Impact of Nutrient density of formula on nutritional intakes in healthy term infants; Influence of home reconstitution.

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This study, is a part of the GIRAFFE study (Growth of Infants who are Formula Fed Exclusively) promoted and funded by Danone Research Utrecht, The Netherlands
Study objective

The study is a part of a prospective, double-blind RCT investigating the nutritional efficacy and suitability of hypo-allergenic formula with lowered protein content until 16 weeks of life in healthy term infants.

- Control product: 2.27 g protein /100 kcal (1.50 g & 66 kcal/dL)
- Investigational product I: 2.0 g protein /100 kcal (1.32 g & 66 kcal/dL)
- Investigational product II: 1.8 g protein /100 kcal (1.19 g & 66 kcal/dL)

• Primary study outcome of the GIRAFFE study is a weight gain equivalent to the WHO reference values.
• Primary outcome of the present study is the influence of home reconstitution on nutritional intakes and growth
Study design

**Diary:** adverse events, medication, concomitant food

**Measurements of weight, length, head circumference** (arm circumference at 4, 12, 16 & 52 weeks)

- **Healthy, full-term aged between 0 and 14 days exclusively formula fed**
- **N=156**
  - **n=52**
  - **n=52**
  - **n=52**

- **Diary:** adverse events, medication, concomitant food
- **Bottle analysis**
- **Blood sample**
- **Body composition**
- **3 days food intake**

- **Investigational product with a protein content of 1.8 g/100 kcal**
- **Investigational product with a protein content of 2.0 g/100 kcal**
- **Control product with a protein content of 2.27 g/100 kcal**

- **Study entry & randomisation**

**Q = Questionnaire:**
- **7 days formula intake, GI tolerance**

**Age:**
- baseline
- 4 weeks
- 8 weeks
- 12 weeks
- 16 weeks
- 52 weeks
Material and Methods

We calculated:
• Protein content of formulas: N*6.25 as well as the formula density: Fat content/Fat labeled
• Protein and energy intakes at 4 and 12 wks from chemical and labeled values considering the mean volume intake during the corresponding 7 days record.
• Weight gain between 4 and 12 wks in g, g/d, g/kg*d

We compared:
• Protein & energy intakes estimated from chemical analysis to that from labeled values.

We evaluated:
• The relationship between formula intake and the formula density.
• The influence of formula density on weight gain between 4 and 12 wks.
Results

• 207 subjects were included in the GIRAFFE study. Of those, 162 subjects completed the intervention period of 16 weeks (PP).

• In all, 333 bottles were collected and analyzed in our laboratory. In 6 bottled, the results of the fat and the nitrogen contents were discordant, and the results were excluded from the final analysis.

• 7 days formula intakes reported as <100 or >220 ml/kg body weight*day were considered as out of ranges (n=29) and were excluded from the final analysis (n=298).

Paired data at 4 and 12 wks were finally obtained in 129 infants.
Protein and energy content of the home prepared Bottles

Formula density range (Chem fat/label fat): (0.87 – 1.14) at 4wks and (0.86 - 1.15) at 12 wks

n=258
## Formula and nutrient intakes at 4 and 12 wks of age

<table>
<thead>
<tr>
<th>Intakes (/kg*d)</th>
<th>4 weeks N=129</th>
<th>12 weeks N=129</th>
<th>Paired t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>168.7 ± 23.0</td>
<td>137.4 ± 19.1</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Label</td>
<td>2.25 ± 0.40</td>
<td>1.83 ± 0.32</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>-Chemical</td>
<td>2.35 ± 0.46 §</td>
<td>1.94 ± 0.36 §</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Label</td>
<td>5.91 ± 0.81</td>
<td>4.87 ± 0.67</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>-Chemical</td>
<td>5.91 ± 0.88</td>
<td>4.81 ± 0.67 §</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Energy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Label</td>
<td>111.4 ± 15.2</td>
<td>90.7 ± 12.6</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>-Chemical</td>
<td>111.4 ± 16.6</td>
<td>91.9 ± 12.7 §</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Density*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-label</td>
<td>1.00</td>
<td>1.00</td>
<td>p=0.001</td>
</tr>
<tr>
<td>-Chemical (Prot.)</td>
<td>1.042 ± 0.080</td>
<td>1.064 ± 0.087</td>
<td>p=0.014</td>
</tr>
<tr>
<td>-Chemical (Fat)</td>
<td>1.002 ± 0.068 §</td>
<td>1.016 ± 0.062 §</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(0.87 – 1.14)</td>
<td>(0.86 - 1.15)</td>
<td></td>
</tr>
</tbody>
</table>

* The difference between the calculated fat density and protein density could be the result of a laboratory slight overestimation of the nitrogen content or to the use of an inadequate conversion factor: Prot = N*6.25

$\text{p<0.05; } \£ \text{p<0.005; } \£ \text{p<0.0001}$
Relationship between volume intakes and the formula density

Vol intake (ml/kg*d)= -0.789 * Formula density + 233.2; r=0.20; p= 0.00057; n=298
## Weight gain during the Study

<table>
<thead>
<tr>
<th>Weight gain 4 to 12 wks</th>
<th>Girls n=65</th>
<th>Boys n=64</th>
<th>Total n=129</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kg/study</td>
<td>1.59±0.37</td>
<td>1.80±0.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.68±0.38</td>
</tr>
<tr>
<td>g/d</td>
<td>27.8±5.1</td>
<td>31.5±7.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.6±6.4</td>
</tr>
<tr>
<td>g/kg*d</td>
<td>3.96±0.64</td>
<td>4.34±0.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.15±0.74</td>
</tr>
<tr>
<td>Prot g/dl</td>
<td>1.37±0.18</td>
<td>1.46±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.39±0.18</td>
</tr>
<tr>
<td>BW at 4 wks (g)</td>
<td>4,073±0,492</td>
<td>4,323±0,426&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4,197±0,475</td>
</tr>
</tbody>
</table>
Body weight Z-Scores compared to WHO Values

![Graph showing body weight Z-Scores compared to WHO values for different groups and ages.](image)
Relationship between weight gain and E intake \((F=24.2)\) is improve when and F density is included in the relation \((F=30.3)\)

\[ WG = \text{WG (g/kg*d)} = 0.029 \times 	ext{E intake (labelled)} + 2.43 \times \text{F density} - 1.236; \]

\(n=129; r=0.44; p \text{ E int}<0.00001; p \text{ F density}=0.026\)
In conclusion

Our study suggests:

- The reconstitution procedure at home significantly influences the nutrient density of the studied formulas.
- Density of powder formula is an additional factor to volume intake to be considered in nutritional studies. It could also influence the sample size evaluation.
- Formula intakes at 4 and 12 wks are inversely related to the formula density.
- Weight gain is related to energy intakes but also to formula density that needs to be considered as an additional factor.

Reconstitution procedure needs to be take into account in the interpretation of the results in nutritional studies using powder formula in healthy term infants.