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Carbohydrate to protein ratio in food and cognitive performance in the morning

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Abstract

The effect of different carbohydrate to protein ratios in food on cognitive functions and the relation between postprandial metabolic and cognitive changes were studied in 15 healthy male students. Subjects were tested in three sessions, separated by 1 week, for short-term changes in mood states, objective cognitive functions, blood parameters, and indirect calorimetry using a repeated-measures, counterbalanced cross-over design. Measurements were made after an overnight fast before and hourly during 3.5 h after test meal ingestion. The isoenergetic (1670 kJ) test meals consisted of three carbohydrate to protein ratios, i.e. a carbohydrate-rich (CHO[4:1]), balanced (BAL[1:1]), and protein-rich (PRO[1:4]) meal, respectively. Overall accuracy in short-term memory was best after the PRO[1:4] meal concomitant to the least variation in glucose metabolism and glucagon to insulin ratio (GIR). Related to changes in glucose metabolism and/or in the ratios of large neutral amino acids (LNAA), respectively, attention and decision times were transiently improved within the first hour after the CHO[4:1] meal, whereas after the first hour the BAL[1:1] and PRO[1:4] meal resulted in improved performance. Overall reaction times of a central task were fastest after the BAL[1:1] meal concomitant to the highest overall tyrosine (Tyr) to LNAA ratio. Our findings suggest that the carbohydrate to protein ratio in food specifically influences higher cognitive functions in the morning. Except for a transient positive effect of rising blood glucose after a carbohydrate-rich meal, a protein-rich or balanced meal seems to result in better overall cognitive performance presumably because of less variation in glucose metabolism and/or higher modulation in LNAA ratios indicated by the overall GIR.

Keywords: Cognitive performance; Protein to carbohydrate ratio; Postprandial metabolism; Glucose; Glucagon to insulin ratio; Large neural amino acids; Tryptophan; Tyrosine

1. Introduction

Carbohydrates and protein appear to have differential effects on mood and simple cognitive functions [24,26,49]. These effects have often been related to the nutrients' effects on the synthesis of brain serotonin and catecholamines, which are mediated through changes in the ratios of plasma tryptophan (Trp) and tyrosine (Tyr) to the other large neutral amino acids (LNAA) [12,22]. Other potential mediating mechanisms for the observed effects of nutrients on mood and simple cognitive functions are changes in the energy supply to nerve cells and fluctuations in plasma metabolite or hormone levels [5,17,31]. The effects of carbohydrate and protein ingestion on complex or 'real-world' [6] cognitive functions, however, have scarcely been studied. Moreover, the few results available are inconclusive and difficult to compare because of marked differences in design and cognitive tasks selected (for review see Ref. [11]).

An 'inverted U'-shaped dose–response relation between individual metabolite or hormone concentrations and cognitive functioning has been observed [5], and stable metabolic conditions seem to stabilize cognitive performance [8]. We recently reported [16] that overall postprandial cognitive performance in the morning was better after pure fat than after pure carbohydrate or protein ingestion. Fat ingestion in that study was followed by stable glucose metabolism and a high and constant state of metabolic activation, as reflected by the glucagon to insulin ratio (GIR). In contrast, pure

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carbohydrate or protein ingestion led to pronounced changes in glucose metabolism and in the GIR. Thus, overall cognitive performance in the morning might be better after a balanced or protein-rich meal than after a carbohydrate-rich meal because of smaller postprandial changes in glucose metabolism and a more constant or higher metabolic activation. Therefore, in the present study we tested whether variations in the protein to carbohydrate ratio of a meal affect specific simple as well as complex cognitive functions. We investigated postprandial metabolic and hormonal alterations (glucose, GIR, Trp/LNAA and Tyr/LNAA in plasma and respiratory indices) as well as the changes in cognitive functions in response to the ingestion of isoenergetic meals with different protein to carbohydrate ratios, but similar volume and sensory properties. The cognitive tests employed targeted motor performance, information processing, short-term memory, and attention as well as the accuracy and efficiency of cognitive tasks.

2. Subjects and methods

2.1. Subjects

Fifteen healthy male students, mean age 26.3 (S.D. 3.6) years, were recruited through advertisements posted at the Swiss Federal Institute of Technology and the University in Zurich. The subjects were informed about the general objectives, the procedure and possible risks of the study. All subjects gave their written informed consent to the study, which was approved by the ethical committee of the Federal Institute of Technology Zurich. They were nonsmokers, not color blind, and had a mean body mass index of 23.9 (S.D. 3.2) kg/m^2. They were not on any medication and did not take any drugs or nutritional supplements the week before and during the experimental period. Their nutritional habits and physical activity were recorded before the study. All were used to eat breakfast and did not consume caffeine daily.

2.2. Design and procedure

A repeated-measures, counterbalanced cross-over design was used [16]. Each subject was tested in three sessions separated by exactly 1 week. Subjects were told that various physiological and psychological effects of food intake were examined, but they were not aware of ingesting different ratios of macronutrients. To avoid interferences of learning with treatment effects, the subjects had to practice all cognitive tests on two separate days in the week before the experimental period. In this way, the subjects also became familiar with the experimental procedure, which simulated stress situations of daily work.

To ensure similar baseline conditions, subjects were not allowed to ingest alcohol or caffeine-containing drinks and foods, and were requested to refrain from strenuous physical exercise the day before each testing. In addition, all subjects consumed an identical prepackaged pasta meal between 1900 and 2100 h on the evening before each testing. The meal consisted of fresh ravioli with a vegetarian filling (250 g), preserved tomato sauce (300 g), fresh carrot salad (160 g), and a canned fruit cocktail (160 g). It provided approximately 4400 kJ, with 74%, 11% and 15% of the energy derived from carbohydrates, protein and fat, respectively, and the composition of the meal suggested a medium glycemic index (GI). The meal was rich in carbohydrates and scheduled late to avoid a depletion of liver glycogen stores over night. Subjects were instructed to ingest the

