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Ingestion of Protein Hydrolysate and Amino Acid–Carbohydrate Mixtures Increases Postexercise Plasma Insulin Responses in Men[1]

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ABSTRACT To optimize the postexercise insulin response and to increase plasma amino acid availability, we studied postexercise insulin levels after the ingestion of carbohydrate and wheat protein hydrolysate with and without free leucine and phenylalanine. After an overnight fast, eight male cyclists visited our laboratory on five occasions, during which a control drink and two different beverage compositions in two different doses were tested. After they performed a glycogen-depletion protocol, subjects received a beverage (3.5 mL · kg^{-1}) every 30 min to ensure an intake of 1.2 g · kg^{-1} · h^{-1} carbohydrate and 0, 0.2 or 0.4 g · kg^{-1} · h^{-1} protein hydrolysate (and amino acid) mixture. After the insulin response was expressed as the area under the curve, only the ingestion of the beverages containing wheat protein hydrolysate, leucine and phenylalanine resulted in a marked increase in insulin response (+52 and + 107% for the 0.2 and 0.4 g · kg^{-1} · h^{-1} mixtures, respectively; $P < 0.05$) compared with the carbohydrate-only trial). A dose-related effect existed because doubling the dose (0.2–0.4 g · kg^{-1} · h^{-1}) led to an additional rise in insulin response ($P < 0.05$). Plasma leucine, phenylalanine and tyrosine concentrations showed strong correlations with the insulin response ($P < 0.0001$). This study provides a practical tool to markedly elevate insulin levels and plasma amino acid availability through dietary manipulation, which may be of great value in clinical nutrition, (recovery) sports drinks and metabolic research. J. Nutr. 130: 2508–2513, 2000.

KEY WORDS: • *insulin secretion* • *amino acids* • *postexercise recovery* • *leucine* • *phenylalanine* • *humans*

Intensive exercise leads to an increase in muscle protein synthesis (Biolo et al. 1995b, Yarasheski et al. 1999) and muscle protein degradation (Biolo et al. 1995b) in the postexercise phase in humans. Biolo et al. (1997) demonstrated that hyperaminoacidemia, resulting from the intravenous infusion of amino acids, increases postexercise muscle protein synthesis rates and prevents the exercise-induced increase in protein degradation. Recent studies have demonstrated that amino acid ingestion, with (Rasmussen et al. 2000) and without (Tipton et al. 1999) carbohydrates, can also increase postexercise muscle protein synthesis and net protein balance (protein synthesis minus protein degradation). As such, postexercise amino acid ingestion may be an effective method to maximize the anabolic effect of exercise. The mechanisms responsible for this amino acid–induced, anabolic response have not yet been established. Potential regulating factors include changes in levels of various hormones, paracrine substances and vasodilators. Insulin has been proposed as an important factor in protein metabolism, because acute physiologic elevations of plasma insulin levels, especially during conditions of hyperaminoacidemia, result in an additional increase in net muscle protein anabolism in vivo in humans (Fryburg et al. 1995, Gelfand and Barrett, 1987, Hillier et al. 1998). However, insulin should not be regarded as a primary regulator because in the absence of elevated amino acid concentrations, insulin levels exert only a modest effect on muscle protein synthesis (Biolo et al. 1995a). In accordance, Anthony et al. (1999 and 2000) reported a stimulating effect of leucine ingestion on postexercise muscle protein synthesis in rats, independent of an increase in plasma insulin levels.

Insulin also stimulates muscle glucose utilization through the activation of glucose transport (Ivy 1997 and 1998, Ivy and Kuo 1998) and glycogen synthase (Bak et al. 1991, Kruszynska et al. 1986), which is generally considered to be the major factor to determine the rate of glycogen synthesis when substrate supply is adequate (Conlee et al. 1978). Therefore, an increase in postexercise insulin response, after the ingestion of protein (and amino acids) in combination with carbohydrates, has been suggested to accelerate muscle glycogen synthesis (van Hall et al. 2000, van Loon et al. 2000b, Zawadzki et al. 1992). Because of the proposed role of insulin and amino acids in promoting postexercise muscle protein anabolism and/or muscle glycogen synthesis, there is increasing interest in nutritional strategies to maximize postexercise insulin levels and to increase plasma amino acid availability.

