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Enhanced anabolic response to milk protein sip feeding in elderly subjects with COPD is associated with a reduced splanchnic extraction of multiple amino acids

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Abstract

Background & Aims—We previously observed in elderly subjects with Chronic Obstructive Pulmonary Disease (COPD) an enhanced anabolic response to milk protein sip feeding, associated with reduced splanchnic extraction (SPE) of phenylalanine. Milk proteins are known for their high Branched-chain Amino Acids (BCAA) content, but no information is present about splanchnic extraction and metabolism of the individual BCAA in COPD.

Objective—To investigate whether BCAA metabolism and SPE of the individual BCAA are altered in COPD during milk protein sip feeding.

Design—In elderly subjects with COPD and in healthy age-matched elderly SPE, endogenous rate of appearance (Ra_{endo}) of the leucine (LEU), isoleucine (ILE) and valine (VAL) were measured before and during sip feeding of a Whey protein meal. To study the effect of aging, the healthy elderly were compared to a group of healthy young subjects. Stable isotopes of L-[²H₃]-LEU, L-[1-¹³C]-ILE and L-[1-¹³C]-VAL were given on two separate test days orally or intravenously. Simultaneously, L-[ring-²H₅]-phenylalanine (PHE) and L-[ring-²H₂]-tyrosine (TYR) were given to determine the whole body protein breakdown (WbPB), synthesis (WbPS) and NetPS.

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CONFLICT OF INTEREST

There is no Conflict of Interest to declare.

STATEMENT OF AUTHORSHIP

Each author has participated sufficiently, intellectually or practically, in the work to take public responsibility for the content of the article, including the concept, design, and conduction of the experiment and for data interpretation (authorship).

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Results—SPE of all BCAA, TYR, and PHE ($p<0.01$) were lower in the COPD group, and the increase in netPS during feeding was higher in the COPD group ($P<0.01$) due to higher values for PS ($P<0.001$). Raendo of all BCAA, PHE and TYR were higher in the COPD than the healthy elderly group ($P<0.05$) before and during feeding ($P<0.001$). Sip feeding resulted in a reduction of Raendo of PHE, ILE and VAL ($P<0.05$). Postabsorptive Raendo was not different for any of the measured amino acids between the healthy elderly and young group, while sip feeding resulted in a reduction of Raendo of PHE. Only SPE of TYR was higher in the elderly ($P<0.05$) and the increase in netPS during sip feeding was independent of aging.

Conclusion—The enhanced anabolic response to milk protein sip feeding in normal-weight COPD patients is associated with a reduced splanchnic extraction of multiple amino acids including all branched-chain amino acids.

Registration ClinicalTrials.gov = NCT01418469

Keywords

splanchnic extraction; branched-chain amino acids; COPD; whey protein; protein turnover

INTRODUCTION

Milk proteins (casein and whey) have been used as therapeutic modality to conserve muscle mass in chronic wasting diseases such as Chronic Obstructive Pulmonary Disease (COPD). Milk proteins are known for their high branched-chain amino acids (BCAA) content, which include leucine (LEU), isoleucine (ILE) and valine (VAL). BCAA are playing critical roles in protein structure, metabolism and regulation (1). Furthermore, there is evidence that BCAA can improve respiratory function in neonates (2) and attenuate ratings of perceived exertion during exercise (3).

BCAA are essential amino acids. We previously observed in patients with COPD an elevated anabolic response to sip feeding of a casein protein meal (4). Moreover, we found that adding free BCAAs to a BCAA-low soy protein sip meal was able to enhance anabolism in COPD patients, but not in healthy elderly (5). We realize that sip feeding is a model of continuous enteral feeding in contrast to bolus meals as practiced by patients in daily life. On the other hand, sip feeding excludes modifying effects of differences in absorption kinetics between proteins. Therefore, sip feeding gives important insight whether differences in the amino acid composition of the protein play a role to its anabolic capacity in COPD. In both studies with sip feeding (4, 5), a reduced splanchnic extraction (SPE) of the essential amino acid phenylalanine was observed in the COPD group. Moreover, the COPD patients were characterized by higher values for phenylalanine turnover, suggesting that alterations in splanchnic extraction and metabolism of phenylalanine might play a role in the mechanism in which anabolism was stimulated in COPD. Whether the reduced SPE in COPD during protein feeding specifically involves PHE or also other amino acids including the BCAA is not known.

