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A novel method to identify fat malabsorption: The Serum Retinyl Palmitate Test

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ABSTRACT

Background: Malabsorptive etiologies of chronic diarrhea are important to identify. The 72-h stool for fecal fat test (FFT), the gold standard for diagnosing fat malabsorption, is fraught with limitations that impact its reliability. Vitamin A, a fat-soluble vitamin, parallels the absorption of lipids. We assessed the feasibility and validate a novel clinical test, retinyl palmitate (RP), for the diagnosis of fat malabsorption, and to compare the results to the FFT.

Methods: Using a case-control study design, patients with chronic diarrhea secondary to suspected malabsorption, and healthy control subjects were identified. A Dietitian taught subjects to consume a 100 g fat diet for the FFT with measurements of stool fat after 72-h. Serum levels of Vitamin A (retinol) and RP were measured by reversed-phase high pressure liquid chomatography. Two-way comparisons were made between the groups using 2 sample Wilcoxon rank-sum tests.

Results: Sixteen patients completed this study (8 cases and 8 control subjects). Fecal fat results were available for 15/16 patients. The sensitivity of the FFT was 100% (identified all cases), but the FFT specificity was 42%, as 4/7 control patients were identified as malabsorbers. Cases with short bowel syndrome had the lowest RP levels but this did not meet statistical significance. There was no significant difference for serum RP levels when comparing cases and control patients' AUC.

Conclusions: Serum RP is useful to identify malabsorption, albeit in severe cases. Furthermore, we have shown that the 72-hour FFT has poor performance characteristics, highlighting the need for more useful diagnostics in identifying malabsorption.

1. Introduction

Chronic diarrhea is a common clinical presentation, which often requires extensive investigation to determine an exact etiology. Diarrhea is defined as >200 g/day of stool, in the presence of altered consistency of bowel movements, and chronic diarrhea is defined as lasting >4-weeks [1]. When symptoms are persistent beyond 4 weeks, pathophysiology could involve secretory, osmotic, motility and malabsorptive mechanisms. Malabsorption, or defective mucosal absorption throughout the gastrointestinal tract, is challenging to discern from other causes of diarrhea. Making an accurate diagnosis is central as the patient may benefit from an effective management strategy. Malabsorptive etiologies of chronic diarrhea are the most important to identify, as the potential for adverse consequences such as nutrient deficiencies and malnutrition are present.

Diseases affecting the stomach, small intestine, liver, biliary tree and pancreas can contribute to malabsorption, with potential deleterious consequences on nutrition status. The differential diagnosis for malabsorptive diarrhea is broad, encompassing luminal, mucosal and lymphatic disorders involving different organ systems. The possibility of undiagnosed systemic endocrine or immunodeficient diseases also highlights the importance of a thorough work-up.

The malabsorption of fat has been the most commonly used indicator of global malabsorption. Among the macronutrients (fat, carbohydrates, and protein), fat absorption is both complex and most sensitive to interference from disease processes. Fat is also calorically the most dense macronutrient, so fat malabsorption contributes to weight loss, a critical factor in most malabsorptive disorders.

Several invasive and noninvasive tests are available to establish the cause of malabsorption when it is suspected. Historically, the 72-h stool for fecal fat (FFT) has been the gold standard for diagnosing fat malabsorption. However, the FFT is cumbersome for patients, and fraught with major limitations impacting the validity and reliability of the test [2].

To conduct the FFT, patients need to adhere to a 100 gram fat per day diet for 3 days to avoid false negative results due to low fat intakes. This may require increasing daily fat intake for individuals, sometimes necessitating the guidance of a dietitian. The test itself is time dependent and successful completion requires collection of stool specimens to coincide with the output from the 100 gram fat diet. The chance of false negative results from the fecal fat test is high if fat intake is

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inadequate and if stool collection is incomplete or not accurately collected [2]. Furthermore, the FFT is largely unpleasant for patients to do the stool collection, as well as for laboratory personnel who are required to homogenize the submitted specimen. Lust et al. assessed the attitudes, knowledge, and practices of Australian gastroenterologists and found the FFT was completed sub-optimally with only 26% of physicians attempting to fat-load patients appropriately [3].

As a result, more novel techniques to diagnose malabsorption have been explored. The absorption of vitamin A, a fat-soluble vitamin, parallels and is governed by factors that determine the absorption of lipids. Vitamin A, from animal-derived foods, is found as long chain acyl esters of retinol and these are digested to free fatty acids and retinol before uptake by the intestinal mucosal cell [4]. The retinol is then reesterified to retinyl esters for incorporation into chlylomicrons and absorbed via the lymphatics or effluxed into the portal circulation facilitated by lipid transport [4].

In the postprandial state intestinal cells thus take up retinol and esterify, store, and secrete retinal esters into the serum [5], which can then be measured. Johnson et al. have previously demonstrated that ingestion of physiologic doses of oil-soluble vitamin A 2400 retinol equivalents (8000 IU) (retinyl palmitate) was a valid test to distinguish patients with malabsorption compared to those without malabsorption [6]. Healthy control patients had robust post-prandial serum retinyl ester responses to vitamin A administration, and gastrointestinal patients with elevated fecal fat excretion had poor responses.