In the 1960s, the synergistically stimulating effect of the combined intake of carbohydrate and protein on plasma insu-

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lin levels was first reported (Pallotta and Kennedy 1968, Rabinowitz et al. 1966) and was later confirmed by Nuttall et al. (1984 and 1985). In addition, the infusion of free amino acids increases plasma insulin levels in humans (Fajans et al. 1962, Floyd et al. 1963, 1966, 1968, 1970a, 1970b). We recently studied the effects of the combined oral intake of carbohydrate ($0.8\ g \cdot kg^{-1} \cdot h^{-1}$) with different amino acids and/or protein (hydrolysates) ($0.4\ g \cdot kg^{-1} \cdot h^{-1}$) on plasma insulin levels in the postabsorptive resting state and observed a twofold increase in insulin response after the ingestion of carbohydrate with a mixture of wheat protein hydrolysate, free leucine and phenylalanine compared with the intake of only carbohydrate (van Loon et al. 2000a). A synergistic increase in insulin response was also observed after the ingestion of this mixture ($0.4 \cdot kg^{-1} \cdot h^{-1}$) with carbohydrate ($0.8\ g \cdot kg^{-1} \cdot h^{-1}$) after exercise in trained athletes (van Loon et al. 2000b). In the same study, we observed that the ingestion of this mixture accelerated postexercise muscle glycogen synthesis compared with the ingestion of only carbohydrate ($0.8\ g \cdot kg^{-1} \cdot h^{-1}$) but not compared with the ingestion of a higher amount of carbohydrate ($1.2\ g \cdot kg^{-1} \cdot h^{-1}$).

The present study was designed to investigate the effects of the ingestion of different amounts of protein hydrolysate, with and without the addition of free leucine and phenylalanine, in combination with a large amount ($1.2\ g \cdot kg^{-1} \cdot h^{-1}$) of carbohydrate, on the postexercise insulin response and plasma amino acid concentration in trained athletes. Our aim was to define the mixture with the strongest insulinotropic properties in combination with increased amino acid availability when ingested in the postexercise phase. Theoretically, this mixture would be the best candidate to explore whether such an insulinotropic mixture can maximize postexercise net muscle protein anabolism and glycogen synthesis rates.

SUBJECTS AND METHODS

Subjects. Eight highly trained male cyclists [age 24.0 ± 0.6 y, body mass 70.0 ± 1.0 kg, body mass index 21.4 ± 0.6 kg/m^2, maximum workload (W_{max})[3] 390 ± 8 W, maximum heart rate 191 ± 3 bpm) participated in this study. Subjects trained at least three times a week for 2 h and had a training history of >5 y. All subjects were informed about the nature and risks of the experimental procedures before their informed consent was obtained. This study was approved by the local ethics committee.

Pretesting. Maximum oxygen uptake capacity ($V_{O_2\ max}$) and W_{max} were measured on an electronically braked cycle ergometer (Lode Excalibur, Groningen, the Netherlands) during an incremental exhaustive exercise test 1 wk before the first experimental trial (Kuipers et al. 1985). These findings were used to determine the power output settings in the glycogen-depletion protocol.

Experimental trials. Each subject participated in five trials, separated by ≥3 d, in which five different beverages were tested. During those trials, subjects first performed a glycogen-depletion protocol (Kuipers et al. 1987). Thereafter, subjects were studied for 3 h while ingesting $1.2\ g \cdot kg^{-1} \cdot h^{-1}$ (60:40% maltodextrin/glucose). Blood samples were taken at 30-min intervals. During those 3 h, subjects remained physically inactive. Beverages were provided in a random order. Both subjects and researchers who were present were unaware of the specific drink being tested. All drinks were vanilla flavored to make the taste similar among the trials. Subjects were instructed to refrain from heavy physical labor and to keep their diet constant the day before the trials. Subjects fasted for 12 h before reporting to the laboratory but were allowed to drink water ad libitum.

Protocol. Subjects reported to the laboratory at 0830 h. Muscle glycogen depletion was established by performing an intense cycle ergometer test (Kuipers et al. 1987). This muscle glycogen-depletion protocol started off with a 10-min warm-up period at a workload of 50% W_{max}. Thereafter subjects were instructed to cycle for 2-min block periods at alternating workloads of 90 and 50% W_{max}. This was continued until subjects were no longer able to complete the 2 min at 90% W_{max}. That moment was defined as the inability to maintain cycling speed at 60 rpm. At that moment, the high intensity blocks were reduced to an intensity equal to 80% W_{max}. Again, subjects had to cycle until they were unable to complete a 2-min block at 80% W_{max}, after which the high intensity block was reduced to 70% W_{max}. Subjects were allowed to stop exercising when they were not able to maintain pedaling speed at >60 rpm at this 70% W_{max}. Subjects were allowed to drink up to 1.0 L of water during the depletion test. After cessation of the exercise, subjects were allowed to take a 5-min shower, after which a Teflon catheter (Baxter BV, Utrecht, the Netherlands) was inserted into an antecubital vein and a resting blood sample was drawn (t = 0 min). Immediately thereafter, subjects drank an initial bolus ($3.5\ mL \cdot kg^{-1}$) of a given test drink. Subjects were seated for the next 3 h, during which they received a beverage volume of $3.5\ mL \cdot kg^{-1}$ every 30 min until t = 150. Blood samples (4 mL) were taken every 30 min for the measurement of plasma glucose, insulin and amino acids until t = 180.