Patients with COPD with a deprived nutritional state are often characterized by reduced plasma BCAA levels in the postabsorptive state (6, 7) without significantly elevated leucine turnover levels (8). Low plasma BCAA levels in COPD are associated with disturbances in muscle energy metabolism during exercise (9), suggesting that preservation of BCAA levels is of importance in COPD. In contrast, COPD patients with preserved body weight have increased leucine turnover (8) and preserved plasma leucine levels (6). These data suggest that a reduced splanchnic BCAA extraction from a meal might be an adaptive mechanism to preserve the plasma BCAA levels in normal weight COPD. It is unclear whether there is a discrepancy in turnover rate and splanchnic extraction between the individual BCAAs in

response to feeding in COPD patients and healthy subjects. As different extraction patterns of the individual BCAAs during milk protein feeding will result in modified delivery of BCAAs to muscle, this information might be important to determine the optimal composition of the individual BCAAs in milk protein to optimize anabolism in COPD.

Therefore, in the present study, we examined whole body turnover and splanchnic extraction of the individual BCAAs; LEU, VAL and ILE in the postabsorptive state and during whey sip feeding using stable isotope methodology. In addition, whole body protein breakdown and (net) protein synthesis rate were studied to determine the anabolic response to whey protein sip feeding in COPD. In this way we were able to compare these data with the previously obtained data using casein and soy protein, taking into account differences in absorption kinetics. As COPD commonly occurs in the elderly, normal-weight COPD patients and a healthy elderly age-matched control group were studied to carefully examine the effects of the disease on protein and BCAA metabolism without a potential direct effect of a deprived nutritional state. Besides a group of healthy elderly, also a group of young individuals were studied to investigate the direct effect of aging.

SUBJECTS AND METHODS

Subjects

A group of eight patients with moderate airflow obstruction (FEV_1 : $50 \pm 4\%$ of predicted), 8 healthy elderly (age-matched) volunteers, and 8 healthy young subjects, all men, were studied. The patients were in clinically stable condition and suffered from moderate COPD (stage 2+ 3) according to the established GOLD guidelines (10). The patients were outpatients, attending the hospital for routine pulmonary control every 6 or 12 months. Exclusion criteria for all groups were malignancy, cardiac failure, recent surgery, and endocrine, hepatic or renal disorders. Also, subjects who were using systemic corticosteroids within 3 months before the study were excluded. Maintenance treatment of the studied COPD patients consisted of inhaled β_2 -agonists, inhaled anti-cholinergics, inhaled corticosteroids, oral theophylline, or a combination. 25% and 62% of the COPD patients and healthy elderly controls were present vs. former smokers resp. 37% of the healthy young subjects were current smokers. Written informed consent was obtained from all subjects and the study was approved by the medical ethics committee of the University Hospital Maastricht.

Pulmonary function tests—Before the study, the healthy elderly and COPD patients underwent spirometry for determination of forced expiratory volume in 1 sec (FEV_1), as a marker of disease severity, with the highest value from at least 3 technically acceptable maneuvers being used. The diffusion capacity for carbon-monoxide (DLCO) as an indirect indicator of emphysema was measured using the single-breath method (Masterlab; Jaeger; Würzburg, Germany). All values obtained were related to a reference value and expressed as percentages of the predicted value (11). The COPD patients had lower values for FEV_1 (COPD vs. healthy elderly patients: $50 \pm 4\%$ pred vs. $110 \pm 5\%$ pred, $P < 0.01$) and DLCO (COPD vs. healthy elderly: $78 \pm 7\%$ pred vs. $104 \pm 9\%$ pred, $P < 0.05$).

Study design—On 2 test days which were at least 3 days apart, subjects came to the metabolic ward of the University Hospital Maastricht after an overnight fast. All subjects were instructed to continue their habitual dietary intake for at least 3 days preceding the study.

Body composition measurements—Body weight was measured using an electronic beam scale with digital readout to the nearest 0.1 kg (model 708; Seca) with the subjects

standing barefoot and wearing light indoor clothing. Body height was measured to the nearest 0.1 cm (model 220, Seca). Whole-body fat-free mass (FFM) was measured in each subject using bioelectrical impedance analyses (Xitron 4000B, Xitron Technologies) to express metabolic data per kilogram of FFM. FFM was calculated using a specific regression equation described by Dey et al. (12).

