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Non-invasive assessment of intestinal permeability in healthy volunteers using transcutaneous fluorescence spectroscopy

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Abstract

The permeability of the intestinal barrier is altered in a multitude of gastrointestinal conditions such as Crohn's and coeliac disease. However, the clinical utility of gut permeability is currently limited due to a lack of reliable diagnostic tests. To address this issue, we report a novel technique for rapid, non-invasive measurement of gut permeability based on transcutaneous ('through-the-skin') fluorescence spectroscopy. In this approach, participants drink an oral dose of a fluorescent dye (fluorescein) and a fibre-optic fluorescence spectrometer is attached to the finger to detect permeation of the dye from the gut into the blood stream in a non-invasive manner. To validate this technique, clinical trial measurements were performed in 11 healthy participants. First, after 6 h of fasting, participants ingested 500 mg of fluorescein dissolved in 100 ml of water and fluorescence measurements were recorded at the fingertip over the following 3 h. All participants were invited back for a repeat study, this time ingesting the same solution but with 60 g of sugar added (known to transiently increase intestinal permeability). Results from the two study datasets (without and with sugar respectively) were analysed and compared using a number of analysis procedures. This included both manual and automated calculation of a series of parameters designed for assessment of gut permeability. Calculated values were compared using Student's T-tests, which demonstrated significant differences between the two datasets. Thus, transcutaneous fluorescence spectroscopy shows promise in non-invasively discriminating between two differing states of gut permeability, demonstrating potential for future clinical use.

1. Introduction

Intestinal permeability is a functional feature of the intestinal wall that defines the degree to which molecules can pass from the intestine into the blood stream at given sites [1]. Two aspects of gut permeability that can be measured are transcellular permeability (representing passage through the water pores of cell membranes) and paracellular permeability (passage through the tight junctions between cells in the intestinal wall) [2]. Paracellular permeability is known to be altered in wide-ranging gastrointestinal (GI) conditions such as coeliac disease, inflammatory

bowel disease (IBD) and environmental enteric dysfunction, and in conditions outside the GI tract such as schizophrenia, autism and Parkinson's Disease [3].

Thus, for the practising clinician, a reliable tool to measure gut permeability could potentially provide a new way to diagnose these conditions and monitor the effectiveness of treatments. Specific to the GI area, such a technique could provide improved monitoring of IBD treatment responses and could also help in developing a better understanding of functional gut disorders (a 'catch all' term for poorly understood GI conditions that are often treated as a diagnosis of exclusion).

At present, there are many options available to measure gut permeability such as the lactulose:mannitol (L:M) test, lactulose:rhamnose (L:R) test, chromium-51 labelled ethylenediamine tetraacetic acid (Cr-EDTA) assay, polyethylene glycol (PEG) test, use of Ussing chambers, analysis of haematological markers such as zonulin, and analysis of bacterial markers such as systemic lipopolysaccharide (LPS) [4]. However, clinical use of these tests has thus far been limited as they are either too cumbersome, too invasive, or because of a lack of standardisation in how the tests are performed [5]. As a result, new technologies that provide improved monitoring of gut permeability could provide significant clinical benefit [6, 7].

To address this challenge, we have developed a novel technique—transcutaneous fluorescence spectroscopy—for non-invasive assessment of gut function using orally ingested fluorescein [8–10]. Fluorescein is a fluorescent contrast agent that is inert, safe and has been used in many clinical settings such as routine ophthalmology procedures (e.g. fluorescence angiography) [11] and confocal laser endomicroscopy during colonoscopy [12]. Furthermore, the potential of fluorescence spectroscopy in gut permeability assessment (using fluorescein or other contrast agents) has been demonstrated both in cells and in animal models [13–17].

Using transcutaneous fluorescence spectroscopy, gut function can be assessed non-invasively through the following process: patients consume an oral dose of fluorescein (or another fluorescent contrast agent) and a wearable probe/sensor is used to detect the fluorescence signal through the skin (transcutaneously) as the contrast agent permeates from the gut into the blood stream. As such, it is possible to measure fluorescein uptake from the gut into the blood stream in real time. Appropriate analysis of the resulting fluorescence versus time curve then allows quantification of multiple elements of GI function including gastric emptying rate and gut permeability.

