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Maud Rizk Rizk-El Zraiby. Etude des facteurs nutritionnels et métaboliques de la cancérogenèse hépatique chez des patients atteints de cirrhose. Médecine humaine et pathologie. Université Bourgogne Franche-Comté, 2019. Français. NNT : 2019UBFCI007 . tel-02421089

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**THESE DE DOCTORAT**

**UNIVERSITE BOURGOGNE FRANCHE-COMTE**

**PREPAREE AU LABORATOIRE INSERM UMR1231**

**« LIPIDES, NUTRITION, CANCER », DIJON**

Ecole doctorale n°554

Environnements-Santé

Doctorat : Science de la Santé- Epidémiologie

Par

Mme El Zraiby Rizk Maud

**FACTEURS NUTRITIONNELS ET MÉTABOLIQUES  
DE LA CANCÉROGENÈSE HÉPATIQUE**

*Etude épidémiologique des facteurs de risque de carcinome hépatocellulaire  
chez des patients atteints de cirrhose*

Thèse présentée et soutenue à Dijon, le 27 Septembre 2019

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## Titre : Facteurs nutritionnels et métaboliques de la cancérogenèse hépatique

**Mots clés :** Carcinome Hépatocellulaire, cirrhose, nutrition, lipides, étude cas-témoin

**Résumé :** Le carcinome hépatocellulaire (CHC) est, dans le monde, le 5<sup>ème</sup> cancer le plus fréquent pour les hommes et le 9<sup>ème</sup> pour les femmes. En Europe, la France a un taux élevé à 7,8/100 000 habitants en 2018 (10 624 nouveaux cas estimés). Malgré les progrès, le pronostic du CHC est encore très sombre : il est la 4<sup>ème</sup> cause de mortalité par cancer au niveau mondial, avec une médiane de survie de 10 à 11 mois.

Le CHC se développe dans 80% des cas sur une cirrhose due à une intoxication alcoolique chronique, une infection virale B ou C, ou plus rarement une pathologie métabolique. Les patients atteints de cirrhose ont un risque très élevé de développer un cancer. Il est donc indispensable d'identifier les facteurs intervenant dans le risque de complications et en particulier de CHC dans cette population pour mettre en place des actions préventives adaptées. L'alimentation est une source importante de composés bioactifs, en particulier pro- et anti-inflammatoires et représente un facteur modifiable potentiel. Les facteurs nutritionnels liés à la cancérogenèse hépatique sont donc encore mal définis. De plus, ces études ne sont pas toujours transposables dans une population aux habitudes alimentaires occidentales, et surtout n'ont pas été réalisées chez des patients atteints de cirrhose, pourtant les plus à risque de développer un CHC mais qui ont sans doute une alimentation spécifique.

L'étude cas-témoin "CiRCE" (investigateur principal : Pr Hillon) offre l'opportunité de travailler sur les facteurs de risques environnementaux, nutritionnels et métaboliques du CHC chez des patients cirrhotiques, toute étiologie confondue.

Cette étude est coordonnée par l'équipe EPICAD du centre de Recherche Inserm UMR 1231 « Lipides, Nutrition Cancer » avec le soutien du Centre d'Investigation Clinique Inserm1432/CHU de Dijon.

Six centres hospitaliers (Besançon, Dijon, Metz, Nancy, Reims, Strasbourg) ont inclus entre 2008 et 2012 des malades atteints de cirrhose compliquée de CHC (428 cas) ou non compliquée de CHC (760 témoins). En plus du recueil standardisé de données cliniques, et d'une collection biologique, un questionnaire alimentaire a permis l'estimation de la consommation de 208 aliments, boissons et plats.

La comparaison des cas et des témoins permet d'étudier le risque de CHC chez des patients atteints de cirrhose. Plusieurs approches ont été abordées :

1/ par aliments permettant de confirmer les hypothèses du WCRF/AICR mais chez des patients atteints de cirrhose.

2/ par profils de consommation alimentaire définis *a priori* permettant une approche plus intégrée de l'alimentation.

3/ Utilisation du Diet Inflammatory Index conçu pour estimer le potentiel inflammatoire de l'alimentation.

4/ Focus les lipides.

Plusieurs groupes alimentaires (viandes rouges, charcuteries, boissons sucrées...) et scores (DII, AHEI) ont montré des différences significatives entre les cas et les témoins soulignant un rôle de l'alimentation sur le risque de CHC chez des patients atteints de cirrhose.

Ces résultats qui manquaient encore dans la littérature pourraient amener à des recommandations nutritionnelles spécifiques à cette population de patients particulière et très à risque de complications graves et en particulier de CHC.

**Title:** Nutritional and metabolic factors of hepatocellular carcinoma among cirrhotic patients

**Keywords:** Hepatocellular carcinoma, cirrhosis, nutrition, lipids, case-control study

**Abstract:** Hepatocellular Carcinoma (HCC) is worldwide the 5<sup>th</sup> most common diagnosed cancer in men and the 9<sup>th</sup> in women and the 4<sup>th</sup> cause of cancer death with a median survival between 10 and 11 months. The incidence rate in France is relatively high (7.8/100 000 with 10 624 estimated new cases in 2018). HCC prognosis is very poor due to the difficulty to diagnose it at an early stage. Around 80% of HCC occur in the setting of cirrhosis, mainly caused by alcoholism, chronic infections with HBV and HCV or metabolic syndrome. Cirrhotic patients have a high-risk to develop chronic inflammation or other liver complications that can progress toward cancer. Thus, it is important to identify the risk factors of these complications, and mainly HCC, in this specific population in order to define preventive tools. Diet is composed of several bioactive components and represents a modifiable factor with preventive potential. Associations between dietary factors and HCC risk have been little investigated and results were conflicting. It has been suggested that some foods, such as fruit, vegetables, fish and white meat, have a protective effect, whereas red and processed meat seem to be associated with an elevated risk of HCC. Strong evidence exists only for the relationship between liver carcinogenesis and coffee consumption. The previous results require confirmation especially in occidental countries and in such a high-risk population suffering from cirrhosis, for which food choices may differ.

The French multicenter case-control study CiRCE (main investigator: Pr Hillon) was conducted to identify the environmental,

nutritional and metabolic risk factors of developing HCC in cirrhotic patients with different etiologies. This study is coordinated by the EPICAD team from the National Institute for Health and Medical Research Inserm UMR1231 “Lipid Nutrition Cancer” with the collaboration of the Clinical Investigation Center of Dijon CHU. Between June 2008 and December 2012, cirrhotic patients with HCC (428 cases) or without HCC (760 controls) were recruited in six academic hospitals of the North-East of France (Besancon, Dijon, Metz, Nancy, Reims and Strasbourg). In addition to clinical data, every patient has to fill a diet history questionnaire that included 208 food items. A biobank has been also constituted.

Several studies were developed in order to:

1/ Confirm the hypothesis of the World Cancer Research Found (WCRF) on food items in cirrhotic patients

1/ Predefine a food consumption pattern

allowing a more integrated approach to food

3/ Apply the Dietary Inflammatory Index that assesses the inflammatory potential of the diet

4/ Focus on lipids.

The comparisons between cases and controls allow investigating the HCC risk in cirrhotic patients. Several food groups (meat, processed meat, carbonated beverages...) and scores (DII, AHEI) showed a significant difference between cases and controls which can have several impacts on HCC risk.

These results will contribute to the development of nutritional recommendations specific to this particular population at risk of serious complications such as HCC.

## **Remerciements**

*Je voudrais, tout d'abord, adresser toute ma gratitude à ma directrice de thèse Docteure Vanessa COTTET, pour sa patience, sa confiance, sa disponibilité et surtout ses judicieux conseils. Elle m'a guidée dans mon travail et m'a transmis des connaissances.*

*J'aimerais aussi remercier Professeure Mona DIAB ASSAF, ma codirectrice, qui, malgré la distance et ses préoccupations a pu m'aider, me soutenir et m'encourager pour accomplir ce travail.*

*Je tiens également à remercier Professeure Dominique Roulot-Marullo et Professeur Bertrand Liagre de m'avoir fait l'honneur d'accepter le rôle de rapporteurs et membres de Jury.*

*Je remercie les responsables et personnels de l'Université de Bourgogne Franche-Comté en générale et de l'Ecole Doctorale Environnement-Santé en particulier.*

*Je remercie l'équipe du laboratoire INSERM 1231 « Lipides, Nutrition, cancer » de Dijon et le Centre d'Investigation Clinique, Inserm 1432/CHU de Dijon, les participants de l'étude, les équipes cliniques et de recherche du Groupe d'Etude CiRCE, le LabEx LipSTIC pour les analyses biologiques, le CRB Ferdinand Cabanne, ainsi qu'à l'équipe E3N du CESP INSERM UMR 1018 (DR MC Boutron-Ruault) et tous ceux qui ont de près ou de loin participé à la réalisation de cette thèse.*



*Enfin, je tiens à remercier*

*Mes parents et mes beaux-parents pour leur soutien tout au long de mes études et de mes voyages.*

*Mon Mari, Mario, de m'avoir épaulée au quotidien durant mes années d'études. Je mesure chaque jour la chance que j'ai de vivre à tes côtés.*

*Mon garçon, Lucas, pour sa patience et son amour.*

*Mes sœurs Mireille, Maya, Manal et mon frère Marwan, pour le soutien et l'amour dont vous m'enveloppez.*

*Mes amies Maya, Rosy, Christelle, Amra et Hélène pour leur amitié.*

*Chacun d'entre vous enrichit ma vie.*

*Merci d'être là.*

## **Productions scientifiques issues du travail de thèse**

### **Articles issus du travail de thèse**

Maud Rizk, Adrien Guilloteau, Thomas Mouillot, Gérard Thieffin, Jean-Pierre Bronowicki, Carine Richou, Michel Doffoel, Mona Diab Assaf, Patrick Hillon, Vanessa Cottet. **Dietary components modulate the risk of hepatocellular carcinoma in cirrhotic patients.** Nutr Res (2018), DOI:10.1016/j.nutres.2018.10.002

Guilloteau Adrien, Rizk Maud, Binquet Christine, Boutron-Ruault Marie-Christine, Jooste Valérie, Bonithon-Kopp Claire, Thieffin Gérard, Barraud Hélène, Bronowicki Jean-Pierre, Richou Carine, Di Martino Vincent, Doffoel Michel, Jouve Jean-Louis, Hillon Patrick, Cottet Vanessa. **Dietary patterns and risk of hepatocellular carcinoma in cirrhotic patients: a case-control study.** (Manuscrit soumis)

Thomas Mouillot, Maud Rizk, Jean-Paul Pais De Barros, Adrien Guilloteau, Amandine Busson, Gérard Thieffin, Hélène Barraud, Jean-Pierre Bronowicki, Carine Richou, Vincent Di Martino, Michel Doffoel, Anne Minello, Marianne Latournerie, Jean-Louis Jouve, Marie-Claude Brindisi, Jean-Michel Petit, Patrick Hillon, Vanessa Cottet. **Fatty acid composition of the erythrocyte membrane and risk of hepatocellular carcinoma in cirrhotic patients.** (Manuscrit en préparation)

Maud Rizk, Thomas Mouillot, Brigitte Bernard-Chabert, Jean-Pierre Bronowicki, Carine Richou, Michel Doffoel, Jean-Louis Jouve, Cyril Boisson, James R. Hebert, Nitin Shivappa, Marie-Christine Boutron-Ruault, Mona Diab Asaf, Patrick Hillon, Vanessa Cottet. **Association between Dietary Inflammatory Index and the risk of hepatocellular carcinoma in a French cirrhotic population.** (Manuscrit soumis)

Alexia Karen Cotte, Vanessa Cottet, Virginie Aires, Thomas Mouillot, Maud Rizk, Sandrine Vinault, Christine Binquet, Jean-Paul Pais de Barros, Patrick Hillon and Dominique Delmas. **Phospholipid profiles and hepatocellular carcinoma risk and prognosis in cirrhotic patients.** Oncotarget (2019), Vol. 10, (No. 22), pp: 2161-2172, DOI: 10.18632/oncotarget.26738.

### **Communications affichées:**

- 06-07 Avril 2017, Beyrouth-Liban, 23<sup>ème</sup> Conférence Scientifique Internationale de l'Association Libanaise pour l'Avancement de la Science (LAAS) « *Dietary components modulate the risk of hepatocellular carcinoma in cirrhotic patients* »
- 1<sup>er</sup> Décembre 2017, Paris, Colloque "Cancer : les actualités de la recherche en nutrition" organisé par le réseau NACRe, « *Dietary components modulate the risk of hepatocellular carcinoma in cirrhotic patients* ».
- 13-15 décembre 2017, Nantes, Journées Francophones de Nutrition « *Dietary components modulate the risk of hepatocellular carcinoma in cirrhotic patients* »

### **Communications orale:**

- 15 juin 2018, Besançon, Forum des Jeunes Chercheurs organisé par l'Ecole Doctorale de l'Université BFC : « *Dietary components modulate the risk of hepatocellular carcinoma in cirrhotic patients* »  
Prix du jury de la meilleure présentation.
- 21-23 Février 2019, Beyrouth-Liban, XII<sup>ème</sup> rencontre scientifique de l'IEA-EMR (International Epidemiological Association- Eastern Mediterranean Regional) organisée par l'Association Libanaise de l'Epidémiologie (LEA) : « *Dietary components modulate the risk of hepatocellular carcinoma in cirrhotic patients* ».
- 4 Avril 2019, Paris, journée NACRe/Partenariat : « *Association between Dietary Inflammatory Index and the risk of hepatocellular carcinoma in a French cirrhotic population* »
- Soumission pour communication orale de l'article « *Association between Dietary Inflammatory Index and the risk of hepatocellular carcinoma in a French cirrhotic population* » aux Journées Francophones de Nutrition (JFN) qui auront lieu à Rennes-France du 27 au 29 Novembre 2019.



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## **Les abréviations :**

- AFP : Alpha-foetoprotéine
- AG : Acides gras
- AGMI : Acides Gras Monoinsaturés
- AGPI : Acides Gras Polyinsaturés
- AGS : Acides Gras Saturés
- AHEI : Alternate Healthy Eating Index
- ASMR : Age Standardized Mortality Rate
- AST : Aspartate amino-transférase
- BCLC : Barcelona Clinic Liver Cancer
- BHT : Butylated hydroxytoluene
- BMR : Basal Metabolic Rate
- CHC : Carcinome Hépatocellulaire
- CIC : Centre d'Investigation Clinique
- CiRCE : Cirrhose et Risque de Cancer hépatocellulaire dans le grand Est
- CRB : Centre de Ressources Biologiques
- CRP : C-reactive protein
- DGA: Dietary Guidelines for Americans
- DHA : Acide docosahexaénoïque
- DII : Dietary Inflammatory Index
- DM : Données manquantes
- EASL-EORTC : European Association for the Study of the Liver-European Organization for Research and Treatment of Cancer
- EIER : Energy Intake and Energy Requirement
- EPA : Acide eicosapentaénoïque
- EPIC : European Prospective Investigation into Cancer and nutrition
- EPICAD : Épidémiologique et recherche clinique en oncologie digestive
- FPID : Food Patterns Equivalents Ingredient Database
- FPED : Food Patterns Equivalents Database
- FRAP : Ferric Reducing Antioxydant Power
- GGT : Gamma-glutamyl transférase

- GLDH : Glutamate déshydrogénase
- HEI : Healthy Eating Index
- HVB : Hépatites virales B
- HVC : Hépatites virales C
- IARC : International Agency for Research on Cancer
- IC : Intervalle de Confiance
- IL-6 : Interleukine 6
- IMC : Indice de Masse Corporel
- INCa : Institut National du Cancer
- InVS : Institut de Veille Sanitaire
- IRM : Imagerie par Résonance Magnétique
- MEIA : Microparticule enzyme immuno assay
- NAFLD : Non-alcoholic fatty liver disease
- NASH : Non Alcoholic SteatoHepatitis
- NCI : National Cancer Institute
- NEAC : Non Enzymatic Antioxydant Capacity
- NIH-AARP : National Institutes of Health- American Association of Retired Persons
- n-3 : Oméga-3
- n-6 : Oméga-6
- n-9 : Oméga-9
- OR : Odd Ratio
- PC : Phosphatidylcholine
- PNNS : Programme Nationale Nutrition Santé
- pPE : Plasmalogène-phosphatidyléthanolamine
- RR : Risque Relatif
- SM : Sphingomyéline
- TRAP : Radical- Trapping Antioxydant Parameter
- USDA : United States Department of Agriculture
- VHC : Virus de l'hépatite C
- VHB : Virus de l'hépatite B
- WCRF/AICR : World Cancer Research Fund/American Institute for Cancer Research
- WHR : Waist-Hip Ratio

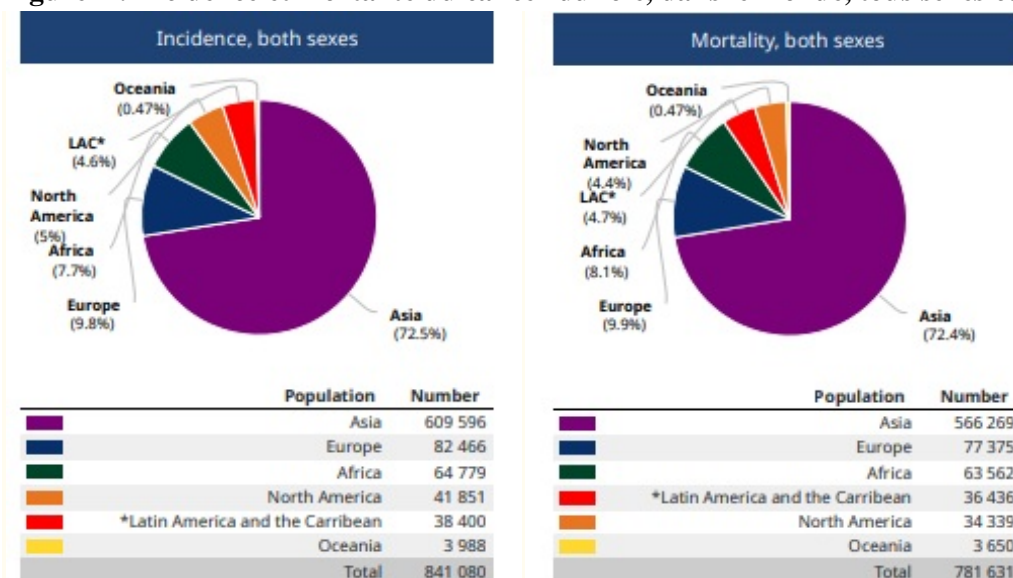
# 1. Introduction

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## 1.1 Epidémiologie du carcinome hépatocellulaire

Le carcinome hépatocellulaire (CHC) est un des cancers les plus fréquents à l'échelle mondiale et constitue un problème de santé publique majeur. Il est la principale tumeur maligne primitive du foie, représentant 85 à 90% des cancers du foie. Le CHC se développe habituellement sur une cirrhose (75 à 80% des cas), plus rarement sur une hépatopathie chronique non cirrhotique, exceptionnellement sur un foie sain. La cirrhose est une maladie du foie caractérisée par une inflammation chronique qui entraîne la destruction des cellules hépatiques et leur régénération anarchique, sous forme de nodules. Les principales causes sont la consommation excessive d'alcool, l'infection par les virus de l'hépatites B ou C et l'obésité. D'autres facteurs peuvent intervenir dans la carcinogenèse hépatique de la cirrhose vers le CHC expliquant une incidence et une mortalité variables selon les régions géographiques (1). De nos jours, les traitements disponibles se divisent en deux selon l'évolution du cancer. Des traitements à visée curatives comme la transplantation hépatique, la résection chirurgicale et la radiofréquence ou bien des traitements à visée palliatifs, appliqués dans les formes évoluées, comme la chimioembolisation, la radio-embolisation, la radiothérapie, la chimiothérapie ou le traitement médical principalement le sorafénib (2,3). L'étude GLOBOCAN 2018, dans 184 pays, a estimé que le CHC était passé de la 2<sup>ème</sup> cause de mortalité par cancer en 2012 à la 4<sup>ème</sup> place en 2018 montrant les progrès en termes de prévention et prise en charge réalisés ces dernières années. Mais, malgré ces progrès, le CHC est encore le 5<sup>ème</sup> cancer le plus fréquent pour les hommes et le 9<sup>ème</sup> pour les femmes (4).

