Effects of Various Dietary Amino Acid Preparations for Phenylketonuric Patients on the Metabolic Profiles along with Postprandial Insulin and Ghrelin Responses

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Key Words

Amino acid mixtures · Ghrelin · Insulin · Phenylketonuria

Abstract

Aim: We investigated the metabolic profiles along with insulin and ghrelin responses following ingestion of various amino acid (AA) substitutes commonly used in the treatment of phenylketonuria to study the effects of added macronutrients. **Methods:** Twenty healthy and 6 phenylketonuric adults ingested AA mixtures with or without carbohydrates and fat (Anamix, Easiphen, or p-am 3; 0.35 g AA/kg body weight); milk powder shakes were used for control purposes. Serum AA, glucose, urea, insulin, and ghrelin were measured over 5 h. **Results:** Peak AA concentrations were achieved at around 60 min postprandially for supplemented AA powders and control shakes, significantly later than for pure AA. Of interest, the mean Phe/Tyr ratio declined by 40–50% in phenylketonuric patients following intake of Easiphen, Anamix, or p-am 3. The insulin peaks, up to 500% as compared with baseline, occurred at 30 min and were approximately 100% higher after intake of AA plus macronutrients. Glucose and urea remained constant. Ghrelin showed a nadir at 60 min, followed by a rise leading to a 30% increase of initial concentrations for pure AA as compared with more constant levels for preparations with macronutrients. **Conclusion:** An oral AA bolus together with macronutrients re-

tards hyperaminoacidemia, displays a higher insulin secretion, normoglycemia, and more stable ghrelin concentrations, whereas the pure AA tested here exerted weaker anabolic effects.

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Introduction

Phenylketonuria (PKU; OMIM*261600) is an autosomal recessive disorder of the amino acid (AA) metabolism characterized by a deficiency of the enzyme phenylalanine (Phe) hydroxylase. The aim of the treatment is to lower the blood Phe concentrations in order to prevent neurological damage. PKU therapy is based on a Phe-restrictive diet in combination with AA mixtures lacking Phe but enriched with vitamins, minerals, and trace elements. Dietary AA preparations are given to maintain adequate physical well-being and an optimal metabolic control in these patients. Although dietary treatment has been established for almost 50 years, optimizing its physiological and metabolic impact, natural efficiency, and convenience is still an issue [1, 2].

Insulin and ghrelin are endocrine mediators of food intake, since insulin is a well-known activator of glucose and AA uptake for peripheral tissues [3], and ghrelin is an activator of appetite and eating behavior [4, 5]. Ghre-

lin is upregulated by fasting or insulin-induced hypoglycemia, and circulating plasma concentrations can be decreased by meals but not by water-induced gastric distension [6, 7]. Concerning the interaction between ghrelin and insulin, conflicting data have been reported in the literature, showing that ghrelin may reduce or stimulate the insulin secretion, depending on the setting [7–9].

Reports on the responses of both insulin and ghrelin to special diets for the treatment of inborn errors of metabolism such as PKU are lacking.

The present study was performed to investigate insulin and ghrelin responses following the ingestion of various Phe-free dietary AA mixtures in fasted healthy volunteers and PKU patients. We tested the hypothesis that postprandial metabolic and anabolic endocrine effects differ between dietary AA mixtures with or without other macronutrients and that, therefore, supplementing the diets of patients with PKU could be of value.

Subjects and Methods

Subjects, Test Dose, and Sample Preparation

Twenty healthy Caucasian adults [age range 22–33 years; 8 males and 12 females; body weight range 47–86 kg, mean body weight 66 kg; body mass index 19.0–24.8 (kg/m²), mean body mass index 21.6 (kg/m²)] and 6 adults with early-treated classical PKU [age range 19–32 years; 4 males and 2 females; body weight range 56–70 kg, mean body weight 67 kg; body mass index 21.1–24.7 (kg/m²), mean body mass index 22.4 (kg/m²)] were recruited, after the protocol had been approved by the local ethics committee and informed consent was obtained.

