Evaluation of dietary reference values for protein

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Background document to:
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01
introduction

This report serves as the background document for the advisory report *Dietary reference values for protein* (in Dutch: *Voedingsnormen voor eiwitten – referentiewaarden voor de inname van eiwitten*), which has been prepared by the Committee on Nutrition of the Health Council of the Netherlands (HCNL). In the advisory report, the Committee evaluated whether the dietary reference values (DRV) for protein for different age groups set by the European Food Safety Authority (EFSA) in 2012[1] could be adopted by The Netherlands.

In this background document, the EFSA's DRVs for protein are presented and discussed, in combination with the current Dutch DRVs for protein of the Health Council of the Netherlands,[2] and the DRVs of the Nordic countries, The German-speaking countries (Deutschland [Germany], Austria, and Confœderatio Helvetica [Switzerland]; DACH), and the World Health Organization (WHO)/Food and Agriculture Organization (FAO)/ United Nations University (UNU).

02 background

This chapter describes background concepts on protein metabolism, mainly based on the reports of EFSA and the WHO. See also Annex A for more details. Dietary proteins are the body's source of nitrogen and indispensable amino acids. Protein metabolism comprises the processes that regulate protein digestion, amino acid metabolism and body protein turnover. These processes include the absorption and supply of both dispensable and indispensable dietary amino acids and the *de novo* synthesis of dispensable amino acids, protein hydrolysis, protein synthesis and amino acid utilisation in catabolic pathways or as precursors of nitrogenous compounds.[1]

The requirement of protein is determined by needs for maintenance (to compensate for protein losses through faeces, urine, sweat) and growth, pregnancy and lactation. The 'obligatory nitrogen loss' is the ongoing loss of nitrogen (N) from the body when dietary intake of nitrogen is zero, and energy and all other nutrients are consumed in adequate amounts. The proportion of nitrogen intake that is retained in the body is called the net protein utilisation (NPU). NPU values are true or apparent, depending on whether the loss of endogenous nitrogen is taken into account or not. NPU refers to the efficiency of conversion from dietary protein to body protein.[3] Based on available data in healthy adults at maintenance the mean optimal NPU value determined as 'net post-prandial protein utilisation' (NPPU) is 70%. This NPPU approach represents the maximal potential NPU efficiency of the dietary protein sources when determined in optimised controlled conditions in healthy adults, and it can be modified by different factors including food matrix, diet and physiological conditions. The usual NPU value as determined from N-balance studies in adults is approximately 47%.[1] N-balance studies involve subjects fed different levels of protein until they attain nitrogen equilibrium, i.e. when intake = loss, and balance = 0. Often a linear regression is used so that the intake for nitrogen equilibrium (the requirement) is defined by an intercept (the nitrogen loss at zero intake) and a slope. The intercept is an estimate of metabolic demands, i.e. the obligatory nitrogen losses. The slope indicates the efficiency of dietary protein utilisation.[3]

The minimum protein requirement is the level of protein intake that is adequate to enable N-balance (in the short and long term). In practice, measurements of the minimum protein requirement have varied widely within and between individuals. For this reason, identifying the minimum protein requirement is inherently difficult.[3] Both in the diet and the body, 95% of the nitrogen is found in the form of protein, and 5% is found in the form of other nitrogenous compounds (non-protein nitrogen; NPN), i.e. free amino acids, urea or nucleotides. A conversion factor of 6.25 is usually applied for the conversion of nitrogen to protein. Total N x 6.25 is called crude protein and [total minus non-protein-N] x 6.25 is called true protein. Unless specifically noted otherwise, "protein" is total N x 6.25 and protein requirements are calculated from nitrogen content.[1]

Of the 20 proteogenic dietary amino acids, nine (proteogenic) amino acids are classified as indispensable in humans (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) as they cannot be synthesised in the human body from naturally occurring precursors at a rate to meet the metabolic requirement. Among the nine indispensable amino acids, lysine and threonine are strictly indispensable since they are not transaminated, and their deamination is irreversible.
In contrast, the seven other indispensable amino acids can participate in transamination reactions. Eleven amino acids are dispensable (alanine, arginine, cysteine, glutamine, glycine, proline, tyrosine, aspartic acid, asparagine, glutamic acid, and serine). This means that these amino acids can, under normal physiological conditions, be synthesised in the body. Some dispensable amino acids can become limiting under special physiological or pathological conditions, such as in premature neonates when the metabolic requirement cannot be met unless these amino acids are supplied in adequate amounts with the diet; they are then called conditionally indispensable amino acids (arginine, cysteine, glutamine, glycine, proline, tyrosine).[1,4,8]

Protein quality refers to the capacity of a protein source to meet both the requirement for nitrogen and the requirement for indispensable amino acids as limiting precursors for body protein synthesis. A score used to determine protein quality is the protein digestibility-corrected amino acid score (PDCAAS). PRIs for protein are based on high-quality protein and protein from mixed diets / PDCAAS=100%.[5]

03 methods

The EFSA established DRVs for protein in 2012.[1] DRVs for indispensable amino acids were not derived since amino acids are not provided as individual nutrients, but in the form of protein. In addition, the EFSA Panel noted that more data was needed to obtain sufficiently precise values for indispensable amino acid requirements. Since 2012, more reports on protein DRVs of multiple countries have been published. In 2014, the Nordic Council of Ministers (NCM) published its report on DRVs and dietary guidelines for the Nordic countries.[6] For protein, the recommendations were based on three systematic reviews (SR): for children, adults and older adults.[7-9] The DACH countries published new DRVs for protein in 2017.[10,11]

In this background document, the EFSA's DRVs for protein are presented and discussed, in combination with the current Dutch DRVs for protein of the HCNL,[2] and the DRVs of the NCM 2012[6] and DACH.[10,11] In addition, the reports on protein and amino acid requirements of the WHO/FAO/UNU (2007) are part of our evaluation, because they traditionally have been, and still are, the basis for many other international reports on protein requirements.[3,5] Other international reports from multiple countries (IOM, Australia/New Zealand, United Kingdom) are older than the WHO/FAO/UNU 2007 report and were therefore not included in the current evaluation.[4,12,13]

For the purpose of harmonisation, the Committee earlier decided to adopt EFSA's terminology, definitions, and age categorisation (see also Annexes B and C).[14,15] In case of major objections against the EFSA's reference values or approach, the Committee has derived alternative values.

Literature update for older adults

The abovementioned reports by HCNL, EFSA, WHO/FAO/UNU, DACH and NCM were the point of departure for updating the Dutch reference values for protein. The Committee did not carry out a systematic update of the literature, with one exception. In recent years, a number of scientific publications, not included in the EFSA report, appeared, discussing whether or not the reference values for proteins for older adults should be higher than for younger adults.[16-22] EFSA's protein requirement for older adults is similar to that of younger adults, whereas the Nordic and German-speaking countries have set higher values for older adults as compared with younger adults.[6,10,11] For the current evaluation of the HCNL DRVs for protein, no scientific reports were available based on sufficiently recent literature or with a sufficiently transparent approach. Therefore, the Committee performed a SR of randomised controlled trials (RCT) in older adults (on average at least 65 years of age) on the effects increasing protein intake has on health outcomes: i.e. muscle mass, muscle strength, physical function, bone health, blood pressure, parameters of glucose and insulin metabolism, blood lipids, kidney

function, and cognitive function. The Committee differentiated between trials with and without additional physical exercise (mostly resistance training); the Committee evaluated 1) RCTs with additional protein compared with no additional protein as well as 2) RCTs with additional protein + additional physical exercise compared with additional exercise alone. A comprehensive overview of methods, results, and conclusions is provided in a separate background document, see background document *Systematic review of health effects of dietary protein in older adults*.
With regard to N-balance, the Committee also checked for recent studies in older adults that were not included in the EFSA report.

04
dietary reference values for adults

4.1 Overview of dietary reference values for adults 18-59 years

Table 1 shows the various existing DRVs for protein for adults and the criteria on which these values are based. In all reports, the protein requirements for adults are based on nitrogen balance (N-balance) data. Age categories differ between the reports, also depending on whether or not different recommendations have been provided for (healthy) older adults. As reference weights for different populations differ, this logically results in differences in the requirements expressed in grams per day between the reports.

