Clinical Nutrition xxx (2011) 1–9

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Effect of a high protein meat diet on muscle and cognitive functions: A randomised controlled dietary intervention trial in healthy men☆

Lene H. Jakobsen [a,b,*], Jens Kondrup [a,b], Maria Zellner [c], Inge Tetens [d], Erich Roth [c]

[a] Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, Denmark
[b] Clinical Nutritional Unit, Rigshospitalet, Denmark
[c] Surgical Research, Medical University Vienna, Austria
[d] Department of Nutrition, National Food Institute, Technical University of Denmark, Denmark

ARTICLE INFO

Article history:
Received 16 July 2010
Accepted 21 December 2010

Keywords:
Protein
Muscle function
Cognitive function
Plasma amino acids

SUMMARY

Background: Recommendations to use other criteria than N-balance for defining protein requirements have been proposed. However, little evidence to support other measures such as physiological functions is available.
Objective: To investigate the effects of a usual (UP) *versus* a high protein (HP) diet on muscle function, cognitive function, quality of life and biochemical regulators of protein metabolism.
Design: A randomised intervention study was conducted with 23 healthy males (aged 19–31 yrs). All subjects consumed a Usual Protein (UP) diet (1.5 g protein/kg BW) for a 1-wk run-in period before the intervention period where they were assigned to either a UP or a High Protein (HP) diet (3.0 g protein/kg BW) for 3-wks with controlled intake of food and beverages. Blood and urine samples were taken along with measurements of physiological functions at baseline and at the end of the intervention period.
Results: The HP group improved their reaction time significantly compared with the UP group. Branched chain amino acids and phenylalanine in plasma were significantly increased following the HP diet, which may explain the improved reaction time.
Conclusion: Healthy young males fed a HP diet improved reaction time. No adverse effects of the HP diet were observed.
This trial was registered at www.clinicaltrials.govas NCT00621231.

1. Introduction

The current WHO/FAO/UNU recommendations for protein intake are based on studies of nitrogen (N) balance.[1] However, the authors recommended that future protein requirements should be based on criteria related to "long-term health and well being" rather than N-balance.

An increasing number of studies suggests a positive role of protein in promoting optimal health at intakes beyond the Dietary Reference Intakes (DRI), e.g. in relation to weight management and satiety, sarcopenia, glucose homeostasis, and bone health.[2] There is limited evidence from intervention studies on the effects of an increased protein intake on performance in physical and mental tests related to quality of life in healthy subjects. A few studies have investigated the effects of different protein intakes on physiological functions.[3,4] One study included healthy elderly women and demonstrated significant losses in immune response and muscle function after a 9-wk period of marginal protein intake (0.45 g protein/kg BW) in comparison to an adequate protein intake (0.92 g protein/kg BW).[3] It was not investigated whether increased dietary protein intake (above 0.92 g protein/kg BW per day) could further improve physiological functions. Another study investigated the effect of different carbohydrate to protein ratios on cognitive function, in which they found improved reaction time after a high protein meal compared to a high carbohydrate meal.[4] The possible effects of a prolonged dietary intake of protein rich foods on these physiological functions have not been investigated in healthy young males, commonly used as a reference population. The purpose of our study was to investigate the effect of a high protein (HP) intake compared to a usual protein (UP) intake on physiological functions, including muscle function, cognitive function including reaction time, and quality of life. The usual protein intake in Denmark and US are 1.5 g

☆ Parts of the work were presented at the ESPEN 2008 congress in Florence, Italy (P232) and at the ESPEN 2009 congress in Vienna, Austria (P086).

* Corresponding author. Clinical Nutritional Unit, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark. Tel.: +45 35 45 81 47; fax: +45 35 45 25 53.
E-mail address: lene.holm@rh.regionh.dk (L.H. Jakobsen).

0261-5614/$ – see front matter © 2010 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
doi:10.1016/j.clnu.2010.12.010

Abbreviations

AA	amino acids
ACE	addenbrooke cognitive examination
BCAA	branched chain amino acids
BIA	bioelectrical impedance analysis
BW	body weight
DRI	dietary reference intakes
E	errors
FAO	Food and Agriculture Organization
GLM	general linear models
HGE	handgrip endurance
HGS	handgrip strength
HGW	handgrip work
HP	high protein
MCS	mental component summary
ms	milliseconds
N	nitrogen
PABA	para amino benzoic acid
PCS	physical component summary
QoL	quality of life
REE	resting energy expenditure
TAP	testbattery for attentional performance
TUG	timed up and go test
UNU	United Nations University
UP	usual protein
WHO	World Health Organization

per kg BW.[5,6] In addition, N losses were evaluated to demonstrate adherence to the protocol and to relate our results to earlier investigations. Blood concentrations of hormones and substrates related to protein turnover were measured to elucidate the mechanisms underlying possible physiological effects. Resting energy expenditure, physical activity and body composition were measured or recorded to evaluate possible changes in energy balance and nutritional status, with the aim of maintaining body weight stability during the study. The diets were composed primarily of animal protein to ensure a high-quality protein intake.

2. Subjects and methods

2.1. Study protocol

The study was a randomised, single blinded, parallel intervention study with virtually complete dietary control, conducted over 3 periods: a pre-study assessment (3-d), a run-in (1-wk), and an intervention period (3-wk). (See Fig. 1).

Subjects were recruited by advertisement via a website at the University of Copenhagen. Only subjects (aged 20–40 yrs) judged to be in good health based on a physical examination at screening were included. Exclusion criteria were smoking, overweight (BMI $\geq$ 25), use of any medication, heavy physical exercise (strenuous physical exercise $>$ 4-h/wk), presence of any chronic disease, and illness or impairment of shoulder, arm and/or hand. All subjects enrolled in the study had stable weights at inclusion and were instructed to remain as such throughout the study period. In addition, the subjects were instructed not to change their habitual physical activity levels.

Our power calculation (See Statistics) was based on the HGS measurements in the study mentioned above.[3] To assure comparable baseline values for HGS, subjects were stratified according to HGS. HGS was measured at the screening session and the overall mean value was used as a stratification criterion.

At the pre-study assessment, subjects were asked to keep a 3-day weighed food record of their spontaneous intake. This was validated by analysis of 3 consecutive 24-h urine collections. After the pre-study assessment, an estimated energy requirement was calculated as the average of the dietary recordings and calculated energy requirements (Harris–Benedict equation $\times$ 1.75[7]). The dietary recordings were also used to control for extreme food habits and to confirm that they consumed a diet with usual protein content.

The subjects were randomised at a meeting 1 week before run-in, where they picked a sealed, opaque envelope containing a piece of paper with the word “Red” or “Blue” corresponding to a control and an intervention group. The randomization envelopes were divided into 2 piles differentiating the subjects that were weaker or stronger than the overall mean HGS. The assigned randomization codes were kept by a member of the kitchen staff, who had to know the randomization codes for practical reasons. The colour code was not revealed to the researchers until the study was completed and the results were analyzed statistically.

The study was approved by the research ethical committee of the Capital region of Denmark (Accept number: H-A-2007-0030). All subjects signed a written informed consent.

