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Nutritional and metabolic factors in the regulation of reproductive hormone secretion in the primate

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Nearly a century ago, Darwin described the fact that well-nourished domestic animals are more fertile than animals receiving a poor diet (Darwin, 1896). Since that time, scores of research articles have testified to the importance of nutritional factors in the regulation of reproduction in virtually every mammalian species, including man. In Western societies over the last 300 years, we have witnessed a dramatic decline in the age of puberty onset, a phenomenon associated with improved diet and socio-economic conditions. Studies by Kennedy & Mitra (1963) in the early 1960s established that the age of puberty onset in the rat is more closely correlated with the animal's body-weight than chronological age. This relation was soon shown to apply to human beings as well. On the basis of epidemiological information, showing an association between body-weight and the onset of menarche in girls, Frisch & Revelle (1970) proposed that a 'critical body-weight' was determinative in controlling the onset of puberty. Further investigation, again based on epidemiological information, led to the suggestion of a 'minimum percentage of body fat' as a possible signal to puberty onset (Frisch & MacArthur, 1974). Excitement and controversy swelled around these findings (Wilen & Naftolin, 1977; Penny *et al.* 1978; Glass & Swerdloff, 1980); the findings were not in question, only the inference that body composition *per se* could somehow trigger the awakening of the reproductive system at the time of puberty. Others, finding exceptions to the rule, were soon to become dissatisfied with the body-composition theory and argued that 'basal metabolic rate' was more likely to serve as a physiological link between the body's energy-regulating components and the reproductive system (Crawford & Osler, 1975). It remains unchallenged that all these variables—body size, body fat and metabolic rate—are somehow associated with the activity of the reproductive axis; however, despite these labours and our collective wisdom, we are left with only descriptive associations—no mechanisms to explain how the body 'knows' its metabolic rate or how big and fat it may be.

Some light has been shed on this problem by studying the effect of metabolic stresses on the reproductive system. For example, in the case of severe weight loss in the adult human, caused by anorexia nervosa, gonadotrophin secretion is markedly reduced owing to an inhibition of gonadotrophin-releasing hormone (GnRH) secretion (Boyar *et al.* 1974). When these patients recover during

nutritional therapy, GnRH secretion is restored and plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels increase coincidently with weight gain (Warren *et al.* 1975; Beumont *et al.* 1980). Malnourished children have reduced plasma gonadotrophin levels and delayed puberty compared with well-nourished children (Dreizen *et al.* 1967; Chakravarty *et al.* 1982). Together, these clinical observations underscore the powerful association between nutrition and the development and maintenance of normal reproductive function.

Other metabolic stresses, besides weight loss, can have profound effects on reproductive function. Severe exercise, particularly when it is associated with low body fat reserves, is commonly associated with reproductive disturbances. Competitive athletes and ballet dancers have delayed pubertal development and menarche compared with other groups (Frisch *et al.* 1980; Warren, 1985). In the case of ballet dancers with amenorrhoea, if their exercise programme is interrupted, due to illness or injury, menstrual cycling may resume without a coincident change in body composition (Warren, 1980). As in the case of anorexia nervosa, the effects of exercise are thought to be mediated at the level of the brain and its control of GnRH secretion. These observations suggest that body composition itself is unlikely to account for the alterations in reproductive function associated with either exercise or weight loss, but rather some derivative of metabolic status (which must reflect not only body composition but demand on the body's energy reserves, as well) must control the activity of the brain–pituitary axis. What are the derivatives of body-weight, body fat or metabolic rate that control GnRH secretion, switching on or off the activity of the entire reproductive system as a function of metabolic status? Could these derivatives be metabolic hormones or substrates, whose circulating levels reflect the functional and reserve status of the animal's metabolic milieu?

The focus of our research has been to identify the factors linking an animal's metabolic status to its reproductive system. Having adopted the working hypothesis that these factors are blood-borne, we launched a campaign to sift among the myriad possibilities to find the critical hormones and substrates.

Effects of dietary restriction on gonadotrophin secretion

As a step toward the goal of identifying the metabolic factors linking the body metabolism and the reproductive system, we studied the effects of restricted food intake on plasma levels of LH and FSH and selected metabolic hormones and substrates in the adult male primate (Dubey *et al.* 1986). We chose castrated, adult rhesus monkeys for the experiment. The animals were housed individually under controlled photoperiod (lights on 06.00–18.00 hours) and surgically fitted with indwelling venous catheters, led to remote sampling ports. This permitted drawing serial blood samples from the animals with neither restraint nor anaesthesia.

First, animals were studied during a 10 d control period, during which each animal received fifty monkey pellets/d (approximately 4800 kJ (1150 kcal)/d) and

baseline determinations of circulating hormones and substrates were made. We measured body-weight and plasma levels of LH, FSH, cortisol, thyroid hormone, insulin, glucose, glycerol, β-hydroxybutyrate, and free amino acids in blood samples at selected intervals throughout the study. Subsequently, animals were placed on an energy-restricted diet of seven to twelve pellets/d (690–1110 kJ (164–265 kcal)/d) for an additional period of 20–34 d, during which hormone and substrate determinations were again made. Following this, animals were returned to their normal control diet and studied for another 30–35 d until their body-weights returned to pretreatment levels.