The HCNL 2001 report adopted the approach of FAO/WHO/UNU 1985,[23] but applied a coefficient of variation (CV) of 15% (instead of 12.5%) to allow for individual variability, resulting in a recommended intake of 0.8 gram per kilogram (g/kg) body weight per day.[2] The DRVs of WHO/FAO/UNU (2007)[3] are based on a re-evaluation of their recommendations from 1985.[23] Based on a meta-analysis of N-balance studies in healthy adults by Rand et al. (2003),[24] which involved studies stratified for a number of subpopulations, settings in different climates, sex, age and protein source, a population average requirement (AR) for protein of 0.66 g/kg body weight per day (105 mg nitrogen/kg body weight/day) resulted as the best estimate for (minimal) protein requirement. The 'safe level of intake' was identified as the 97.5th percentile of the population distribution of protein requirement, which was equivalent to 0.83 g/kg body weight per day (132 mg nitrogen/kg/d).[3] The requirement implies high-quality protein (PDCAAS=1). This value can be applied to common mixed diets in Europe which are unlikely to be limiting in their content of indispensable amino acids. No significant differences between the climate of the study site, adult age class, sex, or source of dietary protein were observed, although there was an indication that women might have a lower requirement than men.[24] This meta-analysis has been the basis for protein recommendations for adults worldwide ever since. Apart from an AR and PRI, it provided a variation coefficient (VC; 12%) and a value for efficiency of dietary protein utilisation for maintenance (47%). Earlier estimations by the WHO/FAO/UNU[23] were 70% for the efficiency of dietary protein utilisation and 12.5% for the VC.

Although the AR and PRI (in g/kg/d) of the HCNL from 2001 (0.6 and 0.8 mg protein/kg/d, consecutively) are very similar to the current AR and PRI estimates in more recent reports, the underlying values for the derivation differ as described above. The advisory reports by EFSA, WHO/FAO/UNU, NCM and DACH all based their protein requirements for adults on the meta-analysis of N-balance studies by Rand et al. (2003).[24] DACH used, in addition to the meta-analysis of Rand et al., a more recent meta-analysis by Li et al., which supported the findings of Rand et al.[25] Differences between the advisory reports are a consequence of different reference weights, and of calculations based on energy percentages (NCM).

4.2 Conclusion for adults 18-59 years of age

The Committee agreed with EFSA's DRVs for high-quality protein and protein from mixed diets for healthy adults (18-60 years of age) and therefore adopts EFSA's AR for adults of 0.66 g protein/kg body weight per day and EFSA's PRI of 0.83 g protein/kg body weight per day.

4.3 Overview of dietary reference values for older adults (60 years and older)

In all evaluated DRV reports, protein requirements for older adults are primarily based on N-balance data (see Table 1). In the HCNL 2001 report, protein requirements for older adults were derived in a similar way compared with younger adults; no additional allowance was considered necessary for adults aged >70 years.[2] WHO/FAO/UNU 2007 also concluded that the available data (based on N-balance) did not provide convincing evidence that the protein requirement of older adults (per kg body weight, age category of 'older adults' not specified) differs from the protein requirements of younger adults.

EFSA separately evaluated older adults.[1] EFSA concluded, based on N-balance data, that data were insufficient to establish that the requirement for (healthy) older adults was different from that of (healthy) younger adults, primarily based on the meta-analysis of Rand et al.[24] Following this meta-analysis, one more recent short-term N-balance study did not show differences between younger (21-46 years) and older (63-81 years) subjects after short-term assessment of N-balance.[26] Evaluated other health outcomes in relation to the PRI were: muscle mass and function, bodyweight control and obesity, insulin sensitivity and glucose control, and bone health. In relation to the upper level (UL) the following outcomes were evaluated: insulin sensitivity and glucose control, bone health, kidney function, capacity of the urea cycle, and tolerance of protein. However, the data was not considered sufficient for any of these functional outcomes to derive a PRI or UL. EFSA concluded that the available data were insufficient to specifically determine the protein requirement in older adults and that at least the same level of protein intake as for young adults is required for older adults. EFSA also noted that, as sedentary older adults have a lower energy requirement, the protein to energy ratio of this subgroup is higher than for younger adults.

Also NCM[6] and DACH[11] evaluated the health effects of additional protein in addition to N-balance data to derive a PRI for older adults. For the NCM 2012 advisory report, an SR[8] was performed including publications from 2000 until December 2011 regarding the health effects of food-based protein intake in healthy elderly populations (mean age of ≥65 years) in settings similar to the Nordic countries (studies with disabled/frail elderly were excluded). The evaluation included a quality assessment with a grading of the individual studies based on guidelines developed by the NNR working group.[27] Included study designs were prospective cohort studies, case-control studies, and intervention studies. The grade of evidence was classified as convincing, probable, suggestive, or inconclusive. The evidence was assessed as probable for an AR of 0.66 g protein/kg body weight/day based on N-balance studies (a value similar to younger adults). Associations on protein intake in relation to both muscle mass (protein 13-20 en% (energy per cent)) and bone mineral density were assessed as suggestive. For the following outcomes, the evidence base was deemed inconclusive: bone loss, risk of fractures, risk of falls, muscle mass or body composition in combination with resistance training, blood pressure, frailty and mortality. In the SR, the following was concluded: “Still, adequate enough data do not exist to estimate an optimal intake of protein based on the main physiological end points in the elderly.” The authors’ overall impression was that “the optimal protein intake may be higher than the estimated RDA assessed from N-balance studies, whereas an exact level cannot be determined.” Regarding harmful effects of a high protein intake, the evidence was considered as inconclusive: “It cannot be ruled out that a high protein intake corresponding to approximately 24 en% or 2 g protein/kg BW/day may affect kidney function negatively among older adults.” In the final advisory report,[6] a protein intake corresponding to 15-20 en% was recommended (as opposed to a range of 10-20 en% for younger adults), corresponding to approximately 1.1 to 1.3 g protein/kg body weight/day provided by a physical activity level (PAL) of 1.6 (average physical activity) for 15 energy per cent (en%) and a PAL of 1.4 (sedentary lifestyle) for 20 en%, respectively. This recommendation was derived from data from N-balance studies in relation to maintenance of muscle mass (supported by some prospective cohort studies and by suggestive health effects). NCM stated that in relation to the age-related decrease in energy intake, a diet with a protein content in the range of 10–14 E% might not sufficiently cover the need for protein in absolute amounts.

The DACH countries[10,11] based their recommendations for older adults (>65 years of age) on meta-analyses of N-balance studies[24,26] and two additional N-balance studies[28,29] that were excluded from the meta-analyses due to methodological limitations. In addition, they conducted a structured literature search in PubMed considering publications (in English and German) between 2000 and 2017 reporting results of original human studies, meta-analyses, and SRs (including cross-sectional studies) focusing on protein or amino acid requirements and metabolic and functional parameters. The results were presented per study. Judgements of study quality or the method of weighing of the totality of evidence were, however, not provided. The DACH countries set a higher reference value (AI=1.0 mg/kg body weight per day) for older adults (>65 years of age).

The current HCNL Committee on Nutrition judged that for older adults, there was a need to add recent literature to the advisory report of EFSA 2012. In recent years, several guidelines of international organisations were published regarding older adults, such as ESPEN 2014[30] and 2019,[21] ESCEO 2014[31] and PROT-AGE 2013,[20] which were considered as a starting point for the literature update. ESPEN 2019 recommended (GRADE evidence judged as 'sufficient') the following protein intake for older adults >65 years: "Protein intake in older persons should be at least 1 g/kg BW/day. The amount should be individually adjusted for nutritional status, physical activity level, disease status and tolerance." This guideline was based on a systematic literature search and included publications until July 2016. The included publications were 1) a position paper by PROT-AGE (2013),[20] 2) a review by ESPEN (2014) with regard to protein in relation to muscle function,[30] and 3) a consensus statement by the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO 2014).[31] Because the Committee judged the available reviews as not sufficiently transparent with regard to followed methodology[11,20,21,30,31] or not sufficiently recent,[8] the Committee systematically searched for SRs of randomised controlled trials (RCTs) and prospective cohort studies on protein intake and health outcomes in older adults. Four SRs including prospective cohort studies were identified.[8,32-34] In the SR by Pedersen et al, the basis of the Nordic recommendations of 2014 regarding older adults, prospective cohort studies, case-control studies, and intervention studies (published from 2000 until December 2011) were combined and graded together. This SR was judged not sufficiently recent anymore (i.e. not much more up to date than the EFSA 2012 report). The SR by Groenendijk et al[32] regarding bone health was based on 12 prospective cohort studies and one RCT. This SRs could not be used, because the protein intake levels of the studies were not sufficiently specific in relation to the levels of the recommended protein intake and the quality of the included studies was limited. The SRs of Coelho-Junior et al only included three prospective cohort studies each, with similar methodological problems.[33,34] Additionally, many of the included studies scored high for risk of bias. Based on the mentioned limitations of cohort studies, especially in relation to the derivation of recommended intakes, the Committee decided to perform an SR of randomised controlled trials (RCTs).

The Committee also performed a literature search for N-balance studies published after the meta-analysis by Rand et al.[24] The Committee searched in PubMed for studies citing the meta-analysis by Rand et al. In addition, the Committee reviewed multiple (recent) expert opinion papers[20,30,35,36] to identify recently performed N-balance studies among older adults. The Committee did not find additional, more recent studies on N-balance compared with the EFSA 2012 report.