Beverages. All beverages contained $68.5\ g \cdot L^{-1}$ glucose, $102.8\ g \cdot L^{-1}$ maltodextrin, $0.20\ g \cdot L^{-1}$ sodium saccharinate, $1.80\ g \cdot L^{-1}$ citric acid and $5.00\ g \cdot L^{-1}$ vanilla cream flavor. In addition, beverages 2 and 3 contained 28.6 and $57.1\ g \cdot L^{-1}$ wheat protein hydrolysate, respectively, whereas beverage 4 contained $14.3\ g \cdot L^{-1}$ wheat protein hydrolysate and $7.1\ g \cdot L^{-1}$ of both free leucine and phenylalanine. Beverage 5 contained twice the level of wheat protein hydrolysate and free leucine and phenylalanine compared with beverage 4. At t = 0, 30, 60 90, 120 and 150 min, subjects received a beverage volume of $3.5\ mL \cdot kg^{-1}$ to ensure a given dosage of $1.2\ g \cdot kg^{-1} \cdot h^{-1}$ carbohydrate (40:60% maltodextrin/glucose) and 0, 0.2 or $0.4\ g \cdot kg^{-1} \cdot h^{-1}$ of a mixture containing wheat hydrolysate or wheat hydrolysate with the addition of free leucine and phenylalanine. Glucose and maltodextrin were obtained from AVEBE (Veendam, the Netherlands), crystalline amino acids were obtained from BUFA (Uitgeest, the Netherlands) and the protein hydrolysate (Hyprol) was prepared by Quest International (Naarden, the Netherlands). The protein hydrolysate is prepared from wheat protein via enzymatic digestion and has a medium chain length of 11 amino acids. The amino acid profile of the wheat hydrolysate is listed in **Table 1**. The maltodextrin used had a medium chain length of 14–16 glycosyl

TABLE 1

Amino acid profile of the wheat protein hydrolysate

Amino acid	$g \cdot 100\ g\ hydrolysate^{-1}$
L-Alanine (Ala)	1.8
L-Cysteine (Cys)	0.9
L-Aspartate (Asp)	0.2
L-Glutamate (Glu)	3.2
L-Phenylalanine (Phe)	4.8
L-Glycine (Gly)	2.8
L-Histidine (His)	1.6
L-Isoleucine (Ile)	2.6
L-Lysine (Lys)	. . .
L-Leucine (Leu)	5.6
L-Methionine (Met)	1.1
L-Asparagine (Asn)	1.9
L-Proline (Pro)	12.3
L-Glutamine (Gln)	29.0
L-Arginine (Arg)	2.2
L-Serine (Ser)	4.4
L-Threonine (Thr)	2.0
L-Valine (Val)	3.0
L-Tryptophan (Trp)	. . .
L-Tyrosine (Tyr)	2.5

[3] Abbreviation used: W_{max}, maximal workload.

units. To make the taste comparable in all trials, sodium saccharinate, citric acid and vanilla cream flavor (Quest International) were added.

Analysis. Blood was collected in EDTA-containing tubes and was centrifuged at 1000 × *g* and 4°C for 5 min. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at −40°C. Glucose (Uni Kit III 07367204; Hoffman-La Roche, Basel, Switzerland) was analyzed with the COBAS FARA semiautomatic analyzer (Hoffman-La Roche). Insulin was analyzed by radioimmunoassay (Insulin RIA 100 kit; Pharmacia, Uppsala, Sweden). Plasma (200 μL) for amino acid analysis was deproteinized on ice with 500 g · L^{-1} 5-sulfosalicylic acid and vortex mixed. The clear supernatant obtained after centrifugation was stored at −80°C until analysis for amino acids. Amino acids were analyzed on an automated dedicated amino acid analyzer (LC5001; Biotronik, München, Germany) using a cationic exchange resin (type BTC2710; Biotronik), a gradient of lithium citrate elution buffers and postcolumn derivatization with ninhydrin, all according to working recipes of the supplier. Same procedures were performed to determine the amino acid composition of the wheat protein hydrolysate except for the use of a different amino acid analyzer (Pharmacia LKB Biotechnology, Roosendaal, the Netherlands). Calibration curves of the amino acids were obtained using commercial amino acid mixtures. Norvaline was used as internal standard and added to all plasma samples before deproteinization.

