Acid steatocrit in the first 2 years of life: diagnostic accuracy and reference limits

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Introduction

Fat excretion and concentration in the 72-hour stool collection by the titrimetric Van de Kamer method is considered the gold standard for the diagnosis of fat malabsorption [1–4]. Although it is a non-invasive, simple and inexpensive test, it is also time-consuming, difficult to perform, especially in infants using diapers, and undesirable for patients and laboratory personnel [3, 5, 6]. Furthermore, this method requires proper training of the patient to improve the reliability of the test. For this reason, other methods have been investigated.

An alternative test is the measurement of acid steatocrit (AS), which was first described by Tran ABSTRACT

Introduction. The measurement of acid steatocrit (AS) is an established method to assess faecal fat balance. However, data regarding AS in healthy infants and toddlers are sparse.

Aim. This study aimed to determine the range of normal values for AS in the first two years of life and evaluate the correlations with other faecal fat balance tests.

Material and Methods. AS (%) was assessed by 72-hour stool collection in 160 children aged 1–24 months (8 groups of 20: aged 1–3, 4–6 months, etc.).

Results. AS was higher in infants < 6 months than in those aged 7–12, 13–18 and 19–24 months (p < 0.05, p < 0.001 and p < 0.001, respectively). The correlations between AS and age and faecal fat concentration (FFC) were statistically significant, but moderate or weak (r = -0.48, p < 0.0001; r = 0.28, p < 0.001, respectively). A 90th/95th percentile nomogram of AS was created based on these results, with values ranging from 23.6/23.9% at 1 month to 12.1/12.9% at 24 months.

Conclusions. Healthy infants have a significantly higher AS than older children. For this reason, we propose an upper limits nomogram, providing detailed reference values for infants and children in their first two years of life. However, it should be noted that AS does not reflect adequately FFE and FFC in this population.

Keywords: exocrine pancreatic function, healthy children, reference intervals, fecal free fatty acids.

Introduction

Fat excretion and concentration in the 72-hour stool collection by the titrimetric Van de Kamer method is considered the gold standard for the diagnosis of fat malabsorption [1–4]. Although it is a non-invasive, simple and inexpensive test, it is also time-consuming, difficult to perform,
et al. [7] in 1994 as a simple screening test for steatorrhea. This method remains not only facile (no 3-day collection) but is also cost-effective. AS is considered very reliable as it correlates well with the coefficient of fat absorption (CFA) and faecal fat excretion (FFE) in defining significant steatorrhea in alcoholic chronic pancreatitis [8, 9]. However, it is not standardised, possibly influenced by dietary fat intake, and unhelpful in patients with mild steatorrhea [2, 10]. It should also be emphasised that despite evidence of the clinical usefulness of AS in cystic fibrosis, chronic pancreatitis, small bowel disease and untreated coeliac disease, data on normal AS in healthy infants and toddlers are sparse [2, 4, 9, 11, 12].

Since the gastrointestinal tract is immature in infants and young children, it may be expected that not only FFE and faecal fat concentration (FFC) but also AS will be higher than in older subjects [13, 14]. Therefore, the aims of the present study were to determine the range of normal AS values in the first two years of life and to evaluate the correlations with other faecal tests assessing fat loss.

Material and Methods

Patients

One hundred and sixty infants and toddlers (82 boys, 78 girls; aged 4 weeks–24 months) were recruited for the purpose of the study, all of whom had normal pancreatic status determined using the faecal elastase-1 concentration as described previously [15]. In all patients, FFC and FFE were defined as described earlier [15]. The children were divided into 8 groups of 20 aged 1–3 months, 4–6 months, etc. The exclusion criteria were prematurity and the presence of comorbidities (e.g., chronic gastrointestinal diseases, inflammatory processes or other). The inclusion criteria were age ≤24 months and agreement to participate in the study. The anthropometric parameters describing the healthy subjects are presented in Table 1.