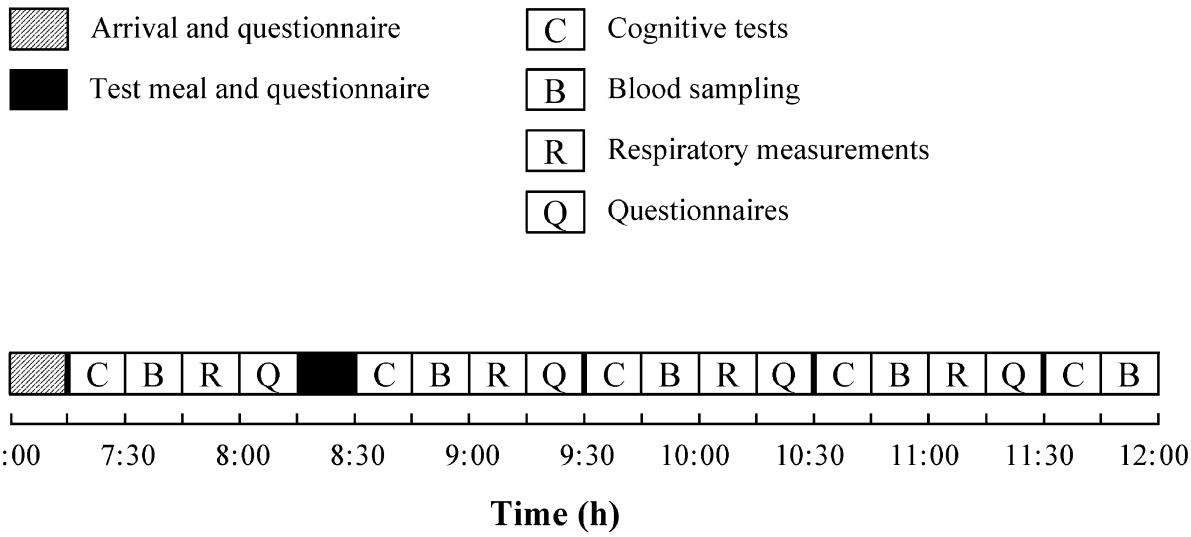

Fig. 1. Schedule of experimental procedures for the first subject on each test morning. The second and third subjects started 15 and 30 min later, respectively, following the same order of procedures.

whole meal and to have no further food or drinks except water before testing. Subjects were also asked to sleep for at least 8 h if possible.

Subjects arrived at the institute by public transport. Three subjects were tested daily, beginning at 0700, 0715 and 0730 h, respectively (Fig. 1). Just after arrival, subjects had to fill out a questionnaire checking their compliance with the restrictions on the day before as well as their sleep quality and their actual mental and subjective physical performance. Before the test meal, baseline assessments were taken in the order (1) cognitive tests, (2) blood samplings, (3) respiratory measurements and (4) questionnaires. Fifteen minutes were scheduled for each of these assessments. Subsequently, the cream-like test meal was served in a dessert bowl. Subjects had 10 min to eat the whole meal with a spoon and afterwards 5 min to fill in a questionnaire concerning the meal's acceptance and sensory properties. For repeated postprandial measurements within the next 3.5 h, subjects rotated each hour through the same stations as at baseline. Subjects had free access to mineral water during the whole study, but no additional food or fluid intake was permitted.

2.3. Test meals

Test meals (Table 1) consisted of isoenergetic (1670 kJ) carbohydrate (CHO) and protein (PRO) suspensions mixed at three different ratios: The CHO-rich meal with a CHO/PRO ratio of 4:1 (CHO[4:1]), the balanced meal with a CHO/PRO ratio of 1:1 (BAL[1:1]), and the PRO-rich meal with a CHO/PRO ratio of 1:4 (PRO[1:4]). All test meals had similar volume and sensory properties (taste, consistency and color). They were freshly prepared by mixing (Bamix M 120, ESGE, Mettlen, Switzerland) appropriate quantities of basic ingredients and water to obtain 400 ml of a foam-like vanilla cream. Different kinds of protein (milk protein and egg white powder) and carbohydrates (glucose, maltodextrin, rice starch) were combined to cover a broad range of the representative compounds that might affect cognitive behavior differentially. The carbohydrate proportion of the test meals suggested a high to medium GI. The profile of the LNAA valine, leucine, isoleucine, phenylalanine, Tyr, and Trp (g/100 g protein) for the milk protein was 6.4, 9.5, 5.2, 4.5, 4.7 and 1.7 and for the egg white powder 7.1, 6.9, 5.8, 4.9, 3.7 and 1.6, respectively. Thus, the total Trp and Tyr content of the three test meals was 1.5 and 3.9 g for the PRO[1:4], 0.9 and 2.4 g for the BAL[1:1], and 0.4 and 1.0 g for the CHO[4:1] meal, respectively. The energy content of the test meals was in the range of the subjects' habitual energy intake for a morning. Acceptance and sensory homogeneity of the three test meals were tested by staff members of our institute before study onset.

Table 1
Composition of the cream-like test meals (1670 kJ; 400 ml)

Basic ingredients (g)[a]	Carbohydrate-rich [4:1]	Balanced [1:1]	Protein-rich [1:4]
Glucose[b]	8.4	5.3	2.1
Maltodextrin[c]	33.7	21.0	8.4
Maltodextrin[d]	33.7	21.0	8.4
Rice starch[e]	8.4	5.3	2.1
Milk protein[f]	18.9	47.3	75.8
Dried chicken egg white powder[g]	2.1	5.3	8.4
Water	150	200	250

[a] For sensory acceptance, 0.5 g/l of a nonenergetic powdered mixture of sweeteners (aspartame:acesulfame K = 1:1; NutraSweet, Zug, Switzerland, Hoechst, Frankfurt/Main, Germany, respectively) as well as 3 ml/l of vanilla flavor (vanille aroma 78506-33, Givaudan-Roure Flavors, Dübendorf, Switzerland) were also added before mixing. The creams were later colored, using an egg-yellow food color (1 ml/l, E104/E110, Werna W. Schweizer, Wollerau, Switzerland), to get an identical appetizing yellow color.

[b] Glucodry 380, commercially available from Blattmann, Wädenswil, Switzerland.

[c] Maltodextrin 15 (Blattmann).

[d] Maltrin M100 (Blattmann).

[e] Becosan FR (Blattmann).

[f] Promilk 852 E, commercially available from Lacto Prospérité, Bern, Switzerland.

[g] Commercially available from Lüchinger + Schmid, Kloten, Switzerland.

2.4. Blood sampling and analyses

A 20-gauge vialon catheter (Insyte-W, Becton Dickinson, Rutherford, NJ) was placed into the antecubital vein, and fasting blood samples were drawn whilst the subject was in a lying position. The first 2 ml of each blood sample was discarded. Using a multiadapter, 2.6 ml blood was collected into a NaF-containing tube (Monovette, Sarstedt, Sevelen, Switzerland) for later determination of plasma glucose. Another 12.4 ml was collected into two EDTA-containing tubes (Monovettes, Sarstedt) for analyses of further metabolites and hormones. For glucagon analysis, 1 ml of the EDTA blood sample was transferred into a glass tube containing 500 KIU trypsin inhibitor (Aprotinin, Böhringer Mannheim, Mannheim, Germany). All blood samples were immediately centrifuged ($1600 \times g$; 4 °C; 12 min), and plasma was stored at − 20 °C. Plasma for glucagon analysis was stored separately in glass tubes.

Plasma glucose and urea were analyzed enzymically at 37 °C (Cobas-Mira analyzer, Roche, Basel, Switzerland) using a commercial kit (Roche). Hormones were analyzed using commercially available radioimmunoassay kits: insulin (CIS Medipro, Geneva, Switzerland) and glucagon (Diagnostic Products, Los Angeles, CA). Plasma free LNAA were analyzed by ion exchange chromatography [48] on a Beckman amino acid analyzer (System 6300, Beckman Coulter, Fullerton, CA).

2.5. Respiratory measurements

The respiratory exchange ratio (RER) was determined during a 15-min period using a half open system (Oxycon

Sigma, Mijnhardt, Bunnik, Netherlands). Subjects walked slowly on a treadmill (2.5 km/h) to ensure a stable respiration. Energy expenditure (EE) in terms of total thermogenesis was calculated according to the Weir formula [55].

