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Am J Physiol Regulatory Integrative Comp Physiol, September 1, 2008; 295 (3): R799-R805.

[Abstract] [Full Text] [PDF]

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S. Soenen and M. S Westerterp-Plantenga
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Blood Glucose Dynamics and Control of Meal Initiation: A Pattern Detection and Recognition Theory

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Campfield, L. Arthur, and Françoise J. Smith. Blood Glucose Dynamics and Control of Meal Initiation: A Pattern Detection and Recognition Theory. *Physiol Rev* 83: 25–58, 2003; 10.1152/physrev.00019.2002.—A new framework for understanding the control of feeding behavior, with special emphasis on the evolution of hunger, the initiation of feeding, and its dependence on patterns of blood glucose, is the subject of this review. A perspective on the current status and future directions of this search for a more complete understanding of the regulation of feeding behavior in laboratory rats and humans is presented including theoretical and experimental components. First, a historical perspective on the role of blood glucose in the control of feeding is presented. Next, the theoretical approaches that have been applied to the control of feeding and had a strong influence on experimental feeding research are summarized. This is followed by a statement and overview of a current theory that has emerged from studies of the role of transient declines in blood glucose in the control of meal initiation. The current working hypothesis that

transient declines in blood glucose are endogenous metabolic patterns that are detected and recognized by the central nervous system and are mapped into meal initiation in rats and are correlated with meal requests in humans are then presented. Then, the experimental studies on meal initiation and its dependence on patterns of blood glucose, first in rats and then in humans, are reviewed in detail. Finally, the future directions of the work, limitations, and the implications for the understanding of the control of feeding behavior and the regulation of energy balance are discussed.

I. PROLOGUE: A NEW FRAMEWORK FOR THE CONTROL OF FEEDING

Curiosity, interest, and questions about the acquisition, preparation, and presentation of food, the cultural importance of food, appropriate diet and menu selection to promote optimal nutrition, states of hunger and satiety, regulation of body fat, body weight, and energy balance naturally arise in most of us from our daily experiences and contact with food. Among scientists with interests and training in physiology, behavior, neuroscience, metabolism, physiological psychology, and/or behavioral neuroscience, many have given at least passing thought and perhaps even a little professional attention and effort to this topic. However, a few become so captivated by this complex, multifactorial regulatory system at the interface between “internal” physiology and the “external” world that they then devote their working scientific lives to grappling with these issues. These scientists from many different disciplines continually try to tease out and identify the fundamental facts, the organs, tissue, cells and molecules, the physiological mechanisms, the levels and patterns of organization, and the decision algorithms and rules involved. Their ultimate goal is to provide a deep and complete mechanistic understanding of the regulation of feeding behavior.

The subject of this review is our perspective on the current status and, what we believe are, promising future directions of this search for a more complete understanding of the regulation of feeding behavior in laboratory rats and humans. We will not be comprehensive in the sense of enumeration of all the ideas or theoretical and experimental approaches that our former and current colleagues have productively or unproductively applied to the problem. Instead, we attempt to provide a new framework for understanding the control of feeding behavior, with special emphasis on the evolution of hunger and the initiation of feeding, including theoretical and experimental components. We will begin by providing, in section II, a historical perspective on the role of blood glucose in the control of feeding. In section IIIA, theoretical approaches that have been applied to the control of feeding and had a strong influence on experimental feeding research are summarized. We then provide a statement and overview of our current theory that has emerged from our studies of the role of blood glucose dynamics or “patterns” in the control of meal initiation in section IIIB. Then, we review

in detail the experimental studies on meal initiation that have been conducted, first in rats (sect. IV) and then in humans (sect. V). Sections A and B of the rat and human data presentations provide the reader with the basic data set upon which our theory is based. The remaining sections may be too specialized for the general reader. We conclude with a discussion of the future directions of the work, limitations, and the implications for the understanding of the control of feeding behavior and the regulation of energy balance in section VI. Section VIA presents a future research agenda specific to meal initiation that may also be too detailed for the general reader. However, section VI, B and C, is intended for both specialist and the general reader and forms the summary of the review. Here a discussion of limitations of previous studies of feeding, obstacles to linking eating to physiological processes, the limitations of our “patterns as signals” concept, and the implications for our theory for the control of food intake and body energy balance are presented. Along the way, we present a mixture of philosophy, theoretical arguments, experimental design issues, as well as formal presentation and discussion of experimental results. We hope that this journey into the complexities of contemporary feeding research will prove interesting and useful to the reader.

At the outset, we want to acknowledge and explicitly state that the ideas, concepts, and implications of the experimental results discussed here have been strongly influenced by the experimental approach, integrative perspective, and brilliant insights into the regulation of feeding of Jacques Le Magnen and his students and colleagues. Professor Le Magnen, who died in May 2002, was a major intellectual force in the field of the regulation of feeding throughout his career. In addition, Donald Novin, Stephen Woods, and G. P. Smith and their students and colleagues as well as the work of many others have also had important influences on our research and thinking. Of course, our research in the area of meal initiation and the physiology of hunger descends from the seminal work of Jean Mayer and his students and colleagues. We have extended his view by adding and emphasizing a dynamic perspective that proposes patterns of blood glucose, rather than glucose utilization, as endogenous signals for meal initiation. Jean Mayer wrote the following about his glucostatic theory that is based on glucose utilization:

As for the glucostatic theory, . . . its reception has illustrated to a certain extent the fate which, William James warned, awaited all new concepts: First people say it is not true; then they say it is of no general significance; and finally, they say that anyway it had been known for a long time. . . . At any rate, what lasting value, if any, it will achieve will be inexorably decided by the test of time.

Jean Mayer (77)

We are truly “standing on the shoulders of those that have gone before” as we take this look, first, backward and, then, forward in time at the complexity of feeding behavior and its regulation.

II. HISTORICAL PERSPECTIVE ON THE ROLE OF BLOOD GLUCOSE IN THE CONTROL OF FEEDING

Among the major issues in the control of food intake, the identification of the biochemical basis for hunger and meal initiation, and the signals controlling these neurobiological processes have been the subject of extensive research and debate for many decades. These studies led to the classical aminostatic, thermostatic, and lipostatic theories of food intake control as well as more recent hypotheses that are discussed in section III (6, 9, 37, 46–50, 70–72, 93, 122). However, all of these theoretical ideas were preceded by the notion that glucose uptake and utilization played a central and metabolically privileged role in the control of hunger, satiety, and the regulation of body energy balance. Although these notions were first discussed and suggested by Carlson in a classic text published in 1916 (37), they were formalized by Jean Mayer into the classical glucostatic theory in the mid 1950s (76, 77).

A. Early Proposals and Conceptualizations

The observation that the sensation of hunger was often associated with discomfort referred to the abdominal region was the basis of early attempts to explain the regulation of food intake. Cannon and Washburn (36) reported that “hunger pangs” were associated with contractions of an empty stomach. Washburn swallowed a flexible tube to which a balloon was attached at the end. Stomach contractions could be monitored because each contraction would increase the pressure in the partially inflated balloon. In general, Washburn’s reports of hunger pangs coincided with measured gastric contractions. This reported relationship between hunger pangs and gastric contractions, together with the observation that injection of glucose reduced gastric contractions, led Carlson (37) to propose that food intake was regulated by feedback from the stomach.

B. Mayer’s Hypotheses, Supportive Evidence, and Its Early Impact

Mayer proposed that decreased glucose utilization, which was detected by the brain at glucosensitive sites, in unspecified locations, represented the stimulus for meal initiation. The glucostatic theory of hunger postulated that reduced glucose utilization in these critical brain regions leads to perception and expression of hunger. He also argued that increased glucose utilization in these same glucosensitive sites leads to decreased hunger and the cessation of eating. Mayer proposed that decreased glucose utilization or “metabolic hypoglycemia,” the point at which the peripheral arteriovenous difference in blood glucose (A-V delta glucose) becomes negligible and glucose is no longer entering “metabolizing cells,” was the signal for meal initiation. He viewed his signal, metabolic hypoglycemia, as reflecting the point at which the energy substrate flux was at a minimum or turning in the direction of increasing fatty acid utilization; in other words, the point of the beginning of carbohydrate depletion. In his 1955 paper, Mayer (77) explicitly argues that the glucostatic theory would account for the short-term regulation of hunger and food intake, while he invoked a lipostatic mechanism to account for the long-term regulation of body weight and energy balance. Mayer observed that the temporal changes in blood glucose concentration were correlated and consistent with the observed time domain of changes in hunger and food intake in rats and humans. From this observation, he argued that changes in blood glucose concentration and/or arteriovenous differences in glucose concentration reflected or mirrored the postulated glucose uptake and utilization in glucosensitive brain areas and could be used as surrogates of these unobservable parameters. He also argued that the changes in body weight would be consistent and controlled by a slower lipostatic mechanism in which increases in fat content will be followed by increased fat utilization, with the resulting sparing effect on carbohydrates (77).

After formulation of the glucostatic hypothesis, there was a blossoming of research interest in the biological basis of hunger and satiety in both humans and laboratory rats. Mayer’s specific hypothesis and its straightforward predictions relating behavior and metabolic processes were disseminated widely, attracted a lot of attention, and quickly became objects of a flurry of research directed at validation and testing of the hypothesis. The initial results of several studies, including studies in humans conducted in Mayer’s laboratory, demonstrated that arteriovenous differences in blood glucose concentration were correlated with hunger ratings and food intake under some circumstances (125, 128). However, other research comparing the arteriovenous differences in blood glucose and hunger ratings or food intake failed to observe a correla-

tion under other situations (2, 129). In other research, hunger ratings and food intake were measured following exogenous infusions of glucose. Again, the results of these investigations were either consistent (7, 125–127) or inconsistent (2, 55, 133) with a role for glucose in the onset of hunger. A review of this research area by one of Mayer's early collaborators, Dr. Ted VanItallie, has provided an important historical perspective (127). Mayer acknowledges the very important contribution of Dr. T. VanItallie to the broadening of his initial theory and its application to different metabolic situations including the hyperphagia of diabetes (76).

C. Later Studies, Revision, and Retreat From the Hypothesis

Numerous experimental studies emphasize the role of decreased glucose utilization or decreased intracellular glucose concentrations rather than the absolute level of blood glucose as the stimulus for meal initiation. The observed induction of feeding by administration of pharmacological doses of insulin (8, 45, 68, 75, 83) or of nonmetabolizable glucose analogs (73, 117, 123), the satiating effects of small glucose infusions or gastric loads (91, 92), and effects of central injections of glucose and 2-deoxyglucose (4, 99, 101, 123) all strongly suggest a role for decreased glucose uptake and utilization, possibly modulated by insulin, at a target site or sites in the control of meal initiation. However, other experimental results appeared inconsistent with the glucostatic theory. When intravenous glucose infusions (using peripheral veins) with or without insulin were administered before meals, no delay in meal initiation or reduction in meal size was observed (2, 55, 86). Furthermore, the observations that large, prolonged decreases in blood glucose were required to induce feeding following insulin administration and that the onset of feeding often occurred when the blood glucose had returned to baseline have also been used as evidence against the glucostatic theory (44, 119, 120).

By the mid 1970s, the weight of the experimental evidence and its contemporaneous interpretation had cast serious doubt on the glucostatic hypothesis. In the absence of a strong advocate (Mayer had left the field and active research by that time) and in the face of strong and vocal attacks, interest in research motivated by the glucostatic hypothesis based on glucose utilization waned. Mayer himself was quite fatalistic about the role of hypoglycemia, or any theory based on hypoglycemia, ever being consistent with the well-established hyperphagia commonly observed in diabetes:

As the possible role of hypoglycemia. . . , its study was pursued no further, especially in the view of the lack

of correlation encountered between hunger and absolute levels of blood glucose and then apparently insurmountable difficulty of diabetic hyperphagia.

Jean Mayer (77)

Both Mayer and VanItallie have argued that the ability of the glucostatic theory to adequately explain the hyperphagia commonly observed in uncontrolled diabetes demonstrated the strength of the theory based on glucose utilization rather than glucose concentration (76, 77, 127). The reasoning was that an uncontrolled, insulin-deficient diabetic would experience hunger because the blood glucose, although high, was not being properly utilized and hyperphagia would follow.

Two other parallel developments in the field also contributed to the abandonment of the glucostatic hypothesis based on glucose utilization. First, the prominence and primacy of the hypothalamus in the control of feeding behavior and regulation of energy balance strongly argued as a critical component of the dual-center hypothesis published by Stellar in 1954 (121) was also being forcefully challenged by scientists advocating the importance of peripheral mechanisms involved in the control of feeding. Second, the demonstration that the gastrointestinal hormone cholecystokinin (CCK) rapidly and potently reduced meal size in rats through a peripheral mechanism (51) caused a major shift in research and conceptual focus from meal initiation and hunger to meal termination and satiety. The combination of these trends led to a paradigm shift in feeding research away from the glucostatic hypothesis and central mechanisms toward meal termination, satiety, and peripheral mechanisms.

D. Technological Innovation and a Rebirth for the Role of Blood Glucose in the Control of Meal Initiation

More recently, a signal for the initiation of freely taken meals in rats with continuous access to familiar food has been identified: a brief fall and rise in blood glucose concentration before ingestion of food (Fig. 1). The identification of this signal was a direct result of a series of technological innovations leading to computer-based continuous monitoring of blood glucose in freely moving rats (14, 74, 84, 120).

The ability to monitor blood glucose continuously in freely behaving animals, in contrast to discrete blood sampling at fixed 10-, 15-, or 30-min intervals, led to renewed consideration of the role of blood glucose in meal initiation. Transient declines in blood glucose were first described by Louis-Sylvestre and Le Magnen (74), who showed that a fall in blood glucose was correlated with meal initiation in the rat. They observed that blood glucose concentration declined 6–8% at 5.0 ± 0.3 min

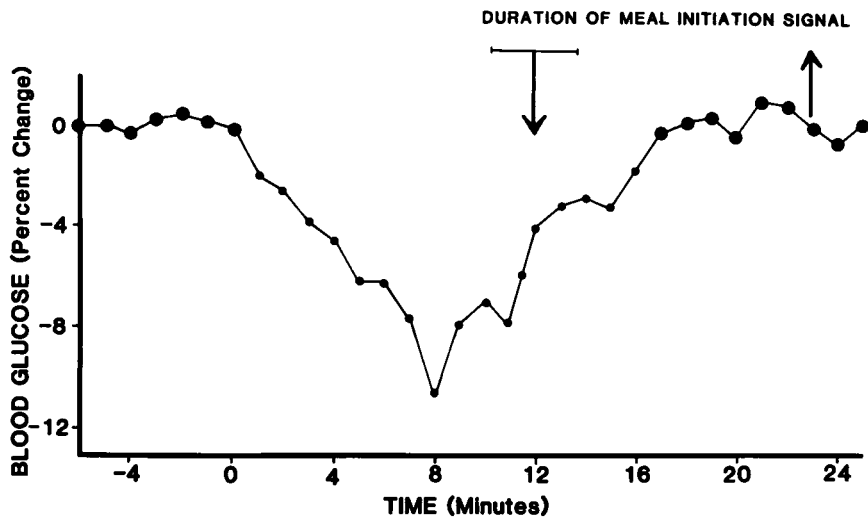


FIG. 1. Composite time course of the functional coupling between transient decline in blood glucose and meal initiation. Average time course of blood glucose concentration in the prevented access condition was expressed as percent change from baseline concentrations. Data are mean values. The latest estimates of the onset and offset times for the meal initiation signal to be above threshold are indicated by downward and upward arrows, respectively. For details, see text. [From Campfield and Smith (18), with permission from Elsevier Science.]

before meal onset in both the dark and light phases of the light-dark cycle.

We have confirmed and extended these initial findings. We have provided experimental evidence supporting the hypothesis that spontaneous, self-resolving transient declines in blood glucose precede and signal meal initiation in free-feeding rats. This evidence was obtained using on-line, computer-based technology for continuous monitoring of blood glucose concentration in freely behaving rats. In nondeprived free-feeding rats, this signal precedes food-seeking behavior and the initiation of a meal but does not predict the size of the meal or the timing of meal termination.

III. RESEARCH STRATEGIES AND STATEMENT OF THE THEORY

A. Research Strategies in the Study of Feeding Behavior: How Are the Critical Signals in the Control of Feeding Represented?

Research on the control of feeding behavior over many decades has been as varied, multidisciplinary, and complex as the phenomena of feeding behavior and its regulation. As in any field of scientific investigation, most of the research has been focused on a variety of major and minor issues over the years. Individual scientists or groups of scientists in one or more laboratories have become interested in an issue and have experimentally addressed that issue by applying the conceptual framework and technology that was available or preferred by them. The list of questions in Table 1 has been among the major issues in the field.

Each of these questions has been further subdivided into classes of factors (e.g., sensory, gastrointestinal, metabolites, hormonal, neural), site(s) of origin or action,

and antecedent or prevailing metabolic states. A set of theoretical models has been employed and has led to several different research strategies being utilized in the field (5, 9, 10, 67, 70–72, 93, 122). These are briefly described below.

