serum binding protein resulted in increased cerebral TRP and 5-HT turnover.
3. Fasted rats had higher levels of serum free TRP, less total serum TRP, more brain TRP, and increased turnover of 5-HT when compared to fed rats.
4. Lithium and amphetamine administration resulted in increases in serum free TRP, brain TRP, and 5-HT turnover. And
5. Increases of brain TRP and 5-HT synthesis were observed with electroconvulsive shock or intracerebral injections of puromycin or cyclohexamide without increases of serum free TRP.

Fernstrom and Wurtman (1974) considered the effects of insulin on plasma TRP. It has been established that insulin raises the levels of plasma TRP in the rat but not in the human. They postulated that as insulin frees fatty acids from the albumin, more TRP is bound to it. This would allow more TRP to diffuse into the plasma from other tissues. The absence of this response in humans was attributed to a lower affinity of albumin for TRP.

One possible source of contradiction in these studies may have resulted from the incubation of serum and plasma

samples during preparation or during the dialysis to separate free TRP. Hutson, Knott, and Curzon (1976) observed that in vitro lipolysis occurred during the incubation of samples from rats being fed high fat diets. The samples had been taken two hours after the feeding started. Incubation of the plasma resulted in increases in NEFA and free TRP in the samples from fat-fed but not in the samples from control-fed animals. NEFA were also observed to be higher in serum than in plasma. The free TRP concentrations of unincubated plasma from fat-fed and control-fed rats were similar although the total TRP was lower in the plasma of the fat-fed rats. Brain TRP was similar in both groups.

An improvement in investigation techniques seems to have resolved the controversy of availability of bound and free plasma TRP to the brain. Pardridge (1979) conducted an in vivo study of the competition of rat albumin and the blood-brain barrier (BBB) for TRP. The innovation was the use of a competitive ligand binding assay. The apparent binding capacity of the BBB for TRP was determined to be 1.9 mM and was calculated from determinations of the concentration of albumin (1.4 mM), the dissociation constant of TRP binding to the albumin (K_c = .13 mM), and the affinity constant of the BBB tryptophan transport system (K_M = .19 mM). This high apparent binding capacity enables the capillary transport system to compete with the

albumin for TRP binding.

Support for the influence of total rather than free plasma TRP on brain TRP levels came from the results of an experiment by Madras et al. (1973). They found that carbohydrate consumption reduced levels of free TRP in the serum of rats and elevated brain TRP. After fasted animals had consumed one of two protein-free diets for two hours, serum and brain samples were taken. One diet contained carbohydrate and fat; the second contained no fat. Both diets caused an increase in serum total TRP, serum bound TRP, and brain TRP. Both also caused a decrease of serum free TRP. Animals given the diet with fat had less of a decrease in NEFA and serum free TRP than those on the fat-free diet. The serum free TRP levels was significantly higher in the animals receiving the fat diet, but brain TRP levels were not different.

A consideration of dietary effects must account for the insulin secretion which results from intake of carbohydarates. One of the effects of insulin is its facilitation of peripheral uptake of the BCAA (Munro, 1964). Difficulties arise when the only insulin effect considered is the release of NEFA from plasma albumin, but when competition for uptake by the BBB is considered, the studies involving carbohydrate intake and insulin administration can be explained more easily by competitive inhibition than by albumin binding of TRP.

Passage of the large neutral amino acids from the capillaries to the brain is facilitated by carrier molecules in the endothelial cells which line the brain capillaries. There is evidence that the same carrier is used for transport of all six amino acids. Therefore, each of the amino acids competes with the others for uptake into the brain (Wurtman, 1982).

Pardridge (1977) studied the kinetics of transport of TRP across the BBB in rats and found an equality of the K_i and K_M that indicates transport of TRP, PHE, LEU, methionine (MET), and VAL is at the same site and that the kinetics of competitive inhibition are followed. Pardridge further suggested that examination of the equation for the kinetics of transport of large neutral amino acids across the BBB indicates that competition does not have much effect until the substrate levels approach the K_M value. Also, at physiological plasma levels, competition probably does not occur at tissues other than the brain because the K_M is about ten times greater in the intestine, liver, kidney, and erythrocyte (Pardridge, 1977). This would mean that large single doses of TRP would affect peripheral metabolism more than the brain and probably would not drastically alter serotonin production. Pardridge's (1977) value for the K_{diss} of albumin binding of TRP was similar to the K_M(app) of BBB transport of TRP, and thus he concluded that a substantial fraction of the protein-bound TRP competes

for BBB transport. Uptake of radioactive amino acids, amines, and hexoses in the rat brain was studied by Oldendorf (1971). His results led to inclusion of PHE, LEU, TRY, ILE, MET, TRP, histidine (HIS), DA, and VAL in the competition for the same BBB carrier system. He found a low uptake by the brain of non-essential amino acids, DA, NE, epinephrine, 5-HT, and histamine injected into the carotid artery. Study of the rat BBB transport system by Oldendorf and Szabo (1976) with radioactive amino acids injected into the carotid artery determined that all the amino acids tested except for proline, alanine, and glycine could be assigned to only one BBB carrier system. The neutral carrier system transported PHE, LEU, TYR, ILE, MET,TRP, VAL, DA, cystine (CYS), HIS, threonine, glutamine, asparagine, and serine. These studies would indicate that the presence of other amino acids and amines in the plasma would inhibit TRP transport into the brain.

A test of the hypothesis that brain TRP, 5-HT, and 5-HIAA levels vary post-prandially as a function of the ratio of serum TRP to the competing amino acids was undertaken by Fernstrom, Faller, and Shabshelowitz (1975). Four diets were used. Diet 1 contained no amino acids. Diet 2 contained TYR, PHE, LEU, ILE, and VAL. Diet 3 contained 12 non-neutral amino acids. Diet 4 was like Diet 3 but TRP was added. The rats were fasted overnight and given one of these diets between 800 and 1000 h. They were then

killed two hours later. In general, the brain TRP, 5-HT, and 5-HIAA levels followed the changes in TRP/ΣNAA in the serum. Diet 1 did not raise serum TRP, but there was an increase over control levels for serum TRP/ΣNAA, brain TRP, 5-HT, and 5-HIAA. Diet 3 had the same effect as Diet 1. Diet 2 lowered serum TRP, serum TRP/ΣNAA, and brain TRP, 5-HT, and 5-HIAA. Diet 4 increased the serum TRP, but there was no change in the other parameters. Fernstrom and his colleagues concluded that these results confirmed the hypothesis that the changes in brain TRP and 5-hydroxyindoles of rats after feeding are parallel to the changes in the serum TRP/ΣNAA ratio and are not necessarily parallel to changes in the serum TRP alone.

Post-prandial effects of dietary amino acids were also studied by Fernstrom and Wurtman (1972). They presented food-deprived rats with a diet containing 18% casein or the equivalent as an amino acid mixture. The rats were allowed to feed for three hours. Two hours after the feeding, the plasma TRP showed an increase over that of the fasted controls, but there were no increases in levels of brain TRP or 5-hydroxyindoles. When the amino acids TYR, PHE, LEU, ILE, and VAL were omitted from the amino acid mixture, however, the increase of plasma TRP was accompanied by increases in brain TRP, 5-HT, and 5-HIAA. With all three diets, correlations of brain TRP, 5-HT, and 5-HIAA with plasma TRP/ΣNAA were stronger than

correlations of brain TRP, 5-HT, and 5-HIAA with plasma TRP alone (Fernstrom & Wurtman, 1972).

Colemenares, Wurtman, and Fernstrom (1975) measured the concentration of TRP, 5-HT, and 5-HIAA in the spinal cords and brains of fasted rats which were fed a carbohydrate and fat meal. Increased levels of all these parameters were seen two hours after the rats started eating. Fernstrom and Faller (1978) also observed changes in the brain levels of aromatic amino acids and BCAA two hours after fasted rats started eating. The rats in this experiment were fed either a carbohydrate and fat diet or a similar one with 18 or 40% protein. The carbohydrate-fat diet elicited increases in brain aromatic amino acids and decreases in the BCAA. The addition of protein to the diet resulted in similar changes of the amino acid levels, but the magnitudes of change were less. There was a correlation of the change of a brain amino acid concentration with the ratio of the serum concentration of that amino acid to the sum of the other neutral amino acids. There was no relationship of the brain and serum concentrations of the neutral amino acids alone. In some cases the changes in the brain and serum went in the opposite direction.

Crandall and Fernstrom (1980) found that the increased brain levels of 5-HT and 5-HIAA seen in normal

rats after carbohydrate ingestion were absent in diabetic rats and could be altered by injection with insulin. They also compared the effects of protein-containing meals in normal and diabetic rats. After these meals, the normal rats had a slight increase in serum TRP/ΣNAA but no change in brain TRP or 5-HT. Brain 5-HIAA was significantly reduced. The diabetic rats responded to the protein meal with a decrease in serum TRP/ΣNAA and brain TRP. There was no alteration of the brain levels of 5-HT or 5-HIAA.

To determine the contributions by meals to changes of plasma and brain TRP levels and of brain 5-HT levels, Li and Anderson (1982b) allowed young rats to freely select from two isocaloric diets which differed in protein concentration. Food was removed from the cages at the beginning of the light period, and the food cups were returned four or eight hours later. Meal consumption was monitored, and 20 minutes after termination of the first meal, the rats were killed. Blood and brain samples were analyzed for amino acids, 5-HT, and 5-HIAA. There were differences in results according to the protein source (gluten or casein). Gluten fed rats exhibited inverse significant relationships between the percentage of calories as protein of the final meal and plasma TRP/ΣNAA and brain TRP. Correlations were not significant between the percentage of calories as protein and brain concentrations of 5-HT and 5-HIAA. The casein fed rats, however, showed no

significant correlations between plasma TRP/ΣNAA and the percentage of calories as protein of the last meal. In contrast to the gluten fed rats, the casein fed rats had significant negative correlations between the percentage of calories as protein of the last meal and the sum of 5-HT and 5-HIAA concentrations in the brain (Li & Anderson, 1982b).

Influences of diet on the rat's brain levels of 5-HT; its precursor, TRP; and its metabolite, 5-HIAA, have been reviewed in this section. TRP administration at dietary levels has been seen to elevate plasma TRP and brain TRP and 5-HT. TRP deficient diets have been found to decrease plasma TRP and brain 5-HT, and this effect was shown to be unrelated to niacin availability. Alterations in the fat content of diets produces changes in the proportion of plasma and serum free TRP but does not result in consistent effects on brain levels of TRP and 5-hydroxyindoles. Insulin effects on brain levels of TRP, 5-HT, and 5-HIAA were seen to be more relevant to the alteration of competitor neutral amino acid levels than to changes in the plasma or serum free TRP concentration. Competition by the large neutral amino acids with TRP for transport across the BBB consistently explains the relationship of plasma or serum TRP and brain TRP, 5-HT, and 5-HIAA. The ratio of TRP/ΣNAA in the plasma or serum is increased by the insulin effects of a carbohydrate diet and decreased by diets

containing the neutral amino acids. Changes in brain concentrations of TRP, 5-HT, and 5-HIAA have been found to parallel changes in the plasma or serum TRP/ΣNAA. With evidence that diet can alter brain serotonin metabolism, the next step in determining whether serotonin is central to a macronutrient regulatory mechanism is to establish the effects of serotonin on food intake.

Feed-back mechanism for regulation of protein or carbohydrate intake

Reduction of total food intake has been observed in rats after administration of TRP (Latham & Blundell, 1979), PCPA (Panksepp & Nance, 1974), 5-HT (Pollock & Rowland, 1981), a combination of ORG 6582 (an inhibitor of 5-HT uptake) and 5-HTP (Sugrue et al., 1978), 5-HTP (Joyce & Mrosovsky, 1964), fluoxetine (Weinberger & Mandell, 1978), and chlorimipramine which arrests raphé cell discharge and increases tryptophan hydroxylase activity (Weinberger & Mandell, 1978).

When rats are given a choice between diets differing in macronutrient content, the resulting changes in 5-HT metabolism can be compared with the intake of specific nutrients as well as total caloric intake. Interest in the protein intake has resulted in several studies in which rats were offered choices between different levels of dietary protein (Peters et al., 1981; Ashley & Anderson,

1977b; Wurtman & Wurtman, 1977a,b, 1979a,b; Hirsch & Wurtman, 1981). In these studies the alteration of protein in the diet also involved an isocaloric change in the carbohydrate content. Investigators have tended to approach the question of macronutrient appetite as either the investigation of protein appetite or the investigation of carbohydrate appetite. The research orientation toward either protein or carbohydrate appetite is frequently the only difference between studies of protein intake and studies of carbohydrate intake.

Protein consumption

Rats which were allowed access to a choice of diets during 12 hours of darkness showed no change in protein or carbohydrate consumption after injections of TRP 30 minutes before the feeding period (Peters et al., 1981). Plasma and brain TRP were observed to rise 4-5 fold, and whole brain 5-HT and 5-HIAA were both elevated. The results of Peters and his colleagues were unusual because other studies have found changes in food choice to occur with alteration of 5-HT metabolic parameters.

Administration of PCPA or 5,7-dihydroxytryptamine (5,7-DHT) to weanling rats resulted in a 30% lower protein consumption than that by controls over a two-week period. Calorie intake was not affected (Ashley & Anderson, 1977b). Wurtman and Wurtman (1979a) trained weanling rats to

consume all their food during the dark cycle. After injections with fenfluramine or MK-212 (a drug which enhances serotonergic transmission) the rats were presented with various dietary choices. Following an injection of MK-212, the animals had a diminished total food intake from dietary choices differing in levels of both protein and carbohydrate; however, because their protein intake remained steady and at control levels, the percentage of calories as protein increased and total carbohydrate intake was reduced. With the available dietary choices varying only in carbohydrate content, and after receiving injections of fenfluramine or MK-212, the animals had a steady intake of protein with a decreased intake of calories and carbohydrates. After fenfluramine injections and dietary options varying only in protein content, the protein intake again remained steady, and the caloric intake was reduced (Wurtman & Wurman, 1979a).

Wurtman and Wurtman (1977b) presented rats with a choice of protein levels after injection with fenfluramine, fluoxetine, or D-amphetamine. Anorectic doses of fenfluramine did not decrease the total protein intake but increased the percentage of calories as protein. Subanorectic doses of fenfluramine increased the proportion of calories consumed as protein when the animals were given a prior treatment with TRP. Fluoxetine decreased calorie, but not protein, intake, and this resulted in an increased

proportion of protein. D-amphetamine reduced protein and calorie consumption proportionately.

These studies (Ashley & Anderson, 1977b; Wurtman & Wurtman, 1977b, 1979a) consistently show that agents which decrease brain 5-HT alter food consumption by decreasing the percentage of calories as protein, and agents which increase brain 5-HT result in an increased percentage of calories as protein.

The timing of food intake measurements has been found to be important. When male rats were given a choice between 5 and 45% casein diets, Wurtman and Wurtman (1977a) observed differences in protein consumption with time during the eight-hour feeding period. The protein consumption was 25% of the calories during the first hour, but this increased to an average of 34% during the following seven hours. After injection of sub-anorectic doses of fenfluramine, the consumption of protein increased to 48% between the second and fourth hours of feeding. Anorectic and lower doses of fluoxetine resulted in a protein intake of 40% during the first three hours. Injection of amphetamine or TRP did not affect the protein consumption.

To determine whether protein and energy intake were affected by the same mechanism, male weanling rats which were allowed an ad lib. choice between 15 and 55% casein diets were given treatments to reduce brain 5-HT content before observation of protein and calorie consumption

(Ashley et al., 1979). The 5-HT reduction treatments were systemic injections of PCPA, central injections of 5,7-DHT, or mid-brain raphé lesions. Treatment with PCPA or 5,7-DHT resulted in no effect on the weight gain or energy intake. Protein intake (total and %) was decreased as were the brain levels of 5-HT and 5-HIAA when the animals were killed between 900 and 1100 h (i.e., after they had eaten all night). 5,7-DHT was less effective than PCPA, and during the last five days of the 14 day experiment, the percentage of calories as protein was the same as that of the controls. When a group of animals reached a weight of 140 g, they were given raphé lesions or sham operations, and food intake was observed for two weeks. The lesions did not affect weight gain or caloric intake, but the total protein and percentage of calories as protein decreased with the greatest decrease occurring in the first two days. Another group of rats was given injections of 6-hydroxydopamine to moderately deplete the brain catecholamines. Monitoring of food selection for the following 14 days indicated there was no effect on the weight gain or protein intake, but energy consumption decreased, and brain levels of NE were diminished. The authors suggested that these experiments supported the hypothesis that energy and protein intake are under separate control mechanisms. It appears that protein intake involves the central nervous system and 5-HT. Ashley et al. (1979) also proposed that the

absence of an effect on caloric intake after administration of 5,7-DHT and PCPA may have occurred because the rats being studied were weanling rather than adult rats. Other investigators had found diminished calorie intakes by adult rats with these agents. Ashley et al. (1979) further observed that a decreased protein intake was not expected to occur with the decrease in brain 5-HT. They explained the results with daily variations in brain 5-HT levels, but their results, although not what they expected, agree with those of other studies in which percentage of calories as protein increased with increased brain 5-HT (Wurtman & Wurtman, 1977a,b, 1979a).

Carbohydrate consumption

Wurtman (1982) proposed a mechanism to explain the effect of food intake on brain serotonin synthesis which in turn would affect carbohydrate consumption. The mechanism is an alteration of that seen in Fig. II-1, and is shown here in Fig. II-3 (p.53).

Young male rats were trained by Hirsch and Wurtman (1981) to consume their daily food intake during the first eight hours of darkness. Their diet was presented in two dishes containing either 5 or 45% casein. Injections of drugs or saline were given i.p. 30 minutes before feeding. The drugs tested were D-, L-, and D,L-fenfluramine and D- and L-norfenfluramine. Food intake was measured 1, 3½, and

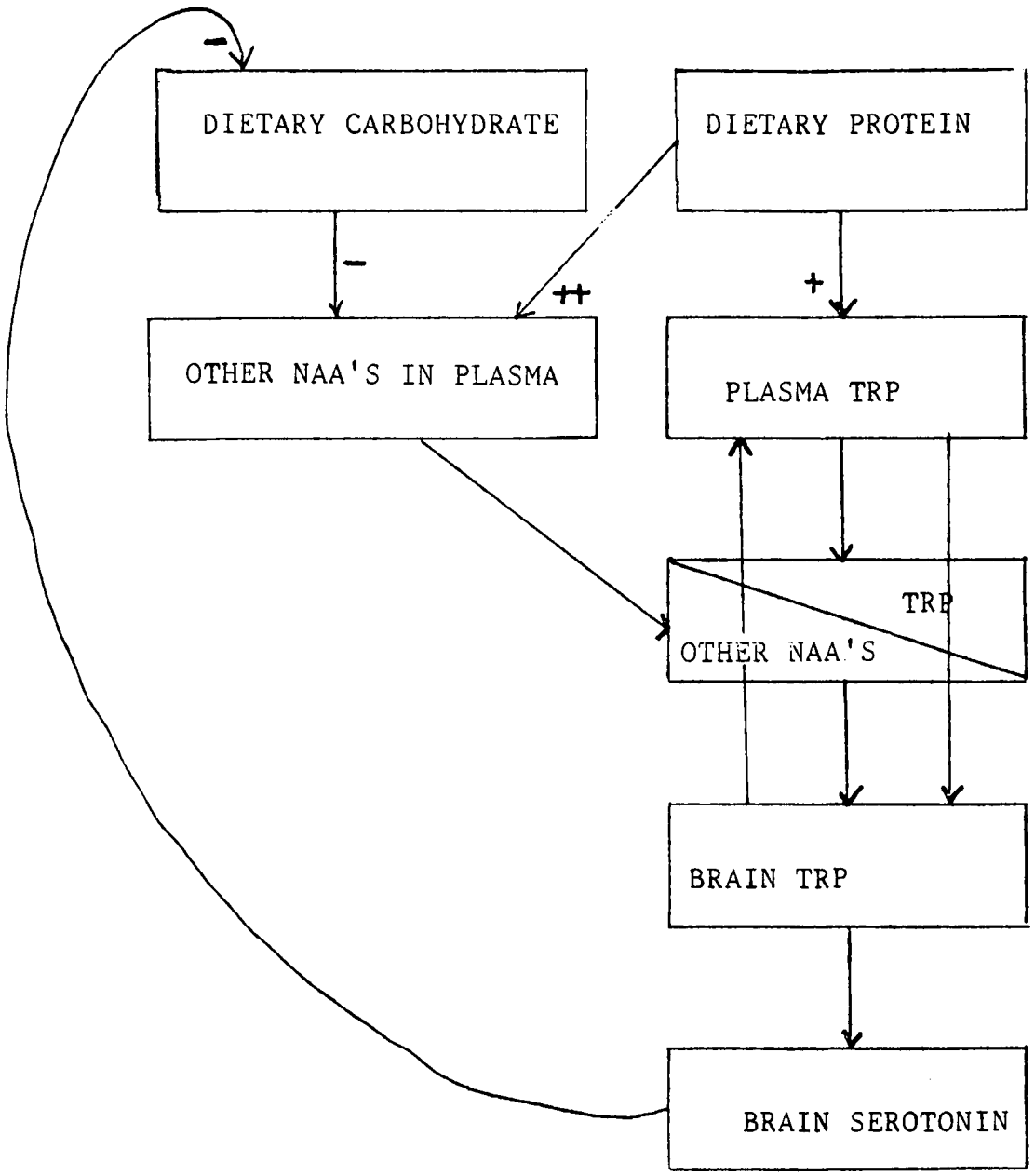


Figure II-3. The effect of food consumption on brain serotonin synthesis. (Wurtman, 1982)

8 hours after the feeding period started. Only the measurements at one hour showed any intake differences. At one hour, but not thereafter, a low (1.25 mg/kg) dose of D-fenfluramine suppressed consumption of the 5% protein food without affecting consumption of the 45% diet. Carbohydrate intake was reduced by 24%, and the protein intake was reduced 10%. D-norfenfluramine had a greater effect on caloric intake, but no specificity for the dietary components was seen. Hirsch and Wurtman observed that D- and D,L-fenfluramine suppressed elective carbohydrate consumption at doses which were barely anorectic. With anorectic doses, the selective modification of food choice no longer occurred. D-norfenfluramine was a more powerful anorectic, resulting in reduced consumption of both the low and high protein diets. The authors concluded that their results supported the hypothesis that 5-HT releasing neurons are involved in elective carbohydrate consumption.

The above studies provide support for a serotonergic mechanism which regulates either the protein or the carbohydrate consumption separately from the caloric intake of the laboratory rat. When manipulations which increase brain 5-HT reduce caloric intake, the protein intake remains steady and the carbohydrate intake decreases. The result is a higher percentage of calories as protein. With treatments which reduce brain 5-HT activity but do not affect caloric intake, the protein intake increases. Intake

of carbohydrate in each case changes in the direction opposite to that of the protein intake change (Table II-1, p.56).

Tryptophan metabolism in the human brain

Because of ethical considerations, there is essentially no direct knowledge of TRP metabolism in the central nervous system of humans. Faulty TRP metabolism has been implicated in depression, and clinical treatments of this disease have given some indirect knowledge of TRP and 5-HT metabolism in the brain.

Oral administration of TRP has been used to treat depression. In a review of the clinical use of TRP, Young and Sourkes (1977) suggested that there is weak evidence for the antidepressive effect of TRP. Overactivity of the adrenal cortex in depression and the TRP load could both contribute to stimulation of pyrrolase activity and thus treatment with lower doses may be more effective than treatment with higher doses of TRP.

Chouianard, Young, Annable, and Sourkes (1979) reported that patients given oral doses of TRP had the greatest improvement of depression when the increases in free plasma TRP were also high. Treatment with a combination of TRP and imipramine, however, had the opposite result. The patients in this treatment group with the lowest increases in plasma free TRP showed the most improvement of depression symptoms. Three factors were found to be

Table II-1. Effects of drugs influencing central serotonergic action on calorie, protein, and carbohydrate consumption by the rat.

Investigators	Drug	5-HT action	Changes in consumption				
			Calories	Protein	%	CHO*	%
Wurtman and Wurtman (1977b)	Fenfluramine (2.5 mg/kg)	potentiates	none	+	+	-	-
	Fluoxetine (10 mg/kg)	potentiates	-		+	-	
	(lower dose)	potentiates	none	+	+	-	-
Wurtman and Wurtman (1979a)	MK-212 (1.5 or 3.0 mg/kg)	potentiates	-	none	+	-	
	Fenfluramine (2.5 mg/kg)	potentiates	-	-		-	-
			-	none		-	
	MK-212 (1.5 mg/kg)	potentiates	-	-		-	-
Wurtman and Wurtman (1977b)	Fluoxetine (5 or 10 mg/kg)	potentiates	-	none	+	-	-
	Fenfluramine (2.5 mg/kg)	potentiates	-		+		-
	(5.0 mg/kg)		-	±	+		-
Ashley et al. (1979)	PCPA	decreases	none	-	-	+	+
	5,7-DHT	decreases	none	-	-	+	+
	raphé lesions	decreases	none	-	-	+	+

* indicates carbohydrate

important in the anti-depressant action of oral TRP. The first was the existence of a therapeutic "window" in that too much or too little TRP resulted in no improvement. The second factor was the patient's initial plasma TRP level. Those with high levels showed more improvement. And third, the responses of bipolar depressives occurred at higher levels of TRP than those of unipolar depressives.

Bourne et al. (1968) measured hindbrains of 23 victims of suicide and 28 victims of other types of death for 5-HT, NE, and 5-HIAA. The 5-HT and NE levels were similar in both groups, but the 5-HIAA levels were lower in the suicide victims. Suicide victims who had symptoms of primary depression did not have different brain parameters from the others.

Møller, Kirk, and Fremming (1976) found that manic-depressive patients whose clinical symptoms were similar could be divided into two groups biochemically. When the plasma TRP/ILE+LEU+VAL was lower than control normals, the response to TRP treatment was an improvement of depression. Those resistant to TRP treatment had ratios in the normal range. Treatment effects were not related to plasma free or total TRP which were found to be in the normal range. Although no correlation between plasma TRP and kynurenine was seen in the controls, a significant positive correlation was found with manic-depressive patients. This study

indicates that although altered TRP metabolism occurs with depression, treatment with TRP is only effective when the entry into the brain can be improved by raising the plasma ratio of TRP to the competing amino acids. Unfortunately, Møller et al. did not report the ratios after treatment.

Autopsy of four phenylketonuria (PKU) and four non-PKU retardates revealed some differences in brain parameters resulting from the high PHE levels in the plasma of PKU subjects (McKean, 1972). The PKU brain stem had less 5-HT and NE, but DA levels were similar. TRP and TYR levels were lower in PKU cortical tissue but higher in the CSF. McKean also measured the rates of 5-HIAA and homovanillic acid (HVA) accumulation in CSF after probenecid administration. As the PKU patients were treated and the levels of PHE in the plasma dropped, an increase in CSF 5-HIAA was observed.

Indirect evidence indicates that the 5-HT content of the brain changes during the menstrual cycle. Cox and Calame (1977) studied fasting plasma amino acid levels in one subject over four cycles. Significant changes in levels occurred during the month. The ΣNAA rose after menses to a high of 517 nmoles/ml at the second week and declined to a low of 417 nmoles during the week prior to menses. Plasma TRP and post-prandial levels were not studied. No studies were found of TRP metabolism during the menstrual cycle. All 18 of the plasma amino acids studied by Cox

and Calame (1977) were at their nadir in the week prior to menstruation, and it might be expected that TRP levels would also decline.

Factors other than availability of plasma TRP to the brain may alter brain 5-HT during the menstrual cycle. A compilation of studies indicating increased levels of brain 5-HT levels prior to ovulation and decreases prior to menses was presented in Medical Hypotheses (Warren, Tedford, & Flynn, 1979). Decreased MAO plasma levels after administration of estrogen and increases in MAO after progesterone could indicate parallel brain level changes which would result in high levels of brain 5-HT and catecholamines at ovulation and lowered levels at the onset of menstruation. Behavioral variability that supports these postulated changes in brain 5-HT levels are cyclic alterations in body temperature, depression, distress symptoms, elation, irritability, motor activity, pain sensitivity, sleep and rest needs, and sexual desire. A more recent study supports the evidence for reduced brain 5-HT levels prior to menstruation. Abramowitz, Baker, and Fleischer (1982) reported increased frequency of admissions for psychiatric acute care for patients suffering from depression but not from schizophrenia on the day before and the first day of menses.

