



Role du fructose dans la physiopathologie du syndrome de l'intestin irritable

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THESE

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Préparée au sein de «L'université de Rouen Normandie»

Rôle du fructose dans la physiopathologie du syndrome de l'intestin irritable

**Présentée et soutenue par
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Résumé

La consommation journalière de fructose est en augmentation croissante, jusqu'à plus de 50g par jour aux Etats-Unis et en Europe de l'Ouest. En effet, à l'échelle mondiale, le fructose est de plus en plus incorporé dans les boissons, les produits laitiers et les conserves, les produits cuisinés ou transformés. Le fructose peut déclencher ou aggraver les symptômes digestifs chez des volontaires sains, mais aussi dans le syndrome de l'intestin irritable. Le rôle de l'hypersensibilité viscérale dans le déclenchement des symptômes, lié à la prise de fructose a été suspecté mais n'a jamais été évalué.

La prévalence de la malabsorption du fructose était mal documentée chez les patients souffrant d'un syndrome de l'intestin irritable, en Europe de l'Ouest, principalement en raison de l'hétérogénéité des méthodes diagnostiques. Le premier objectif de ce travail a été de définir, dans une population de patients souffrant d'un syndrome de l'intestin irritable, la prévalence de la malabsorption du fructose. Notre test de malabsorption du fructose a été défini par une dose de charge de 25g. Il était systématiquement réalisé après avoir éliminé une pullulation bactérienne endoluminale par un test respiratoire au glucose. Chez nos patients souffrant d'un syndrome de l'intestin irritable, 22% présentaient une malabsorption du fructose. Les patients jeunes et de sexe masculin étaient plus à risque de malabsorption du fructose. Nous avons également étudié l'association de la malabsorption du fructose avec d'autres anomalies physiopathologiques connues dans le syndrome de l'intestin irritable. Nous n'avons pas retrouvé d'association entre la présence d'une inflammation digestive (par le dosage de la calprotectine fécale) et la présence ou non d'une malabsorption du fructose. En revanche, une association a été retrouvée entre malabsorption du fructose et hypersensibilité viscérale.

L'efficacité du régime appauvri en fructose dans le syndrome de l'intestin irritable est connue. L'existence ou non d'une malabsorption du fructose associée pourrait être un facteur prédictif d'efficacité d'un tel régime. Le deuxième objectif de ce travail a été de déterminer si le test de dépistage de la malabsorption au fructose (par un test respiratoire) permettait de prédire l'efficacité du régime appauvri en fructose sur les symptômes digestifs des patients souffrant d'un syndrome de l'intestin irritable. Les résultats de notre étude ont confirmé l'efficacité du

régime appauvri en fructose dans le syndrome de l'intestin irritable. En revanche, la présence ou non d'un test respiratoire au fructose positif n'impactait pas l'efficacité du régime. Une des explications à cette absence de différence est l'association possible à une ou plusieurs anomalies physiopathologiques (comme l'hypersensibilité viscérale) qui ne sont pas ciblées par un régime.

Le dernier objectif de ce travail était de modéliser la malabsorption du fructose sur des modèles murins, pour permettre d'identifier les mécanismes physiopathologiques sous-jacents. La modélisation sur 3 modèles murins de malabsorption du fructose (par régime riche en fructose, par délétion des gènes codant les transporteurs du fructose GLUT5 et GUT2) permettait d'induire une hypersensibilité viscérale associée à une augmentation de la perméabilité intestinale, deux anomalies déjà rapportées dans le syndrome de l'intestin irritable. L'étude des mécanismes physiopathologiques sous-jacents a permis d'écarter l'implication d'une inflammation de bas grade qui n'était pas retrouvée chez nos souris. L'augmentation d'activité élastase dans les selles de souris avec malabsorption du fructose était associée à l'hypersensibilité viscérale. Or il a déjà été démontré que l'activité protéasique pouvait être responsable d'une hypersensibilité viscérale et d'une augmentation de la perméabilité intestinale. Les récepteurs associés à la protéase-2 sont connus pour être associés à l'hypersensibilité viscérale et l'augmentation de la perméabilité intestinale. Des travaux sont encore nécessaires pour déterminer son implication dans la malabsorption du fructose associée à l'hypersensibilité viscérale.

Les résultats obtenus dans le cadre de ce travail soulignent le rôle de la malabsorption du fructose, qui entraîne la survenue d'une hypersensibilité viscérale et d'une augmentation de la perméabilité intestinale, dans le syndrome de l'intestin irritable. Un régime appauvri en fructose n'améliore pas de manière ciblée les symptômes des patients souffrant d'un syndrome de l'intestin irritable avec malabsorption du fructose.

Mots clés

Syndrome de l'intestin irritable - malabsorption du fructose - test respiratoire - régime appauvri en fructose - facteur prédictif - GLUT5KO - GLUT2^{ΔIEC} - souris - hypersensibilité viscérale - perméabilité intestinale - activité protéasique

Fructose implication in irritable bowel syndrome pathophysiology

Summary

Fructose intake has increased by up to 50 g per day in the USA and Western Europe. Fructose is increasingly incorporated in beverages, dairy products and canned, baked or processed foods worldwide. Fructose has been shown to trigger or worsen digestive symptoms not only in healthy volunteers, but also in patients with irritable bowel syndrome. The involvement of visceral hypersensitivity has been suspected but has never been assessed.

The prevalence of fructose malabsorption in patients with irritable bowel syndrome in Western Europe remains poorly documented, due to the heterogeneity of available tests. Therefore, the first objective of this present work was to assess the prevalence of fructose malabsorption in patients with irritable bowel syndrome. We assessed fructose malabsorption with a fructose breath test, after a 25 g load. We systematically ruled out small intestinal bacterial overgrowth which could promote false positive. In our irritable bowel syndrome patients, 22% had fructose malabsorption. Young, male patients were more likely to have fructose malabsorption. We also assessed the association between fructose malabsorption and other abnormalities. We did not observe any association between low-grade inflammation (with faecal calprotectin dosage) or fructose malabsorption. In contrast, an association between fructose malabsorption and visceral hypersensitivity was evidenced.

Low fructose diet is known to improve symptoms in patients with irritable bowel syndrome. The presence of fructose malabsorption could be predictive of the efficacy of a low fructose diet. The second objective of this work was to determine if an abnormal fructose breath test was a predictor of symptomatic response to low fructose diet in irritable bowel syndrome. Our study has confirmed the efficacy of low fructose diet on irritable bowel syndrome. However, the results of the fructose breath test had no impact on its efficacy. One explanation for this result could be the presence of other abnormalities (including visceral hypersensitivity) that were not addressed only with a diet.

The last objective of this work was to model fructose malabsorption in mice, in order to identify the underlying mechanisms. We used three models of fructose malabsorption (high fructose diet, invalidation of GLUT5 and GLUT2 coding gene). In these models, fructose malabsorption induced visceral hypersensitivity and increased intestinal permeability, the two abnormalities being reported in irritable bowel syndrome. In our models, there was no low-grade inflammation. Increased elastase activity in mice faeces was associated with visceral hypersensitivity. Protease-activated receptor-2 is known to be associated with visceral hypersensitivity and increases intestinal permeability. Further works are warranted to determine the involvement of protease-activated receptor-2 in fructose malabsorption-associated visceral hypersensitivity.

The results of this work underlined the role of fructose malabsorption in irritable bowel syndrome, in the onset of visceral hypersensitivity and increased intestinal permeability. A low fructose diet is not helpful to improve symptoms of irritable bowel syndrome with fructose malabsorption.

Key-words

Irritable bowel syndrome - fructose malabsorption - breath test - low fructose diet - predictive factor - GLUT5KO - GLUT2^{ΔIEC} - mice - visceral hypersensitivity -intestinal barrier - protease activity

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Listes des articles publiés dans le cadre de ma formation doctorale

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- Melchior C**, Douard V, Atmani K, Guérin C, Bôle-Feysot C, Schmitt C, Zhang X, Coëffier M, Waligora-Dupriet A-J, Grosfeld A, Deraison C, Ducrotté P, Serradas P, Vergnolle N and Gourcerol G. Visceral hypersensitivity and altered intestinal permeability in mouse model of irritable bowel syndrome with fructose malabsorption. (cible gut)

Liste des abréviations

AGCC : acides gras à chaîne courte

ANCA : anticorps anti-cytoplasme des polynucléaires neutrophiles

ASCA: anticorps anti-Saccharomyces Cerevisiae

CCK : cholecystokinine

CH₄ : méthane

CRF : cortisol releasing factor

CRP : C reactive protein

ELISA : Enzymed Linked ImmunoSorbent Assay

FODMAPs : fermentable oligosaccharides, disaccharides, monosaccharides and polyols

H₂ : hydrogène

GF : Germfree

GI : gastro-intestinaux

GLP-1 : glucagon-like-peptide-1

GLUT : transporteur du glucose

GLUT2^{ΔIEC} : délétion intestinale de GLUT2

IRM : imagerie par résonance magnétique

KHK : Kétohexokinase

KO : knockout

MICI : maladie chronique inflammatoire de l'intestin

min : minutes

NFS : numération formule sanguine

PAR : Protease-activated receptor-2

pH : potentiel hydrogène

ppm : partie par million

SGLT1 : cotransporteur sodium-glucose 1

SII : syndrome de l'intestin irritable

SII-C : SII avec constipation

SII-D : SII avec diarrhée

SII-I : SII indéterminé

SII-M : mixte avec alternance diarrhée/ constipation

TFI : troubles fonctionnels intestinaux

TSH : thyroestimuline

T2R : récepteur à la protéine G de la voie de signalisation au goût amer

WT : Wild type

I) Introduction

1) Les troubles fonctionnels intestinaux

Les troubles fonctionnels intestinaux (TFI) constituent un ensemble de pathologies intestinales chroniques caractérisées par la présence de symptômes digestifs (douleur abdominale, ballonnement, distension...) et/ou de troubles du transit (constipation, diarrhée ou alternance des deux). Les TFI se distinguent des autres maladies gastro-intestinales (GI) par leur chronicité (symptômes évoluant depuis plus de 6 mois), leur fréquence (au moins 1 jour par semaine) et l'absence d'anomalie anatomique ou physiologique identifiée par des examens diagnostiques de routine jugés appropriés (1).

Ces pathologies digestives sont très fréquentes et répandues dans le monde. Elles peuvent toucher n'importe quelle personne, indépendamment de l'ethnie, de l'âge, du sexe et du statut social. Leur impact socio-économique (conséquences sur l'inaptitude transitoire au travail) est majeur et le retentissement sur la qualité de vie des malades important.

La dernière classification de Rome IV (1) a revu les critères diagnostiques des TFI et définit cinq catégories de patients (**table 1**) : le syndrome de l'intestin irritable (SII), la constipation fonctionnelle, la diarrhée fonctionnelle, le ballonnement abdominal fonctionnel et les troubles fonctionnels intestinaux non spécifiés. Il est important de souligner l'existence d'un chevauchement entre ces catégories. Il existe également une 6^{ème} catégorie un peu à part : la constipation induite par les opioïdes. Elle est distincte des autres par l'existence d'une étiologie spécifique.

Table 1 : Troubles fonctionnels intestinaux selon la classification de Rome IV (1)

Troubles fonctionnels intestinaux
1) Syndrome de l'intestin irritable
2) Constipation fonctionnelle
3) Diarrhée fonctionnelle
4) Ballonnement abdominal fonctionnel
5) Troubles fonctionnels intestinaux non spécifiés
6) Constipation induite par les opioïdes

2) Le syndrome de l'intestin irritable

a) Définition

Le SII est un des TFI dans lequel la douleur abdominale chronique est associée à la défécation ou à des modifications du transit. Des troubles du transit sont typiquement présents (constipation, diarrhée ou alternance diarrhée/ constipation), de même que les symptômes de distension/ ballonnement abdominaux. Le début des symptômes doit dater d'au moins 6 mois avant le diagnostic et les symptômes être présents sur les 3 derniers mois. Les critères diagnostiques (**table 2**) sont définis selon la classification de Rome IV (1).

Table 2 : Critères diagnostiques du syndrome de l'intestin irritable (classification de Rome IV)
(1)

Critères diagnostiques* pour le syndrome de l'intestin irritable (classification de Rome IV)
Douleur abdominale chronique survenant au moins 1 jour par semaine dans les 3 derniers mois associée à au moins 2 des points suivants : 1) en relation avec la défécation 2) associée à une modification - de la fréquence des selles - de l'aspect des selles *Les symptômes surviennent depuis au moins 3 mois avec un début des symptômes au moins 6 mois avant le diagnostic

b) Critères diagnostiques des sous-types du syndrome de l'intestin irritable

Le SII est classé en 3 principaux sous-types selon le trouble du transit prédominant : SII avec constipation (SII-C), SII avec diarrhée (SII-D) et SII mixte avec alternance diarrhée/constipation (SII-M) (**table 3**). L'échelle de Bristol (2) (**figure 1**) doit être utilisée pour déterminer la consistance des selles . Les patients répondant aux critères de Rome pour le SII mais ne rentrant pas dans l'une des 3 catégories doivent être classés dans SII indéterminé (SII-I) (**table 3**).

Figure 1 : Echelle de Bristol (2)

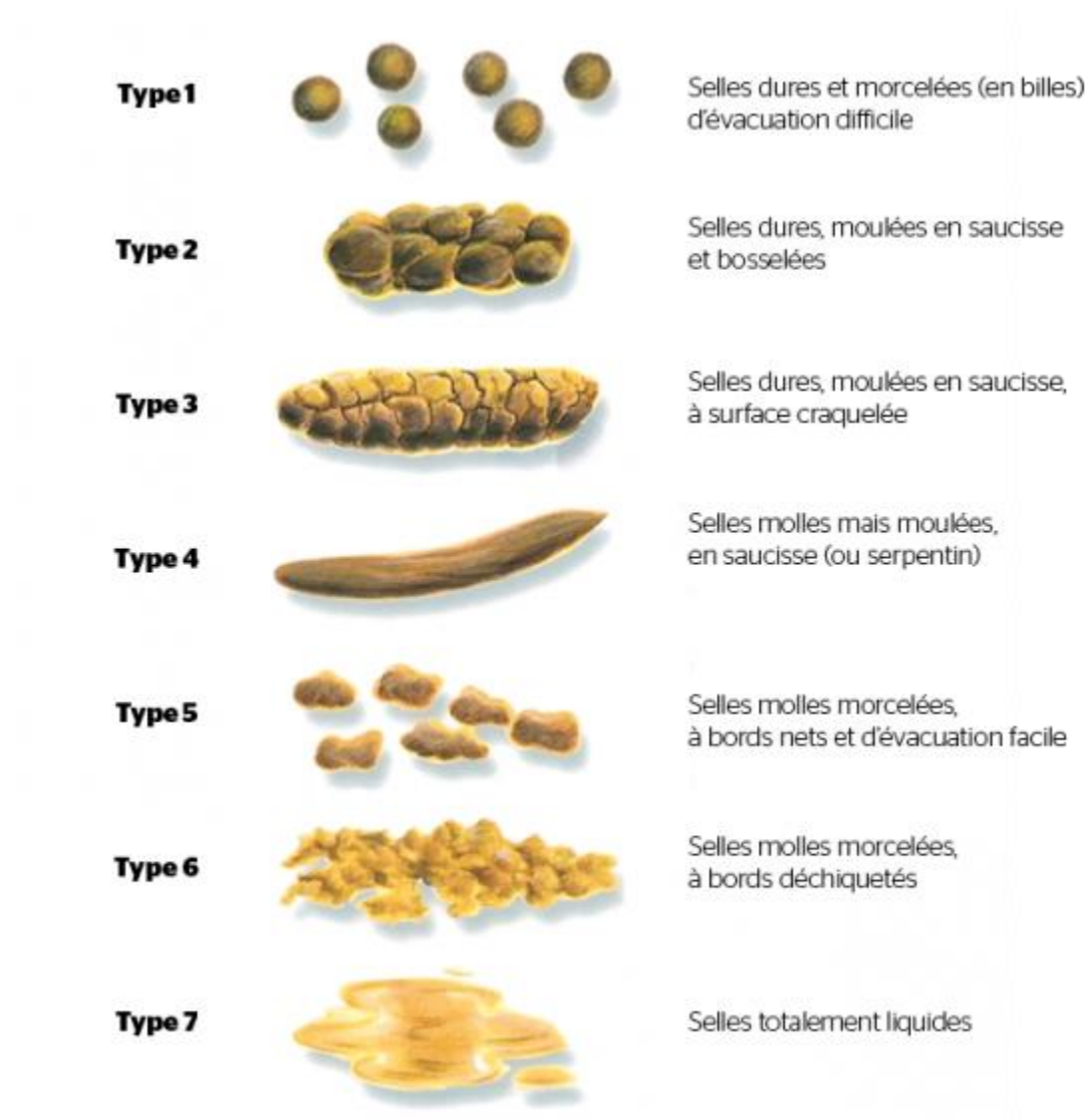


Table 3 : Sous-type de syndrome de l'intestin irritable

Sous type de syndrome de l'intestin irritable (SII)
-SII avec constipation prédominante : plus de 25 % des selles de type 1-2 sur l'échelle de Bristol et moins de 25 % de type 6-7.
-SII avec diarrhée prédominante : plus de 25 % des selles de type 6-7 sur l'échelle de Bristol et moins de 25 % de type 1-2.
-SII avec alternance diarrhée/ constipation : plus de 25 % des selles de type 1-2 sur l'échelle de bristol et plus de 25 % des selles de type 6-7.
-SII indéterminé : patients répondant aux critères de Rome IV pour le SII mais ne pouvant être catégorisés dans les 3 groupes ci-dessus.

c) Epidémiologie

La prévalence mondiale du SII a été abaissée de moitié soit 5.7% depuis l'utilisation des critères de Rome IV en 2016 (3, 4). L'incidence du SII est estimée à 1.5% basée sur une étude longitudinale d'une durée de 10 ans (5). La prévalence est plus élevée chez les femmes comparativement aux hommes et chez les jeunes en comparaison au plus de 50 ans (6). La prévalence varie en fonction des pays mais également des critères diagnostiques utilisés (6). En France elle est estimée à 5%.

d) Impact économique

La chronicité des symptômes et l'altération de la qualité de vie en font un problème de santé publique majeur. L'altération de la qualité de vie peut-être équivalente ou même plus marquée que celle consécutive à des pathologies chroniques comme le diabète ou l'insuffisance rénale chronique hémodialysée (7). Les symptômes amènent à un absentéisme notable et une perte de productivité au travail et conduisent à un recours important aux soins de santé et à des

dépenses de santé dédiées (coûts directs et indirects) élevées (1658 millions de dollars/an aux Etats-Unis) (8). En France, le coût annuel direct a été estimé à 756 euros/patient et environ 8% des patients ont nécessité une hospitalisation dans l'année (9).

e) Diagnostic

Le diagnostic du SII est avant tout clinique. Devant le nombre de pathologies qui peuvent mimer les symptômes du SII (maladies inflammatoires chroniques intestinales (MICI), maladie cœliaque, colites microscopiques), certaines explorations comme la calprotectine fécale peuvent-être requises pour faire la distinction.

A ce jour aucun examen complémentaire ne permet de faire le diagnostic de SII. Le diagnostic est basé sur l'histoire de la maladie, l'examen clinique, quelques tests biologiques et seulement, lorsque nécessaire, par une coloscopie ou d'autres tests diagnostiques. C'est la normalité de l'examen clinique, des tests biologiques et endoscopiques, associée à la positivité des critères de Rome IV qui permettent de poser le diagnostic de SII. Pour la majorité des patients, lorsque les critères diagnostiques de SII sont remplis et en l'absence de signes d'alarme (un antécédent familial de cancer colorectal, un âge supérieur à 50 ans, un saignement digestif ou une anémie, une perte de poids involontaire, des symptômes nocturnes, une modification récente des symptômes), le recours aux examens complémentaires doit être fait avec discernement, leur rentabilité diagnostique étant faible (10). Une numération formule sanguine (NFS) et une C réactive protéine (CRP) sont recommandées pour éliminer une anémie et/ou une inflammation (11). En présence d'un SII-D, un dosage de la thyroïdostimuline (TSH) et des Anticorps anti-transglutaminases est recommandé. Le bilan endoscopique ne sera proposé qu'en cas de signes d'alarme initiaux ou d'anomalie au bilan biologique.

f) Comorbidité

Une variété de symptômes gastro-intestinaux (dyspepsie) ou non gastro-intestinaux (fibromyalgie, syndrome de fatigue chronique, cystite interstitielle, dyspareunie, migraine)

sont fréquemment associés au SII (12, 13). Leur présence concomitante peut aider à orienter le diagnostic (12, 13).

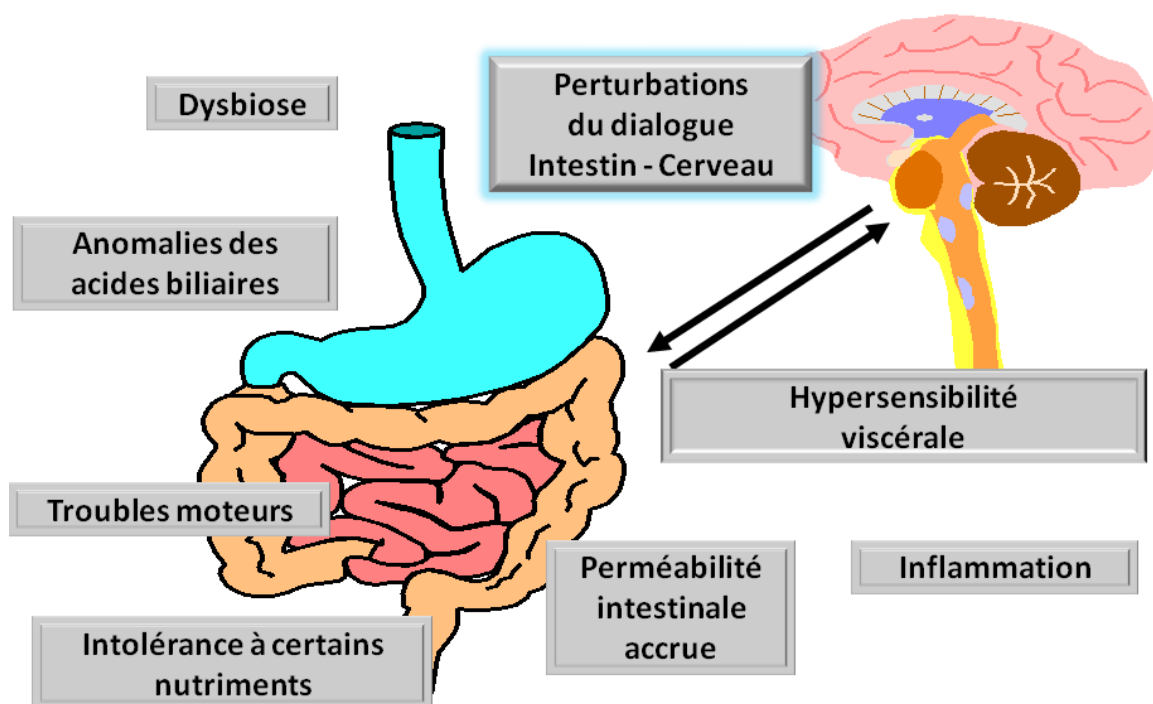
Des troubles psychologiques sont également souvent associés au SII. Les facteurs psychosociaux sont prédictifs de la sévérité et de l'évolution du SII (14). Ils doivent être pris en compte dans la prise en charge des patients. Le SII est associé à plus de troubles psychiatriques, de troubles du sommeil, "une vulnérabilité affective" et "un sur-ajustement à l'environnement" (15).

g) Physiopathologie

Le SII est une pathologie multifactorielle avec une physiopathologie complexe qui demeure imparfaitement comprise. Les facteurs augmentant le risque de développer un SII incluent certains facteurs génétiques, d'environnement et des facteurs psychosociaux. Les facteurs déclenchant le début ou l'exacerbation des symptômes sont : une gastroentérite aiguë, la consommation d'aliments auxquels les malades sont intolérants, un stress aigu ou chronique (16).

Les symptômes sont liés à un dysfonctionnement des communications bilatérales entre le système nerveux central et le système nerveux entérique. Ce dysfonctionnement de l'axe intestin-cerveau peut-être d'origine digestive et/ou centrale. Il en résulte des troubles de la motricité digestive, une hypersensibilité viscérale, une augmentation de la perméabilité intestinale, une activation immune, des modifications de la flore colique, des acides biliaries, une perturbation du dialogue intestin-cerveau (16). Des études de plus en plus nombreuses montrent que le SII est un cadre physiopathologique hétérogène avec des mécanismes différents sous-tendant une expression clinique commune (**figure 2**) (16).

Figure 2 : Différents facteurs impliqués dans la physiopathologie actuelle du SII



Parmi les mécanismes physiopathologiques sous-tendant du SII, les principaux sont: l'hypersensibilité viscérale, la dysbiose du microbiote intestinal, les altérations de la perméabilité intestinale et l'inflammation de bas grade.

Les patients souffrant d'un SII présentent une hypersensibilité viscérale à la distension colorectale. Cette hypersensibilité est associée à la composante douloureuse des symptômes ainsi qu'à la qualité de vie des malades, et constitue le seul marqueur fonctionnel de la maladie (17). Cette anomalie est inconstante, et dépend du seuil diagnostique choisi, mais peut-être retrouvée chez la moitié des malades, et jusqu'à 80% des patients présentant un SII diarrhéique (18).

Des études récentes soulignent l'implication éventuelle d'une dysbiose du microbiote intestinal dans la genèse du SII (19). Il existe des différences qualitatives et quantitatives dans la composition de la flore entre patients SII et sujets contrôles (20). Il a été démontré que le pH colique des patients SII est plus acide que celui de sujets volontaires sains, suggérant une fermentation colique plus importante (21). Cette fermentation intestinale anormale pourrait être un des mécanismes étiologiques dans le SII car l'acidification colique sensibilise les mécano-récepteurs coliques à la distension (22).

L'augmentation de la perméabilité intestinale quant à elle semble présente chez plus de 40% des patients, principalement en cas de sous type diarrhéique (16, 23, 24). L'expression des protéines de jonction serrées, l'occludine et la claudine, est altérée dans le SII, uniquement dans le sous-type diarrhéique et pourrait contribuer à l'hypersensibilité viscérale (23).

Les analyses biopsiques chez les patients atteints du SII n'identifient pas de réaction inflammatoire détectable. La présence d'une inflammation "de bas grade" reste néanmoins suspectée. En effet, l'équipe de Bologne a démontré par microscopie électronique qu'il existait chez les patients SII une augmentation de l'activation mastocytaire, et ce, directement au contact des terminaisons nerveuses digestives (25). Cette activation mastocytaire pourrait contribuer à l'hypersensibilité viscérale. Environ 1/3 des patients SII présente une élévation modérée de la calprotectine fécale possiblement témoin d'une inflammation de bas grade (26).

Le lien entre ces 4 anomalies n'est, à ce jour, pas élucidé. Le modèle actuellement envisagé, serait que l'augmentation de la perméabilité intestinale par atteinte des jonctions serrées favoriserait la pénétration d'antigènes et/ou de pathogènes. Ceci aurait pour conséquence d'induire une inflammation de bas grade qui conduirait à la sensibilisation des afférences viscérales. Ces effets pourraient passer par des modifications du microbiote intestinal qui activeraient la réponse immunitaire innée, au niveau de la muqueuse digestive.

h) Traitements

Le SII demeure, dans beaucoup de cas, un problème dans la pratique des cliniciens. L'objectif principal du traitement est le soulagement de la douleur et/ou de l'inconfort abdominal qui

constitue la plainte principale des malades. L'amélioration de la qualité de vie passe également par une amélioration des troubles du transit qui sont constants et plus nets lors des poussées douloureuses. Le traitement ne permet pas de guérir mais vise à atténuer les symptômes en réduisant la sévérité de la maladie; ceci, afin d'améliorer la qualité de vie des patients et de diminuer les conséquences psycho-sociales des troubles.

Le traitement du SII commence avec l'explication de la maladie aux patients mais également la réassurance sur l'histoire naturelle bénigne. Le traitement doit être basé sur le type de symptôme et la sévérité. Les modifications du style de vie peuvent améliorer les symptômes et peuvent comporter une activité sportive, la réduction du stress et une prise en charge des troubles du sommeil (27).

Les traitements médicamenteux de première intention comprennent les antispasmodiques, les laxatifs et les ralentisseurs du transit qui ont une efficacité inconstante. L'hétérogénéité des patients souffrant d'un SII peut expliquer le médiocre gain thérapeutique de 7 à 15% (28).

L'efficacité des antispasmodiques a fait l'objet de plusieurs études et méta-analyses. Elles ont conclu que les antispasmodiques (comme la trimébutine, le pinavérium ou la mébévérine) soulageaient mieux la douleur que le placebo, avec un gain thérapeutique de l'ordre de 10-12% et un nombre de malades à traiter variant de 3 à 8 selon les méta-analyses (29). Les laxatifs osmotiques peuvent être proposés en cas de constipation ou d'alternance diarrhée-constipation. Le lopéramide est proposé en cas de diarrhée. En cas d'échec, la cholestyramine qui est un chélateur des acides biliaires, peut améliorer les patients dans l'hypothèse d'une diarrhée aux acides biliaires (30).

En cas d'inefficacité des traitements de première ligne, lorsque la sévérité des symptômes et le retentissement sur la qualité de vie sont fortement impactés, les praticiens ont recours à des traitements de seconde intention dont le bénéfice est soit plus controversé, soit les effets secondaires plus importants.

Les antidépresseurs tricycliques et les inhibiteurs de la recapture de la sérotonine agissent sur les troubles thymiques mais également sur l'hypersensibilité viscérale d'origine neuropathique. Ils peuvent être prescrits dans le sous-groupe diarrhéique. Le nombre de

malades à traiter est de 4 mais au prix d'effets indésirables qui ont un impact sur la compliance au traitement (31).

Les probiotiques sont une option pour agir sur la flore. Les résultats des études sont encourageants avec une réduction du risque de persistance des symptômes (32). La comparaison entre les études et les méta-analyses est limitée, les études utilisant le plus souvent des combinaisons de souches bactériennes différentes (33). Le probiotique ayant démontré la meilleure preuve scientifique est le *Bifidobacterium infantis* 35624, dont l'utilisation pendant 4 semaines chez 362 femmes souffrant de SII a permis une amélioration significative des symptômes ($p=0.01$) (34). L'utilisation des probiotiques est ainsi recommandée par les sociétés savantes avec un faible niveau de preuve (avis d'experts).

Les médicaments à action anti-inflammatoire intestinale sont une option séduisante pour réduire l'inflammation de bas grade qui existe chez certains patients SII. Cependant les études sont discordantes, un sous-groupe de patients non défini semble pouvoir en tirer un bénéfice (35).

Dans les options thérapeutiques récentes, le linaclotide agit sur les récepteurs de la guanylate cyclase induisant une sécrétion d'eau, de chlore et de bicarbonate dans la lumière intestinale. Il existe également un effet anti-nociceptif permettant une amélioration symptomatique globale des patients (36). L'eluxadoline, agoniste des récepteurs μ aux opioïdes et antagonistes des récepteurs δ aux opioïdes, permet une amélioration symptomatique dans le SII-D (37). Malheureusement le linaclotide et l'eluxadoline ne sont pas disponibles en France.

Les traitements non médicamenteux font également part entière de l'arsenal thérapeutique (sport, relaxation, psychothérapie cognitivo-comportementale, hypnose...) (38). L'hypnose a démontré son efficacité sur le long terme dans une étude randomisée de 90 patients SII avec une amélioration de la qualité de vie, y compris chez les patients réfractaires (39). L'hypnose a également démontré sa non infériorité en comparaison à un régime pauvre en FODMAPs sur l'amélioration globale des symptômes gastro-intestinaux chez 74 patients SII (40).

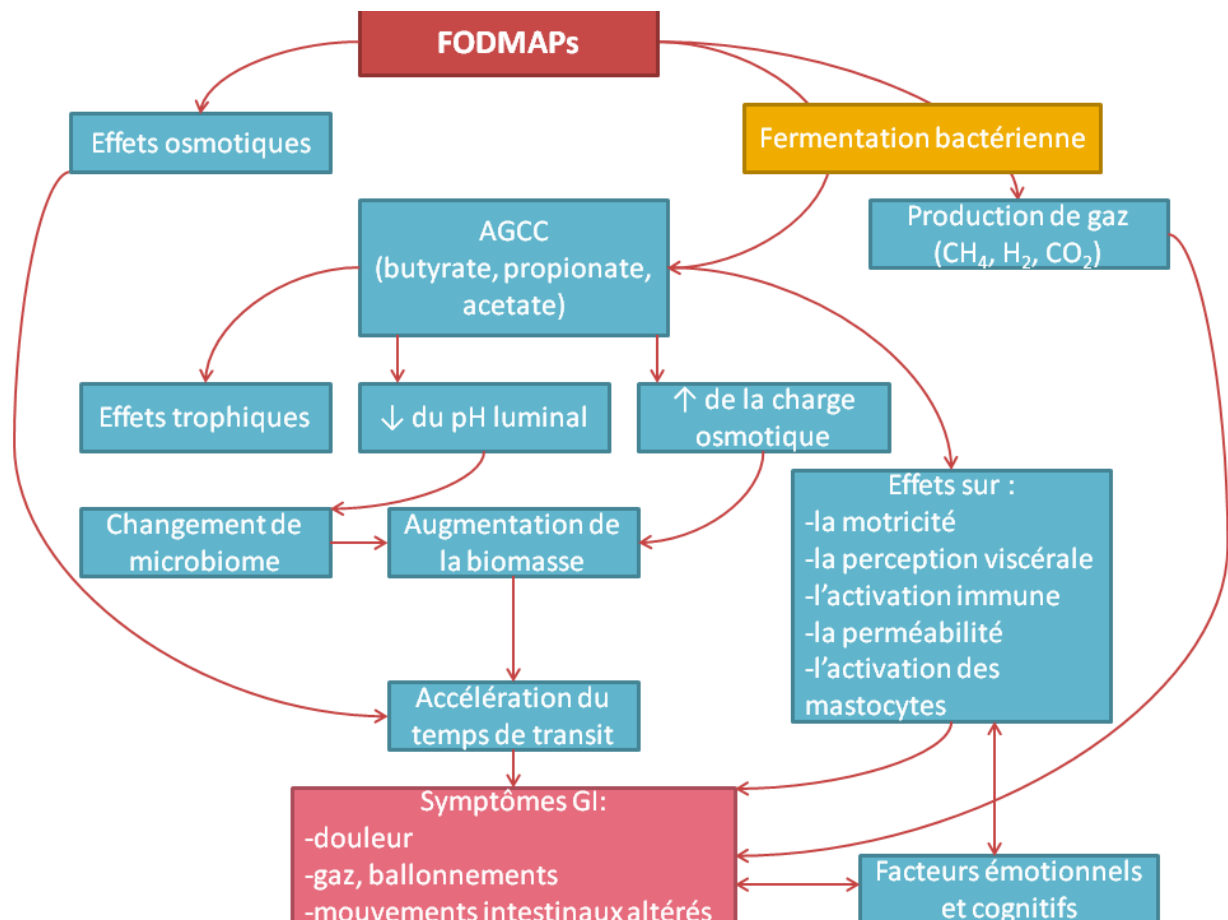
i) Rôle de l'alimentation

La majorité des patients souffrant d'un SII décrivent un lien entre la survenue de leurs symptômes et l'alimentation, cette relation devenant une de leurs préoccupations principales au cours des consultations, la prise alimentaire déclenchant ou aggravant les symptômes (41, 42). Ce lien a été confirmé par des études prospectives (43). La présence d'un lien entre l'alimentation et la symptomatologie du SII est associée à la sévérité des symptômes et à une moins bonne qualité de vie (41).

j) Les FODMAPs

Le rôle délétère potentiel des "FODMAPs" (Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols), tels que les fructo-oligosaccharides, le fructose, les fructanes ou le lactose, est désormais bien admis (44). Ces courtes chaînes de carbone ont pour caractéristique d'être peu absorbées dans l'intestin grêle, d'avoir un effet osmotique et d'être rapidement fermentées par la flore. Ce phénomène entraîne la production d'acide gras à chaînes courtes et de gaz (hydrogène, méthane et dioxyde de carbone) causant de nombreux symptômes comme les ballonnements, la douleur, les nausées et les troubles du transit, de façon plus marquée chez les patients souffrant de SII (45-47) (**figure 3**). La distension intestinale, en stimulant les mécanorécepteurs digestifs, pourrait accentuer la douleur ou la déclencher (46, 48). Le rôle de l'hypersensibilité colique pourrait expliquer l'importance de la symptomatologie chez les patients SII en comparaison de volontaires sains (46).

Figure 3 : Mécanismes par lesquels les FODMAPs peuvent causer des symptômes gastro-intestinaux (47)



D'après Eswaran S et al. Low FODMAP in 2017: lessons learned from clinical trials and mechanistic studies. *Neurogastroenterol Motil* 2017 (47).

FODMAPs : Fermentable Oligosaccharides Disaccharides, Monosaccharides and Polyols,
AGCC: Acides gras à chaînes courtes

La production d'acide gras à chaînes courtes et de gaz est probablement plus marquée en cas de malabsorption du fructose et du lactose; leur fréquence est sans doute sous-estimée (49) alors que les fructo-oligosaccharides, le fructose et les fructanes sont incorporés de plus en plus largement dans les préparations industrielles alimentaires.

Il y a de plus en plus de preuve de l'efficacité d'un régime pauvre en FODMAPs chez les patients souffrant d'un SII. La première étude, contrôlée randomisée en double aveugle ayant démontré un lien entre les FODMAPs et les symptômes, remonte à 2008 par l'équipe australienne de Shepherd et Gibson (48). Chez plus de 70% des 25 patients, une solution de fructose ou de fructanes entraînait la survenue de symptômes en comparaison à 14% avec une solution de glucose (48). Cette approche nouvelle a ouvert la porte à une stratégie diététique excluant un groupe d'hydrates de carbone et non plus un seul type. Ceci a été confirmé par une amélioration symptomatique, après régime pauvre en FODMAPs, chez 52 à 76% des patients souffrant d'un SII sur plusieurs études rétrospectives, randomisées avec des biais d'interprétation (50-54). Le premier essai australien contrôlé randomisé en cross-over, avec une méthodologie robuste avec livraison des repas pendant 3 semaines, a confirmé l'efficacité du régime pauvre en FODMAPs en comparaison à un régime australien typique dans l'amélioration globale des symptômes gastro-intestinaux chez 30 patients souffrant d'un SII (55). Les essais contrôlés randomisés suivants n'ont pas été uniformément positifs, notamment dans des conditions de "vie réelle" avec éducation du patient sans livraison des repas (41, 56). Depuis, l'efficacité du régime pauvre en FODMAPs a été étudiée dans de nombreux essais notamment sur les symptômes digestifs (consistance des selles, besoin impérieux), sur l'anxiété et sur la qualité de vie (57-61). Le niveau de preuve de niveau B autorise aujourd'hui les cliniciens à proposer ce régime en première intention (62).