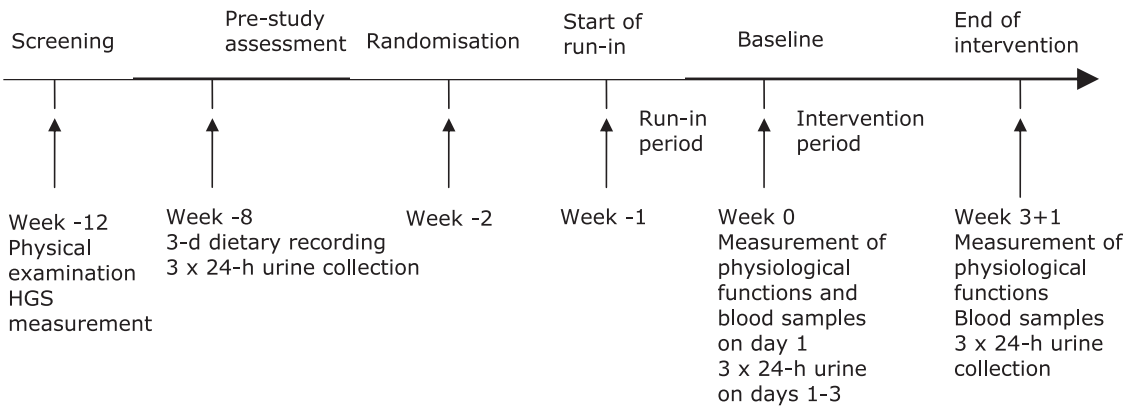

Fig. 1. Time flow of the study. Physiological variables and fasting blood samples were measured/taken at baseline and at the end of the intervention period (Week 3 + 1)in the morning, 12 h after the last meal containing an average of 26 and 79 g protein in the UP and HP group, respectively. Three $\times$ 24-h urine were collected at the pre-study assessment, at baseline and at the end of the intervention period.

Please cite this article in press as: Jakobsen LH, et al., Effect of a high protein meat diet on muscle and cognitive functions: A randomised controlled dietary intervention trial in healthy men, Clinical Nutrition (2011), doi:10.1016/j.clnu.2010.12.010

2.2. Diet

The subjects started the run-in period consuming a diet providing their individually estimated energy requirements. The amount of energy provided was adjusted according to BW changes by incrementally adding or subtracting 0.5 or 1.0 MJ/d. During the 1-wk run-in period, all subjects were kept on a diet with the usual protein content (approx. 1.5 g protein/kg BW).[5,6] Immediately after the run-in period, the subjects were assigned to one of two diets with different amounts of protein according to the randomization (Tables 2 and 3). The control group continued on the UP diet (approx. 1.5 g protein/kg BW) and the intervention group received a HP diet (approx. 3.0 g protein/kg BW) during the 3-wk intervention period. Isoenergetic replacement of carbohydrate with protein was achieved by replacing pasta, rice, bread and juice of the UP diet with an equivalent amount of animal protein (low fat beef, pork, chicken, milk, and cheese) in the HP diet. This replacement also led to significant differences in the intakes of vitamin D, B_2, B_6, B_{12}, and dietary fiber (Tables 2 and 3).

During both the run-in and intervention periods, subjects received all food and beverages from the department and were instructed not to consume anything else but water and salt. On weekdays, lunch was consumed in the department under supervision, whereas beverages, supper, snacks, and breakfast for the next morning were provided daily as a package with instructions for preparation and consumption. Food and beverages for the weekend were provided on Fridays. Leftovers were brought back to the department for weighing and recording. All nutrients were estimated using a national database (Dankost3000; National Food Agency).

2.3. Anthropometric measurements

All physiological functions and blood samples were measured in the morning at baseline (week 0) and in the morning after the final day (week 3 + 1) of the intervention (Fig. 1). The study was blinded to the investigators.

BW was measured twice a week on an electronic scale to the nearest 0.1 kg with light clothing and no shoes. Weight stability was defined as being within 1 kg from baseline BW. At baseline and at the end of intervention, the subjects were weighed in the morning after voiding and an overnight fast, whereas during the run-in and the intervention period the subjects were weighed before lunch. Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer while the subjects were barefooted. BMI was calculated as BW (kg)/height^2 (m^2).

2.4. Muscle function

HGS was measured in the right hand with a Jamar 5030J1 Hydraulic Hand Dynamometer (Sammons Preston Rolyan, Bolingbrook, Illinois, USA) as described in Jakobsen et al.[8]. Handgrip endurance (HGE) and handgrip work (HGW) were measured by an ElectroFluidGraph (Akern, Florence, Italy) and mobility was measured by the Timed Up and Go test (TUG) as previously described by Jakobsen et al.[8] For the HGE test, subjects were instructed to press a handle at 65–75% of their maximal force, for as long as possible. HGW is a measure of the total work produced during the HGE test (HGW = 70% HGS × seconds), calculated as the area under the curve of handgrip force against time.

2.5. Cognitive function

Cognitive function was evaluated using a modified version of the Addenbrooke Cognitive Examination (ACE), which consists of 6 components (orientation, attention/concentration, verbal fluency, memory, language and visuospatial function) of which orientation, verbal fluency, and language have been shown to be related to the reaction time tests applied in this study.[9] Reaction time was investigated using 3 tests with varying degrees of complexity (Intrinsic/Phasic Alertness, Go/No-Go and Sustained Attention) from the Testbattery for Attentional Performance (TAP), (TAP 2.1, PsyTest, Herzoganrath, Germany). The tests were conducted with the participant sitting in front of a computer screen, responding to visual stimuli by pressing a button connected to the computer with the forefinger of their dominant hand. Alertness analyses a subject's ability to react and maintain a high level of attention in anticipation of a visual stimulus without or with a preceding auditory warning stimulus, which is referred to as Intrinsic and Phasic Alertness, respectively. This test mainly reflects nerve conductance or reactivity.[10] Go/No-Go is a 3 min test, which analyzes the subject's ability to react to some complex patterns and to withhold a response to other rather similar complex patterns. It requires central analysis with suppression of undesired responses, i.e. a cognitive effort, in addition to reactivity. Sustained Attention is also a pattern recognition test, but the patterns are simpler to recognize and the test lasts considerably longer (15 min). This test analyzes sustained attention in addition to (less demanding) central analysis with suppression of undesired responses and reactivity.

2.6. Bioelectrical impedance analysis

Bioelectrical Impedance Analysis (BIA) was performed to investigate possible changes in body composition using the ElectroFluidGraph, which applies alternating electric currents of 300 mA at 50 kHz. The ElectroFluidGraph measures impedance and phase angle, from which resistance and reactance are calculate together with lean body mass and fat mass.

2.7. Energy expenditure

Resting Energy Expenditure (REE) was measured to assist in maintaining weight stability. REE was assessed by indirect calorimetry using a ventilated hood system as previously described by Raben et al.[11] In addition, habitual physical activity was assessed by the Bouchard activity diary as described in[8] to evaluate whether the subjects remained at their usual physical activity level.

2.8. Quality of life

QoL was assessed by the Short-Form Health Survey (SF-36)[12] consisting of 36 questions grouped into 8 multi-item scales: physical function, perception of physical role, vitality, general health, mental health, perception of emotional role, social function and bodily pain.[12] The standard American scoring algorithms, as described by Ware et al.[13] were used. The scales of the SF-36 are summarized into two dimensions: Physical Component Summary (PCS) and Mental Component Summary (MCS). The SF-36 has been shown to be valid in discriminating between physical and mental health status in both cross-sectional and longitudinal tests[13] and it has been validated in a healthy Danish population.[14]

2.9. Analysis of blood samples

Fasting blood samples were taken at baseline and at the end of intervention period in the morning, 12 h after the last meal containing an average of 26 and 79 g protein in the UP and HP group, respectively. See Fig. 1. Blood samples were centrifuged immediately at 2800 RPM for 15 min at 4 °C and the plasma was stored at −20 °C