**Figure 1 : Incidence et mortalité du cancer du foie, dans le monde, tous sexes confondus.**



Source: International Agency for Research on Cancer/World Health Organization. GLOBOCAN 2018: Liver Cancer, estimated incidence, mortality and prevalence Worldwide [Internet]. [Cited 2018 Sep 27]. Available from: <http://gco.iarc.fr/today/home/factsheets/cancers/11-Liver-fact-sheet.pdf>

## 1.2 Répartition des taux d'incidence

Les nouveaux cas de cancers hépatiques sont de 841 080 en 2018 alors qu'en 2012 ils étaient estimés à environ 782 500 (4,5) (figure 1) ainsi qu'au niveau des deux sexes (figure 3). Selon l'International Agency for Research on Cancer (IARC), l'incidence du cancer hépatique la plus élevée est en Asie suivie par l'Europe qui a compté 82 466 nouveaux cas en 2018 (4). Comparée aux autres pays européens, la France est considérée comme un pays ayant une incidence élevée; incidence standardisée selon l'âge pour les 2 sexes de 7,8 par 100 000 habitants (figure 2) (6). Il a été estimé par l'Institut de Veille Sanitaire (InVS) et l'Institut National du Cancer (INCa) que le nombre de nouveaux cas de cancer primitif du foie en France était passé de 1 800 en 1980 à 7 100 en 2008, 8 723 en 2012 et 10 624 en 2018. En France ainsi que dans les pays occidentaux, l'incidence a augmenté au cours des 20 dernières années. Cela peut être lié à plusieurs facteurs et notamment l'augmentation du nombre de cas atteints par le virus de l'hépatite C (VHC), l'amélioration des méthodes diagnostiques, le



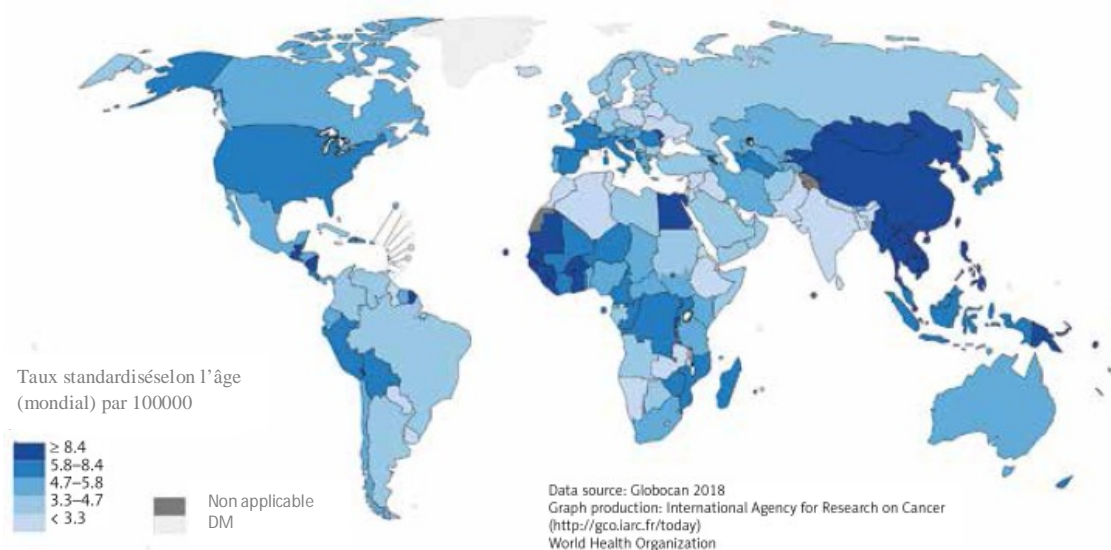
progrès dans la prise en charge des autres complications de la cirrhose, l'augmentation de la consommation d'alcool mais aussi la prévalence de l'obésité et du diabète (7).

### 1.2.1 Selon la région géographique

Le CHC est prévalent surtout dans les pays asiatiques et africains (>20 cas par 100,000 habitants). Le Sud et l'Est de l'Europe ainsi que l'Amérique du Nord ont une incidence moyenne (10–15 cas par 100,000 habitants). Alors que les autres pays de l'Europe sont à faibles risques (<10 cas par 100,000 habitants) (figure 2) (6).

La distribution géographique dépend beaucoup de la distribution des facteurs de risque. Le virus de l'hépatite B (VHB) et l'aflatoxine sont surtout prédominant en Asie orientale et Afrique sub-saharienne. Par contre le VHC est le premier facteur de risque aux Etats Unis (30-50%), Europe (44-75%) et Japon (80-90%)(8,9).

**Figure 2 : Incidence du cancer du foie dans le monde en 2018, taux standardisés selon l'âge.**



Source: International Agency for Research on Cancer/World Health Organization. GLOBOCAN 2018: Estimated age-standardized rates (World) of incident cases/deaths, both sexes, liver cancer, worldwide [Internet]. [Cited 2018 Sep 27]. Available from: <http://gco.iarc.fr/today/online-analysis-map>

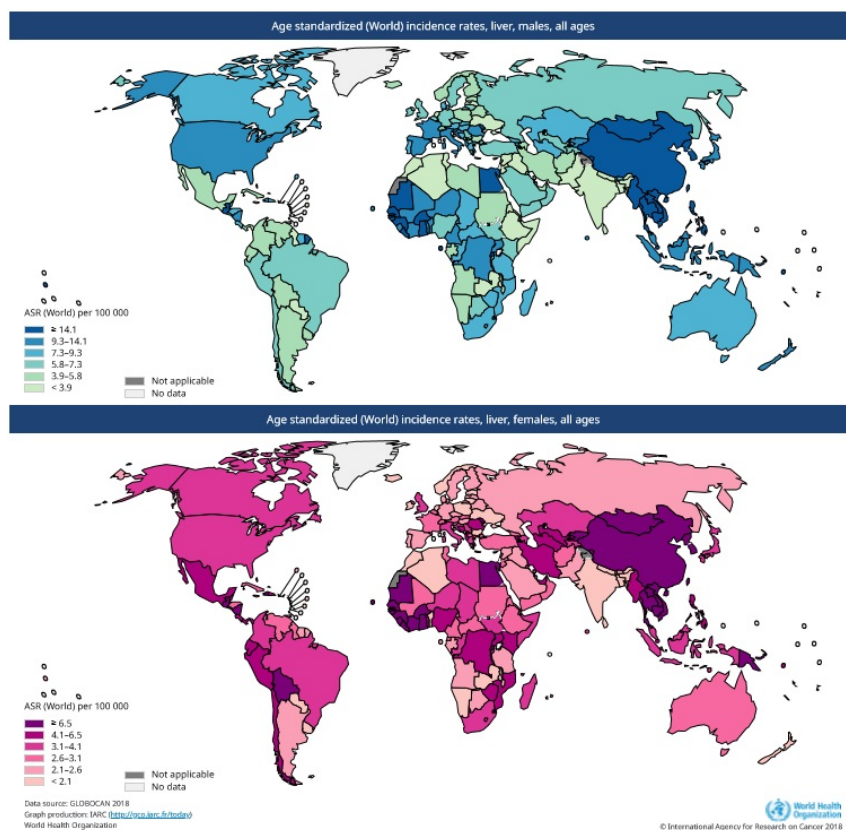
### **1.2.2 Selon l'âge**

Le CHC peut apparaître à tout âge mais il est rarement diagnostiqué avant 40-50 ans. La répartition mondiale du CHC par âge varie selon les régions, le sexe et, éventuellement, l'étiologie. Dans les populations à faible risque, les taux les plus élevés par âge sont observés après 75 ans. En revanche, dans les pays à haut risque de CHC, les taux d'incidence, par âge, augmentent jusqu'à 45 ans, puis se stabilisent. En Europe, la moyenne est entre 63–65 ans (1,9).

### **1.2.3 Selon le sexe**

La différence d'incidence du CHC entre les 2 sexes existe dans presque tous les pays (figure 3). Les hommes présentent un taux d'incidence 2 à 4 fois plus élevé que chez les femmes. Les raisons de cette différence sont encore mal connues mais peuvent en partie être expliquées par la prévalence de certains facteurs de risque selon le sexe mais aussi par les différences au niveau des hormones sexuelles, de l'épigénétique ou de la réponse immunitaire, l'infection chronique par le VHB et le VHC est plus fréquente chez les hommes que chez les femmes, de même que la consommation excessive d'alcool, le tabagisme ou les taux sériques en fer plus élevés. En revanche, les œstrogènes pourraient limiter la transformation maligne des hépatocytes en régulant négativement la libération d'interleukine 6 (IL-6) par les cellules de Kupffer. De plus, les œstrogènes pourraient jouer un rôle protecteur contre la progression des infections à VHB en diminuant la transcription et les effets inflammatoires du VHB grâce aux cytokines. Cependant, toutes ces données n'expliquent pas complètement les différences observées entre les sexes et doivent être confirmées (1,9).

**Figure 3 : Distribution selon le sexe, des taux d'incidence (standardisés selon l'âge), dans le monde en 2018.**



Source: International Agency for Research on Cancer/World Health Organization. GLOBOCAN 2018: Liver Cancer, estimated incidence, mortality and prevalence Worldwide [Internet]. [Cited 2018 Sep 27]. Available from: [www.gco.iarc.fr/today/home/factsheets/cancers/11-Liver-fact-sheet.pdf](http://www.gco.iarc.fr/today/home/factsheets/cancers/11-Liver-fact-sheet.pdf)

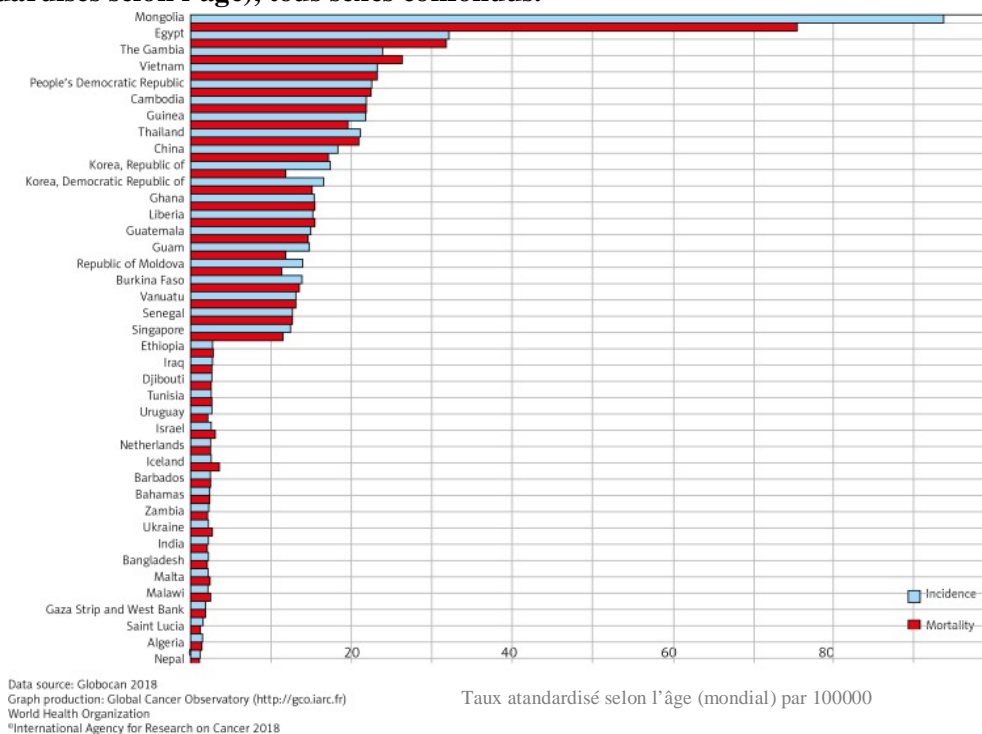
### 1.3 Mortalité

Les décès par cancer hépatique sont de 781 631 personnes en 2018 alors qu'en 2012 étaient estimés à environ 745 500 (4,5) (figures 1). Selon l'IARC, les décès par cancer hépatique en Europe sont de 77 375 personnes en 2018 (4). Comparée aux autres pays européens, la France a un taux de mortalité de 6,4 par 100 000 habitants (6).

Les taux de mortalité standardisés selon l'âge (ASMR : Age Standardized Mortality Rate) du cancer du foie en 2018 étaient les plus élevés en Asie orientale (16,0) et en Afrique du Nord (13,9), suivis de l'Asie du Sud-est (13,2) et de la Micronésie (12,0). L'ASMR le plus faible a

été observé en Asie Centrale du Sud (2,3), suivi de l'Europe du Nord, centrale, et orientale et de l'Asie occidentale (environ 3,8 à 4,0) (figure 4) (4).

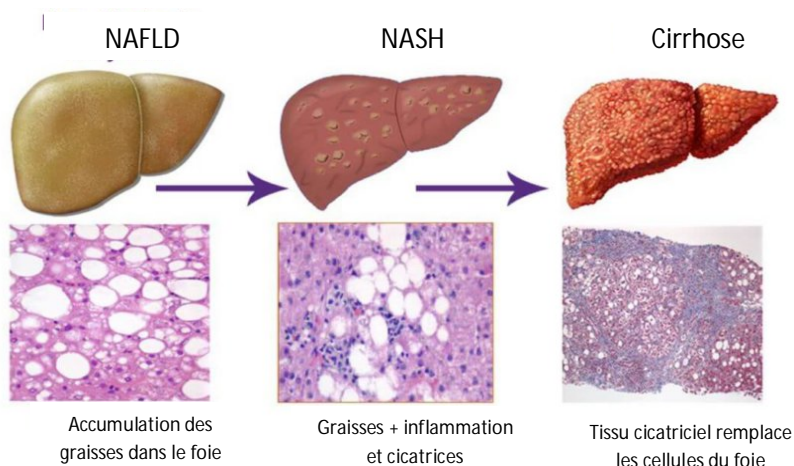
**Figure 4 : Estimation des taux d'incidence et de mortalité, dans le monde en 2018 (standardisés selon l'âge), tous sexes confondus.**



### 1.4 Principales hépatopathies chroniques pouvant évoluer vers une cirrhose

Le foie joue un rôle central dans l'organisme : détoxification, synthèse, dégradation et métabolisme de tous les macronutriments ainsi que stockage du glycogène et de certains micronutriments. Ainsi, une altération de son fonctionnement ou des hépatocytes directement entraîne des effets néfastes, le développement de pathologies aux conséquences graves.

**Figure 5 : Evolution des pathologies hépatiques**



Source: NASH. Canadian Liver Foundation. Available from: <https://www.liver.ca/patients-caregivers/liver-diseases/nash/>

### **1.4.1 Stéatopathies métaboliques**

La NAFLD a pour principale cause l'accumulation de lipides au niveau des cellules hépatiques entraînant une augmentation du volume du foie. Les facteurs de risques sont : l'obésité, l'hypertriglycéridémie, la résistance à l'insuline et d'autres maladies infectieuses (10). Cette maladie est le plus souvent asymptomatique. La prévalence est actuellement de 20-30% de la population adulte américaine. La NAFLD a été diagnostiquée chez 70% des patients atteints de diabète type 2 et 90% des personnes obèses et 10% des enfants (surtout les garçons) (9,11).

La NASH est une complication de la NAFLD et elle se caractérise par l'inflammation des cellules hépatiques contenant un dépôt excessif de graisse avec ou sans fibrose. La progression de cette maladie, non traitée peut aboutir à une cirrhose (11). Une revue de la littérature a suggéré que 60% des patients, âgés de plus que 50 ans, atteints de diabète et/ou obèses, souffrent de NASH et de fibrose avancée (3). Le risque de CHC dans le NASH n'est pas bien défini mais il peut se développer même en absence de cirrhose (9).

### **1.4.2 Hépatopathie alcoolique**

C'est une complication qui induit des lésions histologiques, et dans sa forme grave elle peut causer des encéphalopathies hépatiques, voire le décès, en absence de traitement. Le diagnostic est souvent difficile car il peut être confondu avec l'insuffisance hépatique aigue ou chronique. L'hépatite peut-être due à la consommation excessive d'alcool ou à une inflammation du foie causée par une infection virale due aux VHB ou C et considérées comme des facteurs de risque majeurs de CHC.

L'alcoolisme chronique peut provoquer une stéatose hépatique, une nécro-inflammation, une fibrose, une cirrhose du foie et une malnutrition. Les études faites en Europe ont pu établir qu'une consommation importante d'alcool (supérieur à 7–13 verres/ semaine pour les femmes et 14–27 verres/ semaine pour les hommes), pour des périodes prolongées, augmente le risque de survenue de CHC, qui est d'autant plus important que cette consommation est associée à une infection au VHB ou C (3).

### **1.4.3 Hépatites virales**

Le VHB est transmis par transfusion contaminée, injections intraveineuses et contacts sexuels. La transmission verticale de la mère au fœtus est la principale cause d'infection par le VHB dans le monde. Il est estimé qu'environ 350 millions de personnes (soit 5% de la population mondiale) ont une infection chronique à l'hépatite B, environ 15 millions en Union Européenne. Les porteurs chroniques du VHB ont un risque significatif de développer un CHC au cours de leur vie, estimé entre 10 à 25%. La prévalence de l'antigène du VHB a légèrement diminué en Europe entre 2007 et 2017 grâce aux mesures de prévention appliquées dans les différentes régions. L'incidence du CHC suite à l'infection par le VHB a considérablement diminué grâce à la vaccination contre le VHB (3,8,12).