1. Stores and the depletion/repletion model

The dominant theoretical approach and research strategy to each of these questions has been the so-called “depletion/repletion” model. In this construct, it is postulated that a critical variable, usually associated with the magnitude of a specific “store,” will cause ingestive behavior when it is reduced below an implicit threshold. Feeding behavior will cease when the level of this variable has returned or been “repleted” to the “defended” level. Among the earliest suggestions for such a critical variable was the volume of stomach contents. In this model, depletion was represented by gastric contractions generated by an “empty” stomach and repletion was represented by the meal-related expansion of the volume of the stomach (37). The depletion/repletion model for the control of feeding behavior was derived from a direct translation of the negative-feedback control systems common in engineering to the physiological problem of the regulation of energy balance. Thus changes in carbohydrate, fat, and/or

TABLE 1. Major questions in the field of feeding behavior and its regulation

What Factors Are Responsible for
Initiation of feeding?
Maintenance of feeding behavior once initiated?
Termination of meals?
Duration of the intermeal interval?
Size of individual meals?
Matching food intake to short-term and long-term energetic demands?

protein body stores were postulated to lead to the generation of appropriate "error signals" and appropriate changes in food intake to return the regulated store or stores to the desired level. When the theory was reduced to practice, however, it was appreciated that the body fat store was fairly easy to estimate and manipulate, while the carbohydrate and protein stores were not. Measurable surrogates such as liver and, in some cases, muscle glycogen and lean body or fat-free mass were used to represent carbohydrate and protein stores, respectively. Despite these practical difficulties and limitations, this theoretical model has dominated the field and a large experimental literature has accumulated based on this theoretical approach (5, 9, 10, 67, 70–72, 93, 122). Indeed, in spite of widely appreciated deficiencies, most discussions of the control of feeding behavior begin, and all too often end, with the consideration of only the depletion/repletion model.

The research strategy most often suggested by this model was to experimentally manipulate one of the critical stores, most often body weight, and to measure the resulting change in food intake. This model also stimulated a search for the identification of the "critical or error" signals that lead to the observed change in food intake.

2. Blood levels of metabolic substrates and fuels

The search for a representation of body energy stores in the plasma led to another major theoretical construct in the field: the regulation of blood levels of metabolic substrates or "fuels." The most important example was the glucostatic theory of Mayer (76, 77). Other examples of this type of model were the aminostatic, thermostatic, and lipostatic theories of food intake control (5, 9, 10, 70–72, 93, 122). The glucostatic theory and its experimental evaluation were discussed in detail above in section II. Briefly, this theory held that the rate of glucose utilization in a privileged brain region controlled food intake. Blood glucose concentration and/or its arteriovenous difference were used to infer the rates of glucose utilization. Thus hypoglycemia and its associated decreased glucose utilization led to initiation of feeding, whereas postprandial hyperglycemia and increased glucose utilization resulted in cessation of feeding and a period of satiety. However, as the theory spread throughout the field, much of the experimental focus and debate became centered on blood glucose concentrations rather than on rates of glucose utilization in an unknown brain region.

The lipostatic hypothesis, originally postulated by G. Kennedy (65) and J. Mayer (77), also proposed that metabolic fuels or signals generated from adipose tissue, circulated in the blood, and acted on the brain to match or balance energy intake and energy expenditure and body fat mass over the long term (days or weeks). The candi-

date signal molecules most well studied were the fatty acids and glycerol released from adipose tissue by lipolysis. Thus increased adipose tissue-derived fatty acids and/or glycerol led to feeding, whereas postprandial decrease in lipolysis resulted in cessation of feeding and a period of satiety (70–72).

Another candidate has emerged as a result of the cloning of the obese (*ob*) gene and the identification of adipose tissue as the primary source of its gene product, leptin (also known as OB protein) (141). The existence of a circulating signal from adipose tissue that acts on the brain to reduce food intake was established by the studies of Hervey and Coleman and their colleagues (39–42, 57–59). When cross-circulation (or parabiosis) experiments were performed between lean and obese rats by Hervey and colleagues at the University of Leeds (58, 59), the lean partner reduced its food intake and lost weight while the obese partner continued to gain weight. These studies suggested that the obese partner was producing increased amounts of a factor that was proportional to body fat and the factor crossed into the circulation of the lean partner where it acted on the brain to reduce food intake and body weight. When cross-circulation (or parabiosis) experiments were performed in *ob/ob* and *db/db* mice by Coleman and colleagues at the Jackson Lab (39–42, 57), they also observed that the *ob/ob* partner reduced its food intake and lost weight, while the *db/db* partner maintained both its food intake and body weight. These studies led Coleman to conclude that *ob/ob* mice fail to produce a circulating factor (or factors), perhaps from adipose tissue, which normally acts on their brain to reduce food intake. However, *ob/ob* mice still retain the ability to respond to this factor. He also concluded that *db/db* mice produce this circulating factor, but their brain cannot respond to it (39, 41). Experimental attempts to purify a bioactive factor from the adipose tissue of overfed, obese rats independently conducted by Harris and Martin (56) and Hulse and Martin (61) were not successful. Initial, impure extracts did have biological activity, but this activity was lost after further purification. The insightful hypotheses of Hervey and Coleman have been proven by the emerging biology of the leptin pathway since 1995. Evidence suggests that leptin appears to play a major role in the long-term regulation of adipose tissue mass through coordinated regulation of feeding behavior, metabolism, autonomic nervous system, and body energy balance in rodents, primates, and humans (13, 25–27).

The aminostatic hypothesis, originally postulated by Mellinkoff et al. (81), proposed that amino acids were candidate signals generated from the breakdown of protein stores in muscle, circulated in the blood, and acted on the brain to match or balance energy intake and energy expenditure and body fat mass over the long term (days or weeks). Thus increased muscle catabolism and elevation of amino acids led to feeding, while postprandial

uptake of amino acids from the plasma into muscle resulted in cessation of feeding and a period of satiety. These ideas have been pursued, and the current status of the field has been reviewed by Gietzen (52).

The thermostatic hypothesis, originally postulated by Brobeck (11), also proposed that changes in skin, visceral, and core temperature could induce behaviors that would appropriately alter energy balance and match energy intake and energy expenditure and body fat mass over the long term (days or weeks). Thus hypothermia and decreased thermogenesis and its associated metabolic effects led to feeding, while postprandial hyperthermia resulted in cessation of feeding and a period of satiety. In addition to the very large literature on the role of thermogenesis in energy balance, this concept has been studied and reviewed by Woods and Strubbe (138) and Himms-Hagen (60).

The research strategy most often suggested by this model was to experimentally manipulate one of the putative circulating metabolic substrates or fuels, such as blood glucose, and to measure the resulting changes in food intake. These experiments were most often performed in animals and humans in specific metabolic states with the goal of modifying the anticipated behavioral response to one inappropriate for the prevailing metabolic state (99). For example, food-deprived or "hungry" subjects should eat less than expected following the experimental increase of blood glucose concentration. Although this model was originally conceived to be compatible with several blood-borne metabolic substrates modulating food intake under different circumstances, it unfortunately led to narrowly focused and mutually exclusive, competing searches for the identification of the "critical" or "major" blood-borne metabolite that globally controlled food intake. Although such a single blood-borne signal was not required or implied by this conceptual model, it was often invoked to justify the primacy of one factor over another and to push to the margins of the discussion and debate interesting multifactorial models that required integration of these multiple signals over different temporal domains and metabolic states.

3. *The molecule as signal model*

The shift in focus away from glucose utilization led many in the field to consider the glucose molecule itself to be the signal for the control of food intake. This was consistent with the trend in the field to consider molecules that circulate in the blood (e.g., insulin, free fatty acids, glycerol, glucagon, CCK, gastrin, catecholamines, adipin, enterostatin, leptin) to be candidate control signals for the regulation of food intake (5, 9, 13, 25, 70–72, 93, 122). This concept emerged from endocrinology and metabolic physiology and gave the field a much longer list of candidate signals. Many of these signals could be ac-

counted for or associated with the glucostatic, aminostatic, and lipostatic theories of food intake control. However, other signals emerged from the renewed interest in gastric and intestinal mechanisms in the digestion and absorption of nutrients as well as the control of food intake.

The discovery and elucidation of brain/gut peptides that had powerful and often dramatic effects on food intake, and the striking advances achieved in receptor biochemistry and molecular biology also contributed to the "molecule as signal" paradigm. In recent years, the effects of brain and/or gut peptides, peptide fragments, mixtures of peptides, and peptide receptor antagonists on food intake have been studied (118). The success of molecular biology and its penetration into the field of ingestive behavior have made gene expression studies and the use of antisense nucleotide probes to disrupt expression of neuropeptides, so-called knock-out animals, possible. These techniques have identified new brain neuropeptides and receptors and provided new understanding of well-known neuropeptides, involved in the regulation of food intake such as neuropeptide Y (NPY), proopiomelanocortin (POMC), agouti-related peptide, melanocortin receptors and the interaction of these neuropeptides with leptin (26). Also, studies of neuro- or regulatory peptides have awakened interest in the classical neurotransmitters. Although common to earlier research, specific written descriptions of linkages between molecular signals and body stores are now often sketchy or missing. Thus we study molecules per se and construct theories of feeding control based on molecules, for example, the insulin, CCK, or serotonin hypotheses (63, 104, 122, 135).

One of the most compelling theoretical constructs in the field of feeding and body energy balance is the brain insulin hypothesis developed by Stephen Woods and colleagues at the University of Washington in Seattle, and now at the University of Cincinnati (63, 104, 135). This model accounts for the central integration of a circulating signal related to adipose tissue mass, plasma insulin concentration, and the resultant modulation of daily food intake and, thus, body weight regulation. The current formulation of this evolving theory and the experimental evidence supporting it have been reviewed (63, 104, 105, 135). In the brain insulin hypothesis, the 24-h and day-to-day fluctuation or pattern of plasma insulin concentration, when integrated by brain insulin transport and subsequent insulin-dependent signal transduction, is a critical variable of the control of body weight. Although many other factors are involved in the regulation of body weight, the brain insulin hypothesis has provided important insights.

The recent identification by positional cloning of the genes, and predicted gene products, responsible for single gene mutant models of obesity in rats and mice (e.g., *ob*, *db*, *tub*, *fat*, and *agouti*) has increased the focus on alter-

ations of feeding behavior mediated by a single factor to the exclusion of others (e.g., leptin only, melanocortin receptor only), rather than the integration of multiple factors (13, 25).

The research strategy most often suggested by this modification of the metabolic substrate or fuel model was very similar to that inspired by the original model as discussed above.

4. *The pattern as signal model*

Increasing attention has been focused recently on behavioral sequences or patterns. Well-established orosensory motor patterns have been described by Grill and Norgren (54). Also, stereotypic satiety behavioral sequences have been described by Smith and co-workers (1, 118). Thus complex motor programs underlying feeding are thought to reside in the central nervous system (CNS), and signals related to body stores or specific molecules may “initiate” or “trigger” them under appropriate circumstances.

The consideration of sequences in which a complex temporal and/or spatial pattern fulfills the role of initiator of these motor programs has emerged as an alternative theoretical construct (22, 23). Thus the temporal pattern of a specific molecule (e.g., transient decline in blood glucose), the pattern of several molecules (e.g., nutrient flux across the intestine), the temporal pattern of a specific molecule in a specific context of other patterns (e.g., insulin before, during, or after a meal; oral signals; or gastric distension) or the spatial pattern caused by the passage of a specific molecule through body compartments (e.g., glucose interacting with multiple glucose receptive neural elements, insulin in the brain and cerebrospinal fluid, leptin from adipocytes to the brain) may act as control signals that the CNS uses to organize feeding behavior. Within this conceptual framework, it is not the store or molecule (neither its concentration or absolute amount) but rather the dynamic “pattern” of the molecule that conveys critical “information” to the CNS.

Another key element of the pattern as signal concept is the notion of representation of peripheral events and states within the CNS. The CNS may contain “representations” of metabolic and behavioral states: absorptive and postabsorptive states, hunger, and satiety. Although often thought of in terms of spatial maps, transformations, or homunculi, representation in this context is considered to be a dynamic pattern of activity in a set of neurons that function as a central analog of sensory somatic or visceral events. Thus we seek these representations of peripheral body stores, metabolic state, and the external world and how these representations interact to control feeding behavior.

What form would these representations take? Based on our knowledge of the nervous system and by analogy

to other brain systems involved in sensorimotor integration and chemosensory detection and processing, these representations would probably be patterns of electrical and/or neurochemical activity of one or more neural networks controlling feeding. Thus, in this theoretical construct, patterns in the periphery carry information that is detected and recognized by central neural networks, which results in modified patterns of activity of these neural networks. Specific patterns of activity of these networks are postulated to correspond to the behavioral states associated with meal initiation and meal termination. Transition from the representation corresponding to satiety (a period of no food intake) during intermeal intervals to the representation appropriate for, and corresponding to, meal initiation within the CNS will cause the onset of feeding. Meal termination will occur when the representation corresponding to satiety is restored by reversal of the meal initiation pattern activity by the complementary pattern corresponding to satiation (the active process of meal termination, see Refs. 70–72). If we could capture a visual image of the patterns of activity of the feeding network before, during, and after a meal, we would see a transition from the pattern characteristic of the intermeal interval to that of meal initiation and food ingestion followed by a transition back to the pattern corresponding to satiety. Thus activity patterns corresponding to two active processes, meal initiation and satiation, will be superimposed on the hypothesized passive or low-activity steady state corresponding to satiety (a period of no food intake) each time an animal eats a meal. In this theoretical construct, the components of a behavioral sequence become distinct patterns of activity in discrete neuronal networks and are linked to the underlying physiological and biochemical dynamics of these networks. Mechanistic decomposition of the behavioral sequences of feeding will require the identification and characterization of the major components of the key elements of that sequence: initiation, maintenance, and termination. Perturbation or shaping of feeding behavior would be then translated into modulation of these complex activity patterns and their integration by the brain.

Since formulating this conceptual framework for feeding behavior in a review published in 1990 (23), we have come to believe that its utility both in terms of explanatory power and an ability to tightly link feeding behavior and physiology has been demonstrated. We remain optimistic about its potential to refocus our field on the message rather than the messenger. This point of view also has the potential to synthesize much of our field because, rather than debating the merits of transient declines in blood glucose or hypothalamic norepinephrine or hindbrain CCK or circulating leptin, we can ask how all of these components or elements of the pattern are integrated to elicit specific behaviors underlying feeding. This hypothesis is also focused on the detection and recogni-

tion of these multiple patterns by the widely distributed neuronal networks controlling feeding. Thus the goal of the search is not the *pattern* but rather the *patterns* that together form the central representations of meal initiation, maintenance, and termination. The integrative focus of this conceptualization has much in common with and is a descendent of earlier integrative approaches of the balance of lipogenesis and lipolysis rates (70–72), combining energy flux in the plasma (5, 6) and neurons that integrate specific molecular signals (84). However, this concept has an even broader focus on the totality of central representations of peripheral events related to feeding. An analogy may be that the brain is responding, conditioned on its current state, to the entire “symphony” rather than the “voice of individual sections” of the orchestra. Finally, the ability of the “pattern as signal” construct to integrate the dynamics of blood glucose before, during, and after meals into one or more important messages related to feeding behavior suggests that a similar focus on the patterns of insulin, leptin, and CCK rather than a focus on the location or concentration of these hormones may also yield important insights. The application of this conceptual framework and its related research strategy applied to the problem of meal initiation will be the focus and subject of this review.

B. Pattern Detection and Recognition Theory of Meal Initiation

The experimental studies presented and reviewed in our previous publications (14, 18, 22–24, 29, 34, 35, 111, 112, 114) and to be reviewed below have led to the formulation of a signal detection and recognition theory of meal initiation. The major assertions of this theory are that: 1) transient declines in blood glucose represent “endogenous metabolic patterns,” 2) transient declines in blood glucose are signals in the form of “patterns” that are detected and recognized by the central neural network that controls feeding behavior, and 3) these patterns are “mapped into” meal initiation under free-feeding conditions. The phrase *mapped into meal initiation* is meant to describe the process of transformation, in the mathematical sense, or establishing a unique correspondence between the recognition of the transient decline in blood glucose and the activation of the motor program for feeding within the CNS.

The distinguishing feature of this assertion is that it is the temporal pattern, shape, or waveform of blood glucose dynamics rather than the glucose molecule, or the absolute decrease in blood glucose, or blood glucose concentration or glucose utilization that is detected and contains “critical information” that is extracted by the central nervous system to control meal initiation.

We propose that the processes of detection, recogni-

tion of a transient decline in blood glucose, and its mapping into meal initiation behavior are accomplished by the set of spatially and temporally distributed processes shown in Figure 2. Meal initiation will occur only if a “timing or probe signal” was generated recently and a transient decline in blood glucose is detected in the brain and recognized. Figure 2 depicts the information and signal flow through the set of sequential processes without regard to the anatomical localization of the process. The “timing or probe signal” represents an “inquiry” or “interrogation” or “probing” of the periphery by the CNS regarding its ability and capacity to maintain glucose homeostasis in upcoming time interval. This signal is generated by a signal generator (in the CNS) that controls its frequency or timing. The signal acts on the biochemical subsystem that regulates blood glucose concentration and, depending on the peripheral metabolic state and its ability and capacity to “maintain glucose homeostasis” over the next time interval, a transient decline in blood glucose (TDBG) will be generated or not. An average transient decline in blood glucose is shown in Figure 1.

