

Relationship between animal protein intake and muscle mass index in healthy women

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The amount and the type of dietary protein could play a role in determining the quantity of skeletal muscle mass. The aim was to examine the relationship between the type of protein intake and the level of muscle mass in healthy omnivorous and vegetarian Caucasian women. The design of the present study was an observational and cross-sectional study. Twenty-one omnivores (Om) and nineteen vegetarians (Ve) were recruited. Muscle mass index (urinary creatinine), dietary intake (5 d dietary records) and biochemical analyses (hormone, phyto-oestrogen and lipid profiles) were obtained. We found differences between groups for muscle mass (Ve: 18 kg v. Om: 23 kg; $P=0.010$), muscle mass index (Ve: 6.7 kg/m² v. Om: 8.3 kg/m²; $P=0.002$), animal protein intake in g/d ($P=0.001$) and in g/kg body weight per d ($P=0.003$), plant protein intake in g/d ($P=0.015$) and in g/kg body weight per d ($P=0.007$), the animal:plant protein intake ratio ($P=0.001$) and sex hormone-binding globulin (SHBG) ($P=0.001$). Muscle mass index still correlated with animal protein intake in g/d ($P=0.001$) and in g/kg body weight per d ($P=0.008$), and the animal:plant protein intake ratio ($P=0.007$) even after controlling for SHBG and plant protein intake. Finally, animal protein intake (g/d) was the independent predictor of muscle mass index (adjusted r^2 0.42). Thus, a vegetarian diet is associated with a lower muscle mass index than is an omnivorous diet at the same protein intake. A good indicator of muscle mass index in women seems to be animal protein intake.

Muscle mass index: Animal protein intake: Human nutrition

Studies have demonstrated that nutrition and the quality of nutritional intake could play an important role in the prevention of health-related problems⁽¹⁾. An adequate consumption of protein may contribute to the prevention of skeletal muscle loss (sarcopenia), which occurs after 30 years of age⁽¹⁾. The current RDA for protein among young and older adults is 0.85 g/kg per d⁽²⁾. Castaneda *et al.*⁽³⁾ demonstrated that 0.8 g protein/kg per d was insufficient to maintain muscle mass with age. All the data show that no consensus about the RDA for protein was established despite the wide acceptance that an inadequate dietary protein intake may be an important cause of sarcopenia⁽⁴⁾. In fact, one of the possible mechanisms leading to sarcopenia in old frail individuals is an imbalance between the protein synthesis and the protein breakdown rate, which results in a reduction of the basal rate of muscle protein synthesis⁽⁵⁾. Furthermore, studies have proposed that meat may improve protein synthesis by providing creatine in the diet⁽⁶⁾. Moreover, Lord *et al.*⁽⁷⁾ demonstrated that animal protein could be associated with better preservation of fat-free mass. However, Louis *et al.* observed that creatine has no effects on protein synthesis or breakdown in human subjects⁽⁸⁾. Thus, the role of creatine in protein synthesis is still unclear.

Other mechanisms, such as a reduction in anabolic hormones such as testosterone, as well as in oestrogens and

growth hormone, could also contribute to sarcopenia⁽⁹⁾. One possible factor involved is the level of fasting insulin, because studies have shown that age-associated insulin resistance in muscle proteins reduces muscle anabolic response and thus may lead to sarcopenia⁽⁴⁾. Nevertheless, the conclusions about this mechanism are still controversial and further research is needed to understand the role of insulin in the control of protein synthesis. Baumgartner *et al.* have shown that muscle mass is negatively correlated with age and positively with total fat mass and energy intake, but does not correlate with protein intake, sex hormone-binding globulin (SHBG), testosterone or oestrone levels⁽¹⁰⁾. Nevertheless, others have reported a relationship between dietary protein intake and sarcopenia⁽¹¹⁾. Consequently, it is controversial as to whether the quantity and type of protein intake can affect muscle mass.