2.6. Questionnaires

All answers were given through 7-point bipolar equilateral rating scales ranging (in German words) from *not at all* (-3) to *extremely* ($+3$). For sleep quality and mental as well as subjective physical performance on the mornings four points (i.e. -3, -1, 1, 3) on this scale were labeled with describing terms to help the subjects where to range. To rate the acceptance, pleasantness, and sensory homogeneity of the test meals, the following 11 terms (in German) were used: quantity, tasty, sweet, insipid, pleasant, watery, filling, fatty, satiating, sticky and thirst provoking. For the hourly questionnaires addressing the subjects' subjective performance 20 different German terms were used (calm, relaxed, cold, clearheaded, tired, confused, cramped head, exhausted, feeling sick, nervous, heat, energetic, lively, receptive, stressed, efficient, depressed, thirsty, fullness, can wait to eat) with an equal number of positive and negative terms to reduce response bias [25].

2.7. Cognitive performance tests

Different computer-based cognitive tasks, which simulated variously demanding cognitive tasks of today's working conditions, were performed to evaluate objective cognitive performance. One test session lasted about 15 min.

2.7.1. Choice reaction time

An established version of choice visual and auditory reaction time was measured (Wiener Reaktionsgerät, version 8.00, Dr. G. Schuhfried, Mödling, Austria) as a less demanding decision task to assess motor performance and information processing. The subject had to react on a yellow light that flashed either simultaneously with a red light or simultaneously with a tone by moving his finger as fast as possible from a rest-contact-button to a push-button, 5 cm further forward. If the red light appeared simultaneously with a tone, or if any of the signals appeared alone, the subject should not react. The whole reaction time was split into decision and motoric time, and for both the mean reaction time as well as an error rate for each session were calculated. One session lasted 3 min (48 signals).

2.7.2. Combi test

A bimodal computer-based visual combi test [16,46] simulates complex cognitive tasks by combining a demanding central short-term memory task and a simpler peripheral attention task. This design is believed to be more sensitive than one-dimensional single-task tests because the subject has to concentrate on two tasks simultaneously. The central short-term memory task consists of recognizing defined sequences of colored circles by pressing a button on a keyboard. These colored circles are displayed in the middle of a 17-in monitor and appear one after another on the same place. In the additional peripheral attention task, the subject has to detect rotations of one of four patterned circles that are displayed at the corners of the same monitor by pressing another button on the keyboard. A qualitative accuracy score (correct responses in given responses) as well as a quantitative efficiency score (correct responses in demanded responses) that represents a central attention score were calculated from the central short-term memory task. Similarly, a qualitative attention as well as a quantitative efficiency score were calculated for the peripheral task. In addition, the mean reaction time was determined for the demanding central and simple peripheral task. The whole test lasted 5 min per session.

2.7.3. Multitask test

This highly complex test is an extended version of the combi test. In addition to the two tasks of the combi test, there is a tracking task, which has to be carried out simultaneously. The sequence of the colored circles no longer appears on the same place but moves across the monitor. The subject has to follow this movement with a pin on a digitizing board, which is positioned beside the keyboard. A further score for the accuracy of this tracking task can be calculated.

2.8. Statistics

Statistical analyses were performed with Systat software (version 9, SPSS, Chicago, IL). Using a split-plot model [42], independent variables were the subject, the period of the test day, and the type of macronutrient (main unit) as well as the timing of the repeated measurements during the test morning (split unit). The period was included in the model to control for learning effects between test days. Data were adjusted for baseline measurements by including absolute baseline values as covariates in the model. Repeated-measures analysis of variance (ANOVA) with treatment as a grouping factor and time as a within factor was performed to detect overall meal effects and Meal $\times$ Time interactions. Polynomial contrast analysis was performed for significant Meal $\times$ Time interactions. For significant overall treatment differences, the data were further analyzed with Tukey's post hoc comparisons. To detect differences in acceptance and sensory properties of the test meals, the respective ratings were analyzed by ANOVA and Tukey's post hoc comparisons. To analyze the relation between the overall (area under the curve [0–210 min]) postprandial metabolic and cognitive changes, multiple regression analyses with interactive stepwise modeling as well as simple correlation analyses (Pearson) were applied. Pearson correlation analyses were also performed for changes (area under the curve for 60-min intervals) between individual time points. Statistical significance was set at $P < .05$. LNAA ratios were calculated as described earlier [14].

3. Results

3.1. Metabolites and hormones in plasma

There were significant overall meal effects for all plasma metabolite and hormone concentrations as well as for the GIR and the Trp/LNAA ratio [$F(2,25) < 10.6$, $P < .001$] as well as Tyr/LNAA ratio [$F(2,25) > 4.4$, $P < .05$]. Except for the Trp/LNAA ratio, there were also significant Meal $\times$ Time interactions [$F(6,75) > 6.9$, $P < .001$; $F(6,75) > 4.4$, $P < .01$ for glucagon].

3.1.1. Glucose, insulin, glucagon and GIR (Fig. 2)

Overall, postprandial plasma glucose and insulin concentrations were highest after the CHO[4:1] and lowest after the PRO[1:4] meal, with significant differences between the CHO[4:1] and PRO[1:4] as well as between the CHO[4:1] and BAL[1:1] meals (Fig. 2). The postprandial increases in plasma glucose and insulin as well as the treatment differences were observed at 30 and 90 min after meal onset. The plasma glucagon concentration remained nearly constant after the CHO[4:1] meal, but it increased after the BAL[1:1] and PRO[1:4] meals, resulting in significant overall meal differences between all test meals. The GIR first decreased and later increased in response to all meals with a nadir at 30 min after meal onset. Overall, the GIR was significantly higher with the PRO[1:4] and BAL[1:1] meals than with the CHO[4:1] meal.

3.1.2. Amino acid ratios (Fig. 3) and urea (Fig. 4)

Overall, the Trp/LNAA ratios were significantly higher after the CHO[4:1] meal than after the BAL[1:1] and PRO[1:4] meals (Fig. 3). The Tyr/LNAA ratio was significantly higher after the BAL[1:1] than after the CHO[4:1]