Table 2 presents a summary of the SR's conclusions on health outcomes. The average protein consumption in the control groups of the included RCT's was at least 0.8 gram per kilogram of bodyweight (the PRI of adults based on N-balance studies). No probable or convincing beneficial effects of additional protein intake were observed for any of the outcomes. Nevertheless, the Committee considers it possible that increased protein intake, with isocaloric replacement (usually for carbohydrates), has a beneficial effect on body composition (more lean body mass, less fat mass), that does not involve any change in body weight. The Committee also considers it possible that increased protein intake with concomitant physical exercise (resistance exercise training) has a beneficial effect on muscle strength compared with physical exercise alone. For the other outcomes, the evidence was ambiguous, too limited to draw a conclusion, or an effect was unlikely. Altogether, the Committee concluded that the evidence was insufficient to derive a higher recommended protein intake for older adults. In addition, the evidence to study potential adverse effects (e.g. on kidney function) was also limited. For a comprehensive overview of methods, results, and conclusions of the SR on health outcomes, we refer to background document 'Systematic review of health effects of dietary protein in older adults'.

Table 1. Overview of the dietary reference values for protein for adults and older adults and the criteria on which these values are based

Report	Age range, y	AR N maintenance, mg/kg/d	AR protein, g/kg/d	PRI/RDA protein, g/kg/d	Ref weights, kg		AR protein, g/d		PRI protein, g/d		PRI protein, E%		Method of derivation
					♂	♀	♂	♀	♂	♀	♂	♀	
EFSA 2012[1]	[18-59]	105	0.66	0.83	74.6	62.1			62	52			Method of derivation adopted from WHO/FAO/UNU 2007
=WHO/FAO/UNU 2007[3]	≥60	105	0.66	0.83	73.5	66.1			61	55			*PRI refers to high-quality protein and European mixed diets (PDCAAS=1)
WHO/FAO/UNU 2007[3]	>18	105	0.66	0.83	40				33				*N-balance studies: meta-analysis of Rand 2003[24]
				('safe level of	45				37				*Efficiency of utilisation=47% (NPU)
				intake')	50				42				*VC=12%
					55				46				*'Safe level of intake' refers to high-quality protein (PDCAAS=1)
					60				50				
					65				54				
					70				58				
					75				62				
					80				66				
HCNL 2001[2]	[19-30]	70	0.6	0.8	75	64	47	40	61	52	8	9	Method of derivation adopted from FAO/WHO/UNU 1985[23]
	[31-50]	70	0.6	0.8	72	62	45	39	59	50	8	9	*N-balance studies
	[51-70]	70	0.6	0.8	74	64	46	40	60	52	9	10	*Efficiency of utilisation=70% (NPPU)
	>70	70	0.6	0.8	74	63	46	39	60	51	11	11	*VC=15% (instead of 12.5%[23])
													*PRI refers to high-quality protein and Dutch mixed diets (PDCAAS=1), PRI*1.2 (PDCAAS=84%) for lacto-ovo vegetarian diets, PRI*1.3 (PDCAAS=77%) for vegan diets
NCM 2012[6,8,9]	18-64	105	0.66	0.83	-	-			-	-	10-20		SR[9], data on N-balance adopted from the meta-analysis by Rand 2003[24]
													*Efficiency of utilisation (NPU)=47%
													*VC=12%
	≥65	105		1.1-1.3 (1.2)[a]							15-20 (18)		N-balance[24] + maintenance of muscle mass[8,9]
DACH 2017[11]	[19-25>	105	0.66	0.8	70.8	60.5			57	48			N-balance: 2 meta-analyses (Rand 2003, and Li 2014)[24,25]
	[25-51>		0.66	0.8	70.7	60.0			57	48			resulting in the same AR for N (AR for N = 105 mg N/kg/d)
	[51-65>		0.66	0.8	68.7	58.2			55	47			*Efficiency of utilisation (NPU)=47%
													*VC=12%
	≥65			1.0 (AI)	66.8	57.1			67	57			N balance[24,26] + metabolic and functional parameters

Abbreviations: AI: adequate intake, AR: average requirement, d: day, DACH: German-speaking countries Germany, Austria and Switzerland, E%: percentage of total energy, EFSA: European Food Safety Authority, g: gram, HCNL: Health Council of the Netherlands, N: nitrogen, NCM: Nordic Council of Ministers, NNPU: net post-prandial protein utilisation, NPU: net protein utilisation, PDCAAS: protein digestibility-corrected amino acid score, PRI: population reference intake, RDA: recommended dietary allowance, VC: variation coefficient.

4.4 Conclusion for older adults (60 years and older)

The Committee judged that the evidence was insufficient to recommend a higher PRI for protein for healthy older adults compared with younger adults, based on a literature update on N-balance studies and on health outcomes. The Committee concluded that the PRI of younger adults is also valid for older adults. The Committee agreed with EFSA's DRVs for high-quality protein and protein from mixed diets for healthy adults over 18 years of age and therefore estimates the AR of adults to be 0.66 g protein/kg body weight/day and the PRI of adults to be 0.83 g protein/kg bodyweight/day.

Table 2. Overview of the conclusions on health outcomes of protein intake in older adults[a]

Health outcome	Conclusion, number of RCTs and further details
Lean body mass (in combination with a stable body weight).	Possible beneficial effect (7 out of 18 RCTs reported at least one beneficial effect) No indications for differential effect of protein with or without concomitant physical exercise compared with no additional protein.
Muscle strength, with concomitant physical exercise compared with physical exercise alone	Possible beneficial effect (3 out of 8 RCTs reported at least one beneficial effect)
Muscle strength, protein alone (i.e. without concomitant physical exercise in both intervention and control group)	Likely no effect (7 RCTs)
Bone health	Likely no effect (7 RCTs)
Physical functioning	Likely no effect (12 RCTs)
Kidney function	Too few studies (6 RCTs but without appropriate outcome measures)
Cognitive function	Too few studies (1 RCT)
Blood pressure	Too few studies (4 RCTs, but with limited power)
Glucose and insulin metabolism	Too few studies (6 RCTs, but with limited power)
Blood lipids	Effect unclear (7 RCTs) Beneficial as well as adverse effects were observed, and for different lipid measures.

Abbreviations: d: day, g: gram, kg: kilogram, RCT: randomized controlled trial.
Footnotes:
[a] The average protein consumption in the control groups of the included RCT's was at least 0.8 gram per kilogram body weight (the PRI of adults based on N-balance studies).
[b] Total protein intake is the sum of habitual protein intake and the protein dose of the RCT (intervention group).

05 dietary reference values for pregnant women

5.1 Overview of dietary reference values for pregnant women

Table 3 shows the DRVs for protein for pregnant women from the various advisory reports and the criteria on which these DRVs are based. Requirements for pregnant women are all based on the factorial model, i.e. the additional protein requirement for newly deposited protein and increased maintenance costs (associated with increased bodyweight). The derivation of the amount of protein needed for maintenance is assumed similar to that for non-pregnant women (i.e. based on N-balance studies).

HCNL adopted the protein requirements for maintenance for pregnant women from the WHO 1985 advisory report.[23] An additional requirement was estimated to be 8 mg nitrogen/kg body weight per day, based on protein deposition in the foetus, placenta, and breast tissue of 0.9 kg during pregnancy with an efficiency of protein utilisation of 70%.[23] Requirements were not stratified by trimester of the pregnancy.

EFSA and NCM both used the method of derivation of the WHO/FAO/UNU 2007,[3] but EFSA assumed a higher (47%) value for the efficiency of protein utilisation (for deposition of protein in the foetus and maternal tissue) than used by NCM and WHO/FAO/UNU 2007 (42%). The value of 42% was based on a N-balance study in 10 primiparous teenagers during the last 100 days of pregnancy.[37] EFSA assumed the efficiency of protein utilisation in pregnant women to be at least equally efficient compared with non-pregnant women and used the value of 47% (similar to non-pregnant women). DACH's DRVs are lower than EFSA's, because DACH used a lower gestational weight gain (12.0 kg compared with 13.8 kg) and a concurrently lower value of protein deposition (597 compared with 686 gram).

The Committee preferred to use a gestational weight gain of 13.8 kilograms. This is consistent with the parallel advisory report of the Health Council on 'Dietary guidelines for pregnant women' (in preparation) and supported by a recent individual participant-level meta-analysis using data from 196,670 participants from 25 cohort studies from Europe and North America.[38,39] It is worth noting that the optimal range of gestational weight gain depends on the pre-pregnancy body mass index, with a higher optimal gestational weight gain in case of underweight before pregnancy and a lower optimal gestational weight gain for a higher pre-pregnancy body weight.[38] In conclusion, the Committee agreed with the method of derivation of the protein requirement (high-quality protein and protein from mixed diets) during pregnancy used by EFSA.

5.2 Conclusion for pregnant women

The Committee agreed with the method of derivation of the protein requirement (high-quality protein and protein from mixed diets) during pregnancy used by EFSA, resulting in recommended additional protein intakes of 1 g/d, 9 g/d, and 28 g/d for trimester 1, 2, and 3, respectively.