We tested different Phe-free AA mixtures for dietary purposes, with or without other macronutrients (i.e., Anamix and Easiphen as compared with p-am 3, referred to as p-am; all purchased from SHS International, Heilbronn, Germany; standard test dose 0.35 g AA/kg body weight) in comparison with standardized isocaloric shakes on milk powder basis containing intact milk protein, lactose, and <1% fat (table 1). All AA supplements tested contained free AA, especially Tyr, in comparable quantities and comprised a mixture comparable to a mean of the AA amounts determined for human milk, potato, and chicken egg. We recruited male and female adults to rule out gender-related differences and controlled for the volume-related effects of nutritional challenge by administering the test mixtures in comparable amounts of fluid, i.e., 332 ± 50 ml. All subjects reported to our laboratory at 08.00 h, having fasted for 10–12 h, and were at physical rest prior to and during the study period. An intravenous catheter (Venofix; Braun, Melsungen, Germany) was inserted into an antecubital vein. After collecting the basal blood sample, the volunteers took each dietary AA mixture on different days; the PKU patients received only Phe-free preparations. Serum AA, glucose, urea, and hormones were measured at 0, 15, 30, 45, and 60 min and thereafter at 30-min intervals for 5 h in all. The actual serum Phe level in our volunteers with PKU was 169–1,615 (mean 454 ± 449) μmol/l, covering the entire spectrum of dietary purposes.

Table 1. Composition of the test mixtures and mean test dose

L-Amino acids	23 g AA or milk protein/mean test dose, containing on average 14 g Tyr, Ala, Arg, Asp, Cys, Glu, Gly, His, Pro, and Ser and 9 g essential AA such as Ile, Leu, Lys, Met, Thr, Try, and Val Anamix, Easiphen, and p-am 3 (referred to as p-am) are deficient in Phe All test mixtures contained also minerals, trace elements, and vitamins
Energy supply	Anamix, Easiphen, control group 1 on milk powder basis: 900 kJ/mean test dose, including on average 6 g fat and 22 g carbohydrates, including sucrose; energy supply in relation to protein: 39 kJ/g p-am, control group 2 on milk powder basis: 420 kJ/mean test dose (p-am contains 0 g fat and 0 g carbohydrates, control group 2 received <1 g fat and 7 g lactose/mean test dose for reasons of practicability); energy supply in relation to protein: 18 kJ/g
Standard solution	p-am: 7 g/100 ml (per gram protein: 0 g fat, 0 g carbohydrates) Anamix: 15 g/100 ml (per gram protein: 0.5 g fat, 1.2 g carbohydrates) Easiphen: liquid product (per gram protein: 0.4 g fat, 1.0 g carbohydrates) Milk powder controls: 10 g/100 ml (per gram protein: 0.1–1.0 g fat, 0.7–1.4 g carbohydrates)

The powders yielded approximately 1 kcal/kg body weight for p-am and the isocaloric control 2 and 3 kcal/kg body weight for Anamix, Easiphen, and milk powder control 1 (table 1). No adverse effects were observed after ingestion of the AA preparations such as gastrointestinal discomfort. Blood was collected in plastic tubes without anticoagulants (Sarstedt, Nürnbrecht, Germany) and centrifuged after coagulation at 1,000 g for 5 min at room temperature, and serum aliquots were stored frozen. For blood glucose measurement, 20-μl capillaries were filled and mixed with 1.0 ml of a system solution in reaction vials (Care diagnostica, Voerde, Germany).

Analysis of AA, Blood Glucose, and Urea

AA analysis was performed on a standard AA analyzer (LC 3000; Eppendorf, Hamburg, Germany) after deproteinization with 5-sulfosalicylic acid. Blood glucose was determined with an automated glucose oxidase method according to the manufacturer's instructions (Economic; Care diagnostica). Urea was measured enzymatically using the Cobas Integra biochemical analyzer (Roche Diagnostics, Mannheim, Germany).

Hormone Measurements

Radioimmunoassays for the analysis of ghrelin were purchased from Phoenix (Belmont, Calif., USA). The sensitivity of the assay was 2 ng/l and the intra-assay coefficient of variation 4.0%, as established in our laboratory [10]. Insulin was measured using a commercially available enzyme-linked immunosorbent

assay (DSL, Sinsheim, Germany) with a sensitivity of 1.8 pmol/l and an intra-assay coefficient of variation of 6.2%.

Statistics

Calculation was performed using Prism software (GraphPad, San Diego, Calif., USA). The results are given as mean $\pm$ SD, unless otherwise stated. We calculated the total area under the curve (AUC) for serum concentration versus time for AA and hormones. The hormone concentrations are expressed as percentages of baseline levels in order to describe their dynamic responses. Statistical analysis was performed using Mann-Whitney test or one-way ANOVA with Bonferroni's test. Differences were considered to be significant at $p < 0.05$.

