The effects of whey protein feeding on 24-hour protein metabolism in the elderly.

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Draft study protocol by DJ Baer et al. US Department of Argriculture, Beltsville Human Nutrition Research Center on:

Studies to investigate the effect of whey protein on body composition

Primary research questions:
1a. What is the impact of whey protein consumption on lean body mass in humans?
1b. What are some potential mechanisms by which whey can alter body composition?
2. How does whey protein alter risk factors and biomarkers of chronic degenerative diseases?

Methodology:
Ad 1: Comparison of 4 test products as a dietary intervention (random double-blind design). Study will last 6 to 8 months. Primary outcome: **body composition**
Specific variables that will be assessed: Body weight, body composition, dietary intake, and compliance
Ad 2: Individuals fed controlled diets (double blind, randomized cross-over design). Meals: breakfast and dinner from Monday to Friday
Primary outcome: **substrate oxidation pattern (fat, protein, CHO), and protein (nitrogen) metabolism**

Hypothesis:
Whey protein consumption compared to egg white, soy protein and carbohydrate will favorably alter body composition by increasing fat oxidation and protein synthesis, and improve markers and indices of health

Proposed beverages:
whey protein (at least 40g/d), soy protein (isonitrogenous to whey protein), egg white protein (considered as high quality, complete protein), isonitrogenous to whey, carbohydrate (energy equivalent = isoenergetic to whey).

Study population:
Adult males and females (aged 40-60 y), BMI: 28-35 kg/m^2, Individuals who are at increased risk of disease (mildly hypercholesterolemic, prediabetic, prehypertensive will not be excluded). Individuals with active risk (liver, kidney, cancer etc) will be excluded.

Primary outcome:
1. Body weight and composition (DEXA, water space, bioelectrical impedance spectroscopy)
2. Blood pressure
3. Substrate oxidation pattern (protein fat, CHO oxidation rates from respiratory calorimetry)
4. Amino acid metabolism (using 15N stable isotopes)
5. Thyroid hormones
6. Adipose tissue hormones (eg leptin)

Secondary outcome:

1. Blood lipids and lipoproteins
2. Insulin sensitivity
3. Inflammatory profile (markers immune status, inflammatory diseases such as CHF)
4. Hormones (associated with risk of cancer and associated with hunger and satiety)

Draft study protocol by MPKJ Engelen and NEP Deutz. Dept. Surgery, Maastricht University, Maastricht, The Netherlands:

Studies to investigate the effect of whey protein on 24-hour protein metabolism

Primary research questions:

1a. What is the impact of whey protein consumption on 24 hours protein metabolism in humans?

Possible changes in body composition need nutritional intervention over a long period of time. This means long interventions with substantial costs and a lot of human effort is needed (laborious studies). Moreover, body composition techniques are not always precise in the detection of small changes in muscle mass/fat mass.

To overcome these limitations, Young et al {Forslund, Am J Physiol 1998} has shown that 24 hour measurements of protein metabolism give a good reflection whether and to what level meals are anabolic/catabolic over a day, and thus will predict if and to what extent body mass gain is expected to occur or not after chronic intervention. Until yet no studies have been conducted investigating the 24-h response in protein metabolism when mimicking the 3 meals (breakfast, lunch and dinner) as used in daily life condition. Moreover, the 24-hour protocol is able to investigate the protein requirements of the individuals.

1b. What are the underlying mechanisms?

Insight is given in the underlying mechanisms of the (potential) anabolic response of a meal. Quantitative information will be obtained to what extent whole body protein synthesis and breakdown rates influence after a meal and during a 24-h day period. Moreover insight will be obtained on the metabolic response to feeding in different body compartments such as the splanchnic compartment (known to be a very important organ in times of feeding (as a regulator) but also acting as a protein reservoir in times of fasting) and the non-splanchnic (~muscle) compartment.

2. How does whey protein alter risk factors and biomarkers of chronic degenerative diseases?

Chronic degenerative condition such as aging is known to be associated by changes in body composition (ie increased fat mass (obesity) and sarcopenia), and chronic low-grade inflammation. Moreover, and associated with changes in body composition, aging is often accompanied by the presence of metabolic alterations (such as changes in insulin sensitivity and the occurrence of diabetes mellitus).