Before imposition of restricted food intake, plasma levels of LH and FSH were observed to be in the normal high range, characteristic of the castrated animal. As a result of the energy-restricted diet, mean plasma levels of LH and FSH declined, in some cases to non-detectable levels; however, only the decline in LH was statistically significant ($P<0.05$) during the last 5 d of the reduced-energy diet (Fig. 1). Despite the average decline in plasma gonadotrophin levels, only three of six animals exhibited an unequivocal decrease in plasma LH levels, while two of

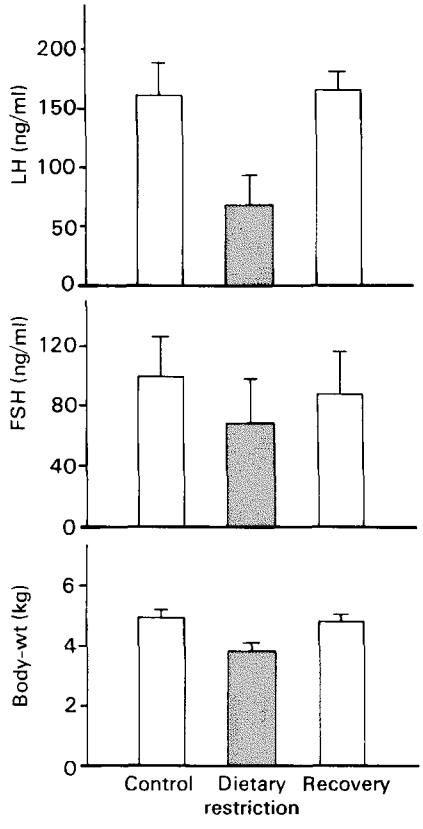

Fig. 1. Plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations and body-weight in six orchidectomized rhesus macaques (*Macaca fascicularis*) before, during and after placing the animals on an energy-restricted diet. Values are means with their standard errors represented by vertical bars. (After Dubey *et al.* 1986.)

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the remaining three animals showed a partial decrease and the last animal demonstrated no effect whatsoever. Body-weight declined significantly during the period on the energy-restricted diet, but we did not find a significant correlation between percentage decline in LH and body-weight reduction. After returning the animals to their normal diet, body-weight and plasma levels of LH and FSH increased to their normal pretreatment values. These observations demonstrate that a prolonged imposition of a restricted-energy diet inhibits gonadotrophin secretion but did not identify whether the dietary effect was mediated at the level of the pituitary or the brain.

In a second experiment we determined whether GnRH could restore gonadotrophin levels once they had been reduced by energy restriction. First, we imposed a restricted-energy diet on another group of monkeys and observed, as in the first experiment, a profound reduction in plasma gonadotrophin levels. Then, while the animals were still maintained on the restricted diet, we initiated a chronic intermittent infusion of GnRH (0·1 μg/min for 3 min/h) and measured its effect on plasma LH and FSH levels. Within 24 h, GnRH restored gonadotrophin secretion, achieving plasma levels comparable to those observed before imposing the nutritional deficit (Fig. 2). These results argue that the dietary restriction exerts its effect at the brain, compromising its ability to secrete GnRH. Still unresolved is what causes the reduction of GnRH secretion. What is the mechanism whereby reduced energy intake thwarts the ability of the brain to secrete GnRH? What are the 'signals' that tell the brain of the body's compromised metabolic status?

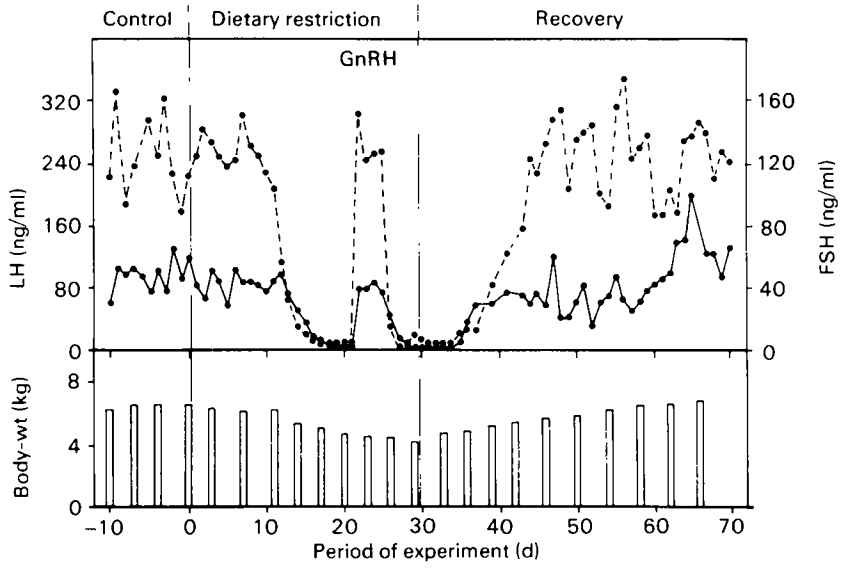

Fig. 2. Plasma luteinizing hormone (LH, – – –) and follicle stimulating hormone (FSH, ———) concentrations and body-weight in a single orchidectomized rhesus macaque (*Macaca fascicularis*) before, during (with and without gonadotrophin-releasing hormone (GnRH)) and after placing the animal on an energy-restricted diet. (After Dubey *et al.* 1986.)