Statistics. All data are expressed as means ± SEM (n = 8). Analysis of variance for repeated measures was performed to study differences in plasma glucose and insulin concentrations over time between groups. A Scheffé post hoc test was applied in case of a significant F-ratio to locate the differences. The plasma glucose, insulin and amino acid responses were calculated as the area under the curve above baseline value (t = 0 min). Statistical analyses of these data were conducted with a two-factor analysis of variance with treatment and subject as the two factors. Differences between drinks were checked for statistical significance using the Tukey post hoc test. Simple regression analysis was performed to calculate correlations between the insulin response and the different plasma amino acid responses. Statistical significance was set at $P < 0.05$.

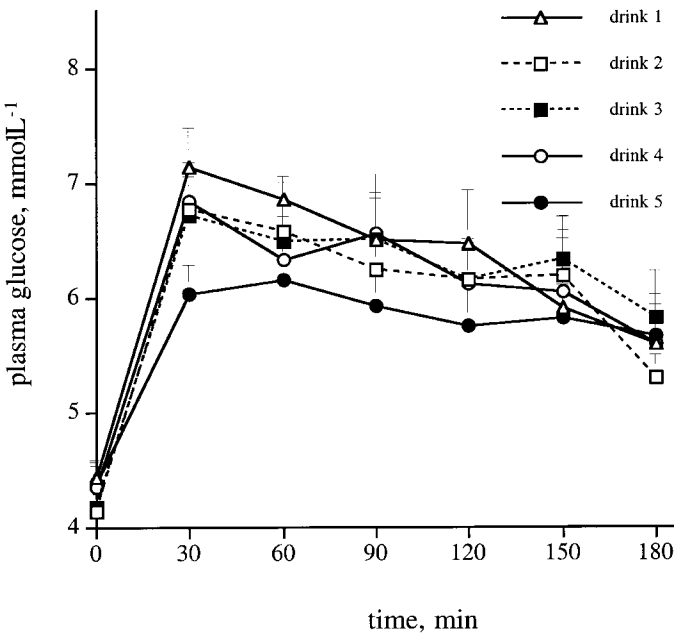

FIGURE 1 Postexercise plasma glucose concentrations after the ingestion of protein hydrolysate/amino acid–carbohydrate mixtures in humans. Test drink 1, carbohydrate only (1.2 g · kg^{-1} · h^{-1}); drink 2, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}); drink 3, carbohydrate with protein hydrolysate (0.4 g · kg^{-1} · h^{-1}); drink 4, carbohydrate with protein hydrolysate (0.1 g · kg^{-1} · h^{-1}), leucine (0.05 g · kg^{-1} · h^{-1}) and phenylalanine (0.05 g · kg^{-1} · h^{-1}); and drink 5, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}), leucine (0.1 g · kg^{-1} · h^{-1}) and phenylalanine (0.1 g · kg^{-1} · h^{-1}). Values are means ± SEM (n = 8). No significant differences between trials, $P < 0.05$.

RESULTS

In all trials, plasma glucose concentrations increased during the first 30 min after beverage ingestion, after which they decreased during the remaining 150 min (**Fig. 1**). After expression of the glucose response as the area under the curve (above baseline) during the entire 3-h period, no significant differences were observed between the different test drink trials.

Plasma insulin concentrations increased in all trials during the first 150 min. In the final 30 min, a plateau developed (**Fig. 2**). The ingestion of drink 5 resulted in significantly higher insulin levels at t = 60, 90, 120 and 150 min compared with drinks 1, 2 and 3. After expression of the insulin response as the area under the curve during the entire 3-h period (**Fig. 3**), insulin responses after the ingestion of drinks 4 and 5 were significantly higher than control (+52 ± 10 and +107 ± 17%, respectively; $P < 0.05$). The ingestion of drinks 2 and 3 did not result in significantly higher postexercise insulin responses compared with the control drink, and responses were significantly lower compared with the insulin responses reported after the ingestion of the free amino acid–containing drinks (drinks 4 and 5). In addition, the ingestion of drink 5 resulted in a significantly higher insulin response (+36 ± 6%; $P < 0.05$) compared with drink 4, in which a lower dose of the same mixture was ingested (0.4 versus 0.2 g · kg^{-1} · h^{-1}, respectively).