We have recently demonstrated the potential of this technique to monitor gut function and to measure gastric emptying rate in studies in healthy volunteers. Lett *et al* [8] demonstrated the use of transcutaneous spectroscopy to measure gastric emptying rate as a feasible alternative to the conventional paracetamol absorption test. Maurice *et al* [9] showed promising preliminary results using the method to measure gut permeability (albeit in a very limited cohort of participants). Importantly, both studies showed that the test is well tolerated and has the potential to produce clinical results within hours rather than days (as data can be analysed immediately rather than requiring analysis of samples in a laboratory). Furthermore, the approach is non-invasive and does not require collection of urine, blood or stool samples (i.e. unlike competitor technologies such as L:M tests and Cr-EDTA assays).
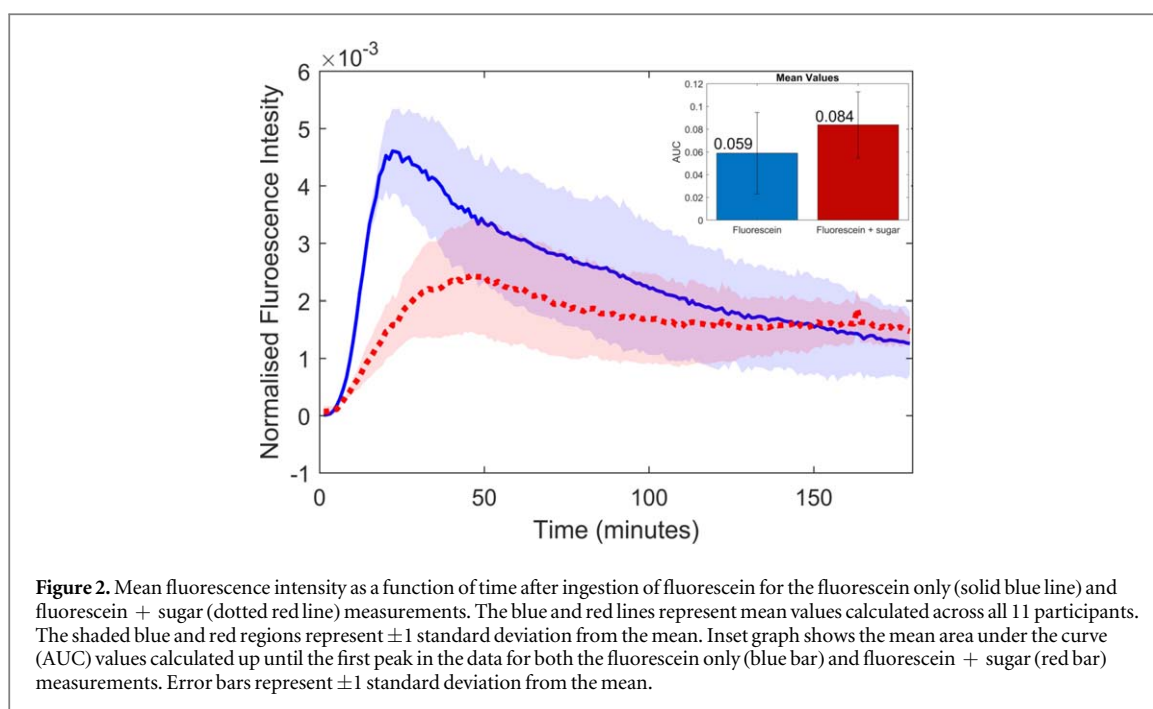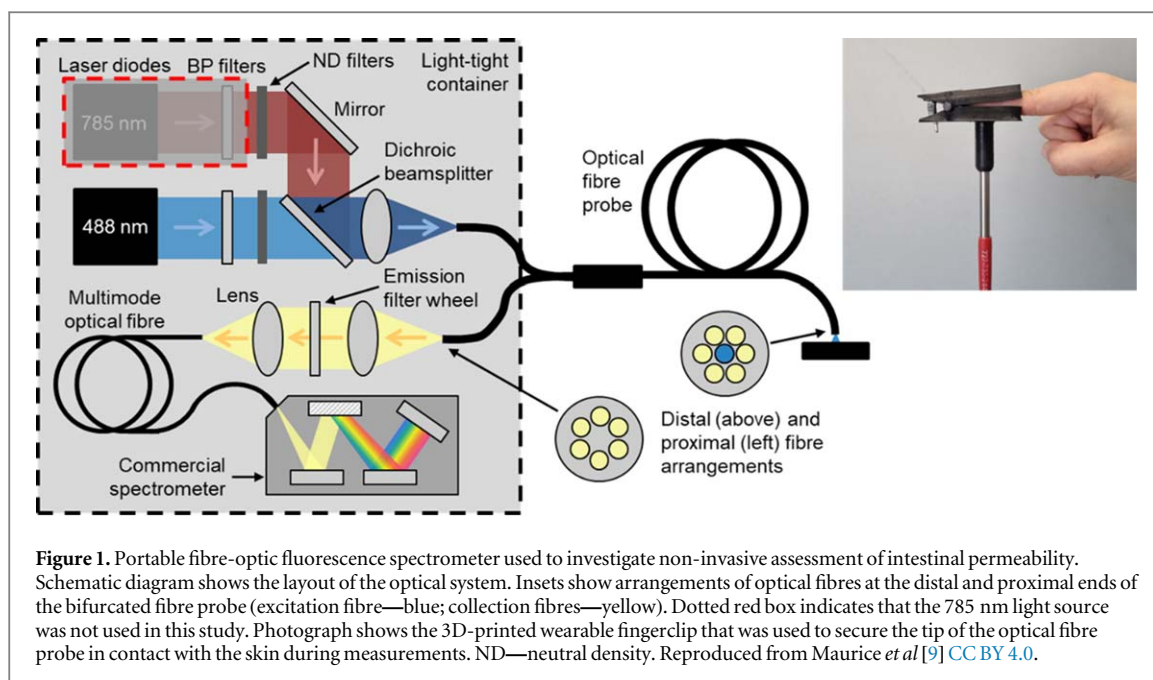
To further demonstrate the clinical potential of transcutaneous fluorescence spectroscopy for non-invasive assessment of gut permeability, this article reports results from clinical experiments in 11 healthy volunteers. All participants took part in two repeat experiments in which they ingested fluorescein dissolved in water (day 1) and fluorescein dissolved in a concentrated aqueous sugar solution (day 2). Ingestion of concentrated sugar solutions is known to drive temporary increases in gut permeability [18, 19]. Thus, by comparing data collected on the two study days, we investigated the potential of transcutaneous fluorescence spectroscopy to non-invasively discriminate between two differing states of intestinal permeability. We investigated a number of data analysis protocols for this purpose—both manual and automated—and demonstrated statistically significant differences between the two datasets, hence indicating the future potential of this technique for clinical assessment of gut permeability.

