RESEARCH PAPER

Bomb calorimetry, the gold standard for assessment of intestinal absorption capacity: normative values in healthy ambulant adults

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Abstract

Background: Intestinal absorption capacity is considered to be the best method for assessing overall digestive intestinal function. Earlier reference values for intestinal function in healthy Dutch adults were based on a study that was conducted in an inpatient metabolic unit setting in a relatively small series. The present study aimed to readdress and describe the intestinal absorption capacity of healthy adults, who were consuming their usual (Western European) food and beverage diet, in a standard ambulatory setting.

Methods: Twenty-three healthy subjects (aged 22–60 years) were included in the analyses. Nutritional intake (energy and macronutrients) was determined with a 4-day nutritional diary. Subsequently, mean faecal losses of energy (by bomb calorimetry), fat, protein and carbohydrate were determined following a 3-day faecal collection. Finally, intestinal absorption capacity was calculated from the differences between intake and losses.

Results: Mean (SD) daily faeces production was 141 (49) g (29% dry weight), containing 891 (276) kJ [10.7 (1.3) kJ g^{-1} wet faeces; 22.6 (2.5) kJ g^{-1} dry faeces], 5.2 (2.2) g fat, 10.0 (3.8) g protein and 29.7 (11.7) g carbohydrates. Mean (SD) intestinal absorption capacity of healthy subjects was 89.4% (3.8%) for energy, 92.5% (3.7%) for fat, 86.9% (6.4%) for protein and 87.3% (6.6%) for carbohydrates.

Conclusions: The present study provides normative values for both stool nutrient composition and intestinal energy and macronutrient absorption in healthy adults on a regular Dutch diet in an ambulatory setting. Intestinal energy absorption was found to be approximately 90%.

Introduction

The absorption of nutrients is an essential function of the gastrointestinal tract, notably of the small intestine. In humans, it is proposed that absorption can be used as a surrogate measure for the whole process of digestion. Absorption is a major physiological function of the intestine. Hence, intestinal absorption capacity may be used as a semi-quantitive marker of intestinal function (Heymsfield *et al.*, 1981).

Clinically evident malabsorption is an issue encountered in daily practice. It is a major clinical challenge when intestinal failure (IF), as defined in accordance with the recently updated definitions (O'Keefe *et al.*, 2006), has been diagnosed. Intestinal failure may be observed in a wide array of clinical problems and is regularly

encountered in general and referral hospitals. Intestinal failure is common in, for example, intensive care unit patients (Strack van Schijndel *et al.*, 2006; Wierdsma *et al.*, 2011), radiation enteritis after surgical treatment, Crohn's disease and other chronic inflammatory intestinal diseases, such as autoimmune enteropathy and refractory coeliac disease. It may lead to a negative balance of energy and proteins, dehydration, deficiencies of vitamins, minerals or trace elements, and a decreased quality of life. In these IF patients, knowledge of intestinal function, as measured by intestinal absorptiometry, is essential. This comprises relevant information with respect to providing adequate dietary advice that aims to adjust to the medical and nutritional care needs of individual patients. In addition, it allows follow-up of the digestive capacity in a quantitative manner. If necessary, it can also be performed regularly until dietetic balance is secured.

Bomb calorimetry, in which faecal energy content is measured by heat of combustion, is regarded as the gold standard laboratory method for quantifying energy losses (Miller & Payne, 1959; Lovelady & Stork, 1970). Bomb calorimetric measurements may be of clinical importance for the early recognition of patients with malabsorption as a consequence of IF. Calculating intestinal absorption as the difference between nutritional intake and faecal losses (as a percentage of the nutritional intake) is a widely accepted method (Heymsfield *et al.*, 1981; Chacko *et al.*, 1984; Messing *et al.*, 1991; Nordgaard, 1998; Jeppesen & Mortensen, 2000). It is generally regarded to be the quantitative gold standard for digestion or intestinal function in clinical practice, being an undisputed biomarker of gastrointestinal functionality. Furthermore, measurement of faecal macronutrient losses can be of additional value in diagnosing and interpreting malabsorptive signs.

However, data on reference values for energy and macronutrient absorption are scarce, especially for adults in an outpatient ambulatory setting, which forms the usual circumstances for dietetic and nutritional interventions or therapy. Based on a small study conducted in an inpatient metabolic unit setting, 'standard' energy absorption is estimated to be at a level of 95% because non-absorbed energy in healthy adults has been reported to be approximately 5% when digesting a standard diet (Southgate & Durnin, 1970).