Plasma amino acid responses were calculated as the areas under the curve above baseline values (**Table 2**). Only the findings most relevant for the aim of this study are reported here. After the postexercise ingestion of the control drink, a decrease was seen in the concentration of all amino acids. The ingestion of drinks 2 and 3 (wheat) resulted in a significantly higher plasma concentration for most amino acids measured compared with the control. The ingestion of drinks 4 and 5 resulted in a substantial increase in plasma leucine, phenylalanine and tyrosine responses compared with the other drinks (Table 2). Regression analysis revealed a strong positive correlation between the size of the insulin response and the change in plasma leucine ($P < 0.0001$, $r = 0.66$), phenylalanine ($P < 0.0001$, $r = 0.62$) and tyrosine ($P < 0.0001$, $r = 0.57$) concentrations. Plasma threonine, asparagine, glycine, alanine, valine, methionine, isoleucine and histidine responses showed a negative correlation with the insulin response ($P < 0.05$, $r = -0.33$ to -0.48) within this postexercise setting.

DISCUSSION

It was recently concluded that the ingestion of beverages containing protein hydrolysate plus carbohydrate is preferred over the ingestion of those containing intact protein plus carbohydrate to stimulate insulin secretion and plasma amino acid availability, because ingestion results in a stronger increase in plasma amino acid levels in the postabsorptive resting state (van Loon et al. 2000a). In addition, the use of an intact protein when ingested as a beverage has another practical disadvantage because most intact proteins are poorly soluble in water. In an attempt to combine gastrointestinal tolerance and palatability with a maximal insulin response, a mixture of wheat hydrolysate with free leucine and phenylalanine was defined (van Loon et al. 2000a). The insulinotropic properties after the ingestion of this mixture in the postabsorptive resting state exceeded those of most other combina-

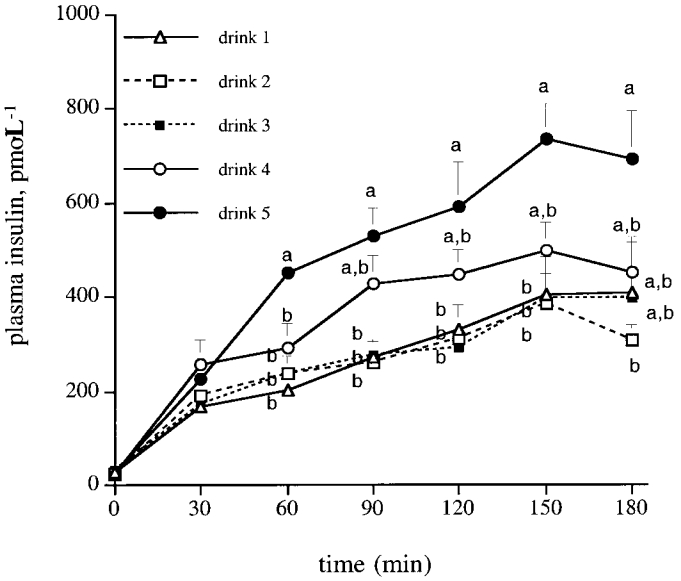

FIGURE 2 Postexercise plasma insulin concentrations after the ingestion of protein hydrolysate/amino acid–carbohydrate mixtures in humans. Test drink 1, carbohydrate only (1.2 g · kg^{-1} · h^{-1}); drink 2, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}); drink 3, carbohydrate with protein hydrolysate (0.4 g · kg^{-1} · h^{-1}); drink 4, carbohydrate with protein hydrolysate (0.1 g · kg^{-1} · h^{-1}), leucine (0.05 g · kg^{-1} · h^{-1}) and phenylalanine (0.05 g · kg^{-1} · h^{-1}); and drink 5, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}), leucine (0.1 g · kg^{-1} · h^{-1}) and phenylalanine (0.1 g · kg^{-1} · h^{-1}). Values are means ± SEM (n = 8). Mean values not sharing a common superscript are different, $P < 0.05$.

tions and did not cause any gastrointestinal or other complaints.