Ce régime induit également des modifications de la flore colique avec une réduction de la proportion et de la concentration en *bifidobactéries* (61, 63), un pH colique plus élevé, une plus grande diversité bactérienne mais une diminution de la charge bactérienne totale (64). En plus de ces effets sur la flore, le régime pauvre en FODMAPs induit des modifications du métabolome (58), avec diminution de la production d'acides gras à chaînes courtes (63), de la perméabilité et de l'immunité intestinale (63). La réponse au régime pauvre en FODMAPs pourrait également être prédite par le profil du microbiote fécal des patients (65). Dans cette étude, les patients répondeurs au régime pauvre en FODMAPs avaient une moindre diversité bactérienne et un plus faible index de dysbiose avant le début du régime (65). Le mécanisme exact par lequel le régime pauvre en FODMAPs améliore les symptômes reste encore à clarifier.

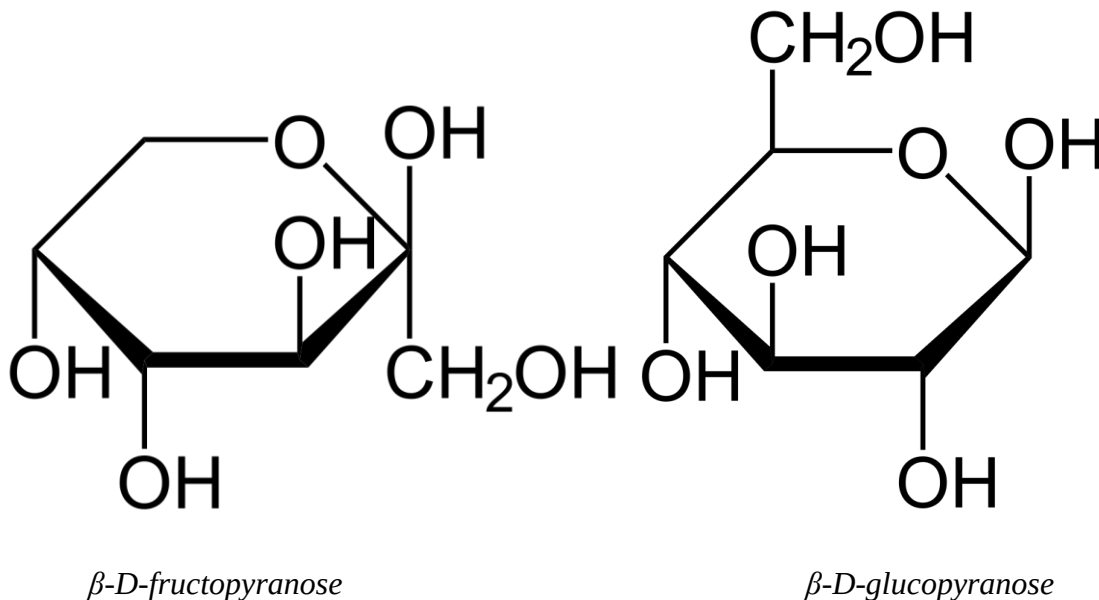
Les limites de ce régime sont tout d'abord les difficultés rencontrées par les patients pour y adhérer de façon stricte, car il s'agit d'un régime très restrictif et contraignant. D'autre part, il n'est pas recommandé de poursuivre ce régime au long cours car les effets notamment sur le microbiote ne sont pas encore connus et le patient s'expose à un risque de carences nutritionnelles (62). Une réintroduction progressive et individuelle de chaque FODMAPs est donc conseillée. Modifier le microbiote, la fermentation et la production d'acides gras à chaînes courtes, dont le butyrate, pourrait avoir un impact direct sur la muqueuse colique avec un renfort de la barrière intestinale et une inhibition de la carcinogénèse colique mais également sur l'environnement luminal, avec une inhibition de l'inflammation et une diminution du stress oxydatif (66). Dans une étude norvégienne en cross-over, après un régime pauvre en FODMAPs, la réintroduction de fructo-oligosaccharides entraîne une annulation de l'ensemble de ces effets (63). Cette étude souligne également la variabilité inter-individuelle de la réponse au régime (63). Il serait donc intéressant, à terme, de connaître des facteurs prédictifs de réponse au régime pauvre en FODMAPs.

Au sein des FODMAPs, le fructose est particulièrement intéressant. Il est présent de plus en plus largement dans l'alimentation des Européens de l'Ouest, la majoration des apports pouvant être à elle seule responsable de l'apparition de symptômes digestifs (67). D'autre part, comme pour le lactose, la malabsorption du fructose peut-être mesurée à l'aide de tests respiratoires. Contrairement au lactose (68, 69), il semblerait exister un lien entre la malabsorption du fructose et l'hypersensibilité viscérale (46).

3) Le Fructose

Le fructose est un hexose qui présente la même formule brute que le glucose $C_6H_{12}O_6$. Sa forme tautomère prédominante est le β -D-fructopyranose (*figure 4*).

Figure 4 : β -D-fructopyranose et β -D-glucopyranose



Le fructose est un sucre alimentaire appartenant aux sucres simples. Il existe dans l'alimentation sous trois formes : en monosaccharides (fructose), en disaccharides : le saccharose (glucose-fructose), ou sous forme polymérisée dans les fructanes. Les fructanes sont de longs polymères de fructose (présents dans les produits à base de blé et d'oignons).

a) Absorption normale du fructose

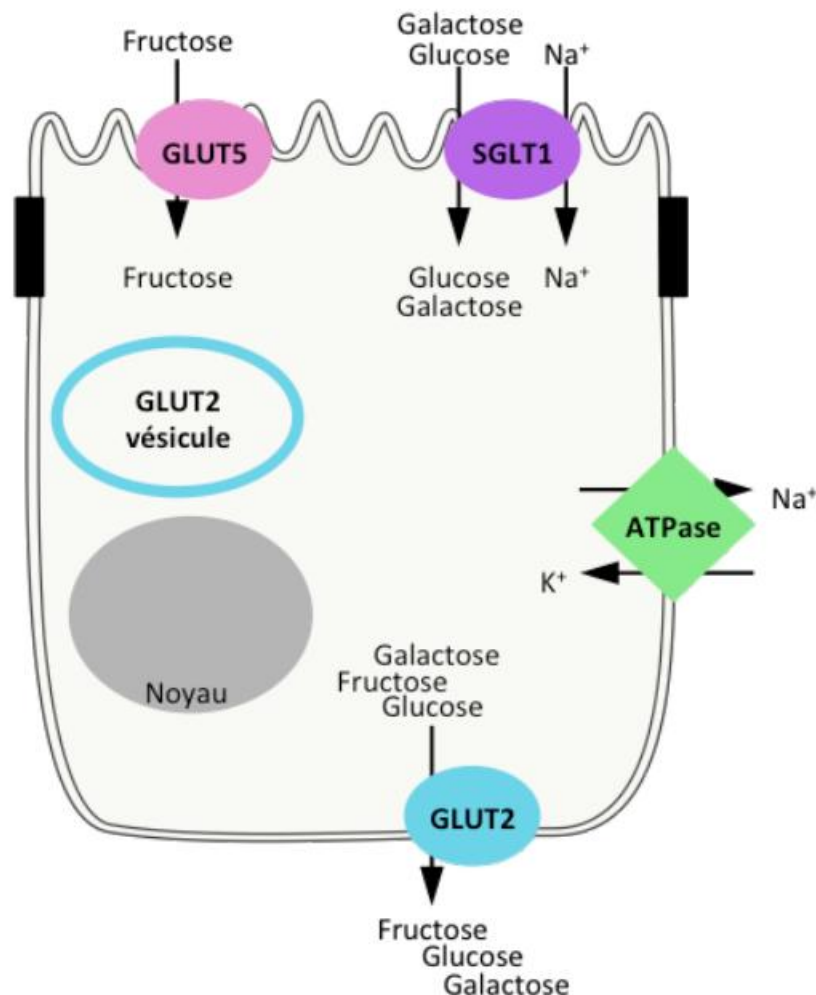
Le fructose, comme tous les glucides, ne peut être absorbé que sous forme de monosaccharide au niveau de l'entérocyte. Les glucides complexes doivent donc être hydrolysés avant de pouvoir être absorbés. C'est le rôle des amylases salivaires et pancréatiques. Les disaccharides sont alors clivés par des enzymes au niveau de la bordure en brosse des entérocytes. Le saccharose (glucose-fructose) et le lactose (glucose-galactose) sont clivés respectivement par la saccharase-isomaltase et la lactase, toutes deux présentes dans la bordure en brosse des entérocytes. Les monosaccharides issus de la digestion sont principalement absorbés dans la partie proximale de l'intestin grêle par les entérocytes (duodénum et jéjunum). Les principaux transporteurs impliqués sont le cotransporteur sodium-glucose 1 (SGLT1), et les transporteurs

du glucose 2 et 5 (GLUT2 et GLUT5). Ils permettent un transport des monosaccharides de la lumière intestinale vers le compartiment sanguin (**figure 5**). Le fructose utilise un transport facilité GLUT5 à travers la membrane apicale des entérocytes tandis que le glucose et le galactose utilisent un transport actif dépendant du sodium : SGLT1. Après son accumulation à l'intérieur de l'entérocyte, le fructose et le galactose passent la membrane basolatérale grâce à un transport facilité, GLUT2, pour rejoindre le milieu extracellulaire. Le glucose peut avoir le même sort mais il peut également être utilisé pour le métabolisme cellulaire entérocytaire.

Le seuil d'absorption du fructose est difficile à estimer chez l'Homme. La malabsorption du fructose peut survenir lorsque les apports dépassent les capacités d'absorption (en prenant en compte la forme du fructose mais également la présence de glucose associé) (70).

La présence de sucre ou de polyols peut avoir un impact direct sur l'absorption du fructose. Le fructose est mieux absorbé en présence de glucose avec un ratio 1/1 ou supérieur (71, 72). A contrario l'erythritol, un polyol, a un effet inverse et diminue l'absorption du fructose (73).

Figure 5: Schéma simplifié de l'absorption intestinale des sucres



D'après Schmitt C. Thèse de doctorat "Implication du transporteur intestinal GLUT2 dans l'absorption des sucres et la fonction entéroendocrine" (74).

b) Régulation des transporteurs du fructose et malabsorption

Ce sont les études animales qui ont permis une meilleure compréhension de ces mécanismes (**figure 7**). Le fructose traverse la bordure épithéliale par un système de transport facilité. Les deux principaux transporteurs du fructose au niveau de l'épithélium intestinal sont GLUT2 et GLUT5 (70). Le principal, GLUT5 permet un transport facilité au niveau apical (75). GLUT5

est localisé au niveau du jéjunum. Il est spécifique du fructose, de faible capacité mais avec une affinité élevée. L'apport de fructose régule de manière positive l'expression de GLUT5 (76, 77). GLUT5 est le principal transporteur responsable de l'absorption du fructose au niveau apical. Ce mécanisme est saturable et en présence d'une forte dose de fructose, il existe un phénomène d'adaptation. D'autre part, le GLUT2 est présent de manière constitutionnelle uniquement au niveau de la membrane basale (en situation de jeûne ou en l'absence de sucres ou d'hydrates de carbone). Il peut être recruté au niveau de la bordure en brosse pour faciliter l'absorption apicale du fructose (78).

L'importance du rôle de GLUT5 a été étudiée dans un modèle de souris GLUT5 knockout (KO) (79). Dans ce modèle, l'absorption de fructose dans le jéjunum est diminuée, de même que les concentrations sériques respectivement de 75 et 90% (79). En présence d'un régime riche en fructose, il en résulte une importante distension intestinale de gaz et de liquides malgré la présence de GLUT2 à la membrane apicale mais qui ne permet pas de compenser l'absence de GLUT5 (79).

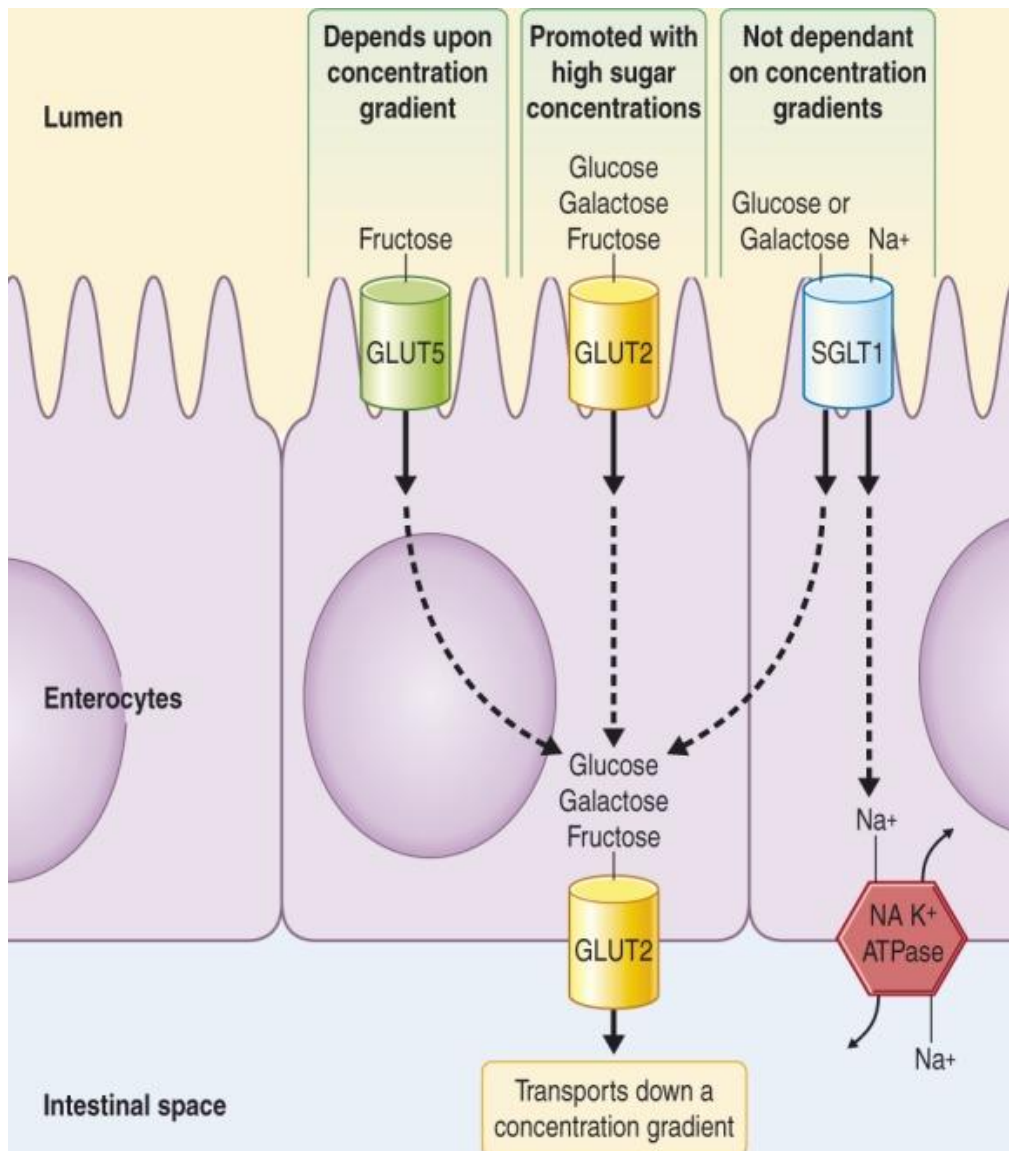
Chez les souris GLUT2-KO, l'absorption du fructose au niveau de la bordure en brosse est diminuée par 2 en comparaison des souris Wild type (WT), le GLUT5 n'étant pas capable de compenser l'absence de GLUT2 (78). En présence d'un régime appauvri en fructose, la totalité du fructose est absorbée par le transporteur GLUT5. L'absorption de fructose par GLUT5 chute à 40% en cas d'un régime riche en fructose, les 60% restants étant assurés par GLUT2 (78). GLUT2 est un intervenant essentiel de l'absorption du fructose dans certaines conditions. La présence de sucres (glucose, fructose et sucrose) entraîne un recrutement rapide et efficace de GLUT2 à la membrane apicale (78). En revanche, le mécanisme par lequel GLUT2 est inséré à la membrane apicale est encore incomplètement compris. Chez l'Homme, la présence de GLUT2 au niveau de la bordure en brosse est difficile à mettre en évidence étant donné que les patients doivent être à jeun avant la réalisation de biopsies (80).

Au niveau de la membrane basolatérale des cellules épithéliales intestinales, le fructose emprunte un transport dépendant de GLUT2 qui est un transport facilité (81). GLUT2 est un transporteur à grande capacité mais de faible affinité. L'expression de GLUT2 est régulée positivement par le glucose et le fructose (82). Il existe également un transport indépendant de

GLUT2 au niveau basolatéral de l'entérocyte, car il persiste une sortie de fructose vers la circulation portale (chez les souris GLUT2 KO) (83).

Ces transporteurs sont tous des transporteurs saturables. Il semble que d'autres transporteurs soient également impliqués dans l'absorption du fructose, notamment le GLUT8 (84). Sur des modèles de cultures cellulaires humaines et chez la souris, les voies de transport impliquant GLUT 8 et GLUT12 permettent une adaptation de l'absorption à de larges apports en fructose (84).

Figure 7: Les principaux transporteurs impliqués dans l'absorption du fructose et leurs mécanismes (85).



Le fructose est lentement absorbé au niveau de l'épithélium intestinal par un mécanisme de diffusion facilitée. GLUT5 est un transporteur à haute affinité, il est indépendant du glucose, de faible capacité et dépend d'un gradient de concentration. GLUT2 a un rôle facultatif de grande capacité, glucose dépendant. Il permet également l'absorption de glucose et galactose. GLUT2 est principalement présent au niveau de la membrane basolatérale. Lorsque le

transporteur SGLT1 transporte du Na^+ et du glucose, il entraîne l'activation et l'insertion de GLUT2 à la membrane apicale.

Chez l'Homme, des mécanismes similaires sont retrouvés au travers de certaines pathologies. Il existe plusieurs syndromes de malabsorption qui fournissent d'importantes informations concernant les rôles de GLUT2 et GLUT5. Ainsi dans le syndrome de Fanconi-Bickel, la présence d'une mutation du gène de GLUT2 résultant en une protéine tronquée inefficace, ne modifie pas la tolérance au fructose chez les patients (86). La perte de fonction de GLUT2 ne porte donc pas atteinte à celle de GLUT5. En revanche, la malabsorption du fructose, tout comme chez les adultes, est fréquemment rencontrée chez les enfants auxquels on donne des jus purifiés riches en fructose, sans qu'ils souffrent d'un déficit en GLUT5. Cette prise de fructose en excès du glucose peut-être responsable de diarrhée par malabsorption (87).

Le mécanisme de la malabsorption du fructose dans le SII n'est pas encore connu. Il semble plus s'agir d'un problème de transporteur étant donné que le fructose est absorbé sous la forme de monosaccharide. Il n'existe pas de mutation de GLUT5 connue à ce jour chez l'Homme (88); le mécanisme sous-jacent de la malabsorption du fructose semble être plutôt en lien avec un problème de régulation ou de localisation de ces transporteurs. En effet, l'équipe de Wilder-Smith a démontré par PCR et western blot que les patients présentant une malabsorption de fructose n'avaient pas de différence d'expression ou de production des transporteurs du fructose dans le duodénum (89).

c) Métabolisme du fructose

Suite à son absorption intestinale, le fructose est libéré dans la circulation porte. Soit il est destiné au foie où il est alors métabolisé en glucose soit il rejoint la circulation générale. Le fructose va rentrer dans les cellules par son transporteur GLUT5 qui est indépendant de l'insuline (contrairement au transporteur du glucose SGLT1); il ne peut pénétrer dans le cerveau ni dans le pancréas qui ne possèdent pas de GLUT5. Le fructose n'induit donc pas de sécrétion d'insuline par les cellules β -pancréatiques (90), une fois dans la cellule, il est phosphorylé en fructose-1-phosphate où il pourra participer à la synthèse lipidique (91).

Ajouté en faible concentration au glucose il va augmenter la synthèse hépatique de glycogène alors qu'en présence de forte concentration, il fournit une source importante d'hydrates de carbone qui seront préférentiellement métabolisés en lipides par le foie (91).

d) Le fructose dans l'alimentation : épidémiologie nutritionnelle

Les principales sources de fructose sont les fruits (pommes et poires principalement), le miel et les produits industriels sucrés avec du sirop de maïs (92). Le fructose est présent sous forme de fructanes dans de nombreux aliments comme le blé, le seigle, l'orge, les oignons... (*table 4*).

Table 4 : Liste des aliments contenant ou non du fructose par catégorie.

ALIMENTS	ALIMENTS CONTENANT DU FRUCTOSE	ALIMENTS A FAIBLE TENEUR EN FRUCTOSE
LAIT ET PRODUITS LAITIERS	Lait sucré Laitages et entremets sucrés Laitages aux fruits, aromatisés Boissons lactées Crèmes desserts Flan, mousse	Lait (poudre ou liquide) Yaourt Fromage blanc nature et sans sucre Petits suisses Entremets sans sucre Fromages
VIANDES POISSONS OEUFS	Charcuterie (Jambon, saucisses, saucissons, pâtés, boudins blancs ou noirs), quenelles, mousse de poisson, plats préparés	Toutes les viandes, volailles Abats Poissons Oeufs
FECULENTS	Produits cuisinés du commerce Pommes de terre cuisinées du commerce Frites surgelées Farines sucrées Légumes secs Céréales entières (avoine, blé, seigle, orge, millet...) Pâtes complètes, pâtes aux légumes Riz complet	Pommes de terre, chips Riz, tapioca, pâte ordinaire, semoule Farine de blé, de sarrasin, d'avoine, de maïs, de riz
PAIN	Pain sucré, autres pains Biscottes, pain grillé Céréales sucrées	Galette de riz
LEGUMES VERTS	Tous les légumes crus Légumes secs Légumes cuisinés du commerce Soja	Sauf légumes cuits: Epinard, laitue, haricots verts, haricots beurre, carotte, céleri, courgette, tomate, cresson, choux fleur, avocat
FRUITS	Tous les fruits frais, crus ou cuits Fruits au sirop Compotes sucrées ou sans sucre	Sauf: Abricot, pamplemousse, citron, nectarine, orange, mandarines, pêche, ananas, rhubarbe



	Fruits secs Fruits confits	
MATIERES GRASSES	Mayonnaise industrielle Sauces industrielles	Beurre Huiles Crème fraîche Mayonnaise maison
SUCRERIES	Sucre ordinaire (de betterave, de canne, d'érable) Miel, confiture, gelée Toutes les pâtisseries du commerce Pâte feuilletée, pâte sablée Toutes les viennoiseries (brioche, croissant, pain au chocolat, pain aux raisins.....) Biscuits sucrés, meringues, pain d'épice Chantilly Glaces et sorbets Confiseries, dragées, nougat, pâtes de fruits Crème de marron Chocolat et barres chocolatées Chocolat en poudre sucré	Cacao non sucré (Van Houten)
BOISSONS	Boissons fermentées (cidre, bière) Vins, apéritifs, digestifs Jus de fruits Sirop de fruit Soda, limonade Eaux aromatisées Boissons lactées sucrées	Eaux plates ou gazeuses Eaux aromatisées édulcorées (voir édulcorants autorisés) Café, tisanes, thé
CONDIMENTS	Sauces industrielles Mayonnaise du commerce Ketchup Moutarde Sauce tomate cuisinée du commerce Vinaigres aromatisés Ail, oignon, échalote	Sel, poivre en grains Gélatine alimentaire Vinaigre non aromatisé



Normandie Université

	Cornichons et câpres Bouillon cube	
DIVERS	Certains édulcorants: -Sucralose	Certains sucres: glucose, lactose, maltose et dextrine maltose Certains édulcorants: Aspartam, saccharine, cyclamate, thaumatine Polyols (mannitol, sorbitol)

Le fructose est de plus en plus présent dans les régimes de l'Ouest de l'Europe et en Amérique du Nord, soit sous forme de sucres ajoutés soit sous forme de jus de fruit (93, 94). Il possède en effet un index glycémique inférieur à celui du glucose. Et son pouvoir sucrant est compris entre 1,1 et 1,7. Aux Etats-Unis les apports moyens en fructose dépassent les 50g/j, représentant environ 10% de l'apport calorique total (93, 94). Le sirop à base de maïs, développé sur les dernières décennies représente plus de la moitié de la consommation calorique sucrée des américains (95). Le sirop de maïs, riche en fructose, fabriqué par isomérisation enzymatique, a été introduit en 1967 ouvrant de nouvelles possibilités à l'industrie des boissons sucrées. Le développement bon marché de ce sirop a permis le remplacement progressif du saccharose et des sucres simples dans notre alimentation. Il représente jusqu'à 40% de la totalité des calories sucrées rajoutées. Il est ainsi devenu le produit de substitution favori du saccharose dans l'alimentation (96). Dans le monde entier, les Etats-Unis sont les principaux utilisateurs de sirop de maïs riche en fructose (95).

e) Implication du fructose dans les pathologies et effet chez l'Homme

Le rôle délétère du fructose alimentaire est envisagé dans l'obésité (97), dans le syndrome d'insulino-résistance (90), dans le SII (98) au cours duquel pourrait exister une malabsorption du fructose, et aussi dans la pullulation bactérienne endoluminale (45) et la dépression (99).

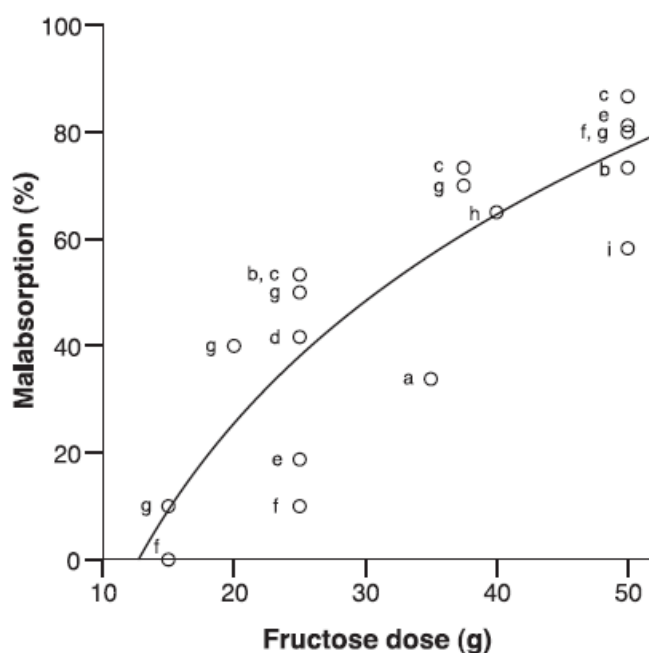
Le mécanisme par lequel survient la malabsorption du fructose n'est pas encore élucidé. Cette malabsorption est majorée par le stress avec un rôle certain du Cortisol Releasing Factor (CRF), prouvé chez le volontaire sain (100). La malabsorption du fructose est responsable d'un cortège de symptômes dans les TFI, incluant les douleurs abdominales et la diarrhée chronique (101). Le fructose non absorbé est fermenté par la flore colique au niveau de l'intestin grêle distal ou dans la lumière colique, entraînant une production de gaz, elle-même responsable d'une distension luminale. Le fructose, de par sa petite taille, a également un effet osmotique créant un appel d'eau et une rétention dans la lumière intestinale (101). Chez les patients SII ayant au premier plan une hypersensibilité viscérale, l'un ou l'autre de ces mécanismes peut-être responsable de ballonnements et de douleurs abdominales (102).

L'apport actuel en fructose est capable d'entraîner des troubles GI dans une population saine (67) de même que dans une population de patients SII (45, 103). La prise de fructose induit chez les volontaires sains (104) comme chez les patients SII, une augmentation du contenu luminal grêlique en eau. Il en peut en résulter une accélération du transit et du péristaltisme (46). L'intensité des symptômes est plus élevée dans le SII malgré des paramètres d'imagerie par résonance magnétique (IRM) fonctionnelle, identique aux volontaires sains. Ces résultats soulèvent la possibilité du rôle de l'hypersensibilité colique dans la genèse des symptômes (46). Le lien entre le fructose et hypersensibilité viscérale ainsi que les mécanismes qui sous-tendent à cette expression n'ont cependant pas encore été identifiés. La flore colique pourrait également contribuer aux symptômes liés à la prise de fructose mais les mécanismes sous-jacent sont incomplètement élucidés (105).

f) La malabsorption du fructose : prévalence chez l'Homme et méthodes diagnostiques

L'absorption du fructose chez l'Homme semble limitée en présence de fortes concentrations, résultant du système de transport facilité qui a une capacité limitée (106). Le fructose qui n'est pas absorbé dans l'intestin grêle, atteint le colon où il est métabolisé par la flore colique, produisant de l'hydrogène. La détection d'hydrogène dans l'air expiré témoigne d'une malabsorption du fructose. Il existe une relation directe entre la dose de fructose et la malabsorption démontrant l'existence d'une capacité limitée d'absorption, dose dépendante même chez les volontaires sains (70) (*figure 6*).

Figure 6 :Relation entre la dose de fructose et la prévalence de la malabsorption du fructose (70)



Relation entre la dose de fructose (g) et le pourcentage de tests respiratoires positifs en faveur d'une malabsorption du fructose chez le volontaire sain. ($r=0.86$, $p<0.001$)(70)

La malabsorption du fructose peut être diagnostiquée par un test respiratoire mais sa précision demeure incertaine (107) et sa valeur diagnostique a longtemps été controversée (108). Il existe une hétérogénéité sur les aspects méthodologiques clés des tests respiratoires : la dose de fructose absorbée et la valeur seuil retenue de production d'hydrogène pour définir la malabsorption (67, 109, 110). Les études chez les volontaires sains soulignent les difficultés de reproductibilité et d'interprétation. Concernant la quantité de fructose absorbable par un sujet sain, un travail de Rao a prouvé que 100, 90 et 20% des sujets sains absorbaient respectivement 15, 25 et 50 grammes (g) de fructose (solution à 10%) (110). Cet article conclue qu'un sujet sain peut absorber 25g de fructose dans la très grande majorité des cas. Dans le cadre d'un test diagnostique, l'utilisation d'une dose supérieure (50g) expose à la survenue de faux positifs dans 40% des cas (110). Certaines études suggèrent la nécessité

d'éliminer une pullulation bactérienne endoluminale pour éliminer de faux positifs (45, 111). Cette pullulation bactérienne endoluminale peut être éliminée par un test respiratoire au lactulose ou au glucose (45, 111). Une estimation fiable de la prévalence de la malabsorption du fructose dans le SII reste donc à réaliser. En effet, les seules données que nous avons à disposition sont pour une dose de charge à 35g, sans test respiratoire au glucose préalable (49).

g) Intérêt d'un régime pauvre en fructose

Il est également bien admis que l'effet symptomatique du fructose est dose dépendant (48). Il existe donc un rationnel pour proposer un régime appauvri en fructose.

L'effet d'un régime pauvre en fructose n'ayant pas été évalué dans des études contrôlées randomisées, un effet placebo ne peut être éliminé (112, 113). Un régime appauvri en fructose et fructanes semble pouvoir maintenir une amélioration symptomatique durable chez les patients souffrant d'un SII avec malabsorption du fructose (48). La réintroduction du fructose ou des fructanes, suite à un régime appauvri, entraîne une récurrence des symptômes dans plus de 70% des cas (48). Pour être efficace, un régime pauvre en fructose doit éliminer le fructose mais également les fructanes (50, 114). Les fructo-oligosaccharides et l'inuline semblent mieux tolérés au niveau symptomatique (50). Néanmoins, ce régime n'a jamais été comparé à un régime appauvri en FODMAPs. L'intérêt d'un régime pauvre en fructose pour améliorer les symptômes des patients souffrant d'un SII reste donc encore à déterminer.

4) Les questions à éclaircir, justifiant mon travail de thèse

a) Quelle est la prévalence de la malabsorption du fructose dans le SII?

La prévalence de la malabsorption du fructose chez les patients SII de l'Europe de l'Ouest est encore mal documentée. La méthode diagnostique exacte avec notamment la dose de charge en fructose reste controversée. Une estimation fiable de la prévalence de la malabsorption du fructose dans le SII reste encore à déterminer.

b) Existe-t-il un lien entre hypersensibilité viscérale et malabsorption du fructose dans le SII?

Le rôle de l'hypersensibilité viscérale est de plus en plus suspecté dans la genèse des symptômes liés aux carbohydrates chez les patients souffrants d'un SII (46). La seule production excessive de gaz est de plus en plus remise en cause. Dans l'étude de Zhu et al. (69), la présence d'une hypersensibilité viscérale au barostat rectal était associée aux symptômes induits par la prise de lactose. L'intolérance au lactose, et non sa malabsorption était associée à une hypersensibilité viscérale et à la survenue des symptômes (68, 69). Certaines équipes comme celle de Gibson sont convaincues du rôle de l'hypersensibilité dans le lien avec les symptômes induits par la prise de FODMAPs (115). Le lien entre malabsorption, intolérance du fructose et hypersensibilité n'a pas encore été démontré. Les mécanismes physiopathologiques restent encore à appréhender.

c) Y a-t-il un intérêt à tester la malabsorption du fructose pour sélectionner les patients candidats à un régime pauvre en fructose dans une population de SII

Le débat sur l'utilité du test respiratoire, notamment pour prédire la réponse à un régime appauvri en fructose, est toujours d'actualité (115, 116). Il existe un rationnel pour proposer un régime éliminant les aliments contenant du fructose en excès, du glucose ou du sorbitol dans le SII pour réduire ou faire disparaître les symptômes digestifs (117). Actuellement, le mécanisme par lequel ce régime est efficace n'est pas connu, de même que le "patient type"

répondant efficacement au régime. Le régime pauvre en fructose étant un régime inconstamment efficace et très contraignant, avec un impact nutritionnel potentiel, il paraît important de cibler les patients pouvant en bénéficier.

Dans une étude en ouvert, un test respiratoire démontrant une malabsorption du fructose avec une dose de charge de 50g de fructose ne semblait pas en lien avec l'efficacité du régime sans fructose (118). Cependant cette étude présentait des faiblesses méthodologiques dont la première était que le régime était évalué en ouvert; de plus, la dose de charge la plus adaptée pour prouver de manière spécifique une malabsorption du fructose semble plutôt à 25g (119). Il y a donc un intérêt à réaliser une étude en aveugle avec un test adapté (dose de charge de 25g).

II) Objectifs

Les objectifs de mon travail de thèse étaient chez les patients SII :

- 1/ d'évaluer la prévalence de la malabsorption du fructose
- 2/ d'étudier l'association entre la malabsorption du fructose et l'hypersensibilité viscérale
- 3/ de déterminer si le test de dépistage de la malabsorption et de l'intolérance au fructose permet de prédire l'efficacité du régime sans fructose sur les symptômes digestifs

Et sur des modèles murins :

- 1/ de reproduire un modèle murin fiable de malabsorption du fructose (GLUT5 KO, GLUT2 KO et souris WT avec un régime high fructose).
- 2/ d'évaluer les répercussions fonctionnelles digestives (perméabilité intestinale, sensibilité viscérale) d'une malabsorption de fructose
- 3/ de trouver les mécanismes physiopathologiques en cause pour mieux appréhender le SII

III) Articles

Article 1 : Symptomatic fructose malabsorption in irritable bowel syndrome : A prospective study.

Le premier objectif de ma thèse de science était de définir dans une population de patients souffrant d'un SII la prévalence de la malabsorption du fructose. En effet la prévalence de la malabsorption du fructose était mal documentée chez les patients SII de l'Europe de l'Ouest principalement en raison de l'hétérogénéité des méthodes diagnostiques. La dose de charge en fructose peut varier de 25 à 50g avec des concentrations différentes.

Notre test de malabsorption du fructose a été défini par une dose de charge de 25g. Il était systématiquement réalisé après avoir éliminé une pullulation bactérienne endoluminale par un test respiratoire au glucose. Les caractéristiques du test respiratoire au fructose utilisé sont celles qui sont actuellement reconnues notamment dans la conférence de consensus américaine de 2017 (120).

Le résultat principal de notre étude était que 22% des patients SII présentaient une malabsorption du fructose. Les patients jeunes et de sexe masculin étaient plus à risque de malabsorption du fructose.

Résumé article 1 : Prévalence de la malabsorption du fructose dans le syndrome de l'intestin irritable : résultats d'une étude prospective

Introduction : Le fructose peut déclencher ou aggraver les symptômes digestifs chez les patients souffrant d'un SII. Le but de notre étude était de définir la prévalence de la malabsorption du fructose dans le syndrome de l'intestin irritable et d'identifier les caractéristiques permettant de distinguer les patients souffrant d'une malabsorption du fructose.

Matériels et Méthodes : Quatre-vingt-dix patients souffrant d'un SII selon les critères de Rome III (SII diarrhéique : 31%, SII constipé : 18% et SII avec alternance : 51%) ont été prospectivement inclus. Après exclusion d'une pullulation bactérienne endoluminale par test respiratoire au glucose, la malabsorption du fructose était recherchée à l'aide d'un test respiratoire au fructose avec une dose de charge de 25g, pendant 5 heures avec recueil des symptômes. Le diagnostic de malabsorption était posé lors d'une élévation de plus de 20 parties par million (ppm) de l'hydrogène (H_2) ou du méthane (CH_4) dans l'air expiré.

Résultats : Le test respiratoire au fructose était positif chez 20 des 90 patients inclus, parmi lesquels 35 étaient intolérants, avec une survenue des symptômes digestifs (inconfort abdominal ou diarrhée) simultanément du pic d' H_2 ou de CH_4 . Aucune caractéristique du SII n'était prédictive de la positivité du test, mais un âge jeune ($p=0.031$) et le sexe masculin ($p=0.029$) étaient à plus haut risque de malabsorption. Par ailleurs, 18 patients présentaient une intolérance sans malabsorption du fructose.

Conclusion : Après une dose de charge de 25 g de fructose, 22% des patients SII présentaient une malabsorption du fructose et 28% une intolérance au fructose.

Symptomatic fructose malabsorption in irritable bowel syndrome: A prospective study

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Abstract

Introduction: Fructose can trigger or worsen symptoms in irritable bowel syndrome (IBS) patients. The aim of this study was to determine the prevalence of symptomatic fructose malabsorption in IBS patients and to test whether the patient's characteristics can help to detect a fructose malabsorption.

Materials and methods: Ninety Rome III IBS patients (predominant diarrhoea (IBS-D): 31%, predominant constipation (IBS-C): 18%, mixed type (IBS-M): 51%) were included prospectively. After exclusion of a small intestinal bacterial overgrowth by a glucose breath test, fructose malabsorption was assessed by a five-hour breath test, with symptom monitoring, after a 25 g load of fructose. An increase of more than 20 ppm of hydrogen (H₂) or methane (CH₄) levels in the exhaled air led to the diagnosis of malabsorption.