Evidence of influence on human appetite by biochemical parameters from the rat appetite model

Although the studies in humans are not as extensive as in rats, there are some reports from studies with human subjects of alterations of appetite and the biochemical indicators of the serotonergic mechanism that has been implicated in the macronutrient intake regulation by the rat.

Shoulson and Chase (1975) administered fenfluramine therapy to seven non-obese human adults who were hospitalized for neurological treatment. Fenfluramine was alternated with placebo treatment. The patients rated their appetite before meals. They were fed the standard hospital diet ad lib. Although the appetite scores did not change, Shoulson and Chase observed decreased caloric intake and a reduction of body weight with the fenfluramine treatment. CSF samples indicated a 53-93% decrease in 5-HIAA after eight days of fenfluramine treatment. CSF samples indicated no change in HVA, a DA product, after treatment. These results agreed with a previous study (Chase & Shoulson, 1975) in which they found no differences in appetite ratings but decreased calorie intake and weight loss with fenfluramine treatment. The authors suggested that the weight loss in these non-obese subjects indicated that the effects of fenfluramine were not limited to obese patients.

Underweight adults were subjects of a double-blind

study of the effects of cyproheptadine hydrochloride, a histamine and 5-HT antagonist. When Noble (1969) compared the drug effects with those of the placebo, he found that the cyproheptadine treatment resulted in increased appetites and weight.

Anderson and Blendis (1981) reported results of an experiment with 11 healthy men as subjects in which percentage of breakfast calories as protein and plasma parameters were measured. Analysis of plasma samples two and four hours after the freely selected breakfast showed an inverse correlation of TRP/ΣNAA with the percentage of breakfast calories as protein. In an abstract (Anderson et al., 1979) which reported results from what appears to be the same study, measures of the percentage of lunch calories as protein were also reported. The lunch was freely selected on the same day as the breakfast. A negative correlation was seen between the percentage of breakfast calories as protein and the percentage of lunch calories as protein. The percentage of lunch calories as protein did not correlate with the TRP/ΣNAA, but negative correlations were seen between the plasma insulin levels and percentage of lunch calories as protein as well as between TRP/ΣNAA and insulin at four hours after the breakfast. The insulin level at four hours correlated positively with the percentage of breakfast calories as protein. Neither study revealed any significant correlations for calorie, fat, or carbohydrate intakes.

Paykel et al. (1973) reported that treatment with amitriptyline, a tricyclic antidepressant, resulted in weight gain and a craving for carbohydrates in all of 51 female patients studied. The association was not accompanied by hypoglycemic responses or reported faintness or dizziness. (The study did not report the definition of carbohydrates, but the illustrations indicate that patients were reporting cravings for sweet foods.) Eleven adult subjects with a demonstrated habit of consuming carbohydrate snacks were given TRP (2g), D,L-fenfluramine (20 mg), or placebos an hour before their usual snacking period (Wurtman & Wurtman, 1981). Snacks which had a predetermined ratio of carbohydrate to protein were allowed. Snack foods were determined from food records and consultation with the subjects. These foods were usually sweet (cookies, seven subjects; candy, one subject; cake, one subject; and crackers, two subjects). Snacks and meals were recorded for the four hours following treatment. Wurtman and Wurtman found that fenfluramine reduced carbohydrate snacking in seven subjects and decreased it in the group as a whole. TRP treatment decreased carbohydrate snacking in four subjects but not in the group as a whole. Fenfluramine decreased the meal-time carbohydrate intake in the six subjects for whom there were sufficient data on meals for analysis. Both TRP and fenfluramine slightly increased the protein consumption.

In a further study, Wurtman, Wurtman, Growdon, Henry,

Lipscomb, and Zeisel (1981) recruited subjects who were obese and reported cravings for carbohydrate snacks. The subjects were given three fixed meals with a total of 1200 or 950 kcal daily and were allowed to choose among protein-rich and carbohydrate-rich snack foods from a vending machine. For two weeks, they received either no treatment or a placebo. For the next two weeks, they received placebo, D,L-fenfluramine, or L-TRP. Fenfluramine administration significantly reduced the carbohydrate snacking in six of nine subjects and in the group as a whole. TRP decreased carbohydrate snacking in three of eight subjects and increased it in one. There was no significant effect by TRP treatment on the group as a whole. The placebo did not affect carbohydrate intake in any of the seven subjects. No treatment significantly changed the temporal pattern of snack consumption, and protein snacks could not be assessed because the consumption of these was too low in the baseline situation. The amount of sweet carbohydrate snacking was not reported.

Wurtman, Wurtman, Growdon, Henry, Lipscomb, and Zeisel (1981) suggested that in obese people the reduction of carbohydrate snacks with treatments of fenfluramine and TRP may indicate a disturbance in the mechanism coupling carbohydrate consumption to increases in brain 5-HT synthesis. The relative rejection of protein snacks and frequent consumption of carbohydrate snacks by their subjects indicated

that some obese people do not simply overeat any food but select particular foods for particular appetites. They suggested that treatment of obesity should include an evaluation of food intake patterns before therapy and an analysis of the types of macronutrients ingested. The times when overeating is likely to occur should also be assessed. If drugs are administered only when overeating is likely to occur, this may delay the development of drug tolerance.

The possibility of decreasing brain 5-HT levels during the post-ovulatory phase of the menstrual cycle (Cox & Calame, 1977) with the resulting nadir just prior to the onset of menstruation has some parallels in studies of eating behavior. An association between depression and cravings for carbohydrates was reported from a survey of 300 nurses by Smith and Sauder (1969). Specific associations were found between the occurrence of cravings for food and/or sweets and premenstrual feelings of tension or depression, between craving for food or sweets at specific times of menstrual periods or depression and the occurrence of premenstrual fluid retention, and the association between the desire to eat compulsively and the tendency to be depressed. This survey concentrated on sweet or spicy food rather than on macronutrients.

Dietary intakes of eight women were compared to their menstrual cycles for 60 days (Dalvit, 1981). Post-ovulation calorie intake was significantly higher than

pre-ovulatory intake. No further analysis of the intakes was reported.

Perhaps the most interesting aspect of menstrual cycle effects on eating behavior is the absence of any studies on carbohydrate intake throughout the menstrual cycle and differences that might exist between women taking oral contraceptives and those who are not.

Dietary influence on human plasma neutral amino acids and central nervous system 5-hydroxyindoles

Knowledge of the dietary influence on human brain metabolism is sparse. Because of the availability of plasma from humans, most of the comparisons of human biochemical parameters and those developed in the rat model for macronutrient regulation are based on the similarities in plasma responses to diet.

Plasma neutral amino acids

Periodicity of amino acids in human blood has been observed by several investigators. Feigin, Klainer, and Beisel (1968) found that the total whole blood amino acids were at maximum levels between 1200 and 2000 h and were at minimum levels between 400 and 800 h. Alterations in dietary protein did not affect the periodicity although a large amount of protein ingested at 800 h increased the

maximum values. The same load given at 2000 h did not diminish the decrease in value between 2000 and 400 h. Physical exercise (an hour of calisthenics and basketball) did not change the amino acid concentrations, and the rhymicity of blood amino acids was not related to body temperature, urine volume, or excretion of sodium or potassium. A twelve-hour shift in the wake-sleep cycle, however, resulted in a rapid (within 48 hours) reversal of periodicity.

Observations of circadian periodicity in whole blood and serum amino acids of 20-23 year-old males by Feigin et al. (1967) also indicated the maximum total level of amino acids occurred between 1200 and 2000 h with the minimum at 400 h. They found that although the concentrations varied from individual to individual, the periodicity patterns were similar. Individuals showed daily variation in concentrations but daily similarities of periodicity patterns.

Milsom et al. (1979) investigated the factors affecting normal adults' plasma amino acid levels in fasting and post-prandial conditions. They found that two hours after a "standard" breakfast there was a high individual variation with no differences because of sex or age. After a 12-hour fast no correlations were observed with age. Sex differences were evident, however. Women had lower levels of THR, ½CYS, VAL, LEU, and ILE than men. Men had lower values of MET, ILE, and PHE in the fasting state than in the non-fasting state, and women had lower values of eleven

amino acids in the fasting state than in the non-fasting state. (TRP values were not measured.) Women's values were not different with oral contraceptives or after menopause. Diurnal variations were observed in male subjects who consumed diets with three levels of protein. With diets reflecting the norm for British populations (1 g protein/kg body weight) and lower (0.7 g/kg body weight) little diurnal variation was observed with the exception of slight increases 2-4 hours after meals. Increasing the protein intake to 1.5 g/kg body weight increased the plasma concentrations after meals and these peaks were observed within 90 minutes of the meals. These differences in response to the higher protein intake were because of increases in plasma values of ½CYS, VAL, proline, ILE, LEU, and MET (Milsom et al., 1979).

Oepen and Oepen (1965) found most amino acids to be lower in women than in men with significantly lower fasting levels of VAL, ILE, and LEU. The fasting levels of women were, however, higher for TAU, CYS, and ½CYS. Armstrong and Stave (1973) observed that fasting levels of plasma amino acids increased with age in children but more in boys than in girls. Armstrong and Stave also observed that the concentrations of CYS and ALA increase throughout life. Growing boys have an increase in the amounts of VAL, ILE, LEU, and PHE with age, but these changes are not seen in girls or adults. TRP levels were not found to be age related.

Møller et al. (1976) reported a significant negative correlation between age and TRP/ΣNAA for subjects aged 25-83 years old. Most of these subjects were women (23 of 25 subjects).

Daily rhythms of plasma amino acids of 18-25 year-old men were not changed by feeding them diets differing in protein level (Wurtman et al., 1968). The peaks of TRP concentration, however, were observed to change with different protein intakes. Two high peaks (at 1030 and 2100 h) occurred with a diet of 1.5 g protein/kg, and only one peak (at 1100 h) occurred on the low protein diet of 0.04 g protein/kg (Wurtman et al., 1968).

Human subjects fed isonitrogenous diets (21.5 g N/day) with different levels of fat and carbohydrate were found to have more urinary nitrogen and a higher post-absorptive concentration of plasma BCAA when fed the high fat-low carbohydrate diet (Swendseid et al., 1967). Comparison of pooled serum samples from young men after they had ingested isocaloric test meals with two levels of protein found no effects of the fat to carbohydrate ratios on amino acids at either level of protein (Yearick & Nadeau, 1967). All the amino acids were higher than fasting levels at 1.5 hours and were lower at 7 hours after eating. MET and TRP levels correlated with the meal content until the seventh hour, but there was no relationship with the other amino acids. The average increase of amino acids was found to be higher

after the high protein meals (Yearick & Nadeau, 1967).

The post-prandial effects of beef and non-protein meals were studied by Nasset and Ju (1969). After men consumed beef, the amino acid levels were maximal in the first or second hours although the levels of some individual amino acids increased up to the fourth hour. At the eighth hour about half the amino acids were below fasting levels. Nasset and Ju observed considerable intra- and interindividual variation. Interestingly, the pattern of the amino acids in the beef was not reflected in the amino acid patterns in the plasma. Palmer, Rossiter, Levin, and Oberholzer (1973) found the sum of plasma amino acids two hours after a meat meal increased to 1.5 times the fasting level.

The responses of amino acid levels to starvation were studied by Adibi and Drash (1970). After one day of starvation, increases in the levels of BCAA and growth hormone were apparent. Decreases were observed in ALA and insulin values. By the second day, BCAA had increased 66-105%, and growth hormone levels were 15 times base values. Refeeding reversed these changes. Except for a slower rise in BCAA, the responses of obese subjects were the same as those of the non-obese.

The responses of plasma amino acids to insulin were studied during a glucose tolerance test in which normal subjects were compared to insulin-dependent diabetics (Zinneman et al., 1966). Two hours after ingestion of a 100 g

glucose load, the normals' values of VAL, MET, ILE, LEU, TYR, and PHE were decreased, and there was no change in the values of the diabetics. TRP levels were not measured. Fasting plasma amino acids were not different in diabetics and normals. Pozefsky, Felig, Tobin, Soeldner, and Cahill (1969) measured the amino acid balance across the forearm before and after two levels of insulin injection in post-absorptive subjects. Before any insulin was given it was noted that amino acids were released from the forearm after an overnight fast. Raising the forearm insulin from post-absorptive levels to high physiological levels stimulated muscle glucose uptake and blocked amino acid release. Consistent declines were seen for plasma levels of LEU, ILE, TYR, PHE, THR, GLY, and alpha-aminobutyric acid. Plasma TRP levels were unchanged by insulin.

Snyderman, Holt, Norton, and Roitman (1968) studied the relationships of plasma levels of amino acids in humans with loading or withdrawal of single amino acids. TRP was unique in that the withdrawal or loading was associated with fewer changes in the plasma levels of the other amino acids. Men who were fed diets with adequate or absent TRP by Young, Hussein, Murray, and Scrimshaw (1969) showed daily fluctuations of plasma TRP that were not dependent on the dietary TRP. When the men were fed six meals a day, the TRP peak observed on a four meal

regimen disappeared. In addition, Hussein, Young, Murray, and Scrimshaw (1971) found that a TRP-free diet did not alter the daily fluctuations of the plasma amino acids other than TRP, but the feeding of six meals rather than four reduced the daily fluctuations of the amino acids.

Fasting plasma amino acid levels were measured in subjects who consumed a wide range of protein in their normal diets. These subjects had recorded their food intake for the three days preceding sampling. Anderson and Blendis (1981) found the most significant correlation was a negative one between the plasma TRP/ΣNAA and the percentage of calories as protein. Energy consumption (kcal/kg body weight) was negatively correlated with plasma ratios of TYR to both ΣNAA and BCAA. A similar study by Anderson, Blendis, Shillabeer, and Krulewitz (1978) also found a negative correlation between the percentage of calories as protein and TRP/ΣNAA. Anderson and Blendis (1981) found a higher than normal TRP/ΣNAA in patients with hepatic encephalopathy who were on protein restricted diets. They concluded that the plasma TRP/ΣNAA is associated with long-term and immediate protein consumption, and the TYR/ΣNAA in the plasma is associated with the long-term energy intake in normal, healthy humans.

Ashley, Barclay, Chauffard, Moennoz, and Leathwood (1982) studied the effects of single meals on plasma amino acids one and two hours after the meals. The subjects had

standard breakfasts and lunches. They were fed dinners containing 500 kcalories and 35% fat. The high-carbohydrate dinner contained 1.6% protein, and the high-protein dinner contained 20% protein. The third type of dinner was the high-carbohydrate meal supplemented with 0.4% TRP. (All percentages refer to weight.) Fasting during the dinner hour resulted in no change in plasma amino acids. BCAA were not different one hour after the high-carbohydrate or the TRP-supplemented dinners, but they were lower in both conditions after the second hour. The

high-protein meal elevated the BCAA after one hour, and they remained elevated after the second hour. TYR and PHE changed in the same manner as the BCAA. Plasma TRP was increased at one and at two hours after the high-protein and the TRP-supplemented dinners and was decreased after the high-carbohydrate dinner. The plasma TRP/ΣNAA was not different after the high-carbohydrate or high-protein dinners, but it was doubled one hour after the TRP-supplemented meal, and the value was tripled at two hours. TYR/ΣNAA and PHE/ΣNAA were not altered by any of the meals.

Diurnal variations in plasma amino acids were studied in subjects fed diets containing 0, 75, or 150 g of egg protein daily. Fernstrom et al. (1979) found that at all times of day the values of the large neutral amino acids varied in direct proportion to the protein content of the diet. The ratios of TRP, TYR, and PHE to the sum of the other neutral amino acids decreased with increasing content of protein in the diet. The peak values of TRP/ΣNAA occurred at different times of day with dietary differences. With no protein in the diet, the peak occurred at 1500 h, with 75 g there was no peak, and with the protein levels at 150 g, the peak occurred at 700 h.

Indicators of brain metabolism

Eccleston et al. (1970) were interested in determining

whether changes in 5-HIAA concentrations in lumbar CSF would reflect changes in plasma and CSF tryptophan levels. Patients with neither gross brain damage nor psychiatric symptoms were given an oral dose (50 mg/kg body weight) of L-TRP in milk. Patients who served as controls were not given the TRP. Blood concentrations of TRP rose to the maximum value at one hour, and the concentration returned to normal after 12 hours. Sampling of CSF by lumbar puncture indicated that CSF tryptophan showed a rise at two hours with the maximum concentration occurring between six and twelve hours, with a return to normal by 18 hours. The concentration of CSF 5-HIAA did not rise until the fourth hour after ingestion. The maximum 5-HIAA concentration occurred between six and twelve hours after ingestion of milk and TRP, and concentrations were normal by 18 hours.

To determine whether the levels of plasma TRP were predictive of TRP metabolism in the human central nervous system, Curzon (1979) took samples of plasma and samples of CSF from the lumbar and ventricular regions of psychiatric patients who were undergoing stereotactic subcaudate tractotomy. There was a high correlation of the CSF tryptophan and 5-HIAA in the ventricular samples, but this was not found in the lumbar samples. In a comparison of plasma samples with CSF samples drawn an hour later, it was found that plasma free but not total TRP

correlated with the CSF concentrations of TRP and 5-HIAA in both the ventricular and lumbar regions. Competing amino acids were not measured in this study.

In another study of the relationships of plasma amino acids and central tryptophan and monoamines, an intravenous injection of saline or L-TRP was given to psychiatric patients before sampling of plasma, lumbar, and ventricular CSF and the frontal cortex (Gillman et al., 1981). The TRP injection resulted in plasma TRP values similar to those of patients being treated for depression or insomnia with oral TRP doses. A six-fold increase in the TRP content of the cerebral cortex and elevated values of CSF 5-HIAA followed the TRP administration. Plasma free TRP was found to be a better predictor of cortex TRP than plasma total TRP. The correlation of the plasma TRP/ΣNAA was less than that of free plasma TRP with the brain TRP and the ventricular CSF tryptophan. The authors suggested that the role of the TRP/ΣNAA ratio at physiological levels may have been over-emphasized; however, because the patients had been fasted for 14 hours, the effects of this TRP load may not have been comparable to dietary effects.

The use of psychiatric patients in these studies may not give a clear indication of TRP metabolism in healthy subjects. Alteration of peripheral TRP metabolism has been indicated in two of the studies (Chouianard et al.,

1979; Young & Sourkes, 1977) of patients with depressive illness. Plasma samples which were taken during a five hour glucose tolerance test were analyzed by Yaryura-Tobias, Chang, Neziroglu, and Bhagavan (1977). They found free TRP levels to be significantly lower in psychotics than in normals and neurotics. DeMyer et al. (1981) studied a group of depressed patients and normal controls. Although the diets of both groups were similar in calories and macronutrients, there was a significantly lower plasma TRP/ΣNAA in the depressed patients. The plasma values of this ratio were observed for five days in the patients and controls. As the Hamilton scores of the patients decreased (i.e., depression improved) the ratios increased. The patients who showed no improvement had no change in TRP/ΣNAA, and an unexplainable decrease was seen in controls during this period.

The studies in which plasma and brain TRP and CNS 5-HIAA have been measured indicate that the plasma levels of TRP can influence 5-HT metabolism in humans. The study by Eccleston et al. (1970) indicates that increases in 5-HT metabolism may not occur until four hours after oral administration of TRP. Curzon (1979) found a correlation between the plasma TRP and the concentration of TRP and 5-HIAA in samples taken an hour later from the brain. Similar results were found by Gillman et al. (1981) with injected doses of TRP. The relationship between plasma

TRP/ΣNAA and brain 5-HT metabolism which has been observed in rats seems to be contraindicated by the findings of Gillman and his colleagues (1981). Gillman et al. measured the plasma TRP/ΣNAA and found less correlation of brain parameters with this ratio than with the plasma free TRP. The metabolism of TRP in psychiatric patients may be abnormal, however, and this may be a poor group to use for study of TRP metabolism in the general population.

Diet and brain tryptophan metabolism

Only one study was found in which the effects of diet on human central nervous system TRP metabolites were determined. Perez-Cruet, Chase, and Murphy (1974) studied subjects with Parkinson's disease, Huntington's chorea, or related neurological disorders. The post-prandial effects on plasma and CSF parameters were measured four hours after the subjects were fed a lunch of 545 kcalories (25 g protein, 25 g fat, and 55g carbohydrate). The samples were compared to those of subjects who remained in a fasting state. The post-prandial plasma samples had higher values of total and bound TRP and lower values of free TRP. The post-prandial CSF samples were lower in TRP and 5-HIAA. A negative correlation was seen between total or bound plasma TRP and both CSF tryptophan and 5-HIAA. There was a significant positive correlation between the plasma free TRP/ΣNAA and CSF

tryptophan or 5-HIAA, but this was not observed with the plasma total TRP/ΣNAA.

There is an abundance of studies in rats which indicate the presence of a macronutrient regulating mechanism that involves serotonergic pathways. The literature contains few human studies with healthy subjects in normal dietary situations. Ethical limitations prohibit use of many of the invasive procedures and manipulations in healthy subjects. What studies exist seem to support the existence in humans of the mechanisms found in the rat. One method of inquiry which can help determine whether further investigation of the question is warranted is to "second guess" evolutionary pressures and to determine whether there is teleological evidence for some value in macronutrient regulation in the human.

Teleological support for macronutrient intake regulation

In an extensive review of the literature on the natural diet of primates, Gaulin and Konner (1977) discussed the natural selection forces on dietary choice. They proposed that natural selection can optimize feeding efficiency only if food choices have an effect on survival or reproductive success. Over time, the individuals which are able to convert environmental resources into offspring at the highest rate will contribute the largest

portion to the gene pools of future generations. Because reproductive success is affected by nutritional status, natural selection strongly favors high feeding efficiency. Gaulin and Konner pointed out that survival of the post-weanling individual has two major types of nutritional threat; poisoning and starvation, but short of death, nutritional factors reduce the individual's fitness by lowering fertility.

Gaulin and Konner (1977) advanced two cautions in considering evolutionary fitness. First, it must not be assumed that the variety of acceptable diets is associated with every individual or population, and second, it must not be presumed that a dietary niche has produced a genetic polymorphism in metabolism.

Protein requirements

It has been found that the protein needs within a species can vary. Growing chicks have shown strain differences for the amounts of amino acids needed (Munro, 1969). Different strains of rats have been found to differ in rates of liver protein synthesis (Rutman et al., 1949). Human metabolic disorders have indicated variation in amino acid needs (Munro, 1969). Mice have shown differences in the ability of different strains to adapt to high protein diets (Munro, 1969). However, fruit flies which are grown on low protein diets exhibit a rapid selection

of individuals adapted to the diet (Robertson, 1962). The effects are significant within only five generations.

Body size is an important factor in dietary requirements. Small animals have high metabolic requirements per-unit-weight but low overall requirements, and they are ideally suited to exploit nutrient-rich but dispersed food items whereas large animals can use low-quality but abundant food because of the high total but low per-unit-weight requirement (Bell, 1970). Thus Gaulin and Konner (1977) propose that body size and diet coevolve.

Munro (1969) has comprehensively reviewed the literature on protein metabolism and developed relationships based on body size of different mammalian species. Two effects which he found on protein metabolism are seen with changes in body size from species to species. The intensity of metabolism per unit body weight is higher in smaller animals, and the relative contribution of different body parts to metabolism will vary. For example, as the size of the animal increases, the weight of visceral organs decreases in the proportion of the body weight, but the muscle proportion remains constant. Further, most mammals have 40-50% of their body weight as muscle. The increasing size, however, means that muscle protein metabolism becomes more important. Responses to insulin are different, and carbohydrate deprivation will have a greater effect on plasma amino acids in larger animals because insulin is

then not facilitating uptake by the muscles (Munro, 1969).

Munro (1969) compared the fasting levels of free amino acids in the blood of mammals ranging in size from the mouse to the cow and found similar values in all species. The turnover rate, however, is higher in the smaller animals. Comparison of human and rat plasma free amino acids shows diurnal rhythms of the same amplitude and duration with adjustments for the light-dark cycle (see Fig. II-4, p. 81). He proposed that changes in

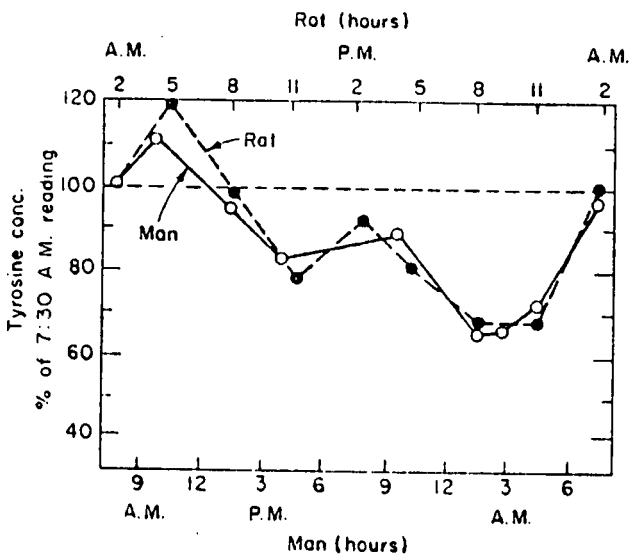


Figure II-4. Comparison of change in free tyrosine content of plasma of man and rat with time. Changes are indicated as a percentage of initial levels which are similar in man and in the rat. Note that the time scales are displaced. (Munro, 1969, p.170)

amino acid concentrations seem to be regulated by the sensitivity of the regulatory mechanisms rather than the

quantities of amino acids added to the pool (Munro, 1969).

In conclusion, Munro stated:

> As a group, mammals are an extension of their evolutionary past. Amino acid requirements and metabolism among the mammalia show no consistent features that distinquish them from other animals. On the other hand, species size has a profound effect on the intensity of metabolism in mammals, irrespective of the phylogenetic relationships of one mammal to another. ...would suggest some caution in transferring such information from a small to a large species. (1969, pp. 175-176)

Need for a mechanism for dietary selection

The feeding survival of animals depends on: (a) finding a sufficient quantity of food; (b) choosing a variety of foods to provide proper nutrients; and (c) avoiding toxic or harmful food (Overman, 1976). Overman's (1976) review brings together the literature on dietary selection and specific hungers, and he points out that there are two major theoretical approaches to food selection for survival. One viewpoint is that learning plays a part in selection, and the other viewpoint is that the behavioral ability to distinguish between foods has been selected for.

A mechanism to keep animals from eating only carbohydrates would ensure some protein intake, and some obese people may suffer from a disturbance of this feed-back mechanism (Wurtman, 1982). Amino acids are needed by the monogastric animal for the replacement of endogenous

losses, the synthesis of tissues which stop growing at the attainment of somatic maturity, and the synthesis of tissues which continue growing throughout life. The amino acids needed for each of these purposes will differ qualitatively and quantitatively (Mitchell, 1962).

A mechanism to prevent over-ingestion of protein would also be useful. Utilization of large amounts of amino acids for energy and the processing of the extra nitrogen are energetically expensive as these increase the work load of the liver and kidney (Harper & Peters, 1981).

Harper (1976) suggests that a system of food intake control based on amino acid need and intake in man would not result in overall body homeostasis. The growing organism would consume excessive energy to compensate for a diet too low in protein. He further indicates that it is difficult to devise a diet from natural foods that does not meet the protein requirement. Evolution may have used a high sensitivity to patterns for selection of appropriate foods, and this may be operating rather than sensitivity to protein content (Harper, 1976).

Although the needs for adequate protein intake are present throughout the life of man, provision of a variety of natural foods may ensure adequate intake. In addition, whereas carbohydrate ingestion to excess may be present in some obese humans, studies have not been published that indicate protein deficiencies occur in these people.

The natural human diet

Homo sapiens is characterized by the wide variety of niches it occupies (Gaulin & Konner, 1977). This variety has called for cultural evolution of a wide variety of feeding strategies. The underlying biological evolution has been of two types: (a) selection established or preserved the adaptability of the species; and (b) individual populations with long-time occupancy of subniches have had differential action of selection. Gaulin and Konner (1977) indicate that we have two methods of studying dietary shifts over evolutionary time. The first is the examination of fossilized fecal material or microwear patterns on fossil teeth. This has many disadvantages with studies of humans, so they suggest the best method with humans is the second, which is the method of comparison with contemporary organisms.

Gaulin and Konner (1977) examined the data of Whiting [Whiting, MG. (1958): A cross-cultural nutrition survey. Doctoral thesis, Harvard School of Public Health]. This study of contemporary pre-agricultural societies reveals

that humans are much more efficient food extractors than other primates, and the diet quality studies in non-industrial societies indicate that protein intakes are usually adequate. The occurrence of shortages in "primitive" economies indicates an evolutionary advantage to individuals who can effectively store surplus in times of plenty, and thus there is a basis for the evolution of obesity (Gaulin & Konner, 1977).