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To gather clues, we measured concentrations of circulating metabolic hormones and substrates and observed how they varied as a function of the dietary regimen and plasma gonadotrophin levels. We found that during dietary restriction, plasma levels of insulin ($P<0.08$), 3,5,3′-triiodothyronine ($P<0.05$), and thyroxine ($P<0.05$) were markedly suppressed, whereas plasma cortisol levels were increased ($P<0.05$) (Fig. 3). Neither the percentage decline in insulin and thyroid hormone nor the increase in cortisol was significantly correlated with the reductions in plasma LH levels.

Plasma levels of glucose, glycerol and β-hydroxybutyrate also declined, but only β-hydroxybutyrate was changed significantly ($P<0.05$) (Fig. 4). Plasma levels of tyrosine, phenylalanine, isoleucine, leucine and valine were similar before and after dietary restriction but were reduced significantly in samples obtained after returning animals to a normal diet. The concentrations of 3-methylhistidine were significantly increased ($P<0.05$), whereas plasma glutamate levels were significantly reduced ($P<0.05$) during the period of dietary restriction compared with the pretreatment control and recovery periods.

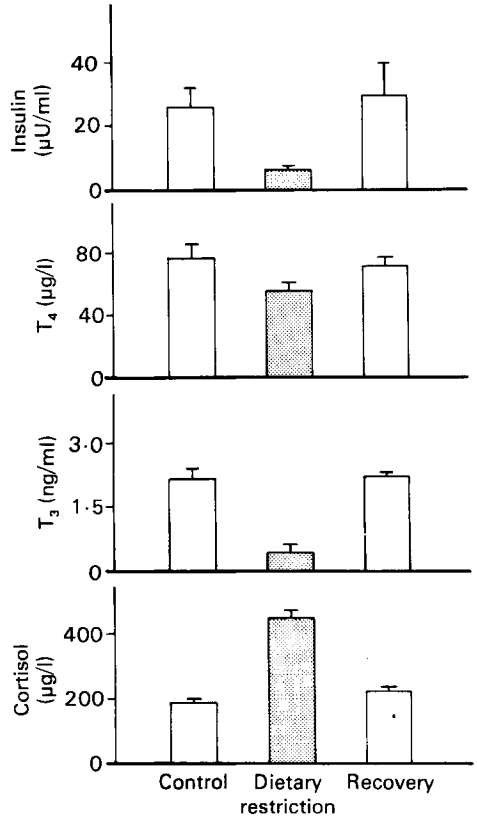

Fig. 3. Plasma concentrations of insulin, thyroxine (T_4), 3,5,3,′-triiodothyronine (T_3) and cortisol in orchidectomized rhesus macaques (*Macaca fascicularis*) before, during and after placing the animals on an energy-restricted diet. Values are means with their standard errors represented by vertical bars for eight to nine animals. (After Dubey *et al.* 1986.)

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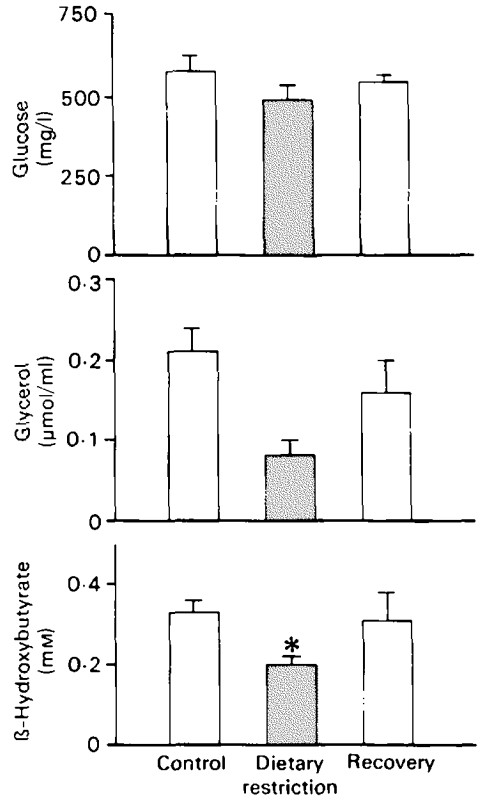

Fig. 4. Plasma concentrations of glucose, glycerol and β-hydroxybutyrate in orchidectomized rhesus macaques (*Macaca fascicularis*) before, during and after placing the animals on an energy-restricted diet. Values are means with their standard errors represented by vertical bars for eight or nine animals. *Significantly different from control period ($P<0.05$). (After Dubey *et al.* 1986.)