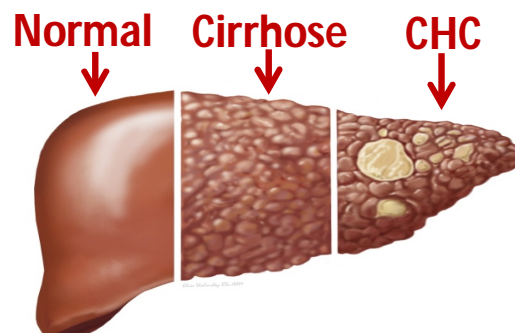
Le VHC est un petit virus à ARN simple brin, qui présente une variabilité génétique élevée avec sept génotypes différents. Les facteurs de risque de transmission sont les transfusions, la consommation de drogue par voie intraveineuse ou par voie nasale, les tatouages ou l'acupuncture, certains soins hospitaliers (dialyse, transplantation...), les personnes exposées au sang, le fait d'avoir un proche porteur du VHC et les rapports sexuels. Dans le monde, à peu près 71 millions de personnes sont porteuses chroniques de VHC selon les dernières données de l'OMS. La prévalence du VHC est de 1.8% en Europe. En France, 150 000 à 200 000 personnes vivraient avec le VHC. Une fois infecté par le VHC, 80% des patients évoluent vers une hépatite chronique et environ 20% développent une cirrhose (8,12,13). Si un patient cirrhotique est atteint des deux hépatites B et C, le risque de CHC augmente avec un odd ratio de 165 contre 17 pour l'hépatite C et 23 pour l'hépatite B seule (3). La consommation d'alcool a, de plus, un effet synergique chez les personnes atteintes de VHC, l'incidence du CHC augmente alors de 1,7 à 2,9 fois. Le risque de CHC est considérablement réduit chez les patients bénéficiant d'un traitement du VHC avec une réduction de 54% de la mortalité toutes causes confondues. Malgré la disponibilité d'anti-viraux efficaces et les progrès des médicaments, le coût et la fréquence élevée des cas non reconnus rend encore difficile le contrôle du virus à l'échelle mondiale. Le développement d'un vaccin anti-VHC est une nécessité pour envisager l'éradication de l'infection (8,11).

#### **1.4.4 Cirrhose**

La cirrhose est une maladie qui altère les fonctions vitales du foie. Elle se caractérise par une inflammation chronique entraînant une fibrose cicatricielle évolutive ou non qui détruit les cellules hépatiques qui se régénèrent de façon anarchique sous forme de nodules. Cette maladie induit une insuffisance hépatocellulaire, une hypertension portale et constitue un état précancéreux (figure 6) (14). L'Europe est considérée comme le continent le plus

touché par les maladies hépatiques au monde, pourtant les causes et l'épidémiologie de ses maladies varient selon les facteurs de risque de chaque région. En France, la prévalence de la cirrhose est estimée entre 2000-3300 cas/million d'habitants. En 2016, en Europe, la prévalence de la cirrhose et des autres maladies hépatiques avait une médiane de 833 par 100 000 habitants, standardisée selon l'âge pour les 2 sexes. Alors que les taux de mortalité, entre 2012-2015, représentaient un total de 151 513 décès (12). La cirrhose est la 13<sup>ème</sup> cause de décès dans le monde. Le diagnostic de cirrhose survient en moyenne à l'âge de 50 ans. Les facteurs de risque de la cirrhose sont : la consommation excessive et prolongée d'alcool (50-75% des cas), les hépatites virales B (5% des cas) et C (15-25%) et le syndrome métabolique associé à un surpoids ou une obésité et entraînant très souvent une NASH. Les autres cas sont liés à des maladies génétiques ou auto-immunes (5% des cas) incluent l'hémochromatose, les hépatites auto-immunes, les cirrhoses biliaires primitives, les cholangites sclérosantes primitives, le déficit en alpha-1-antitrypsine et la maladie de Wilson (14–16).

**Figure 6 : Représentation illustrée de la modification des cellules hépatique selon le stade de la maladie.**



Source : <https://pbs.twimg.com/media/DdZs-DIX4AA5f04.jpg>



## 1.5 Diagnostic

Le CHC peut se présenter de façon asymptomatique ; il est souvent diagnostiqué à un stade avancé. Le dépistage systématique dans une population à haut risque permet de détecter précocement les tumeurs de petite taille et augmente ainsi les chances d'administrer un traitement curatif. Selon les recommandations de l'association européenne pour l'étude du foie (European Association for the Study of the Liver-European Organization for Research and Treatment of Cancer, EASL-EORTC), les patients à risque atteints de cirrhose avec des scores du Child Pugh A/B ou un score C et en attente de transplantation ou ceux non atteints de cirrhose mais porteurs d'hépatites à virus B ou C accompagnés de certains facteurs de risque, doivent être surveillés de façon systématique. Ainsi, le meilleur moyen de diminuer le taux de mortalité est la prévention (11). L'amélioration de l'hygiène sanitaire a réduit le risque de l'hépatite C et de la contamination alimentaire par l'aflatoxine. La vaccination contre l'hépatite B a surtout diminué le risque chez les jeunes. Enfin, le changement vers un mode de vie plus sain comme l'arrêt du tabac, la limitation de la consommation d'alcool et l'augmentation de l'activité physique a diminué l'obésité, la NASH et le diabète (5), facteurs de risque de maladies hépatiques et de CHC.

En Europe, le dépistage et la surveillance du CHC suivent les recommandations de l'EASL-EORTC. La surveillance est recommandée chez les patients non cirrhotiques atteints d'hépatite C chronique ou de fibrose hépatique avancée (F3), car la transition d'une fibrose avancée à une cirrhose ne peut pas être définie avec précision. En outre, un patient cirrhotique avec un score C du Child-Pugh en attente d'une transplantation hépatique doit être soumis à un dépistage afin de définir les critères d'éligibilité et d'exclusion (9).

Le diagnostic du CHC se fait habituellement suite à la découverte à l'échographie d'une lésion focale nodulaire hépatique ou suite à l'apparition de symptômes en cas de tumeur évoluée. Généralement, le diagnostic se base sur des critères non invasifs basés sur l'imagerie

par scanner ou imagerie par résonance magnétique (IRM) ou anatomopathologiques, selon les recommandations de l'EASL-EORTC (2).

## 1.6 Traitement du CHC

De nos jours malgré les progrès, le pronostic du CHC reste sombre et l'efficacité des traitements trop faible. Le choix du traitement dépend du stade de la tumeur défini à l'aide de plusieurs systèmes et scores qui peuvent être appliqués (Okuda, BCLC, CLIP, GRETCH, TNM-AJCC...) (figure 7).

Exemple de classification :

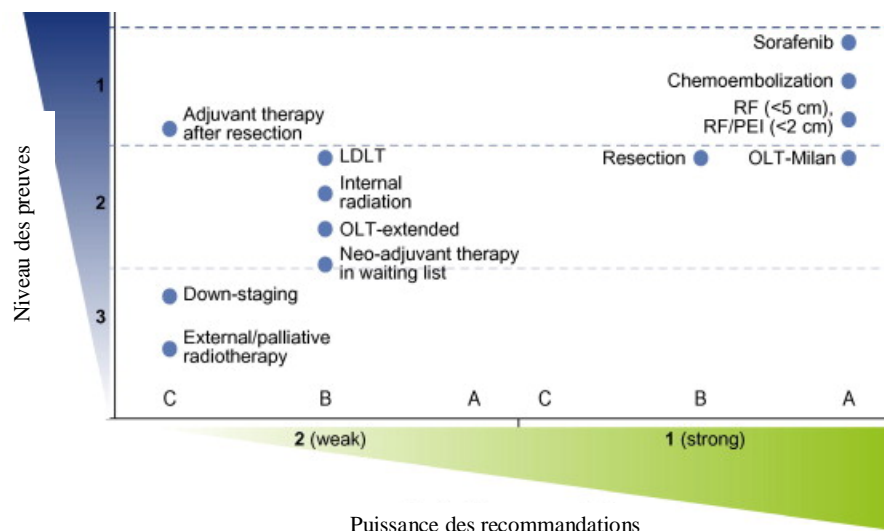
**Figure 7 : Classification «Barcelona Clinic Liver Cancer», BCLC**

Stade	Performance status	Morphologie tumorale	Okuda	Fonction hépatique
<b>A : stade précoce</b>				
A1	0	Unique, < 5 cm	I	Pas d'HTP et bilirubine N
A2	0	Unique, < 5 cm	I	HTP, bilirubine N
A3	0	Unique, < 5 cm	I	HTP, hyperbilirubinémie
A4	0	3 tumeurs, < 3 cm	I-II	Child-Pugh A-B
<b>B : stade intermédiaire</b>				
	0	Multinodulaire	I-II	Child-Pugh A-B
<b>C : stade évolué</b>				
	1-2	Invasion vasculaire Métastases	I-II	Child-Pugh A-B
<b>D : stade terminal</b>				
	3-4	Indifférente	III	Child-Pugh C

*Stade A et B : tous les critères doivent être remplis, Stade C et D : un seul critère suffit.  
htp = hypertension portale.*

Parmi les traitements, une transplantation du foie ou une résection chirurgicale ne sont possibles que pour trop peu de patients éligibles à ce genre de thérapies potentiellement curatives. Dans les stades intermédiaires, la chimioembolisation et la radiofréquence sont une alternative. Le sorafénib est un traitement standard du CHC à un stade avancé avec une fonction hépatique bien préservée (figure 8) (2).

**Figure 8 : Les recommandations de l'association Européenne sur l'étude du foie (European Association for the Study of the Liver) sur le traitement du CHC en fonction du niveau des preuves et de la puissance des recommandations.**



RF: radiofréquence ablation; PEI: percutaneous ethanol injection; OLT: orthotopic liver transplantation; LDLT: living donor liver transplantation

Source: European Association for the Study of the Liver, European Organization for Research and Treatment of Cancer. EASL–EORTC Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 2012 Apr;56(4):908–43.

## 1.7 Facteurs de risque métaboliques et environnementaux

### 1.7.1 Obésité, indice de Masse Corporelle (IMC) et graisses corporelles

Selon le rapport du World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR), l'IMC est un facteur de risque de cancer du foie, avec un niveau de preuve convaincant. La majorité des patients atteints de cancer hépatique développent une ascite d'où la difficulté de bien déterminer le poids corporel, l'IMC et la part de graisses corporelles. Il faut noter que les mesures anthropométriques de la plupart des études sont imprécises car elles ne différencient pas la masse musculaire et la masse grasse. Une étude cas-témoin a mis en évidence une association positive entre l'obésité à l'âge adulte (20-40 ans) et le risque de CHC. Chaque augmentation d'une unité d'IMC au début de l'âge adulte était associée à une diminution de l'âge du diagnostic de CHC de 3,89 mois (17). L'excès de poids et l'obésité

engendrent plusieurs changements métaboliques aboutissant au développement de la NAFLD, NASH et le diabète type 2 qui sont des facteurs de risque du CHC. L'étude prospective Européenne EPIC (European Prospective Investigation into Cancer and nutrition) a montré que le poids, l'IMC, le tour de taille et de hanches, le rapport de ces derniers (Waist-Hip Ratio, WHR) et le changement du poids au fil des années, sont tous des facteurs qui augmentent le risque de CHC (18). Une étude de cohorte de 900 000 personnes menée en Amérique du Nord a montré un taux de mortalité élevé chez les patients atteints de CHC et ayant un  $IMC > 35 \text{ Kg/m}^2$ . Plusieurs études de cohorte et des méta-analyses réalisées dans 3 continents (Etats Unis, l'Europe et l'Asie) ont montré que l'IMC élevé et le syndrome métabolique sont des facteurs de risque pour le CHC. De plus, Starley et al. ont suivi la progression des patients atteints de NASH en cirrhose hépatique durant 5-6 ans. Le résultat a montré que l'obésité et l'IMC élevé sont des facteurs de risque indépendants de la progression de la fibrose hépatique. Finalement, la diminution de l'IMC et de la tour de taille sont associés à la régression de la fibrose hépatique et la diminution de 7% du poids améliore le score du NAFLD (10). L'obésité augmente de 1,5 à 4 fois le risque de CHC. Le risque relatif (RR) du cancer du foie est de 1,17 pour les patients en surpoids et de 1,89 pour les patients obèses (19). Les facteurs de risque qui affectent le développement d'un CHC chez les personnes obèses sont encore mal définis et peuvent être liés à un taux élevé du rapport tour de taille sur tour de hanche, à un taux altéré d'adipokines ou au développement de NASH (20). La masse grasse influe le niveau de certaines hormones comme l'insuline et les œstrogènes et augmente les réponses inflammatoires, ce qui crée un environnement propice à la carcinogénèse. Le contrôle du poids est un facteur important dans la prise en charge des NAFLD/NASH. En limitant la progression de la fibrose hépatique, cela peut améliorer l'incidence élevée du CHC chez les patients atteints du NAFLD/NASH (10). L'obésité, à travers la formation de la stéatose hépatique, est un facteur de risque du carcinome hépatocellulaire. L'obésité produit

un état inflammatoire, caractérisé par des macrophages regroupés autour des adipocytes hypertrophiés et morts, formant une structure sous forme de couronne (21).

### **1.7.2 L'hyperlipidémie**

La consommation excessive de cholestérol et d'acides gras saturés (AGS) est un facteur de risque pour la NAFLD. Le cholestérol sanguin total et le mauvais cholestérol LDL sont souvent élevés chez ces patients. Si les modèles expérimentaux soutiennent cette hypothèse, il n'y a pas d'évidence dans les études menées chez les êtres humains que l'hyperlipidémie contribue à l'apparition d'un CHC (10).

### **1.7.3 Le diabète**

Le foie a un rôle essentiel dans le métabolisme du glucose. En effet, le diabète peut aboutir à une hépatite chronique, un dépôt de graisse au niveau du foie, une insuffisance hépatique et une cirrhose. Le diabète est considéré comme un facteur de risque indépendant pour le CHC. De plus, l'hyperinsulinémie triple le risque de CHC à cause des effets pléiotropiques de l'insuline, qui régule d'une part la cascade anti-inflammatoire mais aussi d'autres voies qui contribuent à la prolifération cellulaire et peuvent influencer la cancérogenèse. Plusieurs études de type transversales, cas-témoin et de cohortes ont montré une association positive (Odd Ratio autour de 2,5) entre le diabète de type 2 et le CHC, indépendamment de la consommation d'alcool et de l'hépatite virale (20).

### **1.7.4 Le tabac**

Les anciennes études ne considéraient pas le tabac comme un facteur de risque indépendant du CHC mais comme un cofacteur. Certains cas de CHC sont apparus chez les fumeurs

n'ayant pas d'autres facteurs de risque, mais comme fumer coïncide souvent avec une consommation excessive d'alcool et/ou une infection virale, les données doivent être interprétées avec prudence (22). Selon le rapport du WCRF/AICR, fumer augmente le risque de cancer du foie en général et surtout s'il est accompagné d'une consommation accrue d'alcool ou chez les patients atteints d'hépatites virales C ou B (HVC ou HVB) comparés à ceux qui ne fument ou ne boivent pas (23). Une méta-analyse récente de 81 études de cohortes et cas-témoins a montré que le tabac augmente l'incidence et la mortalité du CHC. Les produits de la cigarette sont toxiques et la production de radicaux libres entraîne des dégâts et des altérations au niveau de l'ADN ce qui augmente le risque de CHC (24). D'autre part, les fumeurs, et surtout ceux alcooliques ou infectés par le virus de l'hépatites B ou C, ont un risque accru de cancer du foie par rapport à ceux qui ne fument pas et ne boivent pas (23).

### **1.7.5 L'alcool**

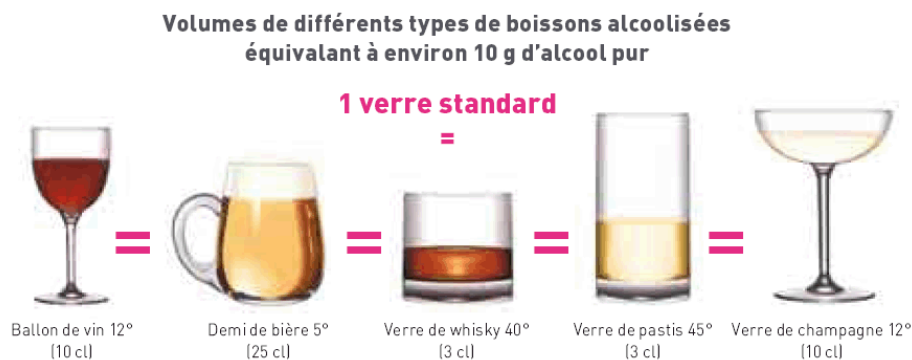
L'abus d'alcool est un des principaux facteurs de risque de la cirrhose et du développement du CHC surtout dans les pays ayant une faible prévalence d'hépatites B et C. L'alcool est le facteur de risque majeur pour le CHC en Amérique du Nord et en Europe du Nord. L'abus d'alcool constitue 40 à 50% des cas atteints de CHC en Europe. Il existe un effet synergique sur le développement de CHC entre l'alcool et les maladies hépatiques surtout les hépatites à virus B et C qui peuvent augmenter le risque de CHC.

Selon la littérature, la relation entre l'alcool et les maladies hépatiques dépend surtout de la quantité consommée tout au long de la vie d'une personne. Une consommation élevée d'alcool augmente le risque de CHC en comparant avec ceux qui ne le consomment qu'occasionnellement. Des études menées en Europe signalent une augmentation du RR des maladies hépatiques lorsque la consommation d'alcool atteint 7-13 verres/semaine pour les femmes et 14-27 verres/semaine pour les hommes. Une méta-analyse de 19 études

prospectives montre que si la consommation d'alcool est > 3 verres/jour le risque de CHC augmente de 16% et si elle est >6 verres/jour le risque augmente de 22% (3).

Par contre, des études avancent l'hypothèse qu'une consommation très modérée de certains types d'alcool peut être bénéfique avec des effets préventifs sur la progression des maladies hépatiques. Le resvératrol, un composant majeur du vin rouge, a beaucoup d'effets positifs sur la santé : il améliore la sensibilité à l'insuline et donc la tolérance au glucose, réduit les lipides plasmatiques et lutte contre le stress oxydatif. De nouvelles études ont pu prouver que le resvératrol peut diminuer le triacylglycérol contenu dans le foie, augmenter l'oxydation des acides gras et prévenir la stéatose hépatique mais il reste à préciser la dose convenable pour qu'il reste efficace sans endommager le foie (25). Selon le rapport du WCRF/AICR, il est évident que l'alcool augmente significativement le risque du cancer hépatique à des doses supérieures à 45g/jour (équivalentes à plus de 3 verres d'alcool). L'étude de la dose-réponse de l'alcool a montré que la consommation d'un verre supplémentaire par jour (équivalent à 10g d'alcool) (figure 9), augmente le risque du cancer du foie de 4%. L'alcool est connu pour provoquer des lésions hépatiques qui engendrent des fibroses et la cirrhose. De plus, l'alcool augmente l'adiposité, surtout centrale, ce qui induit l'obésité et le risque de NAFLD (23).

**Figure 9 : Représentation de la quantité d'alcool par verre équivalente à 10g d'alcool**



### **1.7.6 Le manque d'activité physique**

L'activité physique régulière permet de perdre l'excès de poids pour maintenir un poids corporel sain, augmenter la sensibilité à l'insuline et améliorer l'utilisation du glucose. De plus, elle diminue l'inflammation chronique et le stress oxydatif. Le rapport du WCRF/AICR suggère qu'une activité physique peut diminuer le risque de cancer hépatique à travers les bénéfices déjà cités mais les preuves sont encore limitées et des études supplémentaires sont nécessaires pour confirmer ces résultats (23). Selon certaines études, une activité physique régulière réduit la stéatose et l'inflammation, améliore la sensibilité à l'insuline et réduit la quantité d'acides gras arrivant vers le foie même en absence de perte de poids. Ainsi, elle est inversement associée à la mise en place de NASH et NAFLD. D'autre part, cette activité peut avoir un impact positif après le diagnostic d'un cancer. Elle améliore la qualité de vie, diminue le risque de récurrence du cancer ainsi que la mortalité par cancer (réduction du risque de 50%) chez ceux qui ont survécu d'un cancer du sein, de la prostate et colorectal (26).