2. Methods

This study was approved by the Conjoint Health Research Ethics Board at the University of Calgary and all study participants provided informed consent. Using a case-control study design, patients with chronic diarrhea (defined as persistent symptoms >4 weeks) and suspected malabsorption were identified via referral to either the home parenteral nutrition or University of Calgary GI clinics. Patients from the Home Parenteral Nutrition clinic, if parenteral nutrition dependent, were further classified as having short bowel syndrome (SBS; <200 cm of remaining small bowel). Healthy control patients, in the absence of medical comorbidities, were recruited via invitation.

For the initial 4 subjects, a single capsule of oil-soluble vitamin A, retinyl palmitate (10,000 IU) was administered to both patients and controls after a minimum 10 h overnight fast along with a standardized breakfast containing 20 to 26 g of fat. The subsequent 12 subjects were given five capsules of the oil-soluble vitamin A, retinyl palmitate (50,000 IU), with the same fast and standardized breakfast. An indwelling catheter was inserted into all subjects, and 5 ml blood samples were drawn to measure retinyl palmitate (RP) in the serum.

We requested, and were granted permission, from the Ethics Board to increase our vitamin A dose since our lab was unable to detect retinyl palmitate in any of the samples among our first four subjects. This problem persisted with the higher dose, however, we us found a commercial lab ARUP Laboratories that was able to measure retinyl palmitate without any difficulty.

Blood samples were taken at baseline (0-h) before breakfast was ingested, and after 3 and 4 h. Lunch was then given at 4-h which included a meal with 20–24 g of fat. Further blood samples were drawn at 5, 6 and 7-h post RP ingestion. RP was the chosen measurement as it is the dominant retinyl ester and the only ester commercially available as reference material for assay calibration. The measurement of fatty acyl retinyl esters other than retinyl-palmitate were unavailable by the method used as described below.

2.1. Fecal fat analysis

To improve the sensitivity and specificity of the fecal fat test in its ability to objectively identify the presence of malabsorption, a Research Dietitian taught subjects to consume a 100 g fat diet for the FFT and keep a food diary during the 5-day high fat intake, then analyzed nutrient intakes to estimate actual fat intake (Food Processor 10.7, ESHA Research). Subjects were instructed to collect feces for the final 3 days of their fat intake for the analysis of fecal fat. The Calgary Laboratory Services (Calgary, AB) method is derived from the fecal fat stool procedure (OCB1925/05) from the Hospital for Sick Children (Toronto, ON), which is based on the original method developed by Van de Kamer (1949) [7]. Patients were defined as malabsorbers if the 72-h stool fat excretion was > 5.9 g/day [8].

2.2. Retinyl-palmitate analysis

Venous blood specimens were collected into 10 ml vacutainer collection tubes (Becton Dickson) and processed within 4 to 6 h of collection to separate serum. Patient specimens were stored at −70 °C until analyzed. Serum levels of retinol (vitamin A) and RP were measured by reversed-phase high pressure liquid chomatography (HPLC) as previously described [9]. The detection limit for retinol and retinyl-palmitate were 0.18 mg/L and 0.02 mg/L. Inter-assay imprecision data for low and high levels of retinol and retinyl-palmitate levels of 0.24, 2.05 mg/l and 0.02, 0.59 mg/l were 4.8%, 6.2% and 11.3%, 10.5%, respectively [9].

2.3. Statistical Analysis

The appearance of retinyl palmitate in the serum was summarized as area under the curve (AUC), calculated using a trazepoid calculation. The retinyl palmitate area-under-the-curve (RP-AUC) results were compared using non-parametric Kruskall–Wallis Equality of populations Rank test for the 3 group comparison for the cases and controls. Two-way comparisons were made between the groups using 2 sample Wilcoxon rank-sum tests, using an α of 0.05.

3. Results

A total of 16 patients completed this study with 8 cases and 8 control subjects. Four subjects (2 cases and 2 controls) received the low dose vitamin A. Cases were designated clinically as malabsorbers and further categorized with 6 patients having short bowel syndrome all requiring home parenteral nutrition, 1 patient with pancreatic insufficiency and 1 with rapid intestinal transit. There were no known gastrointestinal

Table 1
Patient characteristics and demographics (median and IQR).

	Controls	Cases	p-value
N	8	8	
Age	37 (31–45)	47 (44–59)	
Females	6 (80%)	7 (88%)	
Health status	No known gastrointestinal issues	6: short bowel syndrome, 1: pancreatic insufficiency, 1: rapid intestinal transit	
Retinyl palmitate (mg/l, AUC)	1.4 (1.0–2.2)	1.0 (0.3–2.6)	0.60
Vitamin A (mg/l, AUC)	4.3 (3.9–4.9)	4.5 (3.2–5.0)	0.83

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