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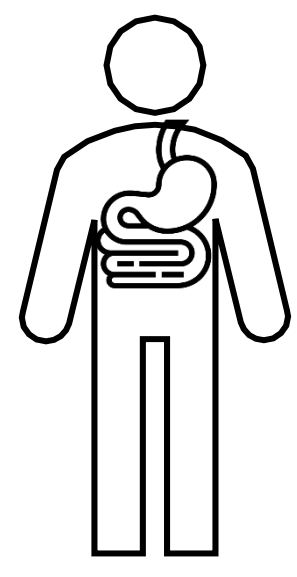
# Determination of true ileal amino acid digestibility of spirulina in healthy volunteers for use as the protein reference in the dual isotope method

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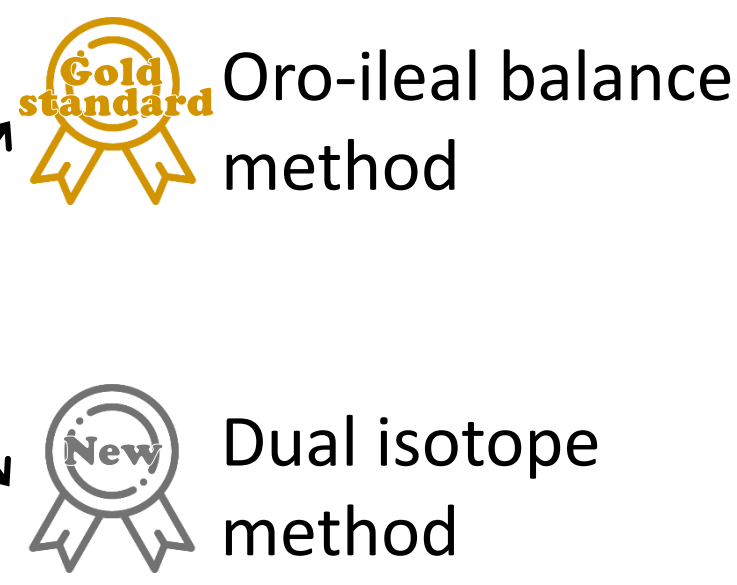
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## INTRODUCTION



Evaluation of amino acid (AA) digestibility in humans



- ✓ Naso-ileal intubation
- ✓ **Direct** evaluation of dietary AA ileal losses
- ✓ **Very invasive** - Not in vulnerable population
- ✓ Use of <sup>15</sup>N-labelled test protein
- ✓ Based on plasma samples
- ✓ **Indirect** evaluation of AA digestibility
- ✓ **Minimally invasive** - Possible in vulnerable population
- ✓ Use of <sup>2</sup>H-labelled test protein and <sup>13</sup>C-reference protein of known digestibility



<sup>13</sup>C-reference protein: Spirulina  
AA digestibility values obtained only in animals or with indirect method

## OBJECTIVE

To determine the true ileal AA digestibility of spirulina using the standard oro-ileal balance method in healthy subjects

## MATERIALS & METHODS

### Volunteers

- Sub-study of VALDIM study (VALidation of the Dual Isotope Method)
- Study approved by the Ethical Committee CPP Nord Ouest III (2022-26) and registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) as NCT05426551
- Main inclusion criteria: Men/women, 18-45 y, BMI 18-30 kg/m<sup>2</sup>  
No digestive or hepatic pathology, no food allergy  
Signed informed consent

Table 1. Subjects characteristics

Variables	Subjects
n	6
Sex	3M, 3F
Age (y)	25.8 ± 5.9
Height (m)	1.7 ± 0.2
Weight (kg)	68.3 ± 9.3
BMI (kg/m <sup>2</sup> )	22.6 ± 1.7

Values are means ± standard deviation (SD)