To our knowledge, no study has examined the association between the types of regular diets (vegetarian v. omnivore) in women and the level of muscle mass. This question is relevant because studies have demonstrated that dietary intake during resistance training programmes could influence the level of skeletal muscle mass⁽¹²⁾. Moreover, in 2006, approximately 2.3% of the US adult population consistently followed a vegetarian diet and avoided eating meat, fish or poultry⁽¹³⁾. The number of female vegetarians aged 45–54 years will most probably increase in the future⁽¹³⁾.

Abbreviations: BW, body weight; SHBG, sex hormone-binding globulin.

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The aim of the present study was to examine the relationship between the type of protein intake and the level of muscle mass in healthy omnivorous and vegetarian Caucasian women.

Materials and methods

Subjects

We recruited two groups of women (twenty-one omnivores and nineteen vegetarians) living in the Helsinki area. Based on their answer to a questionnaire about the frequency of their physical activity, these women were sedentary or moderately physically active (≤ 3 h/week with moderate intensity or ≤ 5 h/week with low intensity). We excluded subjects with a history of breast cancer or any major diseases or using drugs such as oral contraceptives, hormonal replacement therapy or antibiotics.

The omnivorous women consumed all major types of food and the vegetarians were either vegans (consuming no animal products; n 1), lacto-vegetarians (consuming milk products; n 10) or lacto-ovo-vegetarians (consuming milk products and eggs; n 8). To be included as a regular vegetarian, the women were required to have eaten this diet for at least 2 years (mean 12 years).

All subjects gave their informed consent and were initially interviewed by a doctor who explained the study. The ethical committee of the Helsinki University Central Hospital approved the research programme.

Collection of samples

We studied the subjects for 5 consecutive days on four occasions (one visit in each season, 3 months between each visit). Each of these visits included a 72 h collection of urine, 3 d blood samples and a 5 d food record beginning 2 d before the collection of urine and plasma (including at least one weekend day).

The non-menopausal women collected the samples during the mid-follicular phase of the menstrual cycle (days 5–7, ± 2 d). The mean day of the menstrual cycle on which the collections began was for the omnivores 7 ± 1 and 8 ± 1 for the vegetarians.

The 3 d blood samples collected from the fasting subjects were pooled to reduce the number of analyses. For plasma samples, we added 0.1 % ascorbic acid (to prevent oxidation of the hormones) and 0.1 % sodium azide (to prevent bacterial growth).

To prevent oxidation of the hormones, we added 1 g ascorbic acid powder per litre urine volume to each bottle before collection. During the collection, the urine was stored at a temperature between 0 and 10°C, brought to the laboratory every morning, and stored in a refrigerator until completion of the 72 h collection. Thereafter, the 3 d urine collections were pooled, 50 ml were taken, and 0.1 % sodium azide was added to prevent bacterial growth. The urine samples were stored at -20°C until analysed.

Hormones

The plasma sample analyses were carried out in duplicate within 1 year of sample collection with the exception of

the plasma phyto-oestrogens, which were analysed more than 5 years later. Quality-control samples were included in each batch.

As previously described in detail⁽¹⁴⁾, the plasma oestrogens oestrone and oestradiol were determined with RIA after chromatographic separation on a Sephadex LH-20 column (GE Healthcare, Piscataway, NJ, USA). Briefly, ^3H -labelled internal standards were added, and the plasma was extracted with five volumes of 20 % ethyl acetate in petroleum ether (v/v). The organic phase was separated, evaporated to dryness, and reconstructed in 9 % methanol in toluene. The oestrogens were separated using a Sephadex LH-20 column (GE Healthcare) and determined with RIA. The intra-assay CV% of the control serum was 5.1 % for oestrone and 3.9 % for oestradiol. The corresponding inter-assay CV% during a 3-year period (n 15) was 2.0 % for oestrone and 1.4 % for oestradiol⁽¹⁴⁾.

Growth hormone and insulin were measured with commercial RIA kits as described by Hämmäläinen *et al.*⁽¹⁵⁾.