Study protocol—The protocol started at 07:15 AM, after an overnight fast from 00:00 AM. All subjects were in supine position for 3.5 hours. On the 1st test day, a catheter was placed in an antecubital vein of the arm for infusion of the tracer (85 mL/h), according to a primed constant continuous infusion protocol. Primed and constant infusion of the stable isotopes L-[ring-²H₅]-Phenylalanine (PHE; ²H₅-phe; prime: 2.19 μmol/kg bw, infusion: 2.26 μmol/kg BW/h), L-[ring-²H₂]-Tyrosine (TYR; ²H₂-tyr; prime: 0.31 μmol/kg bw, infusion: 0.77 μmol/kg BW/h), L-[5,5,5-²H₃]-Leucine (LEU; ²H₃-leu; prime: 2.19 μmol/kg bw, infusion: 2.4 μmol/kg BW/h), L-[1-¹³C]-Isoleucine (ILE; ¹³C-ile; prime: 0.61 μmol/kg bw, infusion: 1.26 μmol/kg BW/h) and L-[1-¹³C]-Valine (VAL; ¹³C-val; prime: 8.85 μmol/kg bw, infusion: 8.85 μmol/kg BW/h) were infused IV via the antecubital vein catheter to be able to measure and compare whole body rate of appearance of PHE, TYR, LEU, ILE and LEU. In addition, a prime was given of L-[ring-²H₄]-TYR, (prime: 0.31 μmol/kg bw). The stable isotopes were purchased from Cambridge Isotopic Laboratories (Woburn, MA, USA). Before i.v. administration of the priming dose, a venous blood sample was collected to measure baseline enrichment. After administration of the priming dose, a constant continuous tracer infusion was administered (80 mL/h) using calibrated pumps (IVAC corporation, San Diego, USA) until the end of the study day. A second catheter for arterialized venous blood sampling was placed in a superficial dorsal vein of the hand of the contralateral arm, which was placed in a thermostatically controlled hot box (internal temperature: 60°C), at least 20 min before the first blood sampling. The use of the hot box is a technique to mimic direct arterial sampling (13). Triple arterialized-venous blood samples were taken between 80 and 90 min after the start of the infusion. Subsequently, continuous nutrition was started via repeated ingestion (every 20 min) of a carbohydrate-protein drink for 2h, when a tracer steady state was reached. At the end of the ingestion period, triple arterialized-venous blood samples were taken and the test day ended. Arterialized venous blood samples were taken at 80, 85, 90, 200, 205 and 210 minutes after the start of the infusion.

The study design of the 2nd test day was identical to the 1st test day, with the exception that the isotopes were given orally every 20 min instead of i.v. to enable calculation of splanchnic extraction of the individual tracer amino acids. After the priming dose was infused i.v., the tracers were ingested (25 mL/20 min). The tracers were ingested alone (first 90 min) or together with the carbohydrate-protein drink (last 2h).

Composition of the protein-carbohydrate drink—The drink contained 28% whey protein (WPC 80, DMV, The Netherlands) and 72% maltodextrin (Glucodry 210, Amylum Europe, Aalst, The Netherlands), dissolved in ultrapure water. For amino acid composition of the whey protein, see Table 1. All intakes consisted of a fluid ingestion of 0.67 mL/(kg body weight/20 min) and contained 18 mg protein/kg body weight and 46 mg maltodextrin/kg body weight. In total, ±301 mL enteral nutrition (based on 6 ingestions and a 75-kg subject) was supplied during the study. The complete drink supplied 8.1 g protein and 21g maltodextrin (based on a 75 kg subject). The drink was prepared at 60°C, 1h before the start of the experiment and kept at 4°C until use to prevent bacterial growth.

Biochemical analyses—Venous and arterialized venous blood was put in heparinized tubes (Becton Dickinson Vacutainer system, Franklin Lakes, New Jersey, USA), immediately put on ice to minimize enzymatic reactions, and centrifuged (4°C, 3120 × g for

10 min) to obtain plasma. Subsequently, 250 μ L plasma was deproteinized by mixing it with 20 mg sulfosalicylic acid. Samples were frozen in liquid nitrogen and stored at -80°C until analysis. Analysis of plasma amino acid concentrations was performed using a fully automated HPLC (Pharmacia, Woerden, The Netherlands) (14). Tracer enrichments [tracer:tracee ratio (TTR)] were analyzed by LC-MS (Thermoquest LCQ, Veenendaal, The Netherlands) (15). For analysis of urea, glucose, lactate, and ammonia, 900 μ L plasma was deproteinized by mixing with 90 μ L of a 500 g/L trichloroacetic acid solution. Analysis was done spectrophotometrically on a COBAS Mira S (Roche Diagnostica, Hoffman-La Roche, Basel, Switzerland) by standard enzymatic methods (16). All samples were stored at -80°C until further analysis.

Calculations

The sum of amino acids (SUM AA) represents the sum of the concentration of the measurable α -amino acids (glutamine, glycine, threonine, histidine, citrulline, alanine, taurine, arginine, α -amino butyric acid, tyrosine, valine, methionine, isoleucine, phenylalanine, tryptophan, leucine, ornithine and lysine), and BCAA the sum of the three branched-chain amino acids valine (VAL), leucine (LEU) and isoleucine (ILE).