These observations provide some possible clues to the identity of the metabolic factors serving as an interface between body metabolism and the reproductive system, but do not resolve the problem. Insulin is one possible candidate for mediating this function. Insulin was suppressed during dietary restriction in our experiment, as has been previously noted in man during starvation (Cahill, 1970). Insulin binds specifically to receptors in the arcuate nucleus and median eminence of the brain (Van Houten *et al.* 1980), regions regarded to serve important regulatory functions for the control of GnRH secretion. Acting at the brain, insulin influences the availability of substrates that are essential for the synthesis of neurotransmitter systems in the brain, particularly that of norepinephrine and serotonin (Fernstrom, 1983), both of which are thought to interact with GnRH secretory mechanisms. It is therefore possible that by influencing the synthesis of certain neurotransmitters critical for maintaining GnRH secretion, insulin could provide a link between nutritional status and reproductive function. However,

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arguing against a role for insulin is the observation that the magnitude of insulin suppression during dietary restriction was not correlated with the degree of gonadotrophin inhibition. Nevertheless, it is possible that the brain receives and processes the insulin signal as an integrated function of the minute-to-minute plasma insulin levels, an integration not easily determined by measuring peripheral blood levels (Woods *et al.* 1980).

Changes in circulating levels of certain amino acids could also mediate the effects of dietary restriction on GnRH secretion. One possible candidate in this regard is glutamate, an excitatory amino acid thought to serve as a central neurotransmitter. Glutamate levels were markedly suppressed during dietary restriction in our experiment. This fact, together with the observation that peripheral administration to rats or monkeys of either glutamate or a related analogue, *N*-methylaspartate, stimulates GnRH secretion, marks glutamate as another possible molecular interface between the reproductive system and nutritional status (Olney *et al.* 1976; Wilson & Knobil, 1982; Tal *et al.* 1983).

Cortisol levels were significantly elevated during the period of restricted energy intake in our experiment, a phenomenon also noted in malnourished human beings (Alleyne & Young, 1967; Lunn *et al.* 1973). Indeed, administration of hydrocortisone acetate to castrated rhesus monkeys effects an inhibition of LH and FSH secretion through an action mediated at the level of the central nervous system (Dubey & Plant, 1985). These findings suggest that stress and the consequent stimulation of glucocorticoid secretion may play a role in suppressing GnRH secretion during starvation.

There are certain physiological features shared by the prepubertal and the starved adult primate. First, both represent conditions during which GnRH secretion is at a minimum. Second, the metabolic milieu of the prepubertal primate resembles certain features of the adult fasting state. Maybe, by virtue of its high metabolic rate, the prepubertal primate is literally starving the neuroendocrine elements controlling GnRH secretion. Perhaps the impact of even a relatively short, between-meal 'fast' in a young primate exerts an effect on the juvenile's reproductive system equivalent to a more prolonged fast in the adult. Is it possible that the prepubertal primate is literally suspended in a 'fasting' metabolic state, analogous to that of adult fasting states such as anorexia nervosa?

To address these questions, I will first present evidence to support the concept that the onset of puberty reflects merely an amplification of GnRH secretion from a population of neurons present and active long before puberty. Second, I will show evidence that the metabolic transition from the fed to the fasted state occurs more rapidly in juvenile compared with adult primates, giving some credence to the notion that the prepubertal animal may be relatively substrate-limited compared with the adult. And third, I will present evidence that a sustained increase in the availability of plasma amino acids and glucose can amplify GnRH secretion in a prepubertal primate, an animal that would not otherwise show such an increase until the normal time of puberty months later.

Pubertal amplification of GnRH secretion

Childhood in primate species is a time during which the entire reproductive system is relatively quiescent by virtue of the fact that GnRH secretion is minimal. Intermittent infusions of GnRH will activate the reproductive system, demonstrating that neither the pituitary nor gonads are restraining the onset of puberty; only the full awakening of GnRH secretion is required to activate the complete cascade of pubertal maturation (Wildt *et al.* 1980). However, the reproductive axis of the prepubertal primate is not entirely inactive. Indeed, the brain is capable of synthesizing and releasing GnRH long before puberty onset, as evidenced by the fact that administering antisera against GnRH to prepubertal macaques (*Macaca fascicularis*) effects an inhibition of the low levels of circulating LH in these animals (Fig. 5) (Cameron *et al.* 1985*a*). GnRH secretion begins, most likely, even before birth, and continues throughout life, only to be amplified or diminished as a function of life-stage or pathological disturbances, and neurocytological evidence bears this out.

Quantitative morphometric analysis of GnRH-containing perikarya, based on immunocytochemical staining in the medial basal hypothalamus of the male macaque, reveals that the number and distribution of GnRH neurons is similar between prepubertal and adult animals (Cameron *et al.* 1985*a*). However, GnRH-containing perikarya in adult brains are significantly larger in total cross-sectional area and in the cross-sectional area of the cytoplasm than are those measured in prepubertal animals (Fig. 6). The increase in cross-sectional area of GnRH neurons with puberty may reflect cellular hypertrophy associated with

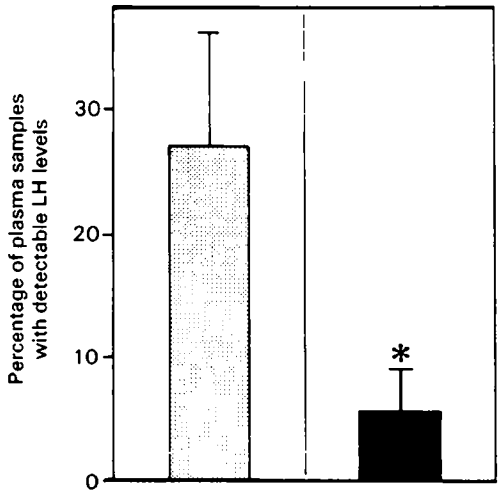

Fig. 5. Percentage of plasma samples with detectable levels of luteinizing hormone (LH) in prepubertal male macaques (*Macaca fascicularis*) before (□) and after (■) administration of gonadotrophin-releasing hormone antiserum. Values are means with their standard errors represented by vertical bars for ten animals. *$P<0.05$. (After Cameron *et al.* 1985*a*.)