Please cite this article in press as: Jakobsen LH, et al., Effect of a high protein meat diet on muscle and cognitive functions: A randomised controlled dietary intervention trial in healthy men, Clinical Nutrition (2011), doi:10.1016/j.clnu.2010.12.010

until subsequent analysis. Bicarbonate, sodium, potassium, chloride, lactate, glucose and amino acids (AA) were analyzed in plasma, whereas, hemoglobin, alkaline phosphatase, creatinine, TSH, T_3, T_4, IGFBP-3, IGF-1, GH, insulin and urea were analyzed in serum. The concentrations of glucose, urea, creatinine, and alkaline phosphatase were analyzed in an ABX Pentra 400, Chemistry Analyzer (Horiba ABX, Montpellier, France). Blood hemoglobin was analyzed by a hematology analyzer (Sysmex, model KX-21, New Jersey, USA). Blood samples for analysis of bicarbonate, lactate, sodium, potassium and chloride were analyzed by a blood gas analyzer (Radiometer ABL 800 Flex, Copenhagen, Denmark). Blood samples were collected for analysis of TSH, T_3, T_4, GH, IGF-1, IGF-1BP, insulin, urea, creatinine and alkaline phosphatase. The hormone concentrations were analyzed by a solid-phase, two-site chemiluminescent immunometric assay in an Immulite 1000 (Siemens, LA, USA). Creatinine clearance was calculated from creatinine in plasma, 24-h urinary creatinine and urine volume. Blood samples for analysis of AA were centrifuged at 2800 g for 10 min at 4 °C. The resulting plasma was transferred to a new tube and centrifuged a second time at 10,000 g for 10 min at 4 °C. Finally, the resulting plasma was transferred to another tube and stored at −80 °C until analysis. AA were analyzed using an automatic precolumn derivatization with ophthalaldehyde and high pressure liquid chromatography separation as in.[15]

2.10. Urine collections

Subjects collected 3 consecutive 24-h urine samples at 3 separate occasions during the study. See Fig. 1. The bladder was emptied immediately prior to the start of each subsequent 24-h collection period. Urine was collected in pre-weighed containers. The subjects were asked to store the containers in a cool, dark place. The volume and density of each 24-h urine collection were determined and samples were frozen at −20 °C until further analysis. During the urine collections, subjects were instructed to consume Para Amino Benzoic Acid (PABA) tablets 3 times a day (240 mg/d), to verify completeness of the 24-h urine collections.[16] Completeness of urine collections was taken as mean PABA recovery ≥90%.[16] Statistical analyses were done with and without incomplete collections.

Samples were taken for analysis of PABA, albumin, creatinine, urea, and N. Urine samples were analyzed for PABA using a spectrophotometer (Stasar, Gilford Instruments Laboratories Inc., Oberlin, OH, USA). The urine content of albumin, creatinine, and urea was measured with the ABX Pentra 400. The urine content of N was determined using an Elementar Vario Max CN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Urinary N losses were expressed as protein equivalents ($protein_{eq}$): $Protein_{eq} = N \times 6.25$ (g protein/kg BW per d) to facilitate comparison with protein intake. N-balance was calculated according to the formula:

$$\text{Balance}\left(\text{protein}_{eq}\right) = \text{Protein intake} - 6.25 \times (\text{Urinary N} + \text{Faecal N} + \text{Miscellaneous N})$$

Urinary N was calculated from the measured N loss. Faecal N loss was calculated as described by Price et al.[17]:

$$\text{Faecal N} = \text{Endogenous faecal N excretion} + \text{N intake} \times (1 - \text{True digestibility})$$

Endogenous faecal excretion was taken to be 0.075 g $protein_{eq}$/kg BW per d[17] and true digestibility was taken to be 0.96 corresponding to a western mixed diet.[17] Miscellaneous N loss was taken to be 0.05 g $protein_{eq}$/kg BW per d.[18]

3. Statistics

Results are reported according to the CONSORT guideline.[19] All data are presented as mean ± SD. Student's paired *t*-test was used to assess changes within groups. The student's *t*-test for unpaired data was used for comparison between groups. General Linear Models (GLM) were used to assess end of intervention between-group differences adjusted for baseline values.[20] In the GLM models, assumptions were checked by testing the residuals for normal distribution and skewed data were transformed by log10, which produced normally distributed residuals.

An acceptable level of statistical significance was established at $p < 0.05$. Area under the curve was calculated using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California, USA. Statistical analysis was carried out using the software package SAS System for Windows Version 9.1.

The power calculation was based on the study mentioned previously.[3] They observed a significant increase in HGS of 3.7 ± 3.2 kg (mean ± SD) in the group receiving adequate protein. To obtain statistical significance of the same increase in HGS in the HP group, considering the higher initial values, with a power of 80% and a type I error of 5%, twelve subjects were considered necessary per study arm. Based on this, we aimed to recruit 26 subjects into the study, which allowed for a few dropouts. Castaneda et al.[3] obtained their results after a 9-wk period, but we chose a 3-wk duration for practical reasons. Power was based on this study only since no long-term nutritional studies with reaction time or cognitive function were available when we planned the study.

4. Results

4.1. Baseline characteristics

The number of persons screened, excluded, randomly assigned, and withdrawn from the study are presented in Fig. 2. Of the 26 subjects randomised, twenty-three completed the study. Consequently, the results shown are for 12 subjects in the control group (UP) and 11 subjects in the intervention group (HP). Characteristics of the 23 subjects who completed the study are summarized in Table 1. There were no obvious differences in anthropometry or energy requirements (Table 1), or in HGS (Table 4), or educational level (mean educational level was a bachelor degree) between the two groups indicating that the groups were comparable at baseline. There were only minor deviations from the estimated energy requirements during the run-in and intervention period.

4.2. Outcome measures

Outcome changes from baseline and differences between the groups are presented in Table 4. The subjects in the two groups were weight stable within 1 kg with no changes in reported physical activity ((estimated as Physical Activity Level): $UP_{baseline}$: 1.74 (1.58−1.83). UP_{end}: 1.62 (1.54−1.90); $p = 0.58$. $HP_{baseline}$: 1.77 (1.59−1.98) HP_{end}: 1.75 (1.54−1.83); $p = 0.39$.). There were no significant changes in the BIA variables (Data not shown). By the end of study, the UP group had significantly decreased their BW and increased their HGW and ACE scores including the total score and the verbal fluency component. The HP group had significantly increased their HGE, HGW and ACE scores including the total score, orientation and verbal fluency components. In addition, they had a decreased reaction time and a decrease in the number of errors in the Go/No-Go test. Mean changes in the ACE component "orientation", reaction time and number of errors in the Go/No-Go test were significantly larger in the HP group than in the UP group. The significant difference in reaction time in the Go/No-Go (ms) test

Please cite this article in press as: Jakobsen LH, et al., Effect of a high protein meat diet on muscle and cognitive functions: A randomised controlled dietary intervention trial in healthy men, Clinical Nutrition (2011), doi:10.1016/j.clnu.2010.12.010