Plasma testosterone was determined with RIA as described in detail by Kuoppasalmi *et al.*⁽¹⁶⁾. SHBG was determined as described by Rosner with slight modifications⁽¹⁷⁾. The intra-assay CV% was 8.3 % for plasma testosterone and 8.8 % for SHBG. The inter-assay CV% was 12.7 % for plasma testosterone and 11.1 % for SHBG.

ApoA1 and apoB were determined with RIA⁽¹⁷⁾ and the apoB:apoA1 ratio was calculated. Plasma daidzein and genistein were measured with time-resolved fluoroimmunoassay⁽¹⁸⁾. The intra-assay CV% for daidzein was 3.2 % and for genistein was 3.8 %⁽¹⁸⁾.

Urinary phyto-oestrogen levels (daidzein, genistein) and urinary oestrogens (oestrone, oestradiol, oestriol) were measured by GC-MS in the selected ion-monitoring mode as described by Fotsis *et al.*⁽¹⁹⁾. The intra-assay CV% was 5.9 % for daidzein, 9.0 % for genistein, 8.9 % for oestrone, 12.6 % for oestradiol and 3.3 % for oestriol. All details were published, including validation of the procedure⁽²⁰⁾.

All the methods have been validated with regard to accuracy, sensitivity, specificity and reproducibility.

Urinary creatinine excretion

Urinary creatinine concentrations were determined by auto-analyser (DCA 2002 + ; Bayer Diagnostics, Tarrytown, NY, USA) using a colorimetric assay based on the Jaffé reaction as described by Wang *et al.*⁽²¹⁾. The intra-assay CV% for creatinine was 8 %. Studies have shown that the skeletal muscle mass:creatinine ratio during 3 consecutive days of urine collection has an inter-assay CV of 6.0 %, suggesting moderate variability⁽²²⁾. Studies have also demonstrated that the urinary creatinine output is directly proportional to the total body creatine content in human subjects⁽²³⁾. In addition, Chinn observed a strong correlation between body creatine content and urinary creatinine excretion⁽²⁴⁾. Heymsfield *et al.*⁽²⁵⁾ demonstrated that this indirect method is valid for measuring fat-free mass and skeletal muscle mass in human subjects, but required certain specific conditions: (1) the consumption of the same diet as normal during data collection; (2) to minimise emotional stress and physical activity during data collection; (3) the absence of severe renal insufficiency; (4) the collection of urine during 3 consecutive days. All of

these conditions were met during the present study. None of the subjects experienced severe renal insufficiency or kidney disease based on the creatinine values, which were in a normal range (7.5 (SD 2.4) v. 5.9 (SD 3.8) nmol/l). We asked the women to record their dietary consumption 2 d before urinary collection and to continue recording during the following 3 d urine collection. In this way, we could control whether our subjects changed their dietary habits during data collection.

The formula used to measure muscle mass (kg), which was developed by Welle *et al.* (26), is the following equation:

$$\begin{aligned} & ((\text{Means of quantity of urine}/100) \\ & \times (\text{means of quantity of creatinine in mg/dl}) \times 21.8)/1000. \end{aligned}$$

Afterward, as with the BMI, we divided the muscle mass variables by height (m²) to obtain the muscle mass index (kg/m²).

Dietary intake

The subjects were instructed to maintain a normal diet throughout the 5 d dietary recordings in each season⁽²⁷⁾. Each subject was provided with a food scale and instructed on how to complete the dietary records. Previous studies have demonstrated that a 3 d dietary record is suitable for estimating dietary intakes in adults without cognitive impairments⁽²⁷⁾. A nutritionist completed the dietary analyses using the 1983 version of the coding system from the Department of Nutrition (University of Helsinki) for total, fat, carbohydrate and protein intake. Total, animal and plant protein intakes were expressed as g/kg body weight (BW) per d and as g/d for all data analyses.