### **1.8 Les facteurs de risque nutritionnels de CHC**

En dehors des maladies hépatiques, des facteurs de risques de CHC ont été identifiés à travers les études épidémiologiques, le plus souvent en comparant des cas de CHC à des individus sans pathologies hépatiques. Il n'est pas toujours facile de savoir si ces facteurs de risque interviennent aussi sur le développement de la cirrhose ou sur les phases tardives de la cancérogenèse.

Le WCRF/AICR réunit de nombreux chercheurs dans le monde entier pour étudier et synthétiser l'ensemble des données scientifiques concernant les facteurs de risques nutritionnelles des cancers. Dans son dernier rapport mise à jour en mai 2018 (figure 10), les experts du WCRF/AICR ont jugé que l'alcool ( $\geq 3$  verres/jour), le surpoids/ l'obésité ainsi que les aliments contaminés par l'aflatoxine augmentaient le risque de cancer du foie avec un



niveau de preuve convaincant, alors que la consommation de café diminue le risque avec un niveau de preuve probable. De plus, la consommation de poissons et l'activité physique pourraient diminuer le risque de ce cancer mais cela demande encore une confirmation (23).

**Figure 10 : les dernières recommandations nutritionnelles du WCRF/AICR concernant le cancer du foie**

2015	DIET, NUTRITION, PHYSICAL ACTIVITY AND LIVER CANCER		
		DECREASES RISK	INCREASES RISK
STRONG EVIDENCE	Convincing		Aflatoxins <sup>1</sup> Alcoholic drinks <sup>2</sup> Body fatness <sup>3</sup>
	Probable	Coffee	
LIMITED EVIDENCE	Limited – suggestive	Fish Physical activity <sup>4</sup>	
	Limited – no conclusion	Cereals (grains) and their products, non-starchy vegetables, fruits, peanuts (groundnuts), meat and poultry, salted fish, tea, green tea, glycaemic index, calcium and vitamin D supplements, vitamin C, water source, low fat diet	
STRONG EVIDENCE	Substantial effect on risk unlikely		

**1** Foods that may be contaminated with aflatoxins include cereals (grains), as well as pulses (legumes), seeds, nuts and some vegetables and fruits.  
**2** Based on evidence for alcohol intakes above around 45 grams per day (about 3 drinks a day). No conclusion was possible for intakes below 45 grams per day. There is insufficient evidence to conclude that there is any difference in effect between men and women. Alcohol consumption is graded by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1) [2].  
**3** Body fatness is marked by body mass index (BMI).  
**4** Physical activity of all types.

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La nutrition peut jouer un rôle dans la prévention mais certains choix alimentaires peuvent, au contraire, être des facteurs de risque sur les maladies hépatiques. Le choix de la qualité et de la quantité des aliments joue un rôle majeur dans le développement, la progression et le traitement des maladies en particulier du cancer. Les études épidémiologiques concernant l'effet des aliments sur le risque de CHC ont montré que certains facteurs nutritionnels

peuvent avoir des effets protecteurs comme la consommation excessive de légumes, poissons, la consommation journalière de café (3-4 verres/jours), la supplémentation en vitamine D et en calcium ; plus globalement, une alimentation saine, variée et équilibrée avec une activité physique régulière sont bénéfiques (11,27–31). A l'inverse, la consommation excessive d'alcool, viandes rouges, charcuteries, sucres et boissons sucrées semblent augmenter le risque (28,32,33). Ces résultats ont besoin d'être confirmés dans de larges études car les données disponibles dans la littérature restent très controversées. Les résultats sur les principaux aliments ou groupes alimentaires qui ont un impact sur le risque de CHC sont détaillés ci-dessous.

### *1.8.1 L'aflatoxine*

L'aflatoxine est une substance toxique et carcinogène produite par certaines souches de moisissures, *Aspergillus flavus* et *A. parasiticus*. Il existe quatre principaux types d'aflatoxine: B1, B2, G1 et G2 parmi lesquelles l'aflatoxine B1 est la toxine la plus fréquemment rencontrée. L'aflatoxine est produite par les espèces d'*Aspergillus* retrouvées dans les graines, le maïs, les arachides et le soja stockés dans des mauvaises conditions (atmosphère chaude et humide). L'aflatoxine exerce un effet synergique avec les hépatites B et C et aggrave le risque de cancer du foie ; le risque est multiplié par 30 en présence d'HVB par rapport à l'exposition à l'aflatoxine seule (3). Ce problème concerne principalement les pays tropicaux et subtropicaux. Pour l'Europe, le problème est surtout lié à l'importation des produits contaminés issus de ces pays. Le rapport du WCRF/AICR a trouvé convaincante l'association entre l'aflatoxine B1 et le cancer du foie. Cette relation peut être expliquée par la production de métabolites de l'aflatoxine qui altèrent l'ADN et surtout le gène du p53 qui a un rôle important dans la régulation de la prolifération cellulaire (23).

### *1.8.2 Viandes rouges et charcuteries*

Les viandes rouges et les charcuteries sont riches en graisses animales et en fer héminique qui peuvent générer des radicaux libres, des composés N-nitrosés et causer des dommages oxydatifs. Les nitrites et les nitrates dans la viande transformée sont probablement cancérigènes pour l'homme. De plus, quand les viandes sont cuisinées à haute température et de façon prolongée, des composés considérés cancérigènes peuvent se former comme les amines hétérocycliques et des hydrocarbures aromatiques polycycliques (34). Dans l'étude EPIC, la consommation de poulet, viande rouge et ses dérivés n'était pas associée significativement avec le risque de CHC. Par contre, substituer 20g de poisson/jour avec de la viande rouge augmentait le risque de CHC de 16%. Quatre études de cohortes japonaises et une américaine (NIH-AARP: National Institutes of Health- American Association of Retired Persons) ont aussi étudié le lien entre la viande et ses dérivées et le risque de CHC. Les résultats étaient controversés : l'étude américaine, a montré que la viande rouge était associée positivement au risque de CHC alors que le poulet pourrait diminuer le risque de CHC. Deux études japonaises ont trouvé une augmentation du risque suite à la consommation élevée de viandes rouges (28).

### *1.8.3 Les lipides*

Des études récentes, analysant le lien entre les graisses et le CHC, ont trouvé des associations intéressantes selon le type de graisses consommé. L'étude européenne EPIC a montré que la consommation totale de lipide était inversement associée au risque de CHC. En analysant les différents types de lipides consommés, seuls les acides gras mono-insaturés (AGMI) étaient associés à la diminution du risque du CHC alors que les graisses saturées étaient liées à l'augmentation du risque mais pas de façon significative (35). L'étude de cohorte américaine (NIH-AARP), par contre, a trouvé une association positive et significative entre les graisses

saturées et le CHC (36). Les graisses saturées induisent l'expression de certains médiateurs inflammatoires associés avec la carcinogénèse. En effet, les lésions lipotoxiques sont provoquées par un flux excessif d'acides gras libres en particulier d'AGS, plutôt que par une simple accumulation de triglycérides. L'excès d'AG libres facilite la production de métabolites lipotoxiques (tels que les céramides, le diacylglycérol, la lysophosphatidylcholine et des espèces réactives), contribuant également au développement du stress oxydatif du foie. Dans la NAFLD et le vieillissement, le tissu adipeux viscéral est infiltré par une quantité accrue de macrophages M1, ce qui déclenche une résistance à l'insuline avec une inflammation du tissu adipeux qui aboutit à la perturbation du profil d'adipokine (diminution de l'adipokine anti-inflammatoire : l'adiponectine ; et augmentation des adipokines pro-inflammatoires telles que la leptine, IL-1 $\beta$ , IL-6 et le TNF- $\alpha$ ), qui induisent finalement une inflammation du foie. Par conséquent, les signaux et les hormones dérivés du tissu adipeux autres que les lipides toxiques pourraient jouer un rôle central dans la NAFLD/NASH (37). D'autre part, la consommation de graisses et surtout de graisses saturées augmente le risque d'obésité qui à son tour est un facteur de risque du cancer. L'OMS recommande de limiter la consommation des graisses totales à 15-30% de l'énergie totale journalière et les graisses saturées à 10% seulement (38).

#### *1.8.4 Les boissons sucrées et les jus*

Les boissons sucrées qui sont riches en fructose et en sucre peuvent influencer négativement le métabolisme de lipides et du glucose ainsi que la fonction hépatique. Une consommation élevée de sucre cause une augmentation des taux d'insuline et accélère la lipogénèse hépatique ce qui peut augmenter l'accumulation des graisses dans le foie. Les résultats de l'étude EPIC ont montré qu'une consommation > 6 cannettes/semaine (6\*330ml) augmente le risque de CHC et de NAFLD de façon significative en les comparant aux personnes qui n'en

consomment pas. D'autre part, une faible consommation de jus de fruits frais (<200ml de jus/semaine) était inversement associée au risque de CHC grâce à la présence des antioxydants, vitamines, minéraux et fibres dans le jus. Par contre, si la dose consommée est supérieure à 200 ml/semaine le risque augmente de façon significative en comparaison avec ceux qui n'en consomment pas car l'effet du sucre consommé annule le rôle protecteur des autres composants du jus (35).

### *1.8.5 Les vitamines et minéraux*

- Fer : l'excès de fer et la consommation élevée d'alcool peuvent agir en synergie et promouvoir la formation de fibrose hépatique. De plus, les personnes atteintes d'hémochromatose, une maladie liée à une surcharge en fer, présentent un risque de cancer du foie considérablement accru (36). Chez les patients atteints de NAFLD, le taux de ferritine est très lié au degré de la fibrose hépatique. L'excès de fer produit un stress oxydatif et par suite aggrave la maladie hépatique. Ainsi, l'excès de fer est un facteur de risque de la progression de la fibrose hépatique et du CHC en cas de NASH (10).
- Sodium : Les rares études publiées sur l'apport en sodium n'ont pas pu mettre en évidence une relation entre le sodium alimentaire et le risque de CHC. Mais des études ont pu montrer une association positive entre le sodium et certains facteurs de risque du CHC tels que le syndrome métabolique, l'obésité, les maladies cardiovasculaires, la NAFLD et la fibrose hépatique. Un régime riche en sel est associé au développement de l'hypertension qui a un effet néfaste sur le développement de la cirrhose et du CHC. De plus, une consommation élevée de sodium peut dérégler le fonctionnement du système rénine-angiotensine. Les études ont montré un rôle possible de ce système dans le développement de l'inflammation hépatique et la fibrose conduisant en

conséquent au développement du NAFLD/NASH. D'autre part, une consommation élevée de sel est souvent associée à une consommation riche en énergie et en graisses induisant une augmentation du tissu adipeux qui à son tour augmente la production de cytokines inflammatoires et de stress oxydatif qui sont aussi impliqués dans le développement de la NAFLD (37–40).

### **Les aliments protecteurs sont :**

#### *1.8.6 Le café, thé et le café décaféiné*

Le café contient de nombreux composants comme des minéraux, antioxydants, composés phénoliques, mélanoidines et diterpènes. Ces derniers renforcent les défenses immunitaires, induisent des capacités de réparation d'ADN et l'apoptose, diminuent l'angiogenèse et l'expression de certains gènes impliqués dans l'inflammation et améliorent la sensibilité à l'insuline (41). Le thé, et surtout le thé vert, est riche en polyphénols connus pour leurs propriétés antioxydantes (42). Cet effet positif a été mis en évidence dans plus de 8 études cas-témoins et 18 études de cohortes. La consommation de 2 tasses de café supplémentaire à la consommation journalière peut diminuer de 35% le risque de CHC (RR= 0,65 [95% IC=0,59-0,72]) (27). L'étude multicentrique européenne EPIC, a montré que la consommation de café et de thé (mais avec moins d'effets selon la dose consommée) diminue significativement l'incidence du CHC. Cet effet n'a pas été remarqué avec le café décaféiné. D'autres études cas-témoins et de cohortes menées en Asie et en Europe ont retrouvé cette association inverse (42). Une étude cas-témoin nichée dans la cohorte EPIC a montré qu'une consommation élevée de café stimule l'immunité chez les malades atteints de NAFLD, diminue la concentration des enzymes aspartate amino-transférase (AST), gamma-glutamyl transférase (GGT) et glutamate déshydrogénase (GLDH) dans le sérum et diminue les doses

d'IL-6 aux effets pro-inflammatoires. De plus, les éléments bioactifs du café comme la caféine, paraxanthine, cafestol, kahweol et acide chlorogénique protègent le foie (43). Selon le dernier rapport du WCRF/AICR, le niveau de preuve est jugé probable concernant la consommation de café inversement associée au risque de cancer hépatique, surtout chez les hommes. La méta-analyse en dose-réponse qui a inclu 6 études a montré une diminution statistiquement significative de 14% pour une augmentation d'une tasse de café/jour (23).

### *1.8.7 Le poisson*

Les poissons sont riches en oméga-3, en AGPI (acides gras polyinsaturés), acide eicosapentaénoïque (EPA) et acide docosahexaénoïque (DHA) qui jouent un rôle important dans l'inhibition de la carcinogénèse en altérant la production de certains précurseurs, l'expression de certains gènes ou la formation de radicaux libres (23). Les études étaient controversées mais l'étude EPIC, l'étude américaine (NIH-AARP) et l'étude de cohorte japonaise où les patients étaient atteints de VHB et VHC ont montré que la consommation totale de poisson était inversement associée de façon significative au risque de CHC (28). Dans le rapport du WCRF/AICR, la méta-analyse montraient une association inverse mais non-significative entre la consommation élevée de poissons et le risque de cancer hépatique. Une autre méta-analyse en dose-réponse a mis en évidence une diminution de 6% du risque de CHC par augmentation de 20g de poissons/jour (23).

### *1.8.8 Les fruits et les légumes*

Les fruits et légumes sont riches en vitamines et minéraux qui ont des propriétés anti-oxydantes et protectrices comme les caroténoïdes, rétinoïdes, acide ascorbique et  $\alpha$ -tocophérol. Ils sont aussi riches en fibres qui augmentent le sentiment de satiété et diminuent le risque d'obésité. L'étude européenne EPIC a montré que les participants qui consommaient plus de légumes ont un risque de développer un CHC diminué de 42% en comparaison avec

ceux qui consomment une faible quantité. De plus, une incrémentation de 100g/jour de légumes est associée à une diminution de 17% de l'incidence de CHC (44). Une méta-analyse menée en 2014, a confirmé l'effet positif de l'incrémentation de 100g/jour de légumes qui a été associée à une diminution du risque de 8% (45). Par contre, l'analyse des résultats concernant les fruits, ne trouvait pas une association significative avec le risque de CHC (44).

#### *1.8.9 Le lait et les produits laitiers*

Le lait et les produits laitiers sont riches en calcium, vitamine D (s'ils en sont enrichis), protéines et graisses (s'ils ne sont pas écrémés). L'étude de cohorte EPIC a rapporté qu'une consommation élevée de produits laitiers (surtout le lait et le fromage mais pas le yaourt) augmente de façon significative le risque de CHC. De plus, le calcium, la vitamine D, les graisses et les protéines de source laitière étaient associés positivement et de façon significative au risque de CHC. En revanche, ces mêmes nutriments d'une autre source alimentaire montraient un effet nul ou inverse et non-significatif (30). L'étude américaine NIH-AARP, n'a pas trouvé de lien significatif dans les deux sexes. Mais une supplémentation en calcium était inversement associée au cancer du foie (29).

#### *1.8.10 Les flavonoïdes*

Les flavonoïdes sont des composés bioactifs qu'on retrouve dans les fruits, légumes, chocolats et boissons... Ils constituent la plus importante catégorie des polyphénols connus pour leurs vertus antioxydantes. Très peu d'études ont analysé l'impact des polyphénols sur le CHC. Dans l'étude EPIC, l'augmentation de la consommation de flavonoïdes diminuait de façon significative le risque de CHC. Cet effet est surtout remarqué avec les flavanols. La consommation d'aliments ayant des capacités antioxydantes non enzymatiques (NEAC : Non Enzymatic Antioxydant Capacity) a été aussi calculée à partir des bases de données du TRAP (Radical- Trapping Antioxydant Parameter) qui contient 207 aliments et FRAP (Ferric



Reducing Antioxydant Power) qui contient 210 aliments. Les sources principales étaient : le café, le thé, les fruits et les légumes. Une association inverse et significative a été trouvée entre la consommation d'aliments NEAC et le risque de CHC mais ces résultats devenaient non-significatifs quand les 2 premières années de suivi des patients étaient exclues (46). Dans une autre étude cas-témoin menée en Grèce, les résultats ont montré une association inverse et significative entre les flavones et le risque de CHC et cela selon diverses étiologies (47). Une étude de cohorte japonaise a analysé l'effet de la consommation d'isoflavones et des produits à base de soja sur le risque de développer un CHC. Les résultats ont montré une augmentation du risque de CHC chez les femmes seulement. Les femmes atteintes d'hépatites devraient diminuer la consommation d'isoflavones (48).

### *1.8.11 Les vitamines et minéraux*

Les études qui investiguent le rôle des micronutriments dans le risque de CHC sont peu nombreuses et non concluantes. La déficience en vitamines dans les maladies hépatiques est liée à un désordre de la fonction hépatique, une diminution des réserves, une diminution des apports et/ou une malabsorption(36). Les doses de certaines vitamines sont à considérer avec précautions car à faibles doses, elles sont bénéfiques et préventives alors qu'à fortes doses, elles peuvent être létales :

- Vitamine A : la déficience en vitamine A est très présente dans l'VHC et chez 50% des patients atteint de cirrhose alcoolique. Une faible consommation de rétinol (taux sérique  $<0,78\mu\text{mol/L}$ ) est associé à la détérioration du foie alors qu'une dose élevée de vitamine A est très toxique pour le foie (36).
- Vitamine D : est connue pour ses propriétés immuno-modulatrices, anti-inflammatoires, anti-fibrotiques. Elle est impliquée dans la prolifération cellulaire, l'apoptose, la différenciation, l'invasion et le développement de la métastase et

l'angiogenèse. Les études récentes ont montré qu'un faible taux sanguin de vitamine D était lié à l'augmentation du taux de mortalité des patients atteints de maladies hépatiques chroniques. Il a aussi été démontré que la carence en vitamine D est liée au développement de NAFLD (36,49).

