Energy and protein metabolism in the elderly

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ENERGY AND PROTEIN METABOLISM IN THE ELDERLY

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Daphne Louise Elise Pannemans

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Contents

Chapter 1

Introduction

The absolute and relative number of elderly people is growing in most Western countries. In 1980 11.5% of the inhabitants of the Netherlands were aged 65 years or over. This percentage rose to 12.0% in 1985 and 12.9% in 1992. It is expected that this figure will increase to 14.7% in 2010 (CBS 1993). Absolute numbers are given in Figure 1.1. The effect of age-related changes of physiological functions on nutritional needs are largely unknown. The nutritional recommendations for the elderly are mainly based upon data gathered in young adults. In view of the important role nutrition plays in health and disease and the growing number of elderly people, it is an essential scientific requirement to study energy and nutrient metabolism in the elderly. Energy intake decreases with age (McGandy et al 1966, The Dutch National Food Consumption Survey 1987-1989, Garry et al 1989). Knowing that the intake of most nutrients depends on total energy intake, this lower energy intake in the elderly could lead to undesirably low intakes of protein, minerals and vitamins. The work presented and discussed in this thesis relates to energy metabolism, protein intake and protein metabolism in young and elderly men and women. In the same subjects the effect of protein intake on calcium excretion and vitamin B6 metabolism was studied in order to determine the interaction between protein intake and calcium and vitamin B6 metabolism.

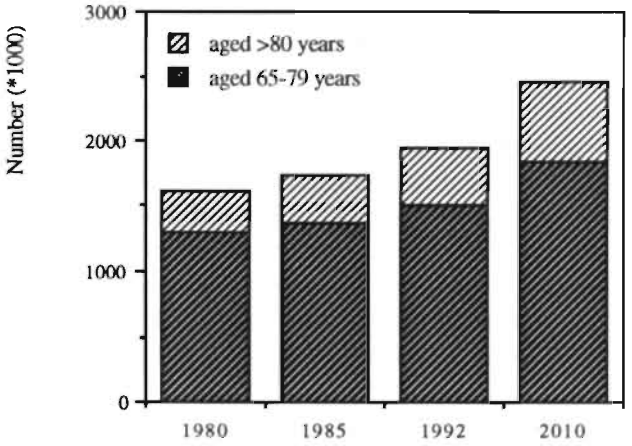

Fig. 1.1. Number of inhabitants aged 65 years or more in the Netherlands as measured in 1980, 1985, 1992 and as expected in the year 2010.

Energy metabolism and aging

In general, the average daily metabolic rate (ADMR) can be divided into three compartments: the basal metabolic rate (BMR), the diet induced thermogenesis (DIT) and the energy costs of physical activity (PA). The BMR comprises the sleeping metabolic rate (SMR) and the energy costs of arousal and can be defined as the minimum rate of energy expenditure of an awake, relaxed person lying on a bed in a thermoneutral environment after an overnight fast (Ravussin 1989). BMR accounts for 60-75% of daily energy expenditure (Poehlman & Horton 1990). The DIT is the increase in energy expenditure as a result of food intake (Garrow 1974). The increase in energy expenditure after food ingestion is due to the energy costs of digestion, transformation and storage of the ingested nutrients. The DIT makes up about 10% of total daily energy intake (Poehlman & Horton 1990). Energy expenditure for PA is the most variable component contributing to ADMR. In inactive humans the energy expended on PA is about 15% of ADMR while this can be 30% or even more in individuals who are regularly engaged in exercise. At energy balance energy intake will equal the ADMR. When energy intake exceeds energy expenditure, energy balance is positive, leading to an increase in body weight. When energy intake is lower than the energy expended, energy balance is negative, leading to weight loss.

Basal metabolic rate

Many studies have demonstrated that BMR decreases with age. Keys et al (1973) measured BMR and fat free mass (FFM) in 63 young men (22±2 y) and in 115 middle aged men (50±3 y). Young men were restudied after 19 years while middle aged men were restudied after 17 years. In the young men average BMR decreased by 3% per decade (9% when expressed per kilogram body weight). BMR of the middle aged men decreased by 1% per decade (3% when expressed per kilogram body weight). It was concluded that the decrease in BMR in the younger group was the effect of changes in body composition (increasing fat mass (FM) and decreasing FFM). In the middle aged men no differences in body composition were measured over a period of 17 years, explaining the relatively constant BMR values. The same conclusion was drawn by Robinson et al (1975) who measured BMR in young men (18-22 y) and repeated these measurements after 22 or 32 years. BMR declined with age, most probably due to the loss of FFM and the increase of FM. Webb et al (1975) measured SMR in male subjects (19-63 years). There was a linear decrease in SMR with age. Unfortunately body composition was not measured. Schofield (1985) described formulas to predict BMR from weight (and height) for six separate age groups (0-3 y; 3-10 y; 10-18 y; 30-60 y; and over 60 y) based on data revealed by reviewing previous work. Predicted BMR values decreased with age, however, equations for estimating BMR in the elderly were based on a rather small sample size, since no more data were available. In the Baltimore Longitudinal Study (1959-1975) BMR was measured in 959 subjects (Tzankoff & Norris 1977). Until the age of 45 years no change in BMR was seen. After the age of 45 BMR decreased significantly. The

authors concluded that the decrease in muscle mass was responsible for the age-related decrease in BMR. Other authors also reported lower BMR values by comparing elderly subjects compared with younger subjects and the similarity of BMR values in both age groups after adjusting for FFM (Calloway & Zanni 1980, Bloesch et al 1988, Poehlman et al 1990).
However, recently some authors have reported that the differences in FFM between young and elderly subjects cannot fully explain the lower BMR in the elderly. Vaughan et al (1991) measured BMR in young adults and in elderly subjects. BMR was significantly lower for the elderly compared with the young subjects even when adjustments were made for FFM. Fukagawa et al (1990), measured BMR and body composition of 24 young men, 24 elderly men and 20 elderly women. BMR was significantly lower for the elderly men compared with the young men even when adjustments were made for FFM. No differences in BMR were found between elderly men and elderly women when corrections were made for differences in FFM. They concluded that BMR was lower for the elderly subjects compared with the young adults, even after adjusting for differences in FFM, suggesting that aging is associated with an alteration in tissue energy metabolism. Fukagawa et al (1990) also suggested that reduction in physical exercise in the elderly may contribute to their observations. This suggestion was confirmed by Poehlman et al (1991) who examined the effect of age and habitual physical exercise on BMR in inactive and active young men and in inactive and active elderly men. Inactive elderly men had a lower BMR, when corrected for differences in FFM, compared with active younger and older men. It was suggested that regular participation in aerobic exercise may attenuate the age-related decline in BMR.by attenuating the loss of skeletal muscle mass. Since Meredith et al (1989) found no increase in BMR after an exercise intervention period of 12 weeks in elderly men, it was suggested that prolonged periods of exercise may be necessary before alterations in BMR can be observed in the elderly.
It can be concluded that BMR decreases with age and that the decrease is closely related to the age-related changes in body composition. However it is unclear whether the decrease in FFM fully explains the lower BMR in the elderly. It has been suggested recently that a more physical active lifestyle in the elderly can possibly partially prevent the age related decrease in BMR.

Diet induced thermogenesis

There are few data available on the effect of age on DIT. Only cross-sectional studies have been performed to examine the effect of aging on DIT. Golay et al (1983) and Bloesch et al (1988) measured glucose-induced thermogenesis in young and elderly subjects. In both studies, DIT was significantly lower in the elderly individuals compared with the young adults. It was speculated that the insulin resistance associated with aging contributed to the lower DIT in the elderly. Morgan &York (1983) studied DIT in young and elderly men in response to two meals with different energy contents. DIT was significantly higher for the young adults compared with the elderly. This was particularly pronounced with the higher energy meal (4 MJ), sug-

gesting that there might be a reduction in the maximum thermogenic response to a meal with age. Schwartz et al (1990) also reported a lower DIT in older subjects compared with young subjects. The authors pointed at the important role of the sympathetic nervous system in DIT since part of the DIT in young men can be accounted for by the meal induced increment in sympathetic nervous system activity. A blunted responsiveness to sympathetic stimulation in the elderly might account for the lower DIT compared with young subjects. Besides the age difference in insulin resistance, the maximum thermogenic response and the sympathetic nervous system activity, it was recently suggested that the level of PA might play a role in the age related difference in DIT (Lundholm et al 1986, Schutz et al 1987, Poehlman et al 1991). Lundholm et al (1986) studied the effect of a liquid meal on DIT in well-trained and sedentary elderly men. DIT was significantly higher for the well-trained subjects probably due to an increased sensitivity of the sympathetic nervous system as a result of the well trained condition. On the other hand, Poehlman et al (1991) found no age related differences in DIT. However they showed differences in DIT between active young and elderly men compared with inactive young and elderly men. These results suggest that PA and not age per se is the influencing factor on DIT in this study. Another study reported the acute effect of PA on DIT in elderly men (Schutz et al 1987). There appears to be no effect on DIT when elderly individuals exercise after ingesting a meal.
Results on the effect of age on DIT are equivocal, although most of the results pointed to a decrease in DIT with age but the difference between older and younger adults was not large. It should also be noted that the size, frequency and composition of the meals affect the DIT, making it difficult to compare the separate studies on the possible impact of aging. The reasons for the age-related differences in DIT are not known. Based on the literature it is speculated that the differences in DIT are caused by differences in insulin resistance, maximum thermogenic response, sympathetic nervous system activity, and PA.

Physical activity

In the past, several studies have reported on the level of PA in elderly subjects estimated by questionnaire or interview (Laporte et al 1983, Dallosso et al 1988, Donahue et al 1988, Voorrips et al 1990, Caspersen et al 1992, Dipietro et al 1993). When comparisons were made with activity levels of younger subjects it was generally concluded that PA decreases with age.
However, the validity of PA questionnaires and interviews is still under debate. In the past decade the doubly labelled water technique has proven to be a very reliable method to measure ADMR under free living conditions. By simultaneous measurement of BMR, it is possible to assess the energy expenditure of daily physical activities by expressing ADMR in multiples of BMR or by expressing the energy expended on PA (plus thermogenesis) as ADMR minus BMR. Only a few studies have reported ADMR in elderly subjects measured with doubly labelled water (Goran & Poehlman, 1992; Roberts et al 1992; Reilly et al 1993). Results of these studies are summarized in Table 1.1.

Table 1.1. Average daily metabolic rate and activity level of elderly subjects (mean±SD)

Reference	N	Sex	Age (y)	BMR (MJ/d)	ADMR (MJ/d)	Activity* (x BMR)	Activity** (MJ/d)
Goran et al (1992)	7	m	68±6	7.18±0.77	11.20±1.65	1.58±0.31	2.90
	6	f	64±5	6.16±0.54	8.76±0.97	1.43±0.23	1.72
Reilly et al (1993)	10	f	73±3	5.11±0.38	9.22±1.48	1.80±0.19	3.18
Roberts et al (1992)	15	m	69±7	5.98±0.66	10.44±1.47	1.75±0.05	3.42

*ADMR/BMR; **ADMR-10% of ADMR-BMR

Goran & Poehlman (1992) reported a wide range of ADMR values among elderly men and elderly women (7.77-13.40 MJ/d). The wide range of ADMR values was explained by level of PA (10-43% of ADMR). Furthermore physical activity (PA) (MJ/d) was negatively correlated with FM ($r=-0.58$; $P<0.05$). Reilly et al (1993) reported much higher activity levels for elderly subjects compared with results of Goran and Poehlman, indicating that the subjects involved in the study were highly physically active, in accordance with the activity questionnaire score. In the study of Roberts et al (1992), also high levels of PA where reported although in this study subjects were characterized as sedentary on the basis of a PA questionnaire. As in the study of Goran & Poehlman, there was a wide range in activity levels (1.2-2.1 x BMR). And in this study also a negative correlation was found between activity level and FM ($r= -0.53$; $P<0.05$).

In order to study whether with increasing age movement efficiency or intensity of the activity decreases, which might affect ADMR, some authors examined the energy costs of specific activities and the time needed to perform specific activities (McGandy et al 1966, Himann et al 1988). Recently Voorrips et al (1993) measured energy expenditure during sitting, sitting with standardized arm activity and walking on a treadmill at 3 km/h in 28 elderly women and 29 middle aged women. Energy expenditure during sitting and sitting with standardized arm activity did not differ between the groups. While the energy costs of walking were higher for the elderly women, suggesting that elderly women walk less efficient. Didier et al (1993) measured energy expenditure of some daily activities in 10 young subjects and in 10 old subjects. Activities were: rising and sitting back on a seat, getting up from and lying down on a bed and getting up from the floor. Energy expenditure and the time necessary for the activities were measured simultaneously. When rising and sitting back down on a seat, elderly subjects expended less energy per kg body weight, while there were no differences in time. Getting up from and lying down on the floor or bed involved the same energy expenditure but took significantly longer for the elderly. However these data are limited and the results are contradictory partly due to the variety of activities that were measured.

From the available data it can be concluded that the energy expended on PA decreases with age, however, data are limited and more research is needed.

Energy intake

As described above, ADMR will decrease with age due to a decrease in BMR, a probably lower DIT and a decrease in PA with age. As a result, energy intake (EI) will decrease. McGandy et al (1966) reported that total EI declines progressively from 11.3 MJ/d in 30 year old subjects to 8.8 MJ/d for those around 80 years; a decrease of 2.5 MJ/d (22%). The Dutch National Food Consumption Survey (1987-1989) also found a decrease in EI with age. Mean EI was 10.4 MJ/d for subjects aged 22-49 years while EI of subjects age >65 was 9.0 MJ/d. Longitudinal results reported by Garry et al (1989) also show a decline of EI in elderly men and women. Elderly subjects may maintain body weight on a relatively low EI simply because they have a low or a very low energy expenditure. Because the intake of most nutrients is strongly related to total EI (van Erp-Baart et al 1989a, van Erp-Baart et al 1989b), elderly people who maintain body weight with a small intake of food may be consuming undesirably low amounts of protein, minerals and vitamins. Especially the very inactive elderly are at risk for malnutrition.

Protein metabolism and aging

The current recommended protein intake for the elderly (aged 65 years or older) is 0.75 g per kg per day as given by the FAO/WHO (1985). The Dutch Nutrition Council recommends 11-12% energy from protein or 0.80 and 0.85 g protein per kg per day for women and men respectively, at the average body weight (men: 70 kg; women 65 kg) and energy expenditure for the elderly (men: 126 kJ/kg; women 120 kJ/kg). This recommended protein intake for the elderly is mainly based on extrapolations of data obtained from healthy young adults obtained with the nitrogen balance technique. Furthermore it is known that subjects can be in nitrogen balance within a wide range of protein intakes. This raises the question not only of what is the minimum requirement of dietary protein but also what is the optimum intake. The nitrogen balance technique gives no information about this optimum. Nitrogen balance is a reflection of overall body protein synthesis and breakdown (protein turnover), and can be achieved within a wide range of protein synthesis and breakdown rates (Fig.1.2.). How rates of body protein synthesis and breakdown are affected by dietary intake is therefore an important step in understanding the metabolic significance of differences in dietary intake.

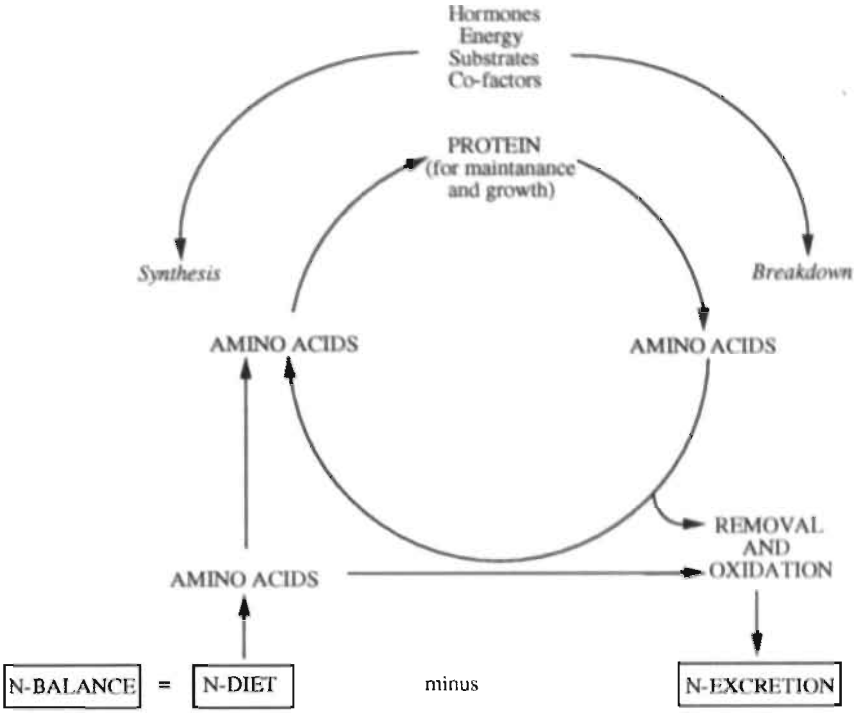

Fig. 1.2. Body and organ protein content is determined by the balance between the rates of protein synthesis and breakdown and each of these phases of protein metabolism is influenced by factors including hormones, substrate (amino acids; nitrogen) and energy supply. A given nitrogen balance can be achieved within a wide range of protein synthesis and breakdown rates. (Adapted from Young et al (1981))

Nitrogen balance

Protein requirement has been estimated using the nitrogen balance method. This method involves feeding different amounts of protein to determine the minimal amount required to balance the nitrogen losses in urine, faeces and sweat, together with minor losses by other routes. In most studies the nitrogen losses in urine and faeces were measured while corrections were made for losses via other routes. In a few studies minimum protein requirements in elderly subjects were measured by means of the nitrogen balance (Cheng et al 1978, Uauy et al 1978, Zanni et al 1979, Gersovitz et al 1982). Cheng et al (1978) determined protein requirements in 15 elderly subjects and recommended a protein intake level of 0.8 g protein/kg.day. Uauy et al (1978) reported the same recommendation for elderly women (0.83 g protein/kg.d) while they established the protein intake for elderly men on 0.70-0.85 g/kg.d. In another study it was estimated that the protein requirements for elderly men were 0.72 g/kg.d (Zanni et al 1979). Gersovitz et al (1982) studied the long term effects of a protein intake of 0.8 g/kg.d (as recommended by the FAO) on nitrogen balance in elderly men and women. Since most of the subjects were in negative nitro-

gen balance it was concluded that the current recommended protein intake was too low for the majority of subjects aged 70 years or more.

Protein turnover

As pointed out earlier it is interesting to study the effect of the daily protein intake on the whole body protein turnover to understand more about the metabolic significance of differences in dietary intake. Protein turnover can be measured with isotopes. Several methods of measuring total protein turnover in humans are available. The methods can differ with respect to the tracer (radio-isotopes versus stable isotopes), the labelled amino acid used (e.g. ^{14}C-leucine, ^{15}N-glycine), whether the tracer is administered orally or intravenous (in one bolus, with intermittent infusion or, with constant infusion) and whether measurements are done in plasma and/or in excreted end products (e.g. excreted urea and ammonia). The methods and their (dis)-advantages are summarized elsewhere (Waterlow et al 1978). There are only a few reports of whole body protein turnover measurements in the elderly (Winterer et al 1976, Uauy et al 1978, Robert et al 1984, Golden et al 1977). Results are summarized in Table 1.2. Whole body protein turnover expressed per unit body weight seems to decrease with age (Uauy et al 1978, Robert et al 1984, Golden et al 1977) but when expressed per unit FFM no differences were seen between young and older subjects. One study reported a decreased leucine flux (g/kg) for elderly women when compared with younger women (Winterer et al 1976). In the same study no differences were found between young and old men. In all of these studies measurements were done on small groups and no distinction was made between active and inactive subjects or between men and women in the same age group. It can be concluded from the published studies, that protein turnover decreases with age, however it is not known whether this is due to changes in body composition (decreased FFM with advancing age) or changes in PA level with age or whether there are gender differences.

Protein intake and protein turnover

Only little is known about the effect of an increased protein intake on protein turnover. Motil et al (1981) studied the effect of protein intake on whole body leucine and lysine metabolism in young men. It was concluded that a change in protein intake from marginal (0.6 g/kg.d) to a surfeit level (1.5 g/kg.d) was associated with an increased leucine and lysine flux. Gersovitz et al (1980) reported the effect of protein intake on glycine metabolism in young and elderly men. Protein intake was adequate (1.5 g/kg.d) or inadequate (0.4 g/kg.d). At the higher protein intake whole body glycine flux was significantly higher (in young and elderly men) when compared with the inadequate protein intake. Recently Garlick et al (1991) reviewed the studies about the influence of dietary protein intake on whole body protein turnover in young adults. It was concluded that dietary protein affects protein turnover at two levels. Firstly an immediate response to the intake of protein in meals. An increase in the protein content of a meal enhanced the response to this meal mainly consisting of a decreased protein breakdown. Secondly, a long term adaptation (1-2 weeks) after a change in

protein intake. The adaptation to higher protein intakes results in an increase in the basal (postabsorptive) rates of both synthesis and degradation. Only speculations can be made about the implication of a higher basal protein turnover. If an increased protein turnover involves an increase in the contribution of the muscle protein turnover, than this probably means that one is better equipped for an unfavourable nutritional status or to other stressful conditions, since muscles contribute to the adaptation in whole body energy and amino acid metabolism during restricted dietary energy and protein intakes (Young 1990). However, the available data, on the effect of protein intake on protein turnover are limited and there is a need for more systematic investigations especially in elderly subjects.

Table 1.2. Whole body protein turnover

Reference	N	Age	Tracer	Method	Diet	Conclusion
Winterer et al (1976)	4 men 5 women 4 men 5 women	65-91 65-91 18-25 18.25	^{15}N-glycine	Orally at 3h intervals for 60 h	1-1.5 g/kg.d protein in 4 meals	Whole body protein turnover decreases with age
Uauy et al (1978)	6 men 5 women 4 men 4 women	68-72 67-91 20-25 18-23	^{15}N-glycine	Orally at 3h intervals for 60 h	1-1.5 g/kg.d protein in 4 meals	Whole body protein turnover is lower in the elderly
Robert et al (1984))	6 men 4 women 10 men 5 women	74±1 76±3 25±1 22±1	^{13}C-leucine	intravenous during 5 h	postabsorptive	Flux is comparable in young and elderly men and lower for elderly compared with young women
Golden et al (1977)	6 subjects	elderly	^{15}N-glycine ^{14}C-leucine	intravenous/ orally 30 h	continuous infusion	Protein turnover is lower in the elderly

Calcium metabolism

There are large discrepancies in the dietary calcium recommendations of national nutrition councils (Miller 1989). In the United States and Canada the recommended dietary allowance for calcium is 800 mg per day for men and women aged 50 years and older. In the United Kingdom the recommended calcium intake is 500 mg per day for the same age groups. In Japan 600 mg calcium per day is recommended for individuals aged 50 years and more, while the Dutch Nutrition Council recommends 800-1000 mg per day. The lowest daily dietary allowance for calcium is given by the WHO (400-500 mg per day for men and women aged >50 years). These allowances are primarily based on calcium balance data (measuring the balance between calcium intake and calcium excretion). The problem is that subjects can reach calcium balance

at a wide range of calcium intakes. However, there is no other method available for determining the calcium requirement. An additional problem in measuring the calcium requirement, is the differences in diet composition. It has been argued that the level of protein (and phosphorus intake) can markedly affect the calcium balance.

Calcium and protein interactions

Increased urinary calcium loss with increased dietary protein is frequently reported, sometimes leading to a negative calcium balance (Johnson et al 1970, Walker & Linkswiler 1972, Anand and Linkswiler 1974, Allen et al 1979, Schuette et al 1980, Hegsted et al 1981). The calciuretic effect of protein is attributable to an increased glomerular filtration rate and filtered calcium load and to a decreased tubular calcium reabsorption (Hegsted & Linkswiler 1982). Dietary phosphorus on the other hand reduces the urinary excretion of calcium by increasing the renal tubular reabsorption and counteracts, at least in part, the calciuretic action of protein. Spencer et al (1978a) reported a decreased urinary calcium excretion with a higher phosphorus intake, regardless of calcium intake. It was suggested that commonly used complex proteins, which contain phosphorus, do not cause calcium loss (Spencer et al 1988). The same group described the effect of high protein (meat) intake on calcium excretion, absorption and retention in male patients (age: 30-67 y) and concluded that a high protein intake, given as meat (complex proteins) does not lead to hypercalciuria and does not induce calcium loss (Spencer et al 1978b, Spencer et al 1983). Although this may be valid for young healthy adults it may not be so in healthy elderly people. Only one study described the protein induced hypercalciuria in older men and women, but no comparisons were made with young adults (Schuette et al 1980).

Vitamin B6 metabolism

The principle metabolic function of vitamin B6 is in amino acid metabolism although vitamin B6 is also associated with glycogen phosphorylase in the muscle, furthermore it has an important role in in the actions of steroid hormones (Bender 1989). The recommended daily amounts of vitamin B6 in various countries vary between 1.5-2.2 mg vitamin B6/d (Bender 1989). However because of the important role vitamin B6 plays in amino acid metabolism vitamin B6 requirements are often expressed per gram protein intake. An allowances of 15 µg/g protein is the basis of the recommended intakes for most countries, although the recommendations in the United States, Australia and the Netherlands are 20 µg/g protein for adult men and women. Several studies investigated the vitamin B6 status in relation to aging. The effect of protein intake on these status parameters was also studied.

Vitamin B6 status and aging

There are several methods to monitor the vitamin B6 status: measurement of plasma pyridoxal phosphate (PLP), coenzyme saturation tests in vitro, metabolic loading tests or measuring urinary 4-pyridoxic acid (4-PA) excretion. These methods are frequently used to measure the effect of aging on vitamin B6 status.

1 Measuring plasma pyridoxal phosphate (PLP).
The plasma level of the metabolically active vitamin B6 vitamer, PLP, seems to provide a reasonable indication of the nutritional status, although it is markedly affected by recent vitamin B6 intake. Rose et al (1976) reported a decline in plasma PLP with age of 3.6 nmol/l per decade. Others also report a decrease in plasma PLP with age (e.g. Guilland et al 1984, Hamfelt & Söderhjelm 1988, Kant et al 1988, Löwik et al 1989, Manore et al 1989, Tolonen et al 1988).

2 Coenzyme saturation tests in vitro.
The coenzyme saturation test is measuring the concentration of PLP-dependent enzymes (e.g. alanine aminotransferase and aspartate aminotransferase) before and after in vitro incubation with a saturating amount of PLP. Results are usually expressed as an activation coefficient, calculated as the ratio of enzyme activity with and without PLP added. Coenzyme saturation tests in young and elderly subjects show conflicting results, partly due to the lack of generally accepted ranges of adequate and deficient values (Porrini et al 1987, Guilland et al 1984, Vir & Love 1979).

3 Metabolic loading tests.
Metabolic loading tests determine the extent to which a subject is able to metabolize a test dose of a physiological substrate with a PLP dependent metabolism (e.g. tryptophan and methionine). Vitamin B6 deficiency gives rise to urinary excretion of metabolites in abnormal ratios. Recently, Ribaya-Mercado et al (1991) measured vitamin B6 requirements of elderly men and women by means of a tryptophan loading test. After a vitamin B6 depletion phase, xanthurenic acid levels returned to baseline at a vitamin B6 intake level of 1.96 and 1.90 mg/d for elderly men and women respectively.

4 Measuring urinary 4-PA excretion.
4-PA is the metabolic end product of vitamin B6 and the urinary excretion of 4-PA is used as a noninvasive vitamin B6 status parameter. Results on age related differences in urinary 4-PA excretion are equivocal. Lee & Leklem (1985) reported higher urinary 4-PA values on normal vitamin B6 intakes for middle-aged women compared with young women, while Kant et al (1988) found no differences in 4-PA excretion in 3 male age groups before and after an oral vitamin B6 load. The differences in results are probably due to the fact that 4-PA reflects recent vitamin B6 intake rather than the underlying state of tissue reserve (Lui et al 1985).

In summary, there are indications for differences in vitamin B6 status between young adults and the elderly: plasma PLP concentrations decrease with age, urinary 4-PA excretion seems to increase with age, while it is unclear whether there are any changes in the activation coefficient of the coenzyme saturation tests, with age. Several sug-

gestions were made to explain the decrease in plasma PLP. First, the lower PLP intake with aging could attribute to a decreased plasma PLP, however, differences were also found when dietary intake was the same in young and elderly subjects. Second, the decrease in plasma PLP could be explained by the increase of plasma alkaline phosphatase activity with age, since the plasma alkaline phosphatase activity is inversely related to the plasma PLP concentration (Kant et al 1988). Third, it was suggested that a decrease in vitamin B6 absorption with age could attribute to the lower PLP concentrations with aging. This seems to be unlikely, since elderly and young subjects show a comparable increase in PLP, when an orally dose of vitamin B6 was given (van den Berg et al 1992; Shultz & Leklem 1985; Ubbink et al 1987), suggesting no effect of age on vitamin B6 absorption. Fourth, it was recently hypothesized that the age dependent decrease in plasma PLP content could be associated with a decrease in tissue body stores due to changes in body composition and/or an effect on PL(P) release from (muscle) protein due to a decrease in protein turnover (Bode & van den Berg 1991a, Bode & van den Berg 1992). It was suggested that vitamin B6 metabolism changes with age. It is unclear whether these changes influence vitamin B6 requirement.

Vitamin B6 and protein interactions

Vitamin B6 requirements for humans are related to the level of dietary protein intake since PLP catalyses a number of biochemical reactions integral to nitrogen metabolism. In the earlier vitamin B6 depletion/repletion studies of Baker et al (1964) a relationship between vitamin B6 requirement and protein intake was demonstrated in young men. Miller et al (1985) confirmed these findings by showing an increased vitamin B6 retention, for increased catabolism of amino acids, in young males with increased intake of dietary protein. Only one study reported the effect of protein intake on vitamin B6 requirement in elderly subjects (Ribaya-Mercado et al 1991). They concluded that elderly subjects required more vitamin B6 to reach baseline vitamin B6 status parameters. It was assumed that increasing the protein intake would lead to an increased retention of PLP (probably in the liver), due to an increased PLP binding to enzymes involved in amino acid metabolism. As a result of the increased PLP retention, plasma PLP would decrease leading to a decreased excretion of urinary 4-PA. More research into the age dependent relationship between indicators of the vitamin B6 status and protein intake is needed.

Interactions with physical activity

The level of PA seems to have a positive effect on BMR, DIT and total energy expenditure as mentioned before. Furthermore it is suggested that PA has an effect on protein turnover. Yarasheski et al (1993) reported increased muscle protein synthesis in elderly men after two weeks of exercise. Calcium metabolism is also effected by the level of PA. Evans & Meredith (1989) reviewed the effect of PA on calcium

metabolism. It was suggested that exercise reduced the rate of bone loss and increased total body calcium and vitamin B6 metabolism. Vitamin B6 metabolism is also affected by exercise. Exercise induced higher plasma PLP values, lower 4-PA excretion in the urine (Dreon & Butterfield 1986, Manore et al 1987). Furthermore, a higher PA level will lead to a higher ADMR. At energy balance, this will lead to a higher EI. As mentioned before, the intake of most nutrients depends on total EI (van Erp-Baart et al 1989a, van Erp-Baart et al 1989b). The increased EI reduces the risk of deficient intakes of protein, calcium and vitamin B6 intake.

Summary

Reviewing the literature with respect to the energy metabolism, it can be concluded that ADMR decreases with age. However more data on total energy expenditure, energy expenditure at rest and energy expenditure during PA are needed since there are many uncertainties. Firstly, BMR decreases with age and is closely related to the age-related changes in body composition. However it is unclear whether the decrease in FFM fully explains the lower BMR in the elderly. It is been suggested recently that other factors, e.g. decreased habitual PA, may contribute to the lower BMR in elderly. Secondly, the results on the effect of age on DIT are equivocal. Furthermore there is no explanation for the age-related differences in DIT. It is speculated that the differences in DIT are caused by differences in insulin resistance, maximum thermogenic response, sympathetic nervous system activity, and PA. Thirdly, it can be concluded that the energy expended on PA decreases with age, however data are limited, and more research is necessary. Furthermore there is much uncertainty about the energy costs of specific activities in elderly subjects and again data are limited and the results are contradictory.