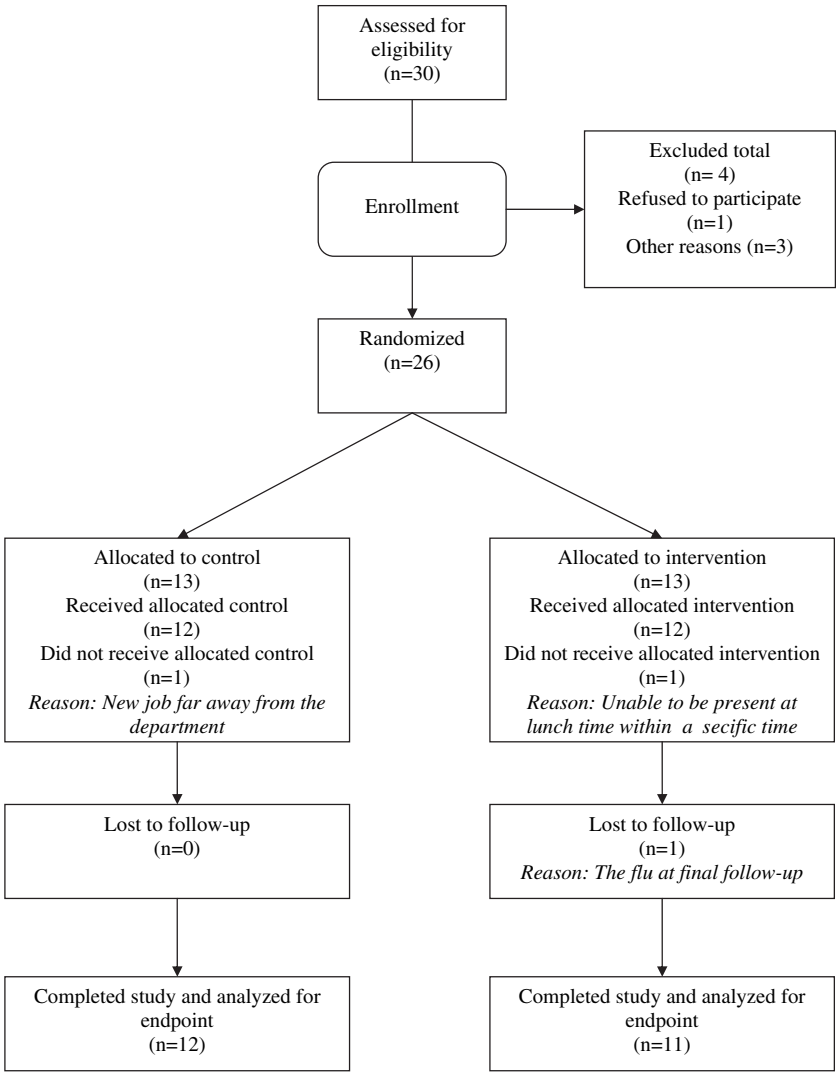

Fig. 2. Subject flow chart.

between the groups persisted after adjusting for baseline value by GLM ($p = 0.040$) (Fig. 3). The baseline Go/No-Go (ms) test in the HP group was insignificantly higher than that of the UP group (Table 4). To rule out a regression-towards-the-mean error, data were re-analyzed by excluding the 2 subjects in the HG group with the highest baseline values, resulting in an average baseline reaction time of 459.9 ms in the HP group. The significant difference between baseline to end values of the groups persisted ($p = 0.031$).

Table 1
Subject characteristics (pre-study assessment).

	Usual Protein ($n = 12$)	High Protein ($n = 11$)
Age (years)	23.7 ± 3.5	24.7 ± 3.6[c]
Height (m)	1.82 ± 0.06	1.84 ± 0.06[c]
Weight (kg)	73.6 ± 8.2	75.9 ± 4.9[c]
BMI (kg/m^2)	22.2 ± 1.7	22.4 ± 1.1[c]
Energy intake (3-d food record) (MJ)	11.9 ± 1.8	12.9 ± 2.5[c]
Estimated energy requirement[a] (MJ)	12.5 ± 1.2	13.2 ± 1.3[c]
Protein intake (3-d food record) (g/kg BW per d)	1.5 ± 0.3	1.7 ± 0.2[c]
Protein$_{eq}$ loss calculated from N losses[b] (g/kg BW per d)	1.4 ± 0.1	1.4 ± 0.2[c]

Data are presented as mean ± SD. Differences between groups assessed by Student's unpaired *t*-test.

[a] Energy requirements were estimated from the average of the mean of 3-day weighed food record and calculated energy requirements (Harris–Benedict equation × 1.75, see text).

[b] Protein$_{eq}$ = N losses calculated from 6.25 × (Urinary N + Faecal N + Miscellaneous N). See text.

[c] No significant difference between groups.

4.3. Blood and urine analyses

There was no significant change between groups in hemoglobin, alkaline phosphatase, creatinine, TSH, T_3, T_4, IGFBP-3, IGF-1, GH, lactate, insulin, glucose, bicarbonate, sodium, potassium, chloride and/or albuminuria.

Three out of 69 (3 × 23) urine collections had a mean PABA recovery <90% and since the results were not different with these samples excluded, these collections were included in the results. The

Table 2
Composition of protein foods (% of total protein intake).

	Usual protein	High protein
Fish	4	5
Dairy products	16	25
Meat	35	55
Egg	7	3
Vegetable	40	13
Total	100	100

Please cite this article in press as: Jakobsen LH, et al., Effect of a high protein meat diet on muscle and cognitive functions: A randomised controlled dietary intervention trial in healthy men, Clinical Nutrition (2011), doi:10.1016/j.clnu.2010.12.010

Table 3
Dietary intake.

	Usual Protein	High protein	*P*
Energy intake (MJ)	12.4 ± 1.2	13.1 ± 1.3	n.s.
Protein (g)	109.6 ± 0.7	230.6 ± 0.8	***
Carbohydrate (g)	427.9 ± 2.3	331.4 ± 1.7	***
Fat (g)	97.1 ± 0.5	102.7 ± 0.7	n.s.
Vitamin A (RE)	835.9 ± 302.0	958.5 ± 371.9	n.s.
Vitamin D (μg)	2.2 ± 0.1	4.1 ± 0.7	**
Vitamin E (αTE)	9.7 ± 1.7	9.3 ± 1.8	n.s.
Vitamin K (μg)	79.2 ± 61.5	86.6 ± 53.8	n.s.
Vitamin B1 (mg)	1.9 ± 0.5	3.1 ± 1.1	n.s.
Vitamin B2 (mg)	1.6 ± 0.3	2.8 ± 0.3	**
Vitamin B6 (mg)	2.1 ± 0.4	3.2 ± 0.4	**
Folacin (μg)	399.5 ± 80.7	387.8 ± 26.0	n.s.
Vitamin B12 (μg)	4.4 ± 1.4	11.3 ± 1.5	***
Vitamin C (mg)	220.3 ± 64.0	144.2 ± 74.6	n.s.
Sodium (mg)	2800 ± 551	2922 ± 596	n.s.
Potassium (mg)	3103 ± 414	4085.0 ± 537	*
Iron (mg)	12.2 ± 2.5	14.5 ± 3.3	n.s.
Zinc (mg)	13.4 ± 0.6	27.0 ± 2.5	***
Iodine (mg)	138.3 ± 15.7	192.3 ± 17.2	**
Selenium (μg)	44.8 ± 6.1	84.0 ± 17.8	**
Dietary Fiber (g)	26.1 ± 4.5	18.3 ± 3.5	*
Saturated Fatty Acids (g)	30.1 ± 2.5	29.6 ± 4.2	n.s.
Monounsaturated Fatty Acids (g)	27.3 ± 1.1	27.7 ± 1.5	n.s.
Polyunsaturated Fatty Acids (g)	11.9 ± 2.0	11.9 ± 2.2	n.s.
EPA[a] + DHA[b] (g)	11.2 ± 2.2	11.4 ± 2.4	n.s.
C22:6 n-3 (g)	0.08 ± 0.1	0.05 ± 0.06	n.s.
Cholesterol (g)	511.0 ± 278.9	693.0 ± 252.9	n.s.

Data are presented as mean ± SD. Student's *t*-test for unpaired data was used for comparison between groups $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
[a] Eicosapentaenoic acid.
[b] Docosahexaenoic acid.