Physical activity level

The level of physical activity was evaluated with a frequency questionnaire (how long and how many sessions per week). We categorised women as physically inactive if they practised physical activity three or fewer times or ≤ 3 h per week. The level of physical activity was considered low or moderate

when the intensity was between 0 and 33.5 kJ/min (0 and 8 kcal/min) or less than 4184 kJ/week (1000 kcal/week).

Statistical analyses

The normality of distribution was determined with the kurtosis test. Data were log-transformed if abnormally distributed. The results are presented as mean values and standard deviations. Because of the number of subjects in each group (twenty-one omnivores and nineteen vegetarians), omnivorous and vegetarian groups were compared with non-parametric Mann–Whitney tests for all variables. We also measured Pearson and partial correlations between muscle mass index and significant variables. Groups were compared using a multivariate general linear model with SHBG and plant protein intake serving as covariables. Afterwards, a stepwise regression model was used to determine the predictor of muscle mass with age, BMI, plasma oestrone, plasma oestradiol, testosterone, dehydroepiandrosterone, SHBG, total protein intake (g/d), plant protein intake (g/d), animal protein intake (g/d) and the animal:plant protein intake ratio included in the model. Once the best model was obtained, we analysed residuals to verify if the postulates of the linear regression were respected. $P \leq 0.05$ were considered statistically significant. Analyses were performed using SPSS 15.0 software (SPSS, Inc., Chicago, IL, USA).

Results

The vegetarian and omnivorous groups were similar in age (48 (SD 12) v. 43 (SD 13) years), BMI (22.1 (SD 2.2) v. 23.5 (SD 3.5) kg/m²), BW (60 (SD 7) v. 63 (SD 10) kg), age of menarche (13 (SD 2) v. 12 (SD 1) years), age of menopause (50 (SD 2) v. 50 (SD 4) years), number of menopausal women (nine v. nine) and level of physical activity (53 v. 57%), but not in muscle mass (18 (SD 4) v. 23 (SD 5) kg; $P=0.010$) and muscle mass index (6.7 (SD 1.2) v. 8.3 (SD 1.6) kg/m²; $P=0.002$), respectively (Table 1).

We also found significant differences between groups for plant protein intake in g/d ($P=0.001$) and in g/kg BW per d ($P=0.003$), plant protein intake in g/d ($P=0.015$) and

Table 1. Anthropometric characteristics of omnivorous and vegetarian groups (Mean values and standard deviations)

	Vegetarians (n 19)		Omnivores (n 21)		P*	P†
	Mean	SD	Mean	SD		
Age (years)	48	12	43	13	0.14	
Body weight (kg)	60	7	63	10	0.34	
Height (cm)	164	6	164	6	0.95	
BMI (kg/m ²)	22.1	2.3	23.5	3.5	0.25	
Menarche (years)	13	2	12	1	0.87	
Age of menopause (years)	50	2	50	4	0.54	
Menopausal women (n)	9		9		0.14	
Muscle mass (kg)	18.2	3.9	22.6	5.0	0.010	0.014
Muscle mass index (kg/m ²)	6.7	1.2	8.3	1.5	0.002	0.009
Physically active (%) [‡]	53		57		0.72	

* P values obtained using the non-parametric Mann–Whitney test and log variables.

† P values obtained using the analysis of covariance test with sex hormone-binding globulin and plant proteins intake as covariables.

‡ Percentage of women who practised moderate physical activity for more than 3 h/week.