All the metabolic data were determined under steady-state conditions. Tracer-tracee ratio of PHE, TYR, LEU, VAL and ILE reached an isotopic steady state within 1.5 hours of infusion in the postabsorptive state and within 2 hours of feeding (data not shown) in all groups.

1. WbBCAA production = Whole body rate of appearance (WbRa=turnover) of the BCAAs ($Ra_{2H3-LEU}$, $Ra_{13C-ILE}$, $Ra_{13C-VAL}$) = infusion rate of the BCAAs/tracer-tracee ratio of the BCAAs in plasma
2. WbPHE or WbTYR production = Whole body rate of appearance (Ra) of the PHE ($Ra_{2H5-PHE}$) or TYR ($Ra_{2H4-TYR}$) (= infusion rate of PHE or TYR/tracer-tracee ratio of PHE or TYR in plasma)

$Ra_{2H3-LEU}$, $Ra_{13C-ILE}$, $Ra_{13C-VAL}$, $Ra_{2H5-PHE}$, $Ra_{2H4-TYR}$ represents whole body rate of appearances of leucine, isoleucine, valine, phenylalanine and tyrosine calculated from I.V. $^2H_3-LEU$, $^{13}C-ILE$, $^{13}C-VAL$, $^2H_5-PHE$ and $^2H_4-TYR$ isotopes.

Splanchnic extraction of BCAAs (SPE_{LEU} , SPE_{ILE} , SPE_{VAL}) and PHE (SPE_{PHE}) and TYR (SPE_{TYR}) represents the fraction (in %) of ingested BCAAs, PHE and TYR taken up by the gut and liver during its first pass and metabolized via oxidation or protein synthesis, calculated as (17, 18):

$$SPE_{BCAA} = [1 - (Ra(BCAA)_{iv} / Ra(BCAA)_{ent})] \times 100 \quad (2)$$

where $Ra(BCAA)_{iv}$ and $Ra(BCAA)_{ent}$ are WbLEU, WbILE, WbVAL production calculated according to Eq. (1) with either the i.v. or enteral tracer, respectively. The Wb rate of appearance of endogenous BCAA ($Ra_{endo-LEU}$, $Ra_{endo-ILE}$, $Ra_{endo-VAL}$) in the fed state represents the amount of LEU, ILE and VAL resp. that is produced in plasma minus the amount of ingested LEU, ILE and VAL resp. that reaches plasma (corrected exogenous BCAA intake): $Wb\ Ra_{endo-BCAA} = Wb\ Ra_{BCAA} - \text{corrected exogenous BCAA intake}$ (3) Corrected exogenous BCAA intake = dietary BCAA intake $\times$ $1 - (SPE_{BCAA}/100)$

Same calculations for SPE and WB Ra_{endo} were also done for PHE and TYR.

Statistical analyses—Results are expressed as mean $\pm$ standard error (SE). The mean value of the measures of BCAA, PHE and TYR turnover in the postabsorptive and the fed state at the time points 80, 85 and 90 min was used as the postabsorptive state, and at 200, 205 and 210 min as the fed state. For the plasma amino acid concentrations, the value at $t=90$ and $t=210$ was used as the postabsorptive and prandial state, respectively. If data failed the normality or equal variance test, they were log-transformed where appropriate. As COPD occurs predominantly in the elderly, the COPD group was compared to a healthy age matched (elderly) control group to determine the COPD effect. Furthermore, a direct comparison was done between the healthy elderly and young subjects to determine the effect of aging. The unpaired Student's t test was used to ascertain whether general characteristics and baseline values of plasma amino acid concentrations and protein and BCAA kinetics and SPE values during sip feeding differed significantly between the COPD and healthy elderly group, and between the healthy elderly and young group. To compare the metabolic effects of whey protein sip feeding on plasma amino acid concentrations and whole body Raendo, the repeated measures (mixed model) analysis of variance (ANOVA) was performed with a group (COPD vs. healthy elderly, healthy elderly vs. young), and sip feeding (postabsorptive and prandial) effect.

Differences were considered significant at $P < 0.05$. The statistical package within Graphpad Prism (Version 5.03) was used for data analysis.

RESULTS

General characteristics

Age and height were not different between the COPD and elderly group (Table 2). BMI and fat mass index (FMI) were higher in COPD patients than in the elderly ($P < 0.05$) but no differences were found in body weight and FFMI. The younger subjects were taller and had lower values for BMI and FMI than the elderly and COPD groups (young vs. elderly: $P < 0.05$, young vs. COPD: $P < 0.01$) but no difference was found in body weight and FFMI.