Results: Fructose test was abnormal in 20/90 patients among whom only 35% were intolerant, with a simultaneous rise of H₂/CH₄ levels and the onset of abdominal discomfort or diarrhoea. IBS characteristics were not predictive even if young ($p = 0.031$) and male IBS patients ($p = 0.029$) were at higher risk of malabsorption. At variance, 18 additional patients experienced intestinal symptoms during the test despite normal fructose absorption.

Discussion: After a 25 g fructose load, symptomatic fructose malabsorption and intolerance without malabsorption were detected in 22% and 28% of IBS patients respectively.

Keywords

Fructose malabsorption, irritable bowel syndrome, FODMAPs, breath tests

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Introduction

Irritable bowel syndrome (IBS) is a chronic disorder characterised by recurrent abdominal pain or discomfort and transit disturbances which are worse during exacerbations of episodes of pain exacerbations.¹ Depending on the type of transit disturbances, IBS can be categorised as predominant diarrhoea (IBS-D), predominant constipation (IBS-C), or mixed type (IBS-M). Current knowledge suggests that IBS is a multifactorial disorder, including visceral hypersensitivity of varying degrees, intestinal motor disturbances and brain-gut axis dysfunction. More recent research has also shown intestinal dysbiosis, altered intestinal permeability and low-grade intestinal inflammation.² Stress, mood disorders and life events influence the patient's capacity to cope with symptoms.³

A link between food intake and the occurrence or the exacerbation of symptom has been confirmed by prospective surveys^{4,5} while the deleterious role of

some fermentable oligo-, di- and mono-saccharides or polyols (FODMAPs), particularly fructose, was recently highlighted⁶ while fructose is increasingly incorporated in beverages, dairy products and canned, baked or processed foods worldwide. When poorly absorbed, these carbohydrates are fermented and have an osmotic effect, promoting gas production and gut distension. Malabsorption is worsened by the concomitant intake of polyols. This relationship between FODMAP intake and symptom onset has led to dietary

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recommendations, including a significant reduction in fructose intake.

However, the prevalence of symptomatic fructose malabsorption in western European IBS patients remains poorly documented. Moreover, the percentage of IBS patients who experience abdominal symptoms in cases of malabsorption and the predictive value of IBS symptom pattern to detect symptomatic fructose malabsorption remain to be assessed.

The aims of this prospective study were to determine the prevalence of a symptomatic fructose malabsorption in IBS patients according to Rome III criteria, following a fructose load, and to assess whether particular clinical characteristics of these patients could be considered predictors of symptomatic fructose malabsorption.

Materials and methods

Patients

All consecutive patients diagnosed with IBS according to Rome III criteria¹, without organic digestive disease or coeliac disease and referred to our Department for IBS management were included in the study. To meet the Rome III criteria, the patients must suffer from recurrent abdominal pain or discomfort at least 3 days per month in the last 3 months associated with 2 or more of the following: 1) improvement with defecation 2) onset associated with a change in frequency of stool 3) onset associated with a change in form (appearance) of stool.

Methods

Clinical assessment

When visiting our unit, all patients were asked to complete the Rome III questionnaire whereas IBS subtype was determined with the Bristol stool scale. Prior to the test, IBS severity was assessed by the IBS symptom severity score (IBS-SSS). In addition, a five-point Likert scale was used to assess symptom frequency. A score of 0 signified 'no symptoms', 1 corresponded to 'infrequent symptoms' (less than once a week), 2 to 'relatively frequent symptoms' (at least once a week), 3 to 'frequent symptoms' (several times a week) and 4 to 'very frequent symptoms' (daily or almost daily). Patients were also asked about their abdominal tolerance to food with a high fructose content according to the list published by Gibson and Shepherd.⁶ No diet restrictions were proposed prior to considering the patient as a true IBS patient.

Levels of anxiety and/or depression were assessed using the hospital anxiety and depression scale (HAD),⁷ and the validated Gastrointestinal Quality of Life Index (GIQLI) assessed quality of life.⁸

Breath tests

Each patient underwent two breath tests. First, a glucose breath test was carried out in order to rule out small intestinal bacterial overgrowth (SIBO), which is a condition that may promote false positive results of sugar breath tests.^{9,10} If negative, a fructose test was then performed after a 25 g fructose load (10% solution). If the glucose breath test was positive, the patient was treated, and then underwent the fructose test if the glucose test was negative.

Before both breath-tests, patients were instructed to follow a diet for 48 h. This diet strictly excluded the following food products: bread, biscuits, cheese, fruits, vegetables, dairy products, fermented drinks, sodas, honey, marmalade, candy, chocolate, ice cream and pastry. Moreover, the evening before each test, patients were given a dinner exclusively composed of rice and meat which are rapidly absorbed. The aim of this regimen was to reduce intestinal gas production that could distort the forthcoming breath tests. In order to reduce the possible metabolic effects of oral flora,¹¹ a careful mouth-wash with a 20% chlorhexidine solution was performed just prior to the test and smoking and exercise were not allowed prior to or during the test.

End-alveolar breath samples were collected using a two-bag system consisting of a mouthpiece, a T-valve and two collapsible bags. The first bag collected the dead space air and the second bag the alveolar air. A breath sample was taken from the second bag using a 20 ml syringe and immediately analysed. The concentrations of carbon dioxide (CO₂), hydrogen (H₂) and methane (CH₄) in the breath samples were determined simultaneously with a gas chromatograph (Quintron Breathtracker SC) and plotted graphically by a trained technician. The chromatograph was calibrated with a CO₂, H₂ and CH₄ reference mixture in compressed air (Quingas). Desiccants in the drying tubes were changed if their colour changed. Results were expressed as parts per million (1 ppm = approximately 0.05 µmol/l for H₂ and CH₄).

Glucose breath test. Both H₂ and CH₄ were measured before the glucose load in order to verify compliance with the instructed diet. Then patients ingested 75 g of glucose dissolved in 250 ml of sterile water. End-alveolar breath samples of expired air were collected every 15 min for 2 h. The test was considered positive if one of the following results occurred: (a) peak of H₂ or CH₄ above 20 ppm, (b) increase of H₂ or CH₄ levels above 10 ppm in two samples by comparison with individual baseline levels, (c) baseline H₂ or CH₄ levels above 20 ppm despite good compliance with the diet. When the glucose breath test was positive, patients received an antibiotic treatment with quinolone or

metronidazole for 10 days per month over two successive months. A second glucose breath test was then performed. The fructose breath test was only carried out in patients in whom the control glucose test was negative.

Fructose breath test. The fructose breath test was carried out using the same gas analyser as the glucose breath test. Patients ingested 25 g of fructose dissolved in 250 cc of sterile water then end alveolar breath samples were collected every 30 min for 5 h. Both H₂ and CH₄ levels were calculated. The test was considered positive and defined a fructose malabsorption in case of a rise of H₂ and/or CH₄ levels above 20 ppm.¹² Moreover, during the test, symptoms such as abdominal pain, bloating, and diarrhoea were closely monitored and were collected by the laboratory technician every 30 min of the breath test. Only the occurrence of gastro-intestinal symptoms was then analysed and defined fructose intolerance but not fructose malabsorption. Patients were asked whether the symptoms occurring during the test reproduced their spontaneous IBS symptoms.

Statistics

A Chi square test was used for the qualitative analyses and Mann-Witney and Wilcoxon tests were used to analyse quantitative variables with Graphpad Prism 5 software. A univariate analysis was carried out with the results of the fructose breath test using Statview software. To determine the factors associated with a positive fructose breath test, a multivariate analysis using a step-by-step logistic regression was performed, with 95% confidence intervals and a level of significance of 0.05. The positive or negative result of the fructose breath test was defined as a dependent variable.

Results

Patients

Ninety consecutive IBS patients were included. Patients' characteristics are reported in Table 1. Eighteen percent of patients had IBS-C, 31% IBS-D and 51% IBS-M. There were no differences for clinical characteristics between the three IBS sub-groups. In no case did the history of symptoms onset suggest a clear-cut association between the onset or the worsening of symptoms and the intake of nutrients with a high fructose content listed by Gibson and Shepherd.^{6,9}

All patients had a negative glucose test except one in whom the glucose test was initially positive then became negative after two months of a sequential antibiotic treatment. This patient then underwent the fructose test.

Table 1. Population characteristics

Characteristics	Population
Patients	90
Age (years)	44.3 ± 1.7
Weight (kg)	70.4 ± 1.9
Height (m)	1.7 ± 0.01
BMI	25.3 ± 0.7
Male gender	19 (21.1%)
Gastroenteritis before occurrence of symptoms	11 (12.2%)
Stress before symptoms occurred	57 (63.3%)
IBS-SSS	285.0 ± 9.9
HADS anxiety	9.2 ± 0.5
HADS depression	4.8 ± 0.4
Frequent abdominal pain	37 (41.1%)
Frequent bloating	53 (58.9%)
Frequent diarrhoea	28 (31.1%)
Frequent constipation	16 (17.8%)
GIQLI score	81.8 ± 2.4
Fructose intolerance	27 (30.0%)

BMI: body mass index; IBS: irritable bowel syndrome; IBS-SSS: IBS-symptom severity score; HADS: Hospital Anxiety Depression Score; GIQLI: Gastrointestinal Quality of Life Index.

Symptoms were considered as frequent with VAS (Visual Analogue Scale) 3–4 on Likert scale (moderate to severe symptoms). Results are presented as mean ± standard error of the mean and number (percentage).

Fructose breath test

The fructose test was positive in 20 patients (22%). In all of these patients, the test was positive due to an increase in H₂ while no CH₄ increase occurred in any patient. Among these 20 patients, H₂ levels higher than 20 ppm in the end-alveolar samples were observed after a mean delay of 80 min while the mean delay for the H₂ peak onset was 114 min. Among patients with a positive fructose breath test, 25% were IBS-C patients, 30% were IBS-D and 45% were IBS-M.

Figure 1 is an example of the result of a five-hour breath test. Twenty-five patients (27.8%) experienced symptoms during the test and were considered as fructose intolerant. In most cases, symptoms were the same as the spontaneous symptoms leading to patients seeking medical advice. Thirty-five percent of the patients with an abnormal fructose breath test had symptoms during the test with an abdominal pain in 89%, a diarrhoea in 44% while an objective abdominal distension was observed in 11%. In contrast, 18 patients (26%) with a normal fructose breath test had abdominal symptoms after fructose ingestion. These symptoms included abdominal pain in 11/18 (61%), diarrhoea in 10/18 (56%) and bloating in 3/18 (17%). In these 18

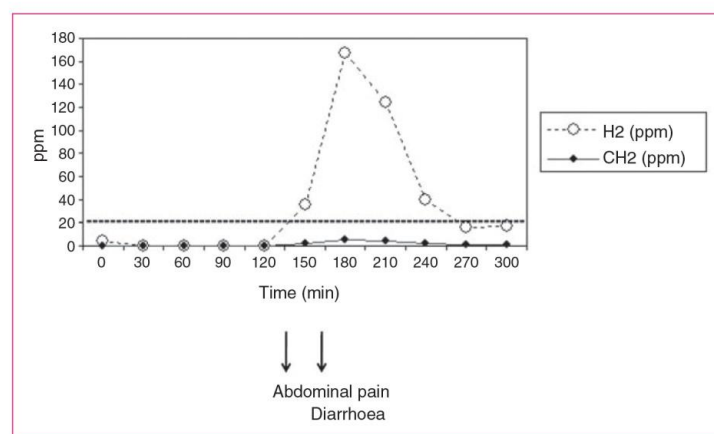


Figure 1. Example of the result of a five-hour breath test. H2: hydrogen; CH4: methane.

cases of poor tolerance to fructose without malabsorption, symptoms occurred after a mean delay of 62 min after fructose intake, but their early onset within the first 30 min of the test was reported by 44% of the patients.

There were no significant differences for either prevalence or pattern of symptoms between patients with positive or negative fructose breath tests. In particular, there was no difference on the time of symptom occurrence during the test.

Comparison of patient characteristics as a function of fructose breath test result according to univariate and multivariate analyses

Characteristics of patients with positive and negative fructose breath tests are shown in Table 2. The univariate analysis showed that patients with symptomatic fructose malabsorption were younger ($p=0.031$), and more often males ($p=0.029$) compared to those without fructose malabsorption according to the breath test. There was no other characteristic significantly different between the two groups.

All characteristics with a p -value <0.03 were included in the multivariate analysis (Table 3). Gender, age, symptoms during the test and the GIQLI score were all included. Height was not included in the logistic regression model because it co-varied with gender ($p < 0.0001$). The results of the multivariate analysis showed that only gender ($p=0.04$) and age ($p=0.009$) were associated with positive fructose breath test.

Correlation between the results of the fructose test and the anxiety and depression levels

Fructose intolerance was neither more frequent in IBS patients with abnormal anxiety or depression levels ($p=0.29$ and $p=0.7532$) (Tables 4 and 5).

Discussion

The dietary recommendation to avoid or at least to reduce the amount of fructose within the diet appears logical for IBS patients with a demonstrated symptomatic fructose malabsorption associated with the onset of intestinal symptoms after fructose intake.

The first aim of this prospective study was to assess the prevalence of a symptomatic fructose malabsorption in all types of IBS patients fulfilling Rome III criteria. Therefore, the experimental protocol was carefully designed to avoid any methodological bias which could interfere with the interpretation of the test. First, we carefully excluded IBS patients with SIBO, a pathological condition during which the colonic bacteria population migrates proximally into the small intestine, gaining access to sugars. This shift in fermentation might lead to a abnormal breath test.¹³ The second major methodological point was the limitation of the fructose intake to 25 g. Indeed, several recent studies have highlighted that 25 g was the highest amount of fructose absorbed by healthy volunteers.^{14,15} According to these studies, a fructose load of 50 g induces false positive results in 40–60% of cases.^{14,15} The collection of breath samples was carried out according to previous methodological recommendations in order to ensure end-alveolar breath samples.¹⁶ We measured both H₂ and CH₄ levels as it has been reported that false negative results occur in 14% of cases when CH₄ is not measured.^{17,18}

Nevertheless, one limitation of this study was the absence of demonstration of production of H₂ or CH₄ with lactulose prior to patient inclusion in the study. However, lactulose does not seem to be a good test to define a non-hydrogen producer.¹⁹ We used a

Table 2. Univariate analysis of conditions and symptoms associated with the detection of a fructose malabsorption by a fructose breath test after a 25 g fructose load

Characteristics	Fructose absorbers (n = 70)	Fructose malabsorbers (n = 20)	p
Age (years)	46.3 ± 2.0	37.3 ± 2.9	0.03
Weight (kg)	70.0 ± 2.3	71.6 ± 2.9	0.72
Height (m)	1.7 ± 0.01	1.7 ± 0.02	0.02
BMI	25.5 ± 0.8	24.8 ± 1.1	0.66
Male gender	11 (15.7%)	8 (40.0%)	0.03
Acute gastroenteritis prior symptoms	8 (11.4%)	3 (15.0%)	0.70
Stress before symptoms	46 (65.7%)	11 (55.0%)	0.70
IBS-SSS	281.0 ± 11.8	304.0 ± 16.2	0.36
HADS anxiety	9.2 ± 0.5	9.3 ± 1.0	0.90
HADS depression	4.6 ± 0.4	5.3 ± 0.8	0.40
Frequent abdominal pain	27 (38.6%)	10 (50.0%)	0.44
Frequent bloating	41 (58.6%)	12 (60.0%)	1.00
Frequent diarrhoea	22 (31.4%)	6 (30.0%)	1.00
Frequent constipation	11 (15.7%)	5 (25.0%)	0.34
GIQLI Score	83.2 ± 2.5	77.1 ± 5.9	0.29
Fructose intolerance	18 (25.7%)	7 (35.0%)	0.11

BMI: body mass index; IBS: irritable bowel syndrome; IBS-SSS: IBS-symptom severity score; HADS: Hospital Anxiety Depression Score; GIQLI: Gastrointestinal Quality of Life Index.

Symptoms were considered frequent when patients scored their frequency 3 or 4 on a Likert scale. Results are presented as mean ± standard error of the mean and number (percentage).

Table 3. Multivariate analysis of conditions and symptoms associated with a positive fructose breath test after a 25 g fructose load

Variable	Odds ratio (95% CI)	p value
Age	−2.59 (0.90–0.97)	0.009
Gender	2.01 (1.03–11.96)	0.04
Symptoms during the test	1.07 (0.59–5.87)	0.28
GIQLI	−1.76 (0.95–1.00)	0.08

CI: confidence interval; GIQLI: Gastrointestinal Quality of Life Index.

Table 5. Relationship between depression and fructose intolerance

Fructose intolerance	Normal HADS (depression score)	Abnormal HADS (depression score)
Yes	23	2
No	61	4

HADS: Hospital Anxiety Depression Score. Depression was pathological when the overall score of the seven questions testing depression was equal or higher than 11/21.

Table 4. Relationship between anxiety and fructose intolerance

Fructose intolerance	Normal HADS (anxiety score)	Abnormal HADS (anxiety score)
Yes	18	7
No	39	26

HADS: Hospital Anxiety Depression Score. Anxiety was pathological when the overall score of the seven questions testing anxiety was equal or higher than 11/21.

five-hour test duration that allowed for increasing the prevalence of fructose malabsorption by 10%. Wilder-Smith et al.²⁰ have underlined the importance of a five-hour test duration. In their data, they would have

missed 16% of fructose intolerance with only a three-hour duration test.

Another potential limitation of this study is the use of a glucose breath test for SIBO detection. The gold standard for the diagnosis of SIBO remains the culture of small bowel aspirates,¹³ even if such aspirates cannot detect a bacterial overgrowth in the distal jejunum or in the proximal ileum. The main reported disadvantage of the sugar breath test, particularly when lactulose is used, is an overestimation of the prevalence of SIBO. However, in the present study, we found only one abnormal glucose breath test in this sample of 90 patients.¹³ This low prevalence of small intestinal

bacterial overgrowth is in accordance with the results of Posserud et al., reporting a 4% prevalence with the results of small bowel aspirate cultures.¹³ The ingestion of fructose alone during the test could be criticised. However, one could argue that simultaneous consumption of glucose and fructose during the test resembles normal food intake and thus more likely reflects conditions in daily life. In addition, fructose malabsorption appears to be partially modulated by the amount of glucose concomitantly ingested and the fructose/glucose ratio of a meal. Indeed glucose reduces fructose malabsorption via the upregulation of the facilitative transporter Glucose Transporter 2 (GLUT2).²¹

Our first result is that a fructose malabsorption was observed in 22% of this sample of IBS patients, without any differences according to the IBS sub-type. This result is significantly lower than the figures of the major Wilder-Smith et al.'s study²⁰ or that of the Barrett et al. trial²² in which the patients were tested with 35 g of fructose. In fact Barret et al. did not report a different prevalence of fructose malabsorption between IBS patients (45%) and healthy volunteers (34%)²² after 35 g of fructose intake. Moreover, in the Wilder-Smith et al. study, fructose malabsorption was defined as an increase of >20 ppm for H₂ and >10 ppm for CH₄, over baseline, in succession. Usually an increase of >20 ppm is used for CH₄ levels.¹² This methodological difference could also explain the higher fructose malabsorption prevalence in Wilder-Smith et al.'s study.²⁰

Our second aim was to assess the percentage of IBS patients with symptomatic fructose malabsorption. One important result of our study was to demonstrate the lack of correlation between the onset of symptoms during the test and impaired fructose absorption: less than half of the malabsorbers were intolerant to fructose. This point is very relevant for clinical practice, reinforcing the need for a diagnostic test including a concomitant symptoms analysis prior to discussing an exclusion diet. In these patients with both intolerance and malabsorption, symptoms always occurred when H₂ levels rose above 20 ppm or at the peak of H₂ rise. Therefore, we can speculate that in these patients, the symptom onset is related to the malabsorption.

Furthermore, 18 patients, without malabsorption, always experienced symptoms during the first hour of the test. The occurrence of symptoms during a sugar test is not rare in patients with functional gastrointestinal disorders, even in the absence of sugar malabsorption. For instance, several studies based on the lactose breath test have demonstrated the lack of a relationship between no lactase and the onset of abdominal symptoms.^{23,24} In our patients who were intolerant to fructose, one possible explanation could be that the symptoms were triggered by other gases (such as CO₂)

which could not be detected with the device we used. Symptoms could be also promoted by visceral hypersensitivity and/or be related to the osmotic effect of fructose rather than the fermentation process leading to H₂ or CH₄ production. Tolerance to the sugar is partly conditioned by gastric emptying, a key factor for the progressive arrival of fructose in the small intestine. The early onset of symptoms in these intolerant patients could be explained by an osmotic effect in relation to gastric emptying.

Our last aim was to determine whether a particular profile of fructose malabsorber did exist. None of the clinical symptoms were predictive of fructose malabsorption and the patients with symptomatic fructose malabsorption did not show a more altered psychological profile. However, in this study, young male IBS patients had a greater incidence of fructose malabsorption. Therefore, a breath test could be particularly indicated for the diagnosis of fructose malabsorption in these patients.

To conclude, our study demonstrates that the prevalence of symptomatic malabsorption of a 25 g fructose load was 22% in IBS patients and lower than that previously reported. Moreover, this 25 g load of fructose triggered bothersome intestinal symptoms in 28% of the patients who were not always malabsorbers. Different mechanisms are implicated in a poor tolerance to fructose. Therefore, further studies are now warranted to determine whether a low-fructose regimen is effective in intolerant to fructose IBS patients, with and without malabsorption.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Article 2 : Does calprotectin level identify a subgroup among patients suffering from irritable bowel syndrome? Results of a prospective study

Dans le premier papier sur la prévalence de la malabsorption du fructose dans le SII, il n'a pas été retrouvé de phénotype clinique du patient "malabsorbeur" du fructose hormis le sexe masculin et un âge plus jeune. En revanche, l'association à d'autres anomalies physiopathologiques, en particulier l'inflammation de bas grade, n'a pas été étudiée.

Cet article montre l'existence d'un sous-groupe de SII avec une inflammation de bas grade chez environ 1/3 de nos patients, confirmant les données de la littérature retrouvant l'existence d'une activation mastocytaire. Dans cette cohorte, il n'existait pas d'association entre l'élévation de la calprotectine fécale et la présence ou non d'une malabsorption du fructose. Ces deux anomalies physiopathologiques ne semblent donc pas être associées et ont probablement des mécanismes sous-jacents différents.

Résumé article 2 : Est-ce que le taux de calprotectine fécale permet d'identifier un sous-groupe de patients souffrant d'un syndrome de l'intestin irritable? Résultats d'une étude prospective

Introduction : Le SII est une maladie multifactorielle. La calprotectine fécale a déjà démontré être un marqueur fiable dans les MICI, son rôle dans le SII est encore débattu.

Le but de cette étude prospective était de mesurer le taux de calprotectine fécale et de caractériser le sous-groupe de patients avec une élévation de la calprotectine fécale.

Matériels et méthodes : Le taux de calprotectine fécale a été mesuré par technique Enzymed Linked ImmunoSorbent Assay (ELISA) chez des patients SII selon les critères de Rome III chez lesquels une coloscopie et les tests appropriés avaient été réalisés en vue d'éliminer une maladie organique. Le taux de calprotectine fécale a été comparé entre les patients SII, des volontaires sains, des patients avec maladie de Crohn active ou quiescente. Lorsque la calprotectine fécale était supérieure à 50µg/g, la normalité des anticorps anti-cytoplasme des polynucléaires neutrophiles (ANCA), des anticorps anti-Saccharomyces Cerevisiae (ASCA) et une exploration de l'intestin grêle étaient requis avant de confirmer le diagnostic de SII. Des scores de sévérité du SII, de qualité de vie, d'anxiété et de dépression ont été systématiquement réalisés de même que des tests respiratoires au glucose et au fructose, un barostat rectal et une évaluation quantitative et qualitative de l'inflammation sur les biopsies coliques.

Résultats : Parmi les 93 patients SII recrutés (73% de femmes, 66.7% de SII-D), 34 (36.6%) avaient une élévation reproductible de la calprotectine fécale. Les patients avec une élévation de la calprotectine fécale tendaient à être plus vieux que ceux avec une calprotectine normale ($p=0.06$), lorsque la calprotectine était élevée, elle était corrélée à l'âge ($p=0.03$, $r=0.22$). Il n'y avait pas d'autre différence entre les deux groupes.

Conclusion : Une élévation de la calprotectine fécale est observée chez 1/3 de nos patients SII, sans aucune association à un phénotype clinique ou paraclinique.

Does calprotectin level identify a subgroup among patients suffering from irritable bowel syndrome? Results of a prospective study

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Abstract

Background: Irritable bowel syndrome is a multifactorial disease. Although faecal calprotectin has been shown to be a reliable marker of intestinal inflammation, its role in irritable bowel syndrome remains debated.

Objective: The aims of this prospective study were to select a subgroup of irritable bowel syndrome patients and to characterise those patients with high faecal calprotectin by systematic work-up.

Methods: Calprotectin levels were determined by enzyme-linked immunosorbent assay test in consecutive irritable bowel syndrome patients fulfilling Rome III criteria in whom normal colonoscopy and appropriate tests had excluded organic disease. Calprotectin levels were compared in irritable bowel syndrome patients, healthy controls and patients with active and quiescent Crohn's disease. When the calprotectin level was higher than 50 µg/g, the absence of ANCA/ASCA antibodies and a normal small bowel examination were required to confirm irritable bowel syndrome diagnosis. Additional explorations included assessment of irritable bowel syndrome severity, anxiety and depression, impact on quality of life, glucose and fructose breath tests, rectal distension test by barostat and quantitative and qualitative assessment of inflammation on colonic biopsies.

Results: Among the 93 irritable bowel syndrome patients (73% women; 66.7% with diarrhoea) recruited, 34 (36.6%) had reproducibly elevated calprotectin. Although they tended to be older than those with normal calprotectin ($P = 0.06$), there were no other differences between the two groups. When elevated, calprotectin was correlated with age ($P = 0.03$, $r = 0.22$).

Conclusions: Elevated faecal calprotectin was observed in one third of patients in this series, without any significant association with a specific clinical phenotype (except age) or specific abnormalities.

Keywords

Faecal calprotectin, irritable bowel syndrome, low-grade inflammation, anti-inflammatory treatment

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Introduction

Irritable bowel syndrome (IBS), according to Rome III criteria,¹ is characterised by chronic abdominal pain or discomfort associated with transit disorders (constipation, diarrhoea or both). These chronic symptoms impair patients' quality of life and lead to a significant burden of the disease, with frequent recourse to health care resources and recurrent periods of absenteeism, both inducing high economic and social costs.

The successful relief of IBS symptoms remains a clinical challenge as existing therapeutic options often yield disappointing results. One explanation is the fact that

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IBS is a multifactorial disease. In addition to visceral hypersensitivity,² motor disorders,³ brain–gut axis dysfunction, enhanced intestinal permeability⁴ and gut dysbiosis,⁵ stress and psychological disorders may further worsen symptoms.⁶ Thus, selecting treatments to target pathophysiological abnormalities remains under discussion. Recent pathophysiological studies in a subgroup of IBS patients have highlighted the existence of a low-grade mucosal immune activity in the intestine, particularly when IBS was post-infectious. Such an inflammation provides arguments to consider drugs with intestinal anti-inflammatory properties as a possible therapeutic option. Recent randomised controlled trials have already tested the effect of prednisone and mesalazine in IBS patients but gave disappointing results, partly explained by the fact that only a subgroup of patients could benefit from this type of treatment.^{7,8}

Calprotectin is a calcium binding protein accounting for 60% of the cytosolic protein content of neutrophils.⁹ Accumulation of neutrophils at the site of inflammation in the gastrointestinal tract results in the release of calprotectin into faeces where it is stable and resistant to bacterial degradation. The faecal calprotectin rate is correlated with the excretion of indium labelled granulocytes.¹⁰ In inflammatory bowel disease (IBD), a clear relationship has been demonstrated between the magnitude of calprotectin elevation and the extent of intestinal inflammation. A meta-analysis of 670 patients has shown that calprotectin had a 93% sensitivity and 96% specificity for the diagnosis of inflammation.¹¹ A faecal calprotectin level of 50 µg/g of faeces, discriminated organic from non-organic intestinal disease with 84.4% sensitivity and 94.5% specificity, in 405 symptomatic patients referred for colonoscopy.¹² Screening the calprotectin level is now indicated as a non-invasive test to identify persistent occult inflammation among IBD patients in clinical remission.¹³ The use of the calprotectin level as a biomarker of intestinal inflammation could represent an interesting targeted therapeutic approach not only to rule out IBD, but also to identify potential IBS candidates for an anti-inflammatory treatment.

The aims of this work were: (a) to assess then compare faecal calprotectin levels in IBS patients, healthy controls and patients with both active and quiescent Crohn's disease; (b) to identify an association between high calprotectin levels and other pathophysiological abnormalities to determine whether the calprotectin level could be a useful marker for indicating anti-inflammatory treatment in IBS patients; and (c) to compare the characteristics of patients with and without high calprotectin levels.

Materials and methods

Patients

This prospective study recruited new consecutive IBS patients according to the Rome III criteria¹ and referred to the gastroenterology department of our tertiary care centre. Clinical examination and standard biological tests including CRP were normal. Total colonoscopy and additional tests when necessary had excluded organic disease.

When the faecal calprotectin level was higher than 50 µg/g, a negative search for ANCA/ASCA antibodies and a normal exploration of the small bowel (by wireless capsule endoscopy, computed tomography enteroclysis or magnetic resonance enterography) were required to confirm IBS diagnosis. Finally, a minimum 1 year clinical follow-up was required to exclude cases of IBD.

We then compared results between the IBS patients, 15 healthy subjects without any digestive symptoms and 35 patients with Crohn's disease, including 20 with active and 15 with quiescent disease.

Ethics and patients' consent

The research was performed according to the Declaration of Helsinki. All patients gave their informed consent. The use of informatics data was declared to the CNIL (no. 817.917).

Methods

Clinical assessment

IBS clinical phenotypes were determined by validated self-questionnaires. IBS severity was assessed by the IBS symptom severity score (IBS SSS)¹⁴ and transit disorders were characterised using the Bristol stool scale.¹⁵ Patients were questioned about a possible history of acute gastroenteritis prior to the onset of IBS suggestive of post-infectious IBS and about a possible worsening of IBS symptoms due to stress. Levels of anxiety and/or depression were assessed using the hospital anxiety and depression scale (HADS).¹⁶ The validated gastrointestinal quality of life index (GIQLI) assessed quality of life.¹⁷

Colonic biopsies

A left-side colonoscopy, without anaesthesia, was performed for sampling in order to detect inflammatory stigmata. After two enemas performed the day before then the morning of the endoscopic procedure, seven colonic biopsy samples were taken in the descending and sigmoid colons. Samples were analysed by standard

histology in the pathology department of our tertiary care centre.¹⁸ Intraepithelial lymphocytic infiltrate and colonic sub-epithelial collagen band were systematically assessed in order to eliminate microscopic colitis. Colonic inflammation was assessed both with qualitative and quantitative criteria after having excluded IBD with the 'PAID' scheme.¹⁹ Inflammation was quantified by our standardised method and scored 0, 1 or 2 for no, moderate, or mild inflammation, respectively. In the presence of inflammation, the cellular infiltrate was then analysed by immunohistochemistry. Monoclonal mouse anti-human CD3 (clone F7.2.38, reference M7254; DAKO, France) and polyclonal rabbit anti-human CD117 (c-kit) (reference A450229; DAKO, France) were used to identify T cells and mast cells, respectively. Then, the extent of T-cell and mast cell infiltrates was quantified (Figure 1) using a scale ranging from 0 (rare or absent), to 3 (severe).

Faecal calprotectin

Faecal calprotectin levels were determined by an enzyme-linked immunosorbent assay (ELISA) test (Bühlmann Labs, Schönenbuch, Switzerland) on 50–100 mg of faeces. Sample collections were stored in plain tubes without chemical or biological additives, between 2°C and 8°C up to 6 days. Dosage was realised on 50–150 µL of faeces extract. Calprotectin offers a dynamic range from 10 µg/g to 1800 µg/g. The test allows for the selective measurement of calprotectin antigen by sandwich ELISA. A monoclonal capture antibody highly specific to calprotectin heterodimeric and polymeric complexes, respectively, was coated to the microtitre plate. Calibrators, as well as control and patient extracts, were incubated at room temperature for 30 minutes. After a washing step, a detection antibody conjugated with horseradish peroxidase detected the calprotectin molecules bound to the monoclonal

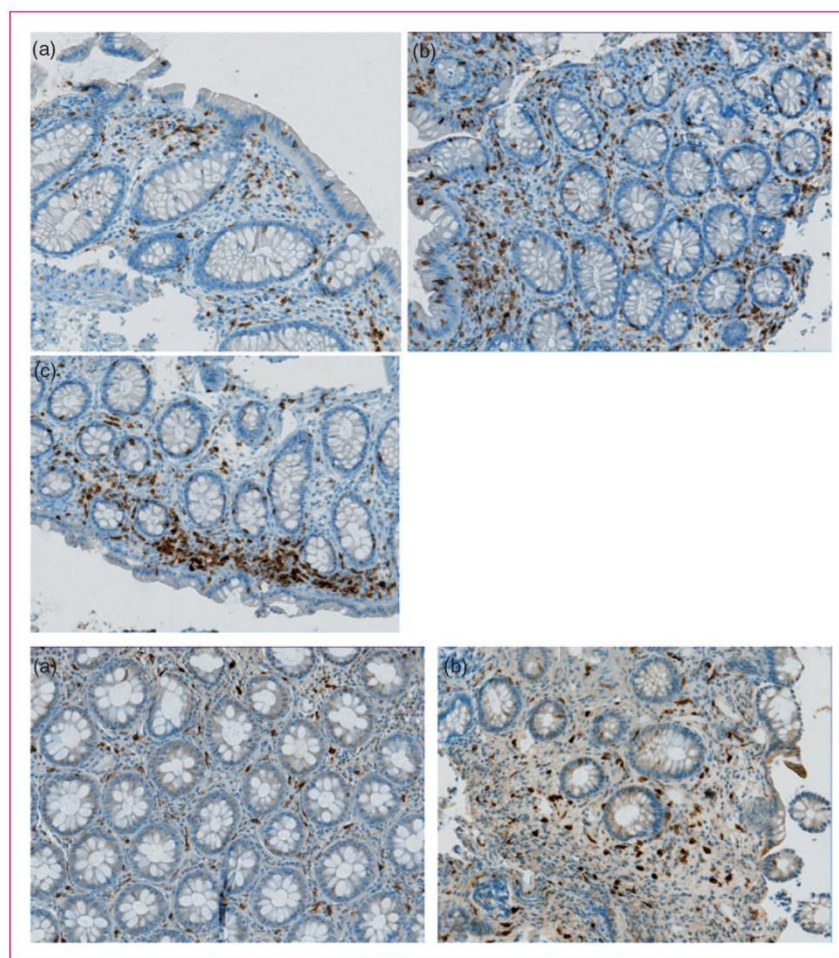


Figure 1. Figure 1: Immunohistochemistry on colonic biopsies. (Magnification X20)
Upper panel: Semi quantitative assessment with CD3 of T Cell infiltrate. A: Moderate, B: Mild and, C: Severe.
Lower panel: Semi quantitative assessment with CD117 of mast cell infiltrate. A: Moderate, B: Mild.



antibody coated on the plate. After incubation and a further washing step, tetramethylbenzidine was added (promoting a blue colour) prior to a stopping reaction (yellow colour). Absorption was measured at 450 nm.

Barostat-assisted distension test for visceral sensitivity assessment

A rectal distension test with an electronic barostat-distender assembly (Distender II; G & J Electronics, Toronto, ON, Canada) was performed to assess visceral sensitivity.²⁰ Distensions were performed automatically from 0 to a maximum pressure limit of 40 mmHg using basic isobaric distensions according to the methods of our department, previously described.²¹ We chose 40 mmHg as the upper rectal distension limit as a pain threshold above this value has been shown to exclude a diagnosis of IBS in 90% of non-IBS cases.²² Patients were asked to report their painful sensations on a 0–10 visual analogue scale (VAS) during the last 30 seconds of each step. The balloon was progressively distended until pain was reported or the last distending step was reached (40 mmHg). Distensions were stopped when the patient scored a painful sensation of at least 3 on a 10-point VAS.

Breath tests for detection of small intestinal bacterial overgrowth and fructose malabsorption and intolerance

Diet prior to breath tests. A strict diet was followed in the 48 hours prior to the breath tests in order to reduce intestinal gas production that could distort the results of the tests. This diet excluded bread, biscuits, cheese, fruits, vegetables, dairy products, fermented drinks, sodas, honey, marmalade, candy, chocolate, ice cream and pastry. In the evening prior to the tests, dinner was exclusively composed of rice and meat.

Glucose breath test. Both hydrogen (H₂) and methane (CH₄) were measured before the glucose load in order to verify compliance with the recommended diet. Then, patients ingested 75 g of glucose dissolved in 250 cc of sterile water. End-alveolar breath samples of expired air were collected every 15 minutes for 2 hours. The test was considered positive if one of the following results occurred: (a) baseline H₂ or CH₄ levels above 20 ppm despite good compliance with the diet; (b) peak of H₂ or CH₄ above 20 ppm after glucose intake; (c) increase of H₂ or CH₄ levels above 10 ppm in two samples by comparison with baseline levels.

Fructose breath test. Previous in vitro and animal studies have suggested that intestinal inflammation may impair sugar absorption. Therefore, we searched for fructose malabsorption, a condition that may promote IBS-like symptoms.²³

The fructose breath test was performed in patients with a negative glucose breath test to avoid false positive results.

When the glucose breath test was positive, patients received antibiotic treatment with quinolone or metronidazole for 10 days per month during two successive months. A second glucose breath test was then performed. The fructose breath test was only carried out in patients in whom the control glucose test was negative.

The fructose breath test was performed with the same gas analyser as for the glucose breath test. Patients ingested 25 g of fructose dissolved in 250 cc of sterile water then end-alveolar breath samples were collected every 30 minutes for 5 hours. Both H₂ and CH₄ levels were calculated. The test was considered positive in the case of a rise in H₂ and/or CH₄ levels above 20 ppm.²⁴ In addition, during the whole test, patients were asked about the onset of abdominal pain, bloating and even diarrhoea.

Statistics

The normality of the quantitative variables was assessed using the d'Agostino–Pearson omnibus test. A chi-square test was used for qualitative analyses and Mann–Whitney and *t* tests were used to analyse quantitative variables with Graphpad Prism 5 software. A *P* value lower than 0.05 was considered significant.

Spearman non-parametric correlation was used to correlate non-parametric data.

Results

Patients

Ninety-three IBS patients were included. They were 41 ± 13.9 years old, predominantly women (73.1%), with a mean body mass index (BMI) of 24.0 ± 5.4. IBS characteristics are described in Table 1. The mean IBS SSS was 258 ± 111.3, impairing their quality of life (GIQLI score 70.9 ± 19.1). Mean HADS scores for anxiety and depression were 10.3 ± 4.8 and 6.7 ± 4.2, respectively.