HP group had significantly higher values of N losses ($protein_{eq}$) at end of intervention compared to the UP group (UP_{end}: 1.3 ± 0.2 g/kg BW per day, HP_{end}: 2.6 ± 0.2 g/kg BW per day; $p < 0.001$). The subjects in the two groups were in positive protein balance throughout the study. Creatinine clearance and 24-h urinary albumin did not change during the study (Data not shown).

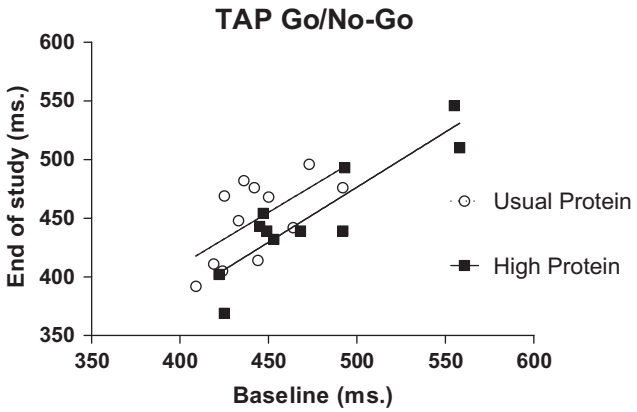

Fig. 3. Effect of diet on reaction time. Significant effect ($p = 0.040$) of diet (1.5 *versus* 3.0 g protein/kg BW) after adjusting for baseline value by a General Linear Model analysis. Estimated difference between HP and UP diet was −25.5 ± 11.6 ms; 95% CI: −49.7;−1.2 ms.

4.4. Amino acids

Plasma AA changes from baseline and differences between groups are presented in Table 5. At the end of the intervention, the UP group had significantly increased concentrations of aspartate, glutamate, methionine and tryptophane and a decreased concentration of histidine. The HP group had significantly increased concentrations of valine, tryptophane, isoleucine, phenylalanine, and branched chain AA (BCAA), and decreased concentrations of glutamine and glycine compared to baseline. Between groups mean changes in glutamine, taurine, valine, phenylalanine, and BCAA were significantly larger in the HP group than in the UP group. The significant differences in valine and BCAA persisted between the groups after adjustment for baseline values by GLM ($p = 0.0002$ and $p = 0.013$, respectively) (Data not shown).

Table 4
Changes in physiologic functions during the intervention period.

	Usual Protein ($n = 12$)		High Protein ($n = 11$)		Usual Protein Difference	High Protein Difference	*P*[a]
	Baseline	End	Baseline	End			
Weight (kg)	73.5 ± 9.0	72.8 ± 8.8**	75.5 ± 5.2	75.5 ± 5.0	−0.7 ± 0.7	0.1 ± 0.4	0.006
BMI (kg/m^2)	22.1 ± 1.8	21.9 ± 1.7**	22.3 ± 1.2	22.3 ± 1.2	−0.2 ± 0.2	0.0 ± 0.1	0.006
HGS Right (kg)	50.1 ± 8.2	51.8 ± 8.9	54.1 ± 5.8	53.8 ± 7.3	1.7 ± 3.6	−0.3 ± 4.5	n.s.
HGE (s)	47.9 ± 18.6	54.7 ± 23.8	53.7 ± 13.4	61.3 ± 11.5*	6.7 ± 14.0	7.6 ± 10.2	n.s.
HGW (sec × force) x10^{-3}	26.0 ± 14.0	30.89 ± 15.8*	32.0 ± 11.1	36.5 ± 9.1*	4.4 ± 6.8	4.5 ± 6.3	n.s.
Timed Up and Go (s)	5.8 ± 0.5	5.6 ± 0.5	5.4 ± 1.0	5.6 ± 0.4	−0.2 ± 0.6	0.2 ± 1.0	n.s.
Intrinsic Alertness (ms)	229.2 ± 18.7	230.2 ± 12.4	230.6 ± 19.2	232.0 ± 20.5	0.9 ± 16.3	1.4 ± 12.9	n.s.
Intrinsic Alertness (E)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	−0.1 ± 0.3	n.s.
Phasic Alertness (ms)	231.0 ± 21.1	231.4 ± 14.8	233.5 ± 22.1	234.2 ± 24.3	0.4 ± 16.2	0.7 ± 20.6	n.s.
Phasic Alertness (E)	0.5 ± 0.7	0.3 ± 0.7	0.8 ± 1.1	0.5 ± 0.7	−0.2 ± 0.7	−0.4 ± 1.0	n.s.
Go/No-Go (ms)	442.6 ± 24.0	448.3 ± 34.9	473.4 ± 47.0	451.5 ± 49.3**	5.7 ± 27.4	−21.9 ± 22.1	0.015[b]
Go/No-Go (E)	0.2 ± 0.4	0.4 ± 0.8	1.0 ± 0.6	0.5 ± 0.5*	0.3 ± 0.9	−0.5 ± 0.5	0.030
Sustained Attention (ms)	419.1 ± 47.6	407.3 ± 43.4	419.7 ± 35.4	416.8 ± 40.4	−11.8 ± 22.9	−2.9 ± 21.5	n.s.
Sustained Attention (E)	3.3 ± 3.9	4.1 ± 4.0	5.1 ± 3.8	2.5 ± 1.8	0.8 ± 3.3	−2.5 ± 4.6	n.s.
ACE (total score)	79.6 ± 5.6	85.6 ± 2.5**	79.0 ± 3.9	85.6 ± 2.6***	6.0 ± 5.0	6.6 ± 2.9	n.s.
Orientation (score)	9.9 ± 0.3	9.9 ± 0.3	9.5 ± 0.5	10.0 ± 0.0*	0.0 ± 0.4	0.5 ± 0.5	0.035
Verbal fluency (score)	13.5 ± 1.7	15.1 ± 1.1**	13.5 ± 1.4	14.4 ± 1.8*	1.6 ± 1.4	0.9 ± 1.1	n.s.
Language (score)	12.8 ± 0.4	12.9 ± 0.3	12.7 ± 0.5	12.9 ± 0.3	0.1 ± 0.5	0.2 ± 0.6	n.s.
REE (kcal/min)	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	−0.0 ± 0.0	−0.0 ± 0.1	n.s.
QoL PCS (score)	56.9 ± 5.0	56.4 ± 4.2	57.2 ± 2.9	57.5 ± 2.6	−0.6 ± 5.2	0.2 ± 1.8	n.s.
QoL MCS (score)	52.2 ± 7.5	53.5 ± 5.2	55.4 ± 2.8	56.2 ± 2.1	1.2 ± 5.2	0.9 ± 2.4	n.s.

Data are presented as mean ± SD. Abbreviations: BMI = Body Mass Index. Ms = milliseconds. E = Number of Errors. HGS = Handgrip Strength. HGE = Handgrip Endurance. HGW = Handgrip Work. ACE = Addenbrooke Cognitive Examination. REE = Resting Energy Expenditure (kcal/min per subject). QoL = Quality of Life. PCS = Physical Component Summary. MCS = Mental Component Summary. Student's *t*-test for paired data was used for comparison within groups $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
[a] Student's *t*-test for unpaired data was used for comparison between groups.
[b] The significant difference in reaction time in the Go/No-Go test between the groups persisted after adjusting for baseline value by GLM ($p = 0.040$).

Please cite this article in press as: Jakobsen LH, et al., Effect of a high protein meat diet on muscle and cognitive functions: A randomised controlled dietary intervention trial in healthy men, Clinical Nutrition (2011), doi:10.1016/j.clnu.2010.12.010

Table 5
Plasma concentrations of amino acids at baseline and at the end of the intervention period.

