Methods and results of fecal lipid determination

by Konrad Seige, and Gerd Muller

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METHODS AND RESULTS OF FECAL LIPID DETERMINATION

By

Seige, Konrad and Müller, Gerd

From the 2nd Medical Clinic and the Polyclinic at the
Martin Luther University in Halle-Wittenberg;
Director of the Clinic: prof. dr. K. Seige

A simple method of quantitative determination of daily excretion of fecal fatty acids, suitable for routine work, has been presented. The method was applied for the determinations carried out in 262 patients. Steatorrhea was observed most frequently in pancreatic as well as in gastric and intestinal diseases. Besides, the relations between fecal fatty acid excretion and nitrogen excretion as well as daily fecal weight have been studied. Positive correlation was found between these parameters. The authors come to the conclusion that greater attention should be paid to quantitative determination of fecal lipid in diagnosis of gastrointestinal diseases.
Quantitative determination of fecal lipid elimination is essential for the diagnosis and control of diseases in which fatty feces occur. Fatty acids are a major component of the fecal lipids and can serve as a representative measure of their elimination. Soaps, glycerides, sterols, sterol esters, phosphatides, bile acids, hydrocarbons, long-chain alcohols and waxes are further representatives of lipids. A complete analysis of all these components is difficult and costly. However, the methods to determine the quantitative elimination of fatty acids are simple and can be employed for routine examination in clinics; in practice they give quite reliable results.

The examination of fecal lipids was carried out in 262 patients under comparable conditions. We employed our own methods. The description of the methods used and the results obtained are presented in this paper.

METHODS

Since the daily quantity of feces undergoes considerable fluctuation, the feces of three days are collected, weighed and mixed. Then, the sample required for analysis

* Numbers in the right-hand margin indicate the corresponding pages in the original.
is separated and weighed. The feces collected for research is kept under refrigeration. The method of Van de Kamer (8), modified by us, was used for the total determination of fatty acids, while our own method, described and published previously (3,4) was used to determine particular lipid fractions in feces.

The method of quantitative determination of fatty acids

5 g of feces with 10 ml of 33% potassium hydroxide (KOH) and 40 ml of ethanol (with the addition of 0.4% amyl alcohol) is placed in a 200 ml flask and heated for 20 minutes under a reversing cooler. Next, the flask is cooled and its contents acidified with 25% hydrochloric acid. The flask is cooled again and 50 ml of paraffin ether added to its contents. After the flask is tightly closed the substance is shaken vigorously for about a minute. 25 ml of the extract undergoes evaporation in a water bath. The remnant left after evaporation is dissolved in 5 ml of ethanol. Fatty acids are titrated with 0.1 N KOH after one drop of 0.05% alcohol solution of Nile blue is added as an indicator.

The quantity of fatty acids in mMol/24 h =

\[ \frac{ml\ 0.1\ N\ KOH \times 2 \times 1.04 \times \text{quantity (g) of feces/24 h}}{10 \times \text{loss in g}} \]

The quantity of fatty acids in mMol/24 h =

\[ \frac{ml\ 0.1\ N\ KOH \times 0.208 \times \text{quantity (g) of feces/24 h}}{\text{loss in g}} \]
Factor 1.04 takes into account the increase in volume of the paraffin ether caused by the addition of ethanol.

**Break-up of fecal lipids**

The feces are homogenized, without being heated, with chloroform, methanol and acetic acid, and water, depending on the water content in the feces. Following the gravimetric determination of all the lipids, analysis is performed to ascertain the content of phosphorus, nitrogen and free fatty acids in the feces. After the isolation of the phosphorides by means of silicic acid, the esterified fatty acids, glycerides, coprosterols and cholesterol are denoted. Chromatographic break-up of fecal lipids is very difficult because of the presence, in great numbers, of free fatty acids and fatty acids with additional polar groups and chromogens. Our own numerous attempts proved that it is extremely difficult to separate successfully free fatty acids from phosphatides and glycerides.

**RESULTS**

**Correlations between the excretion of fatty acids, daily fecal weight and nitrogen excretion.**

In the case of 162 patients examined, a dependence was observed between the excretion of fatty acids in mMol/24 h
and in mMol/100 g of dry fecal mass, between the daily weight of fresh feces and its dry mass, as well as between the nitrogen excretion in g/24 h and g/100 g of the dry fecal mass. Correlations between the quantity of excreted fatty acids in mMol/24 h and in mMol/100 g of dry fecal mass and between the quantity of excreted fatty acids in mMol/24 h and the daily weight of dry fecal mass were observed in the group of patients examined.

