DEVELOPMENT OF A ^{13}C-BASED TEST FOR FAT ABSORPTION IN HUMANS

by
C Alison Lloyd

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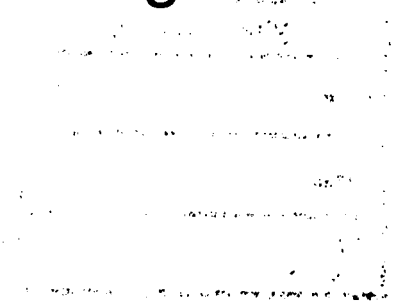

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KEY WORDS

^{13}C-triolein, fat absorption, breath test, humans, BreathMAT, IRMS

ABSTRACT

The assessment of fat absorption forms an important part of the diagnostic process in some patients being investigated for possible malabsorption. This study has assessed the use of ^{13}C-triolein as an alternative to the current ^{14}C-triolein test for fat absorption.

Initial work required an evaluation of performance characteristics of the isotope ratio mass spectrometer, observations on the normal variation in the exhaled breath $^{13}CO_2$:$^{12}CO_2$ ratio over one day, ie, the effect of food and exercise, the investigation of a number of potential test meals to aid isotope delivery and the effect of ^{13}C dose. A protocol has been developed for a test of fat absorption using ^{13}C-triolein as the marker substrate and lemon mousse as the test meal.

Breath samples from 18 healthy volunteers and 26 patients with potential fat malabsorption referred by the gastroenterologists, were studied after the ingestion of lemon mousse and 200 μL ^{13}C-triolein. Breath samples were taken before ingestion and for six hours after ingestion and analysed. This data has been compared with results of samples obtained using the ^{14}C-triolein test of fat absorption performed on the same patients at the same time. There was a significant difference between the peak DOB of the male and female volunteers ($p = <0.05$). In patients, lower peak DOB were observed in those with suspected fat malabsorption, than in those patients with normal fat absorption ($p = <0.005$).

Results are discussed in terms of a ^{13}C-triolein breath test using a lemon mousse fat meal as a simple non radioactive test for fat malabsorption. However, a larger clinical trial of patients is now required to confirm these initial results.

GLOSSARY

BMI	Body mass index
CCK-PZ	Cholecystokinin pancreozymin
CV	Coefficient of variation
Del13PDB	Delta 13 relative to PDB
DOB	Delta over baseline
DRI	Derbyshire Royal Infirmary
IBS	Irritable bowel syndrome
IRMS	Isotope ratio mass spectrometer
MCT	Medium chain fatty acid triglyceride
NEQAS	National External Quality Assurance Scheme
PDB	Pee Dee Belemnite
SD	Standard deviation
SOP	Standard operating procedure

1 AIM

To develop a fat absorption test based on ^{13}C-triolein which could be used in hospitals and Primary Care as an alternative to using radioactive ^{14}C-triolein because of concerns of radioactivity and to improve patient acceptability.

2 INTRODUCTION

The quantitative measurement of fat in the faeces of patients in order to assess malabsorption was first described in 1914 (1). Since that time, the assessment of fat absorption and malabsorption has continued to be used as a marker of gastrointestinal function in clinical practice. In this Introduction, a brief account of dietary fat and the processes involved in fat absorption, are given as background to the more clinical sections. These describe current diagnostic tests for assessing fat absorption and the place of isotopes in the evaluation of gastrointestinal function.

2.1 DIETARY FAT

Humans usually eat a mixed diet which, for continuing good health, must contain the following basic constituents: proteins, fats, carbohydrates, mineral salts, vitamins and water (2). Fat as a food has more energy (39 kJ/g) weight for weight, than any other dietary component (2). Fats are normally stored in the body as an energy reserve; subcutaneous fat also provides individuals with a layer enabling them to retain heat. Certain fats are also essential constituents of cells notably as components of membranes and neurological tissue.

Dietary fats are divided into two main types, animal fats and vegetable fats. Animal fats are largely mixtures of stearic, palmitic and oleic acids combined with glycerol as triglycerides and are obtained from meat and dairy produce. Butter contains 80% fat and double cream is 40% fat (2). Vegetable fats are found in plants, especially in seeds and nuts, for example, sunflower seeds,

olives and rapeseed. They are consumed in the diet in cocoa, chocolate, cooking oils, salad oils and in small amounts in some vegetables. Cooking oils are almost 100% fat and contain a mixture of triglycerides specific to the plant species from which the oil is derived. A comparison of fatty acids found in common cooking oils is shown in Table 2.1.

Table 2.1 – Comparison of fatty acids in some animal and vegetable fats (percentage composition by weight) (4)

%COMPOSITION (BY WEIGHT) OF FATTY ACIDS						
	SATURATED ACIDS			*UNSATURATED ACIDS*		
	PALMITIC	*STEARIC*	*OTHER*	*OLEIC*	*LINOLEIC*	*OTHER*
Animal fats:						
- Chicken fat	25.6	7.0	0.3	39.3	21.8	5.9
- Beef fat	29.2	21.0	3.4	41.1	1.8	3.5
Vegetable oils:						
- Corn/maize	8.1	2.5	0.1	30.1	56.3	2.9
- Olive oil	10.0	3.3	0.6	77.5	8.6	<0.1

On a typical Western diet, a human adult ingests about 100g of fat (40% of the energy required per day), of which 92% to 96% are long chain triglycerides. The rest is mostly phospholipids (1 to 2 g per day), and free and esterified cholesterol (3). Dietary fat is thus mainly composed of triglycerides. In the bowel, dietary lipids are mixed with another 50 to 60 g of endogenous lipids originating from the bile (bile salts, phospholipids and cholesterol), desquamated cells (mixed membrane lipids) and dead bacteria. Cholesterol

in the intestinal lumen is derived from bile salts and from the diet; only about 30% of dietary cholesterol is absorbed (3). The fat in faeces, around 4 g per day in the healthy adult, is derived relatively equally from dietary, biliary, cellular and bacterial sources (7). The low faecal excretion shows the efficiency of the overall process of fat digestion and absorption in the healthy adult. In children who are growing faster, the demand for energy and efficient fat absorption is even greater. The fat intake needed in a newborn baby would be equivalent to an adult ingesting 300 to 500 g of fat per 70 kg of body weight (3).

Fatty acids consist of an alkyl chain with a terminal carboxyl group, the basic formula of completely saturated species being CH_3-$(CH_2)_n$-COOH. Most fatty acids in humans have 16, 18 or 20 carbon atoms. The names and structures of some typical fatty acids that are of importance to humans are shown in Table 2.2.

Table 2.2 – Some fatty acids of importance to humans (5)

COMMON NAME	*NUMERICAL SYMBOL*	*STRUCTURE*	*SYSTEMATIC NAME*
Palmitic acid	16:0	CH_3-$(CH_2)_{14}$-COOH	Hexadecanoic acid
Stearic acid	18:0	CH_3-$(CH_2)_{16}$-COOH	Octadecanoic acid
Oleic acid	18:1(9)	CH_3-$(CH_2)_7$-CH=CH-(CH_2)-COOH	cis-9-Octadecenoic acid
Linoleic acid	18:2(9,12)	CH_3-$(CH_2)_3$-$((CH_2$-CH=CH$)_2$-$(CH_2)_7$-COOH	cis, cis-9-12-Octadecadienoic acid
Linolenic acid	18:3(9,12,15)	CH_3-$(CH_2$-CH=CH$)_3$-$(CH_2)_7$-COOH	cis, cis, cis-9,12,15, Octadecatrienoic acid
Arachidonic acid	20:4(5,8,11,14)	CH_3-$(CH_2)_3$-$(CH_2$-CH=CH$)_4$-$(CH_2)_3$-COOH	cis, cis, cis, cis, 5,8,11, 14-Eicosatetraenoic acid

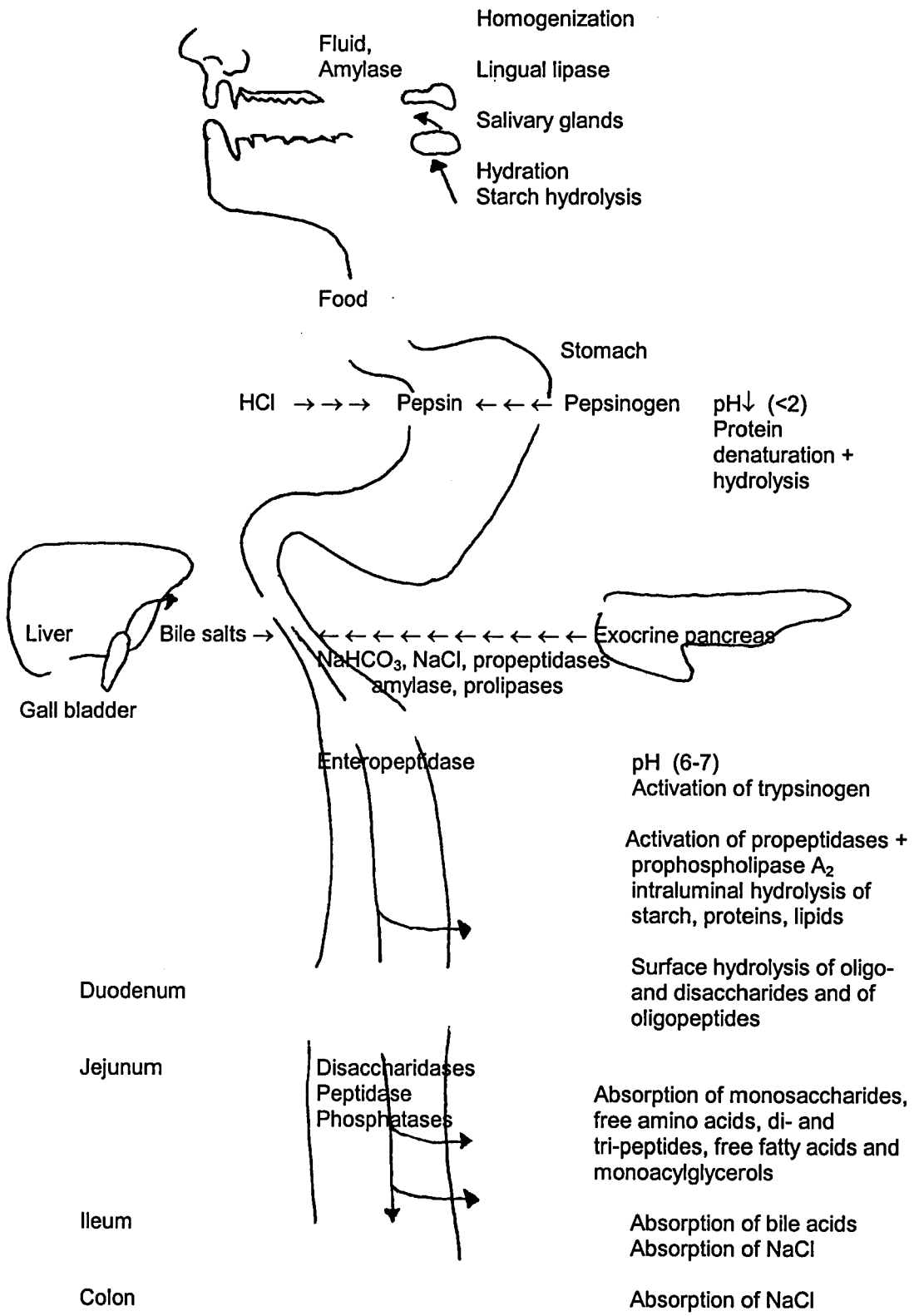
2.2 PROCESSES INVOLVED IN THE DIGESTION AND ABSORPTION OF DIETARY FAT

Normal intestinal absorption of dietary fat in man depends on adequate digestion of food. This requires normal pancreatic function, a normal area of functioning intestinal cells and the presence of bile salts. The absorption of cholesterol, phospholipids and fat soluble vitamins depends on normal triglyceride absorption (6). This process will be expanded on later. Malabsorption of fat, therefore, occurs when there is impaired function of the pancreas, or when the surface area available for absorption is reduced or when the concentration of the luminal bile salts is low.

The gastrointestinal organs and their functions are shown diagrammatically in Figure 2.1. Digestion begins as the mechanical process of the mastication of food, mixed with saliva, starts to reduce the size of the food particles (7). Lingual lipase is secreted by the dorsal surface of the tongue, this enzyme initiates lipid digestion.

In the stomach, secretion of hydrochloric acid in the gastric juice leads to acidification of the stomach contents and the "churning" process further breaks food particles down. Chyme is the name given to the partly digested food as it issues from the stomach into the intestine (4). It is semi liquid in consistency, with a pH of less than 2.

Figure 2.1 - Gastrointestinal organs and their functions (5)



Food is fully digested and absorbed in the small intestine. The common bile duct adds bicarbonate from pancreatic secretions which neutralises the acid from the stomach and increases the pH of chyme to about 6-7. At this pH dietary triglycerides are split by the lipases produced by the pancreas into glycerol and fatty acids. Large globules of fat are reduced in size to a fine emulsion by the action of bile which increases the surface area leading to greater efficiency in the catalytic activity of the lipases at the water/lipid interface.

There are three distinct phases in the process of fat absorption (8), the intraluminal phase, the mucosal phase and the intracellular phase.

2.2.1 The intraluminal phase

The intraluminal phase involves 4 lipases and 1 cofactor in the process of lipolysis.

- Lingual lipase functions in the acid pH of the stomach and plays a significant part in the hydrolysis of dietary fat. It also promotes the binding of colipase to pancreatic lipase and aids in liberating cholecystokinin-pancreozymin (CCK-PZ) from the duodenal mucosa, which stimulates gall gladder contraction and bile flow and increases the secretion of pancreatic enzymes (6).

- Pancreatic lipase functions at a pH of 6 to 6.5 in the duodenum, catalysing the hydrolysis of triacylglyerols to free fatty acids and 2-monoglycerols. It acts at the water/lipid interface of the emulsion

droplets. The concentration of lipase in the duodenal contents during digestion of a test meal is about 0.1 mg/mL. For a meal with a volume of 2,000 mL this is equivalent to 200 mg lipase. 200 mg of lipase can hydrolyse 540 g of triglycerides to diglycerides in 1 minute (8). As a normal meal contains about 30 g of triglycerides there is more than enough lipase to hydrolyse dietary fat in a very short time.

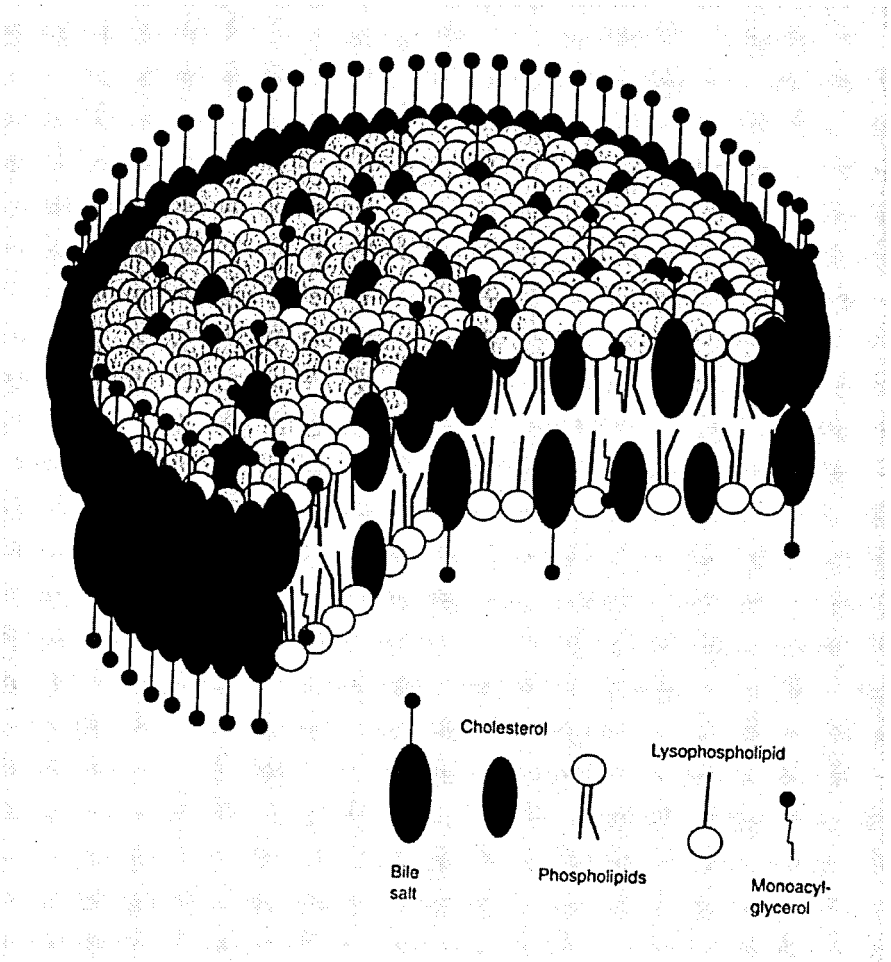
- Colipase binds to pancreatic lipase enabling lipase to compete at the water lipid interface with bile salts. Colipase is initially secreted in the pancreatic juice as a non-active form but is then activated by trypsin. Deficiency of colipase has been reported to lead to malabsorption of fat (8).

- Pancreatic carboxylester lipase, a non specific lipase in pancreatic juice, hydrolyses various neutral lipids, including fat soluble vitamin esters. This lipase is also known as bile salt stimulated lipase, cholesterol ester hydrolase or monoglyceride lipase. An immunologically identical enzyme is present in human milk and the milk of some primates (8).

- Phospholipase A2 is also found in pancreatic juice and is activated by trypsin. It catalyzes the hydrolysis of phospholipids, mainly phosphatidylcholines, to their lyso-forms and to free fatty acids (8).

Micelles are formed when the products of lipolysis, ie, fatty acids, monoglycerides, lysophospholipids and cholesterol, mix with the bile salts in solution in the small intestinal lumen. These micelles range in size between 40 and 60 μm depending on bile salt concentration and the ratio of bile salts to lipids. They are a bilayer disc with a band of bile salts on their outer edge and other more hydrophobic components protected within the interior (See Figure 2.2), (6).

Figure 2.2 - Structure of an intestinal micelle (5)



2.2.2 The mucosal phase

The mucous membrane in the intestine is the surface layer of epithelial cells covered with mucous together with the underlying fibrous tissue. There are two barriers to the uptake of nutrients from the lumen of the small bowel into the epithelial cells of the mucous membrane:

- Firstly, there is a diffusion barrier caused by "unstirred water" over the surface layer of epithelial cells; this prevents transport from the bulk phase of the intestinal contents to the cell membrane. The flux through this layer of water and mucous is a function of the concentration gradient across the layer and the permeability of the layer. The latter is determined by the thickness of the layer and the aqueous diffusion coefficient of the molecules in question.

- The second barrier is the cell membrane itself.

The fatty acids, monoacylglycerol, phospholipids and cholesterol protected within the micelles are able to pass through these two barriers from the lumen of the small bowel into the epithelial cells.

2.2.3 The intracellular phase

Monoglycerides and fatty acids from lipolysis are the main lipids entering the cytoplasm of the cells within the mucosa of the small intestine. Under the electron microscope, fat is initially visualised within the endoplasmic reticulum. A fatty acid binding protein with a molecular weight of 12,000 has been

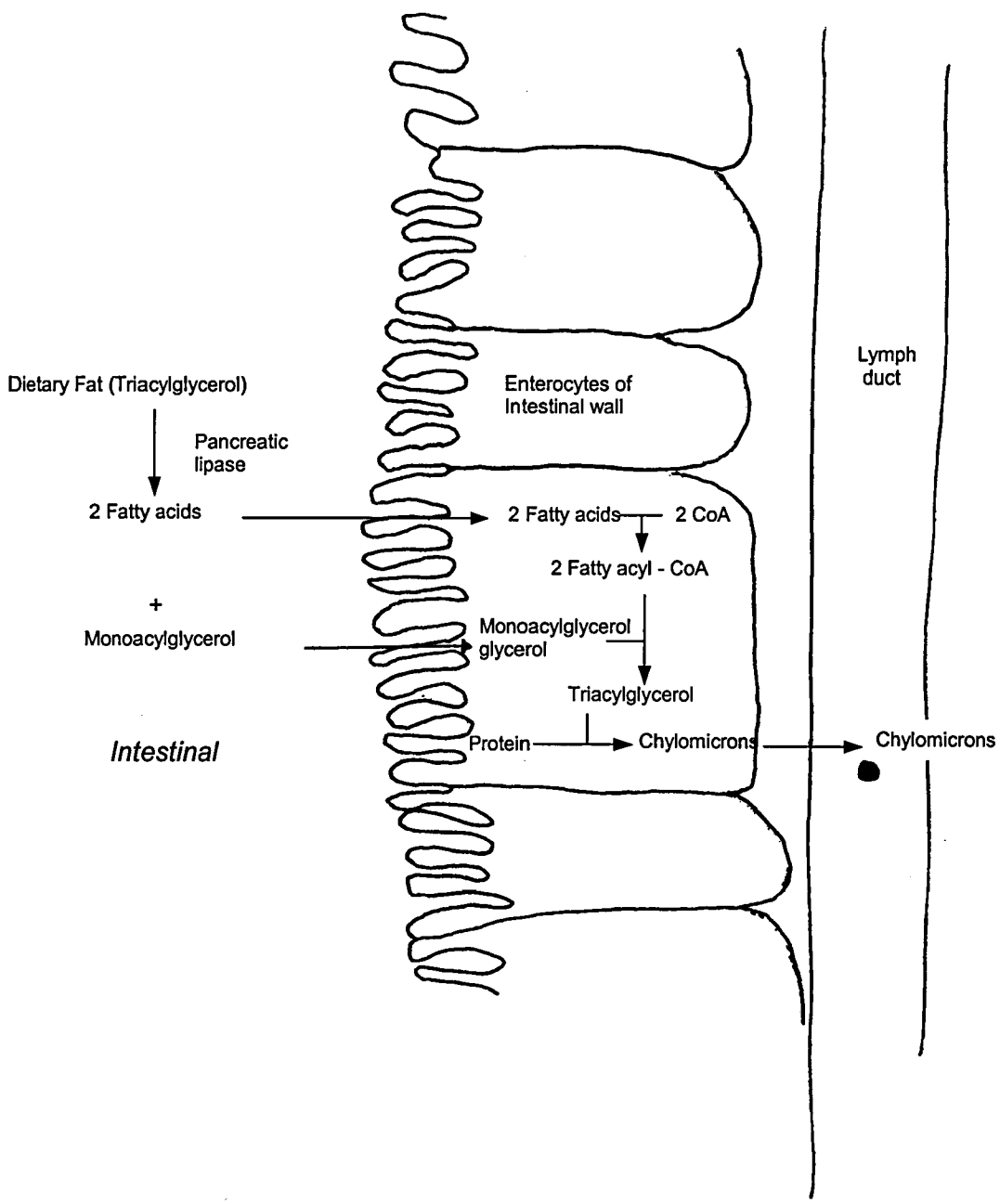
isolated from the enterocyte cystosol, which has a role in the transport of fatty acids from the membrane to the endoplasmic reticulum (8). The intracellular process includes the resynthesis of fatty acids and monoglycerides into triglycerides principally by the monoacylglycerol pathway catalysed by triacylglycerol synthetase shown diagrammatically in Figure 2.3.

The resynthesised triglycerides are delivered to the lymphatic vessels as chylomicrons. Chylomicrons have a diameter of 1 μm and in addition to triglycerides contain other absorbed lipids including cholesterol, fat soluble vitamins, and phospholipids, and some protein. The chylomicrons are transported from the abdominal lymphatic vessels via the thoracic duct to the systemic circulation from where they can then be assimilated into the metabolic processes of the body.

2.2.4 Absorption of medium chain fatty acid triglycerides

The processes described in Sections 2.2.1 to 2.2.3 are applicable to long chain triglycerides, however, the absorption of medium chain fatty acid triglycerides (MCT, triglycerides with fatty acids of chain length C8 to C12), is achieved through a different pathway. MCTs are hydrolysed by pancreatic lipase in the lumen more effectively than long chain triglycerides and their absorption is independent of bile (4).

Figure 2.3 - Processes involved in the transfer of dietary fat from the intestinal lumen to the lymphatic ducts (5)



After entry into the mucosal cells, fatty acids from dietary MCTs are transported to the portal blood and leave the intestine as free fatty acids partly bound to albumin. Of the MCTs, only the C12 fatty acids are found in the chylomicrons and in the adipose tissue or other lipid stores. Shorter chain length fatty acids (C8, C10) are rapidly metabolised to carbon dioxide and water in the liver.

In summary, dietary fat is composed mainly of non polar, long chain triacylglycerols which are virtually water insoluble. In this form fat is not available for digestion in the intestinal tract. The gastrointestinal tract secretes enzymes causing the lipolysis of fat and their conversion to more polar products which are then able to mix with water. Bile disperses the products of lipid digestion and helps overcome the diffusion barrier covering the mucous membrane of the small intestine.

2.3 THE CLINICAL PICTURE OF MALABSORPTION OF FAT AND OTHER DIETARY CONSTITUENTS

A patient with a mild case of malabsorption may complain to the doctor of vague abdominal symptoms and perhaps the common complaint of feeling "tired all the time". Routine laboratory tests will probably show normal results apart from anaemia, ie, a low blood haemoglobin concentration (6).

In severe and long standing generalised fat malabsorption, whether intestinal or pancreatic, the clinical picture may include generalised wasting and malnutrition (due to deficiency of proteins and fat soluble vitamins),

osteoporosis and osteomalacia (protein and calcium deficiency), oedema (hypoalbuminaemia due to protein deficiency) and tetany (hypocalcaemia due to vitamin D and calcium deficiency) (6).

If fat malabsorption is severe, the patient will complain of foul smelling, bulky, pale, greasy stools which are difficult to flush down the toilet. This condition is known as steatorrhoea. Significant steatorrhoea is defined as a daily faecal fat excretion consistently more than 14 g of fat (6).

Typical laboratory findings include (6) increased fat in the stools, anaemia (iron and/or folate and/or vitamin B12 deficiency), hypocalcaemia with hypophosphataemia, raised plasma alkaline phosphatase activity from clinical or sub clinical osteomalacia and rickets, a low concentration of serum albumin and prolonged prothrombin time caused by vitamin K deficiency.

The malabsorption of fat leads to the malabsorption of fat soluble vitamins A, D, E and K contained in the diet.

Vitamin A deficiency is rarely clinically evident in the United Kingdom (UK). In a Western diet, vitamin A is usually present in more than ample quantity and is stored in the liver. Many foods contain vitamin A, including carrots, egg yolk, liver, milk, butter and most green vegetables (6).