Summarizing the literature with respect to protein metabolism it can be concluded that protein turnover decreases with age, however it is not known whether this is due to changes in body composition with age or changes in PA level and whether there are gender differences. Furthermore, an increase in protein intake results in an increase in the basal (postabsorptive) rates of both protein synthesis and protein degradation. However, the available data, on the effect of protein intake on protein turnover are limited and there is a need for more systematic investigations especially in elderly subjects.

Reviewing the literature with respect to the interaction between protein intake and calcium excretion, it can be concluded that an increased protein intake leads to an increased urinary calcium loss. On the other hand dietary phosphorus reduces the urinary excretion of calcium by increasing the renal tubular reabsorption and counteracts, at least in part, the calciuretic action of protein. Although this may be valid for young healthy adults it may not be so in healthy elderly people.

The literature with respect to vitamin B6 status in young and elderly subjects indicates that vitamin B6 status changes with age: plasma PLP concentrations decrease with

age, urinary 4-PA excretion seems to increase with age, while it is unclear whether there are any changes in EAST-activation coefficient with age. Furthermore, it was assumed that increasing the protein intake would lead to an increased retention of PLP, due to an increased PLP binding to enzymes involved in amino acid metabolism. As a result of the increased PLP retention, plasma PLP would decrease leading to a decreased excretion of urinary 4-PA. There is only one study on the effect of protein intake on vitamin B6 status in the elderly. More research into the age dependent relationship between indicators of the vitamin B6 status and protein intake is needed.

Outline of the thesis

The studies described in this thesis were intended to obtain more information on the effect of age on energy metabolism and protein metabolism. It was hypothesized that a reduced PA in the elderly leads to a decreased energy and protein intake which in turn could lead to a decreased protein turnover rate, especially in inactive elderly subjects. In order to determine the interaction between protein intake and protein metabolism, calcium metabolism and vitamin B6 metabolism, the effect of the level of protein intake on protein turnover, calcium excretion and vitamin B6 metabolism was studied in young and elderly subjects with a known activity level.

Since data on EI are often used as a basis for nutrition intervention studies, it is necessary to estimate EI accurate. Chapter 2 describes two methods for measuring EI in elderly subjects in order to recommend an intake to maintain energy balance. After measuring the EI, subjects were fed according to this intake and energy expenditure was measured under free-living conditions to verify the estimates of EI.

Only a few studies are known describing ADMR in elderly subjects as measured under free living conditions. Data on ADMR give useful information on total energy expenditure under free living conditions. Chapter 3 reports on the total energy expenditure and activity level in a group of healthy elderly and young adults under free-living conditions. Since it is still uncertain whether the decrease in FFM fully explains the lower BMR in elderly subjects and because it has been suggested recently that a more physical active lifestyle in the elderly can possibly partially prevent the age related decrease in BMR, the age related differences in body composition and their relation to BMR and activity level are described.

In order to study whether there is a change in energy costs of specific activities with increasing age, the energy costs of controlled daily activities in young and elderly men were measured under experimentally controlled conditions. Results are described in Chapter 4. In addition the age related differences in body composition and their relation to the SMR are also discussed for reasons mentioned above.

As pointed out earlier it is interesting to study the effect of the daily protein intake on the whole body protein turnover to understand more about the metabolic significance of differences in dietary intake. Chapters 5 and 6 report on the effect of the level of

protein intake on whole body protein turnover in elderly and young adults who had a known activity level ranging from sedentary to very active. Comparisons were made between men and women.
Because, both calcium and vitamin B6 metabolism are closely related to the amount of protein intake, the influence of the level of protein intake on the urinary calcium excretion, calcium absorption and calcium balance in young and elderly subjects is described in Chapter 7. Chapter 8 reports on the effect of dietary protein on vitamin B6 metabolism in young and elderly subjects. The age dependent relationship between some indicators of the vitamin B6 status and protein turnover are described.

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Chapter 2

Estimation of energy intake to feed subjects at energy balance as verified with doubly labelled water: a study in the elderly

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Abstract

A study was intended to estimate energy intake (EI) for a nutrition intervention study. Subjects were 17 elderly men (age: 72±5) and 11 elderly women (age: 67±4). Two methods were used to measure EI: a 4-day dietary record (DR-group; n=12) or a dietary questionnaire (DQ-group; n=16). Subjects were fed for three weeks according to this intake during an intervention period and energy expenditure (EE) was measured with doubly labelled water to verify the resulting figure for EI. Body weight and body composition were measured at the beginning of the second week and at the end of the third week and metabolizable energy (ME) was calculated as EI minus energy in faeces and urine. EI, ME and EE do not differ between the DR and DQ-group (mean±sd in MJ/d EI: 10.09±1.21 and 9.29±1.36; ME: 9.09±1.28 and 8.34±1.31; EE: 10.13±1.57 and 9.25±0.35). In both groups ME was significantly lower than EE (DR-group $p<0.05$; DQ-group $p<0.01$. Body weight decreased significantly during the intervention period (mean±sd in kg: DR-group:-0.64±0.50,$p<0.001$; DQ-group: -0.86±0.90, $p<0.01$) The change in body weight was significantly correlated with energy balance (ME-EE; $p<0.05$). As shown from the results of the body composition measurements body weight loss was a decrease of fat mass (FM). In conclusion: Energy intake as measured with a four-day dietary record or with a dietary questionnaire underestimates energy expenditure in elderly men and women. Discrepancy between EI and measured EE is higher in subjects with a higher body mass index ($p<0.05$).

Introduction

Data on energy intake (EI) are often used as a basis for nutrition intervention (Peterson 1992, Westerterp et al 1992, Riumallo et al 1989). Several methods for measuring EI are available. These methods can be divided into retrospective and prospective ones. Retrospective methods include dietary history, frequency of intake and dietary recall. Prospective methods include (weighed) dietary record for 3 or more days and duplicate meal preparation and analysis. Retrospective methods do rely on the subjects memory whereas the prospective methods do not have this problem. Although the dietary record is regarded as the best method to assess EI (Black et al 1991) it has the disadvantage that the subjects have to write down everything they eat for three or more days. This can influence their feeding habits.
Energy balance is reached if EI equals energy expenditure (EE) (EI=EE). If this is not the case the energy stores will change eventually resulting in body weight changes. When subjects are in positive energy balance (EI>EE) body weight will increase, if the opposite is true (EI<EE) body weight will decrease because of negative energy balance.
The purpose of the present study was to assess the EI of elderly men and women in order to feed them in energy balance. With respect to the elderly particular problems can occur in applying the above mentioned methods for measuring EI such as poor vision, hearing and recent memory. Therefore EI was measured with a 4-day dietary record, the golden standard, or a dietary questionnaire as simple and timely as possible. After measuring EI, subjects were fed according to this intake and EE was measured with doubly labelled water to verify the resulting figure for EI.

Subjects and methods

Subjects
Subjects were 28 free-living elderly volunteers. Mean age, height, weight and body mass index (BMI) are presented in Table 2.1. Subjects were recruited with advertisements in local media, and through contacts with alliances for elderly. All subjects were certified to be in good health by a staff physician and gave informed consent to participate in the study after the procedures were explained to them. The protocol was approved by the university ethics committee.

Table 2.1. Physical characteristics of the subjects (mean±SD; range)

N	Sex	Age (y)	Height (m)	Weight (kg)	BMI (kg/m^2)
17	M	72±5	1.72±0.09	73.8±12.2	24.9±3.2
		(65-80)	(1.60-1.91)	(56.5-102.7)	(18.7-28.9)
11	F	67±4	1.61±0.08	67.6±9.2	26.2±3.2
		(63-70)	(1.49-1.72)	(55.0-89.7)	(21.1-32.6)

Experimental design

Subjects were asked to fill in a four-day dietary record or a dietary questionnaire in order to estimate their usual daily EI. Four to six weeks later, when the data were processed and EI was calculated, subjects participated in a nutrition intervention study in which they were fed in energy balance for a period of three weeks based on the results of the dietary intake measurements (see below). During this period all meals, consisting of breakfast, lunch, dinner, beverages and drinks were served at home. The first week was a baseline period in which corrections of intake could be made. Figure 2.1. is a schematic representation of the experimental design. Energy balance was determined during the last two weeks by measuring EI, EE, metabolizable energy (ME) and body weight changes (Δweight).

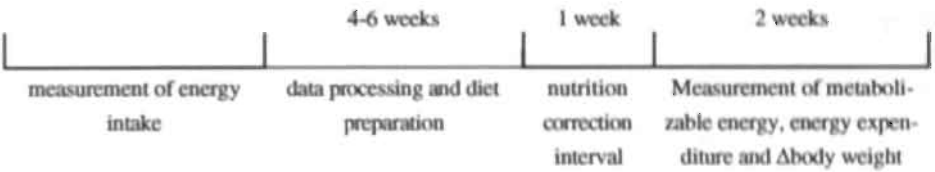

Fig. 2.1. Experimental design

Measurement of energy intake

To estimate the usual EI, subjects were asked to fill in a four-day dietary record (DR-group; n=12) or to answer a dietary questionnaire (DQ-group; n=16). The dietary record was instructed by a dietician by filling in the first day together with the subject. Amounts consumed were recorded in household units, by volume or by weight. Recipes of home-prepared meals were also recorded. The records consisted of three week days and one weekend day. Afterwards the subjects were seen for a second appointment. Then the food record was checked by the dietician for accuracy, completeness and clarity. With a computer program based on food tables the food records were converted into intakes of total energy. The dietary questionnaire asked usual bread consumption (in slices per day), potato consumption (in g per day) and sweet consumption (often, sometimes or never).

Nutrition intervention

For the purpose of the study six energy groups (Table 2.2.) were made with a fixed amount of energy in three product groups: bread plus butter, marmalade and meat; potatoes plus vegetables, meat and gravy; yoghurt, sugar, fruits, juice, milk and cake. Subjects were placed in the best fitting energy group based on the results of the EI measurement with either the dietary record or the dietary questionnaire. With respect to the dietary questionnaire subjects were placed in the best fitting energy group on the basis of bread and potato consumption. If sweet consumption was high it was used to

make a correction to a higher energy group if sweet consumption was low it was used to make a correction to a lower energy group.
During the intervention period all meals were daily provided at home. The subjects were not allowed to eat or drink anything else except for water, tea and coffee. The first week was a correction period in which subjects weighed themselves every morning, before breakfast and after emptying the bladder. When body weight change was observed or when subjects complained of hunger or satiation, the EI was adjusted accordingly by changing to a higher or lower energy group. After this week the same EI was maintained for the last two weeks. The energy content of the food provided during the intervention period was analyzed with bombcalorimetry (Janke & Kunkel, IKA Kalorimeter C-400; adiabatisch, Staufen).

Table 2.2. Energy groups

Group	Energy (MJ/d)*	range (MJ/d)	Bread (g/d)	Potatoes (g/d)
A	7.50	7.125-7.875	150	150
B	8.25	7.875-8.625	165	165
C	9.00	8.625-9.375	180	180
D	9.75	9.375-10.125	195	195
E	10.50	10.125-10.875	210	210
F	11.25	10.875-11.625	225	225

* Based on food tables making allowances for energy losses in faeces and urine

Measurement of energy expenditure
Energy expenditure was measured over the last two weeks of the intervention period with doubly labelled water. Subjects drank a weighed amount of ^{18}O and ^{2}H. The dosage calculation was based on body mass in order to create a ^{2}H excess of 150 ppm and an ^{18}O excess of 300 ppm. The isotope was administered in the evening between 22.00 and 23.00 h, just before the subjects went to sleep, after collecting a background urine sample. Further urine samples were collected 1, 8 and 15 days after drinking the isotope. Isotopes were measured in urine using an isotope ratio mass spectrometer (VG-Isogas Aqua Sira) and CO_2 production was calculated from isotope ratios in baseline, and 1-day, 8-day, and 15-day samples with the equation from Schoeller as described by Westerterp and Saris (1991). CO_2 production was converted to energy expenditure by using a respiratory quotient of 0.85 according to the food quotient of the diet.

Measurement of metabolizable energy
In order to calculate metabolizable energy (calculated as EI minus energy in faeces and urine) the subjects collected 24 h urine for two days and total faeces for three days (during the last week of the intervention period). 24 h urine collection started with the second urine in the morning and included the first voiding of the next day. Daily faeces collection started at 7.00 o'clock in the morning till 7.00 o'clock the next day.

After total volume and total weight of urine and faeces were measured the energy content of both were measured with bombcalorimetry.

Body weight and body composition

Subjects weighed themselves every morning during the baseline period. Furthermore weight was measured twice during the intervention period; at the beginning of the second week and at the end of the third week. Subjects were weighed in underwear in the morning, after voiding and before eating or drinking something, on a balance accurate to 0.1 kg. Body composition was measured at the same days with deuterium dilution.

Results

Energy balance

Energy intake, energy content of faeces and urine, and metabolizable energy (calculated as EI minus energy in faeces and urine) do not differ between both groups (DR-group; DQ-group). In both groups ME intake was significantly lower than EE (Table 2.3.). Metabolizable energy represented 90.7±12.5% of EE in the DQ-group and 90.7±10.4% in the DQ-group. Energy balance (ME-EE) in the DR-group and in the DQ-group was respectively -1.03±1.23 MJ/day and -0.91±0.99 MJ/day. Thus energy balance is negative in both groups but does not differ between both groups.

Table 2.3. Data on energy balance (MJ per day; mean±SD)

	DR-group #	DQ-group
Gross energy intake	10.09±1.21	9.29±1.36
Energy in faeces	0.63±0.18	0.55±0.13
Energy in urine	0.37±0.10	0.40±0.18
Metabolizable energy	9.09±1.28	8.34±1.31
Energy expenditure	10.13±1.57*	9.25±0.35**

Significantly different from metabolizable energy (paired student's t-test): *p<0.05; **p<0.01.

#DR-group: Dietary record group; DQ-group: Dietary questionnaire group

Body weight and body composition

Body weight decreased significantly over the last two weeks of the diet intervention interval in both groups. Mean weight loss in the DR-group and in the DQ-group was respectively 0.64±0.5 kg and 0.86±0.9 kg (Table 2.4.).Weight changes in the DR-group were not significantly different from the weight changes in the DQ-group. Body weight loss was significantly correlated with energy balance as shown in Figure 2.2. ($p<0.05$). From the slope of the regression line it can be calculated that 1 kg body weight loss represents an energy deficit of 43.15 MJ (95% confidence interval:-44.48 to -20.76). It can be concluded that measured weight loss is mainly a decrease

in fat mass (FM). Data of body composition measured with deuterium dilution confirm these findings (Table 2.5.). Since there was no change in total body water (TBW) and therefore no change in fat free mass (FFM), body weight change has to be a decrease in FM.

Table 2.4. Weight changes over 14 days (mean±SD)

	Initial weight (kg)	Final weight (kg)	ΔWeight (kg)
DR-group#	73.1±9.9	72.4±9.8***	-0.64±0.50
DQ-group	70.1±12.5	69.2±11.9**	-0.86±0.90

Significantly different from initial weight (paired student's t-test):**p<0.01; *** p<0.001; #DR-group: Dietary record group; DQ-group: Dietary questionnaire group

Table 2.5. Changes in body composition over 14 days (in kg; mean±SD)

	DR-group #	DQ-group
Initial TBW	38.5±3.8	33.5±7.3
Final TBW	38.8±3.7	33.5±7.3
ΔTBW	0.3±0.7	0.0±0.3
Initial FFM	52.8±5.2	45.9±10.0
Final FFM	53.2±5.0	45.9±10.0
ΔFFM	0.4±1.0	-0.1±0.5
Initial FM	20.3±6.6	24.2±8.5
Final FM	19.3±6.5	23.4±8.2
ΔFM	-1.0±0.9**	-0.8±0.8**

Significantly different from initial value (paired student's t-test):**p<0.01; #DR-group: Dietary record group; DQ-group: Dietary questionnaire group; TBW: Total body water; FFM: Fat free mass; FM: Fat mass

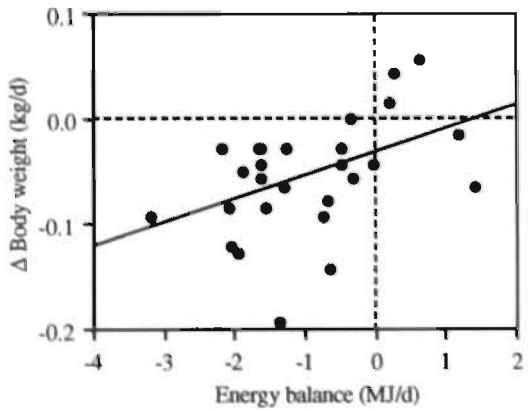

Fig. 2.2. ΔBody weight plotted as a function of the energy balance (metabolizable energy (ME) intake minus energy expenditure (EE)) with the calculated linear regression line: Δbody weight= -0.03+0.02(ME-EE); p<0.05

Discussion

Mean energy balance is negative during the intervention period for the DR-group as well as the DQ-group (ME<EE). In other words: energy balance is negative independent of the method used to estimated EI and despite the baseline period, in which corrections of intake could be made when subjects lost weight or complained of hunger or satiation. During the baseline period three subjects of the DQ-group were placed in a higher energy group because two of them were hungry and one lost weight. Two subjects of the DR-group were placed in a lower energy group because they could not cope with the amount of food of the original energy group. So despite the corrections in the baseline period energy balance was negative. There are several explanations.
A first explanation is that the methods used to determine energy balance are not precise. EE, measured with doubly labelled water, has an accuracy of 1-3% and a precision of 2-8% comparing the method with respirometry (Westerterp & Saris, 1991). Bombcalorimetry, used to determine the energy content of dietary intake, faeces and urine has a repeatability within 2%. The energy balance is also confirmed by the correlation between Δweight and ME-EE ($p<0.05$) and the negative energy balance is likewise seen in an significant decrease of FM.
The second explanation could be underreporting during the EI measurements. Several authors report studies comparing EI (measured mainly with a dietary record and sometimes with a 24-h recall) and energy expenditure measured by the doubly labeled water method. Riumallo et al (1989) report good agreement between reported EI and measured energy expenditure in underweight adults as do Schulz,Westerterp &Brück (1989) in a study with six healthy subjects. Whereas others (DeLany et al 1989; Livingstone et al 1990;) report underreporting ranging from 5-24%. Self reported dietary intake underestimates energy expenditure even more in obese subjects (Bandini, Schoeller & Dietz 1990, Prentice et al 1986, Westerterp et al 1992). Underreporting ranged from 26-41%. The same results are presented in studies with subjects with a very high activity level. Haggarty & McGaw (1987) and Westerterp et al (1986) report underestimation of EI compared with measured energy expenditure in female athletes and male cyclists of respectively 34 and 23%. Recently Livingstone et al (1992) reported a study in children and concluded that EI as measured with a dietary record underestimates energy expenditure with 11% whereas EI as measured with a dietary history overestimated energy expenditure with 8%. The overall conclusion is that self reported EI underestimates energy needs in (obese) adults.
Unfortunately there are no similar studies in elderly available. But it is likely that like younger adults elderly also underreport their habitual EI. It is hard to find the reasons for underreporting. In obese people it could be the wish to eat less and consequently underestimate real EI. In lean subjects there seems to be no reason for underreporting on purpose. The only reason could be that keeping a dietary record possibly influences habitual intake in a negative sense, especially when they have to keep it for longer periods (1-3 weeks). With respect to the elderly, keeping a dietary record or filling in a dietary questionnaire probably is even more difficult because of poor vision and un-

derstanding and most important poor recent memory. Self reported EI underestimated energy expenditure with 9.3% in both groups. Our data show a significant relation ($p<0.05$) between underreporting and body mass index (Figure 2.3.). The difference between ME and EE is related to the degree of overweight as expressed in BMI, although subjects involved in this study are not particular obese (Table 2.1.). These findings are in accordance with data presented by Westerterp et al (1992).

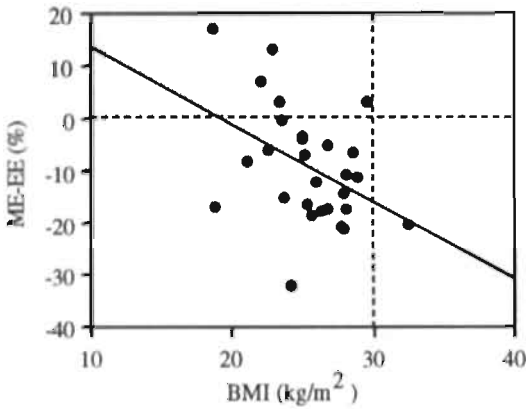

Fig. 2.3. Metabolizable energy (ME) minus energy expenditure (EE) expressed as a percentage of EE plotted against body mass index (BMI); ((ME-EE)= 28.25-1.48(BMI); $p<0.05$)

In conclusion, EI as measured with a four-day dietary record or with a dietary questionnaire underestimates energy expenditure in elderly men and women. The degree of underestimation is independent of the method used to estimate EI but is negatively correlated with BMI.

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Chapter 3

Energy requirements of the elderly

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Abstract

Energy expenditure and therefore energy requirements generally decrease with advancing age because of a decrease in basal metabolic rate (BMR) and because of a decrease in physical activity (PA). The aim of the present study was to measure total energy expenditure (EE) and activity level in a group of healthy elderly and young adults by using the doubly labelled water method in combination with measurements of BMR. Age related differences in body composition and their relation to BMR and activity level were studied by measuring body composition with deuterium dilution. EE was lower in elderly compared with young adults due to a significantly lower BMR. Body weight did not differ between both age groups, however elderly subjects had a significantly higher FM and a significantly lower FFM compared with the young adults. The decrease in BMR was not fully explained by the lower FFM in elderly. The physical activity index (PAI=EE/BMR)) together with the FFM explained respectively 80% and 86% of the variance in BMR in elderly and young subjects. Energy expended (MJ/d) on activity is higher for the younger subjects although there was no significant difference in PAI between both age groups.

Introduction

Several factors contribute to the amount of energy required by an individual: basal metabolic rate (BMR), physical activity (PA) and to a lesser extend the diet induced thermogenesis (DIT). The basal metabolic needs are the energy requirements at complete rest and these are closely related to body composition. The energy required above the basal needs is determined by the level of PA, for example the energy expended during work and leisure. The DIT is the rise in metabolic rate as a consequence of eating and accounts for 10% of the energy intake (EI). In general, EE and therefore energy requirements decrease with increasing age.

Firstly because of a decrease in BMR as shown in many studies (Keys et al 1973; Tzankoff & Norris, 1977; Calloway & Zanni, 1980). BMR decreases as a consequence of decreasing fat free mass (FFM) with aging (Forbes & Reina, 1970). However, it is not clear whether the loss of FFM fully explains the lower BMR in elderly since other physiological factors probably also contribute to the decreased BMR (Fukagawa et al 1990; Vaughan et al 1991; Poehlman et al 1991). Secondly, because elderly tend to be less active (McGandy et al 1966; Dallosso et al 1988). However, these observations are limited to measures obtained from self-recorded PA diaries or motion sensors. Only few studies have reported about total energy expenditure in elderly measured with doubly labelled water (Goran & Poehlman, 1992; Pannemans and Westerterp, 1993; Roberts et al 1992; Reilly et al 1993). Using this technique energy expenditure can be measured under free-living conditions and, by simultaneously measuring BMR, it is possible to assess the energy expenditure for daily activity by expressing PA in multiples of BMR or by expressing the energy expended on PA (plus thermogenesis) as EE minus BMR. In this way the energy requirements can be measured more precise. The aim of the present study is to measure total energy expenditure and activity level in a group of healthy elderly and young adults by using the doubly labelled water method in combination with measurements of BMR. By measuring body composition with deuterium dilution, age related differences in body composition and their relation to BMR and activity level are studied.

Subjects and methods

Subjects

Subjects were 29 young adults and 28 elderly (as described before by Pannemans and Westerterp (1993)). Subjects were recruited with advertisements in local media. Elderly subjects were also recruited through contacts with alliances for elderly. One elderly man and one elderly woman were excluded because of missing values. Mean age, height, weight and body mass index (BMI) are presented in Table 3.1. All subjects were certified to be in good health by a staff physician and gave informed consent to participate in the study after the procedures were explained to them. The protocol was approved by the university ethics committee.

Table 3.1. Characteristics of the subjects

Subjects	Age (y)		Height (m)		Weight (kg)		BMI (kg/m^2)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
16 men	71.3	(4.9)	1.72	(0.09)***	74.0	(12.6)	25.0	(3.3)*
19 men	30.4	(5.0)	1.81	(0.05)	76.3	(8.6)	23.2	(2.0)
10 women	67.6	(4.1)	1.60	(0.08)*	65.4	(5.9)	25.6	(2.6)**
10 women	27.2	(3.9)	1.68	(0.06)	60.8	(7.5)	21.5	(2.0)

*P<0.05; ***P<0.001; ****P<0.0001; differences between elderly and young men and between elderly and young women (unpaired t-test).

Protocol

Energy expenditure, energy balance, basal metabolic rate (BMR) and body composition were measured during a nutrition intervention study as previously reported for the elderly subjects (Pannemans and Westerterp, 1993; Pannemans, Halliday and Westerterp, submitted). Briefly, subjects were given two iso-energetic diets for three weeks each in a cross over design with a "wash-out" period of at least three weeks. Diets contained 12 and 42, and 21 and 33 per cent of total EI from protein and fat respectively. During the experiment subjects were fed according to their estimated EI. Usual daily EI was measured before the start of the experiment with a 4-day dietary record (11 elderly subjects) or with a dietary questionnaire (15 elderly subjects and 29 young adults). During the experiment all meals, consisting of breakfast, lunch, dinner beverages and drinks were daily served at home, and subjects were not allowed to eat or drink anything else except for water, tea and coffee. The first week was a correction period in which EI could be adjusted if necessary. Figure 3.1. is a schematic representation of the experimental design.

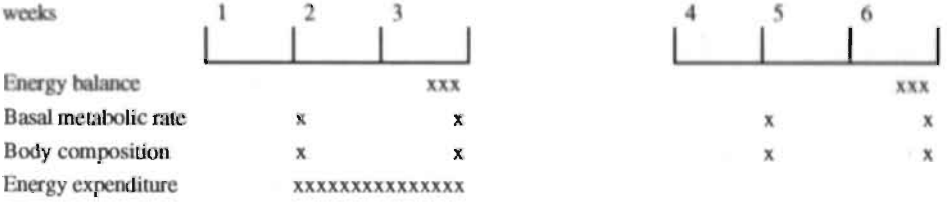

Fig. 3.1. Experimental design

Measurement of energy expenditure

Energy expenditure (EE) was measured over two weeks in the first experimental period (Figure 3.1.) with doubly labelled water. Subjects drank a weighed amount of ^{18}O and ^{2}H. The dosage calculation was based on body mass in order to create a ^{2}H excess of 150 ppm and an ^{18}O excess of 300 ppm. The isotope was administered in the evening between 22.00 and 23.00 h, just before the subjects went to sleep, after collecting a background urine sample. Further urine samples were collected 1, 8 and 15 days after drinking the isotope. Isotopes were measured in urine using an isotope ratio mass spectrometer (VG-Isogas Aqua Sira) and CO_2 production was calculated from isotope ratios in baseline, and 1-day, 8-day, and 15-day samples with the equation from Schoeller as described by Westerterp and Saris (1991). CO_2 production was converted to energy expenditure by using a respiratory quotient of 0.85 according to the food quotient of the diet.

Energy balance

Gross energy intake (GEI) was calculated by measuring the energy content of the food given during the experimental period with bombcalorimetry (Janke & Kunkel,

IKA Kalorimeter C-400; adiabatisch, Staufen). By subtracting the energy content of faeces and urine (collected by the subjects for respectively three and two days at the end of each experimental period) from GEI metabolizable energy (ME) was calculated. Energy balance during the first experimental period was calculated by subtracting ME from EE.

Measurement of basal metabolic rate

A computerized open-circuit ventilated hood system was used to measure BMR. BMR can be calculated by determining the amount of air flowing through the hood and by measuring the oxygen and carbon dioxide concentrations in the incoming and outcoming air. Subjects came to the laboratory in the early morning after an overnight fast of at least 10 hours. Subjects were asked to travel by car, bus or train in order to reduce PA. BMR was measured under thermoneutral temperature conditions, after a period of at least twenty minutes bed-rest, for at least twenty minutes. The subjects were instructed to relax and avoid sleeping during measurements. BMR was measured four times (Figure 3.1.).

Body weight and body composition

Subjects' weight and body composition were measured on the same days as BMR was measured (Figure 3.1.). Subjects were weighed in underwear in the morning, after voiding and before eating or drinking something, on a balance accurate to 0.1 kg. Body composition was measured at the same days with deuterium dilution. Subjects drank a ^2H_2O dilution in the evening after emptying the bladder (baseline sample). The second voiding in the next morning was the second sample. Deuterium was measured in the urine samples with an isotope ratio mass spectrometer as described above. Total body water (TBW) was calculated as the measured deuterium dilution space divided by 1.04 (Schoeller, 1992). FFM was calculated as TBW/0.73.

Statistics

Values are expressed as mean with the SD in parentheses. Data previously obtained in elderly subjects in an identical experimental protocol are used for comparative purposes. Differences between measurements and groups were analyzed using Student's paired and unpaired t-tests as appropriate. Regression analysis was used to assess associations between measured variables. Analysis of co-variance using FFM as the covariate was used to adjust for differences in body weight when comparing BMR values.

Results

Energy balance

The results of the energy balance measurements are given in Table 3.2. GEI, ME and EE were significantly higher for the young adults. As reported before for the elderly

(Pannemans and Westerterp, 1993), ME intake was significantly lower than EE ($P<0.001$ for elderly and $P<0.0001$ for young adults). ME represented 92.0 (SD 10.3) % of EE in the elderly and 87.2 (SD 14.5) % in the young adults. Energy balance, defined as ME-EE, was -0.83 (SD 1.00) MJ/d and -1.62 (SD 1.78) MJ/d for the elderly and the young adults respectively. The energy balance was negative in both groups but did not differ significantly between both age groups. For both groups there is a significant relation between energy balance and the weight change over 14 days in the first experimental period (Figure 3.2.). The regression lines between these two variables do not differ significantly for elderly and young subjects indicating that age has no effect on this relation.

Table 3.2. Energy balance (MJ/d)

	elderly (n=26)		young adults (n=29)	
	mean	SD	mean	SD
Gross energy intake	9.72	(1.35)**	11.30	(1.94)
Energy in faeces	0.57	(0.11)	0.55	(0.19)
Energy in urine	0.39	(0.16)	0.48	(0.10)
Metabolizable energy	8.77	(1.31)**	10.27	(1.85)
Energy expenditure	9.60	(1.56)****	11.89	(1.84)

**P<0.01; ****P<0.0001 differences between elderly and young subjects (unpaired t-test)

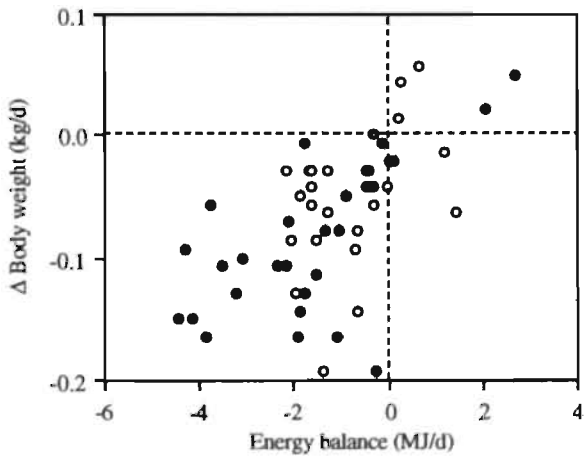

Fig. 3.2. ΔBody weight (Δ BW) plotted as a function of the energy balance (metabolizable energy (ME) intake minus energy expenditure (EE) in a group of 29 young adults (•) and also in a group of 26 elderly subjects (◦) as described previously. Calculated linear regression lines were: young adults: ΔBW=0.02(ME-EE)-0.05 (P<0.001); elderly subjects: ΔBW=0.02(ME-EE)-0.03 (P<0.05); young and old subjects: ΔBW=0.02(ME-EE)-0.04 (P<0.0001).