[μmol/l]	Usual Protein ($n = 12$)		High Protein ($n = 11$)		Usual Protein Difference	High Protein Difference	P^a
	Baseline	End	Baseline	End			
ASP	1.4 ± 0.5	2.2 ± 0.7***	1.6 ± 0.5	1.9 ± 0.9	0.8 ± 0.5	6.0 ± 0.5	n.s
GLU	28.6 ± 9.3	39.2 ± 8.2**	25.6 ± 9.7	30.3 ± 12.1	10.6 ± 10.7	4.7 ± 8.1	n.s
ASN	48.8 ± 5.0	47.8 ± 5.5	48.4 ± 6.5	46.6 ± 11.8	−1.0 ± 3.4	−1.8 ± 8.6	n.s
SER	121.3 ± 12.0	128.5 ± 18.5	110.2 ± 7.7	115.5 ± 24.0	7.1 ± 11.7	5.3 ± 19.4	n.s
GLN	635.3 ± 42.3	634.0 ± 51.1	630.3 ± 58.8	585.9 ± 44.9*	−0.9 ± 34.6	−44.3 ± 52.3	0.027
HIS	99.1 ± 8.2	90.8 ± 7.4*	98.6 ± 16.8	89.7 ± 13.0	−8.3 ± 10.8	−8.9 ± 13.2	n.s
GLY	264.0 ± 113.4	225.7 ± 42.0	241.4 ± 31.1	194.6 ± 29.2***	−38.3 ± 117.2	−46.8 ± 24.2	n.s
THR	139.2 ± 27.8	130.6 ± 24.8	121.9 ± 21.1	106.5 ± 24.7	−8.6 ± 25.9	−15.4 ± 25.8	n.s
ALA	318.0 ± 61.3	312.0 ± 74.2	321.3 ± 68.8	311.8 ± 111.3	−6.0 ± 55.0	−9.5 ± 80.1	n.s
ARG	100.0 ± 17.0	100.3 ± 17.5	97.7 ± 17.0	88.1 ± 10.9	0.3 ± 11.2	−9.6 ± 18.2	n.s
TAU	37.1 ± 5.4	39.7 ± 4.0	37.9 ± 4.5	37.0 ± 4.7	2.6 ± 4.5	−0.9 ± 2.5	0.036
TYR	61.6 ± 9.0	62.9 ± 7.0	68.7 ± 8.5	76.2 ± 18.4	1.3 ± 6.3	7.5 ± 16.1	n.s
VAL	253.0 ± 33.7	253.4 ± 25.0	236.4 ± 22.5	314.5 ± 44.6***	0.4 ± 39.3	78.1 ± 37.2	<0.0001[b]
MET	34.0 ± 3.7	41.2 ± 6.3***	35.6 ± 8.5	41.2 ± 17.2	7.2 ± 5.2	5.6 ± 11.8	n.s
TRP	49.3 ± 5.9	57.4 ± 5.9**	51.6 ± 10.8	61.8 ± 15.7**	8.1 ± 7.9	10.2 ± 8.5	n.s
ILE	73.4 ± 9.6	77.2 ± 10.4	66.9 ± 10.5	77.3 ± 17.8*	3.9 ± 8.9	10.4 ± 14.8	n.s
PHE	53.0 ± 3.6	55.6 ± 6.6	51.5 ± 5.8	60.0 ± 10.8*	2.6 ± 5.3	8.5 ± 9.3	0.045
LEU	146.5 ± 13.4	150.1 ± 15.5	147.6 ± 17.9	160.5 ± 29.0	3.6 ± 15.4	12.9 ± 32.2	n.s
Σ BCAA	472.9 ± 51.3	480.8 ± 48.8	450.9 ± 45.6	552.4 ± 85.8***	7.9 ± 56.0	101.4 ± 76.7	0.003[b]
Σ AA	2482.5 ± 200.4	2467.1 ± 202.1	2412.6 ± 164.4	2414.4 ± 304.4	−15.5 ± 233.0	1.7 ± 300.5	n.s

Data are presented as mean ± SD. Student's *t*-test for paired data was used for comparison within groups $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
[a] Differences between groups assessed by Student's unpaired *t*-test.
[b] The significant differences in valine and total BCAA persisted between the groups after adjustment for baseline values by GLM ($p = 0.0002$ and $p = 0.013$, respectively).

5. Discussion

In the present study, the HP diet had a positive effect on reaction time of healthy subjects compared to the group receiving a UP diet. BCAA and phenylalanine in plasma were increased following the HP diet concomitantly with the improved reaction time found in the HP group. No adverse effects of HP intake were found neither by altered creatinine clearance nor by albuminuria.

5.1. Nitrogen intake and energy balance

Calculated protein intake from N losses at the pre-study assessment was within the range of usual protein intake in Denmark and US[5,6] and the subjects included were therefore considered representative with respect to habitual protein intake. At the end of the study, calculated protein intake from N losses were significantly higher in the HP group. When compared to recorded intake both groups were apparently in positive balance. This agrees with earlier studies[17,21] and it is supposed to be due to AA or other non-protein nitrogenous substances accumulating in response to a HP intake.[22,23] Our study confirms the limitations of the N-balance technique as described in,[24] but it does serve the purpose of documenting dietary compliance.

No significant changes were found in REE, No significant changes were found in REE, physical activity or BIA variables. It has previously been shown that HP meals can increase diet-induced thermogenesis for up to 5-h.[11,25–27] The diet-induced thermogenesis in the HP group apparently did not last long enough to have an effect on REE in the fasted state (approx. 12-h), or on BW. Both groups received approx. 170 kJ/kg BW throughout the study, which indicates that they needed the same amount of energy to maintain BW. These results suggest that the subjects were in energy balance and had an unaltered nutritional status during the study.

5.2. Amino acids

BCAA and phenylalanine concentrations were increased in the HP group. This finding may be due to the lack of increase in degrading enzymes for BCAA, phenylalanine, and tyrosine in contrast to most other amino acid degrading enzymes, which increase during a HP diet.[28] Thus, plasma concentrations of these AA remain elevated after adjustment to a HP diet, whereas concentrations of most other AA decline towards control values,[28,29] confirming that plasma BCAA, phenylalanine and tyrosine concentrations are directly proportional to protein intake.[29]

5.3. Muscle function

No improvements were measured in HGS, HGE, HGW or mobility in the HP group compared to the UP group. The increase in muscle function in both groups may be considered a training effect. Castaneda et al.[3] found a significant decrease in HGS in healthy, elderly women fed an inadequate protein diet (0.45 *versus* 0.92 g/kg BW) after a 9-wk period. We investigated the effect of 1.5 and 3.0 g protein/kg BW, and at least at that interval, there were no further improvements in young healthy males in a 3-wk study.