Pre-agricultural societies in the lower latitudes (where most of human evolution took place) subsist primarily on gathered wild plant food with important contributions of meat and fish (Gaulin & Konner, 1977). The availability of wild foods, even during droughts, indicates that gatherer-hunters are cushioned against severe shortages better than agricultural or pastoral peoples (Lee, 1968).

It is only recently, and still only in a few regions of the world, that a wide variety of foods has been available on a year-round basis (Pilgrim, 1967). Modern hunter-gatherers pursue subsistence strategies similar to those which were probably universal among human ancestors until 10,000 years ago (Gaulin & Konner, 1977). Gaulin and Konner propose that the human organism was evolving and adapting to these strategies and diet. They suggest that a natural diet would be what people ate during the hunting-gathering era which lasted for 99% of the

generations of our genus.

Gaulin and Konner (1977) observe that taste and smell can discriminate certain chemical variables and are ideally located at the site of ingestion to provide preliminary information on the nutritional content of food. The high palatability of sweet substances may be a result of the high caloric value of sweet foods, but Gaulin and Konner suggest that the current great availability of sweet foods, because of advances in processing sugar, may render this preference for sweets maladaptive. Ziegler (1969) found that the increase of height and weight of British men has correlated with an increase of per capita sugar consumption from 1930 to 1965. Evidence of increased pre- and post-natal growth in Canadian Eskimos has been observed (Schaefer, 1970). Schaefer noted that the Eskimos have also decreased their meat intake, whereas other affluent western societies have increased intake of both sugar and animal protein. Some of the increases in height of Eskimo girls would be the result of the decrease in the age of menarche by two years between 1938 and 1968, but because of the Eskimos' reduced glucose tolerance with lowered meat intake, Schaefer suggests that hypoglycemic episodes may place the Eskimos on the borderline of acromegalia from the hypoglycemic stimulation of growth hormone release.

Improvement in growth is frequently used as a

measure of improved nutrition. The case of the Eskimos (Schaefer, 1970), however, indicates that increased growth may be maladaptive because it would be accompanied in this case by lowered glucose tolerance. Schaefer has reported carbohydrate cravings in the Eskimos, and the indications are that it is primarily sweet foods which are craved. (1970).

Dietary selection by children

Because the presence of processed sugars in the diet may contribute to misuse of natural selection mechanisms and because food selection may be strongly influenced by learning, the adequacy of a freely selected diet by humans could be studied if processed sugars were absent and no learning about the diet had occurred. A study published in 1928 by Clara Davis is probably the only study on humans freely selecting a diet with the subjects relatively isolated from cultural clues. This unique experiment was performed with infants who had essentially received only breast milk before their admission to a pediatric ward for long-term care. The infants apparently were not admitted to the hospital because of illness but because they needed foster care by an institution. They were 7½ to 9 months old on admission.

The infants in the Davis (1928) study were offered a variety of wholesome foods at each meal, and although

nurses assisted in the feeding, there was no attempt to influence the children's choices. All the children liked most of the foods that were served, but at any one meal,

they did not usually eat considerable amounts of more than three solid foods. The amounts and kinds of foods selected could not be predicted, but it was observed that they all tended to select certain foods in waves, i.e., a certain food would be eaten in moderate amounts, the quantities would start to increase until very large amounts were eaten in a day, and then the amounts would taper off again.

Throughout the experiment, the infants' appetites were good except for periods of usual childhood illness. Gains in weight and height were above the national averages for that time, and the children were judged to be "smiling, happy, active and full of 'pep', slept soundly, and did not show signs of nervousness" (Davis, 1928).

Analysis of the data from Davis' study in terms of % protein and % carbohydrate indicated that the range of each is great. Subject Earl consumed % protein from 14.6 to 20.1, and % carbohydrate ranged from 27.1 to 43.6. Ranges for Donald were 12.9 to 17.0 for the % protein and 37.2 to 66.9 for the % carbohydrate. For Abraham in the first six months of the study (the period comparable to that for the other subjects), the % protein ranged from 16.0 to 20.4, and the % carbohydrate ranged from 26.0 to 37.4. The intakes changed in a wave-like manner with decreases in % protein occurring during the third month of study. Peaks of % carbohydrate occurred during the fourth or fifth month.

Factors affecting human food choice

A review of the literature on factors influencing human food choice is not intended at this point. A brief summary of these factors will be given, and some studies which suggest the dynamic nature of food choice will be presented.

Cultural factors which can influence food choice are taboos, fuel availability, and the available variety of foods (Pilgrim, 1967). Attitudes also influence food choice, and to the extent that past sensory experiences with food result in current attitudes, these attitudes may be considered as part of the sensory control of eating. But some part of the attitudes toward food are ideational in origin and have no sensory basis. An example of an ideational basis to attitudes would be the common unwillingness to try an unfamiliar food even though it is known that others eat it (Pilgrim, 1967).

Rolls (1981) indicated that few studies have been made of the relationship in adult man of food selection and palatability (the stimulus qualities of food which determine its acceptability). She suggested that the reasons for consuming a varied diet are not clear. The learning of the culture or of the nutritional value of a diet may account for the relationship of food selection and palatability, or there may be changes in the relative palatability of foods as eating proceeds. The changes in

palatability may be affected by learning as well as by sensory factors and the internal state.

Rolls (1981) asked subjects to rate eight food items for degree of "liking". The subjects were then given either cheese on crackers or sausages and asked to eat as much as they wanted. Two minutes after eating, the subjects were again asked to rate the eight foods. The rating for the food eaten declined more than the others. It was also observed that the amount of a particular food eaten declined when the food had been presented in the first course. These results indicated that the palatability of a food declined after it had been ingested.

Rolls (1981) conducted further studies on food choices in normal weight subjects. Subjects who were offered, in succession, sandwiches with four different fillings ate 1/3 more for lunch than when they were offered four successive plates of sandwiches with the same filling. In another study, subjects were offered three flavors of yogurt which differed in taste, texture, and appearance. The subjects ate more than when they were offered one flavor even if that flavor was their favorite. If the three flavors did not differ in appearance or texture, the amount consumed was the same as when only one flavor was presented.

Perhaps the evolution of a desire for some variety in the diet is sufficient for regulating intake when an adequate variety of foods is available in the diet. There is

also the possibility that the changes in palatability are related to serotonergic transmission and that a central feed-back mechanism ensures selection of a variety of foods.

Study of an appetite for specific foods or nutrients may be complicated by the "fleeting" nature of the appetite. If the palatability of a nutrient component of food changes as that food is ingested, measurement of biochemical and physiological responses to food ingestion should be made during the course of eating. "Cravings" for particular food substances may reflect an abnormality in physiological machanisms and may be useful in determining the changes in normal physiological responses that are part of human food choice.

Summary

Because it is easier to study the biochemical and physiological responses of lower animals, it is appropriate to develop a theoretical understanding of factors affecting food choice in other animals. The rat is a good choice of models because it is omniverous and has co-evolved with man, sharing the same food stuffs.

Evidence for a serotonergic mechanism affecting protein or carbohydrate appetite in the rat has been given in this chapter. Parallels in human physiological responses to food ingestion and appetite responses to

drug manipulation have been cited.

The need for a protein or carbohydrate appetite in humans has been discussed with evidence based on conjecture about evolutionary adaptation. Experimental evidence indicates that humans may have a system that ensures a variety in dietary intake, and the author's speculation is that a feed-back mechanism involving protein or carbohydrate appetite may be one aspect of that system.

It is important at this point to determine whether healthy, normal humans respond physiologically to protein and carbohydrate intake in a manner similar to the rat, and to determine whether these humans respond to manipulations of normal dietary components with appetites that provide a compensation for the dietary alterations.

This study was undertaken to examine the plasma neutral amino acid responses to breakfasts of different protein content and to investigate the relationship between these plasma responses and the consequent consumption of protein and carbohydrate in freely-selected meals by healthy, non-obese young men.

CHAPTER III

EFFECTS OF BREAKFASTS WITH LOW AND MODERATE PROTEIN CONTENT ON POST-PRANDIAL PLASMA AMINO ACIDS AND SUBSEQUENT PROTEIN AND CARBOHYDRATE INTAKE OF YOUNG MEN

As submitted to the American Journal of Clinical Nutrition, August, 1983.

Mitchell, Sandra J., M.S.*

Leklem, James E., Ph.D.

Department of Foods and Nutrition, Oregon State University, Corvallis, OR 97331

Support for this study was received from:

American Home Economics Association Foundation

Sigma Xi Society

Running head or foot line:

Effects of breakfast protein content

*Now with California Polytechnic State University, San Luis Obispo, CA 93407.

Abstract

Breakfasts differing in protein (18 g and 4 g) and carbohydrate (105 g and 127 g) were fed to 13 healthy young men. Measurements were made of freely selected food

intake during the day and the plasma neutral amino acids 2 h after breakfast and dinner and 4 h after breakfast (pre-lunch). Comparisons of measurements tested the theory that protein or carbohydrate intake is related to the ratio of plasma tryptophan and the sum of the neutral amino acids (TRP:NAA). Although plasma TRP:NAA was significantly different ($p<.01$) 2 and 4 h after the different breakfasts, lunch intake of calories, protein, and carbohydrate was not different. Daily total intakes did not differ with the exception of a lower mean intake of protein when the low protein breakfast was consumed. No support is given to the postulated feed-back mechanism involving breakfast protein content and calorie, protein, or carbohydrate consumption during the remainder of the day.

Key words

Amino acids; dietary proteins; food selection; tryptophan; appetite regulation.

Introduction

Regulation of protein intake as a percentage of calories (PE) has been observed in the rat (Musten, Peace, & Anderson, 1974). This PE was found to be significantly correlated with the plasma ratio of tryptophan (TRP) to

the sum of the other (leucine, isoleucine, valine, tyrosine, and phenylalanine) neutral amino acids (NAA) between 900 and 1100 h on the final day of the 4 wk feeding period (Ashley & Anderson, 1975a). Because this ratio (TRP:NAA) was found to effect the brain levels of TRP (Fernstrom & Faller, 1978; Wurtman & Fernstrom, 1975) and the subsequent synthesis of the neurotransmitter serotonin (5-HT) (Fernstrom, Faller, & Shabshelowitz, 1975; Fernstrom & Wurtman, 1972; Wurtman & Fernstrom, 1975), Anderson proposed a feed-back mechanism involving 5-HT that regulates the appetite of the rat for protein (1977). Recent studies (Li & Anderson, 1982a,b) have confirmed that a relationship exists in the rat between meal composition, brain 5-HT, and subsequent food selection.

In addition, when rats are presented with dietary choices differing in protein and carbohydrate composition, manipulations of serotonergic transmission with drugs have also indicated that 5-HT metabolism influences the ingestion of protein and carbohydrate by the rat when caloric intake is not altered (Ashley, Coscina, & Anderson, 1979; Wurtman & Wurtman, 1977a) and when caloric intake is diminished (Wurtman & Wurtman, 1977a,b, 1979a). Observations of the effects of such drugs on carbohydrate ingestion led to the proposal by Wurtman and Wurtman that the serotonergic mechanism is part of a feed-back loop which affects carbohydrate consumption (Wurtman & Wurtman, 1979a).

Few studies have been made of the existence of protein and carbohydrate intake regulation in normal, healthy humans. Careful monitoring of the freely selected diets of three infants by Davis (1928) produced data that could be analyzed to determine PE and percentage of calories ingested as carbohydrate (CE). The infants' PE and CE were not constant but varied from month to month in a wave-like fashion. Because changes in the growth rates may have influenced intakes, comparison to data from adults may not be relevant.

Perhaps the absence of adult human data related to normal PE or CE is the result of the few studies in which freely selected meals have been accurately measured. Freely selected snacks were measured in a group of obese subjects who reported cravings for carbohydrates (Wurtman, Wurtman, Growdon, Henry, Lipscomb, & Zeisel, 1981). In many of these subjects, treatment with drugs which potentiate serotonergic transmission reduced carbohydrate snacking below levels observed during placebo treatment.

Recent studies of human plasma amino acid responses to meals and meal components have reported the TRP:NAA (Anderson & Blendis, 1981; Ashley, Barclay, Chauffard, Moennoz, & Leathwood, 1982; Fernstrom, Wurtman, Hammarstrom-Wiklund, Rand, Munro, & Davidson, 1979). Daily diets of different levels of egg protein resulted in an inverse relationship of the TRP:NAA in plasma samples taken throughout the day

and the protein content of the diet (Fernstrom et al., 1979). In another study (Anderson & Blendis, 1981), plasma samples taken 2 and 4 h after freely selected breakfasts indicated that although the TRP:NAA did not differ from fasting levels, the ratios were significantly and inversely correlated with the PE at breakfast. Evening meals with 500 kcal which were either from carbohydrate, carbohydrate and 10% protein, or carbohydrate and 0.4% TRP resulted in changes in the TRP:NAA only when the TRP was added, and this change was an increase in the ratio (Ashley et al., 1982). Fasting plasma TRP:NAA was observed to have a significant negative correlation with the PE of the previous 3 days (Anderson & Blendis, 1981).

Change in plasma free TRP:NAA in humans has been observed to alter central 5-HT metabolism (Perez-Cruet, Chase, & Murphy, 1974), and drug treatment which alters 5-HT metabolism has been shown to alter human appetite (Noble, 1969; Wurtman, Wurtman, Growdon, Henry, Lipscomb, & Zeisel, 1981). It is possible that a feed-back regulatory mechanism similar to that of the rat also exists in humans. This could have many practical applications if alteration of the contents of one meal changed the selection of foods in subsequent meals.

The purpose of this study was to determine whether alteration in the amount of protein consumed at breakfast would affect the amount of protein or carbohydrate chosen

during the remainder of the day by subjects who appeared to have normal appetite regulation. Foods available after breakfast were those the subjects had been consuming for several months. Plasma amino acids were also measured to determine whether relationships observed between plasma amino acids and food consumption in rats were also present in humans.

Methods and materials

Subjects

Thirteen subjects were chosen from male university students who had been eating in university dining halls for at least 2 months. They were by self-report healthy, had no cultural or religious food restrictions, and ate whatever they wanted without concern for weight maintenance. The subjects were 18 to 22 yr old (mean 19.2 yr), and none were considered to be obese (mean height, 180 cm; mean weight 74.3 kg). The experimental procedures were approved by the Oregon State University Committee on Human Subjects, and all the subjects signed consent forms after they were informed of the requirements of the study.

Experimental procedures

Each subject participated in the study for 2 successive weeks. Because of missing data, 2 subjects participated for a 3rd week. Breakfasts were eaten in the nutrition research laboratory dining room Monday through Friday. The breakfasts were composed of 3 oatmeal cookies and as much fruit and fruit juice as each subject wanted. The cookies differed in protein content as described below. The subjects knew there were 2 types of cooky, but they were not told what the differences were. Information on the particular aspect of food intake being investigated was not revealed until the study was completed. Questionnaires administered at the end of the study indicated that none of the subjects had determined the contents of the cookies or the nature of the investigation.

Order of presentation of the 2 types of cooky was alternated with different subjects so that 5 subjects received the high protein cookies the first week, and 8 received the low protein cookies the first week. The subjects consumed each type for a week and thus served as their own controls. The experiment was conducted from November, 1980, to March, 1981, with 2 to 6 subjects participating at any one time.

Prior to the actual study, subjects were given a 2 day trial to become accustomed to the procedure. During

the study and the trial periods, measurements of food intake, as described below, were made on Mondays through Fridays to keep the procedure standard throughout the week, and blood samples were taken on Tuesdays and Thursdays. Because of class schedules of 6 subjects, some of the blood samples (primarily those taken at mid-morning) were taken on Mondays or Fridays. Since there was no significant difference between values obtained on Tuesdays and Thursdays, measurements from these Monday and Friday samples were substituted for those of the nearest day, and comparisons to food intake were based on the food ingested on the day of the blood sampling.

Lunches and dinners were eaten in a university residence hall dining room. On Tuesdays and Thursdays, lunch was consumed 4 h after breakfast. Other meals were consumed at the subjects' convenience during regular dining room hours. Snacks were recorded by the subjects, and it was required that snacks be pre-measured on Tuesdays and Thursdays.

Subjects were encouraged to eat what they felt like eating but were not allowed to eat during the periods between midnight and breakfast, breakfast and lunch, and dinner and the post-dinner blood sampling. If illness altered their appetites, they participated in the study at a later time.

Food composition and measurement

The oatmeal cookies were made from a home recipe. Before baking, the dough was divided in half, and an equal amount by weight of non-fat dry milk powder (Sanalac, Sanna Division, Beatrice Foods Co., Madison, WI 53708) or cornstarch was added to each half. Water was added to produce the appropriate consistency. The moderate protein breakfast included the cookies with milk powder; the 3 cookies served at each breakfast contained 392 kcal, 18 g protein, 60 g carbohydrate, and 9 g fat. The cookies made with cornstarch were consumed in the low protein-high carbohydrate breakfast, and 3 of these cookies contained 393 kcal, 4 g protein, 74 g carbohydrate, and 9 g fat. These values were determined from manufacturer's information and data from food composition tables (Church & Church, 1975). A variety of canned, fresh, and dried fruits (e.g., canned peaches, whole oranges, raisins) and fruit juices (e.g., reconstituted frozen orange juice and grape juice, tomato juice) was always available on the breakfast table, and the types of fruit were provided at the request of the subjects. The fruit and juice was presented in a variety of preweighed portions. Water was available throughout the meal.

Food and nutrient composition of the meals eaten

in the university dining hall was determined from standard recipes, information from the cooks, manufacturers' information, and food composition tables (Church & Church, 1975; Watt & Merrill, 1963). The amount of each food ingested at each meal was determined by weighing the dish and food immediately after selection and subtracting the weight of the dish and waste at the end of the meal. Subjects were encouraged to eat only what they wanted and to return for additional helpings if desired. The quantities of foods (with the exception of the breakfast cookies) were not limited. At each lunch and dinner meal a variety of foods was offered. These offerings included 2 entrees, a salad bar, and other foods. At lunch, a sandwich bar presented a further variety. Many beverages were available at each of these meals and included whole, 2%, and skim milk; assorted soft drinks; and several hot beverages.

The dining hall menu cycle was about 28 days. Only the entrees were rotated on this cycle, and because the entree pairs were not rotated together, the menu was different on each day of the study. The variety of possible food choices was great at each meal. The subjects had eaten in the dining hall long enough to be familiar with all the foods offered during the experimental period.

Blood samples

Subjects rested 10 min before each sample of blood was drawn from the antecubital vein. Blood samples were drawn into heparinized tubes and kept on ice until they could be centrifuged at 4°C. The plasma was aliquoted into glass vials and stored at -40°C. All analysis of samples was performed 3 to 9 months after the experiment.

Blood samples were taken 5 min before breakfast (700 to 830 h), 2 h and 4 h after breakfast began, and 2 h after the evening meal was completed. (The evening meal was consumed between 1700 and 1830 h.)

Analytical methods

Plasma TRP was analyzed with the fluorometric method described by Denckla and Dewey (1967) as modified by Bloxam and Warren (1974). All samples were analyzed in duplicate.

Other neutral amino acids were analyzed with a Beckman 116 amino acid analyzer (Beckman Instruments, Palo Alto, CA 94304). Plasma samples were prepared by mixing 1 ml of plasma with 0.5 ml of 10% sulfosalicylic acid on a vortex mixer. The mixture was centrifuged for 10 min at 4° C and 20,000 G. A 0.7 ml aliquot of the supernate was applied to the amino acid analyzer column. The resin

used was a cation exhange (Na^+) type PA-28 (Beckman Instruments, Palo Alto, CA 94304). Special procedures were used to elute the neutral amino acids in a 4 h period, and only the large neutral amino acids (valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine) were measured. The temperature of the circulating water bath was maintained at 55° C during the entire elution. The first buffer used was 0.2N sodium citrate at pH 3.6 and was replaced after 85 min by the second buffer (0.2N sodium citrate at pH 4.6). A standard amino acid solution was run after every eight samples.

Data were analyzed for differences with a paired *t* test and for correlations. Correlations were tested for significance (Steel & Torre, 1960). Differences and correlations were considered to be significant at $p < 0.05$.

Results

Plasma amino acids

The changes in plasma amino acids 2 h after breakfast reflected the differences in the protein and carbohydrate contents of the breakfasts (Table III-1). TRP remained at fasting levels with the moderate protein breakfast, but after the low protein-high carbohydrate breakfast, the TRP level was significantly lower than fasting

levels and those of the other 2 h samples. The NAA was altered significantly from fasting levels at 2 h, rising with the moderate protein breakfast and declining with the low protein-high carbohydrate breakfast. The resulting raio of TRP:NAA was significantly different with each type of breakfast and had a significant increase from the fasting ratio after the low protein-high carbohydrate breakfast and non-significant decline after the moderate protein breakfast.

Four h after breakfast, both TRP and NAA were significantly lower than fasting plasma levels, and the NAA was significantly lower in samples taken after the low protein-high carbohydrate breakfast than in those taken after the moderate protein breakfast. The TRP:NAA was at the fasting level 4 h after the moderate protein breakfast but was still significantly higher than the fasting ratio 4 h after the low protein-high carbohydrate breakfast. No differences were observed with the different breakfasts 2 h after dinner. In these post-dinner samples, the TRP and NAA were at the highest levels measured during the day, and the ratio of TRP:NAA was at its lowest value.

Food intake

Although the 2 types of breakfast contained significantly different amounts and percentages of calories as protein and carbohydrate (Table III-2), the intakes of calories, protein, and carbohydrates at the other meals

(Table III-2) did not differ. In considering daily intakes which included snacks as well as meals (Table III-3), a significant difference in the daily protein intakes was seen. The difference in the mean protein intake (16 g) on a daily basis was similar to the difference in the protein content of the breakfast cookies (14 g).

To consider the possibility that the intake at one meal affects subsequent intake, correlation coefficients were determined for calories, g protein, g carbohydrate, PE, and CE in each of the meals. Few significant correlations were found and these were all positive. The significant correlations are listed in Table III-4.

Plasma amino acid levels and food intake

The differences in plasma amino acid responses to meals of different protein and carbohydrate content are shown in Table III-1. Significant correlations between meal components and post-prandial amino acids are found in Table III-5. To detemine the predictive value of plasma amino acids for subsequent food intake, correlation coefficients were determined for the plasma amino acids and the caloric, protein, and carbohydrate content of the subsequent meals. Because the protein contents of the breakfasts were predetermined, the only relevant breakfast measures were those of calories. Daily intake was compared to the amino acid values in plasma samples taken before breakfast of that day.

Post-dinner plasma amino acids

Although several significant correlations were observed between post-dinner plasma amino acids and the previous meal and the same day as a whole, only a few relationships between the evening plasma amino acids and the intakes on the following day were significant (Table III-6). The NAA correlated positively with the PE of the next day and negatively with the intake of calories and g carbohydrate. These were reflected in positive correlations between the post-dinner TRP:NAA and the next day intake of calories and g carbohydrates.

Fasting plasma amino acids

Pre-breakfast plasma amino acids had only one significant correlation with breakfast calorie intake, and this was a negative relationship between the TRP:NAA and breakfast calories ($r = -.413$, $p < .01$). When the intake of the day as a whole was considered, again only one significant correlation was observed, and this was a positive relationship between the fasting NAA and the daily caloric intake ($r = .341$, $p < .05$).

Mid-morning plasma amino acids

No significant correlations were found between the mid-morning plasma amino acids and components of the lunch consumed 2 h later.

Pre-lunch amino acids

No significant correlations were observed between the components of the lunch intake and the plasma amino acids sampled about 10 min previous to lunch.

Discussion

The plasma amino acid responses to meals were consistent with those observed by others and can be explained. Fasting plasma values of TRP are relatively high because of the catabolism of body protein (Young & Sourkes, 1977). The lower TRP values 2 h after the low protein-high carbohydrate breakfast and 4 h after both types of breakfast (Table III-1) indicate uptake by the body of ingested TRP. In contrast, after the high intake of protein at dinner (55 g ± 23), the TRP levels were higher than the pre-breakfast fasting levels. Ashley et al. (1982) observed an increase in plasma TRP that was proportional to the TRP content of an evening meal. In a study by Fernstrom and Wurtman (1972), subjects received diets differing in levels of egg protein. Samples of plasma drawn 3 h after breakfasts containing 1 and 25 g protein had lower values of TRP than fasting samples. A breakfast containing 50 g protein, however, resulted in plasma TRP values similar to fasting levels. Anderson and Blendis (1981) observed TRP values similar to fasting levels in samples taken 2 and 4 h after breakfasts containing about 29 g protein.

The correlations observed in the present study between plasma TRP and the g protein ingested at the meal 2 h before (Table III-5) seem to agree with the results of other studies. The lack of correlation 4 h post-prandially

(Table III-5) and the similar values 4 h after both types of breakfast (Table III-1) indicate that plasma TRP no longer reflects the previous meal's protein content after 4 h. The fasting plasma levels of TRP observed in the present study (6.59 μmol/100 ml) are similar to those reported by others; 5-7 μmol/100 ml (Fernstrom et al., 1979) and 6.44 μmol/100 ml (Anderson & Blendis, 1981).

The sum of the neutral amino acids (NAA) in plasma is the result of two major effects on the branched chain amino acids. These amino acids are elevated by protein ingestion or by catabolism during starvation (Adibi, 1976) and are decreased by the muscle uptake which is facilitated by insulin (Zinneman, Nuttall, & Goetz, 1966). A mixed meal (containing moderate levels of both carbohydrate and protein) will add these amino acids to the plasma and because of the resulting insulin secretion will also increase the removal from the plasma. This effect was seen after the moderate protein breakfasts with an increase in NAA over fasting at 2 h; the lowered level at 4 h (Table III-1) indicated that the amino acids had been removed from the plasma. The low protein-high carbohydrate breakfast added a small quantity of amino acids to the plasma, but the cessation of the catabolism which occurred during fasting and the stimulation of insulin by the high carbohydrate intake resulted in the lowered post-prandial plasma NAA (Table III-1). Because of this decrease, the TRP:NAA was increased by the low protein-high carbohydrate breakfast

(Table III-1). The moderate protein breakfast did not alter the ratio from fasting levels. In a previous study (Anderson & Blendis, 1981), when healthy subjects consumed breakfasts with a higher protein content than in this study, the responses were different with the plasma NAA remaining elevated at 4 h after breakfast. The TRP:NAA was, however, as with the moderate protein breakfast in the present study, similar to the fasting ratio 2 and 4 h after breakfast.

In a study by Ashley and his colleagues (1982), no alteration of the TRP:NAA occurred after consumption of evening meals with high protein or high carbohydrate contents. The ratio was increased above the pre-meal ratio, however, when the high carbohydrate dinner was supplemented with TRP. Pre-dinner values were not measured in the present study, but the post-dinner ratio was significantly lower than fasting levels (Table III-1) and correlated inversely with both the dinner calories and the g protein ingested (Table III-5). The previous study (Ashley et al., 1982) considered the effects of a 500 kcal meal, and some differences seen in the present study may reflect the freely selected meal and the higher kcal (mean 1207±414) intake.

A study by Anderson and Blendis (1981) found a significant inverse relationship of PE for the previous 3 days and fasting plasma TRP:NAA. In the present study, Thursday fasting values of TRP, NAA, and TRP:NAA were found

to have no significant correlations with the Tuesday and Wednesday intakes of calories, protein, or carbohydrate. The differences of the results of the two studies may reflect some differences in the subjects as well as methods of determining nutrient intake. The subjects in the Anderson and Blendis (1981) study were older and included women. The nutrient intakes were based on records kept by the subjects of foods which were prepared, weighed, and consumed at home.

In the present study, the intake of calories, protein, and carbohydrate was not different after breakfasts differing in protein and carbohydrate content (Table III-1). Any physiological system containing a feed-back mechanism would be expected to have a time relationship. A feed-back mechanism affecting food intake would seem more likely to involve two adjacent meals rather than meals spaced far apart. However, the number of significant correlations between meal intakes increased as the length of time between the meals increased (breakfast-lunch < lunch-dinner < breakfast-dinner). (See Table III-4.) The significant correlations were, in addition, all positive, and this, rather than indicating a feed-back mechanism operating to establish a constant intake from day-to-day, indicates an eating pattern that is reflected in all the meals consumed during the day. The strict limits placed on breakfast protein intake may have been reflected in the very low correlations between breakfast protein and lunch

or dinner protein intakes. With no restrictions placed on consumption, a significant positive correlation was found between the amount of protein consumed at lunch and dinner (Table III-4).