Two groups of elderly will be compared: a group with an abnormal glucose tolerance (prediabetic) and a group with a normal glucose tolerance. Despite the protein and substrate metabolic response to the protein meals, biomarkers of inflammation, blood profile (hormones, lipids, lipoproteins etc) will be studied.

Methodology:

Ad 1a and b: Comparison of 3 test products as acute dietary intervention (random single-blind design). In total, the study will last 3 complete (24-h) testday a person.
Primary outcome: Protein metabolism during 24-h measurement (3 meals (breakfast, lunch and dinner mimicking daily life condition).

Specific variables that will be assessed: 24-h response of whole body protein synthesis and breakdown rates, and protein (nitrogen) metabolism of different body compartments after a meal (breakfast, lunch, dinner)(i.e. splanchnic area (gut and liver)) and endogenous (muscle) response in protein breakdown. Furthermore, splanchnic extraction of the meals and hormonal changes of the day (i.e. insulin) are studied. In addition, substrate oxidation pattern (fat, protein, CHO) over the day will be investigated.

Ad 2: 2 groups of elderly will be investigated: a group with an abnormal glucose tolerance (prediabetic) and a group with a normal glucose tolerance.
Primary outcome: In both groups the response in protein and substrate metabolism to the different protein meals will be investigated and compared. This response will be related to biomarkers of inflammation, blood profile (hormones, lipids, lipoproteins etc) in both groups.

Hypothesis:
Whey protein consumption compared to casein, and soy protein will favorably alter body composition by positively influencing protein and fat metabolism, and improve markers and indices of health in elderly

Proposed beverages:
Whey protein, casein protein (isonitrogenous to whey protein) and soy protein (isonitrogenous to whey protein). Casein protein is used as, like whey protein, it is a milk base protein and known of its high nutritional value (protein quality). Moreover, several studies have previously been performed using casein, and consistent positive results were found with respect to protein metabolism {Boirie, Proc Natl Acad Sci U S A 1997}. In the three beverages an equal amount of carbohydrates is added to mimick daily life food composition and to enhance protein anabolism

Study population:
Elderly (n=20, age 60 y and older). Individuals with active risk (liver, kidney, cancer, COPD etc) will be excluded.
10 of these elderly subjects have impaired glucose tolerance according to the 1999 WHO criteria (fasting glucose < 7.0 mmol/L; 2hr glucose > 7.8 and <11.1 mmol/L) without medication that interferes with glucose metabolism.
This group is compared with a group (age-matched) with normal glucose tolerance (n=10)

Primary outcome:
1. Protein and amino acid metabolism (using stable isotope methodology)
2. Substrate oxidation pattern (protein, fat, CHO oxidation rates from respiratory calorimetry)
3. Hormonal response (feeding and fasting related such as insulin, GLP, cortisol, glucagon etc)
4. 24h response in glucose, fat and amino acid concentration in blood

Secondary outcome:
1. Body weight and body composition (bioelectrical impedance spectroscopy)

2. Blood pressure
3. Blood lipids and lipoproteins
4. Inflammatory profile (markers immune status)

Experimental design:

The effects of whey protein feeding on 24-hour protein metabolism in the elderly.

Study population:

Option A:
20 elderly will be studied.
10 elderly subjects with impaired glucose tolerance according to the 1999 WHO criteria (fasting glucose < 7.0 mmol/L; 2hr glucose > 7.8 and <11.1 mmol/L) without medication that interferes with glucose metabolism.
This group is compared with a group (age-matched) with normal glucose tolerance (n=10).

Option B:
Dependent on research question and restrictions of financial budget, in option B the study will be limited to 10 healthy elderly individuals with normal glucose tolerance (age 60 years and older).

Screening (only option A)
Before inclusion, the individuals will be screened. After an overnight fast, an oral glucose tolerance test is performed. After measuring body weight with an electronic beam scale with digital readout to the nearest 0.1 kg (model 708; Seca, Hamburg, Germany) and body height with a stadiometer, a catheter will be placed in a superficial dorsal vein of the hand for blood sampling at different time points. After 15 minutes, the first venous blood sample will be taken for determination of venous plasma glucose concentration. Subsequently, a 75-g glucose load (analogous to 82.5 g dextrose monohydrate, Avebe, the Netherlands) in 250 ml plain water is given during the oral glucose tolerance test. After 15, 30, 60, 90 and 120 minutes, a venous blood sample is taken. Moreover, blood will be sampled to exclude subjects who suffer from any other diseases (heart failure, COPD etc) or metabolic disorders (liver failure, renal failure etc).