Vitamin D is formed naturally in the fatty tissues of the body when the skin is exposed to the action of daylight. Vitamin D deficiency, due either to

malabsorption or to dietary deficiency is common in the UK. Cod liver oil is one of the few foods that contain vitamin D naturally. Other foods, for example, margarine and breakfast cereals, contain added vitamin D in order to prevent deficiency. Vitamin D deficiency results in impaired calcium absorption. A low ionised serum calcium concentration stimulates parathyroid hormone secretion, which by causing phosphaturia, leads to hypophosphataemia and then to osteomalacia and rickets, and to osteoporosis (6).

In contrast, to vitamin D, vitamin K is present in many dietary constituents, being found in spinach, leafy vegetables, tomatoes and liver. It is needed for the hepatic synthesis of prothrombin and other clotting factors. A bleeding tendency associated with a prolonged prothrombin time, may develop in patients with severe fat malabsorption. This prothrombin deficiency, unlike that due to liver disease, can be reversed by parenteral dosage of vitamin K (6).

The malabsorption of fats and fat soluble vitamins is often accompanied by the maldigestion or malabsorption of dietary proteins. For example, in pancreatic disease, the digestion of protein is severely impaired due to the deficiency of proteolytic enzymes in addition to the reduction in the output of lipolytic enzymes. This can lead eventually to generalised muscle and tissue wasting, to osteoporosis and a reduced level of all protein fractions in the blood plasma. A low plasma albumin concentration may cause oedema and a reduction of plasma total calcium concentration. Reduced antibody formation

(immunoglobulins) may make a patient more susceptible to infection. In intestinal disease, amino acid and peptide malabsorption may occur in addition to fat malabsorption, due to impaired intestinal transport mechanisms.

2.4 CURRENT TESTS FOR ASSESSING THE ABSORPTION AND MALABSORPTION OF FAT

Fat malabsorption thus has significant immediate and long term clinical consequences. There is therefore a need for clinicians to be able to identify patients with fat malabsorption in order to diagnose and then treat the underlying cause.

A wide variety of methods have been used in clinical laboratories to assess fat absorption. They can be divided into two groups depending on the methods involved. There are those that measure fat excretion in faeces and those that measure fat absorption after an oral "test meal". The latter methods involve measurement of metabolic products in blood, urine or breath after the consumption of the test meal.

2.4.1 Measurement of fat excretion in faeces

Fat in faeces is present principally as triglycerides, free fatty acids or soaps. Triglycerides in faeces contain mainly long chain fatty acids and are derived from dietary fat which has not been absorbed or from intestinal secretions. The latter are the main source in a healthy individual but in steatorrhoea, excess fat is of dietary origin (9). The fatty acids are produced by the luminal

hydrolysis of triglycerides by pancreatic lipase and by bacterial enzyme action. Soaps, present in the faeces, are the sodium, potassium and calcium salts of fatty acids.

Bowel action is irregular and assessment of fat in a 24 hour stool collection does not provide adequate discrimination between normal and abnormal fat excretion. Most quantitative measurements of fat in faeces are therefore made on a 3 day faeces collection. Analyses of total fat content is then carried out on a faecal homogenate with fat being detected by either titration or infrared spectroscopy. In the traditional faecal fat procedure, alkaline hydrolysis (saponification) of neutral fats produces glycerol and soaps. After acidification, the fatty acids are extracted into light petroleum ether and quantified by titration (9).

The use of infrared spectroscopy for faecal fat determination is relatively new (9) and not widely used in clinical laboratories in the UK. In this procedure, after extraction, using acidified petroleum ether-ethanol, the fatty acids are dried and dissolved in chloroform. An infrared spectrum is recorded in the range 4,000-650^{-1} cm and absorbance data used to calculate faecal fat excretion (10).

Another method is the detection of fat globules in a random faecal sample. After vigorous mixing, a small sample of the faeces is placed on a slide, stained with Sudan III and examined under a light microscope for fat globules (11). The "steatocrit" procedure is another simple method. A random faecal

sample is collected and 0.5 g is diluted with two volumes of water. This is homogenised and an aliquot transferred to a haematocrit tube. After centrifugation, three layers are produced; a lower solid layer (S), an intermediate liquid layer and an upper fatty layer (F). The lengths of the layers are measured and the "steatocrit" calculated as a percentage (12) as follows:

Steatocrit = F x 100/ (F + S)

Prior acidification of stool improves the recovery of the fat. The normal adult range is 8-23%. In pancreatitis and in small bowel disease (12) the range is approximately 20-70%.

2.4.2 Measurement of fat absorption after a "test meal"

Tests that assess fat absorption after an oral "test meal" can be divided into those that require the collection of blood or breath samples for analysis. The "butter fat" test involves the measurement of chylomicrons by turbidity or the analysis of triglycerides in blood serum. The butter fat test was developed by Bentley, Eastham and Lane in 1975 and involves the patient fasting for 12 to 14 hours (13). A fasting blood sample is taken and the serum separated. The patient then ingests a meal consisting of 50 mL of unsweetened orange juice, 2 slices of buttered toast (0.5 g butter per kg body weight) and unsweetened tea or coffee. A second blood sample is taken 2 hours later and the serum separated. The sera are then diluted and the Light Scattering Intensity (LSI) measured in a nephelometer which indicates the chylomicron concentration as a measure of absorbed fat (9).

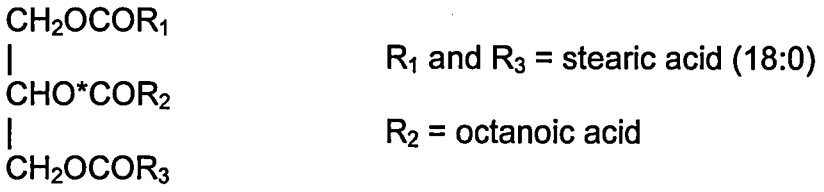
The ^{14}C-triolein breath test (14) assesses malabsorption using a pre-prepared fat meal. This is the current method used by many hospitals including Derbyshire Royal Infirmary (DRI). After fasting overnight, a baseline breath sample is taken followed by ingestion of a meal containing 19.3 g of fat and 185 kBq ^{14}C-triolein. Breath test samples are then collected over the following 6 hours. Levels of labelled $^{14}CO_2$ in the breath samples are measured using a scintillation counter. Generally, ^{14}C-triolein is used as the fat marker in preference to ^{14}C-tripalmitin or ^{14}C-trioctanoin (15). The Duncan survey (16) showed that UK laboratories using this procedure all administer similar amounts of radioactivity, ie, 5 μCi (185 kBq) per patient, although the amount of fat given varies from 0.5 g to 60 g per patient. Triolein breath tests are influenced by the amount and type of calories given in the accompanying meal (17). A fat intake of 0.5 g does not represent a realistic fat challenge but increasing the calorific intake of food either as fat or carbohydrate inhibits metabolism of the labelled fat (18). Newcomer (15) showed that the peak of $^{14}CO_2$ was lower and occurred three hours later when a 50 g fat meal was given instead of 20 g. Duncan in 1992 (17) similarly demonstrated that with a 60 g fat meal, the production of CO_2 was delayed with only 42% of subjects reaching a peak by the ninth hour. Cumulative $^{14}CO_2$ excretion gave a better discrimination. He concluded that the triolein breath test was preferable to faecal fat measurement. He noted that it avoids the unpleasant faecal collection and analysis for the patient and laboratory staff, provides results the following day, is well tolerated by patients and is easily performed on an outpatient basis.

2.4.3 Tests of fat absorption used to assess pancreatic function

The tests described in the preceding 2 sections are all tests which assess the overall process of fat absorption, however, there are also methods that look specifically at pancreatic function. In the Pancreolauryl Test, urine is collected from the patient on two separate days following ingestion of test meals. On the first day, 2 slices of buttered bread are eaten with 350 mg of fluorescein dilaurate which is a substrate for pancreatic lipase. On the second day, 2 slices of buttered bread are eaten with unesterified fluorescein. On both days urine is collected for 10 h following ingestion of the test meals (19). Fluorescein is a harmless, colourless dye which becomes orange green and fluoresces at an alkaline pH, and can be measured by spectrophotometry or fluorimetry. The amount of fluorescein excreted in the urine after oral ingestion of the dilaurate is an indication of pancreatic digestion. The absorption and excretion of free fluorescein on day 2 is used to control for any changes in small bowel absorption or renal excretion of the dye. A normal pancreolauryl excretion index (expressed as fluorescein excreted on Day 1 as a percentage of that excreted on Day 2), suggests that the patient does not have chronic pancreatic disease or at least not in a sufficiently advanced form to reduce the exocrine secretion to less than 5-10% of normal. An abnormal result suggests further investigation is required (ultrasound scanning) to separate the true from the false positives.

Another non invasive test of pancreatic lipase activity in the duodenum is the ^{13}C mixed chain triglyceride breath test, (20). This test uses 1,3-distearyl, 2[carboxyl-^{13}C] octanoyl glycerol which contains a ^{13}C stable isotope labelled

medium chain fatty acid in the 2 position and long chain fatty acids in the 1 and 3 positions. The rate limiting step in the digestion of the mixed triglyceride is hydrolysis of the two stearyl groups by pancreatic lipase.



The pancreatic lipase cleaves the octanoic acid. The absorption of octanoic acid is not dependent on micelle formation. The ^{13}C-octanoic acid is oxidised via acetyl CoA to $^{13}CO_2$. The ^{13}C mixed chain triglyceride breath test assesses the levels of intraluminal pancreatic lipase whereas the ^{14}C-triolein fat absorption test provides a global assessment of fat absorption.

2.4.4 Review of the performance of tests used to assess fat absorption

Analytical performance of most tests carried out in clinical chemistry laboratories in the UK is monitored through the UK National External Quality Assurance Scheme programmes (NEQAS), initially set up in the late 1960s.

Laboratories have to subscribe to this programme as one of the criteria for accreditation with the Clinical Pathology Accreditation Board. The scheme covers most biochemical analytes, so that laboratories can monitor their own analytical performance against other laboratories with the objective of continual quality improvement. Until recently, the NEQAS scheme however, did not cover tests for gastrointestinal function. In order to assess the need to include gastrointestinal tests within the range of procedures included in the

NEQAS scheme, Dr Duncan of Glasgow Royal Infirmary, carried out a comprehensive survey of gastrointestinal function testing in hospital laboratories in 1997 on behalf of the Association of Clinical Biochemists and the British Society of Gastroenterology. A total of 233 laboratories and 330 clinicians took part in the survey which highlighted significant problems in the way that many of these tests for fat absorption were carried out (16).

The survey showed that a wide variety of tests were used to assess fat absorption in the UK. The faecal fat test described by van de Kamer et al. in 1949 (21) was the most widely available test and was used by 67% of gastroenterologists responding to the questionnaire.

In normal health, faecal fat is largely derived from endogenous rather than dietary sources. Adequate dietary fat intake is therefore essential if false negative results are to be avoided. The British Society of Gastroenterology guidelines recommend a diet containing at least 70 g of fat for 6 days (22). However, in the UK only 20% of laboratories attempted to ensure adequate fat intake of patients undergoing this test. A further source of error noted was that patients with steatorrhoea may reduce their fat intake to control their diarrhoea which could lead to falsely low results.

When the titration step of the faecal fat test was evaluated in an external quality assessment, between laboratory CVs for three samples ranged from 31 to 42% (16). The main reason for the wide analytical variation was that 82% of the laboratories surveyed had no internal quality control procedures

for faecal fat analysis. Also as there was no external quality control scheme, the laboratories had no comparative data to use to monitor the validity of their results. Infrared spectroscopy has recently been described as an alternative analytical procedure which should give improved CVs but there will still be problems of dietary input and sample collection (10). The equipment needed for infrared spectroscopy is not available in most clinical laboratories at present in the UK.

The faecal microscopy test was used by 43% of clinicians. A disadvantage of this test is that as faecal fat excretion is a function of dietary fat intake (23), malabsorbing patients on a low fat diet will produce a normal result. The Duncan survey (16) showed that in 91% of laboratories providing the faecal microscopy test, fat intake was not controlled, so that false negative results were likely. This test is qualitative rather than quantitative and the visual examination by microscopy is a subjective measurement. Several members of staff analysing samples also contributed to the wide variability in results (24).

The fat load or butter fat test has some advantages over the other tests in that the procedure is very simple, no complex analytical procedures are required, and there is no need for faecal collections. This test was only available in 21% of laboratories and was used by just 11% of clinicians, and where available was only requested, on average, once every two weeks. Wide inter-laboratory variations in the test protocol with differences in the type of fat used and in the times of blood collection were reported (15). The approved

protocol used 0.5 g fat/kg body weight in the form of butter with collection of blood at zero and 2 hours. This test protocol, however, has been shown (13) to give very unsatisfactory results, with large overlap between normal subjects and patients with malabsorption. When choosing biochemical tests their sensitivity, (the fraction of those with a specific disease that the assay correctly predicts) and their specificity, (the fraction of those without a specific disease that the assay correctly predicts) are compared (25). The fat load test has poor sensitivity unless fat malabsorption is severe and in addition the false positive rate was high. The low sensitivity and specificity of the test make it unsuitable for routine use.

The Pancreolauryl Test was available in 60% of laboratories surveyed (16). Analysis of urinary fluorescein is simple and CVs between laboratories was acceptable at 10%. The test is inconvenient for the patient, because urine is collected over 2 days and the patient must drink large volumes of water on both days of the test. It is however useful in detecting pancreatic exocrine deficiency, with the sensitivity of 85% and specificity of 88%. Clinicians should be aware that false positive results may occur after vagotomy, cholecystectomy or biliary bypass surgery (6). Normal results may be found if pancreatic disease is mild. The Pancreolauryl Test is not a "global" test for fat malabsorption, because it assesses only the intraluminal lipolysis step.

The ^{14}C-triolein breath test is a simple method of assessing fat malabsorption but was only used by 9% of clinicians (16). The main disadvantage is that the test uses a radioactive isotope and is therefore unsuitable for the investigation

of premenopausal women or children. In addition, scintillation counters are not available in many hospitals. Several papers note that the excretion rate of $^{14}CO_2$ is influenced by the endogenous production rate of carbon dioxide (17, 26, 27). They report that this should to be corrected for by measuring carbon dioxide output which can vary considerably between patients. The survey (16) found that no laboratories measured CO_2 output. In practice, CO_2 production was assumed on the basis of body weight (9 mmol/kg/h) or a correction was made for body surface area.

The ^{14}C-triolein breath test has many advantages over other tests for fat malabsorption. As triolein is a long chain triglyceride the test assesses the whole process of fat absorption, ie, it is a global fat absorption test. It is simple to perform and can be easily performed in an outpatient clinic setting. The ^{14}C-triolein test has been used in the author's hospital, Derbyshire Royal Infirmary (DRI), since 1985. In an evaluation of 57 patients, a sensitivity of 85% and a specificity of 93% were found for the detection of fat malabsorption (14). However, with the need to reduce the use of radioactive isotopes the use of this test should be minimised.

2.5 USE AND MEASUREMENT OF ISOTOPES IN GASTROENTEROLOGY

Isotopes are atoms of the same element that differ in atomic mass, due to differences in the number of neutrons. The three most abundant isotopes of carbon are carbon 12 (^{12}C) which contains 6 neutrons; carbon 13 (^{13}C) which has 7 neutrons; and carbon 14 (^{14}C) which has 8 neutrons (28). Having too

few or too many neutrons compared to protons causes isotopes, such as ^{14}C to be unstable. These unstable radioisotopes will decay to stable products. Other isotopes such as ^{12}C and ^{13}C do not decay because their particular combinations of neutrons and protons are stable. On earth 98.89% of carbon found is ^{12}C and 1.11% is ^{13}C (29) with only trace quantities of ^{14}C.

Both radioactive and stable isotopes are currently used in the investigation of gastrointestinal disease. They are regularly used in gastroenterology to label substrates which are then used as tracers for the investigation of metabolic pathways, to measure body composition, energy balance, protein turnover and fuel metabolism (29).

2.5.1 Radioactive isotopes

A list of procedures used in gastroenterology using substrates labelled with radioactive isotopes is shown in Table 2.3. Care has to be taken not to administer them to children, to pregnant women or to women of child bearing age who may become pregnant. There are also many regulations governing all aspects of the use, administration to patients and disposal of radioactive materials (14).

Table 2.3 – Some radioactive isotopes used diagnostically in gastroenterology (30)

ORGAN	*SUBSTRATE*	*MAIN USE*
Small bowel	^{14}C-D-xylose	Bacterial overgrowth
Small bowel	^{75} Selenohomocholyltaurine	Bile acid malabsorption
Intestinal	^{14}C-triolein	Fat absorption
Intestinal	^{14}C-trilinolenin	Fat absorption
Intestinal	^{14}C-tripalmitin	Fat absorption
Intestinal	^{57}Co or ^{58}Co B_{12}	Vitamin B_{12} absorption
Colon	^{111}In white cells	Inflammatory bowel disease

The radioactive vitamin B_{12} test is an example of the use of a nonstable isotope. It is used to identify those patients with a deficiency of intrinsic factor. Vitamin B_{12} contains cobalt and may be labelled by incorporating either ^{57}Co or ^{58}Co into the molecule (30).

Leucocytes labelled with ^{111}Indium are used in the investigation of inflammatory bowel disease, both for establishing the presence of the disease and in assessing its activity, particularly in acute disease or when an abscess or fistula are suspected. The labelled leucocytes have a half life of 7 hours and migrate to the areas of infection thus highlighting the inflammation. Images are taken after 4 hours in cases of inflammatory bowel disease and after 24 hours if an abscess is suspected (30).

As has already been described the ^{14}C-triolein breath test is used to assess fat absorption (13). Following ingestion of ^{14}C-triolein with a test meal,

patients are asked to breathe into a vial containing a fixed amount of alkali (hyamine) solution to trap exhaled CO_2. The vial also contains an indicator to show that the predetermined amount of CO_2 (usually 2 mmol) has been trapped. The amount of ^{14}C present can then be determined by liquid scintillation counting in a beta counter. It is necessary to assume a basal rate of CO_2 production (9 mmol/kg/h) in order to express the results as a percentage of the amount of ^{14}C administered (see Appendix 1).

2.5.2 Stable isotopes

Radioisotopes have been widely used, however, increasing awareness of environmental issues and the regulatory control of these compounds have stimulated the search for alternatives.

Stable isotopes fulfil many of the requirements needed for an ideal marker for use in a clinic setting. They are non radioactive, and can safely be administered to young and old, to male and female. The sample collection is simple, the tests are repeatable and provide a non invasive way of assessing gastrointestinal function (31). The disadvantage is the detection of a small change relative to large background levels of stable isotopes.

Stable isotopes do have limitations, they are more expensive to purchase than radioactive isotopes and require specialist apparatus for testing enrichment. Also $^{13}CO_2$ breath tests are performed with a large natural abundance of ^{13}C (1.1%) which could lead to errors when identifying changes due to ingestion of ^{13}C.

Table 2.4 – Some stable isotopes used diagnostically in gastroenterology (32)

ORGAN	*SUBSTRATE*	*MAIN USE*
Stomach	^{13}C-urea	Detection of Helicobacter pylori
Stomach	^{13}C-acetate	Gastric emptying (liquids)
Stomach	^{13}C-octanoate	Gastric emptying (solids)
Small bowel	^{13}C-lactose	Lactose tolerance
Small bowel	^{13}C-xylose	Small bowel overgrowth
Small bowel	^{13}C-lactose-ureide	Transit time
Liver	^{13}C-aminopyrine	Liver function
Liver	^{13}C-caffeine	Liver function
Intestinal	^{13}C-glycocholic acid	Bile acid malabsorption
Pancreas	^{13}C-hiolein	Pancreatic function test
Pancreas	Glycerol-1,3,distearyl-2-^{13}C-octanoate	Pancreatic function test
Pancreas	Cholesteryl ^{13}C-octanoate	Pancreatic function test
Pancreas	^{13}C-starch	Pancreatic function test

The ^{13}C urea breath test is probably the most widely used stable isotope test. It is used for the detection of *Helicobacter pylori*, a common cause of histological gastritis and the causative organism of most peptic ulcer disease and gastric cancer (33). The bacteria has extremely high urease levels which is the basis of the diagnostic test for detecting abnormal gastric colonisation (34). After an oral dose of the ^{13}C urea, $^{13}CO_2$ rapidly increases in concentration in the breath of *Helicobacter pylori* positive subjects as the organism splits the urea, producing ^{13}C-labelled bicarbonate in the stomach, which is absorbed into the blood and exhaled as $^{13}CO_2$. The breath $^{13}CO_2$, 15 minutes after ingestion of a capsule containing ^{13}C urea, is compared with

the basal breath $^{13}CO_2$. The $^{13}CO_2$:$^{12}CO_2$ ratio is measured by isotope ratio mass spectrometry (IRMS) (see Appendix 2).

The ^{13}C-hiolein breath test (mixed long chain triglyceride uniformly labelled [98%] with ^{13}C) is a non radioactive test for exocrine pancreatic insufficiency (35). The fatty acid composition of the hiolein is shown in Table 2.5.

Table 2.5 – Fatty acid composition of hiolein (35)

FATTY ACID	*%*
Oleic (C18:1)	51
Linoleic (C18:2)	20
Palmitic (C16:0)	17
Other C18	5
Other C16	4
Other (unspecified)	3

This test has similar methodology to the urea breath test. A standard rice snack (1.5 g per kg of patient weight) is ingested with 2 mg per kg labelled hiolein. Then $^{13}CO_2$:$^{12}CO_2$ enrichment in exhaled breath is measured by IRMS.

The lactose breath test is used by clinicians to detect patients who are unable to digest lactose due to lactase deficiency. The ^{13}C -lactose breath test has advantages over unlabelled lactose, which is used in the hydrogen breath test for lactase deficiency The recovery of the carbon isotope in breath $^{13}CO_2$

after digestion, absorption and metabolism of the labelled disaccharide in the small intestine is a more direct measure of lactase activity than the measurement of hydrogen liberated by the fermentation of unabsorbed disaccharide by colonic flora in the large bowel (31). The methodology is similar to the above two examples.

2.5.3 Principles of isotope ratio mass spectrometry

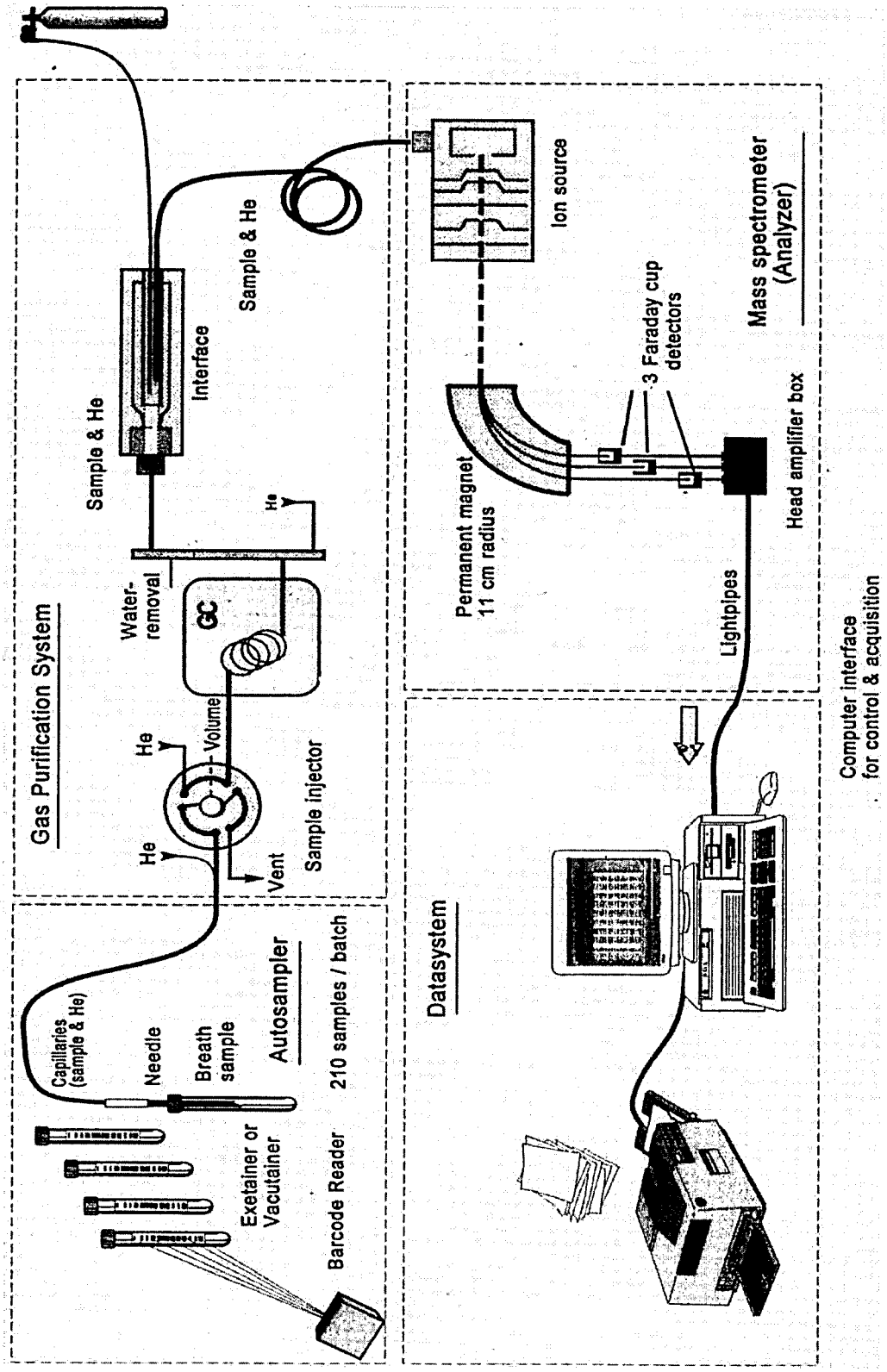
In the 1950s geochemists first began investigating stable isotopes in rocks and soils. Detecting small differences in the natural abundance of stable isotopes can help test hypotheses about the history of the earth. High precision measurements were needed but speed of results was not so important. Geochemists might only analyse 10 samples a day. Biologists have different requirements, they use materials which are artificially enriched with stable isotopes as tracers of natural cycles and pathways. They may not need such high precision instrumentation but they do need the ability to run large numbers of samples with adequate precision.

The basis of mass spectrometry is the production of ions from neutral compounds and the examination of the subsequent fragmentation of those ions (36). A substance can be characterised by investigating the distribution of ions resulting from that substance. As the technique involves a chemical reaction, the sample being investigated is not recoverable but this is not usually a problem as only a very small quantity of the material is needed for the analysis. As with any chemical reaction, the precise outcome, the mass

spectrum, is dependent on a number of factors such as temperature, concentration and effects of the medium (36).