Results

The peak serum concentrations of essential AA were reached 30–60 min after ingestion of pure AA (p-am) which was earlier as compared with the serum AA profiles following intake of AA supplements enriched with other macronutrients (Anamix, Easiphen) or a milk powder shake (fig. 1), with the mean peaks of serum AA occurring after approximately 60 min ($p < 0.05$). Leu and Arg, given at a dose of approximately 0.1 g/g protein in all test preparations, showed their mean peak serum concentrations after 45 min and 60 min for the pure AA substitute p-am versus Anamix or Easiphen ($p < 0.05$), respectively.

For both the healthy volunteers and the PKU patients, the highest AUC values (serum concentration versus time) were seen for the AA supplements enriched with other macronutrients for many essential AA (fig. 2). As compared with the pure AA substitutes, the mean AUC values for, e.g., Thr, Lys, and Try in healthy and PKU volunteers were on average up to 16% higher in both Anamix and Easiphen groups as compared with the p-am group ($p < 0.01$). Since all AA substitutes tested were deficient in Phe, we focused on the Phe/Tyr ratio as an indicator for metabolic control in patients with PKU. As expected, Phe at 0 min was elevated in adults with PKU (range 169–1,615 $\mu\text{mol/l}$). In addition, the mean Phe/Tyr ratio declined by 40–50% in the 6 PKU patients following intake of Easiphen, Anamix, or p-am (fig. 3) and by up to 50% in healthy participants following the ingestion of Phe-free powders, but not after intake of Phe-containing milk powder shakes. The Phe/Tyr ratio declined significantly in the Easiphen group at t_{60} and t_{90} ($p < 0.05$) as compared with the p-am group.

The serum urea concentrations remained stable over the whole study period (mean values ranging from 23 ± 5 to 30 ± 6 mg/dl, NS). There were no differences among

Fig. 1. Peak serum concentrations of the essential AA of 20 healthy and 6 PKU volunteers apart from Phe. * $p < 0.05$ for p-am versus controls, Anamix, and Easiphen.

Fig. 2. Representative serum AA of 20 healthy and 6 PKU volunteers after oral ingestion of various dietary preparations, in terms of AUC, given as percent difference as compared with p-am. ** $p < 0.01$ for Anamix or Easiphen versus p-am.

the various study groups with regard to the mean basal glucose concentrations which ranged from 72 ± 9 to 82 ± 8 mg/dl or peak glucose levels, ranging from 88 ± 7 to 97 ± 6 mg/dl (NS).

The basal insulin serum concentrations for all groups were 7.7 ± 4 to 10.9 ± 5 pmol/l; the maximum insulin peaks were reached 30 min after ingestion of AA supplements or milk-powder-based shakes (54.1 ± 23.7 pmol/l for Anamix, 51.0 ± 26.4 pmol/l for Easiphen, 34.6 ± 17.9 pmol/l for control 1, 27 ± 17.1 pmol/l for p-am, and 37.1 ± 14.5 pmol/l for control 2), corresponding to an increase of approximately 520, 500, 320, 340, or 310% in comparison with baseline values ($p < 0.0001$; fig. 4). Following ingestion of AA mixtures enriched with carbohydrates, the AUC values for insulin serum concentrations

Fig. 3. Serum Phe/Tyr ratios in 6 PKU volunteers in relation to initial values given as percentages after ingestion of different Phe-free preparations and in healthy controls testing milk powder. * $p < 0.05$ for Anamix, Easiphen, or p-am versus control 1 or 2 from 60 min on and * $p < 0.05$ for p-am at t_{60} and t_{90} for Easiphen versus p-am and Anamix.

Fig. 4. Mean serum insulin concentrations following oral intake of a single physiological dose of different AA mixtures in relation to basal values as percentages. The insulin surge after AA ingestion was significantly higher for Anamix and Easiphen as compared with p-am mixture and milk powder controls. ** $p < 0.01$; **** $p < 0.0001$.

versus time were up to 90% higher in the PKU patient group as compared with the healthy controls, as were peak insulin concentrations at 30 min (148 vs. 57 pmol/l for Anamix; $p < 0.05$); following intake of pure AA, however, the insulin concentrations were not significantly different between the groups. The total AUC value for insulin was up to 22% higher for the Anamix and Easiphen groups as compared with the p-am and milk protein cohorts ($p < 0.01$). In the entire study cohort, insulin fell to baseline values after 120 min, and after 90 min the serum insulin courses no longer differed between the groups.