Measurement of body weight and body composition

BW and body composition were measured four times in 53 subjects. Two elderly women were measured twice, being only involved in the first experimental period. Table 3.3. shows the results during both experimental periods. There were no significant differences in BW between young and elderly subjects. However body composition did differ since elderly subjects had a significantly higher FM and a significantly lower FFM compared with the young adults (FM: P<0.01; FFM: P<0.05). BW decreased significantly during both experimental periods in both age groups (P<0.0001 for both age groups during the first experimental period; P<0.001 for elderly and P<0.0001 for young subjects during the second experimental period). As shown in Table 3.3. measured weight loss was due to a decrease in FM. FM decreased significantly during both experimental periods.

Table 3.3. Body weight and body composition during both experimental periods (kg; mean±SD)

		elderly ‡		young adults	
		mean	SD	mean	SD
Body weight	1	70.69	(11.23)†††	70.99	(11.01)††††
Body weight	2	69.98	(10.84)	69.78	(10.69)
Body weight	3	69.90	(11.11)†††	70.61	(10.79)††††
Body weight	4	69.34	(10.83)	69.48	(10.61)
Fat free mass	1	48.47	(8.99)**	55.71	(9.20)
Fat free mass	2	49.3	(9.09)*	55.64	(9.02)
Fat free mass	3	49.62	(9.27)*	55.58	(9.11)
Fat free mass	4	49.78	(8.95)*	55.48	(9.12)
Fat mass	1	21.81	(6.15)††††/****	15.26	(5.56)††††
Fat mass	2	20.85	(6.01)****	14.15	(4.99)
Fat mass	3	20.28	(6.1)†/**	15.44	(5.17)††††
Fat mass	4	19.56	(6.34)***	13.99	(5.24)

‡Body weight, fat free mass and fat mass were measured twice (1+2) in the first experimental period (n=26 elderly and 29 young subjects) and twice (3+4) during the second period (n=24 elderly and 29 young subjects). *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001 differences between elderly and young subjects (unpaired t-test); †P<0.05; †††P<0.001; ††††P<0.0001; differences between the first and the second measurement and between the third and the fourth measurement.

Measurement of basal metabolic rate

BMR was measured at least twice in all subjects (n=55). 53 Subjects were measured three times and 50 subjects were measured four times. Within subject variation (over two, three or four measurements) was 4.6 (SD 2.2) % for elderly and 5.2 (SD 1.9) % for young subjects justifying to take the mean of the available measurements. Mean BMR values for the elderly (6.22 (SD 1.01) MJ/d) were significantly lower than values obtained for the younger subjects (7.14 (SD 0.95) MJ/d; P<0.001). These differences were analyzed in relation to differences in body composition. Analysis of co-

variance was used to derive adjusted BMR values according to a specific relationship between BMR and FFM for elderly and young subjects. This is a better way of comparing BMR data among subjects with different body composition than expressing BMR per kilogram FFM. Since the use of this ratio (BMR/FFM) is only appropriate when the relation between both parameters has no intercept (Ravusssin & Bogardus, 1989; Weinsier et al 1992). Adjusted BMR values were significantly lower for the elderly (6.38 (SD 0.56) and 7.43 (SD 0.40) MJ/d for the elderly and young subjects respectively, P<0.0001). Regression lines between FFM and BMR for young and elderly subjects were compared as shown in Figure 3.3. For both age groups there was a significant relation between both variables (P<0.0001). As tested with analysis of covariance, the y-intercepts, but not the slopes, between young and elderly subjects were significantly different (P<0.05).

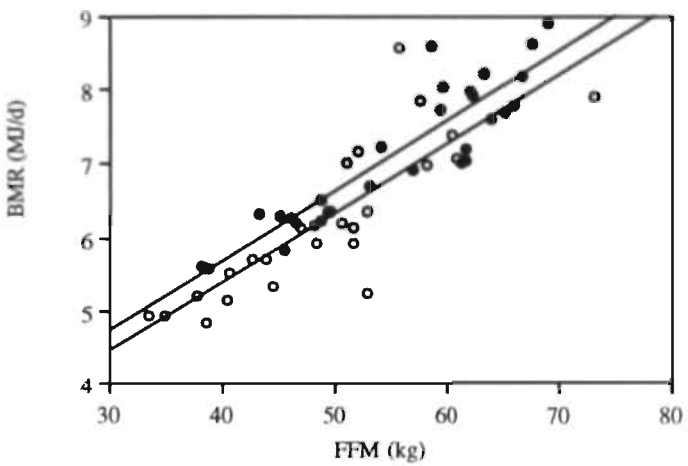

Fig. 3.3. Basal metabolic rate (BMR) plotted as a function of the fat free mass (FFM) in a group of 29 young adults (•) and also in a group of 26 elderly subjects (°) Calculated linear regression lines were: young adults: BMR=0.09 FFM+1.85 (P<0.0001); elderly subjects: BMR=0.09 FFM+1.64 (P<0.0001). Regression lines are significantly different P<0.05.

Measurement of the physical activity level

PA level was calculated as the physical activity index (PAI) given as EE divided by BMR. Mean and ranges of measured PAI are given in Table 3.4. Young men had a significantly higher PAI compared with elderly men. Other differences between sexes or age groups were not seen although there was a tendency for young subjects to have a higher PAI compared with the elderly (P=0.081). There was no significant relation between PAI and body composition (FFM, FM, %FM and BMI)

Multiple regression analysis revealed that the PA level (as given by ADMR/BMR) and the FFM of the subjects contributed significantly to the BMR. FFM and PAI explained respectively 80% and 86% of the variance in BMR in elderly and young

subjects. In the elderly PAI explained 10% extra of the variance in BMR, this was only 3% (though significant) in the young adults.

Table 3.4. Physical activity levels expressed as physical activity index

	PAI‡ mean	SD	range
elderly (n=26)	1.58	(0.21)	1.27-2.05
elderly men (n=16)	1.52	(0.20)*	1.27-2.05
elderly women (n=10)	1.66	(0.20)	1.34-2.00
young adults (n=29)	1.66	(0.16)	1.32-2.06
young men (n=19)	1.66	(0.15)	1.32-1.86
young women (n=10)	1.67	(0.20)	1.42-2.06

‡PAI is physical activity index calculated as energy expenditure divided by basal metabolic rate.
*P<0.05; differences between elderly men and young men (unpaired t-test)

Discussion

Despite the baseline period, in which corrections of intake could be made when subjects lost weight or complained of hunger or satiation, subjects were in negative energy balance. The dietary questionnaire underestimated EE with 12.8% in young adults. This confirms earlier findings in the elderly (Pannemans & Westerterp, 1993) where a 4-day dietary record and the same dietary questionnaire underestimated EE to the same degree. The negative energy balance is corroborated by the significant correlation between ΔBW and energy balance for both age groups, and is also reflected in a significant decrease in FM. Roberts et al (1991) did not find a significant relation between ΔBW and energy balance, this can be explained by the fact that in the present study energy balance was more negative and energy balance was measured over longer periods. It is concluded from our study that it is difficult to feed subjects in energy balance for longer periods (2 weeks) when it is not allowed to eat or drink anything else except the fixed amount that is given during the experiment. Before discussing the other results it has to be noticed that the negative energy balance had no effect on the other results of the study.

The energy requirements of healthy elderly subjects were investigated by measuring EI, total energy expenditure, BMR and the energy needs for physical activity in healthy young and elderly subjects. EE and therefore EI was significantly lower for the elderly. EE is mainly determined by the BMR and by the energy expended by physical exercise.

BMR was measured at least twice in all subjects. The mean coefficient of variation was about 5% for both age groups, a value comparable to other studies (Poehlman et al 1992; Fredrix et al 1990). Absolute BMR values (MJ/d) were lower in the elderly relative to the young adults (±14%). In the past, numerous studies have been reporting lower BMR values in elderly compared with young subjects. In a longitudinal

study reported by Keys et al (1973) BMR declined 2% per decade of age. In cross sectional studies (Tzankoff & Norris, 1977; Calloway & Zanni, 1980). Elderly subjects had a significantly higher FM and a significantly lower FFM compared with the young adults but BMR values were also lower in the elderly when differences in FFM were taken into account using analysis of covariance. These findings support those of Fukagawa et al (1990), Poehlman et al (1991) and Vaughan et al (1991) who also found that differences in FFM cannot fully account for the lower BMR in older subjects.

In the present study the PA level (as given by ADMR/BMR) of the subjects contributed significantly to the BMR. FFM and PAI explained respectively 80% and 86% of the variance in BMR in elderly and young subjects. Fukagawa et al (1990) also suggested that regular physical exercise may attenuate the age-related decline in BMR. Poehlman et al (1992) examined the effect of aerobic capacity, body composition and thyroid hormones on the age related decline in BMR and concluded that maintenance of FFM and VO_2 max by regular PA may attenuate the age related decline in BMR in healthy subjects.

Elderly subjects tended, although not significantly, to be less active in comparison with younger subjects when PA was expressed in multiples of BMR (P=0.081). The current recommendations as given by the WHO/FAO/UNU (1985) for energy requirements in adults with different activity levels are also expressed as multiples of BMR. These recommendations have been questioned recently by Roberts et al (1991, 1992). It was suggested that the current recommended EI for young and elderly subjects may significantly underestimate usual energy requirements since the activity levels of the subjects, who were classified on the basis of a questionnaire as "sedentary", were significantly higher compared with the WHO/FAO/UNU (1985) recommendations (1.75 compared with 1.50 as recommended for the elderly and 1.98 compared with 1.55 or 1.67 for young adults). However, when comparing the PAI values of the present study (1.58 (SD 0.21) for elderly as reported earlier (Pannemans & Westerterp, 1993) and 1.67 (SD 0.17) for the young adults) with those recommended by the FAO/WHO/UNU (1985) no significant differences were seen. Our results are in accordance with results of the study of Goran & Poehlman (1992) who reported mean activity levels of 1.51 for elderly men and women. Compared with other studies (Schulz et al 1989; Livingstone et al 1990) in young adults our PAI values are slightly lower (1.67 compared with respectively 1.92 and 1.82). Recently Reilly et al (1993) reported a mean PAI of 1.80 for elderly women but this rather high activity level was in accordance with the activity questionnaire score, indicating that the subjects involved in the study were more physically active than the mean of a large sample of women of similar age in another study (Dallosso et al 1988). Reilly et al (1993) pointed at the fact that the interpretation of the PAI values requires some caution: PAI may be unjustly raised in elderly subjects since their BMR is reduced relatively to that of young adults (because of a decrease in FFM), at the same level of PA this would raise the PAI with advancing age. When PA (plus thermogenesis) is expressed as EE minus BMR the elderly subjects expended significantly less energy

on activity in comparison with the younger ones (3.46 (SD 1.18) MJ/d for the elderly and 4.73 (SD 1.25) MJ/d for the young adults; P=0.0003). EE-BMR is positively (P<0.01) correlated with the fat free mass index (FFMI=FFM/height^2). In this way corrections were made for differences in height, in analogy with the body mass index (Westerterp et al 1992). These results indicate that there is a tendency that PA has a positive effect on the FFM.

In conclusion, EE is lower in elderly compared with young adults due to a decreasing BMR. The decrease in BMR is not fully explained by the decrease in FFM in elderly. The physical activity level (PAI) together with the FFM explained respectively 80% and 86% of the variance in BMR in elderly and young subjects. The absolute amount of energy expended on activity is higher for the younger subjects although there was no significant difference in PAI between both age groups.

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Chapter 4

24 h Energy expenditure during a standardized activity protocol in young and elderly men

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Abstract

The effect of age on 24h energy expenditure (24h EE), sleeping metabolic rate (SMR), diet induced thermogenesis (DIT) and energy expenditure of physical activity (EE_{act}) were measured in young and elderly men performing an activity protocol in a respiration chamber under strictly controlled conditions. Subjects were 13 young men (26±4 y) and 10 elderly men (74±5 y). SMR as a function of FFM was not different between both age groups. 24h EE during a standardized activity protocol was significantly higher for the young men (young men: 12.85±1.53 MJ/d; elderly men: 10.90±1.12 MJ/d; $P<0.05$). The DIT expressed as MJ/d was significantly higher for the young subjects but similar when expressed as percentage of EI (young men: 13.10±5.44%; elderly men: 9.88±3.86%). The resulting figure for EE_{act} (24h EE-SMR-DIT) was the same for young and elderly men (young men: 3.11±0.71 MJ/d; elderly men: 3.05±0.64 MJ/d) indicating that mean energy costs for sedentary activities (some daily household activities and a bench stepping exercise) were the same for young and elderly men.

Introduction

The average daily metabolic rate (ADMR) of a subject involves the basal metabolic rate (BMR), the diet induced thermogenesis (DIT) and the energy costs of physical activity (PA). The BMR comprises the sleeping metabolic rate (SMR) and the energy costs of arousal. The DIT is the increase in energy expenditure as a result of food intake. The most variable component is the energy expended on PA. Several authors have reported about the effect of age on ADMR and/or its specific components.
Many longitudinal studies have demonstrated that BMR decreases with age (Keys, Taylor & Grande. 1973; Robinson et al 1975; Webb & Hiestrand 1975; Tzankoff & Norris 1977). In cross sectional studies, authors also reported lower absolute BMR

values in elderly subjects compared with younger subjects however, BMR values in young and elderly subjects were similar when adjustments were made for differences in fat free mass (FFM) (Calloway & Zanni 1980; Bloesch et al 1988; Poehlman et al 1990). Recently it has been reported that the differences in FFM between young and elderly subjects can not fully explain the lower BMR in the elderly (Fukagawa et al 1990; Vaughan, Zurlo & Ravussin 1991; Pannemans & Westerterp unpublished data) suggesting that aging is associated with a change in tissue energy metabolism.
There is less extensive research on the effect of age on DIT and results are equivocal. Some studies report lower DIT in elderly subjects compared with younger subjects (Golay et al 1983; Bloesch et al 1988; Morgan &York 1983; Schwartz, Jaeger & Veith 1990). Poehlman et al (1991) did not find age related differences in DIT. Although most of the results are pointing at a decrease in DIT with age, the difference between older and younger adults is not large.
Several studies reported about the PA level of elderly subjects measured with questionnaires or interviews (Laporte et al 1983; Dallosso et al 1988; Donahue et al 1988; Voorrips et al 1990; Caspersen 1992, Dipietro et al 1993). When comparisons were made with activity levels of younger subjects it was generally concluded that PA decreases with age. However, the validity of PA questionnaires and interviews are still under debate. In the past decade the doubly labelled water technique has proven to be a very reliable method to measure PA under free living conditions. So far only few studies have reported about ADMR in the elderly as measured with doubly labelled water (Goran & Poehlman 1992; Roberts et al 1992; Pannemans & Westerterp 1992; Reilly et al 1993). By simultaneously measuring BMR, it is possible to assess the energy expenditure of daily activity by expressing ADMR in multiples of BMR (or SMR) or by expressing the energy expended on PA (plus DIT) as ADMR minus BMR (or SMR). In general it was concluded that, as in young adults, there is a wide range in the level of PA between elderly subjects. Although the use of doubly labelled water technique gives information about the ADMR, it is not known whether there are age related differences in the energetic costs for specific activities. Some authors examined the energetic cost of specific activities and the time needed to perform specific activities (Himann et al 1988; Didier et al 1993; Voorrips et al 1993). However these data are limited and the results are contradictory partly due to the variety of activities that were measured.
Generally, it is assumed that ADMR decreases with age. However more data on energy expenditure at rest (in relation to body composition) and during specific activities are needed, to estimate the energy requirements for the elderly. The present study was performed to study the effect of age on 24h energy expenditure under strictly controlled conditions. SMR, DIT and energy expended on PA were measured in young and elderly men performing an activity protocol, resembling a normal daily activity pattern, in a respiration chamber.

Subjects and methods

Subjects

Subjects were 13 young men and 10 elderly men. They were recruited with advertisements in local media. Mean age, height, weight and body mass index (BMI) are presented in Table 4.1. All subjects were certified to be in good health. Subjects gave informed consent to participate in the study after the procedures were explained to them. The protocol was approved by the university ethics committee. Seven young men were measured at an activity protocol with a moderate intensity while the other six young men were followed an activity protocol with a lower intensity. When measuring the elderly men, it appeared that they were not able to perform the activity protocol with a moderate intensity. So they performed the activity protocol with the lower intensity. Only when comparisons were made with respect to the SMR and its relation to FFM all young men were included (n=13). With respect to all other measurements only six young men were included, those measured at the activity protocol with the lower intensity, which was the same as applied in the elderly men.

Table 4.1. Physical characteristics of the subjects (mean±SD)

	Young men (n=13)	Elderly men (n=10)
Age (y)	26.8±4.3	73.80±4.7
Height (m)	1.83±0.07*	1.75±0.09
Weight (kg)	76.6±12.3	79.4±15.4
BMI (kg/m^2)	22.9±3.2*	25.8±2.5

*P<0.05 differences between young and elderly men

Respiration chamber

Oxygen consumption and carbon dioxide production were measured in a respiration chamber (Schoffelen et al 1984). This chamber measures 14 m^3 and is furnished with a bed, chair, table, TV, radio, telephone, wash-bowl and toilet facilities. The chamber is ventilated with fresh air at 50 l/min. The ventilation rate was measured with a dry gasmeter (Schlumberger, type G6). The concentration of oxygen and carbon dioxide was measured using a paramagnetic O_2 analyser (Hartmann & Braun, type Magnos 6G) and an infrared CO_2 analyser (Hartmann & Braun, type Uras 3G). Ingoing air was analyzed once every 15 min and outgoing air twice every 5 min. The gas sample to be measured was selected by a computer which also stored and processed the data. Energy expenditure (EE) was calculated from O_2 consumption and CO_2 production (Weir 1948). In daytime subjects followed a standard activity protocol (see below).

Energy expenditure, sleeping metabolic rate and diet induced thermogenesis

Figure 4.1. is a schematic presentation of the experimental design. Subjects arrived between 18.30 and 19.30 h at the laboratory. After the procedure was explained to them they stayed in the respiration chamber for 36 hours. 24h Energy expenditure

(24h EE) was calculated from 8.00h to 8.00h. In day time, energy expenditure (EE) during separate activities was calculated at 30 minutes intervals. The PA of the subjects was monitored by means of a radar system, based on the Doppler principle. SMR was calculated over a 3h interval between 2.30h and 7.00h (8h postprandial) in which radar activity was lowest and subjects were asleep. SMR was measured during both nights. The method used for determination of diet induced thermogenesis (DIT) was described previously (Schutz, Bessard & Jéquier 1984, Verboeket-van de Venne, Westerterp & Kester 1993) and is based on simultaneous measurements of PA and EE. The relationship between PA and EE for each individual, both averaged over 30 min. periods, was plotted. Only the intervals after the first meal until bedtime were used, i.e. from 9.30h to 00.00h. The intercept of the regression line at zero activity represents the energy expenditure in the inactive state (EE_0 activity) consisting of two components: BMR and DIT. DIT was obtained by subtracting SMR from EE_0 activity, and corrected for the relevant time interval (see below). Calculated in this way, DIT includes also the energy costs of arousal. Energy expended on physical activity (EE_{act}) was calculated as 24h EE - SMR - DIT (corrected for time). Thus, the separate components of 24h EE were obtained by the following equations:

DIT = EE_0 activity - SMR
DIT (corr.) = (EE_0 activity - SMR) x (postprandial hours/24)
EE_{act} = 24h EE - SMR - DIT (corr.)

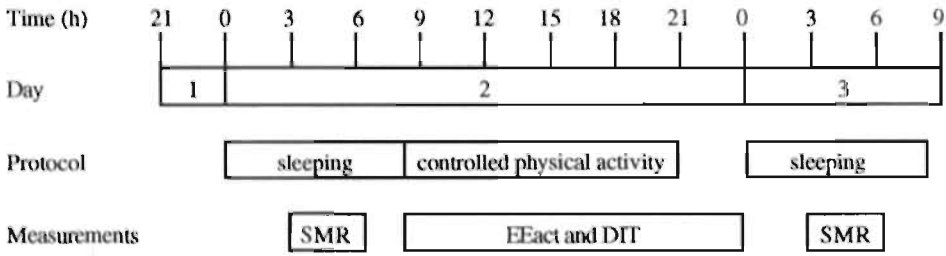

Fig. 4.1. Schematic presentation of the 36h stay in the respiration chamber

Activity protocol
During daytime subjects followed an activity protocol (8.30-21.00h as shown in Table 4.2.). The activity protocol consisted of sedentary activities (desk work, sitting and lying down on the bed), some daily household activities (making the bed, cleaning the room, dish washing) and bench stepping exercise. All activities were done for 30 minutes. The response time of the respiration chamber is 5 minutes, therefore only the last 25 minutes of each 30 minute period were used to calculate mean energy expenditure. Young men were measured first and performed the bench stepping exercise at a rate of 60 steps per minute with a bench height of 33 cm, over intervals of 3 times 5 minutes (i.e. 5 minutes stepping exercise and 5 minutes rest al-

ternately). Elderly men performed the stepping exercise at the same rate and with bench heights of 18 or 33 cm. However, they were not able to perform the bench stepping exercise over intervals of 5 minutes. Therefore they were asked to perform the exercise for 3 times 3 minutes alternated with 7 minutes rest. This procedure was repeated 5 hours later to reach comparable values for the total amount of steps times height in young and elderly subjects.

Table 4.2. Activity protocol for young and elderly men

Time			Young men (n=6)	Elderly men (n=10)
8.30	-	9.00	dressing	dressing
9.00	-	9.30	breakfast	breakfast
9.30	-	10.00	sitting	sitting
10.00	-	10.30	lying	lying
10.30	-	11.00	making the bed	making the bed
11.00	-	11.30	desk work	desk work
11.30	-	12.00	desk work	stepping exercise
12.00	-	12.30	standing/walking	standing/walking
12.30	-	13.00	lunch	lunch
13.00	-	13.30	desk work	desk work
13.30	-	14.00	lying	lying
14.00	-	14.30	sitting	sitting
14.30	-	15.00	desk work	desk work
15.00	-	15.30	desk work	desk work
15.30	-	16.00	cleaning room	cleaning room
16.00	-	16.30	desk work	desk work
16.30	-	17.00	desk work	stepping exercise
17.00	-	17.30	desk work	desk work
17.30	-	18.00	lying	lying
18.00	-	18.30	dinner	dinner
18.30	-	19.00	dish washing	dish washing
19.00	-	19.30	sitting	sitting
19.30	-	20.00	lying	lying
20.00	-	20.30	stepping exercise	desk work
20.30	-	21.00	desk work	desk work

Body weight and body composition

Subjects weighed themselves in underwear in the morning (of day 2 and 3), after voiding and before eating or drinking something, on a balance accurate to 0.1 kg (Seca delta, model 707). In the elderly body composition was measured with deuterium dilution. Before going to bed at night during the first day in the respiration chamber a ^2H_2O solution was drunk after emptying the bladder (baseline urine sample). The dosage calculation was based on body mass in order to create a ^2H

excess of 100 ppm. A second urine sample was collected by the subjects on the next day (day 2) in the morning, from the second voiding between 8.00h and 10.00h. Deuterium was measured in urine samples with an isotope ratio mass spectrometer (VG Aqua Sira). Total body water (TBW) was calculated as the measured deuterium dilution space divided by 1.04 (Schoeller et al 1980). Fat-free mass (FFM) was calculated from TBW assuming a hydration coefficient of 0.73; fat mass (FM) was calculated as body weight minus FFM.
In young men body composition was measured by underwater weighing (at the morning of the third day after the subjects left the respiration chamber). Residual lung volume was measured simultaneously with helium dilution (Volugraph 2000, Mijnhardt). The percentage of body fat was calculated from body density using the equation of Siri (1956).

Energy intake
Energy intake for the maintenance of energy balance was based on the calculated BMR (Harris and Benedict 1919) of the subjects multiplied by 1.45, the estimated activity factor for the chosen activity protocol. The food provided 14 per cent of the total energy content from protein, 27 per cent from fat and 59 per cent from carbohydrate.

Statistics
Values were expressed as mean with the standard deviation. Differences between young and elderly men were analyzed using Student's unpaired t-tests. Comparisons within subjects were made using Student's paired t-tests. Regression analysis was used to assess associations between measured variables. Analysis of co-variance using FFM as the co-variate was used to adjust for differences in body composition when comparing SMR values.

Results

Body weight, body composition and sleeping metabolic rate
Data on body weight, body composition and SMR are given in Table 4.3. There were no differences in body weight for young and elderly subjects. However body composition was different. Elderly subjects had a significantly lower FFM compared with young men ($P<0.05$) and the percentage FM was significantly higher in the elderly ($P<0.0001$). In the elderly SMR was significantly higher during the second night, after the activity protocol (Table 4.3.). This was not the case for the young adults, although there was a tendency for a higher SMR during night 2 ($P=0.054$). For this reason, the SMR of the first night was taken as the true SMR and was used for further calculations. SMR values for the elderly were 6.93± 0.86 MJ/d and 7.50±1.10 MJ/d for the younger subjects. The SMR data were analyzed in relation to body composition. Analysis of covariance was used to derive adjusted SMR values according to a specific relationship between SMR and FFM for elderly and young subjects.

Regression lines between FFM and SMR for young and elderly men were compared as shown in Figure 4.2. For both age groups there was a significant relation between both variables (P<0.0001 for young men; P<0.001 for elderly men). As tested with analysis of covariance, regression lines did not differ between young and elderly subjects.

Table 4.3. Body composition and SMR in young and elderly men

	Young men (n=13)	Elderly men (n=10)
FFM (kg)	65.9±8.1*	57.5±10.4
FM (%)	13.3±7.2****	27.4±2.1
SMR night 1 (J/s)	86.8±12.8	80.2±10.0#
SMR night 2 (J/s)	89.8±11.2	85.1±10.8

****P<0.0001; *P<0.05; differences between young and elderly subjects; #P<0.05; difference between SMR night 1 and SMR night 2

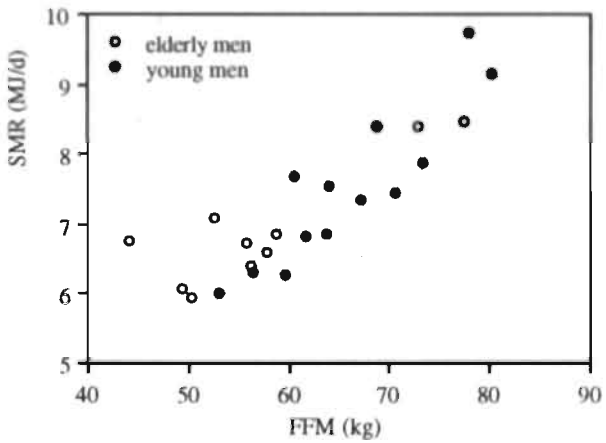

Fig. 4.2. Sleeping metabolic rate (SMR) plotted as a function of the fat free mass (FFM) in young men (•, n=13) and in elderly men (∘, n=10).

24 h Energy expenditure, diet induced thermogenesis and physical activity

24 h Energy expenditure calculated from 8.00 AM-8.00 PM was 12.85±1.53 MJ/d for the young men (n=6) versus 10.90±1.12 MJ/d for the elderly men (n=10; P<0.05). Mean DIT was significantly higher for young men compared with elderly men (young: 1.59±0.59 MJ/d; elderly 0.92±0.42 MJ/d; P<0.05). However, expressed as percentage of energy intake there were no differences between both age groups. DIT was 13.10±5.44% of energy intake for the young men and 9.88±3.86% of energy intake for the elderly. Energy expenditure for physical activity (EE_{act}) was not significantly different between young and elderly men (young: 3.11±0.71 MJ/d; elderly: 3.05±0.64MJ/d). The PA of the subjects was also monitored by means of the radar system. There were no significant differences in radar counts between young

and elderly subjects as registered during each activity. The PA level, 24h EE expressed as a multiple of the SMR, was the same for both age groups (young: 1.58±0.12; elderly: 1.58±0.14).

Discussion

The present study was performed to study the effect of age on 24h energy expenditure under strictly controlled conditions. SMR, DIT and energy expenditure of PA (as multiples of SMR or as EE_{act}) were measured in young and elderly men performing an activity protocol, resembling a daily activity pattern for sedentary conditions, in a respiration chamber. The activity level, calculated as 24 EE divided by the SMR, was equal for young and elderly men (1.58). This figure resembles the age specific energy recommendations for older men (1.51) as given by the WHO/FAO/UNU (1985).
SMR was measured twice during a 36h stay in a respiration chamber. In the elderly SMR was significantly higher during the second night, probably due to the fact that they were not used to perform the imposed activity protocol, although the intensity was not very high (24h EE= 1.58*SMR). In young men the SMR of the second night tended to be higher, this might be attributable to the fact that they performed a stepping exercise relatively late in the evening (20.00-20.30h). Although the impact of post exercise oxygen consumption is still under debate, some studies reported a significant increase in energy expenditure at rest in young subjects performing relatively heavy PA training (Bielinski, Schutz & Jéquier 1985; Molé 1990). For this reason the SMR data of the first night were used for further calculations.
Numerous studies have reported on the relation of BMR to age (Keys, Taylor & Grande 1973; Robinson et al 1975; Webb & Hiestrand 1975; Tzankoff & Norris 1977). Some authors reported similar BMR values in young and elderly subjects when corrections were made for differences in FFM. (Calloway & Zanni 1980; Bloesch et al 1988; Poehlman et al 1990). However, recently Vaughan, Zurlo & Ravussin (1991) and Fukagawa et al (1990) suggested that aging is associated with an alteration in tissue energy metabolism since the differences in FFM between young and elderly subjects could not fully explain the lower BMR in the elderly. Only few authors report about the effect of age on the SMR (ten Hoor, Bergmans & Saris 1987; Vaughan Zurlo & Ravussin 1991, Webb & Hiestand). In the present study SMR, as a function of FFM, was not different between young and elderly men. Since BMR was not measured in the present study, regression lines were compared with previously obtained BMR values in 19 young men and 16 elderly men (Pannemans and Westerterp unpublished data) as measured by means of a ventilated hood system. Analysis of covariance revealed that in young men, BMR values as a function of FFM were significantly higher compared with the SMR values ($P<0.01$). In the elderly no differences were found between BMR and SMR as a function of FFM (Fig.4.3). The results are in accordance with the study of Vaughan, Zurlo & Ravussin (1991). They also found significantly higher BMR values in young adults while no differences were

seen between the SMR values of young and elderly subjects. It can be concluded that in elderly subjects there is no change in energy expenditure from basal to the sleeping metabolic state. In other words: the energy costs of arousal (BMR=SMR+arousal) can be neglected in the elderly.

In the present study, 24h EE in MJ/d during a standardized activity protocol, was significantly higher for young men compared with elderly men.

DIT (MJ/d) was significantly higher for the young men. Expressed as a percentage of the energy intake no difference was found. However, in the present study calculated DIT included the energy costs of arousal since we calculated DIT as EE () activity minus SMR, generally SMR is assumed to be ~5% lower than BMR (Garby et al 1984; Goldberg et al 1988). This means that we overestimated the calculated DIT with a corresponding value of 5% in the young men. Since there seems to be no difference between SMR and BMR in the elderly, calculated DIT in the elderly was not overestimated. Nevertheless, when the DIT was corrected for this difference there still was no difference between young and elderly men (young men: DIT: 12.65±5.18%; elderly men: DIT: 9.88±3.86%).