Table 2. Dietary characteristics of omnivorous and vegetarian groups (Mean values and standard deviations)

Variables	Vegetarians (n 19)		Omnivores (n 21)		P*	P†
	Mean	SD	Mean	SD		
Total dietary intake					0.93	
kJ	7619	1423	7602	1406		
kcal/d	1821	340	1817	336		
Total protein intake (g/d)	58	11	65	11	0.08	
Total protein intake (g/kg BW per d)	0.98	0.21	1.05	0.22	0.78	
Total animal protein intake (g/d)	32	9	44	9	0.001	0.008
Total animal protein intake (g/kg BW per d)	0.54	0.16	0.71	0.18	0.003	0.040
Total plant protein intake (g/d)	26	7	21	5	0.015	
Total plant protein intake (g/kg BW per d)	0.44	0.12	0.34	0.09	0.007	
Animal:plant protein intake ratio	1.23	0.58	2.09	0.64	0.001	0.019
Total fat intake (g/d)	70	16	75	16	0.34	
Total carbohydrate intake (g/d)	241	54	216	52	0.14	
SFA (g/d)	36	8	39	9	0.23	
MUFA (g/d)	21	5	25	6	0.047	0.002
PUFA (g/d)	11	4	9	3	0.22	

BW, body weight.

* P values obtained using the non-parametric Mann-Whitney test.

† P values obtained using the analysis of covariance test with sex hormone-binding globulin and plant proteins intake as covariables.

in g/kg BW per d ($P=0.007$), the animal:plant protein intake ratio ($P=0.001$) and MUFA intake in g/d (MUFA; $P=0.047$; Table 2). Omnivores had a higher level of animal protein intake (44 v. 32 g/d; $P=0.001$) and a lower level of plant protein intake (21 v. 26 g/d; $P=0.015$) than did vegetarians. However, we found no difference in total protein intake (g/d or g/kg BW per d) or in total dietary intake between groups (Table 2).

We found no difference between groups in plasma and urinary hormone levels, except in the plasma level of SHBG ($P=0.001$). In fact, the omnivores presented a lower level of SHBG than did the vegetarians (46 v. 69 nmol/l) (Table 3).

We performed an analysis of covariance with animal protein intake (g/kg BW per d) as a covariable because meat is known to potentially influence creatinine muscle mass

excretion and the present study design included both vegetarian and omnivorous women. However, we observed that the muscle mass index still continued to differ significantly between groups ($P=0.021$) (Fig. 1).

Because plant protein intake⁽²⁸⁾ and SHBG⁽⁹⁾ differed significantly between groups and are known to influence the level of muscle mass, we re-examined the significant variables with analysis of covariance using plant protein intake and SHBG as covariables. We observed a significant difference in muscle mass (Table 1; $P=0.014$), muscle mass index (Table 1; $P=0.009$), total animal protein intake (Table 2; g/d: $P=0.008$ and g/kg BW per d: $P=0.040$) and the animal:plant protein intake ratio (Table 2; $P=0.019$) between groups, even after using plant protein intake and SHBG as covariables.

Table 3. Plasma and urinary hormone and phyto-oestrogen levels in omnivorous and vegetarian groups (Mean values and standard deviations)

Variables	Vegetarians (n 19)		Omnivores (n 21)		P*
	Mean	SD	Mean	SD	
Plasma oestrone (pmol/l)	228	118	214	74	0.95
Plasma oestradiol (pmol/l)	158	122	155	74	0.59
Plasma total testosterone (nmol/l)	1.79	0.61	1.76	0.49	0.95
Plasma sex hormone-binding globulin (nmol/l)	69	15	46	16	0.001
Plasma growth hormone (μ g/l)	3.32	2.44	3.85	2.95	0.61
Plasma dehydroepiandrosterone (nmol/l)	18	8	17	8	0.89
Plasma insulin (μ U/ml)	7.41	2.61	7.21	2.27	0.98
Plasma apoB (g/l)	0.72	0.14	0.79	0.27	0.28
Plasma apoA1 (g/l)	1.59	0.21	1.49	0.19	0.04
Plasma apoB:apoA1	0.46	0.09	0.54	0.18	0.06
Urinary oestrone (nmol/24 h)	8.57	8.73	6.40	5.35	0.74
Urinary oestradiol (nmol/24 h)	22	18	19	14	0.93
Urinary oestriol (nmol/24 h)	14	11	11	7	0.81
Plasma daidzein (nmol/l)	50	69	4	2	0.001
Plasma genistein (nmol/l)	44	47	16	14	0.001
Urinary daidzein (nmol/24 h)	1667	1861	314	343	0.001
Urinary genistein (nmol/24 h)	217	264	7	4	0.004

* P values obtained using the non-parametric Mann-Whitney test and log variables.