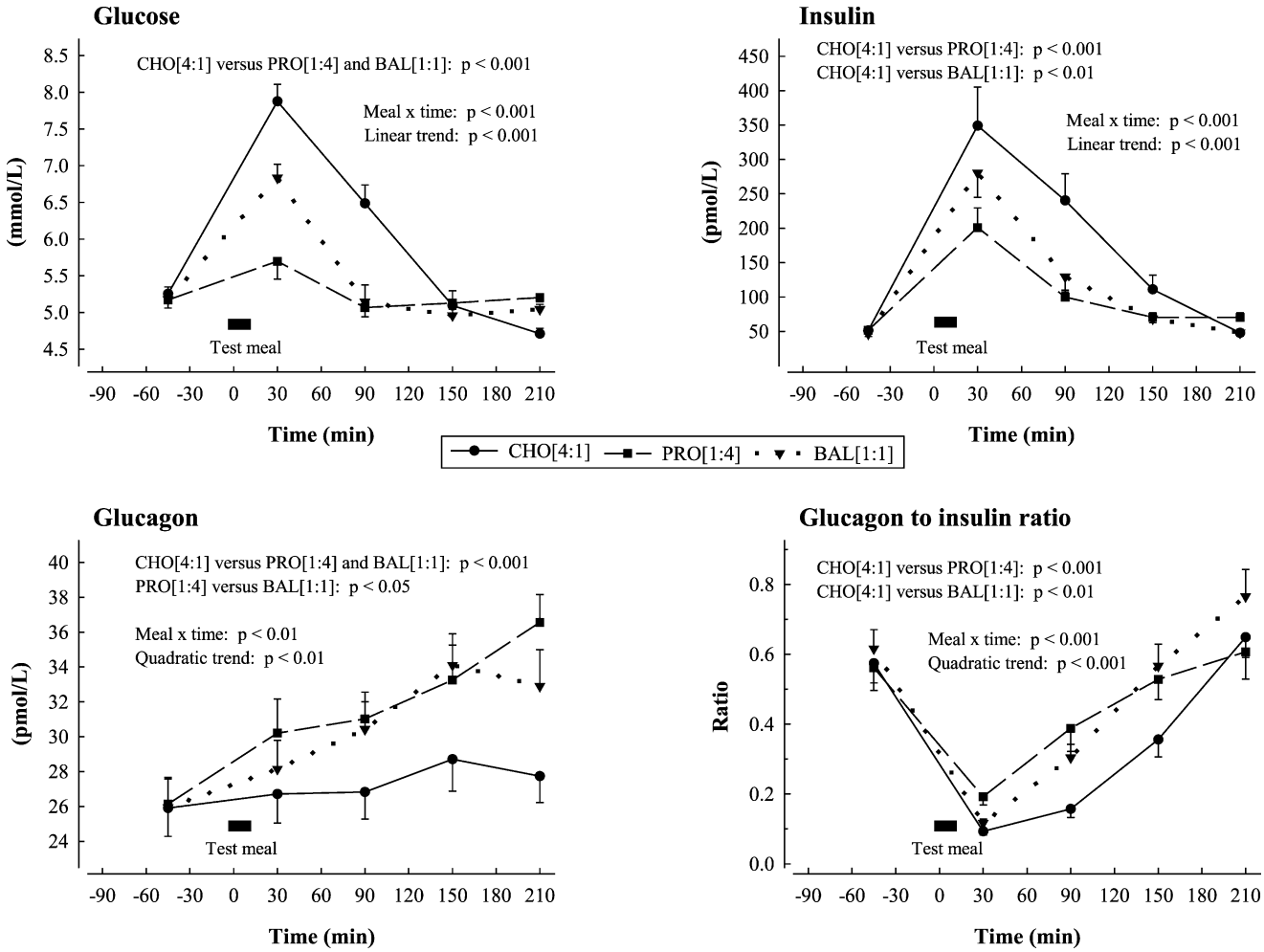

Fig. 2. Temporal profiles for glucose, insulin and glucagon responses as well as for the GIR to a carbohydrate-rich CHO[4:1], protein-rich PRO[1:4] or balanced BAL[1:1] meal. Mean (S.E.M.); $n = 15$. Test meal ingestion (min 0–15) and postprandial temporal pattern of changes (min 30–210) are preceded by a baseline (min −45) measurement. Significant P values are given for Tukey's post hoc comparisons of overall meal effects.

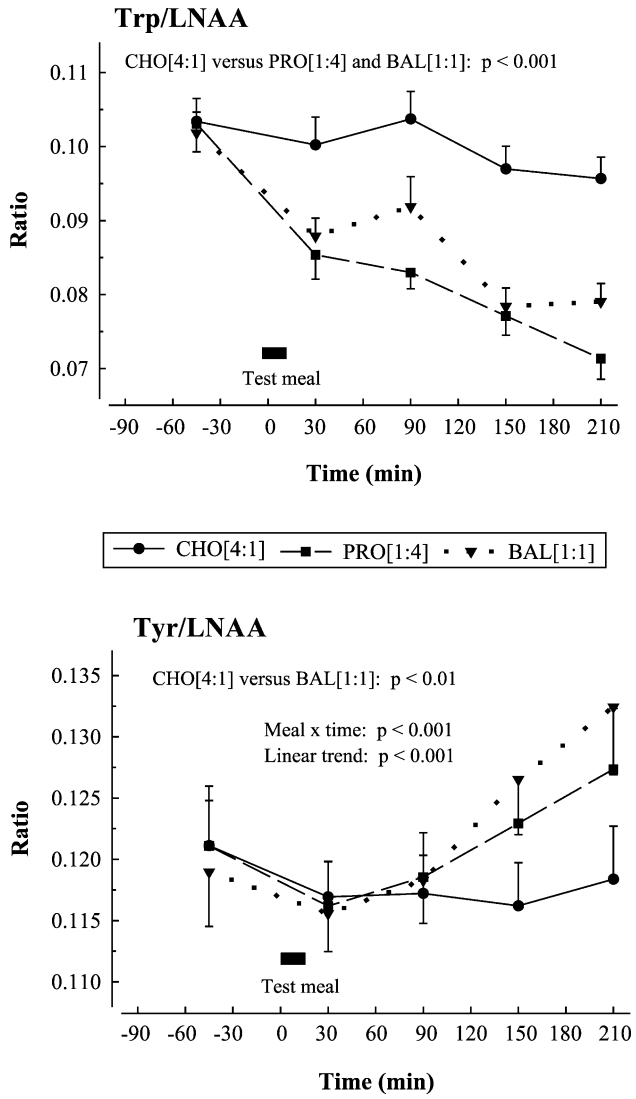

Fig. 3. Temporal profiles for the Trp to LNAA as well as Tyr to LNAA ratios to a carbohydrate-rich CHO[4:1], protein-rich PRO[1:4] or balanced BAL[1:1] meal. Mean (S.E.M.); $n = 15$. Test meal ingestion (min 0–15) and postprandial temporal pattern of changes (min 30–210) are preceded by a baseline (min −45) measurement. Significant P values are given for Tukey's post hoc comparisons of overall meal effects.

meal and was intermediate after the PRO[1:4] meal. This overall difference was mainly due to a postprandial increase after the BAL[1:1] meal.

The plasma urea concentration remained stable after the CHO[4:1] meal, but increased after the BAL[1:1] and PRO[1:4] meals, resulting in significant overall differences between all treatments (Fig. 4).

3.2. Respiratory exchange ratio and energy expenditure

There was a significant overall meal effect on the RER [$F(2,20) = 16.1$, $P < .001$]. The RER increased after all test meals initially (45 min). Overall, RER was highest after the CHO[4:1] and lowest after the PRO[1:4] meal, with significant differences between all treatments (Fig. 4). The calculation of EE did not reveal any significant overall meal or Meal × Time effect (data not shown).