Plasma amino acid concentrations

Postabsorptive plasma PHE, TYR, LEU, ILE and VAL concentrations were not different between the COPD and healthy elderly group, and between the healthy elderly and young subjects. For the COPD vs. elderly comparison, there was a feeding effect for all studied plasma amino acids ((LEU, ILE, sum BCAA, PHE ($P < 0.001$), sum AA ($P < 0.01$), VAL, TYR, ($P < 0.05$)), indicating that feeding increased nearly all studied plasma concentrations. There was no group $\times$ time interaction or group effect for any of the studied amino acids.

For the elderly vs. young comparison, there was a time (feeding) effect for the amino acids (LEU, ILE, ($P < 0.001$), sum BCAA, PHE ($P < 0.01$), sum AA ($P < 0.05$)) but not for VAL and TYR. There was a group $\times$ time interaction for LEU ($P < 0.05$), and a group effect for sum BCAA ($P < 0.01$), LEU, VAL, ILE ($P < 0.05$) but not for PHE, TYR and sum AA, indicating higher BCAA values in the elderly than in the young group, and that plasma LEU levels were higher in the elderly than in the young group after feeding ($p < 0.05$).

In line, significantly higher plasma levels were found in the prandial state for LEU, ILE, sum BCAA, PHE as compared to the postabsorptive state in the elderly and COPD group, but only LEU was significantly higher in the prandial state in the young subjects ($P < 0.01$).

Splanchnic extraction of amino acids

Prandial splanchnic extraction (Figure 1) was significantly lower for all the studied amino acids in the COPD than in the healthy elderly group (SPE_{phe} , SPE_{tyr} , SPE_{ile} , SPE_{val}):

$P < 0.001$, SPE_{leu} : $P < 0.01$). Although SPE tended to be higher for PHE, LEU and ILE in the elderly than in the young subjects ($P < 0.1$), significance was only obtained for SPE_{tyr} ($P < 0.001$).

Whole body endogenous Ra of amino acids during sip feeding

Raendo in the prandial state was derived using the corrected exogenous amino acid intake, whereas in the postabsorptive state Raendo equals WbRa (Figure 2). WbRa in the postabsorptive state was significantly higher in the COPD group than in the elderly group for PHE, LEU ($P < 0.01$) and VAL, ILE and TYR ($P < 0.05$). No differences were present between the healthy elderly and young groups.

For both group comparisons (COPD vs. elderly, elderly vs. young) there was no significant group $\times$ time interaction for Raendo for PHE, TYR, or for the BCAAs LEU, ILE or VAL. For the COPD vs. elderly comparison, there was a group effect for Raendo of PHE, TYR, LEU, ILE and VAL ($P < 0.001$) indicating higher values in the COPD group. Furthermore, a sip feeding effect for Raendo was found for the amino acids PHE, ILE and VAL ($P < 0.05$), with the exception of LEU and TYR, indicating that feeding reduced Raendo of PHE, ILE and VAL ($P < 0.05$) but not that of LEU and TYR.

For the elderly vs. young comparison, there was a group effect for PHE, ILE, VAL ($P < 0.01$), and TYR ($P < 0.05$), indicating that Raendo levels for these amino acids were lower in the elderly than in the young subjects. No group effect was found for Raendo LEU. A feeding effect was found only for Raendo PHE ($P < 0.05$).

Whole protein anabolism—Postabsorptive WbPS was higher in the COPD than in the elderly group ($P < 0.05$) but no difference was present between the healthy elderly and young controls. The change in WbPS after whey sip feeding was lower in the healthy elderly than in the COPD ($P < 0.001$) and young group ($P < 0.05$; Figure 3). The change in WbPB (=Raendo) was not different between the COPD and the healthy elderly group, or between the healthy elderly and young group. The change in net protein synthesis (= protein synthesis– protein breakdown) was significantly higher in the COPD group than in the healthy elderly ($P < 0.01$) but not different between the elderly and young subjects.

DISCUSSION

We observed that whey protein sip feeding resulted in higher stimulation of WbPS and netPS and lower splanchnic extraction of all studied amino acids (LEU, ILE, VAL, PHE, TYR) in COPD patients as compared to age-matched healthy elderly subjects. This suggests that the enhanced anabolic response to milk protein sip feeding in COPD is related to a reduced splanchnic extraction of multiple amino acids including the branched-chain amino acids.

Plasma BCAA levels and turnover rates

The individual BCAA levels were not different in the COPD than in the elderly controls. This was expected as normal-weight patients were studied to exclude the effect of malnutrition on plasma BCAA levels as observed in anorexia nervosa, protein-calorie malnutrition (19, 20) and in depleted COPD (6, 21). All subjects were male thereby eliminating a potential gender effect (22). Postabsorptive BCAA levels generally are within a small range as the main source of BCAA in plasma is appearance from protein degradation. In disease states, skeletal muscle is a major site of BCAA catabolism, and BCAA are used for maintenance of protein quality and repair process of tissues. The

available data on amino acid levels in health and disease are conflicting and reflect variations in methodology, sampling conditions, sex and nutritional status of the subjects.