The similarity of the difference in the mean values of protein in the daily intakes with the two breakfast conditions and the difference in the protein content of the breakfast cookies suggests that the breakfast protein content affects no aspects of the diet other than the breakfast itself.

The absence of evidence for an appetite feed-back mechanism in this study may reflect any of the following: (a) lack of knowledge of the nutrient content of breakfast may inhibit compensatory eating; (b) normal, healthy young men may follow food preference and customary meal patterns without regard to physiological signals; (c) changes in the amino acid levels were not reflected in neurotransmitter synthesis; and (d) appetite mechanisms are established over a period of time longer than a week. (The absence of differences in Tuesday and Thursday intakes does not support a short term adaptation.)

In conclusion, although breakfasts differing in protein and carbohydrate content affected young men's plasma neutral amino acid levels and the TRP:NAA 2 and 4 h after the meal, consumption of calories, protein, and carbohydrate was not altered at other meals during the day.

Table III-1. Plasma TRP*, NAA†, and TRP:NAA of 13 subjects consuming breakfasts differing in protein content

	Fasting	2 h post-breakfast	4 h post-breakfast	2 h post-dinner
TRP (μmol/100 ml)				
Moderate[a] protein breakfast	6.72±0.72‡	6.78±0.79‖	5.82±0.54§	7.52±1.22§
Low protein-high carbohydrate[b] breakfast	6.46±0.88	5.66±0.72§‖	5.83±0.68§	7.45±0.93§
NAA (μmol/100 ml)				
Moderate protein breakfast	69.70±6.50	76.90±10.90§‖	60.69±6.12§‖	94.09±24.4§
Low protein-high carbohydrate breakfast	68.01±8.10	52.50±5.06§‖	54.00±5.47§‖	92.46±16.9§
TRP:NAA				
Moderate protein breakfast	0.097±0.014	0.089±0.012‖	0.097±0.014‖	0.083±0.016§
Low protein-high carbohydrate breakfast	0.096±0.012	0.108±0.015§‖	0.108±0.012§‖	0.082±0.011§

* tryptophan; † leucine + isoleucine + valine + tyrosine + phenylalanine; ‡ mean ± SD; § difference from fasting level significant, $p<.01$; ‖ difference between breakfast conditions significant, $p<.01$; [a] 4 g; [b] 18 g

Table III-2. Mean intake by 13 subjects of meal contents of calories, protein, and carbohydrate with conditions of different breakfast protein content.

Meal component	Breakfast		Lunch		Dinner	
	MPB*	LPHCB†	MPB	LPHCB	MPB	LPHCB
Calories	604±162‡	657±173	1241±423	1272±400	1214±433	1232±419
Protein (g)	18±0.4§	4±0.2§	55±28	55±25	54±24	56±56
PE‖	13±3§	3±1§	17±5	17±5	18±5	19±7
Carbohydrate (g)	105±37§	127±37§	138±51	150±53	127±56	135±64
CE¶	68±7§	77±6§	44±8	45±9	42±11	44±12

* MPB - Moderate protein breakfast

† LPHCB - Low protein-high carbohydrate breakfast

‡ mean + SD

§ Difference in two conditions, $p<.01$

‖ PE - Percentage of calories as protein

¶ CE - Percentage of calories as carbohydrate

Table III-3. Mean daily intake of calories, protein, and carbohydrates of 13 men with different breakfast conditions.

	Moderate Protein Breakfast	Low Protein-High Carbohydrate Breakfast
Calories	3317±860*	3346±799
Protein (g)	133±42†	117±38†
PE‡	16.0±2.7	14.1±3.8
Carbohydrate (g)	409±117	432±126
CE§	49.3±5.6	51.4±7.0

* Mean ± SD

† Difference in values, $p<.05$

‡ Percentage of calories from protein

§ Percentage of calories from carbohydrate

Table III-4. Significant* correlations between meal calorie, protein, and carbohydrate intakes.

	Correlation Coefficient
Breakfast - Lunch	
breakfast g carbohydrate - lunch calories	.35
breakfast calories - lunch g carbohydrate	.34
Lunch - Dinner	
lunch calories - dinner calories	.42
lunch g carbohydrate - dinner calories	.36
lunch calories - dinner g protein	.34
lunch g protein - dinner calories	.39
lunch g protein - dinner g carbohydrate	.34
lunch g protein - dinner g protein	.34
Breakfast - Dinner	
breakfast calories - dinner calories	.66
breakfast g carbohydrate - dinner calories	.68
breakfast calories - dinner g carbohydrate	.66
breakfast calories - dinner g protein	.39
breakfast CE† - dinner calories	.37
breakfast g carbohydrate - dinner g protein	.45
breakfast g carbohydrate - dinner g carbohydrate	.68
breakfast CE - dinner carbohydrate	.36

* $p < .05$

† Percentage of calories from carbohydrate

Table III-5. Significant correlations* between calorie, protein, and carbohydrate components of meals and post-prandial plasma amino acids for 13 men.

Meal (breakfast or dinner) intake	2 h post-breakfast		4 h post-breakfast		2 h post-dinner	
Calories	TRP[†]	-.40[‡]	none significant		NAA[§]	.48
					TRP:NAA	-.36
Protein (g)	TRP	.60			TRP	.58
	NAA	.83	NAA	.50	NAA	.70
	TRP:NAA	-.59	TRP:NAA	-.40	TRP:NAA	-.41
Percentage of calories as protein	TRP	.68			TRP	.35
	NAA	.82	NAA	.50		
	TRP:NAA	-.50	TRP:NAA	-.34		
Carbohydrate (g)	TRP	-.46	none significant		none significant	
Percentage of calories as protein	TRP	-.48	none significant		none significant	
	NAA	-.46				

* $p < .05$

[†] tryptophan

[‡] correlation coefficients were determined from 4 measurements for each variable on each of the 13 subjects

[§] leucine + isoleucine + valine + tyrosine + phenylalanine

Table III-6. Significant* correlations of post-dinner plasma amino acids with intake of calories, protein, and carbohydrate at the previous meal, same day, and next day for 13 men.

	Dinner (2 h previous)		Same Day		Next Day	
TRP†	g protein	.58‡	g protein	.47	none	
	PE§	.35	PE	.40	significant	
NAA‖	calories	.50	calories	.54	calories	-.53
	g protein	.72	g protein	.78		
	PE	.35	PE	.49	PE	.49
			g CHO¶	.34	g CHO	-.59
TRP:NAA	calories	-.36	calories	-.49	calories	.46
	g protein	-.45	g protein	-.62	g CHO	.54

* $p<.05$

† tryptophan

‡ correlation coefficient

§ percentage of calories as protein

‖ leucine + isoleucine + valine + tyrosine + phenylalanine

¶ carbohydrate

CHAPTER IV

FOOD INTAKE MEASUREMENT: UNIVERSITY DINING HALLS AS AN EXTENSION OF THE LABORATORY

To be submitted to Home Economics Research Journal.

Sandra J. Mitchell, Department of Child Development and Home Economics, California Polytechnic State University, San Luis Obispo, CA 93407.

James E. Leklem, Department of Foods and Nutrition, Oregon State University, Corvallis, OR 97331.

Galley proof should be sent to James E. Leklem (503-754-3561).

Research was done in the Department of Foods and Nutrition, Oregon State University.

Research support from grants from the American Home Economics Association Foundation and the Sigma Xi Society.

Abstract

Measurement of dietary intake is complicated by various factors which make accuracy of measurement difficult when the usual dietary intake is maintained. A method used recently by the authors to measure food intakes of subjects in a university residence hall dining room is described. The time costs of this method were shown to be similar to those of metabolic laboratory studies, and the advantages of use of the dining hall method in financial cost and minimal disruption of subjects' food habits are discussed. The need for an accurate but valid measure of food intake as a dependent variable in an experimental situation was met by the dining hall method.

Introduction

Frequently, in nutrition research, objectives include a determination of the dietary intake of individuals. Development of the research design requires consideration of the limitations and advantages of various methods of measuring dietary intake. Some of the considerations and methodologies are presented here and are followed by a description of the method for determining dietary intake used recently by the authors.

We designed an experiment to determine freely-selected food intake by subjects who were fed breakfasts with different quantities of protein. Weighed intakes at breakfast were in the controlled conditions of the metabolic laboratory, and weighed measurements of the foods ingested at the other daily meals were obtained while allowing the subjects to make their usual selection from the variety of foods served in a university dining room. The method used for this determination of freely-selected intake had the advantages of not altering the subjects' usual food habits, providing a central location for the experimenter to weigh the food consumed, permitting access to aliquots of the food actually consumed, controlling variation in cooking methods, and keeping expenses low. Because much of the research involving food intake occurs on university campuses using students as subjects, and because many researchers may feel limited by not having

access to metabolic laboratory facilities or funding for food supplies, this method may be useful in providing an alternative to other less precise methods of determining food intake.

Determining the method of data collection

Young and Trulson (1960) suggested that the objectives of a study must be clearly defined before deciding upon the methods to use for obtaining and processing dietary data. Collection of auxiliary data must be anticipated, and the need for determining either group or individual intakes will influence the decision. The appropriateness of the collection method will also be determined by the time span for which the intake must be assessed, the methods used for converting food intake data into nutrient intake data, and the characteristics of the informants. Selection of the appropriate method is further influenced by the size of the sample and the funds and personnel available (Pekkarinen, 1970).

Many methods of determining usual dietary intake are used in surveys to determine the intakes of a population. Methods appropriate for use in surveys frequently lack the precision necessary in experimental conditions with small numbers of subjects. Unfortunately, many good studies have related precise measures of biochemical, physiological, and behavioral parameters to imprecise (accuracy within 20-25%) measures of food intake such as 24-hour recalls

and records based on estimated quantities of foods of estimated composition. Even with studies based on weighed intakes, precision is lost on factors such as food composition and accuracy of weighing when subjects are eating in their own homes. There is always the risk that the subject will alter his or her dietary intake to facilitate ease in weighing and recording. This decreases the validity of studies in which food intake is a dependent variable.

In general, with all methods, the quality of data depends on the ability of the participants and interviewers as well as the understanding by both parties of the objectives of the study (Adelson, 1960). A method of determining dietary intake which may be very useful in a clinical setting is not necessarily a good choice for use in research. Burke (1947) suggested that the only time a clinical tool should be used for research purposes is when the tool has been designed with research in mind and the accuracy necessary has been incorporated into the design.

In many types of studies, validity and accuracy are the same. The use of the term accuracy here refers to the precision with which the actual food or nutrient intake is determined. Validity refers to how well the measurement quantifies that which is supposed to be measured. Frequently the objective of a study is to measure usual intake, unaltered by the experimental protocol. Thus a very accurate method of determining actual intake may have poor validity because the participants' usual food intakes

are altered by the method of measurement (Stuff, Garza, Smith, Nichols, & Montandon, 1983).

Records based on weighing

It is generally agreed that the most accurate data on food consumption are obtained by weighing the food consumed (Marr, 1971; Pekkarinen, 1970; Trulson & McCann, 1959). Trulson and McCann (1959) found it difficult to get subjects to weigh foods for three or more weeks; additionally, the process may alter eating practices, and food patterns may be simplified for ease in weighing. The validity of weighed food records can be improved by measuring weighed food intake with the subject being unaware that the intake is being observed (Young & Trulson, 1960; Gersovitz, Madden, & Smiciklas-Wright, 1978), but this is rarely feasible.

Because of the accuracy and reliability of weighed dietary intakes, other methods have been evaluated in comparison to weighed intakes.

Records based on estimation

Some precision is lost in having subjects keep records of food consumption in household measures rather than weighed portions, but household measures are frequently used to obtain better subject cooperation. The accuracy of estimated intake records can be improved by having a trained interviewer verify the portion sizes with food

models and determine possible omissions by careful questioning. Food records based on household measures or estimated portions require no special equipment such as scales, but keeping written records requires literacy of the participants, and the measurement technique must be described (Marr, 1971). Todd, Hudes, and Calloway (1983) reported that graduate students preferred verbal recording of dietary intakes on tape recorders to written recording.

Comparison of weighed and estimated records of four consecutive meals showed that the average energy intake calculated from estimated records was very close (94%) to that from weighed records (Morrison, Russell, & Stevenson, 1949). The variability of the values from estimates was great, however; the range was 63 to 125 percent of the values from weighing. Only one of the eight subjects gave estimates that resulted in an energy intake within five percent of the value from weighing. Todd et al. (1983) found smaller co-efficients of interindividual variation for protein and energy intakes when food portions were weighed by the subjects than when the portion sizes were estimated. Because energy intake is commonly reported in all studies, this measure has been used for comparison of methods. Measurements of nutrient intakes usually have more variability than those for kcalories. The variability in different methods differs with the nutrient being measured and this variability should be considered in determining the method for measurement of the specific nutrient being

studied.

24-hour food recalls

Accuracy

Although graduate students reported similar energy and protein intakes with weighed and estimated records, when Todd and her colleagues (1983) asked the 18 students to recall the food they had recorded and eaten in the previous 24 hours, the recalls were useless for determining individual intakes. When group means were considered, however, the recalled intakes were within 15 percent of the recorded intakes. In another study (Adelson, 1960), a group of 59 business and professional men were aided by their wives and dining companions in the recall of the dietary intake of the previous seven days. The recalls were compared to records of weighed intakes. The recalls for energy intakes supplied the most accuracy with 90 percent of the men recalling intakes to provide energy values within 20 percent of those derived from weighed intake records.

Twelve men working on an Antarctic base were asked to recall their dietary intakes of the previous 24 hours. Although these men had weighed and recorded all their food, the estimation of energy intake was 21 percent less than weighed values when the recall was written on special forms. When the men wrote their recalls on plain paper, the under-

estimation was 36 percent (Acheson et al., 1980). The Antarctic workers were not interviewed during the recall, and the common errors contributing to the underestimations were those of omitting one food and underestimating portion sizes. Campbell and Dodds (1967) reported that "prodding" the memories of subjects in institutions increased recalled intakes. The interviewer presented a copy of the previous day's menu after the initial recall, and the food items added to the intakes increased the energy intake 28 to 35 percent in 100 elderly subjects and 12 to 21 percent in 100 younger subjects.

Gersovitz et al. (1978) studied the validity of the 24-hour recall and seven-day diet records. The validity was determined by comparison of the recall and record with luncheon food portions which had been weighed and served to 65 elderly subjects at a congregate meal site. The subjects were unaware that their actual consumption was being measured. The mean recalled intake was higher than the weighed intake with the difference significant only for the calculations of protein. The seven-day record indicated a lower intake for most nutrients calculated, with significantly lower values for calories and thiamin. The validity of the measure of protein intake was rejected by regression criteria. It was found that usable records were submitted only by those participants who had more than nine years of education, and the validity of these usable records declined from high levels early in the week to very poor levels on

the fifth to seventh days. In addition, drop-outs during the record-keeping resulted in demographic biases in data collected on the fifth to seventh days of study (Gersovitz et al., 1978).

Variance

Although group means are similar for 24-hour and one-week intakes, individual intakes have less variation when measured for seven days (Garn, Larkin, & Cole, 1978). Balogh, Kahn, and Medalie (1971) found random 24-hour recalls by clerical and administrative workers to be highly variable. To obtain repeatability with ±20 percent for 95 percent of the recalls by 90 percent of the population, the number of reports needed varies with the nutrient studied. Nine reports are needed for repeatability for calorie intake, but 45 recalls would be required in studies of cholesterol intake (Balogh et al., 1971).

Beaton and his colleagues (1979) reported that the major components of variance in 24-hour intakes are from both inter- and intraindividual variations. When nutrients are considered on a density basis (adjusted for calorie intake) the interindividual but not the intra-individual variance is reduced. Intraindividual variation in intakes prevents improvement of correlation or regression figures by increasing the numbers of subjects. The only way to compensate for this variation is by repeated measures on the same subjects (Beaton et al., 1979).

Metabolic study method

The metabolic study is the most controlled of the methods available for monitoring food intake. An accurate measure of all foods ingested and the metabolic end products is the basic technique used in metabolic balance studies (Donelson, Nims, Hunscher, Shukers, & Macy, 1931). When subjects receive their food in the laboratory, preweighed food portions and strict adherence to a menu of foods which can be analyzed for nutrient composition are usually accompanied by collection of all or some excretions and periodic assessment of body tissues such as blood. Metabolic studies can be performed with subjects selecting, weighing, and submitting aliquots of foods (University of Minnesota, 1958), but this procedure can compromise accuracy and validity. The usual method is to have subjects consume all foods in the laboratory or foods prepared by the laboratory, and this procedure is referred to here as the metabolic study method. Metabolic studies must extend for a period of time that allows subjects to adjust to the stress of the new diet (Meyer, Brown, Wright, & Hathaway, 1955) and other factors of the study that change their usual habits.

The precision achieved in metabolic studies is expensive. Trained personnel are essential for accuracy in food measurement and repeatability in preparation technique. The cost of food and space for long term studies add to the

expenses. The restrictions imposed by the studies often make recruitment of subjects difficult, and, if the subjects are free-living, they have to be trusted to not deviate from the routine. Providing more than one menu or choices from a menu escalates the cost considerably. Hospitals frequently have the facilities to provide a more varied menu, but the concern here is with research conducted in universities without hospital facilities.

Nutrient composition of foods

Determination of food intake with records and recalls has varying degrees of accuracy and validity. These variations are further compounded by problems in determining the nutrient contents of the foods. Direct analysis by laboratory methods for energy and nutrient content of the food eaten can be performed on aliquots obtained during weighing, but with free-living subjects, this type of determination requires aliquots of each food consumed by each subject (University of Minnesota,1958). The usual method of determining food content is by using existing food composition tables. Composition tables have inadequacies because of missing, inaccurate, and outdated values. Poor descriptions of food content or portion size can produce variations in the nutrient calculations by experienced workers using the same tables (Whiting & Leverton, 1960). Stock and Wheeler (1972) found a variation of ±20 percent in measures of energy calculated from food composition

tables and analyzed with a bomb calorimeter. The primary factor in these discrepancies was the variation in fat and water used in food preparation. They suggested that because individual energy intakes will vary ±10 percent from week to week, calculated differences of less than ±30 percent between two groups could not be regarded as significant unless all members of the two groups were eating from the same menu. The proliferation of computer assisted nutrient analysis systems frequently makes nutrient analysis more efficient, but these systems are based on food composition tables and have the same inadequacies. In addition, variations because of coding procedures add to discrepancies in various systems (Hoover, 1982).

Summary of methods

In summary, the method with the greatest accuracy in determining food intake is that of weighing, and some validity may be lost by having the subjects weigh their own food. Record-keeping over a several day period apparently suffers from subjects' loss of motivation. Although estimates by recall make less imposition on life style and fewer requirements of the subjects, interviews must be conducted to get accurate recalls. Twenty-four hour intakes which are usual in the recall method, are highly variable and cannot be used to determine the usual or longer term intake of an individual. Methods used in the metabolic laboratory are expensive because the food must be provided

for the subjects, and facilities on a university campus are frequently inadequate for providing any variety in the menu. Calculations of nutrient intakes from food composition tables introduce errors from reported food content and preparation as well as those in the composition tables.

The limitations of these methods were considered in designing an experiment to measure the effects of breakfasts with differing levels of protein on the freely-selected food intake during the remainder of the day. It was important that the subjects have a variety of foods from which to make their selection, that they have access to foods with which they were familiar, that their daily routines be altered as little as possible, and that their food intakes be determined as precisely as possible.

Procedure

The 13 subjects for the study were selected from men who usually ate all their meals in the dining rooms of the university residence halls. The mean age of the subjects was 19.2 years; mean height, 180 cm; and mean weight, 74.3 kg. A crossover design was used to present the independent variable which was the protein content of breakfasts which were fed to the subjects in the metabolic laboratory. The dependent variables included the protein, carbohydrate, and energy content of the food freely-selected by the subjects during the remainder of each day. The results of the experiment are reported elsewhere (Chapter III).

Collection of food intake data

Lunches and dinners were eaten in a residence hall dining room, and the amounts of the various foods selected were weighed at a table in the adjacent kitchen. The subjects immediately learned to obtain food items on separate dishes (e.g., spaghetti noodles on a plate and the sauce in a bowl). All the items selected were weighed in their containers, and a label was taped on each of these dishes or glasses so that each could be identified later; this procedure took about three to five minutes for each subject. After the subjects finished with their meals, they returned the trays to the weighing table, and the experimenter weighed the containers and waste. If additional portions were desired, the process was repeated. This routine was established with each subject before data for use in the study were collected, and cooperation by the subjects was excellent.

In addition to the weighings, the subjects recorded the approximate quantities of food they ate at meals and the estimated or weighed amounts of their snacks. The lists of food ingested at meals were used to check for omissions in the weighing procedure, and the continual writing down of intake was also used to keep the subjects accustomed to recording everything they ingested so that the recording of snacks would be complete. The intake record of the previous day was verified at breakfast each morning.

On the days of data collection (Tuesdays and Thursdays), the subjects were required to consume only pre-weighed snacks. Occasionally a subject would bring in a food for weighing, but because the subjects were living in the residence halls, they purchased most of their snacks from vending machines. The subjects submitted the wrappers from their snacks with their daily food records, and the weights on the labels were used to determine snack intake.

Determination of nutrient intakes

The protein, carbohydrate, and energy contents of simple foods and ingredients were usually calculated from food composition tables (Church & Church, 1975; Watt & Merrill, 1963). However, many of the foods served in the dining hall are purchased in a complex form (e.g., frozen entrees, mixed vegetables, and baked goods). When nutrition information was not supplied on the labels of these foods, the manufacturers were contacted by mail. The response to these inquiries was about 95%, and only very small companies did not reply (presumably because they had no data on the nutrient contents of their foods). Estimation of added fat was based on estimates by the experimenter and the cooks the first time the item appeared on the menu, and this same estimate was applied throughout unless there was some indication that preparation procedures had been altered.

Basing the food composition of cooked foods on recipe ingredients does not allow for changes in density with

cooking. The density of foods which had been simmered or baked was estimated from the caloric densities of similar foods in the composition tables, and adjustments to the nutrient contents were made accordingly. Because the nutrients studied in this experiment were not ones which would be altered during the cooking process, the only adjustment that had to be made were for the water losses during cooking. Food aliquots could have been analyzed for moisture content or for the various nutrients, but this was not done. Because the subjects freely-selected their meals, a composite sample of a meal such as is usually obtained in metabolic studies would be impractical since samples of each food consumed by each subject would have to combined for separate daily composites. The nutrient being studied would affect the decision on which nutrient determination method is appropriate.

Results

Variability

Although the procedure was followed for three days before the data collected were used, in retrospect it can be seen that this was not long enough to eliminate daily intake differences which seemed to be related to the day of the week and week of the study. Because two subjects did not participate for two consecutive weeks, the data from only 11 subjects have been analyzed according to the day of participation. The mean total daily kcalorie intakes

(±SD) on Tuesday of Week 1, Thursday of Week 1, Tuesday of Week 2, and Thursday of Week 2 were: 3047 ± 768; 3613 ± 870; 3310 ± 953; and 3522 ± 837. As can be seen in Table IV-1, Thursday intakes were higher than Tuesday intakes, but this difference was significant (.05 level) only during the first week of study. These differences were caused mainly by the high variation in snack intakes which were significantly different only on the first and fourth days of data collection. However, the snack contribution to daily caloric intake was minor and was only 6% (SD ± 7%) of the total daily intake.

Costs

Records were not kept on the actual time spent determining food and nutrient intake, but some estimates have been made. The primary time cost is in being available in the dining hall for measurements. If the subjects' meal times are individually scheduled, this would save time spent waiting for the subjects to arrive and to finish eating. During this study, however, this waiting time was used to copy the recipes used in the meal's preparation, to interview cooks on specific preparation procedures, and to retrieve labels. As compared to a study without food choices, the calculation of food and nutrient composition was lengthy because of the variety of foods served and the

variety of manufacturers supplying similar foods.

Although the most subjects participating at one time was seven, it was assumed that ten subjects could participate at one time. Estimates were made on time costs in this procedure and compared to estimates of time costs in our metabolic laboratory with a similar number of subjects. The estimated time costs for the metabolic laboratory were based on seven studies conducted in our laboratory during the past five years. The comparison shown in Table IV-2 indicates that the time costs are not different. The financial costs would differ, however. The subjects in the residence halls paid for their own food. In the metabolic studies, the food is provided for the subjects, and the current estimate of food costs in studies in our laboratory is $2.75-3.25/subject/day. This cost is very low, but it is kept low because the same menu is served every day. In a study requiring a variety of foods similar to those in the usual diet, the costs of food in the metabolic laboratory would be high and the labor would be more intensive.

An additional cost which is incurred in metabolic studies and was not present in the dining hall study is the allocation of space. Metabolic studies must be conducted during the regular school term because of the length of time involved and because recruitment of subjects is

difficult during vacation periods. Because of this, space used for the storage, preparation, and serving of the food must be allocated at a time when usual demands for class use are also present. With the residence hall dining rooms, space was not required for any of the procedures except the weighing. Because this weighing was done at serving times, most of the preparation activities in the kitchen were minimal, and it was always possible to find an empty table or bench for weighing without imposing on the kitchen staff.

Discussion

The methodology of food intake measurement in residence hall dining rooms was not validated by comparison. Instead, the shortcomings of other methods were considered, and this methodology was developed to overcome those problems. This method could be used for experimental purposes in which subjects would ordinarily be eating all their meals in a central foodservice facility. The advantages for many of these purposes can be seen.

Because it is difficult to obtain a measure of a person's freely chosen food intake without sacrificing accuracy, a compromise in the method of data collection is usually necessary (Young & Trulson, 1960). To obtain a good measure of usual intake, the procedure should not influence the food habits of the participants (Gersovitz et al., 1978). Precision is improved by weighing the food

actually consumed rather than estimating the quantity, and having the experimenter do the weighing avoids questions on the ability of the subjects to make accurate measurements (Marr, 1971).

Repeated measures on an individual can reduce the variability found in one day intakes (Balogh et al., 1971; Beaton et al., 1979). Record keeping for several days, however, has been found to have diminished validity after four days (Gersovitz et al., 1978). The method developed here built in continuing interaction between the subjects and the experimenter at meal times, and, in addition, did not rely on the subjects to provide records of intake. These two factors may have prevented the apparent decrease in subject motivation responsible for the diminished validity observed by Gersovitz and his colleagues.

The results of the present study indicated that there was less variability in daily intakes during the second week than during the first week of study. Significant differences were not seen between the meal intakes, but there was a difference in the snack intakes of the first and fourth days. The trends seen in Table IV-1 indicate that lower intakes on Tuesdays reflect some increase in social eating on Thursday nights (most of the snacking was at night). The differences seen in the first week of study could not be attributed to the foods offered in the dining hall because the subjects started the experiment at different times; because of the randomization of

presentation of the independent variable in the experiment, this difference could not be accounted for by the experimental factors. In addition, it was found that differences because of experimental factors were not significant. It appears that the procedure should be followed for at least one week before stability in intakes can be expected. The alternative to waiting for this time is to introduce experimental conditions in a random order.

Differences in food preparation techniques make reliance on food composition tables for nutrient measures a source of error in determining dietary intake (Stock & Wheeler, 1972). The use of standard food preparation procedures in a centralized kitchen allows for greater consistency in nutrient content than could be obtained when subjects eat their meals in a variety of places. Measuring food intake in the dining hall also allows for obtaining aliquots of food which can be analyzed in the laboratory.

Although there is no substitute for the metabolic laboratory when food intake is the independent variable, the procedure relying on free selection of food in the university dining hall can have sufficient accuracy for many purposes. The costs in time have been found to be similar to those of the metabolic laboratory (Table IV-2), but the dining hall method has the advantages of reduced experimental costs and provision of a much greater variety of foods. (The variety of foods available in the residence

hall dining rooms may surprise many researchers who have not been in one since their undergraduate period.)

The determination of actual and usual food intake for experimental purposes can utilize the residence hall dining room method to avoid inaccuracies from estimation of portion sizes, alteration of subjects' eating habits because of the imposition of weighing tasks, and inaccuracies from unknown food composition and preparation methods. It is hoped that this method can help some researchers avoid the use of more inaccurate methods of determining food intake which are chosen only to avoid the expenses involved in a metabolic laboratory study.

TABLE IV-1.