For reasons of practical applicability, the present study aimed to assess faecal energy, subdivided in its major contributors of fat, protein and carbohydrate losses, to quantify standard intestinal absorption capacity in healthy adults on a Western European diet in an ambulatory setting in The Netherlands by using a feasible and unique methodology of intestinal absorptiometry reflecting routine practice.

Materials and methods

Subjects

Twenty-five healthy subjects participated in the present study. Subjects were mainly institutional healthcare workers with specific dietetic and healthcare knowledge. Therefore, they were selected as having skilled competence in adequately registering nutrient intake and meticulously collecting stools. Inclusion was based on voluntary enrolment. The subjects had to meet certain criteria:

- Healthy, defined as absence of gastrointestinal diseases or abnormalities, current or common disease, eating disorders or pregnancy;
- Age ≥ 18 years;
- Regular bowel habits;
- No concomitant use of antibiotics or medication interfering with gastrointestinal motility and;
- Exclusively orally fed without dietary restrictions.

The medical ethical committee of VU University Medical Centre, Amsterdam approved the study protocol and written informed consent was obtained from all subjects.

Methods

During a period of 4 consecutive days, included subjects noted their usual (Western European diet) food and beverage intake; all faecal specimens were collected during the final 3 days. For urinary protein losses, a standardised and fixed correction factor for healthy people, with normal kidney function, was taken into account (Southgate & Durnin, 1970). Subjects collected the data and stools at home in the predefined period, during which they were obliged to continue their habitual diet.

Food and beverage intake
An experienced and dedicated dietitian (NJW) instructed all subjects in advance with respect to accurately weighing all food and drinks using digital electronic scales and recording information, such as brand names, next to cooking methods, if any, for all foods and beverages during the study period. Additionally, the same dietitian interviewed all subjects afterwards to ensure adequate documentation and to check whether all study procedures had been complied with. A computerised food calculation programme (based on the National Dutch Food Composition Table 'NEVO' 2006; Westenbrink *et al.*, 2006) was used to calculate mean nutrient intake (fat, protein and carbohydrates). The total energy intake (TEI) of the diet was determined by using the gross energetic values for fat (39.33 kJ g^{-1}), protein (18.41 kJ g^{-1}, derived from the gross energetic value of 23.64 kJ g^{-1} protein minus the fixed correction for urinary nitrogen loss of 5.23 kJ g^{-1})

(Merill & Watt, 1995) and carbohydrates (17.15 kJ g^{-1}), multiplied by the amount supplied.

Nutrient losses

All faeces were collected during 72 h (day 2–4), as per the protocol, in specifically designed 5-L buckets. Faeces were weighed (faecal wet weight in g day^{-1}), homogenised and immediately stored at <4 °C until analysis. To measure faecal macronutrient content and to calculate intestinal absorption capacity of the healthy subjects, the faeces were analysed for energy, fat and nitrogen content. The faecal fat content (F$_{Fat}$) was determined by the method of Van de Kamer *et al.* (1949). On a sample of wet stools, total nitrogen analysis was performed using the micro-Kjeldahl method to determine faecal nitrogen content (F$_{Nitrogen}$) using previously described catalytic and digestive conditions (Rudman *et al.*, 1975). Faecal protein (F$_{Protein}$) was calculated using a conversion factor, assuming that all of the F$_{Nitrogen}$ was derived from protein: F$_{Protein}$ (g day^{-1}) = F$_{Nitrogen}$ (g day^{-1}) × 6.25. Kilojoules from F$_{Protein}$ were calculated as: F$_{Protein}$ × 18.4 day^{-1}. Subsequently, a sample was taken and freeze dried to be processed by bomb calorimetry. A bomb calorimeter is a type of constant-volume calorimeter used in measuring the heat of combustion of a particular reaction. It consists of a small cup containing the sample, oxygen, a stainless steel bomb, water, a stirrer, a thermometer, the dewar or insulating container (to prevent heat flow from the calorimeter to the surroundings) and an ignition circuit connected to the bomb. The temperature change in the water is accurately measured with a thermometer, and so it is used to calculate the energy given out by the sample burned (in this case, faeces) (Miller & Payne, 1959; Lovelady & Stork, 1970). These calorimetric determinations represented daily faecal energy loss (F$_{Energy}$) in kcal day^{-1} and were performed using a Ballistic bomb calorimeter, (type CBB-33; Gallenkamp Manufactury, Etten-Leur, The Netherlands) at the University of Groningen, The Netherlands. Finally, faecal carbohydrate content (F$_{Carbohydrate}$) was calculated from the nonfat, nonprotein and nonwater fraction of stools (i.e. the faecal 'remaining') and was calculated using the formula: F$_{Carbohydrate}$ (g day^{-1}) = (F$_{Energy}$ − F$_{Fat}$ × 39.3 − F$_{Protein}$ × 18.4)/17.2.