<table>
<thead>
<tr>
<th></th>
<th>( \bar{x} )</th>
<th>( s )</th>
<th>( r )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fatty acids mMol/24 h</td>
<td>8.98</td>
<td>5.37</td>
<td>1—2</td>
<td>0.57</td>
</tr>
<tr>
<td>2. Fatty acids mMol/100 g of dry fecal mass</td>
<td>23.57</td>
<td>18.62</td>
<td>1—3</td>
<td>0.22</td>
</tr>
<tr>
<td>3. Weight of fresh feces g/24 h</td>
<td>185.43</td>
<td>197.49</td>
<td>1—4</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2—3</td>
<td>-0.38</td>
</tr>
<tr>
<td>4. Weight of dry fecal mass g/24 h</td>
<td>31.07</td>
<td>24.32</td>
<td>2—4</td>
<td>-0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3—4</td>
<td>0.63</td>
</tr>
</tbody>
</table>

1. Fatty acids mMol/24 h; 2. Fatty acids mMol/100 g of dry fecal mass; 3. Weight of fresh feces g/24 h; 4. Weight of dry fecal mass g/24 h.
### Table II

**Korelacje między wydalaniem kwasów tłuszczowych a ciężarem kału dobowego u 52 chorych z podwyższonym wydalaniem kwasów tłuszczowych**

**Correlation between the excretion of fatty acids and daily fecal weight in 52 patients with increased excretion of fatty acids**

<table>
<thead>
<tr>
<th>1. Kwasy tłuszczowe mMol/24 h</th>
<th>56,90</th>
<th>46,33</th>
<th>1—2</th>
<th>0,48</th>
<th>&lt;0,001</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Kwasy tłuszczowe mMol/100 g suchej masy kału</td>
<td>86,32</td>
<td>57,10</td>
<td>1—3</td>
<td>0,45</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>3. Ciężar kału świeżego g/24 h</td>
<td>316,31</td>
<td>163,21</td>
<td>1—4</td>
<td>0,49</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>4. Ciężar suchej masy kału g/24 h</td>
<td>70,16</td>
<td>46,93</td>
<td>3—4</td>
<td>0,75</td>
<td>&lt;0,001</td>
</tr>
</tbody>
</table>

1. Fatty acids mMol/24 h; 2. Fatty acids mMol/100 g of dry fecal mass; 3. Weight of fresh feces g/24 h; 4. Weight of dry fecal mass g/24 h.

In cases of steatorrhea it was possible to show a positive correlation between the quantity of excreted fatty acids in mMol/24 h and the daily weight of fresh (unevaporated) feces (Table I & II). In the case of the normal excretion of fatty acids and with a regularized diet the quantity of dry fecal mass is almost constant and may serve as a standard for assessing the excretion of fatty acids. When a regularized diet is not possible, it is recommended that the calculation
of data on the excretion of fatty acids should be based on mMol/24 h; however, daily variations in fecal weight should be taken into consideration and the feces should be collected for, at least, three days.

Table III

Wyniki oznaczeń kwasów tłuszczowych w kale w 36 przypadkach schorzeń trzustki

Results of determinations of fatty acids in feces in 36 patients with pancreatic diseases

<table>
<thead>
<tr>
<th>Wydalenie kwasów tłuszczowych mMol/24 h</th>
<th>≤20,0</th>
<th>20,1-42,0</th>
<th>&gt;42,1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatitis chronica</td>
<td>8</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Cystis, Fibrosis cystica</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Carcinoma, Stat. p. resection.</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Excretion of fatty acids mMol/24 h

The dependence between the excretion of fatty acids and nitrogen was examined in the case of 65 patients with normal excretions and 28 patients with fatty excretions. The correlation between these two parameters in the patients with normal excretion of fatty acids showed a probability error of less than 5%, while in patients with lipid feces, less than 1% (5).
Table IV

Results of determinations of bile acids in 150 patients with gastro-intestinal diseases

<table>
<thead>
<tr>
<th>Wydalenie kwasów tłuszczowych mMol/24 h</th>
<th>1</th>
<th>20,1–42,0</th>
<th>≥42,1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ogółem</td>
<td>100</td>
<td>26</td>
<td>24</td>
</tr>
</tbody>
</table>

1. The excretion of fatty acids mMol/24 h;
2. Total;
3. Examples;

* Note: Table IV has been abbreviated by the Publishers. The complete table, including 24 diseases and disease syndromes presented by the author, is available from the Publishers.
Results of determining fatty acids in various diseases

When examining our data on the patients we observed that lipid feces most frequently occurred in pancreatic diseases. 20 patients out of 36 suffering from various pancreatic diseases were noted to have excreted fatty acids in the amount over 20 mMol/24 h (Table III). The more acute the malfunction of the pancreas' extrasecretory system, the greater and more frequent was the steatorrhea. After a partial
resection of the pancreas in 4 patients, their excretion of fatty acids became normal (below 20 mMol/24 h).