3.3. Questionnaires

The subjects' compliance with the restrictions imposed during the pretest day did not differ significantly between treatments. Sleep quality and subjective mental as well as subjective physical performance on the test mornings were

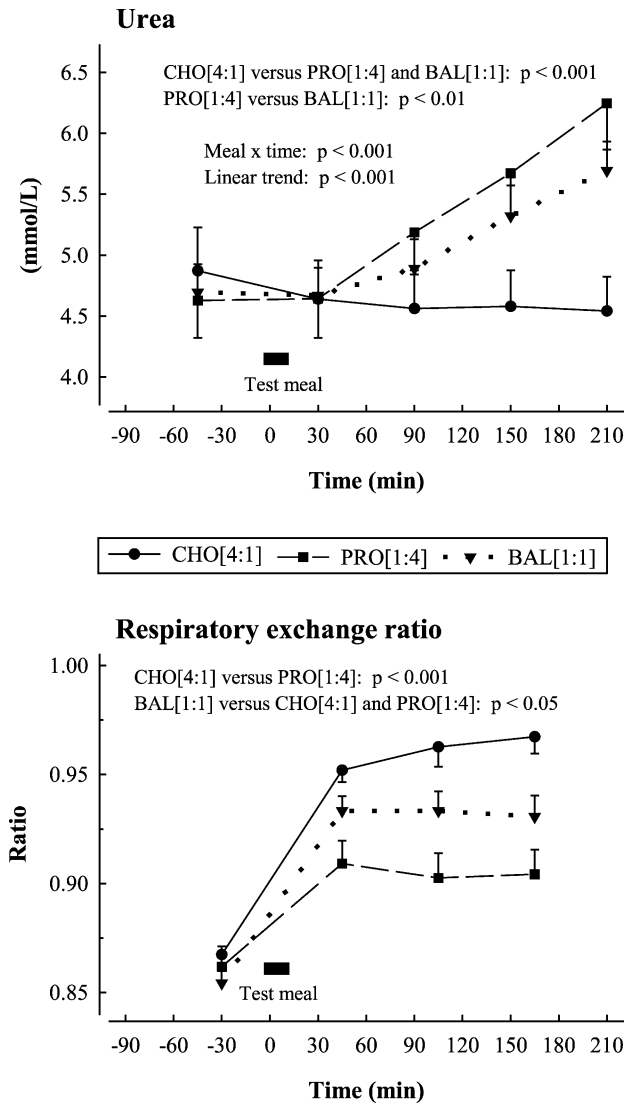

Fig. 4. Temporal profiles for urea responses as well as the RER to a carbohydrate-rich CHO[4:1], protein-rich PRO[1:4] or balanced BAL[1:1] meal. Mean (S.E.M.); $n = 15$. Test meal ingestion (min 0–15) and postprandial temporal pattern of changes for urea and the RER, respectively (min 15–195 and 45–175) are preceded by a baseline (min −60 and −30) measurement. Significant P values are given for Tukey's post hoc comparisons of overall meal effects.

also not significantly different between treatments. Furthermore, the acceptance and sensory impression of the test meals did not differ significantly [$F(2,26) < 3.1$; Tukey $P > .05$]. However, the ratings for 'satiating' were significantly higher immediately after the PRO[1:4] meal than after the CHO[4:1] or BAL[1:1] meal [$F(2,26) = 4.3$, $P < .05$; Tukey $P < .05$], and the CHO[4:1] meal was rated more watery than the other two meals [$F(2,26) = 4.2$, $P < .05$; Tukey $P < .05$] (data not shown). Mean (S.E.M.) ratings for terms that represented the liking/dislike of the CHO[1:4], BAL[1:1] and PRO[4:1] meal, respectively, were 0.0 (0.3), 0.1 (0.3), and −0.1 (0.4) for 'tasty' and 0.0 (0.3), 0.1 (0.3), and −0.3 (0.3) for 'pleasant.' For most terms reflecting the subjective state, no overall meal effect or Meal × Time interaction was detected. Only overall 'can wait to eat' ratings were significantly higher after the PRO[1:4] meal than after the two other meals [$F(2,23) = 3.8$, $P < .05$; Tukey $P < .05$], and there was a Meal × Time interaction for 'feeling sick' with higher mean (S.E.M.) ratings for the PRO[1:4] meal [−2.1 (0.3)] than for the BAL[1:1] [−2.6 (0.2)] and CHO[4:1] [−2.6 (0.2)] meal in the first and second hour and higher ratings after the BAL[1:1] [−2.5 (0.2)] and CHO[4:1] [−2.6 (0.2)] meal than after the PRO[1:4] meal [−2.9 (0.1)] in the third hour [$F(4,44) = 2.7$, $P < .05$].

3.4. Cognitive performance

There was no significant effect for the period on all tasks, indicating that there were no learning effects between test days.

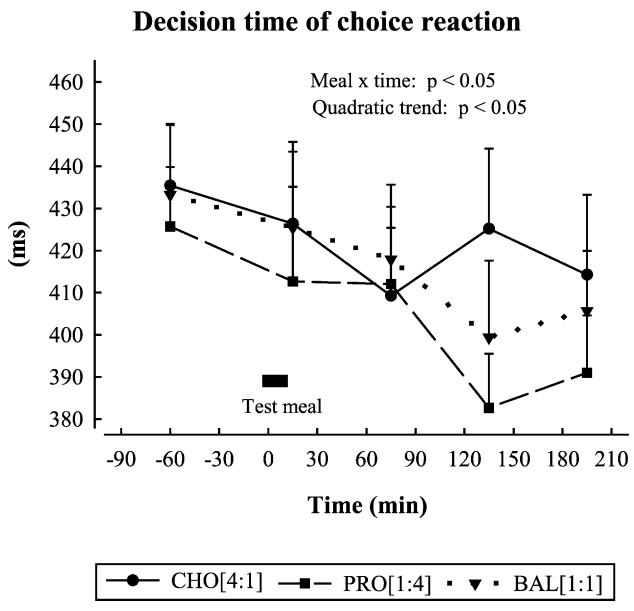

Fig. 5. Temporal profiles for the decision time of choice reaction to a carbohydrate-rich CHO[4:1], protein-rich PRO[1:4] or balanced BAL[1:1] meal. Mean (S.E.M.); $n = 15$. Test meal ingestion (min 0–15) and postprandial temporal pattern of changes (min 15–195) are preceded by a baseline (min −60) measurement. The significant P value is given for the Meal × Time interaction.

3.4.1. Choice reaction time

Despite no overall meal effect on the decision time of choice reaction, there was a significant Meal × Time interaction [$F(3,33) = 2.9$, $P < .05$]. As a result, the decision time of choice reaction appeared to be shorter after the PRO[1:4] than after the CHO[4:1] meal at 135 min after meal onset (Fig. 5). There were no significant meal or Meal × Time effects on the motoric time or the error rate of choice reaction (data not shown).