The intestinal absorption capacity (%) of ingested energy from macronutrients was finally calculated as: (TEI − F$_{Energy}$/TEI) × 100. Specific intestinal malabsorption of energy, fat, protein and carbohydrate was *a priori* defined as an absorption capacity of 85% or less (Southgate & Durnin, 1970; Heymsfield *et al.*, 1981).

Statistical analysis

Data obtained from two subjects were excluded from the data analyses as a result of incomplete data collection.

The data for the remaining 23 subjects are presented as the mean (SD), either range or 95% confidence interval (CI), and box and whisker plots. Subject groups were compared using Students' *t*-test and analysis of variance in the case of more than two groups. Associations between variables were evaluated by Pearson's correlation coefficient (*r*) and the chi-squared test, where appropriate. $P < 0.05$ was considered statistically significant. Outliers were defined as values >3 SD. Statistical analyses were carried out using SPSS, version 18 (SPSS Inc., Chicago, IL, USA). Data were analysed for the total group and separately for men and women.

Results

Twenty-five healthy Dutch adults were enrolled in the present study. Data obtained from two (male) subjects were excluded from the analyses as a result of insufficient thoroughness in documentation as assessed after the collection period in accordance with the study protocol. Table 1 depicts the characteristics of the remaining 23 subjects, including nutrient intake data.

Intestinal absorption capacity

Mean (SD) intestinal absorption capacity (as a percentage of nutritional intake) was 89.4% (3.8%) (95% CI = 87.7–91.0) for energy, 92.5% (3.7%) (95% CI = 90.9–94.2) for fat, 86.9% (6.4%) (95% CI = 84.0–89.8) for protein and 87.3% (6.6%) (95% CI = 84.3–90.3) for carbohydrates.

Table 1 Subject characteristics

	Men	Women	$P*$
Number of subjects	9	14	
Age (years)	43.2 (11.8) (27–57)	42.9 (14.2) (22–60)	NS
Height (m)	1.82 (0.04) (1.76–1.88)	1.72 (0.06) (1.64–1.80)	<0.01
Weight (kg)	84.0 (9.4) (70.1–103.0)	68.9 (8.5) (54.4–88.0)	<0.01
Body mass index (kg m^{-2})	25.3 (2.7) (22.1–30.4)	23.1 (2.2) (20.3–27.2)	<0.01
Nutrient intake			
Total energy intake (kJ day^{-1})	10 163 (946) (8510–11498)	7807 (887) (15 971–9167)	<0.01
Fat (g day^{-1})	89 (16) (65–118)	68 (19) (42–101)	0.048
Protein (g day^{-1})	93 (11) (74–107)	72 (11) (53–91)	<0.01
Carbohydrate (g day^{-1})	286 (64) (169–351)	217 (35) (170–279)	0.03

Values are presented as the mean (SD) (range).
*$P \leq 0.05$ was considered statistically significant.
NS, not significant.

Women had a statistically significantly lower energy absorption capacity compared to men (88.0% versus 91.8%, respectively, $P = 0.02$). A similar trend was seen for fat and carbohydrate absorption, although this was not statistically significant ($P = 0.19$ and 0.06, respectively) (Table 2).

Faecal production and composition

Results of faecal collection and its nutrient contents are presented in Table 3. All subjects confirmed that the number of bowel movements during the 72-h collection period was in accordance with their usual bowel movement pattern. Mean (SD) daily stool production was 141 (49) g (29% dry weight), containing 891 (276) kJ [10.7 (1.3) kJ g^{-1} wet faeces; 22.6 (2.5) kJ g^{-1} dry faeces], 5.2 (2.2) g fat, 10.0 (3.8) g protein and 29.7 (11.7) g carbohydrates. Mean (SD) nutrient contribution to faecal energy content was 23% (10%) for fat, 20% (8%) for proteins and 57% (23%) for carbohydrates. The stools of women contained a lower percentage of water than those of men ($P < 0.05$), and therefore the energy content per gram of wet faeces (kcal g^{-1}) was higher in women than in men ($P < 0.05$). Stool volume (g day^{-1}) and daily faecal nutrient losses were not statistically significantly different between men and women (Table 3).