Percentage of mean four-day intake[a] of kilocalories by young men on different days of two consecutive weeks of study.[b]

	Week 1		Week 2	
	Tuesday	Thursday	Tuesday	Thursday
Meal intake	92 ± 11[c]	107 ± 9	98 ± 10	102 ± 15
Snack intake	66 ± 67*	116 ± 100	57 ± 92	151 ± 76*
Daily total intake	90 ± 12*	107 ± 10*	98 ± 11	105 ± 16

[a] Mean intakes in each category were determined for each subject, and percentages of the mean were determined by subject daily. The means of these were computed and compared for differences with a paired t test, $p < .05$.

[b] All subjects had been following the protocol for three weekdays previous to the first collection day.

[c] Mean ± SD. $n = 11$.

* Starred values in the same row are significantly different, $p < .05$.

TABLE IV-2.

A comparison of time costs in dining hall food intake weighing and metabolic laboratory food procedures.

Activity	Time
Dining hall food weighing[a]	
Weighing (3 meals x 2 hours/meal)	6 hours/day
Determination of food contents and preparation	(no additional time)
Calculation of nutrients	2 hours/day
Total estimated time	approx. 8 hours/day
(one time cost: Subject training, 15 minutes/subject)	
Metabolic laboratory food procedures[b]	
Food purchasing	0.2 hour/day
Food preparation and weighing	6-7 hours/day
Clean up	1½ hours/day
Total estimated time	approx. 8-9 hours/day
(One time costs: Subject training, 15-30 minutes/subject; diet planning and calculation of nutrients, 3-5 hours/study.)	

[a] These costs were estimated for one study involving ten subjects participating at the same time.

[b] These costs were estimated from seven studies involving eight to ten subjects each and which were conducted in our laboratory during the last five years.

CHAPTER V

COMPARISON OF SUBJECTIVE HUNGER RATINGS TO FREELY SELECTED MEAL INTAKE AND PLASMA NEUTRAL AMINO ACID RATIOS

To be submitted to the journal, Appetite.

Sandra J. Mitchell, Department of Child Development and Home Economics, California Polytechnic State University, San Luis Obispo, CA 93407.

James E. Leklem, Department of Foods and Nutrition, Oregon State University, Corvallis, OR 97331.

Galley proof should be sent to James E. Leklem (503-754-3561).

Research was done in the Department of Foods and Nutrition, Oregon State University.

Research support from grants from the American Home Economics Association Foundation and the Sigma Xi Society.

Abstract

This study examined the relationship of subjective hunger ratings on a visual analog scale (VAS) to calorie, protein, and carbohydrate intake values of freely selected meals and to plasma amino acid ratios which may influence central catecholamine and serotonin metabolism. Breakfasts differing in protein and carbohydrate content were fed to 13 men, 18-22 years old and of normal weight. VAS scores were higher before than after meals ($p < .01$) but did not correlate with previous or subsequent meal intakes of calories, protein, or carbohydrate. Although plasma ratios of tyrosine to phenylalanine and trypotophan to the sum of the other neutral amino acids were significantly different ($p < .05$) after the different breakfasts, VAS scores were not different. It is concluded that hunger ratings of normal weight subjects, consuming usual foods, are not reflective or predictive of meal energy, protein, or carbohydrate content and plasma neutral amino acid ratios.

Introduction

Subjective feelings of hunger cannot be measured directly. The method of defining hunger in terms of the time since last eating (Hill, 1974) does not always relate to subjective feelings of hunger. Silverstone (1978) has described rating of subjective hunger on a linear scale which he calls a visual analog scale (VAS). The VAS is a 100 mm line with no divisions and end anchors of "not at all hungry" and "hungry as you have ever felt" (Jain, Kyriakides, Silverstone, & Turner, 1980; Silverstone, 1972; Silverstone & Schuyler, 1975; Silverstone & Stunkard, 1968). Others have used similar scales with various numbers of anchors (Spence & Ehrenberg, 1964; Spiegel, 1973; Strata & Zuliani, 1978). Numerical values from the distance of the rating mark along a line with only two anchors were found to correlate highly with ratings on a nine point verbal scale (Bolton, Heaton, & Burroughs, 1981).

Comparisons with other measures have been made with percentages of each individual's maximal response (Silverstone, 1972), differences in ratings (Silverstone & Fincham, 1978; Silverstone & Stunkard, 1968), and raw values (Hill, 1974; Silverstone, 1972; Silverstone & Schuyler, 1975; Spence & Ehrenberg, 1964; Spiegel, 1973; Strata & Zuliani, 1978; Wooley, Wooley, & Dunham, 1972). Determination of the validity of the VAS has involved comparisons with other measures which might indicate hunger. Comparisons

of VAS scores and caloric intake following treatments with cyproheptadine (Silverstone & Schuyler, 1975), phentermine resinate (Silverstone, 1972), and dexamphetamine (Silverstone & Stunkard, 1968) have resulted in significant correlations of these two measures.

Studies in which drugs have not been used to manipulate appetite have not consistently supported a relationship between the VAS and other indicators of subjective hunger. Wooley and his colleagues (1972) found that after subjects ingested liquid meals differing in caloric density, there was a better relationship between the subjects' hunger ratings and estimates of the caloric value of the meal than between the hunger ratings and the actual caloric value. VAS scores were lowered after ingestion of liquid feedings, but the caloric contents of these feedings were not related to hunger ratings for the next five hours (Spiegel, 1973). Correlations between hunger ratings and the reported time since last eating were not significant (Spence & Ehrenberg, 1964), but ratings one and 18 hours after eating were markedly different (Hill, 1974).

Different relationships between hunger ratings and food intake after treatment with d-amphetamine or fenfluramine (Kyriades & Silverstone, 1979) may indicate differences in the anorectic effects of amphetamine and fenfluramine. The amphetamine mechanism of action is on the catecholaminergic pathways whereas fenfluramine affects the serotonergic pathways in the brain (Garattini, Buczko, Jori, &

Samanin, 1975). Differences in protein and carbohydrate consumption have been observed with fenfluramine treatments of rats at anorectic and subanorectic dosages (Wurtman & Wurtman, 1977b). The types of foods presented to subjects may be important in relating hunger ratings to caloric intake. Carbohydrate snacking has been reduced in humans with treatments of fenfluramine (Wurman & Wurtman, 1981; Wurtman, Wurtman, Growdon, Henry, Lipscomb, & Zeisel, 1981).

Consumption of calories and consumption of protein or carbohydrate may reflect different mechanisms in the brain. The relationship of the serotonergic and catecholaminergic mechanisms to previous food consumption has been studied in rats. Brain serotonin levels are affected by brain tryptophan (TRP) levels which, in turn, are related to the plasma ratio of TRP to the sum of the other neutral amino acids (TRP:NAA); these are tyrosine (TYR), phenylalanine (PHE), leucine (LEU), isoleucine (ILE), and valine (VAL) (Wurtman & Fernstrom, 1975). The plasma TRP:NAA can vary with different amounts of protein and carbohydrate in the diet (Anderson, 1977; Ashley & Anderson, 1975b; Wurtman, Hefti, & Melamud, 1981), and thus the type of food ingested can control the subsequent food intake by alterations in the availability of TRP to the brain (Li & Anderson, 1982a).

Although the synthesis of brain catecholamines may be influenced by the availability of TYR (Gibson &

Wurtman, 1978; Wurtman, Larin, Mostafapour, & Fernstrom, 1974), the relationship between catecholamine synthesis and dietary factors is not well established. Brain levels of tyrosine in rats has been related to the ratio of plasma TYR to the sum of its competitors which are TRP, PHE, LEU, ILE, and VAL (TYR:NAA) (Fernstrom & Faller, 1977). Anderson and Ashley (1977) found that energy intakes of rats correlated more consistently with the plasma TYR:PHE than with the TYR:NAA.

Negative, significant correlations of scaled hunger ratings and serum amino acid nitrogen after meals and infusions have been reported (Mellinkoff, Frankland, Boyle, & Greipel, 1956), but no studies have been found on relationships between plasma neutral amino acid ratios and hunger ratings. In addition, we found no studies indicating validation of the VAS with usual food consumption in normal weight subjects not receiving drug treatment. The present study was undertaken to determine whether subjective hunger ratings by normal weight subjects were predictive of the calories, protein, or carbohydrate consumed in the freely-selected meals which followed the ratings and whether the plasma ratios of TYR:NAA, TYR:PHE, or TRP:NAA were related to simultaneous hunger ratings.

Method

The subjects were 13 men (ages 18 to 22 years, mean 19.2 years) who usually ate their meals in the university residence hall dining rooms. They were, by self report, not concerned with altering their food intake and had no problems maintaining their present weight. None were considered to be obese or underweight (mean height, 180 cm; mean weight, 74.3 kg). After a two-day adjustment period, subjects participated in the study for two weeks. The design included one experimental condition; different amounts (4 g and 18 g) of protein at breakfast in special oatmeal cookies and varying quantities of carbohydrate from fruit or fruit juice in amounts determined by what the subject wanted. Plasma neutral amino acid levels and hunger ratings were measured, and the calorie, protein, and carbohydrate values of measured food intakes were calculated to determine effects of the different breakfasts.

Measurement of food consumption and hunger ratings occurred Mondays through Fridays to maintain a routine for the subjects. Blood samples were taken on Tuesdays and Thursdays, and on these days hunger ratings were made in controlled conditions (with the exception of pre-dinner ratings). Because of these controls and blood samples that corresponded with hunger ratings and food intake, only the data from Tuesdays and Thursdays were analyzed.

For six subjects, because of class schedules, some of the blood samples were taken on Mondays or Fridays. In these cases hunger ratings and meal intakes on these days were substituted accordingly so that all comparisons were made on data from the same days. Because values did not differ significantly from Tuesday to Thursday, the Monday and Friday measurements were considered to be equivalent substitutions.

Hunger Ratings

Subjects rated their hunger on a VAS which was 5 cm long and had no divisions between the two end anchors which were "hungry as I've ever been" and "couldn't eat a thing." The ratings were made five times a day; before breakfast, two hours later (mid-morning), four hours after breakfast (prelunch), before dinner, and two hours after (post-dinner). All the ratings except the one before dinner were made during the ten minute waiting period before blood samplings, and they were made in rooms with no food cues. The blood draws before breakfast and lunch were made immediately before the subjects entered the dining room and made their food selections. The pre-dinner rating was not supervised, and the subjects were requested to mark the scale within the five minutes preceding their entrance to the cafeteria. The scale was marked with an "X," and the distance of the "X" from the end of the line that indicated no hunger was measured in millimeters and

used as the quantitative value of the rating.

Food intake

All the food intake was measured by weight, and the caloric, protein, and carbohydrate contents were determined from recipes, manufacturers' information, and food composition tables (Church & Church, 1975; Watt & Merrill, 1963). Although the breakfast protein contents were controlled by feeding each subject three oatmeal cookies (393 kcal, 4 or 18 g protein, 74 or 60 g carbohydrate), each subject was allowed to consume as much fruit or fruit juice as he wanted. The lunch and dinner meals were eaten in a university dining room which offered a great variety of foods, and these were foods the subjects had been regularly eating for at least two months. They were encouraged to eat only what they wanted and to return for additional helpings if they wanted more. The food and dishes were weighed by the experimenter immediately after the subjects made their selections, and the dishes and waste were weighed at the end of each meal. Snacks were prohibited between breakfast and lunch, for two hours after dinner, and between midnight and breakfast. All snacks were recorded by the subjects,and on Tuesdays and Thursdays, snack quantities were weighed rather than estimated.

Blood collection and plasma processing

Blood samples were drawn immediately before breakfast and lunch and two hours after breakfast and dinner. The pre-lunch sample was scheduled at four hours after breakfast. Blood samples were drawn from the antecubital vein into heparinized tubes. These tubes were kept on ice until the plasma could be separated by centrifugation. Aliquots of plasma were stored in glass vials at $-40^{o}C$ until analysis 3-9 months later.

Amino acid analysis

Plasma tryptophan was analyzed using the procedure of Denckla and Dewey (1967) as modified by Bloxam and Warren (1974). Other neutral amino acids were analyzed with a Beckman 116 amino acid analyzer (Beckman Instruments, Palo Alto, CA 94304). A 0.2 N sodium citrate buffer was used to elute the amino acids. The buffer used for the first 85 minutes had a pH of 3.6, and this was followed by a buffer with a pH of 4.6. The temperature of the circulating water bath was maintained at $55^{o}C$ throughout the elution.

Statistical analysis

Averages of measures for each subject on the two days for each condition were compared with a paired t test. Correlation coefficients were determined using the four values for each subject at each measurement time. Because

the hunger ratings were not different in the different experimental conditions, averages of all four of each subject's hunger ratings were compared for differences before and after meals with a paired t test. Correlations and differences were considered significant if $p < .05$.

Results

Differences in the plasma amino acid ratios of TYR:PHE and TRP:NAA but not TYR:NAA were significant in the mid-morning and pre-lunch samples taken after the different breakfast conditions. Differences in hunger ratings in these two conditions were not significant (see Table V-1).

Correlations between hunger ratings and the caloric, protein, or carbohydrate content of the previous or following meals were not significant. Correlations were not significant between hunger ratings and plasma amino acid ratios. The change in hunger rating from mid-morning to pre-lunch did not correlate with lunch intake. There were significant, positive correlations between same day pre-lunch and pre-dinner hunger ratings ($p < .01$) and same day pre-dinner and post-dinner hunger ratings ($p < .05$). To determine what relationships might exist between pre-meal hunger ratings and meal intakes, each value for pre-lunch hunger rating and the following lunch caloric intake was plotted on a two-dimensional graph.

The two points obtained for each subject in a given week were connected. The slopes of these lines then were categorized as positive and negative. Of the 26 lines, 14 had positive slopes, and 12 had negative slopes.

When differences of the means of the four hunger ratings from each subject were tested with a paired t test, it was found that the mid-morning mean hunger rating of 1.3 cm ± SD 0.8 was significantly lower than that made before breakfast (2.9 cm ± 1.1) or before lunch (3.4 cm ± 0.8). Two hours after dinner the mean hunger rating of 1.8 cm ± 0.8 was significantly lower than that before dinner (3.2 cm ± 1.8). Differences within the two pre-meal ratings and the two post-meal ratings were not significant, $p < .05$.

Discussion

In designing the experiment, there was concern that anticipation of blood sampling would alter subjective hunger. During the experimentation, however, the subjects appeared to have no nervousness about the blood sampling (some subjects withdrew from the study during the adjustment period). The similarity of pre-breakfast and pre-lunch (before blood sampling) to pre-dinner (not connected with blood sampling) hunger ratings indicates that the blood sampling had no effect.

Hunger ratings have been found to be different one hour and 18 hours after eating (Hill, 1974), to decrease

with ingestion (Spiegel, 1973), to increase between meals (Wooley et al., 1972), and to be unrelated to the caloric intake at the last meal (Spiegel, 1973). In all of these studies, drugs were not used to alter appetite. The results of the present study agreed with these findings.

Two studies (Silverstone, 1972; Silverstone & Stunkard, 1968) have reported significant correlations between hunger ratings and calorie intake from a quarter-sandwich meal. In both of these studies, drugs were used to alter appetite, and in neither study were the results of the placebo treatment reported separately. In the present study, the hunger ratings before meals were not predictive of meal calorie, protein, or carbohydrate intake. Silverstone felt that his studies indicated the validity of the VAS (Silverstone & Fincham, 1978), but perhaps the scale is valid only in measuring the effectiveness of drug treatment.

Further mathematical treatment (e.g., conversions to power functions or determining percentage of maximal values) of the measurements in the present study would not have improved correlations because of the apparently random relationship between the directions of change in individuals' hunger ratings and caloric intake on different days.

Silverstone and Fincham (1978) tested the reliability of the VAS by administering tiflorex, an anorectic drug, in a sustained release formulation to five or six subjects on two different occasions. The hunger ratings were

similar on both occasions, and in a third test, ingestion of quarter sandwiches corresponded to the hunger ratings of the previous trials. Subjects' responses were reported in comparison to their responses after placebo treatment. The statistical treatment was not reported to support the authors' conclusion that these tests indicated the reliability of the VAS and the food dispenser measurement. They also did not indicate why the VAS and measurement with the food dispensing apparatus should be expected to be similar when one condition requires continuous fasting and the other allows the subjects to eat.

Wooley et al. (1972) found that post-meal hunger ratings were more closely related to the perceived caloric content of a liquid meal than to the actual caloric content. Possibly subjective hunger is a determinant in perception of caloric content and the feeling of hunger is derived from other factors. The contribution of some aspect of eating to declines in hunger rating is evidenced by the observation of Silverstone and Stunkard (1968) that the decline in hunger ratings with lunch consumption was not affected by dexamphetamine although the caloric consumption in the meal was affected by the drug. Differences between the post-prandial subjective ratings of hunger with isocaloric meals of either whole fruit or fruit juice support the contribution of other factors than calories to subjective hunger (Bolton et al., 1981). That these other factors include neurological alterations

is indicated by the effectiveness of drugs in altering hunger ratings (Kyriakides & Silverstone, 1979; Silverstone, 1972; Silverstone & Schuyler, 1975; Silverstone & Stunkard, 1968). Differences in the types of neurological contributions to hunger ratings and eating behavior are indicated by comparison of treatments with d-amphetamine and fenfluramine (Kyriakides & Silverstone, 1979).

In the present study, mid-morning and pre-lunch plasma amino acid ratios of TYR:PHE and TRP:NAA were affected by the differences in protein and carbohydrate contents of the experimental breakfasts. Although these plasma ratios may have altered the availability of brain tyrosine and tryptophan for catecholamine and serotonin metabolism, there were no differences in hunger ratings in the two breakfast conditions and correlations between the hunger ratings and plasma neutral amino acid ratios were not significant. However, there also was no difference in lunch intakes. The effects of dietary manipulations on hunger ratings and caloric intake in the present study appear to be minor compared to those of drugs in other studies.

The selection and ingestion of food in a normal meal situation may be very complex, and subjective hunger may be only one of many factors contributing to food intake. Alternatively, subjective hunger may be altered during the course of a meal with the result that measurement before

the meal is not a valid predictor of intake if the meal, as chosen, does not have to be completely consumed. It may be that the normal factors contributing to food intake decisions are subordinated to the changes in subjective hunger that are effected by drugs, and in this way drug treatment results in high correlations between VAS measures and subsequent caloric intake.

In the present study, the significantly lower hunger ratings after meals indicate that the VAS measured wide differences in the subjective hunger of subjects. The absence of correlation between hunger ratings and previous or subsequent caloric, protein, or carbohydrate ingestion in the absence of drug manipulation indicates that the subjective feelings of hunger were not related to energy intake at meals.

Table V-1. Mean values of plasma neutral amino acid ratios and hunger ratings with breakfasts differing in protein and carbohydrate content.

Time of Sampling	Breakfast	TRP:NAA	TYR:PHE	TYR:NAA	Hunger (cm)
Pre-Breakfast	A	0.10±.01	1.06±.17	0.13±.02	2.9±1.3
	B	0.10±.01	1.09±.14	0.12±.02	2.9±1.4
Mid-morning	A	0.09±.01[c]	1.18±.18[d]	0.13±.02	1.3±1.1
	B	0.11±.01[c]	1.05±.13[d]	0.12±.01	1.5±1.1
Pre-Lunch	A	0.10±.01[a]	1.16±.20[b]	0.12±.02	3.4±1.1
	B	0.11±.01[a]	1.04±.12[b]	0.11±.01	3.3±1.2
Pre-Dinner	A	------	-----	-----	3.4±1.3
	B	-----	-----	-----	3.0±1.2
Post-Dinner	A	0.08±.02	1.13±.17	0.12±.01	2.0±1.2
	B	0.08±.01	1.18±.17	0.12±.01	1.6±.09

Note: Means ± SD determined with two values in each breakfast condition, 13 subjects. Breakfast A = Mean ± SD values of contents, 604 ± 162 kcal, 18 ± 0 g protein, 105 ± 37 g carbohydrate. Breakfast B = Mean ± SD values of contents 657 ± 173 kcal, 4 ± 0 g protein, 127 ± g carbohydrate. TRP:NAA = ratio of plasma tryptophan to the sum of

(Table V-1, continued)

tyrosine, phenylalanine, leucine, isoleucine, and valine. TYR:PHE = ratio of plasma tyrosine to phenylalanine. TRY:NAA = ratio of plasma tyrosine to the sum of tryptophan, phenylalanine, leucine, isoleucine, and valine. Hunger = rating on a 5 cm visual analog scale.

Means having same superscripts a and b are significantly different in different breakfast conditions, paired t test, $p < .05$. Means having same superscripts c and d are significantly different in different breakfast conditions, paired t test, $p < .01$.

CHAPTER VI

PUPIL RESPONSES TO FOOD PICTURES

The size of the pupils of the eyes is controlled by the autonomic nervous system which activates the iris muscles to contract or constrict for accomodation and for protection of the retina from alterations in light intensities. The sympathetic, or adrenergic, efferent fibers control the dilator muscles of the iris, and the parasympathetic, or cholinergic, fibers innervate the sphincter muscles (Lowenstein & Loewenfeld, 1961). Measurements of the pupillary responses to light have been important in understanding neural dysfunction (Loewenfeld & Rosskothen, 1974).

Changes in pupil size have also been used as measurements of responsiveness to sexual attraction (Hess, 1975a), political statements (Barlow, 1969), lying (Berrien & Huntington, 1943; Hess, 1975b), the taste of foods (Hess & Polt, 1966), and as a measure of the processing of cognitive tasks (Colman & Paivio, 1969; Kahneman, et al., 1969). The validity of many pupillometric (measurement of pupil size) studies, however, has been a subject of controversy (Goldwater, 1972; Janisse, 1974; Tryon, 1975). Controls for visual stimulation, mental processing, and general arousal have not always been incorporated into experimental designs.

Few studies have been conducted on pupillary responses to food. The first was by Hess who reported in 1965 that subjects who had been deprived of food responded to pictures of food with pupil sizes which were 2½ times larger than those of responding subjects who had eaten within the previous hour. Hess (1972) reported an experiment by Seltzer with hypnotized subjects. Pupil response to food pictures was moderate with no hypnotic suggestion; however, after the suggestion that the subjects were very hungry, they responded to food pictures with a greater pupil dilation than to other pictures. The subjects were then led hypnotically through a meal and were told that they were satiated. The response to food pictures was, at this point, pupillary constriction. Beijk and deJong (1971) reported that pupil responses to pictures of food by hungry subjects were significantly larger than those by fed subjects.

Metalis, Hess, and Beaver (1982) studied the responses of overweight and normal weight subjects to pictures of meals and individual foods. Half the subjects had not eaten for over five hours, and the other half had eaten immediately before the testing. The only group that had dilation responses to food pictures were the "hungry" normalweight subjects, and these responses were to complete meals and not to pictures of individual foods. The control pictures in the study by Metalis and his colleagues were pictures of numerals which were matched for brightness with

the food pictures.

The lack of controls for pupillary response to the light intensity, composition, and colors of pictures used as stimuli in these experiments could have made these primarily studies of arousal rather than responses to food in hungry and satiated conditions. To control for the responses to visual stimuli, a method of measuring response to food stimuli was developed; this method included control pictures with brightness, composition, and colors similar to the food pictures (Blaha, 1977). Measures of response to the food pictures were computed as a percentage of variation from the pupillary response to control pictures that immediately preceded or followed the presentation of each food picture. Using this method, I measured the pupillary responses to food pictures by women in the fasting state and 50 minutes after they had eaten a high carbohydrate (1 g/kg body weight) breakfast. Plasma glucose measures were obtained at fasting and 30, 45, and 60 minutes after the breakfast. The change in glucose between 45 and 60 minutes was found to correlate positively and significantly ($p < .05$) with the change in pupil response from fasting to 50 minutes for three pictures: eggs and sausage, cookies, and jelly on toast. Because the decreased response to these pictures of food was related to the physiological parameter of the rate of disappearance of glucose from the blood, it seemed possible that pupillometry had some use as

a measure of desire for food (Blaha, 1977).

The present study was designed to measure several responses to breakfasts containing different quantities of protein and carbohydrate. The hypothesis that differences in breakfast protein and carbohydrate contents would affect subsequent "appetite" for calories, protein, or carbohydrate was tested by measuring three types of responses. The first was lunch intake in calories, protein, and carbohydrate four hours after the breakfasts. The second response was the subjective hunger ratings of the subjects two and four hours after breakfast. The third measure was the two-hour postprandial pupillary response to pictures of foods which had been characterized in a pilot study as high carbohydrate or high protein foods. It was considered that these measures would reflect differences in the plasma neutral amino acid ratios of TRP:NAA. Plasma samples were drawn two and four hours after the breakfasts. Pupil responses to food pictures were measured in conjunction with two other measurements: plasma amino acids and subjective hunger ratings. Comparison to the other measures was for the purpose of determining whether changes in pupil response were related to other measures which were expected to be changed by the experimental conditions.

Method

The subjects and conditions of the experiment are described elsewhere (Chaps. III, IV, and V). The pupillometry measures were made mid-morning (2 hr after breakfast) and followed the subjects' subjective hunger ratings and blood sampling.

Each subject had one pupillometry session on each of four sampling days, two in each experimental condition. During each session the same nine food pictures (each preceded by its control) were viewed. The order of presentation was randomized so that each time the subject participated in the pupillometry the order of the pictures was different.

The method of presentation of the pictures and measurement of the pupil size is the same as that used previously (Blaha, 1977). In brief, the procedure is as follows: The subject is seated in viewing position, and he watches the slides being presented by a rear-illumination projector with an automatic changer. During this time an infra-red lamp shines on his left eye and the reflection is detected by an infra-red sensitive television camera. Electronic interpretation of the size of the pupil is recorded on a kymograph. From the kymograph the mean pupil size can be determined for each picture that is presented.

The tracing of the kymograph is made on paper with

equally spaced horizontal and vertical lines. Calibration of the instrument results in vertical displacement of one space with each one millimeter change of pupil diameter. The speed of the paper is controlled so that horizontal spaces measure one second. Values were determined for the pupil size at each vertical line (i.e., once a second). This meant that eight to nine pupil diameter measures could be determined for each ten seconds of viewing a single slide picture. The means of these pupil diameters were determined for each food and control picture. The percentage change from control size (pupil response) was determined for each food picture with the following calculations:

$$\frac{\text{Mean pupil size (food)} - \text{Mean pupil size (control)}}{\text{Mean pupil size (control)}} \times 100\%.$$

The pictures and controls presented in this study are described in Appendix T. The types of foods presented were chosen because they had different amounts of protein and carbohydrate. Single foods were presented to avoid complications in classification according to protein or carbohydrate content. Because subjects might have a perception of protein and carbohydrate contents of foods that differs from actual contents, a survey of 22 male students, aged 18 to 25 and who had no formal training in nutrition, was conducted. The men were presented with a list of foods and asked their opinions on whether most of the calories in these foods came from fat, protein, or carbohydrate. In

this manner the men generally agreed that protein was the greatest energy source in t-bone steak and eggs. Carbohydrate was named as the primary energy source in bread and jelly, pancakes, baked potato, and oatmeal cookies. The foods actually presented by slide pictures were milk, steak, pancakes, peaches, peas, carrots, egg, baked potato, and oatmeal cookies.

Correlation coefficients were determined for responses to the food pictures (the percentage change from controls) and (a) the plasma amino acid ratios of TRP/ΣNAA, TYR/ΣNAA, and TYR/PHE calculated from measurements of plasma samples taken immediately preceding the pupillometry; (b) the calorie, protein, and carbohydrate contents of the breakfast and lunch of the same day; (c) hunger ratings made about ten minutes prior to the pupillometry; and (d) the responses to the other pictures. Correlations were considered significant if $p < .05$. Mean responses to the food pictures were compared for each breakfast condition with a paired t test. Differences were considered significant if $p < .05$.

Results

Correlations considered significant ($p < .05$) were: response to peas and plasma TYR/PHE ($r = -.375$); response to carrots and hunger rating ($r = -.379$); and response to

peas and response to steak (r = -.401).

The mean responses were compared for the two breakfast conditions. None of these were significantly different in the two conditions. The mean responses are listed in Table VI-1.

Discussion

As can be seen in Table VI-1, the standard deviations of the pupil responses are very high indicating great variations in the responses. The proximity of the mean values to zero with standard deviations having values of several multiples of the mean reflects abundance of both positive and negative responses (i.e., pupil size was either larger or smaller in response to food pictures in comparison to the control slides) for subjects in the same conditions viewing the same slides.

The only other study reporting standard deviations of responses was that by Metalis and his coworkers (1982). They reported standard deviations which were several multiples of the mean responses in all cases but one. This single exception which was the response to main course foods by hungry normal weight subjects was central to all the findings of significance with analysis of variance.