Table 3. Overview of the dietary reference values for pregnant women and the criteria on which these values are based

Report	Trimester	Ref. weight / weight gain, kg	AR N, mg/kg/d	AR protein for maintenance, g/d	AR protein for growth,[a] g/d	AR protein, g/kg/d	PRI protein, g/kg/d	AR protein, g/d	PRI protein, g/d	Method of derivation
EFSA 2012[1]	1 2 3	+0.8[b] +4.8[b] +11[b]		+0.5 +3.2 +7.3	+0[c] +4.0[c] +15.7[c]			+0.5 +7.2 +23	+1 +9 +28	**Factorial model** largely based on WHO/FAO/UNU 2007[3] *Efficiency of utilisation similar to non-pregnant women=47%.
HCNL 2001[2]	N/A	68	M:70 G: 8			0.7	0.9	47	62 (=+10)[d]	**Factorial model** based on FAO/WHO/UNU[23] Maintenance: N for maintenance similar to non-pregnant women (N-balance studies).[23] *Efficiency of utilisation similar to non-pregnant women=70%.[23] *VC=15% Growth: *Efficiency of utilisation=70%. Total average need of protein for foetus, placenta and mammal tissue: 0.9 kg protein = 5 g protein/d = 8 mg/kg/d N.[23]
WHO/FAO/ UNU 2007[3]	1 2 3	+0.8[b] +4.8[b] +11[b]		+0.5 +3.2 +7.3	+0[e] +4.5[e] +17.7[e]			+0.5 +7.7 +24.9	+0.7 +9.6 +31.2	**Factorial model** Maintenance: Based on maintenance costs for increased body weight (mid-trimester weight gain, adult maintenance value of 0.66 mg/kg/d)[3] *Efficiency of utilisation similar to non-pregnant women=47%. Growth: Newly deposited protein (686 g) estimated by total body K accretion. *Average weight gain=13.8 kg *Efficiency of N utilisation=42%. *VC=12%
NCM 2012[6] [8,9]	1 2 3	+0.8[b] +4.8[b] +11[b]		+0.5 +3.2 +7.3	+0[e] +4.5[e] +17.7[e]			+0.5 +7.7 +24.9	+0.7 +9.6 +31.2	**Factorial model** based on WHO/FAO/UNU 2007[3] Maintenance: *Based on maintenance costs for increased body weight (mid-trimester increase, 0.66 mg/kg/d); *Efficiency of utilisation similar to non-pregnant women=47%. Growth Newly deposited protein (686 g) estimated by total body K accretion and an average weight gain of 13.8 kg *Efficiency of N utilisation for growth=42% *VC=12%

Report	Trimester	Ref. weight / weight gain, kg	AR N, mg/kg/d	AR protein for maintenance, g/d	AR protein for growth,[a] g/d	AR protein, g/kg/d	PRI protein, g/kg/d	AR protein, g/d	PRI protein, g/d	Method of derivation
DACH 2017[11]	1			+0.4	+0[c]		0.8 (+0)[f]	+0	+0[f]	**Factorial model**
	2	64.6		+2.7	+2.8[c]		0.9 (+0.1)	+5.5[g]	+7	Maintenance:
	3	70.0		+6.3	+10.9[c]		1.0 (+0.2)	+17.1[g]	+21	*Based on maintenance costs for increased body weight (11% of 12 kg in 1st trimester, 47% of 12 kg in the 2nd trimester, and 42% of 12 kg in the 3rd trimester, and maintenance value of 0.66 mg/kg/d) *Efficiency of protein utilisation similar to non-pregnant women (47%). Growth: Total protein deposition of 597 g for foetus, placenta and mammal tissue = 1.3 g/d (20%) in the 2nd trimester and 5.1 g/d (80%) 3rd trimester; *Average weight gain: 12 kg (11% in 1st, 47% in 2nd, and 42% in 3rd trimester) *Efficiency of protein utilisation=47%. *VC=12%

Abbreviations: AI: adequate intake, AR: average requirement, d: day, DACH: German-speaking countries Germany, Austria and Switzerland, E%: percentage of total energy, EFSA: European Food Safety Authority, G: growth, HCNL: Health Council of the Netherlands, K: potassium, kg: kilogram, M: maintenance, NCM: Nordic Council of Ministers, N: nitrogen, PRI: population reference intake, RDA: recommended dietary allowance, VC: variation coefficient.

Footnotes:

[a] protein needed for the pregnancy.
[b] mid-trimester weight gain.
[c] adjusted for efficiency (47%).
[d] compared with non-pregnant women 19-30 years.
[e] adjusted for efficiency (42%).
[f] the calculated amount of 0.4 is neglected.
[g] compared with mean body weight of non-pregnant women 19-25 years.

06
dietary reference values for lactating women

6.1 Overview of dietary reference values for lactating women

Table 4 shows the various DRVs for protein for lactating women and the criteria on which these values are based. Requirements for lactating women are all based on the factorial model, i.e. the additional protein requirement needed for excretion of breast milk added to the protein needed for maintenance based on N-balance data.

The protein requirements of HCNL 2001 for protein maintenance of lactating women were similar to non-lactating women (based on WHO 1985.[23]) The protein requirements for breast milk production were based on Fomon 1993.[40]

EFSA and NCM both adopted the more recent method of WHO/FAO/UNU 2007,[3] based on maintenance requirements similar to adults (Rand et al., 2003[24]), and requirements for breast milk production based on a WHO report from 2002.[41] Mean production rates of milk produced by well-nourished women exclusively breastfeeding their infants during the first six months postpartum and partially breastfeeding their infants in the second six months postpartum[41] were used together with the mean concentrations of protein in human milk to calculate mean equivalent milk protein output.[3] WHO/FAO/UNU 2007 assumed that the increased nitrogen needs of the lactating woman should cover protein nitrogen, but not the non-protein nitrogen.[3] An additional protein intake of 19 g/day was recommended for the first six months postpartum and an additional protein intake of 13 g/day for the second six months postpartum.

DACH 2017[11] based their protein requirements for breast milk production on an SR on protein content of breast milk (2014). In addition to the protein content of human breast milk[42] (estimated as 1.0 g true protein per 100 millilitre (mL) breast milk), the authors considered the NPN in breast milk to be 25%. They further assumed that 50% of the nitrogen in NPN is newly synthesised and must therefore be added to the protein requirement. The following calculation was provided after personal communication with the authors: Protein content of mature breast milk: 1.0 g/100 mL is equal to 75% of the total nitrogen content of breast milk. Therefore, 1% of the total nitrogen of breast milk is 1.0/75 = 0.013 g/100 mL. If half of the NPN is newly synthesised nitrogen and is required as well, then 12.5% of total nitrogen must be added: 0.013x12.5 = 0.166 per 100 mL. The total is: 1.0 + 0.166 = 1.17 g/100 mL. A breast milk consumption of 750 mL was applied, resulting in an additional protein requirement of 23 g/d. This additional protein requirement is much higher than that of EFSA (WHO/FAO/UNU), which was, after six months, based on the assumption that the amount of breast milk decreases after six months postpartum, when babies are partially rather than exclusively breastfed. The Committee preferred the calculations of WHO/FAO/UNU based on separate calculations for the first six months and the second six months.

In conclusion, the Committee agreed with the method of derivation of the protein requirement (high-quality protein and protein from mixed diets) during pregnancy used by EFSA.

6.2 Conclusion for lactating women

The Committee agreed with the method of derivation of the protein requirement (high-quality protein and protein from mixed diets) during lactation used by EFSA, resulting in a recommended additional protein intake of 19 g/day for 0-6 months postpartum and 13 g/day for >6 months postpartum.