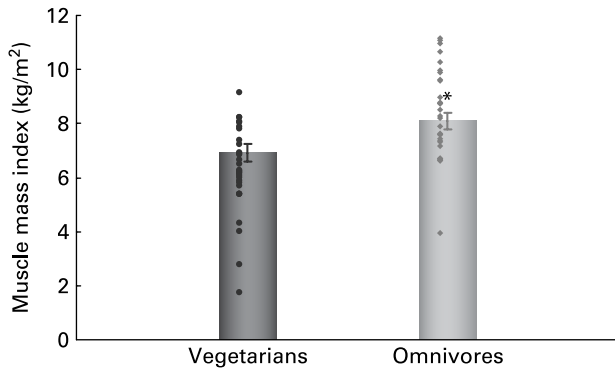


Fig. 1. Difference in muscle mass index between omnivores and vegetarians, with animal protein intake (g/kg body weight per d) as a covariable. Values are means, with standard deviations represented by vertical bars. (●), Individual data for vegetarians; (○), individual data for omnivores. * Mean value was significantly different from that for the vegetarian women ($P=0.021$).

Pearson's correlations were performed between the muscle mass index and all significant variables. We found that the muscle mass index correlated significantly with animal protein intake in g/d ($r\ 0.616$; $P=0.001$), animal protein intake in g/kg BW per d ($r\ 0.474$; $P=0.002$), the animal:plant protein intake ratio ($r\ 0.499$; $P=0.001$) and SHBG ($r\ -0.326$; $P=0.040$). We found no correlation between muscle mass index and total protein intake or plant protein intake. We also performed a partial correlation between animal protein intake (in g/d or g/kg BW per d) or the animal:plant protein intake ratio and the muscle mass index, controlling for SHBG and plant protein intake (g/kg BW per d). We observed that the muscle mass index still correlated with animal protein intake in g/d (Fig. 2; $r\ 0.601$; $P=0.001$), animal protein intake in g/kg BW per d ($r\ 0.422$; $P=0.008$) and the animal:plant protein intake ratio ($r\ 0.431$; $P=0.007$).

As a means of examining in greater depth the relationship between animal protein intake and muscle mass, we performed a stepwise linear regression analysis with age, BMI, plasma oestrone, oestradiol, testosterone, dehydroepiandrosterone, SHBG as well as total protein intake (g/d), plant protein intake (g/d), animal protein intake (g/d) and the animal:plant protein intake ratio all included in the model. We observed an absence of inter-correlation between residuals (Durbin–Watson (DW) statistic = 2.1), showing that the

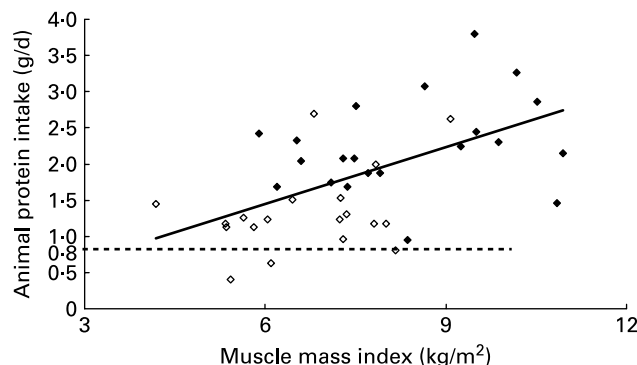


Fig. 2. Partial correlation ($r\ 0.623$, $P=0.001$) between animal protein intake (g/d) and muscle mass index, with sex hormone-binding globulin and plant protein intake as covariables. (◆), Omnivores; (◇), vegetarians; (---), protein RDA (g/d).

model presented no outliers (leverage (h) = 0.005; Cook's distance (d) = 0.001), no problem of multicollinearity between variables (variance inflation factor < 10; tolerance = 1) and that the residuals were normally distributed. Thus, the model respected the postulates of a stepwise linear regression. We found that animal protein intake (g/d) was the independent predictor of muscle mass index, explaining 42% of the variance (adjusted $r^2\ 0.42$; unstandardised coefficients: $\beta = 0.27$; standardised coefficients: $\beta = 0.6$).