2. Methods

2.1. Fibre-optic spectrometer

Following oral ingestion of fluorescein, a fibre-optic fluorescence spectrometer was used to detect fluorescence signals at the fingertip. This system has been described in detail in previous studies [8, 9]. Briefly, it consists of two laser sources (at 488 nm and 785 nm) that are coupled into a bifurcated fibre-optic probe. The fibre-optic probe delivers excitation light to the measurement site (the fingertip, where it is secured using a 3D-printed clip) and also collects the resulting fluorescence signal and delivers it to a spectrometer for detection. In this study, only the 488 nm laser was used, as this provided efficient excitation of fluorescein. The laser power (at 488 nm) at the tip of the fibre-optic probe was limited to 63 μW using a combination of variable neutral density (ND) filters and the laser control software. This ensured that the laser exposure was below the maximum permissible exposure for the skin in all cases [20–22], allowing safe use in the clinic. The optical system and 3D-printed fingerclip are shown in figure 1. The entire system is controlled using a laptop computer running custom-written LabVIEW software and is housed within a wheeled trolley to facilitate use within clinical environments.

The spectrometer records both the fluorescence signal and the directly scattered laser signal (by switching between two filter ports in a motorised filter wheel). The backscattered laser signal is used to normalise the fluorescence data to correct for any variations in laser power or probe position between measurements. This normalisation procedure—which is described in detail in previous studies [8, 9]—then allows for calculation of a fluorescence versus time curve (e.g. see figure 2), which can be analysed to

Figure 1. Portable fibre-optic fluorescence spectrometer used to investigate non-invasive assessment of intestinal permeability. Schematic diagram shows the layout of the optical system. Insets show arrangements of optical fibres at the distal and proximal ends of the bifurcated fibre probe (excitation fibre—blue; collection fibres—yellow). Dotted red box indicates that the 785 nm light source was not used in this study. Photograph shows the 3D-printed wearable fingerclip that was used to secure the tip of the optical fibre probe in contact with the skin during measurements. ND—neutral density. Reproduced from Maurice *et al* [9] CC BY 4.0.

Figure 2. Mean fluorescence intensity as a function of time after ingestion of fluorescein for the fluorescein only (solid blue line) and fluorescein + sugar (dotted red line) measurements. The blue and red lines represent mean values calculated across all 11 participants. The shaded blue and red regions represent ±1 standard deviation from the mean. Inset graph shows the mean area under the curve (AUC) values calculated up until the first peak in the data for both the fluorescein only (blue bar) and fluorescein + sugar (red bar) measurements. Error bars represent ±1 standard deviation from the mean.

extract information regarding gut permeability and other elements of GI function.

For the clinical experiments reported here, the spectrometer was programmed to record the fluorescence and laser signals once every minute for a total of 3 h. This allowed for calculation of fluorescence versus time curves with sufficient temporal resolution to observe the dynamics of the fluorescein signal (e.g. the permeation from the gut into the blood stream and the elimination of the dye from the blood). In turn, this permitted calculation of a set of parameters designed to provide readouts of gut permeability (see more details in Data Analysis section below).

2.2. Clinical trial measurements

Clinical trial measurements were performed in healthy participants at St. Mary's Hospital (London, UK) between February 2021 and October 2021. Participants were recruited and consented in accordance with the clinical study protocol [23], which received ethical approval from the UK Health and Research Authority (HRA) and a local Research Ethics Committee (REC) (IRAS Project ID—242462, REC reference —18/LO/0714/AM04). This study was conducted in line with local Good Clinical Practice (GCP) guidelines and in accordance with the World Medical Association's Declaration of Helsinki. All participants gave

informed consent prior to the study and all were screened before participation to confirm that they did not have a diagnosis of any known conditions that altered gut permeability. All participants were aged 18 and above and had no previous reactions to fluorescein. Participants who were pregnant, were breast feeding, or had taken antibiotics for the last 4 weeks were excluded.

After 6 h of fasting, all participants ingested 500 mg fluorescein sodium (supplied as a 5 ml vial of 100 mg ml^{-1} fluorescein; Fluorescite Antera 10%) dissolved in 100 ml water. A dose of 500 mg was chosen as this was expected to provide strong signal levels in all participants (previous work demonstrated a limit of detection for orally ingested fluorescein of less than 25 mg [9]) and because it represented the clinically approved dose of fluorescein for other medical procedures. Fluorescence measurements were started immediately before ingestion of the fluorescein to allow collection of a baseline measurement. Measurements were taken using the fibre-optic spectrometer discussed above, with the tip of the probe secured in contact with the skin at the fingertip using a 3D-printed mount (see photograph in figure 1). Spectra were then recorded at one minute intervals for a total of three hours to allow investigation of the kinetics of the recorded fluorescence signals.