Prevalence of IBS with high faecal calprotectin levels

Stool samples were always analysed after less than 72 hours of storage. Mean faecal calprotectin was 90.5 ± 180 µg/g and a subgroup of 34 patients (36.6%) had levels higher than 50 µg/g (Figure 2). No patient with abnormal levels had diverticular disease or colorectal adenoma or was taking medications (mainly non-steroidal anti-inflammatory drugs) known to increase the faecal calprotectin level.²⁰ No patient developed IBD during 1 year follow-up.

Table 1. Characteristics of the 93 IBS patients

IBS characteristics	n = 93 (%)
Subtype	
• Diarrhoea	62 (66.7)
• Constipation	14 (15.0)
• Alternating	16 (17.2)
• Undetermined	1 (1.1)
Post-infectious IBS	25 (26.9)
Disease duration (years)	6.9 ± 7.3
Worsening of symptoms by stressful events	46 (49.5)
Nocturnal pain	14 (18.3)
Breakthrough pain	8 (8.6)

Results are presented as number (percentage) and mean ± standard deviation.

IBS: irritable bowel syndrome.

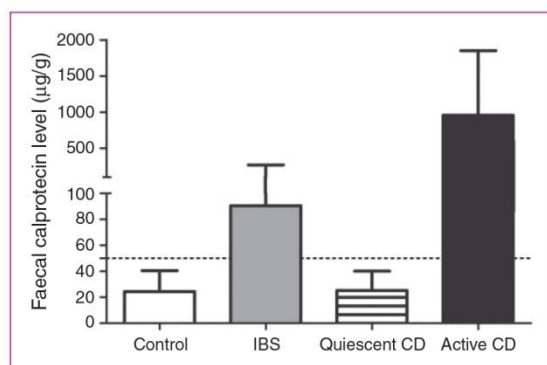


Figure 2. Calprotectin levels according to control subgroup: control, active and quiescent Crohn's disease compared to IBS population.

In the control group without digestive symptoms, the mean faecal calprotectin level was $24.3 \pm 16.1 \mu\text{g/g}$ ($P < 0.01$). In Crohn's disease, the mean level was higher for active lesions (958.4 ± 892.4 , $P < 0.0001$) but levels were lower for quiescent disease, compared to IBS patients (25.2 ± 14.8 , $P < 0.002$). The results are presented in Figure 2.

Faecal calprotectin levels according to the IBS subtype are given in Figure 3.

Results of systematic evaluation of patients

In our IBS population, 13.4% and 30.6% of patients tested had abnormal levels on the glucose breath test and the fructose breath test, respectively. Stigmata of qualitative histological colonic inflammation were found in 46.3%. The results of the test evaluation in patients with high and normal calprotectin levels are presented in Figure 4.

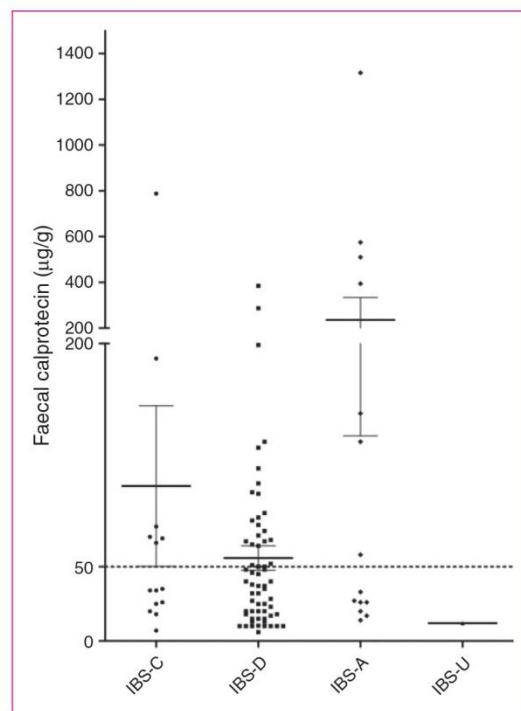


Figure 3. Faecal calprotectin distribution according to IBS subtype.

The figure represents faecal calprotectin distribution according to IBS sub-type. Lines represent mean and error bars standard error of the mean. The dotted line indicates the upper limit of the normal values. IBS: Irritable Bowel Syndrome. C: Constipation. D: Diarrhoea. A: Alternating. U: Undetermined.

Comparison between IBS patients with and without high faecal calprotectin levels

Characteristics of both groups of IBS patients are detailed in Table 2. IBS patients with elevated calprotectin levels tended to be older than those with normal levels (44.2 ± 12.3 years vs. 39.3 ± 14.6 , $P = 0.06$).

Patients with abnormal calprotectin levels had neither abnormal glucose ($P = 0.73$), abnormal fructose breath test ($P = 0.98$), nor rectal hypersensitivity ($P = 1.0$).

Histological findings. Microscopic colitis was excluded in all biopsies. Histological examination found inflammation in 46% of the patients, with mononuclear infiltrate and mixed infiltrate in 74% and 26% of cases, respectively, but without any difference between patients with or without abnormal calprotectin levels ($P = 0.73$). Semi-quantitative histological criteria for colonic inflammation were not different in cases of elevated calprotectin levels ($P = 0.66$).

In the 19 patients with inflammation, the mean density of T cells was 2 ± 0.8 with no difference according to

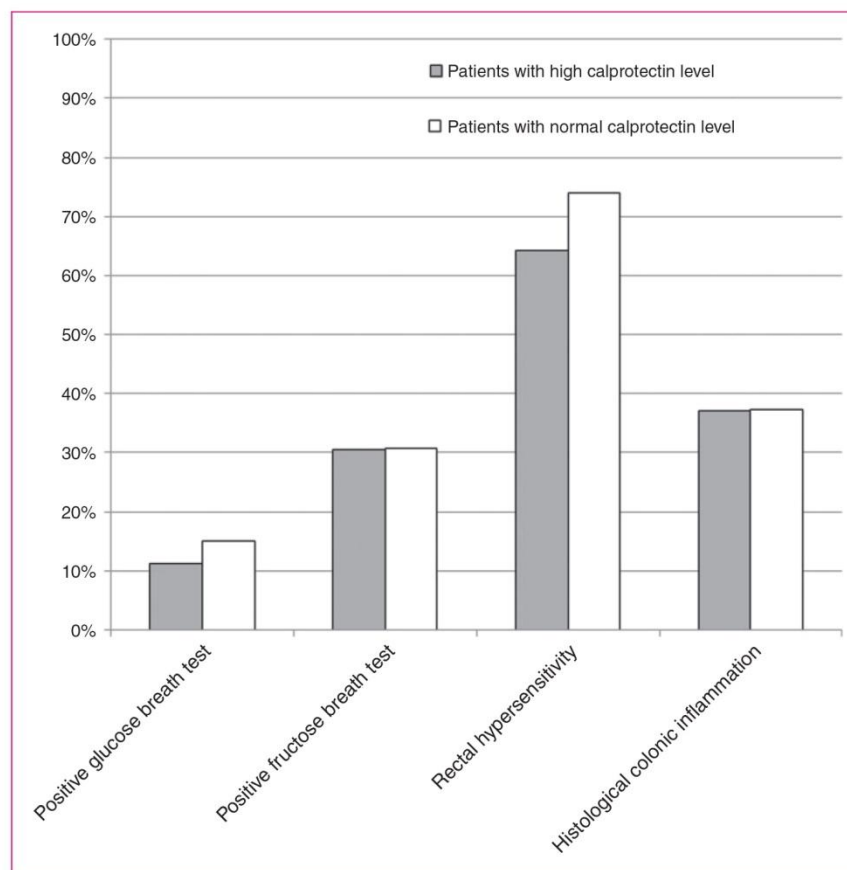


Figure 4. Results of tests in IBS patients with high and normal faecal calprotectin levels. Results are presented as abnormal in percentage for small intestinal bacterial overgrowth, fructose malabsorption, rectal hypersensitivity and histological inflammation.

the calprotectin level ($P=0.85$). Similar results were obtained for mast cells, with a mean intensity of 1.4 ± 0.6 ($P=0.45$). The calprotectin level was not correlated with the extent of T-cell or mast cell infiltrates quantified by immunohistochemistry ($r=0.11$ with $P=0.66$ and $r=0.07$ with $P=0.76$, respectively).

Correlation between elevation level of faecal calprotectin and clinical assessment in patients with abnormal levels

In this subgroup of patients, the highest levels were observed in the oldest patients ($P=0.03$, $r=0.22$). The correlation between a high faecal calprotectin level and other quantitative data are presented in Table 3.

Discussion

At variance with previous papers in which the faecal calprotectin level aimed to differentiate IBS from IBD, we quantified calprotectin levels in IBS patients and

tried to correlate these levels with histological inflammation to identify a subgroup of IBS patients for whom an anti-inflammatory intestinal treatment could be discussed. In this series of 93 IBS patients mainly with moderate to severe disease (mean IBS SSS: 258 ± 111.3) with a significant negative impact on quality of life (mean GIQLI score 70.9 ± 19.1), 36.6% of patients had an elevated level of faecal calprotectin. This level was higher than the upper limit of the normal values taken into account in most published studies¹² and was confirmed in our control group of healthy volunteers. Levels in IBS patients were also higher than those calculated in quiescent IBD. This subgroup of IBS patients with elevated calprotectin tended to be older than patients with normal levels, with a positive correlation between age and abnormal calprotectin levels. In contrast, no significant association was observed between high faecal calprotectin levels and other pathophysiological abnormalities.

The characteristics of our IBS population were comparable with those reported in previous series

Table 2. Characteristics of IBS patients according to faecal calprotectin level

Characteristics	High faecal calprotectin level <i>n</i> = 34 (%)	Normal faecal calprotectin level (<i>n</i> = 59) (%)	<i>P</i> value
Age	44.2 ± 12.3	39.3 ± 14.6	0.06
Gender (male/female)	12 (35.3)/22 (64.7)	13 (22.0)/46 (78.0)	0.16
BMI	23.5 ± 4.6	24.2 ± 5.8	0.86
Subtype			0.84
• Diarrhoea	22 (64.7)	40 (67.8)	
• Constipation	6 (17.6)	8 (13.6)	
• Alternating	6 (17.6)	10 (16.9)	
• Undetermined	0 (0.0)	1 (1.7)	
Post-infectious	9 (26.5)	16 (27.1)	0.95
Disease duration (years)	7.6 ± 8.1	6.4 ± 6.7	0.46
IBS SSS	268.9 ± 110.4	252.1 ± 113.2	0.62
Worsening of symptoms by stressful events	16 (47.1)	30 (50.8)	0.94
Nocturnal pain	7 (20.6)	10 (16.9)	0.66
Breakthrough pain	3 (8.8)	5 (8.5)	1.00
GIQLI score	73.3 ± 17.4	68.9 ± 20.6	0.51
HADS score for anxiety	9.1 ± 5.0	11.0 ± 4.6	0.17
HADS score for depression	6.6 ± 4.0	6.8 ± 4.3	0.82

Results are presented as number (percentage) and mean ± standard deviation.

IBS: irritable bowel syndrome; BMI: body mass index; GIQLI: gastrointestinal quality of life index; HADS: hospital anxiety and depression scale; IBS SSS: IBS syndrome severity score.

Table 3. Correlation between faecal calprotectin level and other quantitative data in patients with abnormal faecal calprotectin level

	Faecal calprotectin level (µg/g)	
	<i>P</i> value	<i>R</i>
BMI	0.65	0.06
Age (years)	0.03	0.22
Disease duration (years)	0.31	0.12
IBS SSS	0.63	0.07
GIQLI score	0.44	−0.13
HADS score for anxiety	0.13	−0.22
HADS score for depression	0.98	−0.00

P < 0.05 is considered significant.

IBS: irritable bowel syndrome; BMI: body mass index; GIQLI: gastrointestinal quality of life index; HADS: hospital anxiety and depression scale; IBS SSS: IBS syndrome severity score.

(predominance of women, median-aged patients) with the exception of over-representation in the IBS with diarrhoea subgroup. The high prevalence (around 25%) of probable post-infectious IBS in this series could contribute to this over-representation. The discomfort related to IBS with diarrhoea, its impact on patients' quality of life and the difficulties of managing

both diarrhoea and pain could be additional explanations for this over-representation in a referral centre.

The determination of the calprotectin level was the milestone of our work. For this, only one sample of faeces was analysed. This can be criticised. However, Tibble et al. have shown in 22 patients a significant correlation between the result of a single stool analysis and the 4-day faecal expression of calprotectin.²⁵ In a recent study in 98 patients with Crohn's disease in clinical remission, a 3-day determination of faecal calprotectin levels showed low variability across samples allowing clinicians to use a single sample.²⁶ Another possible weakness of our study lies in the lack of confirmation of abnormal calprotectin levels by second sampling in all patients, but this control was obtained and the elevation confirmed in patients with abnormal calprotectin levels closest to the upper limit of the normal range.

One important objective of our work was to demonstrate an association between elevated calprotectin levels and anomalies of other pathophysiological tests. Breath test and rectal distension by barostat are validated tests for the detection of fructose malabsorption and visceral hypersensitivity, respectively, which are prevalent in IBS patients. The choice of a breath test to detect a possible small intestinal bacterial overgrowth (SIBO) could be criticised, even though we

selected glucose and not lactulose for this breath test in order to limit the false positive rate related to accelerated transit in the small bowel promoted by the lactulose itself rather than a SIBO.²⁷ In our series, the percentage of patients with a possible SIBO was lower than in other reports.²⁸ One of our pre-study hypotheses was that elevated calprotectin could be explained by SIBO. Indeed, elevated calprotectin levels similar to those obtained in the present study have been reported in systemic sclerosis, a pathological condition in which SIBO is highly prevalent. In a recent study, we were able to show that faecal calprotectin greater than 275 µg/g was predictive of SIBO in a cohort of patients with systemic sclerosis.²⁹ However, in our present cohort of IBS patients, we were unable to demonstrate such a link between SIBO and elevated calprotectin, as SIBO and high calprotectin levels were only associated in three patients. This result is in accordance with Gasbarrini's findings, both in adults³⁰ and children, suggesting that SIBO is not associated with intestinal subclinical inflammatory changes mainly involving the neutrophils. We also speculated that elevated calprotectin would be found more frequently in hypersensitive patients, due to the role of inflammation on enteric sensitive neurons. We were unable to confirm this hypothesis.

The last point is that colonic biopsies did not allow an explanation of abnormal levels. Such a paradoxical result highlights some additional weaknesses of this study: (a) colonic biopsies were performed in only 80% of patients, as some patients refused a new examination after a previous one that had diagnosed IBS; (b) biopsy samples were taken only in the left colon and we cannot conclude as to possible inflammation in the right colon or even in the small bowel despite normal imaging; and (c) biopsy samples were taken routinely, mainly involving the mucosa, but deeper biopsies are needed to study mast cells.³¹ Neither the quantification of standard inflammation nor the qualitative evaluation of mast cells and T cells by immunohistochemistry were able to explain the elevated levels of calprotectin found in a subgroup of patients. Unfortunately, we were unable to measure the production of inflammatory cytokines. Therefore, the reason for elevated calprotectin levels in more than one-third of our IBS patients, including 17% with levels higher than 100 µg/g, remains unclear. One explanation could be increased intestinal permeability. The positive correlation between calprotectin and age is an indirect argument for such a hypothesis.³² In addition, a recent study has demonstrated that in quiescent IBD, IBS-like symptoms related to persistent subclinical inflammation were associated with increased colonic paracellular permeability and could be related to tumour necrosis factor

alpha, which was significantly increased in quiescent IBD with IBS-like symptoms in this study.³³ However, intestinal permeability was not studied in our series due to the lack of a simple and validated test.³⁴ As it is well established that foods, particularly FODMAPs and wheat proteins, can play a central role in triggering inflammation in IBS, it would be interesting to verify whether the raised level of faecal calprotectin can be reverted to normal by dietary interventions such as low FODMAPs and/or gluten-free diet.³⁵

In conclusion, we confirmed our hypothesis that faecal calprotectin was abnormally high in a subgroup of our IBS patients. However, we were unable to explain this high level with quantitative and qualitative analysis of colonic inflammation. Therefore, according to the results of our study, a high faecal calprotectin level cannot be the sole selection criteria to indicate an anti-inflammatory treatment in this subgroup of IBS patients. Further studies are needed to understand this elevation better.

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Article 3 : A prospective controlled study : Is abnormal fructose breath test a predictor of symptomatic response to low fructose diet in irritable bowel syndrome?

Un des objectifs de ma thèse de science était de déterminer si le test de dépistage de la malabsorption au fructose (par un test respiratoire) permettrait de prédire l'efficacité du régime appauvri en fructose sur les symptômes digestifs des patients souffrant d'un SII.

En effet l'efficacité du régime appauvri en fructose dans le SII est connu (117) mais non le patient répondeur type. L'existence ou non d'une malabsorption du fructose associée pourrait être un facteur prédictif d'efficacité d'un tel régime. L'utilité pré-thérapeutique du test respiratoire au fructose, notamment dans cette indication reste toujours à définir (115, 116). Répondre à cette question permettrait de cibler les patients pouvant bénéficier d'un régime pauvre en fructose (régime inconstamment efficace et très contraignant avec un impact nutritionnel potentiel).

Le régime établi dans notre service diminue la quantité de fructose et des fructanes. Il élimine donc tous les aliments contenant du sucre. Pour une meilleure acceptabilité, une portion de 200g de légumes cuits (contenant moins de 2% de fructose) est autorisée par jour de même qu'une portion de 100g de fruit/j (<2% fructose). Une portion journalière de 80 g de pain blanc est également autorisée. Ce régime a été adapté avec l'aide de l'équipe des diététiciennes du service d'hépatogastroentérologie selon les habitudes alimentaires françaises pour une meilleure acceptabilité.

Les résultats de notre étude confirment l'efficacité du régime appauvri en fructose dans le SII avec une amélioration d'environ 70 points du score de Francis. En revanche, la présence ou non d'un test respiratoire au fructose positif n'impacte pas l'efficacité du régime qui peut donc être proposé à tous les patients SII. Ces résultats remettent en cause nos pratiques et l'utilité du test respiratoire au fructose à la dose de 25g, du moins pour prédire l'efficacité d'un régime appauvri en fructose. Une des explications à cette absence de différence est l'association possible à une ou plusieurs anomalies physiopathologiques qui ne sont pas ciblées par un régime seul.

Résumé article 3 : Etude contrôlée prospective : La positivité d'un test respiratoire au fructose est elle prédictive de l'efficacité d'un régime appauvri en fructose dans le syndrome de l'intestin irritable?

Introduction : Un sous-groupe de patients, souffrant de SII, malabsorbe le fructose. Un régime d'éviction pourrait améliorer la symptomatologie digestive des patients SII.

Objectifs : L'objectif de cette étude était de déterminer si la présence d'une malabsorption du fructose détectée par un test respiratoire pourrait être prédictive de l'efficacité d'un régime appauvri en fructose.

Méthodes : Notre étude contrôlée, prospective a inclus 88 patients souffrant d'un SII selon les critères de Rome III. Tous les patients ont passé un test respiratoire au fructose avec une dose de charge de 25g. Un test positif définissait la malabsorption du fructose. Trente-sept patients "malabsorbeurs" et 40 patients "absorbeurs" ont suivi un régime appauvri en fructose pendant 15 jours, en aveugle des résultats du test. Une évaluation clinique et de la qualité de vie par auto-questionnaires validés ont été réalisés avant et après le régime. Le critère de jugement principal a été mesuré par l'amélioration du score de sévérité (score de Francis).

Résultats : Le score de Francis a été significativement amélioré par le régime appauvri en fructose dans le groupe de patients "malabsorbeurs" et "absorbeurs" (-68.0 [-137 ; 0] vs -73.5 [-173 ; -11.5]) sans différence significative entre les 2 groupes (p ajusté=0.9836). La plupart des patients a eu le sentiment d'être amélioré par le régime, 64.9% et 72.1% pour les patients "malabsorbeurs" et "absorbeurs" (p ajusté=0.3241).

Conclusion : Un test respiratoire au fructose positif n'est pas prédictif de l'efficacité d'un régime appauvri en fructose dans le SII.

Original article

A PROSPECTIVE CONTROLLED STUDY: Is abnormal fructose breath test a predictor of symptomatic response to low fructose diet in irritable bowel syndrome?

Short running title: Low fructose diet and IBS

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Authorship statement

AML is the guarantor of the article.

CM and AML designed the research study,

CM, GG, MM, PD and AML performed the research,

CM, LB, LAD, GG and AML collected the data,

EH, CM, GG, AML and PD analysed the data,

CM, GG, AML, and PD wrote the paper,

All authors approved the final version of the article, including the authorship list.

Summary

Background

A subgroup of patients with irritable bowel syndrome poorly absorbs fructose. A fructose restricted diet seems to improve digestive symptoms in these patients.

Aims

The aim of our study was to determine whether fructose malabsorption detected by a fructose breath test could be a predictor of the efficacy of a low fructose diet.

Methods

Our prospective, controlled study included 88 patients with irritable bowel syndrome according to Rome III criteria. All patients had a fructose breath test with a 25 g load. A positive test defined fructose malabsorption. Thirty-seven malabsorber patients and 40 absorber patients followed a low fructose diet for 2 weeks, blinded to their test results. Patients filled self validated-questionnaires for both symptoms and quality of life before and at the end of the diet. The main outcome measurement was the irritable bowel syndrome Symptom Severity Score.

Results

Irritable bowel syndrome Symptom Severity Score was significantly improved in absorber and malabsorber patients after low fructose diet (-68.0 [-137 ; 0] vs -73.5 [-173 ; -11.5]) with no difference according to fructose breath test result (adjusted $p=0.984$). Most patients reported symptom improvement after the low fructose diet, 64.9% and 72.1% of malabsorber and absorber patients, respectively (adjusted $p=0.324$).

Conclusions

A positive fructose breath test is not a predictor of the efficacy of a low fructose diet in irritable bowel syndrome.

Key words

Fructose breath test - irritable bowel syndrome - predictive factor - low fructose diet –
FODMAPs – abdominal pain

Main text

Introduction

Irritable bowel syndrome (IBS) is the main functional disorder worldwide and is characterised by chronic abdominal pain associated with transit disorders. Its prevalence in Western Europe and the USA is around 5-10% (1). The underlying pathogenesis of IBS is considered complex and several functional alterations have been described (2). These include altered visceral sensitivity, bowel dysmotility and secretory dysfunctions, immune intestinal activation, gut dysbiosis, brain gut alterations, somatic and psychiatric co-morbidities (2).

A link between food intake and the occurrence or the exacerbation of IBS symptoms has also been reported (3). Particularly, several studies have underlined the deleterious symptomatic role of fructose malabsorption (FM) (4-8). Fructose is made of a hexose sugar that is commonly present in fruit (mainly pears and apples), vegetables, honey and sweeteners. It has replaced traditional cane sugar over recent years and is increasingly incorporated in beverages, dairy products and canned, baked or processed foods worldwide (9). Fructose absorption is dose-dependent and limited in case of high luminal concentrations (10). Consequently, excessive dietary intake can easily exceed the absorptive capacity of the small bowel, leading to incomplete absorption of fructose and, finally, causing FM (11). The unabsorbed fructose may play a role in osmotic load and is therefore rapidly propelled into the colon, where its contact with anaerobic microbiomes causes fermentation and the production of a gaseous feeling, abdominal bloating, and even diarrhoea (12, 13). A low fructose diet is therefore recommended. Open labelled studies have demonstrated the effectiveness of reducing fructose in patients with IBS, achieving adequate symptom relief in 70-80% of patients (14-16). At present, it is unclear which IBS patients will benefit from a low fructose

diet. The current diagnostic means of FM is the fructose breath test (FBT) (11). However, the ability of the FBT to predict the efficacy of low fructose diet in IBS patients is still debated (14, 17).

Our objective was to study the predictive value of FBT results on the outcome of a low fructose diet in a cohort of patients with IBS using controlled, simple-blind, parallel groups.

Materials & methods

Patients

All consecutive IBS patients referred to the Physiology Unit of Rouen University Hospital (France) for FBT from January 2014 to January 2018 were invited to participate in the trial if they met the inclusion criteria. Inclusion criteria were: IBS defined according to Rome III criteria (18), ≥ 18 years of age; normal clinical examination, standard biological tests (including C Reactive Protein) and colonoscopy (with colonic biopsies) performed in the last 6 months. Exclusion criteria were: small intestinal bacterial overgrowth (SIBO) as determined by breath testing, history of abdominal surgery (except for appendectomy), systemic scleroderma, diabetes, metabolic syndrome, anorexia and pregnancy. Other exclusion criteria were changes to IBS medication during the trial.

The study was approved by the Haute-Normandie Ethics Committee (2013-AOO116-39) and was registered at www.clinicaltrials.gov (NCT02188680). Patients gave written informed consent before participation.

Study design

All IBS patients included in the study performed a FBT. Whatever the results of this test (i.e; FM present or absent), after a consultation with a dietician, patients were instructed to follow a low fructose diet carefully during a period of 2 weeks. Improvement in digestive symptoms was recorded at the end of the 2-week diet period and dietary compliance was evaluated at 1-week and 2-week dietary periods.

Breath tests

Each patient had two breath tests. First, a glucose breath test was carried out in order to rule out SIBO, which is a condition that may promote false positive results of sugar breath tests (11). If negative, a FBT was then performed following a methodology previously described (4). After an overnight fast, hydrogen (H_2) and methane (CH_4) levels were measured by a Microlyzer (Quintron Instr. Company Inc., Milwaukee, WI, USA) in samples of expired air from patients according to the manufacturer's instructions. H_2 and CH_4 levels were collected in end-expiratory breath samples, before administration of a 25 g load of fructose and then collected every half hour for 5 hours (4). FM was defined as an increase of H_2 and/or CH_4 above 20 ppm (19). Both dieticians and participants were blind to FBT results during the entire study duration.

Dietary advice

All included patients were referred to one of two experienced dieticians (M.M. and E.G.) for a standardised 2-week dietary adaptation based on published low fructose diet guidelines (16). We decided to maintain the low fructose diet only for 2 weeks to limit the risk of non-compliance to this restrictive diet. The initial consultation comprised an interview during

which a qualitative patient-defined typical day dietary intake was recorded, reflecting usual eating practices over the past month. Then, individual instructions for the low fructose diet were given both verbally and through written information that included a table in French showing the foods to be restricted (i.e; those containing significant free fructose in excess of glucose, and foods that are a substantial source of fructans) and safe foods (i.e; foods in which fructose and glucose are balanced or have more glucose than fructose) (*Table 1*) (16). The diet authorised 200 g of cooked vegetables (containing less than 2% of fructose), 100 g of fruit (containing less than 2% of fructose) and a single 80 g portion of white bread per day. Patients with IBS already following a diet were eligible as long as they agreed to abandon their diet for the study duration, except for patients with lactose intolerance. In this case, patients were included if they maintained consistent lactose intake during the 2-weeks of the study period.

Dietary compliance was assessed by live telephone interview (“in the last week I have followed the diet”) by the investigators, one week after initiation of the dietary change and during the last assessment visit, two weeks after initiation of the diet. Diet compliance categories were adapted from previous work (16) : never/rarely (>25% of the time), sometimes (25%-50% of the time), frequently (51%-75% of the time) or always (76%-100% of the time). Compliance was considered adequate if patients confirmed they adhered to the dietary guidelines during at least 50% of the meals consumed.

Assessments and end points

The primary outcome was a decrease in self-assessed clinical severity of the IBS symptoms evaluated by the irritable bowel severity scoring system (IBS-SSS) (20) before and at the end of the 2-week dietary period. The IBS-SSS contains five questions, each given a value from 0

(no symptoms) to 100 (most severe) for measuring the severity and frequency of IBS-associated symptoms. The total score is ranged from 0 to 500 (20).

Secondary endpoints were assessed before and at the end of the dietary intervention. They included: mean change from baseline in the quality of life, in the severity of anxiety and/or depression, in the stool consistency, and in the weight and body mass index (BMI). The quality of life was assessed using the Gastrointestinal Quality of Life Index (GIQLI) (21). This questionnaire, validated in French, includes 36 items asking about symptoms, physical status, emotions, social dysfunction, and effects of medical treatment with five response levels to each survey question (0-4, a higher value represents a better outcome). Anxiety and depression levels were assessed using the hospital anxiety and depression scale (HAD) (22). In addition, patients gave a description of stool quality on a scale from 1 to 7 (Bristol stool scale) (23). The opinion of the patients about the low fructose diet was also recorded using two questions: “Do you think that the diet has improved your digestive symptoms?” and “Do you think to stay on the diet at the end of the study?”. Adverse events of any kind were monitored throughout the study period.

Statistical analysis

The sample size calculation was based on the primary end-point (i.e; the mean change of IBS-SSS between the beginning and the end of the low fructose diet period). The mean IBS-SSS in a cohort of 241 IBS patients has been estimated to 298 ± 85 (personal unpublished data). Based on this standard deviation, 40 subjects had to be included in each group (patients with or without FM) to highlight a difference of 60 between the variation of IBS-SSS between the two groups with two-sided 0.05 type I error and to obtain at least 80% power.

Per protocol analysis was performed for the symptom primary outcome using data from all patients completing the study who did not violate the protocol. Patients who did not complete the study were replaced by other patients, limited to 10% of the overall population (8 patients).

Otherwise, demographic and clinical characteristics, GIQLI, Bristol stool scale, anxiety and depression HAD at baseline were compared between IBS patient with or without FM using a Mann–Whitney test. The same test was also used for comparison of score variations (IBS-SSS, GIQLI, Bristol stool scale and HAD) between groups and between the beginning and the end of the diet. These comparisons were adjusted for sex and age (<40 and ≥ 40) variables using a logistic regression.

Results are presented as median with first and third quartile (Q1-Q3). A p value lower than 0.05 was considered significant. These analyses were carried out using SAS 9.3 software (SAS Institute Inc., Cary, NC, USA).

Results

A total of 88 patients were included in the study. Eleven patients (12.5%) were withdrawn from the trial: 2 were noncompliant with the low fructose diet that they found too strict, 1 violated the protocol as they knew their FBT results before the end of the study, 1 was lost to follow-up, 4 withdrew for personal reasons and 3 did not complete the final evaluation. Seventy-seven patients (87.5%) completed their dietary course, 40 patients in the absorber group and 37 in the malabsorber group (*Figure 1*). The different size of the groups was due to the limited number of possible replacements (8 patients).

Patient characteristics are shown in *Table 2*. The sex ratio was significantly different between the absorber and malabsorber group (0.017). Patients in the malabsorber group were significantly older than those in the absorber group (0.019). As age and sex ratio were different between the two groups, further analyses were performed to adjust comparisons for age and sex. There was no other significant difference in any demographic or baseline characteristics between the two groups of patients (*Table 2*).

Sixty-eight patients (77%) attributed their IBS symptoms to food, with high-carbohydrate foods, dairy, beans, foods rich in fats and spices, and lentils most commonly cited. Twenty-three patients among the 88 included (26%) were on special therapeutic diets at the start of the study: low lactose diet (10 patients); low fructose diet (4 patients); hypo-caloric diet (5 patients); gluten free diet (4 patients). There was no difference concerning the frequency of food-related disorders and alimentary restrictions between the fructose absorber and malabsorber groups ($p=0.963$ and $p=0.478$, respectively).

Effect of low fructose diet

There was a significant positive change in reported symptoms between baseline and follow-up after dietary intervention. Low fructose diet significantly reduced both IBS-SSS, GIQLI and

HAD scores whereas no change was observed for stool consistency (*Table 3*). This symptom improvement was concomitant with patients' feelings regarding the diet. Fifty five patients out 88 (62.5%) reported symptom improvement following their diet. Nevertheless, among them, only 34 patients (62.0%) were prepared to continue the low fructose diet after the end of the study, because they considered the diet to be too restrictive. In addition, the diet was associated with weight loss (*Table 3*).

Associations between breath test results and outcome of dietary programme

Figure 2 shows the effects of low fructose diet on IBS-SSS in the absorber and malabsorber groups. The median changes at week 2 versus baseline were -73.5 [-173; -11.5] in the absorber group versus -68.0 [-137; 0] in the malabsorber group ($p=0.984$). We failed to highlight any significant association between the nature of the gas production (i.e peak of H_2 and/or CH_4) recorded during the FBT and the symptom improvement during the low fructose diet because only 4 patients out of 40 (10%) with a positive breath test had a peak concentration of CH_4 . This small sample size did not allow further statistical analysis. Furthermore, the variation in IBS-SSS during the diet was comparable in patients with peak concentration of H_2 compared to others (-70 [-137; 2] versus -72 [-174; 0]; $P=0.774$).

No different effect of the low fructose diet could be detected on quality of life, Bristol Stool Scale, or HAD scores between the two groups (*Table 4*). Similarly, 64.9% of patients in the malabsorber group and 72.1% of patients in the absorber group reported similar improvement following the diet ($p=0.324$). In both groups, the diet was associated with weight loss (-1Kg [-2;0] in both groups, $p=0.562$).

Discussion

In this controlled simple-blind prospective study of patients with IBS, low fructose diet reduced symptom scores independently of FBT results. Of note, even in the fructose absorber group (negative FBT), most patients had significant improvement of their symptom scores.

As far as the authors know, there is only one study reporting the predictive value of the FBT for a symptomatic response to a low fructose diet in IBS patients (14). Bert et al. previously demonstrated that FBT did not discriminate well between IBS patients with and without effect of the low fructose diet (14), but they used a relatively high dose of 50 g of fructose to reduce the risk of false-negative (14). However, the ability of the human intestines to absorb fructose is limited (24). Specific intestinal fructose transporters in the intestines of humans may be easily overwhelmed by fructose > 50 g (12) leading to a variety of gastrointestinal symptoms. When healthy subjects were administered 50 g fructose and had breath tests, 80% tested positive providing evidence of FM (13). The higher the dose and the concentration of fructose, the greater the chance of a false-positive being included biasing the capacity of the FBT to identify the best candidates for a low fructose diet. The optimal dose for FM diagnosis is unclear (24). However, we chose a dose of 25 g because it is closer to the daily intake and has been demonstrated to be effective in FM diagnosis (12). We expected that lower doses of fructose would allow us to identify subjects with severely restricted fructose absorptive capacity who would benefit more from a low fructose diet than others. That was not the case. As suggested by others (14), we cannot exclude the fact that a low dose of fructose increased the risk of false-negative results in our absorber group explaining the symptom improvement on diet in this group.

The lack of predictive value of FBT to identify the best candidates for a low fructose diet is hard to explain. It is possible that it is a useful test but improperly used. Indeed, the

heterogeneity of methodologies used, particularly concerning the cut-off value for breath gas concentration and the ingested dose of fructose, is obvious. A previous study (14) and our present study have demonstrated that a dose of 50 g or 25 g of fructose were not the optimum choice to discriminate IBS patients who would benefit from a low fructose diet from those who would not. However, de Roest et al. demonstrated that a positive FBT performed with a 35 g load of fructose was strongly associated with the efficacy of a low FODMAPs (Fermentable Oligo-, Di- and Mono-saccharides And Polyols) diet that also includes a low fructose diet (17). We can hypothesise that the choice of the dose should not be determined intuitively, but from a load that best discriminates responders and non-responders to diet (11). There is a need for further large studies assessing different doses of ingested fructose and cut-off values for breath gas during the FBT to determine their value to discriminate the best candidates for a low fructose diet.

Another explanation of the low predictive value of FBT could be that FM is not the right target to explain some bowel symptoms in IBS patients. The prevalence of FM in the healthy population appears to be similar as in populations with IBS (11). The main difference between symptomatic and asymptomatic populations is the frequency of symptoms induced after fructose absorption (i.e fructose intolerance) suggesting that the sensitivity of the bowel to the change in luminal conditions induced by FM is the key difference rather than the malabsorption itself (11). As we did not record bowel symptoms after fructose absorption, we do not know if the presence of symptoms provoked during breath testing, called fructose intolerance, associated with FM would improve the predictive value of the breath test. However, previous studies have shown responses to a low-FODMAPs diet to be similar in patients with fructose intolerance with or without malabsorption (5, 25).

Finally, it is not possible to exclude a placebo effect of the low fructose diet registrations in some patients, which could explain the poor predictive value of FBT. The improvement in

anxiety and depression scale scores during the short period of low fructose diet could be an additional argument for the placebo-effect. Placebo-controlled trials are especially important in IBS where the placebo response is known to be considerable (26). However, performing a double-blind randomised study is challenging in dietary intervention trials compared with nutrient or drug trials.

One of the strengths of our study is its controlled simple-blind design which meant that patients could not be influenced by the results of their FBT. In addition, in this study, we chose global relief assessment as the broadest main outcome measure, as changes in specific symptoms incompletely assess the impact of treatment in IBS or changes in quality of life (25). Lastly, the single centre characteristic of this study ensured the consistency of all dietary advice for all included patients.

Nevertheless, our study had several limitations. First, our two groups of fructose absorbers and malabsorbers patients were not identical. Malabsorbers patients were older and more frequently male than in the absorber group. In a previous paper, we already found that male gender was more frequent in the malabsorber group (4). To our knowledge, these findings have never been reported in the literature and we do not have any clear explanation for this. However, we have taken these differences into consideration in our statistical analysis. Second, we performed a diet of short duration to limit the risk of non-compliance and withdrawals whereas a 6-week diet period is recommended (27). We cannot exclude the fact that a 2-week diet could miss a potential effect on fecal microbiota (11). Third, there was a probable lumping together of heterogeneous patient groups according to phenotype.

Conclusion

We have demonstrated that a 2-week low fructose diet significantly improved the bowel symptoms and quality of life of IBS patients whatever their fructose absorption status defined by a FBT performed with a 25 g load of fructose. The FBT as performed in this study cannot be used to predict the beneficial effect of a low fructose diet. As noted in our study, IBS patients often followed a diet which was more or less appropriate and sometimes with other consequences particularly on weight. Further studies are needed to identify the best predictive test that would provide an opportunity to rationally introduce dietary changes with the goal of achieving better symptom control.