Discussion

The aim of the present study was to examine the relationship between the type of dietary protein and muscle mass index in healthy Caucasian women. Even after correcting for total protein intake and SHBG concentration, which are known to be confounding variables^(9,29), we observed a significant difference in the muscle mass index and animal protein intake between omnivorous and vegetarian women. We showed that the muscle mass index is strongly associated with animal protein intake, but not with plant protein intake or total protein intake. These results are interesting because the loss of muscle mass is known to be associated with functional limitations⁽³⁰⁾. In addition, this result is in accordance with the study by Lord *et al.*⁽⁷⁾, who found a positive correlation between animal protein intake and the fat-free mass index in postmenopausal women.

Even though vegetarian groups consumed the same amount of protein, the amount of plant protein intake seems insufficient to counteract the difference in muscle mass between groups. Studies have demonstrated that skeletal muscle is a dynamic tissue that is a constant state of flux with proteins simultaneously being synthesised and degraded⁽³¹⁾. Studies have also shown that 65–80% of amino acids are re-synthesised into proteins and that the availability of amino acids is an important regulator of muscle protein turnover and metabolism⁽³²⁾. An imbalance between synthesis and degradation leads to reduced skeletal muscle mass and muscle protein content⁽³³⁾. The nutritional intake of amino acids contributes to maintain this balance⁽³²⁾. Furthermore, research has shown that the type of protein intake could influence protein synthesis and turnover^(28,31) as well as muscle mass^(34–36). The anabolic action of amino acids on muscle proteins is mainly due to the essential amino acids⁽³⁷⁾. Specifically, leucine is the most potent of the amino acids for the stimulation of muscle protein synthesis⁽³⁸⁾. Plant proteins contain a lower proportion of essential amino acids, particularly leucine, methionine, lysine and tryptophan⁽³⁹⁾. A diet containing fewer than all essential amino acids did not produce a satisfactory effect on muscle protein anabolism^(40,41). Other studies have shown that vegetarians could counteract this deficit by ingesting a combination of high-quality plant proteins such as soya, beans, whole grains and nuts containing all essential amino acids⁽⁴²⁾. Plant proteins containing all essential amino acids could compensate for the lack of animal proteins in the diet⁽²⁾. In the present study, the isoflavone intake did not reach the RDA level⁽⁴⁰⁾ and the plasma and urinary levels of isoflavones in our Caucasian women were much lower than in Asian women^(43,44). The low level of isoflavones and, consequently, the soya protein intake showed that the

vegetarians may have ingested insufficient plant proteins containing all essential amino acids. Thus, the lack of some essential amino acids in a vegetarian diet could lead to reduced protein synthesis, resulting in lower skeletal muscle mass⁽⁴⁵⁾, and could explain the difference observed in the muscle mass index between our groups^(28,37). Nevertheless, the literature on the loss of muscle mass and fat-free mass due to changes in protein synthesis is controversial⁽⁴⁵⁾.