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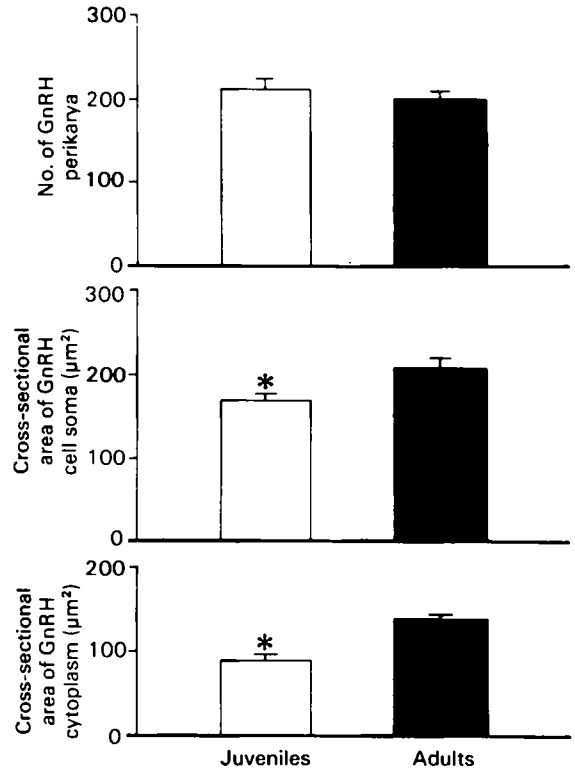

Fig. 6. Number, somal cross-sectional area and cytoplasmic cross-sectional area of gonadotrophin-releasing hormone (GnRH)-containing perikarya in the medial basal hypothalamus of adult (n 3 or 5) and juvenile (n 3 or 5) male macaques (*Macaca fascicularis*). Values are means with their standard errors represented by vertical bars. *$P<0.05$. (After Cameron *et al.* 1985*a*.)

increased protein synthesis, as is known to occur in cells of the anterior pituitary following gonadectomy. These findings suggest that GnRH neurons are in place early in childhood, but are not fully activated.

What amplifies GnRH secretion at the time of puberty? Could the synthesis and secretion of GnRH be limited by the availability of certain substrates in ample supply in the adult, limited only in the juvenile? How do the plasma profiles of metabolic hormones and substrates differ between adult and juvenile primates?

Metabolic correlates of sexual maturation

To identify metabolic hormones or substrates that could herald the onset of puberty by reflecting metabolic rate or body size to the GnRH neurosecretory elements, we measured plasma levels of insulin, glucose, amino acids, β-hydroxybutyrate and glycerol after a meal in prepubertal and adult male macaques (Cameron *et al.* 1985*b*). In addition, based on the idea that plasma ratios of certain amino acids to one another may influence neurotransmitter levels in the brain (e.g. norepinephrine and serotonin) (Fernstrom, 1983) and might thereby

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modulate GnRH secretion, we also tested the hypothesis that the ratios of either tyrosine or tryptophan to other large neutral amino acids (LNAA) may be different between juvenile and adult animals. Thirteen adults and thirteen prepubertal macaques were selected for the study. Each animal was surgically fitted with an indwelling venous catheter connected to a swivelling tether mounted on top of the cage; this permitted the collection of blood samples at frequent intervals without disturbing or upsetting the animals. All animals were given an identical mixed meal, after which blood samples were obtained, beginning 1·5 h and continuing on until 52 h after the meal.

Circulating insulin levels declined in a similar fashion in the two groups until 16 h after the fast had begun, after which basal insulin levels fell more rapidly and were significantly lower in the juvenile compared with the adult animals ($P<0·025$) (Fig. 7). Plasma glucose levels were similar in prepubertal compared with adult animals throughout most of the postprandial period. During the first 20 h of fasting, plasma concentrations of LNAA (i.e. tyrosine, tryptophan, phenylalanine, valine, leucine, isoleucine) declined more rapidly in juvenile compared with adult animals (Fig. 8); however, the ratios of neither tyrosine nor tryptophan to other LNAA differed between adult and juvenile animals. Circulating levels of β-hydroxybutyrate remained low during the first 20 h of fasting and were similar between the age-groups; however, by 24 h after the meal, β-hydroxybutyrate levels increased more rapidly and attained higher absolute plasma levels in juveniles compared with adults (Fig. 9).