Any changes in blood glucose concentration from baseline values are detected by peripheral and central glucose responsive neural elements which form the “TDBG detector” process in Figure 2. The recognition of the presence of a transient decline in blood glucose that meets all the necessary criteria from among the many changes in blood glucose concentration occurring in the peripheral blood is reported by the TDBG recognition process. This process is located in both the CNS and the periphery, and its output acts on the “decision algorithm” process. The decision algorithm unit computes the conditional probability of activating the stored meal initiation program given the state of the three inputs it receives: 1) output from the TDBG recognition process, the recognition of TDBG; 2) peripheral metabolic state; and 3) presence of a recent “probe or timing signal.” If a transient decline in blood glucose is recognized in the presence of a favorable peripheral metabolic state and the recognition of recent generation of the “probe of timing” signal, then the output of the decision algorithm will be yes and the meal initiation program will be activated. By a “favorable metabolic state” we mean the later part of the intermeal interval which would allow a transient decline in blood glucose in response to the probe or timing signal. The transient decline in blood glucose will indicate that additional energy intake from a meal may be required to maintain blood glucose over the coming time interval (see below). An “unfavorable” metabolic state would be just after a meal. Unless other events interfere with the meal initiation program or the act of feeding, the meal initiation program, when activated, generates all of the motor acts required for expressing meal initiation behavior.

Our current working hypothesis is shown in Figure 3 and is as follows. In free-feeding rats, we have shown that

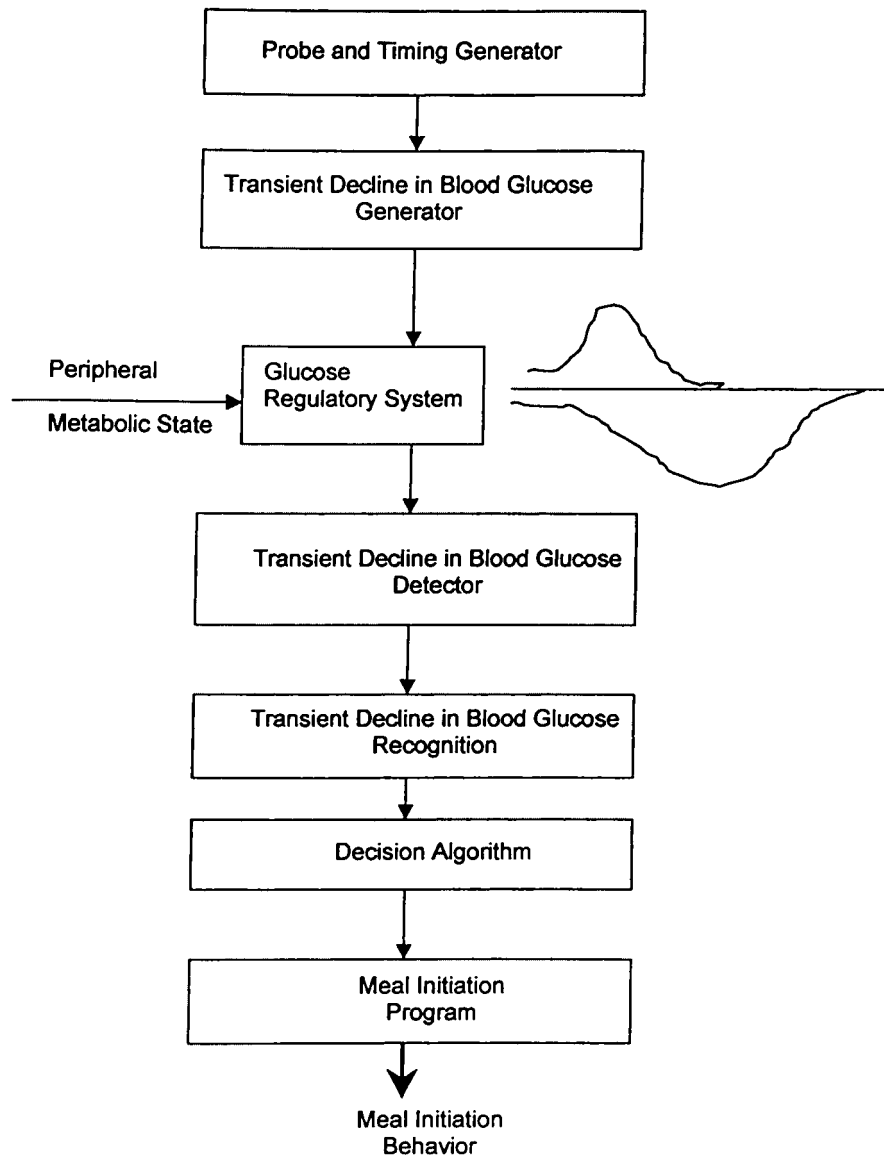


FIG. 2. Proposed information and signal flow in the pattern detection and recognition model. The information flow chart is shown without regard for the anatomic location of each process. The process of meal initiation begins with the generation of a "probe or timing signal" within the brain, continues as this signal "passes through" the peripheral metabolic system acting on the blood glucose regulatory system, and ends with an affirmative decision to activate the "meal initiation program." The blood glucose regulatory system generates an insulin spike and a transient decline in blood glucose (represented by the sketch; *middle*). See text for details.

there are brief, spontaneous plasma insulin peaks antecedent to each decline in blood glucose (19, 29). We postulate that a "probe or timing signal" generated by the CNS results in a brief change in the firing rate of the parasympathetic (vagal) and/or sympathetic nervous system efferents which innervate pancreatic β -cells, liver, adipose tissue, and the gastrointestinal tract. The change in autonomic firing rate causes a brief insulin spike from pancreatic β -cells, along with other responses, which, in the presence of an appropriate peripheral metabolic state, induces a transient decline in blood glucose by decreasing hepatic glucose production and/or increasing peripheral glucose disposal. In the presence of hyperglycemia, the brief insulin spike, along with other responses, may have to be larger in magnitude to induce a transient decline in blood glucose (see sect. *IVB2*). The peripheral metabolic state is postulated to "condition," in a probabilistic sense,

or "gate," in a signal flow sense, the likelihood of a feeding response to the activation of vagal and/or sympathetic efferents. Thus a central/peripheral interaction is proposed in which a centrally generated meal initiation signal must "pass through" the peripheral metabolic system, and it will be mapped into feeding behavior only if a transient decline in blood glucose of the correct shape or pattern occurs in response to a recent probe signal. If the output of the decision algorithm remains in the "no" state, either because of the absence of a transient decline in blood glucose or the failure of a decline to meet all the criteria for meal initiation or an unfavorable peripheral metabolic state (e.g., recent meal), the TDBG recognition unit will be reset to the "no decline" state. If the peripheral metabolic state cannot maintain glucose homeostasis over the next time interval, without additional energy intake from a meal, a transient decline in blood glucose in response to

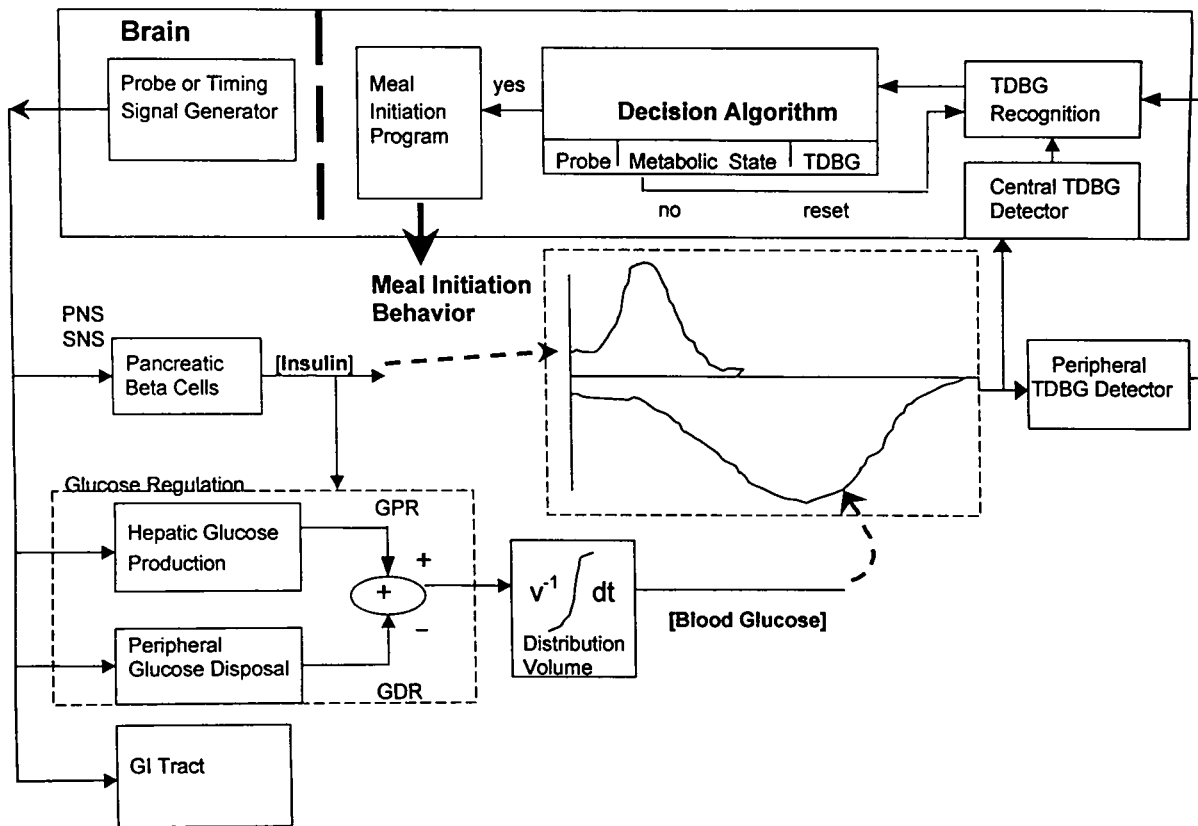


FIG. 3. Block diagram representation of the information and signal flow in our conceptual model of the pattern detection and recognition theory of meal initiation. A “probe or timing signal” is generated within the brain (*top left*) and causes a brief change in firing rate of the parasympathetic (PNS) and/or sympathetic (SNS) afferents which innervate pancreatic β -cells, glucose regulatory system, and gastrointestinal (GI) tract (*middle and bottom left*). If additional energy (a meal) is needed to maintain glucose homeostasis over the next time interval, the probe signal (e.g., insulin spike) will “pass through” the peripheral metabolic system acting on the blood glucose regulatory system and generate a transient decline in blood glucose (represented by the sketch; *middle*). Changes in blood glucose concentration will be detected by peripheral and central glucose responsive neural elements (*middle and top right*) and then the transient decline in blood glucose is “recognized” in the brain (*top right*). If the criteria of the decision algorithm (*top*) are satisfied (transient decline recognized, favorable peripheral metabolic state, recent probe signal), the “meal initiation program” will be activated and meal initiation behavior will be observed (*top left*). If the decision criteria are not satisfied, the “recognition” unit will be reset and meal initiation will not be observed until the next transient decline in blood glucose occurs. TDBG, transient decline in blood glucose; GPR, glucose production rate; GDR, glucose disposal rate.

a probe signal will occur. On the other hand, if glucose homeostasis can be maintained over the next time interval without additional energy intake (e.g., liver glycogen breakdown and utilization), a transient decline in blood glucose will not occur. However, if a transient decline in blood glucose occurs but is ignored and no food is eaten, the only consequence may be the occurrence of the next transient decline in blood glucose sooner than expected. In this case, blood glucose may be maintained through novel involvement or activity of the liver or other organs.

The model assumes that some probe or timing signals will not result in transient declines in blood glucose, and, therefore, meal initiation will not be observed. Therefore, a prediction of our model is that the number of probe or timing signals should be greater than the number of meals. The acetylcholine analog bethanechol, which provokes a brief spike in insulin concentration, was used to

induce transient declines in blood glucose and meal initiation in rats. In these studies, transient declines in blood glucose were observed in 63% of the trials (10 of 16 trials), and meal initiation was observed in 56%. In contrast, 38% of injections of bethanechol (6 of 16 trials) were not followed by a transient decline in blood glucose and meal initiation, thus providing support for this hypothesis (111). In these same experiments, the same dose of bethanechol that induced transient declines in blood glucose in some circumstances (e.g., time during of light-dark cycle), failed to do so in others (e.g., light phase).

The processes of detection and recognition are considered distinct in our formulation because we interpret our experimental results to mean that the minute-by-minute blood glucose concentration is detected by glucose responsive peripheral afferents and central neurons. Thus blood glucose is continuously represented and al-

ways available to the CNS. The process of detection begins with a representation of the relatively steady intermeal concentration of blood glucose ("baseline") and "reports" the detection of all departures in blood glucose concentration from that baseline to the TDBG recognition unit. In contrast, the unique process of recognition that an ongoing pattern of blood glucose concentration "matches" or "fits" the criteria for a transient decline in blood glucose is the function of the TDBG recognition unit. Thus the recognition of a transient decline in blood glucose is the result of a two-step process: 1) detection that the blood glucose concentration is changing and 2) the recognition that the pattern of the blood glucose change meets the criteria for transient declines in blood glucose. Another way of looking at the function of these two processes is that the TDBG recognition unit must recognize the shape or pattern of transient declines, but only transient declines, out of the set of all other changes in blood glucose concentration detected throughout the day.

The output of the decision algorithm was considered to also be yes throughout the duration of the transient decline in blood glucose, but the output is assumed to switch to no after the blood glucose concentration returned to baseline for 6 min, or more. Therefore, the time interval between the output of the decision algorithm switching from the no state to the yes state, following the initial recognition of the transient decline in blood glucose, to the output switching back from yes to no states was of short duration (~12 min in rats) and persisted <6 min after the end of a blood glucose decline.

The necessary conditions for a fall and rise in blood glucose concentration in rats to be recognized as a transient decline in blood glucose have also been calculated from our experimental data set. The following necessary conditions must be met sequentially by all transient declines in blood glucose that signal meal initiation in rats: 1) the slope of the falling phase must be within -0.4 and -1.5 mg/dl \cdot min; 2) the nadir of the decline must be at least 6% below baseline and must occur between 40 and 60% of the total duration of the decline, and the total duration of the transient decline must be longer than 6 min; and 3) the slope of the rising phase must be within 0.5 and 1.5 mg/dl \cdot min.

These necessary conditions, which specify an approximately symmetrical fall and rise in glucose concentration, shown in Figure 1, provide a first-order approximation of the criteria for recognition of a transient decline in blood glucose by the TDBG recognition unit in our model.

The conditions required for a yes output of the decision algorithm and activation of the meal initiation program are proposed to be as follows: the recognition of a transient decline in blood glucose that was generated by a receptive or favorable peripheral metabolic state in

response to the "recent" generation of a probe or timing signal by the CNS.

As discussed above, if the output of the decision algorithm remains in the no state, because these criteria were not met, the TDBG recognition unit will be reset to the no decline state.

Although the conceptual basis and a basic structure of our working hypothesis has been represented in our model shown in Figure 3, many aspects of the model remain to be determined in quantitative terms. However, we feel that this model, and the hypotheses on which it is based, provide a new framework for the understanding of feeding behavior, with special emphasis on the evolution of hunger, the initiation of feeding and its dependence on patterns of blood glucose.

IV. PHYSIOLOGICAL AND BEHAVIORAL STUDIES OF TRANSIENT DECLINES IN BLOOD GLUCOSE AS A SIGNAL FOR MEAL INITIATION: STUDIES IN RATS

A. Basic Studies Under Free-Feeding Conditions in Rats

1. Transient declines in blood glucose as a signal for meal initiation

Blood glucose concentration and meal pattern were continuously monitored in rats feeding freely using methods described previously in detail (14). Since all of the studies reviewed have utilized these same techniques and the evidence supporting a role for glucose in meal initiation is critically dependent on these technological innovations, it is important that the reader has a clear understanding of these methods. Thus these methods will be briefly described.