As can be seen in Table 2.4 substances labelled with ^{13}C are commonly used in clinical investigations. In organic matter about 1.1% of carbon is ^{13}C. When a relatively ^{13}C rich substrate is ingested, it will be digested and absorbed and the ^{13}C will then enter into oxidative metabolic pathways leading to enrichment of bicarbonate, protein, fat and carbohydrate within the body. The breath will be enriched with $^{13}CO_2$ relative to the basal situation. Breath can be collected and treated to separate CO_2 from other components, and the proportion of $^{13}CO_2$ measured by gas isotope mass spectrometry. Figure 2.4 shows a schematic of the BreathMAT IRMS used in this study.

Figure 2.4 - Schematic of BreathMAT IRMS (37)



3 OBJECTIVES

To develop a test that will:

- Eliminate the use of radioactive material
- Be as accurate as the radioactive method (see Appendix ^{14}C fat absorption test.
- Be simple in method to enable any health care professional to perform the test.
- Be "patient friendly"
- Simple sample collection

The current study set out to assess the possibility of using a ^{13}C-labelled long chain triglyceride as a marker of fat absorption and malabsorption in the clinical setting. Previous local experience has been with a comparable 14C-labelled substrate (Appendix II), which was used as a "gold standard" in studies in patients described in this thesis.

The objective of this thesis has been to develop a ^{13}C-based test for fat absorption in humans.

The use of ^{13}C substrate would eliminate the use of radioactive materials. The aim has been to develop the test which would be as accurate as the current radioactive method (^{14}C fat absorption test, see Appendix II). The test should also be simple in method and sample collection to enable any health care professional to perform the test. The test needs to be "patient friendly" to ensure attendance and compliance with dietary restrictions.

4 METHODS AND MATERIALS

4.1 MATERIALS

4.1.1 Chemicals

^{13}C-Triolein: Glycerol [1,1'1"-^{13}C] trioleate, catalogue number CLM-163. Cambridge Isotope Laboratories, Andover, Mass, USA.

^{14}C-Triolein; glycerol tri [1-^{14}C] oleate, code CFA 258, 9.25 MBq, 3.7 MBq/mL. Amersham Pharmacia Biotech, Little Chalfont, Bucks.

Carbon Dioxide, 652 Grade 4.5, (reference CO_2 for BreathMAT), Air Products plc, Basingstoke.

Ethyl alcohol, absolute. Hayman Limited, Eastways Park, Witham, Essex.

Helium 689 Grade A. BOC, Manchester.

Hydroxide of hyamine 10x (diisobutyl-cresoxyethoxyethyl) dimethylbenzyl-ammonium hydroxide, 1M solution in methanol, Canberra Packard, Brook House, Pangbourne.

Olive oil, Co-op, extra virgin.

Scintillant, Emulsifier Safe, Canberra Packard, Pangbourne, Berks.

Thymolphthalein crystalline (5',5"-diisopropyl-2',2"-dimethyl-phenolphthalein), Sigma, Aldrich Company Limited, Fancy Road, Poole, Dorset. Prepared as a 500 mg/L solution in ethyl alcohol.

4.1.2 Foods used to aid isotope delivery

Lemon mousse, see Appendix VII.

Chunky Kit Kat (55 g). Nestle.

Duobar, natural flavour (45 g). SHS International Limited, Liverpool.

Scandishake, strawberry flavour (85 g). SHS International Limited, Liverpool.

4.1.3 Apparatus

BreathMAT Plus, Isotope ratio mass spectrometer. Finnegan MAT, GmbH, Bremen, Germany.

Drinking straws. Labco Limited, Bucks.

Exetainers, 10 mL glass tubes with gas tight caps. Labco Limited, Bucks.

Hair dryer.

One way valves, XCO 160 4/7 OD mm. Analytical Supplies Limited, Derby.

Pipette 200 μL capacity. Fisher Scientific UK, Loughborough, Leics. Pipette tips, Universal disposable 0-300 μL. Fisher Scientific UK.

Scintillation Counter, TRICARB 2300TR. Canberra Packard.

Scintillation Vials, Poly Q vials. Beckman R11C Limited, High Wycombe, Bucks.

Teaspoon (household), stainless steel.

Weighing boats, plastic.

4.2 METHODS

4.2.1 Collection of breath samples

The patient was instructed to inhale normally, then to hold their breath for 5 seconds before exhaling. These instructions were identical for the collection of breath samples for the analysis of $^{14}CO_2$ and $^{13}CO_2$.

4.2.1a Collection of breath samples for $^{14}CO_2$ analysis

The patient exhaled through a straw assembly (see Appendix II) into 4 mL of a 50:50 mixture of 1 mol/L hyamine hydroxide and ethanol containing 100 μL of thymolphthalein solution in a scintillation vial. When the solution changed from blue to clear, 2 mmol CO_2 had been trapped.

4.2.1b Collection of breath samples for $^{13}CO_2$ analysis

The subject exhaled through a straw into the bottom of an Exetainer tube. When condensation appeared on the sides of the tube, and exhalation was complete, the tube was capped and labelled.

4.2.2 Analysis of $^{14}CO_2$ in breath samples

In the ^{14}C-triolein breath test, 185 kBq (5 μCi) ^{14}C-triolein was given to the patient in a test meal of lemon mousse containing 19.3 g of fat. End expiratory breath CO_2 was trapped by exhalation into hyamine hydroxide as described above. 10 mL of the scintillant Emulsifier Safe was added to each vial and ^{14}C activity measured by liquid scintilliation counting. For further information, see ^{14}C-triolein breath test Standard Operating Procedure (SOP) (see Appendix II).

4.2.3 Analysis of $^{13}CO_2$ in breath samples

4.2.3a BreathMAT IRMS

After collection of breath samples in Exetainers, the levels of $^{13}CO_2$ were analysed using the BreathMAT IRMS. For further information, see BreathMAT IRMS SOP, Appendix IV.

4.2.3b Expression of $^{13}CO_2$ values

Stable isotope abundances are expressed as the ratio $^{13}C/^{12}C$ in the sample compared with the same ratio in an international standard.

The international standard for carbon is Pee Dee Belemnite (PDB). This is calcium carbonate ($CaCO_3$) from a cretaceous belemnite, *Belemnitella americana* from the Pee Dee formation in South Carolina, USA (37). The generally accepted absolute ratio of $^{13}C/^{12}C$ for PDB is 0.0112372 (29). The isotopic abundance for $^{13}CO_2$ in a standard gas or breath sample is expressed as the ratio of $^{13}C/^{12}C$ in parts per thousand ("per mil", ‰) compared to the CO_2 produced from PDB calcium carbonate. The "delta" (δ) notation, in units per mil (‰) is used to express the difference in abundance between samples; thus PDB has a value of 0 and materials with ratios of more than 0.0112372 have positive delta values and those with ratios of less than 0.0112372 have negative delta values (29).

The BreathMAT software uses the abbreviation Del13PDB to denote the difference of the sample $^{13}CO_2$ abundance from to PDB. This abbreviation is

therefore used throughout this thesis; alternatives in the literature are δ^{13}PDB, del13PDB or δ^{13}C.

For comparison of sequential breath samples from one individual, the convention is to express the $^{13}CO_2$ abundances in parts per thousand relative to the baseline sample. The change from the baseline is described as DOB (ie, the change in the Del13PDB value of an individual sample from the Del13PDB of baseline sample; the "delta over baseline").

4.2.3c Maintaining analytical performance of $^{13}CO_2$ measurement

The performance and precision of the BreathMAT has to be continually monitored to ensure accurate results.

Peak shape stability and high voltage scan: the peak shape (see Figure 4.1) shows the intensity (mV) of the signals arriving at each cup (for mass 44, 45 and 46) over a range of high voltage 2,765 to 2,810 volts. The upper trace is the ratio of masses 45/44. This upper trace can be expanded to observe the flatness of the ratio trace. This procedure was carried out every 3 months or after maintenance.

Time scan: the signal stability (the drift or noise) can be observed using the time scan mode over 600 seconds. This procedure was completed every time the BreathMAT was used. The time scan should be horizontal (ie, no change in ratio over the 10 min period) and the amplitude (noise) of the signal should not exceed 0.001 (Figure 4.2).

Figure 4.1 - Demonstration of BreathMAT peak shape stability

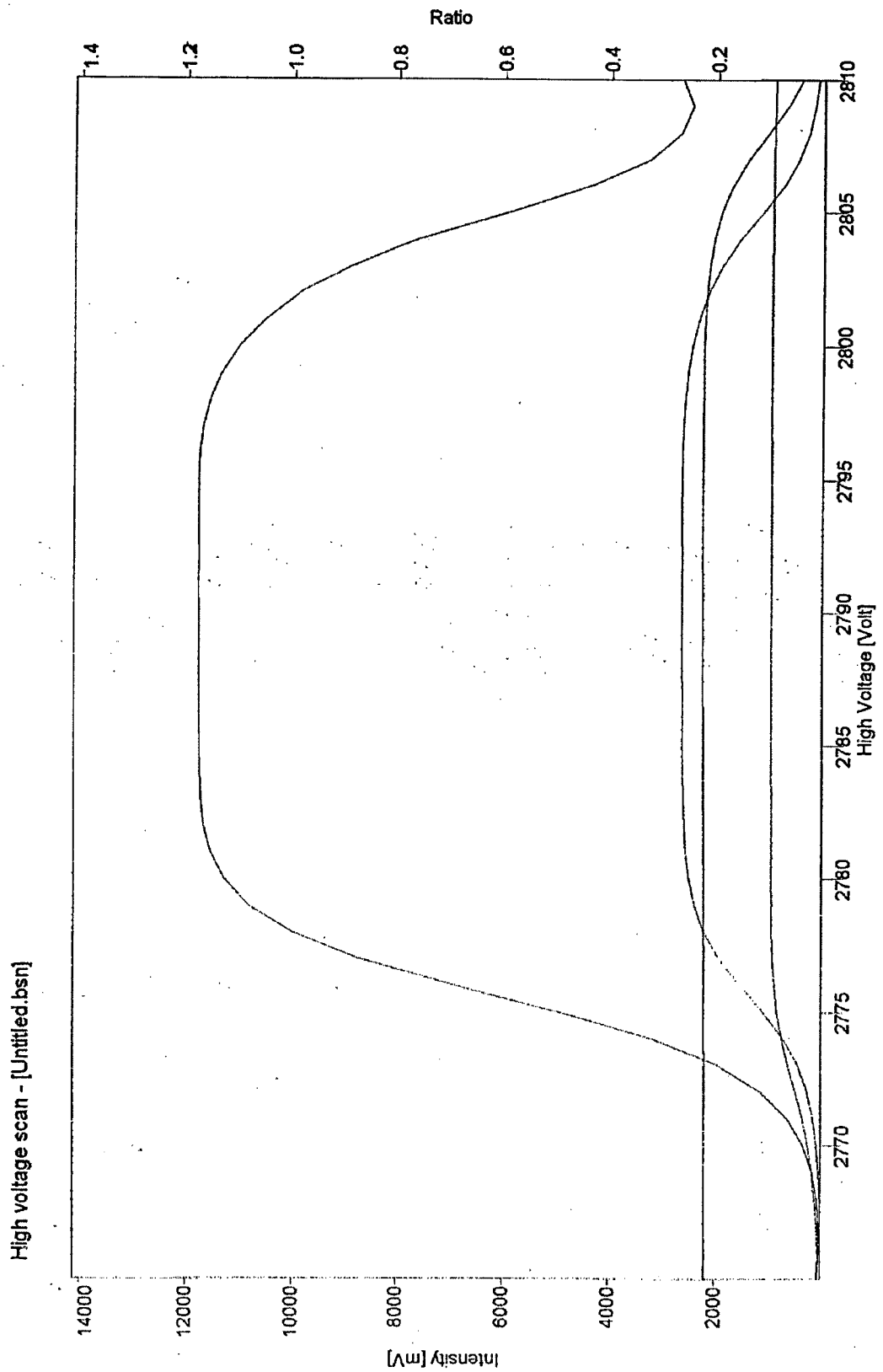
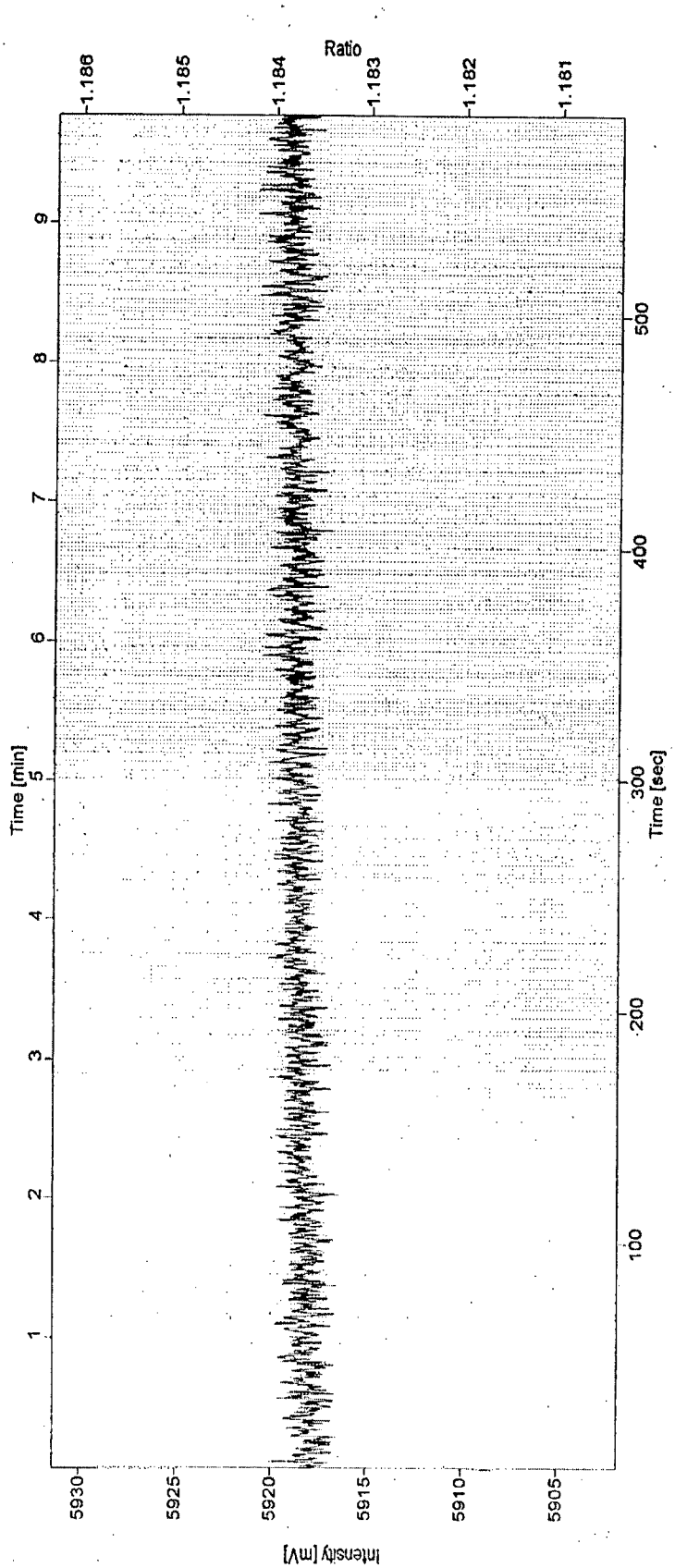


Figure 4.2 - Time scan - signal stability of BreathMAT



Zero enrichment (internal reproducibility): to check internal reproducibility, the BreathMAT pre set sequence (CO_2zero 20) was run. The reference CO_2 gas was sampled 20 times in sequence. This procedure was carried out every 3 months or after maintenance.

Precision (10 replicates of the same breath sample): to ensure the precision of the results the operator's own breath was collected in the prescribed manner into 10 separate Exetainers. These were sampled one after the other on the BreathMAT.

Linearity correction: the measured sample Del13PDB value may be influenced by the total CO_2 concentration of that sample. To ensure the precision of results over a range of CO_2 concentrations, a single sample of the operator's breath was sampled up to 10 times using a BreathMAT pre set sequence ("Multimet"), to provide a range of CO_2 concentrations.

When Del13PDB results were found to vary with CO_2 concentration, a "linearity correction" was made following the manufacturer's instructions either manually or using software now available on the analyser.

Between batch precision: to ensure that variation between batches of samples fell within acceptable ranges, the operator's own breath and three patients samples (DOB values <1, 1.0-2.0 and >5) were evaluated in 5 consecutive batches.

4.2.4 Development of fat absorption test using ^{13}C-triolein

4.2.4.1a Assessment of the precision of dispensing 200 μL of olive oil

The successive increases in weight as 200 μL samples of olive oil were dispensed from a pipette on to a weighed plastic weighing boat were recorded. The mean, standard deviation and coefficient of variation were calculated.

4.2.4b Breath $^{13}CO_2$:$^{12}CO_2$ ratios; natural variation over one day

The natural variation in $^{13}CO_2$:$^{12}CO_2$ ratios in breath samples over one day was determined by collecting breath samples in the prescribed manner (4.2.1b) from a single subject every 30 minutes over the course of 1 day. Food and drink were consumed as normal. A note was made of the type of food consumed and of activities undertaken by the volunteer. All samples were collected in duplicate. Samples were evaluated on the BreathMAT IRMS, and the mean Del13PDB was calculated for each pair of samples.

The first sample was taken as the baseline (the subject had fasted for 8 hours). All subsequent samples were compared to this baseline value and results expressed as DOB ‰.

4.2.4c Breath $^{13}CO_2$:$^{12}CO_2$ ratios; changes after ingesting various test meals to aid isotope delivery

The subjects fasted overnight then after collecting a baseline breath sample, the test meal (see 4.1.2) was eaten and breath samples were collected over

the next 6 hours. The breath samples were collected in the prescribed manner, (4.2.1.b).

4.2.4d Breath $^{13}CO_2$:$^{12}CO_2$ ratios; changes after ingesting 200 μL ^{13}C triolein with lemon mousse or Scandishake as the test meal

The volunteers were identified and written consent obtained. In order to ensure that an adequate response was observed with a 200 μL ^{13}C-triolein dose, breath samples were collected and analysed in the prescribed manner over a period of 6 hours. They were allowed to eat a light lunch after completion of the 4 hour breath samples.

4.2.5 Protocol for ^{13}C-triolein fat absorption test

The patient or healthy volunteer was identified and a letter given explaining the test and fasting requirements (subject fasted from midnight the night before the test, if the test is scheduled to start at 9.00 am). The subject was allowed to have a cup of tea before the test and a light lunch 4 hours after the start of the test; smoking was not permitted at any time. The amount of exercise during the time of the test was restricted. The breath sample containers were labelled clearly with bar codes and patient identifier. The collection of breath samples was explained to the patient, ie, take breath in, hold for 5 seconds, then blow into the tube. The first sample was taken and when condensation was seen on the glass and exhalation was complete, the cap was replaced. A duplicate sample was taken. These two samples were at time zero minutes.

The lemon mousse was obtained from the Catering Department at Derbyshire Royal Infirmary. To facilitate flow, 1 cm was cut from the end of the delivery pipette tip. 200 μL ^{13}C-triolein was dispensed into the spoon, which was placed in the lemon mousse. The patient ate the mousse and was asked to lick the spoon well to ensure complete ingestion of the substrate. Then 50 mL of water was drunk to wash the fat meal and substrate from the mouth. Duplicate breath samples were collected from the patient every hour for 6 hours. A note was made of the patient's sex, age, weight and height. The breath samples were analysed on the BreathMAT IRMS. The protocol was approved by the Southern Derbyshire Local Research Ethics Committee and each subject provided written consent prior to undertaking the test.

4.2.6 Evaluation of proposed protocol

4.2.6a Healthy volunteers

Twenty volunteers (all NHS employees) were recruited and written consent obtained. The ^{13}C-triolein fat absorption test was carried out as above (4.2.5). The volunteer's Body Surface Area (BSA) and Body Mass Index (BMI) were noted. Using patients weight, height and a nomogram (51) BSA was calculated in square metres and BMI was calculated as weight (Kg)/height (m^2).

Two of the volunteers were re tested after a period of a few months to assess the reproducibility of the test. One ingested lemon mousse as the test meal, the other ingested Scandishake.

4.2.6b Patients

Patients referred by gastroenterologists for the ^{14}C-triolein fat absorption test as part of their clinical investigations, were asked for their written consent to also ingest ^{13}C-triolein. Breath samples were then collected for both tests simultaneously, as described in the next section (4.2.6c).

4.2.6c Method for the comparison of the ^{14}C-triolein and ^{13}C-triolein fat absorption breath tests

The patients had been identified by consultant gastroenterologists as suitable for the fat absorption breath test (the patients were referred by Drs Freeman and Cole at Derby City General Hospital and Drs Holmes and Norton at the Derbyshire Royal Infirmary). Patient information sheets explaining the tests, a consent form and date of appointment were sent to the patient (Appendix V). The patient was allowed to have a cup of tea before the test and a light lunch 4 hours after beginning the test.

On arrival at the clinic, both tests were explained to the patient and written consent obtained. The sex, age, weight and height of the patient were noted. The breath sample containers were labelled clearly. The collection of breath samples was explained to the patient. At time 0 minutes, duplicate breath samples were collected following the prescribed procedures (see 4.2.1a and 4.2.1b) to allow evaluation of baseline $^{14}CO_2$ and $^{13}CO_2$ levels. 50 μL ^{14}C-triolein was dispensed on to a teaspoon. The carrier toluene was evaporated using a hair dryer on the cold setting. The patient ate half the lemon mousse with the ^{14}C triolein coated spoon, 200 μL ^{13}C-triolein was dispensed on to the

same spoon and the rest of the mousse was eaten. The spoon was licked well and 50 mL water was drunk to wash the mousse down and rinse any residue from the mouth. Breath samples were collected every hour in duplicate for both isotopes. 10 mL of scintillation fluid, Emulsifier Safe was added to ^{14}C samples and ^{14}C levels counted on a scintillation counter in Vascular Medicine Department at Derbyshire Royal Infirmary. The samples contained within the Exetainers were tested in the Chemical Pathology Department at Derbyshire Royal Infirmary on the BreathMAT IRMS to give the $^{13}CO_2$:$^{12}CO_2$ ratios. The ^{14}C results were calculated as in the ^{14}C-triolein SOP (Appendix II) and a report was sent to the consultant who had requested the test. Results for ^{13}C-triolein breath test are presented as DOB, and cumulative DOB at 1-4 hours 4-6 hours and 1-6 hours, after ingestion of labelled triolein. ^{13}C-triolein results were also expressed as peak percentage dose recovered (PDR) and cumulative PDR at 1-4 h, 4-6 h and 1-6 h post dose. Calculations for PDR for both isotopes included assumed CO_2 production rate of 9 mol/kg/h.

5 RESULTS

5.1 MAINTAINING ANALYTICAL PERFORMANCE OF $^{13}CO_2$ MEASUREMENT

5.1.1 Zero enrichment (internal reproducibility)

An example of a BreathMAT print out, after the pre set sequence "CO_2 zero 20" has been run, is shown in Figure 5.1. The standard deviation of the Del13PDB values (with reference gas as baseline with Del13PDB - 29.00) was 0.054‰.

Figure 5.1 – An example of a BreathMAT printout after the pre-set sequence "CO_2 zero 20" has been run.

No.	Group No	Date of Acq	Del 45	Del 46	Ratio 45	Ratio 46	Del 13	Del 13 PDB	mV
1	1	2000/11/06-09:46:	0.06	-0.05	1.183823	0.420025	0.06	-28.94	3254.3
2	1	2000/11/06-09:47:	-0.01	-0.01	1.183799	0.420089	-0.01	-29.01	3256.7
3	1	2000/11/06-09:48:	0.06	0.04	1.183885	0.420080	0.06	-28.94	3253.6
4	1	2000/11/06-09:50:	0.01	-0.09	1.183867	0.420074	0.01	-28.99	3252.8
5	1	2000/11/06-09:51:	0.12	0.03	1.183913	0.420095	0.12	-28.88	3250.2
6	1	2000/11/06-09:52:	0.03	0.03	1.183915	0.420075	0.03	-28.97	3252.1
7	1	2000/11/06-09:53:	-0.06	0.04	1.183792	0.420119	-0.07	-29.06	3250.8
8	1	2000/11/06-09:54:	-0.02	0.00	1.183908	0.420088	-0.02	-29.02	3249.8
9	1	2000/11/06-09:55:	0.02	0.01	1.183890	0.420107	0.02	-28.98	3246.8
10	1	2000/11/06-09:56:	-0.07	0.05	1.183828	0.420107	-0.08	-29.07	3246.2
11	1	2000/11/06-09:57:	0.06	0.01	1.183916	0.420097	0.07	-28.93	3246.1
12	1	2000/11/06-09:58:	0.07	0.03	1.183965	0.420095	0.07	-28.93	3247.3
13	1	2000/11/06-09:59:	-0.01	0.08	1.183907	0.420145	-0.02	-29.02	3245.2
14	1	2000/11/06-10:00:	0.04	-0.13	1.183935	0.420094	0.04	-28.96	3243.9
15	1	2000/11/06-10:01:	-0.05	0.02	1.183899	0.420111	-0.05	-29.05	3243.3
16	1	2000/11/06-10:02:	0.02	0.02	1.183967	0.420102	0.02	-28.99	3241.9
17	1	2000/11/06-10:03:	-0.05	0.01	1.183908	0.420138	-0.05	-29.05	3243.7
18	1	2000/11/06-10:04:	0.01	0.12	1.183957	0.420157	0.01	-28.99	3241.4
19	1	2000/11/06-10:05:	0.02	-0.09	1.183947	0.420117	0.02	-28.98	3242.6
20	1	2000/11/06-10:06:	0.05	-0.03	1.183995	0.420131	0.05	-28.95	3241.6
21	1	2000/11/06-10:07:	0.08	-0.02	1.184009	0.420140	0.08	-28.92	3239.4
22	1	2000/11/06-10:08:	-0.05	0.03	1.184006	0.420141	-0.06	-29.05	3240.5

5.1.2 Use of linearity correction

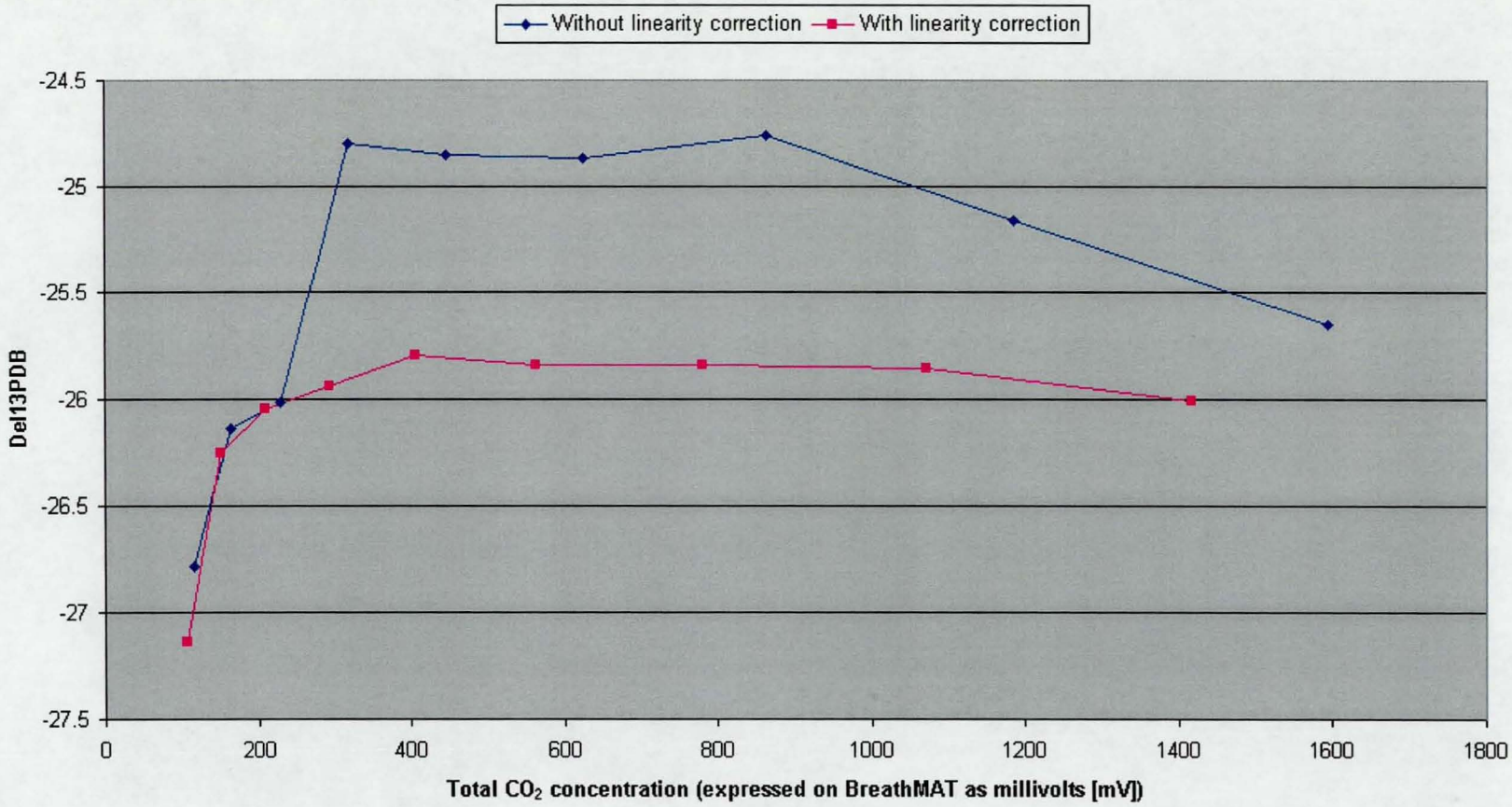
Figure 5.2 shows the relationship between CO_2 concentration (as mV) and apparent Del13PDB with and without the use of "linearity" correction software. The figure shows that the linearity correction software corrects the results so that Del13PDB is independent of carbon dioxide concentration over a wider

range of carbon dioxide concentrations. Table 5.1 shows the effect of using the linearity correction software on precision of Del13PDB on another occasion when the same sample was analysed over a wide range of CO_2 concentrations. Linearity correction software was used for all subsequent analyses, except for those in Section 5.3.