Among 150 patients with gastro-intestinal diseases (Table IV), 50 were observed to have fecal lipids. Following the Billroth II operation, 23 out of 49 patients operated on had fecal lipids. The highest excretion of fatty acids of about 300 mMol/24 h was observed in one patient who was suffering from hyperchlorhydria (with basic excretion of over 40 mEq HCl/h) and was suspected of having the Zellinger-Ellison syndrome. A very large quantity of fatty acids, 200 mMol/24 h, occurred in two patients suffering from exudative enteropathy. In the case of female patient who, suffering from fibro-sarcoma, had all her small intestine removed except for 30 cm, the very high excretion of fatty acids of 192 mMol/24 h was noted. Temporary steatorrhea was observed in 5 out of 15 patients suffering from acute enteritis of the large intestine. One patient had about 12 bowel movements daily and excreted fatty acids in the amount of 70 mMol/24 h. In the cases of irritable colon and other disorders of enteric canal we did not observe any lipids in the feces. Among 36 patients with liver and biliary tract diseases 4 had light steatorrhea (Table V). One patient suffering from liver cancer and, due to it, chronic jaundice had steatorrhea of 88 - 123 mMol of fatty acids excreted daily. However, occasional fecal lipids could be observed
in a group of 40 patients who were unlikely to have any of the enteric canal diseases. Two of these patients were suffering from cancer of the bronchi; one had, in addition, parapemphigus. A temporary increase in the excretion of fatty acids also occurred in one female patient suffering from pyelonephritis. A patient with Pfeiffer-Weber-Christian syndrome and fecal lipids of 95 mmol of fatty acids per 24 h was noteworthy.

In the cases of abundant lipid feces it was possible to detect by immunoelectrophoresis the presence of serum albumin in feces.

We did not succeed in establishing correlations between the excretion of fatty acids and the level of lipids in blood, neither did we succeed with the xylose test or with the age, sex and weight of the examined patients.

The results of the research into particular fecal lipid fractions

Table VI and VII* present the mean values and standard deviations of lipid fractions in the feces of 40 patients with normal excretion of fatty acids and 40 patients with increased excretion of fatty acids of various origins.

* Table VII, containing the detailed results of lipid break-up in every patient examined, is available from the publishers.
In cases of steatorrhea not only was a general increase in the excretion of all the lipids and non-esterified fatty acids observed, but also, in a highly statistically significant manner (\( p < 0.001 \)), of sterols and phospholipids. The probability of error related to the value of the increased excretion of esterified fatty acids in the group of patients with steatorrhea was less than 5% as compared with the group of patients with a normal excretion of fatty acids. The probability of error in relation to triglycerides was 5% (6).

**DISCUSSION**

It is often believed that the mixture of lipids excreted in feces consists solely of unresorbed food fats. However, in reality, a considerable part of the fecal lipids is made up of lipid components of secretions produced by digestive glands, and also from flaked off cells of enteric epithelium; a certain amount of the fecal lipids is of bacterial origin. It seems that in the case of steatorrhea the lipids excreted in the feces are mostly of endogenous origin. The excretion of lipids may, in this case, surpass the intake of fats in food, or it may continue to be high in spite of a reduced intake of fats in the diet. Since fecal lipids appear in many gastro-intestinal diseases they are of relatively frequent occurrence. A quantitative
### Table VI

Wartości średnie (ś) i odchylenia standardowe (s) frakcji lipidowych w kale u chorych z normalnym i podwyższonym wydalaniem kwasów tłuszczowych

<table>
<thead>
<tr>
<th></th>
<th>Chorzy z normalnym wydalaniem</th>
<th>Chorzy z podwyższonym wydalaniem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>s</td>
</tr>
<tr>
<td>1. Lipidy ogółem g/24 h</td>
<td>5,82</td>
<td>2,95</td>
</tr>
<tr>
<td>2. Niezestrzyfikowane kwasy tłuszczowe mMol/24 h</td>
<td>9,57</td>
<td>7,41</td>
</tr>
<tr>
<td>3. Zestrzyfikowane kwasy tłuszczowe mMol/24 h</td>
<td>0,71</td>
<td>0,72</td>
</tr>
<tr>
<td>4. Glicerydy mMol/24 h</td>
<td>0,32</td>
<td>0,40</td>
</tr>
<tr>
<td>5. Fosfor zawarty w lipidach mg/24 h</td>
<td>4,61</td>
<td>6,93</td>
</tr>
<tr>
<td>6. Azot zawarty w lipidach mg/24 h</td>
<td>53,87</td>
<td>69,83</td>
</tr>
<tr>
<td>7. Sterole g/24 h</td>
<td>1,23</td>
<td>1,06</td>
</tr>
</tbody>
</table>