The fasting ghrelin concentrations of all participants were between 72 and 123 ng/l. Following the test mixture ingestion, the ghrelin levels dropped in all groups by 20–30%, with a low at 60 min. After the nadir, a continuous rise leading at 210 min to a 30% increase from initial concentrations was shown for free AA as compared with more constant levels following ingestion of AA substitutes plus carbohydrates and fat or milk powder controls (fig. 5). Serum ghrelin was highest at 300 min following the ingestion of pure AA lacking other macronutrients for both patients and healthy volunteers as compared with Anamix, Easiphen, or the controls (fig. 5).

Fig. 5. Ghrelin concentrations at t_0 , t_{60} , and t_{300} in human subjects after ingestion of different AA powders (Anamix, Easiphe, p-am). Note significant differences between the t_0 and t_{300} values and the nadir at t_{60} : * $p < 0.05$.

Discussion

Treatment of PKU consists of a 'diet for life' that is low in daily Phe, combined with a Phe-free protein substitute, enriched with vitamins, minerals, and trace elements, which is the source of approximately 75% of the daily protein equivalent [2]. However, optimizing its physiological impact, e.g., on anabolism, nitrogen (N) balance, and serum Phe concentrations, is still an issue.

Concerning the N balance, it is known that increased energy intake at a constant N intake increases the N balance [11] and that carbohydrates or fat modulate splanchnic retention and peripheral availability of dietary N [12]. However, branched-chain AA are especially potent modulators of the protein turnover [13]. Leu and Phe, in particular, are able to activate cellular pathways of protein synthesis, to inhibit proteolysis, and to decrease glucose disposal, while inhibiting early steps in insulin action and thereby furthering a bidirectional modulation of the insulin effects [14]. High-protein diets in animals have been shown to increase fasting blood glucose levels by increasing the hepatic glucose production and by decreasing the glucose disposal in peripheral tissues [15]. During this study, the blood glucose levels remained stable, indicating that there was no acute impairment of the glucose tolerance. Higher doses of AA (1.5 g/kg), however, have been reported to decrease the blood glucose concentrations in healthy volunteers [16].

In general, AA kinetics to a large extent depend on application form, test setting, and dosage. It has been reported that the highest blood AA levels after ingesting a whole meal are found after 3–5 h [17] as compared with 1 h following the consumption of a pure AA mixture [16]. Since the dietary regimen of PKU patients contains a Phe-free protein substitute given at three to four doses per day together with the meals, we here studied an oral challenge. Catabolic processes, leading to increased throughput of N in the hepatic urea cycle, are of minor importance for the disposal of ingested AA in our experimental approach, since the blood urea concentrations remained unaffected throughout the study period. But in general, the larger the dose of AA ingested, the higher both the oxidative utilization and the urinary N loss [18, 19]. Therefore, we next focused on the anabolic and anticatabolic hormone insulin. Peak insulin levels in the serum occurred already 30 min after ingestion of the test mixtures. The insulin concentrations returned to baseline levels 120 min after the AA challenge. The increase of serum insulin explains the absent rise of blood glucose concentrations in our volunteers. However, administra-

tion of gluconeogenic AA prevented hypoglycemia, especially when given in combination with carbohydrates (e.g., Anamix, Easiphen). Generally, the postprandial decline in protein breakdown can be attributed to insulin effects or an increase in protein synthesis, however, depends mainly on the availability of free AA. An increased AA intake may also lead to an enhanced oxidative catabolism which lowers the anabolic effects of a high protein intake [18]. Hyperinsulinemia in combination with hyperaminoacidemia stimulates AA oxidation, especially in the absence of nonprotein energy, therefore, reducing the efficiency of AA supplementation [20]. This effect may be unfavorable in the treatment of patients with inborn errors of metabolism such as PKU.

AA are not 'forbidden' substrates for energy production; they are able to compete with glucose for oxidative use, as has been formerly shown in clamp studies [20]. However, insulin should not be regarded as the primary regulator; its effects on protein anabolism, e.g., in skeletal muscle, depend on the availability of glucose and AA and on the presence of anti-insulinotropic hormones. Certain AA, e.g., Leu, may exert stimulating effects on protein synthesis independently of increased insulin levels and mainly as a consequence of the net protein balance, especially in postexercise skeletal muscle [21]. However, to rule out the effects of physical exercise, our volunteers were at physical rest prior to and during the study period. It is well known that certain insulinotropic AA, e.g., Leu, Phe, and Arg, facilitate postprandial insulin response [22]. There may be a strong correlation of plasma Phe, Tyr, or Leu concentrations with the insulin response after protein intake [21, 22]. Along these lines, the AUC values for insulin and also the peak insulin concentrations were significantly higher following intake of Anamix and Easiphen for the PKU patient group as compared with the healthy controls which is indicative of a combined insulinotropic stimulus of Phe, glucose, and also other AA.