Adult rats were housed in individual cages with free access to food and tap water (Fig. 4). Powdered rat food was placed in a food cup fitted with strain gauge weighing apparatus. Animals were kept in a temperature-controlled room with 12:12-h light-dark schedule. Rats were implanted with chronic cannulas in the right atria of heart. After a seven-day recovery period, characterized by resumption of consistent gain in body weight and normal meal pattern, experimental studies were conducted in freely moving rats in their home cages. Heparin sodium (200 U) was injected intravenously, and 45 min later, blood withdrawal (25 μ l/min) for continuous blood glucose monitoring was initiated. Venous blood was withdrawn from freely behaving rats through polyethylene tubing attached to the cardiac cannula and injected into the sample chamber of a glucose analyzer (YSI model 23A or 27). The analog outputs from both the food cup and the glucose analyzer were sampled 8–10 times a minute, am-

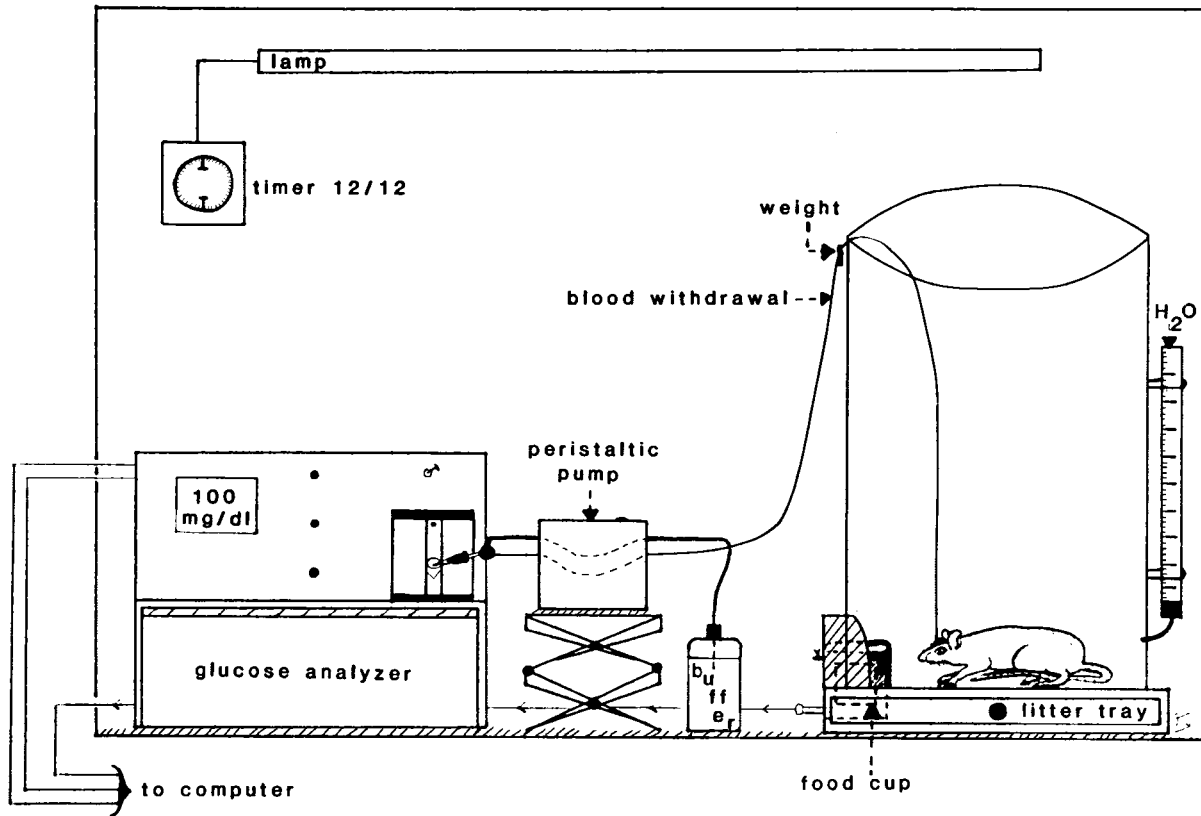


FIG. 4. Experimental set-up for studies of continuous recording of blood glucose concentration and meal initiation. Note that the animal remains in its home cage during blood glucose recording. [From Campfield et al. (14), with permission from Elsevier Science.]

plified, digitized, and interfaced to a microcomputer. Blood glucose monitoring was continued for up to 3 h. Experiments have been conducted throughout the light-dark cycle (14).

When the blood glucose concentration and meal pattern were monitored continuously in free-feeding rats, a transient fall and rise in blood glucose was observed before each meal independent of the light-dark cycle. The average time course of blood glucose in nine early experiments is shown in Figure 5. The blood glucose concentration was expressed as percent change from baseline; the *time 0* reference point was chosen as the minimum blood glucose, and the data points were averaged at 1-min increments before and after the nadir. During an average decline, blood glucose concentration fell gradually to $11.6 \pm 1.2\%$ below baseline. Blood glucose concentration began to decline 12.1 ± 1.7 min before the onset of food intake and continued to decline to a minimum at 5.4 ± 1.5 min before meal onset. Note that these average times were measured from the beginning of the meal as opposed to the minimum glucose concentration. Correlations between meal size and percent maximum decline in blood glucose concentration and the total duration of the decline were both nonsignificant. Thus

transient declines in blood glucose predict meal initiation but not meal size.

Random fluctuations in blood glucose occurred in these experiments that were not related to feeding. Analysis of the lower limits of transient declines in blood glucose that were associated with meal initiation indicated that blood glucose decreases with magnitudes $>6\%$, and durations of >6 min invariably preceded food intake. When these criteria are applied to our large data set, we conclude that no such transient decline in blood glucose has been observed in the absence of food intake nor has feeding been observed in the absence of such a transient decline in blood glucose concentration.

These findings are consistent with and confirm the original observations of a premeal decline in blood glucose by Louis-Sylvestre and LeMagen (74). They also are consistent with the decrease in *metabolisme de fond* before meal initiation reported by Nicolaidis and Even (85). The striking similarity to the time course of blood glucose and *metabolisme de fond* before meals suggests that blood glucose could be a major contributor to this measure of the resting metabolism of the rat.

These results are also consistent with the premeal patterns in both liver and skin temperature reported by

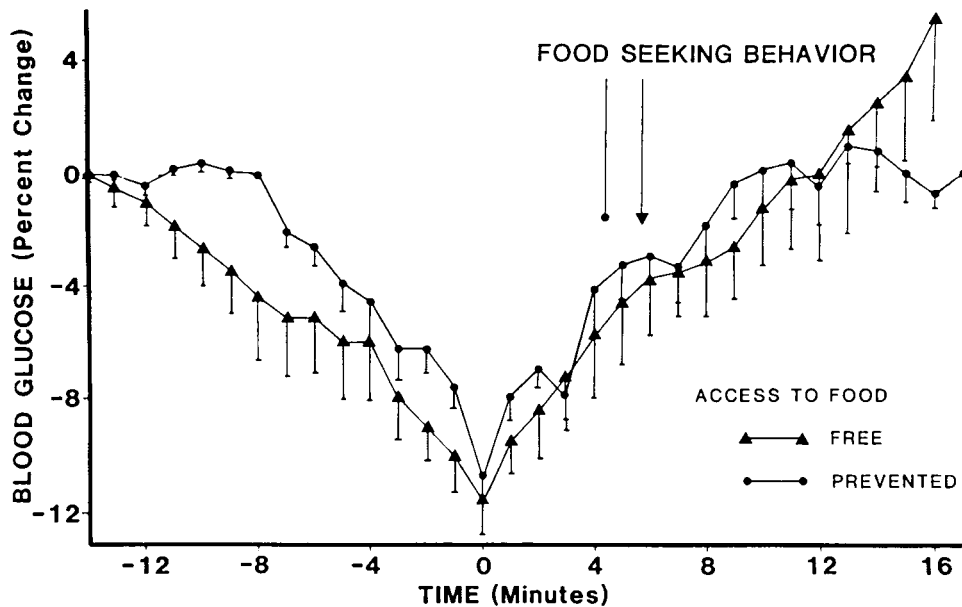


FIG. 5. Average time course of the transient decline in blood glucose when access to food was free or prevented. Blood glucose concentrations are expressed as percent change from the intermeal baseline concentrations in this figure. The minimum glucose concentration has been taken as the *time 0* reference. Data were selected each minute from the reference point and averaged in each of nine experiments. Data are means \pm SE. The onset of food-seeking behavior is indicated by the vertical arrows in free access (\blacktriangle) and prevented access (\bullet) groups. Mean baseline glucose concentrations were 104 ± 5 and 96 ± 3 mg/dl, respectively. Note the similar time course of blood glucose and onset of food-seeking behavior when food access was free and prevented. [From Campfield and Smith (18), with permission from Elsevier Science.]

Woods and Strubbe (138). They have observed a rise in liver and skin temperature from the intermeal minimum temperature preceding spontaneous meal initiation in rats. Although meals of different size began at different temperatures, all meals terminated at constant liver and skin temperatures. Our results also permit the reevaluation of the reports that meal initiation after insulin administration occurred when blood glucose was near or at baseline levels (44, 119, 120). Although these results have been used to argue against the glucostatic theory of Mayer (76, 77), the temporal relation between blood glucose and meal initiation is remarkably similar following both intravenous insulin and spontaneous transient declines in blood glucose.

2. Nature of the signal

Having documented the temporal relation between transient declines in blood glucose and meal initiation in free-feeding conditions, the next set of questions addressed the nature of this systemic signal for meal initiation. Furthermore, the magnitude or strength and the time course of the functional coupling between blood glucose dynamics and feeding behavior were also investigated. These questions were addressed by preventing access to food before and during declines in blood glucose, restoring access to food at various times following the beginning of the decline in blood glucose, and measuring the latency to feeding and the next decline in blood glucose (18). In experiments conducted across the light-dark transition, the food cup was covered before and during the transient fall of blood glucose and was uncovered only after blood glucose had returned to the baseline concentration. The time course of blood glucose concentration

before an expected meal, expressed as percent change from baseline, in these experiments is compared with similar experiments in which rats had free access to food in Figure 5. The time course of blood glucose concentration in both groups was very similar. Food seeking behavior (i.e., orienting and/or moving to the food cup, sniffing, trying to remove the cover) occurred with a latency comparable to that seen in free-feeding animals. Food-seeking behavior was transient and ended as the glucose concentration increased toward baseline.

In similar experiments, the cage and food cup were thoroughly cleaned before the experiment, and no food was placed in the food cup ($n = 6-9$). The time course of blood glucose and the latency to food-seeking behavior observed were not different in the total absence of food. Thus neither preventing access to nor the absence of food affected the time course of the transient fall and rise of blood glucose before an expected meal and the latency to food-seeking behavior.

In these experiments, the food cup was uncovered 6-8 min after blood glucose had returned to the baseline concentration and the latency to meal initiation was measured ($n = 6-9$). In all cases, food-seeking behavior ceased before uncovering the food cup. Feeding was not observed immediately after the uncovering of the food cup but rather after a normal intermeal interval and a second transient fall in blood glucose and rise toward baseline. Meal initiation following the second decline in blood glucose occurred an average of 84 ± 8 min after the beginning of the first decline in blood glucose. This compares with an average intermeal interval for these rats at this phase of the light-dark cycle of 98 ± 7 min. The mean coefficient of variation of blood glucose concentration

during the interdecline interval was $2.1 \pm 0.5\%$. The size of the meal was not different from meals eaten at this time by these rats in the free access condition. In other experiments, the food cup was uncovered 4–9 min before blood glucose returned to baseline. In these experiments, rats moved to the food cup and feeding began within 2 min after removal of the food cup cover. These experiments indicate that when access to food was restored as the blood glucose was rising toward baseline, meal initiation occurred as expected.

Combination of these studies and our studies in the free-feeding condition allowed calculation of the approximate temporal evolution of the functional coupling between blood glucose and meal initiation. The resulting composite time course is shown in Figure 1. The latency to meal initiation in the free-feeding condition (12.1 ± 1.7 min from the beginning of the decline in glucose) was taken to be the latest time that the glucose-dependent signal for meal initiation exceeded its threshold and coupled blood glucose to feeding behavior (downward arrow). Because meals were initiated with a short latency when access to food was restored while the glucose was rising toward baseline, the meal initiation signal was considered to be above threshold throughout this period. The termination of the functional coupling was then taken as 6 min after blood glucose concentration returned to baseline because when food access was restored at this time, or later, a normal intermeal interval and a second transient decline in blood glucose preceded meal initiation (upward arrow). Therefore, the functional coupling between blood glucose and feeding behavior was of short duration (~ 12 min) and persisted < 6 min after the end of a blood glucose decline. The transient nature of this coupling required a second decline in blood glucose to initiate feeding when the rat was unable to eat within this narrow temporal window.

In other experiments, a novel food (orange slice, potato chip, or chocolate chip cookie) was presented 30 min after the food cup was uncovered. Access to powdered food had been restored 8 min after the end of the transient decline in blood glucose. The novel food was eaten in seven experiments with an average latency of 2.5 ± 0.9 min after presentation but without a prior decline in blood glucose. These studies demonstrate that novel foods with strong sensory qualities can be eaten without any prior changes in blood glucose concentration (18).

In addition to transient declines in blood glucose, other physiological and metabolic changes can occur in the premeal period. When rats are presented food on fixed time schedules for several days, gastric contractions increase just before anticipated periods of food availability (43). Increased gastric contractions have also been observed following hypoglycemia (83, 125, 126). Because transient declines in blood glucose also occur prior to food availability in conditioning studies in rats (see be-

low), it is probable that gastric contractions occur in association with blood glucose dynamics and meal initiation. Additional metabolic changes are discussed in section 1vD.

In summary, the nature of the signal controlling meal initiation that we are proposing is very different from that proposed by Mayer. In his glucostatic theory, Mayer proposed that decreased glucose utilization or metabolic hypoglycemia, the point at which the peripheral arteriovenous difference in blood glucose (A-V delta glucose) becomes negligible and glucose is no longer entering metabolizing cells, was the signal for meal initiation. In contrast, we propose that it is a specific pattern of blood glucose concentration, the transient decline in blood glucose, that signals and controls meal initiation under most conditions. Mayer viewed his signal, metabolic hypoglycemia, as reflecting the point at which the energy substrate flux was at a minimum or turning in the direction of increasing fatty acid utilization, in other words, the point of the beginning of carbohydrate depletion. However, we view transient declines in blood glucose as signals that “interrogate” the peripheral metabolic state and signal meal initiation if additional energy intake will be needed to maintain glucose homeostasis for the coming time interval. Thus transient declines in blood glucose are not the result of a change in direction or magnitude of metabolic flux indicating depletion, but rather are metabolic patterns that signal energy intake to avoid depletion of metabolic fuels. Another important difference is that, theoretically at least, dynamic patterns of blood glucose could explain meal initiation and hunger in the context of a variety of diet patterns and metabolic conditions, while the Mayer glucose utilization, which dependent on changes in glucose utilization and metabolism, lacks this property. Finally, rather than a difference in glucose utilization (a metabolic process) in central glucoreceptors, as represented by peripheral glucose concentration difference across metabolically active tissues, the transient decline of blood glucose is a dynamic pattern that is detected and recognized by central and peripheral glucose-sensitive neural elements and mapped into meal initiation.

3. Evidence for causality

Based on their initial studies, Louis-Sylvestre and LeMagen (74) concluded that blood glucose concentration was among the feedback signals in the regulation of feeding and body energy storage, and the observed decline in blood glucose concentration before meal onset was either the signal for meal initiation or a consequence of the true signal. In either case, a causal relation between the decline in blood glucose concentration and feeding was proposed (70). This proposal has proven to be quite controversial. Several investigators argued that the pre-

meal decline in blood glucose concentration was correlated with rather than causally related to meal initiation (see commentaries in Ref. 70). Motivated by the controversy over the causality of the observed premeal decline in blood glucose, we conducted a series of experiments designed to determine whether transient declines in blood glucose induce meal initiation.

Our first approach was to infuse glucose intravenously to partially block the premeal decline in blood glucose and assess the effect on subsequent feeding behavior. In some experiments, 10% glucose (up to 0.2 ml) was infused over a 5-min period beginning as soon as a fall in blood glucose prior to an expected meal was "recognized." Glucose infusions were administered each time a decline in glucose was recognized. Isotonic saline also was administered in separate experiments to control for the nonspecific effects of infusions (14). The transient declines in blood glucose observed in these experiments are summarized in Figure 6. Comparison of the results obtained indicates that although the initial rate of decline, the timing, and the magnitude of the glucose nadir were not significantly affected by the glucose infusions, the duration of the decline was decreased and the latency to the anticipated meal was increased markedly (318 ± 94 compared with 12.1 ± 1.7 min). It is important to note that 0.2 ml of 10% glucose contains at most 9% of the calories consumed in the smallest meal. The very long latency to the anticipated meal in glucose-infused rats may have been overestimated. The sensitivity of the chart recorder used to monitor the meal pattern was lower (minimum meal size approximately ≥ 0.2 g) in these early studies than the computer-based recording system used in later studies. Thus earlier small meals may have failed to be detected leading to an overestimate in the latency. Despite this potential overestimate, these studies indicate that glucose infusions that change the shape of the transient declines in blood glucose delay meal initiation (14).

These studies suggest that it is the shape or pattern of the transient declines in blood glucose that affects meal initiation. Further support for this hypothesis was pro-

vided by the results of additional experiments in which glucose was infused during the rising phase at the end of the transient decline. In these experiments, the anticipated meal occurred with normal latency despite the glucose infusion. These observations suggest that it is not glucose itself that uncouples the transient declines in blood glucose from meal initiation but rather a modification of the shape of the transient decline in blood glucose. Taken together, these results offer an explanation for the previously reported failure of intravenous glucose infusions to delay the onset of feeding or reduce meal size (44, 119, 120). Our studies demonstrate that to block meal initiation, a glucose infusion must occur during the early phase of the transient decline in blood glucose. Thus meal initiation would not have been blocked if glucose had been infused either before the decline or toward its end (14).

Additional experiments were performed in which glycine, β -hydroxybutyrate, or fructose was infused intravenously instead of glucose. The effects of these infusions on the parameters of the transient decline and the latency to meal initiation were minimal; only glucose infusions blocked meal initiation. Although it would have been interesting, glucosamine and glucagon were not studied. This result suggests that changes in blood glucose rather than any other nutrient tested provides, or generates, the signal for meal initiation.

