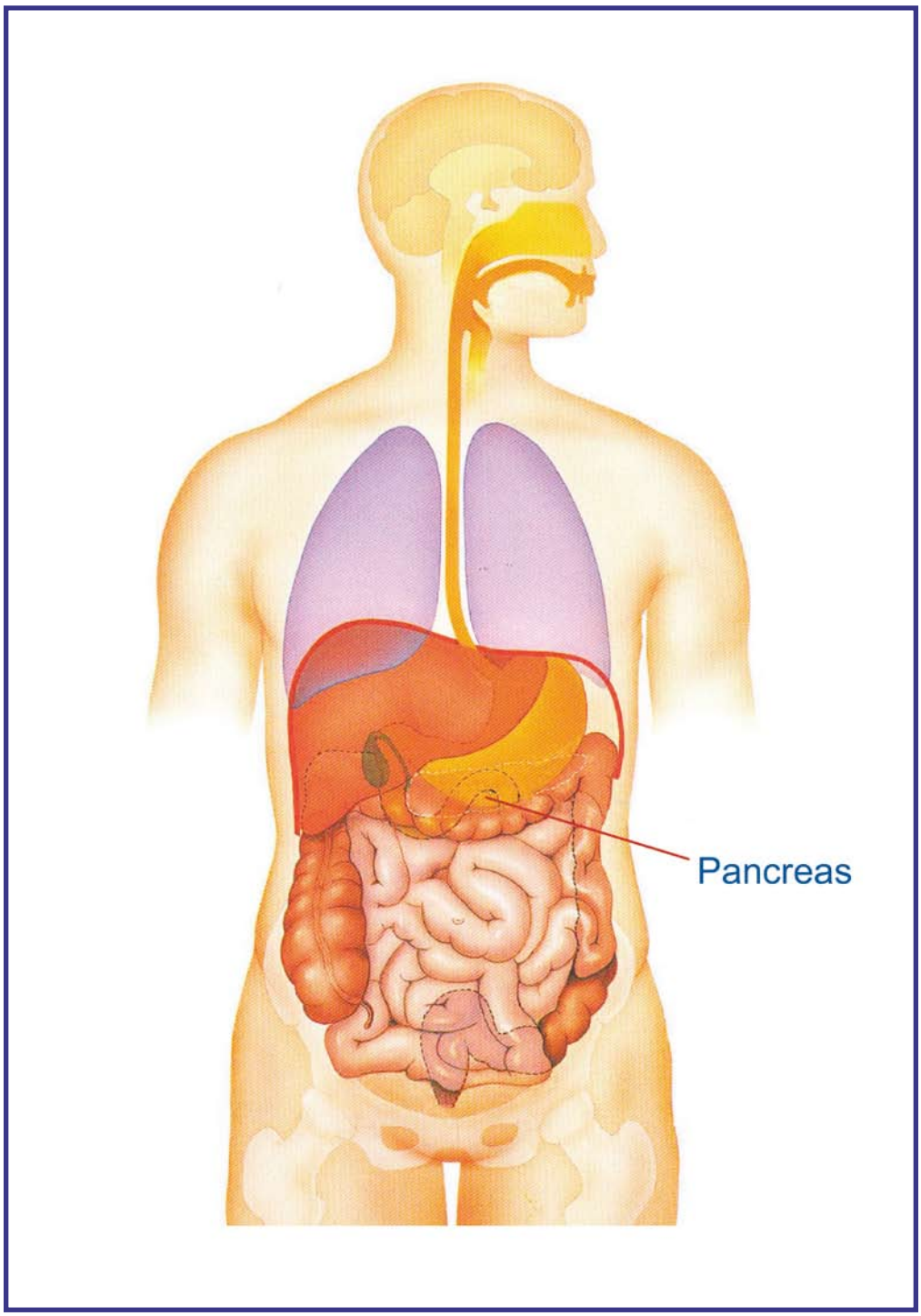
Protocols - ^{13}C Breath Tests - PANCREAS



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Introduction

^{13}C Breath Test protocols

This folder contains a set of protocols describing the principles and general test procedures for today's most relevant ^{13}C Breath Tests to study specific functions of the liver, pancreas, stomach and intestine. The list will be updated regularly adding additional tests or additional information on the already described tests.
The information is meant as a start to enter the field of stable isotope ^{13}C Breath testing initiated by the interest in a specific test. To actually be able to introduce a test in your hospital you must familiarize yourself with basic knowledge of breath testing with ^{13}C substrates and the existing knowledge on the particular application of interest. There is no such thing as a standard protocol for all tests.