Although differences in the pupillary responses in the present study were not seen with the different breakfast conditions, differences were also not observed in hunger ratings or in lunch caloric, protein, or carbohydrate

Table VI-1. Mean values of percentage change in pupil responses to food pictures in different breakfast conditions.

Food Pictures	Moderate Protein-Moderate Carbohydrate Breakfast	Low Protein-High Carbohydrate Breakfast
Milk	1.17 ± 9.74%[a]	-1.52 ± 9.00%
Steak	0.13 ± 11.6	2.19 ± 11.5
Pancakes	0.02 ± 12.7	2.57 ± 12.1
Peaches	-6.24 ± 10.6	-1.89 ± 10.5
Peas	0.09 ± 13.5	-1.05 ± 8.48
Carrots	-1.72 ± 13.0	-2.86 ± 12.0
Egg	2.12 ± 8.87	-1.02 ± 9.35
Baked Potato	1.65 ± 12.0	1.80 ± 11.0
Oatmeal Cookies	-3.06 ± 10.0	-2.88 ± 15.6

[a] Mean ± S.D.

intakes. The responses to the food pictures appear to vary randomly. Larger mean responses were observed with three pictures (steak, pancakes, and peaches), and the mean responses were smaller with four pictures (milk, peas, carrots, and egg) after the low protein-high carbohydrate breakfast than after the moderate protein-moderate carbohydrate breakfast. Mean responses were similar for baked potatoes and oatmeal cookies after both breakfast conditions.

Considering that 172 correlations were determined with a significance level of 0.05, it would be expected that eight correlations would lie in the range of significance. Because of this, the three correlations that actually did come into the significance range cannot be considered as indicators of underlying relationships.

Other such studies of response to visual stimuli have not used control slides which were similar to the stimulus pictures but have presented a cross (Beijk & deJong, 1971), numerals (Hess, 1972; Metalis et al., 1982), or asterisks (Barlow, 1969; Janisse, 1974). Changes observed with dissimilar controls may indicate a response to the shape or form of the stimulus. For this reason, in the present study, the form and composition of control pictures were similar to those of the food pictures.

Most authors have used black and white visual stimuli. They have done this to avoid responses of the pupil to colors. Longer wavelengths elicit greater pupil dilation (Bouma, 1962). If, however, the colors elicit a response

to the stimuli, the colors in the controls should also elicit a response. If these colors are not changed from the control to the stimulus, the response to the stimulus would not be different merely because of color. Black and white stimuli are appropriate if the subject of the picture is one which is normally seen in black and white (e.g., printed words or newspaper photographs), but food is never presented for eating in black and white, and the achromatic presentation of food stimuli is a very unnatural condition.

The only other study with similar types of control pictures was that done by the author (Blaha, 1977). Unfortunately, the design of the present study did not allow for a measurement of change in pupil responses from fasting to postprandial conditions. Such measures would have allowed for comparison of the present results with those of the previous study. The large variations in measure of pupil size seen here indicate that reliability between subjects and between measures on the same subject on different days is low. Correction for fasting values on the same day may be necessary to obtain meaningful data.

Further study of pupillometry as a measure of appetite should include measures of pupil response prior to and after a freely selected meal. Validation has been attempted to this point with indirect measures of interest in food. Validation studies should control for measures of interest in foods by presenting control stimuli in the same form, composition, and color as the food stimuli.

CHAPTER VII

SUMMARY

This study tested the hypothesis that normal weight young men would have different lunch intakes of protein and/or carbohydrates after consuming breakfasts differing in protein and carbohydrate content.

An experiment was conducted with thirteen young men as subjects. These men received breakfasts containing three oatmeal cookies (392 kcal) and as much fruit or fruit juice as they desired. The cookies were fortified with milk powder or cornstarch so that the breakfasts contained either 18 g or 4 g of protein. Each type of breakfast was consumed for a week, and each subject consumed both types of breakfast. The order of presentation of the type of breakfast cookies was randomized.

Subsequent food intake during the day was measured by weighing the food consumed in a university residence hall cafeteria and from reported weighed snack intake. On two days of each week, plasma samples were obtained before breakfast, two hours after breakfast, four hours after breakfast (immediately before lunch), and two hours after dinner. Plasma was analyzed for levels of the neutral amino acids tryptophan, valine, isoleucine, leucine, tyrosine, and phenylalanine. Calculations determined the ratio of tryptophan to the sum of the other neutral amino acids

(TRP/ NAA). Subjective hunger ratings on a Visual Analog Scale (VAS) were made by the subjects before each meal, two hours after breakfast, and two hours after dinner. Pupil responses to pictures of food were measured two hours after breakfast twice a week.

Plasma neutral amino acid levels were significantly different ($p < .01$) after the two types of breakfast. Plasma TRP and ΣNAA were higher two hours after the 18 g protein (moderate protein) breakfast than after the 4 g protein (low protein-high carbohydrate) breakfast. The ΣNAA continued to be higher in the samples taken four hours after the moderate protein breakfast. The plasma TRP/ΣNAA was significantly higher two and four hours after the low protein-high carbohydrate breakfast than after the moderate protein breakfast. Significant changes from fasting levels were also observed. Plasma TRP was lower than fasting levels four hours after the moderate protein breakfast and two and four hours after the low protein-high carbohydrate breakfast. Although the plasma ΣNAA was elevated two hours after the moderate protein breakfast it was significantly lower than fasting levels two hours after the low protein-high carbohydrate breakfast and four hours after both types of breakfast. The plasma TRP/ΣNAA was significantly elevated from fasting two and four hours after the low protein-high carbohydrate breakfast. The elevation of this ratio resulted in significantly higher values than after the moderate protein breakfast.

These differences in plasma neutral amino acids were not accompanied by changes in any of the measures which were used to determine interest in food. Food intakes did not differ with the two types of breakfast at subsequent meals or in considering the food intakes for the remainder of the day. Total daily intakes of protein in grams were higher with the moderate protein breakfast. The difference in mean intakes in the two conditions was 16 g which was similar to the difference in breakfast protein content of 14 g. Subjective hunger ratings were not different after the two types of breakfast, but VAS scores also were found to have no relationship to intakes of calories, protein, or carbohydrate at meals immediately following such ratings. Pupillometric responses did not differ when measured two hours after the different breakfasts, but the high variability in responses indicated difficulties in using pupil responses as measures of food interest.

A comparison of the method of determining food intake by weighing freely-selected meal consumption in the university cafeteria with the method of providing meals in a metabolic laboratory suggested that although there were similar labor-time costs in both methods of study, in studies using freely-selected food intakes as a dependent variable, the weighed cafeteria meals presented advantages in monetary costs and the availability of a greater variety of foods with which the subjects were familiar.

CHAPTER VIII

SUGGESTIONS FOR FURTHER STUDY

The design of this experiment could be altered in several ways to further examine the existence of a protein or carbohydrate feed-back mechanism in humans.

1. Subjects. Other categories of subjects such as obese adults or younger children could be used. Young women could serve as subjects through two menstrual cycles to determine changes which might occur with different hormonal levels.

2. Breakfasts. Using a similar design but controlling the percentage of calories as protein at breakfast would be feasible if subjects' usual breakfast intakes were observed for one or two weeks before experimentation. Being able to anticipate each subject's usual caloric intake at breakfast would enable the researcher to serve a greater portion of the breakfast as experimental cookies, and the wide range of fruit intakes would be avoided. Another change in the breakfasts could be to use food stuffs with contents known to the subjects. This could test the role of cognition in meal selection. This experimental design could be used, but rather than adding starch and milk powder to the cookies, the cookies could be made with the usual recipe. The subjects could

consume milk or a starch drink with the cookies.

3. Measurement of food intake. Food intake could be measured at various times throughout the morning. Presentation of mid-morning snacks with a range of protein and carbohydrate contents would determine whether the four hour time span was too long to examine the feed-back mechanism.

4. Measurement of food selection. Initial quantities of foods chosen at meals might be more revealing of a feed-back mechanism than actual consumption. Changes in food interest may occur during eating. Some practicalities would confuse this measure, however. Plate sizes, served portions, and usual portion sizes all contribute to the amounts of foods initially selected. Some of the men in this study ate more in one meal than could be placed on the cafeteria tray at one time. They may habitually choose several "helpings" because of this limitation. Subjects' speeds of eating and desire for foods at appropriate temperatures would also affect the amounts placed on the tray at any one time.

5. Length of study. It may take some time for food habits to change in response to a physiological feed-back mechanism. Extension of the study so that each breakfast was served every day for 3 to 4 weeks might result in some changes in subsequent food consumption that would be apparent. The practical limitations of scheduling, however, may present some problems. Scheduling during this study

required flexibility because of illness and a champion basketball team.

6. Observation rather than experimentation. Using the method of food intake measurement, longer term studies of usual eating patterns could be observed in larger numbers of subjects. Periodic blood sampling in a random fashion could elucidate the relationship of plasma amino acid ratios and usual eating patterns.

7. Pupillometry. Tests of the intrasubject reliability of pupil responses should be made. There may be more reliability in same day measurements than in those made on different days. If this is the case, then the inclusion of pupillometry should provide baseline, e.g., fasting values, measures for comparison to avoid the variations which were found here.

We may never be able to understand the complete relationship between physiology and behavior, especially in humans. Rat models of behavior with regard to food are lacking both a genetic structure which has the unique evolutionary pressure of the human culture and the behaviors learned in many years of socialization. Sociocultural factors may play a part in human food selection that we may acknowledge but not yet be able to measure. Katz (1982) suggests that although we have developed considerable understanding of the biological system and the central nervous system of humans,

> there is little systematic understanding about analogous ways in which the sociocultural system develops, changes, stores, transforms, and communicates (i.e., evolves) its information, particularly in response to the needs of the biological system. (p. 175)

Food patterns developed in human subjects may be very resistant to alteration by physiological cues until these cues are very strong as in drug manipulation or illness.

BIBLIOGRAPHY

Abramowitz ES, Baker AH, Fleischer SF. Onset of depressive psychiatric crises and the menstrual cycle. Am J Psychiatry 1982;139:475-478.

Acheson KJ, Campbell IT, Edholm OG, Miller DS, Stock MJ. The measurement of food and energy intake in man: an evaluation of some techniques. Am J Clin Nutr 1980;33:1147-1154.

Adelson SG. Some problems in collecting dietary data from individuals. J Am Diet Assn 1960;36:453-461.

Adibi SA. Metabolism of branched-chain amino acids in altered nutrition. Metabolism 1976;25:1287-1302.

Adibi SA, Drash AL. Hormone and amino acid levels in altered nutritional states. J Lab Clin Med 1970;76:722-732.

Anderson GH. Regulation of protein intake by plasma amino acids. Adv Nutr Res 1977;1:145-165.

Anderson GH, Ashley DVM. Correlation of the plasma tyrosine to phenylalanine ratio with energy intake in self-selecting weanling rats. Life Sci 1977;21:1227-1234.

Anderson GH, Blendis LM. Plasma neutral amino acid ratios in normal man and in patients with hepatic encephalopathy: correlations with self-selected protein and energy consumption. Am J Clin Nutr 1981;34:377-385.

Anderson GH, Blendis LM, Kessel BR, Dietrich L, Jeejeeboy KN. Metabolic determination of food selection in man. Fed Proc 1979;38:607 (Abs. 2004).

Anderson GH, Blendis LM, Shillabeer G, Krulewitz J. Correlation between the plasma tryptophan to neutral amino acid ratio and dietary protein-energy selection in man. Fed Proc 1978;37:788.

Armstrong MD, Stave U. A study of plasma free amino acid levels. III. Variations during growth and aging. Metabolism 1973;22:571-578.

Ashley DV, Anderson GH. Correlation between the plasma tryptophan to neutral amino acid ratio and protein intake in the self-selecting weanling rat. J Nutr 1975a;105:1412-1421.

Ashley DVM, Anderson GH. Food intake regulation in the weanling rat: effects of the most limiting essential amino acids of gluten, casein, and zein on the self-selection of protein and energy. J Nutr 1975b;105:1405-1411.

Ashley DVM, Anderson GH. Protein intake regulation in the weanling rat: effects of addditions of lysine, arginine, and ammonia on the selection of gluten and energy. Life Sci 1977a;21:1235-1244.

Ashley DVM, Anderson GH. Selective decrease in protein intake following drug-induced brain serotonin depletion in the self-selecting weanling rat. Fed Proc 1977b;36:1153.

Ashley DV, Barclay DV, Chauffard FA, Moennoz D, Leathwood PD. Plasma amino acid responses in humans to evening meals of differing nutritional composition. Am J Clin Nutr 1982; 36:143-153.

Ashley DVM, Coscina DV, Anderson GH. Selective decrease in protein intake following brain serotonin depletion. Life Sci 1979;24:973-984.

Ashley DV, Curzon G. Effects of long-term low dietary tryptophan intake on determinants of 5-hydroxytryptamine metabolism in the brains of young rats. J Neurochem 1981; 37:1385-1393.

Balogh M, Kahn HZ, Medalie JH. Random repeat 24-hour dietary recalls. Am J Clin Nutr 1971;24:304-310.

Barlow JD. Pupillary size as an index of preference in political candidates. Percept Mot Skills 1969;28:587-590.

Beaton GH, Milner J, Corey P, McQuire V, Cousins M, Stewart E, deRamos M, Hewitt D, Brambsch PV, Kassim N, Little JA. Sources of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. Am J Clin Nutr 1979;32:2546-2559.

Beijk J, DeJong MA. De Reacties van Pupil en Hart op Aangename en Onaangename Stimuli. Nederlands Tijdschrift voor de Psychologie en Haar Grensgebieden 1971;26:615-638.

Bell RHV. The use of the herb layer by grazing ungulates in the Serengeti. In: Watson A, ed. Animal populations in relation to their food resources. Oxford:Blackwell; 1970:111-123.

Berrien FK, Huntington GH. An exploratory study of pupillary responses during deception. J Exp Psych 1943;26:443-449.

Blaha, SJM. Measurements of pupil size in response to pictures of food with changes in the percentage of blood glucose in underweight, normal weight, overweight, and obese women. Unpublished master's thesis, Oregon State University;1977.

Bloxam DL, Warren WH. Error in the determination of tryptophan by the method of Denkla and Dewey: a revised procedure. Anal Biochem 1974;60:621-625.

Blundell JE, Leshem MB. The effect of 5-hydroxytryptophan on food intake and on the anorexic action of amphetamine and fenfluramine. J Pharm Pharmacol 1975;27:31-37.

Bolton RP, Heaton KW, Burroughs LF. The role of dietary fiber in satiety, glucose, and insulin: studies with fruit and fruit juice. Am J Clin Nutr 1981;34:211-217.

Booth DA. Acquired sensory preference for protein in diabetic and normal rats. Physiolog Psychol 1974a;2:344-348.

Booth DA. Food intake compensation for increase or decrease in the protein content of the diet. Behav Biol 1974b;12:31-40.

Booth DA, Toates FM, Platt SV. Control system for hunger and its implications in animals and man. In: Novin D. Wyrwicka W, Bray GA, eds. Hunger: basic mechanisms and clinical implications. New York:Raven Press, 1976:127-143.

Bouma H. Size of the static pupil as a function of wavelength and luminosity of the light incident on the human eye. Nature 1962;293:690-691.

Bourne HR, Bunney WE, Colburn RW, Davis JM, Davis JN, Shaw DM, Coppen AJ. Noradrenaline, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in hindbrains of suicidal patients. Lancet 1968;2:805-808.

Bricker ML, Shively RF, Smith JM, Mitchell HH, Hamilton TS. The protein requirements of college women on high cereal diets with observations on the adequacy of short balance periods. J Nutr 1948;37:163-183.

Burke BS. The dietary history as a tool in research. J Am Diet Assn 1947;23:1041-1046.

Campbell VA, Dodds ML. Limitations of the 24-hr recall: collecting dietary information from groups of older people. J Am Diet Assn 1967;51:29-33.

Chase TN, Shoulson I. Behavioral and biochemical effects of fenfluramine in patients with neurologic disease. Postgrad Med J 1975;51(Suppl. 1):105-109.

Chouianard G, Young SN, Annable L, Sourkes TL. Tryptophan-nicotinamide, imipramine, and their combination in depression. Acta Psychiat Scand 1979;59:395-414.

Church CF, Church HN. Food values of portions commonly used (12th ed.). Philadelphia:Lippincott, 1975.

Clineschmidt BV. 5,6-Dihydroxytryptamine: suppression of the anorexigenic action of fenfluramine. Eur J Pharmacol 1973;24:405-409.

Collier G, Leshner AI, Squibb RL. Dietary self-selection in active and non-active rats. Physiol Behav 1969;4:79-81.

Colemenares JL, Wurtman RJ, Fernstrom JD. Effects of ingestion of a carbohydrate-fat meal on the levels and synthesis of 5-hydroxyindoles in various regions of the rat central nervous system. J Neurochem 1975;25:825-829.

Colman FD, Paivio A. Pupillary response and galvanic skin response during an imagery task. Psychonom Sci 1969;16: 296-297.

Coscina DV, Stancer HC. Selective blockade of hypothalamic hyperphagia and obesity in rats by serotonin depleting midbrain lesions. Science 1977;195:416-419.

Costa E, Neff HN. Estimation of turnover rates to study the metabolic regulation of the steady-state level of neuronal monoamines. In: Lathja A, ed. Handbook of neurochemistry. Vol IV. New York:Plenum Press;1970:45-90.

Cox BD, Calame DP. Changes in plasma amino acid levels during the human menstrual cycle and in early pregnancy: a preliminary report. Horm Metab Res 1978;10:428-433.

Crandall EA, Fernstrom JD. Acute changes in brain tryptophan and serotonin after carbohydrate or protein ingestion by diabetic rats. Diabetes 1980;29:460-466.

Culley WJ, Saunders RN, Mertz ET, Jolly DH. Effect of a tryptophan deficient diet on brain serotonin and plasma tryptophan level. Proc Soc Exp Biol Med 1962;113:645-648.

Curzon G. Relationships between plasma, CSF and brain tryptophan. J Neural Trans 1979;15:81-92.

Curzon G, Knott PJ. Fatty acids and the disposition of tryptophan. In: Wurtman RJ, ed. Aromatic amino acids in the brain. Ciba Foundation Symp. Amsterdam:Elsevier-North Holland;1974:217-229.

Dalvit SP. The effect of the menstrual cycle on patterns of food intake. Am J Clin Nutr 1981;34:1811-1815.

Davis CM. Self-selection of diets by newly weaned infants: an experimental study. Amer J Dis Child 1928;36:651-689.

DeMyer MK, Shea PA, Hendrie HC, Yoshimura NN. Plasma tryptophan and five other amino acids in depressed and normal subjects. Arch Gen Psychiatry 1981;38:642-646.

Denckla WD, Dewey HK. The determination of tryptophan in plasma, liver, and urine. J Lab Clin Med 1967;69:160-169.

Donelson E, Nims B, Hunscher HA, Shukers CF, Macy IG. Simple methods for metabolic balance studies and their interpretation. J Home Ec 1931;23:267-272.

Eccleston D, Ashcroft GW, Crawford TB, Stanton JB, Wood D, McTurk PH. Effect of L-tryptophan administration on 5-HIAA in cerebrospinal fluid in man. J Neurol Neurosurg Psychiat 1970;33:269-272.

Erikson EH. Childhood and society (2nd ed.). New York: Norton;1963.

Feigin RD, Klainer AS, Beisel WR. Circadian periodicity of blood amino acids in adult men. Nature 1967;215:512-514.

Feigin RD, Klainer AS, Beisel WR. Factors affecting periodicity of blood amino acids in man. Metab Clin Exp 1968; 17:764-775.

Fernstom JD, Faller DV. Neutral amino acids in the brain: changes in response to food ingestion. J Neurochem 1978; 30:1531-1538.

Fernstrom JD, Faller DV, Shabshelowitz H. Acute reduction of brain serotonin and 5-HIAA following food consumption: correlation with the ratio of serum tryptophan to the sum of competing neutral amino acids. J Neur Trans 1975;36: 113-121.

Fernstrom JD, Hirsch JM, Madras BK, Sudarsky L. Effects of skim milk, whole milk and light cream on serum tryptophan binding and brain tryptophan concentrations in rats. J Nutr 1975;105:1359-1362.

Fernstrom JD, Larin F, Wurtman RJ. Correlation between brain tryptophan and plasma neutral amino acid levels following food consumption in the rat. Life Sci 1973;13:517-524.

Fernstrom JD, Wurtman RJ. Brain serotonin content: physiological dependence on plasma tryptophan levels. Science 1971;173:149-152.

Fernstrom JD, Wurtman RJ. Brain serotonin content: physiological regulation by plasma neutral amino acids. Science 1972;178:414-416.

Fernstrom JD, Wurtman RJ. Nutrition and the brain. Sci Am 1974;230(2):84-91.

Fernstrom JD, Wurtman RJ, Hammarstrom-Wiklund B, Rand WM, Munro HN, Davidson CS. Diurnal variations in plasma concentrations of tryptophan, tyrosine and other neutral amino acids and effect of dietary protein intake. Am J Clin Nutr 1979;32:1912-1922.

Fuller RW, Snoddy HD, Hemrick SK. Effects of fenfluramine and norfenfluramine on brain serotonin metabolism in rats (40021). Proc Soc Exp Biol Med 1978;157:202-205.

Gallagher DW, Aghajanian GK. Inhibition of firing of raphe neurons by tryptophan and 5-hydroxytryptophan: blockade by inhibiting serotonin synthesis with Ro-4-46021. Neuropharmacology 1976;15:149-156.

Garattini S, Buczko W, Jori A, Samanin R. The mechanism of action of fenfluramine. Postgrad Med J 1975;51(Suppl 1): 27-35.

Garn SM, Larkin FA, Cole PE. The real problem with 1-day diet records. Am J Clin Nutr 1978;31:1114-1116.

Gaulin SJC, Konner M. On the natural diet of primates, including humans. In: Wurtman RJ, Wurtman JJ, eds. Nutrition and the brain. Vol 1. New York:Raven Press, 1977:1-86.

Gersovitz M, Madden JP, Smiciklas-Wright H. Validity of the 24-hr. dietary recall and seven-day record for group comparisons. J Am Diet Assn 1978;73:48-55.

Gessa GL, Taliamonte A. Serum free tryptophan: control of brain concntrations of tryptophan and of synthesis of 5-hydroxytryptamine. In: Wurtman RJ, ed. Aromatic amino acids in the brain. Ciba Foundation Symp. Amsterdam: North Holland;1974:207-216.

Gibson CJ, Wurtman RJ. Physiological control of brain norepinephrine synthesis by brain tyrosine concentration. Life Sci 1978;22:1399-1406.

Gillman PK, Bartlett JR, Bridges PK, Hunt A, Patel AJ, Kantameneni BD, Curzon G. Indolic substances in plasma, cerebrospinal fluid, and frontal cortex of human subjects infused with saline or tryptophan. J Neurochem 1981;37:410-417.

Goldwater BC. Psychological significance of pupillary movements. Psychol Bull 1972;77:340-355.

Goudie AJ, Thornton EW, Wheeler TJ. Effects of Lilly 110-140, a specific inhibitor of 5-hydroxytryptamine uptake, on food intake and on 5-hydroxytryptophan induced anorexia. Evidence for serotoninergic inhibition of feeding. J Pharm Pharmacol 1976;28:318-320.

Grahme-Smith D. How important is the synthesis of brain 5-hydroxytryptamine in the physiological control of its central function? Adv Biochem Psychopharmac 1974;10:83-91.

Greenberg DM. Metabolic pathways. Vol 11. New York:Academic Press;1961:pp. 159-161.

Harper AE. Protein and amino acids in the regulation of food intake. In: Novin D, Wyrwicka W, Bray GA, eds. Hunger: basic mechanisms and clinical implications. New York:Raven Press;1976:103-113.

Harper AE, Peters JC. Amino acid signals and their integration with muscle metabolism. In: Cioffi LA, James WPT, vanItallie TB, eds. The body weight regulatory system: normal and disturbed mechanisms. New York:Raven Press;1981:33-38.

Harper HA, Rodwell VW, Mayes PA. Review of physiological chemistry, 16th Ed. Los Altos, CA:Lange Medical Publications;1977:p. 386.

Hess EH. Attitude and pupil size. Sci Am 1965;212(4): 46-54.

Hess EH. Pupillometrics: a method of studying mental, emotional and sensory processes. In: Greenfield NS, Sternbach RA, eds. Handbook of psychophysiology. New York:Reinhart and Winston;1972:491-531.

Hess EH. The role of pupil size in communication. Sci Am 1975a;233:110-119.

Hess EH. The tell-tale eye. New York:Van Nostrand Reinhold;1975b.

Hess EH, Polt JM. Changes in pupil size as a measure of taste difference. Percept Motor Skills 1966;23:451-455.

Hill SW. Eating responses of humans during dinner meals. J Comp Physiol Psychol 1974;86:652-657.

Hirsch J. Obesity: a perspective. In: Bray GA, ed. Recent advances in obesity research: II. London: Newman; 1978:1-5.

Hirsch JA, Wurtman RJ. Effects of D- or L-isomers of fenfluramine and norfenfluramine on food choice by rats. In: Garattini S, Samanin R, eds. Anorectic agents: mechanisms of action and tolerance. New York:Raven Press;1981:159-168.

Hoover LW. Comparison of data banks. In: Bealand VA, Lewis MJ, eds. Proceedings of the symposium on dietary data collection, analysis, and significance (research bull no 675). Amherst:Massachusetts Agricultural Experiment Station;1982:59-64.

Hussein MA, Young VR, Murray E, Scrimshaw NS. Daily fluctuation of plasma amino acid levels in adult men: effect of dietary tryptophan intake and distribution of meals. J Nutr 1971;101:61-70.

Hutson PH, Knott PJ, Curzon G. Control of brain tryptophan concentration in rats on a high fat diet. Nature 1976;262: 142-143.

Jain S, Kyriakides M, Silverstone T, Turner P. The effect of small and moderate doses of d-amphetamine on hunger, mood, and arousal in man. Psychopharmacology 1980;70:109-111.

Janisse MP. Pupillometry: some advances, problems and solutions. In: Janisse MP, ed. Pupillary dynamics and behavior. New York:Plenum Press;1974:1-8.

Joyce D, Mrosovsky N. Eating, drinking and activity in rats following 5-hyroxytryptophan (5-HTP) administration. Psychopharmacologia 1964;5:417-423.

Kahneman D, Tursky B, Shapiro, D, Crider A. Pupillary, heart rate, and skin resistance changes during a mental task. J Exper Psychol 1969;79:164-167.

Kanarek RB, Marks-Kaufman R, Lipeles BJ. Increased carbohydrate intake as a function of insulin administration in rats. Physiol and Behav 1980;25:779-782.

Katz SH. Food, behavior and viocultural evolution. In: Barker LM, ed. The psychobiology of human food selection. Westport, CT: AVI;1982:171-188.

Knapp S, Mandell AJ. Coincidence of blockade of synaptosomal 5-hydroxytryptamine uptake and decrease in tryptophan hydroxylase activity: effects of fenfluramine. J Pharmacol Exp Ther 1976;198:123-132.

Knott PJ, Curzon G. Free tryptophan in plasma and brain tryptophan metabolism. Natur 1972;239:452-453.

Kratz CM. Response to protein dilution in the self-selecting rat. Fed Proc 1977;36:1152(Abs 4555).

Kuhar MJ, Aghajanian GR, Roth RH. Tryptophan hydroxylase activity and synaptosomal uptake of serotonin in discrete brain regions after midbrain raphe lesion: correlations with serotonin levels and histochemical fluorescence. Brain Res 1972;44:165-176.

Kyriakides M, Silverstone T. Comparison of the effects of d-amphetamine and fenfluramine on hunger and food intake in man. Neuropharmacology 1979;18:1007-1008.

Latham CJ, Blundell JE. Evidence for the effect of tryptophan on the pattern of food consumption in free feeding and food deprived rats. Life Sci 1979;24:1971-1978.

Leathwood PD, Ashley DV. Nutrients as regulators of food choice. In: Cioffi LA, James WPT, VanItallie TB, eds. The body weight regulatory system: normal and disturbed mechanisms. New York:Raven Press;1981:263-269.

Lee RB. What hunters do for a living or how to make out on scarce resources. In: Lee RB, Devore I, eds. Man the hunter. Chicago:Aldine;1968:30-55.

Lehninger AL. Biochemistry, 2nd ed. New York:Worth;1975: p.717.

Li ETS, Anderson GH. Meal composition influences subsequent food selection in the young rat. Physiol Behav 1982a;29:779-783.