Daily faecal production was positively correlated with faecal energy loss in kcal day^{-1} (Pearson's $r = 0.80$, $P < 0.001$; Fig. 1) and negatively correlated with intestinal energy absorption capacity (%) (Pearson's $r = -0.46$, $P < 0.05$; Fig. 2).

Discussion

The present study represents the first analysis of a representative population of healthy subjects on their usual (Western European) food and beverage intake in a standard ambulatory setting. The data assess intestinal absorption and faecal composition by bomb calorimetry, which is the gold standard for measuring digestive intestinal function. The data presented may be used as normative values for the evaluation of patients presenting with symptoms of intestinal failure in a standard ambulatory setting. The calculated intestinal energy absorption in these Dutch adults was found to be almost 90% (95% CI = 87.7–91.0), which is lower than the earlier reported absorptive capacity from a metabolic setting.

Reference values for the described method of IF quantification are crucial when treating the previously described (usually malnourished) IF patients. Only seven small studies have reported on stool nutrient contents and intestinal nutrient absorption in healthy subjects. Two studies determined energy and nutrient stool contents of healthy children (Rivero-Marcotegui et al., 1998; Van den Neucker et al., 2003). Four studies focused on nutrient absorption in healthy adults, of which one study compared the nutrient absorption of European adolescents with that of elderly individuals (66–78 years) on diets with different energy densities in a metabolic unit setting (Southgate & Durnin, 1970). Three other studies were performed in small groups, in non-Dutch adults or only in women (Bo-Linn et al., 1983; Chacko et al., 1984; Murphy et al., 1993). Finally, one study reported on absorption and faecal composition in different groups of non-malabsorptive patients instead of in healthy subjects (Heymsfield et al., 1981). The data obtained from these patients cannot be extrapolated to healthy adults and therefore cannot be used as reference values. The interpretation of results of bomb calorimetric measurements is difficult without suitable reference values obtained in a comparable clinical setting.

When comparing our presented results with those of earlier studies, it is notable that the faecal composition

Table 2 Absorption capacity (%) of energy and macronutrients in healthy Dutch adults, subdivided by sex

		Sex		
	Total group	Men	Women	P*
Energy absorption	89.4 (3.8) (80.3–94.8)	91.8 (2.3) (88.3–94.8)	88.0 (3.8) (80.3–94.0)	0.018
Fat absorption	92.5 (3.7) (85.0–97.2)	93.9 (3.1) (87.4–97.2)	91.7 (3.9) (85.0–96.9)	NS
Protein absorption	86.9 (6.4) (68.3–93.8)	87.7 (4.5) (79.8–93.0)	86.4 (7.5) (68.3–93.8)	NS
Carbohydrate absorption	87.3 (6.6) (72.1–94.8)	90.2 (3.2) (84.9–94.8)	85.5 (7.6) (72.1–94.1)	NS

Values are presented as the mean (SD) (range).
*$P \leq 0.05$ was considered statistically significant.
NS, not significant.