The results of other studies on the effect of age on DIT are not consistent. Golay et al (1983) and Bloesch et al (1988) measured glucose-induced thermogenesis in young and elderly subjects. In both studies, DIT was significantly lower in the elderly individuals compared with the young adults. Morgan &York studied DIT in young and elderly men in response to two meals with different energy contents. DIT was significantly higher for the young adults compared with the elderly. Schwartz et al (1990) also reported a lower DIT in older subjects compared with young subjects. On the opposite, Poehlman et al (1991) and Vaughan, Zurlo & Ravussin (1991) did not find age related differences in DIT. It should also be noted that the size, frequency and composition of the meals affect the DIT, making it difficult to draw a definitive conclusion concerning the possible impact of aging. The EE_{act} was calculated as 24h EE minus SMR minus DIT. Again no differences were found between young and elderly men. It can be concluded that the energy costs for PA during a standardized activity protocol were similar for young and elderly men. Furthermore the PA level expressed as multiples of SMR was the same for young and elderly men. No other studies are known, measuring energy expenditure during an standardized activity protocol in young and old men. Vaughan, Zurlo & Ravussin (1991) did not find differences in spontaneous PA in young and elderly. Only few reports are known reporting about the energy costs of specific activities in the restricted environment of a respiration chamber. Voorrips et al (1993) measured energy expenditure during sitting, sitting with standardized arm activity and walking on a treadmill at 3 km/h in 28 elderly women and 29 middle aged women. EE during sitting and sitting with standardized arm activity did not differ between the groups. While the energy costs of walking were higher for the elderly women, suggesting that elderly women walk less efficiently. Didier et al (1993) measured EE of some daily activities in 10 young subjects and in 10 old subjects. Activities were: rising and sitting back on a seat, getting up

from and lying down on a bed and getting up from the floor. EE and the time neces-

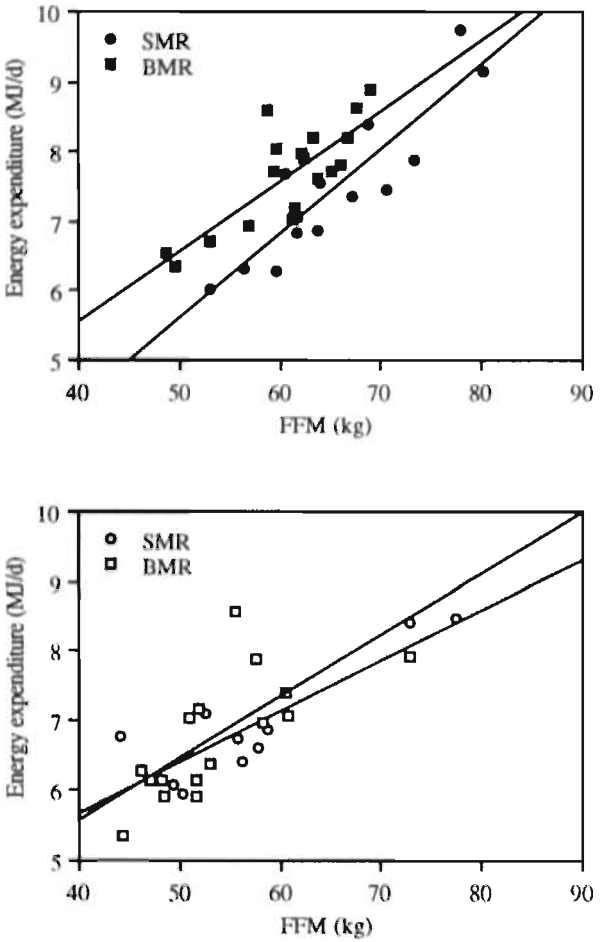

Fig. 4.3. Sleeping metabolic rate (SMR) and basal metabolic rate plotted as a function of the fat free mass (FFM) in young and elderly men with the calculated linear regression lines :• young men (n=13):SMR=0.12 FFM-0.54 (P<0.0001); ■ young men (n=19):BMR=0.10 FFM+1.44 (P<0.0001); ○ elderly men (n=10):SMR=0.07 FFM+2.78 (P<0.001); ❑ elderly men (n=16):BMR=0.09 FFM+2.06 (P<0.001).

sary for the activities were measured simultaneously. When rising and sitting back down on a seat, elderly subjects expended less energy per kg body weight, while there were no differences in time needed to perform the activity. Getting up from and lying down on the floor or bed involved the same EE but took significantly longer for the elderly. It can be concluded that the data are limited and the results are contradictory partly due to the variety of activities measured.

In summary, SMR as a function of FFM was not different between young and elderly men. 24h EE during a standardized activity protocol was significantly higher for the young men. The DIT expressed as MJ/d was significantly higher for the young subjects but similar when expressed as percentage of energy intake. The resulting figure for EE_{act} (24h EE-SMR-DIT) was the same for young and elderly men indicating that mean energy costs for sedentary activities (some daily household activities and a bench stepping exercise) were the same for young and elderly men.

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Chapter 5

Whole body protein turnover in elderly men and women: responses to two levels of protein intake

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Abstract

In this study the effect of the level of protein intake (12 and 21 per cent of total energy intake, diet A and B resp.) on nitrogen balance and on whole body protein turnover (PT) was measured in 17 elderly men and 11 elderly women (mean±SD: 74±12 y; 68±9 y resp.) with different levels of physical activity (PA). Mean nitrogen balance (mean±SD: diet A -0.004±0.027 g/kg.d; diet B 0.011±0.064 g/kg.d) did not differ significantly from zero during either diet. PT increased significantly when the protein content of the diet increased from 12 to 21 percent of total energy ($P<0.0001$). There was a positive correlation between protein intake and PT (diet A synthesis and breakdown $P<0.01$; diet B synthesis $P< 0.001$, breakdown $P<0.0001$). PT rates were significantly higher for men when compared with women, even when corrections were made for differences in body composition ($P<0.05$).

Introduction

The current recommended protein intake for the elderly (aged 65 years or older) is 0.75 g per kg per day as given by the FAO/WHO (1985). The Dutch Nutrition Council recommends 11-12% energy from protein or 0.80 and 0.85 g protein per kg per day for women and men respectively, at the average body weight and energy expenditure for the elderly. This recommended protein intake for the elderly is mainly based on extrapolations of data obtained from healthy young adults. However, total energy expenditure (EE), and therefore energy intake (EI), decreases with age because of a decline in fat free mass (FFM) (Forbes & Reina 1970) and a diminished physical activity (PA) (McGandy et al 1966; Garry et al 1989) in the elderly compared with young adults. Total EI declines progressively from 11.3 MJ/d in 30 year old subjects

to 8.8 MJ/d for those around 80 years; a decrease of 2.5 MJ/d (22%). The decrease in FFM accounts for 0.8 MJ and the decreased PA accounts for 1.7 MJ (McGandy et al 1966). If the protein content of the food remains unchanged (e.g. 11-12% of total EI), this lowered energy intake will lead to a lower protein intake especially in those who are very inactive. It is known that subjects can be in nitrogen balance within a wide range of protein intake. Nitrogen balance is a reflection of overall body protein synthesis and breakdown (Young et al 1989). So the question is what consequences do the daily protein recommendations have for the whole body protein turnover in the elderly. There are only a few reports of whole body protein turnover measurements in the elderly (Robert et al 1984, Golden& Waterlow 1977, Winterer et al 1976, Uauy et al 1978). Whole body protein turnover expressed per unit body weight seems to decrease with age (Golden& Waterlow 1977, Winterer et al 1976, Uauy et al 1978) and expressed per unit FFM no differences were seen between young and older subjects. One study reports a decreased leucine flux (g/kg) for elderly women when compared with younger women (Robert et al 1984). In the same study no differences were seen between young and old men. In all of these studies measurements were done on small groups and no distinction was made between active and inactive subjects or between men and women of one age group. The purpose of the present study was to measure whole body protein turnover in elderly subjects who had a known activity level ranging from low to high. Additionally the effect of the level of protein intake was studied in a cross over design with two diets with protein as 12 or 12 per cent of energy intake. Comparisons were made between men and women.

Subjects and methods

Subjects

Seventeen free living elderly men and eleven free living elderly women participated in this study. Mean age, height, weight, body mass index (BMI) and physical activity index (PAI) are presented in Table 5.1. The subjects' PAI was calculated by dividing their average daily metabolic rate (ADMR) by their basal metabolic rate (BMR). ADMR was measured with doubly labeled water as described before (Pannemans & Westerterp 1993) and BMR with a computerized open-circuit ventilated hood system (unpublished data). Subjects were recruited with advertisements in the local media, and through contacts with alliances for the elderly. All subjects were certified to be in good health by a staff physician and gave informed consent to participate in the study after the procedures were explained to them. The protocol was approved by the university ethics committee.

Table 5.1. Physical characteristics of the subjects (mean±SD; range in parentheses)

N	Sex	Age (years)	Height (m)	Weight (kg)	BMI (kg/m^2)	PAI*
17	M	72±5	1.72±0.09	73.8±12.2	24.9±3.2	1.5±0.2
		(65-80)	(1.60-1.91)	(56.5-102.7)	(18.7-28.9)	(1.3-2.1)
11	F	67±4	1.61±0.08	67.6±9.2	26.2±3.2	1.6±0.2
		(63-78)	(1.49-1.72)	(55.0-89.7)	(21.1-32.6)	(1.3-2.0)

*PAI: physical activity index measured as subjects' average daily metabolic rate divided by their basal metabolic rate

Diet

All subjects were given two different diets for three weeks allocated according to a cross over design with a 'wash-out' period of at least three weeks. There was an iso-energetic exchange between protein and fat. In diet A 12 per cent of the total energy (en%) was protein, 43 en% fat and 46 en% carbohydrate whereas in diet B 21 en% was protein, 35 en% fat and 46 en% carbohydrate. For the purpose of the study six energy levels (7.5 - 11.25 MJ/d) were produced. Subjects were fed according to their estimated energy intake (Pannemans & Westerterp 1993). Daily food intake contained: bread plus butter, marmalade and meat; potatoes plus vegetables, meat and gravy; yoghurt, sugar, fruits, juice, milk and cake. Iso-energetic exchange between protein and fat was achieved by using low fat milk, meat and bread enriched with protein (sodium caseinate), and by using low fat yoghurt and gravy in diet A to replace the fat milk, meat, bread, yoghurt and gravy of diet B. During the intervention all meals were provided daily at home. The subjects were not allowed to eat or drink anything else except for water, tea and coffee. The first week of each period was a preliminary period in which subjects could get used to the diet and in which, if necessary, adjustments in energy intake could be made.

Protein metabolism

Nitrogen balance

In order to determine nitrogen balance (calculated as nitrogen intake minus nitrogen in feces and urine) the subjects collected 24 h urine for two days and total feces for three days (during the last week of each diet period). 24 h urine collection started with the second urine in the morning and included the first voiding of the next day. Daily feces collection started at 7.00 o'clock in the morning till 7.00 o'clock the next day. After total volume and total weight of urine and feces were measured the nitrogen content of both were measured with a Heraeus analyzer (type CHN-O-rapid). Corrections were made for other obligatory losses (8 mg N/kg body weight (FAO/WHO 1985)).

Protein turnover

- *Procedure*

  Whole body protein turnover was measured with ^{15}N-glycine given orally (Cambridge Isotope Laboratories; 200 mg; 99 atom %) at the last day of each diet

period. Rates of protein breakdown and synthesis were estimated from the urinary excretion of ^{15}N in ammonia and urea during the following 11 h. Subjects were not allowed to eat or drink from at least three hours before administration of the ^{15}N-glycine and during the following 11 h of the study period.

- *Measurement of urinary ammonia, urinary and plasma urea and total urinary nitrogen*
  Urinary ammonia and urea concentrations were determined spectrophotometrically by standard enzymatic methods on a centrifugal analyzer system (Cobas Bio; Roche Diagnostics, Hoffmann La Roche, Basle, Switzerland) using commercial kits (Janssen et al 1988, Bergmeyer 1974). Urea values were corrected for ammonia. Total nitrogen in urine was measured with a Heraeus analyzer (type CHN-O-rapid).
- *Determination of ^{15}N enrichment of urinary ammonia and urea and of plasma urea*
  ^{15}N enrichment of urinary ammonia and urea and of plasma urea was measured using a sodium/potassium form of a cationic ion-exchange which specifically binds ammonia from neutral solutions, as described by Read, Harrison and Halliday (Read et al 1982). The resin-ammonia complex was reacted directly with alkaline hypobromite to produce a quantitative yield of molecular nitrogen which was directly analyzed in a mass spectrometer.
- *Calculations of the rate of protein turnover*
  Nitrogen flux (= catabolism since no food was ingested during the measurements) was calculated from the equation (Fern et al 1981): $Q=E_x*d/e_x$ where Q is the rate of nitrogen flux (g of nitrogen/11h); E_x is the excretion of ammonia or urea (g of nitrogen/11h); d is the dose of isotopic nitrogen (g of ^{15}N); e_x is the amount of isotope excreted in the urine as ammonia in 11 h or in the case of urea, is the sum of the amount excreted in 11 h and the amount retained in the urea pool of the body at the end of 11 h (g of ^{15}N). The rate of ammonia excretion was taken to equal the actual amount of ammonia nitrogen excreted in the urine during the experimental period. The rate of urea excretion was taken to equal the amount of urea nitrogen excreted in the urine after adjusting for changes in the body urea pool. The size of this pool was calculated as the sum of the plasma urea concentration and its volume of distribution (represented by the total body water -TBW). TBW was measured by deuterium dilution during the protein turnover measurements. Rates of protein synthesis and breakdown in the whole body were derived from the expression Q=E+Z=I+B, were E is the rate of excretion of total nitrogen in urine, Z is the rate of whole body protein synthesis, I is the rate of intake of nitrogen from the diet and B is the rate of whole body protein breakdown. As these studies were conducted in the fasting state protein breakdown is equal to the calculated flux. All units are expressed as g of nitrogen/11h. A factor of 6.25 was used to convert g of nitrogen into g of protein. Whole body protein turnover was taken as the harmonic average of the estimates of flux based on urea and ammonia (Fern et al 1984a).

Statistics

When comparisons were made between diet A and diet B a paired Student's t-test was used since all subjects acted as their own control. When comparisons were made between men and women (on diet A or diet B) an unpaired Student's t-test was used. Gender and diet interaction was tested by repeated measure two way ANOVA.

Results

Nitrogen balance

Table 5.2. presents the results with respect to the nitrogen balance. Nine women and sixteen men completed both diets. One woman completed only diet A and one woman and one man completed only diet B. When comparing diet A with diet B these subjects were excluded, when comparing men and women these subjects were included.

Nitrogen balance data were expressed per kg body weight to facilitate interpretation given body mass differences between men and women. Because of the 12 en% and 21 en% protein of diets A and B, respectively, nitrogen intake was significantly higher on the latter diet. The nitrogen content of the urine was significantly higher on diet B but this had no effect on the nitrogen balance. There were differences between men and women as well. During diet A the nitrogen intake was significantly higher for the men and with diet A, the nitrogen content of the urine was also significantly higher for the men. There were no differences in nitrogen balance between men and women. The PA level had no effect on the nitrogen balance.

Protein turnover

Results on protein turnover measurements are presented in Table 5.3. Protein turnover was measured in the postabsorptive state and not surprisingly protein breakdown was higher than protein synthesis. Repeated measure two way ANOVA revealed that no interaction exists between diet and gender. In other words: the effect of the amount of protein in the diet on protein turnover rates did not differ for men and women. When comparing diet A with B protein turnover (breakdown and synthesis) increases significantly when changing from 12 en% protein to 21 en% protein in the diet ($P<0.0001$). When comparing men with women protein turnover (g protein/d) is significantly higher for the men during both diet A and diet B ($P<0.0001$). These differences could be explained by a significantly higher protein intake (g/d; diet A $P<0.001$; diet B $P<0.0001$) for the men compared with the women. When expressed as g/kg.d protein intake is the same for both sexes. At a same level of protein intake protein turnover is significantly higher for the men (diet A $P<0.0001$; diet B $P<0.001$). When corrections were made for body composition by expressing protein turnover in g/kg.FFM this difference still exists (diet A and B $P<0.05$). Age and activity level in elderly men and women did not contribute to the explained variation in turnover rates.

Table 5.2. Nitrogen balance (mean±SD)

		Diet A			Diet B	
	Total (n=25 ‡)	Men (n=16)	Women (n=10)	Total (n=25 ‡)	Men (n=17)	Women (n=10)
N-intake (g/kg.d)	0.142±0.028****	0.510±0.028†	0.127±0.020	0.246±0.045	0.256±0.44	0.227±0.037
N-urine (g/kg.d)	0.116±0.029****	0.126±0.025†	0.098±0.028	0.206±0.067	0.208±0.072	0.185±0.065
N-faeces (g/kg.d)	0.021±0.006	0.021±0.004	0.021±0.004	0.021±0.009	0.023±0.010	0.021±0.009
N-balance§ (g/kg.d)	-0.004±0.027	-0.004±0.029	-0.005±0.024	0.011±0.064	0.018±0.073	0.014±0.061

Diet A contains 12 en% protein; Diet B contains 21 en% protein; ‡ 16 men and 9 women who completed both diets; ****P<0.0001; differences between diet A and diet B for the whole group; paired t-test; †P<0.05; differences between men and women during diet A or diet B; unpaired t-test; § corrections of 8 mg N/kg.d are made for other obligatory losses

Table 5.3. Protein intake and protein turnover rates (mean±SD)

		Diet A			Diet B	
	Total (n=25 ‡)	Men (n=16)	Women (n=10)	Total (n=25 ‡)	Men (n=17)	Women (n=10)
PI (g/d)	62.0±10.2****	67.3±7.8††††	53.1±6.1	107.3±16.3	114.5±13.2††††	92.2±9.7
Breakdown (g/d)#	224.1±67.7****	257.4±58.1††††	164.6±33.8	323.6±114.6	373.0±102.6††††	212.6±51.1
Synthesis (g/d)	181.5±61.2****	211.2±52.3††††	127.5±32.9	258.2±111.5	306.3±97.5††††	148.5±54.9
PI (g/kg.d)	0.9±0.2****	0.9±0.2	0.8±0.1	1.5±0.3	1.6±0.3	1.4±0.2
Breakdown (g/kg.d)	3.2±0.8****	3.6±0.7††††	2.4±0.3	4.6±1.6	5.2±1.5†††	3.3±0.8
Synthesis (g/kg.d)	2.6±0.8****	2.9±0.7††††	1.9±0.3	3.7±1.6	4.3±1.5†††	2.3±0.9
PI (g/kg FFM.d)	1.3±0.2****	1.3±0.2	1.3±0.2	2.2±0.3	2.1±0.3	2.3±0.3
Breakdown (g/kg FFM.d)	4.5±0.9****	4.8±0.9†	4.0±0.5	6.6±1.8	7.0±1.9†	5.4±1.3
Synthesis (g/kg FFM.d)	3.6±0.9****	3.9±0.9†	3.1±0.6	5.2±1.9	5.7±1.8†	3.8±1.5

Diet A contains 12 en% protein; Diet B contains 21 en% protein; PI= protein intake; ‡ 16 men and 9 women who completed both diets; #Protein turnover rates are calculated as the harmonic mean of ammonia and urea results; ****P<0.0001; differences between diet A and diet B for the whole group; †P<0.05; †††P<0.001; ††††P<0.0001; differences between men and women during diet A or diet B

As shown in Figure 5.1 there is a positive relation between protein intake (g/d) and protein turnover (g/d) during both diets (diet A breakdown and synthesis: $P<0.01$; diet B breakdown: $P<0.001$ synthesis: $P<0.0001$).

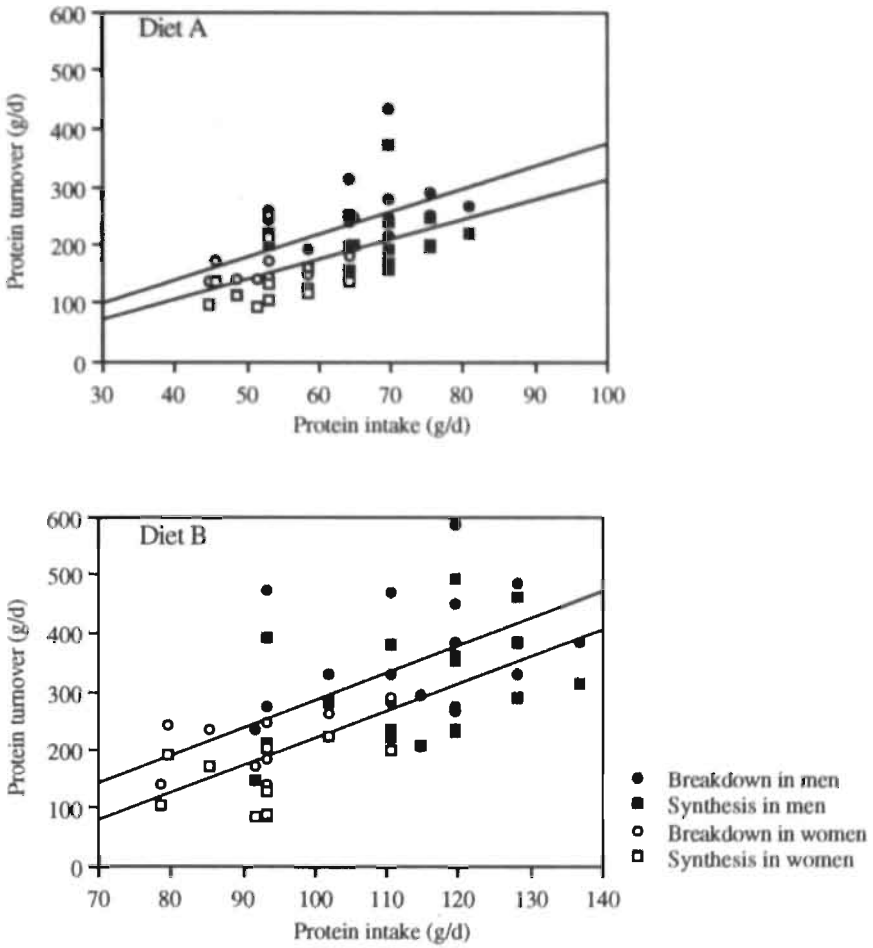

Fig. 5.1. Protein turnover (PT) plotted as a function of protein intake (PI)with the calculated linear regression lines: Diet A protein breakdown=3.92(PI)-20.78 ($P<0.01$); Diet A protein synthesis=3.45(PI)-34.37 ($P<0.01$); Diet B protein breakdown=4.71(PI)-186.95 ($P<0.001$); Diet B protein synthesis=4.71(PI)-252.34 ($P<0.0001$)

Discussion

Mean nitrogen balance does not differ significantly from zero during both diets. In other words when elderly subjects were fed according to the recommended protein

intake (12 percent of energy intake) or a higher protein diet (21 percent of energy intake) for three weeks, they were in nitrogen balance at the end of both diet periods. It is assumed that these measurements were performed "perfectly". Because creatinine excretion in urine during diet A correlates well with creatinine excretion during diet B ($P<0.0001$) and the mean coefficient of variation (CV) was 11% it can be concluded that the urine collection was complete. However nitrogen balance measurements reflect only the balance between overall body protein synthesis and breakdown rates and a given nitrogen balance may be achieved within a wide range of protein synthesis and breakdown rates (Young et al 1989). Therefore protein turnover was also measured.

Protein turnover can be measured in several ways (Waterlow et al 1978a). Waterlow et al (1978a) compared methods of measuring total protein turnover and concluded that all methods are probably measuring essentially the same thing, and that any one of them is likely to be adequate for comparative measurements. In this study protein turnover was measured in the postabsorptive state with an orally given dose (200 mg) of ^{15}N-glycine. Protein turnover was calculated on the basis of excreted end products urea and ammonia. This method was used because of its simplicity and its convenience for use outside the laboratory especially when elderly people are involved. Fern et al (1984b) reported about the precision of this method in the fed state. The overall precision was between 5 and 11 per cent (CV) when based on ammonia or urea. For synthesis the variation was slightly larger, up to 15 per cent for ammonia and urea. Table 5.4. shows the results of protein turnover based on urea and ammonia. Protein turnover (breakdown and synthesis) is always significantly lower based on ammonia compared with results based on urea (during diet A and B $P<0.01$). Fern et al (1984a) and Waterlow et al (1978b) have reported about the differences in protein turnover when based on different end products. The overall conclusion is the same: rates given by ammonia are almost always lower than rates given by urea. However, precision of the method used increases when turnover rates of ammonia and urea are taken together in the harmonic or arithmetic average (Fern et al 1984a). The precision for breakdown rates is between 3 and 6 per cent when based on the arithmetic or harmonic average of rates given by these two end products and between 5 and 7 per cent for synthesis when using the two end product averages. In the above mentioned article of Fern et al (1984b) it is stated that even though the harmonic average and the arithmetic average are based on different assumptions, their estimates of whole body protein turnover were very similar (less than 2 per cent). The results of this study are comparable when using the arithmetic or the harmonic average of the end product, and results are presented as the harmonic average of both end products. Protein turnover increases significantly when changing from 12 to 21 en% protein in the diet ($P<0.0001$). These findings in the elderly are in accordance with studies of the effects of dietary protein in young adults, as reviewed by Garlick et al (1991). The conclusion, with respect to a longer term adaptation to higher intakes of protein, is that there is a modification of the basal (fasting) level of protein turnover, both synthesis and degradation are increased in the postabsorptive state. It is not known what the impli-

Table 5.4. Protein turnover rates for urea and ammonia (mean±SD)

		Diet A			Diet B		
		Total (n=25 ‡)	Men (n=16)	Women (n=10)	Total (n=25 ‡)	Men (n=17)	Women (n=10)
Breakdown (g/d)	urea	329±167***	358±171	278±147	447±208	522±222†	321±90
	NH_3	190±86****	227±87††	124±21	282±127	328±124††	269±79
Synthesis (g/d)	urea	290±169**	315±175	247±147	389±208	462±227†	269±79
	NH_3	151±78****	184±80††	91±21	223±122	267±118††	119±65
Breakdown (g/kg.d)	urea	4.8±2.9***	5.2±3.4	4.0±1.5	6.5±3.7	7.5±4.3	4.9±1.3
	NH_3	2.7±1.1****	3.2±1.1††	1.9±0.3	4.0±1.7	4.6±1.7††	2.7±1.0
Synthesis (g/kg.d)	urea	4.2±2.9**	4.6±3.4	3.6±1.6	5.7±3.8	6.7±4.3	4.1±1.2
	NH_3	2.1±1.0****	2.6±1.0††	1.4±3.1	3.2±1.7	3.7±1.6††	1.8±1.0
Breakdown (g/kg FFM.d)	urea	6.7±3.2***	6.7±3.4	6.6±2.7	9.2±4.4	9.9±5.0	8.2±2.6
	NH_3	3.8±1.4****	4.3±1.5†	3.0±0.5	5.7±2.1	6.2±2.3†	4.3±1.6
Synthesis (g/kg FFM.d)	urea	5.9±3.2**	5.9±3.5	5.8±2.8	8.1±4.5	8.8±5.1	6.9±2.4
	NH_3	3.0±1.3****	3.4±1.4†	2.2±0.6	4.5±2.1	5.0±2.2†	3.0±1.7

Diet A contains 12 en% protein; Diet B contains 21 en% protein; PI= protein intake; ‡ 16 men and 9 women who completed both diets; **P<0.01; ***P<0.001; ****P<0.0001; differences between diet A and diet B for the whole group; †P<0.05; ††P<0.01; differences between men and women during diet A or diet B

cation of the increased protein turnover is. It is known from literature (Young 1984; Munro & Young 1980) that the contribution of the skeletal muscle to whole body protein turnover decreases with age with a relatively larger contribution from the visceral organs in the elderly. Recently Welle et al (1993) studied myofibrillar protein synthesis in young and elderly men. The major finding of this study was that (fractional and total) myofibrillar protein synthesis was slower in elderly subjects (>60 y) compared with young adults (<35 y). Yarasheski et al (1993) reported about the acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. It was concluded that muscle protein synthesis was lower in elderly men and women compared with young men and women. However, after only two weeks of resistance training muscle protein synthesis increased in young and elderly persons and no differences were found between both age groups. The implications of the lower contribution of the skeletal muscle to whole body protein turnover are not yet understood. It is speculated (Young 1990), because muscles contribute to the adaptation in whole body energy and amino acid metabolism during restricted dietary energy and protein intakes, that a reduced contribution of the muscle to whole body protein metabolism might diminish the capacity of the elderly individual to respond successfully to unfavourable dietary situations. The increased protein turnover after the 21 en% diet probably means that one is better equipped for an unfavourable nutritional status or to other stressful conditions. Further studies should investigate whether this increased protein turnover arises from a greater contribution from skeletal muscles: an outcome that may well be favourable in the elderly.

Furthermore, the absolute difference between breakdown and synthesis was higher during diet B ($P<0.0001$) compared with diet A and the absolute difference between breakdown and synthesis is also higher for the elderly men during diet A compared with the women. During diet B there were no differences. When expressed relatively (%) there were no differences between the diets and also no differences between the sexes, indicating that the increase in protein breakdown and synthesis is the same. Further interpretation needs protein turnover measurements during the day to see if the net protein breakdown during the night is compensated during the day when synthesis is higher than breakdown because of feeding, as has been described before in obese women (Garlick 1980) and normal adults (Millward et al 1990).

During diet A (12en%), the nitrogen loss (as measured during the nitrogen balance) was positively correlated ($P<0.01$) with whole body protein breakdown (Figure 5.2.). This has been previously reported by Uauy et al (1978). This is not seen during diet B, probably due to larger variation. The differences in protein turnover between elderly men and women are remarkable. At the same level of protein intake (in en%, g/kg.d, g/kg FFM.d) protein breakdown and synthesis are significantly lower in elderly women (Table 5.3.) during both diets. Only Winterer et al (1976) reported a lower protein turnover for women (young and elderly) compared with men (young and elderly). In the other studies no distinction was made between the sexes (Robert et al 1984, Golden & Waterlow 1977, Uauy et al 1978). It is not known why these differences exist but it probably results from hormonal control as suggested by Smith

and Rennie (1990). Male and female sex hormones (testosterone and oestrogens) stimulate protein synthesis. In women oestrogen levels decrease during and after menopause while in men testosterone levels remain constant.

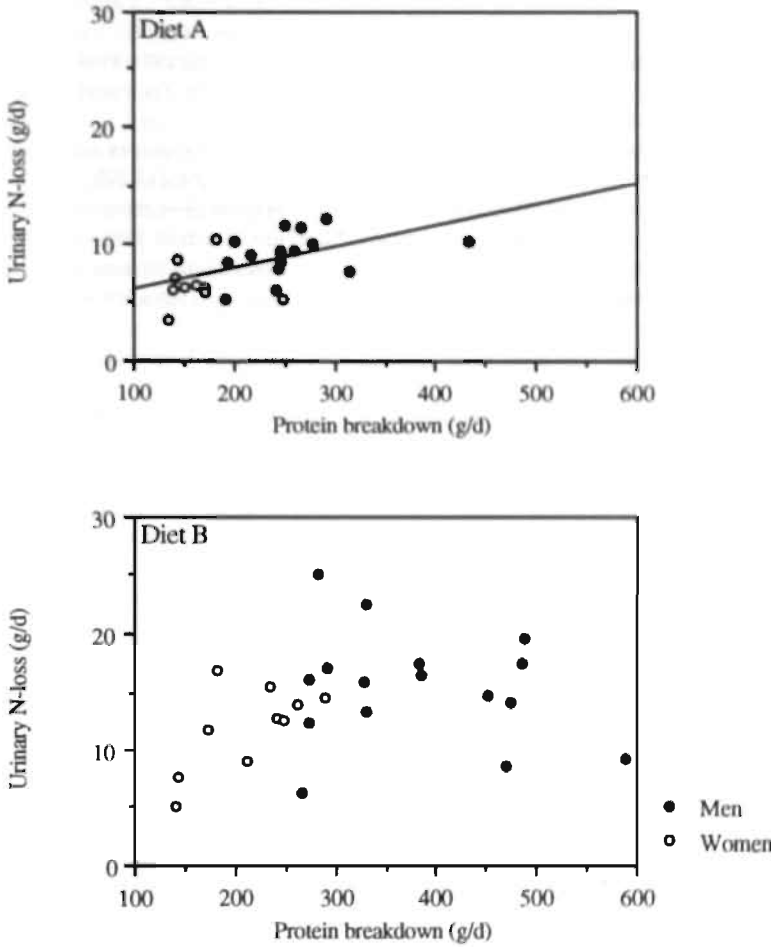

Fig. 5.2. Urinary nitrogen loss plotted as a function of protein breakdown during diet A with the calculated regression line: urinary N-loss= 0.018(protein breakdown)+4.25 ($P<0.01$)

In conclusion, protein turnover increases significantly when changing from diet A (12en%) to diet B (21 en%) while there was no effect on the nitrogen balance. There is a positive correlation between protein intake and protein turnover. Furthermore elderly women have lower protein turnover rates compared with elderly men. It is not possible to compare the absolute protein turnover data of this study with other studies because different techniques were used to measure protein turnover (Robert et al 1984, Golden& Waterlow 1977, Winterer et al 1976, Uauy et al 1978). To interprete

these results in more detail it is necessary to repeat the same experiment in young adults.