Li ETS, Anderson GH. Self-selected meal composition, circadian rhythms and meal responses in plasma and brain tryptophan and 5-hydroxytryptamine in rats. J Nutr 1982b;112: 2001-2010.

Loewenfeld IE, Rosskothen HD. Infrared pupil camera: a new method for mass screening and clinical use. Am J Opthalmol 1974;78:304-313.

Lovenberg W, Beselaar GH, Bensinger RE, Jackson RL. Physiological and drug-induced regulation of serotonin synthesis. In: Barchas J, Usdin E, eds. Serotonin and behavior. New York:Academic Press;1973:49-54.

Lowenstein O, Loewenfeld IE. Influence of retinal adaptation upon the pupillary reflex to light in normal man: Part II. Effect of adaptation to dim illumination upon pupillary reflexes elicited by bright light. Am J Opthalmol 1961;51:644-654.

Madras BK, Cohen EL, Fernstrom JD, Larin F, Munro HN, Wurtman RJ. Dietary carbohydrate increases brain tryptophan and decreases serum-free tryptophan. Nature 1973;244:34-35.

Madras BK, Cohen EL, Messing R, Munro HN, Wurtman RJ. Relevance of free tryptophan in serum to tissue tryptophan concentrations. Metabolism 1974;23:1107-1116.

Marr JW. Individual dietary surveys: purposes and methods. World Rev Nutr Diet 1971;13:105-164.

Maslow AH. The farther reaches of human nature. New York: Viking Press; 1971.

McKean CM. The effects of high phenylalanine concentrations on serotonin and catecholamine metabolism in the human brain. Brain Res 1972;47:469-476.

Mellinkoff SM, Frankland M, Boyle D, Greipel M. Relationship between serum amino acid concentration and fluctuations in appetite. J Appl Physiol 1956;8:535-538.

Metalis SA, Hess EH, Beaver PW. Pupillometric analysis of two theories of obesity. Percept Motor Skills 1982;55:87-92.

Meyer FL, Brown ML, Wright HJ, Hathaway ML. A standardized diet for metabolic studies. Washington, DC:U.S. Dept of Agriculture;1955.

Milsom JP, Morgan MY, Sherlock S. Factors affecting plasma amino acid concentrations in control subjects. Metabolism 1979;28:313-319.

Mitchell HH. The maintenance requirement of protein. In: Mitchell HH, ed. Comparative nutrition of man and domestic animals. Vol 1. New York:Academic Press;1962:129-191.

Loewenfeld IE, Rosskothen HD. Infrared pupil camera: a new method for mass screening and clinical use. Am J Opthalmol 1974;78:304-313.

Lovenberg W, Beselaar GH, Bensinger RE, Jackson RL. Physiological and drug-induced regulation of serotonin synthesis. In: Barchas J, Usdin E, eds. Serotonin and behavior. New York:Academic Press;1973:49-54.

Lowenstein O, Loewenfeld IE. Influence of retinal adaptation upon the pupillary reflex to light in normal man: Part II. Effect of adaptation to dim illumination upon pupillary reflexes elicited by bright light. Am J Opthalmol 1961;51:644-654.

Madras BK, Cohen EL, Fernstrom JD, Larin F, Munro HN, Wurtman RJ. Dietary carbohydrate increases brain tryptophan and decreases serum-free tryptophan. Nature 1973;244:34-35.

Madras BK, Cohen EL, Messing R, Munro HN, Wurtman RJ. Relevance of free tryptophan in serum to tissue tryptophan concentrations. Metabolism 1974;23:1107-1116.

Marr JW. Individual dietary surveys: purposes and methods. World Rev Nutr Diet 1971;13:105-164.

Maslow AH. The farther reaches of human nature. New York: Viking Press; 1971.

McKean CM. The effects of high phenylalanine concentrations on serotonin and catecholamine metabolism in the human brain. Brain Res 1972;47:469-476.

Mellinkoff SM, Frankland M, Boyle D, Greipel M. Relationship between serum amino acid concentration and fluctuations in appetite. J Appl Physiol 1956;8:535-538.

Metalis SA, Hess EH, Beaver PW. Pupillometric analysis of two theories of obesity. Percept Motor Skills 1982;55:87-92.

Meyer FL, Brown ML, Wright HJ, Hathaway ML. A standardized diet for metabolic studies. Washington, DC:U.S. Dept of Agriculture;1955.

Milsom JP, Morgan MY, Sherlock S. Factors affecting plasma amino acid concentrations in control subjects. Metabolism 1979;28:313-319.

Mitchell HH. The maintenance requirement of protein. In: Mitchell HH, ed. Comparative nutrition of man and domestic animals. Vol 1. New York:Academic Press;1962:129-191.

Møller SE, Kirk L, Fremming KH. Plasma amino acids as an index for subgroups in manic depressive psychosis: correlation to effect of tryptophan. Psychopharmacol 1976;49: 205-213.

Morrison SD, Russell FC, Stevenson J. Estimating food intake by questioning and weighing: a one-day survey of eight subjects. Br J Nutr 1949;3:v.

Munro HN. General aspects of the regulation of protein metabolism by diet and hormone. In: Munro HN, Allison JB, eds. Mammalian protein metabolism. Vol 1. New York: Academic Press;1964:381-481.

Munro HN. Evolution of protein metabolism in mammals. In: Munro HN, ed. Mammalian protein metabolism. Vol 3. New York:Academic Press;1969:133-182.

Musten B, Peace D, Anderson GH. Food intake regulation in the weanling rat: self-selection of protein and energy. J Nutr 1974;104:563-572.

Nasset ES, Ju JS. Amino acids and glucose in human blood plasma after beef and non protein meals. Proc Soc Exp Biol Med 1969;132:1077-1080.

Nathanson JA, Greengard P. "Second messengers" in the brain. Sci Am 1977;237(2):108-119.

Noble RE. Effect of cyproheptadine on appetite and weight gain in adults. JAMA 1969;209:2054-2055.

Oepen H, Oepen I. Geschecuts-spezifiscle Konzentration suhtherscheide den Serum-amino Sauren. Klin Wochenschr 1965;43:211-214.

Oldendorf WH. Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. Am J Physiol 1971;221:1629-1639.

Oldendorf WH, Szabo J. Amino acid assignment to one of three blood-brain barrier amino acid carriers. Am J Physiol 1976;230:94-98.

Overman SR. Dietary self-selection by animals. Psychol Bull 1976;83:218-235.

Palmer T, Rossiter MA, Levin B, Oberholzer VG. The effect of protein loads on plasma amino acid levels. Clin Sci Mol Med 1973;45:827-832.

Panksepp J, Nance DM. Effects of para-chlorophenylalanine on food intake in rats. Physiol Psychol 1974;2:344-348.

Pardridge WM. Kinetics of competitve inhibition of neutral amino acid transport across the blood-brain barrier. J Neurochem 1977;28:103-108.

Pardridge WM. Tryptophan transport through the blood-brain barrier: in vivo measurement of free and albumin-bound amino acid. Life Sci 1979;25:1519-1528.

Paykel ES, Mueller PS, delaVergne PM. Amitriptyline, weight gain and carbohydrate craving: a side effect. Br J Psychiat 1973;123:501-507.

Pekkarinen M. Methodology in the collection of food consumption data. World Rev Nutr Diet 1970;12:145-171.

Peng Y, Meliza LL, Vavich MG, Kemmer AR. Effects of amino acid imbalance and protein content of diets on food intake and preference of young, adult and diabetic rats. J Nutr 1975;105:1395-1404.

Perez-Cruet J, Chase TN, Murphy DL. Dietary regulation of brain tryptophan metabolism by plasma ratio of free tryptophan and neutral amino acids in humans. Nature 1974;248: 693-695.

Peters JC, Tews JK, Harper AE. L-Tryptophan injection fails to alter nutrient selection by rats. Trans Am Soc Neurochem 1981;12:404(Abstr).

Pilgrim FJ. The components of food acceptance and their measurement. Am J Clin Nutr 1957;5:171-175.

Pilgrim FJ. Human food attitudes and consumption. In: Code DF, ed. Handbook of physiology, Sec 6, Alimentary canal, Vol 1, Control of food and water intake. Washington DC:American Physiological Society;1967:139-146.

Pollock JD, Rowland N. Peripherally administered serotonin decreases food intake in rats. Pharmacol Biochem Behav 1981;15:179-183.

Pozefsky T, Felig P, Tobin JD, Soeldner JS, Cahill GF,Jr. Amino acid balance across tissues of the forearm in post-absorptive man. Effects of insulin at two dose levels. J Clin Invest 1969;48:2273-2282.

Reinhard JF,Jr, Wurtman, RJ. Relation between brain 5-HIAA levels and the release of serotonin into brain synapses. Life Sci 1977;21:1741-1746.

Robertson FW. Genetic variation in the nutrition of Drosophila melanogaster: some general inferences. Proc Nutr Soc 1962;21:169-178.

Rolls BJ. Palatability and food preference. In: Cioffi LA, James WPT, vanItallie TB, eds. The body weight regulatory system: normal and disturbed mechanisms. New York: Raven Press;1981:271-277.

Rozin P. Are carbohydrate and protein intakes separately regulated? J Comp Physiol Psychol 1968;65:23-29.

Rozin P. The selection of foods by rats, humans, and other animals. In: Rosenblatt J, Hinde RA, Beer C, Shaw E, eds. Advances in the study of behaviour. Vol 6. New York: Academic Press;1976:21-76.

Rutman RJ, Demptster E, Tarver H. Genetic differences in methionine uptake by surviving tissue. J Biol Chem 1949; 177:491-492.

Saavedra JM. Distribution of serotonin and synthesizing enzymes in discrete areas of the brain. Fed Proc 1977;35: 2134-2141.

Samanin R, Bendotti C, Miranda F, Garattini S. Decrease of food intake by quipazine in the rat: relation to serotoninergic receptor stimulation. J Pharm Pharmacol 1977; 29:53-54.

Schaefer O. Pre- and post-natal growth acceleration and increased sugar consumption in Canadian Eskimos. Can Med Assoc J 1970;103:1059-1068.

Schoenfeld TA, Hamilton LW. Multiple factors in short-term behavioral control of protein intake in rats. J Comp Physiol Psychol 1976;90:1092-1104.

Shoulson I, Chase TN. Fenfluramine in man: hypophagia associated with diminished serotonin turnover. Clin Pharmacol Ther 1975;17:616-621.

Siegel PS. The completion compulsion in human eating. Psychol Rept 1957;3:15-16.

Silverstone JT, Stunkard AJ. The anorectic effect of dexamphetamine sulphate. Br J Pharmacol Chemother 1968;33: 513-522.

Silverstone T. The anorectic effect of a long-acting preparation of phentermine. Psychopharmacologia 1972;25:315-320.

Silverstone T, Fincham J. Experimental techniques for the measurement of hunger and food intake in man for use in the evaluation of anorectic drugs. In: Garattini S, Samanin R, eds. Central mechanisms of anorectic drugs. New York: Raven Press;1978:375-382.

Silverstone T, Schuyler D. The effect of cyproheptadine on hunger, calorie intake and body weight in man. Psychopharmacologia 1975;40:335-340.

Smith SL, Sauder C. Food craving, depression and premenstrual problems. Psychosom Med 1969;31:281-287.

Snyderman SE, Holt LE,Jr., Norton PM, Roitman E. Effect of high and low intakes of individual amino acids on the plasma aminogram. In: Leathem JH, ed. Protein nutrition and free amino acid patterns. New Brunswick, NJ:Rutgers Univ Press;1968:19-31.

Spence DP, Ehrenberg B. The effects of oral deprivation and responses to subliminal and supraliminal verbal food stimuli. J Abnorm Soc Psychol 1964;69:10-18.

Spiegel TA. Caloric regulation of food intake in man. J Comp Physiol Psychol 1973;84:24-37.

Steel TGD, Torre JH. Principles and procedures of statistics. New York:McGraw-Hill;1960.

Stock AL, Wheeler EF. Evaluation of meals cooked by large-scale methods: a comparison of chemical analysis and calculation from food tables. Br J Nutr 1972;27:439-448.

Strata A, Zuliani U. Amphetamines in the treatment of obesity. In: Garattini S, Samanin R, eds. Central mechanisms of anorectic drugs. New York:Raven Press;1978:405-418.

Stuff JE, Garza C, Smith EO, Nichols BL, Montandon CM. A comparison of dietary methods in nutritional studies. Am J Clin Nutr 1983;37:300-306.

Sugrue MF, Charlton KG, Mireylers SE, McIndewar I. Drug-induced anorexia and monoamine reuptake inhibition. In: Garattini S, Samanin R, eds. Central mechanisms of anorectic drugs. New York:Raven Press;1978:191-203.

Swendseid ME, Yamada C, Vinyard E, Figueroa WG, Drenick EJ. Plasma amino acid levels in subjects fed isonitrogenous diets containing different proportions of fat and carbohydrate. Am J Clin Nutr 1967;20:52-55.

Todd KS, Hudes M, Calloway DH. Food intake measurements: problems and approaches. Am J Clin Nutr 1983;37:139-146.

Trulson MF, McCann MB. Comparison of dietary survey methods. J Am Diet Assoc 1959;35:672-676.

Tryon WW. Pupillometry: a survey of sources of variation. Psychophysiology 1975;12:90-93.

University of Minnesota Agricultural Experiment Station. Methods used for human metabolic studies in the North Central Region (tech bull no 225). University of Minnesota Agricultural Experiment Station; May, 1958.

Vartiainen I, Bastman-Heiskanen L. Increased appetite for protein after administration of alloxan. Endocrinology 1950;47:134-138.

Warren DE, Tedford WH,Jr., Flynn WE. Behavioral effects of cyclic changes in serotonin during the human menstrual cycle. Med Hypotheses 1979;4:359-364.

Watt BK, Merrill AL. Composition of foods: agriculture handbook no 8. Washington, DC:U S Dept of Agriculture; 1963.

Weinberger SB, Mandell AJ. Raphe neuron firing rate and serotonin release versus serotonin biosynthesis as modulators of food intake in the rat. Abstract volume, Soc for Neurosci, 8th Ann Meeting 1978;4:Abstr 561.

Weissman A. Behavioral pharmacology of p-chlorophenylalanine (PCPA). In: Barchas J, Usdin E, eds. Serotonin and behavior. New York:Academic Press;1973:235-248.

Whiting MG, Leverton RM. Reliability of dietary appraisal: comparisons between laboratory analysis and calculation from tables of food values. Am J Pub Health 1960;50:815-823.

Wooley OW, Wooley SC, Dunham RB. Can calories be perceived and do they affect hunger in obese and nonobese human? J Comp Physiol Psychol 1972;80:250-258.

Wurtman JJ, Wurtman RJ. Experimental model for examining changes in food choice caused by drugs: effects of fenfluramine and fluoxetine. Fed Proc 1977a;35:1132(Abstr 4554).

Wurtman JJ, Wurtman RJ. Fenfluramine and fluoxetine spare protein consumption while suppressing caloric intake by rats. Science 1977b;198:1178-1180.

Wurtman JJ, Wurtman RJ. Drugs that enhance central serotoninergic transmission diminish elective carbohydrate consumption by rats. Life Sci 1979a;24:895-904.

Wurtman JJ, Wurtman RJ. Fenfluramine and other serotoninergic drugs depress food intake and carbohydrate consumption while sparing protein consumption. Curr Med Res Opin 1979b;6(Suppl 1):28-33.

Wurtman JJ, Wurtman RJ. Suppression of carbohydrate consumption as snacks and at mealtime by DL-fenfluramine or tryptophan. In: Garrattini S, Samanin R, eds. Anorectic agents: mechanisms of actions and of tolerance. New York: Raven Press;1981:169-182.

Wurtman JJ, Wurtman RJ, Growdon JH, Henry P, Lipscomb A, Zeisel SH. Carbohydrate craving in obese people: suppression by treatments affecting serotonergic transmission. Int J Eating Disorders 1981;1:2-15.

Wurtman RJ. When--and why--should nutritional state control neurotransmitter synthesis? J Neural Transm 1979; Suppl 15:69-79.

Wurtman RJ. Nutrients that modify brain function. Sci Am 1982:246(4):50-59.

Wurtman RJ, Fernstrom JD. Control of brain monoamine synthesis by diet and plasma amino acids. Am J Clin Nutr 1975;28:638-647.

Wurtman RJ, Hefti F, Melamud E. Precursor control of neurotransmitter synthesis. Pharmacol Rev 1981;32:315-325.

Wurtman RJ, Larin F, Mostafapour S, Fernstrom JD. Catechol synthesis: control by brain tyrosine concentration. Science 1974;185:183-184.

Wurtman RJ, Rose CM, Chou C, Larin FF. Daily rhythms in the concentrations of various amino acids in human plasma. N Engl J Med 1968;179:172-175.

Yaryura-Tobias JA, Chang A, Neziroglu F, Bhagavan HN. Changes in serum tryptophan, glucose and related measures in psychotics and neurotics. Fed Proc 1977;36:1132(Abstr 4557).

Yearick ES, Nadeau RG. Serum amino acid response to isocaloric test meals. Am J Clin Nutr 1967;20:338-344.

Young CM, Trulson MF. Methodology for dietary studies in epidemiological surveys: II. Strengths and weaknesses of existing methods. Am J Pub Health 1960;50:803-814.

Young SN, Sourkes TL. Tryptophan in the central nervous system: regulation and significance. Adv Neurochem 1977; 2:133-191.

Young VR, Hussein MA, Murray E, Scrimshaw NS. Tryptophan intake, spacing of meals, and diurnal fluctuations of plasma tryptophan in men. Am J Clin Nutr 1969;22:1563-1567.

Zambotti F, Carruba M, Vicentini L, Mantegazza P. Selective effect of a maize diet in reducing serum and brain tryptophan contents and blood and brain serotonin levels. Life Sci 1975;17:1663-1670.

Ziegler E. Height and weight of British men. Lancet 1969; 1:1318.

Zinneman HH, Nutall FQ, Goetz FC. Effect of endogenous insulin on human amino acid metabolism. Diabetes 1966;15: 5-8.

APPENDICES

APPENDIX A

Table A. Information on subjects.

Subject Number	Age (years)	Height (cm.)	Weight (kg.)
11	19	188	74.4
12	21	180	72.5
13	19	180	73.1
14	18	186	79.6
21	18	176	78.9
22	18	175	63.5
24	21	189	71.8
25	18	178	63.4
30	22	170	70.8
31	19	178	69.1
36	18	174	77.5
42	20	185	94.1
44	18	184	66.5
Mean	19.2	180	74.3

APPENDIX B

Table B. Plasma amino acid levels in pre-breakfast samples, moderate protein-moderate carbohydrate breakfast condition.

Subject	μmole/100 ml								
	TRP	VAL	ILE	LEU	TYR	PHE	TYR/ΣNAA	TRP/ΣNAA	TYR/PHE
11[a]	6.0	26.7	10.7	18.6	7.7	8.8	0.105	0.079	0.881
	6.6	28.3	10.6	18.9	7.0	7.8	0.097	0.091	0.900
12	7.6	27.2	11.3	21.1	9.1	6.4	0.124	0.100	1.409
	6.8	32.0	12.4	20.6	10.5	8.5	0.130	0.081	1.229
13	6.4	23.5	7.1	14.2	5.6	6.7	0.097	0.113	0.842
	8.0	24.0	7.9	17.1	6.1	6.6	0.095	0.130	0.915
14	7.0	27.3	9.0	18.4	9.7	7.4	0.141	0.097	1.325
	8.6	27.6	9.4	18.3	9.6	6.8	0.135	0.120	1.415
21	6.7	26.8	9.7	16.9	9.0	8.3	0.132	0.095	1.082
	7.1	30.2	10.5	20.7	8.4	8.5	0.109	0.090	0.987
22	7.6	30.7	7.2	16.0	3.5	3.6	0.054	0.125	0.986
	5.5	33.1	9.2	17.2	7.1	8.3	0.097	0.073	0.854
24	6.7	23.2	8.1	16.0	7.8	7.1	0.127	0.108	1.096
	6.6	23.2	7.6	14.9	7.5	6.3	0.128	0.111	1.188
25	6.6	27.8	8.9	15.9	8.5	7.3	0.128	0.097	1.158
	7.2	28.7	9.8	17.2	8.7	7.6	0.124	0.100	1.156
30	6.8	28.2	8.9	16.0	6.7	7.6	0.099	0.100	0.876
	5.6	25.5	8.3	14.1	6.5	6.8	0.108	0.091	0.955
31	6.6	28.6	10.6	18.4	10.4	9.6	0.140	0.085	1.077
	6.6	29.0	8.6	16.4	8.2	7.1	0.121	0.096	1.156
36	6.8	29.5	8.5	16.4	7.1	8.4	0.101	0.098	0.842
	5.8	29.6	7.9	15.3	6.8	7.1	0.104	0.087	0.957
42	5.9	30.9	10.2	18.6	8.1	7.2	0.112	0.078	1.129
	5.8	28.0	11.4	17.8	6.4	6.0	0.093	0.084	1.074
44	6.6	28.5	11.3	17.4	7.9	7.6	0.122	0.100	1.042
	7.1	29.0	7.6	15.9	6.6	6.9	0.090	0.098	0.954

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX C

Table C. Plasma amino acid levels in pre-breakfast samples, low protein-high carbohydrate breakfast condition.

Subject	μmole/100 ml								
	TRP	VAL	ILE	LEU	TYR	PHE	TRP/ΣNAA	TYR/ΣNAA	TYR/PHE
11[a]	5.2	19.7	8.3	11.8	6.9	7.2	0.096	0.133	0.969
	6.4	25.4	10.1	17.7	7.0	7.5	0.094	0.105	0.937
12	5.6	27.3	10.6	17.7	8.9	7.6	0.078	0.129	1.165
	6.9	31.6	9.6	19.9	9.9	7.6	0.088	0.131	1.297
13	7.3	28.7	10.0	18.3	7.8	7.5	0.101	0.109	1.041
	8.0	29.5	8.4	17.0	6.8	7.0	0.117	0.098	0.972
14	8.1	30.3	9.6	18.8	9.4	8.3	0.106	0.125	1.135
	8.0	30.7	10.4	19.6	9.2	7.7	0.103	0.120	1.197
21	6.3	23.8	8.7	15.8	6.6	6.5	0.103	0.108	1.014
	6.9	26.0	8.9	16.8	8.2	7.6	0.102	0.125	1.086
22	6.6	29.2	7.6	18.2	6.2	6.0	0.098	0.092	1.042
	7.2	29.0	8.4	18.1	6.0	6.5	0.106	0.086	0.918
24	5.4	23.8	7.8	11.4	7.8	6.4	0.094	0.143	1.229
	5.6	18.8	5.7	10.6	6.4	5.4	0.119	0.138	1.182
25	6.1	21.0	7.6	12.6	8.5	7.1	0.107	0.156	1.196
	6.9	26.3	8.1	14.3	9.0	6.2	0.109	0.145	1.455
30	5.9	24,9	8.6	14.5	7.4	7.4	0.094	0.121	1.001
	5.4	30.6	8.3	15.5	7.4	7.3	0.078	0.110	1.005
31	7.1	34.5	10.1	19.4	7.2	7.7	0.090	0.092	0.945
	7.2	32.1	9.7	19.2	9.5	8.3	0.092	0.124	1.146
36	6.0	29.8	9.0	19.0	7.9	8.8	0.081	0.109	0.900
	4.9	29.0	8.4	17.0	7.1	7.6	0.071	0.107	0.933
42	6.1	28.8	10.4	18.2	9.3	7.1	0.083	0.132	1.318
	6.4	28.5	10.1	17.2	8.0	6.9	0.091	0.115	1.162
44	5.9	27.3	8.6	14.5	6.3	6.8	0.094	0.108	1.078
	6.5	28.4	9.0	18.8	7.7	7.5	0.091	0.109	1.015

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX D

Table D. Plasma amino acid levels in mid-morning (2 hrs post breakfast) samples, moderate protein-moderate carbohydrate breakfast condition.

Subject	μmole/100 ml								
	TRP	VAL	ILE	LEU	TYR	PHE	TRP/ΣNAA	TYR/ΣNAA	TYR/PHE
11[a]	6.7	28.8	9.8	18.2	7.9	8.4	0.092	0.110	0.944
	7.2	25.1	11.4	14.1	7.4	7.4	0.110	0.114	0.996
12	6.9	37.3	15.5	26.2	13.0	11.5	0.067	0.134	1.134
	7.5	31.9	12.4	22.2	12.9	9.1	0.085	0.155	1.415
13	6.7	31.1	8.4	17.7	9.1	7.1	0.091	0.128	1.273
	8.0	30.2	8.5	17.3	7.3	6.9	0.114	0.103	1.059
14	7.3	31.7	10.1	20.8	11.7	6.9	0.089	0.152	1.700
	8.0	32.0	11.1	22.0	12.1	7.9	0.094	0.149	1.154
21	5.9	22.8	6.9	13.3	8.8	6.6	0.101	0.158	1.337
	6.9	28.5	8.4	16.8	9.6	8.2	0.096	0.140	1.167
22	7.8	35.1	9.2	20.0	9.2	8.6	0.096	0.113	1.058
	5.6	30.7	10.1	20.1	8.0	7.8	0.073	0.108	1.039
24	7.3	28.5	9.5	19.9	8.2	6.8	0.101	0.114	1.214
	6.7	28.2	9.4	18.7	8.8	6.6	0.093	0.126	1.323
25	5.8	29.2	11.2	19.4	10.7	8.0	0.074	0.145	1.326
	7.3	41.5	11.3	21.8	10.0	8.3	0.079	0.111	1.210
30	8.0	34.7	11.5	20.7	10.2	9.8	0.093	0.121	1.042
	5.7	25.1	8.4	14.2	7.0	7.0	0.092	0.115	0.996
31	7.2	30.0	9.9	18.3	11.2	8.1	0.093	0.153	1.386
	6.6	30.2	8.8	17.6	9.8	7.5	0.089	0.139	1.307
36	5.8	29.5	7.6	15.0	7.2	7.8	0.086	0.109	0.916
	5.7	33.2	8.4	17.2	9.3	8.3	0.074	0.127	1.115
42	6.0	34.7	11.2	21.4	10.2	7.8	0.070	0.126	1.312
	6.9	37.7	15.5	25.1	9.4	7.7	0.073	0.101	1.222
44	6.8	26.1	10.1	16.4	7.2	6.4	0.103	0.109	1.120
	6.0	28.7	5.9	14.9	7.2	7.1	0.094	0.116	1.018

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX E

Table E. Plasma amino acid levels in mid-morning (2 hrs post breakfast) samples, low protein-high carbohydrate breakfast condition

Subject	μmole/100 ml								
	TRP	VAL	ILE	LEU	TYR	PHE	TRP/ΣNAA	TYR/ΣNAA	TYR/PHE
11[a]	5.8	16.8	6.0	7.6	5.4	5.1	0.141	0.131	1.058
	5.6	21.7	6.6	11.3	5.0	5.7	0.110	0.099	0.878
12	5.4	25.8	7.5	13.0	7.0	5.9	0.090	0.122	1.120
	6.0	27.2	5.4	10.3	7.8	6.1	0.106	0.142	1.277
13	7.1	21.9	6.9	11.9	5.6	5.6	0.137	0.110	1.050
	7.2	25.2	6.7	13.1	5.9	6.3	0.126	0.101	0.944
14	6.8	24.2	6.0	12.8	6.5	6.0	0.122	0.117	1.088
	5.5	23.1	6.3	11.8	6.6	5.9	0.103	0.126	1.122
21	5.5	20.0	5.5	10.6	5.2	5.5	0.119	0.110	0.943
	5.5	21.8	6.9	12.2	6.2	6.3	0.104	0.118	0.986
22	5.6	28.6	6.9	13.7	4.7	5.1	0.095	0.079	0.918
	6.4	21.8	5.6	10.6	4.6	5.0	0.134	0.093	0.925
24	5.1	19.0	5.1	9.4	5.9	4.6	0.116	0.136	1.273
	6.2	22.3	7.2	14.1	6.9	6.2	0.109	0.123	1.106
25	5.2	21.1	5.7	12.7	7.0	5.4	0.100	0.139	1.292
	4.4	21.7	6.2	10.4	5.8	4.9	0.090	0.121	1.175
30	5.7	23.6	7.4	11.8	7.3	7.0	0.099	0.132	1.038
	5.9	22.2	5.6	10.3	5.7	6.1	0.118	0.114	0.939
31	6.2	24.6	5.4	12.1	7.1	6.8	0.111	0.129	1.053
	5.2	24.0	5.1	11.0	6.6	5.7	0.099	0.130	1.157
36	4.7	23.5	5.6	11.6	5.3	6.1	0.089	0.103	0.863
	4.6	22.2	5.3	9.9	4.9	5.9	0.095	0.103	0.840
42	5.9	23.2	6.5	11.4	7.1	5.9	0.109	0.134	1.196
	5.4	22.5	6.6	12.0	6.5	6.6	0.100	0.123	0.989
44	4.7	21.0	5.4	9.7	4.6	4.5	0.105	0.102	1.034
	5.6	24.4	8.2	12.4	8.3	8.3	0.090	0.141	1.000

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX F

Table F. Plasma amino acid levels in pre-lunch (4 hrs post breakfast) samples, moderate protein-moderate carbohydrate breakfast condition.