Table 4. Overview of the reference values for lactating women and the criteria on which these values are based

Report	Age of baby	Reference weight, kg	AR N, g/kg/d	AR protein for growth[a] adjusted for efficiency, g/d	AR protein, g/kg/d	PRI protein, g/kg/d	AR protein, g/d	PRI protein, g/d	Method of derivation
EFSA 2012[1]	0-6 mo ≥6 mo							+19 +13	**Factorial model** adopted from WHO/FAO/UNU 2007[3]
HCNL 2001[2]	Not specified	64	70(M)+18(G)= 88		0.8	1.0	50	65= 52+13	**Factorial model** Maintenance: N for maintenance similar to non-pregnant women (N-balance studies, adopted from FAO/WHO/UNU 1985.[23] Milk production: Average protein excretion in breast milk based on Fomon 1993 = 7 g/d[40] and additional N requirement=18 mg/kg/d; *Efficiency of N utilisation for both maintenance and growth 70% (NPPU). *VC=12.5%
WHO/FAO/ UNU 2007[3]	0-6 mo			1 mo pp: 16.2 2 mo pp: 15.6 3 mo pp: 14.8 4 mo pp: 14.3 5 mo pp: 14.4 6 mo pp: 15.5				+19	**Factorial model** Maintenance: N for maintenance similar to non-pregnant non-lactating women. Milk production: *Breast milk intake[41] and the protein concentration of breast milk[3] *Efficiency of N utilisation=47%. *VC=12.5%[b]
	≥6 mo			6-12 mo pp: 10.0				+13	
NCM 2012[6,8,9]	0-6 mo 6-12 mo			14.3-16.2 10				+18-20 +12.5	**Factorial model** adopted from WHO/FAO/UNU 2007[3]

Report	Age of baby	Reference weight, kg	AR N, g/kg/d	AR protein for growth[a] adjusted for efficiency, g/d	AR protein, g/kg/d	PRI protein, g/kg/d	AR protein, g/d	PRI protein, g/d	Method of derivation
DACH 2017[11]	Not specified	60.5		18.6		1.2= 0.8+0.4		71= 48+23	**Factorial method** Maintenance: N for maintenance similar to non-pregnant non-lactating women. Milk production: Excretion of 8.8 g protein/d based on excretion of 750 mL breast milk[43] with protein content of 1.17 g/100 mL[42] *Efficiency of N utilisation=47%. *VC=12%

Abbreviations: AI: adequate intake, AR: average requirement, d: day, DACH: German-speaking countries Germany, Austria and Switzerland, E%: percentage of total energy, EFSA: European Food Safety Authority, g: gram, G: growth (=milk production), HCNL: Health Council of the Netherlands, kg: kilogram, M: maintenance, mL: millilitre, N: nitrogen, NCM: Nordic Council of Ministers, NPPU: net post-prandial protein utilisation, pp: postpartum, PRI: population reference intake, RDA: recommended dietary allowance, VC: variation coefficient.

Footnotes:

[a] protein needed for breast milk production.

[b] The WHO/FAO/UNU calculations[3] state that a variation coefficient of 12% was used to derive the PRI. However, the Committee's recalculations suggest that a variation coefficient of 12.5% was used instead.

07
dietary reference values for infants and children

7.1 Overview of dietary reference values for infants up to six months of age

Table 5 shows the existing DRVs for protein for infants up to six months and the criteria on which these values are based. HCNL 2001[2] derived an adequate intake for fully formula-fed infants, and concluded that adequate intakes for fully breastfed infants are equal to the average protein intake through breast milk. Compared with HCNL 2001, DACH 2017[11] used more recent numbers for the composition of breast milk to derive DRVs. WHO/FAO/UNU 2007 and EFSA did not derive reference values for protein for infants below six months given that requirements will refer to amounts of nutrients provided by breast milk.[1,44] Also NCM does not provide DRVs for infants up to six months either; infants are either breastfed (protein content is considered adequate) or formula-fed (regulated by EC legislation). The Committee agreed with EFSA to not derive DRVs for infants up to 6 months of age.

The composition of infant formula

The composition of infant and follow-on formula is regulated by the European Union.[45] The underlying scientific data is based on the latest scientific advice of EFSA in its opinion on the essential composition of infant and follow-on formulae.[46] The values for protein used in the EFSA report on infant and follow-on formulae have been adopted from the EFSA report on nutrient requirements and dietary intakes of infants and young children in the European Union.[47] In this latter EFSA report, the Panel provides advice on the levels of nutrients which it considered adequate for healthy, term, normal-weight infants and young children, explicitly mentioning that no DRVs were derived.[47]

7.2 Conclusion for infants up to six months

The Committee agreed with EFSA to not derive DRVs for infants up to six months of age for similar reasons as EFSA.

Table 5. Overview of the reference values for infants up to 6 months of age and the criteria on which these values are based

Report	Age	Reference weight, kg		AR protein, g/kg/d	PRI protein, g/kg/d	AR protein, g/d		PRI protein, g/d		Method of derivation
		♂	♀			♂	♀	♂	♀	
EFSA 2012[1]	0-6 mo									No DRVs were derived
HCNL 2001[2]	Exclusively breast fed									Protein content of breast milk[40]
	0-2 mo[a]	5.0	4.5	N/A	1.2 (AI)					
	3-5 mo	7.0	6.5	N/A	1.2 (AI)					
	Formula fed[a]:									VC=15%; no further details provided on the derivation of the AR
	0-2 mo	5.0	4.5	1.4	1.8	7	6	9	8	
	3-5 mo	7.0	6.5	1.1	1.4	8	7	10	9	
WHO/FAO/UNU 2007[3]										No DRVs were derived
NCM 2012[6]	0-6 mo	N/A								No DRVs were derived
DACH 2017[11]	0 to under 1 mo	3.3	3.2		2.5			8 (AI)		Protein content of breast milk[42]
	1 to under 2 mo	4.5	4.2		1.8			8 (AI)		Daily average breast milk intake: 600 mL/d
	2 to under 4 mo	6.0	5.5		1.4			8 (AI)		(0-1 mo), 694 (1-2 mo), 723 (2-4 mo)[11]

Abbreviations: AI: adequate intake, AR: average requirement, d: day, DACH: German-speaking countries Germany, Austria and Switzerland, EFSA: European Food Safety Authority, g: gram, HCNL: Health Council of the Netherlands, kg: kilogram, mL: millilitre, mo: month, N: nitrogen, N/A: not applicable, NCM: Nordic Council of Ministers, PRI: population reference intake, RDA: recommended dietary allowance, VC: variation coefficient.

Footnotes:

[a] Exclusively bottle-fed.

7.3 Overview of dietary reference values for infants and children of 6 months to 18 years of age

Table 6 shows the various DRVs for protein for infants from six months to 18 years of age and the criteria on which these values are based. All reports used the factorial model as developed by WHO/FAO/UNU,[3] although the HCNL in 2001[2] used the report of 1985,[23] whereas the DRVs of EFSA[1] and DACH[11] are adopted from the report of 2007.

The protein requirements for maintenance of WHO/FAO/UNU 2007[2] were based on a regression analysis of N-balance studies with children from six months to 18 years. The protein requirements for growth of WHO/FAO/UNU 2007[2] were based on two studies: 1) A longitudinal study in which Butte et al. (2000) followed 76 children from birth to two years, with measurements of whole-body potassium at 0.5, 3, 6, 9, 12, 18, and 24 months;[48] 2) Cross-sectional data of 856 healthy European-American, African-American, and Mexican-American children aged 4-18 years of Ellis et al. (2000).[49]

NCM derived DRIs for only three age groups (6-11m, 12-23m, 2-17 y), which were based on their earlier report of 2004;[50] details of the method of derivation were not provided.[6,50]

WHO/FAO/UNU 2007 did not provide PRIs in g/d. For children, EFSA defined the reference weight for each age group as the median of the body weights of European children.[51] HCNL 2001 used data of Dutch growth studies (based on a representative sample for the Netherlands of 14,500 babies, infants, and adolescents).[52-55] For the current DRVs, the reference body weights of Dutch children were updated. Body heights, obtained from the Fifth Growth Study,[56] were combined with body weights from the Third Growth Study, based on weight for height growth charts[57,58] and additional details as provided by dr. Schönbeck and dr. Van Buuren (TNO Healthy Living, the Netherlands). Because the prevalence of childhood obesity has increased over the years, protein recommendations would be too high if they were based on the most recently measured body weights.

7.4 Conclusion for infants of 6 months to 18 years of age

The Committee agreed with EFSA's DRVs in g/kg body weight for high-quality protein and protein from mixed diets for infants and children from 6 months to 18 years of age. For the DRVs in grams per day, the Committee used Dutch growth data.