Furthermore, the present results showed that the amount of total protein (g/kg BW per d) ingested in all groups exceeded the RDA⁽³⁾, and that vegetarian women had the same total protein intake as their omnivorous counterparts. Thus, the present results are in accordance with previous studies demonstrating that ovo-lacto-vegetarians and lacto-vegetarians have an adequate protein intake⁽⁴⁶⁾, as at least defined by the RDA⁽⁴⁷⁾. That plant proteins from cereals and legumes (beans) are digested and absorbed less than are animal proteins could explain the difference between vegetarians and omnivores in muscle mass⁽⁴⁸⁾. In fact, studies have demonstrated that some plant proteins are less bioavailable than are animal proteins for the same protein intake^(49,50). Studies have also shown that a decrease in the bioavailability of all essential amino acids could induce changes in the nutritional value of foods and specifically of plant proteins⁽⁵¹⁾. Thus, to prevent and to counteract the impact of the loss of muscle mass, it could be important to establish a specific protein RDA for vegetarians and to show that the most important factor is not the total amount of protein taken in per d but the type of dietary protein taken in. Furthermore, it is important to note that the present results appear uninfluenced by other factors (hormones; see Table 3) considered important in the regulation of skeletal muscle mass^(10,11).

The present study does have some limitations. First, it is an observational study, and it would be interesting to follow the evolution of muscle mass in omnivorous and vegetarian women. In addition, our vegetarian women included all the type of vegetarians (lacto-, ovo-lacto-vegetarians and vegans). We had a small sample size of about twenty subjects in each group, yet the findings were sufficiently precise that we were able to rule out random variation as a source of between-group differences. In addition, our ability to generalise is restricted because our data specifically concern our relatively young women. Furthermore, we had no other measurements of fat-free mass estimated from dual-energy X-ray absorptiometry, MRI scans or computerised tomography (CT) scans, considered as 'gold standard' methods for estimating fat-free mass and skeletal muscle mass. Moreover, the equations used are not specifically validated for the Finnish diet, and it could be interesting to develop specific equations for omnivores and vegetarians to predict muscle mass based on creatinine excretion. However, Proctor *et al.*⁽⁵²⁾ showed that creatinine excretion correlated strongly with dual-energy X-ray absorptiometry to estimate muscle mass in younger (r^2 0.76), middle-aged (r^2 0.72) and older (r^2 0.66) men and women. Welle *et al.*⁽²⁶⁾ demonstrated that creatinine excretion correlated closely with cross-sectional areas of the upper arm (r 0.85), thigh (r 0.88) and total fat-free mass (r 0.96) determined with MRI, and could also be used to evaluate fat-free mass in healthy women aged over 60 years (r 0.63). Furthermore, Proctor *et al.* showed that creatinine excretion was the only independent

body composition method to demonstrate a reduction in fat-free mass across age groups⁽⁵²⁾. This finding suggests that urinary creatinine excretion is sufficiently sensitive to detect age-associated changes in metabolically active muscle mass. In addition, the physical activity questionnaire used in the present study was invalidated. However, our subjects were equally active in each group and the activity was low or moderate throughout the study period. None of the participants practised physical activity with high intensity or was considered an athlete. Intense physical activity is known to potentially influence the level of creatinine and, indirectly, muscle mass^(10,53). The four 3 d urinary collection periods for each individual during each season most probably eliminated the effect of any substantial variation in creatinine excretion related to physical activity.

To our knowledge, the present study is the first to compare the effect of regular omnivorous and vegetarian diets on the muscle mass index in healthy Caucasian women. A great advantage of the present study is the collection of many samples across all four seasons and the total of 20 d dietary records. We conclude that a vegetarian diet seems to be associated with a lower muscle mass index in Caucasian women than is an omnivorous diet at the same protein intake. Furthermore, we report that a good indicator of muscle mass index in women seems to be the animal protein intake and not the total protein intake. In light of these results, it would be interesting to generalise these results and to verify the following: whether the prevalence of sarcopenic women is higher in vegetarians than in omnivores and whether vegetarian women require a higher protein intake from defined sources to maintain fat-free mass and skeletal muscle mass. Finally, further research using more precise methods for fat-free mass measurements, specifically in the vegetarian population and older women (aged 65 years or older), is required to confirm our observations.

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The authors contributed equally to the article. M. A.-L. was responsible for the statistical analysis and wrote the manuscript. H. A. was responsible for the study design, the data collection and corrected the manuscript.

There are no conflicts of interest.

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