14C triolein breath test: an assessment in the elderly

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SUMMARY 14C triolein breath tests are highly sensitive in detecting fat malabsorption in adults, but experience in the elderly is sparse. We have evaluated 48 ‘normal’ subjects over the age of 65 years and compared the results with those of 46 normal subjects under 65 from a previous study. Results were calculated as eight hour cumulative values and expressed as percentages of the administered dose. Reference ranges have been calculated separately for the following age groups: 65 years or less (201 to 460), 66 to 75 (182 to 405) and over 75 (141 to 336). A clear age related decline in eight hour cumulative values was noted. The values fell by 23% between the ages of 17 and 65 years and by 25% between the ages of 65 and 87 years. Pancreatic insufficiency or bacterial colonisation of the small bowel is unlikely, but delayed intestinal absorption or the effects of slower metabolic rate cannot be excluded. This test is simple and practicable in the elderly and the study emphasises the importance of age in the interpretation of results.

Malnutrition is increasingly recognised as a major health problem in the elderly and malabsorption may be a significant contributing factor. The clinical diagnosis of malabsorption in the elderly individual is not easy as presentation is often atypical compared with that seen in younger patients. Malabsorption has been found in patients presenting with problems such as ‘off-feet’, mental deterioration, heart failure, and gangrene.

Tests currently available for the detection of malabsorption require careful consideration when applied to the elderly. There is deterioration of renal function with age, and this complicates the interpretation of tests of absorption dependent upon serum or urine measurements such as the ‘D-xylene’ tolerance test or the Schilling test. The estimation of faecal fat is traditionally regarded as the standard test for fat absorption but collection of faeces requires patient co-operation. Incomplete collections are a problem even with young subjects and the elderly have a particular resistance to the collection of stools. In the elderly, who are more likely to be infirm, complete collection requires constant supervision over several days, which is not practicable. Moreover, the diagnostic efficiency is only 80%, the test unpleasant and expensive as admission is normally required.

There is an increased interest in radioactive breath tests of fat absorption. Many have reported their experience with 14C labelled triolein breath tests. The simplicity of such tests makes them an attractive diagnostic tool for application to the elderly. It may not be appropriate, however, to extrapolate conclusions from these studies, so far drawn from predominantly young and middle aged adults, to the elderly.

We have previously evaluated the version of a 14C triolein breath test devised by West et al and examined the reproducibility of the procedure in subjects of 65 years or less. We have reported a decline of 20% in eight hour cumulative values between the ages of 17 and 70. In the current study subjects over the age of 65 have been examined to answer the following questions: (1) is the test equally simple to carry out in elderly subjects, (2) does the absorption pattern differ quantitatively or qualitatively from that of young subjects, and (3) is the test reproducible in this age group?
**Methods**

**SUBJECTS**

Tests were conducted with the written consent of the subjects and with hospital ethical committee permission.

Forty nine normal subjects were entered for the study, which 48 completed. There were 33 women and 15 men with an age range of 66 to 87 years (mean 73.2). For comparison we have taken the results of the 46 normal subjects who were under 65 in our previous study.

Subjects were recruited from the Age Concern ‘Pop-in’ Centre (n=19) and the geriatric outpatient clinics and the day hospital (n=29). They were included in the study after an assessment which established that the following criteria had been met:

- Absence of gastrointestinal symptoms, clinical chronic respiratory disease, and diabetes mellitus.
- Normal haematological profile with haemoglobin ≥12 g/l.
- Normal thyroid and liver function tests.
- Normal anthropometric measurements, as judged by normal weight for height, mid-arm circumference and triceps skin fold thickness.

In principle, a defined meal of known fat content containing $^{14}C$ triolein is administered and hourly samples of exhaled CO$_2$ examined for $^{14}CO_2$ activity. This is calculated as a percentage of the administered dose and an eight hour cumulative value of $^{14}CO_2$ is used to express fat absorption. Five microcurie (0.185 MBq) of $^{14}C$ triolein was added on the morning of the test to the 60 g meal as formulated by West et al (excluding the buttered toast or bread). The $^{14}C$ triolein test was conducted as previously described and is essentially the same as that of West et al.

Modifications to the collection system during the present study included a simple fluid trap to obviate any risk of aspirating collection fluid. This comprised a small perspex plate to which were clipped two plastic ‘Universal’ containers and the scintillation vial. These were linked by short pieces of plastic tubing (cut from an air inlet set) inserted into the caps of the bottles through rubber grommets to ensure an airtight seal. Expired air was blown over solid calcium chloride in the first container, through the empty second container and finally bubbled through the collection fluid in the scintillation vial.

For the purposes of reproducibility a second test was performed on nine individuals who agreed to have a repeat test after an interval of greater than six weeks.

Mean hourly values were plotted as a function of time for patients aged 65 years or less and those in the older age group. Additionally, the individual $^{14}CO_2$ exhalation patterns were examined to determine whether a peak exhalation had occurred before eight hours.

In selected patients $^{14}C$ glycocholic acid breath tests were performed as described by James et al, with the exclusion of the Lundh meal, and Pancreolauryl tests using fluorescein dilaurate in accordance with the instructions of the manufacturers of a diagnostic ‘kit’ (Simco Ltd, Guernsey, British Isles).

**STATISTICAL ANALYSIS**

Student’s $t$ test was used for univariate comparisons between groups and Hotelling’s $T^2$ test was used for multivariate comparisons. The methods of Deming and Morgan were used for regression analysis. Differences between proportions were tested by treating them as samples from binomial populations. Values in parentheses after mean values and regression coefficients indicate the 95% confidence intervals for the parameters. Unless otherwise indicated, significance testing has been performed at the 5% level.