After a minimum of 7 days, participants were invited back to St. Mary's hospital for a repeat study, this time ingesting 500 mg fluorescein sodium with 60 g sugar in 100 ml water. Fluorescence data was collected in an identical manner to that described above. As discussed briefly above, the concentrated sugar solution is expected to have a hyperosmolar effect that drives a transient increase in gut permeability that is broadly comparable to the changes observed in coeliac disease [18, 19] (and preliminary results reported by Maurice *et al* were in accordance with this [9]). Thus, we hypothesised that if we could differentiate between the data collected with and without sugar, this would indicate potential for non-invasive assessment of intestinal permeability. Data recorded using the fibre-optic spectrometer were therefore compared across the two study days.

A statistical power calculation was used to estimate that a minimum of 10 participants would be required in this study to obtain statistical power of over 80%. Thus, in total, 13 participants were recruited and included in the study to account for the possibility of participant dropout/withdrawal. Data from two participants was removed from the analysis as one participant was later found to have a health condition that was expected to impact gut permeability and the second participant did not complete the second study day (meaning that their dataset was incomplete). Of the remaining 11 participants, 4 were female and 7 were male, and the median age (of all 11 participants) was 29 years (range: 24–50 years). All 11 participants included in the analysis had no previous conditions that would affect gut permeability and did not have any

Table 1. Demographics of participants included in the study. BMI—body mass index (kg m^{-2}); M—male; F—female.

Patient	Age	Sex	Ethnicity	BMI
1	32	M	Caucasian	28
2	50	M	Caucasian	31
3	28	M	Caucasian	25
4	31	M	Asian	30
5	24	F	Caucasian	23
6	28	M	Asian	25
7	29	F	Asian	29
8	33	M	Asian	26
9	27	F	Asian	23
10	28	M	Asian	28
11	30	F	Asian	25

bowel symptoms. The participant demographics—age, sex, ethnicity and body mass index (BMI)—are tabulated in table 1.

2.3. Data analysis

To assess whether the transcutaneous fluorescence data could be used to differentiate between two differing states of intestinal permeability (i.e. between the fluorescein only data and the fluorescein + sugar data), we investigated three methods of data analysis. In each case, the first peak in the fluorescence versus time curve was identified and we calculated the area under the curve (AUC) up to that point. The AUC value was then 'corrected' for differences in gastric emptying rate by either dividing by the average gradient (slope) of the first stage of the fluorescence versus time curve (i.e. the section of the curve up until the first peak) or by multiplying by the time at which the first peak value was observed. This correction was required as a later peak (which indicates slower gastric emptying) means that more fluorescein would have been eliminated from the body by the time the peak had been reached, thus reducing the AUC value.

Having calculated these three parameters—AUC, AUC/slope, and AUC*time—we then compared them across the two datasets to assess which exhibited the clearest changes in response to induced changes in permeability. This comparison was performed using both scatter plots (showing the values observed for each individual participant for both the fluorescein only and fluorescein + sugar experiments) and box plots to visualise the distribution on an individual level and across the cohort as a whole (see section 3.2). In each case, statistical significance was quantified using the Student's T-test.

Box plots showed the median values, 25th and 75th percentiles, most extreme data points excluding outliers, and outliers for the fluorescein only and fluorescein + sugar measurements. Outliers were defined as points that fell below the 25th percentile or above the 75th percentile by more than 1.5 times the interquartile range, and were plotted for visualisation

purposes only. All calculations (e.g. of mean values, median values, etc) and all statistical tests were performed based on the data points from all 11 participants (i.e. including outliers) in all cases.

The three parameters (AUC, AUC/slope, and AUC*time) were also calculated using three different computational methods. These three approaches were tested to optimise the accuracy of the identification of the peak time and to provide varying degrees of automation. Following identification of peak time, all three methods calculated the AUC using trapezoidal numerical integration. Detection of peak times and calculation of AUC parameters are depicted for each method in supplementary figures S1-3 (see supplementary information, available online at stacks.iop.org/MAF/10/044014/mmedia) using three example fluorescence versus time curves that were representative of the dataset as a whole. Furthermore, the fluorescence versus time curves for all participants are presented in supplementary figures S4 and 5 (for the fluorescein only and fluorescein + sugar measurements respectively; see supplementary information).

The first method was the simplest and least automated. The raw data was manually inspected to identify the times at which the first peak was reached in each dataset. The AUC values were then calculated up to the obtained times using the raw data.

The 2nd method implemented a filtering step (median filter of order $n = 10$) to reduce noise and provide more accurate identification of the first peak in the data. Peak times were again identified manually (this time based on the filtered data) and AUC values were then calculated up to the manually detected time points (again, based on the filtered data).