These observations demonstrate that during the postprandial period, plasma levels of insulin and certain amino acids decline more rapidly in juvenile than in adult macaques, suggesting that the metabolic transition from the fed to the fasted

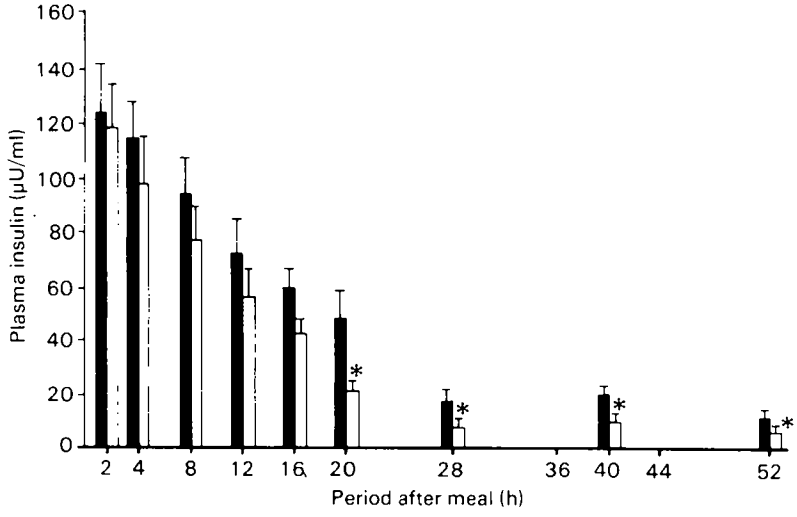

Fig. 7. Plasma insulin concentrations in juvenile (n 13, □) and adult (n 13, ■) male macaques (*Macaca fascicularis*) from 0 to 52 h after a meal. Values are means with their standard errors represented by vertical bars. *$P<0·05$. (After Cameron *et al.* 1985*b*.)

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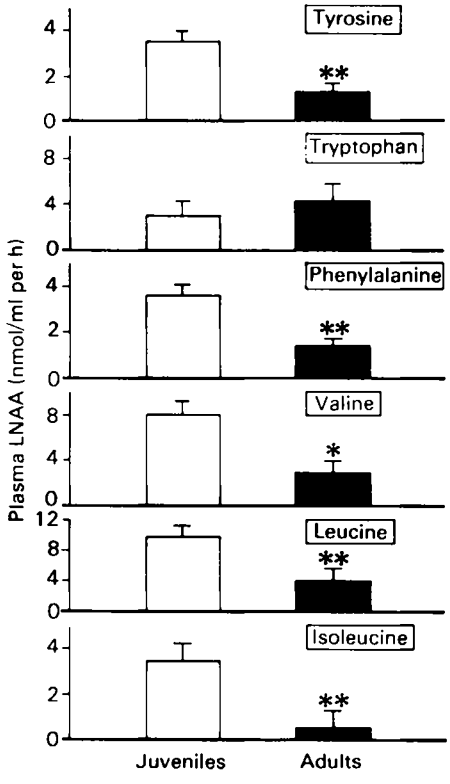

Fig. 8. Rates of decline in plasma concentrations of large neutral amino acids (LNAA) in juvenile (n 13, □) and adult (n 13, ■) male macaques (*Macaca fascicularis*) during the first 20 h after a meal. Values are means with their standard errors represented by vertical bars. $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$. (After Cameron *et al.* 1985*b*.)

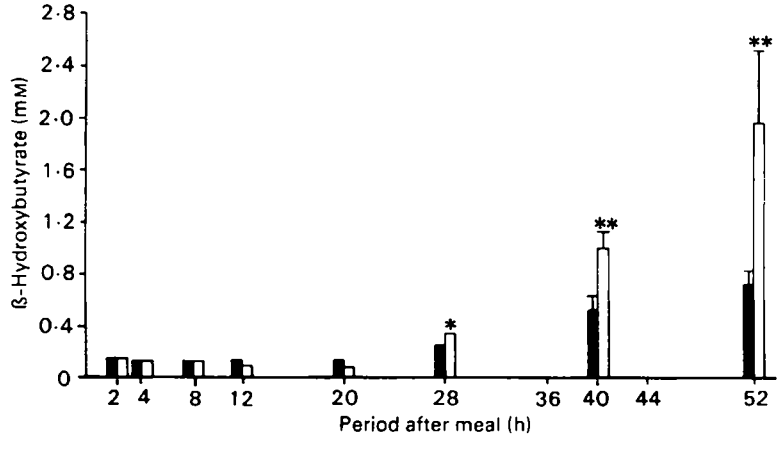

Fig. 9. Plasma concentrations of β-hydroxybutyrate in juvenile (n 13, □) and adult (n 13, ■) macaques (*Macaca fascicularis*) from 0 to 52 h after a meal. Values are means with their standard errors represented by vertical bars. $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$. (After Cameron *et al.* 1985*b*.)

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state develops more rapidly in younger animals. Similar differences in the rate of adaptation to fasting have been reported between children and adult human beings (Chaussain *et al.* 1977; Haymond *et al.* 1982; Kerr *et al.* 1983). The metabolic response to fasting may occur more rapidly in juveniles as a result of their increased rate of energy consumption and decreased substrate availability. During the postabsorptive phase, children exhibit a higher glucose flux rate than do adults, perhaps reflecting the greater brain:body-mass ratio of children, the brain being the body's primary glucose sink (Bier *et al.* 1977; Haymond *et al.* 1983).