Table 3 Faecal composition of healthy Dutch adults, subdivided by sex and age

		Sex			Age categories			
	Total group	Men	Women	P*	<30 years	30–50 years	>50 years	P*
Number of subjects	23	9	14		7	7	9	
Faecal wet weight (g day^{-1})	141 (49) (44–221)	151 (36) (111–221)	134 (56) (44–212)	NS	150 (54)	144 (59)	131 (40)	NS
Faecal dry weight (%)	29 (6) (20–43)	26 (4) (20–31)	31 (6) (20–43)	0.038	27 (5)	32 (8)	28 (4)	NS
Wet faeces (kJ g^{-1})	6.7 (1.3) (4.2–10.9)	5.9 (1.3) (4.2–7.9)	7.1 (1.3) (4.6–10.9)	0.040	6.3 (0.8)	7.1 (2.1)	6.7 (1.3)	NS
Dry faeces (kJ g^{-1})	22.6 (2.5) (15.1–25.1)	22.2 (2.5) (17.6–26.4)	22.6 (2.5) (15.1–25.1)	NS	23.0 (1.26)	21.3 (3.3)	23.0 (2.5) 23.0 (1.3)	NS
F$_{Energy}$ (kJ day^{-1})	891 (276) (481–1536)	894 (180) (644–1092)	916 (331) (481–1536)	NS	954 (335)	920 (322)	824 (205)	NS
F$_{Fat}$ (g day^{-1})	5.2 (2.2) (2.4–10.7)	5.3 (2.6) (2.4–10.7)	5.2 (2.1) (2.5–9.7)	NS	6.3 (2.8)	4.3 (1.5)	5.2 (2.1)	NS
F$_{Nitrogen}$ (g day^{-1})	1.6 (0.6) (0.7–2.8)	1.8 (0.5) (1.2–2.8)	1.5 (0.6) (0.7–2.7)	NS	1.7 (0.5)	1.8 (0.8)	1.4 (0.5)	NS
F$_{Carbohydrate}$ (g day^{-1})	29.7 (11.7) (14.9–53.0)	25.8 (7.5) (16.7–41.9)	32.3 (13.5) (14.9–53.0)	NS	30.7 (13.1)	32.4 (13.8)	26.8 (9.4)	NS

Values are presented as the mean (SD) (range).
*$P \leq 0.05$ was considered statistically significant.
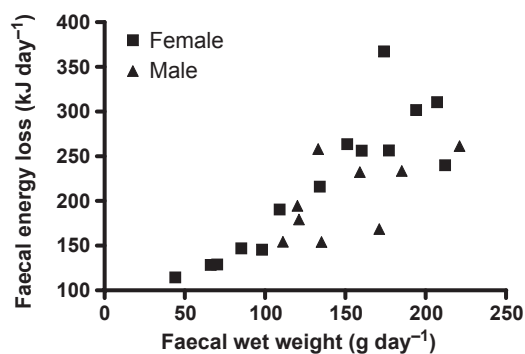NS, not significant.

Figure 1 Relation between faecal energy loss in kJ day^{-1} measured by bomb calorimetry and faecal wet weight (g day^{-1}) ($n = 23$). Daily faecal production is positively correlated with faecal energy loss (Pearson's $r = 0.80$, $P < 0.001$).
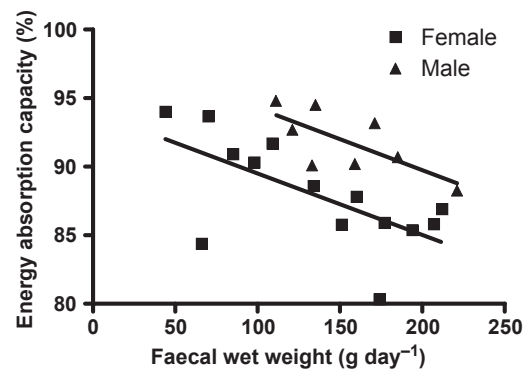

Figure 2 Daily faecal production (g day^{-1}) negatively correlated with intestinal energy absorption capacity (% of the energy intake) ($n = 23$) (Pearson's $r = -0.46$, $P < 0.05$ for the total group, $r = -0.65$, $P < 0.05$ for women and $r = -0.71$, $P = 0.05$ for men).

matches broadly, although the studies were performed in various populations, such as adolescents, the elderly (Southgate & Durnin, 1970) and Southern Indian inhabitants (Chacko *et al.*, 1984). A normal daily faecal production between 100 and 200 g with a faecal water content of approximately 70–75% was reported previously (Wyman *et al.*,1978; Murphy *et al.*, 1993), whereas faecal fat excretion (Southgate & Durnin, 1970; Chacko *et al.*, 1984), nitrogen excretion (Southgate & Durnin, 1970) and energy contents of dry faeces (Murphy *et al.*, 1993) showed similar quantities to those reported in the present study (Table 3).

The faecal carbohydrate fraction of stools is estimated to be 19%. Of this fraction, 3% is truly carbohydrate loss, 15% is fibre and 1% (partly volatile) is lactic acid (Zarling *et al.*, 1986). To our knowledge, carbohydrate absorption cannot be directly measured and no reference values exist. In the present study, mean faecal carbohydrate was calculated from the nonfat, nonprotein and nonwater fraction of stools (i.e. the faecal 'remaining' energy) and found to be 30 (12) g day^{-1}. Thus, it included indigestible fibres and the lactic acid fraction, with the latter being small in healthy subjects.