Table 5.1 - The effect of linearity correction on precision of Del13PDB

	mV RANGE	*Del13 PDB(‰)*	
		Mean	*SD*
Without linearity correction	623-1593	-25.11	0.40
With linearity correction	564-1416	-25.89	0.08

Figure 5.2 The relationship between CO_2 concentration (mV) and the apparent Del13PDB, without and with the use of "linearity" correction .



5.1.3 Precision of breath measurements

Table 5.2 shows the results (Del13PDB and mV) for 10 replicates of the same breath sample. The mean of the Del13PDB results was -25.29 (SD = 0.12, CV = 0.47%).

Table 5.2 - Precision of breath measurement

SAMPLE NUMBER	*Del13PDB*	*mV*
1	-25.44	3036
2	-25.34	2821
3	-25.20	2683
4	-25.12	2713
5	-25.23	2244
6	-25.35	2841
7	-25.27	2848
8	-25.15	2749
9	-25.31	2829
10	-25.49	3077

5.1.4 Between day analytical precision

Table 5.3 shows the Del13PDB values for pairs of breath samples from the operator over 20 days. The mean difference was 0.0025‰ (SD = 0.14‰).

Table 5.3 - Reproducibility of Del13PDB values for pairs of breath samples from the operator over 20 days.

DAY	*SAMPLE 1 Del13PDB‰*	*SAMPLE 2 Del13PDB‰*	*DIFFERENCE (DOB ‰)*
1	-23.67	-23.71	-0.04
2	-25.91	-25.89	0.02
3	-26.35	-26.5	-0.15
4	-25.53	-25.3	0.23
5	-26.18	-26.37	-0.19
6	-26.24	-26.27	-0.03
7	-26.02	-26.08	-0.06
8	-26.45	-26.35	0.10
9	-25.58	-25.41	0.17
10	-25.80	-25.89	-0.09
11	-26.16	-26.32	-0.16
12	-26.63	-26.36	0.27
13	-24.47	-25.39	0.08
14	-23.91	-23.89	0.02
15	-24.45	-24.74	-0.29
16	-25.08	-24.94	0.14
17	-26.76	-26.80	-0.04
18	-26.93	-27.00	-0.07
19	-27.14	-27.10	0.04
20	-27.14	-27.04	0.10

Table 5.4 shows the DOB for pairs of patient breath samples enriched with $^{13}CO_2$. These were tested with 5 consecutive batches of patient samples on different days confirming acceptable precision over a wide range of carbon dioxide concentrations.

Table 5.4 - Between day analytical precision for DOB (‰) for 2 pairs of patient samples re-tested in 5 batches of samples

RE-TESTS	*PATIENT 1*	*PATIENT 2*
1	1.13	10.59
2	1.28	10.48
3	1.09	11.49
4	1.12	11.18
5	1.21	11.46
Mean	1.17	11.04
SD	0.08	0.48

5.2 ASSESSMENT OF THE PRECISION OF DISPENSING 200 μL OF OLIVE OIL

Olive oil was used for this assessment as it has similar properties to ^{13}C-triolein. The plastic pipette tip was reduced by 1 cm to ease the flow caused by the viscous nature of the olive oil. The reverse pipetting technique was used to ensure accuracy; the oil was dispensed to the first pipette "stop", after which the pipette was held vertically for 10 seconds and the oil then dispensed again three times. This was repeated 10 times from the same olive oil sample with a new pipette tip each time.

Table 5.5 shows the weight of olive oil when 200 μL aliquots were pipetted ten times. The mean weight was 0.1847 g (SD = 0.0027 g, CV = 1.46%).

Table 5.5 - Assessment of precision of dispensing olive oil. The weight (g) of olive oil of ten 200 μL aliquots is recorded.

1	0.1890 g	6	0.1857 g
2	0.1857 g	7	0.1855 g
3	0.1841 g	8	0.1820 g
4	0.1859 g	9	0.1787 g
5	0.1848 g	10	0.1854 g

5.3 BREATH $^{13}CO_2$:$^{12}CO_2$ RATIOS: NATURAL VARIATION OVER ONE DAY

In a healthy volunteer, after fasting overnight, the breath sample Del13PDB value was -24.33. The volunteer noted all food ingested and activities undertaken over the course of the day, as shown in Table 5.6. Breath samples were collected in duplicate at intervals throughout the day. DOB values varied from -1.97‰ to +0.5‰, relative to sample number 1, (Figure 5.3).

The data demonstrates the effect on precision due to variations in CO_2 concentrations between duplicates when linearity correction software is not used. Table 5.7 shows the variation in Del13PDB values between the duplicates. The difference in Del13PDB between duplicate breath samples varied from -0.51‰ to 0.36‰ (mean difference -0.057‰). The differences in total CO_2 concentration (expressed as mV) between the duplicates varied from 47 to 777 mV. Table 5.8a and 5.8b demonstrates that precision improves when the variation between CO_2 concentration in duplicates is low.

Figure 5.3 Variation of DOB over one day

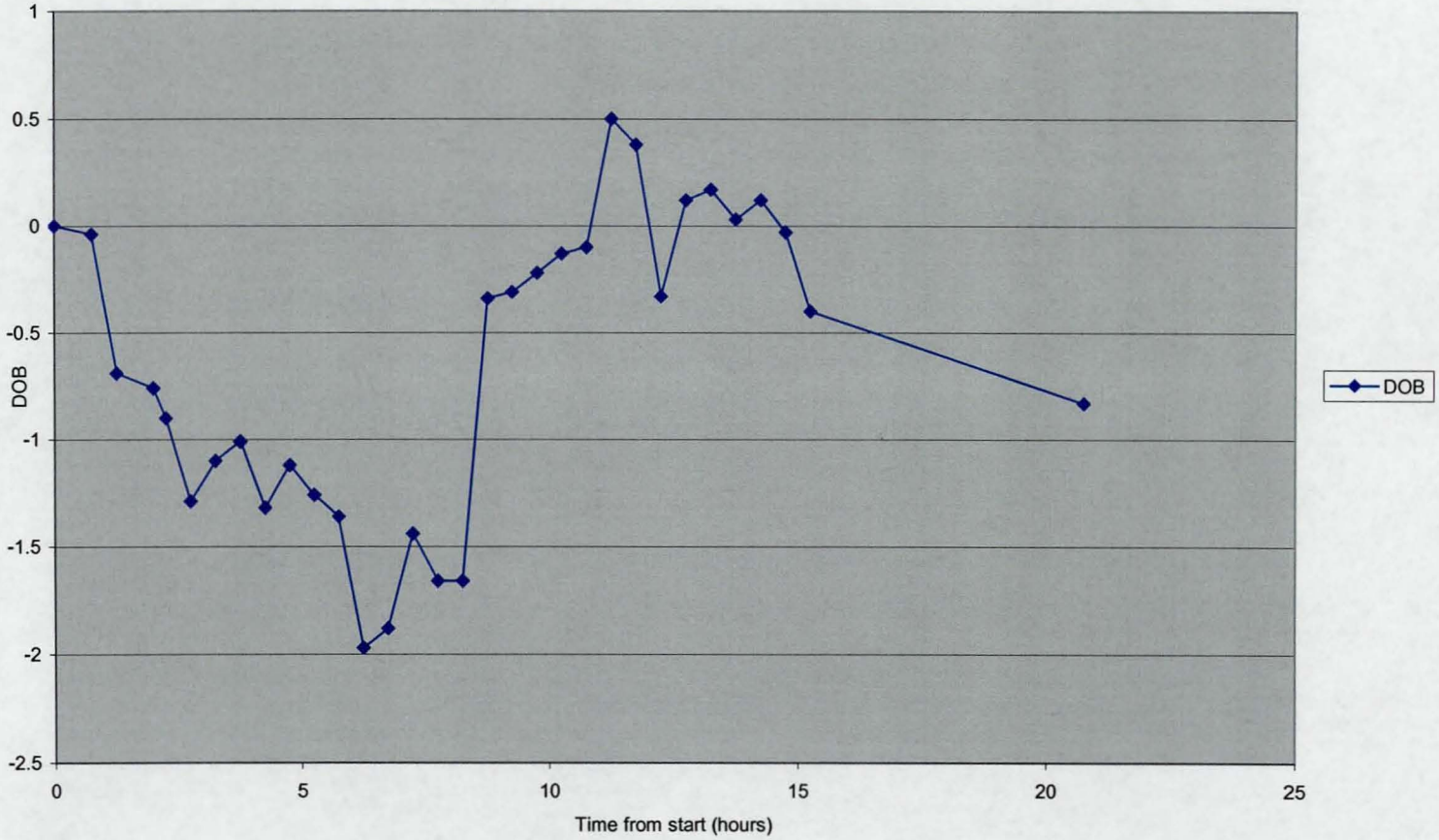


Table 5.6 - The variation of Del13PDB and DOB over one day in a healthy volunteer (V1)

SAMPLE No	*TIME FROM START*	*TIME*	*AVERAGE Del13PDB VALUES*	*VALUE RELATIVE TO BASELINE SAMPLE (DOB ‰)*	*FOOD*	*ACTIVITY*
1	0	0645	-24.33	0		Awoke
2	0.75	0730	-24.37	-0.04		
3	1.25	0800	-25.02	-0.69		Changing bed
4	2	0845	-25.09	-0.76	Granary bread/tea/marmite/marmalade	
5	2.25	0900	-25.23	-0.9		Washed teeth
6	2.75	0930	-25.62	-1.29		At work
7	3.25	1000	-25.43	-1.1		At work
8	3.75	10.30	-25.34	-1.01		At work
9	4.25	1100	-25.65	-1.32	Banana/coffee	At work
10	4.75	1130	-25.45	-1.12		At work
11	5.25	1200	-25.59	-1.26		At work
12	5.75	1230	-25.69	-1.36		At work
13	6.25	1300	-26.30	-1.97		At work
14	6.75	1330	-26.21	-1.88		Driving/shopping
15	7.25	1400	-25.77	-1.44	Granary bread/cheese/pork pie mustard/tea/lime cordial/choc	
16	7.75	1430	-25.99	-1.66		

Table 5.6 (continued) - The variation of Del13PDB and DOB over one day in a healthy volunteer (V1)

SAMPLE No	*TIME FROM START*	*TIME*	*AVERAGE Del 13PDB VALUES*	*VALUE RELATIVE TO BASELINE SAMPLE (DOB ‰)*	*FOOD*	*ACTIVITY*
17	8.25	1500	-25.99	-1.66		Shopping
18	8.75	1530	-24.67	-0.34		Wrapping presents
19	9.25	1600	-24.64	-0.31	Tea	Wrapping presents
20	9.75	1630	-24.55	-0.22		Wrapping presents
21	10.25	1700	-24.46	-0.13		Wrapping presents
22	10.75	1730	-24.47	-0.1		Reading
23	11.25	1800	-23.83	0.5	Baked beans/toast/sausages lime cordial/bioyoghurt/choc	
24	11.75	1830	-23.95	0.38		
25	12.25	1900	-24.66	-0.33		
26	12.75	1930	-24.21	0.12		Ironing
27	13.25	2000	-24.16	0.17		
28	13.75	2030	-24.30	0.03	Coffee/milk/2 apples	
29	14.25	2100	-24.21	0.12		
30	14.75	2130	-24.36	-0.03	Ice cream/choc sauce	
31	15.25	2200	-24.73	-0.4		To bed
32	20.75	0330	-25.17	-0.83		Bathroom

Table 5.7 – The difference in Del13PDB and total CO_2 concentration (mV) values between duplicate samples over one day in a healthy volunteer (V1, *this value is expressed as the modulus).

Del13PDB‰			*mV**
SAMPLE 1	*SAMPLE 2*	*SAMPLE 1-SAMPLE 2*	*SAMPLE 1-SAMPLE 2*
-24.14	-24.51	-0.37	394
-24.23	-24.50	-0.27	192
-24.98	-25.06	-0.08	67
-25.04	-25.13	-0.09	222
-25.19	-25.26	-0.07	125
-25.65	-25.59	0.06	103
-25.33	-25.52	-0.19	114
-25.42	-25.25	0.17	95
-25.65	-25.78	-0.13	224
-25.30	-25.6	-0.3	211
-25.4	-25.78	-0.38	402
-25.76	-25.61	0.15	307
-26.41	-26.19	0.22	229
-25.95	-26.46	-0.51	601
-25.61	-25.93	-0.32	518
-25.96	-26.02	-0.06	374
-25.9	-26.08	-0.18	85
-24.67	-24.67	0	58
-24.61	-24.66	-0.05	111
-24.5	-24.59	-0.09	131
-24.49	-24.43	0.06	20
-24.61	-24.33	0.28	254
-24.01	-23.65	0.36	777
-23.82	-24.07	-0.25	255
-24.45	-24.21	0.24	224
-24.28	-24.13	0.15	145
-24.09	-24.23	-0.12	47
-24.22	-24.38	-0.16	162
-24.16	-24.26	-0.1	107
-24.36	-24.36	0	101
-24.75	-24.71	0.04	71
-25.26	-25.08	0.18	426

Table 5.8a - Variation in SD (of results shown in Table 5.7) due to non linearity of BreathMAT.

	DIFFERENCES IN mV BETWEEN DUPLICATES				
	0-200 mV	*0-300 mV*	*0-400 mV*	*0-500 mV*	*0-800 mV*
Mean	-0.049	-0.036	-0.042	-0.046	-0.057
SD	0.121	0.162	0.169	0.181	0.21
n	17	24	27	29	32

Table 5.8b – Variation in SD, showing that precision improves when the variation between CO_2 concentrations in duplicates is low.

	DIFFERENCES IN mV BETWEEN DUPLICATES		
	0-200 mV	*200-400 mV*	*300-800 mV*
Mean	-0.049	-0.031	-0.119
SD	0.121	0.239	0.321
n	17	10	8

5.4 BREATH $^{13}CO_2$:$^{12}CO_2$ RATIOS; CHANGES AFTER INGESTING VARIOUS TEST MEALS TO AID ISOTOPE DELIVERY

The properties of potential test meals, which could be used to aid isotope delivery to the small bowel, are shown in Table 5.9. On the basis of the data, it was decided to assess changes in breath $^{13}CO_2$:$^{12}CO_2$ ratios after ingesting Chunky Kit Kat, Scandishake, Duobar and lemon mousse.

Table 5.9 - Foods assessed for suitability as a test meal (foods in bold are those tested in vivo)

FOOD	*MADE BY*	*WT (g)*	*ENERGY (kJ)*	*PROTEIN g (%)*	*CARBOHYDRATE g (%)*	*FAT g (%)*
Lemon mousse	**DRI**	**73**		**1.4 (4.6%)**	**10 (33%)**	**19.3 (63%)**
Mars	Mars, UK	65	1302	2.9 (5%)	47.8 (74%)	11.9 (19%)
Fudge choc	Cadburys	24.5	480	0.7 (4%)	18.4 (75%)	4.2 (17%)
Twix	Mars, UK	58	1203	2.7 (5%)	37.6 (65%)	11 (24%)
Crunchie	Cadburys	39	825	1.8 (5%)	30.3 (78%)	7.6 (20%)
Milk choc biscuit	McVities		366	1.2		
Organic fruit/ nut bar	Shepherdboy Ltd	50	919	8.4 (17%)	20.4 (41%)	11.6 (23%)
Full fat milk	Natural	11.4	264	3.2 (28%)	4.7 (41%)	3.5 (34%)
Penguin biscuit	McVities	25.5	572	1.4 (5%)	16.4 (64%)	7.3 (28%)
Scandishake	**Nutricia SHS**	**83**	**1831**	**4 (4.8%)**	**58 (70%)**	**21(25%)**
Chunky Kitkat	**Nestle**	**55**	**1181**	**3.8 (7%)**	**32.9 (59%)**	**15.2 (27%)**
Duobar	**Nutricia SHS**	**45**	**1212**	**<1**	**22.4 (50%)**	**22.4 (50%)**

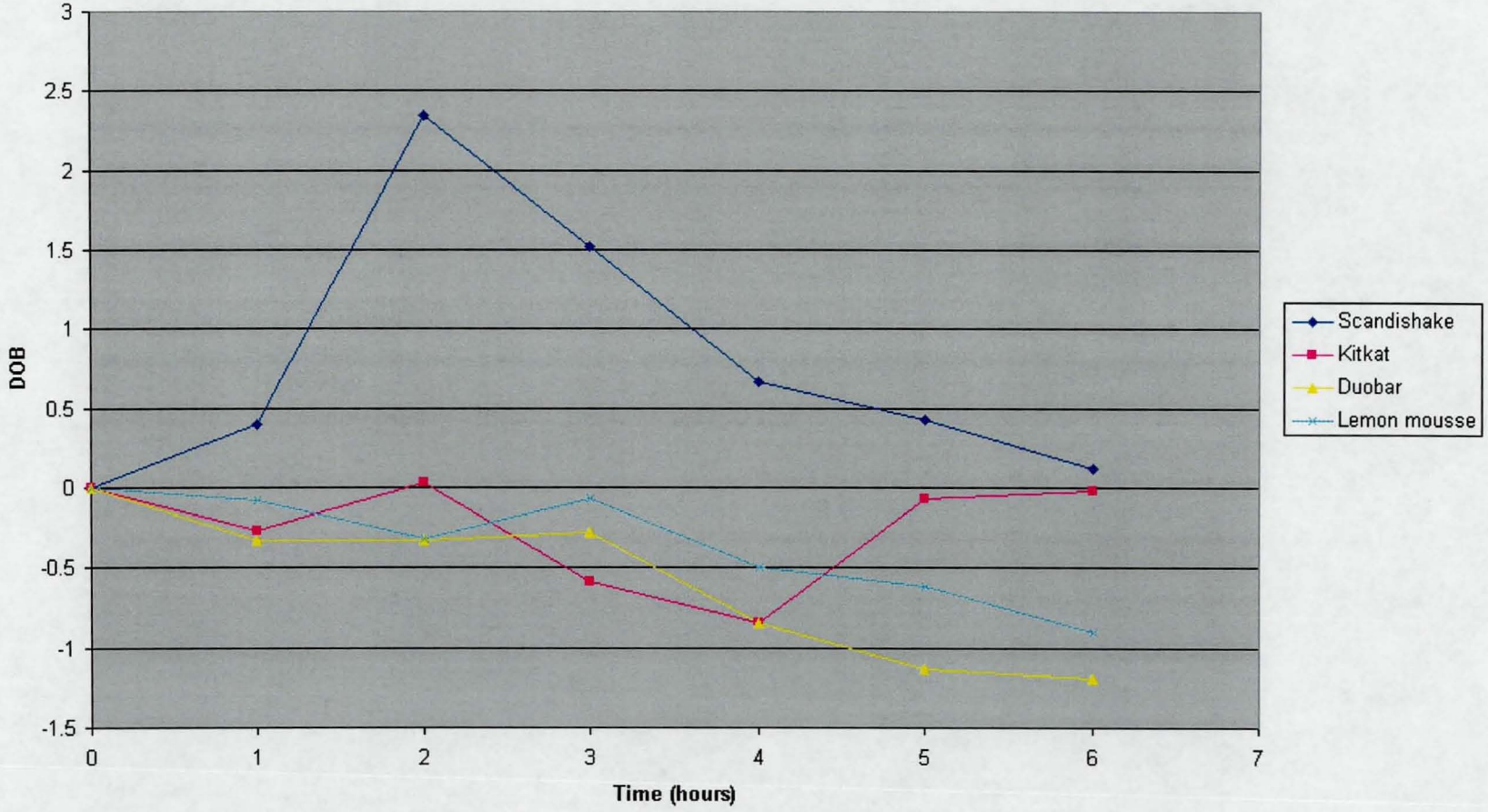
The $^{13}CO_2$:$^{12}CO_2$ ratios, expressed as DOB, following ingestion of the four test meals by a single subject (V1) on 4 separate days are shown in Figure 5.4. The same activities were performed over the test period on each occasion. The result in Figure 5.4 shows marked differences in DOB responses following ingestion of the different test meals.

Breath samples were collected from 4 patients, routinely referred for the ^{14}C-triolein fat absorption test, after ingestion of lemon mousse to confirm the isotopic neutrality of this test meal. However, unlike the volunteer, these patients had been allowed to have a light snack after the fourth hourly breath collection. Of these four patients, 3 were male and 1 female, their ages ranged from 36 to 65. Other variations in their profiles can be seen in Table 5.10. The results from two additional patients were excluded due their BMI being greater than 29.

Table 5.10 - Profiles of patients ingesting lemon mousse

CODE	*SEX*	*AGE*	*WT (kg)*	*HT (m)*	*BSA (m^2)*	*BMI*
A	M	59	79	1.86	1.88	22.8
B	M	59	80	1.83	1.86	23.9
C	M	65	58	1.85	1.62	16.9
D	F	36	60	1.65	1.55	22.1
Range		36-65	58-80	1.65-1.86	1.55-1.88	16.9-23.9
Mean		55	69	1.80	1.73	21.4

Figure 5.4 Changes in DOB (‰) with time after ingestion of 4 test meals by a single subject (V1)



The results from the four patients are shown in Table 5.11 and confirm little change in DOB following ingestion of lemon mousse. The results from the volunteer who ingested the four meals and the mean of the four patients who ingested lemon mousse are shown in Table 5.12.

Table 5.11 - Changes in DOB (‰) with time in four patients following ingestion of lemon mousse, without ^{13}C-triolein.

	CHANGE IN DOB (‰) WITH TIME AFTER INGESTION OF LEMON MOUSSE					
PATIENT CODE	*1 h*	*2 h*	*3 h*	*4 h*	*5 h*	*6 h*
A	0.11	0.42	0.06	-0.26	-0.16	0.56
B	0.3	0.21	0.27	0.41	0.05	0.09
C	-0.04	0.11	0.07	-0.02	0.08	-0.2
D	0	0.31	0.22	-0.2	-0.45	-0.44
Mean	0.09	0.26	0.16	-0.02	-0.12	0

Table 5.12 - DOB (‰) following ingestion of 4 test meals by a volunteer. The mean DOB for 4 patients following ingestion of lemon mousse is shown for comparison (data shown in Table 5.11).