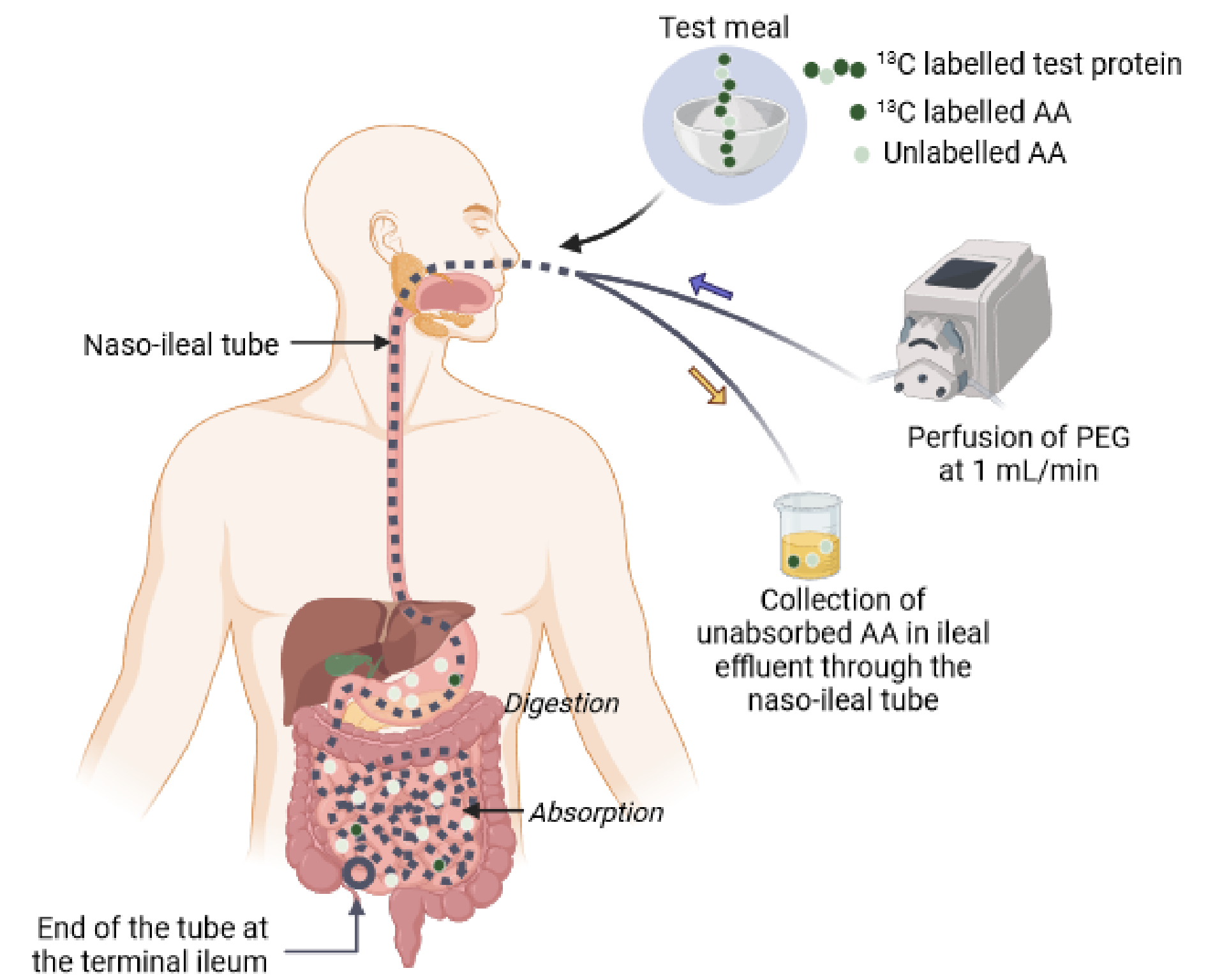
### Clinical investigation

- 2 days of hospitalisation in Centre de recherche sur Volontaires (CRV) of Avicenne Hospital (Bobigny, France)
- Test-meal: <sup>2</sup>H-labelled eggs (0.85 g/kg) with tracer dose of uniformly <sup>13</sup>C-labelled spirulina whole cells (U-<sup>13</sup>C, 97%; 12 mg/kg)

Table 2. Composition of the test meal

Ingredients	Quantity (g/kg bw)	For a 70-kg subject (g)
<sup>13</sup> C-spirulina whole cell	0.012	0.84
<sup>2</sup> H-lyophilized egg powder	0.85	59.5
Water	3.4	238
Olive oil	0.17	11.9
Provencal herbs	0.02	1.4

Bw, body weight.



$$\text{Ileal AA true digestibility (\%)} = \frac{\text{Dietary AA intake} - \text{Ileal dietary AA flow}}{\text{Dietary AA intake}} \times 100$$

Figure 1. Schematic representation of the principle of the oro-ileal balance method to measure ileal AA digestibility