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Chapter 6

Effect of variable protein intake on whole body protein turnover in young and elderly men and women

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Abstract

The effect of the level of protein intake (12% and 21%of total energy intake; diet A and diet B respectively) on nitrogen balance and on whole body protein turnover (PT) was measured in 19 young men and 10 young women (aged: 30±5 y and 27±4 y resp.). Comparisons were made with earlier findings in elderly subjects. In young adults, mean nitrogen balance was approximately zero during diet A while it was positive during diet B. In young adults, PT was significantly higher during diet B in comparison with diet A. This was likewise seen in the elderly. During diet A, young adults had higher PT rates compared with elderly subjects. During the higher protein intake (Diet B), PT of young men was comparable with PT of elderly men while young women still had higher PT rates compared with elderly women (even when corrections were made for differences in body composition).

Introduction

Very little is known about protein needs and protein metabolism in the elderly and the current recommended protein intake for people aged over 65 years is mainly based upon data obtained in young adults (FAO/WHO 1985). However protein metabolism might be expected to change with old age because of a decrease in energy expenditure. Energy expenditure decreases with age because of a decrease in basal metabolic rate (according to a decrease in fat free mass (FFM) (Forbes 1970)) and because of a decrease in physical activity (PA) (McGandy 1966; Garry 1989). The decrease in energy expenditure will lead to a decrease in energy intake. This lower energy intake might lead to a lower protein intake, and therefore to lower protein turnover rates, especially

in the very inactive elderly. In the past only a few authors reported about protein turnover rates in elderly subjects. In general it was concluded that protein turnover rates (expressed per kg body weight) decrease with advancing age (Winterer et al 1976; Uauy et al 1978; Golden & Waterlow 1977). In the study of Robert et al (1984) no differences in protein turnover were found between young and elderly men while elderly women had lower protein turnover rates compared with young women. When corrections were made for body composition, by expressing protein turnover per kg FFM, no differences were found between young and elderly subjects. The above mentioned studies were done on small groups and no distinction was made between men and women of one age group. Recently we studied whole body protein turnover rates in elderly men and elderly women and their response to two levels of protein intake (Pannemans et al, in press). Protein turnover rates were significantly higher for men when compared with women, even when corrections were made for differences in body composition ($P<0.05$). Furthermore the results indicated that protein turnover increases significantly when the protein content of the diet was increased from 12 to 21% of total energy intake ($P<0.0001$). It was difficult to compare these data with other studies undertaken in young or elderly subjects because of the different techniques used to measure protein turnover. For a better understanding of the results in the elderly, the experiment was repeated in young adults as described the present study.

Subjects and methods

Subjects

Nineteen young men and ten young women participated in this study. Mean height, weight, body mass index and physical activity index (calculated as subjects average metabolic rate divided by their basal metabolic rate, unpublished data) are presented in Table 6.1. Subjects were recruited with advertisements in local media. Subjects gave informed consent to participate in the study after the procedures were explained to them. The protocol was approved by the university ethics committee. Comparisons were made with seventeen elderly men and eleven elderly women of whom physical characteristics have been described earlier (Pannemans et al in press). For comparison reasons physical characteristics of the elderly were also added to Table 6.1.

Table 6.1. Physical characteristics of the subjects (mean±SD)

	Young men (n=19)	Young women (n=10)	Elderly men (n=17)	Elderly women (n=11)
Age (years)	30±5	27±4	72±5	67±4
Height (m)	1.81±0.05	1.68±0.06	1.72±0.09	1.61±0.08
Weight (kg)	76.3±8.6	60.8±7.5	73.8±12.2	67.6±9.2
BMI (kg/m^2)	23.2±2.0	21.5±2.0	24.9±3.2	26.2±3.2
PAI*	1.7±0.1	1.7±0.2	1.5±0.2	1.6±0.2

*PAI: physical activity index measured as subjects' average daily metabolic rate divided by their basal metabolic rate

Diet

All subjects were given two different diets for three weeks allocated according to a cross over design with a 'wash-out' period of at least three weeks as described before (Pannemans et al in press). There was an iso-energetic exchange between protein and fat. In diet A 12 per cent of the total energy (en%) was protein, 43 en% fat and 46 en% carbohydrate whereas in diet B 21 en% was protein, 35 en% fat and 46 en% carbohydrate. Subjects were fed according to their estimated energy intake (Pannemans & Westerterp 1993). The first week of each period was a preliminary period in which subjects could get used to the diet and in which, if necessary, adjustments in energy intake could be made.

Nitrogen balance

In order to determine nitrogen balance (calculated as nitrogen intake minus nitrogen in feces and urine) the subjects collected 24 h urine for two days and total feces for three days (during the last week of each diet period). After total volume and total weight of urine and feces were measured the nitrogen content of both were measured with a Heraeus analyzer (type CHN-O-rapid). Corrections were made for other obligatory losses (8 mg N/kg body weight (FAO/WHO,1985)).

Protein turnover

- *Procedure*

  Whole body protein turnover was measured with ^{15}N-glycine given orally (Cambridge Isotope Laboratories; 200 mg; 99 atom %) at the last day of each diet period. Rates of protein breakdown and synthesis were estimated from the urinary excretion of ^{15}N in ammonia and urea during the following 11 h. Subjects were not allowed to eat or drink from at least three hours before administration of the ^{15}N-glycine and during the following 11 h of the study period.

- *Measurement of urinary ammonia, urinary and plasma urea and total urinary nitrogen*

  Urinary ammonia and urea concentrations were determined spectrophotometrically by standard enzymatic methods on a centrifugal analyzer system (Cobas Bio; Roche Diagnostics, Hoffmann La Roche, Basle, Switzerland) using commercial kits (Janssen et al 1988, Bergmeyer 1974). Urea values were corrected for ammonia. Total nitrogen in urine was measured on a Heraeus analyzer (type CHN-O-rapid).

- *Determination of ^{15}N enrichment of urinary ammonia and urea and of plasma urea*

  ^{15}N enrichment of urinary ammonia and urea and of plasma urea was measured using a sodium/potassium form of a cationic ion-exchange which specifically binds ammonia from neutral solutions, as described by Read, Harrison and Halliday (Read et al 1982). The resin-ammonia complex was reacted directly with alkaline hypobromite to produce a quantitative yield of molecular nitrogen which was directly analyzed in an isotope ratio mass spectrometer.

- *Calculations of the rate of protein turnover*

  Calculations of the rate of protein turnover were made as described previously (Pannemans et al in press) In short: nitrogen flux was calculated from the equation (Fern et al 1981): $Q=E_x*d/e_x$ where Q is the rate of nitrogen flux (g of nitrogen/11h); E_x is the excretion of ammonia or urea (g of nitrogen/11h); d is the dose of isotopic nitrogen (g of ^{15}N); e_x is the amount of isotope excreted in the urine as ammonia in 11 h or in the case of urea, is the sum of the amount excreted in 11 h and the amount retained in the urea pool of the body at the end of 11 h (g of ^{15}N). Rates of protein synthesis and breakdown in the whole body were derived from the expression Q=E+Z=I+B, were E is the rate of excretion of total nitrogen in urine, Z is the rate of whole body protein synthesis, I is the rate of intake of nitrogen from the diet and B is the rate of whole body protein breakdown. As these studies were conducted in the fasting state protein breakdown is equal to the calculated flux. All units are expressed as g of nitrogen/11h. A factor of 6.25 was used to convert g of nitrogen into g of protein. Whole body protein turnover was taken as the harmonic average of the estimates of flux based on urea and ammonia (Fern et al 1984).

Statistics

When comparisons were made between between diet A and diet B a paired students t-test was used since every subject acted as their own control. When comparisons were made between elderly men, elderly women, young men and young women (on diet A or diet B) one factor ANOVA (with age and gender combined in one factor) was used. Significance values were adjusted for multiple comparisons according to Bonferroni. Diet and gender interactions were tested by two factor ANOVA repeated measures. Data are given as mean±SD.

Results

Nitrogen balance

In young adults, mean nitrogen intake was as expected higher during diet B (diet A: 11.0±1.8 g/d; diet B: 19.8±3.2 g/d). Urinary nitrogen excretion was significantly higher during the higher protein intake (diet A: 8.4±2.0 g/d; diet B: 14.4±3.0 g/d; $P<0.0001$), while nitrogen excretion in faeces remained constant (diet A: 1.5±0.5 g/d; diet B: 1.5±0.6 g/d). Nitrogen balance was more positive during diet B (diet A: 0.6±1.9 g/d; diet B: 3.3±2.9 g/d; $P<0.0001$). Table 6.2. shows the nitrogen balance data expressed per kg body weight for young men and women and elderly men and women. During diet A and B no differences in nitrogen intake, urinary nitrogen excretion, fecal nitrogen excretion and nitrogen balance were seen between young men and young women and there were no differences in nitrogen balance. When comparisons were made between young men and old men and between young women and

old women no differences were seen except for a higher nitrogen intake in young women compared with elderly women.

Protein turnover

In young adults, protein turnover increased when protein intake increased from 12% to 21% of total energy intake (diet A: protein breakdown 321.0±97.0 (g/d); protein synthesis 270.3±89.9 (g/d); diet B: protein breakdown 393.5±81.1 (g/d); protein synthesis 311.8±77.9 (g/d); $P<0.001$ and $P<0.05$ resp.). Table 6.3. shows the protein turnover data for young men and women and elderly men and women. In the young subjects, protein turnover rates (expressed in g/d) are significantly higher for the young men. However when protein turnover is expressed per kilogram body weight (g/kg.d) or per kilogram FFM (g/kg FFM.d) no differences were found between both sexes (during diet A as well as diet B). As shown in Table 6.3. young men and women had higher protein turnover rates compared with elderly men and women when expressed in grams per day. However these differences could be due to the significantly higher protein intake (g/d) in the young adults. When corrections were made for differences in body composition by expressing protein intake and protein turnover per kilogram FFM (g/kg FFM.d) protein intake did not differ for all groups while protein turnover was still significantly lower for the elderly women compared with the young women during diet A and B. Elderly men had significantly lower protein turnover rates compared with young men during diet A, no differences were seen during diet B.

Protein breakdown as well as protein synthesis were positively correlated with protein intake (diet A: $P<0.05$ diet B: $P<0.001$) as shown in Figure 6.1. Comparisons with the regression lines in the elderly (Pannemans et al in press) revealed that, during diet A, protein turnover was significantly higher in the young adults (synthesis as well as breakdown $P<0.05$) when corrections were made for differences in protein intake (g/d). During diet B no differences were found. Figure 6.2. shows the regression lines of protein intake versus protein breakdown in young and elderly subjects during diet A and diet B (regression lines with protein synthesis show the same results -for clarity reasons they were not included).

Table 6.2. Nitrogen balance in young and elderly men and women (mean±SD)

	Diet A				Diet B			
	Young adults		Elderly		Young adults		Elderly	
	Men (n=19)	Women (n=10)	Men (n=16)	Women (n=10)	Men (n=19)	Women (n=10)	Men (n=17)	Women (n=10)
N-intake (g/kg.d)	0.158±0.027	0.159±0.022**	0.151±0.028†	0.127±0.020	0.285±0.047	0.285±0.038**	0.256±0.044	0.227±0.037
N-urine (g/kg.d)	0.122±0.023	0.122±0.039	0.126±0.025†	0.098±0.028	0.202±0.050	0.224±0.043	0.208±0.072	0.185±0.065
N-faeces (g/kg.d)	0.023±0.031	0.021±0.010	0.021±0.004	0.021±0.007	0.022±0.010	0.021±0.011	0.023±0.010	0.021±0.009
N-balance (g/kg.d)§	0.008±0.031	0.013±0.024	-0.004±0.029	-0.005±0.024	0.053±0.034	0.034±0.048	0.018±0.073	0.014±0.061

Diet A contained 12% of total energy from protein; Diet B contained 21% of total energy from protein; †P<0.05; differences between elderly men and elderly women during diet A; one factor ANOVA using Bonferroni correction; **P<0.01; differences between elderly women and young women during diet A and diet B; one factor ANOVA; § corrections of 8 mg N/kg.d were made for obligatory losses

Table 6.3. Protein intake and turnover rates in young and elderly men and women (mean±SD)

	Diet A				Diet B			
	Young adults		Elderly		Young adults		Elderly	
	Men (n=19)	Women (n=10)	Men (n=16)	Women (n=10)	Men (n=19)	Women (n=10)	Men (n=17)	Women (n=10)
PI (g/d)	73.8±10.3††††	59.6±4.7	67.3±7.8††††	53.1±6.1	132.5±18.2$^{\dagger\dagger\dagger\dagger/***}$	106.5±8.5	114.5±13.2†††	92.2±9.7
Breakdown (g/d)$^{\#}$	357.4±93.8$^{\dagger\dagger\dagger/***}$	251.7±59.7**	257.4±58.1††	164.6±33.8	429.0±7.57††	332.0±46.7**	373.0±102.6††††	212.6±51.1
Synthesis (g/d)	304.0±86.6$^{\dagger\dagger\dagger/***}$	207.0±57.4*	211.2±52.3††	127.5±32.9	344.2±72††	250.2±45.4**	306.3±97.5††††	148.5±54.9
PI (g/kg.d)	1.0±0.2	1.0±0.1**	0.9±0.2	0.8±0.1	1.8±0.3	1.8±0.2**	1.6±0.3	1.4±0.2
Breakdown (g/kg.d)	4.8±1.2***	4.2±0.8****	3.6±0.7††	2.4±0.3	5.8±1.0	5.6±0.5****	5.2±1.5††††	3.3±0.8
Synthesis (g/kg.d)	4.1±1.1***	3.5±0.8****	2.9±0.7†	1.9±0.3	4.6±0.9	4.2±0.5***	4.3±1.5††††	2.3±0.9
PI(g/kg FFM.d)	1.2±0.2	1.3±0.1	1.3±0.2	1.3±0.2	2.2±0.3	2.2±0.3	2.1±0.3	2.2±0.3
Breakdown (g/kg FFM.d)	5.9±1.6**	5.5±1.1**	4.8±0.9†	4.0±0.5	7.0±1.9	7.3±0.9**	7.0±1.0††	5.4±1.3
Synthesis (g/kg FFM.d)	5.0±1.4*	4.6±1.1**	3.9±0.9†	3.1±0.6	5.6±1.0	5.5±0.8**	5.7±1.8††	3.8±1.5

Diet A contained 12% of total energy from protein; Diet B contained 21% of total energy from protein; $^{\#}$Protein turnover rates are calculated as the harmonic mean of ammonia and urea results; ††††P<0.0001; †††P<0.001; ††P<0.01; †P<0.0125; differences between young men and young women and between elderly men and elderly women during diet A and diet B; ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05; differences between young men and young women and between elderly men and elderly women during diet A and diet B (One factor ANOVA using Bonferroni correction)

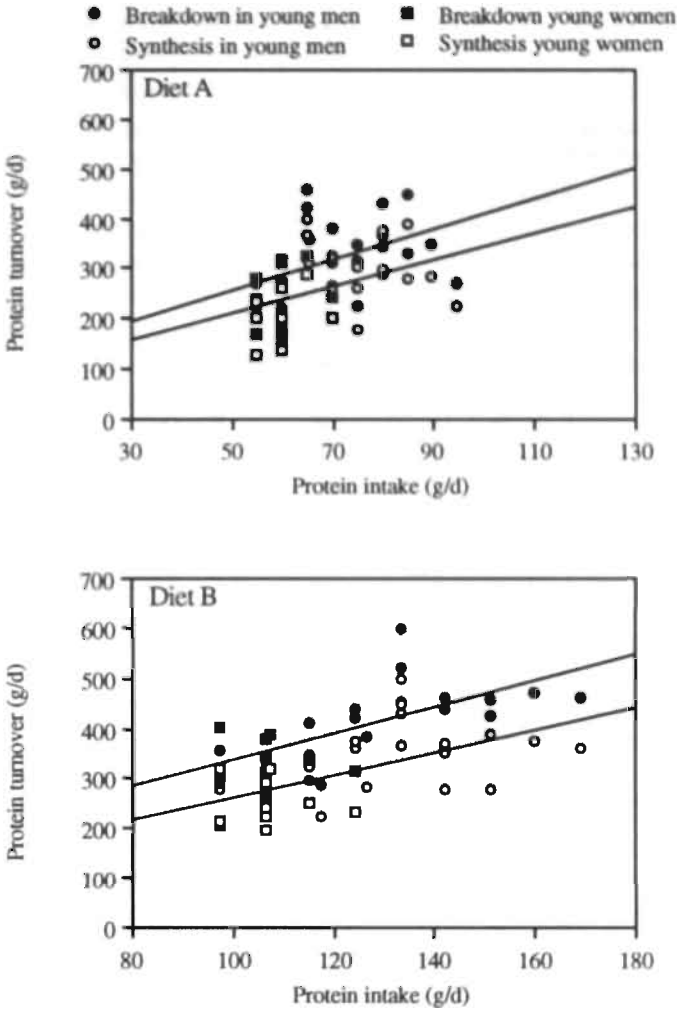

Fig. 6.1. Protein turnover (PT) plotted as a function of protein intake (PI) with the calculated linear regression lines: Diet A protein breakdown=3.08(PI)+97.93 ($P<0.05$); Diet A protein synthesis=2.66(PI)+77.26 ($P<0.05$); Diet B protein breakdown=2.60(PI)+74.4 ($P<0.001$); Diet B protein synthesis=2.27(PI)+31.74 ($P<0.001$)

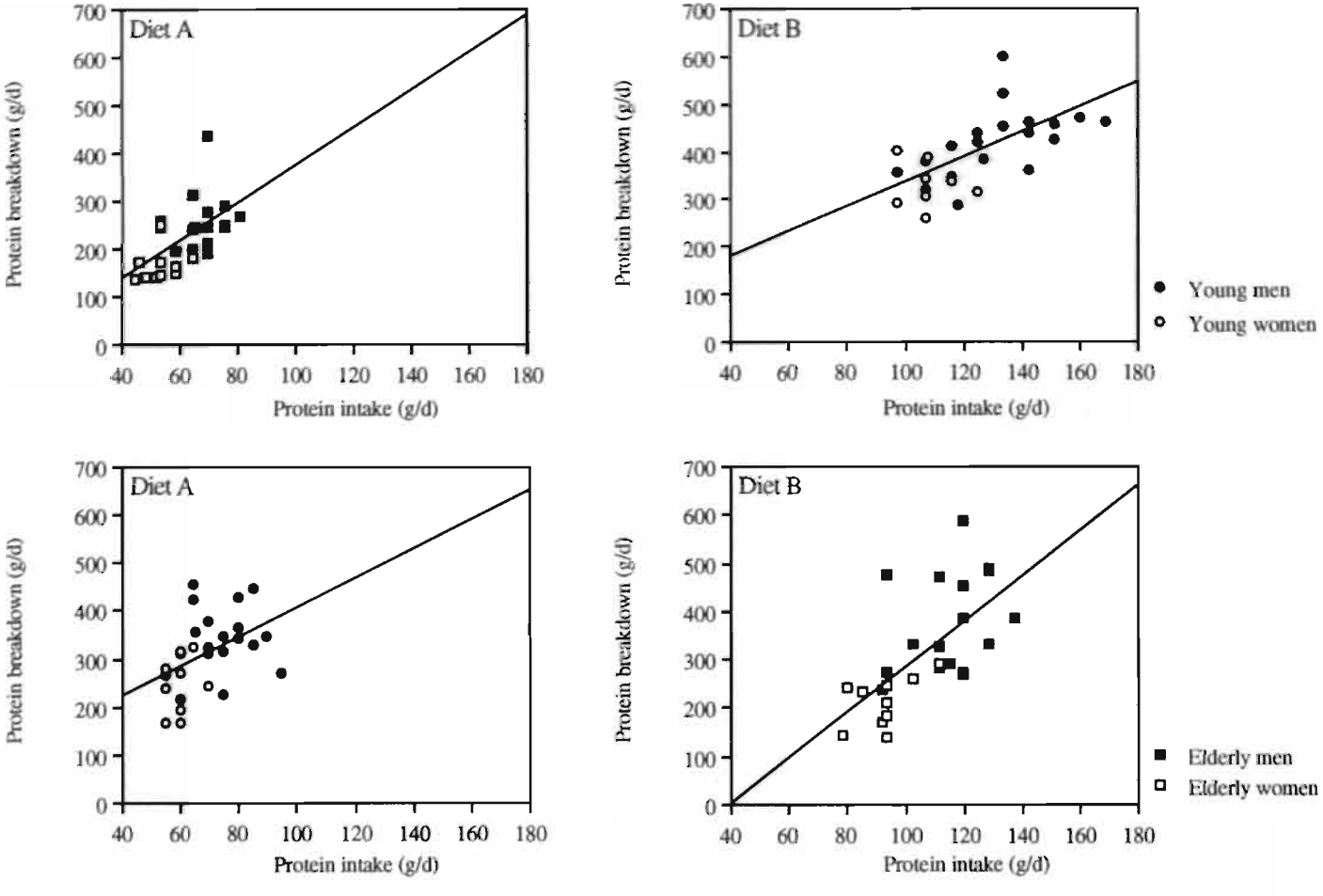

Fig. 6.2. Protein breakdown (PB) plotted as a function of protein intake (PI) with the calculated linear regression lines: Diet A: young adults PB=3.08(PI)+97.93 (P<0.05); Diet A: elderly PB=3.92(PI)-20.78 (P<0.01); Diet B: young adults PB=2.60(PI)+74.4 (P<0.001); Diet B: elderly PB=4.71(PI)-186.95 (P<0.0001)

Discussion

Adults are able to maintain body nitrogen balance over a wide range of dietary intakes of protein. As seen in the present study, young adults as well as the elderly subjects were in nitrogen balance at the end of each dietary regimen. In the elderly mean nitrogen balance did not differ significantly from zero after both diets (12% or 21% of total energy given by protein). In young adults this was true during diet A while during the higher protein intake (diet B) nitrogen balance was positive.

There is no ready explanation for this positive nitrogen balance but this observation has been made many times in the literature (Winterer et al 1976, Motil et al 1981; Young et al 1981) even after prolonged periods of time when levels of protein intake are above physiological needs (Oddoye & Margen 1979). Urine collection seemed to be complete since urinary creatinine excretion during diet A correlated well with creatinine excretion during diet B.

Since miscellaneous nitrogen loss was not measured, it is possible that the allowance assumed for this loss was less than actual and nitrogen balance was overestimated. Nitrogen balance is the net result of protein synthesis and protein breakdown, i.e. protein turnover. Therefore the effect of two levels of protein intake on protein turnover was also measured.

Protein breakdown and synthesis were measured in the postabsorptive state with an orally administered dose (200 mg) of ^{15}N-glycine. Protein turnover was calculated on the basis of label excreted in the end products urea and ammonia. The results were expressed as the harmonic average of both end products (Pannemans et al in press). The ^{15}N-glycine method was used because of its simplicity and its convenience for use outside the laboratory especially when elderly people are involved. No gender differences were observed in young adults during diet A and diet B. While in the elderly, men had significantly higher protein turnover rates compared with elderly women, even when corrections were made for body weight and body composition (during both diets). As suggested before (Pannemans et al in press), this gender difference in the elderly probably results from hormonal control.

At a normal protein intake (12% of total energy intake) protein turnover rates were significantly higher in the young adults compared with the elderly. Protein breakdown and synthesis were higher for young men and women compared with elderly men and women even when corrections were made for body weight and body composition by expressing protein turnover per kg FFM. When the protein content of the diet was increased from a usual protein level (12%) to 21% of total energy intake, no differences were seen between young men and elderly men while elderly women still had a lower protein turnover compared with young women. To date, few reports regarding protein turnover in young and elderly subjects have appeared. The rate of whole body protein turnover, expressed per unit of body weight, was less in elderly subjects compared with young adults (Winterer et al 1976; Uauy et al 1978). When corrections were made for body composition by expressing protein turnover per unit of body cell mass, no differences were found between both age groups (Uauy et al 1978). Winterer et al

(1976) reported higher turnover rates for elderly men compared with young men when protein turnover was expressed per unit body cell mass. Golden et al (1977) compared two methods for measuring protein synthesis in 6 elderly subjects. The results were compared with data in young adults and it was concluded that protein turnover was decreased with 20-30% in the elderly. Robert et al (1984) studied whole body leucine metabolism in young and elderly subjects. Whole body leucine-flux (per kg body weight) was significantly lower for elderly women compared with young women while no differences were seen between young and elderly men. Expressed per unit lean body mass no statistically significant differences were found between both age groups although leucine flux remained slightly lower in elderly women compared with young women. It is difficult to compare the data of the above studies with the results of our present study because of the different techniques used to measure protein turnover and body composition. Furthermore, differences in protein intake before the start of the study and sometimes during the study (fed state), hamper comparisons between the studies. The difference in protein turnover between young and elderly women is probably also due to differences in hormonal status (as discussed before with respect to the difference in protein turnover between elderly men and elderly women). Male and female sex hormones (testosterone and oestrogens) stimulate protein synthesis. In women oestrogen levels decrease during and after menopause while in men testosterone levels tend to remain constant.

In young adults the protein turnover increased significantly when the protein content of the diet increased from 12 to 21% of total energy. These results were in accordance with earlier findings in the elderly (Pannemans et al in press). The increase in both synthesis and breakdown did not differ between young and elderly subjects. Motil et al (1981) studied the effect of protein intake on whole body leucine and lysine metabolism in young men. It was concluded that a change in protein intake from marginal (0.6 g/kg. d) to a surfeit level (1.5 g/kg.d) was associated with an increased leucine flux. Gersovitz et al (1980) reported on the effect of adequate (1.5 g/kg.d) or inadequate (0.4 g/kg.d) protein intake on glycine metabolism in young and elderly men. At the higher protein intake whole body glycine flux was significantly higher (in young and elderly men) compared with the inadequate protein intake. Recently Garlick et al (1991) reviewed the studies about the influence of dietary protein intake on whole body protein turnover in young adults. It was concluded that the adaptation to higher protein intakes results in an increase in the basal (postabsorptive) rates of both synthesis and degradation. It is not known what the implication of this higher protein turnover is. It is known that with aging the contribution of the skeletal muscle to whole body protein turnover decreases with a relatively larger contribution from the visceral organs (Munro et al 1980; Young 1984). Recently Welle et al (1993) and Yarasheski et al (1993) reported that muscle protein synthesis (fractional and total) was lower in elderly compared with young adults. Unfortunately it is not known whether the increase in protein turnover during the higher protein intake (diet B) arises from an increased contribution from skeletal muscle to whole body protein turnover. Since muscles contribute to the adaptation in whole body amino acid metabolism

during restricted dietary energy and protein intakes, an increase in muscle protein turnover may well be favored, especially in the elderly.

Protein breakdown as well as protein synthesis were positively correlated with protein intake. Comparisons of the regression lines of young and elderly subjects revealed that, during diet A, protein turnover was significantly higher in the young adults when corrections were made for differences in protein intake (g/d). During diet B no differences were found.

In conclusion, protein breakdown and protein synthesis of elderly and young subjects increased significantly when the protein content of the diet increased from 12 to 21% of total energy intake. In young adults mean nitrogen balance was approximately zero during diet A while it was positive during diet B. There was a positive correlation between protein intake and protein turnover (for young and elderly subjects during both diets). Furthermore, at a normal protein intake (12% of total energy intake) elderly subjects had lower protein turnover rates compared with young adults. At the higher protein intake (21% of total energy intake), protein turnover of elderly men was comparable with protein turnover of young men while elderly women still had lower protein turnover rates compared with young women.

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Chapter 7

Calcium excretion in young and elderly subjects: influence of protein intake

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Abstract

The present study was conducted to investigate the effect of dietary protein on urinary calcium excretion, calcium absorption and calcium balance in young and elderly subjects. 29 Young adults and 26 elderly consumed diets containing 12% (diet A) and 21% (diet B) of total energy in protein for three weeks according to a randomized cross over design. Calcium excretion in faeces (as % of calcium intake) was lower during the higher protein intake (Diet A: 106.34±7.18%; diet B: 85.72±6.55%; $P<0.05$) as a result of a higher intestinal absorption for the elderly. In young adults faecal calcium excretion tended to be be lower during diet B (Diet A: 94.00±5.00%; diet B: 83.19±5.90%; $P=0.09$). Urinary calcium excretion was increased during the higher protein intake in young adults as well as in the elderly (Diet A: 150±11, 131±12; diet B: 192±16, 163±15 mg/d for the young and elderly subjects respectively $P<0.0001$, $P<0.05$). Increasing the protein intake from 12% to 21% of total energy intake had no negative effect on calcium excretion and calcium balance. Moreover there were no differences between both age groups with respect to the interaction between protein intake and calcium excretion, calcium absorption and calcium balance.

Introduction

Several investigators have reported an increased urinary calcium loss when dietary protein is increased, sometimes leading to a negative calcium balance (Allen et al 1979, Anand and Linkswiler 1974, Hegsted et al 1981, Johnson et al 1970, Schuette et al 1980, Walker and Linkswiler 1972). The calciuretic effect of protein is attributable to an increased glomerular filtration rate and filtered calcium load and to a

decreased tubular calcium reabsorption caused by sulphate that originates from S-containing amino acids. Dietary phosphorus on the other hand reduces the urinary excretion of calcium by increasing the renal tubular reabsorption and counteracts, at least in part, the calciuretic action of protein. Phosphorus has been shown to decrease urinary calcium regardless of calcium intake (Spencer et al 1978). Spencer et al (1988) have put forward that commonly used complex proteins, which contain phosphorus, do not cause calcium loss. The same group described the effect of a high protein (meat) intake on calcium excretion, absorption and retention in male patients (age: 30-67 y) and concluded that a high protein intake, given as meat (complex proteins) does not lead to hypercalciuria and does not induce calcium loss (Spencer et al 1978, Spencer et al 1983). Although this may be valid for young healthy adults it may not be so in healthy elderly people, who are already confronted with a negative calcium balance. Since a higher protein intake is probably preferable for the elderly (Pannemans et al, unpublished data) it is necessary to investigate the effect of a higher protein intake on calcium metabolism. It was the aim of the present study to investigate the effect of dietary protein on urinary calcium excretion, calcium absorption and calcium balance in young and elderly subjects.

Subjects and methods

Subjects

Subjects were 29 young adults and 28 elderly. They were recruited with advertisements in local media. Elderly subjects were also recruited through contacts with alliances for elderly. Mean age, height, weight and body mass index (BMI) are presented in Table 7.1. Two elderly women did not complete the whole study and they were excluded from this analysis. All subjects were certified to be in good health by a staff physician and gave informed consent to participate in the study after the procedures were explained to them. The protocol was approved by the university ethics committee.

Table 7.1. Physical characteristics of the subjects (mean±SEM)

	Young subjects		Elderly subjects	
	men	women	men	women
n	19	10	17	9
Age (y)	30.4±1.2††††	27.2±1.2††††	71.6±1.2	67.3±1.5
Length (m)	1.81±0.01†††	1.68±0.02†	1.72±0.02	1.61±0.03
Weight (kg)	76.3±2.0	60.8±2.4	73.8±3.0	68.3±3.0
BMI (kg/m^2)	23.2±0.4†	21.5±0.6†††	24.9±0.8	26.5±1.1

††††P<0.0001; †††P<0.001; †P<0.05 differences between young and old men and between young and old women.