The aim of the present study was to maximize postexercise insulin levels and to increase plasma amino acid availability in trained athletes. Our data did not show an increase in postexercise insulin response after the ingestion of a wheat protein hydrolysate only (at an intake of 0.2 or 0.4 g · kg^{-1} · h^{-1}) in combination with carbohydrate (1.2 g · kg^{-1} · h^{-1}) compared with the ingestion of only carbohydrate. This is in contrast to earlier findings in the postabsorptive resting state (van Loon et al. 2000a), during which considerable, but nonsignificant, increases in insulin response were observed after the ingestion of carbohydrate (0.8 g · kg^{-1} · h^{-1}) combined with pea, whey or wheat protein hydrolysate in comparison with the ingestion of only carbohydrate. This could be related to the preceding exercise in the present study, because muscle contraction stimulates glucose transport via GLUT4 translocation (Ivy 1997), which is likely to result in a reduction in postexercise insulin response. In addition, endurance trained athletes exhibit a markedly reduced secretory insulin response after glucose administration (Lohmann et al. 1978, Rodnick et al. 1987). However, a significant, additional increase in plasma insulin level occurs after the ingestion of carbohydrate (0.8 g · kg^{-1} · h^{-1}) with a whey or wheat protein hydrolysate (0.4 g · kg^{-1} · h^{-1}) in endurance trained athletes after exercise (van Hall et al. 2000). Therefore, the apparent contradictory findings should be explained by the higher carbohydrate ingestion rate (1.2 versus 0.8 g · kg^{-1} · h^{-1}) that was applied in the present study.

We observed a substantial additional increase in insulin response after the ingestion of the mixtures containing wheat protein hydrolysate in combination with free leucine and phenylalanine. The addition of these free amino acids clearly led to a significant increase in the insulin response (the area under the curve) compared with the control and wheat protein–only trials (Fig. 3). A dose-effect relationship existed in that doubling the ingestion rate of the hydrolysate–amino acid mixture up to 0.4 g · kg^{-1} · h^{-1} resulted in a substantial increase in insulin response (the area under the curve) compared with the ingestion of only 0.2 g · kg^{-1} · h^{-1} of the same mixture.

Recently, we studied the effects of ingestion of carbohydrate with this mixture of wheat protein hydrolysate, free leucine and phenylalanine on postexercise insulin levels and muscle glycogen synthesis rates in trained athletes (van Loon et al. 2000b). We demonstrated a substantial, additional increase in insulin response after the ingestion of this mixture (0.4 g · kg^{-1} · h^{-1}) in addition to normal postexercise carbohydrate consumption rates (0.8 g · kg^{-1} · h^{-1}). In accordance with Zawadzki et al. (1992), we reported a significant acceleration of muscle glycogen synthesis rates compared with the ingestion of only carbohydrate (0.8 g · kg^{-1} · h^{-1}). However, these increased muscle glycogen synthesis rates were not significantly higher than synthesis rates observed after the ingestion of larger amounts of carbohydrate (1.2 g · kg^{-1} · h^{-1}). Future research will be necessary to investigate whether muscle glycogen synthesis can be further accelerated by ingesting an insulinotropic protein hydrolysate (and amino acid) mixture in combination with a carbohydrate intake of ≥1.2 g · kg^{-1} · h^{-1}.

Consistent with recent findings in the postabsorptive resting state (van Loon et al. 2000a), the magnitude of the in vivo insulin response correlated with the increase in plasma leucine, phenylalanine and tyrosine concentrations. Regression analysis showed a strong positive correlation between plasma leucine, phenylalanine and tyrosine concentrations and the insulin response. This suggested relationship is in accordance with the effects of leucine and phenylalanine in vitro in studies with incubated β cells of the pancreas (Blachier et al. 1989a and 1989b, Hutton et al. 1980, Malaisse