The digestibility of energy has been studied previously in small groups of both healthy subjects, as well as in the

healthy elderly, and was calculated to be as high as 95% (range 93–97%) (Southgate & Durnin, 1970) and 96% (range 89–99%) (Heymsfield et al., 1981), respectively. The differences between these results and those of the present study may result from the different methodologies employed in that Southgate & Durnin (1970) applied both fixed diets and faecal markers (carmine).

Additionally, determination of the intake of nutrients may have introduced several possible flaws. The 'double portion method', which is the method of preference in absorption studies, is a balance study in which all subjects receive the same prepared diet with a known and measured composition, whereas the uneaten fraction is analysed for remaining energy and nutrients. This was not applied in the present study because we considered it too cumbersome and time-consuming for a 'standard ambulatory setting', requiring extra cooking and the recording of wasted food. Additionally, we anticipated that subjects were likely to change their eating habits under conditions of the 'double portion method' because of the need to throw away the uneaten portion. The applicability of our findings to daily ambulatory practice, a prerequisite for the intended purpose, is likely to be hampered by this, thus affecting the presumed generalisibility. Our purpose was to introduce normative values for an absorptiometry method that can be used on a regular and daily basis in ambulatory patients as well. The described double portion method is not feasible for this target group. That is why we used estimated dietary records that are considered to be one of the best techniques for providing quantitatively accurate dietary intake information, although this method does have limitations and assumptions (Thompson & Byers, 1994). Errors could be made regarding food content and the natural variation in foods throughout the year. To optimise the exact documentation of food intake, healthy subjects were instructed to weigh all foods that were consumed, instead of providing an estimate of the amount, and to record information such as brand names, as well as recording cooking methods, for all foods and beverages. The applied faecal collection period of 3 days aimed to correct for day-to-day variation in excretion, taking mean transit time and sex differences into account as reported and advocated previously (Graff et al., 2001). Because the healthy subjects mainly comprised healthcare workers, with specific dietetic or healthcare knowledge, we expected them to collect specimens and to record the food and beverages correctly. Unexpectedly, a wide range of protein absorption was found (68.3–93.8%; Table 3), whereas the faecal nitrogen excretion and its range appeared to be reliable. This may be explained by an under-reporting of protein intake in this specific case because one female subject reported only 53 g proteins day^{-1}. Under-reporting has been associated with this kind of dietary assessment methods previously (Bingham et al., 1994; Bingham, 2002).

Stools consist of many components, such as shedded intestinal cells, bacterial fractions of the more than 800 bacterial species of the intestinal microbiota, and non-absorbed nutritional elements. Together with water, bile salts and minerals, they contribute to a faecal composition that is considered as normal. Therefore, we presumed this as being unlikely to affect the results of the present study. Additionally, the energy content of faeces partly originates from biological materials such as bacterial mass. Repelled intestinal cells were of little influence in these measurements, both absolutely and relatively, because this type of cell loss contributes only a small fraction to the completely collected stools (Achour et al., 2007). Little information is also available concerning the possible amount of energy loss via the bacterial fraction in human faeces. In two small human studies, the faecal bacterial fraction has been quantified in g day^{-1} (Huijsdens et al., 2002; Achour et al., 2007). The measurement error introduced by the ignoring the faecal bacterial fraction appeared to be small in healthy volunteers (Huijsdens et al., 2002; Achour et al., 2007).

Remarkably, sex influenced the percentage of faecal dry weight in the present study; women had more concentrated faeces despite an equal fluid consumption between sexes (data not shown). As a consequence, the energy content of faecal wet weight (kJ g^{-1}) was higher and intestinal energy absorption significantly lower in women compared to men. Also, in previous studies, more concentrated faeces have been reported in women, which was ascribed to a more prolonged colonic transit time (Degen & Phillips, 1996a,b). Because daily faecal energy (kJ day^{-1}) and nutrient loss (g day^{-1}) remained equal between sexes in these series, we do not recommend formulating reference values by sex.

In conclusion, the present study provides normative values for faecal energy and macronutrient losses and, subsequently, the intestinal absorption capacity of healthy adults on a regular Western European diet, as obtained in a standard ambulatory setting. The calculated standard for energy absorption in healthy Dutch adults was found to be approximately 90%. This normative data can be used to evaluate the intestinal absorption capacity of patients presenting with symptoms of intestinal failure.

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