Protocol

Subjects consumed two iso-energetic diets, for three weeks each, in a cross over design with a "wash-out" period of at least three weeks. Diets contained 12 and 42 (diet A), and 21 and 33 (diet B) per cent of total energy intake from protein and fat respectively. During the experiment subjects were fed according to their estimated energy intake. All meals, consisting of breakfast, lunch, dinner beverages and drinks were daily served at home, and subjects were not allowed to eat or drink anything else except for water, tea and coffee. They were asked to keep the coffee and tea as well as the sodium consumption constant during the intervention study. Since every subjects was his own control this has no effect on the results. The first week was a correction period in which energy intake could be adjusted if necessary. Figure 7.1. is a schematic presentation of the experimental design.

	week 1 preliminary period	week 2 intervention period	week 3 intervention period
Diet A or B* (provided)	<---------->	<---------->	<---------->
Food samples		x	x
Urine collection			xx
Faeces collection			xxx

Fig. 7.1. The protocol of the study. *Diet A contained 12% of total energy as protein; diet B contained 21% of total energy as protein. Diets were given according to a cross over design.

Nutrition intervention

For the purpose of the study fixed energy groups were made, ranging from 7.5-15.0 MJ/d). The subjects were placed in the best fitting energy group according to their estimated energy intake as measured with a dietary record or with a dietary questionnaire (Pannemans & Westerterp 1993). During the nutrition intervention period the subjects were given a basal diet supplemented with specific products: milk, yoghurt, bread, minced meat and sausage. During diet B part of the fat in these products was replaced by purified proteins (sodium caseinate in diary products, gluten in bread and soy in minced meat) and sausage was replaced by smoked beef.

Calcium excretion

Samples of total diet, given during the intervention period, were collected and analyzed for calcium with atomic photon spectrophotography. Subjects collected total faeces for three days and total urine for two days at the end of each third week of the intervention period. Daily faeces collection started at 7.00 o'clock in the morning till 7.00 o'clock the next day. 24 h urine collection started with the second urine in the morning and included the first voiding of the next day. After total weight and total volume of faeces and urine were measured the calcium content was measured with

atomic photon spectrophotography. Calcium balance was calculated as calcium intake minus calcium in faeces and urine.

Statistics

Values were expressed as mean with the SEM in parentheses. Differences between measurements and groups were analyzed using Student's paired and unpaired t-tests as appropriate. Regression analysis was used to assess associations between measured variables.

Results

Diets

Table 7.2. shows the nutrient intake during the experimental diets. Energy intake was the same during diet A and diet B for both age groups. Nitrogen intake was, as planned, significantly higher during diet B. Nitrogen intake increased 78% and 76% respectively for the elderly and the young subjects. Although diets were formulated to contain equal amounts of calcium, calcium intake appeared higher during the high protein diet (B). This was mainly attributable to a higher calcium content of the minced meat and the smoked beef. Phosphorus intake increased also during diet B. However the increase (expressed as % of intake during diet A) in nitrogen, calcium and phosphorus intake was the same for the young adults and the elderly. Since the energy intake of the young subjects was higher, the absolute amount of nitrogen, calcium and phosphorus intake was also higher compared with the elderly.

Table 7.2. Energy, nitrogen, calcium and phosphorus intake (mean±SEM)

	Young subjects (n=29)			Elderly subjects (n=26)		
	Diet A*	Diet B	Δ (%)	Diet A	Diet B	Δ (%)
Energy	††	††				
(MJ/d)	11.26±0.36	11.30±0.36	0	9.75±0.27	9.75±0.27	0
Nitrogen	####/†	††		####		
(g/d)	11.03±0.33	19.77±0.59	76	9.85±0.32	17.08±0.51	78
Calcium	####/††††	††††		####		
(mg/d)	1081±32	1215±37	12	873±28	985±24	13
Phosphorus	####/††††	††††		####		
(mg/d)	1519±46	1841±52	21	1250±45	1501±47	21

*Diet A contained 12 en% protein; Diet B contained 21 en% protein; ####P<0.0001 differences between diet A and diet B ; ††††P<0.0001; ††P<0.01; †P<0.05 differences between the young and the elderly subjects

Calcium metabolism

Data on calcium intake, calcium excretion and calcium balance are shown in Table 7.3. As already shown in Table 7.2. calcium intake was significantly higher during diet B (P<0.0001). Calcium excretion in faeces (in grams per day) was not significantly different during diet A and diet B for both age groups. However when faecal calcium excretion expressed as percentage of calcium intake (to correct for the difference in calcium intake) for the elderly calcium excretion was significantly lower during the higher protein diet (P<0.05). For the young adults there was a tendency for a lower faecal calcium excretion during the higher protein intake (P=0.09). Consequently net calcium absorption in the elderly (calculated as calcium intake minus faecal calcium excretion) was significantly increased during diet B (Diet A: -38±61 mg/d; diet B: 139±64 mg/d; P<0.05). In young adults there was a tendency for a higher calcium absorption during the higher protein intake (Diet A: 75±56 mg/d; diet B: 213±74 mg/d; P=0.0564). There were no significant differences between the young adults and the elderly subjects.

Urinary calcium excretion was significantly increased during the higher protein intake in both age groups (Table 7.3.). Expressed as percentage of calcium intake, no significant difference was seen in the elderly. In young adults urinary calcium excretion (%) was significantly higher during diet B. There were no significant differences between the young adults and the elderly.

Calcium balance was not significantly different between young adults and elderly subjects (Table 7.3.). In young adults calcium balance was also not influenced by the amount of protein in the diet since there were no differences between calcium balance measured during diet A or diet B. During both diets, calcium balance did not differ significantly from zero. In the elderly calcium balance was significantly less positive during diet A. To correct for the difference in calcium intake calcium balance was also expressed as percentage of calcium intake. The calcium balance during diet A was still significantly more negative compared with calcium balance during diet B, despite the higher urinary calcium excretion during diet B.

Table 7.3. Calcium metabolism during diet A and diet B (mean±SEM (% calcium of intake))

	Young subjects		Elderly subjects	
	Diet A* (n=29)	Diet B (n=29)	Diet A (n=26)	Diet B (n=26)
Intake (mg Ca/d)	1081±32$^{\dagger\dagger\dagger\dagger/\#\#\#\#}$	1215±37††††	873±28$^{\#\#\#\#}$	985±24
	(100%)	(100%)	(100%)	(100%)
Faeces (mg Ca/d)	1007±56	1002±72	911±58	846±69
	(94%)	(83%)	(106%$^{\#}$)	(86%)
Urine (mg Ca/d)	150±11$^{\#\#\#\#}$	192±16	131±12$^{\#}$	163±15
	(14%$^{\#}$)	(16%)	(15%)	(17%)
Balance (mg Ca/d)	-75±59	21±74	-169±60$^{\#}$	-24±68
	(-8%)	(1%)	(-22%$^{\#}$)	(-2%)

*Diet A contained 12 en% protein; Diet B contained 21 en% protein; $^{\#\#\#\#}$P<0.0001; $^{\#}$P<0.05 differences between diet A and diet B; ††††P<0.0001 differences between young and elderly subjects

Discussion

There are many studies on effects of protein intake on the absorption and urinary excretion of calcium and on calcium balance showing a negative effect of protein intake on calcium balance (Allen et al 1979, Anand and Linkswiler 1974, Hegsted et al 1981, Hegsted and Linkswiler 1981). However, no research is done concerning the effect of age on the interaction between protein intake and calcium metabolism. Only one study described the protein induced hypercalciuria in older men and women, but no comparisons were made with young adults (Schuette et al 1980). The aim of the present study was to investigate the effect of dietary protein on calcium excretion and calcium balance in young adults and in elderly.

The results showed that a protein intake of 21% of total energy resulted in a significant higher urinary calcium excretion compared with the a protein intake of 12% of total energy, in the young as well as in the elderly subjects. For young adults this effect of protein intake on urinary calcium excretion has been described before (Chu et al 1975, Johnson et al 1970, Kim & Linkswiler 1979, Lutz & Linkswiler 1981, Margen et al 1974, Walker and Linkswiler 1972, Zemel et al 1981). The hypercalciuria at a high protein intake is mainly caused by a decrease in fractional tubular reabsorption of calcium (Allen et al 1979, Kim & Linkswiler 1979, Schuette et al 1980, Zemel et al 1981) but there is also an increase in glomerular filtration rate (Allen et al 1979, Chu et al 1975, Kim & Linkswiler 1979, Schuette et al 1980, Zemel et al 1981). Spencer et al (1978, 1983) did not find an effect of (meat) protein intake on urinary calcium excretion. The authors suggested that commonly used complex proteins, which contain phosphorus, do not cause calcium loss. In the present study calcium excretion in urine (mg/d) was increased in the young adults and in the elderly while phosphorus intake was also increased. Schuette et al (1982) also found a higher urinary calcium excretion during a high protein diet with high phosphorus intake. There were no differences in urinary calcium excretion between both age groups. When urinary calcium excretion was expressed as percentage of calcium intake (to correct for the difference in calcium intake), in young adults calcium excretion was still significantly higher during diet B although there was no difference in urinary calcium excretion (%) for the elderly.

Faecal calcium excretion (in mg/d) did not differ during both diets. However when faecal excretion was expressed in percentage of calcium intake, excretion was significantly lower during the higher protein intake, (whole group: $P<0.01$; elderly $P<0.05$; young adults $P=0.09$). Other authors also report a lower faecal calcium excretion when protein intake is increased (Chu et al 1975, Walker and Linkswiler 1972) while others did not (Allen et al 1979, Anand and Linkswiler 1974). As a consequence of the lower faecal calcium excretion, apparent absorption (calculated as the difference between intake and faecal excretion) was significantly higher during diet B for the elderly and tended to be significantly higher for the young adults ($P=0.0564$). These results indicate that the higher urinary calcium excretion was compensated by a higher intestinal absorption of calcium (probably attributable to the higher phosphorus

intake). These results are in agreement with the results described by Schuette et al (1982). When protein intake increased (with simultaneously supplementation of calcium and phosphorus) urinary calcium excretion increased but calcium retention increased, resulting in a increased calcium balance (Schuette et al 1982). However measuring calcium excretion in faeces is subject to rather large errors since large errors can occur when subjects fail to collect all of the faeces (leading to false positive balances). To overcome this problem faecal calcium excretion was also expressed in mg calcium per gram faeces. Expressed in this way no differences in faecal calcium excretion were seen (elderly: diet A 7.26±0.55 mg/g; diet B 7.92±0.63 mg/g (P=0.15); young adults: diet A 9.12±0.65 mg/g; diet B 10.06± 0.66 mg/g (P=0.63)).

Calcium balance was calculated as intake minus excretion in urine and faeces. No corrections were made for calcium loss via sweat and dermal calcium losses since these losses are very small and are not affected by the level of protein and calcium intake (Chu et al 1975). There were no statistically differences in calcium balance between the young and the elderly subjects. A higher protein intake (diet B) seems to have no effect on the calcium balance of young adults, despite the higher urinary calcium excretion. In the elderly, calcium balance was significantly less negative during diet B. However as mentioned, before balance studies are susceptible to rather large errors since balance is calculated as the difference between two relatively large values, neither of which can be obtained with high precision. And although calcium balance was carried out as precise as possible the results should be interpreted with care. Nevertheless it can be concluded that changing the protein intake from 12% to 21% of total energy had no negative effects on calcium balance in healthy young and elderly people. Moreover there were no differences between both age groups with respect to the interaction between protein intake and calcium excretion, absorption and balance. Others reported a significant decrease in calcium balance as a result of a higher protein intake in young and middle aged subjects (Allen et al 1979, Anand and Linkswiler 1974, Hegsted et al 1981, Hegsted and Linkswiler 1981) while others didn't find an effect (Johnson et al 1970, Spencer et al 1978, Spencer et al 1983 Schuette et al 1980). Several factors have to be considered comparing the results with other studies with respect to the effect of protein on calcium metabolism in humans, such as the type of protein (purified or complex proteins), the duration of the study (long term or short term), whether the studies were carried out in out patient volunteers or or under strictly controlled conditions, whether the phosphorus intake was higher, lower or the same during the intervention and the age of the subjects. Since the study conditions of the different studies do differ it is hard to compare their results.

In conclusion it can be said that urinary calcium excretion was increased in young and elderly subjects when changing from 12% to 21% of total energy as protein. This increase in calcium excretion in urine was compensated by an increased calcium absorption. For the elderly this results in a less negative calcium balance during the higher protein intake whereas for the young adults there was no effect of protein intake on calcium balance. However for reasons mentioned above these data should be interpreted with care. Nevertheless it can be concluded that changing the protein intake

from 12% to 21% of total energy has no negative effects on calcium balance in healthy young and elderly people. Moreover there were no differences between both age groups with respect to the interaction between protein intake and calcium excretion, absorption and balance.

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Chapter 8

Vitamin B6 metabolism in young and elderly humans: influence of protein intake

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Abstract

Vitamin B6 metabolism was studied as a function of dietary protein intake. Subjects were 29 young adults (29±1 y) and 26 elderly (70±1 y) who consumed standardized diets containing 12% (diet A) and 21% (diet B) of total energy in protein for three weeks each, according to a randomized cross over design. Vitamin B6 intake for young and elderly subjects was respectively 1.52±0.08 mg/d (21.74±0.45 µg/g protein) and 1.47±0.05 mg/d (23.81±0.08 µg/g protein) during diet A and 1.79±0.07 mg/d (14.49±0.11 µg/g protein) and 1.73±0.05 mg/d (16.24±0.06 µg/g protein) during diet B. Plasma pyridoxal phosphate (PLP), pyridoxal (PL) and total vitamin B6 concentrations were significantly lower in the elderly compared with the young adults during both diet periods. In the elderly, PLP was significantly higher during diet B (diet A: 27.42±2.54 nmol/L; diet B: 31.58±2.84 nmol/L) while the level of protein intake had no significant effect on plasma PLP in the young adults (diet A: 47.00±5.82 nmol/L; diet B: 45.14±5.01 nmol/L). Plasma PL and plasma total vitamin B6 concentrations were not influenced by the amount of protein intake in young and elderly subjects. Relative urinary pyridoxic acid (4-PA) excretion did not differ significantly in the elderly (diet A: 36.9±2.9%; diet B: 42.8±2.5%), while 4-PA excretion was lower in young adults when diet B was consumed (diet A: 46.1±2.6%; diet B: 37.5±1.7%; $P<0.001$). The results of this study suggest an age-dependent difference in the protein intake related vitamin B6 needs, whereby the elderly apparently need less vitamin B6 at a higher protein intake as compared with young adults.

Introduction

Several authors report about the effect of aging on vitamin B6 status as measured by plasma pyridoxal phosphate (PLP) coenzyme saturation tests in vitro, metabolic

loading tests and by urinary 4-pyridoxic acid (4-PA) excretion. Measurement of vitamin B6 status is often used as an indicator for vitamin B6 requirement.
Plasma PLP, the metabolically active B6 vitamer, has been shown to decrease with advancing age (e.g. Guilland et al 1984, Hamfelt & Söderhjelm 1988, Kant et al 1988, Löwik et al 1989, Manore et al 1989, Tolonen et al 1988). Rose et al (1976) reported a decline in plasma PLP of 3.6 nmol/L per decade as determined in 617 noninstitutionalized men aged 18 to 90.
Coenzyme saturation tests in vivo are also often used to measure vitamin B6 status. Measurements of alanine aminotransferase (EC 2.6.1.12) and aspartate aminotransferase (EAST; EC 2.6.1.1) in elderly show conflicting results, partly due to the lack of generally accepted ranges of adequate and deficient values (Bode 1992), usually expressed as an activation coefficient. Defined deficient values for the EAST-activation coefficient ranged from 1.80 (Porrini et al 1987) to 2.28 (Tolonen et al 1988) while the percentage of deficient values ranged from 3% in free living elderly subjects (Porrini et al 1987) to 86% in institutionalized elderly women (Guilland et al 1984). Vir & Love (1979) found 42% deficient values (based on alanine aminotransferase-activation coefficient>1.15) in a heterogeneous elderly population.
Vitamin B6 status assessment with metabolic loading tests are determining the extent to which a subject is able to metabolize a test dose of a physiological substrate which metabolism is PLP-dependent (e.g. tryptophan and methionine). Vitamin B6 deficiency gives rise to urinary excretion of metabolites in abnormal ratios. Recently, Ribaya-Mercado et al (1991) measured vitamin B6 requirements of elderly men and women by means of a tryptophan load test. After a vitamin B6 depletion phase, xanthurenic acid returned to baseline levels at a vitamin B6 intake level of 1.96 and 1.90 mg/d for elderly men and women respectively.
Results on urinary excretion of the metabolic end-product 4-PA, as a noninvasive vitamin B6 status parameter, are equivocal. Lee & Leklem (1985) reported higher urinary 4-PA values on normal vitamin B6 intakes (2.3-2.4 mg/d) for middle-aged women, while Kant et al (1988) found no differences in 4-PA excretion in 3 male age groups before and after an oral vitamin B6 load. The differences in results are probably due to the fact that 4-PA will reflect recent vitamin B6 intake rather than the underlying state of tissue reserve (Lui et al 1985).
Some of the above mentioned studies indicate that there are indeed differences in vitamin B6 status between young adults and the elderly: plasma PLP concentrations decrease with age, urinary 4-PA excretion seems to increase with age, while it is unclear whether there are any changes in EAST-activation coefficient with age. Several suggestions were made to explain these age differences. First of all the higher prevalence of abnormal indices of vitamin B6 status may be explained by a lower vitamin B6 intake among the elderly. However, this could not explain the differences in B6 status under controlled dietary intake of vitamin B6. Secondly it was suggested that there is a causal inverse relationship between plasma alkaline phosphatase (AP; EC 3.1.3.1) activity and plasma PLP concentrations. Since the AP concentrations increase with age this could explain the lower plasma PLP concentration in the elderly (Kant et al

1988). Thirdly, it could be that vitamin absorption is affected by aging. However when vitamin B6 is given orally as a supplement, the plasma PLP increase is comparable between young and elderly subjects (van den Berg et al 1992; Shultz & Leklem 1985; Ubbink et al 1987) suggesting no effect of age on vitamin B6 absorption. It was recently hypothesized that the age dependent decrease in plasma PLP content could be associated with a decrease in tissue body stores due to changes in body composition and/or an effect on PL(P) release from (muscle) protein due to a decrease in protein turnover (Bode & van den Berg 1991a, Bode & van den Berg 1992).

Vitamin B6 requirements for humans are related to the level of dietary protein intake since PLP catalyses a number of biochemical reactions integral to nitrogen metabolism. The earlier vitamin B6 depletion/repletion studies reported by Baker et al (1964) with young adult males fed two different diets, containing 30 and 100 g protein per day respectively, already demonstrated the relationship between vitamin B6 requirement and protein intake. Miller et al (1985) confirmed this relationship and showed an increased vitamin B6 retention in young males with increased intake of dietary protein. Recently, Ribaya-Mercado et al (1991) studied the effect of protein intake on vitamin B6 requirement. They concluded that at a higher protein intake, elderly subjects required more vitamin B6 to reach baseline vitamin B6 status after depletion phase. However this conclusion was mainly based on tryptophan loading tests and only elderly subjects were involved in this study.

The objective of the present study was to determine the effect of dietary protein on vitamin B6 metabolism in young and elderly subjects and to investigate the age dependent relationship between indicators of the vitamin B6 status and protein turnover. It was hypothesized that vitamin B6 requirement (as measured by plasma PLP, EAST activation and urinary 4-PA excretion) increases when the protein intake increases (as a result of an increased protein turnover).

Subjects and methods

Subjects

Subjects were 29 young adults and 28 elderly. They were recruited with advertisements in local media. Elderly subjects were also recruited through contacts with alliances for elderly. Mean age, height, weight and body mass index (BMI) are presented in Table 8.1. Two elderly women did not complete the whole study and they were excluded from this analysis. All subjects were certified to be in good health by a staff physician and had normal clinical chemical profiles. Subjects were not taking any drugs known to affect vitamin B6 metabolism. Subjects gave informed consent to participate in the study after the procedures were explained to them. The protocol was approved by the university ethics committee.

Table 8.1. Physical characteristics of participating subjects [1,2]

	Young subjects		Elderly subjects	
	Men	Women	Men	Women
n	19	10	17	9
Age (y)	30.4±1.2	27.2±1.2	71.6±1.2	67.3±1.5
Height (m)	1.81±0.01***	1.68±0.02*	1.72±0.02	1.61±0.03
Weight (kg)	76.3±2.0	60.8±2.4	73.8±3.0	68.3±3.0
BMI (kg/m^2)	23.2±0.4*	21.5±0.6***	24.9±0.8	26.5±1.1
AP[3](U/L)	54.3±2.8**	40.6±5.4****	75.3±6.1	75.8±3.3

[1]Values are means ± SEM. Values that are significantly different between young and elderly men and between young and elderly women are indicated: ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05.
[2]Abbrevations used: BMI, body mass index; AP: plasma alkaline phosphatase. [3]U/L represents rate hydrolyses of p-nitrophenyl phosphate per liter of plasma.

Protocol

Subjects consumed two iso-energetic diets, for three weeks each, in a cross over design with a "wash-out" period of at least three weeks. Diets contained 12 and 42 (diet A), and 21 and 33 (diet B) per cent of total energy intake from protein and fat respectively. During the experiment, subjects were fed according to their estimated energy intake. All meals, consisting of breakfast, lunch, dinner beverages and drinks, were daily served at home, and subjects were not allowed to eat or drink anything else except for water, tea and coffee. The first week was a correction period in which energy intake could be adjusted if necessary. Figure 8.1. is a schematic presentation of the experimental design. Subjects were not allowed to use vitamin supplements during the experiment (starting six weeks before the start of the study).

	week 1 preliminary period	week 2 intervention period	week 3 intervention period
Diet A or B* (provided)	<----------	<----------	<----------
Food samples		x	x
Urine collection			xx
Blood collection			x
Protein turnover			x

*Diet A contained 12% of total energy as protein; diet B contained 21% of total energy as protein. Both diets were given according to a cross over design

Fig. 8.1. The protocol of the study

Nutrition intervention

Fixed energy groups, ranging from 7.5-15 MJ/d, were made for the purpose of the study. The subjects were placed in the best fitting energy group according to their es-

timated energy intake. Energy intake of the elderly was measured with a dietary record or with a dietary questionnaire (Pannemans & Westerterp 1993). Energy intake of the young adults was measured with a dietary questionnaire. During the nutrition intervention period the subjects were given a diet containing: wheaten bread plus diet margarine (Becel), marmalade (Hero) and meat (sausage or smoked beef); potatoes plus vegetables (cabbage, beans), minced meat and gravy (Knorr); yogurt (Campina), sugar, fruit cocktail (Queens), orange juice (Jaffa), milk (Campina) and cake (Puts). The higher protein content of diet B was reached by an isocaloric exchange between protein and fat. The isocaloric exchange was achieved by using low fat milk, low fat yogurt, bread and meat enriched with protein (sodium caseinate (DMV Campina Veghel) in dairy products, gluten in bread (Excelsior) and soybean (Ralston Purina) in minced meat), and by replacing sausage by smoked beef in diet B.

Pyridoxine intake, plasma pyridoxal phosphate, urinary 4-pyridoxic acid excretion
Samples of the total diet, given during the intervention period, were collected and analyzed for total vitamin B6 using HPLC with fluorometric detection (Coburn & Mahuren 1983). A venous blood sample was obtained, from subjects who fasted for at least 10 hours, on the morning of the last day of each three week intervention period. Plasma PLP was measured by a modification of the L-tyrosine apodecarboxylase assay of Chabner and Livingstone (1970). Pyridoxal (PL) was measured by a modification of the HPLC method described by Schrijver et al (1981). Erythrocyte aspartate aminotransferase (EAST) activity before and after in vitro stimulation by PLP was measured with a kinetic assay on a Hitachi 705 automatic analyser (Hitachi, Tokyo, Japan) and expressed as an activation coefficient (EAST-activation coefficient) (Vuilleumier et al 1983). Subjects collected total urine for two days at the end of each three week intervention period. Urine collection (24 h) started with the second urine in the morning and included the first voiding of the next day. After total volume of urine was measured the urinary 4-pyridoxic acid (4-PA) content was assessed by a modification of the HPLC method described by Gregory & Kirk (1979). All measurements were carried out in the same laboratories of the TNO Toxicology and Nutrition Institute.

Protein turnover
Whole body protein turnover was measured with ^{15}N-glycine given orally (Cambridge Isotope Laboratories; 200 mg; 99 atom %) at the last day of each experimental period. Rates of protein breakdown and synthesis were estimated from the urinary excretion of ^{15}N in ammonia and urea during the following 11 h as measured with an isotope ratio mass spectrometer. Urinary ammonia and urea concentrations were determined spectrophotometrically by standard enzymatic methods on a centrifugal analyzer system (Cobas Bio; Roche Diagnostics, Hoffmann La Roche, Basle, Switzerland) using commercial kits (Bergmeyer 1974, Janssen et al 1988). Urea values were corrected for ammonia. Total nitrogen in urine was measured with a

Heraeus analyzer (type CHN-O-rapid). Protein synthesis and breakdown rates were calculated as described before (Fern et al 1984, Pannemans et al in press).

Statistics

Values are expressed as mean with SEM. Analysis of variance was performed on all data using 2x2 factorial design with the factors sex (man/woman) and age (young adults/elderly). A probability level <0.05 was considered statistically significant. Since sex appeared to have no influence on the outcome of the data, differences between measurements and age groups were analyzed using Student's paired and unpaired t-tests as appropriate. Regression analysis was used to assess associations between measured variables. Analysis of co-variance using protein intake as the covariate was used to adjust for differences in the absolute amount of protein intake when comparing urinary 4-PA excretion.

Results

Diets

Table 8.2. shows the nutrient intake during the experimental diet periods.

Table 8.2. Energy, nitrogen and vitamin B6 intake of participating subjects[1,2]

	Young subjects (n=29)			Elderly subjects (n=26)		
	Diet A	Diet B	Δ (%)	Diet A	Diet B	Δ (%)
Energy (MJ/d)	11.26±0.36**	11.30±0.36**	0	9.75±0.27	9.75±0.27	0
Nitrogen (g/d)	11.03±0.33$^{*/\dagger}$	19.77±0.59**	76	9.85±0.32†	17.08±0.51	78
Vitamin B6 (mg/d)	1.52±0.08†	1.79±0.07	16	1.47±0.05†	1.73±0.05	16
Vitamin B6 (µg/g protein)	21.74±0.45$^{***/\dagger}$	14.49±0.11***	-32	23.81±0.08†	16.24±0.06	-33

[1]Values are means ± SEM. Values that are significantly different between young and elderly subjects are indicated: ***P<0.0001; **P<0.01; *P<0.05. Values that are significantly different between diet A and diet B are indicated: †P<0.0001. [2]Diet A: contained 12% of total energy intake as protein; Diet B: contained 21% of total energy intake as protein.

Energy intake was the same during diet A and diet B for both age groups. Nitrogen intake was, as planned, significantly higher during diet B. Nitrogen intake increased 78% and 76% respectively for the elderly and the young subjects. Although diets were formulated to contain equal amounts of vitamin B6, intake (expressed in mg/d and in mg/g protein) appeared higher during the high protein diet (B). This was partly attributable to a higher vitamin B6 content in the gluten enriched bread and in the

soybean enriched meat and partly due to a higher vitamin B6 content of the smoked beef. However, the greater nitrogen and vitamin B6 intake during diet B (expressed as % of intake during diet A) was the same for the young adults and the elderly. Since the energy intake of the young subjects was higher, the absolute amount of nitrogen intake was also higher, compared with the elderly.

Vitamin B6 metabolism

In the elderly, plasma PLP increased significantly when protein intake increased ($P<0.01$), while in the young adults there was no effect on plasma PLP concentration. (Table 8.3.). Plasma PLP and PL were significantly lower in the elderly subjects compared with the young adults during diet A ($P< 0.01$) as well as during diet B ($P<0.05$; Figure 8.2., Table 8.3.).

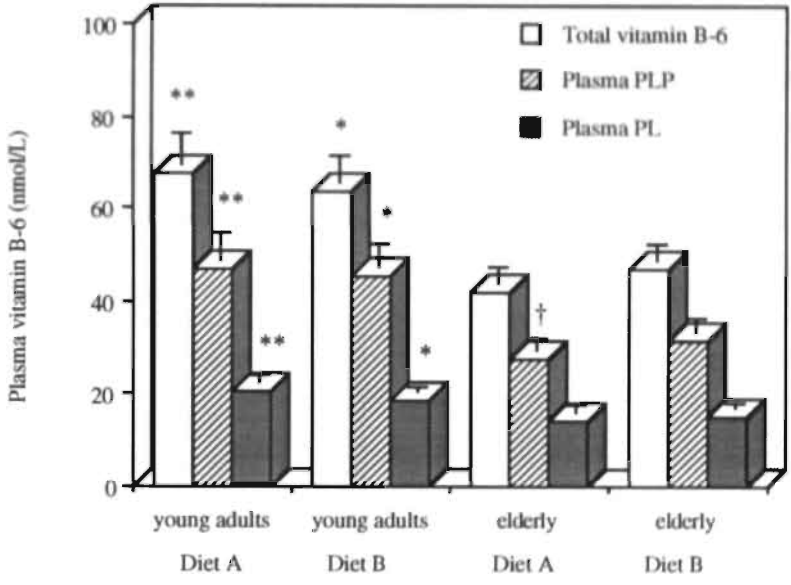

Fig. 8.2. Concentrations of plasma total vitamin B6 (white bars) pyridoxal phosphate (PLP; hatched bars) and pyridoxal (PL; grey bars) (means±SEM) of young and elderly subjects who received two levels of dietary protein. Diet A contained 12% of total energy as protein and diet B contained 21% of total energy as protein. For the elderly PLP concentration was significantly higher during diet B $^{\dagger}P<0.01$). Plasma total vitamin B6, PLP and PL are significantly higher for the young adults during both diets (Student t-test: Diet A: $^{**}P<0.01$; diet B: $^{*}P<0.05$)

There was no effect of protein intake and age on basal EAST activity ($EAST_0$) (without PLP added to the reaction mixture).The EAST activation coefficient was significantly higher for the young adults during both diets ($P<0.01$; Table 8.3.).

Table 8.3. Vitamin B6 intake, 4-pyridoxic acid excretion (4-PA), plasma pyridoxal phosphate (PLP), plasma pyridoxal (PL) and erythrocyte aspartate aminotransferase (EAST) activity [1,2]

	Young subjects (n=29)		Elderly subjects (n=26)	
	Diet A	Diet B	Diet A	Diet B
Vitamin B6 (μg/g protein)	21.74±0.45$^{***/\dagger\dagger}$	14.49±0.11***	23.81±0.08††	16.24±0.06
4-PA (μmol/d)	3.67±0.18*	3.61±0.16	2.98±0.26†	4.05±0.27
Plasma PLP (nmol/L)	47±6**	45±5*	27±3†	32±3
Plasma PL (nmol/L)	21±9**	19±6*	14±8	15±7
$EAST_0$	66.1±1.9	67.4±2.6	67.0±2.6	67.7±3.3
EAST-AC	2.1±0.0**	2.07±0.0**	1.91±0.0	1.93±0.0

[1]Values are means ± SEM. EAST was only measured in serum of 25 young and 25 elderly subjects because of coagulation fragments in the serum of 4 young and 1 elderly subject. Values that are significantly different between young and elderly subjects are indicated: $^{***}P<0.0001$; $^{**}P<0.01$; $^{*}P<0.05$. Values that are significantly different between diet A and diet B are indicated: $^{\dagger\dagger}P<0.0001$; $^{\dagger}P<0.01$. [2]Diet A: contained 12% of total energy intake as protein; Diet B: contained 21% of total energy intake as protein.