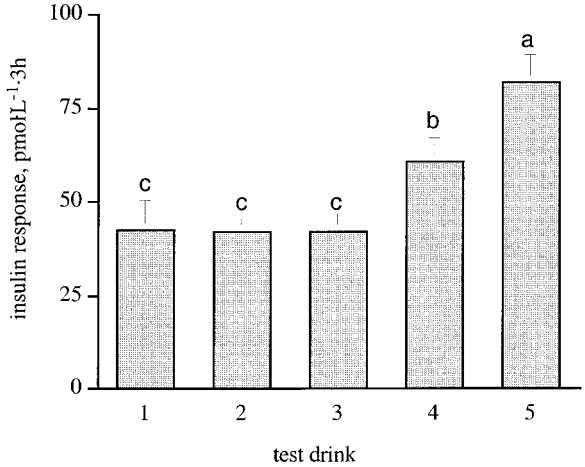

FIGURE 3 Postexercise plasma insulin responses after the ingestion of protein hydrolysate/amino acid–carbohydrate mixtures in humans. Test drink 1, carbohydrate only (1.2 g · kg^{-1} · h^{-1}); drink 2, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}); drink 3, carbohydrate with protein hydrolysate (0.4 g · kg^{-1} · h^{-1}); drink 4, carbohydrate with protein hydrolysate (0.1 g · kg^{-1} · h^{-1}), leucine (0.05 g · kg^{-1} · h^{-1}) and phenylalanine (0.05 g · kg^{-1} · h^{-1}); and drink 5, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}), leucine (0.1 g · kg^{-1} · h^{-1}) and phenylalanine (0.1 g · kg^{-1} · h^{-1}). Values are means ± SEM (n = 8). Mean values not sharing a common superscript are different, $P < 0.05$.

TABLE 2

Plasma amino acid responses in humans after the ingestion of carbohydrate and protein (hydrolysate) with or without free amino acids[1]

	Trials[2]				
Amino acids	Drink 1 control	Drink 2 wheat-0.2	Drink 3 wheat-0.4	Drink 4 wheat/Leu/Phe-0.2	Drink 5 wheat/Leu/Phe-0.4
			mmol · L^{-1} · 3 h^{-1}		
Threonine[3]	−3.36 ± 0.69	−0.32 ± 0.38[a]	2.06 ± 0.70[a]	−2.67 ± 0.56[c]	−2.33 ± 0.34[c]
Serine	−3.20 ± 0.59	−0.08 ± 0.40[a]	2.95 ± 0.94[a,b]	−2.56 ± 0.45[c]	−1.56 ± 0.24[c]
Asparagine	−2.22 ± 1.08	−0.21 ± 0.20	0.96 ± 0.48[a]	−1.67 ± 0.43[c]	−1.42 ± 0.27
Glutamate	−2.45 ± 1.05	0.97 ± 1.02	−0.01 ± 0.84	−0.33 ± 1.51	−0.56 ± 0.82
Glutamine	−13.93 ± 4.03	−2.85 ± 3.03	6.63 ± 3.72[a]	−8.42 ± 4.84	−5.03 ± 2.38
Proline	−4.64 ± 1.05	7.82 ± 1.14[a]	16.86 ± 2.26[a,b]	−0.92 ± 0.70[b,c]	2.78 ± 0.50[a,c]
Glycine	−4.46 ± 1.05	0.29 ± 0.47[a]	2.99 ± 0.77[1]	−4.07 ± 1.06[b,c]	−4.49 ± 0.84[b,c]
Alanine	−11.42 ± 1.76	−4.88 ± 1.19	−1.46 ± 2.55[a]	−8.66 ± 1.90	−9.58 ± 1.43
Citrulline	−3.07 ± 0.57	−0.73 ± 0.24[a]	0.30 ± 0.25[a]	−0.64 ± 0.22[a]	0.63 ± 0.15[a,b]
α-Aminobutyrate	−0.55 ± 0.41	0.21 ± 0.10	0.48 ± 0.12[a]	−0.24 ± 0.11	−0.31 ± 0.06
Valine[3]	−6.10 ± 0.89	0.40 ± 0.64[a]	6.45 ± 1.19[a,b]	−9.14 ± 1.05[b,c]	−6.10 ± 0.71[b,c]
Methionine[3]	−1.63 ± 0.48	−0.88 ± 0.13	−0.44 ± 0.26	−1.42 ± 0.09	−1.38 ± 0.17
Isoleucine[3]	−3.21 ± 0.56	0.74 ± 0.24[a]	3.57 ± 0.53[a,b]	−3.40 ± 0.43[b,c]	−1.84 ± 0.30[b,c]
Leucine[3]	−5.30 ± 0.76	1.81 ± 0.46	7.34 ± 0.98[a]	31.51 ± 1.08[a,b,c]	66.68 ± 3.29[a,b,c,d]
Tyrosine[3]	−2.95 ± 0.69	0.20 ± 0.19	1.97 ± 0.53[a]	3.79 ± 0.47[a,b]	8.26 ± 1.15[a,b,c,d]
Phenylalanine[3]	−2.48 ± 0.73	2.17 ± 0.32	4.30 ± 0.47	23.14 ± 2.27[a,b,c]	53.17 ± 4.14[a,b,c,d]
Tryptophan[3]	−0.69 ± 0.65	0.74 ± 0.62	1.57 ± 0.55	−1.97 ± 1.96	−0.77 ± 0.54
Ornithine	−1.74 ± 0.54	1.81 ± 0.24[a]	3.27 ± 0.44[a]	0.51 ± 0.17[a,c]	1.86 ± 0.24[c]
Lysine[3]	−3.82 ± 0.81	−2.03 ± 0.35	−1.64 ± 0.58	−3.15 ± 0.78	−2.98 ± 0.73
Histidine[3]	−2.03 ± 0.60	0.68 ± 0.19[a]	1.70 ± 0.35[a]	−1.09 ± 0.22[b,c]	−0.68 ± 0.30[c]
Arginine	−4.14 ± 0.80	−1.19 ± 0.44	1.24 ± 0.36[a]	−1.45 ± 0.58[a,c]	−0.04 ± 0.68[a]