(Adapted from Bandyopadhyay et al. Adv Nutr 2021, created in Biorender)

### Analyses

- Determination of dietary C from spirulina in ileal digesta with measurements of <sup>13</sup>C enrichment by EA-IRMS
- Determination of dietary AA in ileal digesta collected with measurements of <sup>13</sup>C enrichment in individual AA by GC-C-IRMS

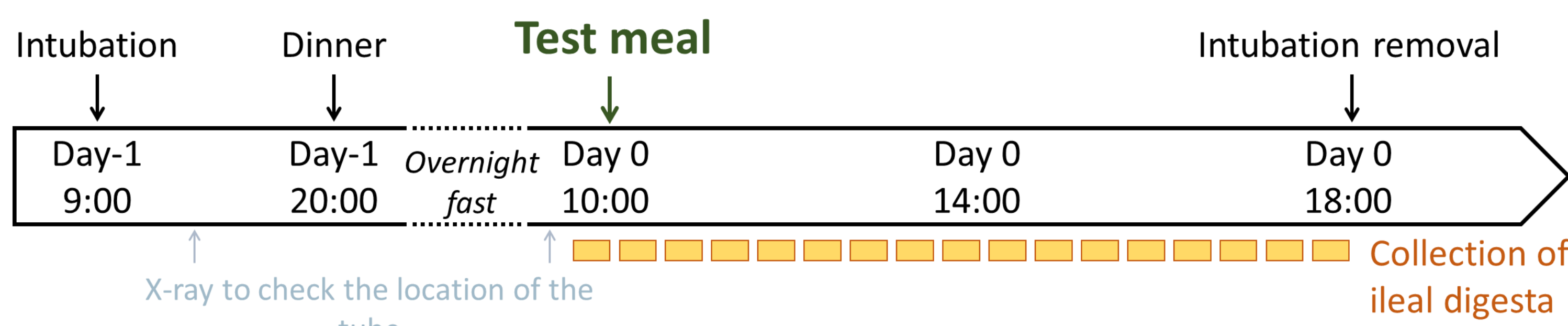


Figure 2. Experimental design of the study

## RESULTS

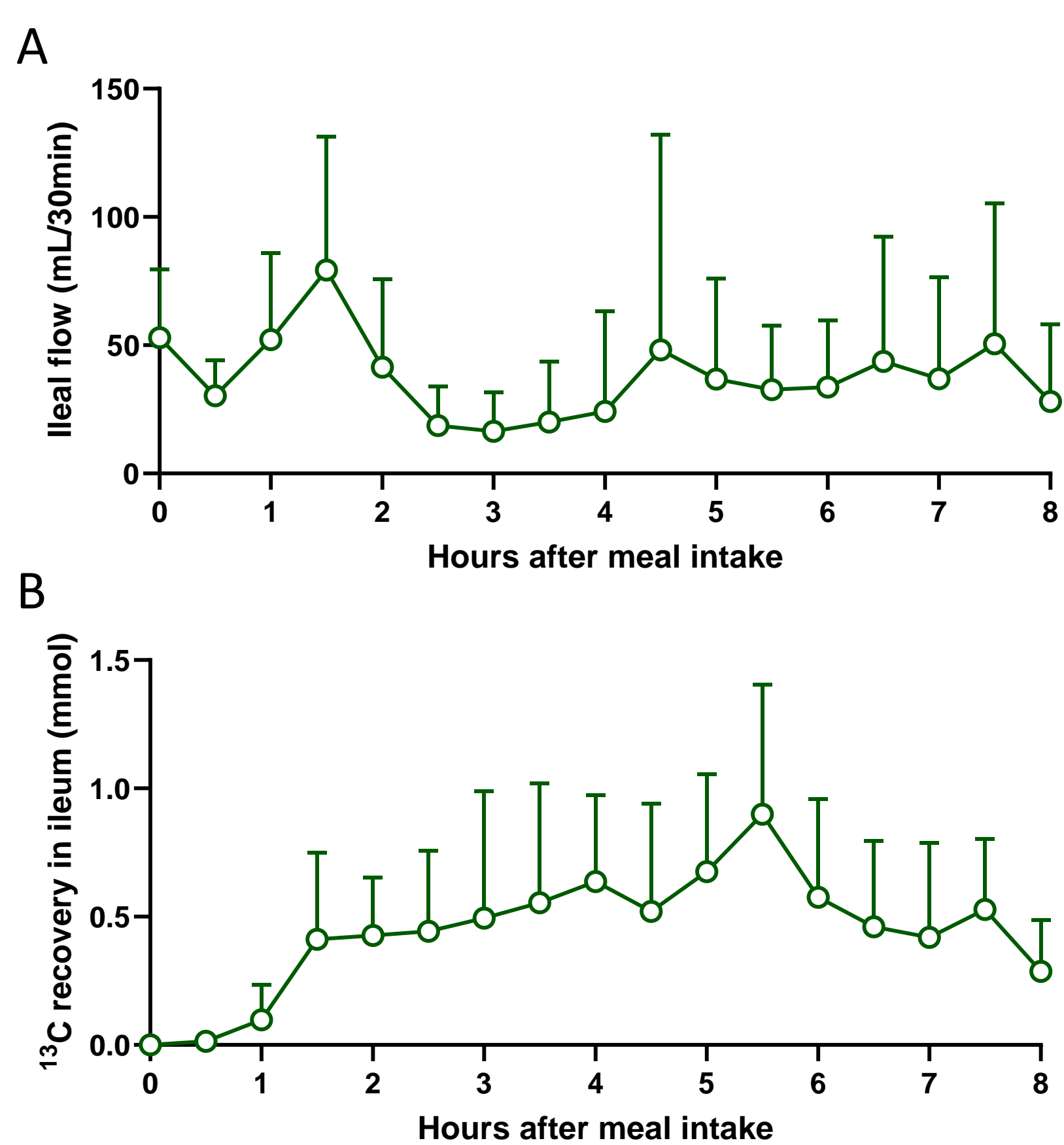


Figure 3. Liquid ileal flow rate (A) and <sup>13</sup>C flow in the ileum (B) after test meal intake

Values are means ± SD

→ The average liquid ileal flow rate was 38 ± 25 mL/30min

→ <sup>13</sup>C-spirulina appeared in the ileum 1h after meal intake, the peak being observed at 5.5h

→ <sup>13</sup>C-spirulina digestibility was 78.1 ± 6.8%

Table 3. True ileal digestibility of AA in spirulina whole-cell protein

Amino acids	Digestibility (%)
<b>Indispensable AA</b>	
Histidine	95.1 ± 2.7
Isoleucine	88.0 ± 2.9
Leucine	88.6 ± 3.0
Lysine	91.8 ± 1.6
Methionine	94.9 ± 1.0
Phenylalanine	90.7 ± 2.9
Threonine	84.4 ± 3.5
Valine	87.0 ± 3.5
<b>Mean IAA</b>	<b>88.7 ± 2.7</b>
<b>Dispensable AA</b>	
Alanine	87.2 ± 3.8
Aspartate + asparagine	85.3 ± 3.3
Glutamate + glutamine	91.6 ± 2.6
Glycine	83.7 ± 3.9
Proline	88.7 ± 2.6
Serine	81.0 ± 4.2
Tyrosine	88.8 ± 3.2