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Tables

Table 1: Low fructose diet

<u>FOOD</u>	<u>NOT RECOMMENDED</u>	<u>RECOMMENDED</u>
<u>MILK AND DAIRY PRODUCTS</u>	Sweetened milk Dairy based sweetened desserts Dairy based fruit desserts, flavoured Milk based drinks Custard desserts Egg custard, mousse	Milk (powder or liquid) Yoghourt Cottage cheese } natural or without added sugar Petits suisses/fromage blanc Unsweetened desserts Cheese
<u>MEAT</u> <u>FISH</u> <u>EGGS</u>	Cold meats (Ham, sausage, saucisson, pâtés, white and black pudding,), quenelles, fish mousse, ready made meals	All meat, poultry Offal Fish Eggs
<u>CARBOHYDRATES</u>	Ready made meals, Manufactured cooked potato products Manufactured deep frozen fries Sweetened flour based products Dried vegetables and pulses Whole grains (oat, wheat, rye, barley, millet etc.) Whole-grain pasta, vegetable pasta Whole-grain rice	Potatoes, chips Rice, tapioca, pasta, semolina Wheat, buckwheat, oat, corn rice flour
<u>BREAD</u>	Sweetened bread, other bread crackers, toast Sweetened cereals	White bread (80 g/day), Rice crackers
<u>GREEN VEGETABLES</u>	Raw vegetables Dried vegetables Manufactured cooked vegetables Bean sprouts	Except cooked vegetables (200g/day): Spinach, lettuce, green beans, butter beans, carrots, celery, courgettes, tomatoes, water cress, cauliflower, avocado
<u>FRUIT</u>	All fresh fruit, cooked or not Fruit in syrup Stewed fruit, with or without sugar Dried fruit Candied fruit	Except (1 per day): Apricot, grapefruit, lemon, nectarine, orange, mandarin, peach, pineapple, rhubarb



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<u>FATTY PRODUCTS</u>	Manufactured sauces Manufactured mayonnaise	Butter Oil Fresh cream Homemade mayonnaise
<u>SWEET PRODUCTS</u>	Sugar Honey, marmalade, jam, jelly Pastries Puff pastry, shortcrust pastry Bread pastries (brioche, croissant, pain au chocolat, pain aux raisins, etc.) Sweets biscuits, meringues, gingerbread Whipped cream Ice cream and sorbets Confectionery, dragees, nougat, fruit jellies Chestnut mousse Chocolate and chocolate bars Sweetened chocolate powder	Unsweetened cocoa
<u>DRINKS</u>	Fermented drinks (cider, beer) Wine, aperitifs, liqueurs Fruit juices Fruit syrup Soda, lemonade Flavoured water Dairy based sweetened drinks	Still and sparkling water Flavoured water (with artificial sweeteners) Coffee, herbal infusions, tea
<u>CONDIMENTS</u>	Manufactured sauces Manufactured mayonnaise Tomato ketchup Mustard Manufactured tomato sauce Flavoured vinegar Garlic, onion, shallots Pickles and capers Stock cubes	Salt, pepper Gelatine Non-flavoured vinegar
<u>MISCELLANEOUS</u>	Some artificial sweeteners: -Sucralose	Some sugars: glucose, lactose, maltose and dextrine maltose Some artificial sweeteners: aspartame, saccharine, cyclamate, thaumatococine polyols (mannitol, sorbitol)

Table 2: Demographics and baseline variables for all included IBS patients

	Total (n=88)	Negative fructose breath test (n = 48)	Positive fructose breath test (n = 40)	Adjusted p
Female n(%)	73 (83)	44 (91.7)	29 (72.5)	0.017
Age (years)	45.8 [31.2; 57.1]	41.2 [29; 51.2]	49.6 [39.9; 58.8]	0.019
Weight (kg)	63.5 [55; 74.5]	62.5 [55.5; 71]	64.5 [55; 82.5]	0.397
Height (cm)	166 [160; 170.5]	165 [160; 170]	166 [160.5; 171]	0.703
BMI	22.9 [21.1; 26.6]	22.7 [21.1; 26]	23.3 [21.2; 28.1]	0.348
IBS-SSS	274.5 [193; 308.5]	277.5 [166.5;309.5]	269 [215; 303.5]	0.633
Bristol Stool Scale	4 [2.5; 6]	4 [2; 6]	4 [3; 6]	0.956
GIQLI	87.5 [67.5;98]	88 [70; 97]	87 [66; 101.5]	0.621
HAD-A	9 [7; 13]	11 [8; 14]	9 [6; 12.5]	0.072
HAD-D	5 [3; 8]	5.5 [3; 8.5]	5 [2.5; 8]	0.506

BMI: Body mass index. IBS-SSS: IBS symptom severity score. GIQLI: Gastrointestinal Quality of Life Index. HAD-A and B: Hospital anxiety and depression scale (HAD)
Results were presented as number (percentage) and median with first and third quartile [Q1-Q3].

Table 3: Effect of fructose restricted diet on digestive symptoms, quality of life, Bristol Stool Scale, HAD scores, weight and body mass index for all included IBS patients

	Before diet N=88	End of 2-week diet N=77	p
IBS-SSS	274.5 [193; 308.5]	168 [112; 253]	1.10^{-4}
Bristol Stool Scale	4 [2.5; 6]	4 [3; 5]	0.166
GIQLI	87.5 [67.5;98]	98 [81; 112]	1.10^{-4}
HAD-A	9 [7; 13]	9 [6; 13]	0.018
HAD- D	5 [3; 8]	4 [2; 7.5]	0.005
Weight (kg)	63.5 [55; 74.5]	63 [55; 73.5]	1.10^{-4}
BMI	22.8 [21.1; 26.6]	22 [19.9; 26]	1.10^{-4}

BMI: Body mass index. IBS-SSS: IBS symptom severity score. GIQLI: Gastrointestinal Quality of Life Index. HAD-A and B: Hospital anxiety and depression scale (HAD)
Results are presented as number (percentage) and median with first and third quartile [Q1-Q3].

Table 4: Comparison of the variations of Bristol Stool Scale, GIQLI and HAD scores, evaluated after 2-week low fructose diet between patients with and without fructose malabsorption

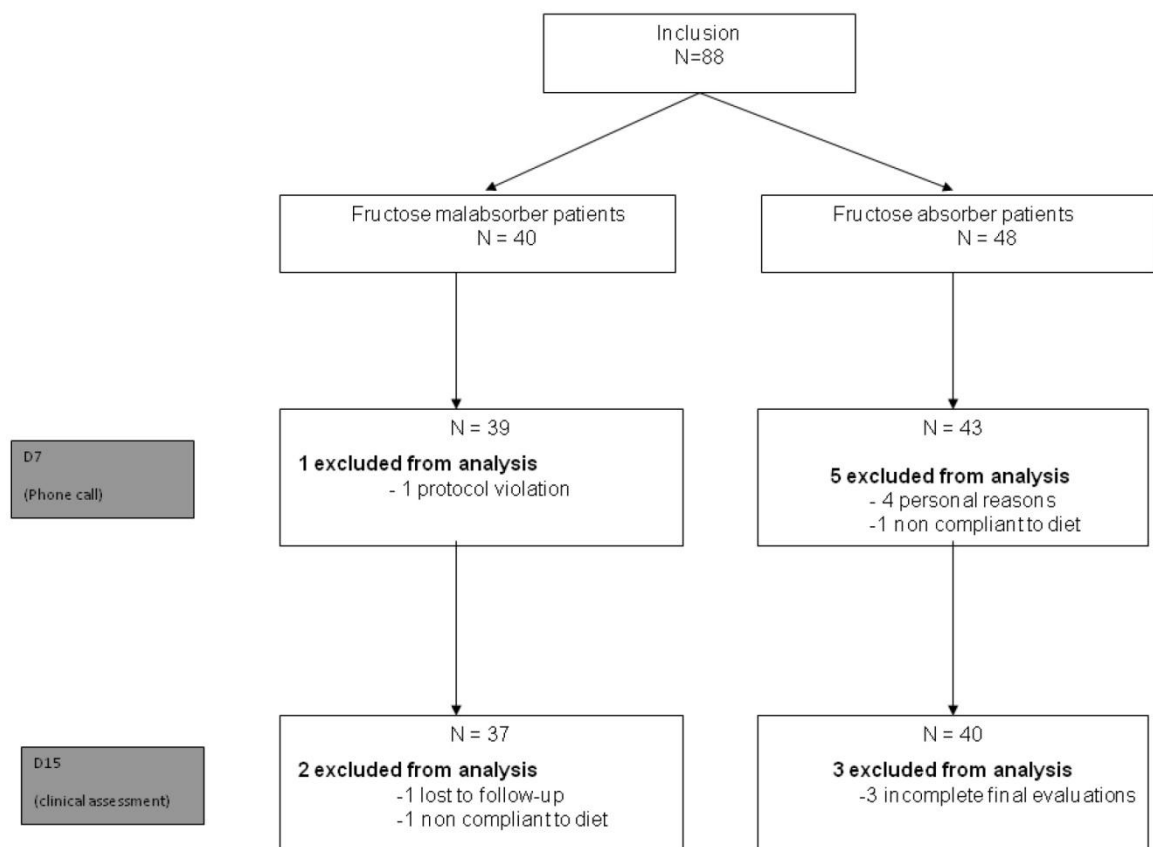
	Absorber group (N = 48)	Malabsorber group (n = 40)	Adjusted p
Bristol Stool Scale	0 [-1.5; 1]	0 [-2; 1]	0.8635
GIQLI	16.5 [7; 26]	8 [-2; 17]	0.0976
HAD-A	-1.5 [-3.5; 0.5]	0 [-1; 2]	0.0533
HAD-D	-1 [-3; 1]	0 [-2; 1]	0.1410

GIQLI: Gastrointestinal Quality of Life Index. HAD-A and B: Hospital anxiety and depression scale (HAD)

Results are presented as number (percentage) and median with first and third quartile [Q1-Q3].

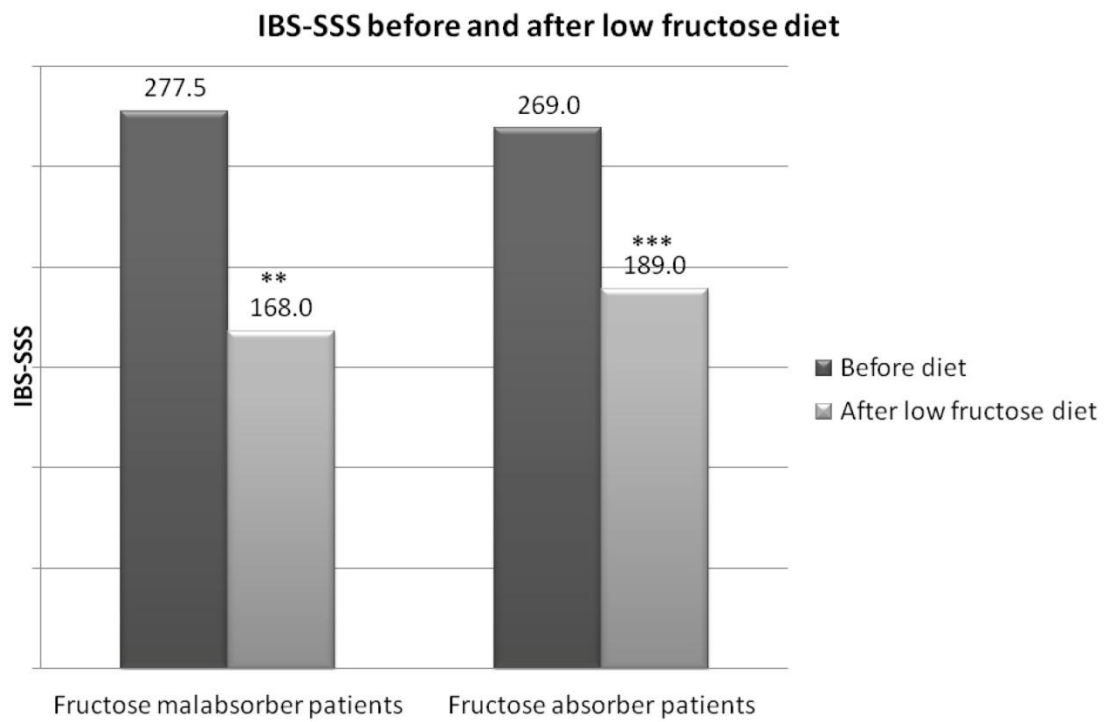
Figure legends

Figure 1: Flow shart



D: Day

Figure 2: IBS-SSS before and after low fructose diet



IBS-SSS: IBS symptom severity score

****:** $p < 0.01$ *****:** $p < 0.001$

Article 4 : Visceral hypersensitivity and altered intestinal permeability in mouse model of irritable bowel syndrome with fructose malabsorption

Cet article englobe la majorité des résultats fondamentaux de ma thèse de science. Un des objectifs était d'étudier l'association entre malabsorption du fructose et l'hypersensibilité chez les patients souffrant d'un SII. Une association a été retrouvée puisqu'1 patient sur 2 avec malabsorption du fructose était hypersensible pour seulement 1 sur 10 en l'absence de malabsorption du fructose. La modélisation sur 3 modèles murins de malabsorption du fructose (par régime riche en fructose, par délétion des transporteurs du fructose GLUT5 et GUT2) permettait de reproduire ce même phénotype associé à une augmentation de la perméabilité intestinale. L'étude des mécanismes physiopathologiques sous-jacents a permis d'écarter l'implication d'une inflammation de bas grade qui n'était pas retrouvée chez nos souris. En revanche, une activité élastase augmentée a été retrouvée dans les selles de 2 de nos modèles de souris avec malabsorption du fructose (GLUT5 KO et régime riche en fructose) en comparaison aux contrôles. Or il a déjà été démontré que l'activité protéasique pouvait être responsable d'une hypersensibilité viscérale et d'une augmentation de la perméabilité intestinale (121). Cette augmentation d'activité élastase pourrait être d'origine pancréatique et être induite par l'augmentation de la cholécystokinine (CCK) (Résultats V. Douard souris KHK/-). Ces résultats nous permettent de mieux comprendre le phénotype et les mécanismes physiopathologique dans le SII. Il reste encore à élucider par quel mécanisme le fructose va entraîner une augmentation de CCK ainsi que l'implication du microbiote.

Contrairement à des souris WT dont le microbiote a été déplété par antibiotique, nous avons pu démontrer que des souris axéniques mise sous régime riche en fructose ne développaient pas d'hypersensibilité viscérale, cependant leur activité protéasique dans les selles était également élevée (mais elle l'est également de base, Résultats V. Douard). L'absence de sensibilisation pourrait être expliquée par l'absence de système nerveux entérique mature en l'absence de microbiote (122).

Résumé article 4 : Hypersensibilité viscérale et augmentation de la perméabilité de la barrière intestinale dans des modèles murins de syndrome de l'intestin irritable avec malabsorption du fructose

Introduction : L'association entre malabsorption du fructose et hypersensibilité viscérale n'a jamais été démontrée.

Objectifs : Notre objectif était de déterminer si la malabsorption du fructose est associée à une hypersensibilité viscérale chez l'Homme. Nous avons également modélisé la malabsorption du fructose sur des modèles de souris pour essayer d'identifier les mécanismes physiopathologiques sous-jacents.

Méthodes : Nous avons étudié l'association entre malabsorption du fructose et hypersensibilité viscérale (mesurée à l'aide d'un barostat rectal) chez des patients souffrant d'un SII, selon les critères de Rome III. Nous avons reproduit 3 modèles de la malabsorption du fructose : GLUT5^{-/-}, invalidation intestinale de GLUT2 (GLUT2^{ΔIEC}) et souris WT soumises à un régime riche en fructose. Dans ces modèles nous avons évalué l'hypersensibilité viscérale par barostat rectal, la perméabilité intestinale dans le sérum après gavage de FITC-dextran, l'inflammation colique par le dosage de l'activité MPO et du taux d'ARNm de certaines cytokines et l'activité protéasique dans le colon et les fèces.

Résultats : Trente-huit patients SII ont été étudiés (81.6% de femme de moyenne d'âge 41.5 ± 14.7 ans). L'hypersensibilité viscérale était associée à la malabsorption du fructose dans 45.4% des patients et seulement à 11.1% des patients absorbeurs (p=0.03). Nos 3 modèles de malabsorption du fructose présentaient une hypersensibilité viscérale (WT avec régime riche en fructose, GLUT5^{-/-}, GLUT2^{ΔIEC}, p<0.0001, p=0.05 and p=0.009) et une augmentation de la perméabilité intestinale (WT avec régime riche en fructose, GLUT5^{-/-}, GLUT2^{ΔIEC}, p<0.05 en comparaison aux contrôles). L'activité élastase dans les fèces mais pas dans le colon était plus élevée chez les WT avec régime riche en fructose et les GLUT5^{-/-} en comparaison aux contrôles (23.2 U/mg ± 28.7 and 35.8 U/mg ± 30.8 vs 4.6 U/mg ± 10.3, p=0.02 and p=0.01). L'hypersensibilité viscérale n'était pas associée à l'inflammation chez les patients SII (dosage de calprotectine fécale identique) de même que chez les souris.

Conclusion : Chez les patients SII et chez les souris, la malabsorption du fructose est associée à une hypersensibilité viscérale et une augmentation de la perméabilité intestinale qui pourrait être induite par un mécanisme PAR-2 avec une augmentation de l'activité élastase dans les fèces.

**Visceral hypersensitivity and altered intestinal permeability in
mouse model of irritable bowel syndrome with fructose
malabsorption**

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Abstract :

Objective : Our aim was to determinate whether fructose malabsorption could be a cause of visceral hypersensitivity in irritable bowel syndrome (IBS) patients and to identify the mechanisms triggering the nociceptive response to fructose using mouse models.

Design : We assessed visceral hypersensitivity using a rectal barostat in IBS patients according to Rome III criteria. We then used 3 mouse models of fructose malabsorption, namely GLUT5^{-/-}, intestinal invalidation of GLUT2 (GLUT2^{ΔIEC}) and high-fructose fed (HF) mice. In these models, we assessed visceral pain response to colorectal distension, intestinal permeability by measuring serum FITC-dextran after gavage, intestinal inflammation using MPO assays and cytokine mRNA levels and protease activity both in colon and faeces.

Results : Thirty-eight IBS patients were studied (81.6% of women with mean age of 41.5 ± 14.7 years). 45.4 % of IBS patients with fructose malabsorption showed visceral hypersensitivity compared to 11.1% without fructose malabsorption (p=0.03). Visceral hypersensitivity was unrelated to intestinal inflammation in IBS patients since faecal calprotectin levels were similar in patients with or without fructose malabsorption. Fructose malabsorption induced visceral hypersensitivity in 3 mouse models (HF, GLUT5^{-/-} and GLUT2^{ΔIEC}, p<0.0001, p=0.05 and p=0.009). This was associated with increased intestinal permeability (HF, GLUT5^{-/-} and GLUT2^{ΔIEC} compared to controls, p<0.05). Intestinal inflammation markers (MPO and intestinal cytokine levels) remained unchanged in mouse models of fructose malabsorption. Elastase activity in faeces, but not in colonic mucosa was increased in fructose malabsorption models of HF and GLUT5^{-/-} compared to control mice (p=0.02 and p=0.01 respectively).

Conclusion: In IBS patients, fructose malabsorption is associated with visceral hypersensitivity independently of intestinal microinflammation. In mouse models, fructose malabsorption increases visceral hypersensitivity that was associated with increased intestinal permeability and faecal elastase activity, while intestinal inflammatory markers remains unmodified. Further studies should be performed to evaluate whether limitation of fructose consumption improves visceral hypersensitivity in IBS patients.

Key words : Irritable bowel syndrome -Visceral hypersensitivity - Fructose malabsorption - Intestinal permeability - Intestinal invalidation GLUT2 -GLUT5 KO - Elastase activity - faecal calprotectin

Introduction

The deleterious role of fermentable, oligo-, di- and mono-saccharides or polyols (FODMAPs) as one of the key trigger of gastro-intestinal symptoms was recently evidenced (1). Among FODMAPs, fructose intake is increasingly consumed worldwide (2). The two major transporters for fructose are GLUT2 and GLUT5. Fructose absorption is dose-dependent due to the limited absorption capacity of these transporters, even in healthy subjects (3). In western Europe and USA, fructose intake increased up to 50g per day and represents 10% of the total caloric intake (4). Eighty percent of healthy subjects are unable to absorb a fructose load of 50g per day, while 90 % of healthy volunteers absorb a 25g load (5). By contrast, we have previously shown that patients with irritable bowel syndrome (IBS) displayed fructose malabsorption for a load of 25g in 22% of case (6). Non absorbed fructose is broken down by colon bacteria into short fatty acids, CO₂ and H₂ (7). Consequences of fructose malabsorption include increased osmotic loading leading to abdominal pain and diarrhoea. However, while fructose malabsorption -induced gut distension is similar in asymptomatic subjects and IBS patients, the latter report more symptoms (7). This suggests that other mechanisms may be involved in fructose malabsorption -triggered symptoms, including visceral hypersensitivity, impaired intestinal permeability or low grade inflammation. Recent pathophysiological studies have highlighted the existence of a low-grade mucosal immune activity in the intestine of a sub-group of IBS patients (8, 9). In fact, these three features have been pointed out as key mechanisms generating IBS symptoms (10). Recently, the role of proteases have been underlined in IBS and could promote visceral hypersensitivity through a protease-activated receptor-2-dependent mechanism (PAR2) (11).

To date, the association between fructose malabsorption and visceral hypersensitivity in Human is not known. Therefore, we assessed in the present study whether fructose malabsorption was associated with visceral hypersensitivity in an IBS population. We then

modelled this feature in mouse models of fructose malabsorption in order to identified mechanism underlying visceral hypersensitivity induced by fructose malabsorption.

Material and Methods

-1) Patients

Our prospective study involved thirty-eight consecutive IBS patients according to Rome III criteria (12) with standard tests, including at least flexible colonoscopy and standard biological tests, to rule out organic disease. The research was performed according to the declaration of Helsinki. All patients gave their informed consent. The use of data was declared to the French Committee for the use of electronic data (CNIL) (n° 817.917). Demographic data (age, sex, weight and size) were collected and IBS phenotypes were determined by validated self-questionnaires filled by the patient on a touch pad. IBS severity was assessed by the IBS symptom severity score (IBS-SSS). Anxiety and depression levels were assessed using the hospital anxiety and depression scale (HAD) (13). The validated Gastrointestinal quality of life index (GIQLI) assessed quality of life (14).

Fructose breath test

A strict diet administrated during the 48 hours prior to fructose breath test in order to reduce intestinal gas production that could distort the results of the test. This diet excluded bread, biscuits, cheese, fruits, vegetables, dairy products, fermented drinks, sodas, honey, marmalade, candy, chocolate, ice cream and pastry. In the evening prior to the tests, dinner was exclusively composed of rice and meat.

The fructose breath test (FBT) was performed in patients with a negative glucose breath test to avoid false positive results due to small intestinal bacterial overgrowth as previously described (6). Patients ingested 25g of fructose dissolved in 250 cc of sterile water then end alveolar breath samples were collected every 30 minutes for 5 hours. Both H₂ and CH₄ levels

were calculated. The test was considered as positive in case of a rise in H₂ and/or CH₄ levels above 20 ppm. In addition, during the whole test, patients were asked about the onset of abdominal symptoms.

Rectal barostat testing

A rectal distension test with an electronic barostat-distender assembly (Distender II; G & J Electronics, Toronto, ON, Canada) was performed to assess visceral sensitivity as previously described (15). A progressive isobaric rectal distension protocol with ascending method of limits was performed. Patients were asked to report their painful sensations on a 0-10 VAS during the last 30 seconds of each step. The balloon was progressively distended until pain was reported or the last distending step was reached (40 mmHg). Distensions were stopped when the patient scored a painful sensation of at least 3 on a 10-point VAS. Visceral hypersensitivity was defined by a pain threshold lower than 24 mmHg (16).

Faecal calprotectin

Faecal calprotectin levels were determined by an ELISA test (Bühlmann labs, Schönenbuch, Switzerland) on 50 to 100mg of faeces. Dosage was realized on 50-150 µL of faeces extract. Calprotectin offers a dynamic range from 10 to 1800µg/g. The test allows for the selective measurement of calprotectin-antigen by sandwich ELISA. Absorption was measured at 450 nm as previously described (9).

-2) Mice

Animals

Animal care and experimentation were approved by the local Committee on the Ethics of Animals Experiments (CENOMEXA n°54 and by the Ministry of Research (Ethical agreement number: n°05354.04) and by the local animal ethical committee (COMETHEA at Jouy-en-Josas, France, APAFIS#1620-2015102618572930v2).

All experiments were carried out on male C57BL/6J mice (Janvier Labs, Le Genest-St-Isle, France) or genetically modified mice as described below. Mice were housed in a controlled environment with a 12:12-h light-dark cycle and allowed access to diet and water ad libitum. Mice aged of 5-7 weeks were either fed with 1/ a normal-fructose diet contained around 1.7g fructose/100g of fructose (AO3, SAFE DIETS, Augy, France); or 2/ with a HF contained 70 Kcal % fructose (D08040107, research diet, New Brunswick, USA); or 3/ with a fructose-free diet which contained no fructose (D12450K, research diet, New Brunswick, USA) (**Table 1, supplementary data**). All mice were fed with specific diet at least 3 weeks before experimental procedures.

GLUT2^{ΔIEC} mice.

Slc2a2^{FLOX/FLOX} × Villin CreERT2^{+/−} and *Slc2a2^{FLOX/FLOX} × Villin CreERT2^{−/−}* mice were generated in INSERM UMR-S 1138 (Centre de Recherche des Cordeliers, Paris, France) (17). In the *Slc2a2^{FLOX/FLOX} × Villin CreERT2^{+/−}* mice, Cre recombinase was activated by tamoxifen gavage (1 mg/mouse; Sigma-Aldrich) once a day for three consecutive days to induce a specific *Slc2a2* deletion in intestinal epithelial cells. *Slc2a2^{FLOX/FLOX} × Villin CreERT2^{−/−}* received the same tamoxifen gavage and were used as controls littermate. All mice were on a C57BL/6J background. Mice were genotyped as previously described (17) using the following PCR primers: CreVillin F: 5'-CAAGCCTGGCTCGACGGCC-3'; R: 5'-

CGCGAACATCTTCAGGTTCT-3'; Glut2Flox F: 5'-
TCTTGGCTTTGGGTGAAAATGAGCA-3'; R2: 5'-GAACTTCGGTTCCGGCGCCA-
3' (floxed allele, 239 bp) ; R7: 5'-AGGAGCAGTGGTTTGGATGGCA-3' (wild-type allele,
201 bp). GLUT2^{AIEC} mice and their controls littermate received normal-fructose diet.

GLUT5^{-/-} mice

GLUT5^{-/-} mice (18) and their controls littermate (WT on the C57BL/6J background) were kindly provided by Dr. Douard (INRA Micalis Jouy-en-Josas). Heterozygous (F1) were generated by crossing GLUT5^{-/-} mice with C57BL/6J mice (Charles River, France). In addition, F2 offspring were generated by intercrossing F1 mice, and F3 WT and F3 GLUT5^{-/-} offspring were generated by intercrossing WT F2 mice and GLUT5^{-/-} F2 mice, respectively. GLUT5^{-/-} and their controls littermate received fructose-free diet until they were 5-7 weeks old, then normal-fructose diet.

Colorectal distension

We measured visceral pain to colorectal distension using a non-invasive method as previously reported in mice (19). An infinitely compliant distension bag (diameter 0.7cm) was made using a polyethylene bag attached to a PE-50 catheter (Intramedics, France) drilled in its end and taped 2 cm below the pressure sensor of a miniaturized pressure transducer catheter (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston, TX, USA). Ligature points (silk 4.0) were covered with parafilm to prevent any air leak.

On experimental day, mice were briefly anesthetized with isoflurane (3% in O₂) and the lubricated “balloon-pressure sensor” was introduced into the colorectum, so that the balloon

was inserted 2 cm past the anal marge into the colon. Each mouse was placed in an adjustable mouse restrainer (30 mm diameter × 90 mm length, LE5016, In vivo research instruments, USA), and left to rest for 30 min before the colorectal distension (CRD) procedure. The balloon was then secured to the tail with tape and connected to an electronic barostat (G&J Electronics, Toronto, Canada) to perform isobaric graded CRD.

At the start of each experiment, each “balloon-pressure sensor” was calibrated. The CRD protocol consisted of a set of graded phasic distensions to constant pressures of 15, 30, 45, and 60 mmHg (two times each, 20 s duration, 4 min inter-stimulus interval) (Protocol Plus Deluxe, G&J Electronics, Toronto, Canada). Voltage output was converted to pressure using CED digital analogue converter (Micro 1401, Cambridge Electronic Design, Cambridge, UK) and Spike 2 software (CED, Ltd., Cambridge, UK). The pressure sensor allowed the measurement of visceral pain by a custom-made script which allowed to specifically extracting signals from abdominal muscle contractions (excluding colonic contractions).

Intestinal permeability

Intestinal permeability was assessed by luminal gastro-intestinal gavage of FITC-dextran (4 kDa; Sigma-Aldrich) as previously described (20). Intestinal permeability was measured in GLUT2^{ΔIEC}, GLUT5^{-/-} and their controls (normal-fructose diet) and in WT mice (HF). Mice were fasted 6 hours and for the remainder of the experiment. Mice were gavaged with FITC-dextran (0.6 mg/ g body weight) 4 hours before bleeding. A serum of WT mice was used as control. The concentration of FITC-dextran in serum was quantified by spectrophotofluorometry (485/535 nm).

Myeloperoxidase assay

Myeloperoxidase (MPO) activity, a marker of polynuclear neutrophils, was measured in colon of normal-fructose fed GLUT2^{ΔIEC} mice and controls as described previously (21). Briefly, frozen pieces of the distal colon (50 mg/ml) were homogenised in a phosphate buffer (50 mmol/L, pH = 6) containing hexadecyl trimethyl ammonium bromide (0.5% w/v) with a tissue lyser II (Qiagen, France). The homogenates were submitted to 3 cycles of 30 minutes freezing/thawing (Liquid N₂, 1 min/37 °C, 10 min) and then further disrupted with a sonicator (Bioblock scientific, France) and then centrifuged (6000 g at 4 °C for 15 min). Supernatants were collected for measuring MPO activity and total protein contents. Samples were diluted into a reaction buffer containing O- dianisidine dihydrochloride (1 mg/mL) and hydrogen peroxide (3×10^{-4} % v/v). Human neutrophil MPO was used as a standard. The absorbance was measured after 10 min of incubation at 450 nm. Total protein content was assessed from the supernatants according to Lowry's method (Bio Rad DC Protein Assay, France).

Colonic cytokines mRNA levels measured using RT-qPCR

RNA of IL1 β , CxCl1 and TNF α were measured by RT-qPCR in pieces of colon in normal-fructose fed GLUT2^{ΔIEC} mice and their controls. After reverse transcription of 1.5 μ g total RNA into cDNA by using 200 units of SuperScript™ II Reverse Transcriptase (LifeTechnologies) as previously described, qPCR was performed by SYBR™ Green technology on BioRad CFX96 real time PCR system (BioRad Laboratories, Marnes la Coquette, France) in duplicate for each sample. GAPDH was used as the endogenous reference gene. We used previously described specific primers for IL1 β : F:

CCCAAAAGATGAAGGGCTGC and R: AAGGTCCACGGGAAAGACAC, for TNF α : F:

TGTCTACTCCTCAGAGCCCC and R: TGAGTCCTTGATGGTGGTGC and for CxCl1: F: ACTCAAGAATGGTCGCGAGG and R: GGGACACCTTTTAGCATCTTTTGG.

Trypsin and elastase activity in colonic tissues and faeces

Proteolytic activity (trypsin-like and elastase/PR-3 activity) were measured in colon and faeces in normal-fructose diet fed Glut5^{-/-} and GLUT2^{ΔIEC} and in HF fed WT. Trypsin-like and elastase/PR-3 activities were measured as previously described (22) using tosyl-Gly-Pro-Arg-*p*-nitroanilide (150 μmol/L; Sigma, Saint Quentin Fallavier, France) and MeO-succinyl-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μmol/L; Sigma, Saint Quentin Fallavier, France), respectively, as substrates. Samples (20 μL for trypsin activity or 10 μL for elastase/PR-3 activity) were resuspended in their respective buffer: 100 mmol/L Tris/HCl, 1 mmol/L CaCl₂, pH = 8 for trypsin activity and 50 mmol/L Tris-HCl, 500 mmol/L NaCl, 0.1% Triton X-100 for elastase/PR-3 activity. The change in absorbance at 405 nm was determined over 30 minutes at 37°C with a microplate reader (NOVOstar; BMG Labtech, Champigny, France). Activity was compared with known standard dilution of trypsin from porcine pancreas (Sigma) or human NE (Sigma). Protein concentration was determined using a BCA kit (Pierce, Thermo Scientific, Courtaboeuf, France).

Statistical analysis

Statistical comparisons were performed using GraphPad Prism 5 software. Data are expressed as mean ± SEM and number (percentage). Gaussian distribution was checked with Kolmogorov-Smirnov's test. Parametric quantitative data were analyzed with Student's t-test and non parametric quantitative data with Mann Whitney's test. Qualitative data were

analyzed with chi-square test and Fisher's test for low number ($n < 5$). Differences among group were assessed using two-way ANOVA followed by Bonferroni's multiple comparison tests or Dunn's multiple comparison tests. Differences were considered as significant at $P < 0.05$.

Results

Fructose malabsorption is associated with visceral hypersensitivity in IBS patients

Patient characteristics are provided *in supplementary table 1*. Fructose malabsorption and visceral hypersensitivity were detected in 11 (28.9 %) and 8 (21.0%) of the 38 IBS patients, respectively. There was no demographic differences between patients with or without fructose malabsorption. Visceral hypersensitivity was observed in 5 (45.4%) of patients with fructose malabsorption and in 3 (11.1%) of patients with normal fructose absorption ($p=0.03$) (*figure 1*).

Fructose malabsorption is associated with visceral hypersensitivity in mice

In WT mice, 3 weeks of HF induced visceral hypersensitivity to colorectal distension compared to fructose-free fed mice ($p<0.0001$) (*figure 2A*). Likewise, normal-fructose fed GLUT2^{ΔIEC} mice developed visceral hypersensitivity to colorectal distension when compared to control fed with the same diet ($p=0.009$) (*figure 2B*). Lastly, 3 weeks of normal-fructose fed GLUT5^{-/-} mice developed visceral hypersensitivity to colorectal distension when compared to fructose-free fed GLUT5^{-/-} mice ($p=0.05$) (*figure 3*).

Fructose malabsorption is associated with altered intestinal permeability in mice

Normal-fructose fed WT mice served as controls. Intestinal permeability was increased in WT mice fed with HF compared to controls (4.81 ± 0.02 mg/ml versus 0.92 ± 0.12 mg/ml, $p<0.05$). A smaller increase of intestinal permeability was observed in GLUT5^{-/-} mice fed with normal diet when compared to controls (2.40 ± 1.44 mg/ml versus 0.92 ± 0.12 mg/ml, $p<0.05$).

$p < 0.05$). A smaller increase of intestinal permeability was noted in GLUT2^{ΔIEC} mice when compared to controls ($1.31 \text{ mg/ml} \pm 0.38$ vs $0.92 \text{ mg/ml} \pm 0.12$, $p = 0.02$) (*figure 4*).

Fructose malabsorption is not associated with intestinal inflammation neither in IBS patients nor in GLUT2^{ΔIEC} mice

Faecal calprotectin was not different between patients with or without fructose malabsorption ($56.1 \pm 41.0 \text{ } \mu\text{g/g}$ and $76.1 \pm 91.6 \mu\text{g/g}$, $p = 0.82$).

Normal-fructose fed WT mice served as controls. In normal-fructose fed GLUT2^{ΔIEC} mice, MPO activity was not different in colon compared to controls ($0.008 \text{ U/mg protein} \pm 0.006$ versus $0.009 \text{ U/mg protein} \pm 0.009$, $p = 0.80$). Likewise, colonic IL1 β ($p = 0.19$), CxCl1 ($p = 0.63$) and TNF α ($p = 0.90$) mRNA levels were not different between GLUT2^{ΔIEC} mice and controls.

Fructose malabsorption is associated with increased faecal elastase activity in mice

Normal-fructose fed WT mice served as controls. Elastase activity in faeces was higher in HF fed WT mice compared to controls ($23.2 \text{ U/mg} \pm 28.7$ vs $4.6 \text{ U/mg} \pm 10.3$, $p = 0.02$). Likewise, faecal elastase activity increased in GLUT5^{-/-} compared to controls ($35.8 \text{ U/mg} \pm 30.8$ vs $4.6 \text{ U/mg} \pm 10.3$, $p = 0.01$). However, faecal elastase activity was not different between GLUT2^{ΔIEC} mice and controls ($3.7 \text{ U/mg} \pm 7.6$ vs $4.6 \text{ U/mg} \pm 10.3$, $p = 0.28$) (*figure 5*). By contrast, faecal trypsin activity was not different between WT (HF), GLUT5^{-/-}, GLUT2^{ΔIEC} and controls ($68.7 \text{ U/mg} \pm 84.4$, $45.4 \text{ U/mg} \pm 30.5$, $64.6 \text{ U/mg} \pm 56.1$ and $29.9 \text{ U/mg} \pm 20.9$, $p = 0.30$). Colonic elastase activity in colon was lower in WT (HF) compared to controls ($0.014 \text{ U/mg} \pm 0.007$ vs $0.028 \text{ U/mg} \pm 0.011$, $p = 0.02$) but was not different in GLUT5^{-/-},

GLUT2^{ΔIEC} and controls (0.017 U/mg ± 0.015, 0.027 U/mg ± 0.004 and 0.028 U/mg ± 0.011, p= NS). Trypsin activity in colon was lower in GLUT5^{-/-} compared to controls (0.17 U/mg ± 0.23 vs 0.41 U/mg ± 0.25, p=0.05) but was not different between WT (HF), GLUT2^{ΔIEC} mice and controls (0.29 U/mg ± 0.38, 0.66 U/mg ± 0.46 and 0.41 U/mg ± 0.25, p=NS).

Discussion

In the present work, we choose to focus on fructose rather than oligofructosacharides. This was because oligofructosacharides are not physiologically absorbed. Therefore, all IBS patients as asymptomatic subject may have diet-increased intraluminal content of oligofructosacharides leading to water osmotic load and to gas production due to fermentation. Consequently, there are currently no routine tests to identify patients susceptible to report oligofructosacharides-induced symptoms neither from patients without such association nor from normal subjects. By contrast, mechanisms of fructose absorption have been clearly established. Moreover, routine tests are available to diagnose fructose malabsorption and fructose malabsorption induced symptoms.