3.4.2. Combi test

Some data for the central task of the combi test are presented for only 12 subjects because 3 subjects did not react to the requested sequences of colored circles. For the accuracy in short-term memory a significant overall meal effect [$F(2,19) = 3.6$, $P < .05$] was detected. Subjects reached higher scores after the PRO[1:4] meal than after the BAL[1:1] or CHO[4:1] meal (Fig. 6). The central attention task revealed a quadratic Meal × Time interaction [$F(6,57) = 2.3$, $P < .05$], which mainly resulted in slightly higher scores at 75 min after the CHO[4:1] than after the BAL[1:1] meal. The competing peripheral attention task also revealed a quadratic Meal × Time interaction [$F(3,30) = 3.7$, $P < .05$] (Fig. 6); while scores were higher for the BAL[1:1] than for the CHO[4:1] meal immediately after meal end (15 min), the opposite was true at 135 min. Reaction times of the simpler peripheral task did not reveal a significant meal or Meal × Time effect (data not shown). The central reaction times of the demanding central task, however, showed a significant overall meal effect [$F(2,25) = 4.0$, $P < .05$] (Fig. 6) with slower reaction times after the CHO[4:1] than after the BAL[1:1] meal.

3.4.3. Multitask test

Scores for the central and peripheral task of the multitask test showed similar patterns as the corresponding scores of the combi test. Yet, both tasks and the tracking task did not reveal any significant meal or Meal × Time effect (data not shown).

3.5. Correlation and regression analyses

Overall cognitive effects usually correlated better with quadratic metabolic changes, whereas cognitive changes between individual time points correlated better with linear metabolic changes (data not shown). This basic information was applied in the subsequent correlation and regression analyses.

In simple correlation analyses of changes between individual time points (Table 2), changes in the accuracy of short-term memory were positively related to changes in glucose and insulin concentrations. Changes in central and peripheral attention were positively related to glucose and insulin concentrations and/or negatively to the GIR and the Trp/LNAA ratio, respectively. While changes in the central reaction time were negatively related to changes in glucose

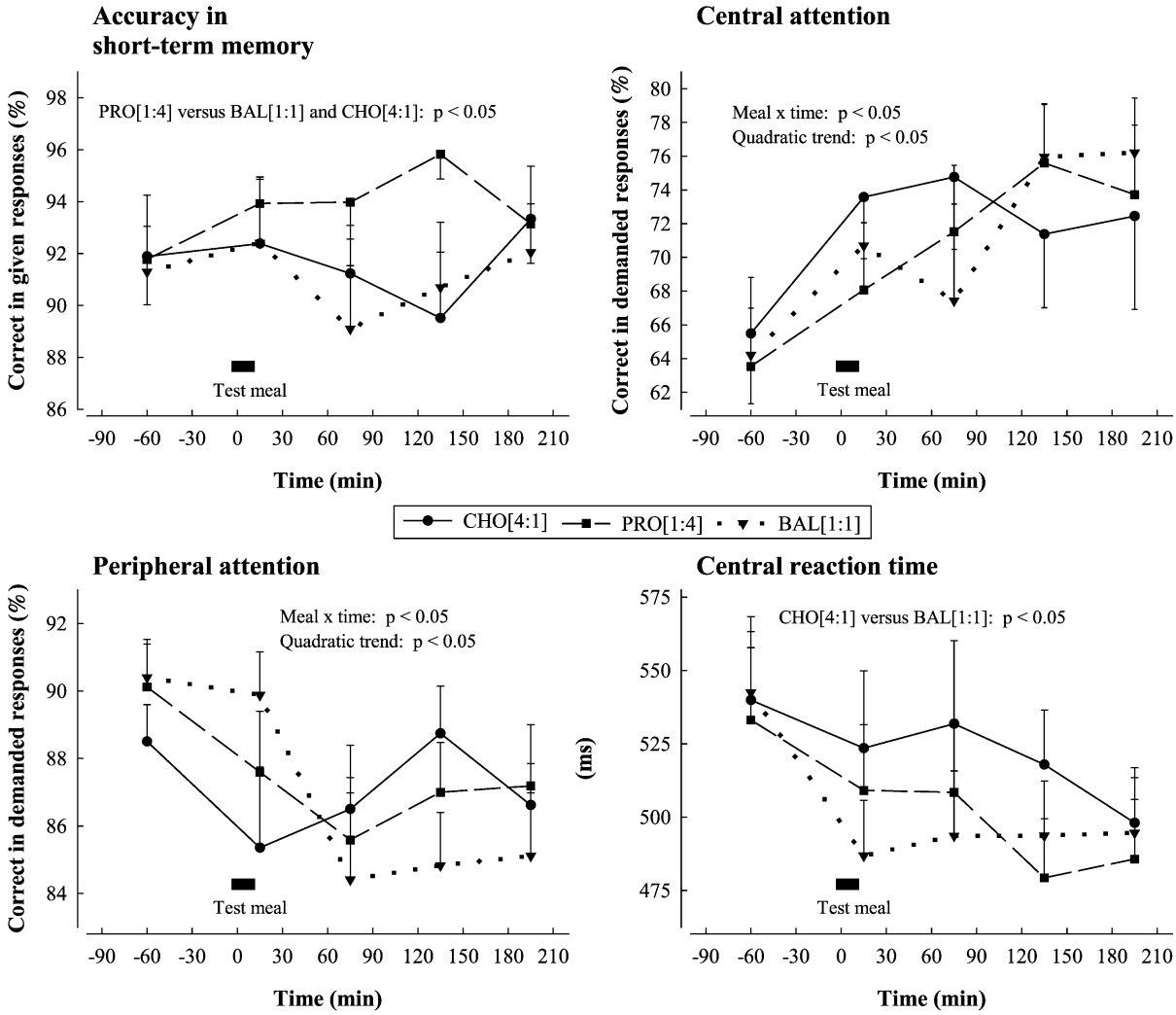

Fig. 6. Temporal profiles for the accuracy in central short-term memory, central and peripheral attention scores as well as for reaction times of the central task of the combi test to a carbohydrate-rich CHO[4:1], protein-rich PRO[1:4] or balanced BAL[1:1] meal. Mean (S.E.M.); $n = 15$. Test meal ingestion (min 0–15) and postprandial temporal pattern of changes (min 15–195) are preceded by a baseline (min −60) measurement. Significant P values are given for Tukey's post hoc comparisons of overall meal effects and for Meal × Time interactions.

and insulin concentrations as well as the Tyr/LNAA ratio, changes in the decision time of choice reaction were negatively related to the Tyr/LNAA as well as positively to the Trp/LNAA ratio. In multiple regression analyses (Table 3), overall changes in the accuracy of short-term memory were related to overall changes in glucagon concentration and the GIR and to changes in the Trp/LNAA ratio. For central and peripheral attention, overall changes were related to overall changes in the insulin and glucagon concentration and/or the GIR, respectively. Overall changes in the central reaction time were related to overall changes in the Tyr/LNAA and to changes in the Trp/LNAA ratio. Overall changes in the decision time of choice reaction were related to overall changes in the GIR as well as the glucagon concentration. Pearson correlations (values in brackets) between overall cognitive changes and overall changes of single metabolic parameters revealed similar relations.