Finally, the 3rd method involved automatic identification of the peak times using the median filtered data. In order to detect the time points at which the first peak was reached, the 3rd method used a predefined MATLAB function ('*findpeaks.m*') to detect local maxima. AUC values were then calculated up to these time points using the median filtered signal. This approach served to reduce the risk of manual/observer bias in the identification of peak times and also provided fully automated analysis of the collected data.

3. Results

3.1. Fluorescence intensity versus time

Figure 2 presents the mean fluorescence intensity as a function of time for all participants for both the first study day (fluorescein only) and second study day (fluorescein + sugar). With fluorescein alone, a peak value of 0.0044 was observed in the normalised fluorescence data at a time of 24 min. Following ingestion of 60 g sugar, the peak value was observed to be lower (0.0024) and to occur later in time (49 min).

This was attributed to slower gastric emptying in the sugar experiments. This meant not only that the

peak signal level occurred later (due to slower emptying of fluorescein from the stomach into the gut) but also that the absolute value at the peak was reduced due to elimination of fluorescein from the blood stream. Fluorescein can be expected to be eliminated from the body at an approximately constant rate across the two measurement days. Thus, it follows that the later the peak in the signal, the greater the degree of elimination that will have taken place by that point, thereby reducing the absolute value of the peak signal.

Despite the observed reduction in peak fluorescence intensity, we found that the mean area under the curve (AUC) calculated up until the first peak in the data (which can be considered as an estimate of the total amount of fluorescein absorbed) was higher in the sugar measurements than in the fluorescein only measurements (figure 2 (inset)). While this result was not statistically significant (see figure 3 and section 3.2), it nonetheless revealed differences between the two datasets and suggested that correcting for changes caused by alterations in gastric emptying rate may permit accurate detection of changes in permeability.

3.2. Discrimination of differing states of gut permeability

To further explore the ability to correct for gastric emptying rate and elimination of fluorescein from the body, we investigated a number of analysis procedures (both manual and automated) to assess whether the data could be used to identify changes in gut permeability.

As described in section 2.3, to achieve this, we used three methods of analysis to calculate three parameters designed to provide readouts of gut permeability (AUC, AUC/slope, and AUC*time).

Using the 1st method (manual analysis; figure 3), we found that the AUC up to the first peak was higher following ingestion of sugar (compared to the fluorescein only measurements) in 9 out of 11 participants (figure 3(a)). However, this change was not statistically significant (figure 3(d); $p = 0.088$). Conversely, the AUC/slope and AUC*time values were significantly higher with ingestion of sugar than without (figures 3(b), (c) and (e), (f); $p = 0.0037$ and $p = 0.0052$ respectively). This was attributed to the additional degree of correction that the AUC/slope and AUC*time parameters provided for the impact of elimination of fluorescein from the body.

The 2nd and 3rd methods of analysis (see figures 4 and 5 respectively) demonstrated similar results to the manual analysis. AUC values alone did not show significant differences between the fluorescein + sugar and fluorescein only measurements (2nd method— $p = 0.067$, figures 4(a) and (d); 3rd method— $p = 0.53$, figures 5(a) and (d)). However, we found that the AUC/slope and AUC*time values were significantly higher with ingestion of sugar than without (2nd method—$p = 0.0044$ (AUC/slope), $p = 0.0048$

Figure 3. Measuring gut permeability using transcutaneous fluorescence spectroscopy and 1st method of analysis (manual analysis). (a)–(c) Scatter plots showing the parameter values calculated for each participant as readouts of gut permeability (red asterisks— fluorescein only; blue circles—fluorescein + sugar). (a) area under the curve (AUC) at first peak in the data (Peak Area). (b) AUC divided by average gradient of graph in region up until first peak (Peak Area/Slope). (c) AUC at first peak multiplied by time of first peak (Peak Area * Peak Time). (d)–(f) Box plots showing median values (red lines), 25th and 75th percentiles (upper and lower bounds of blue box), most extreme data points excluding outliers (whiskers), and outliers (red crosses) for the fluorescein only (F) and fluorescein + sugar (F + sugar) measurements. Outliers were defined as points that fell below the 25th percentile or above the 75th percentile by more than 1.5 times the interquartile range. Outliers were plotted for visualisation purposes only. All calculations (e.g. of means, medians, etc) and all statistical tests were performed based on the data points from all 11 participants (i.e. including outliers) in all cases. (d) AUC up until first peak (Peak Area). (e) AUC divided by average gradient (Peak Area/Slope). (f) AUC at first peak multiplied by time of first peak (Peak Area * Peak Time). P-values are shown below each graph indicating the results of Student's T-tests comparing the values obtained from the fluorescein only and fluorescein + sugar measurements using the three parameters above (Peak Area, Peak Area/Slope, and Peak Area * Peak Time).