Visceral hypersensitivity to colorectal distension is a key feature of IBS. This parameter is currently considered to be the most robust functional assay in IBS patients. Indeed, visceral hypersensitivity is correlated with symptom scores and quality of life, and is a strong predictor of therapeutic response (23). Using colorectal distension with the barostat technique, we found an association between fructose malabsorption and visceral hypersensitivity both in IBS patients and mouse models. Although previous works suggested that oligofructosacharides could induce visceral hypersensitivity in mice (24), our study is the first to evidence visceral hypersensitivity in IBS patients with fructose malabsorption. In fact, such association was already suspected in the work of *Major et al.* In this work, 29 IBS patients

and 29 healthy volunteers underwent MRI after fructose intake. Fructose increased small-bowel water in the two groups, but IBS patients had higher symptoms scores (25). In addition, we demonstrated that fructose malabsorption induced visceral hypersensitivity in three different mouse models. We use two models of fructose malabsorption, with the deletion of fructose transporters GLUT2 and GLUT5 (26, 27) and one model with fructose malabsorption induced by high fructose intake. These results are of relevance since traditional models of fructose malabsorption -induced symptoms in IBS relies on water osmotic load and to gas production due to fermentation. Visceral hypersensitivity may therefore explain why, given similar gut distension induced by fructose malabsorption, IBS patients report more painful and/or discomfort symptoms than healthy volunteers (25). Previous works have shown in IBS patients, as well as in murine models, that visceral hypersensitivity was associated with altered intestinal permeability (10). Altered intestinal permeability may favour the penetration of antigens and/or microorganisms into the gut wall, leading to primary afferent nerve sensitization. In fact, whether fructose induces altered intestinal permeability is controversial. Previous studies (28) have shown that HF was associated with altered permeability of the small bowel while we reported that GLUT2^{ΔIEC} mice had unaltered of small bowel permeability (17). In the present work, we found that visceral hypersensitivity was associated with altered whole gut permeability. Considering previous works, our study therefore suggests that colonic, rather than small bowel permeability is altered in the three fructose malabsorption mouse models we used. One explanation to these discrepant results may rely on blood glucose that has recently been shown to modify intestinal permeability (29). We did not measured blood glucose in our study as the report from *Thais et al.* was not published at the time we were running permeability assays (29). Further works are therefore warranted to elucidate whether fructose malabsorption -induced altered permeability in these mouse models relies on blood glucose or not.

Involvement of micro-inflammation in IBS has been reported although this remains undetectable to routine tests. For instance, *Bashashti et al* found that mast cells and T cells were increased in colonic biopsies of patients with IBS. However, these changes were segmental and IBS-subtype dependent (30). By contrast, *Boeckxstaens et al.* reported that the number of mast cells, macrophages and T cells was decreased in IBS patients in comparison to healthy volunteers and was not associated with the presence of visceral hypersensitivity (16). In our study, we found that fructose malabsorption was unrelated to mucosal inflammation as demonstrated by MPO assay or mucosal cytokine levels measured using RT-PCR in mice, and by using faecal calprotectin in IBS patients. By contrast, *Zhou et al.* observed in mice that enriched oligofructosacharides diet was associated with gut dysbiosis and elevated faecal LPS levels (24). In this study, these alterations were associated with visceral hypersensitivity (24). Whether IBS patients with fructose malabsorption have elevated faecal LPS levels remains to be further demonstrated.

The role of proteases has been underlined in IBS and could promote visceral hypersensitivity and increased in intestinal permeability through a protease-activated receptor-2 (PAR-2)-dependent mechanism (11). Especially, Trypsin 3 in intestinal epithelial cells increases intestinal permeability and cause visceral hypersensitivity (31). The transient receptor potential vanilloid-4 (TRPV4) seems to play a pivotal role in the nociceptive response to PAR-2 (32). In the present study, increased faecal elastase activity was found in fructose malabsorption mouse models. In these models, increased intraluminal concentration of non absorbed fructose is likely to have stimulated pancreatic juice secretion and therefore pancreatic enzyme, including elastase and trypsin. The pancreatic, rather than intestinal, origin of elastase in our mouse models is strongly suggested by the increase in faecal and the decrease of colonic mucosal elastase activity associated with fructose malabsorption. Indeed,

fructose malabsorption is associated with higher levels of amylase and lipase concentrations in the serum (33). Whether PARs are involved in colonic sensitisation during fructose malabsorption remains to be established both in mice and in IBS patients.

In conclusion, we showed that fructose malabsorption is associated with visceral hypersensitivity both in IBS patients and in mouse models of fructose malabsorption. Fructose malabsorption -associated visceral hypersensitivity was associated with altered intestinal permeability, while it was unrelated with intestinal inflammation. Last, fructose malabsorption -associated visceral hypersensitivity can be associated with increased elastase activity. Further studies are warranted to assess PARs involvement in fructose malabsorption - associated visceral hypersensitivity.

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Tables

Table 1 : IBS patients characteristics

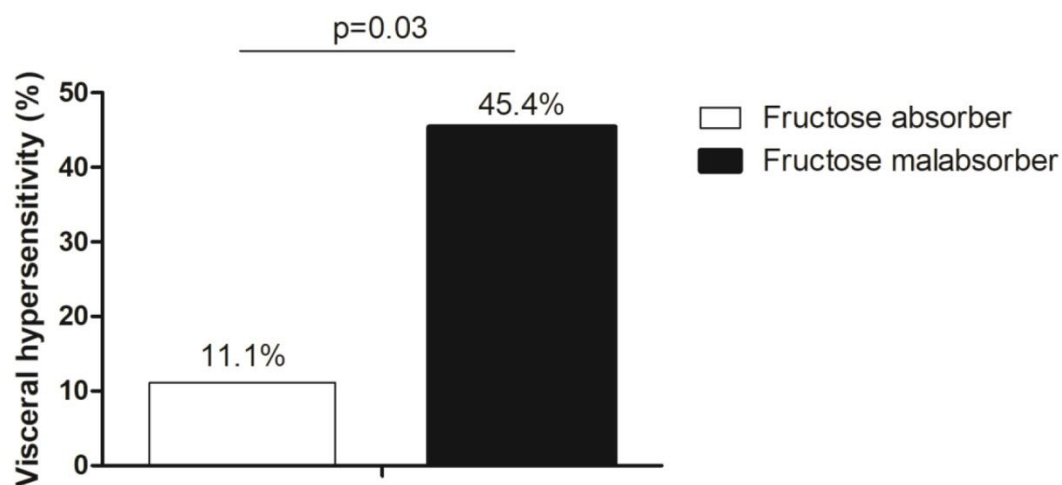
Characteristics	Population (n=38)
Female gender (%)	31 (81.6)
Age (years old)	41.5 $\pm$ 14.7
BMI (units)	23.2 $\pm$ 5.5
Subtypes (%):	
-Diarrhoea	-21 (55.3)
-Constipation	-6 (15.8)
-Alternating	-10 (26.3)
Post-infectious IBS (%)	4 (10.5)
Stress before symptoms occurred	13 (34.2)
IBS-SSS	274.6 $\pm$ 94.4
GIQLI score	72.3 $\pm$ 18.4
HADS anxiety	10.3 $\pm$ 3.9
HADS depression	6.8 $\pm$ 3.8

BMI : body mass index; IBS : irritable bowel syndrome. IBS-SSS : IBS-symptom severity score, HADS : Hospital Anxiety Depression Score, GIQLI : Gastrointestinal Quality of Life Index.

Results are presented as number (percentage) and mean $\pm$ standard deviation.

Figures

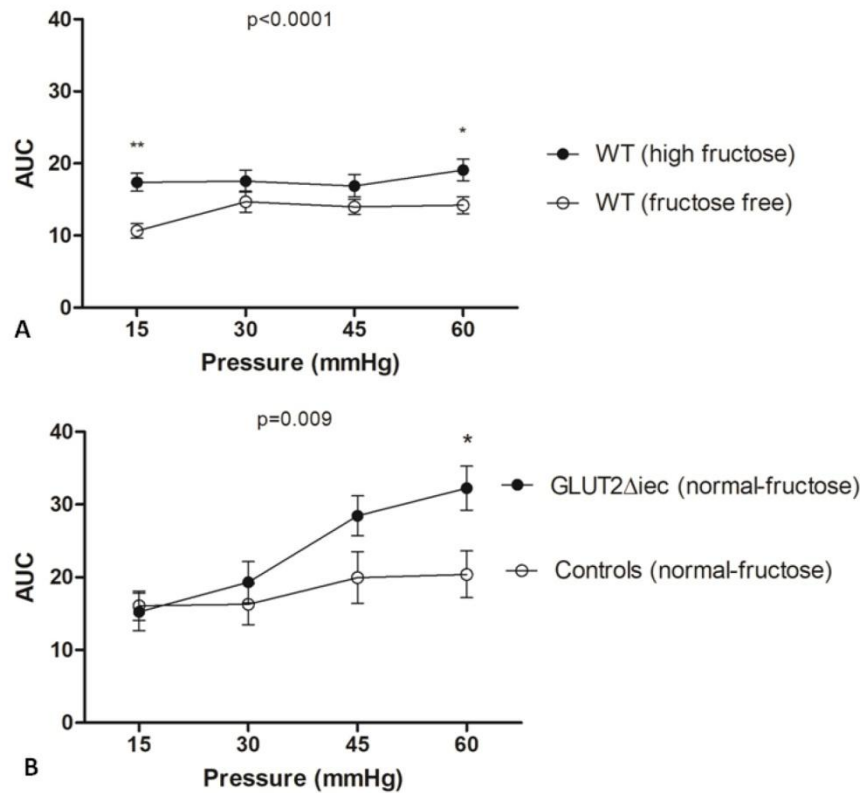
Figure 1 : Visceral hypersensitivity in IBS patients.



The percentage of IBS patients with visceral hypersensitivity in presence or not of fructose malabsorption.

IBS : irritable bowel syndrome

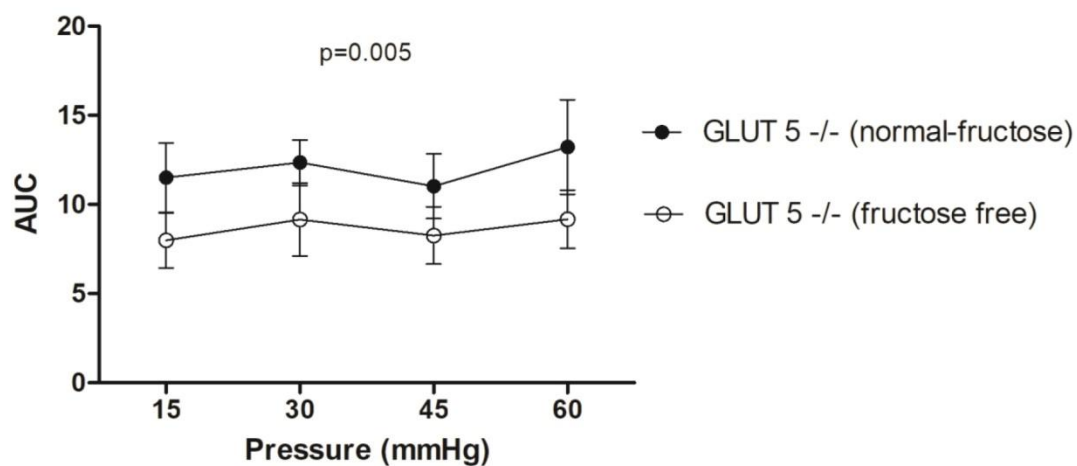
Figure 2 : Visceral pain response in high fructose diet fed mice and in $GLUT2^{AIEC}$ mice.



Visceral pain response was expressed in area under the curve (AUC) of the intra-colonic pressure. A) WT mice were exposed to high fructose diet and fructose-free diet 3 weeks prior the distension protocol. B) $GLUT2^{AIEC}$ mice and their controls were exposed to normal-fructose diet 3 weeks prior the distension protocol. Results are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$

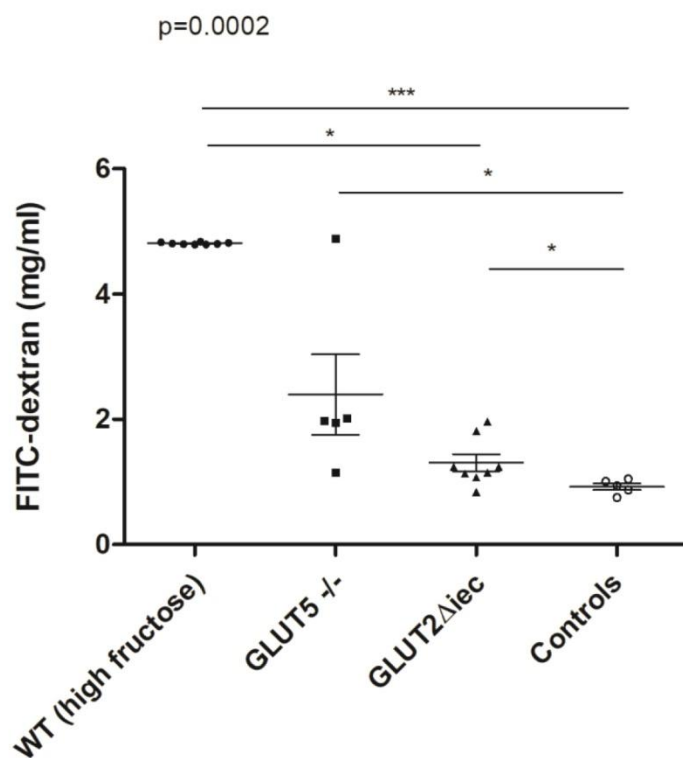
AUC : area under the curve.

Figure 3 : Visceral pain response in GLUT5 $-/-$ mice.



Visceral pain response was expressed in area under the curve (AUC) of the intra-colonic pressure. GLUT5 $-/-$ mice were exposed to fructose-free diet then to normal-fructose diet 3 weeks prior the distension protocol. Results are expressed in mean $\pm$ SEM.

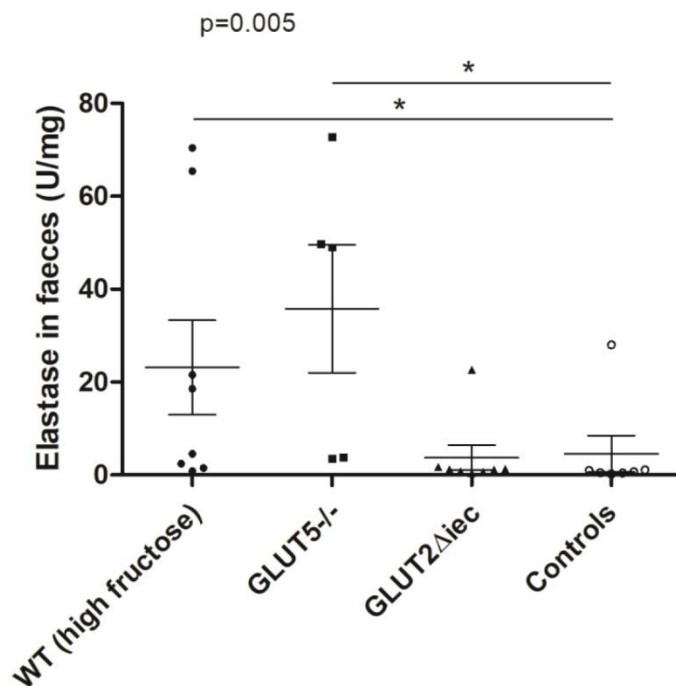
Figure 4 : Intestinal permeability.



FITC concentrations in serum (mg/ml) in each group in WT with high fructose diet and in GLUT5^{-/-}, GLUT2 ^{Δ IEC} mice and controls mice, after 3 weeks with normal-fructose diet.

*Results are expressed in mean $\pm$ SD. * $p < 0.05$, *** $p < 0.001$*

Figure 5 : Faecal elastase activity in mice.



Elastase activity was measured in faeces of wild type (WT) mice fed with high fructose diet (HF), *Glut5*^{-/-} mice, *GLUT2*^{ΔIEC} mice and in WT fed with normal-fructose diet (controls). Results are expressed in mean $\pm$ SD. * $p < 0.05$.

Supplementary data

Table 1 : Diet description

Product	Normal-fructose diet A03, SAFE DIETS	High-fructose diet D08040107, research diet	Fructose-free diet D12450K, research diet
Protein (Kcal%)	21.4	20.0	20.0
Carbohydrates (Kcal%)	73.5	70.0	70.0
Including fructose (Kcal%)	1.8	69.0	0.0
Fat (Kcal%)	5.1	10.0	10.0
Caloric intake (Kcal/g)	2.8	3.8	3.8

Article 5 : Intestinal invalidation of the glucose transporter GLUT2 delays tissue distribution of glucose and reveals an unexpected role in gut homeostasis

Cet article résulte de la collaboration que j'ai eue au cours de ma thèse de sciences avec le centre de recherche des Cordeliers. Il faisait partie intégrante de la thèse de Charlotte Schmitt intitulée "Implication du transporteur intestinal GLUT2 dans l'absorption des sucres et la fonction entéroendocrine". J'ai travaillé dans cet article sur l'étude de la perméabilité intestinale dans le modèle de souris avec délétion intestinale de GLUT2 (GLUT2^{ΔIEC}).

Au cours de ma thèse de science, je me suis intéressée aux répercussions fonctionnelles digestives (perméabilité intestinale, sensibilité viscérale et dysbiose) liées à la malabsorption de fructose. Il a déjà été démontré sur des modèles de souris KO en GLUT2 que les capacités d'absorption du fructose était diminuées de 60% et étaient associées à une perte d'adaptation à l'exposition préalable par fructose (78). Ce travail démontre que le GLUT2 intestinal module l'absorption de glucose et constitue une étape de contrôle de la distribution du sucre alimentaire dans les tissus. J'ai participé à ce travail en montrant que la perméabilité jéjunale, mesurée par chambre de Ussing, était diminuée chez les souris GLUT2^{ΔIEC}.

Résumé article 5 : L'invalidation intestinale du transporteur du glucose GLUT2 retarde la distribution tissulaire du glucose et révèle un rôle insoupçonné dans l'homéostasie intestinale

Objectifs : L'absorption intestinale de glucose est orchestrée par des transporteurs spécialisés tel SGLT1 et GLUT2. Cependant, le rôle de GLUT2 dans la régulation de l'absorption du glucose reste encore à élucider.

Méthodes : Nous avons voulu évaluer le rôle de GLUT2 dans l'absorption du glucose et l'homéostasie du glucose après invalidation intestinale dans un modèle de souris (les souris GLUT2^{ΔIEC}).

Résultats : Comme attendu, la délétion intestinale de GLUT2 a provoqué une malabsorption du glucose, représentée par un retard de la distribution tissulaire du sucre oral. Les conséquences de l'invalidation intestinale de GLUT2 chez les souris GLUT2^{ΔIEC} étaient une limitation de la prise de poids malgré une prise de nourriture normale, une amélioration de la tolérance au glucose et une augmentation de la production de corps cétoniques. Ces résultats rappellent les conséquences d'une restriction calorique. Les autres mécanismes adaptatifs de la délétion intestinale de GLUT2 étaient une réduction de la longueur des microvillosités, une dysbiose, qui étaient associés à une amélioration du statut inflammatoire. De plus, une réduction de la densité des cellules exprimant le glucagon-like-peptide-1 (GLP-1) qui était compensé par une augmentation de GLP-1 par cellules L, suggérant une fonction entéroendocrine conservée chez les souris GLUT2^{ΔIEC}.

Conclusion : Le GLUT2 intestinal module l'absorption de glucose et constitue une étape de contrôle de la distribution du sucre alimentaire dans les tissus. En conséquence, le métabolisme et l'homéostasie intestinale sont améliorés en l'absence de GLUT2 fonctionnel dans l'intestin, reproduisant les effets d'une restriction calorique.

Intestinal invalidation of the glucose transporter GLUT2 delays tissue distribution of glucose and reveals an unexpected role in gut homeostasis

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ABSTRACT

Objective: Intestinal glucose absorption is orchestrated by specialized glucose transporters such as SGLT1 and GLUT2. However, the role of GLUT2 in the regulation of glucose absorption remains to be fully elucidated.

Methods: We wanted to evaluate the role of GLUT2 on glucose absorption and glucose homeostasis after intestinal-specific deletion of GLUT2 in mice (GLUT2^{ΔIEC} mice).

Results: As anticipated, intestinal GLUT2 deletion provoked glucose malabsorption as visualized by the delay in the distribution of oral sugar in tissues. Consequences of intestinal GLUT2 deletion in GLUT2^{ΔIEC} mice were limiting body weight gain despite normal food intake, improving glucose tolerance, and increasing ketone body production. These features were reminiscent of calorie restriction. Other adaptations to intestinal GLUT2 deletion were reduced microvillus length and altered gut microbiota composition, which was associated with improved inflammatory status. Moreover, a reduced density of glucagon-like peptide-1 (GLP-1) positive cells was compensated by increased GLP-1 content per L-cell, suggesting a preserved enteroendocrine function in GLUT2^{ΔIEC} mice.

Conclusions: Intestinal GLUT2 modulates glucose absorption and constitutes a control step for the distribution of dietary sugar to tissues. Consequently, metabolic and gut homeostasis are improved in the absence of functional GLUT2 in the intestine, thus mimicking calorie restriction.

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Keywords Malabsorption; Glucose homeostasis; Intestinal adaptation; Microbiota; GLP-1

1. INTRODUCTION

The intestine responds to the external environment by complex interplays between cells in the epithelium, the immune system, and microbes. Gut homeostasis allows a permanent adaptation to nutritional, metabolic, or bacterial signals through continuous renewal and differentiation of epithelial cells. After carbohydrate intake and digestion, sugars are absorbed in the proximal small intestine by several specialized transporters. The Na⁺/glucose cotransporter SGLT1 triggers glucose absorption at the food-facing membrane. GLUT2

facilitates the passage of dietary sugars, glucose, fructose, and galactose towards the bloodstream [1]. In enterocytes, GLUT2 is consistently located in the basolateral membrane but, during a sugar rich meal, can be recruited transiently to the apical membrane, where it remains permanently in case of insulin resistance [2–4]. GLUT2 is expressed in enteroendocrine cells, especially in L-cells [5]. L-cells express nutrient transporters and receptors that can sense luminal nutrients at their apical membrane and thereby influence their own functions [6,7]. Enteroendocrine cells are scattered in the intestinal epithelium and constitute the largest population of hormone-producing

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Abbreviations: 2FDG, 2-deoxy-2-[¹⁸F]fluoro-D-glucose; DPP-IV, dipeptidyl-peptidase IV; GLP-1, glucagon-like peptide-1; GLUT1-7, glucose transporter 1–7; IEC, intestinal epithelial cells; IL, interleukin; IPGTT, intraperitoneal glucose tolerance test; ITT, insulin tolerance test; OGTT, oral glucose tolerance test; PET-CT, Positron Emission Tomography-Computed Tomography; SGLT1, sodium-glucose transporter 1

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Original Article

cells of the body. L-cells produce incretins such as glucagon-like peptide-1 (GLP-1), a potent activator of glucose-induced insulin secretion [8]. It is now thought that sufficient L-cells are present in the proximal intestine to account for early rises of GLP-1 after a meal. However, when absorption is reduced, the higher nutrient delivery to distal intestine may bring more L-cells in contact with nutrients and/or their microbial fermentation products, resulting in increased plasma GLP-1 levels [9].

Physiological studies of genetically modified mice have revealed a role for GLUT2 in several regulatory mechanisms. A total knockout of GLUT2 in mouse indicated that GLUT2 is dispensable for intestinal glucose absorption because of the existence of compensatory mechanisms [10]. However, *Glut2*^{-/-};RIPGlut1 mice rescued by GLUT1 expression in pancreatic β cells displayed impaired plasma GLP-1 levels after glucose gavage, which was attributed to reduced intestinal GLP-1 content [11], suggesting that GLUT2 is of importance in L-cell function. In the present study, our hypothesis was that intestinal GLUT2 could contribute to both metabolic and gut homeostasis. Impaired glucose absorption in the small intestine could cause disturbed glucose delivery, modified gut microbiota composition, and altered L-cell function. To test this hypothesis, we generated an inducible model of GLUT2 invalidation in intestinal epithelial cells.

2. MATERIAL AND METHODS

2.1. Generation of transgenic mice with targeted deletion of *Slc2a2* in the intestine

Heterozygous *Slc2a2*^{tm1a(KOMP)Wtsi} mice were generated by the KnockOut Mouse Project (KOMP) at the University of California, Davis (Project ID#CSD40514), using the targeting vector described in Figure 1A(a). This vector contains the lacZ expressing cassette with polyA tail inserted between Exon 3 and Exon 4 of *Slc2a2*, generating a null allele that produces β -galactosidase in cells with active *Slc2a2* gene transcription (Figure 1A(a)). We could observe that heterozygous mice survived and expressed β -galactosidase in GLUT2 expressing tissues including the intestine (data not shown). These founder mice were crossed with mice that express the Flippase recombinase to remove the lacZ and neomycin cassettes generating *Slc2a2*^{FLOX/FLOX} mice (Figure 1A(b)). *Slc2a2*^{FLOX/FLOX} mice were then crossed with a *Villin-CreERT2* mouse line [12] (gift from S. Robine) to generate *Slc2a2*^{FLOX/FLOX} $\times$ *Villin-CreERT2*^{+/-} and *Slc2a2*^{FLOX/FLOX} $\times$ *Villin-CreERT2*^{-/-} mice. In the *Slc2a2*^{FLOX/FLOX} $\times$ *Villin-CreERT2*^{+/-} mice, Cre recombinase was activated by tamoxifen gavage (1 mg/mouse; Sigma–Aldrich) for 3 consecutive days to induce a specific *Slc2a2* deletion in intestinal epithelial cells (GLUT2 Δ IEC mice, Figure 1A(c)).

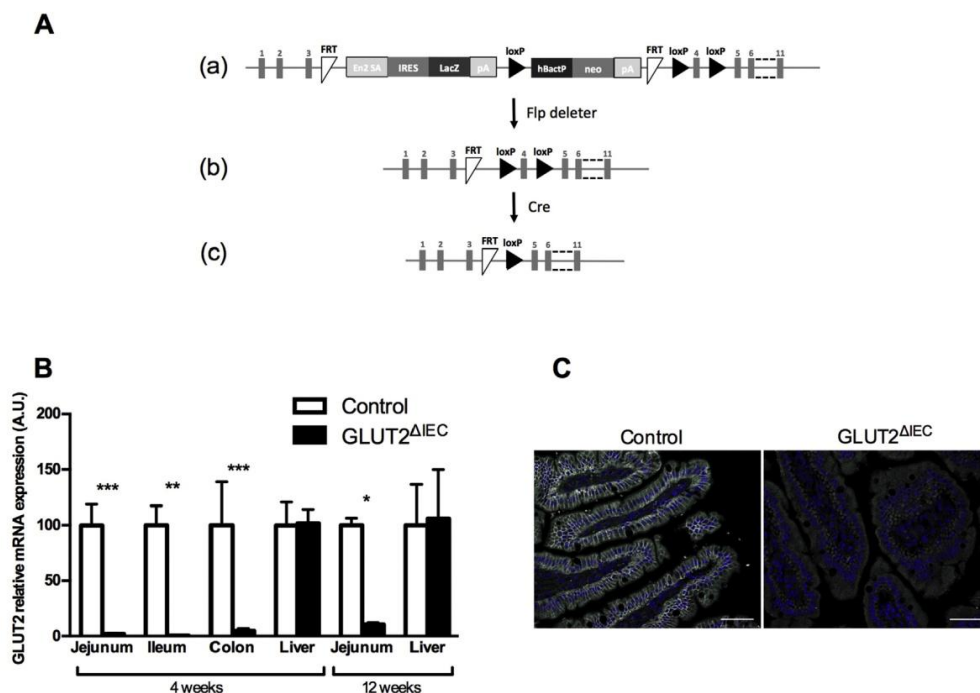


Figure 1: Generation of GLUT2 Δ IEC mice. (A) The *Slc2a2*/lacZ knock-in allele (*Slc2a2*(KOMP)Wtsi) is shown to illustrate the mutated *Slc2a2* gene, in which the lacZ and neomycin expression cassettes were inserted between Exon 3 and Exon 4 and which is flanked by FRT (Flippase Recognition Target) sites. This cassette contains the splice acceptor of mouse engrailed 2 exon 2 (En2 SA), an internal ribosome entry sequence (IRES) to initiate lacZ translation, and polyadenylation (pA) to terminate transcription after the lacZ gene. The neo gene is driven by human beta actin promoter (hBactP) and contains its own pA. Additionally, Exon 4 is flanked by loxP sites. (b) The resulting *Slc2a2* conditional knockout gene structure is shown to illustrate removal of the lacZ and neomycin cassettes; it is generated by crossing *Slc2a2*^{tm1a(KOMP)Wtsi} mice with mice that express the Flp recombinase. (c) The resulting *Slc2a2* knockout allele generated after crossing with Cre expressing mice and activation by tamoxifen gavage is shown. (B) Relative mRNA levels of sugar transporter GLUT2 in the jejunum, ileum, colon, and liver 4 weeks after tamoxifen administration and in the jejunum and liver 12 weeks after tamoxifen administration in control (white bars, n = 4–8) and GLUT2 Δ IEC (black bars, n = 3–9) mice. Values are means $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001; control vs GLUT2 Δ IEC mice (Mann–Whitney U test). (C) Jejunal sections from control (left panel) and GLUT2 Δ IEC (right panel) mice 4 weeks after tamoxifen administration were immunostained for GLUT2 (white) and DAPI (blue). Scale bars = 50 μ m. Note the absence of GLUT2 labeling in jejunum of GLUT2 Δ IEC mice.

Slc2a2^{FLOX/FLOX} × *Villin-CreERT2*^{-/-} received the same tamoxifen gavage and were used as littermate controls (control mice). All mice were on a C57BL/6J background. Mice were housed in the Cordeliers research center animal facility (Paris, France) with a 12:12-h light–dark cycle and allowed access to standard chow diet (SAFE R03) and water *ad libitum*. Mice were genotyped using the following PCR primers: CreVillin F: 5'-CAAGCCTGGCTCGACGGCC-3'; R: 5'-CGCGAATCTTCAGGTTCT-3'; *Glut2*^{Flox} F: 5'-TCTTGGCTTTGGGTGAAATGAGCA-3'; R2: 5'-GAACCTCGGTTCCGGCGCCA-3' (floxed allele, 239 bp); R7: 5'-AGGAGCAGTGGTTGGATGGCA-3' (wild-type allele, 201 bp).

All animal use followed the French ethical guidelines for animal studies and was approved by the Regional Animal Care and Use Committee (CREEA Ile de France No. 3, agreement number 05210.02).

Body weight measurements and oral glucose tolerance tests were performed weekly until the 12th week after tamoxifen administration. All other experiments were performed 4 and/or 12 weeks after tamoxifen administration.

2.2. Glucose and insulin tolerance tests

All experiments were performed on conscious mice. For oral glucose tolerance test (OGTT) or intraperitoneal glucose tolerance test (IPGTT), overnight fasted (16 h) mice received a glucose load (4 g/kg body weight) orally or intraperitoneally, respectively. For insulin tolerance test (ITT), mice fasted for 6 h were injected intraperitoneally with 1 U/kg insulin (Actrapid, Novo Nordisk). For both tests, blood glucose was measured at the tail vein with an AccuCheck Performa glucometer (Roche Diagnostics) at 0, 5, 10, 30, 60, and 120 min. Glucose absorption through the intestine was quantified by calculating the glycaemic slope between 0 and 5 min after an oral glucose load.

2.3. Plasma hormones and metabolites

GLP-1 and insulin levels were measured immediately before and 30-min after 2 g/kg *D*-glucose and 100 μ L olive oil challenge to mimic a meal in mice fasted overnight (16 h). Blood samples were collected from the tail vein in EDTA-precoated tubes containing DPP-IV inhibitor (DPP4-010, Millipore). Plasma active GLP-1 levels were determined using high sensitivity GLP-1 active chemiluminescent ELISA kit (EMD Millipore Corporation). Plasma insulin levels were measured using ultrasensitive insulin ELISA kit (Alpco). Plasma β -hydroxybutyrate was measured using an automatic chemistry analyzer AU400 (Olympus Diagnostics) in blood samples collected from non-fasted mice.

2.4. Imaging intestinal glucose absorption and biodistribution using 2FDG-PET-CT

Control and *GLUT2* ^{Δ IEC} mice were fasted overnight and subjected to Positron Emission Tomography-Computed Tomography (PET-CT) using the radiotracer 2-deoxy-2-[¹⁸F]fluoro-*D*-glucose (2FDG) to assess intestinal glucose absorption and biodistribution in peripheral tissues. Mice were anesthetized with 1–2% isoflurane (IsoVet 100%, Centravet) in 100% O₂ during the whole experiment. PET acquisitions were acquired in the PET-CT scanner (nanoScan PET-CT, Mediso) 1 min after oral gavage of 10 MBq 2FDG (Gluscan, Advanced Applied Applications) in a saline solution containing 4 g/kg of *D*-glucose. Body temperature and respiration were registered. List-mode PET data were collected between 1 and 60 min post-injection, binned using a 5-ns time window, a 400- to 600-keV energy window, and a 1:5 coincidence mode. At the end of the PET acquisitions, CT scans were performed using the following parameters: mode semi-circular, tension of 39 kV, 720 projection full scan, 300 ms per projection, binning 1:4. After the *in vivo* scans, mice were sacrificed and the whole intestine was isolated and flushed to remove intraluminal 2FDG for *ex vivo* PET-

CT acquisition of 5 min. *In vivo* PET acquisitions were reconstructed in 12 frames of 5 min using the Tera-Tomo reconstruction engine (3D-OSEM based manufactured customized algorithm) with expectation maximization iterations, scatter, and attenuation correction. Images were analyzed using the software PMOD (PMOD Technologies LLC). Standardized Volume of Interest (VOI) was drawn in each organ, and Standardized Uptake Values (SUV) were calculated by dividing the mean tissue radioactivity concentration by the total orally administered 2FDG and body weight.

2.5. Intestinal transit time

Carmine red was given by gavage to mice fasted for 6 h (10 mg/mL of water, 10 μ L/g body weight). The total intestinal transit time was measured by determination of time between ingestion of carmine red and first appearance of the dye in feces.

2.6. Jejunal permeability

Jejunal permeability was assessed by measuring FITC-dextran (4 kDa, FD4) fluxes in Ussing chambers with an exchange surface of 0.07 cm² (Harvard Apparatus). FD4 (5 mg/mL) was placed at the mucosal side. After 3 h at 37 °C, medium from the serosal side was removed and stored at –80 °C. The fluorescence level of FD4 in serosal side (excitation at 485 nm, emission at 535 nm) was measured in 96-well black plate with spectrometer Chameleon V (Hidex). Values were converted to concentration using a standard curve.

2.7. Metabolic and stool analysis

Control and *GLUT2* ^{Δ IEC} mice were housed individually in metabolic cages (Techniplast), 3 weeks after tamoxifen administration. After a 5- to 7-day adaptation period with *ad libitum* access to water and powdered chow diet, experiments were performed for a period of 7 days. Body weight, food and water intakes, and urine and fecal outputs were recorded daily. Stools were collected daily and stored at –20 °C. Stools collected for 2 days were pooled and analysed on homogenized samples. Total energy content was determined by calorimetry (PARR 1351 Bomb Calorimeter, Parr Instrument Company). 24-hour fecal calorie loss represented the proportion of ingested energy recovered in stool output.

2.8. Fecal microbiota analysis

Total DNA was extracted from frozen fecal samples using the chemical guanidine thiocyanate and the mechanical bead-beating method as described previously [13]. TaqMan[®] qPCR was used to quantify total bacterial population and the dominant (>1% of fecal bacterial population) bacterial groups, and genera: *Clostridium* cluster IV (*Clostridium leptum* group) and *Bacteroides/Prevotella* group. qPCR using SYBR-Green[®] was performed to quantify *Lactobacillus/Leuconostoc/Pedococcus* group, *Akkermansia muciniphila*, *Staphylococcus* and *Enterococcus* sp. (primers and probes reported in Supplementary Table 1). Standard curves were obtained from serial dilutions of a known concentration of plasmid DNA containing a 16S rRNA gene insert from each species or group. The coefficients of correlation between log₁₀ CFU and rRNA gene copy numbers for each species and group were obtained from rrnDB (<https://rrnodb.umms.med.umich.edu/>), which allowed the calculation of the number of CFU/g of feces. When species or targeted taxonomic groups were not detected, the arbitrary value of 1.5 log₁₀ CFU/g of feces was used.

2.9. Glucose transporter and cytokine tissue expression analysis

Jejunal, ileal, colonic mucosa, and liver were collected, snap frozen, and kept at –80 °C until RNA extraction using RNeasy Mini kit

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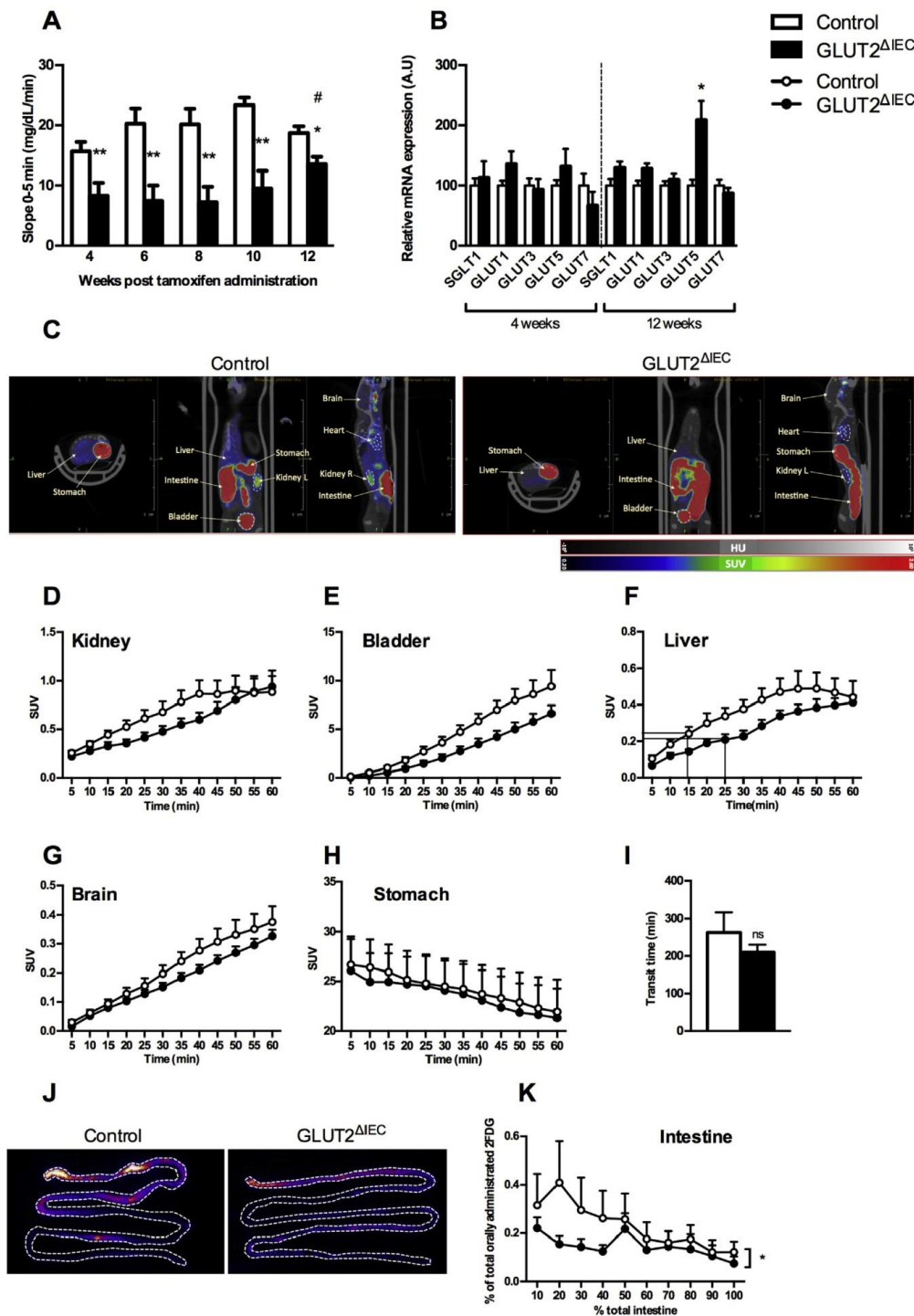


Figure 2: GLUT2^{ΔIEC} mice display glucose malabsorption. (A) Glucose absorption index was evaluated by the glycemic slopes calculated using blood glucose levels measured between 0 and 5 min after oral glucose bolus (4 g glucose/kg) in control (white bars, n = 10) and GLUT2^{ΔIEC} mice (black bars, n = 11), from 4 to 12 weeks after tamoxifen administration. Values are means ± SEM. *P < 0.05; **P < 0.01; Control vs GLUT2^{ΔIEC} (Mann–Whitney U tests). #P < 0.05; GLUT2^{ΔIEC} 4 vs 12 weeks post-tamoxifen

(Qiagen). For interleukin mRNA gene transcription assessment, ileal lamina propria lymphocytes were enriched using Lamina Propria dissociation kit (Miltenyi Biotec) prior to RNA extraction. One microgram of RNA was reverse transcribed with 200 units of reverse transcriptase using the Superscript II kit (Invitrogen) according to manufacturer's recommendations. RT-qPCR analysis was performed with a LightCycler 480 instrument (Roche Applied Science), and cDNA was amplified using SYBR[®] Green PCR Master Mix (Roche Applied Science) and specific primers reported in Supplementary Table 2. Relative gene expression was expressed in arbitrary units (A.U.) as a ratio of target transcripts normalized to murine cyclophilin or GAPDH mRNA level.