Products of β-oxidation of free fatty acids (e.g. β-hydroxybutyrate) increase more rapidly in children compared with adults. Within the first few hours after eating, children display higher circulating levels of branched-chain amino acids (valine, leucine, isoleucine) than do adults; however, as fasting continues, branched-chain amino acid levels decline to lower levels in children, most likely because of their limited stores. In addition, after prolonged periods of fasting, children have lower plasma levels of alanine and glutamine than adults. A more rapid onset of amino acid utilization from muscle protein stores and free fatty acids from fat depots would provide substrates for gluconeogenesis and permit children to maintain euglycaemia in the face of greater glucose demand. However, as fasting continues children run out of reserve more quickly than adults and are more likely to develop hypoglycaemia (Chaussain *et al.* 1977; Haymond *et al.* 1982).

Having described differences in the metabolic adaptation to fasting between juvenile and adult primates, we ask whether these metabolic differences could help to explain the difference in the activity of the reproductive systems of the juvenile and adult animal. Is it possible that changes in metabolism occurring near the time of puberty, perhaps reflecting increased body-weight, percentage fat or decreased metabolic rate, could provide a signal to augment GnRH secretion and stimulate sexual maturation?

In the earlier discussion of fasting in the rhesus macaque, I suggested that insulin may be considered as a candidate in linking metabolic status and reproductive function. Increases in body adiposity are positively correlated with plasma insulin levels (Bagdade *et al.* 1967), and certainly body adiposity is strongly correlated with puberty onset (Frisch, 1984). Studies by Woods *et al.* (1979) have suggested that basal insulin levels, slowly integrated by the cerebral spinal fluid, may be monitored by the hypothalamus and serve as a long-term feedback signal to regulate appetite in the primate. We have shown that plasma insulin levels are lower in juvenile primates compared with adults. Is it possible that changing insulin level (or its metabolic sequelae), occurring near the time of puberty, could provide an important signal, relating body size to the endocrine reproductive system? While these are intriguing associations, they by no means establish a causal relation between insulin or any other metabolic factor and puberty onset. Indeed, puberty is unlikely to be determined by one factor but is more likely served by a multifactorial plan through which a variety of physiological signals contribute to the overall outcome. Despite this, we remained intrigued with the possibility that the quiescence of the reproductive system before puberty may, in some

measure, reflect the reduced availability of certain metabolic substrates or hormones.

Stimulation of gonadotrophin secretion by glucose and amino acids

To test the hypothesis that GnRH secretion during prepubertal life is limited by the availability of certain key substrates or insulin, we infused a mixture of glucose and amino acids to juvenile macaques and studied its effect on LH and FSH secretion (Steiner *et al.* 1983; Cameron *et al.* 1985*c*). We selected six prepubertal male macaques, *Macaca fascicularis*, for the study. The animals were all castrated to eliminate possible interference by negative feedback effects of gonadal hormones. Despite being castrated they had extremely low circulating LH and FSH levels, as would be expected for prepubertal primates (<31 months of age and <2·5 kg body-weight). Puberty in this species normally occurs between 36 and 52 months of age. To permit blood sampling without anaesthesia, each animal was surgically fitted with an indwelling venous catheter, attached to a swivelling tether, mounted on top of the cage. Saline (9 g sodium chloride/l) was administered intravenously during a 2-week control period, after which animals received a constant infusion of glucose and amino acids at a rate of 190 ml/d, which was sustained for 4–6 weeks. Before beginning the infusion, the animals' normal energy intake averaged 1280 kJ (305 kcal)/d. The infusion provided the animals with 740 kJ (177 kcal)/d which, added to a reduced oral intake (approximately 1080 kJ (258 kcal)/d) raised their average energy intake to 1820 kJ (435 kcal)/d. This represented a 24% increase in daily energy intake during the infusion period over that of the control period. Animals gained an average of 5% in body-weight during the infusion period. During the course of the experiment, we obtained multiple blood samples each week to measure plasma hormone and substrate levels.

Over the course of the glucose and amino acid infusion, plasma LH levels increased significantly in three of six animals ($P<0·05$) (Fig. 10). The increase in LH was not correlated with age, body-weight, weight gain or daily energy intake. The three other animals showed no change in plasma LH levels over the course of the study. From the three animals showing an LH increase, we obtained blood samples at 30-min intervals on 1 d during both the control and infusion periods. Plasma LH levels were all below the limits of assay detectability during the control period, whereas during the infusion LH levels were high and indicated some tendency to exhibit a pulsatile secretion pattern (Fig. 11). In a single animal responding to the glucose–amino acid infusion, we examined the effects of alternating periods of saline followed by glucose–amino acids. During the first saline period, plasma LH levels ranged between 0·2 and 0·3 μg/ml and increased to 0·9 (SE 0·3) μg/ml while on the first glucose–amino acid trial. After returning the animal to saline, LH levels declined to control levels within 1 week, and remained there for the next 2 weeks. Subsequently, after reinstating the glucose–amino acid infusion, plasma LH levels again increased to a mean value of 1·6 (SE 0·2) μg/ml (Fig. 12).