As the plasma level of an amino acid does often not reflect its turnover rate (23), we simultaneously infused the stable isotopes of the individual BCAAs to be able to calculate the whole body turnover rate of LEU, VAL and ILE. Higher postabsorptive turnover values were found for all BCAA ($P<0.05$) in the normal weight COPD group, indicating that plasma LEU, VAL or ILE levels in COPD do not reflect the underlying changes present in BCAA metabolism in these patients. Elevated turnover values were also found for TYR and PHE in the COPD group. These data suggest a general up-regulation of metabolism of all BCAAs and of TYR that well reflects the elevated basal protein turnover as assessed by PHE tracer in these patients. Increased protein turnover, which is an energy consuming process (24), has been observed previously in COPD (4, 5) and might be linked to the enhanced inflammatory status (25).

BCAA turnover and splanchnic extraction of amino acids during whey protein sip feeding

SPE of LEU, VAL and ILE ($p<0.01$) were lower in the COPD than in the healthy elderly group. All BCAAs are generally absorbed after intake by the small intestinal epithelial cells *via* discrete amino acid carriers, transported to the liver, and then released into the systemic circulation. Intestinal absorption capacity rate is often increased in stress states due to inflammation (26). However, SPE of the individual BCAA were lower in the COPD than in the control subjects. Also SPE of PHE and TYR were lower during whey protein sip feeding which is in line with previous findings during casein protein sip feeding in COPD (4). The reduced SPE for BCAA, PHE and TYR suggest a general SPE reduction of all essential and likely of all alimentary amino acids in COPD leading to higher systemic availability of alimentary amino acids for peripheral protein anabolism.

Despite lower SPE in COPD, no differences were found in plasma amino acid levels between the COPD and the healthy elderly during whey protein sip feeding suggesting increased amino acid uptake and/or reduced endogenous protein breakdown in COPD. Muscle and other non-splanchnic tissues are the major sites of postprandial uptake of BCAA (27). A feeding effect was present for Raendo ILE and VAL but not for LEU, indicating that the reduced SPE of ILE and VAL during whey sip feeding was associated with a reduction in Raendo in the COPD group. This discrepancy for LEU is of interest as whey protein consists of high BCAA levels mainly because of high LEU levels. Also the reduced SPE of LEU during sip feeding in the COPD was not associated with higher plasma LEU level suggesting that LEU was immediately oxidized or incorporated into protein (for protein synthesis).

Increased LEU oxidation in skeletal muscle is one of the metabolic responses that compensates for the increased energy expenditure in catabolic states (24, 28). Although LEU oxidation was not measured in the present study, it is expected to be increased in our normal weight COPD group as recently higher LEU oxidation rates have been found in COPD with preserved BMI than in those with low BMI (8). Enhanced BCAA oxidation rates have been observed on whole body and skeletal muscle level during sepsis, endotoxin or tumor necrosis factor treatment (29), and in chronic wasting diseases characterized by enhanced inflammatory mediators (30). Low grade systemic inflammation was also present in our studied COPD patients (COPD: 8.7 ± 10.9 mg/L, healthy controls: 1.7 ± 1.1 mg/L, healthy young: 0.6 ± 0.3 mg/L). Epiphenomena of COPD such as reduced skeletal muscle oxidative capacity may contribute to an increased BCAA oxidation in these patients (31). Because BCAAs share common membrane transport systems and metabolic enzymes it is possible that the individual BCAA can influence each other's kinetics and requirements. As a high LEU intake has been shown to result in an increased VAL oxidation (32), it might

explain the unaltered VAL plasma levels found in all the study groups during whey sip feeding as whey protein consists of high levels of LEU and relatively low levels of VAL. More research is necessary to unravel the factors responsible for the discrepancy in the metabolic response to protein sip feeding among the individual BCAA. There was a tendency towards higher values for SPE of the studied amino acids of interest in the elderly than in the young healthy subjects, but only SPE of TYR reached statistical significance. Higher SPE levels during feeding in the elderly are in line with previous studies (17, 18).