5.4. Cognitive function and reaction time

Both groups improved equally in their total ACE score, which probably is a training effect. A significant increase in the ACE component "orientation" in the HP group was found when compared to the UP group. In a descriptive study,[9] we found that the ACE components: orientation, verbal fluency and language were related to the Go/No-Go test. These 3 components are considered basic cognitive processes, which serve as building blocks for the development of higher intellectual abilities.[30] A significant improvement in reaction time (Go/No-Go test) in the HP group was found compared to the UP group. The Go/No-Go test has previously been shown to be sensitive to omega-3 fatty acids supplementation.[10] Alertness mainly reflects speed of nerve conduction.[10] The information processing required in the Sustained Attention is less demanding than that of the Go/No-Go test, cf. that reaction time was slightly longer in Go/No-Go compared to Sustained Attention. Thus, the HP diet seems to have a positive effect on central processing in a demanding test but no effect in less demanding tests, characterized by simpler tasks of longer duration (Alertness and Sustained Attention). Several studies have investigated the effect of single

Please cite this article in press as: Jakobsen LH, et al., Effect of a high protein meat diet on muscle and cognitive functions: A randomised controlled dietary intervention trial in healthy men, Clinical Nutrition (2011), doi:10.1016/j.clnu.2010.12.010

meals on reaction time. Fischer et al.[31] investigated test meals of either pure carbohydrate, protein or fat, and they found that protein ingestion resulted in improved reaction times in complex (but not simple) reaction time tasks, whereas ingestion of pure carbohydrate slowed reaction time.[31] Fischer et al.[4] and Smith et al.[32] investigated the effect of high carbohydrate or high protein meals on complex reaction time, in which they found improved reaction time after the high protein meal compared to the high carbohydrate meal. In our study, the improved reaction time in the HP group may be explained by the higher concentrations of phenylalanine and tyrosine compared to the UP group. Phenylalanine is a precursor for tyrosine, from which the neurotransmitters dopamine and norepinephrine are synthesized. The rate of synthesis and release of these neurotransmitters are modified by the brain concentrations of their AA precursors, which in turn are influenced by their availability in blood.[33] Tyrosine supplementation has been shown to improve complex reaction time in subjects exposed to stressful conditions.[34] The increased values of valine and total BCAA in the HP group may also contribute to the improved reaction time. In rat studies, BCAA increase in brain as protein intake increases, and valine is increased more than leucine and isoleucine.[35] BCAA is known to reduce fatigue and improve tests of mental function after heavy exercise.[36] This has been suggested to occur via inhibited cerebral uptake of tryptophan and the following reduced synthesis of the inhibitory neurotransmitter 5-hydroxytryptamine, but other modes of action are also possible. BCAA may by themselves act as neurotransmitters or BCAA may be converted to (as yet) unidentified metabolites which act as excitatory neutrotransmitters.[36]

The HP group had a significantly higher intake of vitamin D, B_2, B_6 and B_{12} compared with the UP group. B vitamins (Folic acid, vitamins B_6 and B_{12}) have been shown to improve cognitive function in healthy elderly supplemented with mega doses (corresponding to 375%, 600% and 5357% of DRI) for 5-wk's and in elderly patients with impaired cognitive function supplemented with moderate amounts of vitamins and trace elements for at least 1 year.[37] The effect of increased intake of B vitamins on reaction time has not been investigated. It cannot be ruled out that the surplus intake of vitamins B_6 and B_{12} contributed to the improved reaction time.

Also, the lower carbohydrate intake may have contributed to the improved reaction time, rather than the increased protein intake. However, the possible explanation given by the higher concentrations of BCAA and phenylalanine makes the role of higher protein intake more likely. In addition, the small (0.7 kg), but significant weight loss in the UP group could contribute to the final difference between groups, but since the difference was due to improvement in the HP group, rather than worsening in the UP group, this is not the most likely explanation.

Furthermore, it cannot be ruled out that the improved reaction time is a result of multiple comparisons. With the $p = 0.015$ in the Go/No-Go test, only 3 comparisons are allowed according to the Bonferroni correction to become insignificant. However, the pattern of changes in reaction time and cognition (ms, *E*, Orientation) in conjunction with the higher concentrations of BCAA and phenylalanine, together with the earlier meal studies discussed above, does not render a bias due to multiple comparisons the most likely interpretation of our results.

5.5. Quality of life

No changes in QoL single scales or in Component Summaries, PCS and MCS were found in any of the intervention groups. Our results indicate that improved reaction time in the Go/No-Go test or 'orientation' in the cognitive function test did not improve QoL in these individuals who had high baseline scores in PCS and MCS, as compared to normative data.[38]

5.6. Adverse effects of a high protein diet

No adverse effects of the HP diet were observed in the present study. HP intake significantly increased serum urea concentrations into the upper normal range and also increased N, urea and creatinine excretion in urine, but did not alter creatinine clearance or albuminuria. This is in accordance with.[39] No change in bicarbonate in the HP group suggesting that there was no acid accumulation. There were no dietary effects on insulin concentrations or IGF-1. Neither TSH nor T_3 concentrations changed with dietary protein intake which is in accordance with.[40]

5.7. Limitations of the study

This study has certain limitations. The sample size was relatively small and we may have lacked the sensitivity to detect subtle changes. To generalize our results, a study with a larger number of subjects is essential. Young healthy males seem to be less sensitive to nutritional intervention than elderly, women[41] or stress-prone individuals.[42] Therefore, the observed effects might be more pronounced in older, vulnerable or malnourished[43] populations.

6. Conclusion

Reaction time in young healthy young males was improved when they were on a HP diet for 3 wks. The effects may be explained by the concomitant increased plasma levels of BCAA and phenylalanine. No adverse effects of the HP diet were observed.

Conflict of interest

The authors have no financial or other relations that could lead to a conflict of interest.

Acknowledgments

The Danish Meat Association sponsored this study. Akern/RJL Systems (Florence, Italy) sponsored the ElectroFluidGraph (EFG). Study sponsors were not involved in the study design, data collection, analysis or interpretation of data, writing of the manuscript, or in the decision to submit this manuscript for publication.

The authors would like to thank the staff from Department of Human Nutrition, Faculty of LIFE Sciences, University of Copenhagen, Denmark for excellent assistance including research assistants, Janice Marie Sorensen, Fatma Ahmad Shaker Al-Abaiji, and Ingeborg Krarup Rask; laboratory assistants, Soren Andresen, Kristina Moller Kristensen, and John Gargul Lind; kitchen staff, Kira Holst Bjerre Hamann, Karina Graff Rossen, and Charlotte Kostecki; dieticians Bettina Belmann Mirasola, and Hanne Mary Jensen. Furthermore, we wish to thank Rita Babeluk, Surgical Research, Medical University Vienna, Austria, for excellent laboratory assistance.

LHJ carried out the study, performed the statistical analysis and drafted the manuscript.

JK and IT conceived of the study, participated in its design and helped with the manuscript.

MZ and ER were responsible for the plasma amino acid analysis and helped with the manuscript.

All authors have read and approved the final manuscript.

References

1. World Health Organization. Protein and amino acid requirements in human nutrition: report of a joint WHO/FAO/UNU expert consultation. Geneva, World Health Organization technical report series, no. 935; 2007.
2. Rodriguez NR, Garlick PJ. Introduction to Protein Summit 2007: exploring the impact of high-quality protein on optimal health. *Am J Clin Nutr* 2008;**87**: 1551S–3S.