Table 6. Overview of the reference values for infants 6 months to 18 years of age and the criteria on which these values are based

Report	Age	AR N for maintenance, mg/kg/d	AR N for growth, mg/kg/d		AR protein for maintenance, g/kg/d	AR protein for growth, g/kg/d		AR protein, g/kg/d		PRI protein, g/kg/d		Reference weight, kg		AR protein, g/d		PRI protein, g/d		PRI protein, E%		Method of derivation
			♂	♀		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
EFSA 2012[1]	0.5	-	-		0.66[a]	0.46		1.12		1.31		7.7	7.1	-		10	9	-		**Factorial method** Numbers adopted from WHO/FAO/UNU 2007[3], except for the age category of 18 years (18 years is included in the PRI for adults)
	1					0.29		0.95		1.14		10.2	9.5			12	11			
	1.5					0.19		0.85		1.03		11.6	10.9			12	11			
	2					0.13		0.79		0.97		12.7	12.1			12	12			
	3					0.07		0.73		0.90		14.7	14.2			13	13			
	4					0.03		0.69		0.86		17.0	16.4			15	14			
	5					0.03		0.69		0.85		19.2	18.7			16	16			
	6					0.06		0.72		0.89		21.5	21.1			19	19			
	7					0.08		0.74		0.91		24.3	23.8			22	22			
	8					0.09		0.75		0.92		27.4	26.8			25	25			
	9					0.09		0.75		0.92		30.6	30.0			28	28			
	10					0.09		0.75		0.91		33.8	33.7			31	31			
	11					0.09	0.07	0.75	0.73	0.91	0.90	37.3	37.9			34	34			
	12					0.08	0.06	0.74	0.72	0.90	0.89	41.5	42.6			37	38			
	13					0.07	0.05	0.73	0.71	0.90	0.88	46.7	47.5			42	42			
	14					0.06	0.04	0.72	0.70	0.89	0.87	52.7	51.6			47	45			
	15					0.06	0.03	0.72	0.69	0.88	0.85	59.0	54.6			52	46			
	16					0.05	0.02	0.71	0.68	0.87	0.84	64.1	56.4			56	47			
	17					0.04	0.01	0.70	0.67	0.86	0.83	67.5	57.4			58	48			
HCNL 2001[2]	6-11 mo	63	36		-	-		0.9	0.9	1.2	1.2	9	8.5	8	8	10	10	6	6	**Factorial method** Maintenance: N-balance studies WHO 1985[23] *Efficiency of utilisation for maintenance=70% Growth: *Percentage of body protein,[59] Body weights based on growth data of Dutch children[52-55] *Efficiency of utilisation for growth=70% *VC=15%
	1-3 y	70	14					0.8	0.7	0.9	0.9	14	13.5	11	10	14	13	5	5	
	4-8 y	70	9	8				0.7	0.7	0.9	0.9	24	23.5	17	16	22	21	5	5	
	9-13 y	70	8	7				0.7	0.7	0.9	0.9	40	41	28	28	36	37	6	6	
	14-18 y	70	4	2				0.7l	0.6	0.8	0.8	65	59	43	38	56	49	7	8	

Report	Age	AR N for maintenance, mg/kg/d	AR N for growth, mg/kg/d		AR protein for maintenance, g/kg/d	AR protein for growth, g/kg/d		AR protein, g/kg/d		PRI protein, g/kg/d		Reference weight, kg		AR protein, g/d		PRI protein, g/d		PRI protein, E%		Method of derivation
			♂	♀		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
WHO/FAO/UNU 2007[3]	0.5				0.66[a]	0.46		1.12		1.31		7.7	7.1	-		-		-		Maintenance:
	1					0.29		0.95		1.14		10.2	9.5							Maintenance requirement
	1.5					0.19		0.85		1.03		11.6	10.9							similar to adults (0.66)[24]
	2					0.13		0.79		0.97		12.7	12.1							(Efficiency of utilisation for
	3					0.07		0.73		0.90		14.7	14.2							maintenance=47%)
	4					0.03		0.69		0.86		17.0	16.4							*VC=12%
	5					0.03		0.69		0.85		19.2	18.7							Growth:
	6					0.06		0.72		0.89		21.5	21.1							*Based on whole-body
	7					0.08		0.74		0.91		24.3	23.8							potassium deposition
	8					0.09		0.75		0.92		27.4	26.8							*Efficiency of utilisation
	9					0.09		0.75		0.92		30.6	30.0							for growth: 58%
	10					0.09		0.75		0.91		33.8	33.7							*PRI based on combined SD
	11					0.09	0.07	0.75	0.73	0.91	0.90	37.3	37.9							(maintenance and growth)
	12					0.08	0.06	0.74	0.72	0.90	0.89	41.5	42.6							
	13					0.07	0.05	0.73	0.71	0.90	0.88	46.7	47.5							
	14					0.06	0.04	0.72	0.70	0.89	0.87	52.7	51.6							
	15					0.06	0.03	0.72	0.69	0.88	0.85	59.0	54.6							
	16					0.05	0.02	0.71	0.68	0.87	0.84	64.1	56.4							
	17					0.04	0.01	0.70	0.67	0.86	0.83	67.5	57.4							
	18					0.03	0.00	0.69	0.66	0.85	0.82	74.6	62.1							
NCM 2012[6]= NCM 2004[50]	6-11 mo				0.66					1.1						0.4-0.9		7-15		No details provided
	12-23 mo				0.66					1.0						0.6-0.9 g/100 kJ		10-15		
	2-18 y				0.66					0.9						N/A		10-20		
DACH 2017[11]	4 to under 12 mo				0.66			1.12		1.3[b]		8.6	7.9			11				Adopted from factorial method
	1 to under 4 y							n.r.		1.0		13.9	13.2			14				of WHO/FAO/UNU 2007[3], with
	4 to under 7 y							n.r.		0.9		20.2	20.1			18				some age categories collapsed
	7 to under 10 y							n.r.		0.9		29.3	28.7			26				and DACH reference weights
	10 to under 13 y							n.r.		0.9	0.9	41.0	42.1			37	38			
	13 to under 15 y							n.r.		0.9	0.9	55.5	54.0			50	49			
	15 to under 19 y							n.r.		0.9	0.8	69.2	59.5			62	48			

Abbreviations: AI: adequate intake, AR: average requirement, d: day, DACH: German-speaking countries Germany, Austria and Switzerland, E%: percentage of total energy, EFSA: European Food Safety Authority, g: gram, G: growth, HCNL: Health Council of the Netherlands, M: maintenance, mg: milligram, mo: month, N/A: not applicable, NCM: Nordic Council of Ministers, N: nitrogen, n.r.: not reported, PRI: population reference intake, RDA: recommended dietary allowance, SD: standard deviation, VC: variation coefficient, y: year.

Footnotes:

[a] N requirement originally estimated as 110; the value of 105 (similar to adults was applied) for all ages.

08 tolerable upper intake level

8.1 Overview of tolerable upper intake levels

The tolerable upper intake level is defined as the maximum intake of substances in food, such as nutrients or contaminants, that can be consumed daily over a lifetime without adverse health effects.[60]

In 2001, HCNL derived a tolerable upper intake level, with some caution, of 25 en% for all people aged 4 years and older.[2] It was stated that for protein intakes higher than 20 en%, little evidence was available. In addition it was stated that for infants, excessively high protein intakes are undesirable, because the kidneys are still developing. Upper levels for infants were derived as 10 en% for infants aged 0-5 months, 15 en% for infants aged 6-11 months, 20 en% for children aged 1-3 years. HCNL also mentioned that excessively high protein intakes are harmful to people with impaired kidney function. However, this group is not the target group of DRVs, as the DRVs are derived for the healthy population.

In 2007, the WHO concluded that knowledge about the relationship between protein intake and health was insufficient to enable clear recommendations about either optimal intakes for long-term health or to define a safe upper limit.[3] In addition, EFSA concluded (2012) that data was insufficient to establish a tolerable upper intake level for protein.[1] EFSA evaluated data on bodyweight control and obesity, insulin sensitivity and glucose control, bone health, kidney function, capacity of the urea cycle and tolerance of protein, and concluded that the available data was not sufficient to establish a tolerable upper intake level for protein.[1] EFSA stated that “Data from food consumption surveys show that actual mean protein intakes of adults in Europe are at, or more often above, the PRI of 0.83 g/kg body weight per day. In Europe, adult protein intakes at the upper end (90-97.5th percentile) of the intake distributions have been reported to be between 17 and 27 en%. In adults, an intake of twice the PRI is considered safe.”[1] Regarding infants, EFSA reported that a very high protein intake (around 20 en%) could severely impair the water balance, particularly when no other liquids are consumed and/or external water losses are increased. Consequently, such high protein intakes should be avoided in the first year of life.” This statement was based on the following: “Because the protein content of the diet is, as a rule, the main determinant of the potential renal solute load, which needs water for excretion, a very high protein intake (around 20 en%, e.g. through exclusive consumption of cow’s milk), with a consecutive increased production of urea, can severely impair the water balance of infants, particularly when no other liquids are consumed and/or extrarenal water losses, e.g. through diarrhoea, are increased.”[1] The committee agreed with this statement of EFSA to limit the protein intake to 20 energy percent regarding infants.

NCM concluded that no *upper level* could be derived based on the present evidence.[6] NCM maintained its *upper range* of 20 en% as established in 2004,[8,50] According to NCM, this recommendation took into account the potential harmful effects of a long-term dietary protein intake above 20-23 en% seen in studies with protein *per se* and with low-carbohydrate/high-protein and/or high-fat diets, the caveat from renal function studies, and a consideration of the recommendations for fat and carbohydrates. Although possible negative consequences of a high-protein intake had not been clearly demonstrated in infants and children, a decrease in the upper levels for the ages of 6 to 23 months was deemed prudent.[6] The following upper *ranges* for protein intake were suggested, assuming sufficient intake of other nutrients: 0-6 months, 10 en%; 6-11 months, 15 en%; 12-23 months, 17 en%; and 2 years and older, 20 en%,[6] based on convincing evidence that the risk of obesity in childhood and adolescence increases with increased protein intake during infancy and early childhood.[7] DACH did not derive or discuss upper levels of protein.[11]

8.2 Conclusion for tolerable upper intake levels

The Committee agreed with the EFSA’s conclusion that data is insufficient to establish a tolerable upper intake level.

