Clinical monitoring of steatorrhoea in cystic fibrosis

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Abstract
In 100 patients with cystic fibrosis the severity of steatorrhoea was assessed by three separate methods. Using chemical faecal fat assay as the gold standard, two other rapid and inexpensive methods were compared with it. The steatocrit method proved unreliable in our hands and gave little indication of the presence or severity of steatorrhoea. The more simple microscopy method was highly sensitive (97%) and only three of 80 patients with steatorrhoea were missed. All patients with severe steatorrhoea (>60 mmol fat/day) were clearly demonstrated. The method is applicable to spot faecal samples and can readily be carried out on an outpatient basis. In centres where faecal fat assays are not available, the simple and cheap microscopic examination will give some indication of the response to enzyme treatment and may also help to identify non-compliant individuals.

Chemical measurement of faecal fat in carefully collected faecal samples is the traditional method of assessing fat absorption. Repeated measurements are extremely helpful in assessing the effectiveness of pancreatic enzyme treatment. Adequate facilities for processing samples do not exist in many laboratories and, for this reason, many centres do not have faecal fat assays available to them. Consequently most patients with cystic fibrosis in the United Kingdom do not have faecal fat estimated.

Many other alternative methods to chemical fat estimation have been described, but most have various shortcomings. Some involve the administration of isotopes, $^{13}$I or $^{14}$C, and measurement of the isotope in either faeces or expired air. Other methods involve blood sampling after a fat load and measurement of serum triglyceride. None of these procedures is particularly suitable in children. A simple, non-invasive screening test, which would give reliable qualitative measure of faecal fat loss, would be particularly useful in children.

We have compared the results obtained from chemical faecal fat assays with microscopic examination after fat staining and with the recently described 'steatocrit' method in 100 patients with cystic fibrosis.

Patients and methods
One hundred patients with proved cystic fibrosis (age range 6 months–27 years) were studied as outpatients. Each patient received a constant fat diet for a seven day period; during this period they were also administered eight radio-opaque pellets three times per day, with each main meal. After five days equilibration, stools were collected for a further two days into strong polythene bags. The bags were x rayed and the pellets counted to assess the completeness of collections. The faecal samples were then homogenised with two volumes of water and the faecal fat determined chemically. For the purpose of comparison, faecal fat results are defined as: normal, <20 mmol/day; mild, 20–39 mmol/day; moderate, 40–59 mmol/day; or severe steatorrhoea, >60 mmol/day.

The homogenates were also examined after staining with Sudan III as follows, for neutral and split fats.

NEUTRAL FATS
One drop of faecal homogenate or a small pellet of faeces plus one drop of water was placed on a glass slide and one drop of 95% ethanol added and mixed with a coverslip. Two drops of Sudan III, saturated solution in 95% ethanol, were then applied and well mixed. The slide was then examined under low power (×150) for the presence of three triglycerides and soaps; these appear in various forms as wads, plaques, occasionally globular or almost crystalline, and generally stain a deep orange.

SPLIT FATS
Samples were applied to a glass slide as above and one drop of 36% acetic acid added and then well mixed using a coverslip. Two drops of Sudan III were then added and mixed in well. The slide was then heated gently, almost to boiling, three times and then examined while warm for split fats. The free fatty acids appear as intensely red-orange stained globules with great variation in size.

In 19 samples the microscopic fat examination of a pellet of solid stool before homogenisation was compared with the result in the same sample after homogenisation (table).

The steatocrit method was carried out on 50 of the chemically assayed faecal fat samples. A standard haematocrit tube (75 mm Dade Co) was filled with faecal homogenate and spun in a Hawksley haematocrit centrifuge for 15 minutes. The tubes were removed and carefully examined. Usually a lower solid layer of faecal debris and liquid layer above was evident, and sometimes an upper fatty layer above this. The lower solid (S) and upper fatty (F) layers were measured...
and the steatocrit expressed as a percentage using the formula:

\[ S = \frac{F}{S+F} \times 100 \]

Results

Microscopic examination was graded as negative where only traces of fat were seen, or +, ++, +++, +++ for increasing quantities of fat staining, either as neutral or split fats. Neutral fat usually stained as clumps rather than as discrete globules as seen with split fat. A one + corresponded to 10 or more fatty globules of 10 μm diameter or greater. Two ++ corresponded to a significant excess of fat and this result was given when the sample showed material with 20–100 fatty clumps or globules in the range 10–50 μm in diameter. Three +++ indicated appreciable excess fat and was given when examination showed very large numbers (>100) of fatty clumps or globules, many of large diameter.