[1] Plasma amino acid response expressed as the area under the curve above baseline (means ± SEM; $n = 8$); [a,b,c,d] significant difference in plasma amino acid response when compared to a specific trial (drink 1, 2, 3, and 4, respectively) ($P < 0.05$).

[2] The applied drinks contain drink 1, carbohydrate only (1.2 g · kg^{-1} · h^{-1}); drink 2, carbohydrate with wheat protein hydrolysate (0.2 g · kg^{-1} · h^{-1}); drink 3, carbohydrate with wheat protein hydrolysate (0.4 g · kg^{-1} · h^{-1}); drink 4, carbohydrate with wheat protein hydrolysate (0.1 g · kg^{-1} · h^{-1}), leucine (0.05 g · kg^{-1} · h^{-1}) and phenylalanine (0.05 g · kg^{-1} · h^{-1}); and drink 5, carbohydrate with wheat protein hydrolysate (0.2 g · kg^{-1} · h^{-1}), leucine (0.1 g · kg^{-1} · h^{-1}) and phenylalanine (0.1 g · kg^{-1} · h^{-1}).

[3] Essential amino acids.

et al. 1991, Malaisse Lagae et al. 1971, Sener et al. 1989 and 1981, Sener and Malaisse, 1980 and 1981, Varnier et al. 1995) and with the in vivo studies by Floyd and coworkers (Fajans et al. 1962, Floyd et al. 1963, 1966, 1968, 1970a and 1970b) in which several (combinations of) amino acids with and without glucose were infused. The positive correlation observed with plasma tyrosine concentrations may be explained by the fact that tyrosine, the hydroxylation product of phenylalanine in the liver, is formed when large amounts of phenylalanine are ingested (Pogson et al. 1985). Furthermore, we observed a less substantial but significant negative correlation between the insulin response and plasma threonine, asparagine, glycine, alanine, valine, methionine, isoleucine and histidine concentrations. These negative correlations could be explained by an increased amino acid uptake after an increase in insulin level. Interestingly, the amino acids that revealed significant negative correlations included all of the essential amino acids (of course, with the exclusion of the supplemented amino acids leucine, phenylalanine and its product tyrosine). Plasma amino acid concentrations were generally lower after the ingestion of drinks 4 and 5 compared with the control trial, although in the latter, considerable amounts of protein and amino acids were ingested, which would normally increase the plasma amino acid response as shown in trials 2 and 3. This seems to suggest that tissue amino acid uptake and possibly also postexercise net muscle protein balance were increased after the ingestion of this insulinotropic mixture. This would be in line with several studies demonstrating that an increase in plasma insulin concentration, during conditions of hyperaminoacidemia, further increases net muscle protein balance in vivo in humans (Fryburg et al. 1995, Gelfand and Barrett, 1987, Hillier et al. 1998). Such a stimulating effect on net protein balance may in part also be a consequence of a stimulating effect of leucine on skeletal muscle protein synthesis, independent of an increase in insulin levels (Anthony et al. 1999 and 2000). However, the potential of insulinotropic protein hydrolysate and amino acid mixtures to stimulate postexercise net muscle protein anabolism, and the mechanisms involved, remains to be investigated.

The present study shows that the ingestion of a mixture of wheat protein hydrolysate, free leucine and phenylalanine, in combination with carbohydrate, results in a substantial, additional increase in the postexercise insulin response compared with the ingestion of only carbohydrate. Furthermore, it is demonstrated that the magnitude of this increase in insulin response is dose dependent. Consequently, this mixture provides a practical tool to strongly elevate postexercise insulin levels via dietary manipulation only. This mixture has previously been shown to stimulate glycogen synthesis after exercise when added to a carbohydrate-containing solution (0.8 g · kg^{-1} · h^{-1}) and may also serve to increase net protein balance in the postexercise phase and be applied as a tool in metabolic research investigating the effects of high plasma insulin concentrations.

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