**Results**

There was no difference in cumulative eight hour value, age, height, and weight between the hospital group (n=29) and the community group (n=19) by Hotelling’s $T^2$ test, therefore, the data were pooled.

Multiple regression analysis was used to test the effect of age, sex, height, and weight on the cumulative eight hour value in the pooled younger and older subjects:

- eight hour value = $239-16.1 (34.54) \times \text{sex}$
- $-1.89 (0.77) \times \text{age}$ (p<0.05)
- $+1.21 (1.95) \times \text{height}$
- $-0.55 (0.91) \times \text{weight}$

The eight hour cumulative value fell (p<0.05) with increasing age (Fig. 1) independent of sex, height, and weight, which had no effect on the value.

Simple regression analysis was carried out on subjects above and below 65 years of age. In both groups there was a significant decline in the cumulative eight hour values: (age 65 or less: eight hour value = 239 - 1.749 (1.17) × age); (age over 65: eight hour value = 486 - 3.085 (2.89) × age). The estimated decline between the ages of 17 and 65 years was 23%, with a further estimated 25% decline between the ages of 65 and 87. There was, however, no significant difference between these two regression coefficients, although the mean values in the two groups were significantly different. 310.8 for the younger age group and 260.9 for the older age group (Student’s $t$ test: p=0.0001).

The individual results and the two regression lines are illustrated in Figure 1. Significantly, seven subjects over the age of 64 had subnormal values by our previously estimated reference range (207–435.
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Fig. 1 Relationship between eight hour cumulative value and age in 94 normal individuals. The two regression lines are for individuals aged 65 or less and for those aged more than 65 years. Horizontal lines represent reference ranges for the following age groups: 65 years or less, 66 to 75 years and over 75 years.

95% confidence limits) for young and middle aged subjects. In an attempt to find a cause for these low values, we performed 14C glycocholic acid breath tests on five of these subjects and all produced normal results. We also performed pancreolauryl tests on

Fig. 2 14CO2 exhalation profiles in 46 normal individuals aged 65 years or less and in 48 individuals aged more than 65 years. Vertical bars represent two standard errors above or below the mean values.

four and no definite pancreatic deficiency was detected.

Reference ranges for the cumulative eight hour values were calculated for the following age groups: 65 years or less, 66 to 75 and over 75. These were 201 to 460, 182 to 405 and 141 to 336, respectively. As in our previous study, the data were logarithmically transformed before calculation of the ranges.

Mean hourly results were plotted against time for those patients aged 65 years or less, and for those in the older age group (Fig. 2). There was a significant difference between the two lines (Hotelling's T2 test, p=0.005), the younger patients exhaling a greater amount of 14CO2 each hour for the first seven hours. In the younger age group, 25 of 46 subjects (54-3%) reached a peak of 14CO2 exhalation before eight hours, whereas only 13 of 48 patients (27-1%) in the older age group did. There is a significant difference between these two ratios (p=0.0084).

Reproducibility Studies

The results are illustrated in Figure 3.

In an attempt to quantify reproducibility, a mean value has been calculated from the results of the first and second test. The calculated difference between either test result and the mean has been expressed as a percentage of the mean value. The average difference of a test value from the mean of each respective pair was 10-35% (1.2%) for the nine pairs. The same calculation was applied to the reproducibility figures in our earlier study of younger subjects (nine pairs) and a value of 4-2% (1.6%) was obtained. The differences from the mean are significantly greater in the elderly (p=0.0035, Student's t test). Reproducibility was therefore significantly lower in the elderly.
Discussion

All subjects found the meal acceptable and all but one completed the test under supervision without major difficulties. One subject accidentally aspirated the trapping agent and proceeded no further in the test. With reversion to a fluid trap no such problems arose subsequently.

The results in Figure 1 imply that fat absorption diminishes with age. There is some support in the literature for this. Becker et al showed definite diminution in the rate of absorption of fat by aged people using a chylomicron technique. Webster et al showed that the pattern of absorption in elderly subjects was significantly different from that of younger controls. Our study illustrates, within the limits of the procedure, that the decline in fat absorption with age is a continuing process and that the decline is apparently steeper in the over 65 age group. The question arises as to whether the decline is a normal function of aging, or whether it represents an increasing prevalence of pathological abnormalities in the 'normal' aging population - giving rise to 'abnormal' fat absorption as reflected by the 14C triolein breath test. Several possible factors relating to reduced absorption have been incriminated. Impaired uptake, delayed transintestinal transport, reduction in mucosal surface area, delayed expression of several enzymes in epithelial cells on the intestinal villus, pancreatic insufficiency and bacterial colonisation of the small bowel, even in the absence of a stagnant loop, are the factors that could, either in isolation or in combination, cause malabsorption in the elderly. Our results, in the small number of subjects tested revealed no evidence of pancreatic insufficiency or bacterial colonisation of the small bowel to account for the low values. Our finding that more subjects over 65 years do not reach an obvious peak within eight hours would support the possible hypothesis of impaired uptake and delayed transintestinal transport as a cause for low absorption in the elderly. Therefore, overall delayed absorption, rather than a true decline in absolute absorption may account for the low values. Alternatively the observations may be explained by the differences in basal metabolic rate as it is known that this declines throughout adult life. The investigation, as it stands, cannot distinguish between impaired and delayed absorption and the effects of declining metabolism.

As a further consideration, the possibility exists that the difference between younger and older patients may be an artefact related to the respiratory response to exercise. Higgs et al showed that CO2 production in response to exercise was greater in 'less fit' than 'fit' subjects. This implies that CO2 produc-

References


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