Additional supportive evidence was obtained from experiments in which fructose was infused intravenously in free-feeding rats to produce transient declines in blood glucose observed before meal initiation (114). Random sequences of fructose (doses range 0.05–0.2 ml of 10%), separated by at least 30 min, were infused intravenously over 2 min during intermeal intervals. During the early dark phase, fructose was followed by slight decreases or increases in blood glucose (-4 to $+10\%$ at 6 min). In the light phase, however, three types of dose-dependent declines in blood glucose were observed. The first pattern was a fall to a suppressed level (-6%) that was maintained for at least 30 min. The second pattern was a

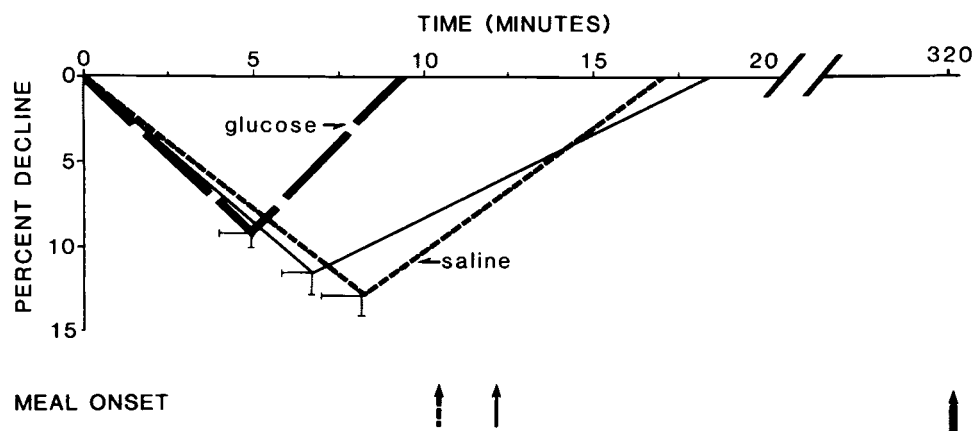


FIG. 6. Effects of glucose clamp on transient decline in blood glucose and latency to the anticipated meal. Comparison of the timing and magnitude of the maximum percent decline and time of the return to baseline in experiments without infusions (solid line), with saline infusions (dotted line), and with glucose infusion (heavy dotted line). The arrows represent the time of onset of the next meal in each group. Note decreased decline duration and increased latency to meal initiation with glucose infusion. [From Campfield et al. (14), with permission from Elsevier Science.]

transient fall (-10% at 8 min) and return to baseline at 28 min. However, the third pattern mimicked the transient decline in blood glucose observed before meal initiation: a transient fall (-9% at 8 min) and return to baseline at 17 min, during which meal initiation occurred with a latency within the normal range for spontaneous meals following transient declines in blood glucose. No feeding was observed after the other two blood glucose response patterns induced by fructose infusion. The observation of feeding during a period of low probability for spontaneous meals only following a fall and rise in blood glucose induced by fructose that mimicked the transient decline in blood glucose provides further evidence that patterns in blood glucose dynamics are a causal signal for meal initiation (114).

The observation of a transient spike of insulin before the transient decline in blood glucose (see sect. IV C) suggested another way to induce transient declines in blood glucose. If so, then meal initiation should result if the induced decline mimicked the spontaneous transient declines in blood glucose. Perhaps a brief release of insulin from the pancreas could be used to induce such a transient decline. Intravenous infusion of the peripherally acting acetylcholine analog carbamyl- β -methylcholine chloride (bethanechol) caused a brief spike of insulin release (110) that was very similar to the insulin spike observed before feeding (19, 109). Therefore, a series of experiments was conducted in which various doses of acetylcholine analog were infused intravenously in female Wistar rats during the long intermeal intervals in the light phase (111). Random sequences of up to four doses were infused at least 20 min apart until a fall in blood glucose, if any, was observed. In nine experiments, meal initiation occurred when analog infusion was followed by a transient decline in blood glucose with a shape similar to that observed before meals. In six other experiments, blood

glucose changed by $<5\%$ after a smaller average dose of the analog and no feeding occurred.

Analysis of these data revealed a clear state dependence for meal initiation. The frequency of induction of a transient decline that mimicked the spontaneous premeal glucose transient averaged 28% in the early light phase (a period of low probability for meal initiation) and rose to 79% in the late light and light-dark transition (a period of high probability of feeding). These studies demonstrate the experimental induction of meal initiation during the long intermeal intervals of the light phase in nondeprived rats but only following a brief fall and rise in blood glucose that mimicked the spontaneous blood glucose transients before meal initiation (Fig. 7). The latency of meal initiation following infusion of acetylcholine analog corresponds to the temporal relation between the spike in plasma insulin and meal initiation in free-feeding rats. These studies provide strong support for a causal relation between transient declines in blood glucose and meal initiation under free-feeding conditions (111).

Combining the experimental induction of meal initiation using two different probes, acetylcholine analog and fructose, probably acting through different mechanisms (fructose is not a potent stimulus for insulin secretion), underscores the importance not of the glucose molecule nor blood glucose concentration but instead of the temporal pattern of blood glucose dynamics in the control of meal initiation.

4. Detection and recognition of the signal

In an attempt to identify a role for the parasympathetic nervous system in the origin of the decline in blood glucose and/or its detection by the hepatic glucose receptive elements described by Nijijima (87–89), rats receiving transection of the subdiaphragmatic vagus nerve or its hepatic branch were studied (29). Completeness of vagot-

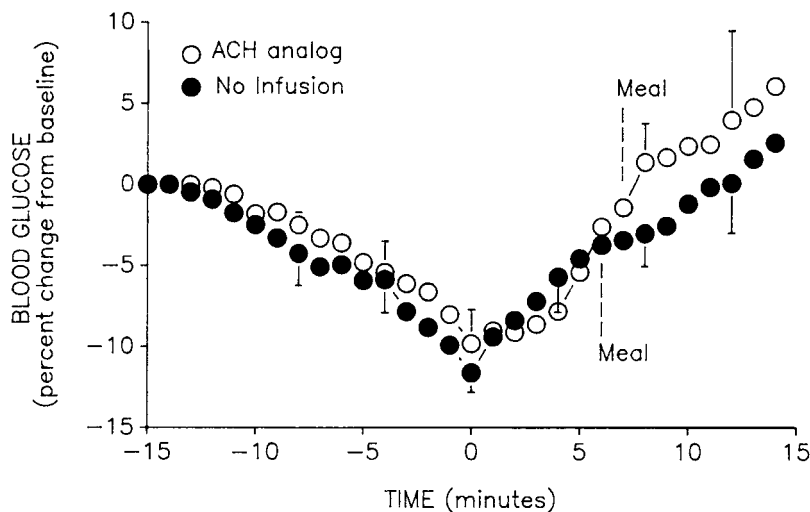


FIG. 7. Transient declines in blood glucose and meal initiation induced by acetylcholine (ACh) analog infusion during the light phase. Blood glucose concentrations are expressed as percent change from the intermeal baseline concentrations. The minimum glucose concentration has been taken as the *time 0* reference in the ACh-induced (\circ) and spontaneous (\bullet) transient declines. Data are means \pm SE ($n = 9$). [From Smith and Campfield (111).]

omy was verified by a combination of behavioral and physiological measures (33). Transient declines in blood glucose qualitatively similar to those observed in intact rats occurred before meals in both vagotomized groups. However, the major finding of these studies was the failure of transient declines in blood glucose to reliably predict meal initiation in 45% of the trials in both vagotomized groups. This is the first experimental situation we have studied in which glucose declines that satisfy the necessary conditions fail to predict meal initiation. These studies suggest that detection of the transient declines by peripheral glucoreceptive elements, innervated by the vagus nerve, is important in the robust coupling between blood glucose dynamics and meal initiation observed in free-feeding intact rats.

Although these studies indicate that central glucoreceptive elements are able to detect and recognize a transient decline in blood glucose and participate in the neural network that maps or transforms that signal into meal initiation, they also demonstrate that the resultant coupling between blood glucose dynamics and meal initiation was much less reliable. Thus these results suggest that peripheral receptors, possibly through an effectively lower threshold, provide faithful coupling between blood glucose and meal initiation. These findings provide the rationale for proposing both a peripheral and a central "TDBG detector" in our working model (Fig. 3). In these experiments, we observed an increased frequency of transient declines in both vagotomized groups that just met or fell below the criteria (magnitude, slope, duration, and/or area below baseline) for transient declines that mapped successfully into meal initiation. This observation is consistent with the notion that the peripheral glucoreceptors have the equivalent of a lower threshold for detection of the systemic signal compared with the central detection elements [e.g., nucleus tractus solitarius (NTS), hypothalamus]. The importance of central integration of peripheral signals in the control of appetite is further demonstrated in these experiments (18).

The mechanism underlying the observed functional coupling between transient declines in blood glucose and meal initiation remains elusive. The hepatic glucoreceptive afferents described by Nijima (87–89) and the central glucoreceptive neurons described by Oomura et al. (95, 96) provide possible afferent pathways for the detection, measurement, and/or relay of control information contained in the transient premeal decline in blood glucose to the caudal brain stem (54, 90) and the lateral hypothalamic neuronal systems (95, 96, 103) that are thought to initiate and sequence the motor programs for feeding (90, 94, 103). A well-described pathway in the brain stem has been shown to be activated by reductions in blood glucose concentration (99, 101). This pathway acts to activate feeding and to return blood glucose to baseline values. One possibility consistent with our stud-

ies is that the afferent elements recognize the pattern of blood glucose dynamics, perhaps by integrating the area of the glucose excursion below baseline, and food seeking and feeding are initiated once the critical pattern or component of the pattern is detected.

To determine if central glucose-responsive neural elements could detect and recognize transient declines in blood glucose and provide a representation of them within the CNS, electrophysiological studies were conducted in rats. Extracellular recordings in the NTS, the first sensory and vagal afferent relay in the CNS, were conducted in anesthetized rats using standard techniques. Units (individual nerve cells or tightly coupled nerve cells) were identified and isolated (by tracking the sinusoidal inflation of a gastric balloon filled with saline). Units in the caudal NTS were activated to change firing rate by electrical stimulation of one or more vagal subdiaphragmatic branches, or brief 10–20% increases in hepatic-portal blood glucose concentration, or perturbations in systemic blood glucose concentration. Glucose-sensitive units were observed that displayed transient "on" or "off" responses with and without accommodation, or sustained increases and decreases in firing rate as a function of glucose concentration. Some units received convergent inputs from multiple vagal branches or from changes in glucose and gastric distension, while other units appeared to respond to only one vagal branch or sensory modality. The firing patterns of these glucose-sensitive NTS neurons were similar to those glucose-sensitive and glucose-responsive units described in the hypothalamus and basal ganglia (64, 95). These electrophysiological recording studies demonstrate that glucose-sensitive neurons in the NTS are capable of detecting transient declines in blood glucose occurring spontaneously and providing a representation of the transient declines in blood glucose within the hindbrain (69).

Given the importance of the hypothalamus in the neural network controlling food intake and body energy balance, electrophysiological recording studies were performed using hypothalamic slices from rat brains. After placing the extracellular electrode next to a unit, the glucose concentration of the chamber was increased or decreased by 10%, and the firing rate was recorded as a function of time. Units that decreased or increased firing rate in response to increases in glucose concentration were observed. Other glucose-sensitive units were observed that displayed transient on or off responses. The firing patterns of these hypothalamic glucose-sensitive neurons were similar to those glucose-sensitive and glucose-responsive units we found in the NTS and the glucose-sensitive units described in the hypothalamus and basal ganglia (64, 95).

Another approach to determining the role of glucose-sensitive neurons in the detection of transient declines in blood glucose was meal initiation studies with a glucose

analog, 2-buten-4-olide (2B4O). 2B4O is a synthesized analog of an endogenous three-carbon sugar compound thought to play a role in the central control of food intake. When 2B4O was injected onto hypothalamic neurons, it mimicked the effects of glucose on the firing rate of those same neurons.

When 2B4O was infused during intermeal intervals, doses of 10–40 μM did not change systemic blood glucose concentration (<5%). When 2B4O at doses of 10, 20, and 40 μM was infused during a transient decline in blood glucose, the shape of the transient decline was not different from when saline was infused. However, meal initiation was completely blocked at 20 and 40 μM , but only partially blocked (33%) after 10 μM 2B4O. This is the second experimental condition in which meal inhibition was dissociated from transient declines in blood glucose, vagotomy being the other. These studies strongly suggest that 2B4O mimicked the effects of a glucose infusion on the glucose-sensitive neural elements. Although a transient decline in blood glucose, with a normal shape, occurred in the peripheral circulation, its detection was blocked by the action of 2B4O on central and peripheral glucose-sensitive neuronal, and meal initiation did not occur (113). These results, together with the results of vagotomy and the presence of transient declines in blood glucose in hyperglycemic rats (see below), indicate that at least one of the roles of glucose-sensitive neuronal elements is to detect and map these specific patterns of blood glucose dynamics into meal initiation.

B. Studies in Genetic and Experimentally Obese and Diabetic Rats

1. Transient declines in blood glucose in ventromedial hypothalamus-lesioned rats and obese Zucker rats

Following the characterization of the role of transient declines in blood glucose in signaling meal initiation, we were interested in extending these studies to rats with altered feeding patterns. After bilateral electrolytic lesions of the ventromedial hypothalamus (VMH), rats display well-characterized alterations in feeding behavior including increased meal size and frequency, particularly during the light phase, which results in increased daily food intake. VMH-lesioned rats also have altered patterns of autonomic neural activity, which is thought to lead to increased pancreatic hormone secretion, altered gastrointestinal function and altered metabolic state (10, 98).

Blood glucose and meal pattern were monitored continuously in female Wistar rats that had received successful bilateral electrolytic VMH lesions (30 days after surgery). Transient declines in blood glucose were observed before meal initiation in VMH-lesioned rats; the parameters of these declines in blood glucose were within the

ranges previously observed in normal rats. However, the striking difference in the VMH-lesioned group was the significantly earlier onset of feeding; meal initiation occurred at the nadir of the transient decline rather than during the rising phase of the decline. When the individual blood glucose and meal initiation records were analyzed, it was discovered that meal initiation occurred as the blood glucose began to decline in six experiments and as the blood glucose rose toward baseline in six other experiments. Both temporal patterns were observed in different trials in the same animal (109).

Similar studies were conducted in genetically obese Zucker (*fa/fa*) rats. The pattern of blood glucose concentrations before meal initiation resembled the pattern observed in obese Wistar rats (Fig. 8, *bottom*). The behavior of the rats before, during, and after transient declines in blood glucose was scored. These results clearly demonstrated that increased physical activity was not a predictor of meal initiation, nor did it correlate with changes in blood glucose (116).

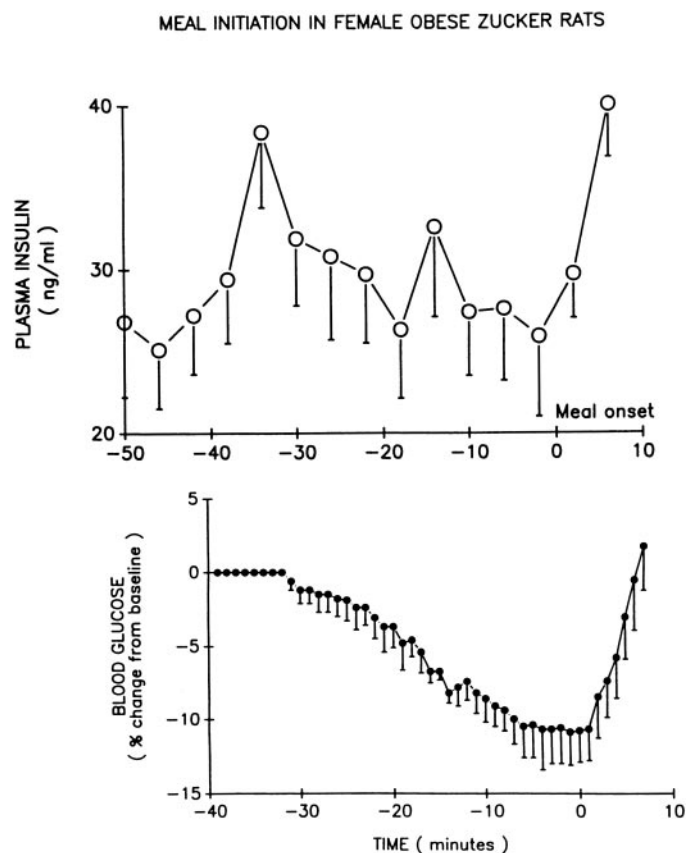


FIG. 8. Plasma insulin and blood glucose concentration profiles before meal initiation in obese Zucker rats. *Time 0* indicates the time of meal initiation. The figure is a composite; the continuous blood sampling for insulin (*top*) and continuous recording of blood glucose (*bottom*) were performed in different experiments. Note the cycles in plasma insulin before meal initiation.

2. Transient declines in blood glucose in experimentally induced diabetic rats

As mentioned above, Jean Mayer felt that decreased rate of glucose utilization in diabetes could adequately explain the hyperphagia commonly observed in this metabolic disease (76, 77). However, he was extremely negative about the ability of any theory based on hypoglycemia to explain the hyperphagia of experimental diabetic rats or humans (see quote in sect. II C). Thus monitoring of blood glucose dynamics before meal initiation in rats with experimental hyperglycemia would provide a robust test of our hypothesis for meal initiation. Specifically, we wanted to determine whether transient declines in blood glucose occurred and signaled meal initiation in hyperglycemic rats (112). Experimental hyperglycemia was induced in rats by administration of streptozotocin, which destroys glucose-sensitive cells in the pancreatic islets and the brain. After stabilization of the diabetic state, cannulas were implanted for continuous blood glucose monitoring. Baseline blood glucose was elevated as expected (range 200–400 mg/dl). During recording, meals of powdered food were initiated only after a transient decline in blood glucose of a larger magnitude than observed in euglycemic rats (nadir-range 20–40 mg/dl; Fig. 9B). However, when expressed as percent change in blood glucose from baseline, the nadir was again 10–12% (Fig. 9A). These results indicate that destruction of glucose-sensitive elements within the CNS by streptozotocin was followed by a resetting upward of the mean baseline blood glucose concentration around which regulation occurred. It has been shown that some aspects of glucose regulation, or the response to hypoglycemia, remain functional following induction of hyperglycemia by streptozotocin or alloxan treatment (100). They also demonstrate that enough of these glucose-sensitive elements in the CNS remained functional so that transient declines of blood glucose, of similar shape as that observed in euglycemic rats, could be detected and mapped into meal initiation (112). Thus, despite Mayer's pessimism, the continuous monitoring of blood glucose dynamics before meal initiation in rats with experimental hyperglycemia provided very strong support for our hypothesis that transient declines in blood glucose signal meal initiation.