		CHANGE IN DOB (‰) WITH TIME AFTER INGESTION OF TEST MEAL					
TEST MEAL	*SUBJECT*	*1 h*	*2 h*	*3 h*	*4 h*	*5 h*	*6 h*
Scandishake	Volunteer V1	0.4	2.34	1.52	0.67	0.43	0.12
Kit Kat	Volunteer V1	-0.27	0.03	-0.59	-0.84	-0.07	-0.02
Duobar	Volunteer V1	-0.33	-0.33	-0.28	-0.84	-1.13	-1.19
Lemon mousse	Volunteer V1	-0.08	-0.32	-0.07	-0.5	-0.62	-0.9
Lemon mousse	Mean of 4 patients	0.09	0.26	0.16	-0.02	-0.12	0

5.5 BREATH $^{13}CO_2$:$^{12}CO_2$ RATIOS; CHANGES AFTER INGESTING 200 μL ^{13}C-TRIOLEIN WITH LEMON MOUSSE OR SCANDISHAKE

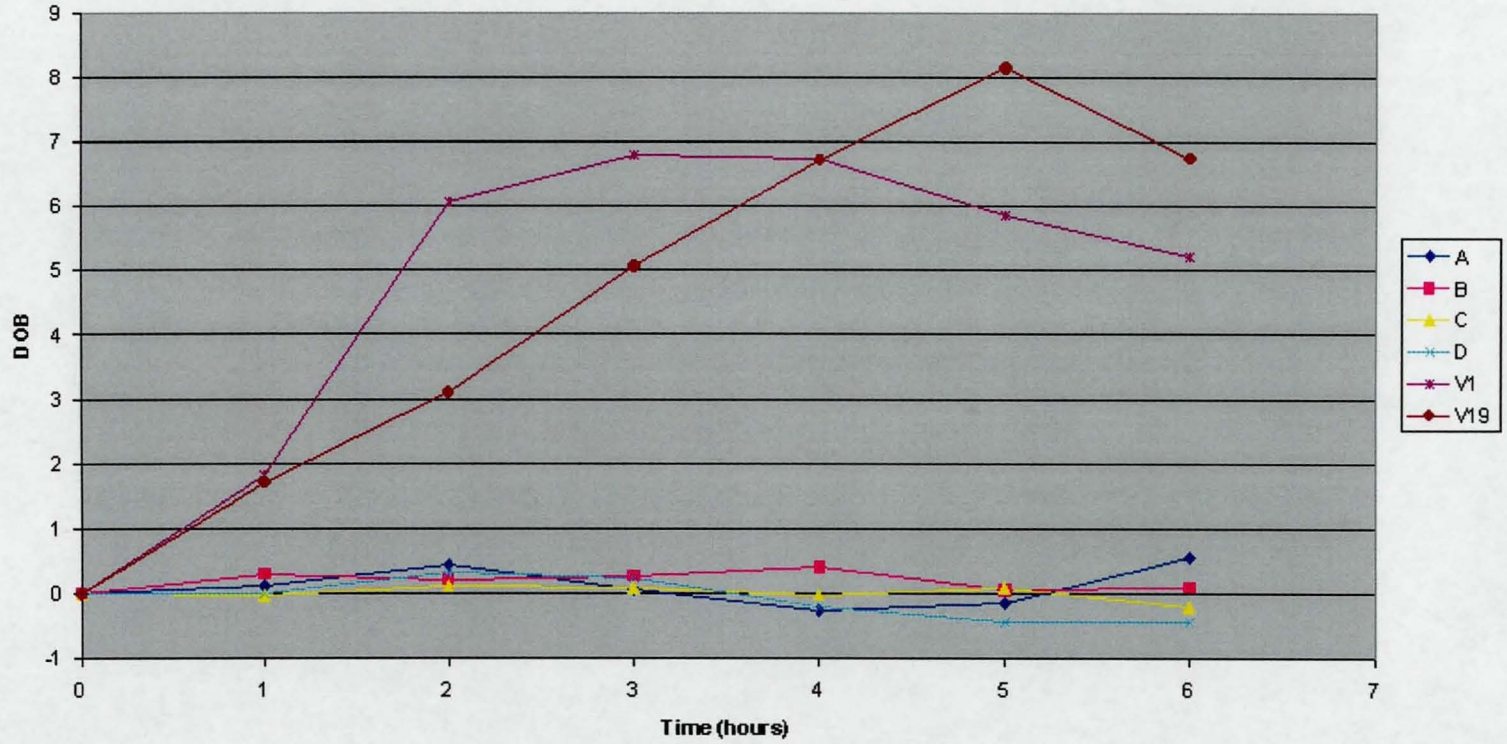
The Del13PDB values were measured and DOB calculated over a 6 hour period in 2 healthy volunteers who ingested 200 μL ^{13}C-triolein added to lemon mousse. Table 5.13 shows the DOB values after ingesting lemon mousse with and without ^{13}C-triolein as the substrate.

Table 5.13 - Comparison of DOB (‰) in two volunteers 1-6 h after ingesting 200 μL ^{13}C-triolein and lemon mousse

	DOB (‰) WITH TIME AFTER INGESTING ^{13}C-TRIOLEIN AND LEMON MOUSSE					
SUBJECT	*1 h*	*2 h*	*3 h*	*4 h*	*5 h*	*6 h*
V1	1.80	6.07	6.78	6.73	5.86	5.21
V19	1.71	3.1	5.07	6.71	8.14	6.74

The results, compared with the changes observed in 4 patients who ingested lemon mousse without ^{13}C-triolein (data from Table 5.11), are shown in Figure 5.5. The peak DOBs following ingestion of ^{13}C-triolein and lemon mousse were +6.8 and +8.2‰ compared to changes of -0.5 to +0.6‰ in the patients ingesting lemon mousse alone.

Figure 5.5 Changes in DOB (‰) in two volunteers (V1 and V19) who ingested ^{13}C-triolein and lemon mousse, compared to the changes in 4 patients (A,B,C and D) who ingested lemon mousse with no ^{13}C-triolein.

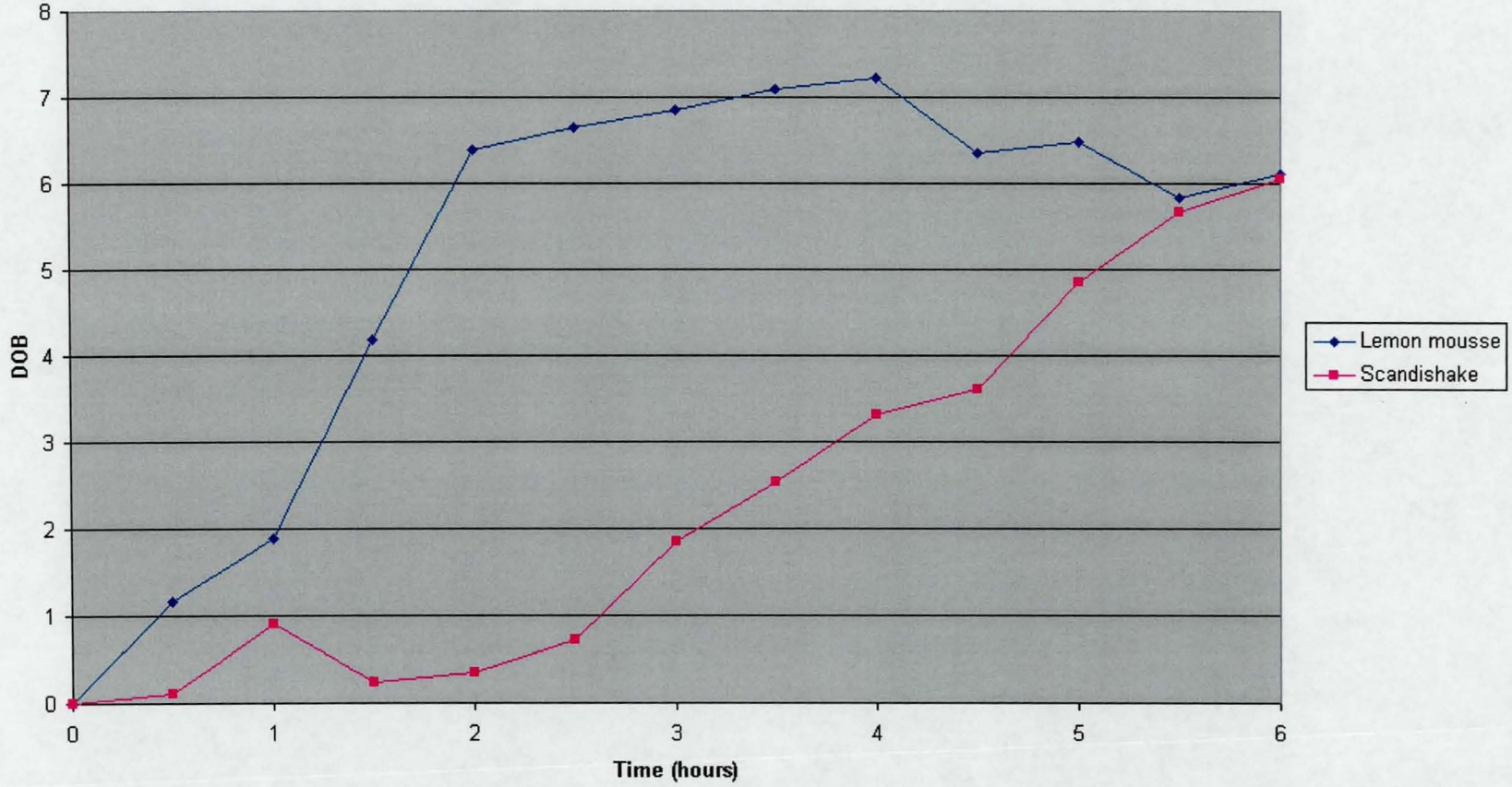


In order to directly compare lemon mousse and Scandishake as potential carriers for the isotope in the fat absorption test, one volunteer (V1) ingested Scandishake with and without the addition of ^{13}C-triolein (200 μL) and lemon mousse, with and without the addition of ^{13}C-triolein (200 μL). The DOB values are shown in Table 5.14. Table 5.14 and Figure 5.6 also show the DOB expressed as the difference between test meal +^{13}C-triolein and the test meal alone. Although the DOB at 6 h was identical for the 2 test meals, the shapes of the response curves are very different.

Table 5.14 - Changes in DOB (‰) in a single volunteer (V1) for 0.5 h-6 h after separately ingesting lemon mousse and Scandishake with and without the addition of ^{13}C-triolein (200 μL).

	CHANGES IN DOB (‰) WITH TIME (h) AFTER INGESTING TEST MEAL ALONE AND WITH ^{13}C-TRIOLEIN					
TIME (h)	*LEMON MOUSSE*	*LEMON MOUSSE +^{13}C*	*DIFFERENCE*	*SCANDISHAKE*	*SCANDISHAKE +^{13}C*	*DIFFERENCE*
0	0	0		0	0	
0.5	-0.27	0.9	1.17	-0.02	0.09	0.11
1	-0.08	1.8	1.88	0.4	1.03	0.9
1.5	-0.18	4	4.18	2.23	1.98	0.25
2	-0.32	6.07	6.39	2.34	2.7	0.36
2.5	-0.29	6.35	6.64	2.12	2.84	0.72
3	-0.07	6.78	6.85	1.52	3.38	1.86
3.5	-0.34	6.75	7.09	1.27	3.8	2.53
4	-0.5	6.73	7.23	0.67	3.99	3.32
4.5	-0.49	5.86	6.35	0.55	4.17	3.62
5	-0.62	5.86	6.48	0.43	5.28	4.85
5.5	-0.43	5.4	5.83	0.17	5.83	5.66
6	-0.9	5.21	6.11	0.12	6.18	6.06

Figure 5.6 Changes in DOB(‰) expressed as difference between ingestion of test meal plus 13C-triolein and the test meal alone for lemon mousse and Scandishake in a single volunteer (V1).



5.6 EVALUATION OF PROPOSED PROTOCOL FOR ^{13}C-TRIOLEIN TEST OF FAT ABSORPTION

5.6.1 Healthy volunteers

The objective of this part of the study was to establish the normal range for the ^{13}C-triolein test in healthy volunteers. The 18 subjects were all employed at the Derbyshire Royal Infirmary. 14 were from the Biochemistry Department, the remaining 4 were from the Vascular Medicine Department. Seven were male and 11 were female, their ages ranged from 22-61 years, with a mean of 41. The details of subjects are shown in Table 5.15.

Table 5.15 - Profiles of healthy volunteers

SUBJECT	*SEX*	*AGE (y)*	*WT (kg)*	*HT (m)*	*BSA (m^2)*	*BMI*
V1	F	45	71	1.69	1.66	24.9
V2	F	30	57	1.73	1.54	19.0
V3	M	35	73	1.73	1.71	24.4
V4	F	51	84	1.61	1.72	32.4
V5	F	46	70	1.64	1.61	26.0
V6	F	49	66	1.78	1.66	20.8
V7	F	47	70	1.68	1.64	24.8
V8	F	52	76	1.58	1.63	30.4
V9	M	46	98	1.88	2.06	27.7
V10	F	50	77	1.60	1.66	30.1
V11	M	22	80	1.78	1.83	25.2
V12	F	44	62	1.60	1.52	24.2
V13	M	30	85	1.76	1.85	27.4
V14	M	40	80	1.75	1.81	26.1
V15	F	61	82	1.67	1.75	29.4
V16	M	48	79	1.72	1.78	26.7
V17	M	22	97	1.90	2.08	26.9
V18	F	26	51	1.55	1.35	21.2
Range		22-61	51-98	1.55-1.90	1.35-2.08	19.0-32.4
Mean		41	75	1.70	1.71	26.0

The DOB values for the 18 subjects are shown in Table 5.16 for samples collected at 30 minute intervals over a 6 hour period, after ingesting the lemon mousse and ^{13}C-triolein.

Table 5.17 shows the peak DOB ‰ and the cumulative (ie, sum of DOB ‰ for hourly values) data for 1-4 h and 4-6 h with results uncorrected for BMI and corrected to a BMI of 25. In all cases, correction for BMI widened the standard deviation for the results. A wide variation in results was noted, for example, the peak DOB range was 1.0-7.4. The 3 lowest results were in subjects with BMI >29, and in general results in females were higher than in males. Table 5.18 therefore shows the data separately for males and females and excludes subjects with BMI >29. The t-test confirmed that results for men are lower than in women ($p < 0.05$). The ranges for volunteer males and females expressed as mean ±2SD is shown in Table 5.19 (not corrected for BMI).

Table 5.16 - DOB (‰) in 18 volunteers 0.5-6 h after ingesting ^{13}C-triolein and lemon mousse (figures in bold = peak values)

SUBJECT	*DOB (‰) IN VOLUNTEERS 0.5-6 h AFTER INGESTING ^{13}C-TRIOLEIN AND LEMON MOUSSE*											
	0.5 h	*1.0 h*	*1.5 h*	*2.0 h*	*2.5 h*	*3.0 h*	*3.5 h*	*4.0 h*	*4.5 h*	*5.0 h*	*5.5 h*	*6.0 h*
V1	0.57	1.54	3.17	4.24	4.93	4.94	4.68	4.8	4.47	4.42	4.43	**4.56**
V2	-0.13	0.20	0.45	0.93	1.63	3.39	4.56	6.44	6.39	**6.77**	5.47	5.39
V3	0.8	1.29	1.20	1.32	1.47	1.33	1.78	2.07	1.63	2.06	3.34	**3.54**
V4	-0.09	0.15	0.32	0.26	0.93	0.82	**1.20**	1.01	0.94	1.04	0.78	0.77
V5	0.33	0.62	0.95	0.80	1.26	3.28	4.42	**5.23**	4.81	4.92	4.55	4.30
V6	0.09	0.90	1.27	1.74	2.0	2.85	3.76	4.28	**4.57**	4.35	3.51	3.53
V7	-0.05	0.25	2.19	2.33	3.16	5.08	5.91	**6.08**	5.96	4.76	4.69	3.97
V8	0.40	0.58	0.42	0.64	0.32	0.37	0.96	1.34	1.90	2.20	2.48	**2.48**
V9	0.20	0.54	1.08	1.27	1.73	1.88	2.22	2.89	3.37	2.70	**3.64**	3.57
V10	0.14	0.75	1.00	1.50	2.03	2.54	3.45	4.06	4.61	**5.08**	4.94	4.77
V11	0.02	0.10	0.34	0.40	0.56	0.99	1.38	2.41	3.13	4.28	**4.71**	4.50
V12	0.40	0.87	2.79	3.76	5.57	6.17	7.21	7.42	**7.50**	7.31	6.87	6.18
V13	-0.09	0.38	0.40	0.35	0.71	1.05	1.19	2.02	2.52	2.81	2.99	**3.22**
V14	0.31	0.38	0.50	0.56	1.18	1.53	1.70	2.02	2.54	2.72	3.01	**3.58**
V15	0	0.39	0.47	0.66	0.99	0.98	1.42	1.46	1.48	**1.90**	1.67	1.55
V16	-0.06	0.56	1.05	1.35	2.06	3.17	3.69	**3.77**	3.42	2.88	2.39	2.66
V17	0.56	0.59	0.75	1.06	1.38	1.25	0.88	1.68	2.39	**3.07**	2.63	2.57
V18	0.38	0.60	2.04	2.54	3.41	3.58	3.06	4.69	**5.33**	4.86	4.12	4.57

Table 5.17 - DOB (‰) in 18 volunteers following ingestion of ^{13}C-triolein and lemon mousse. Results are expressed as peak and cumulative DOB (‰) for 1-4 h and 4-6 h, with and without correction for BMI (corrected for BMI = observed DOB x 25/actual BMI)

SUBJECT	*PEAK DOB‰*	*PEAK DOB ‰*		*CUMULATIVE DOB (‰)*			*CUMULATIVE DOB (‰)*	
		CORRECTED FOR BMI		*1-4 h*	*CORRECTED FOR BMI*		*4-6 h*	*CORRECTED FOR BMI*
V1	4.9	4.9		15.5	15.6		13.8	13.9
V2	6.8	8.9		11.0	14.5		18.6	24.5
V3	3.5	3.6		6.0	6.1		7.7	7.9
V4	1.0	0.8		2.2	1.7		2.8	2.2
V5	5.2	5.0		9.9	9.5		14.5	13.9
V6	4.4	5.3		9.8	11.8		12.2	14.7
V7	6.1	6.1		13.7	13.8		14.8	14.9
V8	2.5	2.5		2.9	2.4		6.0	4.9
V9	3.6	3.3		6.6	6.0		9.2	8.3
V10	5.1	4.2		8.9	7.4		13.9	11.5
V11	4.5	4.5		3.9	3.9		11.2	11.1
V12	7.4	7.6		18.2	18.8		20.9	21.6
V13	3.2	3.2		3.8	3.5		8.1	7.4
V14	3.6	3.4		4.5	4.3		8.3	8.0
V15	1.9	1.6		3.5	3.0		4.9	4.2
V16	3.8	3.5		8.9	8.3		9.3	8.7
V17	3.1	2.9		4.6	4.3		7.3	6.8
V18	4.9	5.8		11.4	13.4		14.1	16.6
Mean	**4.19**	**4.28**		**8.07**	**8.24**		**10.98**	**11.17**
SD	**1.64**	**2.00**		**4.64**	**5.22**		**4.76**	**5.91**

Table 5.18 – DOB ‰ in 14 volunteers (all with BMI <29) following ingestion of ^{13}C-triolein and lemon mousse. Results are expressed as peak and cumulative DOB (‰) for 1-4 h and 4-6 h, with and without correction for BMI (corrected for BMI = observed DOB x 25/actual BMI)

MALE

SUBJECT	*PEAK DOB‰*	*PEAK DOB ‰ CORRECTED FOR BMI*	*CUMULATIVE DOB (‰) 1-4 h*	*CUMULATIVE DOB (‰) CORRECTED FOR BMI*	*CUMULATIVE DOB (‰) 4-6 h*	*CUMULATIVE DOB (‰) CORRECTED FOR BMI*
V3	3.5	3.6	6.0	6.1	7.7	7.9
V9	3.6	3.3	6.6	6.0	9.2	8.3
V11	4.7	4.5	3.9	3.9	11.2	11.1
V13	3.2	3.2	3.8	3.5	8.1	7.4
V14	3.6	3.4	4.5	4.3	8.3	8.0
V16	3.8	3.5	8.9	8.3	9.3	8.7
V17	3.1	2.9	4.6	4.3	7.3	6.8
Mean	**3.6**	**3.5**	**5.5**	**5.2**	**8.7**	**8.3**
SD	**0.5**	**0.5**	**1.8**	**1.7**	**1.3**	**1.4**

FEMALE

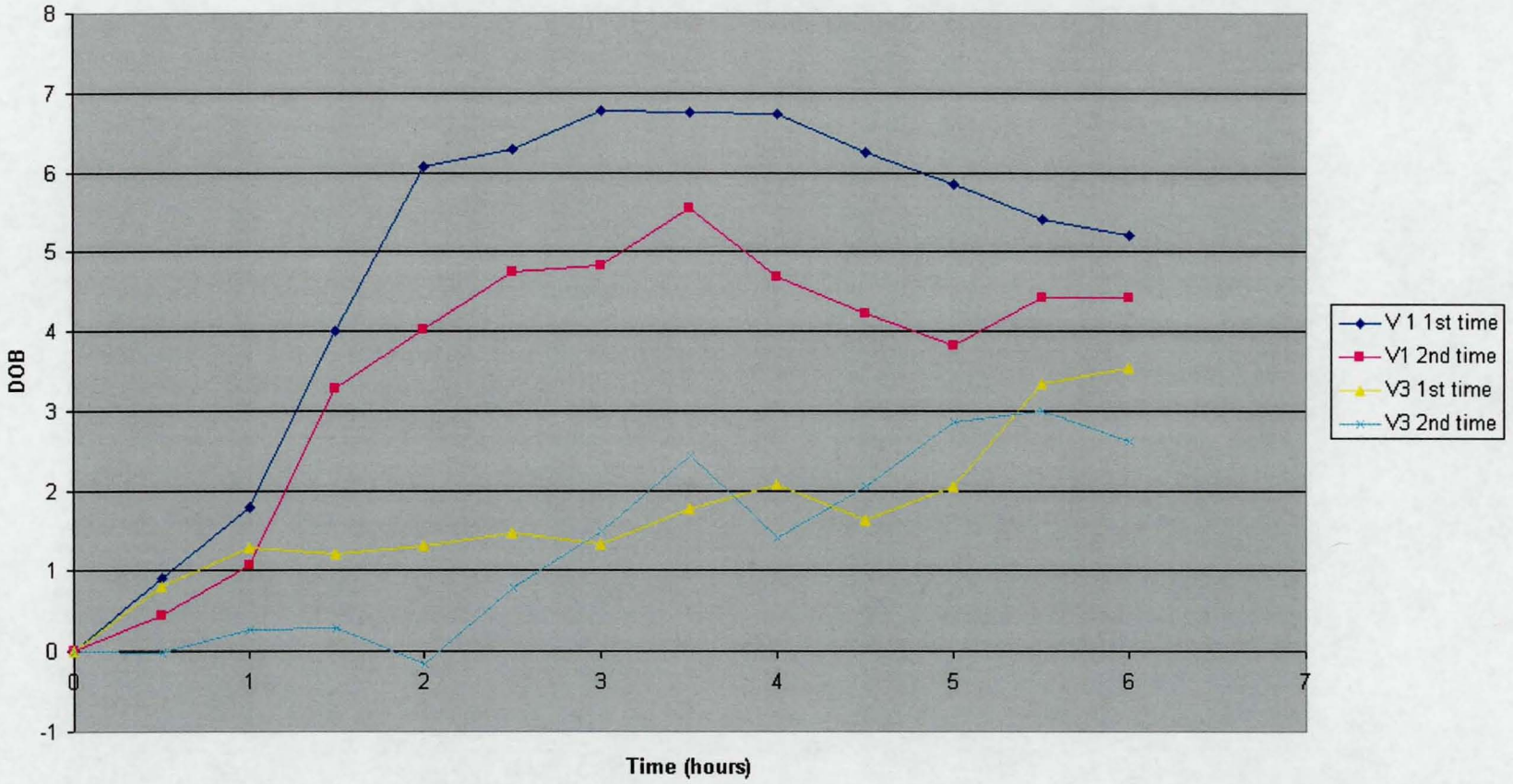
V1	4.9	4.9	15.5	15.6	13.8	13.9
V2	6.8	8.9	11.0	14.5	18.6	24.5
V5	5.2	5.0	9.9	9.5	14.5	13.9
V6	4.4	5.3	9.8	11.8	12.2	14.7
V7	6.1	6.1	13.7	13.8	14.8	14.9
V12	7.4	7.6	18.2	18.8	20.9	21.6
V18	4.9	5.8	11.4	13.4	14.1	16.6
Mean	**5.7**	**6.2**	**12.8**	**13.9**	**15.6**	**17.2**
SD	**1.1**	**1.5**	**3.2**	**2.9**	**3.1**	**4.2**

The reproducibility of the results was assessed in 2 volunteers (V1 and V3) by repeating the procedure after intervals of 6 months for V1 and 5 weeks for V3. Figure 5.7 shows the reproducibility of the results in the 2 volunteers.

Table 5.19 - DOB (‰) expressed as mean ±2SD for males and females for peak, and 1-4 h and 4-6 h breath samples.

	PEAK (DOB ‰)	*1-4 h (DOB ‰)*	*4-6 h (DOB ‰)*
Male	2.6-4.6	1.9-9.1	6.1-11.3
Female	3.5-7.9	6.4-19.2	9.4-21.8

Figure 5.7 Change in DOB (‰) with time after ingestion of lemon mousse and ^{13}C-triolein in two volunteers (V1 and V3) tested on two separate occassions.



5.6.2 Patients

The objective of this part of the study was to compare results by the ^{14}C-triolein and ^{13}C-triolein absorption tests using the proposed ^{13}C-triolein protocol.

Twenty six patients referred by the gastroenterologists for the ^{14}C-triolein test agreed to simultaneously undertake the ^{13}C-triolein test. 15 were male and 11 were female, their ages ranged from 20 to 73 years with a mean of 47 years. Details of the patients are shown in Table 5.20.

In order to evaluate the proposed protocol, the patients were classified into the following 4 groups on the basis of the diagnostic information available:

Group 1	:	Diagnosis consistent with normal fat absorption.
Group 2	:	Diagnosis consistent with fat malabsorption.
Group 3	:	Patients excluded because of other factors which may interfere with the test, for example, diabetes, BMI >29, or no clinical information available.
Group 4	:	No reason to exclude; but insufficient information to assign to Groups 1 or 2.

The DOB ‰ values for the 26 patients assigned to Groups 1, 2, 3 or 4 are shown in Table 5.21 for samples collected at 1 hour intervals over a 6 hour period following ingestion of the ^{13}C-triolein.

Table 5.20 – Information on patients undergoing investigations for fat malabsorption.