Anabolic response to whey protein sip feeding

A sip feeding protocol was used to measure whole body PS and PB during whey protein feeding and was comparable to that used in our previous study during casein and soy protein feeding in COPD (4, 5) to make the absorption characteristics of the proteins comparable. We realize that this pattern of sip protein feeding does not reflect the “bolus” type meal intake as practiced by patients in daily life. However the data will give important insight whether the amino acid composition of whey protein besides its fast absorption kinetics alone contributes to its anabolic capacity in COPD. The manner of drinking has an important effect on the anabolic response on whole body level and in the muscle level compartment (33, 34) as it influences the delivery of amino acids and therefore the presence of hyperaminoacidemia. Rapid aminoacidemia is known to be an important factor in promoting muscle anabolism by increasing muscle (myofibrillar) protein synthesis (35). The tracer design used (PHE M+5 tracer was provided either IV or orally on two different test days) was slightly different to that used previously (4, 5) (IV PHE M+5 tracer and oral PHE M+1 tracer (together with the meal) were provided simultaneously). The methodology used in the present study was more time consuming, however it resulted in less variability in TTR data.

No difference was present in net anabolic response to whey protein sip feeding in the healthy young as compared to the elderly group. However the change in whole body protein synthesis was higher in the young group. Previously it was suggested that in young subjects slow (e.g. casein) proteins are far better than fast (e.g. whey) proteins with respect to prandial protein gain (36). The present study confirms that when you provide fast proteins via sips and thus make them “slow” they will do better in young subjects than in the healthy elderly. The COPD patients responded to whey sip feeding more anabolic than the healthy elderly, which is in line with our previous data showing an enhanced anabolic response to casein and soy (with and without BCAA) sip feeding in COPD (4, 5). It remains unclear whether the positive anabolic effects in COPD are reflecting increased muscle anabolism, increased plasma (acute phase) or splanchnic protein synthesis. Recent studies show that whole body protein gain was greater with whey protein than with casein protein, suggesting that a ‘fast’ protein might be more beneficial than a ‘slow’ one to limit protein losses during aging (37). Also on muscle level, fast protein is beneficial in terms of postprandial muscle accretion (38) in older men. More studies are needed to unravel the exact contribution of the muscle vs. the splanchnic compartment to the increased whole body anabolic response to protein feeding in patients with COPD.

In conclusion, alterations are present in BCAA metabolism despite normal plasma levels in normal weight COPD. The higher BCAA and protein turnover in the postabsorptive state indicates an overall turnover upregulation in COPD. Protein sip feeding was not able to normalize the elevated BCAA turnover in COPD despite a reduced BCAA splanchnic extraction. The high anabolic response in COPD make milk proteins an important therapeutic modality to conserve muscle mass in COPD. Whether the anabolic capacity of milk proteins can be further enhanced in COPD by adding specific amino acids (i.e. leucine to reduce leucine breakdown) in combination with physical activity to reduce protein turnover and energy consumption in COPD (25) needs to be studied.

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Figure 1. Splanchnic extraction of phenylalanine (SPE-PHE, fig 1a), tyrosine (SPE-TYR, fig 1b), leucine (SPE-LEU, fig 1c), isoleucine (SPE-ILE, fig 1d), and valine (SPE-VAL, fig 1e) in young (n=8, striped bar) and elderly healthy controls (n=8, closed bar) and COPD patients (n=8, open bar) during enteral intake of the whey protein meal. Mean values $\pm$ SE are shown. Significance of difference between the COPD and healthy elderly group **: $P < 0.01$, ***: $P < 0.001$. Significance of difference between the healthy elderly and young group ###: $P < 0.001$.

Figure 2.

Raendo in the postabsorptive state (=WbRa, open bar) and the prandial state (closed bar) of phenylalanine (PHE, fig 2a), tyrosine (TYR, fig 2b), leucine (LEU, fig 2c), isoleucine (ILE, fig 2d) and valine (VAL, fig 2e) in young (n=8) and elderly healthy controls (n=8), and in COPD patients (n=8) during enteral intake of the whey protein meal. Mean values $\pm$ SE are shown. Significance of difference in postabsorptive Raendo between the COPD and the healthy elderly group *: $P < 0.05$, **: $P < 0.01$.

For the COPD vs. elderly comparison, there was a group effect for Raendo PHE, TYR, LEU, ILE and VAL ($P < 0.001$) and a feeding effect for Raendo PHE, ILE and VAL ($P < 0.05$). For the elderly vs. young comparison, there was a group effect for PHE, ILE,

VAL ($P < 0.01$), and TYR ($P < 0.05$) and a feeding effect for Raendo PHE ($P < 0.05$). There was no significant group $\times$ time interaction for Raendo for Phe, Tyr, or the three BCAAs Leu, Ile or Val.

Figure 3.