C. Plasma Insulin Dynamics During the Premeal Period

1. Male and female lean rats

To evaluate the role of insulin in meal initiation, another series of studies characterized the time course of plasma insulin concentrations before, during, and after meal initiation. In these and related studies, blood was

continuously withdrawn from freely moving male and female Wistar rats at the rate of 25 μ l/min and pooled over 4-min intervals. Sampling began at least 40 min before an anticipated meal and was continued up to 100 min. Plasma insulin was stable during intermeal intervals and then briefly increased by \sim 50% and then returned to basal levels. The peak of the insulin spike occurred at 26 or 13 min (males and females, respectively) before meal initiation and preceded the transient decline in blood glucose. The plasma insulin concentration in female rats before meal initiation is shown in Figure 10. However, in experiments in which feeding did not occur, plasma insulin remained constant (coefficient of variation 11%) throughout the sampling period. Hepatic vagotomy abolished the insulin spike. Plasma insulin reached its lowest point just before the meal in most experiments as reported originally by Strubbe et al. (124). These data suggest that a spike of insulin in the plasma, probably vagally mediated, may play a role in the origin of the transient declines in blood glucose that precede meal initiation (19, 29). However, the presence of the insulin spike is not required for meal initiation.

2. Obese Zucker rats

To further explore the role of insulin in the control of meal initiation, the time course of plasma insulin concentrations before, during, and after meal initiation in genetically obese Zucker rats was determined. In these studies, blood was continuously withdrawn from freely moving female obese Zucker rats at the rate of 25 μ l/min and pooled over 4-min intervals. Sampling began at least 40 min before an anticipated meal and was continued up to 120 min. Integrated plasma insulin concentrations during the 40 min preceding meal initiation were significantly larger (150%) than during intermeal intervals in these recordings (Fig. 8, top). Multiple cycles (2–4) of plasma insulin concentrations were observed in each record; the magnitude of plasma insulin concentrations was larger before meals than during intermeal intervals. In addition, plasma insulin concentrations were lower and tended to decrease during intermeal intervals. These findings, together with the blood glucose patterns (see sect. IV B1), suggest that a correlation exists between plasma insulin dynamics and blood glucose patterns before meal initiation in obese Zucker rats (116).

D. Profile of Plasma Substrates Preceding Meal Initiation

In addition to transient declines in blood glucose, other physiological and metabolic changes can occur in the premeal period. Studies were conducted to determine

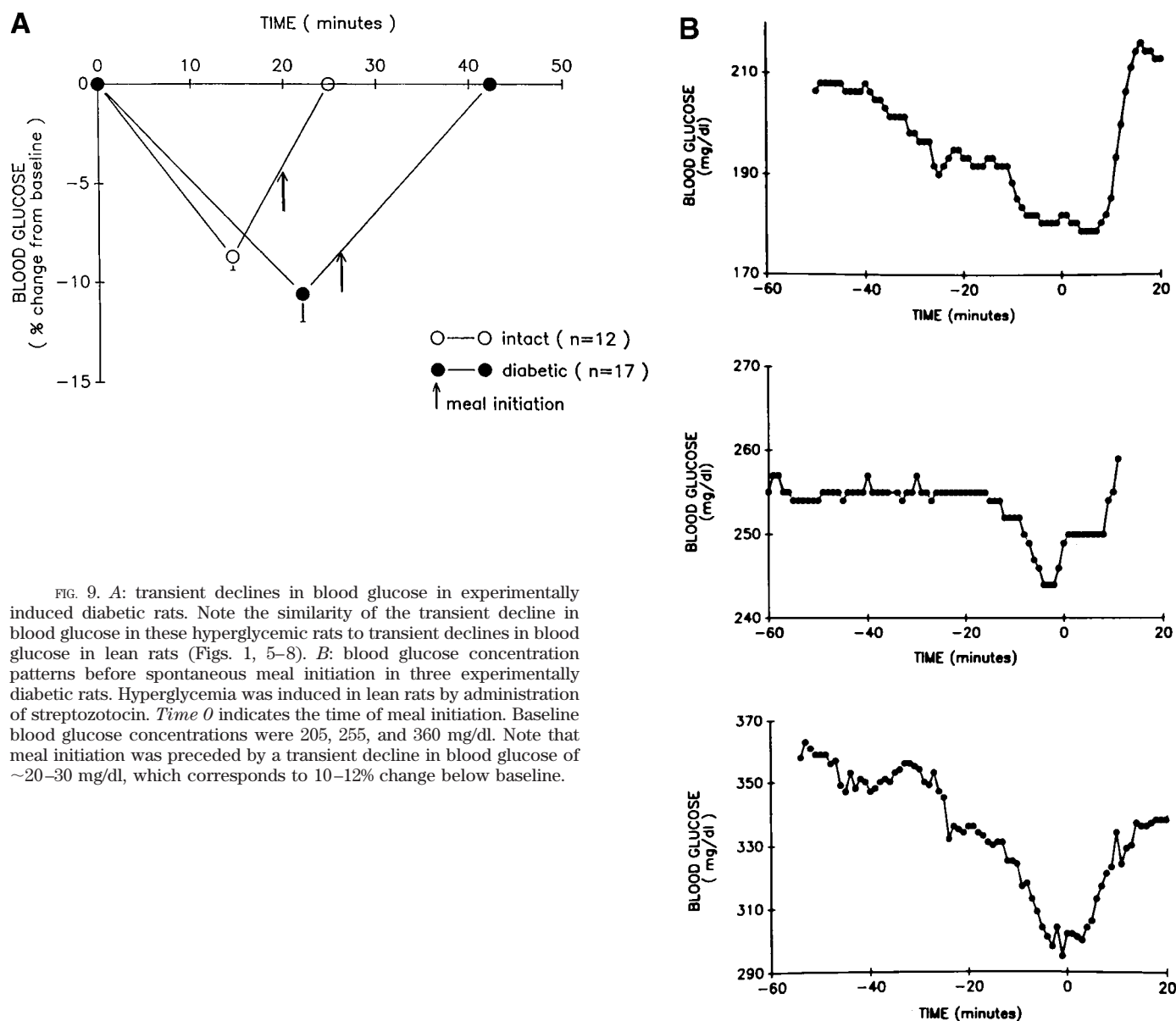


FIG. 9. *A*: transient declines in blood glucose in experimentally induced diabetic rats. Note the similarity of the transient decline in blood glucose in these hyperglycemic rats to transient declines in blood glucose in lean rats (Figs. 1, 5–8). *B*: blood glucose concentration patterns before spontaneous meal initiation in three experimentally diabetic rats. Hyperglycemia was induced in lean rats by administration of streptozotocin. *Time 0* indicates the time of meal initiation. Baseline blood glucose concentrations were 205, 255, and 360 mg/dl. Note that meal initiation was preceded by a transient decline in blood glucose of ~20–30 mg/dl, which corresponds to 10–12% change below baseline.

additional metabolic changes during this period. The concentrations of plasma substrates (glycerol, free fatty acids, triglycerides, and ketones) were determined in chronically cannulated, freely moving free-feeding female Wistar rats using similar design. Feeding occurred in 13 experiments, whereas in 6 other experiments meals were not observed.

Analysis of the individual profiles by aligning the peak value of each substrate and averaging the time to meal initiation revealed the following pattern of plasma substrates before meals: a significant decrease (~50%) in triglycerides at 11 min followed by peaks in glycerol (~50%) and triglycerides (~80%) at 6.5 min before meal initiation. A rise in plasma free fatty acids (~30%) also occurred at 6 min before meal initiation, which corresponds to the nadir of the transient decline in blood

glucose. However, plasma ketones were significantly lower before meals than during intermeal intervals.

These data suggest that transient changes occur in plasma triglycerides, glycerol, and free fatty acids during the transient decline in blood glucose (30). These changes in plasma substrates were consistent with the observed transient changes in plasma insulin and glucose during the same time interval. Insulin administration has been shown to reduce plasma ketone body concentration (48, 53). These results suggest that changes in plasma substrates before meal initiation may be in response to the antecedent perturbations in plasma insulin that signal meal initiation in free-feeding rats. However, the participation of one or more of these metabolic fuels in the generation of transient declines in blood glucose or in the control of meal initiation cannot be excluded.

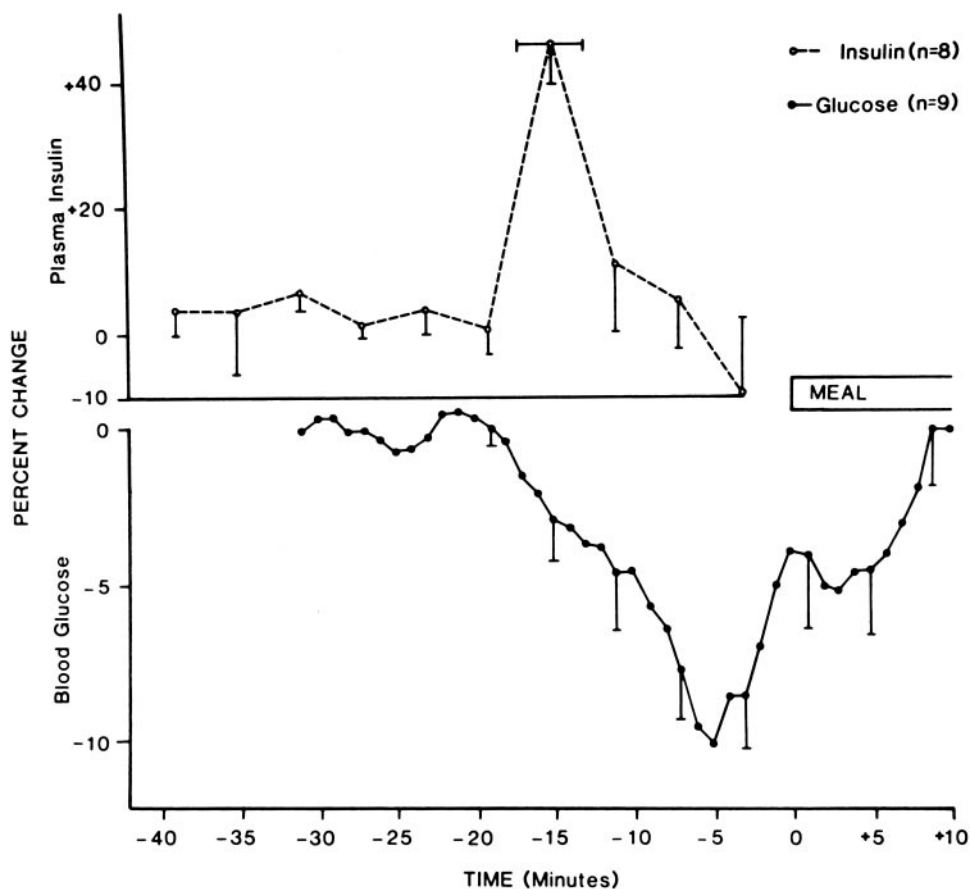


FIG. 10. Plasma insulin and blood glucose concentration profiles before meal initiation in lean female rats. The figure is a composite; the continuous blood sampling for insulin (*top*) and continuous recording of blood glucose (*bottom*) were performed in different experiments. Note the spike in plasma insulin before meal initiation.

E. Role of Blood Glucose Dynamics as a Determinant of the Intermeal Interval

It was also noted that the intermeal interval was much shorter in the experiments in which meal initiation occurred earlier during the transient decline in blood glucose. This analysis suggested an association between the slope of the blood glucose trajectory during feeding and the intermeal interval.

To test this suggestion, the slope of blood glucose concentration during the initial 10 min of feeding and subsequent intermeal intervals were computed in experiments in VMH-lesioned and intact rats. A significant, positive correlation was found between the slope of the blood glucose trajectory and intermeal interval. Thus, if blood glucose concentration was constant or fell during the initial 10 min of feeding, subsequent feeding would occur with a short latency (17.8 ± 4.8 min), whereas if blood glucose rose during feeding, as one might expect, the longer (98 ± 6.8 min) intermeal interval would be observed. Negative slopes in blood glucose during feeding were observed in only ~20% of meals in intact rats but during 50% of the meals in VMH-lesioned rats. These studies suggest that the CNS is monitoring not only excursions of blood glucose below baseline prior to feeding

to signal meal initiation, but also the blood glucose trajectory during feeding as a determinant of the intermeal interval. These results support the importance of the pattern of blood glucose dynamics, including during feeding, and the peripheral and/or central detection and processing of glucose related inputs by the CNS in the control of ingestive behavior (21).

F. Studies With a Palatable and Preferred Carbohydrate Option

Sclafani (107) has shown that polyose, an easily digestible hydrolyzed starch, is highly preferred by many species including rats. They have shown that it can be used to support robust conditioned flavor preferences (107). Experimental studies were conducted in which blood glucose was continuously recorded while the test rat could freely choose to eat powdered food diet only, polyose only [in powder or gel (32%) form], both diet options, or nothing. Groups of rats were habituated to powdered food and either powdered polyose or polyose gels for 1 wk before surgical implantation of the blood withdrawal cannula. Experiments were conducted after complete recovery (4–7 days after surgery).

Daily caloric intake and meal pattern were not sig-

nificantly changed by the presence of the polycose diet option. However, the majority of ingested calories in individual meals (64–100%) were from polycose. No polycose or powdered food meals were observed during periods of stable blood glucose concentration. After transient declines in blood glucose similar to those seen in other studies, the initiation of either polycose alone or polycose and powdered food meals were observed. These studies demonstrate that the ingestion of a highly preferred, familiar carbohydrate food option either alone, or combined with rat food, was signaled by the temporal pattern of blood glucose dynamics. It should be noted, however, that signaled meals of different composition were preceded by similar transient declines in blood glucose.

The observation that polycose-containing meals occurred only after transient declines in blood glucose may indicate that polycose, which initially was a novel food, had become a familiar food with experience over time. However, the significant preference for either form of polycose over powdered food was retained. Thus it is tempting to speculate that the transition from novel food item to incorporation into the set of habitual food items may involve blood glucose dynamics. Further research will be required to test this hypothesis (15).

G. Studies in Food-Deprived Rats

The studies of meal initiation in free-feeding rats suggested the following question: If the recognition of the transient decline in blood glucose was totally uncoupled from food intake by completely preventing feeding, would the transient declines in blood glucose continue to occur or would they disappear or “extinguish”? In this study, blood glucose was continuously recorded in nonconditioned rats fasted for 24 h. Baseline blood glucose concentrations were significantly lower in the deprived rats than in free-feeding rats studied previously (78 ± 2 vs. 104 ± 5 mg/dl, respectively, $P < 0.05$). During the light phase of the light-dark cycle, brief declines in blood glucose (8%, <8-min duration) were observed that were not associated with overt feeding behavior ($n = 6$). At the light-dark transition and during the beginning of the dark cycle, transient declines in blood glucose with a magnitude similar to those observed in free-feeding rats occurred (nadir = -12% at 20 min, $n = 11$), and food-seeking behavior was observed ~ 22 min after the beginning of the transient declines in blood glucose. In contrast to studies in free-feeding rats, the durations of the declines were prolonged (40 min), and blood glucose did not always completely return to the baseline, but rather to a slow, linearly decreasing asymptote; that is, the posttransient decline “baselines” were slowly decreasing, straight lines over time. These studies suggest that

transient declines in blood glucose continue to occur even after 24 h of complete food deprivation. These results imply that the processes that generate transient declines in blood glucose and, thus, signal meal initiation are independent of feeding behavior, are persistent, and do not extinguish within 1 day (115).

H. Studies in Rats Working For Food

To determine if meal initiation in nonconditioned rats working for food would also be signaled by transient declines in blood glucose, female rats housed in operant cages were trained to bar press for food pellets (continuous reinforcement). Rats were required to earn all of their food by bar pressing; one 45-mg pellet was dispensed with each bar press. After training, cannulas were implanted for continuous blood glucose monitoring. Blood glucose concentration was stable during intermeal intervals, and meals were preceded by transient declines in blood glucose. Bouts of bar pressing were initiated ~ 15 min (range: 5–24 min) after the beginning of the transient decline in blood glucose. The parameters of these declines were similar to those observed before meals of powdered food in the free-feeding condition. These results suggest that meal initiation in rats working for food is predicted by transient declines in blood glucose and provide further support for the relationship between blood glucose dynamics and meal initiation (82).