- Vitamine E : les maladies hépatiques d'origine alcoolique entraînent souvent une déficience en vitamine E. La vitamine E est connue pour ses effets antioxydants. Ainsi, les études ont montré qu'une supplémentation en vitamine E améliore de façon significative les caractéristiques pathologiques du NASH (36).
- Zinc : une déficience est fréquente dans les maladies hépatiques. Il joue un rôle important dans la prolifération, le développement et la différenciation des cellules. Un essai clinique contrôlé randomisé en double aveugle a montré qu'une faible supplémentation en zinc prévenait la détérioration de l'état clinique et améliorait la fonction hépatique des patients cirrhotiques (36).
- Magnésium : une déficience est fréquente dans les maladies hépatiques. Cette déficience est associée à une résistance périphérique à l'insuline. Un essai clinique randomisée a montré qu'un traitement en magnésium améliorait le taux des enzymes hépatiques (36).

L'ensemble de ces données montrent qu'il existe des facteurs susceptibles d'influencer le risque de maladies hépatiques et leur aggravation en CHC. Néanmoins, il existe très peu d'études épidémiologiques auprès de patients atteints de cirrhose qui ont pourtant des caractéristiques bien particulières.

## 2. Objectifs de la thèse

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Les patients atteints de cirrhose ont un risque très élevé de développer un CHC. Il est donc indispensable d'identifier les facteurs intervenant dans le risque de complications et en particulier de CHC dans cette population pour mettre en place des actions préventives adaptées.

L'alimentation est une source importante de composés bioactifs, en particulier pro- et anti-inflammatoires et représente un facteur modifiable potentiel. Les études sur les liens entre facteurs nutritionnels et CHC sont peu nombreuses et souvent discordantes. Les facteurs nutritionnels liés à la carcinogenèse hépatique sont donc encore mal définis. De plus, ces études ne sont pas toujours transposables dans une population aux habitudes alimentaires occidentales, et surtout n'ont pas été réalisées chez des patients atteints de cirrhose, pourtant les plus à risque de développer un CHC et qui ont sans doute une alimentation spécifique. Notre objectif était donc d'étudier le rôle des facteurs nutritionnels sur le risque de CHC chez des patients atteints de cirrhose.

Nous avons envisagé plusieurs approches:

- 1/ par aliments permettant de confirmer les hypothèses du WCRF/AICR mais chez des patients atteints de cirrhose
- 2/ par profils de consommation alimentaire définis *a priori* et *a posteriori* permettant une approche plus intégrée de l'alimentation.
- 3/ par utilisation du Diet Inflammatory Index conçu pour estimer le potentiel inflammatoire de l'alimentation.
- 4/ par un focus sur un groupe alimentaire : les lipides.

### 3. Matériels et Méthodes

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Nos travaux s'appuient sur l'étude cas-témoin "CiRCE" (« Cirrhose et Risque de Carcinome hépatocellulaire dans le grand Est ») qui offre l'opportunité de travailler sur les facteurs de risques environnementaux, nutritionnels et métaboliques du CHC chez des patients cirrhotiques, toute étiologie confondue. Cette étude a été initiée par le Professeur Patrick Hillon, investigateur principal, et coordonnée par l'équipe « Épidémiologique et recherche clinique en oncologie digestive » (EPICAD) du centre de Recherche Inserm UMR1231 «Lipides, Nutrition Cancer » avec le soutien du Centre d'Investigation Clinique (CIC) (Inserm 1432/CHU de Dijon). Le recrutement a eu lieu entre Juin 2008 et 2012 dans six centres hospitaliers : Besançon, Dijon, Metz, Nancy, Reims et Strasbourg situés dans le Nord Est de la France. Le protocole de cette étude a été approuvé par la Comité de Protection des Personnes Est I et la Commission Nationale de l'Informatique et des Libertés (agrément n°2008/09 du Comité de Protection des Personnes Est et n° 2008-A00023- 52 de l'AFSSAPS). Elle a été enregistrée sur clinicaltrials.gov sous le numéro NCT01798173.

L'étude CiRCE était déjà initiée avant le début de mon doctorat. Les laboratoires, les centres de recherches et les hopitaux avaient déjà collecté les données individuelles, démographiques et cliniques. A mon arrivée, des analyses biologiques ont été confiées à des laboratoires partenaires, notamment la plateforme de Lipidomique et les laboratoires du LabEx LipSTIC (lauréat ANR/PIA 2012 laboratoire d'excellence Lipoprotéines et Santé – renouvelé en 2019). Mon rôle était de collecter, d'organiser et d'analyser les données reçues à l'aide des programmes statistiques puis d'interpréter les données avec ma directrice de thèse et l'équipe de recherche.

### **3.1 Population d'étude**

L'étude CiRCE a inclu des personnes atteintes de cirrhose de toutes les étiologies, les cas avaient un CHC nouvellement diagnostiqué. Les témoins étaient atteints de cirrhose sans CHC. Ces patients étaient pris en charge dans les services d'hépatogastroentérologie des 6 centres d'investigation.

#### **Critères d'inclusion**

- Personne atteinte de cirrhose alcoolique, virale B ou C ou métabolique
- Personne de 35 ans et plus
- Patient ayant donné son consentement écrit
- Patient affilié à un régime de sécurité sociale

#### **Critères d'exclusion**

- Patient atteint d'un cancer extra hépatique en évolution
- Patient atteint d'un cancer du foie autre qu'un CHC
- Patient atteint d'un CHC sans cirrhose
- Patient atteint d'une hépatite alcoolique aiguë
- Personne souffrante d'une maladie somatique ou psychiatrique grave
- Personne porteuse du VIH
- Personne de moins de 35 ans
- Personne sous tutelle ou sauvegarde de justice

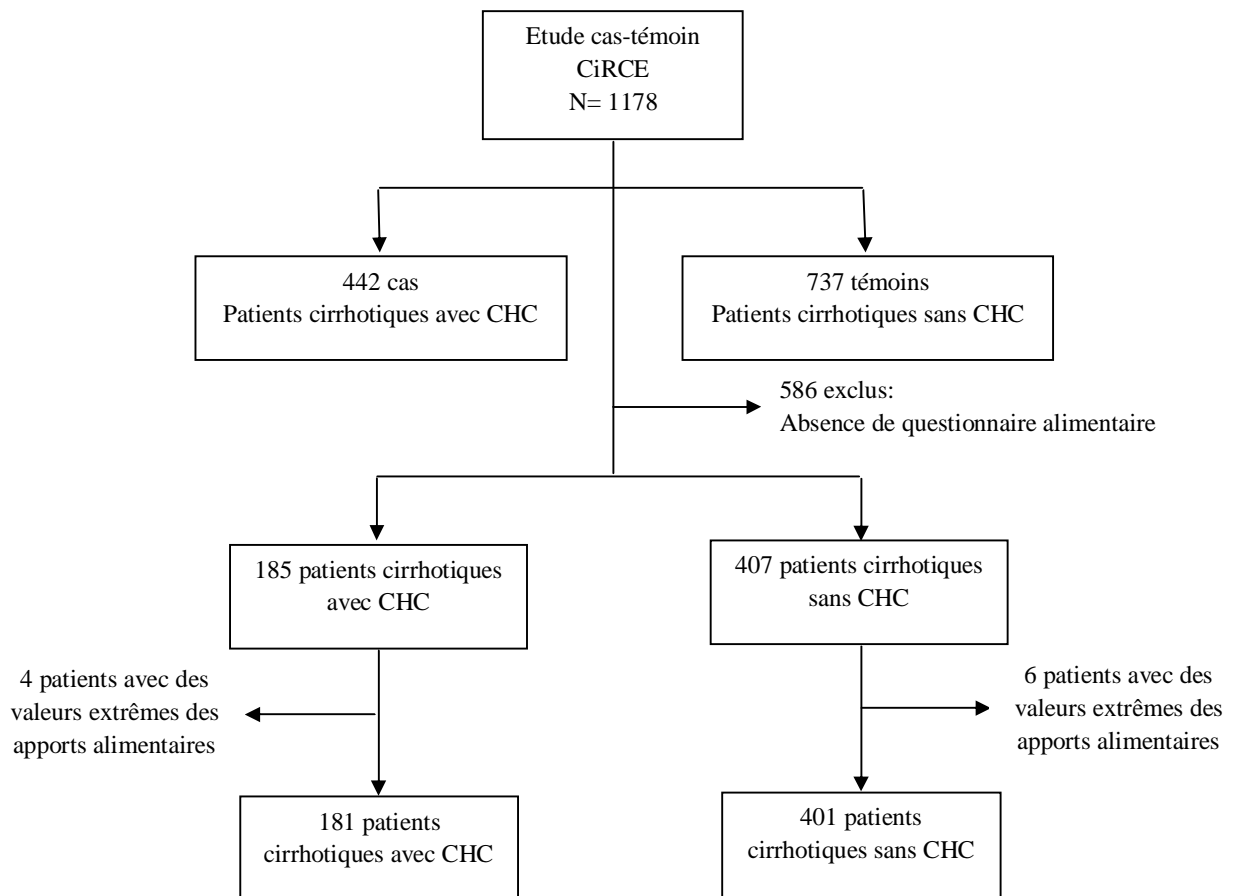
Au total, 1178 patients ont été recrutés dont 444 patients cirrhotiques atteints de CHC (cas) et 734 patients cirrhotiques non atteints de CHC (témoins). Le diagnostic de cirrhose pour tous les patients était posé sur biopsie ou en cas d'absence de biopsie, sur un faisceau d'arguments cliniques, biologiques, morphologiques, hémodynamiques ou endoscopiques. Les cas étaient des patients cirrhotiques avec un CHC qui a été diagnostiqué dans les centres médicaux par histologie, échographie abdominale, scanner, IRM ou AFP (alpha-fetoprotéine) avec un taux

supérieur à 100 ng/ml. Le diagnostic était confirmé selon des critères définis par l'EASL-EORTC (2). Parmi ces 1178 participants, 592 patients ont répondu au questionnaire alimentaire dont 185 cas et 407 témoins. Les 586 patients restants ont été exclus des travaux présentés ici. Nous avons comparé les 592 ayant répondu au questionnaire alimentaire aux 586 patients exclus (table 1). Les participants étaient plus jeunes (médiane d'âge de 62,2 [54,1-70,7] pour les non-participants et de 60,3 [53,4-66,5] pour les participants), le plus souvent des cas, plus éduqués, plus actifs et des consommateurs d'alcool. Ils étaient atteints de cirrhose souvent liée à une infection virale avec un score du Child-Pugh A en comparaison avec les non participants (table 1).

Les patients présentant des valeurs extrêmes (1<sup>er</sup> et 99<sup>ème</sup> percentiles) pour le rapport entre les apports énergétiques et les besoins énergétiques (EIER : Energy Intake and Energy Requirement) ont été exclus de l'analyse finale (n = 10). L'EIER et le métabolisme de base (BMR: Basal Metabolic Rate) ont été calculés en fonction de la taille, du poids, de l'activité physique et de l'activité physique au travail. Ainsi, 181 malades cirrhotiques atteints de CHC (cas) et 401 malades cirrhotiques sans CHC (témoins) étaient finalement inclus dans nos études. Les malades atteints de CHC (cas) seront comparés à des malades sans CHC (témoins).

Le flow chart (figure 11) présente les autres choix de sélection que nous avons fait.

**Figure 11 : Flow chart illustrant le recrutement des participants dans l'étude CiRCE**



**Table 1 : Comparaison des principales caractéristiques des participants et des non participants dans l'étude.**

Variables	Non participants	Participants	P valeur
	n=586	n=592	
<b>Age, années</b>	62,2 [54,1-70,7]	60,3 [53,4-66,5]	0,006
<b>Sexe</b>			0,13
Homme	449 (76,6)	431 (72,8)	
<b>Niveau d'éducation<sup>1</sup></b>			0,008
≤12 ans d'études	529 (91,0)	509 (86,1)	
Plus de 12 ans d'études ou diplôme des grandes écoles/universitaire	52 (9,0)	82 (13,9)	
<b>Activité physique professionnelle<sup>2</sup></b>			0,39
Aucune ou faible	433 (74,8)	435 (74,1)	
Modérée	78 (13,5)	93 (15,8)	
Importante	68 (11,7)	59 (10,1)	
<b>Activité physique de loisir<sup>3</sup></b>			<0,001
Aucune ou faible	335 (57,5)	277 (46,8)	
Modérée	91(15,6)	135 (22,8)	
Importante	156 (26,9)	180 (30,4)	
<b>Indice de Masse Corporelle<sup>4</sup></b>			0,52
Maigre ou normal	216 (36,9)	200 (33,8)	
Surpoids	217 (37,1)	227 (38,3)	
Obèse	152 (26,0)	165 (27,9)	
<b>Consommation tabac</b>			0,07
Jamais	140 (23,9)	149 (25,2)	
Actuelle	224 (38,2)	189 (31,9)	
Ancienne	222 (37,9)	254 (42,9)	
<b>Consommation d'alcool<sup>5</sup></b>			0,035
Jamais	44 (7,5)	34 (5,7)	
Actuelle	140 (24,1)	180 (30,4)	
Ancienne	398 (68,4)	378 (63,9)	
<b>Cas</b>	259 (44,2)	185 (31,3)	<0,001
<b>Témoins</b>	327 (55,8)	407 (68,7)	
<b>Ancienneté de la cirrhose, années</b>			0,11
<2	297 (50,7)	272 (46,0)	
≥2	289 (49,3)	320 (54,0)	
<b>Etiologie de la cirrhose</b>			0,021
Infection virale associée ou non avec abus d'alcool	80 (13,7)	111 (18,8)	
Abus d'alcool	376 (64,2)	330 (55,7)	
Métabolique	60 (10,1)	66 (11,1)	
Autres	70 (12,0)	85 (14,4)	
<b>Score du Child-Pugh<sup>6</sup></b>			0,002
A	285 (49,2)	332 (58,6)	
B	189 (32,7)	168 (29,6)	
C	105 (18,1)	67 (11,8)	
<b>Diabètes<sup>7</sup></b>	212 (37,6)	219 (38,8)	0,69

Comparaison univariée en utilisant le test de chi2 pour les variables qualitatives et le test de Student pour les variables continues. Les valeurs sont présentées en n (%) ou médiane [IQR].

<sup>1</sup>Niveau d'éducation: 6 données manquantes

<sup>2</sup>Activité physique professionnelle: 12 données manquantes

<sup>3</sup>Activité physique de loisir: 12 données manquantes

<sup>4</sup>Indice de Masse Corporelle : 1 donnée manquante

<sup>5</sup>Consommation d'alcool: 4 données manquantes

<sup>6</sup>Score du Child-Pugh: 22 données manquantes

<sup>7</sup>Diabètes: 49 données manquantes



### **3.2 Déroutement de l'étude**

Le médecin investigateur informait ses patients sur le protocole et les objectifs de la recherche. Les personnes acceptant de participer à l'étude étaient alors interrogées par le médecin ou une personne autorisée au cours d'une consultation ou d'une hospitalisation après avoir recueilli leur consentement éclairé, signé. Chez les malades atteints de CHC, le protocole était proposé à distance de la consultation d'annonce.

Dans tous les centres, les enquêteurs ont collecté des informations relatives à la cirrhose et au CHC à l'aide de questionnaires standardisés. La collecte de données concernaient des données individuelles et démographiques (âge, sexe, niveau d'éducation, activité physique, consommation d'alcool passée et actuelle, statut tabagique) et des données cliniques (mesures anthropométriques, premier diagnostic, examen clinique, médicaments actuels ou déjà pris, antécédents médicaux du patient et de sa famille, traitement médical et données du diagnostic du CHC) (annexe 4).

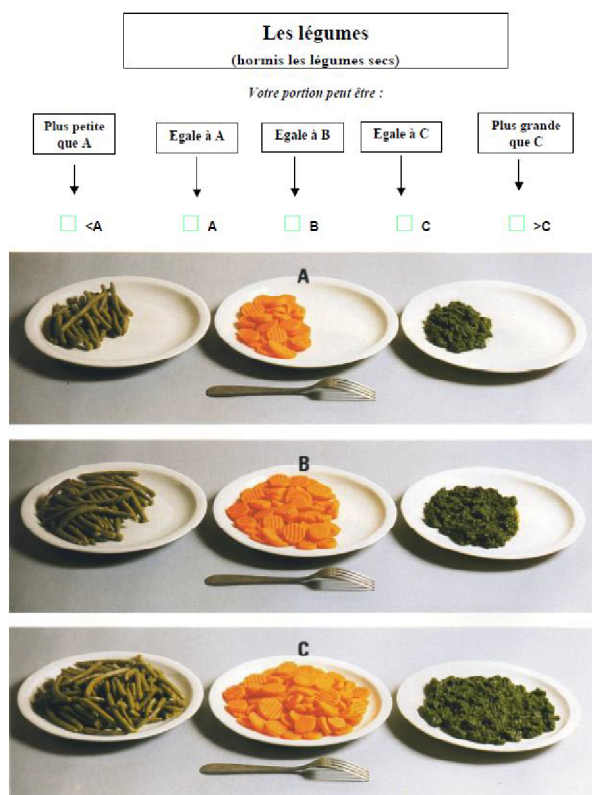
De plus, les participants ont été soumis à un auto-questionnaire alimentaire permettant l'estimation de la consommation de 208 aliments, boissons et plats. Le questionnaire utilisé, évaluait les consommations en fonction des habitudes alimentaires des patients au cours de l'année précédant l'inclusion (56,57), et donc tient compte des variations saisonnières. Le questionnaire est composé de deux parties, l'une quantitative, l'autre qualitative. La première partie (figure 12), quantitative, décrit les fréquences de consommations et les tailles de portions habituellement consommées d'une liste de 66 aliments ou groupes d'aliments.

**Figure 12 : Première partie du questionnaire alimentaire**

QUESTIONNAIRE ALIMENTAIRE – PREMIERE PARTIE														
Décrivez votre alimentation au cours de l'année précédente. Voir les recommandations ci-dessus.														
Réveil, Petit Déjeuner	Combien de fois ? Cochez la case correspondante											Inscrivez ici vos initiales : .....		
	Jamais	1, 2, 3 fois / mois			OU	1, 2, 3,...7 fois par semaine							En quelle quantité ? Inscrivez la lettre correspondant à la photo et le nombre de portions ou d'unités correspondantes	
		1	2	3	1	2	3	4	5	6	7			
Eau : Robinet <input type="checkbox"/> Douteille <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Type de verre : photo page 33	<input type="checkbox"/>	Nombre : <input type="text"/> , <input type="text"/>
Jus de fruits (fruits pressés ou jus de fruits du commerce)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Type de verre : photo page 33	<input type="checkbox"/>	Nombre : <input type="text"/> , <input type="text"/>
Chicoree : Au lait <input type="checkbox"/> Nature <input type="checkbox"/> (Riciné, Niesconé, )	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Type de bol ou de tasse : photo page 2	<input type="checkbox"/>	Nombre : <input type="text"/> , <input type="text"/>
Café : Au lait <input type="checkbox"/> Normal <input type="checkbox"/> Moulu <input type="checkbox"/> Nature <input type="checkbox"/> Décaféiné <input type="checkbox"/> Soluble <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Type de bol ou de tasse : photo page 2	<input type="checkbox"/>	Nombre : <input type="text"/> , <input type="text"/>

Cette partie est structurée autour de 8 repas journaliers : le petit déjeuner, la collation de la matinée, l'apéritif précédant le déjeuner, le déjeuner, la collation de l'après-midi, l'apéritif précédant le dîner, le dîner et la collation suivant le dîner. Pour chaque item, le patient doit préciser sa fréquence de consommation parmi 11 modalités proposées (jamais ou moins d'une fois par mois, 1 à 3 fois par mois, 1 à 7 fois par semaine) et la quantité consommée, estimée soit en unité standard, soit en portions, avec l'aide d'un livret de photographies en couleurs validé et associé au questionnaire (figure 13) pour faciliter l'estimation des portions consommées (51).