3. Castaneda C, Charnley JM, Evans WJ, Crim MC. Elderly women accommodate to a low-protein diet with losses of body cell mass, muscle function, and immune response. *Am J Clin Nutr* 1995;**62**:30–9.
4. Fischer K, Colombani PC, Langhans W, Wenk C. Carbohydrate to protein ratio in food and cognitive performance in the morning. *Phys Behav* 2002;**75**:411–23.
5. Fulgoni III VL. Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003–2004. *Am J Clin Nutr* 2008;**87**: 1554S–7S.
6. Pedersen AN, Fagt S, Groth MV, Christensen T, Biltoft-Jensen A, Matthiessen J, et al. *Dietary habits in Denmark 2003–2008*. 1st ed. Soeborg, Denmark: National Food Institute Technical University of Denmark; 2010.
7. World Health Organization. *Energy and protein requirements: report of a joint FAO/WHO expert consultation*. Geneva: World Health Organization; 1985.
8. Jakobsen LH, Rask IK, Kondrup J. Validation of handgrip strength and endurance as a measure of physical function and quality of life in healthy subjects and patients. *Nutrition* 2010;**26**:542–50.
9. Jakobsen LH, Sorensen JM, Rask IK, Jensen BS, Kondrup J. Validation of reaction time as a measure of cognitive function and quality of life in healthy subjects and patients. Nutrition, in press.
10. Fontani G, Corradeschi F, Felici A, Alfatti F, Migliorini S, Lodi L. Cognitive and physiological effects of omega-3 polyunsaturated fatty acid supplementation in healthy subjects. *Eur J Clin Invest* 2005;**35**:691–9.
11. Raben A, Agerholm-Larsen L, Flint A, Holst JJ, Astrup A. Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. *Am J Clin Nutr* 2003;**77**:91–100.
12. Ware JE, Kosinski M, Bjorner JB, Turner-Bowker DM, Gandek B, Maruish Mark E. *User's manual for the SF-36v2 health Survey*. Lincoln, RI: Quality Metric Incorporated.; 2007.
13. Ware JE, Gandek B, Kosinski M, Aaronson NK, Apolone G, Brazier J, et al. The equivalence of SF-36 summary health scores estimated using standard and country-specific algorithms in 10 countries: results from the IQOLA Project. *J Clin Epidemiol* 1998;**51**:1167–70.
14. Bjorner JB, Damsgaard MT, Watt T, Groenvold M. Tests of data quality, scaling assumptions, and reliability of the Danish SF-36. *J Clin Epidemiol* 1998;**51**: 1001–11.
15. Spittler A, Sautner T, Gornikiewicz A, Manhart N, Oehler R, Bergmann M, et al. Postoperative glycyl-glutamine infusion reduces immunosuppression: partial prevention of the surgery induced decrease in HLA-DR expression on monocytes. *Clin Nutr* 2001;**20**:37–42.
16. Bingham SA. Urine nitrogen as a biomarker for the validation of dietary protein intake. *J Nutr* 2003;**133**(Suppl. 3):921S–4S.
17. Price GM, Halliday D, Pacy PJ, Quevedo MR, Millward DJ. Nitrogen homeostasis in man: influence of protein intake on the amplitude of diurnal cycling of body nitrogen. *Clin Sci (Lond)* 1994;**86**:91–102.
18. Rand WM, Pellett PL, Young VR. Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. *Am J Clin Nutr* 2003;**77**: 109–27.
19. Moher D, Schulz KF, Altman D. The CONSORT Statement: revised recommendations for improving the quality of reports of parallel-group randomized trials 2001. *Explore (NY)* 2005;**1**:40–5.
20. Van Breukelen GJ. ANCOVA versus change from baseline: more power in randomized studies, more bias in nonrandomized studies [corrected]. *J Clin Epidemiol* 2006;**59**:920–5.
21. Cheng AH, Gomez A, Bergan JG, Lee TC, Monckeberg F, Chichester CO. Comparative nitrogen balance study between young and aged adults using three levels of protein intake from a combination wheat-soy-milk mixture. *Am J Clin Nutr* 1978;**31**:12–22.
22. Fisher H, Brush MK, Griminger P, Sostman ER. Nitrogen retention in adult man: a possible factor in protein requirements. *Am J Clin Nutr* 1967;**20**:927–34.
23. Garlick PJ, McNurlan MA, Patlak CS. Adaptation of protein metabolism in relation to limits to high dietary protein intake. *Eur J Clin Nutr* 1999;**53**(Suppl. 1): S34–43.
24. Millward DJ. Metabolic demands for amino acids and the human dietary requirement: Millward and rRvers (1988) revisited. *J Nutr* 1998;**128**:2563S–76S.
25. Johnston CS, Day CS, Swan PD. Postprandial thermogenesis is increased 100% on a high-protein, low-fat diet versus a high-carbohydrate, low-fat diet in healthy, young women. *J Am Coll Nutr* 2002;**21**:55–61.
26. Mikkelsen PB, Toubro S, Astrup A. Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate. *Am J Clin Nutr* 2000;**72**:1135–41.
27. Westerterp KR, Wilson SA, Rolland V. Diet induced thermogenesis measured over 24 h in a respiration chamber: effect of diet composition. *Int J Obes Relat Metab Disord* 1999;**23**:287–92.
28. Harper AE, Miller RH, Block KP. Branched-chain amino acid metabolism. *Annu Rev Nutr* 1984;**4**:409–54.
29. Harper AE, Peters JC. Protein intake, brain amino acid and serotonin concentrations and protein self-selection. *J Nutr* 1989;**119**:677–89.
30. Strub RL, Black FW. *The mental status examination in neurology*. 2nd ed. Davis: Philadelphia, F.A; 1985.
31. Fischer K, Colombani PC, Langhans W, Wenk C. Cognitive performance and its relationship with postprandial metabolic changes after ingestion of different macronutrients in the morning. *Br J Nutr* 2001;**85**:393–405.
32. Smith A, Leekam S, Ralph A, Mcneill G. The influence of meal composition on post-lunch changes in performance efficiency and mood. *Appetite* 1988;**10**:195–203.
33. Fernstrom JD, Fernstrom MH. Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *J Nutr* 2007;**137**:1539S–47S.
34. Banderet LE, Lieberman HR. Treatment with tyrosine, a neurotransmitter precursor, reduces environmental stress in humans. *Brain Res Bull* 1989;**22**: 759–62.
35. Gustafson JM, Dodds SJ, Burgus RC, Mercer LP. Prediction of brain and serum free amino acid profiles in rats fed graded levels of protein. *J Nutr* 1986;**116**: 1667–81.
36. Newsholme EA, Blomstrand E. Branched-chain amino acids and central fatigue. *J Nutr* 2006;**136**:274S–6S.
37. Manders M, de Groot LCPG, van Staveren WA, Wouters-Wesseling W, Mulders AJMJ, Schols JMGA, et al. Effectiveness of nutritional supplements on cognitive functioning in elderly persons: a systematic review. *J Gerontol A Biol Sci Med Sci* 2004;**59**:1041–9.
38. Ware JE, Kosinski M, Gandek B, Aaronson NK, Apolone G, Bech P, et al. The factor structure of the SF-36 health survey in 10 countries: results from the IQOLA Project. *J Clin Epidemiol* 1998;**51**:1159–65.
39. Skov AR, Toubro S, Bulow J, Krabbe K, Parving HH, Astrup A. Changes in renal function during weight loss induced by high vs low-protein low-fat diets in overweight subjects. *Int J Obes Relat Metab Disord* 1999;**23**:1170–7.
40. Pacy PJ, Price GM, Halliday D, Quevedo MR, Millward DJ. Nitrogen homeostasis in man: the diurnal responses of protein synthesis and degradation and amino acid oxidation to diets with increasing protein intakes. *Clin Sci (Lond)* 1994;**86**:103–16.
41. Spring B, Maller O, Wurtman J, Digman L, Cozolino L. Effects of protein and carbohydrate meals on mood and performance: interactions with sex and age. *J Psychiatr Res* 1982;**17**:155–67.
42. Markus CR, Panhuysen G, Tuiten A, Koppeschaar H, Fekkes D, Peters ML. Does carbohydrate-rich, protein-poor food prevent a deterioration of mood and cognitive performance of stress-prone subjects when subjected to a stressful task? *Appetite* 1998;**31**:49–65.
43. Pollitt E, Cueto S, Jacoby ER. Fasting and cognition in well- and undernourished schoolchildren: a review of three experimental studies. *Am J Clin Nutr* 1998;**67**:779S–84S.

Please cite this article in press as: Jakobsen LH, et al., Effect of a high protein meat diet on muscle and cognitive functions: A randomised controlled dietary intervention trial in healthy men, Clinical Nutrition (2011), doi:10.1016/j.clnu.2010.12.010