I. Conditioning Studies

Rats that have learned to associate cues with food delivery reliably initiate meals upon subsequent exposure to these learned signals for meal initiation (130, 139). Based on the pioneering studies of conditioning on glucose and insulin concentrations by Woods et al. (139), we explored whether the transient decline of blood glucose that is causally related to spontaneous meal initiation was also evident in cases of conditioned meal initiation. Over a 12-day period, male rats were conditioned to associate a tone-light conditioning stimulus (CS+), presented at 3-h intervals, with food and the opportunity for ingestion by providing six such signaled meals per day (130, 132). After conditioning, rats were maintained ad libitum except for ~ 3 -h food deprivation imposed before tests examining the effects of the CS+ on meal initiation and blood glucose. Although the CS+ reliably elicited food anticipatory behavior and meal initiation, its presentation did not result in any systematic change of blood glucose, as measured with continuous monitoring of blood glucose. However, measurement of blood glucose during the intermeal interval revealed the presence of a transient decline of blood glucose at times that, as a result of the conditioning schedule, rats had learned to anticipate a meal. The pa-

rameters of this blood glucose dynamic were similar to the transient decline of blood glucose associated with the initiation of spontaneous meals. Control rats that had not been provided with an opportunity to learn that meals were provided every 3 h showed no analogous changes in blood glucose concentration during the intermeal interval. These studies further define the occasions under which meal initiation is associated with transient declines of blood glucose and suggest that this dynamic may be related to meals initiated in response to endogenous signals as opposed to meals instigated by external signals such as exteroceptive CS+ or the presence of palatable foods (131). These results are consistent with the earlier studies of Woods et al. (139). They found similar results and offered a similar conclusion.

V. PHYSIOLOGICAL AND BEHAVIORAL STUDIES OF TRANSIENT DECLINES IN BLOOD GLUCOSE AS A SIGNAL FOR MEAL REQUESTS AND INCREASED HUNGER RATINGS: STUDIES IN HUMANS

A. Meal Requests and Increased Hunger Ratings Are Preceded by Transient Declines in Blood Glucose in Humans

The purpose of these human studies was to examine the hypothesis that hunger and meal initiation in humans could be related directly to patterns of blood glucose. Our specific objective was to answer the following questions: 1) Do transient declines in blood glucose concentration occur in human subjects? 2) If so, do they precede changes in hunger ratings and meal requests? A major limitation of the previous human experiments on the role of blood glucose in hunger has been the necessity of discrete blood sampling on experimenter-determined schedules (2, 7, 55, 97, 125–129, 133).

Each of 18 healthy adults (9 males and 9 females) was housed individually in a room isolated from time cues the night before and during the study. They were informed that blood glucose and other biorhythms including hunger and thirst would be monitored. Following an overnight fast, a double-lumen cannula for blood withdrawal was placed in the antecubital vein; blood and heparin mixture was withdrawn at a rate of 55 $\mu\text{l}/\text{min}$ (blood withdrawal rate = 25 $\mu\text{l}/\text{min}$), and blood glucose concentration was monitored over a 2- to 6-h period. Breakfast was not served. Subjects controlled the room lighting and rested, slept, read, or wrote during the experiment. Visual analog ratings of internal state including hunger and satiety were completed approximately twice each hour using a quasi-random schedule when the subjects were not asleep. Subjects could request a meal at any time but were not required to do so. A verbal (spo-

ken) request was required to obtain a meal. The experiment ended when the meal was presented to the subject. The methodology utilized in these studies is described in more detail elsewhere (35).

The average time course of blood glucose concentration during the experiment is shown in Figure 11A. Overall, in 83% of the 18 subjects, both changes in hunger ratings and spoken meal requests were preceded by, and significantly correlated with, spontaneous, brief transient declines in blood glucose (nadir: -10% below baseline at 27 min). The recordings of blood glucose concentration during the experiment for two subjects are shown in Figure 11, B and C. Unchanged hunger ratings were associated with stable blood glucose concentrations. The association between meal requests and/or change in hunger ratings and declines in blood glucose was significant ($X^2 = 5.96$, $P \leq 0.02$). The pattern, magnitude, and time course of these declines was similar to those observed in rats (35).

B. Evidence for Causality: Induction of Transient Declines in Blood Glucose and Changes in Hunger Ratings

The significant association between increased expression of hunger and transient declines in blood glucose observed was tested in a second study in which insulin infusions were used to induce transient declines in blood glucose that mimicked the spontaneous transient declines observed before meal requests.

Subjects were admitted to a metabolic research room isolated from food and time cues in the Clinical Research Center of The Rockefeller University Hospital where they remained for 3 days. Each subject was studied twice. After an overnight fast, cannulas for blood withdrawal and intravenous infusion were placed in the subject's veins, blood glucose concentration was monitored, and visual-analog hunger ratings were obtained over a 2- to 4-h period. Breakfast was not served. After a stable blood glucose baseline was obtained, a sterile solution of either saline or insulin (5 mU/kg) was injected intravenously in <2 min. Hunger ratings were obtained up to four times an hour. This dose of insulin was selected based on pilot studies in other healthy subjects. Two days later, the experiment was repeated and the other solution was infused.

Combining the results in five subjects indicates that hunger ratings increased (mean delta = 22 ± 5 mm) after insulin-induced transient declines in blood glucose concentrations (nadir = -11% at 38 min). These preliminary results support and strengthen the conclusion that the transient decline in blood glucose represents a temporal pattern that reflects an antecedent physiological event or provides a signal related to the expression of hunger in

BLOOD GLUCOSE DYNAMICS PRIOR TO MEAL REQUEST IN HUMAN SUBJECTS

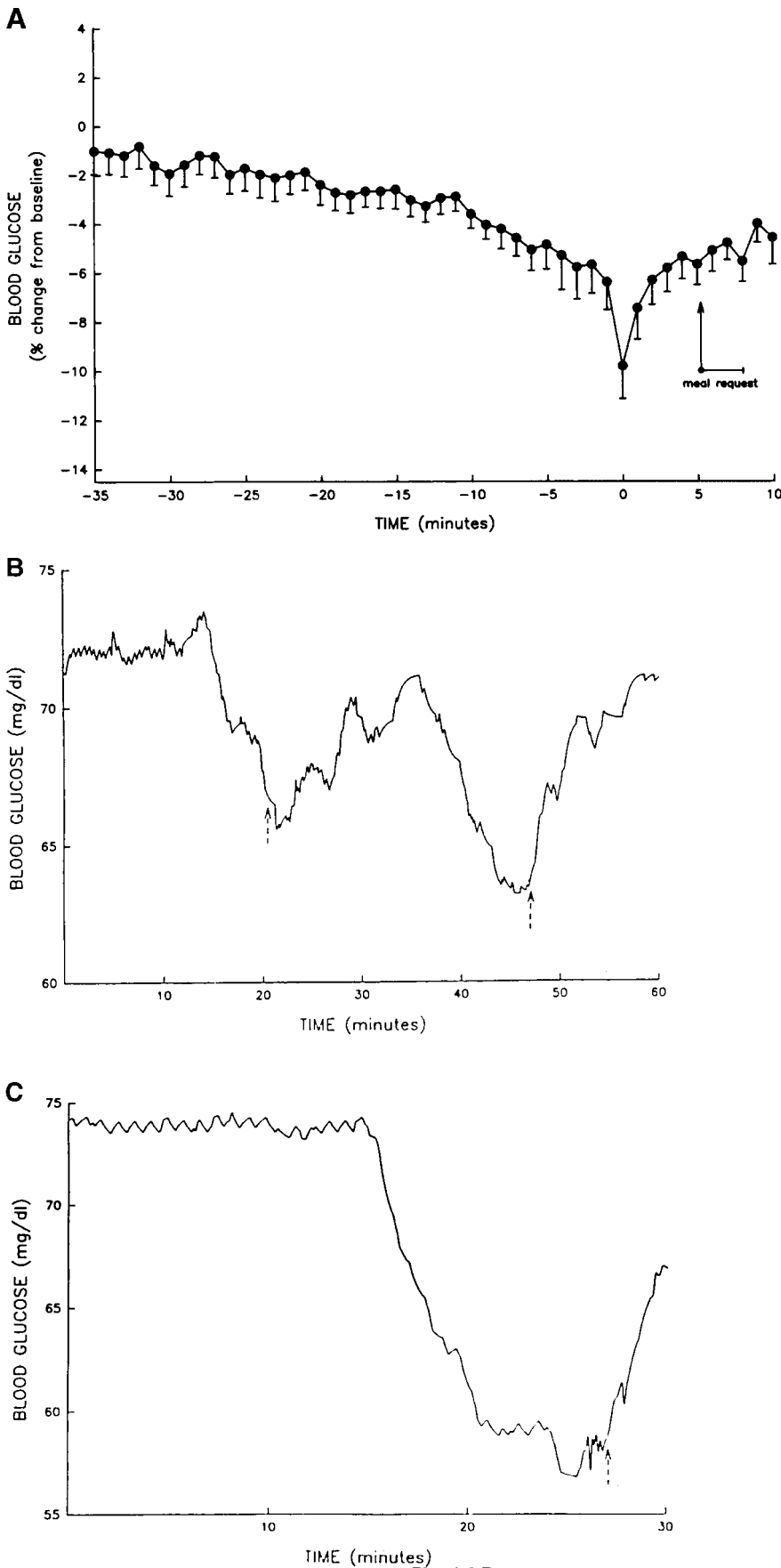


FIG. 11. A: average time course of the transient decline in blood glucose in time isolated humans. Blood glucose concentrations are expressed as percent change from the baseline concentration in this figure. The minimum glucose concentration has been taken as the *time 0* reference. Data were selected each minute from the reference point and averaged in each of 18 experiments. Data are means \pm SE. The meal request is indicated by the vertical arrow. Mean baseline glucose concentrations were 83.3 ± 3 mg/dl. B and C: examples of temporal evolution of blood glucose concentration before meal requests in two subjects. Blood glucose concentration is shown on the ordinate and is expressed as mg/dl. The abscissa is time in minutes. Spoken meal requests are indicated by the dotted arrow. [From Campfield et al. (35), with permission from Elsevier Science.]

humans. These results are consistent with many reports in the literature of hunger after insulin administration (8, 45, 68, 75, 83).

C. Recently Completed Human Studies

The human studies described above were all conducted in the fasting state to begin the experiment with a stable blood glucose concentration. Recently, studies of continuous monitoring of blood glucose concentration in humans have been completed in the absorptive or postprandial state in the laboratory of Professor Wim Saris at the University of Maastricht in The Netherlands (78–80). In the first study, male volunteers were time-blinded beginning in the morning and they were free to request meals at any time while their blood glucose was continuously monitored. As expected, transient declines in blood glucose were observed that predicted spontaneous meal requests ($X^2 = 8.29$; $P < 0.01$). Subjects were then given either isoenergetic (1 MJ) high-fat or simple carbohydrate beverages. Meal requests were also observed after rapid falls in blood glucose from a meal-induced peak in blood glucose (80). [In rats, meal initiation was also observed after similar rapid falls from meal-induced peaks in glucose concentration (23).] Meal requests in the subjects were separated by intermeal intervals twice as long after high-fat intake than after simple carbohydrate intake (80). In the second study, three beverages of identical volume, isoenergetic high-fat or simple carbohydrate or aspartame, were offered after the first meal request, and high-fat or high-carbohydrate foods were available for the remainder of the study. Blood glucose patterns were predictive of the next intermeal interval. For all beverages combined, transient declines in blood glucose and meal initiation were significantly associated ($X^2 = 19.0$, $P < 0.001$), and the duration of blood glucose responses and intermeal intervals were significantly correlated ($X^2 = 16.8$; $P \leq 0.001$) (79). In the third study, the men performed an exercise designed to deplete glycogen in the evening then ate a low-carbohydrate evening meal and went to bed in the laboratory. The next morning, blood glucose was monitored continuously and, after the baseline blood glucose and respiratory quotient were determined, the subjects had free access to high-fat and high-carbohydrate foods. Insulin and glucagon concentrations were not measured in this study. After glycogen-depleting exercise, the first meal request of the next day was not related to transient declines in blood glucose (in 8 of 10 requests). However, after the initial meals were eaten during the monitoring of blood glucose, transient declines in blood glucose from a stable baseline preceded the next meal request in five of six requests ($X^2 = 4.93$; $P < 0.05$). These results suggest that depletion of glycogen stores may dissociate meal requests from transient

declines and allow meal requests (and meals) to occur during periods of blood glucose stability. After refeeding, the predictable relationship between transient declines in blood glucose and meal requests and meals was reestablished (78). It should be noted that the ingestion of calorie-containing beverages and meals in these three studies caused a dynamic response in blood glucose that made the assessment of the relationship between subsequent meal requests and blood glucose pattern impossible until the baseline blood glucose concentration was again reestablished.

D. Discussion of Human Studies

These combined behavioral and metabolic experiments answer both of the specific questions we posed in the affirmative under these experimental conditions. These experiments, using continuous, on-line monitoring and visual analog ratings of hunger, have demonstrated an association between transient declines in blood glucose concentration and meal requests and changes in hunger ratings in human subjects isolated from food and time cues. This association was observed following both spontaneous and insulin-induced transient declines in blood glucose. Most of the deviations in blood glucose observed closely resemble the patterns of blood glucose dynamics shown to precede and signal meal initiation in rats (14, 18–23, 109, 111–114, 116). This suggests that, at least under these experimental conditions, a signal for hunger in humans is associated with transient declines in blood glucose that is similar to that observed in rats.

These results are consistent with some, but not all, previous studies of the relationship of blood glucose and human hunger as described above (see sect. 11B). Two additional reports on this topic have appeared in the literature. The first was a retrospective analysis of blood samples collected at random intervals averaging 20 min before and after lunch from seven subjects living in time isolation for several days (97). The result failed to demonstrate a significant deviation in plasma glucose concentration before meal requests. This failure in the absence of continuous monitoring of blood glucose, based on discretely measured and mathematically interpolated plasma glucose levels, does not imply that no such correlation exists and does not invalidate the extension of the hypothesis, supported by these results summarized here, that blood glucose dynamics play a role in the onset of human hunger. The second study was conducted using techniques and an experimental design similar to those reported here and has only been reported in abstract form (38). The authors reported transient declines in blood glucose or periods of stable blood glucose concentration before meal requests. They described their findings as a “preprandial glycemic phenomenon.” Although these data

cannot be evaluated in detail, these findings appear to be consistent with the present results.

Although there is evidence, reviewed above, supporting the proposition that the transient decline in blood glucose is causally related to meal initiation in rats and meal request in humans, the "true" signal for meal initiation may be 1) an "originating event," neuronal and/or biochemical, that causes the transient decline in blood glucose concentration; 2) the "transient decline in blood glucose concentration"; 3) a covarying "metabolic and/or neural signal"; or 4) an "event," neuronal and/or biochemical, "in response" to the transient decline in blood glucose concentration.

The determination of which of these alternatives is correct will require further research on meal initiation.

Nevertheless, the studies reviewed here strongly suggest that transient declines in blood glucose 1) represent an "endogenous signal" for meal initiation; 2) their detection and mapping into meal initiation can be viewed as a "pattern detection and recognition problem"; 3) may be useful "probes," or experimental interventions, to elucidate the operation, nature, and decision algorithms of the widely distributed neural network that controls meal tak-

ing and, thus, feeding behavior; and 4) play an important role in the regulation of individual meals and by interacting with longer-term regulatory systems, such as the brain insulin system (63) or the leptin pathway (25, 26, 28, 31, 106, 137), also may play an important role in the regulation of body energy storage.

VI. FUTURE DIRECTIONS OF THE WORK, LIMITATIONS, AND IMPLICATIONS

A. Future Research Directions

Among the important directions for future research to further test the signal detection and recognition theory of meal initiation are the four major areas listed in Table 2.

B. Limitations

1. Limitations of previous studies based on earlier conceptualization

Despite numerous studies of human feeding behavior in which physiological and/or biological mechanisms or

TABLE 2. *Future research directions*

Research Area	Objectives
Mechanistic studies of the origin, detection, and recognition of patterns of blood glucose	Metabolic studies using stable isotopes should be directed at determining the sequence of steps that leads to transient declines in blood glucose. Among the likely possibilities are the following: 1) an autonomic neural- and/or hormonal-mediated transient decrease, followed by increase, in the rate of hepatic glucose production, and/or 2) an autonomic neural- and/or hormonal-mediated transient increase, followed by a decrease, in the rate of peripheral glucose utilization. Other studies should be directed at the neural mechanisms responsible for the detection of transient declines in blood glucose concentrations. Glucose-sensitive neurons, capable of detecting and transducing the transient declines in blood glucose, have been identified in vagal afferents, area postrema-nucleus tractus solitarius, parabrachial nucleus, paraventricular, ventromedial, and lateral hypothalamic neurons. Detailed mapping studies of the location and relative abundance of glucose-sensitive neurons in these areas should be completed.
Neurophysiological, neurochemical, and molecular studies on glucose-sensitive neurons in the periphery, the brain stem, and the hypothalamus	The molecular mechanisms by which local concentrations of glucose act on glucose-dependent channels and determine the membrane potential of these neurons must be identified. The structure, pharmacology, and dynamics of these glucose-dependent channels must also be determined. One possibility is ATP channels linked with sulfonylurea binding sites in the central nervous system. Multisite electrical recording and microinjection studies using multibarrel electrodes should provide additional information to test this theory.
Studies directed at elucidating the algorithms by which the detection of the transient declines in blood glucose is mapped into meal initiation	These studies will require the application of newly emerging technology to experimentally determine the activity of neuron ensembles in widely distributed multiple neural networks and to develop appropriate dynamical system models and to perform the necessary computational analyses. These technological innovations will be required to identify and characterize these algorithms, decision rules, conditional probabilities, and molecular mechanisms that are responsible for the central and peripheral integration that lies at the center of the regulation of feeding behavior and body energy balance.
Additional studies of the onset of hunger and meal requests in humans with both normal and disordered feeding behavior	These studies will require the application of the type of experimental paradigm presented in the human studies described above and elsewhere (35) and should identify the biological and physiological bases of the diverse range of disordered feeding behavior displayed by humans (e.g., eating disorders, obesity, and diabetes). The elucidation of the mechanisms responsible for transient declines in blood glucose will also be very important for increased understanding of hunger in humans. Whether a similar sequence of insulin and glucose patterns occur before meal requests in humans must await further research.

factors have been examined, many of these previous studies may have been flawed by several categories of limitations in design and execution (12). The categories and examples of these limitations are listed in Table 3.

