Monitoring Changes in Exhaled Volatile Organic Compounds Following Iron Supplementation for Anemia Treatment

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Aims

• To investigate changes in VOCs (volatile organic compounds) due to iron supplementation between Day 1 and Day 28 of the study. This will assess VOC changes due to iron supplementation.

• To investigate changes in VOCs following lactulose ingestion. This will assess VOC changes along the gastrointestinal tract.

1. Background and Objectives

Iron deficiency anaemia (IDA) affects approximately >1.2 billion people worldwide[1,2,3]. In the UK, it can be the reason for up to 13% of referrals to gastroenterologists[4]. Furthermore, The World Health Organisation recognizes IDA as one of the most expensive diseases due to its negative impact on productivity.

IDA can be treated with both oral supplements or IV infusions which are both effective at restoring iron levels in patients. Unabsorbed iron can have unintended side-effects such as enriching intestinal bacteria that result in bloating due to production of gases. These gases can diffuse into the lungs via the blood and are then detectable on exhaled breath.

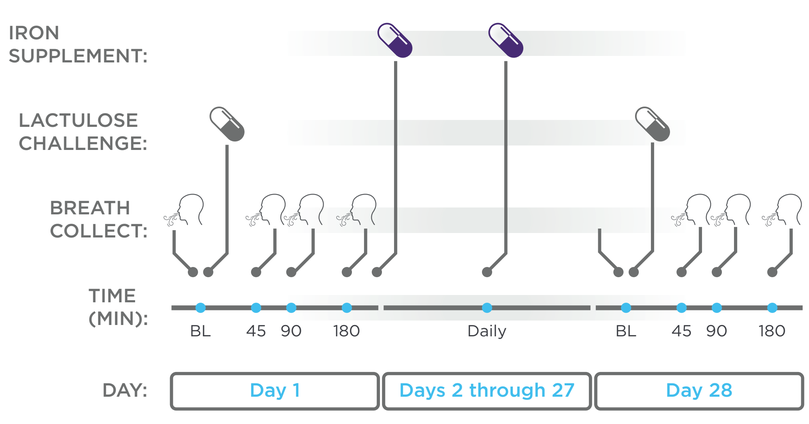
Hydrogen and methane are two gases that have been associated with IDA following consumption of the fermentable carbohydrate, lactulose[5]. This research aims to extend this knowledge by exploring whether other gases, volatile organic compounds (VOC), are associated with oral iron supplementation using the lactulose test.

2. Method

This project was based on VOC changes caused by 28 days of iron supplementation in healthy volunteers.

Owlstone Medical and The Functional Gut Clinic (FGC) were interested in identifying novel breath biomarkers that change in response to oral iron supplementation, and whether production of these biomarkers are related to intestinal geography.

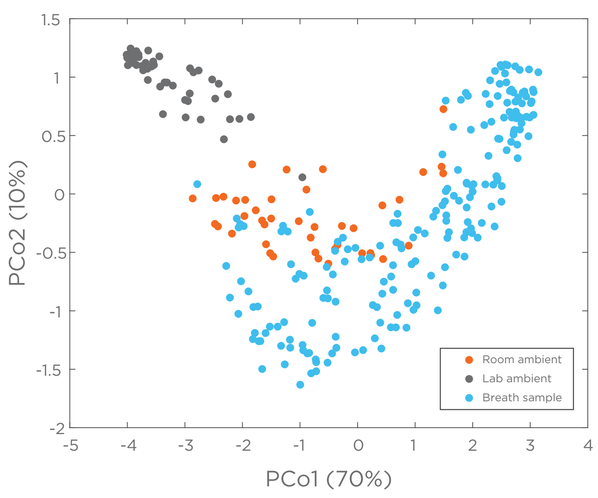
Samples were collected at multiple time points before and after the iron supplementation process, thus each subject served as their own control. Breath samples were collected in breath bags and analysed at Owlstone Medical Inc. using SIFT-MS technology. Targeted analyses were performed, and compounds deemed statistically significant if they were more than two standard deviations from the lab ambient



Day	Description	Sampling Time Point (n)			
		T=0 Baseline Before Lactulose Challenge	45 min	90 min	180 min
1	Pre-iron suppl.	N=25	N=25	N=25	N=25
28	Post-iron suppl.	N=25	N=25	N=25	N=25

Figure 1: Experimental Design: This was a single-centre, longitudinal study with a population of healthy volunteers monitored before and after exposure to iron supplementation [ClinicalTrials.gov identifier (NCT number): NCT04705662]. 25 adult healthy volunteers were recruited for breath sampling for breath collection using polyvinylidene difluoride (PDVF) breath bags. The site of volunteer induction and samplecollection was The Functional Gut Clinic, Manchester. Each volunteer underwent sampling on day 1 before and after administration of lactulose to measure baseline of fermentation levels. After the day 1 visit, volunteers took iron supplements daily and kept a record of any gastrointestinal (GI) tract symptoms experienced. Each volunteer underwent sampling on day 28 ± 2d or sooner if GI symptoms were severe (follow-up clinic visit) before and after administration of lactulose to measure follow-up levels of fermentation.

3. Results and Discussion



From the 25 healthy volunteers that participated in this study, 2 were excluded due to incomplete samples. Ambient (blank) samples were collected, but not for all patients/visits/timepoints.

Data was symmetrically distributed therefore further mathematical transformation was unnecessary.

Figure 2: PCA analysis showing breath samples are distinct from both lab and room ambient samples using targeted compounds. Pentanoic, butanoic, propanoic, acetic 3-methylnutanoic and hexanoic acids, ethanol, hydrogen sulphide, methane, indole, isoprene, cresols, 2,3-butanedione, trimethylamine, acetone, limonene and phenol were selected in targeted analysis. Lab ambient and room ambient samples show divergent composition, with room ambient resembling the composition of breath samples more than lab samples.