Methods

A 72-hour stool collection was performed on all children. Faecal samples were weighed and homogenised before analysis using the method of Tran et al. [7] to determine AS. After centrifugation, the individual layers were measured and the percentage AS was calculated using the formula:

\[
\text{AS (\%)} = \frac{\text{Fatty layer length}}{\text{Fatty layer length} + \text{Solid layer length}}
\]

The flow chart showing the accuracy of AS compared with other faecal fat tests was prepared based on STARD 2015 guidelines for reporting diagnostic accuracy studies [16]. Abnormal values were assumed in flow chart A: AS higher than 20%, FFE higher than 5 g/day and FFC higher than 5% (AS values lower than 10% were considered normal). In flow chart B every result higher than the 90th percentile was considered as abnormal.

Statistical methods

The study size was calculated using G•Power v. 3 (University of Dusseldorf, Dusseldorf, Germany). The unpaired comparisons between subgroups of size not smaller than 17 were determined to be sufficient assuming power of the test at 80% and a 5% significance level.

GraphPad Prism 5.01 software (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analysis. AS values are given as medians and 1st–3rd quartiles. Differences between groups were evaluated by the Kruskal-Wallis test with post-hoc testing (Dunn’s multiple comparison test). The influence of FFC, FFE, faecal elastase-1

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### Table 1. Basic anthropometric data of the studied healthy infants and young children. The number of children in each group was 20

| Age (months) | 1–3 | 4–6 | 7–9 | 10–12 | 13–15 | 16–18 | 19–21 | 22–24 | p |

| Median values (1\textsuperscript{st}-3\textsuperscript{rd} quartiles) |
|---|---|---|---|---|---|---|---|---|---|
| Z-score for body weight | -0.03 | -0.05 | 0.14 | 0.09 | -0.14 | 0.08 | 0.02 | 0.01 | Ns. |
| [0.46–1.04] | [0.90–0.85] | [0.49–0.55] | [0.44–0.65] | [0.73–0.47] | [0.35–0.89] | [0.92–0.53] | [0.61–0.43] | Ns. |
| Sex ratio (male/female) | 8 / 12 | 10 / 10 | 10 / 10 | 10 / 10 | 7 / 13 | 11 / 9 | 14 / 6 | 12 / 8 | Ns. |
concentration, age, sex, Z-score for body mass on AS value was assessed using multiple linear logistic regression. Correlations were evaluated using the Pearson test and \( p \)-values < 0.05 were considered statistically significant.

For the graphical presentation of AS, results were smoothed by the least squares weighted distances method (Stata/SE 15.0 64 bit for Windows, College Station, USA). To determine the reference values for AS, the 90/95th percentile was calculated and estimated on the nomogram according to the respective percentile for each age group.

Ethical considerations

The study protocol was approved by the Ethical Committee of the Poznań University of Medical Sciences, Poland (decision no. 1275/05). Written informed consent was obtained from all the subjects’ parents. The study was carried out in accordance with the revised Declaration of Helsinki.

Results

Differences in AS between younger and older age groups were found and are presented in Table 2. AS values in the first 6 months were higher (\( p < 0.05 \), \( p < 0.001 \) and \( p < 0.001 \), respectively) than in children aged 7–12, 13–18 and 19–24 months (median [1st–3rd quartile], 14.6% [12.1–18.0], 11.7% [9.2–14.0], 9.9% [6.8–12.5], 9.8% [6.9–12.0], respectively) and in the first year of life higher than in the second year (13.3% [10.7–15.5] vs 9.9% [6.8–12.2]; \( p < 0.0001 \)) (Figure 1). The dependence of AS values on age smoothed using the LOWESS method is shown in Figure 2.

The relationship between AS and age and FFC/FFE is depicted in Figure 3. The correlations between AS and age as well as FFC were statistically significant but moderate or weak (\( r = -0.4788, p < 0.0001; r = 0.2812, p < 0.001 \); respectively). There were no correlations between AS and faecal elastase-1 or FFE.

In multiple regression analysis, values of AS were predicted by age (\( p \) model < 0.000001, \( p = 0.000042, b = -0.25453 \)).

All infants aged 1–3 months, 75% of those aged 4–6 and 7–9 months, and 55% of those aged 10–12 months had higher AS than normal values proposed for older children (<10%) [2]. For this reason, a nomogram representing 90th/95th percentiles of AS was generated (Table 3). The reference percentile curves for AS are shown in Figure 4 AB.

A flow diagram according to the STARD initiative describing the accuracy of AS to determine FFE and FFC was also created (Figure 5 AB).