It is important to recognize that descriptive studies have 1) revealed interesting effects on the amount consumed as a function of the stimulus properties and method of food presentation, 2) reinforced clinical observations of the striking degree of disordered eating observed in pathological states, and 3) optimized the design of preload experiments. However, it is our opinion that solely descriptive studies should not continue ad infinitum while question or hypotheses driven experiments are not performed or are delayed. Rather than conduct additional descriptive studies, it may be preferable for those with resources and opportunity to study human feeding to devote that time and energy to the careful design and performance of a single more complex, difficult, and important question or hypothesis-based investigation. In the least, human feeding studies should combine both descriptive and hypothesis-based components.

Although these limitations and the specific examples were taken from experimental studies of human feeding, these same limitations would apply to studies with rats and other animals. Even a superficial review of food intake studies in rats would reveal a common reliance on investigator-determined time schedules, clock-based measurements, and "test meals" in "testing cages." However, it is true that some of these studies also include hypothesis-driven components. The multiple repetition of primarily descriptive, rather than more question- or hypothesis-driven, studies of food intake of rats has surely limited our true "understanding" of the physiological and biochemical mechanisms of the control of food intake. Per-

haps adding hypothesis-based components to descriptive studies of food intake would advance our understanding.

2. Obstacles to linking eating to physiology

Several other barriers that may have blocked additional experimental demonstrations linking human eating to physiology are listed in Table 4 (12).

3. Limitations on the patterns as signal conceptualization

The fact that transient declines in blood glucose specifically signal meal initiation but do not predict the duration or size of the signaled meals can be viewed as its strongest virtue. At the same time, this specificity can also be viewed as a major limitation. This all or none character of the control of meal initiation is entirely consistent with the model which assumes that the processes of meal initiation, maintenance, and termination of meals are separate components of a behavioral process or pattern and result from separately controlled decisions of the same widely distributed neuronal network which regulates feeding behavior. This model, which forms the heart of our theoretical formulation, predicts that feeding behavior is multifactorial and more complex than the previous single factorial models of the control of feeding. This apparent limitation will be easily overcome once the other patterns that signal maintenance of feeding and meal termination are identified and are incorporated into a multiple pattern formulation for the control of single meals.

Another limitation of the present state of our signal detection and recognition formulation is that the control of meal initiation in rats, or meal requests in humans, is

TABLE 3. *Limitations of previous studies of human feeding*

Limitations	Examples
Experimental settings that were not appropriate for the expression of feeding-related behavior	Study environments ranging from eating laboratories with multiple stations (may preclude physiological measurements) to metabolic ward hospital rooms set-up for feeding studies to specially designed isolated testing environments (which may suppress the expression of the "normal" feeding behavior) may not be appropriate for combined physiological and behavioral studies.
Investigator-determined time schedules	Experimenter-determined "sampling" schedules for drawing blood samples, completing visual-analog scales, and/or questionnaires, administering preloads, and presenting test meals.
Clock-based measurements in which the experimental manipulation or measurements were made on a time schedule predetermined by the investigator	The study of "lunch" in the lab regardless of antecedent perceptions of hunger/satiety or habitual diet or customary eating schedule of the subjects. The perceptions and experience brought to the experiment by the subject are not collected and are often ignored. Also, postexperiment data are often ignored due to short study periods.
Emphasis on descriptive studies	Studies of stimulus characteristics of the food items presented to the subjects: form, composition, calorie content, palatability, and degree of novelty of food items on the amount of food consumed. Studies of patients with disordered eating and the compelling need for effective treatment for these conditions contribute to focus and overemphasis on descriptive studies. Differences in the subjective perceptions of hunger and satiety using visual-analog rating scales and the amount consumed between subjects with disordered and normal eating are described. Preload studies of caloric compensation: variation of the stimulus characteristics of the preload and/or the test meal and the degree of caloric compensation has been determined.

TABLE 4. *Obstacles to linking eating to physiology*

Barrier	Comments
Negative opinions about the validity of subject self-report of hunger/satiety	Dissociation of subjective ratings and amount eaten in patients with eating disorders and the difficulty of accurately recalling the number, timing, and composition of meals hours or days later causes the validity of any self-report of subjective ratings, composition, and amount eaten in specific meals and daily intake to be doubted. However, studies using contemporaneous recording of meals in food diaries have shown that, under appropriate circumstances and with sufficient motivation, free-living and eating human subjects can accurately self-report intake and subjective hunger/satiety ratings over a period of weeks.
Negative impact of frequently observed uncoupling of self-reported perceptions of hunger/satiety and metabolic state in patients with "disordered eating"	Uncoupling of metabolic state and hunger ratings in patients with disordered eating does not imply that subjects with more ordered perceptual worlds and eating behavior cannot provide accurate and meaningful self-reports. Even studies with independent measures of energy expenditure (e.g., doubly labeled water) that document a systematic underreporting of caloric intake also show that the self-reported intakes are proportional to the estimated actual intake. Our studies of blood glucose and meal requests in humans have demonstrated the utility of subject self-reports.
Poor correlation between hunger/satiety ratings and amount eaten in some studies	Assumption that the processes of meal initiation, maintenance, and termination are not separate components of a behavioral process or pattern, but rather that the perception of hunger is tightly and deterministically linked to meal size. Little or no allowance made for the modulation of meal size by the perceived or experienced taste and/or palatability of the presented test meal and/or its "postingestional consequences," that is, the physiological responses to the test meal. An extreme extrapolation of this view would suggest that identical perceptions of hunger would accurately predict identical meal sizes. However, the view that the antecedents of the perception of hunger are a distinct component of the behavioral sequence of eating and would predict only a variable correlation between hunger ratings and meal size would exist within and across individual subjects.
Excessive reliance on amount of food consumed in laboratory studies of human feeding	This reliance requires that changes in subject's perception of hunger/satiety of cognitive and/or physiological origin must reach a "behavioral" threshold that leads to changes in the amount consumed. Factors correlated or causally linked to the evolution of hunger or satiation or the subtle modulation of palatability are eliminated from investigation because these aspects require using other dependent measures in addition to amount consumed.
Relative scarcity of studies of spontaneous or subject-initiated eating	Although more complex and demanding than some clock-based or investigator-determined studies, experimental investigations of spontaneous or subject-initiated eating are required to understand human eating and the relative roles of cognitive and physiological factors. Because the factors controlling initiation and determining the number of meals would be obvious therapeutic targets to modify food intake, the elucidation of these factors is a high-priority goal of human feeding research, and its achievement will require studies of subject-initiated single and multiple meals and unrestricted intermeal intervals.

not explicitly linked to a longer-term regulation of body energy balance; that is, it is not clear how the meal-to-meal regulation determined by the transient declines in blood glucose concentration is linked to the 24-h regulation of daily food intake and the regulation of body energy balance and body weight over a 1- to 3-day interval. A critical part of our working hypothesis is that transient declines in blood glucose interact in an as yet unspecified manner with the brain insulin system of Woods and colleagues (63) and the leptin pathway (25, 26, 28, 137) to achieve the daily regulation of food intake and regulation of body weight over 1–3 days. As was discussed above, the dynamic pattern of plasma insulin concentration is an integral part of the temporal sequence of patterns that occur in the premeal period leading to meal initiation. Woods and colleagues (63, 104) have clearly and repeatedly demonstrated that excursions in plasma insulin con-

centration are reflected in the patterns of brain insulin concentration which have predictable and robust effects on daily food intake and body weight regulation. The current understanding of the biology of leptin suggests that it is also an important long-term regulator of body energy balance (25, 26, 28, 31, 137). Therefore, a functional interaction between the patterns in plasma insulin and blood glucose concentrations that signal the initiation of each individual meal and the longer term regulation and modulation of daily food intake and body weight controlled by brain insulin and leptin could be linked through the dynamical patterns of plasma insulin and leptin. The experimental demonstration of this hypothesized linkage and interaction must await further research.

Our current formulation is also limited by the fact that we have not yet defined the origin of the transient declines in blood glucose. Thus we cannot state how the

observed transient declines in blood glucose are themselves initiated. We are confronted with a series of nested questions not unlike the well-known sets of nested Russian dolls; answer one and another question arises that demands an answer. Thus meal initiation is caused by the transient decline in blood glucose which may be caused by transient changes in hepatic glucose production and peripheral glucose disposal which is in turn caused by x , which is caused by z , and so on. Possible mechanisms for the origin of the transient decrease in systemic blood glucose include decreased glucose absorption from the intestine (70), decreased glucose production from the liver, increased central and/or peripheral glucose utilization, or a combination of transient alterations in both glucose production and utilization. The suggestion that decreased glucose absorption across the intestine is responsible for the transient declines in blood glucose is not supported by our observation that transient declines occur frequently during the dark phase when the stomach remains at least partially full and the occurrence of transient decline in 24-h fasted rats. A role for plasma insulin is suggested by studies described above, and other hormonal or neural signals involved in glucoregulation also may play a role (108). The identification of the mechanism or mechanisms and the nature of the efferent signals responsible for these modifications in glucose homeostasis must await further experimentation. Whether the transient decline in blood glucose was a reflection of a change in brain utilization of glucose or an event of peripheral origin or both also cannot be determined by these studies and must await further research.

However, one mechanistic possibility for the origin of the transient declines in blood glucose that will be experimentally tested in future studies is plasma insulin concentration and its modulation by descending autonomic neural inputs (3, 16, 17, 32, 33, 62, 98, 110, 136). As described above, a brief peak in plasma insulin concentration occurs just before the beginning of the transient decline of blood glucose in lean and obese rats (19, 29). Not only is the temporal sequence of changes in plasma insulin consistent with the hypothesis, but also the biological effects of insulin, to cause increased peripheral glucose uptake and disposal and to suppress hepatic glucose production. In addition to causing a peak in plasma insulin, descending parasympathetic, and possibly sympathetic, descending neural inputs to the intestine and liver can entrain the many biochemical processes that are meal related (digestive enzymes, transporters, key enzymes in pathways of carbohydrate, amino acid and lipid metabolic pathways) (66, 102, 108, 140). Since numerous studies have demonstrated an important role for experimental conditioning in the regulation of gastrointestinal responses and ingestive behavior, the antecedents for the tight coupling between transient declines in blood glucose and meal initiation may not be discernible in every

meal but, instead, may be the result of a complex set of conditioned biochemical and physiological processes (130, 132, 134, 138). Therefore, even though transient declines in blood glucose occur in vagotomized rats without insulin peaks, this does not mean that the premeal insulin peak and other vagal-mediated biochemical events that occur in the normal sequence, or pattern of meal initiation in intact rats are not mediators of the transient decline in blood glucose.

Once the descending autonomic neural modulation of plasma insulin and other processes is tested and other mechanisms are experimentally identified, further refinement of our formulation can occur that will reduce the degree of uncertainty. As each of the other topics listed above under future directions of the research is identified through experimental studies, further refinements in our formulation will be made.

A final limitation of our formulation is that the continuous measurement of blood glucose concentration in human subjects, upon which it is based, requires a certain degree of restriction of the subject's freedom of movement and a limitation of the range of possible experimental settings under which feeding can be observed. This is a direct result of the absolute requirement of the invasive nature of the measurement of blood glucose. If in the future a less invasive method of continuous measurement of blood glucose is developed, then these limitations and restrictions can be relaxed. However, in practical terms, the extensive list of research questions related to meal initiation in rats and meal requests and the onset of hunger in humans that invite experimental study using our current methodology should fully occupy many investigators for the foreseeable future.

C. Implications

The identification of a potential signal for meal initiation imbedded in blood glucose dynamics is significant because it provides a biological basis, and, thus, additional justification, for the continued experimental study of feeding behavior in humans in both the laboratory and metabolic ward. This finding, therefore, gives added confidence to the investigation of metabolism and biochemistry as related to human feeding behavior. The existence of a biochemical and physiological signal for hunger and meal requests in humans argues strongly for renewed interest in the experimental study of hunger and the development of biologically based strategies for the reshaping of disordered feeding behavior. This new research may provide important information about the interaction and relative importance among the several factors in the decision-making process that ultimately leads to the perception and behavioral expression of hunger in humans. These results may provide a biological foundation for a

more complete and complex understanding of human feeding behavior that should emerge from future, biologically based behavioral research. It is also hoped that this increased understanding will allow more precise diagnosis of disordered feeding and improved and more efficacious treatment of these devastating conditions.

On a more speculative note, transient declines in blood glucose may also play a role in the behavioral and metabolic adaptation or "learning" that occurs following alterations in food availability and lighting schedules, food and diet options, cost of working for food, as well as the conditioned feeding situation. The studies reviewed above on rats working for food and in conditioned feeding suggest such a role, but additional studies will be required to fully explore this possibility.

Is the "pattern as signal" conceptualization advocated here simply a complementary theoretical construct to the other more established concepts in the control of feeding mentioned in the introduction or is it a competing, alternative notion with more explanatory power? The ultimate ability of this construct to explain adequately meal initiation and/or the intermeal interval or other aspects of feeding behavior remains to be determined by further research. However, the successful extension of this paradigm in the last several years to additional animal models of disordered feeding (e.g., experimentally diabetic and genetically obese rats) and the successful extension to the domain of human meal requests and changes in hunger ratings demonstrate the explanatory power, the applicability of a general theoretical construct from rats to humans, and provide increased confidence in this approach. These patterns of blood glucose and plasma insulin concentration in rats and humans must be subjected to further and more rigorous experimental tests, and additional patterns have to be identified and tested. The impressive initial successes of this approach suggest to the authors that its application to the numerous disparate and competing findings in feeding research may provide insight and, possibly, facilitate the development of a more complete understanding.

VII. FINAL THOUGHTS

Has the description and appreciation of the pattern of blood glucose concentration that precedes and signals meal initiation in rats and meal requests in humans led us to a new conceptual "world view" and established the "pattern as signal paradigm" for future consideration of the problem of the regulation of feeding behavior? Alternatively, has the path we followed to our current formulation just led us full circle back to a more complex, but not substantially different, formulation of Mayer's glucostatic hypothesis? The answer to these questions is both yes and no. Mayer's original proposal was consistent

with the state of knowledge regarding the regulation of metabolism with its emphasis on tissues utilization of metabolic substrates in the early 1950s. During this period of rapid growth of metabolic biochemistry, the mechanistic details of the utilization of these substrates or "fuels" in newly emerging metabolic pathways in the peripheral tissues were being described. Yet Mayer made the insightful and essential link between peripheral metabolism, brain metabolism, and the regulation of feeding behavior and body energy balance by defining as critical the rate of glucose utilization in an undefined brain region. Our current theoretical formulation still contains as a critical element the peripheral and central detection and recognition of not the utilization of glucose but rather the pattern of blood glucose concentration. Yet it makes a much more explicit and dynamic statement about the shape of transient declines in peripheral blood glucose that are required to initiate spontaneous feeding behavior in rats or the perception of hunger in humans. We have now defined the precise antecedent conditions required, in terms of the shape of the transient declines in blood glucose, for meal initiation or meal requests. Although the precise pattern required to be detected and recognized by the central and peripheral nervous system has been specified, the neural mechanisms that underlie this detection and the algorithms used to perform this pattern recognition and map this pattern into the initiation of feeding or meal requests remain to be elucidated by future research. In our formulation we have specifically defined the "what," the transient decline in blood glucose, that signals a transition in the prevailing behavioral state to meal initiation, but leaves the "how," the origin of the transient decline in blood glucose and its detection, as a theoretical construct. This situation is in marked contrast to the glucostatic theory, in which both the signals responsible for organizing feeding behavior (what) and the origin and the detection of those signals (how) were only vaguely, at best, specified. Whether this theoretical construct will serve the same important role as an organizing principle and have same positive impact on the focus, direction, productivity, and increased understanding as Mayer's glucostatic hypothesis based on glucose utilization remains to be seen as the future of our field emerges.

We hope that the unraveling and identification of the mechanisms responsible for the origins and the detection of transient declines in blood glucose will be an important focus of the feeding research in the coming years. However, this search will only be a special case of the more general and, in our opinion, the most critical and important problem in our field: the central and peripheral integration of both peripherally and centrally generated signals into the exquisite and elegant regulation of feeding behavior and body energy balance. If the theoretical concept presented here proves to be helpful in resolving this essential problem and continues to be useful in thinking

about feeding behavior and its physiological bases, then a small change in blood glucose will have indeed had a large effect on behavior.

The ideas, concepts, and implications of the experimental results discussed here have been strongly influenced by the experimental approach, seminal integrative perspectives, major concepts, and brilliant insights into the regulation of feeding of Jacques Le Magnen, Director of the Laboratoire de Neurophysiologie Sensorielle et Comportementale, Collège de France in Paris, France, who died in May 2002.

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