Ghrelin is involved in the regulation of food ingestion, since it increases during fasting and stimulates the initiation of food intake [23, 24]. In addition, ghrelin also has a growth hormone releasing function [25] and may have an important role in coordinating energy needs with the growth process. We found different courses of the serum ghrelin concentration in our two study groups. The more pronounced increase shown in the subjects following the ingestion of pure AA may be understood as a response to the lack of an adequate energy supply. Since ghrelin increases naturally during fasting and exhibits considerable heterogeneity in its preprandial surges [26], we had in former studies investigated the effects of fasting and

the physiological responses following a test dose of essential AA in fasted volunteers [27, 28]. We here observed a stronger suppression of ghrelin responses during the study period after intake of AA mixtures containing carbohydrates and fat as well, along with higher insulin levels than after intake of pure AA. A single bolus of a Phe-free AA mixture is, therefore, not sufficient to induce satiety and to drive anabolic pathways. Comparing the effects of different macronutrients, it has been recently found in rats that isocaloric administration of glucose or AA suppresses ghrelin more rapidly than fat meals [29]. In our study, the ghrelin levels in both healthy volunteers and PKU patients exhibited an initial decrease at 60 min, followed by an increase over baseline concentrations at 210 min, while the insulin levels had returned to basal values at 120 min, underlining that the nutrient-related suppression of gastric ghrelin secretion is not quantitatively driven by circulating insulin concentrations, but also involves enteroendocrine mediators or neural mechanisms. In PKU patients, normal ghrelin plasma concen-

trations have been found in diet-controlled individuals, including our small cohort, but the ghrelin levels are significantly reduced in poorly controlled patients [30] which may also be indicative of a dysregulation of the neuroendocrine system in affected patients off diet.

Taken together, a dietary strategy of AA substitution as close as possible to the natural state [31], such as the combination with additional macronutrients, is mandatory for the treatment of patients with inborn errors of metabolism such as PKU, in order to achieve an optimal nutritional status along with physiological endocrine and metabolic profiles.

Acknowledgments

We are grateful to Patricia Schmid and Norbert Meier, Laboratory of Metabolism at the Children and Youth Hospital in Erlangen, for their excellent technical assistance and to Jana Weber for her technical support. We thank the patients and all other volunteers for their participation in this study.

References

- Rutherford P, Poustie V: Protein substitute for children and adults with phenylketonuria. *Cochrane Database Syst Rev* 2005;(4): CD004731.
- Knerr I, Herwig J, Bohles HJ, Forster H: Biochemical and nutritional status in early treated phenylketonuria patients. *Aktuel Ernährungsmed* 1997;22:206–211.
- Liao K, Lane MD: Expression of a novel insulin-activated amino acid transporter gene during differentiation of 3T3-L1 preadipocytes into adipocytes. *Biochem Biophys Res Commun* 1995;208:1008–1015.
- Lawrence CB, Snape A, Baudoin F, Luckman SM: Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. *Endocrinology* 2002;143:155–162.
- Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR: Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001;86:5992–5995.
- Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, Kangawa K, Matsukura S: Upregulation of ghrelin expression in the stomach upon fasting insulin-induced hypoglycemia and leptin administration. *Biochem Biophys Res Commun* 2001;281:1220–1225.
- Broglia F, Gottero C, Arvat E, Ghigo E: Endocrine and non-endocrine actions of ghrelin. *Horm Res* 2003;59:109–117.
- Broglia F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R, Ghigo E: Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab* 2001;86:5083–5086.
- Lee HM, Wang G, Englander EW, Kojima M, Greeley GH Jr: Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. *Endocrinology* 2002;143:185–190.
- Groschl M, Wagner R, Dotsch J, Rascher W, Rauh M: Pre-analytical influences on the measurement of ghrelin. *Clin Chem* 2002;48:1114–1116.
- Metges CC, Petzke KJ, Young VR: Dietary requirements for indispensable amino acids in adult humans: new concepts, methods of estimation, uncertainties and challenges. *Ann Nutr Metab* 1999;43:267–276.
- Fouillet H, Gaudichon C, Mariotti F, Bos C, Huneau JF, Tome D: Energy nutrients modulate the splanchnic sequestration of dietary nitrogen in humans: a compartmental analysis. *Am J Physiol Endocrinol Metab* 2001;281:E248–E260.
- Fouillet H, Mariotti F, Gaudichon C, Bos C, Tome D: Peripheral and splanchnic metabolism of dietary nitrogen are differently affected by the protein source in humans as assessed by compartmental modeling. *J Nutr* 2002;132:125–133.
- Patti ME, Brambilla E, Luzi L, Landaker EJ, Kahn CR: Bidirectional modulation of insulin action by amino acids. *J Clin Invest* 1998;101:1519–1529.
- Rossetti L, Rothman DL, DeFronzo RA, Shulman GI: Effect of dietary protein on in vivo insulin action and liver glycogen repletion. *Am J Physiol* 1989;257(2 Pt 1):E212–E219.
- Monch E, Herrmann ME, Brosicke H, Schoffer A, Keller M: Utilisation of amino acid mixtures in adolescents with phenylketonuria. *Eur J Pediatr* 1996;155(suppl 1):S115–S120.
- Stegink LD, Filer LJ, Brummer MC, Baker GL, Krause WL, Bell EF, Ziegler EE: Plasma amino acid concentrations and amino acid ratios in normal adults and adults heterozygous for phenylketonuria ingesting a hamburger and milk shake meal. *Am J Clin Nutr* 1991;53:670–675.
- Darmaun D: Role of nutrients in the regulation of in vivo protein metabolism in humans. *Acta Paediatr Suppl* 1999;433:92–94.
- Herrmann ME, Brosicke H, Keller M, Monch E, Helge H: Dependence of the utilisation of a phenylalanine-free amino acid mixture on different amounts of single dose ingested. *Eur J Pediatr* 1994;153:501–503.