4. Discussion

Our data reveal significant overall meal effects as well as Meal × Time interactions for specific cognitive functions after the ingestion of meals with different carbohydrate to protein ratios in the morning. Overall cognitive performance was usually better after a balanced or protein-rich meal than after a high to medium glycemic carbohydrate-rich meal. In addition, changes in cognitive functions were related to postprandial changes in glucose metabolism, in the GIR, and in the LNAA ratios. All test meals were moderately liked by the subjects. As energy content, volume, acceptance, and sensory properties of our test meals were matched, the observed effects on metabolic and cognitive indices can be attributed to the ratio of carbohydrates to protein ingested. When interpreting cognitive effects after macronutrient ingestion, the energy content, sex, age and the

Table 2
Correlations of metabolic and cognitive changes between individual time points (area under the curve for 60-min intervals) for the combi test

	Accuracy in short-term memory	Central attention	Peripheral attention	Central reaction time	Decision time of choice reaction
Pearson correlation coefficient[a]					
GIR	− 0.06	− 0.15*	− 0.16 *	0.08	0.04
Glucose	0.22**	0.28***	− 0.06	− 0.16*	0.04
Insulin	0.25***	0.27***	0.03	− 0.22**	0.03
Glucagon	− 0.10	0.05	0.03	0.05	− 0.10
Trp/LNAA	− 0.11	− 0.24**	− 0.22* *	0.08	0.14*
Tyr/LNAA	0.06	0.08	− 0.02	− 0.22**	− 0.16*

[a] For repeated measurements: $n = 180$.
* Significance of the correlation coefficients: $P < .05$.
** Significance of the correlation coefficients: $P < .01$.
*** Significance of the correlation coefficients: $P < .001$.

constitution of the subjects as well as the time of day must be considered [16,67]. Our subjects were healthy young male students, and the test meals matched their habitual breakfast size. The subjects' glycogen stores were presumably not completely depleted because of the carbohydrate-rich meal on the evening before the test day. Since the GI of the evening meal was approximately medium, it may not have influenced the glucose tolerance of the subjects at the following morning significantly. Young healthy males seem to be less sensitive to nutritional variables than older people and females [49] or stress-prone individuals [26]. Therefore, the observed effects might be even more pronounced in vulnerable or malnourished [35] population groups. The 3.5-h postprandial interval was used to evaluate cognitive effects of the 1670-kJ meals assuming that digestion and absorption had almost ceased by then, and that the next meal or snack would be imminent.

4.1. Metabolic changes

The RER and the plasma urea concentrations indicate that the extent of carbohydrate and protein oxidation was positively related to their proportion in the test meals. The EE, however, was similar for all test meals. Therefore, nutrient effects on cognitive functions may be attributed to qualitative rather than quantitative differences in total substrate oxidation. The test meals caused metabolic changes within the usual physiological range. Consistent with the carbohydrate content and GI of our test meals, plasma glucose and insulin concentration as well as the GIR changed less after the PRO[1:4] and BAL[1:1] meals than after the CHO[4:1] meal suggesting a lower GI for the BAL[1:1] and particularly for the PRO[4:1] meal than for the CHO[1:4] meal. These differences were most pronounced in the first hour and disappeared in the third hour after test meal ingestion. Since the brain is very sensitive to changes in nutrient supply [31], even small metabolic changes can influence behavior [8]. Changes in plasma metabolite concentrations rather than absolute levels seem to be critical for the modulation of cognitive functions [2,29]. In addition, an 'inverted U'-shaped dose–response curve was reported for the effects of plasma glucose [19,33], epinephrine [4,18] and insulin concentration [5,20] on cognitive functions. The optimum of such a relation seems to be an individually variable range

Table 3
Statistics for most important metabolic changes of multiple stepwise regression for overall (area under the curve [0–210]) cognitive functions of the combi test

	Accuracy in short-term memory	Central attention	Peripheral attention	Central reaction time	Decision time of choice reaction
Multiple regression[a]					
GIR	*** [0.56]	*[− 0.37]	*** [− 0.37]	–	*[− 0.33]
Glucose	–	–	–	–	–
Insulin	–	*[− 0.35]	–	–	–
Glucagon	**	*	–	–	**[− 0.30]
Trp/LNAA	*[− 0.37]	–	–	*	–
Tyr/LNAA	–	–	–	**[− 0.29]	–
Multiple R	0.72	0.56	0.47	0.58	0.64
F statistics[b]	8.1***	5.0**	12.4***	6.9**	13.4***

[a] Quadratic overall changes of metabolic indices were the independent variables of the regression model. Variables were removed from the model if $P > .15$ for the respective regression coefficients. Values in brackets indicate Pearson correlations for simple regression.
[b] F statistic of ANOVA for the whole regression model.
* Significance of metabolic indices within the multiple regression model and of the F statistic: $P < .05$.
** Significance of metabolic indices within the multiple regression model and of the F statistic: $P < .01$.
*** Significance of metabolic indices within the multiple regression model and of the F statistic: $P < .001$.

[19,29,33] and is presumably close to the homeostatic concentration during the initial stage of fasting for some metabolites [16,36]. It is likely that the ascending or descending part of an 'inverted U' relation represents the acute positive and negative effects of a rise or fall [17,29,30] in blood glucose concentration on specific cognitive functions. For a longer time period, however, constant metabolic conditions [41] might optimize overall cognitive performance [8,16].

Meal-induced differences in the plasma Tyr/LNAA and Trp/LNAA ratio were generally most pronounced in the third hour after the PRO[1:4] and BAL[1:1] meals. Test meal composition may influence the Trp/LNAA ratio directly (through the meals' LNAA content) or indirectly (through postprandial changes in insulin and glucagon [15]). The decrease in the Trp/LNAA ratio by about 0.03 (i.e. 30%) after the PRO[1:4] meal and the absence of a change after the CHO[4:1] meal are in line with other findings in tests of similar amounts or ratios of carbohydrates and protein [1,7,26]. The failure of the CHO[4:1] meal to increase the Trp/LNAA ratio is in line with findings suggesting that as little as 5% protein in a meal are sufficient to block the postprandial rise in the Trp/LNAA ratio [52,57]. It is unclear how much exactly the Trp/LNAA ratio must change to influence brain serotonin synthesis, but the >20% decreases observed after the PRO[1:4] and BAL[1:1] meals may be sufficient [11]. Similarly, the postprandial increase in the Tyr/LNAA ratio after the BAL[1:1] meal may be sufficient to cause a change in brain catecholamine synthesis [56].