Subject	μmole/100 ml								
	TRP	VAL	ILE	LEU	TYR	PHE	TRP/ΣNAA	TYR/ΣNAA	TYR/PHE
11[a]	5.3	24.0	7.2	16.5	6.3	6.9	0.087	0.105	0.908
	6.2	22.0	8.5	11.8	6.1	6.5	0.112	0.111	0.947
12	6.7	23.4	8.9	16.0	7.6	5.5	0.108	0.127	1.393
	5.7	25.4	9.7	16.6	10.0	6.9	0.083	0.156	1.445
13	6.4	22.1	5.9	11.5	6.0	6.4	0.123	0.114	0.936
	6.8	23.2	7.1	15.7	6.0	5.8	0.117	0.102	1.033
14	6.4	24.9	7.0	14.8	7.9	5.5	0.107	0.135	1.443
	5.6	27.1	7.7	15.5	9.4	5.5	0.086	0.153	1.711
21	5.3	27.3	7.9	15.2	9.4	6.9	0.080	0.150	1.353
	6.5	27.2	7.6	15.2	8.0	7.3	0.099	0.126	1.102
22	6.0	30.7	9.2	17.6	7.0	6.7	0.084	0.100	1.046
	4.9	27.2	7.4	14.6	5.6	5.1	0.081	0.095	1.094
24	5.5	25.5	7.4	15.4	6.1	5.7	0.092	0.102	1.070
	5.6	25.3	7.4	14.7	7.1	5.6	0.094	0.122	1.287
25	6.2	25.1	6.5	12.2	7.1	5.7	0.110	0.127	1.239
	5.7	21.4	4.8	9.5	5.0	4.6	0.126	0.110	1.093
30	5.8	27.3	7.9	13.8	7.0	7.0	0.092	0.113	1.007
	6.0	23.4	7.2	12.7	6.4	6.7	0.106	0.115	0.960
31	5.7	26.1	7.2	14.2	9.3	6.7	0.089	0.155	1.391
	5.5	22.2	5.8	11.6	6.2	5.2	0.108	0.124	1.187
36	5.3	25.4	6.9	13.2	5.6	6.2	0.093	0.098	0.894
	5.4	25.7	6.1	12.5	6.8	6.5	0.093	0.121	1.045
42	4.8	27.8	8.0	14.8	7.2	5.8	0.075	0.118	1.255
	5.5	30.3	10.4	17.5	7.2	5.9	0.077	0.103	1.220
44	6.7	23.9	7.5	12.1	7.6	5.6	0.100	0.115	1.091
	6.1	26.3	6.9	13.8	7.4	6.8	0.100	0.123	1.092

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX G

Table G. Plasma amino acid levels in pre-lunch (4 hrs post breakfast) samples, low protein-high carbohydrate breakfast condition.

Subject	μmole/100 ml								
	TRP	VAL	ILE	LEU	TYR	PHE	TRP/ΣNAA	TYR/ΣNAA	TYR/PHE
11[a]	5.7	19.4	7.0	10.3	5.6	6.5	0.117	0.115	0.867
	5.1	22.2	7.4	12.6	5.6	6.2	0.095	0.104	0.898
12	5.0	23.5	6.8	11.9	5.9	5.2	0.094	0.113	1.130
	5.7	24.2	5.1	10.1	6.8	6.0	0.108	0.134	1.136
13	7.1	24.5	8.2	14.4	5.6	6.6	0.120	0.093	0.852
	5.8	25.6	7.7	14.8	6.6	6.8	0.094	0.109	0.982
14	6.8	23.7	6.5	13.4	7.1	6.3	0.119	0.125	1.121
	6.6	24.7	7.5	13.7	7.0	6.1	0.113	0.119	1.144
21	5.6	23.9	6.6	12.2	5.4	5.7	0.104	0.100	0.952
	5.2	17.8	6.1	11.9	6.2	5.8	0.109	0.133	1.079
22	5.9	25.6	6.2	12.4	4.3	4.5	0.112	0.079	0.947
	5.8	21.6	5.8	11.5	5.9	4.7	0.117	0.120	1.270
24	5.4	20.1	6.3	11.4	6.3	5.4	0.109	0.130	1.173
	5,4	17.4	5.4	9.7	5.4	4.9	0.125	0.126	1.095
25	6.0	27.8	9.2	14.9	9.6	8.2	0.086	0.145	1.166
	5.7	20.5	5.6	10.2	6.1	6.1	0.118	0.127	1.010
30	5.4	22.2	7.0	11.6	6.6	6.2	0.101	0.127	1.066
	6.5	25.0	6.8	11.9	7.0	7.0	0.112	0.123	0.997
31	5.6	26.0	6.5	13.8	6.1	6.9	0.094	0.103	0.877
	6.4	24.1	7.5	12.1	6.3	6.1	0.115	0.111	1.024
36	5.1	23.4	6.3	13.3	5.5	6.2	0.093	0.101	0.886
	4.8	22.7	5.1	11.3	4.5	5.5	0.097	0.092	0.827
42	5.8	23.4	7.5	13.4	7.0	5.9	0.101	0.125	1.187
	7.5	21.4	7.3	13.0	6.2	5.0	0.143	0.114	1.238
44	5.4	20.4	6.1	11.6	5.1	5.0	0.113	0.105	1.018
	6.3	25.8	6.4	10.5	6.2	6.0	0.115	0.113	1.032

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX H

Table H. Plasma amino acid levels in post-dinner (2 hrs post dinner) samples, moderate protein-moderate carbohydrate breakfast condition.

Subject	μmole/100 ml								
	TRP	VAL	ILE	LEU	TYR	PHE	TRP/ΣNAA	TYR/ΣNAA	TYR/PHE
11[a]	7.0	28.3	11.3	18.1	8.4	8.7	0.093	0.114	0.961
	7.3	27.6	10.3	15.3	9.0	8.5	0.103	0.131	1.063
12	7.0	24.7	7.2	16.1	7.5	8.7	0.109	0.118	0.869
	6.2	30.5	15.2	23.1	10.5	8.8	0.070	0.126	1.194
13	8.0	32.9	12.3	21.8	9.2	8.5	0.094	0.111	1.091
	7.0	42.1	16.6	30.3	11.8	8.2	0.064	0.113	1.439
14	7.7	30.1	10.4	18.7	9.9	7.3	0.101	0.134	1.360
	9.5	32.7	11.6	20.3	9.7	7.4	0.116	0.119	1.315
21	6.9	33.9	12.6	21.9	9.5	8.0	0.081	0.114	1.191
	6.9	30.4	9.7	16.5	9.7	9.3	0.091	0.134	1.051
22	6.5	40.5	16.1	26.2	8.1	9.6	0.065	0.082	0.846
	5.4	23.1	8.1	14.1	6.7	6.5	0.092	0.118	1.040
24	6.5	35.1	10.9	19.8	9.3	8.1	0.078	0.116	1.145
	7.9	40.2	14.3	26.2	10.9	9.0	0.079	0.111	1.209
25	5.9	20.5	7.3	10.6	5.1	5.7	0.121	0.102	0.893
	8.9	50.0	17.0	28.8	13.6	11.3	0.074	0.118	1.202
30	7.8	43.1	17.6	28.6	13.1	12.5	0.068	0.119	1.050
	7.5	39.7	14.9	23.8	11.2	10.8	0.075	0.116	1.035
31	7.9	50.5	15.7	28.0	16.1	11.4	0.065	0.142	1.411
	11.7	65.1	25.3	42.6	19.7	14.0	0.070	0.124	1.406
36	8.1	49.3	14.8	28.2	11.6	11.1	0.070	0.104	1.047
	7.8	43.2	12.8	24.5	11.4	10.6	0.076	0.115	1.075
42	7.6	40.2	14.8	25.3	12.4	9.8	0.074	0.126	1.259
	7.3	48.8	16.6	28.6	13.1	11.0	0.061	0.116	1.193
44	7.8	34.0	14.6	23.7	9.6	9.3	0.085	0.108	1.034
	7.6	35.4	14.9	21.0	8.8	9.4	0.084	0.100	0.936

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX I

Table I. Plasma amino acid levels in post-dinner (2 hrs post dinner) samples, low protein-high carbohydrate breakfast condition.

Subject	μmole/100 ml								
	TRP	VAL	ILE	LEU	TYR	PHE	TRP/ΣNAA	TYR/ΣNAA	TYR/PHE
11[a]	8.6	33.2	15.2	23.8	10.4	6.2	0.093	0.114	1.057
	7.8	35.4	14.6	26.9	10.5	9.6	0.081	0.111	1.096
12	5.6	24.9	9.3	11.9	7.3	7.2	0.089	0.120	1.015
	6.1	33.4	12.6	20.1	8.8	8.2	0.073	0.110	1.076
13	8.3	45.3	16.8	29.0	10.3	9.0	0.075	0.095	1.146
	7.4	46.9	18.1	31.6	14.8	9.8	0.061	0.130	1.500
14	9.0	33.3	13.0	22.9	11.3	8.4	0.101	0.130	1.335
	8.0	29.8	10.8	18.5	9.8	7.9	0.105	0.130	1.236
21	6.8	33.5	12.2	20.9	10.2	9.1	0.080	0.123	1.116
	7.4	33.2	13.2	21.8	11.6	7.1	0.085	0.140	1.624
22	6.4	28.0	10.9	17.0	5.8	6.1	0.094	0.085	0.960
	6.8	30.6	12.7	19.4	8.9	8.5	0.084	0.114	1.000
24	8.0	35.9	13.1	22.5	10.8	8.4	0.088	0.123	1.284
	7.0	31.9	12.1	20.7	9.8	7.8	0.084	0.124	1.255
25	7.4	31.7	14.5	21.5	10.1	9.3	0.085	0.120	1.085
	7.6	38.5	13.7	24.0	12.1	9.3	0.077	0.130	1.300
30	7.8	39.3	13.4	22.6	11.7	10.5	0.069	0.126	1.112
	8.0	44.7	17.1	27.3	13.5	11.9	0.070	0.124	1.135
31	7.8	42.0	15.1	25.9	13.3	10.6	0.073	0.131	1.258
	8.0	46.4	15.4	28.2	15.3	11.3	0.068	0.140	1.352
36	7.5	43.7	14.7	27.7	11.6	10.6	0.069	0.111	1.096
	8.6	47.2	18.4	31.5	12.2	11.0	0.071	0.105	1.109
42	9.2	42.7	15.8	28.0	13.5	9.8	0.084	0.128	1.376
	5.9	31.0	10.5	16.6	7.8	7.4	0.080	0.109	1.067
44	6.7	27.8	10.4	18.0	6.7	7.1	0.096	0.095	0.939
	7.0	32.6	11.1	15.2	8.3	8.0	0.093	0.112	1.035

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX J

Table J. Tuesday and Thursday breakfast intakes of kcalories, protein, and carbohydrate in moderate protein-moderate carbohydrate breakfast condition.

Subject	Kcalories	Protein(g)	Carbohydrate(g)	Protein(% Kcal)	Carbohydrate(% Kcal)
11[a]	531	18	76	13.6	57.1
	576	18	82	12.6	56.8
12	624	19	116	12.3	74.0
	610	19	114	12.4	74.6
13	392	18	59	18.5	60.4
	458	18	76	15.8	66.4
14	476	18	79	15.2	66.6
	449	18	74	16.1	65.7
21	573	18	90	12.6	62.6
	569	18	88	12.7	61.6
22	505	18	73	14.3	58.2
	567	18	88	12.8	62.1
24	473	18	78	15.3	66.1
	549	19	100	13.8	72.3
25	444	18	72	16.3	65.3
	547	18	99	13.2	72.3
30	743	18	120	9.8	64.4
	860	19	154	8.8	71.7
31	770	19	154	10.0	80.0
	1044	19	210	7.3	80.4
36	910	19	189	8.3	83.2
	787	18	128	9.2	65.0
42	586	18	91	12.4	62.2
	539	18	94	13.4	69.7
44	695	18	114	10.4	65.3
	655	18	126	11.1	77.0

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX K

Table K. Tuesday and Thursday breakfast intakes of kcalories, protein, and carbohydrate in low protein-high carbohydrate breakfast condition.

Subject	Kcalories	Protein(g)	Carbohydrate(g)	Protein(% Kcal)	Carbohydrate(% Kcal)
11[a]	580	4	109	2.8	75.4
	546	4	100	2.9	73.1
12	870	5	168	2.2	77.2
	720	4	121	2.2	67.2
13	475	4	94	3.4	79.4
	393	4	74	4.1	74.8
14	593	4	120	2.7	81.2
	597	4	122	2.7	81.5
21	570	4	102	2.8	71.9
	778	4	121	2.1	62.2
22	525	4	94	3.0	71.4
	509	4	87	3.1	68.6
24	455	4	88	3.5	77.6
	542	4	108	3.0	80.1
25	555	4	114	2.9	82.0
	572	4	118	2.8	82.6
30	518	4	103	3.1	79.1
	564	4	116	2.8	82.2
31	945	4	194	1.7	82.3
	1107	4	227	1.4	82.2
36	776	4	169	2.1	87.0
	865	4	176	1.8	81.2
42	828	4	157	1.9	76.0
	732	4	123	2.2	67.5
44	798	4	157	2.0	78.8
	713	4	137	2.2	77.0

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX L

Table L. Tuesday and Thursday lunch intakes of kcalories, protein, and carbohydrate in moderate protein-moderate carbohydrate breakfast condition.

Subject	Kcalories	Protein(g)	Carbohydrate(g)	Protein(% Kcal)	Carbohydrate(% Kcal)
11[a]	937	30	105	12.6	44.9
	1274	45	196	14.2	61.5
12	977	37	121	15.1	49.4
	1783	82	186	18.4	41.7
13	1237	41	133	13.1	43.1
	2381	103	302	17.3	50.7
14	1175	53	122	18.0	41.6
	682	43	64	25.0	37.5
21	976	43	113	17.7	46.3
	914	47	97	20.4	42.4
22	1396	43	151	12.2	43.2
	1628	54	191	13.2	47.0
24	905	38	87	17.0	38.3
	784	40	69	20.3	35.2
25	789	19	105	9.6	53.4
	1103	21	149	7.5	54.1
30	1191	38	149	12.9	50.1
	1108	54	93	19.3	33.4
31	2225	136	141	24.5	25.4
	1969	83	230	16.9	46.8
36	1258	80	132	25.4	41.9
	1198	87	143	29.0	47.8
42	1335	85	121	25.6	36.2
	1306	74	105	22.6	32.0
44	930	32	141	13.9	60.6
	1289	32	147	9.9	45.6

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX M

Table M. Tuesday and Thursday lunch intakes of kcalories, protein, and carbohydrate in low protein-high carbohydrate breakfast condition.

Subject	Kcalories	Protein(g)	Carbohydrate(g)	Protein(% Kcal)	Carbohydrate(% Kcal)
11[a]	686	36	67	20.9	39.3
	1278	52	109	16.1	34.1
12	1655	33	185	8.0	44.7
	1480	36	245	9.9	66.2
13	1840	113	116	24.5	25.3
	1817	103	158	22.6	34.8
14	1284	47	152	14.6	47.5
	1632	55	206	13.6	50.4
21	1558	59	144	15.1	37.0
	1282	50	166	15.7	51.7
22	772	24	99	12.3	51.1
	1621	48	206	11.9	50.9
24	839	43	76	20.6	36.4
	1023	40	121	15.5	47.1
25	952	37	98	15.4	41.2
	1214	49	148	16.0	48.8
30	1016	64	99	25.3	38.9
	1404	42	205	12.1	58.4
31	2371	107	279	18.1	47.0
	1900	97	230	20.3	48.4
36	1490	69	130	18.4	35.0
	1214	41	136	13.4	44.7
42	904	58	122	25.6	53.9
	1496	81	161	21.5	43.0
44	1200	24	153	8.1	50.9
	928	38	111	16.4	47.7

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX N

Table N. Tuesday and Thursday dinner intakes of kcalories, protein, and carbohydrate in moderate protein-moderate carbohydrate breakfast condition.

Subject	Kcalories	Protein(g)	Carbohydrate(g)	Protein(% Kcal)	Carbohydrate(% Kcal)
11[a]	1065	46	115	17.3	43.1
	1220	61	126	19.9	41.4
12	1243	35	179	11.3	57.6
	1118	44	153	15.8	54.8
13	1184	61	112	20.6	37.7
	869	61	83	28.0	38.1
14	903	39	71	17.2	31.6
	583	30	66	20.3	45.2
21	733	52	41	28.5	22.6
	1081	52	102	19.3	37.7
22	791	42	62	21.2	31.6
	1420	55	95	16.6	26.8
24	662	44	62	26.9	37.4
	1177	59	72	20.0	24.3
25	768	25	96	12.9	50.0
	1215	68	104	22.4	34.3
30	1307	56	111	17.0	34.1
	1246	44	152	14.0	48.8
31	1604	50	166	12.4	41.4
	2659	155	260	23.3	39.2
36	1500	68	163	18.0	43.6
	1488	62	252	16.7	67.8
42	1102	41	152	14.9	55.0
	1696	72	195	17.1	46.0
44	975	35	129	14.3	52.9
	1273	43	177	13.6	55.6

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX O

Table O. Tuesday and Thursday dinner intakes of kcalories, protein, and carbohydrates in low protein-high carbohydrate breakfast condition.

Subject	Kcalories	Protein(g)	Carbohydrate(g)	Protein(% Kcal)	Carbohydrate(% Kcal)
11[a]	1080	47	132	17.4	49.0
	972	71	50	29.1	20.5
12	904	11	137	4.7	60.4
	1277	70	163	21.8	51.0
13	1287	73	120	22.6	37.3
	744	47	89	25.1	47.7
14	849	44	77	20.9	36.3
	861	52	75	24.1	34.7
21	892	48	81	21.6	36.4
	1142	47	59	18.8	23.5
22	1057	48	97	18.2	36.5
	820	37	122	18.2	59.6
24	985	64	65	26.1	26.2
	989	52	89	21.1	36.1
25	1072	31	114	11.5	42.5
	1050	87	124	33.1	47.1
30	1052	57	137	21.7	52.1
	1557	69	109	17.6	28.0
31	2409	79	239	13.2	39.7
	1899	105	245	22.2	51.7
36	1920	92	278	19.1	57.9
	1482	74	144	20.0	38.8
42	863	62	106	28.7	49.4
	1406	12	188	3.4	53.5
44	1283	34	205	10.7	63.8
	1989	41	259	8.3	52.1

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX P

Table P Tuesday and Thursday snack intakes of kcalories, protein, and carbohydrates in moderate protein-moderate carbohydrate breakfast condition.

Subject	Kcalories	Protein(g)	Carbohydrate(g)	Protein(% Kcal)	Carbohydrate(% Kcal)
11[a]	272	4	43	6.2	63.0
	765	10	139	5.1	72.8
12	178	3	21	7.8	70.0
	126	2	26	5.8	81.1
13	270	2	61	3.6	90.4
	-0-	-0-	-0-	---	----
14	-0-	-0-	-0-	---	----
	-0-	-0-	-0-	---	----
21	56	0	14	1.4	100.0
	240	5	31	8.3	51.7
22	-0-	-0-	-0-	---	----
	146	-0-	37	-0-	102.6
24	368	3	60	3.8	65.1
	1082	31	156	11.5	57.6
25	-0-	-0-	-0-	---	----
	-0-	-0-	-0-	---	----
30	-0-	-0-	-0-	---	----
	424	4	101	4.1	95.2
31	-0-	-0-	-0-	---	----
	360	-0-	37	-0-	41.6
36	-0-	-0-	-0-	---	----
	225	6	39	11.2	69.3
42	957	23	135	9.6	56.4
	63	3	13	21.2	84.7
44	666	4	84	2.7	50.4
	181	2	16	3.7	35.2

[a]First line for each subject indicates Tuesday values, second line is Thursday values.

APPENDIX Q

Table Q. Tuesday and Thursday snack intakes of kcalories, protein, and carbohydrates in low protein-high carbohydrate breakfast condition.

Subject	Kcalories	Protein(g)	Carbohydrate(g)	Protein(% Kcal)	Carbohydrate(% Kcal)
11[a]	157	-0-	41	-0-	104.1
	539	3	60	2.5	44.4
12	-0-	-0-	-0-	---	----
	188	4	29	9.0	61.2
13	-0-	-0-	-0-	---	----
	226	3	47	4.5	83.7
14	-0-	-0-	-0-	---	----
	-0-	-0-	-0-	---	----
21	-0-	-0-	-0-	---	----
	156	2	23	4.6	59.3
22	-0-	-0-	-0-	---	----
	244	-0-	62	-0-	102.5
24	-0-	-0-	-0-	---	----
	640	12	102	7.7	63.7
25	196	4	49	8.2	99.6
	-0-	-0-	-0-	---	----
30	160	-0-	41	-0-	103.5
	-0-	-0-	-0-	---	----
31	-0-	-0-	-0-	---	----
	190	4	24	8.4	51.4
36	81	-0-	21	-0-	103.7
	32	-0-	8	-0-	100.0
42	300	6	33	7.4	43.3
	-0-	-0-	-0-	---	----
44	188	4	29	9.0	61.2
	264	6	40	10.0	60.7

[a]First line of each subject indicates Tuesday values, second line is Thursday values.

APPENDIX R

Table R. Tuesday and Thursday daily intakes of kcalories, protein, and carbohydrates in moderate protein-moderate carbohydrate breakfast condition.

Subject	Kcalories	Protein(g)	Carbohydrate(g)	Protein(% Kcal)	Carbohydrate(% Kcal)
11[a]	2804	98	339	14.0	48.3
	3834	134	543	14.0	56.6
12	3022	95	436	12.5	57.7
	3636	147	478	16.2	52.6
13	3083	122	365	15.8	47.4
	3707	182	460	19.6	49.7
14	2554	110	273	17.2	42.7
	1714	90	203	21.1	47.5
21	2338	134	258	19.5	44.7
	2804	122	317	17.4	45.3
22	2693	103	287	15.3	42.6
	3761	127	412	13.5	43.8
24	2408	104	287	17.3	47.6
	3593	149	396	16.6	44.0
25	2001	62	274	12.4	54.8
	3167	142	349	17.9	44.1
30	3241	112	330	13.8	46.9
	3639	121	500	13.3	55.0
31	4599	205	461	17.9	40.1
	6032	257	738	17.1	48.9
36	3668	166	484	18.1	52.8
	3698	173	562	18.8	60.8
42	3980	167	498	16.8	50.0
	3604	168	407	18.6	45.1
44	3225	90	480	11.1	59.5
	3439	95	453	11.0	52.7

[a]First line of each subject indicates Tuesday values, second line is Thursday values.

APPENDIX S

Table S. Tuesday and Thursday daily intakes of kcalories, protein, and carbohydrates in low protein-high carbohydrate breakfast condition.

Subject	Kcalories	Protein(g)	Carbohydrate(g)	Protein(% Kcal)	Carbohydrate(% Kcal)
11[a]	2502	87	350	13.9	55.9
	3335	130	318	15.6	38.2
12	3429	48	489	5.6	57.1
	3665	114	558	12.5	60.9
13	3602	189	331	21.0	36.8
	3180	156	368	19.6	46.2
14	2725	95	350	13.9	51.4
	3090	111	402	14.4	52.1
21	3019	111	328	14.7	43.4
	3225	104	369	12.8	45.8
22	2354	76	289	12.8	49.1
	3193	90	478	11.2	59.9
24	2279	111	229	19.6	40.2
	3194	108	420	13.5	52.6
25	2775	75	374	10.9	54.0
	2836	140	390	19.7	55.0
30	2746	125	380	18.2	55.4
	2997	117	305	15.7	40.8
31	5724	190	712	13.3	49.8
	5361	198	747	14.8	55.8
36	4268	164	598	15.4	56.1
	3593	118	463	13.2	51.6
42	2895	129	418	17.9	57.8
	3634	97	472	10.6	52.0
44	3468	67	543	7.7	62.7
	3894	90	547	9.3	56.2

[a]First line of each subject indicates Tuesday values, second line is Thursday values.

APPENDIX T

Table T. Description of pictures presented on slides for pupil measurements.

Food Item	f/c[a]	Control	f/c
Glass of milk	4.6	Spool of string	4.6
T-bone steak	3.8	Clothes brush	5.2
Pancakes	3.8	Brown leaves	3.2
Canned peaches	2.1	Rolls of yellow tape	2.0
Peas	4.5	String of green beads	5.5
Carrot sticks	3.0	Cut-up orange napkin	3.1
Fried egg	5.4	Daisy	5.6
Baked potato	3.8	Plastic bottles	3.9
Oatmeal cookies	4.5	Brown leaves	3.0

[a] foot-candles at position of subject

APPENDIX U

Table U. Significant correlations not previously mentioned in the main text.

Plasma amino acids	Previous meal intake	r
Mid-morning TYR	Breakfast CHO (% kcal)	-.618**
Pre-lunch TYR	Breakfast Protein (g)	.400*
Pre-lunch TYR	Breakfast Protein(% kcal)	.408*
Post-dinner TYR	Dinner Calories	.450*
Post-dinner TYR	Dinner Protein (% kcal)	.352*
Mid-morning TYR/PHE	Breakfast Protein(% kcal)	.455**
Pre-lunch TYR/PHE	Breakfast Protein (g)	.365*
Pre-lunch TYR/PHE	Breakfast Protein(% kcal)	.413*
Post-dinner TYR/PHE	Dinner Protein (g)	.350*
Post-dinner TYR/PHE	Dinner Protein (% kcal)	.442**
Post-dinner TYR/PHE	Daily Protein (g)	.509**
Post-dinner TYR/PHE	Daily Protein (% kcal)	.503**

Same meal intakes

Meal	Intake	Intake	r
Breakfast	Calories	CHO (g)	.873**
Breakfast	Calories	Protein(% kcal)	-.434*
Breakfast	Calories	CHO (% kcal)	.394*
Breakfast	Protein (g)	CHO (g)	-.519**
Breakfast	Protein (g)	Protein(% kcal)	.922**
Breakfast	Protein (g)	CHO (% kcal)	-.839**
Lunch	Calories	Protein (g)	.758**
Lunch	Calories	CHO (g)	.805**
Lunch	Protein (g)	CHO (g)	.373*
Lunch	Protein (g)	Protein(% kcal)	.634**
Lunch	Protein (g)	CHO (% kcal)	-.455**
Lunch	CHO (g)	CHO (% kcal)	.488**
Lunch	Protein(% kcal)	CHO (% kcal)	-.562**
Dinner	Calories	Protein (g)	.646**
Dinner	Calories	CHO (g)	.823**
Dinner	Protein (g)	CHO (g)	.373*
Dinner	Protein (g)	Protein(% kcal)	.518**
Dinner	CHO (g)	Protein(% kcal)	-.438**
Dinner	CHO (g)	CHO (% kcal)	.660**
Dinner	protein(% kcal)	CHO (% kcal)	-.464**

Table U. (Continued)

Plasma amino acids in the same blood sample

	TRP/ΣNAA	ΣNAA	TYR	TYR/PHE
TRP	PB .625** PL .593** PD .683**	PB .337* MA .656**	MA .600** PD .703**	PD .457**
TRP/ΣNAA	----	PB -.518** MA -.720** PL -.696** PD -.753**	MA -.637** PL -.460** PD -.614**	-0-
ΣNAA	----	----	PB .572** MA .892** PL -.729** PD .912**	MA .657** PL .366* PD .480**
TYR	----	----	----	PB .664** MA .662** PL .730** PD .678**

Plasma amino acids in different blood samples

PB TRP MA TRP r = .552**

Note: CHO = carbohydrate
PB = pre-breakfast sample
MA = mid-morning sample
PL = pre-lunch sample
PD = post-dinner sample

Mid-morning samples were taken 2 hrs after breakfast.

Pre-lunch samples were taken 4 hrs after breakfast.

Post-dinner samples were taken 2 hrs after dinne.

* $p < .05$ ** $p < .01$