- 20 Ferrannini E, Bevilacqua S, Lanzzone L, Bonadonna R, Brandi L, Oleggini M, Boni C, Buzzigoli G, Ciociaro D, Luzi L, DeFronzo RA: Metabolic interactions of amino acids and glucose in healthy humans. *Diabetes Nutr Metab* 1988;3:175–186.
- 21 Van Loon LJ, Kruijshoop M, Verhagen H, Saris WH, Wagenmakers AJ: Ingestion of protein hydrolysate and amino acid-carbohydrate mixtures increases postexercise plasma insulin responses in men. *J Nutr* 2000;130:2508–2513.
- 22 Van Loon LJ, Saris WH, Verhagen H, Wagenmakers AJ: Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. *Am J Clin Nutr* 2000;72:96–105.
- 23 Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S: A role for ghrelin in the central regulation of feeding. *Nature* 2001;409:194–198.
- 24 Horvath TL, Diano S, Sotonyi P, Heiman M, Tschop M: Minireview: ghrelin and the regulation of energy balance – a hypothalamic perspective. *Endocrinology* 2001;142:4163–4169.
- 25 Date Y, Murakami N, Kojima M, Kuroiwa T, Matsukura S, Kangawa K, Nakazato M: Central effects of a novel acylated peptide, ghrelin, on growth hormone release in rats. *Biochem Biophys Res Commun* 2000;275:477–480.
- 26 Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS: A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001;50:1714–1719.
- 27 Knerr I, Groschl M, Rascher W, Rauh M: Endocrine effects of food intake: insulin, ghrelin, and leptin responses to a single bolus of essential amino acids in humans. *Ann Nutr Metab* 2003;47:312–318.
- 28 Groschl M, Knerr I, Topf HG, Schmid P, Rascher W, Rauh M: Endocrine responses to the oral ingestion of a physiological dose of essential amino acids in humans. *J Endocrinol* 2003;179:237–244.
- 29 Overduin J, Frayo RS, Grill HJ, Kaplan JM, Cummings DE: Role of the duodenum and macronutrient type in ghrelin regulation. *Endocrinology* 2005;146:845–850.
- 30 Schulpis KH, Papassotiropoulos I, Vounatsou M, Karikas GA, Tsakiris S, Chrousos GP: Morning preprandial plasma ghrelin and catecholamine concentrations in patients with phenylketonuria and normal controls: evidence for catecholamine-mediated ghrelin regulation. *J Clin Endocrinol Metab* 2004;89:3983–3987.
- 31 Cleary MA, Feillet F, White FJ, Vidailhet M, MacDonald A, Grimsley A, Maurin N, Ogier de Baulny H, Rutherford PJ: Randomised controlled trial of essential fatty acid supplementation in phenylketonuria. *Eur J Clin Nutr* 2006;60:915–920.