09
comparison of 2001 and 2021 DRVs for protein

A comparison with the previous DRVs is shown in table 7 for boys and men and in table 8 and for girls and women.

Table 7. Comparison of 2001 and 2021 DRVs for protein for boys and men

Age or category	AR (g/kg/d) 2001	AR (g/kg/d) 2021	PRI (g/kg/d) 2001	PRI (g/kg/d) 2021	PRI (g/d) 2001	PRI (g/d) 2021[a]
0 up to and including 2 mo	1.4	-	1.8		9	
3 up to and including 5 mo	1.1	-	1.4		10	
0.5 y		1.12		1.31		10
6 up to and including 11 mo	0.9		1.2		10	
1 y	0.8	0.95	0.9	1.14	14	12
1.5 y		0.85		1.03		12
2 y		0.79		0.97		13
3 y		0.73		0.90		14
4 y	0.7	0.69	0.9	0.86	22	15
5 y		0.69		0.85		17
6 y		0.72		0.89		20
7 y		0.74		0.91		22
8 y		0.75		0.92		25
9 y	0.7	0.75	0.9	0.92	36	28
10 y		0.75		0.91		30
11 y		0.75		0.91		34
12 y		0.74		0.90		37
13 y		0.73		0.90		42
14 y	0.7	0.72	0.8	0.89	56	46
15 y		0.72		0.88		51
16 y		0.71		0.87		57
17 y		0.70		0.86		58
18 yr		-		-		-
19 up to and including 30 y	0.6	-	0.8		61	
18 up to and including 29 y	-	0.66	-	0.83	-	63
31 up to and including 50 y	0.6	-	0.8	-	59	-
30 up to and including 39 y	-	0.66	-	0.83	-	61
40 up to and including 49 y	-	0.66	-	0.83	-	61
51 up to and including 70 y	0.6	-	0.8	-	60	-

Age or category	AR (g/kg/d) 2001	AR (g/kg/d) 2021	PRI (g/kg/d) 2001	PRI (g/kg/d) 2021	PRI (g/d) 2001	PRI (g/d) 2021[a]
50 up to and including 59 y	-	0.66	-	0.83	-	63
60 up to and including 69 y	-	0.66	-	0.83	-	60
>70 y	0.6	-	0.8	-	60	-
≥70 y	-	0.66	-	0.83	-	61

Abbreviations: AR: average requirement, g/kg/day: gram per kilogram body weight per day, g/d: gram per day, mo: month, PRI: population reference intake, y: year.
Footnotes:
[a] methods regarding the updated reference weights are described in the main report (*Dietary reference values for protein*).

Table 8. Comparison of 2001 and 2021 DRVs for protein for girls and women

Age or category	AR (g/kg/d) 2001	AR (g/kg/d) 2021	PRI (g/kg/d) 2001	PRI (g/kg/d) 2021	PRI (g/d) 2001	PRI (g/d) 2021[a]
0 up to and including 2 mo	1.4	-	1.8		8	
3 up to and including 5 mo	1.1	-	1.4		9	
0.5 y		1.12		1.31		9
6 up to and including 11 mo	0.9		1.2		10	
1 y	0.7	0.95	0.9	1.14	13	11
1.5 y		0.85		1.03		11
2 y		0.79		0.97		12
3 y		0.73		0.90		13
4 y	0.7	0.69	0.9	0.86	21	15
5 y		0.69		0.85		16
6 y		0.72		0.89		19
7 y		0.74		0.91		22
8 y		0.75		0.92		25
9 y	0.7	0.75	0.9	0.92	37	28
10 y		0.75		0.91		31
11 y		0.73		0.90		35
12 y		0.72		0.89		38
13 y		0.71		0.88		42
14 y	0.6	0.70	0.8	0.87	49	44
15 y		0.69		0.85		45
16 y		0.68		0.84		49
17 y		0.67		0.83		48
18 y		-		-		-
19 up to and including 30 y	0.6	-	0.8	0.83	52	

Age or category	AR (g/kg/d) 2001	AR (g/kg/d) 2021	PRI (g/kg/d) 2001	PRI (g/kg/d) 2021	PRI (g/d) 2001	PRI (g/d) 2021[a]
18 up to and including 29 y	-	0.66	-	0.83	-	54
31 up to and including 50 y	0.6	-	0.8	-	50	-
30 up to and including 39 y	-	0.66	-	0.83	-	52
40 up to and including 49 y	-	0.66	-	0.83	-	52
51 up to and including 70 y	0.6	-	0.8	-	52	-
50 up to and including 59 y	-	0.66	-	0.83	-	53
60 up to and including 69 y	-	0.66	-	0.83	-	52
>70 y	0.6	-	0.8	-	51	-
≥70 y	-	0.66	-	0.83	-	52
Pregnant women	0.7		0.9		62 (52+10)	1st trimester: +1 2nd trimester: +9 3rd trimester: +28
Lactating women	0.8		1.0		65 (52+13)	0-6 mo pp: +19 6-12 mo pp: +13

Abbreviations: AR: average requirement, g/kg/day: gram per kilogram body weight per day, g/d: gram per day, mo: month, pp: postpartum, PRI: population reference intake.
Footnotes:
[a] methods regarding the updated reference weights are described in the main report (*Dietary reference values for protein*).

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annexes

A concepts of protein metabolism and dietary reference values of protein

Terms and principles	Explanation
Biological value (BV)	A measure of how well the absorbed amino acid profile matches that of the requirement.
Digestibility (apparent vs. true)	The proportion of food nitrogen that is absorbed. If the correction for endogenous nitrogen loss is not made, the value is termed ‘apparent digestibility’ (measured as nitrogen content of foods minus nitrogen content of faeces). If the correction for endogenous nitrogen losses is made, the value is termed ‘true digestibility’.
Dispensable amino acids (DAA) also called ‘non-essential amino acids’	Of the 20 proteogenic dietary amino acids, 11 are dispensable (alanine, arginine, cysteine, glutamine, glycine, proline, tyrosine, aspartic acid, asparagine, glutamic acid and serine). This means that these amino acids can, under normal physiological conditions, be synthesised in the body. Some dispensable amino acids can become limiting under special physiological or pathological conditions, such as in premature neonates when the metabolic requirement cannot be met unless these amino acids are supplied in adequate amounts with the diet; they are then called conditionally indispensable amino acids (arginine, cysteine, glutamine, glycine, proline, tyrosine) (IoM, 2005; NNR, 2004).[1]
Efficiency of (dietary protein) utilisation	The efficiency of conversion from dietary protein into body protein. Based on estimating the extent to which dietary protein is absorbed and retained by the organism and is able to balance daily nitrogen losses. Nitrogen losses are determined by measuring faecal, urinary and miscellaneous nitrogen losses. As the post-prandial phase is critical for dietary protein utilisation, the measurement of the immediate retention of dietary nitrogen following meal ingestion represents a reliable approach for the assessment of protein nutritional efficiency. In the net post-prandial protein utilisation (NPPU) approach, true dietary protein retention is directly measured in the post-prandial phase based on experiments using 15N-labelled dietary proteins. Dietary proteins are considered to have a mean NPPU value of 70%. This NPPU approach represents the maximal potential NPU efficiency of the dietary protein sources when determined in optimised conditions in healthy adults, and it can be modified by different factors including food matrix, diet and physiological conditions. From N-balance studies, a net protein utilisation (NPU) of 47% was derived from the slope of the regression line relating nitrogen intake to retention for healthy adults at maintenance, and no differences were found between the results when the data were grouped by sex, diet or climate.[1] From available data in healthy adults at maintenance, the mean optimal NPU determined as NPPU is 70%, and the usual NPU value as determined from N-balance studies is ~47%. Different values are used for efficiency of dietary protein utilisation for maintenance (47%) and for tissue deposition/growth in different populations and age groups, including infants, and pregnant or lactating women.
Endogenous nitrogen losses	Endogenous intestinal (faecal) and metabolic (urinary) nitrogen losses can be estimated with a protein-free diet, be derived from the y-intercept of the regression line relating nitrogen intake to retention at different levels of protein intake (N-balance studies), or be directly determined from experiments using isotopically labelled dietary proteins.[1]
Indispensable amino acids (IAA), also called ‘essential’ amino acids	Nine (proteogenic) amino acids are classified as indispensable in humans (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) as they cannot be synthesised in the human body from naturally occurring precursors at a rate to meet the metabolic requirement. Among the nine indispensable amino acids, lysine and threonine are strictly indispensable since they are not transaminated and their deamination is irreversible. In contrast, the seven other indispensable amino acids can participate in transamination reactions.[1]
Metabolic demand of protein	Is determined by needs for maintenance (to compensate for protein losses) and growth, pregnancy and lactation.