<b>Mean DAA</b>	<b>87.4 ± 2.6</b>
<b>Mean all AA</b>	<b>88.1 ± 2.5</b>

→ True ileal digestibility of AA in spirulina whole-cell protein varied depending on the AA

→ Digestibility ranged from 83.7 ± 3.9% for glycine to 95.1 ± 2.7% for histidine

→ The mean ileal digestibility of AA in spirulina was 88.1 ± 2.5%

Values are means ± SD. Abbreviations: AA, amino acid; DAA, dispensable AA; IAA, indispensable AA. Mean digestibility values of IAA, DAA and all AA were weighted by the proportion of each AA in spirulina.

## CONCLUSIONS

- ❖ Our study provides original data on ileal AA digestibility of spirulina determined with the standard and direct oro-ileal balance method in humans.
- ❖ The ileal digestibility values of AA in spirulina obtained with the standard oro-ileal balance method were within the same range as those obtained with the indirect dual isotope method in humans (mean AA digestibility 85-88% in Devi et al. AJCN 2018 and Kashyap et al. J Nutr 2019).
- ❖ The true individual AA ileal digestibility of spirulina obtained in the present study can be applied in the dual isotope method when spirulina is used as the reference protein. This method is very promising as it can be applied in vulnerable population, such as elderly, children or in pathophysiological conditions for which protein and AA digestibility data are lacking.