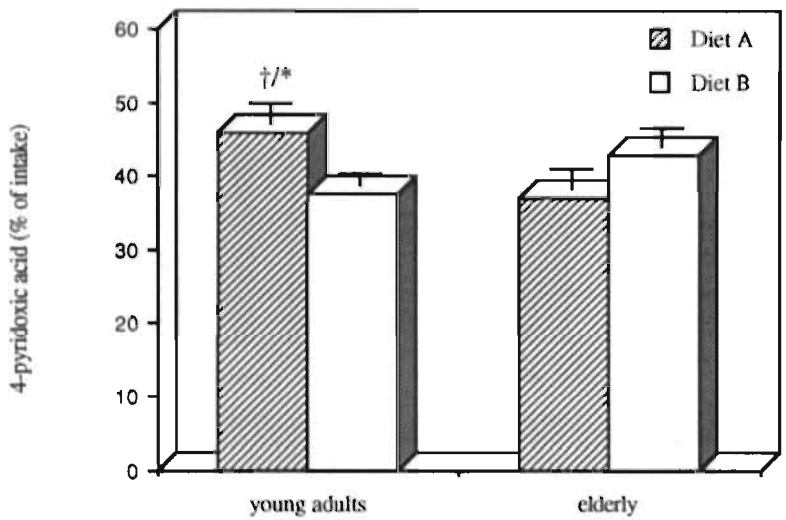

Fig. 8.3. Urinary excretion of 4-pyridoxic acid (4-PA) of young and elderly subjects who received two levels of dietary protein. Diet A (hatched bars) contained 12% of total energy as protein and diet B (white bars) contained 21% of total energy as protein. Since vitamin B6 intake was higher during diet B 4-PA excretion was expressed as percentage of vitamin B6 intake. 4-PA excretion of the young subjects was significantly higher during diet A compared with diet B and was also significantly higher compared with 4-PA excretion of the elderly during diet A (Student t-test: $^{\dagger}P<0.001$; $^{*}P<0.05$)

When the level of protein intake was increased, from 12% to 21% of total energy intake, the urinary excretion of 4-PA increased significantly in the elderly subjects ($P<0.01$) while 4-PA excretion remained stable in young adults. During the lower protein intake young adults excreted significantly more 4-PA compared with elderly subjects ($P<0.05$) (Table 8.3.). To make a correction for the higher vitamin B6 intake during diet A, 4-PA excretion was also expressed as a percentage of vitamin B6 intake (Figure 8.3.). In this case 4-PA excretion tended to be higher during diet B for the elderly (diet A: 36.9±2.9%; diet B: 42.8±2.5%; $P=0.09$), while 4-PA excretion decreased significantly for the young adults with increasing protein intake (diet A: 46.1±2.6%; diet B: 37.5±1.7%; $P<0.001$). 4-PA excretion during diet A was significantly higher for young adults compared with elderly subjects ($P<0.05$).

Vitamin B6 metabolism and protein metabolism

During diet A, protein breakdown and synthesis were respectively 224.1±67.7 g/d and 181.5±61.2 g/d in the elderly and 321.0±97.0 g/d and 270.6±90.0 g/d in young adults. Protein breakdown and synthesis were 323.6±114.6 g/d and 258.2±111.5 g/d in the elderly and 395.5±81.1 g/d and 311.7±77.9 g/d in young adults during diet B. Data on protein turnover were described in detail elsewhere (Pannemans et al in press and unpublished data). Correlation coëfficients and P-values between protein intake and turnover, and 4-PA and PLP were presented in Table 8.4. No correlations were found between protein turnover and the vitamin B6 status parameters as measured in the present study. Although there was a tendency for a negative correlation between protein breakdown and plasma PLP in the elderly during diet B ($P=0.09$). In the elderly no correlation was found between protein intake and vitamin B6 status parameters while in young adults, 4-PA excretion (as percentage of vitamin B6 intake) was negatively correlated with protein intake during diet A and diet B (Figure 8.4., diet A: $P<0.001$; diet B: $P<0.05$).

Table 8.4. Correlation coefficients and P-values between protein intake and turnover, and 4-pyridoxic acid excretion and plasma pyridoxal phosphate as measured in participating subjects[1,2]

	Diet A				Diet B			
	4-PA (%)		PLP (nmol/L)		4-PA (%)		PLP (nmol/L)	
	r	P	r	P	r	P	r	P
Elderly (n=26)								
Protein intake (g/d)	0.13	0.54	0.05	0.81	0.03	0.90	0.20	0.32
Protein breakdown (g/d)	0.15	0.47	0.22	0.29	0.20	0.32	0.33	0.09
Protein synthesis (g/d)	0.14	0.50	0.20	0.33	0.16	0.43	0.21	0.30
Young adults (n=29)								
Protein intake (g/d)	0.59	0.001	0.26	0.18	0.38	0.04	0.11	0.57
Protein breakdown (g/d)	0.23	0.24	0.02	0.93	0.17	0.38	0.07	0.72
Protein synthesis (g/d)	0.21	0.27	0.01	0.99	0.20	0.29	0.04	0.85

[1]Diet A: contained 12% of total energy intake as protein; Diet B: contained 21% of total energy intake as protein. [2]Abbrevations used: 4-PA, 4-pyridoxic acid; PLP, pyridoxal phosphate.

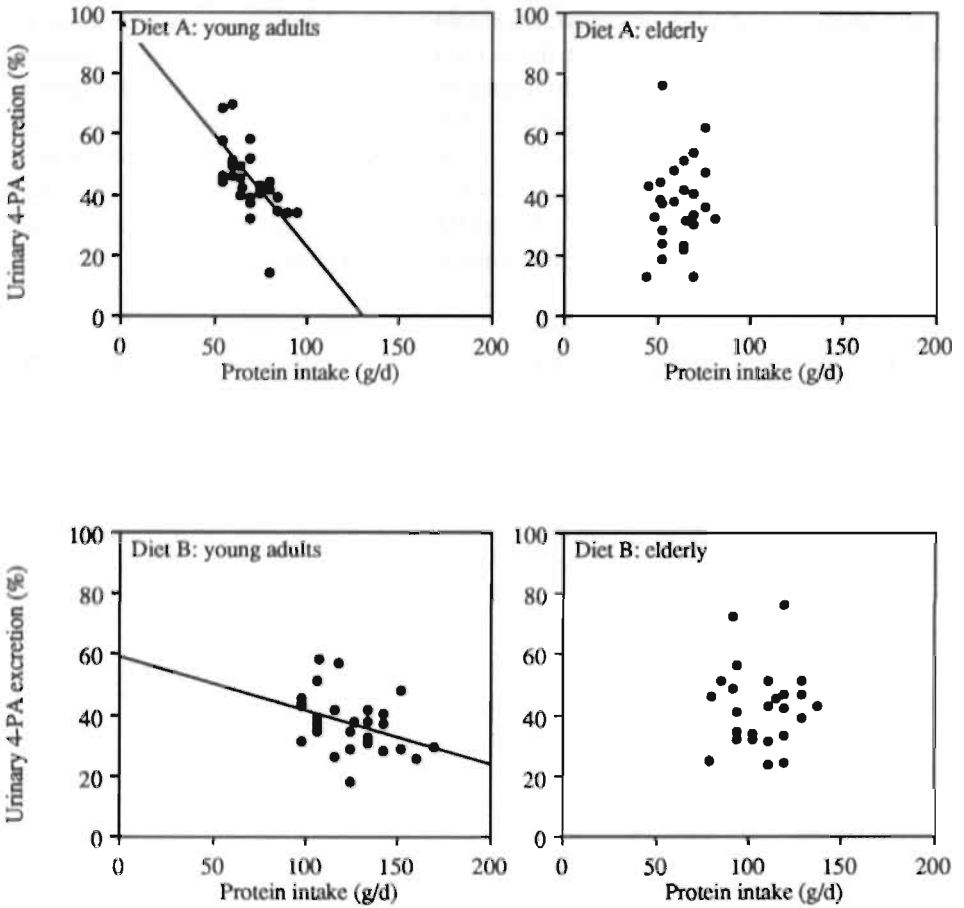

Fig. 8.4. Urinary 4-pyridoxic acid (4-PA) excretion plotted as a function of protein intake (PI) for young and elderly subjects with calculated linear regression lines: Diet A: young adults:4-PA =97.70-0.75(PI) ($P<0.001$); Diet B: young adults: 4-PA=59.15-0.18(PI) ($P<0.05$).

Discussion

In the present study, plasma PLP, plasma PL, plasma total vitamin B6, EAST stimulation test in vitro and urinary 4-PA excretion were measured. Comparisons were made between young and elderly subjects and the effect of the dietary protein intake

was studied. As described before (Guilland et al 1984, Hamfelt & Söderhjelm 1988, Kant et al 1988, Löwik et al 1989, Manore et al 1989, Rose et al 1976, Schrijver et al 1985, Tolonen et al 1988), plasma PLP concentrations were significantly lower for the elderly subjects compared with the young adults during diet A and during diet B. Plasma total vitamin B6 was also lower for the elderly compared with the young adults as described before (Kant et al 1988, Lee & Leklem 1985). In spite of the lower plasma PLP levels, the EAST-activation coefficient was significantly lower for the elderly, suggesting that the lower PLP levels were not associated with functional impairment at the level of EAST saturation (activity). Urinary 4-PA excretion (in absolute or relative amounts) was significantly lower for the elderly during diet A, while no differences were seen during diet B. As already mentioned, Lee & Leklem (1985) reported higher 4-PA excretion in elderly women compared with young women, while Kant et al (1988) found no difference in urinary 4-PA excretion between young, middle aged and elderly men. Differences could be due to sex differences as reported before in animals (Bode 1992) although no difference between men and women was found in the present study.

When comparing the vitamin B6 status of young healthy adults with the vitamin B6 status of healthy elderly subjects the conclusion is threefold. Firstly, urinary 4-PA excretion was lower or equal for the elderly subjects. It can not be excluded that urine collection was incomplete although creatinine excretion during diet A correlates well with creatinine excretion during diet B ($P<0.0001$ for young adults as well as for the elderly) and the mean variation coëfficient was 11% (for both age groups). The 4-PA/creatinine ratio in young and elderly subjects did not differ between both diets (diet A: $4.79*10^{-4}\pm1.50*10^{-4}$; diet B: $5.16*10^{-4}\pm1.46*10^{-4}$). Nevertheless, our results indicate that the rate of vitamin B6 catabolism was not greater in the elderly. Secondly, the lower EAST-activation coefficient results in the elderly do not suggest a functional impairment for the elderly (at least at the level of EAST-activity). Thirdly, the plasma PLP level was significantly lower for the elderly compared with the young adults, although vitamin B6 intake was the same. As already mentioned, the age-dependent decrease in plasma PLP concentration has been associated with concurrent changes in AP activity and plasma albumin content. However, studies in the rat show a similar decrease in plasma PLP content with increasing age without a concurrent change in plasma AP activity (Bode & van den Berg 1991b). Besides, most plasma PLP is bound to albumin, protecting PLP from hydrolysis to PL. A decrease in serum albumin could lead to lower PLP levels. However this is unlikely to be a major cause of lower PLP in the elderly since the PLP binding capacity of albumin is known to be far in excess of available PLP. A lower absorption rate seems also unlikely, although a lower bioavailability of food-bound vitamin B6 cannot be excluded. Based upon earlier studies in rats (Bode & van den Berg 1991b), showing an age-related redistribution of vitamin B6 body stores which is possibly related to age induced differences in fractional protein turnover, we were especially interested in the relationship between protein intake (and protein turnover) and vitamin B6 metabolism for younger and elderly age groups.

Vitamin B6 metabolism was measured in young adults and elderly at two levels of protein intake. In the elderly, plasma PLP was significantly higher at diet B compared with the lower protein diet (diet A). In young adults, plasma PLP was not significantly different between both diets. Plasma PL and total vitamin B6 in plasma were the same for young and elderly subjects when comparisons were made between diet A and diet B. Of course these results are affected by the higher vitamin B6 intake (ca. 0.27 mg/d) during the higher protein diet (diet B). However it can be argued that if the vitamin B6 intake had been similar in both periods the mentioned difference between both age groups remained, i.e. a stronger decrease in plasma PLP (and probably total plasma vitamin B6) in the young adults compared with elderly. Our results for the young adults are in accordance with those reported by Miller et al (1985). In the study of Ribaya-Mercado et al (1991) subjects were repleted with respectively 0.015, 0.022 and 0.033 mg/(kg.d) of vitamin B6 after a 20 day vitamin B6 depletion period. Plasma PLP concentrations reached baseline levels at vitamin B6 intakes of 0.0225 mg/d for men in the high protein group (n=4; 1.2 g protein/(kg.d)). In women (n=4), baseline plasma PLP levels were not obtained when vitamin B6 intake was 0.015, 0.022 and 0.033 mg/(kg.d). At the low protein diet (0.8 mg/(kg.d)), the elderly subjects needed 0.015 mg vitamin B6/(kg.d) to reach baseline plasma PLP levels (1 man and two women). It was concluded that subjects who ingested less protein required less vitamin B6 to normalize plasma PLP values after the vitamin deficiency period. In other words it can be said that (at a same level of vitamin B6 intake) plasma PLP tended to be lower during the higher protein intake. Unfortunately only elderly subjects were involved in this study so no comparisons could be made with young adults.

The level of protein intake had no effect on basal EAST activity ($EAST_0$) and EAST-activation coefficient. In the study of Ribaya-Mercado et al (1991) also no effect of protein intake on EAST activity was found.

Urinary 4-PA excretion was significantly greater during the higher protein intake (diet B) for the elderly, while 4-PA excretion remained unchanged in young adults. To correct for the higher vitamin B6 intake during diet B the relative urinary 4-PA excretion was calculated. As expected, relative 4-PA excretion was significantly lower during the higher protein intake for the young adults while there was a tendency for higher urinary 4-PA excretion in the elderly. The inverse relationship between protein intake and 4-PA excretion is already described by Miller et al (1985). In the already mentioned study of Ribaya-Mercado et al (1991) no effect of protein intake on 4-PA excretion in elderly men and women was apparent.

It is assumed that with an increased protein intake, more PLP is retained in the tissues, presumably the liver, due to increased PLP binding to enzymes involved in amino acid metabolism, induced by the higher protein intake. This increased retention results in decreased plasma PLP and lower urinary 4-PA excretion. Our findings suggest that this effect is more pronounced in younger subjects than in the elderly, i.e. the (protein induced) increase in tissue PLP binding capacity was less or even absent in elderly as compared to that in younger adults. In the present study protein

metabolism was studied in terms of protein intake and protein breakdown and synthesis as measured during an overnight fast. No correlations were found between protein turnover and the vitamin B6 status parameters as measured in the present study. Although there was a tendency for a negative correlation between protein breakdown and plasma PLP in the elderly during diet B. In the elderly no correlation was found between protein intake and vitamin B6 status parameters while in young adults, 4-PA excretion (as percentage of vitamin B6 intake) was negatively correlated with protein intake during diet A and diet B. These results are in accordance with the results described by Miller et al (1985), who also found a negative relation between 4-PA and protein intake in young adults. However interpretation of the associations between vitamin B6 metabolism and protein metabolism is complex and needs further investigation.

In conclusion, the data presented here indicate a difference in the protein intake dependent tissue PLP binding capacity and plasma PLP release between young and elderly subjects. The results suggest a relatively lower vitamin B6 requirement at a higher protein intake in the elderly.

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Chapter 9

General discussion

Energy intake decreases with age (McGandy et al 1966, The Dutch National Food Consumption Survey 1987-1989, Garry et al 1989). Knowing that the intake of most nutrients depends on total energy intake, this lower energy intake in the elderly could lead to undesirably low intakes of protein, minerals and vitamins. The research in this thesis has focussed on the effect of aging on energy and protein metabolism. It was hypothesized that a reduced physical activity (PA) in the elderly leads to a decreased energy and protein intake which in turn could lead to a decreased protein turnover rate, especially in inactive elderly subjects. Energy intake (EI), average daily metabolic rate (ADMR), basal metabolic rate (BMR), sleeping metabolic rate (SMR), diet induced thermogenesis (DIT) and energy costs of PA were studied in a group of healthy young and elderly subjects. Age related differences in body composition and their relation to BMR and SMR are described. In addition, the effect of the level of protein intake on whole body protein turnover in elderly and young men and women with a known activity level, was investigated. Finally, the effect of protein intake on protein metabolism, the effect of protein intake on calcium excretion and vitamin B6 metabolism was described.

Energy metabolism

Energy intake

For nutrition intervention studies in which subjects have to be in energy balance, it is important to know the energy requirements of the subjects. However feeding subjects to energy balance is not easy to accomplish. In the studies described in this thesis (Chapter 2 and 3) it was concluded that energy intake measurements underestimated energy expenditure in young and elderly men and women. The result was independent of the method used to estimate energy intake (a 4-day dietary record or a dietary questionnaire). Despite the baseline period, in which corrections of intake could be made when subjects lost weight or complained of hunger or satiation, subjects were in negative energy balance. Several studies compare EI (as measured by dietary record or 24h-recall) and energy expenditure (as measured with the doubly labelled water method). Results vary from good agreement in underweight and healthy subjects (Riumallo et al 1989; Schulz, Westerterp & Bruck) to underreporting of energy intake ranging from 5-24% in healthy subjects (DeLany et al 1989; Livingstone et al 1990) or extreme underreporting (26-41%) in obese subjects (Bandini, Schoeller & Dietz 1990, Prentice et al 1986, Westerterp et al 1992). Recently Goran & Poehlman (1992)

also described underreporting of energy intake as verified by measurements of energy expenditure in healthy elderly subjects (21% of energy intake). In the present study subjects underreported energy intake up to 10% with a higher discrepancy, between reported intake and measured energy expenditure, for those with a higher BMI. It was concluded that it is difficult to feed subjects in energy balance for longer periods (2 weeks) when they are not allowed to eat or drink anything except the fixed amount that is provided during the study. Moreover, it has to be noted that subjects did not complain about hunger although they were in negative energy balance. This was probably due to energy density of the food products provided during each diet. Since subjects got most of their daily food as meals with bread, potatoes and vegetables, the volume was probably higher than they were used to. The greater volume probably attributed to the absence of hunger feelings. To overcome the problem of the negative energy balance it probably would be better to provide more food as snacks with an high energy density. Before discussing the other results it has to be mentioned that all experiments described below had a randomized cross-over design. Therefore the negative energy balance did not affect the conclusions from the results presented.

Changes in BMR and SMR with aging

Age related changes in BMR and SMR are usually related to body composition, i.e. fat free mass (FFM). In the studies presented in this thesis (Chapter 2-6), FFM of the elderly subjects was derived from measures of total body water (TBW) as measured with deuterium dilution. The use of TBW in measurements of body composition implies a two compartment model: fat mass (FM) and FFM (consisting of extracellular water, intracellular water and skeletal structures (Fukagawa et al 1990). The analysis assumes that the ratio TBW/FFM is independent of age (~73%). Recently Schoeller (1989) reported data on this topic in relation to age. It was concluded that there is little or no change in the average hydration of FFM in normal aging. Comparing the body composition data of young and elderly subjects revealed that, although there were no differences in body weight, elderly subjects had a lower FFM (absolute and relative) compared with young adults. This finding has been reported previously (e.g. Forbes & Reina 1970; Fukagawa et al 1990).

BMR values of young adults were significantly higher compared with elderly persons (Chapter 3). The difference could partly be attributed to the difference in FFM. BMR values corrected for differences in FFM were still significantly higher in young adults compared with elderly subjects. It was concluded that the decrease in BMR with aging was not fully explained by the decrease in FFM. These results were in accordance with some recent studies (Vaughan et al 1991, Fukagawa et al 1990), although others did not find differences in BMR with age after correction for differences in FFM (Calloway & Zanni 1980, Bloesch et al 1988, Poehlman et al 1990).

In Chapter 4 the effect of age on SMR was studied. In contrast to the findings with respect to BMR values, no differences were found in SMR values (expressed as a function of FFM) between young and elderly men. Combining the results obtained in Chapters 3 and 4, it appeared that in elderly men regression lines of BMR as a

function of FFM and SMR as a function of FFM did not differ. In young men BMR values (as a function of FFM) were higher compared with SMR values (as a function of FFM). Although the interpretation of these results is difficult we hypothesized that in elderly men there is no change in energy expenditure from sleeping to basal metabolic rate which is normally found in young adults. Vaughan et al (1991) reported similar results: SMR as a function of FFM was the same for young and elderly subjects while BMR as a function of FFM was higher for young adults. They also reported that SMR was equal to BMR in the elderly while BMR was higher than SMR in young adults. The authors suggested that, although aging is associated with an increase in sympathetic nervous system (SNS) activity, as reflected by higher basal plasma norepinephrine concentrations or norepinephrine appearance rate, aging is also marked by a blunted response to sympathetic activation (Heinsimer & Lefkowitz 1985; Schwartz et al 1987; Vaughan et al 1990). This may partly explain the decreased BMR in the elderly. The fact that SNS activity as a determinant of energy expenditure is most important in the awake state, may explain why SMR did not differ between young and elderly men.

Diet induced thermogenesis

The effect of age on DIT was described in Chapter 4. The absolute DIT was significantly higher for the young men. This difference could be attributed to the higher energy intake of the young men since DIT, expressed as percentage of energy intake, did not differ between young and elderly men. Results of other studies are equivocal. Golay et al (1983), Bloesch et al (1988); Morgan &York (1983), Schwartz et al (1990) reported lower DIT in elderly subjects compared with young adults while Poehlman et al (1991) did not find age related differences in DIT. The conflicting results are probably due to differences in meal size, meal composition and meal frequency; important factors affecting DIT. It was concluded from the present study that there were no differences in DIT between young and elderly men. However we have to take into account that the variability in DIT is high. Recently Westrate et al (1993) that sample sizes lower than 10 subjects, with one measurement per subject and per treatment, have power levels lower than 80% for assessing true between groups differences in DIT.

Level of physical activity

There are two ways to express the level of PA of a subject when data are available with respect to ADMR and BMR (or SMR). PA can be expressed either by ADMR/BMR; ADMR/SMR or by ADMR-DIT-BMR; ADMR-DIT-SMR-(arousal).
Under free living conditions (Chapter 3), ADMR/BMR tended to be lower in elderly subjects compared with young men and women (P=0.081). As shown in Figure 9.1. the level of PA covers a wide range in both young and elderly subjects The distribution of ADMR/BMR showed a clear shift to higher values for the young subjects. Expressed as ADMR-BMR-estimated DIT, energy costs of PA were higher for the young subjects (P<0.0001). Under controlled conditions, when subjects were con-

fined to a metabolic chamber performing the same activity protocol (Chapter 4) no differences in ADMR/SMR between young and elderly men and no differences in absolute level of energy expenditure for PA were found.
When the level of PA is expressed as multiples of BMR some caution is required when comparisons are made between young and elderly subjects: when the energy costs of PA remain constant, the lower BMR of the elderly would raise the ADMR/BMR ratio with advancing age. Furthermore, since BMR in the elderly equals SMR, PA may be artificially raised in the elderly since in the young adults BMR is SMR plus ~5% for arousal, which leads to a lower PA ratio.
Under free living conditions the energy expended on PA (MJ/d) was higher in the young adults (Chapter 3) even when expressed per kg body weight. Under strictly controlled conditions, that means when performing a PA protocol, the energy expended on PA was similar in young and elderly men (Chapter 4). From the latter study it can be concluded that the energy costs for specific activities were the same for young and old men. Combining these results it can be concluded that the participation of elderly subjects in physical activities is lower compared with young adults or that the elderly participate in physical activities with a lower intensity. However, it should be noticed that results in Chapter 4 are based on small groups and other studies do report higher energy costs of specific activities (Voorrips et al 1993, Didier et al 1993).

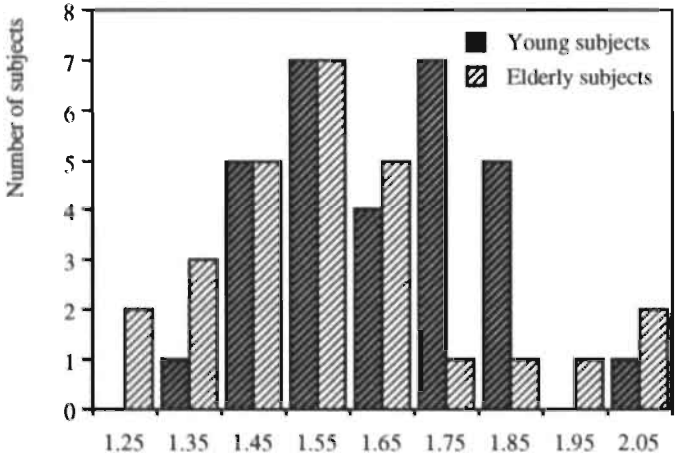

Fig. 9.1. Frequency distribution of the measured ADMR/BMR values as measured during the study described in Chapter 3.

Measurement of ADMR with doubly labelled water
As described in Chapter 3 and 4, the ADMR (MJ/d) of young adults was higher in comparison with the ADMR of elderly subjects. In view of the results described above, it seems that the decrease in BMR (MJ/d), the decrease in energy expended on

PA (MJ/d) and the lower absolute values of DIT (in MJ/d) explain the decrease in ADMR. Only few studies have been published measuring ADMR under free living conditions (with doubly labelled water) in elderly persons (Reilly et al 1993, Roberts et al 1992, Goran & Poehlman 1992). The reported ADMR values were in accordance with the present findings, and as found in our study, ADMR covered a wide range. The current recommendations as given by the WHO/FAO/UNU (1985) for energy requirements in adults with different activity levels are also expressed as multiples of BMR. In the present study, mean ADMR/BMR value of the elderly (1.58±0.21) did not differ significantly from the recommendation as given by the FAO/WHO/UNU (1985) for sedentary elderly people, namely 1.50. Our results are in accordance with results of the study of Goran & Poehlman (1992) who reported mean activity levels of 1.51 for elderly men and women. Roberts et al (1991, 1992) questioned the recommendations since the activity levels of the subjects in their studies, who were classified on the basis of a questionnaire as "sedentary", had significantly higher ADMR/BMR values compared with the WHO/FAO/UNU (1985) recommendations (1.75 compared with 1.50 as recommended for the elderly and 1.98 compared with 1.55 or 1.67 for young adults).

It can be concluded that ADMR decreases with age. At energy balance, this lower ADMR will result in a decrease in energy intake. Several studies indeed report a decrease in energy intake (McGandy et al 1966, The Dutch National Food Consumption Survey 1987-1989, Garry et al 1989). Because the intake of most nutrients depends on total energy intake, this lower energy intake in the elderly could lead to undesirably low intakes of protein, minerals and vitamins. This is especially true in the inactive elderly who are at risk for malnutrition.

Protein metabolism

Nitrogen balance

The nitrogen balance method is used to determine the minimum protein requirement. This technique gives no information about the optimum protein intake since nitrogen balance can be achieved within a wide range of protein intake based on the principles of adaptation (Waterlow 1986) as shown in the study presented in this thesis. In our study nitrogen balance was approximately zero when the protein content of the diet was 12% (Diet A) of total energy intake (Chapter 5 and 6). This protein content corresponded the recommended daily protein intake as given by the Dutch Nutrition Council (1989). When the protein intake of the diet was increased to 21% (diet B) of total energy intake the nitrogen balance was again approximately zero in the elderly. In young adults nitrogen balance was positive during diet B. There was no clear explanation for this positive nitrogen balance, although other authors also reported positive nitrogen balances of the same magnitude (Motil et al 1981, Winterer et al 1976, Oddoye & Margen 1979) even after prolonged periods of time when levels of protein intake were above physiological needs (Young et al 1981).

Protein turnover

As described above, nitrogen balance can be achieved within a wide range of protein intake. As a consequence the nitrogen balance technique gives no information about an optimum level of protein intake. Nitrogen balance is a reflection of overall body protein synthesis and breakdown (protein turnover), a given nitrogen balance may be achieved within a wide range of protein synthesis and breakdown rates. In Chapter 5 and 6 the effect of the recommended daily protein intake (12% of total energy intake) on protein turnover (protein synthesis and protein breakdown) was described. Protein breakdown and protein synthesis were significantly higher in young adults compared with elderly men and women. Protein turnover was lower in elderly men and women compared with young men and, even when corrections were made for body weight or FFM, while no differences were seen between young men and women. Only few reports are known, studying the effect of age on protein turnover. In general, whole body protein turnover decreases with age (Golden & Waterlow 1977; Uauy et al 1978; Winterer et al 1976; Robert et al 1984). However when corrections were made for body composition results are equivocal. Winterer et al (1976) found significantly higher protein turnover rates, expressed per kg body weight, in elderly men compared with young men and the same tendency (although not significant) was found in elderly women compared with young women. Uauy et al (1978) reported no differences in protein breakdown in young and elderly subjects when expressed per kg body cell mass. Robert et al (1984) also found no difference although there was a tendency towards a lower leucine flux in elderly subjects expressed per kg lean body mass. The discrepancies between the results of the studies are probably due to differences in techniques used to measure protein turnover and body composition, differences in protein intake before the start of the study and sometimes during the study (postabsorptive versus fed state). We are aware of the fact that the choice of the tracer would probably influence the absolute values for protein breakdown and synthesis. However, in the studies described in Chapter 5 and 6, measurements were all done with the same technique using the same tracer and protocol. This allows us to make comparisons between both age groups. The lower protein turnover in elderly women, compared with elderly men and young women is probably due to differences in hormonal status. Male and female sex hormones (testosterone and oestrogens) stimulate protein synthesis. In women oestrogen levels decrease during and after menopause while in men testosterone levels tend to remain constant (Griffiths 1981).

It was concluded from the studies described in Chapter 5 and 6 that protein turnover decreases with age, even when corrections were made for differences in body weight and FFM. In this study, only whole body protein turnover was measured. Other authors suggest a change in the distribution of whole body protein turnover with age, with muscle making a lower contribution to whole body protein turnover in elderly subjects compared with young subjects. Welle et al (1993) and Yarasheski et al (1993) reported lower muscle protein synthesis in elderly subjects compared with young adults. It is speculated (Young 1990), because muscles contribute to the adaptation in whole body energy and amino acid metabolism during restricted dietary

energy and protein intakes, that a reduced contribution of the muscle to whole body protein metabolism might diminish the capacity of the elderly individual to respond successfully to unfavourable dietary situations. The lower protein turnover of the elderly in our study might be due to a lower contribution of the muscles to whole body protein turnover, resulting in a diminished capacity to respond successfully to unfavourable dietary situations or stress due to illness. How rates of body protein synthesis and breakdown are affected by dietary intake is therefore an important step in understanding the metabolic significance of differences in dietary intake.

Protein intake and protein turnover

The effect of increasing the level of protein intake from 12 to 21% of total energy intake on whole body protein turnover in elderly and young adults was described in Chapter 5 and 6. Protein breakdown and protein synthesis of young and elderly subjects increased significantly when the protein content of the diet increased from 12 to 21% of total energy intake. There were no differences in the relative and absolute increase in protein breakdown and synthesis in young and elderly subjects. As reported previously during diet A, there was also a positive correlation between protein intake and protein turnover for young and elderly subjects during diet B. Furthermore, during diet B protein turnover of elderly men was comparable with protein turnover of young men while elderly women still had lower protein turnover rates compared with young women. Again elderly women had significantly lower protein turnover rates compared with elderly men and young women, even when corrections were made for differences in body weight and body composition. The relative increase in protein breakdown and protein synthesis was the same for young and elderly subjects. Motil et al.(1981) and Gersovitz et al (1980) reported on the effect of protein intake (from marginal to adequate) on leucine, lysine and glycine metabolism. It was concluded that the amino acid flux increased with increasing protein intake. Recently Garlick et al (1993) reviewed the studies on the influence of dietary protein intake on whole body protein turnover in humans. Our conclusion from the present study in young and elderly subjects, is in accordance with their conclusion, which was mainly based on data in young adults: the adaptation to higher protein intakes involves an increase in the basal (post absorptive) rates of both synthesis and breakdown. Only speculations can be made about the implication of a higher basal protein turnover in young and elderly subjects. Since we measured whole body protein turnover we can only speculate about the contribution of the muscles. If the increased protein turnover involves an increase in the contribution of the muscle protein turnover, then this probably means that one is better equipped for an unfavourable nutritional status or to other stressful conditions (for reasons above mentioned). Further studies should investigate whether this increased protein turnover arises from a greater contribution from skeletal muscles: an outcome that may well be favourable in the elderly, because muscles contribute to the adaptation in whole body energy and amino acid metabolism during restricted dietary energy and protein intakes.