(AUC*time), figures 4(b), (c) and (e), (f); 3rd method —p = 0.017 (AUC/slope), p = 0.037 (AUC*time), figures 5(b), (c) and (e), (f).
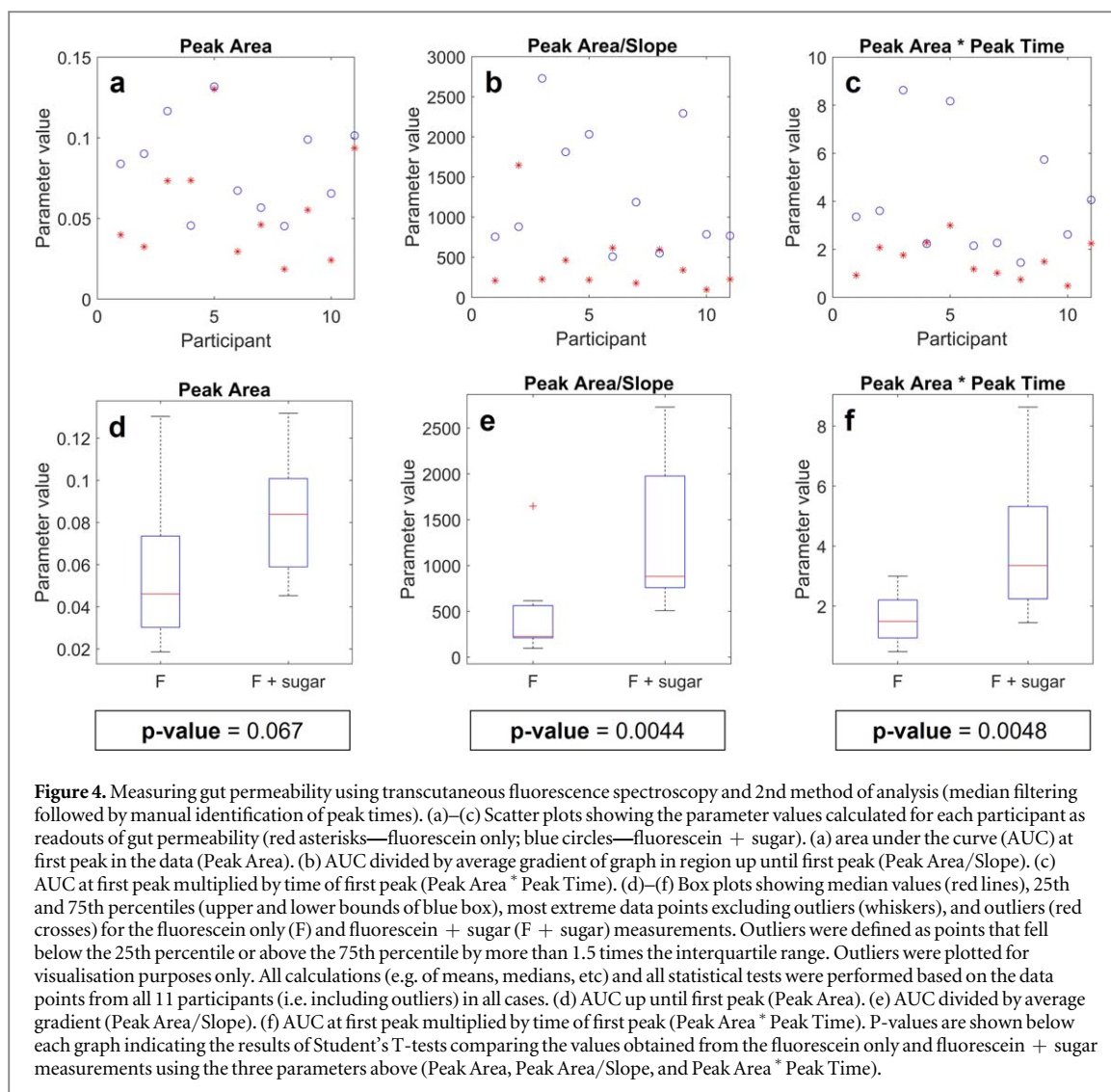
Furthermore, the AUC/slope and AUC*time values were higher following ingestion of sugar in 8/11 participants (AUC/slope) and 10/11 participants (AUC*time) using the 2nd method (figures 4(b) and (c)) and in 10/11 participants (for both parameters) using the 3rd method (figures 5(b) and (c)). Together with the results above, this demonstrates the potential of the AUC/slope and AUC*time parameters to provide rapid, non-invasive readouts of intestinal permeability (using all three methods of analysis).

4. Discussion

This study has reported a series of analysis techniques for assessment of intestinal permeability using non-invasive (transcutaneous) fluorescence spectroscopy. Our data demonstrate that three methods of analysis (ranging from manual to fully automated) produce similar results for the three parameters investigated (AUC, AUC/slope and AUC*time). The parameters

AUC/slope and AUC*time were effective in differentiating between states of normal and (transiently) increased gut permeability. Crucially, this is true both when analysing the data in a fully manual manner and when doing so using a completely automated (computational) technique. This demonstrates that the results are not caused by observer/manual bias in the analysis. Moreover, as data can be automatically analysed immediately following collection without further user input, this highlights the potential for future use in rapid diagnostics and therapy monitoring for diseases such as IBD.

Interestingly, the sugar challenge model used in this study to generate states of increased intestinal permeability has previously been shown to induce changes that are broadly similar to those observed in coeliac disease [19]. While there are limited reports comparing the effects of sugar (and other) challenge models with disease states, this nonetheless further indicates the potential of this technology for clinical monitoring of gut permeability, as the changes observed in diseases such as IBD and coeliac disease are likely to be of a similar order to those observed here.