PATIENT	*SEX*	*AGE (y)*	*WT (kg)*	*HT (m)*	*BSA (m^2)*	*BMI*	*^{14}C-TRIOLEIN*	*DIAGNOSIS*
1	M	73	86	1.72	1.84	29.1	4.5	Diabetes, jejunal diverticulosis
2	M	61	84	1.78	1.96	26.5	4.1	?Bile salt malabsorption
3	M	33	86	1.8	1.9	26.5	3.1	No information available
4	M	44	73	1.75	1.73	23.9	3.6	Pancreatic insufficiency, alcohol induced
5	F	20	48	1.57	1.33	19.5	2.7	No diagnosis; probable fat malabsorption
6	M	43	70	1.55	1.55	29.2	3.1	(With Creon); no info available
7	M	55	73	1.65	1.65	26.8	4.9	Probable Irritable bowel syndrome(IBS)
8	M	39	72	1.98	1.88	18.4	5.9	No information available
9	M	54	79	1.8	1.83	24.4	4.5	Probable IBS
10	F	33	71	1.57	1.58	28.9	5.0	Probable IBS
11	M	39	70	1.67	1.65	25	4.7	Jogger's diarrhoea
12	M	53	95	1.8	1.95	29.3	1.9	Probable IBS
13	M	31	77	1.78	1.79	24.3	2.5	No information available
14	F	36	76	1.65	1.69	27.9	3.5	Lactose intolerance
15	M	48	80	1.83	1.85	23.9	2.6	Diabetes, partial villous atrophy, lactose intolerance, no final diagnosis
16	F	56	63	1.57	1.5	25.6	3.4	Probable IBS
17	F	57	61	1.57	1.48	25.6	2.1	No diagnosis; probable fat malabsorption
18	F	68	61	1.57	1.48	25.6	4.5	IBS
19	M	56	56	1.65	1.47	20.6	1.1	Coeliac disease; fat malabsorption
20	F	23	82	1.57	1.68	33.3	3.2	Probable IBS
21	F	29	45	1.4	1.2	22.9	1.7	Coeliac disease; fat malabsorption
22	M	45	89	1.72	1.85	30.1	4.8	No diagnosis
23	F	66	90	1.7	1.85	31.1	3.3	Diabetes; metformin induced diarrhoea
24	F	39	50	1.65	1.4	18.5	3.5	Chronic pancreatitis (alcohol induced)
25	F	64	68	1.6	1.53	26.6	1.3	Coeliac disease; pancreatic insufficiency
26	M	65	55	1.7	1.49	19	2.4	Coeliac disease; untreated
Range		20-73	45-95	1.40-1.98	1.20-1.96	18.4-3.33		
Mean		47	72	1.68	1.66	25.50		

The ^{13}C-triolein results are also expressed as the cumulative DOB ‰ for 1-4 hours, 4-6 hours and 1-6 hours and compared with ^{14}C-triolein expressed as peak CO_2 excretion/hour as % of dose given. The results for the patients in Groups 1 and 2 are shown graphically in Figure 5.8 and show that overall DOB values are lower in those patients with fat malabsorption (Group 2). The mean for the cumulative DOB values for Groups 1 and 2 were compared by the t-test. The results shown in Table 5.22 demonstrate that the results for Group 2 are significantly lower than Group 1. Patients in Groups 3 and 4 were not evaluated further but the results are shown in Figure 5.9.

Correlation coefficients for the ^{13}C-triolein breath test (expressed as ^{13}C DOB and ^{13}C percentage dose recovered (PDR)) compared with ^{14}C-results expressed as peak ^{14}C PDR were as follows:

	^{13}C (DOB) vs peak ^{14}C PDR		*^{13}C (PDR) vs peak ^{14}C PDR*	
	All patients	*Groups 1 & 2*	*All patients*	*Groups 1 & 2*
At peak	0.50	0.56	0.59	0.72
Cum 1-4 h	0.26	0.61	0.25	0.63
Cum 4-6 h	0.49	0.62	0.57	0.68
Cum 1-6 h	0.43	0.65	0.45	0.70

The PDR comparisons for peak, 1-4 h and 4-6 h are shown in Figures 10a, 10b and 10c for Groups 1 and 2.

Table 5.21a - ^{13}C-triolein breath test results for 26 patients assigned to diagnostic groups. (Group 1: Diagnosis consistent with normal fat absorption, Group 2: Diagnosis consistent with fat malabsorption, Group 3: Patients excluded because of diabetes, BMI >29 or no clinical information available, Group 4: No reason to exclude; but insufficient information to assign to Groups 1 or 2).

		DOB (‰) HOURS POST DOSE						*CUMULATIVE DOB (‰)*		
	Patient	*1*	*2*	*3*	*4*	*5*	*6*	*1-4 h*	*4-6 h*	*1-6 h*
Group 1	7	0.51	1.19	2.02	2.42	3.58	4.09	6.14	10.09	13.81
	9	0.46	0.87	2.04	4	3.78	3.56	7.37	11.34	14.71
	10	0	0.45	2.39	3.01	3.42	5.07	5.85	11.50	14.34
	11	0.57	1.54	2.85	4.83	6.15	5.51	9.79	16.49	21.45
	14	0.62	1.33	1.05	2.98	3.74	4.34	5.98	11.06	14.06
	16	0.42	0.52	1.2	3.73	6.69	5.89	5.87	16.31	18.45
	18	0.30	0.80	1.13	1.20	2.56	4.38	3.43	8.14	10.37
Group 2	4	0.74	1.01	1.45	1.76	1.86	0.23	4.96	3.85	7.05
	5	-0.99	0.02	0.32	1.52	2.03	0.55	0.87	4.10	3.45
	17	-0.53	0.54	1.85	2.02	1.65	1.09	3.88	4.76	6.62
	19	0.57	1.17	1.76	1.68	2.66	2.79	5.18	7.13	10.63
	21	-0.51	0.27	1.08	2.47	1.94	1.05	3.31	5.46	6.30
	25	0.44	0.46	0.24	0.93	1.44	2.21	2.07	4.58	5.72
	26	0.11	0.40	1.13	1.74	1.26	1.66	3.38	4.66	6.30
Group 3	1	-0.04	0.18	0.41	1.33	2.03	3.01	1.88	6.37	6.92
	3	0.16	0.22	0.67	1.03	1.82	3.45	2.08	6.30	7.35
	6	-0.67	-0.54	-0.65	-0.55	2.32	3.61	-2.41	5.38	3.52
	8	0.44	1.3	2.26	3.47	4.09	5.32	7.47	12.88	16.88
	12	0.45	1	1.88	1.42	1.7	3.39	4.75	6.51	9.84
	13	1.29	1.99	2.91	3.64	4.08	4.27	9.83	11.99	18.18
	15	-0.08	0.09	0.68	0.98	0.65	0.51	1.67	2.14	2.83
	20	0.52	0.54	0.24	0.63	2.61	3.91	1.93	7.15	8.45
	22	-0.12	0.24	0.94	0.98	2.34	3.51	2.04	6.83	7.89
	23	-0.09	-0.12	-0.63	0.3	1.19	2.3	-0.54	3.79	2.95
Group 4	2	0.1	0.11	0.6	1.25	2.13	2.44	2.06	5.82	6.63
	24	0.25	0.25	1.49	3.27	5.09	4.37	5.26	12.73	14.72

Table 5.21b – Peak % dose recovered for ^{13}C and ^{14}C-triolein breath tests and cumulative % dose recovered for ^{13}C-triolein for 26 patients assigned to diagnostic groups (as Table 5.21a).

		PEAK % DOSE RECOVERED		*CUMULATIVE PERCENTAGE DOSE RECOVERED*		
	Patient	*^{13}C-triolein*	*^{14}C-triolein*	*1-4 h*	*4-6 h*	*1-6 h*
Group 1	7	4.83	4.9	5.79	8.06	13.86
	9	5.11	4.5	6.86	9.65	16.51
	10	5.82	5.0	4.99	8.56	13.55
	11	6.96	4.7	8.34	12.81	21.15
	14	5.33	3.5	5.52	9.09	14.61
	16	6.81	3.4	4.10	11.71	15.81
	18	4.43	4.5	2.69	5.33	8.02
Group 2	4	2.19	3.6	4.81	3.37	8.18
	5	1.57	2.7	0.05	2.38	2.43
	17	3.23	2.1	4.24	3.16	7.40
	19	2.53	1.1	3.93	4.43	8.36
	21	1.80	1.7	1.51	2.69	4.20
	25	2.43	1.3	1.76	3.31	5.07
	26	1.55	2.4	2.23	2.63	4.86
Group 3	1	4.18	4.5	1.69	5.84	7.53
	3	4.80	3.1	2.18	5.64	7.82
	6	4.08	3.1	-1.28	5.49	4.20
	8	6.19	5.9	6.67	9.87	16.55
	12	5.21	1.9	6.36	6.46	12.81
	13	5.31	2.5	9.97	10.00	19.97
	15	1.27	2.6	1.51	1.80	3.32
	20	5.18	3.2	2.14	6.47	8.61
	22	5.05	4.8	2.22	6.58	8.80
	23	3.35	3.3	-1.0	3.62	2.62
Group 4	2	3.31	4.1	1.95	5.40	7.35
	24	4.11	3.5	2.93	7.20	10.13

Table 5.22a – Comparison of cumulative DOB values for Groups 1 and 2

	CUMULATIVE DOB, MEAN (AND SD) FOR TIME PERIODS SHOWN		
	GROUP 1	*GROUP 2*	*p-VALUE*
1-4 h	6.35 (1.92)	3.38 (1.53)	0.0042
4-6 h	12.13 (3.13)	4.93 (1.10)	0.0004
1-6 h	15.31 (3.58)	6.58 (2.13)	0.0001

Table 5.22b – Comparison of peak % dose recovered and cumulative percentage dose recovered 1-4 h, 4-6 h and 1-6 h in Groups 1 and 2.

	GROUP 1	*GROUP 2*	*p-VALUE*
Peak	5.61 (0.97)	2.49 (0.61)	<0.001
1-4 h	5.47 (1.83)	2.65 (1.73)	0.0194
4-6 h	9.32 (2.45)	3.14 (0.68)	<0.001
1-6 h	14.79 (3.93)	5.79 (2.24)	0.001

Figure 5.8 ^{13}C-triolein breath test results for Group 1 (normals fat absorption, solid lines) and Group 2 (fat malabsorption, dotted lines)

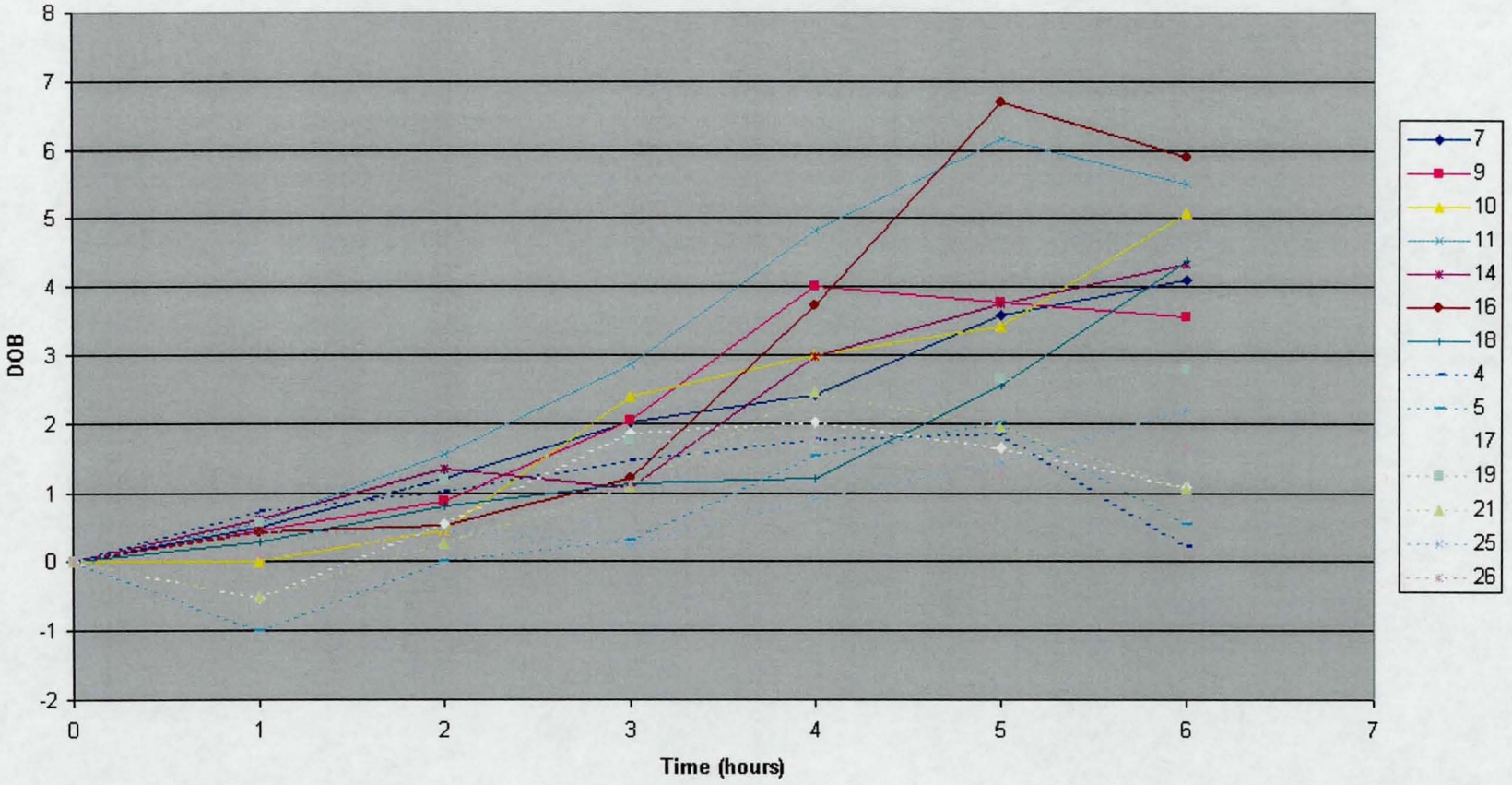


Figure 5.9 ^{13}C-triolein breath test results for Group 3 (patients excluded from Groups 1 and 2) and Group 4 (insufficient information, patients 2 and 24)

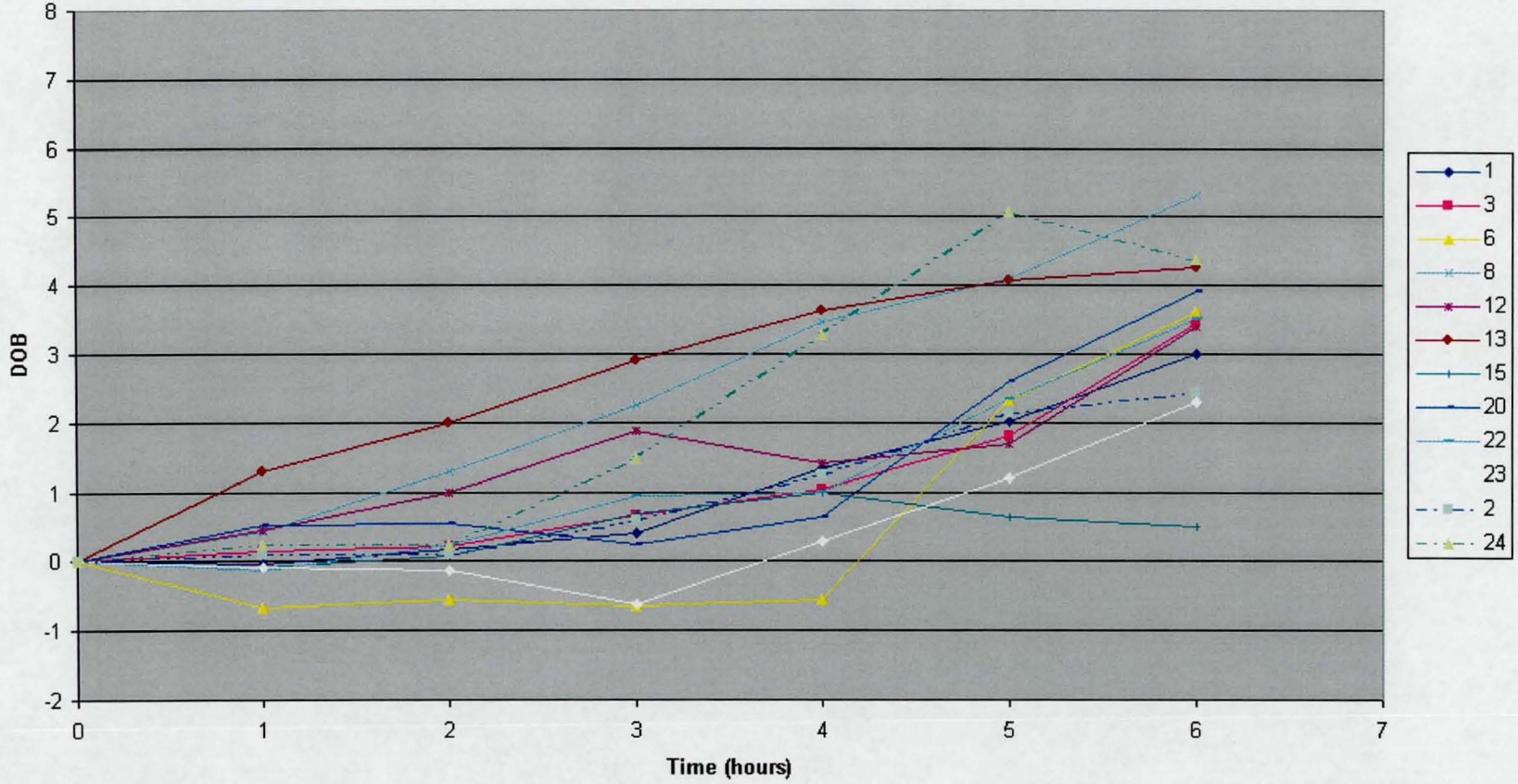


Figure 5.10a Comparison of ^{14}C Peak PDR and ^{13}C Peak PDR in Group 1 and Group 2

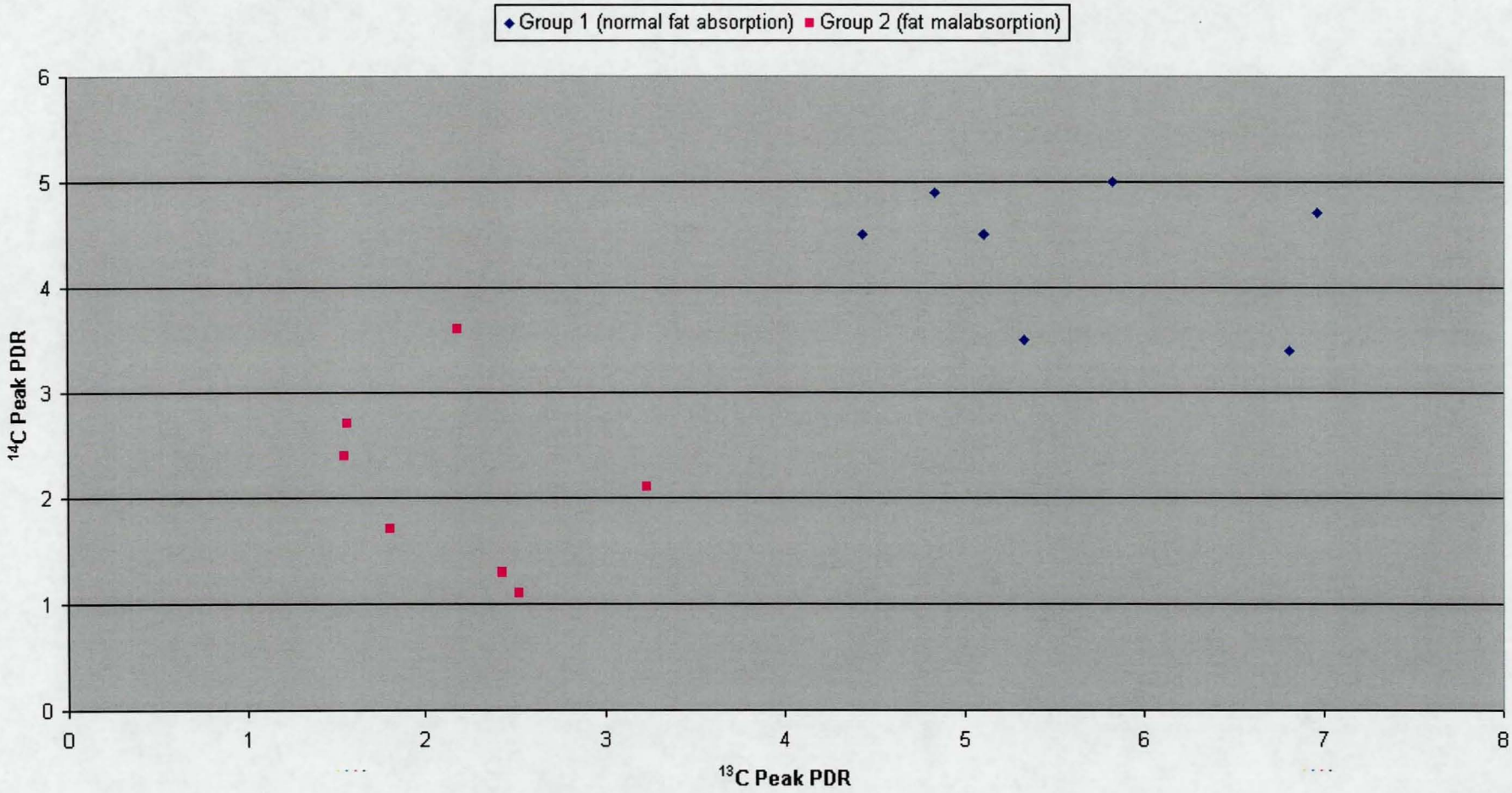


Figure 5.10b Comparison of ^{14}C Peak PDR and ^{13}C Cumulative 1-4h PDR in Group 1 and Group 2

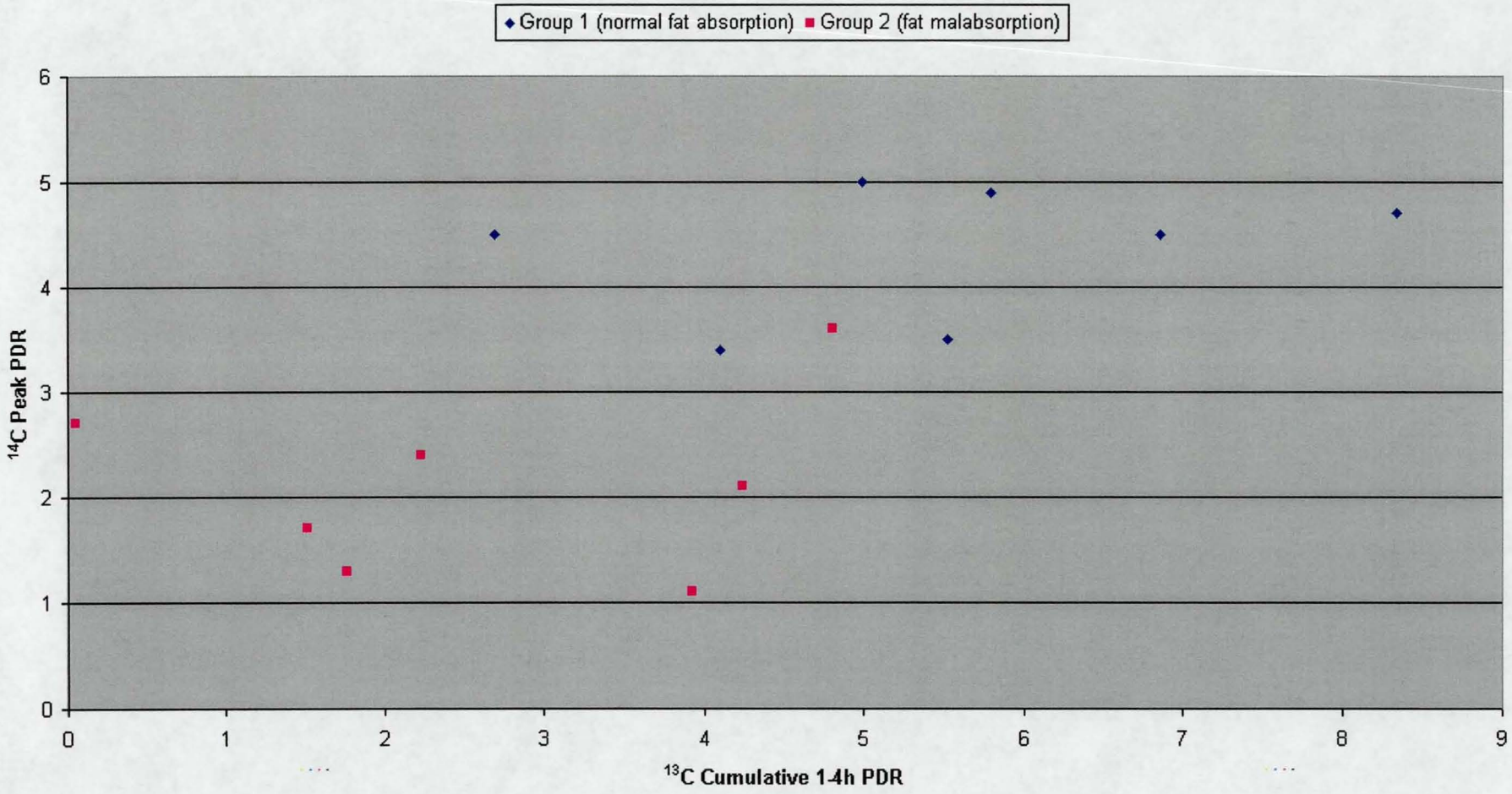
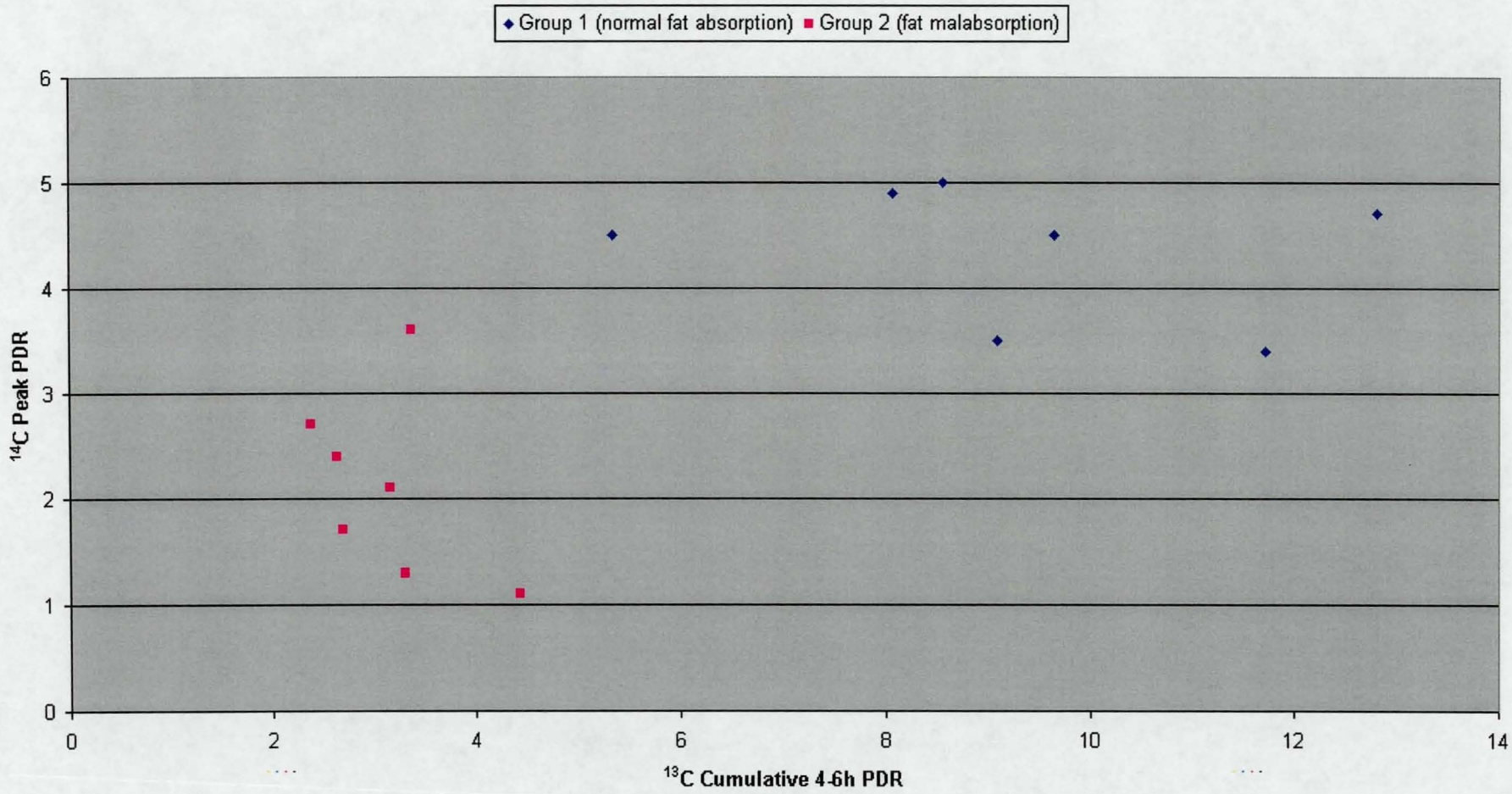


Figure 5.10c Comparison of ^{14}C Peak PDR and ^{13}C Cumulative 4-6h PDR in Group 1 and Group 2



6 DISCUSSION

In Southern Derbyshire, which has a population of 500,000, 4,000 patients are referred annually to the Gastoenterology clinics at Derbyshire Royal Infirmary and Derby City General Hospital for further investigations. 5-10% of these patients will require tests for the assessment of small bowel disorders. In about 1% of cases, a test for fat absorption forms a valuable part of the investigation as it covers a range of metabolic processes of digestion.