Experimental design (option A and B)
In total, the experiment involves three complete test days. All of them obtain the same experimental design, only different protein enriched drink are ingested every test day. The experimental design is described below:

After an overnight fast, body weight will be measured by using an electronic beam scale with digital readout to the nearest 0.1 kg (model 708; Seca, Hamburg, Germany) with the subjects standing bare-foot and wearing light indoor clothing.

Body height will be measured to the nearest 0.1 cm (model 220, Seca). Whole body fat free mass (FFM) will be determined by bioelectrical impedance spectroscopy. Subsequently, at 6.30 h a catheter will be placed in an antecubital vein of the arm for infusion of the tracers, according to a primed-constant and continuous infusion protocol. Whole-body protein metabolism will be assessed via combined infusion of stable isotopes L-[*ring*-$^{2}H_5$]PHE, L-[^{15}N]TYR, and L-[1-^{13}C]LEU, [$^{2}H_3$]3-methyl-histidine. Moreover, a bolus dose of L-[*ring*-$^{2}H_4$]TYR will be administrated to prime the PHE-derived plasma tyrosine pool. The arm will be positioned approximately 10 cm above the level of the heart, so that the heart will receive a uniform dose of tracers throughout the experiment.

A second catheter for arterialized venous blood sampling will be placed in a superficial dorsal vein of the hand of the contra-lateral arm, which is placed in a thermostatically controlled hot box (internal temperature: 60°C), a technique to mimic direct arterial sampling.

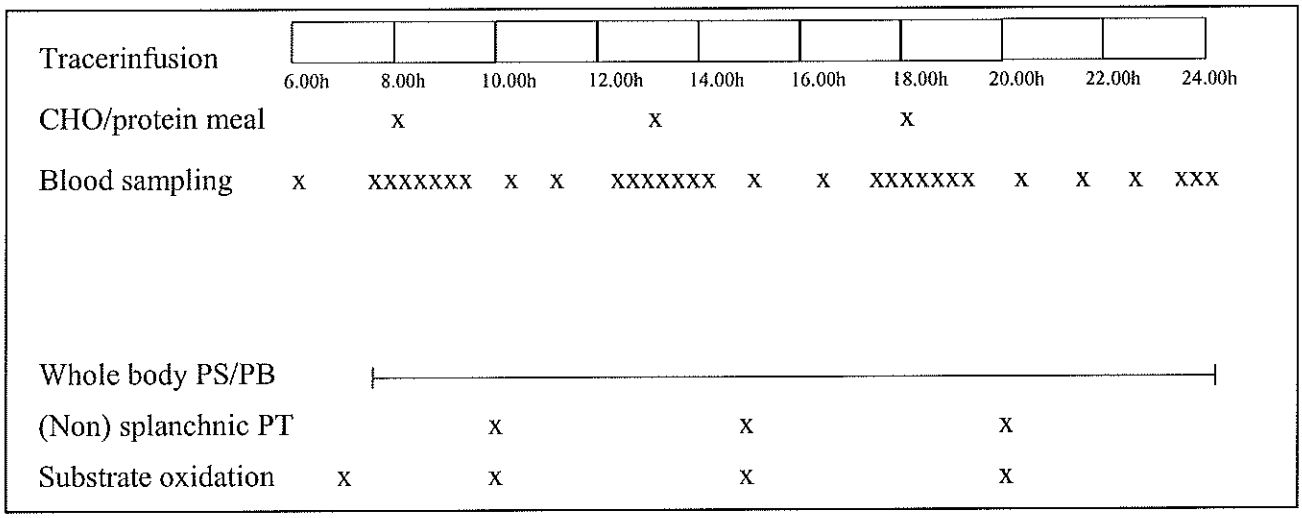
Before administration of the priming dose, venous blood will be collected for measurement of the natural (baseline) enrichment of amino acids. Arterialized venous blood will be sampled at 80, 85, 90 min to reassure a tracer steady state. After 1.5 hour, at 8.00 h subjects will start sipping a test meal as a bolus protein meal. This test meal is reflecting the protein and carbohydrate composition of a standard breakfast meal. Subsequently and 5 hours apart, at 13.00 h and 18.00 h the subjects will ingest another carbohydrate protein meal reflecting a standard lunch and dinner meal. All meals are taken as a bolus and ingestion will be completed within 5-10 minutes. The test meals at one experimental day involve either a casein-based protein meal (CAPM), a whey-based protein meal (WPM) or a soy-based protein meal (SOPM) and are ingested in a randomized single blind order. For the amino acid composition of the test meals, see addendum. The dose of the test meals at breakfast, lunch, and dinner time still needs to be determined but the protein level is in accordance to their habitual intake as assessed by a strict dietary questionnaire. After the dinner meal, each subject will stay in the hospital until the last blood sample at 24.00 hour.