Figure 4. Measuring gut permeability using transcutaneous fluorescence spectroscopy and 2nd method of analysis (median filtering followed by manual identification of peak times). (a)–(c) Scatter plots showing the parameter values calculated for each participant as readouts of gut permeability (red asterisks—fluorescein only; blue circles—fluorescein + sugar). (a) area under the curve (AUC) at first peak in the data (Peak Area). (b) AUC divided by average gradient of graph in region up until first peak (Peak Area/Slope). (c) AUC at first peak multiplied by time of first peak (Peak Area * Peak Time). (d)–(f) Box plots showing median values (red lines), 25th and 75th percentiles (upper and lower bounds of blue box), most extreme data points excluding outliers (whiskers), and outliers (red crosses) for the fluorescein only (F) and fluorescein + sugar (F + sugar) measurements. Outliers were defined as points that fell below the 25th percentile or above the 75th percentile by more than 1.5 times the interquartile range. Outliers were plotted for visualisation purposes only. All calculations (e.g. of means, medians, etc) and all statistical tests were performed based on the data points from all 11 participants (i.e. including outliers) in all cases. (d) AUC up until first peak (Peak Area). (e) AUC divided by average gradient (Peak Area/Slope). (f) AUC at first peak multiplied by time of first peak (Peak Area * Peak Time). P-values are shown below each graph indicating the results of Student's T-tests comparing the values obtained from the fluorescein only and fluorescein + sugar measurements using the three parameters above (Peak Area, Peak Area/Slope, and Peak Area * Peak Time).
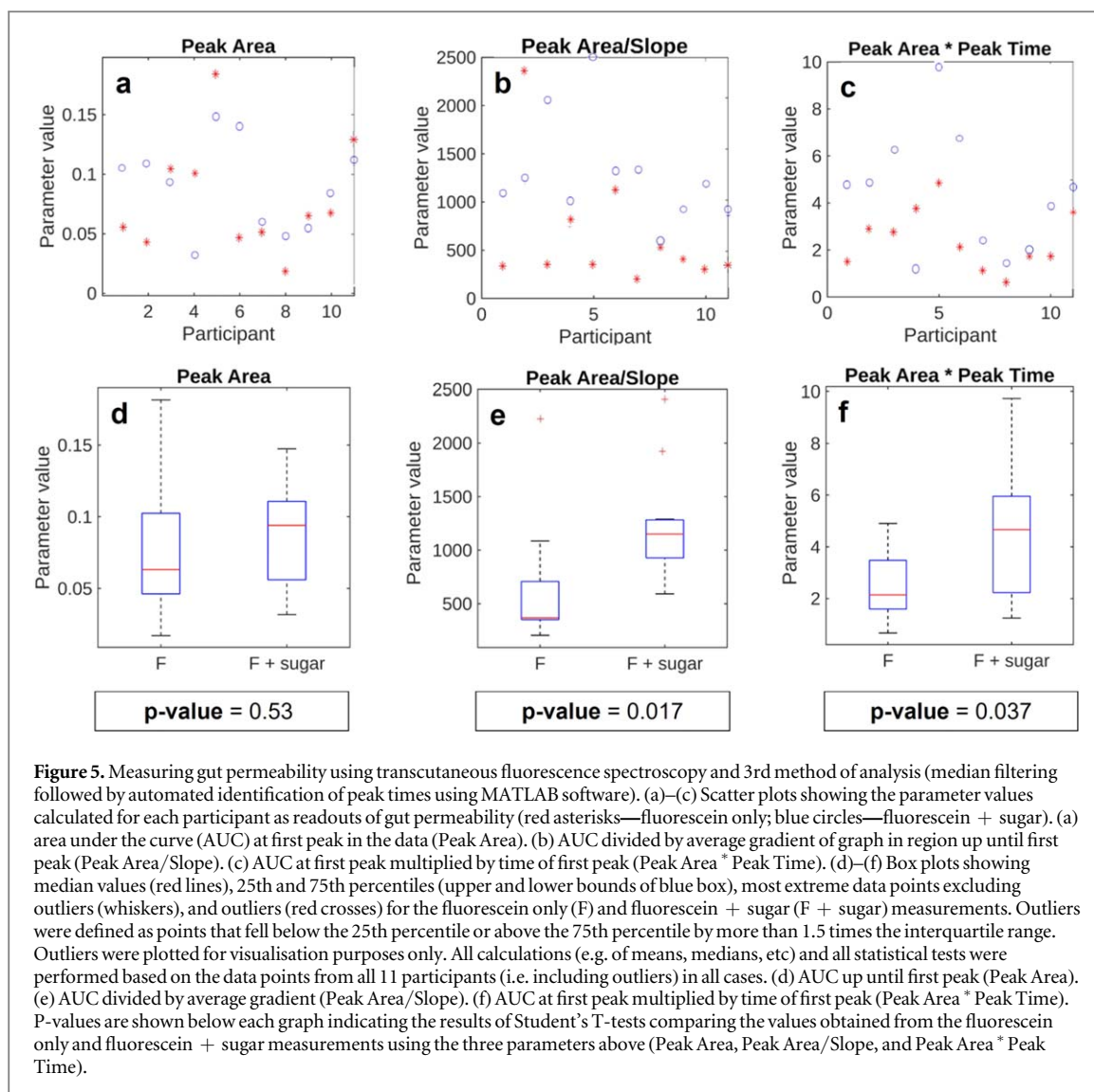
There are of course limitations associated with this study. The sample size is relatively small and there is also a risk of heterogeneity in gut permeability among the study participants (both in their gut permeability across the two study days and in how they respond to the hyperosmolar solution). Indeed, in a small minority of participants, some of the calculated gut permeability parameters (i.e. AUC, AUC/slope and/or AUC*time) were found to stay the same or decrease following ingestion of sugar (rather than increasing as expected). This was tentatively attributed to natural variations in physiology (e.g. variations in intestinal permeability, gastric emptying rate and/or rate of elimination/clearance) in the relevant participants from one study day to the next. The gut is an extremely complex organ, which exhibits functional changes in response to subtle and wide-ranging factors such as diet and exercise. As such, it is not unexpected that the data exhibited variations across participants. Nonetheless, this highlights the need to further validate this technology in larger cohorts/populations.