Interactions with basal metabolic rate and physical activity

We tried to calculate the contribution of protein turnover to BMR in young and elderly subjects. FFM explained 78% of the variation in BMR (taking young and elderly subjects together). When protein breakdown was added 81% of the variation was explained. Other authors reported an explained variation in BMR by protein turnover up to ~10% (Waterlow & Jackson 1981). However there is much uncertainty about this estimate since it is difficult to quantify the energy costs of protein synthesis. The lower contribution of protein turnover to BMR in our study is probably due to the fact that we measured protein turnover during the night. In the fasted state protein breakdown is higher than protein synthesis. We were mainly measuring protein breakdown, while protein synthesis is the main energy requiring process.

There was no effect of the level of PA (expressed as ADMR/BMR or as ADMR-DIT-BMR) on protein turnover, probably due to the fact that only whole body protein turnover was measured. Yarasheski et al (1993) reported an increase in muscle protein turnover after two weeks of resistance training in elderly subjects, while there was no effect on whole body protein turnover. We found a significant correlation between protein intake and protein turnover in both young and elderly subjects (diet A, diet B). When ADMR is increased because of an increased level of PA, this will lead to an increased energy intake and protein intake leading to a higher protein turnover.

Calcium metabolism

Calcium and protein interactions

As described above, a higher protein intake is probably beneficial for elderly subjects. However one should take into account the possible negative effect of an increased protein intake on calcium excretion. In the study described in Chapter 7 we investigated the influence of increasing the level of protein intake (from 12 to 21% of total energy intake) on the urinary calcium excretion, calcium absorption and calcium balance in young and elderly subjects. Absolute urinary calcium excretion increased in young and elderly subjects with increasing protein intake. Relative calcium excretion (as percentage of calcium intake, to correct for differences in calcium intake) increased during diet B in young adults, while it remained constant in the elderly. Many authors have reported increased urinary calcium loss with increased protein intake (Allen et al 1979, Anand and Linkswiler 1974, Hegsted et al 1981, Johnson et al 1970, Margen et al 1974, Schuette et al 1980, Walker and Linkswiler 1972). The calciuretic effect of protein is caused by an increased glomerular filtration rate, an increased filtered calcium load and by a decreased tubular reabsorption (Allen et al 1979, Chu et al 1975, Kim & Linkswiler 1979, Schuette et al 1980, Zemel et al 1981). However, Spencer et al (1978a) reported that phosphorus reduces urinary calcium excretion by increasing the tubular reabsorption. The same group reported that increasing the protein intake by giving complex proteins (which contain much phosphorus) did not lead to hypercalciuria in adults (Spencer et al 1978b, Spencer et al 1983). In the present study the

phosphorus intake also increased during diet B in comparison with diet A, the magnitude of this increase was similar in young and elderly subjects (21%). The increased phosphorus intake (during diet B) is probably contributing to the constant urinary calcium excretion in diet B compared with diet A (as percentage of calcium intake) in the elderly. The higher phosphorus intake did not prevent urinary calcium loss in young adults. However it is speculated that the increase in urinary calcium during diet B probably would have been higher when the phosphorus intake had been equal during both diets.

Relative calcium absorption (the difference between intake and faecal excretion as percentage of calcium intake) increased when the protein intake of the diet increased from 12 to 21% of total energy intake in the elderly and the same tendency was seen in young adults. Results on the effect of protein intake on calcium absorption are equivocal. Some authors report an increased absorption (Chu et al 1975, Walker and Linkswiler 1972, Lutz & Linkswiller 1981) while others found no effect (Allen et al 1979, Anand and Linkswiler 1974). The results described in Chapter 7 indicate that the increased calcium excretion in the urine was compensated by an increased calcium absorption.

As a result there was no effect of increasing the protein intake on calcium balance in young adults while there seemed to be a positive effect of increased protein intake on calcium balance in the elderly. These results are in accordance with the study of Schuette et al (1982). They studied the effect of an increased protein intake, (with simultaneously supplementation of calcium and phosphorus). The protein intake was increased by adding meat (or simulated meat with purified proteins) and dairy products. The authors reported that urinary calcium excretion increased with a simultaneous increased calcium retention, resulting in a positive effect on calcium balance (Schuette et al 1982). In our study the same effect was found with a relatively lower increase in calcium and phosphorus. The increase in protein, calcium and phosphorus intake was respectively ~38%, ~43% and ~43% in the study of Schuette et al (1982) versus respectively ~78%, 12% and 21% in the present study. However, balance studies are susceptible to rather large errors since the outcome is calculated as the difference between calcium intake and calcium excretion in faeces (and urine), neither of which can be measured with high precision when subjects live on their own, compared to observations in a metabolic ward. Although calcium balance was carried out as precisely as possible the results should be interpreted with care. It was concluded that increasing the protein intake from 12% to 21% of total energy intake had no negative effect on calcium excretion and calcium balance in young and elderly subjects. Furthermore there were no differences between the two age groups with respect to the interaction between protein intake and calcium excretion, calcium absorption and calcium balance.

Vitamin B6 metabolism

Vitamin B6 status in young and elderly subjects

Several studies reported age related differences in vitamin B6 status (Guilland et al 1984, Hamfelt & Söderhjelm 1988, Kant et al 1988, Löwik et al 1989, Manore et al 1989, Tolonen et al 1988, Rose et al 1976, Lee & Leklem 1985). The effect of aging on vitamin B6 status was measured in young and elderly subjects by means of plasma pyridoxal phosphate (PLP), erythrocyte aspartate aminotransferase (EAST) activity and urinary 4-pyridoxic acid (4-PA) measurements (Chapter 8). Consuming the average protein diet (diet A) plasma PLP and plasma PL were significantly lower in the elderly. A decrease of plasma PLP with age was described before (e.g. Guilland et al 1984, Hamfelt & Söderhjelm 1988, Kant et al 1988, Löwik et al 1989, Manore et al 1989, Tolonen et al 1988, Rose et al 1976). Several suggestions were made to explain this decrease. First, the causal inverse relationship between plasma alkaline phosphatase activity and plasma PLP concentration; the increase of plasma alkaline phosphatase activity with age could explain the decrease in plasma PLP (Kant et al 1988). Second, it was suggested that a decrease in vitamin B6 absorption with age could attribute to the lower PLP concentrations with aging. However, this seems to be unlikely, since elderly subjects showed an increase in PLP when an orally dose of vitamin B6 was given, which was comparable with young adults(van den Berg et al 1992; Shultz & Leklem 1985; Ubbink et al 1987), suggesting no effect of age on vitamin B6 absorption. Third, it was recently hypothesized that the age dependent decrease in plasma PLP level could be associated with a decrease in tissue body stores due to changes in body composition and/or an effect on PL(P) release from (muscle) protein due to a decrease in protein turnover (Bode & van den Berg 1991a, Bode & van den Berg 1992). The latter suggestion was studied in the experiment described in Chapter 8. The PLP concentration was related to protein turnover rate as measured during diet A. However, no correlation was found between protein turnover and plasma PLP in young and elderly subjects.

Despite the decreased PLP concentrations in the elderly, EAST activity was lower in the elderly subjects, indicating that the lower PLP values were not associated with functional impairment at the level of EAST saturation. Other studies relating to EAST saturation in the elderly showed conflicting results, partly due to the lack of generally accepted ranges of adequate and deficient values of the activation coefficient (Bode 1992).

In our study, 4-PA excretion was significantly lower in the elderly with an intake of ~1.5 mg/d. This result was in contrast with results of Lee & Leklem (1985) who found higher 4-PA excretion in elderly women on normal vitamin B6 intake (2.3-2.4 mg/d) and with results reported by Kant et al (1988) who found similar 4-PA excretion in young, middle age and elderly men (vitamin B6 intake was ~2.00 mg/d). The fact that 4-PA excretion reflects recent vitamin B6 intake instead of the underlying state of tissue reserves might explain the differences (Lui et al 1985).

The conclusion from the study described in Chapter 8 with respect to age differences in vitamin B6 status is threefold: Firstly, plasma PLP concentration was lower in the elderly compared with the young subjects, although vitamin B6 intake was the same (mg/d). Secondly, the lower EAST activity in the elderly did not suggest a functional impairment for the elderly on the level of EAST activity. Thirdly, the lower 4-PA excretion of the elderly indicated that vitamin B6 catabolism was not increased in the elderly.

Vitamin B6 and protein interactions

Due to the fact that protein and vitamin B6 metabolism are closely linked to each other we studied the effect of an increased protein intake on vitamin B6 status in young and elderly subjects (Chapter 8). It was assumed that increasing the protein intake would lead to an increased retention of PLP (probably in the liver), due to an increased PLP binding to enzymes involved in amino acid metabolism. As a result of the increased PLP retention, plasma PLP would decrease leading to a decreased excretion of urinary 4-PA.

With respect to the age related differences in vitamin B6 status mentioned above it can be concluded that they were the same during the higher protein intake except for urinary 4-PA excretion. 4-PA excretion was similar in young and elderly subjects during the diet B, indicating that vitamin B6 catabolism was not increased in the elderly.

Plasma PLP concentration increased in the elderly during diet B while plasma PLP remained constant in young adults. The results were affected by the higher vitamin B6 intake during diet B and we can only speculate about the effect of the increased protein intake when vitamin B6 intake had been constant: when vitamin B6 intake had been constant, the PLP concentrations would have been only slightly increased or remained stable in the elderly, while in young adults the decrease in plasma PLP would have been even more pronounced. Our results with respect to the young adults were in accordance with results reported by Miller et al (1985). However, our results are in contradiction with the results of Ribaya-Mercado et al (1991). They studied the effect of protein intake on plasma PLP in elderly subjects and concluded that elderly subjects ingesting less protein required less vitamin B6 to normalize plasma PLP after a depletion period.

The level of protein intake had no effect on the EAST activity in young and elderly subjects. This was in accordance with the study of Ribaya-Mercado et al (1991) who also reported that the amount of protein intake had no effect on the EAST activity (Ribaya-Mercado et al 1991).

Urinary 4-PA excretion increased with increasing protein intake in the elderly while it remained constant in young adults. To correct for the differences in intake during both diets 4-PA excretion was also expressed as percentage of vitamin B6 intake. Expressed in this way, 4-PA excretion tended to increase in the elderly during diet B while 4-PA excretion was decreased in young adults. Miller et al (1985) also described a decrease in 4-PA excretion with increasing protein intake in young adults, while Ribaya-Mercado found no effect of protein intake on 4-PA excretion in elderly

subjects. Furthermore in young adults the amount of 4-PA excretion (%) was negatively correlated with protein intake (during diet A and diet B) while no relation was found in the elderly. Miller et al (1985) also reported a negative correlation between protein intake an 4-PA excretion in young adults.
Furthermore, as during diet A no correlations were found between protein turnover and the vitamin B6 status parameters as measured in the present study during diet B. Although there was a tendency for a negative correlation between protein breakdown and plasma PLP in the elderly during diet B. This latter finding was in accordance with the already mentioned hypothesis that the age dependent decrease in plasma PLP content could be associated with an effect on PL(P) release from (muscle) protein due to a decrease in protein turnover (Bode & van den Berg 1991a, Bode & van den Berg 1992).
It was assumed that increasing the protein intake would lead to an increased retention of PLP (probably in the liver), due to an increased PLP binding to enzymes involved in amino acid metabolism. As a result of the increased PLP retention, plasma PLP would decrease leading to a decreased excretion of urinary 4-PA. The results suggest that this effect was more pronounced in young adults than in elderly subjects. The results indicated an age related difference in protein intake dependent tissue PLP binding capacity and suggest a relatively lower vitamin B6 requirement at higher protein intakes in the elderly.

Interactions with physical activity

Since PA is likely to decrease with age, this would lead to a lower energy intake in elderly making them more vulnerable for insufficient nutrient intake. The insufficient nutrient intake can lead to health problems. Therefore PA was one of the key issues in this project.
For the studies described in this thesis, subjects were recruited with advertisements in local media. Elderly subjects were also recruited through contacts with alliances for the elderly. Subjects were asked to fill in an activity questionnaire (Caspersen 1992). When the questionnaire was filled in accurately and completely, they were ranked from very active to very inactive. The most active and inactive subjects were asked to participate in our experiments. However, the activity questionnaire did not predict the individual activity level accurately (r=0.42). Furthermore, not all subjects were willing to participate in the diet intervention study, in which they were not allowed to eat anything else except for the food provided. Therefore also moderately active subjects were asked to participate. For these reasons, the mean activity level of the subjects involved in our studies was moderate. However as mentioned before, the activity levels covered a wide range.
In the studies described in this thesis the level of PA was assessed by measuring ADMR and expressing ADMR as multiples of BMR (or SMR) or as the energy expended on PA (see above). The effect of the PA level on BMR (SMR), protein

turnover, calcium balance and vitamin B6 status parameters were studied. No effect of the PA level on BMR was found. Other studies do report an increased BMR in subjects with a high PA level (Fukagawa et al 1991; Poehlman et al 1992). We also found no effect of the level of PA on protein turnover, probably due to the fact that we measured whole body protein turnover was measured. Yarasheski et al (1993) reported an increase in muscle protein turnover after two weeks of resistance training in elderly subjects, while they also found no effect of PA on whole body protein turnover. In the study described in Chapter 7, no effect of the level of PA on calcium balance was found. The positive effect of PA was reported on the level of bone mass and total body calcium (Evans & Meredith 1989). Only at the long term, the changes in calcium balance would result in changes in bone mass and total body calcium. In the elderly, the level of PA (expressed as multiples of BMR or in energy expended on PA) was positively correlated with plasma PLP during diet A and diet B, while no correlations were found in young adults. Manore et al (1987) reported an increased basal plasma PLP in young trained women compared with young untrained and postmenopausal untrained women. In conclusion, no major effects of the level of PA on BMR, protein turnover and calcium balance were found. There are several explanations for this unexpected result. First, one can question whether the difference in activity level was high enough. Although the range in activity level was wide and comparable in young and elderly subjects, it probably would be better to select a very active and a very inactive group in order to identify effect related to PA. Second, with respect to the probable training effects of the subjects, we have to take into account that in this thesis no information is available about the intensity of the PA and the training status of the subjects involved. Most of the effects on metabolism are related to the intensity of the PA, probably explaining why no effects were found in our study.

Nevertheless, when ADMR is increased because of an increased level of PA, this will lead to an increased energy and nutrient intake, making especially the elderly less vulnerable for inadequate energy and nutrient intake.

Conclusions

1 ADMR decreased with age due to changes in BMR, DIT and energy expenditure for PA. BMR as a function of FFM was significantly lower in elderly subjects compared with young adults suggesting that aging is associated with an alteration in tissue energy metabolism. Absolute DIT values were significantly lower in elderly men compared with young men this was due to the lower energy intake in elderly men since relative DIT (as a percentage of energy intake) did not differ between young and elderly men. Under free living conditions, PA (expressed as ADMR divided by BMR) tended to be lower in elderly subjects compared with young adults while energy expended on PA (absolute or per kg body weight) was higher in young adults. Under controlled conditions, e.g. performing an activity protocol (simulating sedentary living conditions), no differences were found in the

level of PA (expressed as multiples of SMR or as energy expended on PA) indicating that the energy costs of specific activities were the same for young and elderly men.

2 During diet A protein turnover was lower in elderly men and women compared with young men and women, even when corrections were made for differences in body composition. Furthermore protein turnover was lower in elderly women compared with elderly men even when corrections were made for differences in body composition. Increasing the protein intake to 21% of energy intake resulted in an increased protein turnover in both young and elderly subjects. During diet B, protein turnover (corrected for differences in body composition) was comparable in young and elderly men while elderly women still had lower turnover rates compared with elderly men and young women.

3 Increasing the protein intake from 12% to 21% of total energy intake had no negative effect on calcium excretion and calcium balance in young and elderly subjects. There were no differences between both age groups with respect to the interaction between protein intake and calcium excretion, calcium absorption and calcium balance.

4 At the same level of vitamin B6 intake, plasma PLP concentration was lower in the elderly compared with the young subjects, 4-PA excretion was lower (diet A) or equal (diet B) in the elderly compared with young adults. Furthermore EAST activity was lower in the elderly. The results indicated an age related difference in protein intake dependent tissue PLP binding capacity and suggest a relatively lower vitamin B6 requirement at higher protein intakes in the elderly.

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Summary

Energy intake decreases with age. Since the intake of most nutrients depends on total energy intake, a reduced energy intake in the elderly could result in insufficient intakes of protein, minerals and vitamins. The studies presented and discussed in this thesis were intended to obtain more information about the age related changes in energy metabolism and protein metabolism. The effect of protein intake on protein metabolism, calcium excretion and vitamin B6 metabolism was also investigated in young and elderly men and women, in order to determine the interaction between protein intake and protein, calcium and vitamin B6 metabolism.

Data on energy intake are often used as a basis for nutrition intervention studies. Chapter 2 describes a study, which was intended to estimate energy intake for a nutrition intervention study. Energy intake as measured with a four-day dietary record or with a dietary questionnaire underestimated energy expenditure in elderly men and women independent of the method used. The discrepancy between energy intake and measured energy expenditure increased with increasing body mass index.

The aim of the study, presented in Chapter 3, was to measure average daily metabolic rate and the components basal metabolic rate and physical activity of healthy elderly and young adults. Age related differences in body composition and their relation to basal metabolic rate and activity level were also studied. At the same body weight, elderly subjects had a significantly higher fat mass and a significantly lower fat free mass compared with the young adults. The average daily metabolic rate was lower in elderly subjects compared with young adults partly due to a significantly lower basal metabolic rate. When relating the basal metabolic rate to the differences in body composition it revealed that the lower basal metabolic rate was not fully explained by the lower fat free mass in the elderly. It was suggested that aging is associated with an alteration in tissue energy metabolism. The energy expended on physical activity (calculated as the average daily metabolic rate minus basal metabolic rate) was higher for the young adults and positively correlated to the fat free mass index (in both age groups), indicating that there was a positive effect of physical activity on body composition.

The energy costs of controlled daily activities in young and elderly men were measured in order to study whether there is a change in energy costs of specific activities with increasing age (Chapter 4). Total energy expenditure, sleeping metabolic rate, diet induced thermogenesis and energy expended on physical activity were measured under strictly controlled conditions. Total energy expenditure during a standardized activity protocol was significantly higher for the young men. Sleeping metabolic rate as a function of fat free mass was not different between both age groups. The diet induced thermogenesis expressed as MJ/d was significantly higher for the young subjects but similar when expressed as percentage of energy intake. The resulting figure for the energy expended on physical activity (total energy expenditure minus sleeping metabolic rate and diet induced thermogenesis) was the same for young and

elderly men indicating that mean energy costs of sedentary activities were the same for young and elderly men.

To understand more about the metabolic significance of differences in protein intake the effect of the daily amount of protein intake on whole body protein turnover and nitrogen balance was studied in elderly men and women (Chapter 5). Protein turnover increased significantly when the protein content of the diet increased from 12 to 21 percent of total energy. Furthermore, the protein turnover rate was significantly higher for elderly men when compared with elderly women, even when corrections were made for differences in body composition. Mean nitrogen balance did not differ significantly from zero during either diet.

To interprete the above mentioned results in more detail the study was repeated in young adults (Chapter 6). As reported earlier for elderly subjects, the protein turnover rate was significantly higher when the protein content of the diet was increased from 12% to 21% of total energy intake. During the 12% protein diet, young adults had a higher protein turnover rate compared with elderly subjects. During the higher protein intake (21% of total energy intake), protein turnover of young men was comparable with the protein turnover of elderly men while young women still had a higher protein turnover rate compared with elderly women (even when corrections were made for differences in body composition). In young adults, mean nitrogen balance was approximately zero during the 12% protein diet while it was positive during the higher protein intake. It was concluded that a higher protein intake resulted in increased basal (post absorptive) rates of both synthesis and breakdown.

Chapter 7 reports on the effect of two levels of dietary protein intake on urinary calcium excretion, calcium absorption and calcium balance in young and elderly men and women. In young and elderly subjects, urinary calcium excretion was higher when 21% of total energy was given as protein compared with the lower protein diet (12% of total energy as protein). The higher urinary calcium excretion was compensated by an increased calcium absorption. For the elderly this resulted in a less negative calcium balance during the higher protein intake whereas for the young adults there was no effect of protein intake on calcium balance. It was concluded that increasing the protein intake from 12% to 21% of total energy had no negative effects on calcium balance in healthy young and elderly people. Moreover, the interaction between protein intake and calcium excretion, absorption and balance did not differ between both age groups.

The effect of protein intake on vitamin B6 metabolism was studied and the results are described in Chapter 8. Plasma pyridoxal phosphate, pyridoxal and total vitamin B6 concentrations were significantly lower in the elderly compared with the young adults during both diet periods (containing either 12% of 21% of total energy intake as protein). In the elderly, the pyridoxal phosphate concentration was significantly higher during the higher protein intake while the level of protein intake had no significant effect on plasma pyridoxal phosphate concentration in the young adults. Plasma pyridoxal and total vitamin B6 concentrations were not influenced by the amount of protein intake in young and elderly subjects. Relative urinary pyridoxic acid excretion

did not differ significantly in the elderly, while urinary pyridoxic acid excretion was lower in young adults during the higher protein intake (21% of total energy intake as protein). The results of this study suggested an age-dependent difference in the protein intake related vitamin B6 needs, whereby elderly subjects apparently need less vitamin B6 at a higher protein intake as compared with young adults.

Samenvatting

De energie-inname neemt af met de leeftijd. Aangezien de inname van de meeste nutriënten afhankelijk is van de totale energie-inname, zou een verlaagde energie-inname kunnen resulteren in een te lage inname van eiwit, mineralen en vitamines. De in dit proefschrift beschreven onderzoeken zijn uitgevoerd teneinde meer informatie te verkrijgen omtrent de leeftijdsgebonden veranderingen in het energie- en eiwitmetabolisme. Het effect van de eiwitinname op het eiwitmetabolisme, de calciumexcretie en het vitamine B6-metabolisme is eveneens onderzocht in een groep gezonde bejaarden en jongvolwassenen, om zo de interactie tussen eiwitinname en eiwit-, calcium- en vitamine B6-metabolisme te bepalen.

Gegevens met betrekking tot de energie-inname worden vaak gebruikt als basis voor voedingsinterventie-onderzoek. In het in hoofdstuk 2 beschreven onderzoek werd de energie-inname geschat om als basis te dienen voor een voedingsinterventie-onderzoek. De energie-inname, zoals die werd geschat met behulp van een vierdaags-eetdagboekje of met een korte voedingsvragenlijst, onderschatte het energiegebruik van bejaarde mannen en vrouwen; dit was onafhankelijk van de gebruikte methode. De discrepantie tussen de energie-inname en het energiegebruik nam toe naarmate de body-mass-index van de proefpersonen toenam.

Het doel van het in hoofdstuk 4 beschreven onderzoek was het meten van het totale dagelijkse energiegebruik en de componenten energiegebruik in rust en lichamelijke activiteit, in een groep gezonde jongvolwassenen en bejaarden. De leeftijdsgebonden verschillen in lichaamssamenstelling en hun relatie met het energiegebruik in rust en de lichamelijke activiteit werden eveneens onderzocht. Bij een vergelijkbaar gewicht hadden de bejaarden een significant hogere vetmassa en een significant lagere vetvrije massa in vergelijking met de jongvolwassenen. Het totale dagelijkse energiegebruik was lager voor de bejaarden in vergelijking met de jongeren. Dit werd gedeeltelijk veroorzaakt door het lagere energiegebruik in rust van de bejaarden. Wanneer het energiegebruik in rust gerelateerd werd aan de verschillen in lichaamssamenstelling bleek dat het lagere energiegebruik in rust niet volledig verklaard kon worden door de lagere vetvrije massa van de bejaarden. Gesuggereerd werd dat veroudering gepaard gaat met een verandering in het energiemetabolisme van het weefsel. Het energiegebruik voor lichamelijke activiteit (berekend als het totale dagelijkse energiegebruik verminderd met het energiegebruik in rust) was hoger voor de jongvolwassenen en positief gecorreleerd met de vetvrije-massa-index (in beide leeftijds-categorieën), duidend op een positief effect van lichamelijke activiteit op de lichaamssamenstelling.

De energiekosten voor dagelijkse activiteit onder gecontroleerde omstandigheden werden gemeten om na te gaan of er een verandering optrad in de energiekosten voor specifieke activiteiten met de leeftijd (hoofdstuk 4). Het totale energiegebruik, het energiegebruik tijdens slaap, de dieetgeïnduceerde thermogenese en het energiegebruik voor lichamelijke activiteit werden gemeten onder strikt gecontroleerde omstandigheden in een groep jonge en bejaarde mannen. Het totale energiegebruik tijdens

een gestandaardiseerd activiteitenprotocol was significant hoger voor de jonge mannen. Het energiegebruik tijdens slaap als functie van de vetvrije massa was niet verschillend voor beide leeftijdsgroepen. De dieetgeïnduceerde thermogenese, uitgedrukt in megajoule per dag, was significant hoger voor de jonge mannen. Wanneer de dieetgeïnduceerde thermogenese uitgedrukt werd als percentage van de energie-inname werden geen verschillen waargenomen. Het energiegebruik voor lichamelijke activiteit (berekend als het totale energiegebruik verminderd met het energiegebruik tijdens slaap en de dieetgeïnduceerde thermogenese) was gelijk voor jonge en bejaarde mannen. Op basis van deze resultaten kan gezegd worden dat de gemiddelde energiekosten van weinig intensieve dagelijkse activiteiten gelijk zijn voor jonge en bejaarde mannen.

Om meer inzicht te krijgen in de metabole significantie van het eiwitinname-niveau werd het effect van de dagelijkse eiwitinname op de totale lichaamseiwitturnover en de stikstofbalans gemeten in bejaarde mannen en vrouwen (hoofdstuk 5). De eiwitturnover was significant hoger wanneer de hoeveelheid eiwit in de voeding steeg van 12% naar 21% van de totale energie-inname. Gedurende beide dieetperiodes hadden de bejaarde mannen een hogere eiwitturnover in vergelijking met de bejaarde vrouwen, ook wanneer gecorrigeerd werd voor verschillen in lichaamssamenstelling. De gemiddelde stikstofbalans verschilde niet significant van nul tijdens beide dieetperiodes.

Teneinde de resultaten van het hierboven beschreven onderzoek beter te kunnen interpreteren, werd het onderzoek herhaald bij jongvolwassen (hoofdstuk 6). Evenals bij de bejaarden was de eiwitturnover hoger tijdens de hogere eiwitinname (21% van de totale energie-inname). Tijdens het dieet met 12 energie% eiwit hadden jongvolwassenen een significant hogere eiwitturnover in vergelijking met de bejaarden. Tijdens het dieet met het hogere eiwitgehalte (21% van de totale energie-inname) was de eiwitturnover van de jonge en oude mannen vergelijkbaar, terwijl de eiwitturnover van de bejaarde vrouwen nog steeds lager was in vergelijking met de jonge vrouwen (ook wanneer gecorrigeerd werd voor verschillen in lichaamssamenstelling). De stikstofbalans van de jongvolwassenen was nagenoeg nul tijdens het dieet met 12 energie% eiwit en positief tijdens de hogere eiwitinname. Geconcludeerd werd dat een hogere eiwitinname resulteerde in een hoger basaal niveau van eiwitafbraak en eiwitsynthese.

In hoofdstuk 7 wordt het effect beschreven van twee eiwitinname-niveaus op de calciumuitscheiding in de urine, de calciumabsorptie en de calciumbalans van jonge en bejaarde mannen en vrouwen. Voor beide groepen gold dat de calciumexcretie in de urine significant hoger was tijdens het 21 energie% dieet in vergelijking met het 12 energie% dieet. De hogere calciumuitscheiding in de urine werd gecompenseerd door een verhoogde calciumabsorptie. Voor de ouderen resulteerde dit in een minder negatieve calciumbalans tijdens de hogere eiwitinname terwijl de calciumbalans van de jongeren niet beïnvloed werd door de hoeveelheid eiwit in de voeding. Geconcludeerd werd dat een toename in de eiwitinname van 12 naar 21% van de totale energie geen negatieve effecten had op de calciumbalans van gezonde jonge en bejaarde personen.

Verder waren er geen verschillen tussen de leeftijdsgroepen met betrekking tot de interactie tussen eiwitinname en calciumexcretie, calciumabsorptie en calciumbalans.
De resultaten van het onderzoek naar het effect van de eiwitinname op het vitamine B6-metabolisme worden beschreven in hoofdstuk 8. Tijdens twee dieetperioden (waarin 12% of 21% van de totale energie geleverd werd door eiwit) waren de plasma pyridoxaalfosfaat-, de pyridoxaal- en de totale vitamine B6-concentratie significant lager voor de bejaarden in vergelijking met de jongvolwassenen. De pyridoxaalfosfaatconcentratie van de bejaarden was significant hoger tijdens het eiwitrijke dieet, terwijl het eiwitgehalte in de voeding geen effect had op de pyridoxaalfosfaatconcentratie van de jongeren. Voor beide leeftijdsgroepen gold dat de plasma pyridoxaal- en totale vitamine B6-concentratie niet beïnvloed werden door de hoeveelheid eiwit in de voeding. De relatieve pyridoxinezuurexcretie van de bejaarden veranderde niet significant tijdens de twee diëten, terwijl deze significant lager was voor de jongeren tijdens het eiwitrijke dieet in vergelijking met het dieet waarin 12% van de totale energie geleverd werd door eiwit. De resultaten van dit onderzoek suggereerden een leeftijdsgebonden verschil in de aan eiwitinname gerelateerde vitamine B6-behoefte, waarbij bejaarden ogenschijnlijk minder vitamine B6 nodig hadden tijdens een hoge eiwitinname in vergelijking met jongvolwassenen.

Abbreviations

ANOVA	analysis of variance
ADMR	average daily metabolic rate
AP	alkaline phosphatase
BMR	basal metabolic rate
BMI	body mass index
BW	body weight
CO_2	carbon dioxide
CV	coefficient of variation
d	day
diet A	diet with a protein content of 12% of total energy
diet B	diet with a protein content of 21% of total energy
DIT	diet induced thermogenesis
DR-group	dietary record group
DQ-group	dietary questionnaire group
EAST	erythrocyte aspartate aminotransferase
EE	energy expenditure
24h EE	24 hour energy expenditure
$EE_{0\ activity}$	energy expenditure in the inactive state
EE_{act}	energy expended on physical activity
EI	energy intake
GEI	gross energy intake
F	female
FM	fat mass
FFM	fat free mass
FFMI	fat free mass index
$^2H_2{}^{18}O$	doubly labelled water
^2H_2O	deuterium
J	joule
kg	kilogram
kJ	kilojoule
L	liter
m	meter
M	male
mg	milligram
ME	metabolizable energy
MJ	megajoule
µg	microgram
n	number
N	nitrogen
nmol	nanomol
O_2	oxygen

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Curriculum vitae

Daphne Pannemans werd geboren op 19 juli 1967 te Heerlen. In 1985 behaalde zij het Atheneum-B diploma aan het Grotius College te Heerlen. In datzelfde jaar begon zij haar studie Gezondheidswetenschappen, met als afstudeerrichting Biologische Gezondheidkunde aan de Rijksuniversiteit Limburg te Maastricht, een studie die zij in september 1989 voltooide. Per 1 april 1990 trad zij in dienst als assistent in opleiding bij de vakgroep Humane Biologie, alwaar het in dit proefschrift beschreven onderzoek werd uitgevoerd (prof. dr. ir. W.H.M. Saris, prof. dr. D. Halliday, dr. K.R. Westerterp, dr. ir. G. Schaafsma). Vanaf 1 mei 1994 is zij werkzaam als wetenschappelijk medewerker binnen een samenwerkingsverband tussen de Rijksuniversiteit Limburg (vakgroep Humane Biologie) en de afdeling Humane Voeding van TNO-Toxicologie en Voeding te Zeist.

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