^{13}C Breath Testing: principle and requirements

A ^{13}C Breath Test consists of the administration to a patient of a ^{13}C labeled substrate that is metabolized by a specific enzyme system resulting in $^{13}CO_2$ as the end product. To monitor the enzyme response ^{13}C enrichment is measured in breath CO_2.
The total procedure of ^{13}C Breath testing includes the definition of the preparation of the patient before the test, administration of the ^{13}C labeled substrate, collection of breath samples, measurement of ^{13}C enrichment in breath CO_2 and the calculation of an end result.

Preparation of the patient

In general, tests will be performed in the fasting state and the patient should be at a low and stable level of natural ^{13}C abundance. Therefore, the patient must be instructed to avoid eating ^{13}C enriched foods such as corn products, cane sugar, pineapple and tequila the last days before the test and to come to the clinic fasted. In certain cases (^{13}C Lactose-Ureide breath test) the patient must be pretreated with unlabeled substrate to stimulate the involved enzyme system.

Administration of ^{13}C labeled substrate

The test substrate may be administered as a simple solution in water with or without a standardized test meal. Sometimes it needs to be incorporated into a specific ingredient of the meal. The test meal and the dose of substrate may be different for adults and children.

Collection of breath samples

Every protocol has its own time schedule of breath collections. The number of samples may be as small as 2 or more than 20. To define the ^{13}C enrichment in breath CO_2, it is also necessary to obtain at least two breath samples before the ingestion of the ^{13}C substrate to determine the natural background of ^{13}C abundance. The methodology of collecting breath samples is dependent on the technology to determine the ^{13}C enrichment. The protocols are based on Continuous Flow Isotope Ratio Mass Spectrometry as the analytical technique. In this case breath samples are simply blown through a straw into special 10 ml gas collection tubes that directly fit into the sample tray of the instruments. In case of Infrared technology special bags provided by the instrument manufacturer must be used.

Measurement of ^{13}C enrichment

To determine the ^{13}C abundance in breath CO_2 you need the availability of Isotope Ratio Mass Spectrometry (IRMS) or specialized Infrared instrumentation. The protocols are based on Isotope Ratio Mass Spectrometry. For a number of tests (Aminopyrine, Methacetin, Urea) Infrared Spectroscopy has proven to be a valid alternative analytical technique. For other tests Infrared technology has not yet been validated so far. In principle the test substrate is not a determinant of the validity of the anlalytical technique. It is the level of ^{13}C enrichment that determines the analytical requirement. Validation of Infrared analysis for other application is recommended, as it is recommended to validate any breath test in your own clinical laboratory. You may have instrumentation available or contact a service center for the analyses.

Calculation of the end result

For some tests the only calculation needed is the subtraction of the natural background value from the measured value at a defined time. In other cases it is necessary to calculate the amount of ^{13}C that is recovered in breath during the experimental period. In a third type of application the time course of the enrichment appearance is of importance requiring calculation of the rate of appearance.

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Applications

In the present update the following tests have been described:

Table of contents of applications

Literature

Included is a list of literature references that will introduce you to the most important articles describing aspects of the different tests described in the protocols.

Note

Great care has been taken over the composition of the text, figures and tables. The possibility of errors however, cannot be excluded completely. Therefore Campro Scientific GmbH and the authors cannot accept any legal or other liability with respect to incorrect details and their consequences. The authors would be grateful to receive suggestions for improvements and information about errors.

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Test Protocols Pancreas

1. Lipase Activity
^{13}C-Mixed Triglyceride Breath Test

Principle
^{13}C-Mixed Triglyceride consists of a Triglyceride containing 2 Stearic Acid molecules at the sn-1 and sn-3 position and one Octanoic Acid molecule at the sn-2 position. The Octanoic Acid molecule is labeled with ^{13}C at the carboxyl carbon. The official terminology is 1,3-Distearyl-2-^{13}C-Carboxy-Octanoyl Glycerol. After administration of ^{13}C-Mixed Triglyceride with a meal, ^{13}C -Mixed Triglyceride will be transported to the small intestine where it is digested by pancreatic lipase.
After digestion ^{13}C-Octanoic Acid Monoglyceride is effectively absorbed and oxidized, preferentially in the liver. The rate of appearance of ^{13}C in breath CO_2 is considered a reflection of the rate of digestion, i.e. pancreatic lipase activity.

Applicability of ^{13}C-Mixed Triglyceride Breath Test
^{13}C-Mixed Triglyceride Breath Test has so far been applied to adults, children and neonates.

Applications
^{13}C-Mixed Triglyceride Breath Test is used to detect diminished pancreatic lipase activity in the small intestine by comparison with a control range. The result reflects overall lipase activity. The response of patients to pancreatic lipase supplementation can be documented using the ^{13}C-Mixed Triglyceride Breath Test.