In the Derby hospitals the test used since 1987 is the ^{14}C-triolein breath test. The current study has set out to develop a ^{13}C-based test for fat absorption as an alternative to ^{14}C-triolein and which can be used routinely in an out patient department of any hospital.

In the ^{13}C breath tests as in the ^{14}C breath tests, administration of labelled substrate results in the production and exhalation of labelled CO_2 as the substrate is oxidised. Unlike ^{14}C tests in which the isotope background is low, ^{13}C tests are performed against a large background or natural abundance of 1.1% ^{13}C (32).

The study investigated the effect of food, exercise, ^{13}C dose and analytical precision of the BreathMAT during the development of the ^{13}C-based test for fat absorption.

Effect of food

The results shown in Figure 5.3 demonstrate the variation in DOB over the course of one day with measured Del13PDB values varying from –26.30 to –23.83‰. The changes are due both to the type of food ingested and the activities of the subject (Table 5.6). For a satisfactory ^{13}C-breath test, the pre-dose ^{13}C enrichment of CO_2 in the breath should be low and stable, ie, foods that are abundant in ^{13}C must be avoided, for example tuna or pineapple (44). A rice based diet has been used in some ^{13}C-breath tests (32) as rice has a low ^{13}C content. This is in contrast to foods containing maize and cane sugars that contain high levels of ^{13}C (44). Rice gives a value of -24 (expressed as the difference from the PDB standard per mil) which is similar to values typically found in subjects on a European diet. By contrast, maize flour has a value of -10. North Americans typically have breath $^{13}CO_2$ values of –22 reflecting the higher amounts of maize, and cane sugar, in their diets.

Patient preparation prior to the test is therefore very important. The patients in this study were required to fast from the midnight before the morning of the test although they could have a cup of tea before the test and a light lunch 4 hours after beginning the test. Discrimination between normal fat absorbers and fat malabsorbers might be improved if dietary restrictions were imposed for the last meal on the evening prior to the test date. For example, patient preparation for the breath hydrogen test requires that nothing to be eaten from 7.00 pm until the test has finished the following morning, but drinks may be consumed. In the last meal before 7.00 pm, the patient must not eat beans, bread, pasta or any other food containing wheat flour. Imposing these

restrictions has not lead to any problems with the acceptability of that test to patients at the Derbyshire Royal Infirmary.

In the context of fat absorption tests, Schoeller et al. (47) showed that a patient who had fasted overnight before a breath test would have been producing CO_2 mostly from fatty acid oxidation whereas subjects who had ingested a meal containing carbohydrates and proteins would have a greater abundance of $^{13}CO_2$ in breath. Löser et al. (48) mentioned that his subjects fasted for 12 hours overnight before evaluation of the ^{13}C mixed triglyceride breath test, but did not indicate any additional dietary restrictions.

In the present study, various foods were investigated as possible meals in the fat test. All those selected had similar amounts of fat (Table 5.9) and fulfilled our requirement for a test meal to contain about 20 g fat. Lemon mousse showed the least change in ^{13}C values from baseline over 6 hours, with the mean change in DOB 1-6 hour after ingestion varying from only –0.12 to +0.26‰ in 4 subjects (Table 5.10 and 5.11). Figure 5.4 compares the other three test meals (Scandishake, Duobar and Kitkat) with lemon mousse in a single subject (Table 5.12). The shape of the response after ingesting Scandishake is distinct from the other meals in that the DOB values rises to 2.3, two hours after the meal, whereas the other meals do not rise above zero. This difference is probably due to the larger amount of carbohydrate contained in the Scandishake, which is rapidly absorbed and metabolised after fasting. It also contains corn syrup solids which will lead to enrichment of breath $^{13}CO_2$. The sugar used in the recipe for the lemon mousse is refined

from sugar beet which has a low ^{13}C level of –27‰ (44), and is probably the reason the DOB value does not rise above zero after ingesting lemon mousse. The lemon mousse thus most closely fulfils the requirement for an isotopically neutral test meal to be used as a carrier for the labelled substrate.

During the procedure, we have been measuring the "change" from normal baseline values for that particular individual. It is important that the patient fasts before the test to give a stable baseline. Withholding food for 8 hours before and throughout the breath test eliminates or reduces the baseline shifts in ^{13}C abundance (43). The volunteers did fast before and during the test (see Results 5.6), although the patients were allowed a light meal 4 hours after ingestion of the test meal as permitted in the ^{14}C-triolein procedure. Turner et al. (14) showed significant discrimination between normal fat absorbers and fat malabsorbers when using ^{14}C labelled triolein and patients ingesting a light meal after 4 hours.

However, the ^{13}C breath test is likely to be more sensitive to food ingestion. Table 5.11 shows the results of four patients after lemon mousse, no ^{13}C-triolein and a light lunch after 4 hours. There is little change in DOB over the 6 hours. There are greater differences between patient's DOB at six hours than earlier in the test suggesting that the ingestion of food at 4 hours could have had a variable effect on breath $^{13}CO_2$. Figure 5.4 shows a trend towards a negative DOB in a volunteer who ingested lemon mousse but no lunch at 4 hours; however, this volunteer did not rest during the period of the test.

The ^{14}C triolein breath test protocol as described by Turner et al. (14) states that the patient needs to fast from midnight on the night before the test, ie, for nine hours before the test. The experience of using this test in Derby has proved that this fasting regime is acceptable to patients.

Effect of exercise

Another factor to be taken into consideration is the activity of the patient before and during the test. Schoeller et al. (47) showed that mild exercise was found to increase the variations in $^{13}CO_2$ output. This variation is shown in the Results section 5.3 where the natural variation of $^{13}CO_2$:$^{12}CO_2$ ratios was observed over a day. Exercise will increase the percentage of CO_2 production in the muscle mass and increase the mobilisation of glycogen and change the percentage of carbohydrate, protein and lipid being oxidised to CO_2. The increase in CO_2 will dilute the labelled CO_2 and so reduce the sensitivity of the test. The volunteers in the study were NHS employees who continued with their normal activities whereas the patients were relatively sedentary for the period of the test. This is an area which could be investigated further by assessing the length of time taken to reach a stable baseline after patients have travelled to the hospital on the morning of the test and by comparing changes in breath $^{13}CO_2$:$^{12}CO_2$ ratios in volunteers who rest completely after ingesting the lemon mousse with those who continue their normal activity. But at present the variation in the levels of exercise by both groups in the study means that the volunteer data could not be used to derive a reference range for this test of fat absorption.

Whether or not to measure rate of CO_2 production has been debated in many papers. Duncan et al. (17) states that assuming a uniform CO_2 production rate can introduce errors. Amarri et al. (31) states that measuring CO_2 production rate is vitally important to ensure accurate calculation of PDR (percentage $^{13}CO_2$ dose recovered). Ling et al. recently used a ventilated hood indirect colorimeter to measure carbon dioxide production in a ^{13}C-mixed triglyceride breath test in children (52). Vantrappen et al. (20) measured CO_2 and showed a difference in CO_2 production rate between normal subjects and in patients with pancreatic disease. He quoted a simplified procedure which assumed a production rate of 300 mmol per unit body surface area per hour.

Turner et al. (14) assumed the CO_2 production rate of 9 mol/kg/h when developing their test for fat absorption which showed good discrimination between normal patients and fat malabsorbers. In the current study, instrumentation was not available to test CO_2 production levels so the rate of 9 mol/kg/h was assumed. Schoeller et al. (47) states that the SD of CO_2 production rates is less than 25% for resting adults and children, and concluded that the diagnostic value will not be significantly reduced if a CO_2 production rate correction factor is not used as the responses of normal and diseased patients usually differ by a factor of 2.

Effects of the type of fat source

The type of labelled fat used for the test is important. Dietary triacylglycerols can be absorbed only after they have been hydrolysed by gastrointestinal lipases to give fatty acids and monoacylglycerols (4). In vivo, different

triacylglycerols are hydrolysed at different rates. For example, triacylglycerols that contain long chain, saturated fatty acids are hydrolysed more slowly (and therefore absorbed more slowly) than medium chain triglycerols (41). The site of absorption depends on the rate of absorption; triglycerides that are more slowly absorbed, for example those with long chain saturated fatty acids, tend to have a more distal site of absorption.

As described in the Introduction, triolein is considered to be the best substrate for assessment of all the processes of fat absorption, ie, a "global assessment". Therefore, triolein, a long chain triglyceride, was used as the substrate in this study to evaluate the overall process of fat absorption. Using triolein as the labelled fat ensures a direct comparison with the existing ^{14}C-triolein test for fat absorption.

Effect of ^{13}C dose

The ingestion of a sufficiently high dose of substrate is essential to ensure clear discrimination between normal and abnormal results but cost restricts the amount used. The dose of 200 μL (185 μg) of ^{13}C-triolein costs about £40; a balance has to be struck between a large ^{13}C-enrichment in breath CO_2 and cost. The cost of ^{14}C-triolein used currently at Derbyshire Royal Infirmary is £30, but there are also administration costs involved when using radiological materials.

The accurate dispensing of the substrate was essential to ensure reproducibility between patients. The repetitive pipetting of olive oil using a

pipette tip with 1 cm removed gave a CV = 1.46% (Table 5.4). Thus the reproducibility of dispensing the dose was deemed to be acceptable for this study.

The aim of this research has been to use a dose of ^{13}C-triolein that will show an adequate response (enrichment), that is adequate production of $^{13}CO_2$ in exhaled breath in a normal working day. Then the test will be able to be performed at a single outpatient visit.

It has been shown by Lembcke (32) that a dose of 2 mg/kg patient weight, ie, 120 mg for an average 60 kg patient, is adequate for the ^{13}C-hiolein breath test. Vantrappen et al. (20) used 240 mg ^{13}C-mixed triglyceride for their fat absorption test. Löser et al. (48) used both 200 mg and 250 mg of ^{13}C-mixed triglycerides as an indirect pancreatic function test. A trial dose of 200 μL of ^{13}C-triolein, equivalent to 185 μg, was used in the protocol and this proved to give an adequate response as shown in the Results (Figure 5.5, Table 5.13), with peak DOBs of 8.14 and 6.78 in 2 volunteers. Table 6.1 shows the comparison of peak ‰ dose/hour recovered in normal subjects using different ^{13}C-labelled fats. It should be noted that the lower dose used and the higher percentage recovered by Lembcke is due to the use of hiolein, an algal product with uniformly ^{13}C-labelled triglyceride (98%). The percentage dose recovered after the use of ^{13}C-triolein compares favourably with previous research.

Table 6.1 – Comparison of peak ‰ dose/hour recovered in normal subjects using different ^{13}C-labelled fats.

TEST	*DOSE*	*PEAK ‰ DOSE/HR IN NORMAL SUBJECTS*	*AUTHOR*
^{13}C-hiolein	120 mg	10‰	Lembcke (32)
^{13}C-mixed triglyceride	240 mg	8‰	Vantrappen (20)
^{13}C-mixed triglyceride	200 mg	6‰	Löser (48)
^{13}C-triolein	185 mg	5‰	This study

The quantity of fat in the test meal is important because gastric emptying will affect the rate at which the substrate leaves the stomach and is available for digestion in the small intestine. Gastric emptying is affected by the osmolality of the meal and a large fat meal will delay stomach emptying.

In the Introduction, it was noted that the amount of fat present in test meals used to assess fat absorption ranged up to 60 g. Duncan et al. (16) state that 20 g of fat is the optimum quantity, as too much fat in the test meal can delay gastric emptying. The four meals (Sandishake, Duobar, Kitkat and lemon mousse) were expressed as shown in Figure 5.4. Over the 6 hours, the test would be carried out, the response to Scandishake gave a DOB range from 0 to 2.4, Kitkat gave a DOB response of 0 to –0.8, Duobar 0 to –1.2 and lemon mousse 0 to –0.8.

Initial thoughts were that a commercial product could be used as the test meal. If a suitable product had been found the test could be performed more easily in any hospital and the samples returned to a central laboratory for

testing. Table 6.2 shows the comparison of two Penguin biscuits bought at an interval of two months which shows the total weight has reduced, percentage of protein and fat decreases and an increase in carbohydrate. As can be seen, products can vary significantly over a short time span. Pharmaceutical products are likely to vary to a lesser extent due to tighter restrictions and regulations but no satisfactory product could be found.

Table 6.2 - Comparison of composition (declared by the manufacturer) of Penguin biscuits A and B purchased over a 2 month period.

	A	*A (%)*	*B*	*B(%)*
Weight (g)	25.5		24.6	
Energy (kj)	572		549	
Protein (g)	1.4	5.5	1.3	5.3
Carbohydrates (g)	16.4	64.3	16.1	65.5
Sugar (g)	10.3		10.1	
Fat (g)	7.3	28.6	6.8	27.6
Saturates (g)	4.9		4.6	
Fibre (g)	0.4	1.6	0.4	1.6
Sodium (g)	<0.1		<0.1	

Therefore, lemon mousse was used in this study, as the recipe includes 20 g fat, it is isotopically neutral and is made locally in the Derbyshire Royal Infirmary Catering Department.

To conclude, there are a number of factors which will affect the sensitivity of the test, ie, food ingested prior to the test, fat meal used, effect of exercise before the baseline sample taken, type and quantities of labelled fat.

Analytical quality

In 1998, a European inter-laboratory comparison of breath $^{13}CO_2$ analysis was performed by Stellard and Geypens (49). This survey showed a large number of analytical variables (suppliers, models, inlet systems and procedures) but as long as each laboratory ensured regular calibration of the system and an standardised analytical procedure was used, the results obtained by the Finnegan BreathMAT were comparable with other IRMS analyses currently available.

Acceptable analytical quality is essential to ensure confidence in the validity of the results produced and must be demonstrated over the time period of the study. The routine checks of analytical quality of the BreathMAT include multiple analysis of the same sample, ie, the reference gas. This is carried out as a preset analytical sequence and variability of the Del13PDB result reported. Acceptable precision is indicated by an SD of less than 0.1‰. Figure 5.1 gives an example of this sequence, with an SD of 0.054‰. Over the time of the study this gave consistently acceptable standard deviation values. Unlike the reference gas, breath samples have variable CO_2 concentrations and Figure 5.2 and Table 5.1 demonstrate the importance of the linearity correction software in reducing the imprecision of Del13PDB measurements when CO_2 concentration varies for the same breath sample. "Linearity" software enables the relationship between the CO_2 concentration (as mV) and the apparent Del13PDB concentration to be independent of each other (Figure 5.1). The standard deviations improved significantly from 0.40‰ to 0.08‰. Table 5.2 shows acceptable precision for Del13PDB (SD = 0.12‰)

when 10 replicate breath samples from a volunteer were collected over a period of a few minutes in the manner described (section 4.2.1b).

All the results quoted in the study are expressed as the difference from the base line Del13PDB value at a certain time after ingesting the ^{13}C substrate, ie, as DOB. The analytical detection limit is defined as the smallest value that can be distinguished from zero with a defined degree of confidence. For this study, it was therefore necessary to assess the precision of measuring DOB in order to determine what increase in DOB is significantly different from background DOB. Paired breath samples collected from the operator within a few seconds should have a DOB of zero. Table 5.3 shows the variability in DOB in samples collected in this way on 20 different days and therefore included any additional analytical variability due to "between day" factors such as temperature or variability in ion source. The mean difference from zero was +0.0025% with an SD of 0.14‰. In clinical biochemistry, the analytical detection limit is usually defined as zero plus 3SD, ie, a change in DOB of >0.42% represents a significant change from the baseline (50).

The data collected over the course of one day by a volunteer (Table 5.6) shows the effect on precision due to variations in CO_2 concentrations between duplicates when linearity correction software is not used (Table 5.7). Tables 5.8a and 5.8b demonstrate that precision improves when the variation between CO_2 concentrations in duplicates is low. This again shows the importance of using the linearity software program during the course of this study.

A protocol for monitoring analytical quality over the period of this study has been developed. Each day when samples were analysed, pairs of the operators own breath samples were analysed at the beginning and end of each "run". In addition, patients samples were repeated with each run (Table 5.4), and mean and SD evaluated. Every three months, precision was checked using the CO_2 zero 20 method (Figure 5.1) with the reference gas.

These procedures give confidence in the validity of the DOB results, ie, the DOB results after 185 mg labelled triolein was administered remembering that the absolute PDB levels at that particular time were not relevant as DOB is calculated as (post dose Del13PDB – pre dose Del13PDB).

Evaluation of proposed protocol

The aim of this study has been to develop a test of fat absorption that can be performed in an outpatient setting, within the normal working day.

An adequate response was noted when two volunteers ingested 200 μL ^{13}C-triolein and lemon mousse compared with four patients, who ingested lemon mousse alone (Figure 5.5). As a comparison, the same volunteer ingested Scandishake alone and Scandishake plus 200 μL ^{13}C-triolein (Table 5.14, Figure 5.6). Again there was a response to the ^{13}C-triolein but a different shaped response curve. This is probably due to the different proportions of fat, protein and carbohydrate in the meal.

Although the results from the volunteers (V1-V18, Table 5.15) cannot be directly compared to the patients because they continued with normal activity during the test, the results demonstrate the effect of Body Mass Index (BMI) on the Del13PDB values. Those with BMI >29 kg/m^2, ie, those defined as obese were observed to have lower peak DOB values, eg, volunteers V4, V8 and V15, (Table 5.16). When volunteers results were corrected to a BMI of 25 (Table 5.17) the distribution of results around the mean was greater than without correction, and those with BMI >29 remained as outliers. Differences were noted between the male and female volunteers test results (Table 5.18), with males having significantly lower peak DOBs than females. Table 5.18 compares results for males and females, and results for volunteers with BMI >29 were excluded. In Table 5.19, results for male and female volunteers are expressed as mean ±2SD for peak DOB and for the cumulative 1-4 hour and 4-6 hour DOB values. However, these could not be used as "normal" ranges as the subjects were not resting during the period of the test.

Two volunteers were retested using the same protocol after intervals of 6 months and 5 weeks (Figure 5.7). The response curves for the 2 volunteers showed excellent reproducibility both in the shape of the curves and peak DOB values.

The patients were classified into four groups on the basis of review of the medical notes and the clinical information available (Table 5.20). This review was carried out by a member of the medical staff who was unaware of the ^{13}C-triolein results. Group 1 included those patients with gastrointestinal

disorders not usually associated with fat malabsorption, for example, probable IBS, lactose intolerance and Joggers diarrhoea. Group 2 consisted of patients with disorders which would be expected to lead to fat malabsorption, for example, pancreatic insufficiency and untreated coeliac disease, the fat malabsorbers. Group 3 included patients with diabetes or BMI >29. Either of these could lead to a false interpretation of the breath test results. This group also included patients for whom no clinical information was available. In a further 2 patients, (Group 4) there was insufficient information to assign them to Group 1 or 2 as their disorder can have variable effects on fat absorption.

The results for all 26 patients studied are shown in Table 5.21. Patients in Group 2, ie, those with fat malabsorption, had lower peak DOB (1.74-2.79) when compared with patients in Group 1, ie, those with normal fat absorption (4.0-6.69). Table 5.22 compares the curve DOB values for these two groups of patients, and shows significant differences ($p = <0.005$) for all three time periods assessed. The test as developed in this study is therefore able to distinguish between patients with and without fat malabsorption.

The shape of the response curves produced by the patients (Figure 5.8 and Figure 5.9) shows three groups; those whose $^{13}CO_2$ values were still rising at 6 hours (11 patients), those who had reached a low peak and then fallen (mainly Group 2 patients, the fat malabsorbers), and those who reached a high peak and then were falling at the 6 hour point. Lembcke using ^{13}C-hiolein (32) noted that the peak occurred 6-9 hours after ingestion of the labelled test meal, using a meal comprising a similar amount of fat. They

suggested using a measurement at 8 hours after substrate ingestion, however, this would be less convenient in an outpatient setting. Turner et al. (14) using ^{14}C-triolein and lemon mousse prepared as described in this study, observed peak $^{14}CO_2$ excretion within 6 hours in 97% of 61 subjects with normal fat absorption. Similar results would be expected when ^{13}C-triolein is used instead of ^{14}C-triolein.

The correlation between the two tests was examined. Figures 5.10a, 5.10b and 5.10c compare ^{14}C-triolein expressed as the peak % dose recovered with ^{13}C-triolein expressed cumulatively for the three time periods, 1-4 h, 4-6 h and 1-6 h. A wide scatter of results was noted but the cumulative 4-6 h appeared to most satisfactorily separate Group 1 and Group 2 patients and gave the highest correlation coefficient (0.48) when the two tests were compared in all patients studied.

The data analysis focused on Group 1 and Group 2, the patients in the other groups could not be classified as normal fat absorbers or malabsorbers. Cumulative data are shown graphically in Figures 5.10a, 5.10 b and 5.10c. This study has shown the cumulative DOB 4-6 hours gave the greatest separation between the groups of patient results. In future protocol developments, collection of data at 0, 4, 5, 6 h post dose or at a single post dose time could be sufficient to give discrimination between the groups.

This study could be extended further by investigating the effect of restricting the type of food eaten the night before the test and ensuring that no further

food is ingested until the end of the test. The activity of the patient prior to and during the test is another important factor, this could be investigated further by resting the patient before beginning the test and then taking two basal breath samples 30 minutes apart. Activity throughout the test should be restricted. Additional subjects with normal and abnormal fat absorption need to be studied to increase the confidence in the procedure.

In conclusion, this work has showed that this method using 200 μL ^{13}C-triolein with lemon mousse as the fat meal can distinguish normal fat absorbers from fat malabsorbers and with the collection of data from more patients, this method could be one of the routine tests used in a clinical setting for the detection of fat malabsorption.

7 CONCLUSION AND FUTURE DEVELOPMENTS

This study has shown that the protocol using 200 μL ^{13}C-triolein with lemon mousse as the fat meal can distinguish normal fat absorbers from fat malabsorbers. The cumulative DOB data collected 4-6 hours post dose gave the greatest separation between groups of patients results.

Future workers may wish to restrict further the type of food eaten the night before the test and also ensure that no other food is ingested until the end of the test. They may also wish to restrict the activity of the patient before and during the test. The actual point of testing post dose could also be investigated further, only one breath sample at 6 hours post dose may be sufficient to discriminate normal fat absorbers from fat malabsorbers.

During the time of the study, the low numbers of patients referred by the Gastroenterologists has hindered data collection. Future workers may wish to recruit a doctor who could be more active in recruiting suitable patients for a larger study.

Therefore with the collection of data from a larger group of patients and further restrictions in diet and exercise, this method could become one of the routine tests used in a clinical setting for the detection of fat malabsorption.

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9 APPENDICES

APPENDIX I

Southern Derbyshire Local Research Ethics Committee approval

Southern Derbyshire NHS
Health Authority

Southern Derbyshire
Local Research Ethics Committee

Derwent Court
Stuart Street
Derby
DE1 2FZ

Chairman: Dr A W A Crossley MB ChB FRCA
Acting Administrator: Adrian Thorpe ext 6210 (direct dial from Derby hospitals 16-6210)
Direct fax: 01332 363963
email: adrian.thorpe@mail.sderby-ha.trent.nhs.uk

Tel: 01332 626300
Fax: 01332 626350

4th May 2000

Dr P G Hill
Consultant Biochemist
Department of Chemical Pathology
DRI

Dear Dr Hill

SDLREC REF: 0003/154
DEVELOPMENT OF A FAT ABSORPTION TEST USING A STABLE ISOTOPE LABELLED TRIGLYCERIDE (^{13}C-TRIOLEIN) AS A MARKER SUBSTRATE

The Southern Derbyshire Local Research Ethics Committee considered the above protocol on 28th March 2000. I am pleased to be able to inform you that your study was approved on the understanding that you will follow the protocol as agreed. However, the Committee would be grateful if the title on the patient information sheet could be simplified. May I remind you that indemnity arrangements and financial implications need to be agreed with all appropriate Trust management(s) **before commencing the trial.**

Please note that the SDLREC will require:

- to be advised immediately of any adverse report or changes to the protocol or if the study is abandoned;
- a progress report on an annual basis or at the end of the study if this is a lesser time;
- copies of all published reports.

For your information, the SDLREC complies with the ICH Harmonised Tripartite Guidelines for Good Clinical Practice. In line with Department of Health guidance it has an executive sub committee which meets twice a month specifically to consider MREC-approved applications.

[redacted]

Yours sincerely

A W A Crossley
Chairman
Southern Derbyshire Local Research Ethics Committee

cc Dr T Grieve, R & D Manager, DCGH

APPENDIX II

Standard Operating Procedure: ^{14}C-triolein fat absorption breath test

Derby Pathology Chemical Pathology	SOP - ^{14}C Triolein Breath Test No of pages: 6 **Expiry date: 30 June 2004**
AUTHORISED STANDARD OPERATING PROCEDURE	Authorised by: **M R Hopton** Signature 
File Code: - SOPTRIO Work area:- d5	

^{14}C - Triolein Breath Test

INTRODUCTION

In this test fat absorption is assessed by measuring the $^{14}CO_2$ exhaled in the breath over a 6 h period after a small oral dose of ^{14}C labelled triglyceride.