Compound	Adjusted difference	p-value
3-methylbutanoic acid	0.675	0.017
butanoic acid	15.486	0.047
propanoic acid	4.707	0.026
2,3-butanedione	9.793	0.045
limonene	1.307	0.007
hydrogen sulfide	-22.667	0.026
cresol	0.194	0.005

Table 1: Table shows compounds that significantly change after iron supplementation. Linear Mixed models were fitted to evaluate the evolution overtime of the compounds' intensities. These models allow evaluation of both the effect of iron supplementation and that of lactulose challenge, also accounting for the observations' dependence due to repeated measurements from the same HV. For iron supplementation, the model shows that baseline, i.e. time pt. 0, levels of several compounds are significantly different between Day 1 and Day 28.

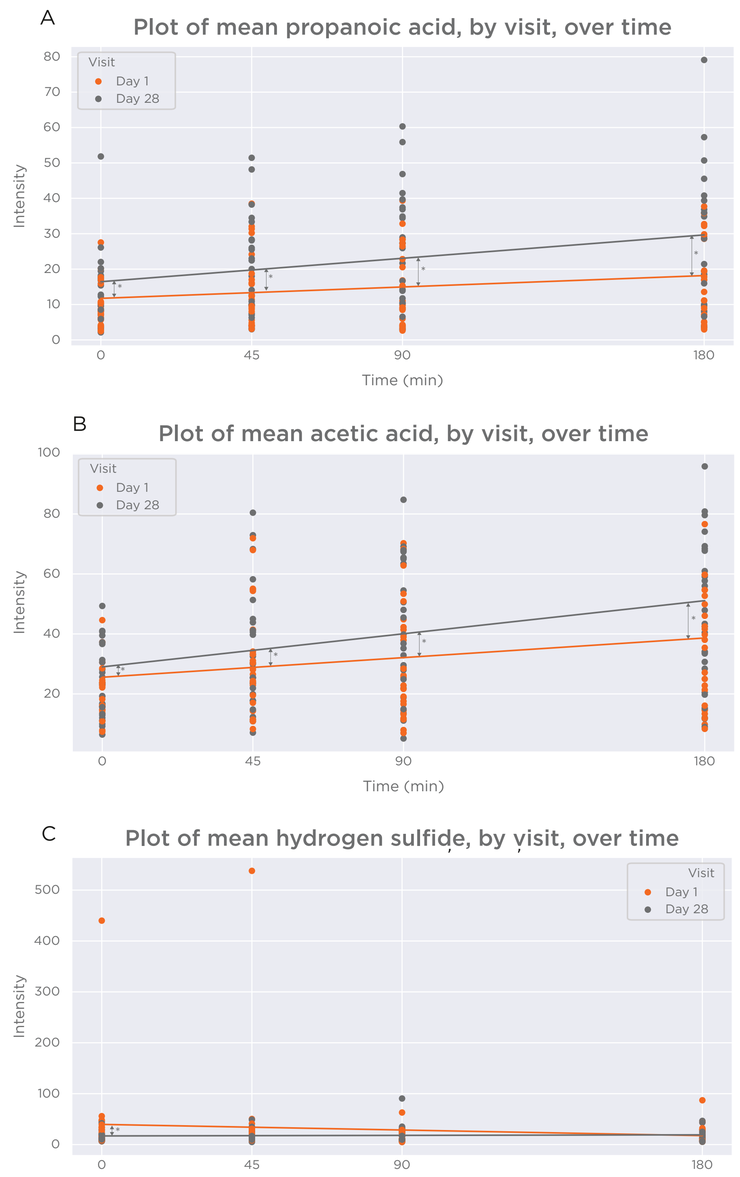


Figure 3: Graphs showing compounds with significant changes after iron supplementation and/or lactulose challenge. For both (A) propanoic and (B) acetic acids, the trends over time are significant and also different by day of the challenge (either 1 or 28). (C) Hydrogen sulphide, however, significantly decreases after 28 days of iron supplementation. This change is not affected by lactulose ingestion. *p value <0.05.

4. Conclusions

Some short chain fatty acids (SCFAs), butanoic, propanoic and acetic acids, increased after 28 days of iron supplementation following lactulose ingestion: Increases in SCFA has been linked to increased gut health[5]. They have been shown to maintain colonocyte development, promote metabolic health and speculated to play a key role in neuro-immunoendocrine regulation[6,7]. The significant increase in these SCFAs indicate a positive effect of iron supplementation in this cohort.

SCFAs propanoic and acetic acids are associated with geography specific fermentation: Relatively higher levels of these compounds were observed at 180m post lactulose ingestion indicating colonic fermentation[8]. These findings are supported by previous evidence showing SCFAs are the main metabolites produced in the colon by bacterial fermentation[9].

Hydrogen sulphide (H2S) was significantly decreased after 28 days of iron supplementation following lactulose ingestion. H2S is considered to be detrimental to gut health thus decreases in this compound is beneficial[10].

It should also be noted that a limitation of this study was that blank measurements were done at the clinic site by drawing ambient air into a bag via a syringe. This air may have atmospheric contamination due to cleaning agents, perfumes etc. Thus, the room ambient and breath samples may be noisy. The statistically significant changes were calculated as two standard deviations from lab ambient.

Another limitation of this study was that not all subjects were healthy. After filling out clinical questionnaires, it was determined that subjects showed signs of small intestinal bacterial overgrowth (SIBO) or irritable bowel syndrome (IBS). These underlying conditions would have likely impacted VOCs produced.

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