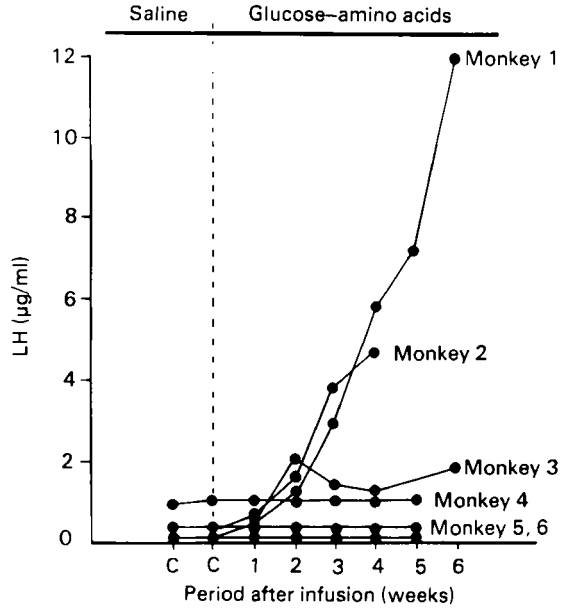

Fig. 10. Plasma luteinizing hormone (LH) levels in six prepubertal male macaques (*Macaca fascicularis*) before and during receipt of an intravenous infusion of saline (control (C) period) and glucose–amino acids. (After Cameron *et al.* 1985*c*.)

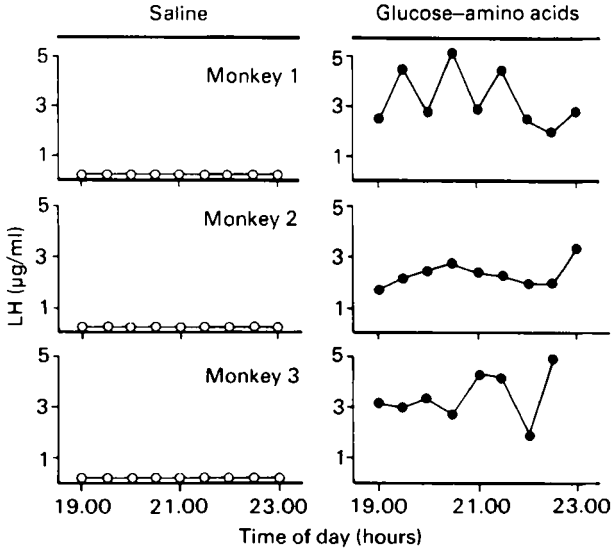

Fig. 11. Plasma luteinizing hormone (LH) levels in serial samples taken from three prepubertal male macaques (*Macaca fascicularis*) before and during receipt of an intravenous infusion of saline (control period) and glucose–amino acids. (After Cameron *et al.* 1985*c*.)

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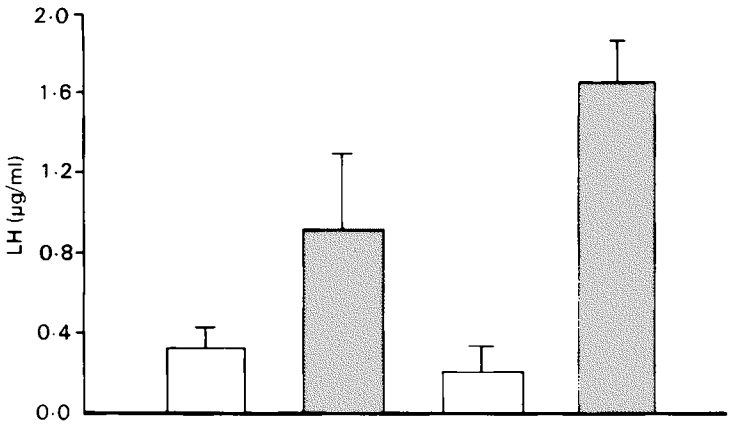

Fig. 12. Plasma luteinizing hormone (LH) levels in samples collected from a single prepubertal male macaque (*Macaca fascicularis*) while receiving alternating infusions of saline (9 g sodium chloride/l) (□) and glucose–amino acids (⊡). Values are means with their standard errors represented by vertical bars for nine observations. (After Cameron *et al.* 1985*c*.)

We do not know why only three of six animals responded to the treatment. Clearly, the treatment is a far cry from Mother Nature, and may not be optimal. Perhaps delivering the glucose–amino acid mixture intermittently or maintaining the treatment for longer periods may have made some difference. Notwithstanding, the study demonstrates that blood-borne metabolic factors, in particular glucose, amino acids and insulin (stimulated by the substrates), can augment LH secretion in immature primates, who would not otherwise show such an increase until the normal time of puberty many months later.

We can only speculate about the mechanism by which this phenomenon is induced. Perhaps the sustained availability of amino acids, glucose and insulin increases the rate of neurotransmitter synthesis required for GnRH secretion or enhances pituitary responsiveness to GnRH. While the latter remains a remote possibility, preliminary evidence suggests that acute exposure to neither glucose nor insulin alters pituitary responsiveness to GnRH in prepubertal monkeys, leaving the brain as the likely target for processing metabolic cues (R. A. Steiner, unpublished results).

Whatever the mechanism, these results argue that normal changes in the body's metabolism during the transition from the juvenile to the mature adult may influence the rate of maturation of the neuroendocrine system and thereby the timing of puberty onset in the primate. And as a corollary, the loss of these same cues may underlie the failure of the reproductive system in adults faced with malnutrition, simple weight loss or severe exercise.

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