Arterialized venous blood samples will be taken 3 times during the first resting period, several times before and within 1 hour after intake of each test meal, and several times between the test meals.

Substrate oxidation pattern (protein fat, CHO oxidation rates from respiratory calorimetry) will be assessed in the postabsorptive state (7.00h) and 2 hours after each meal. (Non) splanchnic protein metabolism will be measured via oral tracer ($^{2}H_2$-PHE and $^{2}H_3$-LEU) ingestion 2 hours after each meal when there is a steady state in plasma PHE and LEU enrichment and concentration.

In plasma 24 response will be examined of several feeding and fasting related hormones (ie insulin, GLP, cortisol, glucagon etc), the substrates glucose and fat (glycerol, FFA) and the amino acid profile. Moreover, blood lipids, lipoproteins and the inflammatory profile will be examined at several time points during the study.

Summary of the protocol:



Addendum 1: Practical implications study: screening and experiment

Option A:

20 study subjects (age: 60+): 10 impaired glucose tolerance, 10 controls

Screening: *OGTT (2hr)*

Expected number of subjects to be tested to obtain 10 IGT subjects: 40

Screening costs per test subject:

Blood samples for glucose (insulin), exclusion metabolic disorders

Glucose (75-g / testday)

Experiment:

1. 3 experiments/person
2. 20 study subjects
3. 1-2 experiments/day
4. Number of test days: 3*20 = 60
5. Number of test days/week = 2 → => data collection during 7 months

Option B:

10 study subjects (age: 60+): 10 healthy subjects

Experiment:

1. 3 experiments/person
2. 10 study subjects
3. 1-2 experiments/day
4. Number of test days: 3*10 = 30
5. Number of test days/week = 2 → => data collection during 3-4 months

Option A and B:

Per test day (n=60 vs 30):

1. Meals
    a. Whey protein
    b. Casein protein (isonitrogenous to whey protein)
    c. Soy protein (isonitrogenous to whey protein).
2. Body composition (BIS) for measurement body composition

3. Whole body protein metabolism: Intravenous stable isotope infusion for 18 hours of the stable isotopes
    a. L-[*ring*-$^{2}H_5$]PHE
    b. L-[^{15}N]TYR
    c. L-[1-^{13}C]LEU
    d. [$^{2}H_3$]3-methyl-histidine
    e. L-[*ring*-$^{2}H_4$]TYR (prime)
4. Splanchnic protein metabolism: Oral stable isotope intake for 4*2 hours of the stable isotopes
    a. L-$^{2}H_2$-PHE
    b. L-$^{2}H_3$-LEU
5. Substrate oxidation

Analysis

1. Blood samples a test day
    a. Tracer-tracee ratio + amino acid concentration + $^{13}CO_2$ (n=35)
    b. Substrates: glucose and fat (ie FFA, glycerol) (n=20)
    c. Hormones: (ie insulin, GLP, cortisol, glucagon) (n=10)
    d. Blood lipids and lipoproteins (n=20)
    e. Inflammatory profile (CRP, IL6) (n=4)
2. Disposables

Addendum 2: Time schedule of the study

Option A:

Permission of the study	Feb 2005
Permission MEC (3 months)	May 2005
Set-up of the study, recruitment of patients and control subjects	June-Sept 2005
Screening	Oct-Nov 2005
Data collection (60 test days, 1-2 times a week)	Dec 2005-June 2006
Sample analyses (6 months)	July-Dec 2006
Data evaluation and statistics (3 months)	Jan-March 2007