1. Total of lipids g/24 h ;  
2. Non-esterified fatty acids mMol/24 h ;  
3. Esterified fatty acids mMol/24 h ;  
4. Glycerides mMol/24 h ;  
5. Phosphorus content in lipids mg/24 h ;  
6. Nitrogen content in lipids mg/24 h ;  
7. Sterols g/24 h ;  
8. Patients with normal excretion of fatty acids ;  
9. Patients with increased excretion of fatty acids.

The analysis of the fatty acids excreted in feces is the most reliable method of determining the fecal lipids. The method of measuring the lipid resorption by means of
isotopes is uncertain (2). Unsaturated fatty acids can be marked with the following isotopes: $^{125}\text{I}$, $^{131}\text{I}$ and $^{132}\text{I}$. Following the oral application of the marked lipids the radioactivity of plasma and feces is examined. Though easy to apply, this method permits possibilities of error. The marked preparations of triolein and oleic acid that are available on the market are usually impure and not durable. The duration of the marked fats in a stomach varies and depends on the activity of the stomach and pylorus. It is not certain whether in the short period of time required for the partial break-up of the end products of lipolysis, the isotope is still with the fatty acid molecule or has become a part of other compounds. The separation of iodine from the iodine-marked compounds takes place in the intestine, liver and blood. In the case of triolein the separation is twice as great as in the case of oleic acid. Due to the resorption and excretion in urine of the radioactive iodine not bound with lipids, the calculated amount of the excreted lipids is less than the actual amount. With this method, in contrast with the chemical method of quantitative determination of the lipids in feces, one should allow for a 25% margin of error. The permanent marking of fatty acids can be achieved by $^{14}\text{C}$ and $^3\text{H}$. By measuring the amount of the exhaled $^{14}\text{CO}_2$ it is possible to assess the utilization of fatty acids by the organism rather than their resorption in the enteric canal.
The methods used up until now to measure the resorption of lipids are of limited value. They do not suit physiological conditions and, therefore, should be defined as tests of lipid utilization. Cases of steatorrhea are most frequently classified according to their original and secondary forms or their congenital and acquired forms or even to steatorrhea's being caused by maldigestion or malabsorption. The transformation of lipids in the enteric canal is a very complex process and it can be disturbed in many places. Many causal factors, both pathophysiological and biochemical, have to be considered in the study of the pathogenesis of steatorrhea (7). A clear-cut distinction between maldigestion and malabsorption is, in our view, still unlikely to be achieved in the present state of knowledge and the present diagnostic possibilities. The hydrolysis and resorption of food components obviously occur at the same time and place.

In view of the facts stated above, the pathogenic factors of steatorrhea should not be studied separately but together with the whole complex of phenomena.

The following are pathogenic factors:

A. Disorders of lipolysis.

1) Deficiency of lipase (extrasecretory disorder of pancreas).
2) Inhibition of the changes in lipolysis due to pH by means of antibiotics or by the deficiency of bile acids.
B. Deficiency of detergents (conjugated bile acids, phosphatides, mono- and di-glycerides).
   1. Decrease in the synthesis and production of bile acids.
   2. Hydrolysis and oxidation of the conjugated bile acids.
   3. Disorders in reversible absorption of fatty acids (diseases or resection of the ileum, treatment with cholestyramine).

C. Disorders in motor activities (enteritis, hyperthyroidism, gastro-intestinal disorders following enterectomy, symptoms of dyspepsia).

D. Disorders in absorption caused by the diminished surface of intestinal mucosa.
   1. Adherence or atrophy of the villi on the mucous membrane of the small intestine.
   2. Enterectomy, internal fistulas, gastroileostomies.

E. Disorders in the secretion of mucus.

F. Inhibition of the enzyme activity caused by cystostatics, antibiotics or X-ray treatment.

G. Special enzymatic defects (enteropathies caused by gliadin, enteropathies caused by milk albumin, deficiencies of the enzymes that break up disaccharides).

H. Inhibition of chylomicron formation by disturbing the synthesis of beta-lipoproteins.

I. Disorders in the lymph outflow (lymphadenitis, fistulas
through lymphatic vessels into the intestines, tumorous
diseases, congenital anomalies).
J. Disorders in blood supply.
K. Disorders in the control of hormones.

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Adres: II Medizinische Universitätsklinik, DDR — 402 Halle, Leninallee 2