2.10. Immunofluorescence microscopy for intestinal GLUT2

Jejunal paraffin-embedded sections of five-micrometer thickness were unmasked in citrate buffer pH 6.0 for 10 min at 97 °C and incubated with primary antibody raised against GLUT2 (custom made, targeting the COOH-terminal; 1:500). Incubation with secondary antibodies (Cy3-conjugated donkey anti-rabbit: 1:1000; DAPI 1:1000 for nuclei staining) was performed. Localization of GLUT2 was examined using confocal microscopy (LSM710, Zeiss; 633 oil lens, 0.8 mm depth of field).

2.11. Immunohistochemistry for intestinal GLP-1

Jejunal and colon sections were treated as previously described [14]. Revelation was performed using the biotinylated secondary antibody/streptavidin-HRP amplification system (BIOSPA) and AEC (Vector laboratories, Inc). Sections were mounted in Glycergel Mounting Medium (Dako). Images were obtained using slide scanner (Axio Scan Z1, Zeiss). Measurement intestinal tissue area was performed using NIH Image J software.

2.12. Electron microscopy

Jejunum (morphological analyses) or colon (immunoelectron microscopy for intestinal GLP-1) samples were fixed with glutaraldehyde and paraformaldehyde after dissection. Jejunal ultra-thin sections (65 nm) were postfixed in osmic acid, then dehydrated in graded alcohol, embedded in Epon resin (Polysciences Inc), and counterstained with uranyl acetate and lead citrate using an LKB 2168 ultrastainer. After alcohol-graded dehydration, colon ultra-thin sections were embedded in polyhydroxy-aromatic acrylic LR-White resin and incubated with anti-GLP-1 antibody (Peninsula) followed by 12 nm gold particle-donkey anti-rabbit IgGs (Jackson ImmunoResearch Laboratories, Newmarket). For both stainings, sections were analyzed using Philips EM10 electron microscope coupled with an Erlangshen 1000 camera and software (Gatan; Roper Scientific). Measurement of microvillus length and quantifications of secretory granules and GLP-1 gold beads were performed with NIH Image J software.

2.13. Intestinal GLP-1 protein content

Proximal jejunum, distal ileum, and the whole colon were sliced into small pieces. Tissues were homogenized in ethanol/acid (100% ethanol: sterile water: 12N HCl 74:25:1 v/v) solution (5 mL/g tissue) and incubated overnight at 4 °C. The homogenates were centrifuged and supernatants were collected for total GLP-1 content measurement using ELISA method (GLP-1 Total ELISA kit, Millipore).

2.14. Statistical analysis

Results are expressed as mean $\pm$ SEM and median (range) for microbiota results, and the significance between the mean of continuous parameters was evaluated by 2-way ANOVA followed by Bonferroni post-test multiple comparisons or Mann–Whitney *U* test using PRISM software. All probabilities were two-tailed with significance set at $P < 0.05$.

3. RESULTS

3.1. Inducible invalidation of GLUT2 in mouse intestine

To induce GLUT2 invalidation specifically in the intestine, we administered by gavage tamoxifen in *Slc2a2*^{FLOX/FLOX} $\times$ *Villin-CreERT2*^{+/-} and *Slc2a2*^{FLOX/FLOX} $\times$ *Villin-CreERT2*^{-/-} mice. We measured GLUT2 mRNA expression in the intestine and liver, another GLUT2 expressing tissue, up to 12 weeks post-invalidation. As expected, 4 weeks after tamoxifen administration, *Slc2a2* mRNA expression was markedly suppressed in the jejunum, ileum, and colon of GLUT2 ^{Δ IEC} mice as compared to control littermates without any change in the liver (Figure 1B). *Slc2a2* mRNA expression remained low in the intestine and unchanged in the liver even 12 weeks after tamoxifen administration (Figure 1B). *Slc2a2* mRNA loss led to an absence of GLUT2 protein at the membrane of jejunal epithelial cells in GLUT2 ^{Δ IEC} mice (Figure 1C).

3.2. Metabolic phenotype

3.2.1. Intestinal GLUT2 invalidation reduces glucose absorption

We first questioned the contribution of GLUT2 to intestinal glucose absorption in GLUT2 ^{Δ IEC} mice. *In vivo*, intestinal glucose absorption can be evaluated by the slope of increase in glycemia between 0 and 5 min after glucose gavage, which reflects the rate of glucose appearance in the bloodstream. Glycemic slopes were significantly decreased in GLUT2 ^{Δ IEC} throughout the 12-week invalidation period (Figure 2A), indicating glucose malabsorption in GLUT2 ^{Δ IEC} as compared to control mice.

Glucose malabsorption was still observed 12 weeks after tamoxifen administration but to a lesser extent, suggesting that compensatory mechanisms occurred (Figure 2A) such as overexpression of other sugar transporters. The expression of glucose transporters SGLT1,

administration (two-way ANOVA followed by Bonferroni correction for multiple comparisons). (B) Relative mRNA levels of sugar transporters SGLT1, GLUT1, GLUT3, GLUT5, and GLUT7 in the jejunum of control (white bars, $n = 9-14$) and GLUT2 ^{Δ IEC} (black bars, $n = 7-13$) mice, 4 (right panel) and 12 weeks (left panel) after tamoxifen administration. Values are means $\pm$ SEM. * $P < 0.05$; Control vs GLUT2 ^{Δ IEC} mice (Mann–Whitney *U* test). (C) Representative transversal, coronal, and sagittal PET-CT images of 2FDG accumulation in liver, kidney, bladder, stomach, intestine, heart, and brain of control (right panel) and GLUT2 ^{Δ IEC} (left panel) mice, 30 min after gavage of the tracer. (D–H) 60-min tracer activity detected in major target organs and tissues including kidney (D), bladder (E), liver (F), brain (G), and stomach (H) in control (white circles, $n = 7$) and GLUT2 ^{Δ IEC} mice (black circles, $n = 9$), 4 weeks after tamoxifen administration. Values are expressed as mean tissue radioactive concentration normalized by total orally administered 2FDG and body weight $\pm$ SEM (SUV: standardized uptake values). (I) Intestinal transit time. First appearance of dye in the feces after carmine red gavage given to control (white bar, $n = 6$) and GLUT2 ^{Δ IEC} mice (black bar, $n = 5$) after 6 h of fasting. Values are means $\pm$ SEM. ns: non-significant. (J) Representative PET-CT images of 2FDG accumulation in *ex vivo* flushed small intestine of control (right panel) and GLUT2 ^{Δ IEC} (left panel) mice, 60 min after gavage of the tracer. Delineation of small intestine with dotted lines. (K) *Ex vivo* tissue profiling for glucose tracer contents along the flushed small intestine of control (white circles, $n = 6$) and GLUT2 ^{Δ IEC} (black circles, $n = 6$) mice, 60 min after an intragastric glucose bolus (4 g/kg) containing 2FDG. Values are means $\pm$ SEM. * $P < 0.05$; control vs GLUT2 ^{Δ IEC} mice (two-way ANOVA).

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GLUT1, GLUT3, and GLUT7 remained unchanged in the jejunum 4 and 12 weeks after tamoxifen administration in GLUT2^{ΔIEC} mice (Figure 2B). Interestingly, the expression level of fructose transporter GLUT5 was significantly increased 12 weeks after tamoxifen administration, supporting a compensatory mechanism for fructose absorption in GLUT2^{ΔIEC} mice (Figure 2B).

3.2.2. Short-term distribution of oral sugar (2FDG) is delayed in tissues of GLUT2^{ΔIEC} mice

To investigate the consequences of glucose malabsorption, we evaluated sugar distribution in tissues of GLUT2^{ΔIEC} mice. We gave orally 2FDG in a highly concentrated glucose bolus and recorded 2FDG biodistribution by PET imaging (Figure 2C). 2FDG biodistribution curves

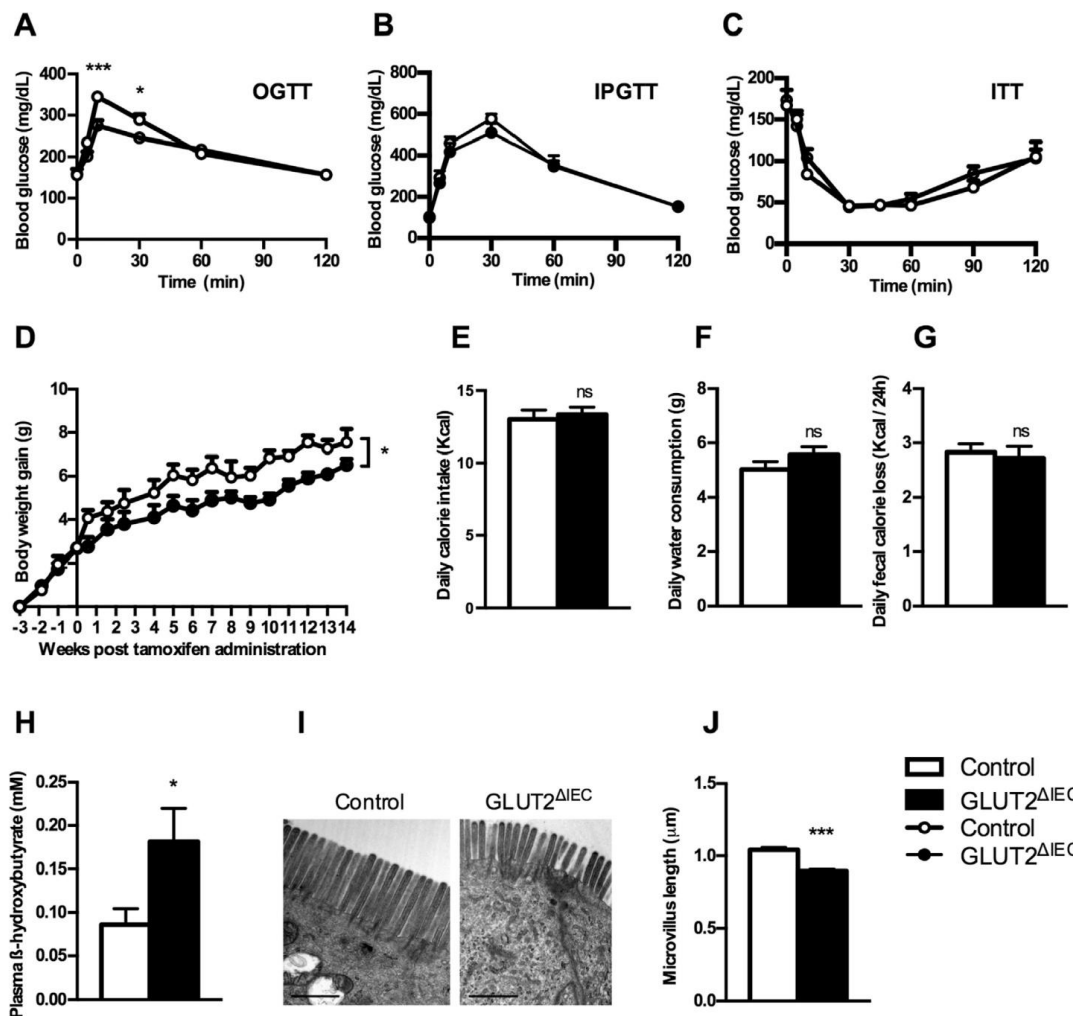


Figure 3: Intestinal GLUT2 invalidation improves mouse metabolism. (A) Oral glucose tolerance test (4 g glucose/kg) was performed in control (white circles, n = 16) and GLUT2^{ΔIEC} mice (black circles, n = 13) after 16 h of fasting, 4 weeks after tamoxifen administration. Values are means ± SEM; *P < 0.05; ***P < 0.001; control vs GLUT2^{ΔIEC} mice (two-way ANOVA followed by Bonferroni correction for multiple comparisons). (B) Intraperitoneal glucose tolerance test (4 g glucose/kg) was performed in control (white circles, n = 6) and GLUT2^{ΔIEC} mice (black circles, n = 10) after 16 h of fasting, 4 weeks after tamoxifen administration. Values are means ± SEM. (C) Insulin tolerance test (1 U insulin/kg) was performed in control (white circles, n = 9) and GLUT2^{ΔIEC} mice (black circles, n = 8) after 6 h of fasting, 4 weeks after tamoxifen administration. Values are means ± SEM. (D) Body weight gain follow-up 14 weeks after tamoxifen administration in control mice (white circles, n = 5) and GLUT2^{ΔIEC} mice (black circles, n = 6). Values are means ± SEM. *P < 0.05; control vs GLUT2^{ΔIEC} mice (Two-way ANOVA). (E, F) Daily calorie intake (E) and daily water consumption (F) measured in metabolic cages in control (white bars, n = 5) and GLUT2^{ΔIEC} mice (black bars, n = 6), 4 weeks after tamoxifen administration. Values are means ± SEM. ns: non-significant. (G) Loss of fecal calories was determined by bomb calorimetry analyses in 24 h feces collected from control (white bar, n = 10) and GLUT2^{ΔIEC} mice (black bar, n = 11), 4 weeks after tamoxifen administration. Values are means ± SEM. ns: non-significant. (H) Plasma levels of β-hydroxybutyrate were measured in fed control (white bars, n = 11) and GLUT2^{ΔIEC} mice (black bars, n = 15), 4 weeks after tamoxifen administration. Values are means ± SEM. *P < 0.05; control vs GLUT2^{ΔIEC} mice (Mann–Whitney U test). (I) Representative images of brush border electron microscopy from the jejunum of control (left panel) and GLUT2^{ΔIEC} mice (right panel), 4 weeks after tamoxifen administration. Scale bar = 1 μm. (J) Measurement of microvillus length from electron microscopy microphotographs of control (white bar) and GLUT2^{ΔIEC} mice (black bar), 4 weeks after tamoxifen administration. 230–250 microvilli were measured for each genotype. Values are means ± SEM. ***P < 0.001; Control vs GLUT2^{ΔIEC} mice (Mann–Whitney U test).

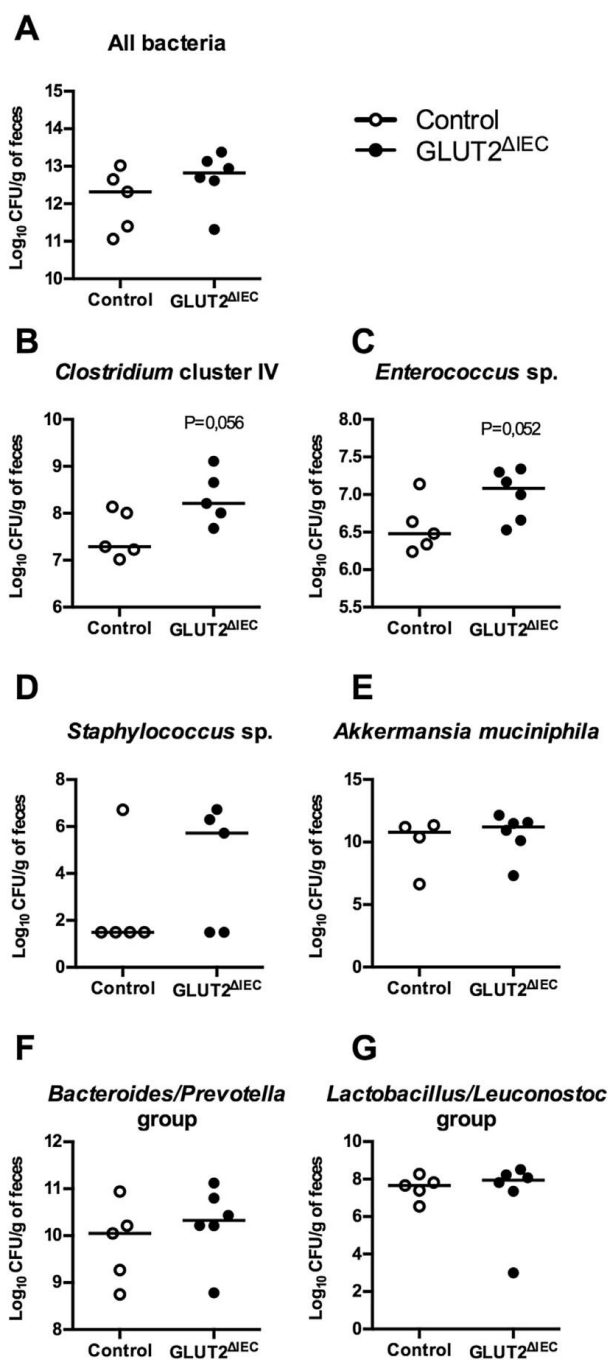


Figure 4: Intestinal GLUT2 invalidation changes fecal microbiota composition. (A) Total fecal bacterial counts in control (white circles, n = 5) and GLUT2^{ΔIEC} mice (black circles, n = 6), 4 weeks after tamoxifen administration. Values are median (range). (B–G) Fecal quantification of *Clostridium* cluster IV (B), *Enterococcus* sp. (C), *Staphylococcus* sp. (D), *Akkermansia muciniphila* (E), *Bacteroides/Prevotella* group (F) and *Lactobacillus/Leuconostoc* group (G) in control (white circles, n = 4–5) and GLUT2^{ΔIEC} mice (black circles, n = 5–6), 4 weeks after tamoxifen administration. Values are medians (range). P values are presented according to the Mann–Whitney U test.

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from kidney (Figure 2D), bladder (Figure 2E), liver (Figure 2F), brain (Figure 2G), and stomach (Figure 2H) enabled the calculation of 2FDG accumulation slopes every 5 min over the first 30-min period. These measurements highlighted that 2FDG appeared more slowly in bladder ($P = 0.0444$) and in kidney ($P = 0.139$) of GLUT2^{ΔIEC} compared to control mice. Slopes of 2FDG appearance in bladder can be considered as an index of 2FDG apparition in the bloodstream due to the lack of 2FDG reabsorption by the kidneys. Furthermore, we also observed a 2FDG accumulation delay in liver ($P = 0.0507$) in GLUT2^{ΔIEC} compared to control mice. Indeed, we calculated a 10-min delay to achieve 50% of maximal 2FDG incorporation in liver in GLUT2^{ΔIEC} compared to control mice (Figure 2F). Interestingly, we could not observe any difference in the brain between the 2 groups of mice ($P = 0.2616$), suggesting a preferential glucose biodistribution to the brain compared to other tissues.

Differences in 2FDG biodistribution could be a consequence of delayed intestinal glucose absorption, which could not be attributed to a defect in gastric emptying (Figure 2H) or intestinal transit duration (Figure 2I). *Ex vivo* examination of flushed intestine showed a significant reduction of 2FDG accumulation the first half small intestinal mucosa of GLUT2^{ΔIEC} compared to control mice ($P = 0.0187$) (Figure 2J,K). This corresponds to the duodenum, jejunum, and very proximal ileum, where GLUT2 is mostly expressed in control mice. This experiment was performed with a high dose of glucose (10 MBq 2FDG in a saline solution containing 4 g/kg of D-glucose), which results in apical GLUT2 recruitment. Moreover, 2FDG is poorly transported through SGLT1. Therefore, 2FDG enters through GLUT2 in enterocytes of control mice, which could not occur in GLUT2^{ΔIEC} mice.

Thus, we propose that sugar absorption and distribution were delayed and moderately decreased by intestinal GLUT2 invalidation in mice.

3.2.3. Intestinal GLUT2 invalidation improves glucose tolerance and limits body weight gain

To investigate the consequences of intestinal GLUT2 invalidation on glucose homeostasis, we evaluated glucose tolerance and insulin sensitivity. GLUT2^{ΔIEC} mice display significantly improved oral glucose tolerance compared to control littermates (Figure 3A). However, intraperitoneal glucose tolerance was similar in both groups (Figure 3B). Improved oral glucose tolerance was independent of insulin sensitivity, which did not differ between groups (Figure 3C). Intestinal GLUT2 invalidation caused a decrease in body weight gain in GLUT2^{ΔIEC} compared to control littermates throughout the 14 weeks of experiment ($P = 0.0486$) (Figure 3D). This occurred despite similar food intake (Figure 3E), water intake (Figure 3F), and total fecal calorie loss (Figure 3G) in both groups. Decreased body weight gain, therefore, could be attributed to glucose malabsorption.

Metabolite plasma measurements showed a significantly higher level of β-hydroxybutyrate in GLUT2^{ΔIEC} compared to control mice (Figure 3H, $P = 0.0433$) with no change in other plasma metabolites measured (data not shown). Ketone body production such as β-hydroxybutyrate in GLUT2^{ΔIEC} mice is reminiscent of fasting state or caloric restriction.

3.3. Gut phenotype

3.3.1. Absorption surface is reduced in the intestine of GLUT2^{ΔIEC} mice

Importantly, we observed a significant decrease ($P < 0.001$) of microvillus length in the jejunum of GLUT2^{ΔIEC} as compared to control mice (Figure 3I and J), mimicking a condition of food deprivation. This

15% diminution in microvillus length reduces the absorbing epithelial surface and, thus, could further limit the absorption of nutrients.

3.3.2. Intestinal GLUT2 invalidation changes microbiota composition

The signs of glucose malabsorption described in GLUT2^{ΔIEC} mice suggest glucose persistence in the intestinal lumen that might affect gut bacterial composition. Despite similar total bacteria counts (Figure 4A), *Clostridium* cluster IV (Figure 4B, $P = 0.056$) and *Enterococcus* sp. (Figure 4C, $P = 0.052$), levels were increased in fecal microbiota from GLUT2^{ΔIEC} compared to control mice. Concerning *Staphylococcus* sp. (Figure 4D), two populations appeared in each group: mice are either not colonized or colonized at high level with *Staphylococci*. Other studied species (i.e. *Akkermansia muciniphila*), or groups (i.e. *Bacteroidetes/Prevotella* and *Lactobacillus/Leuconostoc*) remained unaffected (Figure 4E–G).

3.3.3. Intestinal GLUT2 invalidation decreases gut permeability and improves gut inflammatory status

Because modulation of gut microbiota is often associated with modification in gut barrier function, we investigated the impact of intestinal

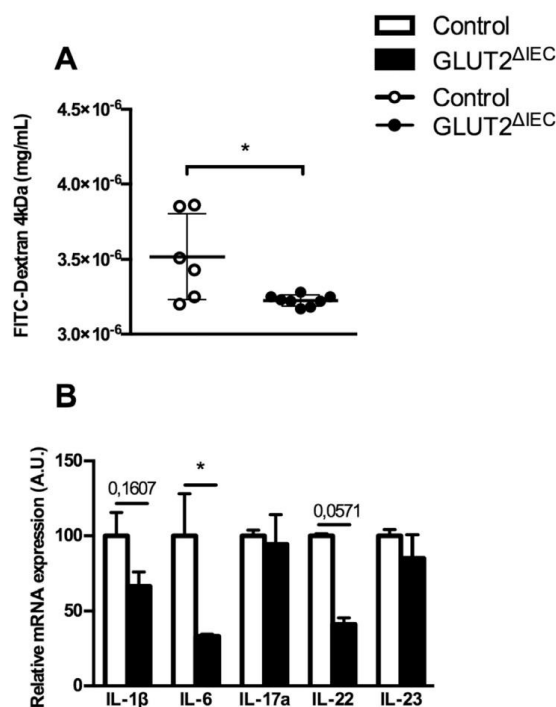


Figure 5: Intestinal GLUT2 invalidation reduces jejunal permeability and inflammatory status. (A) Jejunum permeability was measured in control mice (white circles, $n = 6$) and GLUT2^{ΔIEC} mice (black circles, $n = 8$) by Ussing Chamber method. FD4 concentration was measured in serosal compartment after a 180-min period. Values are means $\pm$ SEM. * $P < 0.05$; control vs GLUT2^{ΔIEC} mice (Mann–Whitney U test). (B) Relative mRNA levels of pro-inflammatory cytokines IL-1β, IL-6, IL-17, IL-22 and IL-23 in ileal lamina propria lymphocytes of control (white bars, $n = 4–5$) and GLUT2^{ΔIEC} mice (black bars, $n = 3$), 4 weeks after tamoxifen administration. Values are means $\pm$ SEM. * $P < 0.05$; Control vs GLUT2^{ΔIEC} mice (Mann–Whitney U test).

GLUT2 invalidation on permeability using Ussing Chamber method (Figure 5A). Interestingly, there was a significantly decreased permeability in the jejunum of GLUT2^{ΔIEC} compared to control mice. Permeability is also affected by inflammatory signals and we highlighted here a decrease of mRNA coding for pro-inflammatory cytokines IL-6 ($P = 0.0357$) and IL-22 ($P = 0.0571$) but no change for the expression of IL-1 β ($P = 0.1607$), IL-17a ($P = 0.8571$) or IL-23 ($P = 0.2143$) in the ileum of GLUT2^{ΔIEC} mice compared to that of control mice (Figure 5B). The gut inflammatory status of GLUT2^{ΔIEC} mice was thus slightly decreased after the invalidation of intestinal GLUT2 suggesting that sugar availability is coupled with permeability and inflammation in gut.

3.3.4. Intestinal GLUT2 invalidation reduces density but improves function of GLP-1 positive cells

As GLUT2 is also expressed in GLP-1 positive cells, we investigated L-cell homeostasis in GLUT2^{ΔIEC} mice. We first measured the density of GLP-1 positive cells (L-cells) in the intestine of GLUT2^{ΔIEC} and control mice (Figure 6A and B). Surprisingly, there was a significant decrease of L-cell density in jejunum (50%, $P = 0.0053$) and a tendency to lower density in colon (20% reduction, $P = 0.147$) of GLUT2^{ΔIEC} as compared to control mice (Figure 6B). Despite this lower L-cell density in GLUT2^{ΔIEC} mice, both intestinal total GLP-1 content (Figure 6C) and plasma active GLP-1 concentration (Figure 6D) remained unaltered when compared to control mice. The preservation of enteroendocrine

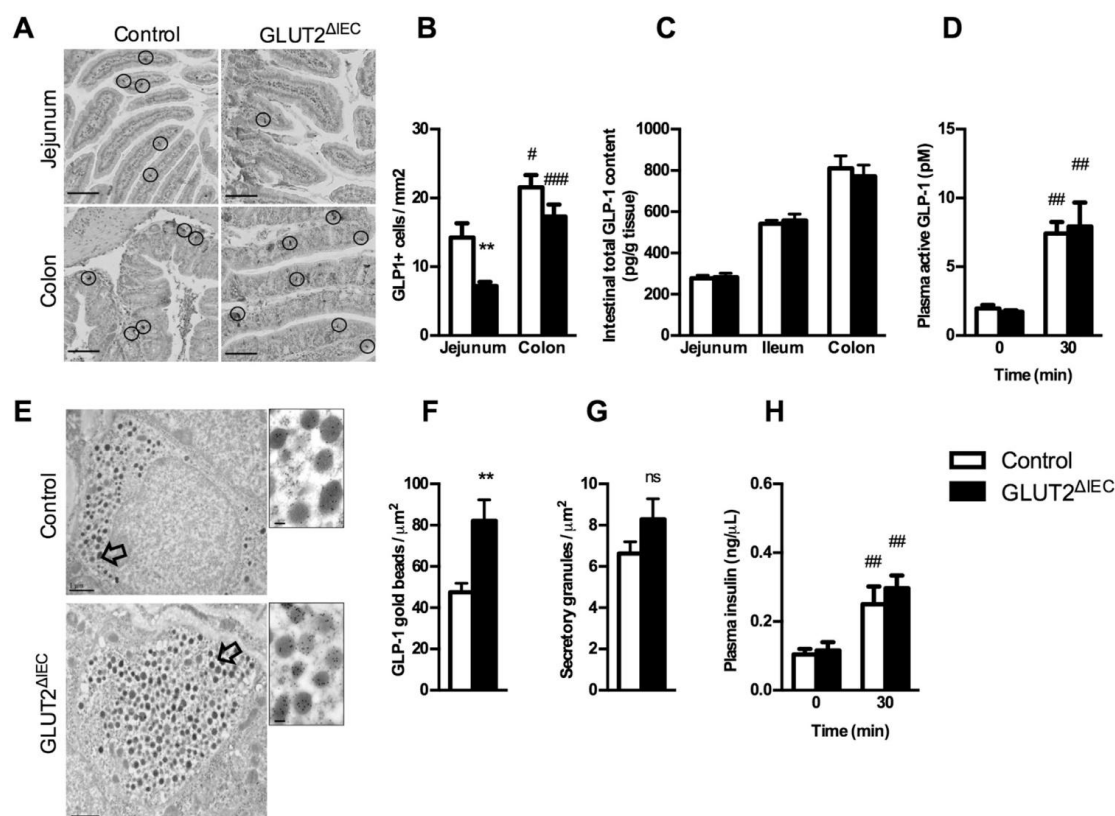


Figure 6: Intestinal GLUT2 invalidation reduces GLP-1-producing cell density in the jejunum. (A) Representative images of GLP-1 immunostaining in jejunum and colon sections from control (left panel) and GLUT2^{ΔIEC} mice (right panel), 4 weeks after tamoxifen administration. GLP-1 immunoreactive cells are circled. Scale bar = 100 μ m. (B) Number of GLP-1 immunoreactive cells per mm² in the jejunum and colon of control (white bars, $n = 15$) and GLUT2^{ΔIEC} mice (black bars, $n = 11$), 4 weeks after tamoxifen administration. Values are means $\pm$ SEM. ** $P < 0.01$; control vs GLUT2^{ΔIEC} mice. # $P < 0.05$; ### $P < 0.001$ jejunum vs colon (Mann–Whitney U tests). (C) Intestinal total GLP-1 contents (pmol/g of tissue) were measured in jejunum, ileum, and colon samples of control (white bars, $n = 9$) and GLUT2^{ΔIEC} mice (black bars, $n = 9$), 4 weeks after tamoxifen administration. Values are means $\pm$ SEM. (D) Plasma active GLP-1 concentrations after a bolus of 2 g glucose/kg + 200 μ L olive oil were measured in control (white bars, $n = 10$) and GLUT2^{ΔIEC} mice (black bars, $n = 11$), 4 weeks after tamoxifen administration. Values are means $\pm$ SEM. ## $P < 0.01$; 0 vs 30 min (Mann–Whitney U test). (E) Representative electron microscopy images of enteroendocrine L-cells labeled with anti-GLP-1-coupled immunogold beads from colon of control (top panel) and GLUT2^{ΔIEC} mice (bottom panel). Scale bar: 1 μ m (entire image) and 100 nm (magnification). (F) Number of anti-GLP-1-coupled immunogold beads (included in a secretory granule) per μ m² in the colon of control (white bar, 29 L-cells) and GLUT2^{ΔIEC} mice (black bar, 14 L-cells), 4 weeks after tamoxifen administration. Values are means $\pm$ SEM. ** $P < 0.01$; control vs GLUT2^{ΔIEC} mice (Mann–Whitney U test). (G) Number of secretory granules per μ m² in the colon of control (white bar, 30 L-cells) and GLUT2^{ΔIEC} mice (black bar, 14 L-cells), 4 weeks after tamoxifen administration. Values are means $\pm$ SEM. ns: non-significant. (H) Basal and stimulated plasma insulin concentrations after an oral challenge of 2 g glucose/kg + 200 μ L olive oil assessed in control (white bars, $n = 10$) and GLUT2^{ΔIEC} mice (black bars, $n = 11$), 4 weeks after tamoxifen administration. Values are means $\pm$ SEM. ## $P < 0.01$; 0 vs 30 min (Mann–Whitney U test).

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function could be explained by an 80% higher GLP-1 content per L-cell in GLUT2^{ΔIEC} mice as compared to control mice (Figure 6E and F). However, secretory granule density per L-cell was similar in the 2 groups of mice (Figure 6G) demonstrating a higher GLP-1 content per granule in GLUT2^{ΔIEC} mice. These data suggested that the residual L-cells present in the epithelium produce more GLP-1 to fulfill GLP-1 secretion in GLUT2^{ΔIEC} mice. Plasma GLP-1 was preserved in GLUT2^{ΔIEC} mice leading to a conserved plasma insulin concentration in GLUT2^{ΔIEC} mice as compared to control mice (Figure 6H).

4. DISCUSSION

We showed that intestinal GLUT2 invalidation provokes glucose malabsorption that delayed glucose biodistribution to tissues. The consequences of intestinal-specific invalidation of GLUT2 on mouse phenotype were limited weight gain and improved glucose tolerance and gut barrier function, which was associated with reduced gut inflammation. All these features have been reported in calorie restriction conditions [15]. Moreover, we showed that gut epithelium homeostasis was challenged by sugar malabsorption. Indeed, microvillus length was reduced, and the change in microbiota composition, possibly related to persistence of malabsorbed glucose in the lumen of the distal intestinal segment, favored *Clostridium* cluster IV, a commensal *Clostridium* known to display protective roles on gut homeostasis. In addition, L-cell density was reduced in the absence of intestinal GLUT2 expression, but the GLP-1 content per L-cell was increased, suggesting preserved enteroendocrine L-cell function. Therefore, we propose that blocking intestinal GLUT2 function is a

possible strategy to temper sugar biodistribution and, surprisingly, modulate gut epithelial homeostasis.

GLUT5 mRNA was doubled in the jejunum 12 weeks after tamoxifen administration, supporting a compensatory mechanism for fructose absorption in GLUT2^{ΔIEC} mice as previously suggested in *Glut2*^{-/-}; *RIPGlut1* mice [2]. However, we could not observe any compensatory mechanism concerning intestinal glucose transport by overexpression of other glucose transporters. This could be explained by the existence of vesicular glucose transport that occurs in absence of GLUT2 as shown in *Glut2*^{-/-}; *RIPGlut1* mice [10]. However, this mechanism is not sufficient to fully compensate for the absence of GLUT2 leading to moderate glucose malabsorption and reduced body weight gain in GLUT2^{ΔIEC} mice.

Inactivating mutations of GLUT2 are reported in patients [16]. Only double heterozygous or homozygous mutations in the *SLC2A2* gene encoding GLUT2 (<http://omim.org/entry/227810>) have been found in infants with Fanconi–Bickel syndrome. These patients suffer from failure to thrive, glucose-galactose malabsorption, gross urinary loss of glucose, glycogen accumulation in liver and kidney, and, in some patients, diabetes. To restore patient growth, a ketogenic diet is proposed to Fanconi–Bickel patients. It has been reported that intestinal regulatory function of GLUT2 could be compensated by a ketogenic diet (frequent boluses of slowly absorbed carbohydrates, such as uncooked cornstarch). Caloric restriction without malnutrition is indeed characterized by ketosis and a slow-down of biological processes such as body weight gain, glucose metabolism [15], and gut inflammation [17–19] as well as decreased microvillus length [20]. Here, the doubling of plasma ketone body β-hydroxybutyrate in

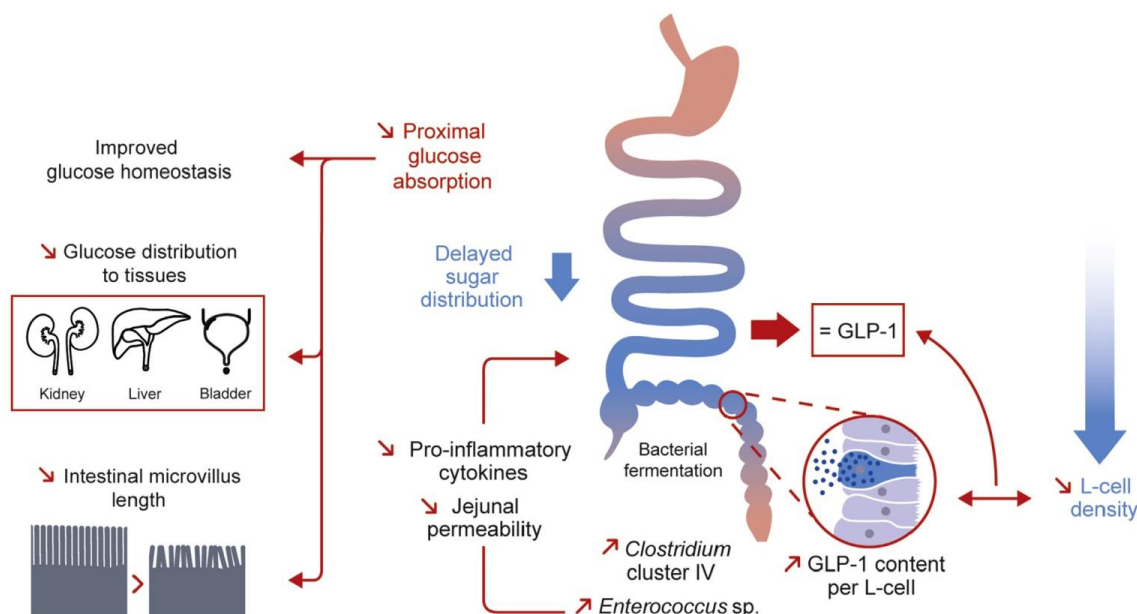


Figure 7: Intestinal GLUT2 plays a role in glucose and gut homeostasis in mice. Intestinal invalidation of GLUT2 in mice reduces proximal intestinal absorption of glucose. This sugar malabsorption modifies body homeostasis by means of 1) improving glucose homeostasis, 2) delaying tissue distribution of glucose to peripheral tissues, and 3) reducing intestinal microvillus length, which could result in a global nutrient malabsorption, mimicking caloric restriction. Our hypothesis is that blocked proximal glucose absorption causes an increased glucose delivery to distal intestine, giving new fermentable energy sources to the distal gut microbiota. Thus, we observed increased levels of commensal *Clostridium* cluster IV and *Enterococcus* sp. in our model. These bacteria show positive impact on gut homeostasis, including a reduced expression of pro-inflammatory cytokines through butyrate production and reduced gut permeability. Surprisingly, intestinal GLUT2 invalidation leads to a strong loss in enteroendocrine L-cell density, with no impact on GLP-1 plasma levels thanks to increased GLP-1 content per GLUT2^{ΔIEC} L-cell.