**Figure 13 : Livret pour aider à estimer les quantités consommées**



La seconde partie du questionnaire (figure 14) est qualitative et permet de détailler les consommations de groupes d'aliments décrits dans la première partie du questionnaire.

Une table de composition alimentaire rattachée au questionnaire E3N a été élaborée afin que les consommations alimentaires puissent être converties en apports nutritionnels journaliers pour les 208 aliments et 23 nutriments. Ce sont les données nutritionnelles du Ciqual qui ont servi de référence. Le Ciqual est une base de données nationale regroupant des informations sur plus de 2 600 aliments différents et 61 constituants. La nouvelle version 2017 regroupent 2807 aliments dont 180 nouveaux aliments (52).

**Figure 14 : La deuxième partie du questionnaire alimentaire**

6. Les légumes chauds (sauf pommes de terre et légumes secs) : en consommez-vous habituellement ?									
<input type="checkbox"/> Non, jamais (ou moins d'une fois par mois), passez question 7.									
<input type="checkbox"/> Oui. Lesquels ? Faire une moyenne sur l'année écoulée (voir comment remplir : page 27 de ce questionnaire)									
	0	+	++	+++		0	+	++	+++
Chou	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Haricots verts	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chou-fleur	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Carottes cuites	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Choux de Bruxelles	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Poireaux	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Brocolis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Salsifis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Epinards	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Tomates cuites	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Endives	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Aubergines	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Champignons	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Poivrons cuits	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Céleri en branche	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Courgettes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bettes (ou cardons)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Petits pois	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fenouil cuit	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Autres (précisez) : .....				<input type="checkbox"/>

Les questionnaires alimentaires, complétés par les patients, ont été soumis à une lecture optique. Plusieurs programmes SAS ont été appliqués sur l'ensemble des données pour rassembler de façon cohérente ; les portions des aliments et boissons ont été converties en g/jour avec calcul des différents nutriments et apport alimentaires par patient grâce à la table de consommation alimentaire.

Les résultats des explorations radiologiques étaient fournis par les médecins radiologues alors que les résultats des dosages biologiques étaient fournis par les laboratoires chargés des dosages. Les prélèvements biologiques pour la biobanque ont été envoyés aux Centre de Ressources Biologiques (CRB) de Dijon où ils sont conservés et utilisés selon le besoin.

Une enquête de suivi a été réalisée chaque année pendant 5 ans pour les derniers patients inclus, soit jusqu'en février 2018 permettant de recueillir les informations de décembre 2017 pour les inclusions de fin 2012. Les informations concernaient la survenue de complications de la cirrhose, l'apparition d'un CHC chez un témoin, les modalités du traitement du CHC et la survenue du décès.

### 3.3 Données disponibles et méthodes de dosage

#### 3.3.1 Prélèvements biologiques

Des prélèvements de sérums, plasma, ADN, ont été réalisés chez les cas et les témoins puis conditionnés et congelés à -80°C. Les collections étaient ensuite transférées trimestriellement au CRB de Dijon (INSERM-DHOS) pour y être stockées. Les prélèvements de sang ont été réalisés à jeun en ambulatoire ou en cours d'hospitalisation. Chez les malades atteints de CHC, le prélèvement biologique a été fait avant les traitements spécifiques du cancer, dans le mois suivant l'inclusion du malade dans l'étude.

#### 3.3.2 Méthode de dosages

- Le dosage de l'Alpha foeto-protéine a été fait par immuno-enzymologie en utilisant un kit commercial (Roche Diagnostica).
- Le dosage de la vitamine B12 et des folates était fait par la technique MEIA (microparticule enzyme immunoassay) en utilisant le système Abbott IMX automated Benchtop analyzer system (Abbott Diagnostic, France).
- Les acides gras et l'alpha tocophérol ont été dosés en plusieurs étapes :
  - *Composition en acides gras* : les lipides totaux étaient extraits des membranes érythrocytaires en utilisant une technique dérivée de la méthode de Folch. Les phospholipides étaient dissous dans une solution de 200 µL de chloroform-méthanol, purifiées par chromatographie puis transformés en acides gras par la méthode Méthyle-Prep II (Alltech, Templemars, France). Les acides gras étaient analysés par chromatographie en phase gazeuse.
  - *Quantification en tocophérol des membranes érythrocytaires* : les membranes érythrocytaires étaient homogénéisées dans un micropotter de 500 µl de sérum salé contenant 50 mM d'acide ascorbique et 500 µl de solution d'éthanol contenant

50 mg/litre de BHT (butylated hydroxytoluene). L'alpha-tocophérol était analysé par spectroscopie de masse en phase liquide.

- L'insulino-résistance était évaluée par la méthode HOMA après dosages de l'insuline par radioimmuno-assay (CIS Biointernational, Gif sur Yvette, France) et de la glycémie par méthode de glucose oxydase en utilisant un Vitros 750 analysé (Johnson & Johnson Clinical Diagnosis, Rochester, NY, USA).
- Les dosages d'adiponectine, de leptine, de resistine, de VEGF et de récepteurs solubles I and II du TNF étaient faits par technique Elisa (Quantikine, R&D Systems, Wiesbaden, Allemagne).

### **3.4 Méthodes statistiques**

Les méthodes statistiques sont développées dans la partie analyse statistique de chaque objectif. Toutes les analyses ont été effectuées avec le logiciel SAS<sup>®</sup>, versions 9,3 ou 9,4 (SAS Institute Inc., Cary, Caroline du Nord) et le logiciel R version 3,1,2 (R-Core Team 2014, Vienne).

## 4. Résultats

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<i>Premier objectif de thèse</i>
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Analyse de l'effet des aliments sur le risque de CHC chez des patients atteints de cirrhose

Le WCRF/AICR dans son analyse de la littérature existante a identifié quelques rares aliments impliqués dans la survenue de CHC. La conclusion précisait un manque de données pour confirmer les hypothèses, les premiers résultats et proposer des recommandations. De plus, ces études ne concernaient pas les patients atteints de cirrhose. L'alimentation peut être un moyen de prévention intéressant mais les résultats d'études dans ce domaine restent rares et divergents. Les études précédentes qui analysaient l'impact de l'apport alimentaire sur le risque de CHC comparaient des patients atteints de CHC à des patients qui ne souffrent pas de maladies hépatiques. Cependant, les patients atteints de cirrhose ont certainement des besoins nutritionnels spécifiques et des profils de consommation différents du reste de la population. Notre travail visait à identifier les facteurs alimentaires associés au risque de CHC dans une population à haut risque souffrant de cirrhose.

***Maud Rizk, Adrien Guilloteau, Thomas Mouillot, Gérard Thieffin, Jean-Pierre Bronowicki, Carine Richou, Michel Doffoel, Mona Diab Asaf, Patrick Hillon, Vanessa Cottet. Dietary components modulate the risk of hepatocellular carcinoma in cirrhotic patients. Nutr Res (2018), DOI:10.1016/j.nutres.2018.10.002***

## **Introduction**

Nous avons émis l'hypothèse que le régime alimentaire influence l'évolution de la cirrhose et en particulier le développement du CHC. Les patients cirrhotiques ont un risque plus élevé de complications et ont donc besoin d'une surveillance et de recommandations spécifiques.

## **Objectifs**

Cet article avait pour but d'étudier les liens entre différents facteurs alimentaires et le risque de CHC dans une population française atteinte de cirrhose. Cela nous aidera à confirmer les hypothèses du WCRF chez une population spécifique atteinte de cirrhose.

## **Méthodes**

Cet article se base sur l'étude CiRCE, seuls les participants qui ont complété le questionnaire alimentaire ont été inclus (592 patients). Le flow chart illustrant la sélection des participants inclus dans les analyses est présentée en figure 11. Ainsi, l'analyse a inclus 401 patients cirrhotiques sans CHC (témoins) et 181 patients cirrhotiques avec CHC (cas).

Dans la présente étude, les différents aliments consommés ont été regroupés dans 27 groupes d'aliments prédéfinis. Les boissons étaient classées en 5 groupes : les différents types d'eau, les boissons alcoolisées, les boissons gazeuses sans sucre et les bières légères, les boissons sucrées et finalement le café/thé/chicorée. La catégorie du sucre inclut le sucre naturel, les édulcorants, le miel et les confitures. La catégorie des desserts regroupent tous les pâtisseries, les viennoiseries, les bonbons et les chocolats. Les fruits et légumes regroupent les produits frais, congelés et en conserves.



La méthode des résidus a été appliquée ainsi qu'un découpage en tertiles. Pour les analyses univariées des quantités consommées, le test du Chi<sup>2</sup> était appliqué (test exact de Fisher si les conditions d'application le nécessitaient) pour les variables qualitatives et le test de Student (test de Wilcoxon si nécessaire) pour les variables quantitatives. Les Odds Ratio (OR) et leurs intervalles de confiance (CI) à 95% ont été obtenus par régression logistique multivariée. L'ajustement a été fait sur les variables suivantes : âge (continu), sexe, énergie totale de sources non alcooliques, ancienneté de la cirrhose, score de Child-Pugh, diabète, étiologie de la cirrhose, consommation d'alcool, activité physique au travail avec une stratification selon le centre.

### **Résultats**

Les résultats ont montré que les 181 cas étaient plus âgés (64,4 ans vs 59 ans,  $p < 0,001$ ), plus souvent de sexe masculin ( $p < 0,001$ ), et diabétiques ( $p < 0,001$ ) que les 401 témoins. Après ajustement, une association positive était mise en évidence entre le risque de CHC et la consommation de boissons gazeuses sucrées (Odd Ratio (OR)<sub>Tertile3vsTertile1</sub>=2,44 [Intervalle de Confiance (IC) à 95% = 1,17-5,09]  $p$ -trend=0,021), de céréales (OR<sub>T3vsT1</sub>=1,87 [IC à 95% = 1,09-3,22]  $p$ -trend=0,035), de charcuteries (OR<sub>T3vsT1</sub>=1,97 [IC à 95% = 1,14-3,41]  $p$ -trend=0,028) mais aussi de sodium (OR<sub>T3vsT1</sub>=2,00 [IC à 95% = 1,14-3,53]  $p$ -trend=0,043). A l'inverse, les consommations de fibres (OR<sub>T3vsT1</sub>=0,49 [IC à 95% = 0,28-0,86]  $p$ -trend=0,012), vitamine E (OR<sub>T3vsT1</sub>=0,52 [IC à 95% = 0,30-0,89]  $p$ -trend=0,017), vitamine B9 (OR<sub>T3vsT1</sub>=0,56 [IC à 95% = 0,33-0,95]  $p$ -trend=0,036), manganèse (OR<sub>T3vsT1</sub>=0,56 [IC à 95% = 0,32-0,97]  $p$ -trend=0,038) et potassium (OR<sub>T3vsT1</sub>=0,44 [IC à 95% = 0,25-0,76]  $p$ -trend=0,004) étaient significativement plus faibles chez les cas que chez les témoins.

Cet article a été publié dans la revue Nutrition Research avec mise en ligne le 12 octobre 2018, DOI : 10.1016/j.nutres.2018.10.002.

## **Conclusion**

Ces résultats, qui manquaient encore dans la littérature, doivent être confirmés dans des études prospectives plus larges. Cette étude a montré plusieurs résultats qui pourraient, après confirmation, servir à élaborer des recommandations nutritionnelles pour les patients cirrhotiques pour leur permettre de prévenir les complications, améliorer leur qualité de vie en plus de diminuer le risque de CHC. A l'issue de la revue de la littérature et des résultats de cette analyse, il nous a donc paru important de poursuivre le travail par la réalisation des études sur des profils alimentaires pour mieux analyser les habitudes alimentaires des patients cirrhotiques.

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## Dietary components modulate the risk of hepatocellular carcinoma in cirrhotic patients



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### ARTICLE INFO

#### Article history:

Received 3 April 2018

Revised 7 September 2018

Accepted 5 October 2018

#### Keywords:

Diet

Cirrhosis

Hepatocellular carcinoma

Case-control study

### ABSTRACT

Eighty percent of hepatocellular carcinoma (HCC) cases occur after cirrhosis from various etiologies. The association between diet and cancer is well accepted, but the links with cirrhosis progression and HCC risk have been poorly investigated. However, we hypothesized that diet could be a modifiable preventive factor for HCC. Thus, the aim of our study was to explore the relationships between dietary factors and the risk of HCC in a population of cirrhotic patients. A total of 582 cirrhotic patients were studied: 401 without HCC (controls) and 181 with HCC (cases). These patients were recruited between 2008 and 2012 for the “CiRCE” case-control study conducted in six French university hospitals. Information about the consumption of 208 food items and 23 nutrients were collected through a diet history questionnaire. Unconditional multivariate logistic regressions were performed for each residual food group and nutrients in tertiles. HCC patients were more often men, diabetic and older than controls. After adjustment, a significant positive association was found between HCC risk and carbonated beverages (OR<sub>Tertile3vsTertile1</sub> = 2.44 [1.17–5.09] p-trend = 0.021), total cereals (OR<sub>T3vsT1</sub> = 1.87 [1.09–3.22] p-trend = 0.035), processed meat (OR<sub>T3vsT1</sub> = 1.97 [1.14–3.41] p-trend = 0.028) and sodium (OR<sub>T3vsT1</sub> = 2.00 [1.14–3.53] p-trend = 0.043). Conversely, the consumption of fiber (OR<sub>T3vsT1</sub> = 0.49 [0.28–0.86] p-trend = 0.012), vitamin E (OR<sub>T3vsT1</sub> = 0.52 [0.30–0.89] p-trend = 0.017), vitamin B9 (folate and

Abbreviations: AFP, alpha-Fetoprotein; AICR, American Institute of Cancer Research; BMI, Body Mass Index; CI, Confidence Intervals; CiRCE, Cirrhosis and Risk of Hepatocellular Carcinoma in the East; CRC, Colorectal Cancer; EASL-EORTC, European Association for the Study of the Liver-European Organization for Research and Treatment of Cancer; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; HCC, Hepatocellular Carcinoma; IQR, Interquartile Range; NAFLD, Non-Alcoholic Fatty Liver Disease; NASH, Non-Alcoholic Steato-Hepatitis; ORs, Odds Ratios; WCRF International, World Cancer Research Fund International.

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<https://doi.org/10.1016/j.nutres.2018.10.002>

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folinic acid) ( $OR_{T3vsT1} = 0.56 [0.33-0.95]$  p-trend = 0.036), manganese ( $OR_{T3vsT1} = 0.56 [0.32-0.97]$  p-trend = 0.038) and potassium ( $OR_{T3vsT1} = 0.44 [0.25-0.76]$  p-trend = 0.004) were significantly lower in HCC patients compared with cirrhotic controls. Although these findings must be confirmed in prospective studies, using dietary patterns or biological parameters, they suggest that certain dietary components may modulate HCC risk in cirrhotic patients.

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## 1. Introduction

Hepatocellular carcinoma (HCC) represents 70% to 90% of the total liver cancer burden worldwide. There were around 782 500 new cases and 745 500 deaths from liver cancer worldwide in 2012 according to GLOBOCAN. In men, liver cancer is the fifth most common cancer and the second most frequent cause of cancer death [1]. In women, it is the seventh most common cancer and the sixth cause of cancer death [2]. HCC is most prevalent in Asian countries and Western Africa. Europe is considered at low to moderate risk of HCC (5–10 per 100 000/year) with the exception of Southern Europe where the incidence increases to 12 per 100 000/year. The geographic distribution of HCC depends on the risk factors of each region: chronic hepatitis B and C infection (HBV and HCV), aflatoxin B1 and alcoholism [3].

The majority of HCC occurs in cirrhotic patients, and between 5% and 30% of these patients develop HCC within the first 5 years [4]. Cirrhosis is the consequence of long-term liver damage with functional alterations due to chronic inflammation. Cirrhosis is considered the fourth cause of death in central Europe (170 000/year) [5] and 13th in the world with a mortality rate that increased by 45.6% between 1990 and 2013 [6]. The highest rates of liver cirrhosis are found in the south-east and north-east of Europe [7]. The main etiologies of cirrhosis are alcohol abuse, HBV, HCV and non-alcoholic steatohepatitis (NASH) [8]. More recently, other HCC risk factors have been recognized such as type II diabetes and metabolic syndrome. These diseases generally have the same mechanisms: accumulation of liver fat due to metabolic disorders, oxidative stress, and inflammation, which is responsible for the liver cell proliferation and dysplasia [2,8].

In the last 20 years, the incidence of HCC has increased dramatically in France and other Western countries. This may be due to an increased prevalence of HCV infection, improved survival of cirrhotic patients, and obesity [4]. Though therapies for HCC have been developed, the prognosis remains poor, underlining the importance of prevention – through HBV vaccination, treatment of HCV infection, and the prevention and treatment of alcoholism and obesity [3,9].

Nutrition is a potential preventive tool since dietary factors may also affect the development of liver cancer. Some studies have investigated the link between food and the risk of HCC, but the results were conflicting. Alcohol abuse is the most important risk factor for HCC in North America and Northern Europe. A higher intake of dairy products, soft drinks and juices high in sugar and fructose has also been associated with an increased risk of HCC [10,11]. On the other hand, regular consumption of certain types of foods, such as vegetables, fish and coffee (3–4 cups per day), seems to limit the risk of HCC [12–14]. Other dietary components such as vitamin D, calcium,

the phenols contained in red wine, green tea, or olive oil may also play a protective role [10,15–18], but these data require confirmation. The majority of the previous studies on dietary intake and HCC risk compared patients with HCC and patients free of liver disease. However, patients with cirrhosis certainly have different dietary consumption profiles. We hypothesized that diet influences the progression of cirrhosis, especially the development of HCC. Cirrhotic patients are at higher risk of complications and thus, need more attention, special care and specific nutritional recommendations.

The aim of the present study was to identify dietary factors associated with HCC risk in a high-risk population diagnosed with cirrhosis. After the study population was defined, the consumption of selected food groups was compared in cases (cirrhotic patients with HCC) and controls (cirrhotic patients without HCC) in order to define dietary factors and nutritional advice that could potentially prevent the development of HCC in cirrhotic patients.