The microscopic results in the 19 samples where both solid stool and homogenised samples were compared are shown in the table. Overall, there was excellent agreement for split fat microscopy, whether a solid stool sample or a sample of the resulting homogenate was used. In only two samples (Nos 2 and 18) was a slight discrepancy seen. Good agreement was also seen when neutral fat was examined, only three samples showing a slight discrepancy (Nos 3, 9, and 15).

**NEUTRAL FATS (FIG 1)**

Of 100 patients, 80 had steatorrhoea and 11 of these were negative on microscopy, giving a sensitivity of 86% and a positive predictive value of 91%. Of the 20 patients without steatorrhoea seven were positive on microscopy, giving a specificity of 60% and a negative predictive value of 56%.

**SPLIT FATS (FIG 2)**

Of the 80 patients with steatorrhoea, 77 were positive on microscopy, giving a sensitivity of 96% and a positive predictive value of 90%. Nine of the 20 patients without steatorrhoea...
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were positive on microscopy, giving a specificity of 55% and a negative predictive value of 79%.

In our hands the steatocrit method proved unreliable and, in many samples, we failed to get a meaningful result. Frequently it was impossible to obtain a clear fat layer at the top of the haematocrit tubes and the fat appeared to be retained by the solid layer at the bottom of the haematocrit tube. In 45 samples where a steatocrit value was obtained, there was little association with either the chemical faecal fat result (fig 3) or the microscopic staining and, using this method, neither the exclusion of steatorrhoea or assessment of its severity was possible.

Discussion
Proving dietary intake is adequate, a major factor in determining the nutritional state of patients with cystic fibrosis is their ability to digest and absorb the food components of the diet. Fat malabsorption is common in these patients and the resultant steatorrhoea is often severe. The only reliable method of assessing the severity of steatorrhoea is a measure of faecal fat loss, and this is also required for assessing the effects of enzyme treatment.

Faecal fat assays are unpopular with patients and ward and laboratory staff, and some centres do not have the assay available. While the chemical faecal fat assay, when available, will remain the gold standard for assessing fat malabsorption, a simple and rapid, qualitative or semiquantitative assay would be extremely useful, particularly in those centres without the necessary methodology, and where frequent changes in enzyme dose are made.

The initial description of the steatocrit method reported very encouraging results, and correlated well with chemical faecal fat assays. To our knowledge, only one other brief report has been published using this method, and the results were also satisfactory in excluding steatorrhoea. In our study we encountered considerable technical difficulties with this method even though, as far as we can determine, the method has been performed exactly as originally described. Some samples were analysed fresh without storage and again after storage, but storage time had no effect on the method. Various methods of homogenisation were attempted but, again, did not influence the results. The major technical difficulty was an inability to obtain clear separation of the fatty, aqueous, and solid layers after centrifugation. Frequently fat remained entrapped in the lower solid layer, demonstrated by Sudan III staining of the haematocrit tube contents. In the 45 samples where a result was obtained, there was no meaningful association between the steatocrit result and either the chemical faecal fat (fig 3) or the microscopic fat results. We have no explanation why our results are so different from the Italian workers, but in our hands this method would not serve as a screening test.

Stool microscopy has been reported by various authors previously, and the results have generally been encouraging. Drummey et al described the method as being simple, rapid and a valid method for screening for steatorrhoea, while Ghosh et al found the method useful in excluding steatorrhoea in children. More recently, Luk and Bin et al reported the method as both specific and sensitive. Indeed, a more recent report suggests that Sudan staining is specific for triglyceride and fatty acid and the method may be more specific than chemical faecal fat assay for assessing triglyceride and fatty acid losses.

The present results, particularly using split fat, showed a high sensitivity of 97% but specificity was only 46%. Of the 80 patients with steatorrhoea, only three were not detected by microscopy and all three had mild (20–40 mmol/day) steatorrhoea. The poor specificity of this technique will result in some patients without steatorrhoea being investigated further for non-existent steatorrhoea. There was some correlation between the microscopic grading and the severity of steatorrhoea; all patients with severe steatorrhoea (>60 mmol/day) were graded ++ or +++ by microscopy. Thus microscopic examination of faecal samples will detect the vast majority of patients with steatorrhoea and may give an indication of its severity.

It is suggested that information provided by regular faecal microscopy in patients with cystic fibrosis will detect those with severe steatorrhoea, which still occurs despite modern microsphere enzyme preparations. In those centres where faecal fat measurements are not available, monitoring of adequacy of pancreatic enzyme treatment by faecal microscopy will lead to better control of steatorrhoea and general nutrition.
M P Walters and J Gilbert were supported by the Cystic Fibrosis Research Trust.