Absolute change (prandial-postabsorptive) in whole body protein synthesis (PS, fig 3a), endogenous rate of appearance (Raendo, fig 3b) and NetPS (=PS-Raendo, fig 3c) during intake of the whey protein meal in young (n=8, striped bar) and elderly healthy controls (n=8, closed bar) and COPD patients (n=8, open bar). Mean values $\pm$ SE are shown. Significance of difference between the COPD and healthy elderly group **: $P < 0.01$, ***: $P < 0.001$. Significance of difference between the healthy elderly and young group #: $P < 0.05$.

Table 1

Amino acid composition of the whey protein meal (WPC 80 DMV)

	g/100 g protein
ASP	10.10
ASN	
GLU	19.20
GLN	
SER	5.00
GLY	1.50
THR	7.10
HIS	1.50
ALA	5.50
ARG	2.10
TYR	2.40
VAL	5.40
MET	2.40
ILE	5.50
PHE	3.00
TRP	1.40
LEU	10.70
LYS	8.80
CYS	2.20
PRO	6.10
Sum BCAA	21.60
Sum EAA	41.30

ASP: aspartate, ASN: asparagine, GLU: glutamate, GLN: glutamine, SER: serine, GLY: glycine, THR: threonine, HIS: histidine, ALA: alanine, ARG: arginine, TYR: tyrosine, VAL: valine, MET: methionine, ILE: isoleucine, PHE: phenylalanine, TRP: tryptophan, LEU: leucine, LYS: lysine, CYS: cysteine, PRO: proline. Sum BCAA: sum of LEU, ILE and VAL. Sum EAA: sum of THR, VAL, MET, ILE, TRP, LEU, and LYS.

Table 2

General characteristics of the healthy young and elderly subjects, and COPD patients

		Young (n=8)		Elderly (n=8)		COPD (n=8)	
Age	y	22	±	63	±	68	± 4 ^a
Height	M	1.82	±	1.74	±	1.74	± 0.03 ^b
Weight	Kg	73.4	±	77.5	±	81.8	± 3.5
BMI	Kg/m ²	22.3	±	25.4	±	27.2	± 0.8 ^a
FFMI	Kg/m ²	18.4	±	19.2	±	18.9	± 0.2
FMI	Kg/m ²	3.9	±	6.3	±	8.4	± 0.8 ^a

Values are means ± SE, *n* = 8/group. Within a row, means with superscripts without a common letter differ, *P* < 0.05.

Table 3

Plasma amino acid concentrations

		Postabsorptive T=0h ²			Prandial T=2h			COPD vs. healthy elderly	Elderly vs. healthy young
	$\mu\text{mol/L}$							T ^{***}	T ^{***} , G [*] , GxT [*]
<i>LEU</i>									
Young		126	±	5	171	±	8 ^{**}		
Elderly		131	±	6	209	±	15 ^{***}		
COPD		128	±	10	199	±	10 ^{***}		
<i>VAL</i>	$\mu\text{mol/L}$							T [*]	G [*]
Young		229	±	17	236	±	13 ^a		
Elderly		260	±	13	277	±	12 ^b		
COPD		251	±	12	270	±	10 ^b		
<i>ILE</i>	$\mu\text{mol/L}$							T ^{***}	T ^{***} , G [*]
Young		61	±	3	75	±	3		
Elderly		65	±	5	97	±	8 ^{***}		
COPD		65	±	8	91	±	5 ^{**}		
<i>Sum BCAA</i>	$\mu\text{mol/L}$							T ^{***}	T ^{**} , G ^{***}
Young		417	±	22	483	±	15 ^a		
Elderly		456	±	24	583	±	34 ^{***b}		
COPD		446	±	27	561	±	24 ^{***b}		
<i>PHE</i>	$\mu\text{mol/L}$							T ^{***}	T ^{**}
Young		58	±	2	62	±	2		
Elderly		58	±	1	67	±	2 ^{**}		
COPD		59	±	2	68	±	3 ^{***}		
<i>TYR</i>	$\mu\text{mol/L}$							T [*]	
Young		58	±	3	61	±	3		
Elderly		55	±	2	59	±	3		
COPD		56	±	2	63	±	2		

		Postabsorptive T=0h ²		Prandial T=2h		COPD vs. healthy lderly	Elderly vs. healthy young
Sum AA	$\mu\text{mol/L}$					T ^{**}	
Young		2868	±	63	3085 ±	102	
Elderly		2835	±	89	3179 ±	137	
COPD		2789	±	151	3153 ±	152	

¹Values are means ± SE, *n* = 8/group.

²Postabsorptive values (T0) and values after 2h of carbohydrate-protein drink ingestion (T2).

³Significant effects listed as Group (G), Time (T), and G X T. Means for a variable in a column with superscripts without a common letter differ, *P* < 0.05. Asterisks indicate a difference from time zero:

* *P* < 0.05,
 ** *P* < 0.01,
 *** *P* < 0.001