Furthermore, only Asian and Caucasian volunteers participated in this study (see table 1), meaning that the range of skin tones investigated was limited. Skin tone would of course be expected to affect the collected data, with darker skin tones leading to lower absolute signal levels. However, good signal-to-noise ratios were observed in all measurements (see supplementary figures S4 and 5) indicating that the fluorescein dose used here was sufficient for non-invasive permeability sensing in all cases. In addition, once fluorescence is detected, the normalisation procedure (see section 2.1) acts to correct for variations in skin tone (and other factors that affect absolute signal levels such as laser power and probe orientation) between participants. As such, while further investigation of the effect of skin tone would be beneficial, the results presented here still demonstrate the potential of transcutaneous fluorescence spectroscopy for non-invasive permeability sensing.

To address the above limitations and to further progress this project, our future work will focus on validating this technique in larger populations (with a wider range of skin tones) and in patient cohorts where increased intestinal permeability is expected (e.g. Crohn's and coeliac disease). We also aim to

Figure 5. Measuring gut permeability using transcutaneous fluorescence spectroscopy and 3rd method of analysis (median filtering followed by automated identification of peak times using MATLAB software). (a)–(c) Scatter plots showing the parameter values calculated for each participant as readouts of gut permeability (red asterisks—fluorescein only; blue circles—fluorescein + sugar). (a) area under the curve (AUC) at first peak in the data (Peak Area). (b) AUC divided by average gradient of graph in region up until first peak (Peak Area/Slope). (c) AUC at first peak multiplied by time of first peak (Peak Area * Peak Time). (d)–(f) Box plots showing median values (red lines), 25th and 75th percentiles (upper and lower bounds of blue box), most extreme data points excluding outliers (whiskers), and outliers (red crosses) for the fluorescein only (F) and fluorescein + sugar (F + sugar) measurements. Outliers were defined as points that fell below the 25th percentile or above the 75th percentile by more than 1.5 times the interquartile range. Outliers were plotted for visualisation purposes only. All calculations (e.g. of means, medians, etc) and all statistical tests were performed based on the data points from all 11 participants (i.e. including outliers) in all cases. (d) AUC up until first peak (Peak Area). (e) AUC divided by average gradient (Peak Area/Slope). (f) AUC at first peak multiplied by time of first peak (Peak Area * Peak Time). P-values are shown below each graph indicating the results of Student's T-tests comparing the values obtained from the fluorescein only and fluorescein + sugar measurements using the three parameters above (Peak Area, Peak Area/Slope, and Peak Area * Peak Time).

develop a miniaturised, portable version of the device to allow more widespread deployment in larger clinical trials. Together with the results reported here, this will help to further validate transcutaneous fluorescence spectroscopy as a clinical tool for non-invasive monitoring of intestinal permeability.

5. Conclusions

This study reveals the potential of transcutaneous fluorescence spectroscopy for non-invasive assessment of intestinal permeability. Our results demonstrate the ability of this technique to differentiate between two states of gut permeability induced in healthy volunteers using a sugar challenge. As permeability is known to be altered in a wide range of GI and other conditions (e.g. in Crohn's disease, coeliac disease and malnutrition), this suggests that transcutaneous fluorescence spectroscopy may have clinical potential, for example in both diagnostic and therapy/intervention monitoring applications. Crucially, the method reported here is non-invasive, does not require collection of biological samples such as blood or

urine, and involves analysis procedures that can be performed in a fully automated manner. Furthermore, the approach uses fluorescein as a contrast agent, which is low-cost and already approved for other clinical procedures. Together, this suggests significant advantages over existing permeability tests (e.g. L:M tests, Cr-EDTA assays, etc) in terms of both invasiveness and time to report results, and implies the potential for rapid clinical translation. In turn, this would offer opportunities to improve patient outcomes in some of the above conditions, for example by providing earlier diagnosis and improved monitoring of therapy responses.

Acknowledgments

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Disclosures

A J T is inventor of a patent relevant to the use of transcutaneous fluorescence spectroscopy as a tool for non-invasive monitoring of gut permeability and gastric emptying rate and the potential recipient of royalty payments related to this method. All other authors declare no competing interests.

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