### Table 2. Acid steatocrit in healthy infants and young children. The number of subjects in each group was 20

<table>
<thead>
<tr>
<th>Fecal test</th>
<th>Age (months)</th>
<th>Median values [1st–3rd quartiles]</th>
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</thead>
<tbody>
<tr>
<td>Acid steatocrit (%)</td>
<td>1–3</td>
<td>15.5bcde [13.7–18.7]</td>
</tr>
<tr>
<td></td>
<td>4–6</td>
<td>13.2f [10.6–15.7]</td>
</tr>
<tr>
<td></td>
<td>7–9</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>10–12</td>
<td>10.8a [8.1–13.7]</td>
</tr>
<tr>
<td></td>
<td>13–15</td>
<td>9.9b [6.9–12.2]</td>
</tr>
<tr>
<td></td>
<td>16–18</td>
<td>9.3c [6.9–13.1]</td>
</tr>
<tr>
<td></td>
<td>19–21</td>
<td>10.4d [8.4–12.6]</td>
</tr>
<tr>
<td></td>
<td>22–24</td>
<td>8.7e [6.2–10.8]</td>
</tr>
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</table>

\( a p < 0.05, b p < 0.01, c p < 0.05, d p < 0.001 \)

### Table 3. Nomogram for the assessment of acid steatocrit (AS) in the first two years of life. The 90th and 95th percentiles are shown. Smoothing for AS was performed using the method of least squares weighted distances

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
<th>13</th>
<th>15</th>
<th>17</th>
<th>19</th>
<th>21</th>
<th>23</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>P90th AS (%)</td>
<td>23.6</td>
<td>20.9</td>
<td>19.4</td>
<td>17.7</td>
<td>16.2</td>
<td>15.6</td>
<td>15.1</td>
<td>15.2</td>
<td>15.4</td>
<td>14.9</td>
<td>13.6</td>
<td>12.6</td>
<td>12.1</td>
</tr>
<tr>
<td>P95th AS (%)</td>
<td>23.9</td>
<td>22.8</td>
<td>21.5</td>
<td>19.8</td>
<td>19.0</td>
<td>19.7</td>
<td>17.3</td>
<td>16.2</td>
<td>15.9</td>
<td>15.3</td>
<td>14.2</td>
<td>13.3</td>
<td>12.9</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>1</th>
<th>3</th>
<th>5</th>
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<th>9</th>
<th>11</th>
<th>13</th>
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<th>19</th>
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</thead>
<tbody>
<tr>
<td>P90th AS (%)</td>
<td>21.7</td>
<td>20.0</td>
<td>18.3</td>
<td>16.4</td>
<td>15.9</td>
<td>15.4</td>
<td>15.1</td>
<td>15.3</td>
<td>15.2</td>
<td>14.9</td>
<td>13.6</td>
<td>12.6</td>
<td>12.1</td>
</tr>
<tr>
<td>P95th AS (%)</td>
<td>23.2</td>
<td>22.2</td>
<td>20.6</td>
<td>19.2</td>
<td>19.0</td>
<td>18.0</td>
<td>16.7</td>
<td>16.0</td>
<td>15.7</td>
<td>14.6</td>
<td>13.8</td>
<td>13.3</td>
<td>12.9</td>
</tr>
</tbody>
</table>
Figure 1. Acid steatocrit (AS; %) value in healthy infants and young children in different age subgroups.

Figure 2. The dependence of acid steatocrit (AS; %) on age smoothed using the LOWESS method (bandwidth = 0.8)
Figure 3. Correlation of acid steatocrit value (AS; %) and fecal fat concentration (FFC; \( p < 0.001, r = 0.2812 \)), fecal fat excretion (FFE; \( p = 0.3790 \)) and age of children (\( p < 0.0001, r = -0.4788 \))
Figure 4. The reference 90th (A) and 95th (B) percentile curves generated for acid steatocrit (AS) smoothed by the least squares weighted distances method.
A

160 children
AS = index test

Abnormal
n=5
AS>20%

Normal
n=69
AS<10%

Inconclusive
n=86
AS = 10-20%

FFE as a standard test. Abnormal fat excretion present (>5g/day)?

FFC as a standard test. Abnormal fat concentration present (>5%)?