GLUT2^{ΔIEC} mice as a sign of a glucose restriction for tissue, could be attributed to delayed glucose delivery to tissues due to intestinal GLUT2 depletion.

GLUT2^{ΔIEC} mice displayed a reduction of both microvillus length and inflammation. This could be due to changes in microbiota composition caused by disturbed glucose abundance in the lumen. Indeed, *Clostridium* cluster IV, belonging to the Firmicutes phylum, is increased in GLUT2^{ΔIEC} mice. These commensal bacteria show a positive impact on gut homeostasis [21], including a reduced expression of pro-inflammatory cytokines through butyrate production [22,23]. *Enterococci* are also increased. Some members of this genus show anti-inflammatory properties, suggesting that this increase also can be associated with a suitable gut homeostasis. On the other hand, enteroendocrine cells are one of the cell types for which density in the gut epithelium is dependent on nutritional factors [14]. In our model, endocrine L-cell homeostasis was modulated by intestinal GLUT2 inactivation. Despite reduced L-cell density, enteroendocrine function seemed to be preserved through higher intracellular GLP-1 content. Recent papers showed that during OGTT in various types of metabolic diseases, GLP-1 release is delayed along with prolongation of the postprandial hyperglycaemia [24–26]. In our study, GLP-1 secretion was measured belatedly, 30 min after GLP-1 secretagogue administration. Therefore, we cannot exclude that the lack of intestinal GLUT2 is retarding glucose-induced GLP-1 release from the jejunum and therefore has consequences on glucose and gut homeostasis. The use of various metabolic stimuli and the measurement of plasma GLP-1 at earlier time points in GLUT2^{ΔIEC} to challenge L-cell function would be informative for enteroendocrine function.

5. CONCLUSION

Intestinal GLUT2 is known as a means to transfer glucose and fructose from the lumen to the bloodstream and, thereby, to provide sugar to tissue. Here, we extended the role of intestinal GLUT2 in sugar distribution to tissues and glucose homeostasis. In addition, inactivation of GLUT2 also revealed unexpected function for GLUT2 in regulating gut homeostasis such as modulating enteroendocrine L-cell density, microvillus length, gut permeability, and inflammation (Figure 7). Therefore, specifically blocking intestinal GLUT2 activity by the mean of drugs could be a strategy to protect against weight gain and metabolic perturbations.

AUTHORS' CONTRIBUTIONS

C.C.S., A.G. and P.S. designed experiments, acquired, analyzed data, and wrote the manuscript. T.A., T.V., A.J.W., C.M., O.R., and N.K. acquired data and contributed to the discussion. D.C. acquired and analyzed data. E.B.L. and M.L.G. generated GLUT2^{ΔIEC} and contributed to the discussion. A.Let. contributed to the discussion and wrote the manuscript. G.G., A.Leh., and B.T. contributed to the discussion.

PRIOR PRESENTATION

Part of these data were presented at 51st Annual Meeting of the European Association for the Study of Diabetes 2015 [27].

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.molmet.2016.10.008>.

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IV) Discussion

I) Synthèse de mes résultats

Au cours de ma thèse, j'ai pu démontrer que la malabsorption du fructose était présente chez 22% des patients souffrant d'un SII. Les patients souffrant de malabsorption étaient plutôt des hommes, dans 40% des cas, alors que le sexe masculin ne représentait que 21% de la population de patients SII. Ils étaient également jeunes, 37 ans en moyenne, pour une population âgée de 44 ans en moyenne. Une élévation de la calprotectine fécale, signant une inflammation digestive de bas grade a été retrouvée chez 1/3 de nos patients souffrant d'un SII. L'élévation de la calprotectine fécale était corrélée à l'âge des patients. En revanche, cette anomalie n'était pas associée aux autres anomalies physiopathologiques (test respiratoire au glucose positif en faveur d'une pullulation bactérienne endoluminale, test respiratoire au fructose positif en faveur d'une malabsorption du fructose, barostat rectal en faveur d'une hypersensibilité viscérale ou présence d'une inflammation sur les biopsies coliques). En particulier, l'inflammation de bas grade n'était pas associée à la malabsorption du fructose. De manière intéressante, la présence d'une malabsorption du fructose était associée à la présence d'une hypersensibilité viscérale mesurée par un barostat rectal.

Ces résultats ont été reproduits chez la souris dans 3 modèles de malabsorption du fructose (régime riche en fructose, souris GLUT5 KO et délétion intestinale de GLUT2). Ces modèles murins de malabsorption du fructose présentaient également une hypersensibilité viscérale ainsi qu'une augmentation de la perméabilité intestinale. En revanche, aucune inflammation digestive n'a été retrouvée sur nos modèles de souris. Ces modèles nous ont permis de mieux appréhender les mécanismes physiopathologiques sous-jacents. Il existe en effet une augmentation de l'activité protéasique dans les fèces de 2 de nos modèles murins de malabsorption du fructose (régime riche en fructose et GLUT5 KO). L'élévation de l'activité protéasique pourrait être d'origine pancréatique (devant l'importance de l'élévation mais également une activité normale dans les tissus) ou du microbiote. On constate une augmentation de la CCK au niveau du caecum et de l'iléon dans d'autres modèles de malabsorption du fructose (modèle de souris KHK^{-/-}, Thèse de science de X. Zhang, Micalis,

INRA, Paris-Saclay, France). L'activité protéasique, par l'intermédiaire de PAR-2 est connue pour induire notre phénotype (hypersensibilité viscérale associée à une augmentation de la perméabilité intestinale) (123-125).

Un régime appauvri en fructose et dérivés a permis d'améliorer significativement les patients souffrant d'un SII, mais de manière indépendante du résultat du test respiratoire au fructose et donc de la présence ou non d'une malabsorption du fructose.

II) Apports au regard des connaissances de la littérature

Article 1 : Prévalence de la malabsorption du fructose dans le syndrome de l'intestin irritable : résultats d'une étude prospective

Notre étude sur la prévalence de la malabsorption du fructose dans le SII a permis de définir à l'aide d'un test respiratoire au fructose avec une dose de charge de 25g, la prévalence exacte, en France, de la malabsorption du fructose dans le SII : 22%. Parmi ces patients, seuls 35% sont symptomatiques et définissent l'intolérance vraie au fructose. Notre méthodologie est validée, notamment par le consensus Nord Américain (120). Le jour du test, le patient devait être à jeun (y compris le tabac) et ne pas avoir pratiqué de sport juste avant le test. Le test était effectué après 48 heures de régime spécifique pour diminuer la production gazeuse intestinale. Il était systématiquement réalisé après avoir éliminé une pullulation bactérienne endoluminale par un test respiratoire au glucose préalable (dose de charge 75g). En effet cette condition pathologique dans laquelle la population bactérienne colique remonte dans l'intestin grêle peut conduire à de faux positifs du test respiratoire au fructose (126). Le monitoring de l'air expiré était effectué toutes les 30 minutes pendant 5 heures; le test était considéré comme positif et définissait la malabsorption du fructose en cas d'élévation de plus de 20ppm des gaz expirés, en accord avec les recommandations récentes (120). Cette prévalence est plus faible que celle démontrée par Wilder-Smith (qui utilisait des tests respiratoire à 35g de fructose et un seuil de positivité différent et non validé ($\text{CH}_4 > 10\text{ppm}$)) mais reste plus spécifique (49). Avec un tel seuil, la prévalence de la malabsorption du fructose ne différait pas entre les patients souffrant

d'un SII et les volontaires sains, cette absence de différence ne semble pas cliniquement pertinente (127). Notre étude n'a pas permis de déterminer de profil type du patient qui malabsorbe le fructose, cependant la population de patients "malabsorbeurs" concernait plus souvent des patients de sexe masculin et plus jeunes.

Article 2 : Est-ce que le taux de calprotectine fécale permet d'identifier un sous-groupe de patients souffrant d'un syndrome de l'intestin irritable? Résultats d'une étude prospective

Notre étude sur *Le dosage de la calprotectine fécale permet-il d'identifier un sous-groupe de patients dans le SII?* est la première à utiliser le dosage de la calprotectine fécale pour identifier une sous-population de SII avec inflammation de bas grade. Dans la littérature, le dosage de la calprotectine fécale permet habituellement de différencier les patients souffrant d'une MICI des patients souffrant d'un SII (128). Une élévation de la calprotectine fécale, signant une inflammation de bas grade est présente chez 1/3 de nos patients SII. Cette élévation n'était pas corrélée à l'intensité de l'inflammation colique en histologie ni à la présence de cellules inflammatoires (mastocytes ou lymphocytes T). Ces résultats sont à prendre avec réserve car seulement 80% des patients ont pu avoir des biopsies coliques qui intéressaient le plus souvent uniquement le colon gauche. D'autre part, les biopsies n'intéressaient que la muqueuse, or les mastocytes sont souvent retrouvés dans les couches plus profondes. Une des critiques de notre travail était le dosage unique de la calprotectine fécale. Cependant dans la littérature, il a été démontré la faible variabilité du dosage de la calprotectine fécale autorisant l'utilisation d'un dosage unique (129, 130). Une de nos hypothèses était que l'élévation de la calprotectine fécale pouvait être expliquée par la présence d'une pullulation bactérienne endoluminale. Dans notre cohorte, seulement 3 des 93 patients présentaient une pullulation bactérienne endoluminale (test respiratoire au glucose). L'absence de lien entre pullulation bactérienne endoluminale et élévation de la calprotectine fécale dans notre étude, conforte les résultats de *Gasbarrini et al.*, où la pullulation bactérienne endoluminale n'était pas associée à l'inflammation intestinale médiée par les neutrophiles (131). Nous n'avons pas pu mettre en évidence d'association entre inflammation

et hypersensibilité viscérale (barostat rectal). Une des explications à l'élévation de la calprotectine fécale pourrait être une augmentation de la perméabilité intestinale. Malheureusement, l'étude de la perméabilité intestinale n'existe pas en routine clinique et n'a pas pu être évaluée chez nos patients.

Article 3 : Etude contrôlée prospective : La positivité d'un test respiratoire au fructose est elle prédictive de l'efficacité d'un régime appauvri en fructose dans le syndrome de l'intestin irritable?

Un des objectifs de ma thèse était de déterminer si le test respiratoire au fructose pourrait permettre de prédire l'efficacité du régime appauvri en fructose sur les symptômes digestifs des patients souffrant d'un SII. Cette question était toujours débattue dans la littérature (115, 116). Malheureusement les résultats de notre étude contrôlée sont négatifs : un test respiratoire au fructose positif ne permet pas de prédire l'efficacité du régime appauvri en fructose chez des patients souffrant d'un SII. Les patients "absorbeurs" comme "malabsorbeurs" avaient une amélioration significative des symptômes digestifs alors qu'on aurait pu s'attendre à une différence. L'efficacité d'un test respiratoire à 50g avait déjà été étudiée en ouvert avec des résultats identiques (118). Nous avons préféré choisir une dose de charge de 25g en nous basant sur la capacité intestinale normale d'absorber 25g et non 50g (110). Le test respiratoire à 25g est très spécifique mais peu sensible et peut entraîner de faux négatifs. La dose de 25g est également plus proche des apports quotidiens en fructose et semble efficace pour diagnostiquer la malabsorption du fructose (119). Nous nous attendions à ce qu'une dose de fructose plus faible nous permette d'identifier les sujets avec une absorption plus limitée du fructose et qui pourraient d'avantage bénéficier du régime. Nous ne pouvons exclure la présence de faux négatifs dans le groupe de patients "absorbeurs" ce qui pourrait expliquer l'amélioration des patients dans ce groupe. L'inefficacité du test respiratoire au fructose pour identifier les patients candidats à un régime appauvri en fructose est difficile à expliquer. L'hétérogénéité des méthodologies, sur la dose de charge de fructose, la concentration de gaz, est importante. De Roest et al. ont démontré l'efficacité d'un test à 35g sur l'efficacité d'un régime pauvre en FODMAPs (51). Nous avons besoin de plus larges

études évaluant différentes doses de charge et cut-off de gaz expirés afin de déterminer le meilleur test pour prédire l'efficacité d'un régime pauvre en fructose. Une autre explication à nos résultats serait que la malabsorption du fructose ne soit pas la bonne cible pour expliquer les symptômes des patients. Il n'est pas possible non plus d'exclure un effet placebo du régime. Une amélioration de l'anxiété et de la dépression durant cette étude de courte durée pourrait être un argument supplémentaire en faveur de l'effet placebo. Les points positifs de notre régime ont été sa bonne observance, raison pour laquelle il avait été réalisé sur 15 jours. Un régime de 6 semaines semble plus approprié, mais plus difficile à appliquer de manière stricte pour les patients (132). Nous pouvons envisager qu'une durée courte du régime ne soit pas bénéfique sur le microbiote intestinal, comme cela a été démontré dans les FODMAPs après plus de 3 semaines de régime (64). Il serait intéressant d'étudier le profil de la flore colique et les composés organiques volatiles comme facteur prédictif de réponse au régime pauvre en fructose puisque cela a été démontré pour le régime pauvre en FODMAPs (65, 133).

Nos résultats ne nous permettent donc pas de recommander ce régime chez nos patients mais une étude contrôlée contre un autre régime serait utile pour déterminer son efficacité dans le SII.

Article 4 : Hypersensibilité viscérale et une augmentation de la perméabilité de la barrière intestinale dans un modèle murin de syndrome de l'intestin irritable avec malabsorption du fructose

Nous avons retrouvé une association entre la malabsorption du fructose et l'hypersensibilité viscérale chez nos patients SII. En effet, 1 patient "absorbeur" sur 10 avait une hypersensibilité viscérale pour 1 patient "malabsorbeur" sur 2. En partant de ce constat, nous avons utilisé 3 modèles murins pour modéliser la malabsorption du fructose et étudier les mécanismes physiopathologiques sous-jacents. Deux modèles avec délétion des transporteurs du fructose GLUT2 et GLUT5 et un modèle avec augmentation des apports en fructose (78, 79) ont été utilisés. Nous avons pu reproduire l'hypersensibilité viscérale dans ces 3 modèles murins de malabsorption du fructose. L'hypersensibilité viscérale était également associée à

une augmentation de la perméabilité intestinale. Plus la malabsorption du fructose était importante, plus la perméabilité intestinale était élevée (régime riche en fructose > GLUT5-/- > GLUT2^{ΔIEC}). Une des explications possibles à ce résultat pourrait être l'hyperglycémie. Il a été récemment démontré par Thaïss *et al.* que l'hyperglycémie jouait un rôle central dans les altérations de la barrière intestinale (134). Malheureusement la glycémie n'a pas été dosée dans notre étude.

Nos modèles n'étaient pas associés à une élévation des paramètres inflammatoires. Ces résultats sont en discordance avec ceux de Zhou *et al.* qui retrouvaient qu'un régime riche en FODMAPs entraîne une dysbiose et une augmentation du LPS fécal qui modulerait la sensibilité viscérale dans le SII (135). Contrairement à Zhou *et al.*, nous n'avons pas d'élévation des ARNm d'IL1 β et de TNF α , ni d'augmentation de l'activité MPO. Chez les patients, nous avons utilisé des méthodes de dosage différentes (calprotectine fécale dans notre étude et biopsies coliques dans l'étude de Zhou). D'autre part, nos patients SII avaient tous les sous-types connus, tandis que Zhou *et al.* n'a étudié que 6 patients du sous-type diarrhéique.

Chez nos souris GLUT5KO et avec régime riche en fructose, il existait une augmentation de l'activité élastase dans les fèces. Le rôle des protéases a déjà été souligné dans le SII et pourrait promouvoir l'hypersensibilité viscérale et l'augmentation de la perméabilité intestinale par un mécanisme PAR-2 dépendant (123). Une de nos hypothèses serait que cette activité élastase provienne du pancréas. Nous n'avons pas encore pu doser la CCK dans nos modèles, mais elle est augmentée dans l'iléon et le caecum d'un autre modèle de malabsorption du fructose (KHK-/-, V. Douard données personnelles). Il existe également un travail chez l'Homme montrant que la malabsorption du fructose était associée à une augmentation des enzymes pancréatiques amylase et lipase (99). L'activité élastase peut également être modulée par le microbiote. Nous n'avons malheureusement pas pu étudier le microbiote dans l'ensemble de nos modèles. Chez les souris GLUT2^{ΔIEC}, nous avons déjà montré une augmentation de *clostridium cluster IV* et *enterococcus species* (74). Nous n'avons pas pu reproduire ce résultat dans cette étude, mais les souris avec et sans délétion partageaient la même cage. L'absence d'élévation de l'activité protéasique dans le groupe de

souris GLUT2^{ΔIEC} pourrait être expliquée par un transfert de microbiote entre les souris des 2 groupes. Pour mieux comprendre le rôle du microbiote dans les modèles de malabsorption du fructose, il serait intéressant d'étudier le microbiote dans l'ensemble des groupes mais également d'avoir un recours de transfert de microbiote de patients SII, après avoir dosé l'activité protéasique dans les fèces (136). Le régime riche en fructose n'a pas pu sensibiliser des souris axéniques (données personnelles), mais ce modèle ne nous semble pas le plus pertinent car l'absence de microbiote intestinal est responsable d'une immaturité du système nerveux entérique (122). Sur des souris déplétées du microbiote par antibiotiques, nous n'avons pas pu reverser l'hypersensibilité (données personnelles). Néanmoins et indépendamment de l'absorption du fructose, les antibiotiques peuvent également être à l'origine d'une hypersensibilité viscérale (137).

III) Perspectives futures

1) Quel est le mécanisme de la malabsorption du fructose chez les patients souffrant d'un syndrome de l'intestin irritable?

Le mécanisme de la malabsorption du fructose dans le SII n'est pas encore connu. Il pourrait s'agir d'un problème de transporteur puisque le fructose est absorbé sous la forme de monosaccharide.

Chez l'Homme, seule la mutation du gène de GLUT2 est connue, résultant en une protéine tronquée inefficace. Il s'agit du syndrome de Fanconi-Bickel, pathologie dans laquelle la tolérance au fructose n'est pas modifiée (86); d'autre part, ce type de mutation est très rare alors que la malabsorption du fructose touche 22% des patients souffrant d'un SII (138). Il n'existe pas de mutation de GLUT5 connue à ce jour chez l'Homme (88). Une mutation des transporteurs ne semble pas pouvoir expliquer le phénotype de malabsorption du fructose chez nos patients.

L'équipe de Wilder-Smith a démontré qu'il n'y avait pas de différence d'expression ou de production des principaux transporteurs du fructose GLUT5 et GLUT2 dans le duodénum de

patients TFI avec malabsorption du fructose en comparaison à des contrôles (89). Le mécanisme sous-jacent de la malabsorption du fructose semblerait être en lien avec un problème de régulation ou de localisation de ces transporteurs.

Sur des données personnelles, nous n'avons pas trouvé de différence de localisation de GLUT2 et GLUT5 sur des biopsies duodénales de patients souffrant d'un SII avec malabsorption du fructose en comparaison avec des patients sans malabsorption. Notre effectif étant faible ($n=8$ et $n=7$) et les biopsies réalisées à jeun, GLUT2 était donc localisé au pôle basal et non apical. Il semblerait intéressant de pouvoir étudier la localisation de ces transporteurs chez des patients SII sans et avec malabsorption du fructose, hors période de jeun ainsi que l'activation de ces transporteurs.

Un autre mécanisme qui pourrait également participer à la malabsorption du fructose est le métabolisme du fructose. Dans l'entérocyte, le fructose est métabolisé par la Kétohexokinase (KHK); la KHK intervient également au niveau du foie et des organes périphériques et permet, entre autre, de réguler l'arrivée du fructose au niveau systémique. Sur des modèles de souris transgéniques avec délétion du gène codant pour la KHK, les KHK^{-/-}, l'absence de KHK ne modifie pas le transport du fructose (139). En revanche, il en résulte une malabsorption du fructose. Il n'existe pas de mutation de la KHK connue à ce jour chez l'Homme. Dans le métabolisme du fructose, il existe également l'aldolase qui transforme le fructose-1-phosphate en glycéraldéhyde et dihydroxyacétone phosphate. Le bon fonctionnement du métabolisme du fructose est indispensable à l'absorption du fructose (139). Des modifications de l'activité de l'aldolase ont déjà été rapportées chez l'Homme en cas de malabsorption du fructose (140).

De manière intéressante, le facteur de transcription ChREBP régule le métabolisme du fructose (141). Il régule l'expression de l'ARN de GLUT2, GLUT5 et de KHK qui sont indispensables au métabolisme du fructose (142). Dans des modèles murins de ChREBP KO, il en résulte une malabsorption du fructose avec distension colique et des modifications du microbiote (142). Une altération de ChREBP pourrait être un mécanisme expliquant les phénotypes de malabsorption du fructose chez les patients. En effet chez la Souris, il a été montré que ChREBP joue un rôle central dans le transport du fructose et le métabolisme du

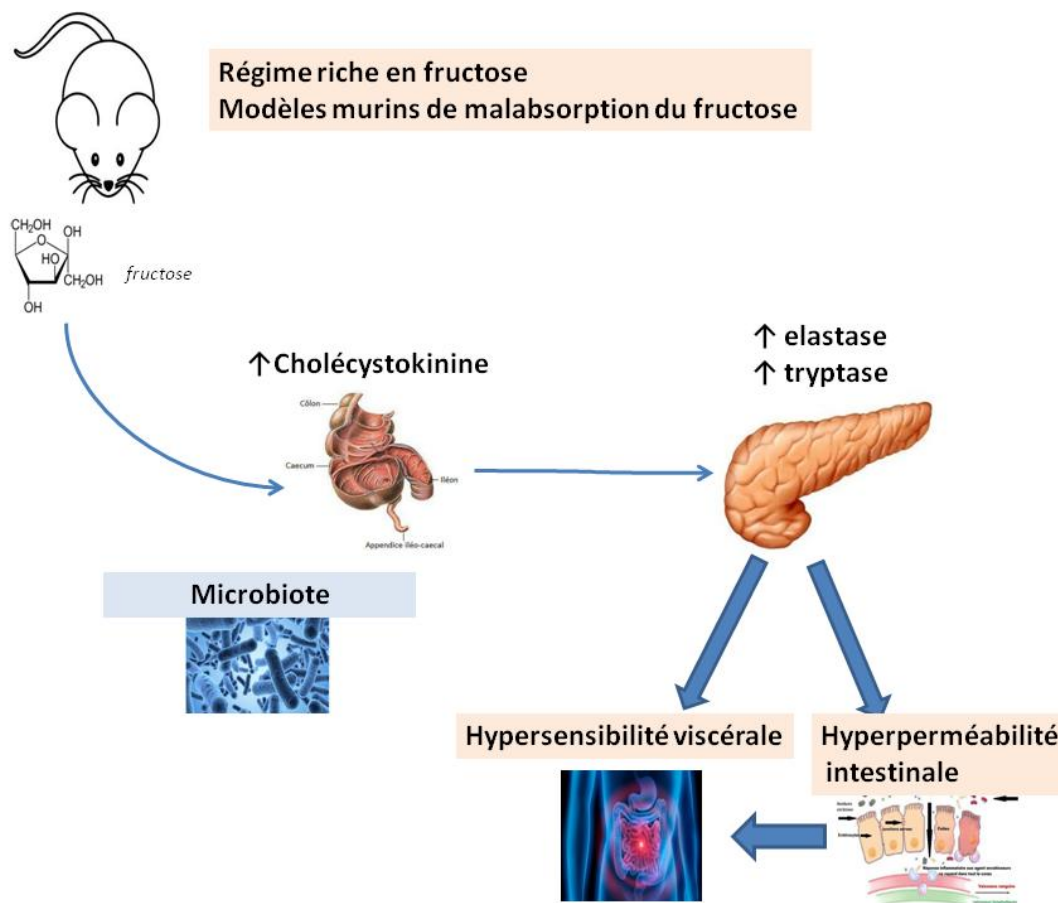
fructose. Un modèle de ChREBP KO soumis à un régime riche en fructose présente à une diarrhée et une induction insuffisante de GLUT5 dans l'intestin (143).

Dans un travail de Murray *et al.* l'absorption du fructose a été étudiée, après injection de CRF et de placebo, chez des volontaires sains. L'injection de CRF majorait la malabsorption du fructose en comparaison à l'injection de placebo (100). Ces résultats démontrent le rôle du stress dans la malabsorption du fructose chez l'Homme (100) or son rôle est bien connu dans le SII et pourrait donc participer à ce phénotype, en revanche. Il a déjà été démontré dans des modèles animaux, que le stress était capable d'inhiber le transport de GLUT2 vers le pôle apical (144). Ce mécanisme pourrait expliquer le problème de malabsorption du fructose en période de stress.

2) Comment la malabsorption du fructose induit une hypersensibilité viscérale?

Les résultats obtenus au cours de ma thèse de science ont permis de montrer l'association entre malabsorption du fructose et hypersensibilité viscérale chez les patients souffrant d'un SII. Les modèles murins de malabsorption du fructose reproduisant une hypersensibilité viscérale nous ont permis d'avancer sur la compréhension des mécanismes physiopathologiques sous-jacents (**figure 8**).

Figure 8 : Mécanismes physiopathologiques expliquant la malabsorption du fructose chez les patients souffrant d'un SII. Synthèse des résultats obtenus au cours de ma thèse de science.



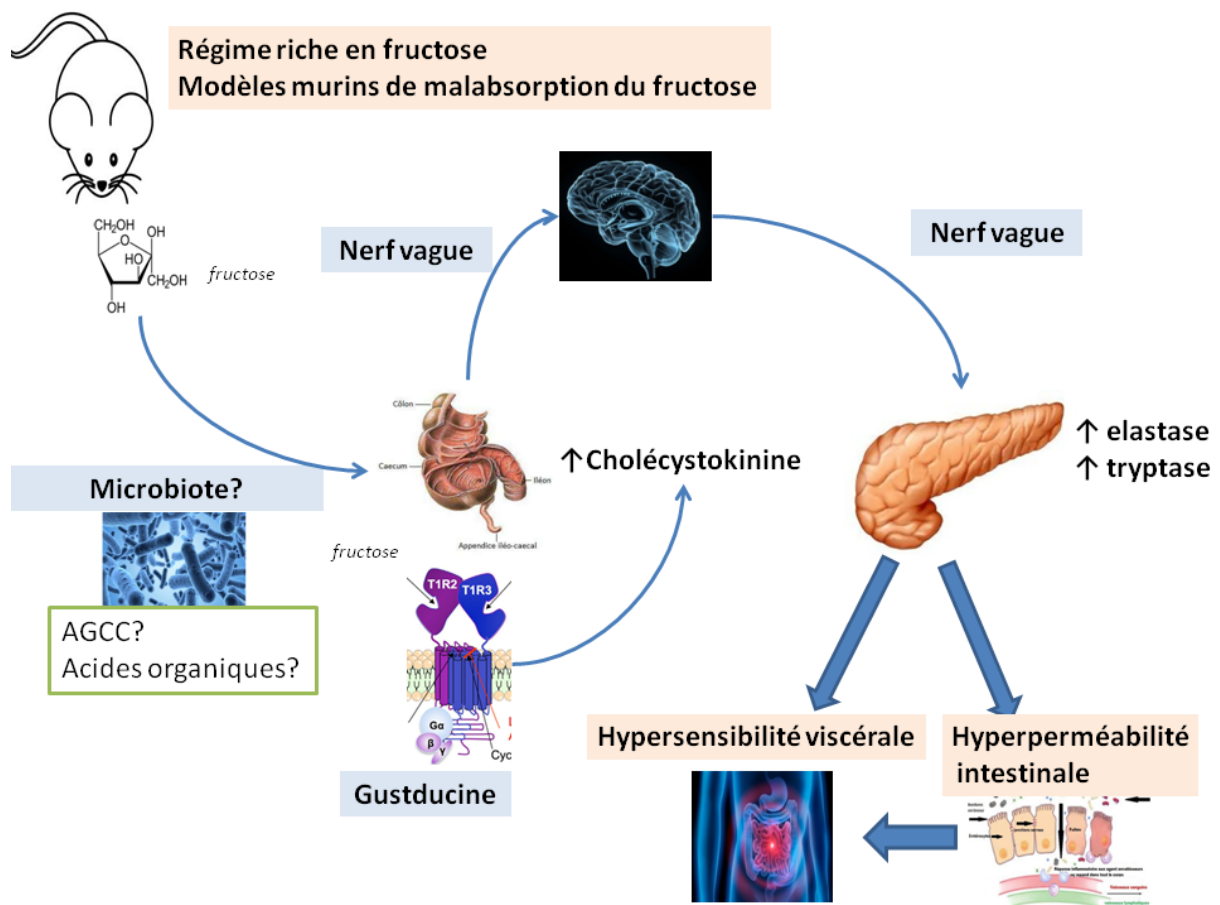
Le fructose malabsorbé est métabolisé par la flore colique au niveau de l'iléon terminal et du caecum. Il est difficile de savoir si les bactéries sont responsables de ce phénotype chez nos patients. Leur identification et leurs implications exactes restent encore à définir. Il reste à déterminer en quel composé est métabolisé le fructose, possiblement en AGCC comme le butyrate (145) ou en acides organiques. Le butyrate a déjà été impliqué dans la survenue d'une hypersensibilité viscérale (146). Récemment, il a également été montré que le fructose en excès était métabolisé dans le colon en glucose et acides organiques (lactate et glycérate) (147). Ces composés sont ensuite retrouvés au niveau de la circulation portale et pourraient également participer à ces mécanismes.

La voie de transduction du goût sucré avec l' α -gustducine est coexprimée au niveau des cellules entéroendocrines avec le peptide CCK (148). L'activation du récepteur de l' α -gustducine et de sa voie de signalisation entraîne une sécrétion de CCK par les cellules entéroendocrines (149). Le fructose est capable d'activer cette voie de signalisation (150). Il est intéressant de noter que le fructose est également capable d'activer directement les récepteurs aux goûts, présents sur les cellules β du pancréas qui modulent la sécrétion d'insuline (151). Ensuite, l' α -gustducine active la voie de signalisation en activant le récepteur couplé à la protéine G (T2Rs); ce qui va entraîner une augmentation de la sécrétion de CCK par un mécanisme calcium dépendant (Ca^{2+}) (152). Il serait intéressant d'étudier le rôle exact de la gustducine sur des modèles de KO soumis à un régime riche en fructose.

La CCK va alors activer les neurones du noyau du tractus solitaire en passant par la voie du nerf vague (153). L'importance de l'axe intestin-cerveau dans la modulation des enzymes pancréatiques est prouvée par une vagotomie qui abolit les effets induits par la sécrétion de CCK (154). L'étude de la sensibilité viscérale dans un modèle de souris avec vagotomie, soumis à un régime riche en fructose permettrait de confirmer cette hypothèse. Une population de patients avec malabsorption du fructose (test respiratoire à 25g) présentait une augmentation de la lipase et de l'amylase sérique en comparaison aux patients sans malabsorption (99). L'axe intestin-pancréas semble donc être au cœur des mécanismes physiopathologiques qui nous intéressent. Dans notre modèle, ces mécanismes induisent ensuite une augmentation de la production de CCK au niveau de l'iléon terminal et du caecum, lieu de la fermentation colique. La CCK stimule les sécrétions enzymatiques pancréatiques (155), raison pour laquelle l'activité protéasique est augmentée dans les fèces de nos souris. Or il a déjà été démontré que l'activité protéasique via la voie PAR-2 entraîne une hypersensibilité viscérale et une augmentation de la perméabilité intestinale, par activation des neurones sensitifs (123). Cette activation est dépendante du récepteur TRPV4 (156). Au niveau du tissu colique des patients SII-D, la présence de métabolites des acides gras polyinsaturés serait capable de stimuler les neurones sensitifs et de générer une hypersensibilité viscérale par activation de TRPV4 (157). L'étude de la sensibilité viscérale, dans un modèle de PAR KO soumise à un régime riche en fructose permettrait de déterminer l'implication de la voie PAR-2 dans notre phénotype. L'implication de chacun de ces

mécanismes, dans notre modèle de malabsorption du fructose, reste encore à être confirmée (*figure 9*).

Figure 9 : Mécanismes physiopathologiques à confirmer dans notre hypothèse sur la malabsorption du fructose chez les patients souffrant d'un SII.



AGCC : Acides gras à chaînes courtes

3) Quel intérêt d'un régime appauvri en fructose dans le syndrome de l'intestin irritable? Quels pourraient être les facteurs prédictifs d'efficacité d'un régime appauvri en fructose?

Le régime pauvre en FODMAPs est validé dans le SII et recommandé par les sociétés savantes dans la prise en charge des patients (62). Bien que ce régime contienne du fructose, le régime pauvre en fructose n'est pas encore validé ni recommandé en pratique clinique courante dans la prise en charge du SII. Il existe cependant un rationnel scientifique sur l'intérêt d'un tel régime, éliminant les aliments contenant du fructose en excès du glucose ou du sorbitol, dans le SII, pour réduire ou faire disparaître les symptômes digestifs (117). Bien que le mécanisme de son efficacité ne soit pas bien connu, nos résultats semblent aller dans le même sens, notre étude n'a malheureusement pas été construite pour répondre à cette question. L'absence de régime contrôle ne nous permet pas de mesurer l'effet placebo. Nous pourrions à l'avenir comparer l'efficacité du régime pauvre en fructose à celle du régime pauvre en FODMAPs chez des patients souffrant de SII dans une étude contrôlée d'équivalence qui prouverait son efficacité.

Devant l'efficacité inconstante, le caractère contraignant et l'impact nutritionnel potentiel des régimes, nous sommes toujours à la recherche du "patient type" répondeur au régime. Nos résultats permettent d'avancer dans le débat sur l'utilité d'un test respiratoire positif pour prédire la réponse à un régime appauvri en fructose (115, 116). Un test respiratoire au fructose positif en faveur d'une malabsorption du fructose ne permet pas de prédire l'efficacité du régime appauvri en fructose chez des patients souffrant d'un SII. La présence de symptômes digestifs au cours du test respiratoire définit l'intolérance. L'intolérance au fructose pourrait être prédictive de l'efficacité d'un régime appauvri en fructose. Wilder-Smith a étudié la présence de facteurs prédictifs de réponse à un régime pauvre en FODMAPs dans une population de patients TFI avec intolérance au fructose et au lactose. Le sous-type diarrhéique et la positivité du test respiratoire au fructose (en particulier avec un pic de méthane) en faveur d'une malabsorption étaient prédictifs de la réponse au régime chez les patients intolérant au fructose (158). Ces éléments soulignent l'importance de la fermentation, du

métabolisme et de la composition du microbiome. La malabsorption du fructose ou plutôt l'intolérance au fructose pourrait-être prédictive de la réponse à un régime. Une étude contrôlée menée avec le régime appauvri en fructose y répondrait en prenant comme critère l'intolérance au fructose. Plusieurs facteurs prédictifs de l'efficacité du régime pauvre en FODMAPs ont récemment été soulignés comme le microbiome avec une meilleure capacité saccharolytique chez les enfants (159), le profil de la flore colique mais également la présence de composés organiques volatils (65, 133). Une manière simple d'étudier les modifications de flore colique est la mesure du PH colique qui peut-être réalisée à l'aide d'une smarptill® (160). Le pH colique des patients SII est plus acide que celui de sujets volontaire sains suggérant une fermentation colique plus importante (21). Cette fermentation intestinale anormale pourrait être un des mécanismes étiologiques dans le SII car l'acidification colique sensibilise les mécano-récepteurs coliques à la distension (22). L'efficacité des régimes n'a jamais été selon la présence d'une fermentation colique accrue. Il est important de savoir si les patients présentant une fermentation colique accrue, secondaire aux modifications de leur microbiote sont meilleurs répondeurs à un régime pauvre en fructose. La malabsorption du fructose semble induire une hypersensibilité viscérale, mais lorsque cette hypersensibilité est installée le régime n'est probablement plus efficace; il existe en effet une persistance de l'hypersensibilité viscérale lorsqu'on induit une déplétion du microbiote après que les souris aient été soumises à un régime riche en fructose (données personnelles). Il semblerait plus logique de cibler alors l'hypersensibilité. L'absence d'hypersensibilité viscérale pourrait peut-être être prédictive de la réponse au régime pauvre en fructose.

4) Quelle est la place du microbiote dans la malabsorption du fructose?

Nous n'avons pu étudier de manière optimale le microbiote dans nos modèles de malabsorption du fructose. Seul le modèle de souris GLUT2^{ΔIEC} a été étudié, nous avons montré une augmentation de *clostridium cluster IV* et *enterococcus species* sur des PCR (74). En revanche, l'analyse complète du microbiote a été assez décevante et ne retrouvait pas ces anomalies, probablement en lien avec les conditions d'étude où les souris sans et avec délétion étaient mélangées dans les cages. Des modifications du microbiote intestinal sont rapportées

dans d'autres modèles de malabsorption du fructose que nous n'avons pas utilisés. Dans le modèle de malabsorption du fructose ChREBP KO, le métabolisme du fructose au niveau colique induit des modifications de la flore colique (142). Il existe une augmentation de la production de lactates, entraînant une augmentation des *lactobacillales* et des *bifidobacterium* et une diminution des *clostridium cluster XIVa* (142). Dans le modèle KHK KO, il existe une augmentation de *lactobacillus jensenni* après 3 semaines de régime contenant du fructose qui se maintient dans le temps (KHK-/-, V. Douard données personnelles). Même si nous ne l'avons pas étudié, il ne faut pas négliger la possibilité du rôle d'une dysbiose fongique, comme cela a déjà été rapporté dans le SII avec hypersensibilité (161).

Pour répondre de manière définitive à cette question il faudrait réaliser un transfert complexe de microbiote de patients SII, sans et avec malabsorption du fructose, vers des souris axéniques au début de leur développement, pour voir s'il existe bien une différence de sensibilité entre les 2 groupes.

V) Conclusion

Les résultats obtenus dans le cadre de ma thèse de sciences soulignent le rôle de la malabsorption du fructose, qui entraîne la survenue d'une hypersensibilité viscérale et d'une augmentation de la perméabilité intestinale, dans le SII. Ce phénotype semble expliqué par une augmentation de la sécrétion de CCK, capable d'induire une activation de la voie PAR-2, en présence d'une malabsorption du fructose. Un régime appauvri en fructose n'améliore pas de manière ciblée les patients SII avec malabsorption du fructose, un traitement ciblant l'hypersensibilité semblerait plus intéressant.

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