Protocol
Adults: The ^{13}C -Mixed Triglyceride Breath Test is performed after an overnight fast.
A dose of 5 mg/kg body weight of ^{13}C-Mixed Triglyceride is administered. For this purpose the ^{13}C-Mixed Triglyceride is mixed into habitual breakfast or preferentially into a standardized breakfast. The meal may consist of 100 g toast with 0.25 g butter per kg body weight. The ^{13}C-Mixed Triglyceride is mixed into the butter. Breath samples are collected before (2x) and every 30 minutes for 360 minutes (6 h) after ingestion of the ^{13}C-Mixed Triglyceride. ^{13}C enrichment in breath CO_2 is determined by Isotope Ratio Mass Spectrometry (IRMS). The cumulative percentage of ^{13}C recovered in breath during the 360 minute collection period is used as the diagnostic parameter.

Children: A dose of 10-20 mg/kg body weight is used for children below 30 kg body weight, 5mg/kg is applied to children with body weight of 30 kg over. The dose is administered in a liquid test meal (Calogen, Nutri-mix) giving about 0.7 g/kg of fat. For neonates the label is administered directly into the mouth dissolved in olive oil during the milk feeding. The same time schedule for breath collections can be used. The sample collection technique must be adapted to the age of the children.

Interpretation of test results
It is advised to obtain your own internal control values. Generally a cut-off value of 22% for the 6 hours cumulative recovery may serve as a starting point in the case of adults and children. Values below 22% indicate for a diminished lipase activity.

Precautions
No side effects have been reported so far. It needs to be mentioned that in exceptional cases the absorption of long chain fatty acids may be disturbed leading to fat malabsorption. This will not affect the results of the 13C-Mixed Triglyceride Breath Test.

Summary

	Dose	Samples	
Adults	5 mg/kg body weight 13C -Mixed Triglyceride	2	Before administration
		12	Every 30 minutes for 360 minutes after administration (6 hours)
Children	10-20 mg/kg body weight for children below 30 kg body weight, 5 mg/kg body weight for children above 30 kg body weight		The sample collection technique has to be adapted to the age of the child

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2. Exocrine Pancreatic Insufficiency
^{13}C-Cholesteryl Octanoate Breath Test

Principle
(1-^{13}C)-Cholesteryl Octanoate is a natural cholesterol ester labeled with the non-radioactive isotope ^{13}C in the Octanoic Acid part of the molecule. After oral administration Cholesteryl (1-^{13}C) Octanoate is hydrolyzed by pancreatic cholesterol esterase. ^{13}C-Octanoic Acid is released, absorbed and effectively oxidized primarily in the liver. The degree of appearance of ^{13}C in breath CO_2 reflects the degree of pancreatic cholesterol esterase activity and therewith pancreatic function.

Applicability of ^{13}C-Cholesteryl Octanoate Breath Test
^{13}C-Cholesteryl Octanoate Breath Test has so far been applied to adults.

Applications
^{13}C-Cholesteryl Octanoate Breath Test is used to detect exocrine pancreatic insufficiency by comparison with a control range.

Protocol
Adults: The ^{13}C-Cholesteryl Octanoate Breath Test is performed after an overnight fast.
A dose of 500 mg (1-^{13}C)-Cholesteryl Octanoate together with 800 mg of unlabeled material is administered orally after dissolution in a liquid meal containing emulsifiers such as glycerol, lecithin and vegetable broth in order to solubilize the hydrophobic substrate. Breath samples are collected before (2x) and every 15 minutes for 180 minutes (3 h) after ingestion of the (1-^{13}C)-Cholesteryl Octanoate. ^{13}C enrichment in breath CO_2 is determined by Isotope Ratio Mass Spectrometry (IRMS). The cumulative percentage of ^{13}C recovered in breath during the 180 minutes collection period is used as the diagnostic parameter

Interpretation of test results
It is advised to obtain your own internal control values. Generally a cut-off value of 6% for the 3 hours of cumulative recovery may serve as a starting point in the case of adults. Values below 6% indicate for a diminished pancreatic function.

Precautions
No precautions have been described so far.

Summary

	Dose	Samples	
	500 mg Cholesteryl (1-13C) Octanoate + 800 mg unlabeled material	2	Before administration
		12	Every 15 minutes for 180 minutes after administration (3 h)

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Literature

Pancreas

1. Lipase Activity
^{13}C-Mixed Triglyceride Breath Test

Recommended literature

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2. Exocrine Pancreatic Insufficiency
^{13}C-Cholesteryl Octanoate Breath Test

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