Option B:

Permission of the study	Feb 2005
Permission MEC (3 months)	May 2005
Set-up of the study, recruitment of patients and control subjects	June-Sept 2005
Screening	Oct
Data collection (30 test days, 1-2 times a week)	Nov 2005-Feb 2006
Sample analyses (3 months)	March-May 2006
Data evaluation and statistics (3 months)	June-Aug 2006

Addendum 3: Composition of the protein products

- ***Test product 1***: casein (gr/100gr)

	MW	alfas1-cas	alfas2-cas	beta-cas	kappa-cas	gamma-cas	**caseine**
%		0,38	0,1	0,36	0,13	0,03	
ASP	133,1	3,99	2,13	2,26	2,8		2,9068
ASN	132,1	4,49	7,27	2,77	4,89		4,0661
GLU	147,1	15	14,56	11,03	10		12,4268
GLN	146,2	9,36	8,63	12,87	10,82		10,4596
SER	105,1	7,15	7,04	7,04	7,15		6,8849
GLY	75,1	2,85	0,6	1,58	0,83		1,8197
THR	119,1	2,5	7,03	4,53	8,81		4,4291
HIS	155,2	3,26	1,86	3,26	2,48		2,9208
ALA	89,1	3,39	2,85	1,87	7,04		3,1616
ARG	174,2	4,36	4,18	2,96	4,53		3,7293
TYR	181,2	7,61	8,7	3,08	8,52		5,9782
VAL	117,2	5,51	6,45	9,26	6,8		6,9564
MET	149,2	3,13	2,39	3,73	1,64		2,9844
ILE	131,2	6,17	5,77	5,51	8,92		6,0648
PHE	165,2	5,62	3,96	6,28	3,47		5,2435
TRP	204,2	1,63	1,63	0,82	1,02		1,2102
LEU	131,2	9,45	6,82	12,07	5,51		9,3345
LYS	146,2	8,63	13,89	6,73	6,87		7,9843
CYS	121,2	0	0,97	0	1,33		0,2699
PRO	115,13	8,29	4,6	16,8	12,08		11,2286
BCAA							22,3557

- ***Test product 2:*** whey (gr/100gr)

	MW	alfa-LA	beta-LG	BloodserumA	proteose	IG	Whey protein
%		0,19	0,5	0,06	0,13	0,13	
ASP	133,1	8,39	7,32	8,39	?	?	5,7575
ASN	132,1	8,32	3,57	2,25	?	?	3,5008
GLU	147,1	8,24	12,94	13,83	?	?	8,8654
GLN	146,2	5,12	7,16	3,8	?	?	4,7808
SER	105,1	5,15	3,99	4,41	?	?	3,2381
GLY	75,1	3,15	1,65	1,73	?	?	1,5273
THR	119,1	5,84	5,24	6,07	?	?	4,0938
HIS	155,2	3,26	1,71	4,04	?	?	1,7168
ALA	89,1	1,87	7,31	6,33	?	?	4,3901
ARG	174,2	1,22	2,79	6,1	?	?	1,9928
TYR	181,2	5,07	3,99	5,25	?	?	3,2733
VAL	117,2	4,92	5,74	6,33	?	?	4,1846
MET	149,2	1,04	3,28	0,9	?	?	1,8916
ILE	131,2	7,35	7,22	2,76	?	?	5,1721
PHE	165,2	4,63	3,63	6,44	?	?	3,0811
TRP	204,2	5,72	2,25	0,61	?	?	2,2484
LEU	131,2	12,07	15,74	15,74	?	?	11,1077

LYS	146,2	12,43	11,99	13,3	?	?	9,1547
CYS	121,2	6,79	3,27	6,42	?	?	3,3103
PRO	115,13	1,61	5,06	4,83	?	?	3,1257
BCAA							20,4644

- ***Test product 3:*** soy (gr/100gr)

ASP	8,51
ASN	0,00
GLU	8,84
GLN	5,26
SER	3,81
GLY	3,06
THR	2,94
HIS	1,87
ALA	3,12
ARG	5,47
TYR	2,89
VAL	3,66
MET	1,08
ILE	3,51
PHE	3,87
TRP	0,91
LEU	5,82
LYS	4,54
CYS	1,07
PRO	3,95
BCAA	12,99