PRINCIPLE

In the normal process of fat digestion, absorption and assimilation, dietary triglycerides are emulsified by bile salts in the small bowel and hydrolysed by lipase to free fatty acids and monoglycerides which are absorbed. Re-esterification to triglycerides occurs in the enterocytes.

After transport via the lymphatic and blood systems to the liver, the triglycerides are oxidised with the production of CO_2 which is subsequently exhaled.

In the ^{14}C - triolein breath test, a small amount of ^{14}C - triolein (the triglyceride, glyceryl - trioleate) is given in a test meal of lemon mousse containing 20 g of fat. The ^{14}C - triolein is incorporated in the body's fat pool and oxidised with the exhalation of $^{14}CO_2$.

Breath CO_2 is trapped by exhalation into hyamine hydroxide; scintillant is added and ^{14}C activity measured by liquid scintillation counting.

As the test uses ^{14}C, it should only be carried out within the first 28 days after the LMP in pre-menopausal women. It is unsuitable for subjects under 16 years of age, and in adults with diabetes, thyroid disease, obesity or severe pulmonary disease.

SPECIMEN TYPE

End expiratory breath collected into hyamine hydroxide, a CO_2 trapping solution. It is important to collect the end expiratory air as the tidal volume will contain largely room air and will thus not reflect CO_2 released from fatty acid metabolism.

After assembling the tube and scintillation vial containing hyamine hydroxide (see figure), the patient should be instructed carefully to breath in, to hold their breath for about 5 seconds, to breath out normally and then, without breathing in , to blow the air remaining in their lungs, gently through the tube to bubble through the hyamine hydroxide solution.

Note that 4 - 5 breaths may be required to saturate the solution and change the colour from blue to colourless.

REAGENTS AND MATERIALS

1. Mousse: in freezer in diet section of DRI kitchen. Ask dietician in office if unsure. The recipe for the mousse is described in Gut (1987); 28: 694 - 700, ^{14}C - triolein absorption: a useful test in the diagnosis of malabsorption. Turner J. M. et al. (copy attached).

2. ^{14}C - triolein; glycerol tri [1 - ^{14}C] oleate, code CFA 258, Amersham International; 9.25 MBq, 3.7 MBq/mL.

   This must be used in accordance with the local rules for the use of carbon^{14}

   Keep in the fridge in Day Case Unit laboratory (key to fridge in key locker in Biochem 1 office). Replace lid promptly and tightly after use to prevent evaporation of toluene.

3. Hyamine hydroxide, 1mol/L (note: the Gut paper erroneously describes this reagent as hyamine hydroxide 2 mol/L).

HAZARD
Hyamine hydroxide: Avoid contact with skin. Wear suitable gloves and eye protection, use in fume cupboard. If hyamine hydroxide comes into contact with the skin wash with plenty of water.

4. Ethyl alcohol, absolute.

5. Hyamine hydroxide (0.5 mol/L): mix equal volumes of hyamine hydroxide (1 mol/L) and ethyl alcohol (absolute). 4 mL of this solution contains 2 mmol of hyamine hydroxide and will trap 2 mmol of CO_2.

6. Thymolphthalein (500 mg/L), in ethanol.

7. Scintillant - EMULSIFIER SAFE obtained from Canberra Packard Ltd. Brook House, 14 Station Road, Pangbourne, Berks. RG8 7DT.

8. Breathing tubes: See Appendix 1.

9. Scintillation vials: Poly - Q vials from Beckman RIIC Ltd, Progress Road, Sands industrial Estate, High Wycombe, Bucks HP12 4JL.

10. **Bottled water: supplied by catering stores using requisition book.**

STANDARDS

The efficiency of the liquid scintillation counter must be checked every 6 months.

CONTROLS

Not available.

METHOD DETAILS

You should only carry out this procedure after proper training.

1. **Make up 7 vials per patient. Pipette 4.0 mL of hyamine hydroxide (0.5 mol/L) and 100 µl thymolphthalen solution into each vial.**

2. **Take mousse out of freezer, allow to thaw in food fridge overnight.**

3. **Dispense 185 kBq (5uCi), i.e. 50µL, of ^{14}C - triolein onto stainless steel teaspoon. Record batch number. Evaporate the toluene from spoon using cold hair dryer.**

4. **Put spoon into mousse.**

5. Wash hands thoroughly to avoid possible transfer of ^{14}C on to tubes or vials.

6. Ensure that the patient has understood and followed the instructions described in the letter to the patient (attached).

7. Take basal sample BEFORE giving mousse. Ensure that patient breaths out as far as possible (i.e. collect end expiratory air). If solution is mistakenly sucked into mouth, wash well with water.

8. Give mousse. Ensure that patients eats it ALL and that the spoon has been well licked.

9. Give patient a drink of water (to ensure no residual radioactivity is left in the mouth).

10. After 1 hour collect another breath sample, and continue at hourly intervals until a total of 7 samples have been collected.

11. A light lunch is permitted 4 hours after the start of the test.

12. Ensure that patient's height and weight are recorded.

13. The patient may eat and drink normally after lunch. Smoking is NOT permitted at anytime during the test.

14. **Add 10 mL scintillant (EMULSIFIER SAFE, Canberra Packard Ltd) and leave overnight. Carry out in a fume cupboard and wear gloves. Send to DCGH for counting.**

CALCULATION

The peak CO_2 excretion/h is expressed as % of the dose given, based on peak cpm. It is calculated as:

$$\frac{\text{peak cpm} \times 9 \times \text{body weight in kg} \times 100}{2 \times 1.11 \times 10^7}$$

In borderline cases CO_2 excretion/3 h expressed as % of the dose, based on the sum of 4 h, 5 h, and 6 h cpm values, may provide additional interpretative information. It is calculated as:

$$\frac{(4\text{ h} + 5\text{ h} + 6\text{ h cpm}) \times 9 \times \text{body weight in kg} \times 100}{2 \times 1.11 \times 10^7}$$

The derivation for the formulae is given in Gut 1987; 28: 694 -700.

SPECIAL REQUIREMENTS FOR REAGENT AND SPECIMEN DISPOSAL

1. For reagent and/or specimen disposal - pipette tips, other radioactive waste from administering the ^{14}C - triolein and the scintillation vials after counting must be disposed of according to the local rules for the use of Carbon^{14}

2. All other reusable equipment must be sterilised for 24 hours in 125 ppm sodium hypochlorite solution.

TECHNICAL DOCUMENTATION AND VALIDATION

A written log must be kept of receipt, usage and disposal of ^{14}C - triolein.

Ensure that a record is kept of patient details, including body weight (kg), and cpm for basal and subsequent samples.

Enter the peak CO_2 excretion/h expressed as % of the dose into the computer. Set code is BCO2. Add appropriate CTEXT comment;

BCO2A: Normal fat absorption is indicated by a value of 2.8% or more. Result indicates malabsorption of fat.

or

BCO2N: Normal fat absorption is indicated by a value of 2.8% or more. Result indicates normal absorption of fat.

Set heading is ^{14}C TRIOLEIN ABSORPTION TEST.

CLINICAL AUTHORISATION

1. Results will be authorised by a senior member of staff prior to printing.

OUT OF HOURS REQUESTS

The test is not available out of hours.

RANGES

Normal fat absorption is indicated by a peak CO_2 excretion/h expressed as % dose of 2.8% or more. A cumulative 3 h results of 6.9% or more indicates normal fat absorption.

TURN ROUND TIME

Appointments for out patients are normally made within 2 weeks of receiving requests. The test will be done on in-patients only when there are clear reasons why an OP visit is inappropriate.

Results are available within 48 hours of completion of the test.

EXTERNAL QA SCHEMES

Not available

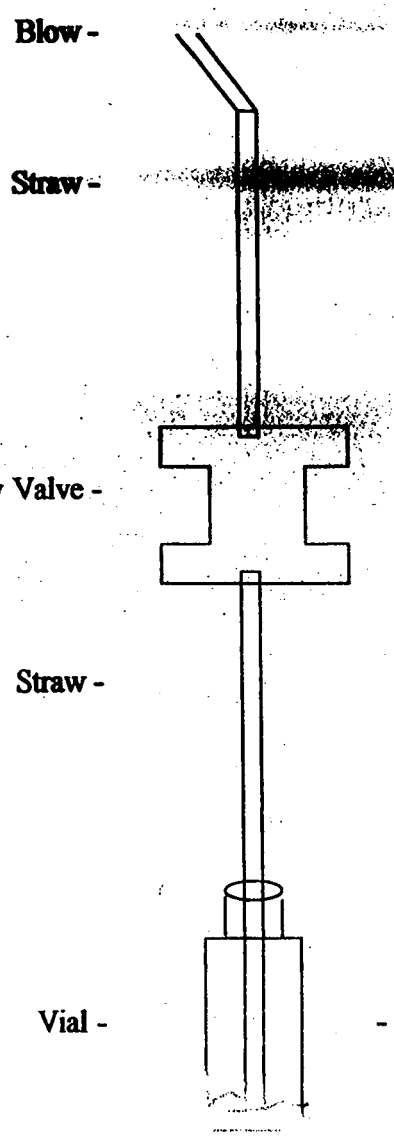
COSHH STATEMENT

These reagents and their vapours will not cause severe or immediate health effects or require immediate recognition to prevent subsequent effects when:

1. Used and stored as recommended by the manufacturers instructions.
2. Disposed of in quantities routinely in use.
3. Spilled in quantities routinely in use.
4. Normal precautions required for handling laboratory reagents are exercised and any control procedures as indicated are observed.

Author: Dr P G Hill		
Reviewed by:	Date:	Expiry date:
A Lloyd	August 1997	June 1998
A Lloyd	June 1998	June 1999
Dr P G Hill	August 1999	June 2000
Dr P G Hill	September 2000	June 2002
V Porter	August 2002	June 2004

Appendix 1


Blow -
Straw -
One Way Valve -
Straw -
Vial -
- Hyamine hydroxide/ absolute alcohol

DERBY PATHOLOGY

29 October 1996

Chemical Pathology Department
Derbyshire Royal Infirmary NHS Trust
London Road, Derby, DE1 2QY

Tel: 01332 347141
Please ask for extension 2521
Fax: 01332 254924

Patient's address

Dear Patient

Dr X has asked me to get in touch with you in connection with a test of fat absorption.

I would be grateful if you could attend the Day Case Unit at Derbyshire Royal Infirmary on Thursday 10 October 1996 at 9.00 am. On arrival at the hospital, follow signs to the Day Case Unit, (see enclosed map), and present the enclosed form at the reception area there. Please do not have anything to eat from midnight on Wednesday, ie, 9 hours before coming for the test.

The test consists of eating some mousse after which we will collect breath samples from you hourly until about 3.30 pm. Details about the test are enclosed. You can have a cup of tea before coming for the test and you can have a light lunch 4 hours after beginning the test and for this it may be convenient to bring some sandwiches. A book or some other means of passing the time would help the day to pass more quickly.

If it is not possible for you to attend on this date, could you ring extension 2521, at the Derbyshire Royal Infirmary (347141) as soon as possible to arrange another date, and so that your appointment time can be offered to someone else.

Yours sincerely

P G Hill, PhD, MCB, FRSC, FRCPath
Consultant Biochemist

DERBY PATHOLOGY

29 October 1996

Chemical Pathology Department
Derbyshire Royal Infirmary NHS Trust
London Road, Derby, DE1 2QY

Tel: 01332 347141
Please ask for extension 2521
Fax: 01332 254924

WOMEN <50 YEARS

Patient's address

Dear Patient

Dr X has asked me to get in touch with you in connection with a test of fat absorption.

I would be grateful if you could attend the Day Case Unit at Derbyshire Royal Infirmary on Thursday 24 October 1996 at 9.00 am. On arrival at the hospital, follow signs to Day Case Unit (see enclosed map) and present the enclosed form at the reception area there. Please do not have anything to eat from midnight on Wednesday, ie, 9 hours before coming for the test. However, as with many other investigations, it is important that this test is not performed if there is any possibility that you are pregnant and we would not wish to do the test if your period is overdue or clearly missed. Please let me know if this is the case.

The test consists of eating some mousse after which we will collect breath samples from you hourly until about 3.30 pm. You can have a cup of tea before coming for the test and you can have a light lunch 4 hours after beginning the test and for this it may be convenient to bring some sandwiches. A book or some other means of passing the time would help the day to pass more quickly.

If it is not possible for you to attend on this date, could you ring extension 2521, at the Derbyshire Royal Infirmary (347141) as soon as possible to arrange another date, and so that your appointment time can be offered to someone else.

Yours sincerely

P G Hill, PhD, MCB, FRSC, FRCPath
Consultant Biochemist

Patient Information

DERBY PATHOLOGY

FAT ABSORPTION TEST

The test which your doctor has requested is used to assess the absorption of fat in the bowel.

You will be asked to blow into a tube, after which you will be given a lemon mousse to eat. A very small amount of radioactive fat is added to the mousse.

Your doctor has given approval for the use of the radioactive fat, which is an unlicensed product, but has been used by Derby Pathology since 1982 and is widely used in the UK and other countries for this purpose.

Radioactivity in subsequent breath samples is used to assess the absorption of fat in the bowel. The radiation dose is very small and equivalent to less than the radiation from a chest x-ray.

If you have any concerns about the test, please ask your doctor before coming for the test, or contact Dr Hill's secretary at the Derbyshire Royal Infirmary (01332 347141, Extension 2521), or speak to the health professional who will conduct the test.

Chemical Pathology Departments - Southern Derbyshire Acute Hospitals NHS Trust

PAT/0296/0011/VERSION1

APPENDIX III

Standard Operating Procedure: ^{13}C-urea breath test

Derby Pathology Chemical Pathology	SOP – ^{13}C-Urea Breath Test No of pages: 4 **Expiry date: 28 February 2004**
AUTHORISED STANDARD OPERATING PROCEDURE	Authorised by: **M R Hopton** Signature
File Code: - 13cubt Work area: - d5	

^{13}C-Urea Breath Test (capsules)

INTRODUCTION

Helicobacter pylori is the most common cause of histological gastritis and probably the causative organism of peptic ulcer and gastric cancer. The bacteria has extremely high urease levels which is the basis of diagnostic tests for detecting abnormal gastric colonisation.

This procedure may only be carried out by staff authorised by the group protocol to administer ^{13}C-Urea.

PRINCIPLES OF METHOD

After an oral dose of ^{13}C-urea, $^{13}CO_2$ rapidly increases in concentration in the breath in H pylori positive subjects.

The breath $^{13}CO_2$ 15 minutes after ingestion of a capsule containing ^{13}C-urea is compared with the basal breath $^{13}CO_2$ level.

$^{13}CO_2$ is always present in breath samples and is measured as the ratio of $^{13}CO_2$: $^{12}CO_2$, i.e. the ratio of mass 45:44 (with corrections for ^{17}O). The UBT result is expressed as the difference between the ratio at T_0 and T_{15}, multiplied by 1000 (i.e. not percent, but per mil):

(ratio at T_{15min} – ratio at $T_{0\,min}$) x 1000 = UBT result.

The $^{13}CO_2$: $^{12}CO_2$ ratio is measured by isotope ratio mass spectometry (IRMS).

SPECIMEN TYPE

End expiratory breath sample collected into 10 mL glass or plastic tube with gas-tight cap (eg Exetainer).

REAGENTS

1. ^{13}C-Urea (99 atom %); Cambridge Isotope Laboratories, USA. Available in the UK from Torbet Laboratories, Chatham, Kent ME4 4TE (Tel 01634 817790). This is a pharmaceutical and must be ordered via Pharmacy DRI.

2. ^{13}C-Urea capsules for administration to patients. Ordered via Pharmacy, DRI, current order is for 150 per month (to end of March 2001). Store in the drugs fridge in the Pathology Suite of the Day Case Unit.

3. Exetainers LABCO Ltd, Brown Works, Copyground Lane, High Wycombe, Buckinghamshire, England HP12 3 HE (Tel 01494 459741)

4. Carbon dioxide, grade 4.5, Air Products PLC, Special Gases Group, Weston Road, Crewe Cheshire (Tel 08457 778800).

5. Helium BOC, product code 689.

STANDARD

The IRMS standardisation is based on PDB (see Breathmat Manual). For the UBT, absolute values are not required and the ratios are calculated relative to the standard CO_2 which is measured between each breath sample.

CONTROLS

The IRMS operators breath sample is used as a control.

METHOD DETAILS

1. Ensure that the patient has understood and followed the instructions described in the letter to the patient (attached).

2. Collect 2 baseline samples as follows. Instruct the patient to hold their breath for a few seconds and then to blow through the straw into the base of the Exetainer tube (zero time); withdraw the straw as condensation appears inside the tube. Replace cap quickly (do not over tighten). Write the patient's name on the tube. Repeat with the second tube.

3. Give the patient ONE ^{13}C-urea capsule, to be swallowed with 20 mL water. They should stand while they swallow the capsule and then sit for the 15 minute period.

4. Collect 2 breath samples as described above, at 15 min after swallowing the capsule.

5. Check names/bar codes on pairs of zero and 15 min tubes. The test is now completed for the patient.

6. Keep the individual patients tubes together and return them to the laboratory for analysis.

SPECIAL REQUIREMENTS

^{13}C-Urea is not radioactive; there are no special requirements for the disposal of waste materials for this procedure.

TECHNICAL DOCUMENTATION AND VALIDATION

^{13}C-Urea is administered as a pharmaceutical. A group protocol authorises this medicine to be administered by Biomedical Scientists or Clinical Scientists. A copy is kept in the Pathology Suite, Day Case Unit DRI and in Special Investigations DRI.

The ^{13}C-UBT set heading includes the dose of ^{13}C-Urea administered, which is a requirement of the group protocol.

ISOTOPE RATIO MASS SPECTOMETER

The Breathmat IRMS is used to measure the breath samples. The analyser software calculates the difference between the 15 minute and zero samples

The adequacy of the breath collection is assessed from the Breathmat readout.

DATA ENTRY

The set for the capsule based UBT is C13UBT. The breath test result from the IRMS printout (column marked DOB) is added in SRES to the test field 13CUBT.

Report minus results as <0.1 (seek advice if any minus results are beyond the range of -0.1 to -0.6).

Check that the result has been entered correctly.

CLINICAL AUTHORISATION

1. Results will be authorised by a senior member of staff prior to printing.

2. The following CT codes are available for authorising results:

   13N: for results <0.7
   13A: for results >0.7

   Please note that for project sample specific codes may be in use.

OUT OF HOURS REQUESTS

The test is not available out of hours.

RANGES

A result greater than 0.7‰ indicates the presence of gastric Helicobacter.

TURN ROUND TIME

Appointments for out-patients are normally made within 2 weeks of receiving the request unless it is clear that the patient has recently been on Losec (or other PPI's) or antibiotics. The test will be done on in-patients only when there are clear reasons why an OP visit is inappropriate. Results are normally available within 4 working days of completion of the test.

EXTERNAL QA SCHEMES

Not available.

COSHH STATEMENT

These reagents and their vapours will not cause severe or immediate health effects or require immediate recognition to prevent subsequent effects when:

1. Used and stored as recommended by the manufacturers instructions.
2. Disposed of in quantities routinely in use.
3. Spilled in quantities routinely in use.
4. Normal precautions required for handling laboratory reagents are exercised and any control procedures as indicated are observed.

Author: P G Hill June 2000		
Reviewed by:	Date:	Expiry date:
A Lloyd	May 2002	February 2004

APPENDIX IV

Standard Operating Procedure: BreathMAT IRMS

Standard Operating Procedure for BreathMAT IRMS

^{13}C breath analysis is the most common stable isotope measurement made in nutrition and medicine. Measuring ^{13}C enrichment in breath is a routine procedure for laboratories using stable isotopes to study human and animal nutrition or performing ^{13}C diagnostic breath tests.

The BreathMAT uses continuos flow isotope ratio mass spectrometry to determine ^{13}C enrichment in breath samples.

Samples of breath stored in septum capped gas containers (Labco Exetainer) are sampled directly by a needle probe. Container contents are flushed onto a gas chromatograph which separates CO_2 from N_2 and O_2.

Water is removed by a chemical trap. The pure CO_2 flows directly to an on line stable isotope analyser for measurement of its $^{13}C/^{12}C$ ratio.

Routine running of BreathMAT

Machine on standby,is always switched on with Helium gas set at low flow rate (1.0 bar), CO_2 turned off

- Check quantity of Helium gas (outside lab in Medical gas store, key on the lab notice board) and CO_2 gas cylinder next to the machine
- Turn Helium up to 3.0bar

- Turn CO_2 on check pressure is 4.5 bar
- Double click instrument control to screen titled BreathMAT control

add copy of Fig 8 page 11 of Quick start manual

This shows two windows:

Instrument control window showing cup signal intensities, status messages, high voltage and vacuum readout (lower half of screen)

Scan window showing a graph space with intensity and ratio labels. High voltage scans or time scans are displayed here

- Check voltage ~ 2500 V
- Check vacuum ~ 1.19 E - 6
- Check green light on valco load, source, HV, emission, sample available
- Check ion source on
- Toggle standard valve to on
- Check green light on standard
- Check Cup 1 ~ 2658 mV
- Check Cup 2 ~ 3111 mV
- Check Cup 3 ~ 1111 mV

- Wait 30 minutes to stabilize
- Toggle to select Scan mode
- Perform Time Scan 0 to 600 seconds (expand scale using "rubber band" to give vertical ratio scale of 1o/oo ratio)
- The "noise " ratio trace should not exceed 1 - 1.5 o/oo

- Close instrument control

Loading the machine with samples

- Double click on BreathMAT sequence
- Open options then evaluation
- Check the correct barcode reader is set
- Check LED indicator on the autosampler is in the green mode(magazine exchange) red is measurement mode

(when green light shows racks can be removed to load samples)

- Tubes must be positioned with the opening left
- DO NOT mix types of tubes in one magazine
- A QC sample in duplicate is positioned at start and end of run (operators own breath)
- Run the sequence module, results will appear
- Any barcode miss reads will be ejected, their numbers need to be edited in at the end of the run
- Any samples that give an mV value of less than 500mV will need to be run again using the duplicate sample
- At end of run click sequence then evaluate groups
- Save work
- Print results

APPENDIX V

Patient information sheets

APPENDIX VI

Consent form

DERBY PATHOLOGY

DEVELOPMENT OF A NEW NON RADIOACTIVE FAT ABSORPTION TEST

Information sheet

We are inviting you to take part in a research study to assist us to develop a new test of fat absorption. Our current standard test has been in use for 12 years and involves eating a lemon mousse to which a very small amount of radioactive fat has been added. The fat from the lemon mousse plus the radioactive fat is absorbed, and metabolised by the body to produce carbon dioxide. This can be detected as radioactivity in the breath. The new test uses exactly the same principle, but does not use any radioactive substances. Instead it uses a non radioactive fat marker which can also be detected as breath carbon dioxide.

You have been asked to participate in the study because:

***1 Your doctor has asked us to arrange tests to assess your fat absorption**

***2 Other tests show that you have normal fat absorption**

***3 Other tests show that you may have a problem with fat absorption.**
(*Delete as appropriate)

We would be very grateful for your help, but you are, of course, free not to take part. This will not affect the standard of care you receive. If you are willing to help us:

* you will need to come to the Derbyshire Royal Infirmary for the test

* the test will be carried out at the same time a the routine test of fat absorption which your doctor has requested
(*Delete as necessary)

The test starts at 9.00 am and finishes at 4.00 pm and is not at all unpleasant, although boring! It involves eating a small meal and blowing into a tube to collect a breath sample every hour. We recommend that you bring a book to read to help pass the time. There are no side effects to the test.

We hope that you will feel able to help us with this study which has been approved by the Southern Derbyshire Ethics Committee. If you have any questions, please contact Dr Hill's secretary at the Derbyshire Royal Infirmary, (01332 347141, extension 2521). A copy of this form is enclosed for you to keep.

If you have any questions or concerns about this study, you should discuss them with the doctor or nurse involved in your care. If you have any concerns about the way this study is being conducted, you are welcome to contact the Chairman of the Southern Derbyshire Ethics Committee, via the Committee's Administrator, Jill Marshall (telephone 01332 363821).

DERBY PATHOLOGY

DEVELOPMENT OF A NEW NON RADIOACTIVE FAT ABSORPTION TEST

Consent form

Name of researcher: Dr P G Hill

Please initial box

1 I confirm that I have read and understand the information sheet dated 09 03 00 for the above study and have had the opportunity to ask questions. ☐

2 I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3 I understand that sections of any of my medical notes may be looked at by responsible individuals from Derby Pathology or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

4 I agree to take part in the above study. ☐

.....	.....	.....
Name of patient	*Date*	*Signature*
.....	.....	.....
Name of person taking consent (if different from researcher)	*Date*	*Signature*
.....	.....	.....
Researcher	*Date*	*Signature*

1 for patient, 1 for researcher, 1 to be kept with hospital notes

APPENDIX VII

Explanation letter to volunteers

DERBY PATHOLOGY

EXPERIMENT TO SHOW THE $^{13}CO_2$:$^{12}CO_2$ RATIOS IN HEALTHY VOLUNTEERS AFTER A TEST FAT MEAL

<u>The rules</u>

1 No food after 12 midnight but you can drink water!

2 You can have a cup of tea before the start of the test.

3 Brush your teeth before coming to work.

4 At time 0 minutes, a breath sample is taken into two glass tubes.

5 A lemon mousse is eaten with 200 microlitres of ^{13}C triolein (a triglyceride labelled with a non radioactive stable isotope).

6 Breath samples will be taken every 30 minutes until 3.00 pm.

7 From 3.00 pm you may eat and drink as normal.

This test will show how the fat in the lemon mousse is digested over the 6 hours. If, as you are all healthy volunteers, the fat is digested normally, $^{13}CO_2$ will be detected in your breath. The quantity of $^{13}CO_2$ will increase gradually until about 5 hours from the start then decrease.

Hopefully, with your help, we will be able to stop using radioactive ^{14}C-triolein as in the present fat absorption test. The test you have taken part in today will replace it.

Thank you for your help.

Alison Lloyd
Senior MLSO

14 May 2001

The study has the approval of the Trust's Research and Development Committee and of the Southern Derbyshire Ethics Committee.

APPENDIX VIII

Lemon mousse recipe

DERBY PATHOLOGY

RECIPE FOR LEMON MOUSSE USED IN THE FAT ABSORPTION BREATHTEST (14)

Recipe to make 10 portions

100 g sugar (Silver Spoon produced from sugar beet)
2-3 lemons, grated rind and strained juice
400 mL double cream
30 g ground gelatine
2 egg whites
300 mL water

Method

1 Dissolve gelatine in some of the measured water.

2 Add remaining water to lemon juice.

3 Dissolve sugar in lemon juice, add gelatine and lemon rind. Cool.

4 Fold in slightly whipped cream.

5 Beat whites of egg until stiff.

6 Fold into mixture.

7 Divide into 10 equal portions.

8 Label "FAT TEST" and date, freeze.

Analysis per portion

Carbohydrate 10 g
Fat 19.3 g
Protein 1.4 g