Glucagon and insulin are the primary counterregulating factors against moderate hypo- [40] and hyperglycemia [51], respectively, and their concentration is controlled by the carbohydrate and protein content of a diet [54]. In rat liver cells, GIR ratios $< 0.03-0.04$ were necessary for the stimulation of glycogen synthesis by insulin, and GIR ratios >0.1 showed a maximal glycogenolytic effect [27], whereas ratios >1.0 seemed to markedly decrease glycogenolysis [39]. Further, GIR ratios >0.2 appear to be necessary for stimulation of gluconeogenesis by glucagon [32]. This highlights the role of the GIR as an index for metabolic state and plasma glucose regulation [45,51]. Since cognitive functions change shortly after a prevailing metabolic state is disrupted and counterregulation begins [8,44], changes in the GIR may be closer related to changes in cognitive functions than changes in glucose concentration. The GIR also affects the plasma Trp/Tyr ratio via the effects of insulin and glucagon on plasma LNAA [15] and can be regarded as an early metabolic index for activation of the sympathetic nervous system, resulting in metabolic and thus potentially also in cognitive arousal [5,28]. Overall, the GIR in our study was higher after the BAL[1:1] and PRO[1:4] meals than after the CHO[4:1] meal. Assuming that GIR ratios are similarly effective for glucose regulation in man and rat, this suggests a more activated glycogenolysis and gluconeogenesis after the PRO[1:4] and BAL[1:1] than after the CHO[4:1] meal together with a higher Tyr/LNAA and lower Trp/LNAA ratio and thus potentially higher arousal.

4.2. Cognitive changes

4.2.1. Subjective state

Only few of the subjective ratings in our study were influenced by the macronutrient composition of the test meals. The higher overall ratings for 'can wait to eat' as well as for 'feeling sick' in the first hour after the PRO[1:4] meal compared with the two other meals may reflect the strong satiating effect of protein [37]. Protein ingestion is assumed to decrease the Trp/LNAA ratio and to facilitate catecholamine synthesis by precursor supply [56], resulting in a more alert and tense state [23]. However, a minimal change of delta 0.07 in the Trp/LNAA ratio was reported to be necessary to influence mood [13], and such a change was not reached in our study. All in all, the failure of the carbohydrate to protein ratio to affect depression, tiredness or other aspects of central fatigue in our study may therefore be related to the facts that the protein content in our CHO[4:1] meal was still too high [52,57], that the treatment difference in the Trp/LNAA was too small [13] and that young men were tested in the morning [24,38,49].

4.2.2. Objective performance

The effects of macronutrient ingestion on mood and subjective performance are not necessarily related to the effects on objective cognitive performance [16,34]. Carbohydrate as well as protein ingestion have been shown to influence reaction times. As in our previous study with pure macronutrient meals [16], we found no effect of the CHO[4:1] or PRO[4:1] meal on the simple peripheral reaction time of the combi test. A simple decision to react on a signal or not was presumably not sensitive enough to detect differences between our test meals. Others, however, reported slower reaction times for simple reaction after carbohydrate-rich meals containing less than 5% of protein [7,23]. The Meal × Time interaction in decision time of choice reaction between the CHO[4:1] and PRO[1:4] meals was similar to that of pure carbohydrate and protein ingestion [16]. It may be explained by the positive effect of a rise in plasma glucose [29] in the first hour after the test meals as well as by different synthesis or interactions of serotonin and catecholamines after the second hour. This last assumption is supported by the relation between decision time and changes in the plasma Trp/LNAA and Tyr/LNAA ratios. Fastest overall reaction times for the demanding central reaction of the combi test after the BAL[1:1] meal were related to the highest overall Tyr/LNAA but not to the lowest overall Trp/LNAA ratio. Tyr administration was consistently found to affect motor performance [22], potentially by influencing dopamine [3] and/or norepinephrine [21] synthesis, whereas Trp application only influenced mood states. Thus, the positive effect of the BAL[1:1] meal on overall central reaction times may also have resulted from a combined effect of simultaneous carbohydrate and protein ingestion.

Central attention was improved shortly after the CHO[4:1] meal and later on after the BAL[1:1] and

PRO[1:4] meals. These effects might be explained by the accompanying changes in glucose metabolism, in the GIR, and in the LNAA ratio, and/or by subsequent changes in the synthesis of serotonin or catecholamines [43]. Scores for the competing peripheral attention task appeared to show an inverse temporal profile, consistent with a trade-off between central and peripheral performance [16,47]. Together, the reported interactions suggest a time-dependent effect of carbohydrate and protein ingestion on objective cognitive performance and emphasize the importance of repeated measurements (at least for 3–4 h) to assess acute effects of macronutrient ingestion on cognitive functions.

The highest accuracy in short-term memory after the PRO[1:4] meal, concomitant with the least variation in glucose metabolism and in the GIR, was in line with previous findings after pure fat ingestion [16]. The high GIR after the PRO[1:4] meal suggests a high state of metabolic activation and glucose production by liver glycogenolysis and gluconeogenesis. Glucose seems to enhance memory (for review see Ref. [11]) specifically by cholinergic mechanisms [10], with an optimum effect around a dose of 25 g carbohydrates [33], which is similar to the carbohydrate content of our PRO[1:4] meal. The moderate increase in the Tyr/LNAA ratio and decrease in the Trp/LNAA ratio may also have influenced short-term memory after the PRO[1:4] meal [9,50,53].

The failure of the multitask test to reveal significant meal effects may be related to the complexity of this test. Within-subjects variability for individual functions was too large to reliably detect treatment-induced differences. Thus, while less demanding tasks such as simple reaction times seem to be less sensitive in detecting effects of macronutrient ingestion, multitasking may confound cognitive effects. This indicates that in addition to the type of cognitive function, the complexity and the demand of a cognitive test is crucial to detect effects of macronutrient ingestion.

4.3. Relation between metabolic and cognitive changes

The weak correlations between metabolic and cognitive changes at individual time points were presumably due to the fact that the cognitive effects occurred later than the underlying metabolic changes. The relatively higher *R* values for overall effects in the multiple regression may therefore be explained by the elimination of such time differences and/or by a better description of cognitive functions by a set of influencing factors. Even then, however, the *R* values were not very high, which suggests that in a theoretical regression model synergism and antagonism of influencing factors [50] are not considered and/or that further influencing factors are missing in the model.

Regarding 60-min changes, plasma glucose and insulin concentrations as well as the LNAA ratios seem to be linearly related to cognitive functions, potentially as a small section of an 'inverted U' relation. For overall 210-min changes in short-term memory, attention as well as decision time, the GIR, as a summarizing index for glucose regulation and potentially also for changes in the LNAA, seems to be quadratically related to cognitive functions. This also suggest an 'inverted U'-shaped relation. For the central reaction time, however, overall changes were only related to changes in the LNAA, suggesting that for a 210-min postprandial period changes in neurotransmitter synthesis are more crucial than changes in glucose metabolism.

5. Conclusion

In summary, in healthy young men the ingestion of different carbohydrate to protein ratios in the morning specifically influenced cognitive functions. Except for a short transient positive effect of the CHO[4:1] meal on attention, the BAL[1:1] and PRO[1:4] meals resulted in better overall cognitive performance. Overall changes in several cognitive functions were related to the GIR. Our findings suggest that overall postprandial cognitive performance in the morning is better after a balanced or protein-rich meal than after a high to medium glycemic carbohydrate-rich meal, presumably because of less variation in glucose metabolism and/or higher metabolic activation and modulation in neurotransmitter synthesis. It remains to be investigated, whether this also holds for a low GI carbohydrate-rich meal and whether the effects are different for more vulnerable population groups or other daytimes.

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