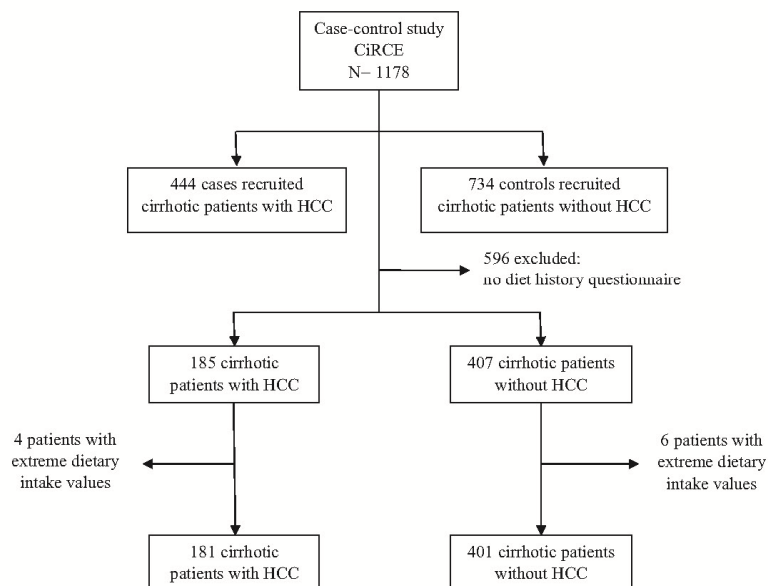
## 2. Methods and materials

### 2.1. Study sample

The French multicenter CIRCE case-control study (“Cirrhosis and Risk of Hepatocellular Carcinoma in the East”) was conducted to identify the environmental, nutritional and metabolic risk factors for HCC among cirrhotic patients. Between June 2008 and December 2012, 1178 patients were recruited in six university hospitals located in North-East France (Besancon, Dijon, Metz, Nancy, Reims and Strasbourg). Cirrhosis was confirmed through liver biopsy, fibroscan or from typical clinical and biological data. Patients were excluded if they were younger than 35 years old, had progressive extrahepatic cancer, primary liver cancer other than HCC, HCC without cirrhosis, HIV infection, acute alcoholic hepatitis or severe psychiatric illness.

Controls were cirrhotic patients without HCC at inclusion. Cases were cirrhotic patients with HCC diagnosed in medical centers through histology, echography, CT scan, MRI or Alpha-fetoprotein (AFP) according to criteria defined by the European Association for the Study of the Liver (EASL), and the European Organization for Research and Treatment of Cancer (EORTC) [19].

For the present study, only participants who completed the dietary questionnaire were included ( $n = 592$ ). Patients with extreme values (1st and 99th percentiles) for the ratio between their energy intake and energy requirement (EI/ER) were excluded from the final analysis ( $n = 10$ ). EI/ER and basal metabolic rate were calculated based on height, weight, physical activity and occupational physical activity. Thus,



**Fig. 1 - The study population. The flow diagram illustrates the selection of the study participants included in the analyses, Patients with extreme values (1st and 99th percentiles) for the ratio between their energy intake and energy requirement (EIER) were excluded from the final analysis (n = 10).**

the analysis included 401 cirrhotic patients without HCC (controls) and 181 cirrhotic patients with HCC (cases) (Fig. 1). All patients read and signed an informed consent form before inclusion. The study protocol was approved by the local ethics committee (Comité de Protection des Personnes Est I) and by the French legal authorities (Commission Nationale de l'Informatique et des Libertés). This study was registered with [clinicaltrials.gov](https://clinicaltrials.gov) as NCT01798173.

## 2.2. Data collection

In all centers, the investigators collected information related to cirrhosis and HCC using standardized questionnaires. Data collection included individual and demographic data (age, gender, educational level, physical activity, past and current alcohol consumption, smoking status), clinical data (anthropometric measurements, first diagnosis, clinical exam, current and past medication records, patient and family medical history, medical treatment and HCC diagnosis data) and information about diet.

## 2.3. Dietary data

At inclusion, every patient had to complete a self-administered, validated diet history questionnaire. It evaluated quantitative and qualitative food consumption according to the patient's eating habits during the year before inclusion [20,21], allowing for seasonal variations. It recorded all meals

and snacks throughout the day. It was accompanied by a booklet of photographs to help patients assess portion sizes for 208 food items. Food portions and the frequency of consumption (11 possibilities, from never to 7 times per week) were converted into daily intake in grams, total energy intake, alcohol and the breakdown for 23 nutrients (protein, carbohydrates, fat, vitamins, minerals...) using the French CIQUAL composition table (2008).

In the present analysis, food variables were measured for 27 predefined food groups. Beverages were classified into 5 groups: all types of water, alcoholic beverages, artificially sweetened soft drinks and light beer, carbonated/soft drinks and finally coffee/tea/chicory. The category for sugar included natural sugar, artificial sweeteners, honey and jam. The dessert category included all kind of sweets, pastries, candies and chocolate. Fruits and vegetables included fresh, frozen and canned products.

## 2.4. Statistical analyses

Two groups were established for educational level:  $\leq 12$  years of schooling or up to 12 years and high-school diploma. Occupational physical activity and leisure time physical activity were categorized as: inactive ( $< 1$  h/week), moderately active (1 h-3 h30/week) and active ( $> 3$  h30/week). Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters and respondents were considered as: underweight, normal weight, overweight, or

Table 1 – Baseline socio-demographic, clinical and medical characteristics of included and non-included patients			
Variables	Non included n = 596	Included n = 582	P
Age, years	61.5 [46.2–76.8]	60.4 [47.3–73.5]	.08
Gender			.11
Male	457 (76.7)	423 (72.7)	
Education level <sup>1</sup>			.004
<12 years of schooling	539 (91.2)	499 (85.9)	
Up to 12 years or high-school diploma	52 (8.8)	82 (14.1)	
Occupational physical activity <sup>2</sup>			.43
Inactive	440 (74.7)	428 (74.2)	
Moderately active	80 (13.6)	91 (15.8)	
Active	69 (11.7)	58 (10.0)	
Leisure-Time Physical activity <sup>3</sup>			<.001
Inactive	340 (57.5)	272 (46.7)	
Moderately active	92 (15.5)	134 (23.0)	
Active	160 (27.0)	176 (30.3)	
Body Mass Index <sup>4</sup>			.57
Underweight or normal	219 (36.8)	197 (33.8)	
Overweight	220 (37.0)	224 (38.5)	
Obese	156 (26.2)	161 (27.7)	
Smoking status			.06
Never	141 (23.7)	148 (25.4)	
Current	228 (38.2)	185 (31.8)	
Former	227 (38.1)	249 (42.8)	
Alcohol status <sup>5</sup>			.031
Never	44 (7.4)	34 (5.8)	
Non abusive	142 (24.0)	178 (30.6)	
Abusive	406 (68.6)	370 (63.6)	
Cases	263 (44.1)	181 (31.1)	<.001
Control patients	333 (55.9)	401 (68.9)	
Cirrhosis diagnosis, years			.15
<2	301 (50.5)	268 (46.0)	
≥2	295 (49.5)	314 (54.0)	
Cirrhosis Etiology			.010
Viral Infection	80 (13.4)	111 (19.1)	
Alcohol abuse	384 (64.4)	322 (55.3)	
Viral infection associated with alcohol abuse	60 (10.1)	66 (11.3)	
Other	72 (12.1)	83 (14.3)	
Child-Pugh Score <sup>6</sup>			.001
A	288 (48.9)	332 (58.6)	
B	195 (33.1)	168 (29.6)	
C	106 (18.0)	67 (11.8)	
Diabetes <sup>7</sup>	215 (37.5)	216 (38.8)	.65

Univariate comparisons were done with chi-square test for qualitative variables or Student's test for continuous variables. Values are presented as n (%) or median [IQR].

<sup>1</sup> Education level: 6 missing data.

<sup>2</sup> Occupational Physical activity: 12 missing data.

<sup>3</sup> Leisure-Time Physical activity: 12 missing data.

<sup>4</sup> Body Mass Index: 1 missing data.

<sup>5</sup> Alcohol status: 4 missing data.

<sup>6</sup> Child-Pugh Score: 22 missing data.

<sup>7</sup> Diabetes: 43 missing data.

obese (respectively:  $\leq 25$  kg/m<sup>2</sup>,  $>25$  and  $< 30$  kg/m<sup>2</sup>,  $\geq 30$  kg/m<sup>2</sup>). Time from diagnosis of cirrhosis was dichotomized at the median:  $<2$  or  $\geq 2$  years. The underlying cause of cirrhosis was divided into 4 categories: viral infection, alcohol abuse, viral infection associated with alcohol abuse, and other causes (Autoimmune hepatitis, hemochromatosis or Wilson's disease, rare viral infections of the liver, parasitic infection, toxic chemicals, and some medications). Smoking status was categorized as: never, current or former (former smoker was considered as having quit smoking for a period  $>6$  months before inclusion). Smoking duration among smokers (former and current) was

defined as  $<10$ , 10–30, or  $\geq 30$  years. Finally, alcohol status was divided in 3 categories: never, non-abusive, or abusive. Abusive or heavy drinker was defined as more than 2 glasses per day for women and 3 glasses per day for men, and the duration of excessive alcohol consumption was divided at the median:  $<20$  or  $\geq 20$  years.

Continuous variables were expressed as medians with interquartile ranges, and quantitative variables as percentages. Univariate comparisons between cases and controls were performed with a chi-square test (Fisher's exact test if conditions were not met) for qualitative variables and the

**Table 2 – Baseline socio-demographic, clinical and medical characteristics of HCC patients and controls**

Variables	Controls n = 401	Cases n = 181	P
Age, years	59.0 [51.8–64.4]	64.4 [58.1–70.1]	<.001
Gender			<.001
Male	267 (66.6)	156 (86.2)	
Education level			.10
≤12 years of schooling	337 (84.3)	162 (89.5)	
Up to 12 years or high school diploma	63 (15.7)	19 (10.5)	
Occupational physical activity <sup>1</sup>			.005
Inactive	285 (71.4)	143 (80.3)	
Moderately active	76 (19.1)	15 (8.4)	
Active	38 (9.5)	20 (11.3)	
Leisure-time physical activity			.47
Inactive	192 (47.9)	80 (44.2)	
Moderately active	94 (23.4)	40 (22.1)	
Active	115 (28.7)	61 (33.7)	
Body mass index			.19
Underweight or normal (<25 kg/m <sup>2</sup> )	138 (34.4)	59 (32.6)	
Overweight (>25 and <30 kg/m <sup>2</sup> )	161 (40.2)	63 (34.8)	
Obese (≥30 kg/m <sup>2</sup> )	102 (25.4)	59 (32.6)	
Energy consumption (non-alcohol sources), Kcal	2629.4 [1963.4–3572.2]	2705.0 [1875.8–3507.2]	.40
≤2192.3	132 (32.9)	55 (30.4)	
2192.32–3182.3	134 (33.4)	71 (39.2)	
≥3182.3	135 (33.7)	55 (30.4)	
Smoking status			.057
Never	112 (27.9)	36 (19.9)	
Current	129 (32.2)	56 (30.9)	
Former	160 (39.9)	89 (49.2)	
Smoking duration, years <sup>*</sup>			.48
<10	23 (8.0)	15 (10.3)	
10–30	121 (41.9)	53 (36.6)	
≥30	145 (50.1)	77 (53.1)	
Alcohol status			.11
Never	28 (7.0)	6 (3.3)	
Non abusive	127 (31.7)	51 (28.2)	
Abusive	246 (61.3)	124 (68.5)	
Excessive alcohol consumption, years			.11
<20	119 (48.4)	49 (39.5)	
≥20	127 (51.6)	75 (60.5)	
Cirrhosis diagnosis, years	2.0 [1–6]	1.0 [0–5]	<.001
<2 years	160 (39.9)	108 (59.7)	
≥2 years	241 (60.1)	73 (40.3)	
Cirrhosis etiology			.26
Viral infection	79 (19.7)	32 (17.7)	
Alcohol abuse	215 (53.6)	107 (59.1)	
Viral infection associated with alcohol abuse	43 (10.7)	23 (12.7)	
Other	64 (16.0)	19 (10.5)	
Diabetic status <sup>2</sup>	128 (33.5)	88 (50.6)	<.001
Child-Pugh score <sup>3</sup>			.006
A	210 (54.1)	122 (68.2)	
B	126 (32.5)	42 (23.5)	
C	52 (13.4)	15 (8.4)	

Univariate comparisons were done with chi-square test for qualitative variables or Student's test for continuous variables. Values are presented as n (%) or median [IQR].

<sup>\*</sup> among smokers.

<sup>1</sup> Occupational physical activity: 5 missing data.

<sup>2</sup> Diabetic status: 9 missing data.

<sup>3</sup> Child-Pugh score: 15 missing data.

Student's test (Wilcoxon test, in case of non-normal variable) for continuous variables. The residual method [22] was applied and calculated from the regression of the food group or nutrient intake on total energy from non-alcohol intake. This method takes into account the global energy intake and

thus better evaluates the specific effect of every food group or nutrient. Then, unconditional logistic regression models were done for each residual food group in tertiles (on the basis of the distribution in controls) to estimate associations with the risk of HCC among cirrhotic patients. Odds ratios (ORs) and

**Table 3 – Associations between case–control status (presence of HCC at inclusion) and different selected food group consumption**

Food groups	Crude model		p-Trend	Residual model		p-Trend
	OR	[95% CI]		OR	[95% CI]	
Alcoholic Beverages (ml/d) *			.011			.27
–373.22 – –254.88 vs < –373.22 <sup>a</sup>	0.98	[0.62–1.56]		0.81	[0.41–1.61]	
≥ –254.88 vs < –373.22 <sup>b</sup>	1.58	[1.03–2.43]		1.18	[0.67–2.10]	
Red Wine (ml/d) *			.05			.32
–146.34 – –135.72 vs < –146.34	0.82	[0.52–1.29]		0.76	[0.38–1.50]	
≥ –135.72 vs < –146.34	1.31	[0.86–2.00]		1.08	[0.58–1.99]	
Carbonated beverages (ml/d)			.68			.021
–66.72 – –15.17 vs < –66.72	1.01	[0.65–1.56]		1.33	[0.68–2.63]	
≥ –15.17 vs < –66.72	1.09	[0.71–1.68]		2.44	[1.17–5.09]	
Coffee, Tea and Chicory (ml/d)			.47			.47
–237.96 – 27.52 vs < –237.96	1.08	[0.71–1.65]		0.86	[0.51–1.45]	
≥ 27.52 vs < –237.96	0.87	[0.56–1.35]		0.81	[0.48–1.39]	
Total cereals (g/d)			<.001			.035
–63.62 – 14.04 vs < –63.62	1.30	[0.81–2.08]		1.69	[0.95–3.02]	
≥ 14.04 vs < –63.62	2.05	[1.32–3.19]		1.87	[1.09–3.22]	
Cereals and Bread (g/d)			.06			.28
–59.02 – 24.17 vs < –59.02	1.27	[0.81–1.99]		1.34	[0.77–2.33]	
≥ 24.17 vs < –59.02	1.53	[0.99–2.37]		1.37	[0.80–2.35]	
Whole grains C&B (g/d)			.56			.73
–16.11 – –13.22 vs < –16.11	1.18	[0.77–1.83]		0.87	[0.41–1.86]	
≥ –13.22 vs < –16.11	1.15	[0.74–1.77]		1.05	[0.52–2.13]	
Added sugar (g/d)			.48			.57
–13.97 – 3.85 vs < –13.97	0.94	[0.61–1.44]		1.41	[0.79–2.52]	
≥ 3.85 vs < –13.97	0.86	[0.56–1.32]		1.27	[0.72–2.24]	
Desserts and pastries (g/d)			.001			.30
–67.64 – 28.89 vs < –67.64	0.74	[0.49–1.12]		0.92	[0.54–1.57]	
≥ 28.89 vs < –67.64	0.47	[0.30–0.74]		0.75	[0.43–1.31]	
Total dairy products (g/d)			.20			.35
–53.42 – –203.64 vs < –203.64	0.87	[0.56–1.37]		1.65	[0.96–2.83]	
≥ 165.41 vs < –203.64	1.32	[0.87–2.01]		1.42	[0.83–2.45]	
All meat (g/d)			.89			.67
–40.47 – 20.19 vs < –40.47	0.92	[0.60–1.42]		1.17	[0.69–1.98]	
≥ 20.19 vs < –40.47	0.96	[0.63–1.47]		0.92	[0.53–1.57]	
Processed meat (g/d)			.038			.028
–24.47 – 4.18 vs < –24.47	1.44	[0.92–2.27]		1.72	[0.97–3.04]	
≥ 4.18 vs < –24.47	1.65	[1.06–2.57]		1.97	[1.14–3.41]	
Red meat products (g/d)			.36			.42
–26.38 – 9.30 vs < –26.38	0.75	[0.49–1.16]		0.90	[0.53–1.52]	
≥ 9.30 vs < –26.38	0.80	[0.52–1.23]		0.81	[0.48–1.38]	
Giblets (g/d)			.002			.24
–9.46 – –3.15 vs < –9.46	1.23	[0.77–1.96]		1.21	[0.68–2.18]	
≥ –3.15 vs < –9.46	1.92	[1.24–2.99]		1.40	[0.81–2.41]	
White meat products (g/d)			.86			.93
–14.22 – 1.70 vs < –14.22	0.80	[0.52–1.23]		0.73	[0.43–1.22]	
≥ 1.70 vs < –14.22	0.93	[0.61–1.42]		0.94	[0.55–1.61]	
Fish and Shellfish (g/d)			.84			.69
–16.36 – 3.05 vs < –16.36	1.01	[0.65–1.55]		0.82	[0.48–1.41]	
≥ 3.05 vs < –16.36	1.04	[0.68–1.60]		1.06	[0.62–1.80]	
Eggs (g/d)			.52			.61
–19.82 – –1.48 vs < –19.82	0.92	[0.59–1.42]		0.85	[0.50–1.44]	
≥ –1.48 vs < –19.82	1.11	[0.73–1.70]		1.09	[0.65–1.83]	
Fruit (g/d)			.34			.08
–187.18 – 44.97 vs < –187.18	1.32	[0.96–2.00]		1.25	[0.73–2.13]	
≥ 44.97 vs < –187.18	0.85	[0.54–1.33]		0.65	[0.37–1.14]	
Juices (g/d)			.05			.28
–125.67 – –3.63 vs < –125.67	0.69	[0.45–1.05]		0.73	[0.39–1.37]	
≥ –3.63 vs < –125.67	0.63	[0.41–0.97]		0.69	[0.39–1.22]	
Fruit compote or syrup (g/d)			.72			.97
–22.02 – –6.93 vs < –22.02	1.15	[0.75–1.76]		1.45	[0.80–2.65]	

(continued on next page)



Table 3 (continued)

Food groups	Crude model		p-Trend	Residual model		p-Trend
	OR	[95% CI]		OR	[95% CI]	
≥ -6.93 vs < -22.02	0.98	[0.63–1.53]		1.15	[0.66–1.98]	
Vegetables and juices (g/d)			.33			.90
-118.53 – 52.58 vs < -118.53	1.20	[0.78–1.86]		1.28	[0.74–2.21]	
≥52.58 vs < -118.53	1.26	[0.81–1.95]		1.02	[0.59–1.77]	
Legumes (g/d)			.63			.99
-25.14 – -3.46 vs < -25.14	1.08	[0.71–1.66]		0.84	[0.49–1.45]	
≥ -3.46 vs < -25.14	0.92	[0.59–1.42]		0.95	[0.56–1.62]	
Nuts and Seeds (g/d)			.90			.88
-25.14 – -3.46 vs < -25.14	1.07	[0.69–1.64]		1.05	[0.59–1.89]	
≥ -3.46 vs < -25.14	0.98	[0.64–1.52]		0.97	[0.57–1.68]	
Rice and Pasta (g/d)			.72			.73
-58.35 – 23.88 vs < -58.35	0.70	[0.45–1.08]		0.72	[0.42–1.24]	
≥23.88 vs < -58.35	0.89	[0.59–1.35]		1.04	[0.63–1.72]	
Potatoes (g/d)			.37			.66
-33.67 – 8.79 vs < -33.67	0.99	[0.64–1.54]		0.95	[0.55–1.64]	
≥8.79 vs < -33.67	1.20	[0.78–1.83]		1.11	[0.66–1.86]	
Fries and Chips (g/d)			.79			.19
-12.56 – 1.27 vs < -12.56	1.11	[0.72–1.71]		1.53	[0.86–2.72]	
≥1.27 vs < -12.56	1.08	[0.70–1.67]		1.57	[0.91–2.73]	
Added Animal Fat (g/d)			.55			.53
-22.66 – 10.43 vs < -22.66	0.90	[0.59–1.38]		1.36	[0.80–2.32]	
≥10.43 vs < -22.66	0.87	[0.57–1.34]		0.88	[0.52–1.51]	
Added Vegetable Fat (g/d)			.12			.44
-495.65 – -62.86 vs < -495.65	0.71	[0.46–1.08]		0.66	[0.36–1.22]	
≥ -62.86 vs < -495.65	0.71	[0.47–1.09]		0.75	[0.40–1.39]	

The residual method was used to take the total energy intake into account in the calculation of consumption. Tertile intervals were defined in controls. ORs, 95% CI and p for linear trends across tertiles for selected food groups were calculated using logistic regression. Unconditional logistic regression models were adjusted for age (continuous), gender, center, total energy from nonalcoholic sources, cirrhosis diagnosis, Child-Pugh score, diabetes, etiology of cirrhosis, alcohol consumption and occupational physical activity.  
\* Alcoholic beverages and wine were adjusted for age (continuous), gender, center, total energy from nonalcoholic sources, cirrhosis diagnosis, Child-Pugh score, diabetes, etiology of cirrhosis and occupational physical activity.