Terms and principles	Explanation
Minimum protein requirement	The level of intake adequate to enable N-balance (in the short and long term). It will involve the highest efficiency of utilisation. In practice, measurements of the minimum protein requirement have varied widely within and between individuals – and to a greater extent than observed with measurements of the obligatory nitrogen loss. For this reason, identification of the minimum protein requirement is inherently difficult.[3]
Net post-prandial protein utilisation (NPPU)	See efficiency of utilisation
Net protein utilisation (NPU)	The proportion of nitrogen intake that is retained. NPU values are true or apparent, depending on whether or not the loss of endogenous nitrogen is taken into account. See also ‘efficiency of utilisation’.
Nitrogen balance	A measure of protein requirement. Studies that estimate N-balance involve subjects fed different levels of protein until they attain nitrogen equilibrium, i.e. when intake = loss, and balance = 0. Often a linear regression is used, so that the intake for nitrogen equilibrium (the requirement) is defined by an intercept (the nitrogen loss at zero intake) and a slope. The intercept is an estimate of metabolic demands, i.e. the obligatory nitrogen losses. The slope indicates the efficiency of dietary protein utilisation, which incorporates both digestibility and biological value: i.e. net protein utilisation.[3]
Nitrogen conversion factor	Both in the diet and in the body, 95% of the nitrogen (N) is found in the form of proteins, and 5% is found in the form of other nitrogenous compounds, i.e. free amino acids, urea or nucleotides. A conversion factor of 6.25 is generally used for the conversion of nitrogen into protein for labelling purposes, assessment of protein intake and for protein reference values. In this report, unless specifically mentioned, “protein” is total N x 6.25 (also called ‘crude’ protein) and protein requirements are calculated based on nitrogen content.
Nutritional value	The nutritional value of dietary proteins is related to their ability to satisfy nitrogen and amino acid requirements for tissue growth and maintenance. According to current knowledge, this ability mainly depends on the digestibility of protein and amino acids, and the dispensable and indispensable amino acid composition of the proteins.[1]
Obligatory nitrogen loss	The ongoing loss of nitrogen from the body when dietary intake of nitrogen is zero, and energy and all other nutrients are consumed in adequate amounts.
PDCAAS	Protein digestibility-adjusted amino acid score, a score used to determine protein quality.
Protein content	Both in the diet and in the body, 95% of the nitrogen is found in the form of proteins and 5% is found in the form of other nitrogenous compounds, i.e. free amino acids, urea or nucleotides. A conversion factor of 6.25 is usually used for the conversion of nitrogen to protein for labelling purposes, assessment of protein intake, and for protein reference values. Total N x 6.25 is called crude protein and [total minus non-protein-N] x 6.25 is called true protein. Unless specifically mentioned, “protein” is total N x 6.25 and protein requirements are calculated from nitrogen content.[1]
Protein quality	The capacity of a protein source to meet both the requirement for nitrogen and the requirement for indispensable amino acids as limiting precursors for body protein synthesis.[1] PRIs for protein are based on high-quality protein and protein from mixed diets/PDCAAS=100% (WHO, EFSA).
Protein requirement	The amount of protein or its constituent amino acids, or both, that must be supplied in the diet in order to satisfy the metabolic demand and achieve nitrogen equilibrium. Requirement = metabolic demand/efficiency of utilisation.[3]
Reference pattern of amino acids	The pattern of amino acids in a reference protein. The reference pattern of amino acids for infants <0.5 years is the amino acid pattern of human milk. The reference pattern of amino acids (mg/g protein) for the assessment of protein quality for adults is derived from proposed data on the requirement for individual indispensable amino acids (WHO/FAO/UNU, 2007) by dividing the requirement (mg amino acid/kg body weight per day) by the average requirement for protein (g/kg body weight per day) Age-specific scoring patterns for dietary proteins can be derived by dividing the requirement of each indispensable amino acid by the protein requirement of the selected age group (WHO/FAO/UNU, 2007, Table 8)[1,3]

B abbreviations

Abbreviation	Meaning	Short explanation (of relevance), mainly based on the EFSA reports on dietary reference values
AI	Adequate Intake	The AI is the level of (nutrient) intake adequate for virtually all apparently healthy people in a population. The AI is established when the AR (and thus the PRI/RDA) cannot be determined.
AR	Average Requirement	The AR is the level of (nutrient) intake adequate for half of the apparently healthy people in a population, given a normal distribution of requirement.
BMR	Basal Metabolic Rate	BMR is the energy expenditure in a physically and psychologically undisturbed state (but not asleep), post-absorptive, in a thermally neutral environment.
CV	Coefficient of Variation	In this report, CV is generally used as the coefficient of variation of the nutrient requirement, expressed as a percentage. If the nutrient requirement is normally distributed, the PRI/RDA/RI is calculated as (1 + [2 x CV/100]) times the average requirement (AR).
DACH	Deutschland (Germany), Austria, and Confœderatio Helvetica (Switzerland)	DACH (or D-A-CH) are the German-speaking countries that establish dietary reference values together.
DRV	Dietary Reference Value	A DRV is a quantitative reference value (such as AR, PRI, AI) for nutrient intakes for healthy individuals and populations which may be used for assessment and planning of diets.
EAR	Estimated Average Requirement	The EAR is IOM's and HCNL's reference value equivalent to EFSA's AR.
EFSA	European Food Safety Authority	EFSA is the agency of the European Union (EU) that provides independent scientific advice and communicating on existing and emerging risks associated with the food chain, including the establishment of dietary reference values.
HCNL	Health Council of the Netherlands	HCNL is an independent Dutch scientific advisory body tasked with advising the government and parliament about matters in the areas of public health and medical research, including dietary reference intakes.
IOM	Institute of Medicine	IOM is the institute that establishes the DRVs for the USA and Canada. IOM is the former name of the Health and Medical Division programme of the National Academy of Medicine (NAM). The NAM is the American non-profit, non-governmental organisation, that provides national advice on issues relating to biomedical science, medicine and health, and serves as an adviser to the nation to improve health. The NAM is a part of the National Academies of Sciences, Engineering, and Medicine, along with the National Academy of Sciences (NAS), National Academy of Engineering (NAE) and the National Research Council (NRC).
NCM	Nordic Council of Ministers	NCM is a geo-political inter-parliamentary forum for cooperation between the Nordic countries Denmark, Finland, Iceland, Norway and Sweden, as well as the autonomous areas of the Faroe Islands, Greenland and the Åland Islands. NCM develops the Nordic Nutrition Recommendations (NNR).
P50, P97.5	50th and 97.5th percentiles of a distribution	A percentile (or a centile) is a measure used in statistics that indicates the value below which a given percentage of observations in a group of observations falls. The dietary reference values AR and PRI/RDA are set respectively at the P50 and P97.5 of the distribution of requirements.
PAL	Physical Activity Level	The PAL is a person's energy expenditure over a 24-hour period, divided by their basal metabolic rate (BMR).
PRI	Population Reference Intake	The PRI is EFSA's reference value for the level of (nutrient) intake adequate for virtually all apparently healthy people in a population, on the condition that this value is established based on the average requirement (AR).
RDA	Recommended Daily Allowance	The RDA is IOM's and HCNL's reference value equivalent to EFSA's PRI.
RI	Recommended Intake	The RI is NCM's, DACH's and WHO/FAO's reference value equivalent to EFSA's PRI.
RIVM	Rijksinstituut voor Volksgezondheid en Milieu	RIVM is the Dutch National Institute for Public Health and the Environment.
UL	Tolerable upper intake level	The maximum level of chronic daily intake of a nutrient (from all sources) judged to be unlikely to pose a risk of adverse health effects in humans.
WHO/FAO	World Health Organization/Food and Agriculture Organization	WHO and FAO are specialised agencies of the United Nations. WHO is specialised in international public health; FAO in food and agriculture.

C terms used for the reference values in the five reports

European Food Safety Authority (EFSA)	Dietary Reference Values	Average Requirement (AR)	PRI	Adequate Intake (AI)	Tolerable upper intake level
HCNL	Dietary Reference Intakes; In Dutch: voedingsnormen	Estimated Average Requirement (EAR); In Dutch: gemiddelde behoefte	Recommended Daily Allowance (RDA); In Dutch: aanbevolen hoeveelheid	Adequate Intake (AI); In Dutch: adequate inname	Tolerable upper intake level (UL); In Dutch: aanvaardbare bovengrens van inneming
NCM	Dietary Reference Values	Average Requirement (AR)	Recommended Intake (RI)	Recommended Intake (RI)	Upper intake level (UL)
DACH	Reference values for nutrient intake	Average Requirement	Recommended intake	Estimated value for nutrient intake	N/A
WHO/FAO	-	Estimated Average Requirement (EAR)	Recommended Nutrient Intake (RNI) Safe level of intake	Recommended Nutrient Intake (RNI)	Safe upper limit

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