B

160 children
AS = index test

Abnormal
n=15
AS>90th percentile

Normal
n=145
AS<90th percentile

FFE as a standard test. Abnormal fat excretion present (>90th percentile)?

FFC as a standard test. Abnormal fat concentration present (90th percentile)?

Figure 5. Flow diagram of diagnostic accuracy of acid steatocrit (AS) and stool collection (fecal fat excretion (FFE) & fecal fat concentration (FFC))
Discussion

This study provides new AS reference values and evaluated the applicability of AS in the first two years of life. The preceding literature described AS only in the context of significant steatorrhea in patients with gastrointestinal diseases compared to healthy subjects. However, the produced data could not be reliably extrapolated to healthy infants because the studies involved small groups of children with a large age range (in none of the manuscripts the infants were delineated as an individual subgroup). This is the first study to propose reference intervals based on a large number of healthy infants and toddlers. Nonetheless, this study is limited as the effects of diet (breast-fed vs non-breast-fed infants) on the obtained results were not taken into consideration.

AS has been described as a reliable tool in screening for steatorrhea in a paediatric population [17]. A few studies [7, 9, 17, 18] documented its high correlation with FFE and/or FFC in cystic fibrosis or chronic pancreatitis patients. Although three of these articles described healthy subjects as a control group, these results cannot be used for creating reference values. The first study included only 6 children aged from 3 to 12 years (mean age of 5.8 years) [7], showing a high correlation between faecal fat content and AS (0.81) and a lower AS value in healthy children (3.8%) as compared with cystic fibrosis patients (26.9%), but infants and toddlers were not included in the study. In the second study, Amann et al. [9] only analysed stool samples from 15 healthy adults. In the third study, the median AS [10th–90th percentile] in 29 healthy children was 7.2% [3.0%–15%] [17], but interpretation of the results is difficult due to the broad age range (0.6–16 years). All subjects were analysed as one group, therefore no age-related differences in FFE could be analysed.

Van de Neucker et al. [11] compared AS values in healthy children with those of sick children without (asthma patients) or with gastrointestinal involvement (patients with cystic fibrosis, untreated coeliac disease, various gastrointestinal problems). The control group comprised 25 boys and 25 girls with a mean age of 3.9 years (the youngest infant was 6 months old) and their median AS was 3.3% [5th–90th percentile: 0–21.0%]. It should be emphasised that very high AS up to 44.8% were also found, however no analysis of the influence of age on AS values was performed. It is worth noting that in the present study, the obtained results were also higher than the earlier suggested cut-off level of 10% (median AS for all children up to 1 years was 13.3%; maximum was 24.8%) [2, 3].

The evaluation of the applicability of AS in cystic fibrosis patients without or with mild steatorrhea (< 10 g/day) was the main goal of our previous study [2]. In the 72-hour stool collection, there were rather weak or moderate correlations between AS and FFE/FFC (r = 0.394, p < 0.005; r = 0.454, p < 0.001, respectively). Using two cut-off values for normal AS (10%) and abnormal AS (20%), the sensitivity, specificity, negative, and positive predictive values in the determination of abnormal FFC and FFE were not satisfactory. Therefore, it was concluded that AS has a limited practical value in patients without or with only mild steatorrhea. Undoubtedly, healthy infants are an interesting group for similar analysis. The limited usefulness of AS was reflected in the results obtained in the present study (flow chart), with no regard to the cut-off level values of AS and FFC/FFE (Figure 5). Overall, AS does not seem to be useful in subjects other than those with severe steatorrhea.

In conclusion, healthy infants have a significantly higher AS than older children. For this reason, we propose an upper limits nomogram, providing detailed reference values for infants and children in their first two years of life. However, it should be noted that AS does not reflect adequately FFE and FFC in this population.

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Conflict of interest statement
The authors declare no conflict of interest.

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Author Contributions
Conceptualization — MWF, SDC & JW; design of the work — MWF, SDC, DW, JSz, AMC & JW; acquisition of data — MWF, SDC, JSz, ZS; analysis and interpretation — MWF, SDC, DW, JAM & JW; data visualization — MWF, SDC, JAM; writing — original draft preparation — MWF, SDC & JW; writing — review & editing — DW, JSz, ZS, AMC; funding acquisition — JW.
References


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