95% confidence intervals (CI) were calculated by using the lowest tertile as the reference category and expressed as (OR<sub>T2vsT1</sub> and OR<sub>T3vsT1</sub>). P values for linear trends were estimated using the median values within each tertile of a dietary pattern, thereby taking the unequal distances between tertiles into account. To account for potential confounders, we constructed models with a backward stepwise logistic regression that included all variables with a significance level below 0.20 in univariate analyses, as well as variables reported to be associated with HCC risk in the literature. Energy consumption from non-alcoholic sources, alcohol consumption and occupational physical activity were forced into the final model.

Interactions between the different food groups and gender or cirrhosis etiology were evaluated in separate analyses by including interaction terms formed by the product of variable categories and food intake. P values and p-trends were considered statistically significant based on 2-sided probability of .05. Statistical analyses were performed with SAS 9.4 (SAS Institute Inc, Cary, NC, USA).

### 3. Results

The 582 patients included in our study were compared to the 596 non-included cirrhotic patients who did not answer the diet history questionnaire (Table 1): they were more often

cases, mainly with Child-Pugh score of A and cirrhosis etiology was more often linked to viral infection. They were also significantly more educated, more active and more often alcohol drinkers than non-included patients.

Table 2 compares the main socio-demographic, clinical and medical characteristics of the 401 controls (cirrhotic patients without HCC) and 181 cases (cirrhotic patients with HCC) of the study sample. HCC patients were more often male and older than controls, with a median age of 64.4 years and 59.0 years, respectively ( $P < .001$ ). For smoking status, the differences between the two groups were borderline ( $P = .057$ ). In the HCC group, 40% were former smokers, 32% were current smokers and 28% had never smoked compared with 49%, 31% and 20%, respectively, in the control group. Smoking duration was generally higher than 30 years for both groups; the difference in duration between groups was not significant ( $P = .48$ ). Concerning alcohol consumption, 61.3% of controls and 68.5% of HCC groups were heavy drinkers who had consumed alcohol excessively for  $\geq 20$  years; the difference was not statistically significant ( $P = .11$ ). Occupational physical activity was associated with HCC status (cases were more often inactive than controls;  $P = .005$ ) but neither educational level ( $P = .10$ ) nor leisure-time physical activity ( $P = .47$ ) were. Diabetes was more frequent in HCC patients (50.6%) than in controls (33.5%;  $P < .001$ ). The time between diagnosis of cirrhosis and inclusion in CirCE study was significantly lower in HCC patients than in controls ( $P < .001$ ). The majority

**Table 4 – Effect of fiber, macronutrients and selected micronutrients among controls and cases on HCC risk**

Food groups	Crude model		p-Trend	Residual model <sup>a</sup>		p-Trend
	OR	[95% CI]		OR	[95% CI]	
Fiber (g/d)			.26			.012
–4.03 – 2.95 vs < –4.03a	1.08	[0.71–1.64]		0.78	[0.46–1.32]	
≥2.95 vs < –4.03b	0.78	[0.50–1.22]		0.49	[0.28–0.86]	
Macronutrients						
Carbohydrates (g/d)			.94			.28
–24.67 – 22.95 vs < –24.67	0.87	[0.56–1.35]		1.03	[0.61–1.77]	
22.95 vs < –24.67	0.98	[0.64–1.50]		1.31	[0.78–2.22]	
Lipids (g/d)			.64			.33
–10.98 – 10.86 vs < –10.98	1.21	[0.79–1.84]		1.17	[0.70–1.96]	
≥10.86 vs < –10.98	0.90	[0.58–1.40]		0.77	[0.45–1.33]	
Proteins (g/d)			.25			.99
–9.43 – 5.37 vs < –9.43	0.81	[0.51–1.27]		0.83	[0.48–1.43]	
≥5.37 vs < –9.43	1.24	[0.82–1.89]		0.98	[0.58–1.65]	
Micronutrients						
Fat-soluble vitamins						
Retinol (mg/d)			.038			.42
–861.22 – 225.22 vs < –861.22	1.22	[0.77–1.91]		0.99	[0.56–1.73]	
≥ – 252.22 vs < –861.22	1.58	[1.02–2.44]		1.20	[0.71–2.03]	
Vitamin D (mg/d)			.77			.88
–0.81 – 0.35 vs < –0.81	1.29	[0.84–1.99]		1.08	[0.63–1.86]	
≥0.35 vs < –0.81	1.10	[0.71–1.72]		1.06	[0.62–1.81]	
Vitamin E (mg/d)			.046			.017
–4.14 – 1.73 vs < –4.14	0.75	[0.49–1.14]		0.89	[0.52–1.54]	
≥1.73 vs < –4.14	0.64	[0.41–0.98]		0.52	[0.30–0.89]	
Water soluble vitamins						
Vitamin B1 (mg/d)			.09			.60
–0.13 – 0.14 vs < –0.13	0.83	[0.55–1.27]		1.05	[0.62–1.75]	
>0.14 vs < –0.13	0.69	[0.45–1.06]		0.86	[0.50–1.47]	
Vitamin B2 (mg/d)			.74			.26
–0.38 – 0.12 vs < –0.38	0.87	[0.57–1.34]		0.62	[0.36–1.08]	
≥0.12 vs < –0.38	0.92	[0.60–1.41]		0.71	[0.42–1.21]	
Vitamin B3 (mg/d)			.19			.70
–4.75 – 1.21 vs < –4.75	1.16	[0.74–1.81]		1.20	[0.70–2.04]	
≥1.21 vs < –4.75	1.34	[0.60–1.41]		0.95	[0.56–1.62]	
Vitamin B5 (mg/d)			.74			.85
–0.83 – 0.29 vs < –0.83	0.98	[0.64–1.52]		0.94	[0.55–1.61]	
≥0.29 vs < –0.83	1.07	[0.70–1.64]		0.94	[0.56–1.59]	
Vitamin B6 (mg/d)			.77			.21
–0.22 – 0.15 vs < –0.22	0.92	[0.62–1.45]		0.74	[0.43–1.26]	
≥0.15 vs < –0.22	0.94	[0.61–1.44]		0.70	[0.41–1.19]	
Vitamin B9 (μg/d)			.39			.036
–62.19 – 38.04 vs < –62.19	0.92	[0.60–1.41]		0.79	[0.46–1.36]	
≥38.04 vs < –62.19	0.83	[0.54–1.27]		0.56	[0.33–0.95]	
Vitamin B12 (μg/d)			.039			.57
–3.96 – 0.46 vs < –3.96	1.52	[0.97–2.40]		1.17	[0.67–2.03]	
≥ –0.46 vs < –3.96	1.66	[1.06–2.60]		1.18	[0.69–2.04]	
Vitamin C (mg/d)			.10			.25
–52.55 – 19.95 vs < –52.55	0.81	[0.53–1.23]		0.84	[0.49–1.44]	
≥19.95 vs < –52.55	0.70	[0.45–1.07]		0.71	[0.41–1.22]	
Minerals						
Calcium (mg/d)			.14			.07
–161.31 – 105.22 vs < –161.31	0.69	[0.45–1.06]		0.66	[0.39–1.12]	
≥105.22 vs < –161.31	0.73	[0.48–1.11]		0.61	[0.36–1.05]	
Iron (mg/d)			.26			.06
–2.83 – 1.51 vs < –2.83	0.63	[0.41–0.98]		0.47	[0.27–0.82]	
≥1.51 vs < –2.83	0.77	[0.50–1.17]		0.56	[0.33–0.96]	
Magnesium (mg/d)			.86			.07
–48.28 – 17.25 vs < –48.28	1.20	[0.78–1.83]		0.94	[0.55–1.59]	
≥17.25 vs < –48.28	0.99	[0.64–1.54]		0.62	[0.36–1.07]	
Manganese (mg/d)			<.001			.038
–1.18 – 0.77 vs < –1.18	0.62	[0.41–0.94]		0.79	[0.47–1.33]	

(continued on next page)

Table 4 (continued)

Food groups	Crude model		p-Trend	Residual model <sup>a</sup>		p-Trend
	OR	[95% CI]		OR	[95% CI]	
≥0.77 vs < -1.18	0.44	[0.28–0.69]		0.56	[0.32–0.97]	
Sodium (mg/d)						
–402.26 – 187.08 vs < –402.26	1.94	[1.21–3.11]	.002	2.11	[1.18–3.78]	.043
≥187.08 vs < –402.26	2.15	[1.35–3.43]		2.00	[1.14–3.53]	
Phosphorus (mg/d)						
–147.80 – 68.32 vs < –147.80	0.98	[0.63–1.52]	.57	1.04	[0.60–1.79]	.73
≥68.32 vs < –147.80	1.12	[0.73–1.72]		0.93	[0.55–1.56]	
Potassium (mg/d)						
–526.33 – 397.83 vs < –526.33	0.93	[0.62–1.42]	.08	0.61	[0.36–1.04]	.004
≥397.83 vs < –526.33	0.68	[0.43–1.05]		0.44	[0.25–0.76]	
Iodine (mg/d)						
–17.27 – 13.95 vs < –17.27	0.56	[0.36–0.86]	.041	0.48	[0.28–0.84]	.14
≥13.95 vs < –17.27	0.65	[0.43–0.99]		0.67	[0.40–1.13]	

The residual method was used to take the total energy intake into account in the calculation of consumption. Tertile intervals were defined in controls. ORs, 95% CI and p were calculated using logistic regression for linear trends across tertiles for selected nutrients. Unconditional logistic regression models were adjusted for age (continuous), gender, center, total energy from nonalcoholic sources, cirrhosis diagnosis, Child-Pugh score, diabetes, etiology of cirrhosis, alcohol consumption and occupational physical activity.

of cases (59.7%) had been diagnosed with cirrhosis less than 2 years previously, while controls had been diagnosed more than 2 years previously (60.1%). The majority of cases (68.2%) had a Child-Pugh score A compared to only 54.1% of controls ( $P = .006$ ).

The dietary intake of cases and controls is compared in Table 3 with ORs and 95% CI and p for linear trends across tertiles. Several food groups were significantly associated with HCC risk: carbonated beverages ( $OR_{Tertile3vsTertile1} = 2.44$ ; 95% CI = 1.17–5.09; p for trend = 0.021), the total cereals group ( $OR_{T3vsT1} = 1.87$ ; 95% CI = 1.09–3.22; p for t = 0.035) and the processed meat group ( $OR_{T3vsT1} = 1.97$ ; 95% CI = 1.14–3.41; p for t = 0.028). Cases tended to consume less fruit than controls although the association was only close to significance ( $OR_{T3vsT1} = 0.65$ ; 95% CI = 0.37–1.14; p for t = 0.08). The total dairy products group was not significantly associated with HCC risk even if dairy products were separated into high-fat ( $OR_{T3vsT1} = 1.41$ ; 95% CI = 0.82–2.43; p for t = 0.36) and low-fat categories ( $OR_{T3vsT1} = 1.01$ ; 95% CI = 0.58–1.76; p for t = 0.82).

Table 4 presents the associations, ORs and 95% CI for macronutrients, selected micronutrients and fiber among controls and cases. HCC cases had a lower intake of fiber than controls ( $OR_{T3vsT1} = 0.49$ ; 95% CI = 0.28–0.86; p for t = 0.012). Similarly, vitamin E was inversely associated with HCC risk ( $OR_{T3vsT1} = 0.52$ ; 95% CI = 0.30–0.89; p for t = 0.017) as well as vitamin B9 ( $OR_{T3vsT1} = 0.56$ ; 95% CI = 0.33–0.96; p for t = 0.036), manganese ( $OR_{T3vsT1} = 0.56$ ; 95% CI = 0.32–0.97; p for t = 0.038) and potassium ( $OR_{T3vsT1} = 0.44$ ; 95% CI = 0.25–0.76; p for t = 0.004). Conversely, sodium intake was higher in HCC patients than in controls ( $OR_{T3vsT1} = 2.00$ ; 95% CI = 1.14–3.53; p for t = 0.043).

No statistically significant interactions were observed between food groups and gender or cirrhosis etiology.

#### 4. Discussion

This case-control study investigates the association between dietary intake and the risk of HCC in cirrhotic patients. Only few studies about the nutritional factors of HCC risk in

cirrhosis have been published, and these in a European population. However, the potential of diet in the prevention of this type cancer is of special interest. In the present study, several food components were consumed less by HCC cases than by controls. These findings confirm our hypothesis that some dietary factors may have a preventive effect while others are risk factors for HCC in a high-risk population suffering from cirrhosis.

Among the main results of our study, HCC patients consumed significantly more carbonated beverages than controls. Sugar-sweetened beverages are usually high in fructose and sugar which can negatively influence lipid and glucose metabolisms, and liver function. High sugar intake causes a rapid increase in insulin levels and accelerates lipogenesis in the liver which may contribute to hepatic lipid accumulation. The consumption of high-fructose beverages has been associated with metabolic abnormalities and the development of NAFLD [23]. The EPIC cohort study found a significant relationship between high sugar intake and HCC risk with a 20% increase in risk for every increment of a soft drink portion [11]. Several studies (mainly animal studies) have showed that dietary fructose can cause NAFLD or increase the risk of liver cancer [23–25].

Results from the meat category showed a significant association between the intake of processed meats and the risk of HCC. There was a weak positive association for consumption of giblets in the crude analyses, but it did not persist after multivariate adjustments. Consumption of red meat and processed meat could be considered a risk factor for HCC because of the heterocyclic amines produced when meat is prepared at high temperatures and the high levels of saturated fat [26]. A meta-analysis that included 7 cohort and 10 case-control studies did not show significant association between the intake of red meat, processed meat or total meat and HCC risk. On the contrary, a higher intake of white meat or fish significantly reduced the risk of HCC [27]. Results of the NIH-AARP study showed that red meat was associated with increased HCC incidence while white meat (chicken, turkey and fish) was significantly inversely associated [28].

None of the few published studies on sodium intake found a relation between dietary sodium and HCC risk. A high-salt diet is associated with hypertension, and could also have an indirect effect on metabolic syndrome, obesity, cardiovascular diseases, NAFLD and hepatic fibrosis [29–33]. All these conditions are related to HCC and may explain the significant association between high salt intake and the risk of HCC in our study.

Unexpectedly, we found a significant positive association between cereal intake and the risk of HCC. Previous studies mostly found no significant relationship between cereal groups and IICC risk, but all the results indicated a trend of increased risk [16,18,26,34]. Only one Italian study from 1988 found a significant inverse association [35]. Another Italian study evaluated the association of adherence to the Mediterranean diet with HCC risk and showed an inverse association with legumes (OR = 0.66; 95% CI = 0.47–0.92) and cereals (OR = 0.65; 95% CI = 0.46–0.91) when they focused on individual score components [36]. In our study, total dietary fiber consumption was inversely associated with HCC risk. Eating a high fiber diet has a number of beneficial effects such as lowering calorie intake and appetite. Furthermore, dietary fiber regulates post-prandial glucose levels and the blood lipid profile by increasing the degradation of intestinal bile acid in the colon. It stimulates the use of liver cholesterol to synthesize new bile acid which might be involved in hepatocarcinogenesis [15,26]. The EPIC study also showed that a higher consumption of dietary fiber significantly decreased HCC risk (HR = 0.70, 95% CI = 0.52–0.93, per 10 g/d).

In our study, the association between HCC and “coffee, tea and chicory” intake was not statistically significant. It may be due to the limited number of participants and perhaps a lower consumption than other populations. The majority of studies that have explored the relation between coffee and HCC risk were larger (>1400 participants). A recent meta-analysis with 18 cohorts and 8 case-control studies found that the risk of HCC was 35% lower with an increase of 2 cups of coffee per day [13]. Coffee is rich in several components with antioxidant, anti-mutagenic and anti-inflammatory effects. Tea, specifically green tea, is rich in polyphenols and catechins which have antioxidant and anti-inflammatory properties as well as an important role in lipid and glucose absorption and metabolism [26].

In the present study, alcohol beverages and red wine consumption were not statistically associated with the risk of HCC in the multivariate models. However, HCC patients reported longer periods of excessive alcohol than controls (mean = 23 years vs 19 years,  $P = .03$ ). Some previous studies also failed to show a significant positive association between alcohol consumption and HCC. In fact, the risk depends on the quantity and duration of alcohol consumption which acts synergistically or in addition to factors such as gender, age, presence of viral hepatitis, or metabolic syndrome [37]. In both genders, the alcohol-attributable fraction is between 18% and 33% in liver cancer. Regular heavy drinking presented the strongest association with cancer risk. The fourth edition of the European code against cancer presented evidence from several cohort studies and meta-analysis of a linear dose-response relationship between average alcohol consumption and liver cancer. The risk of HCC is significantly increased by

about 30% to 40% with prolonged heavy alcohol consumption (60–80 g/d) [38]. Moderate alcohol consumption was also a significant risk factor for liver disease [39].

Cigarette smoking is another recognized risk factor for developing HCC as well as other cancers [1]. Cigarettes contain carcinogenic components, such as N-nitrosodimethylamine, 4-aminobiphenyl, arsenic, and vinyl chloride which may contribute to liver carcinogens. Smokers were 1.5 times more likely to develop HCC than control subjects, a risk close to that of obesity, with a dose-response effect for cigarettes per day [40]. Moreover, a cohort study found that smokers, independent of alcohol consumption, were more likely to develop cirrhosis [41].

We also observed a significant inverse association between HCC risk and manganese, potassium, vitamin B9 (folate and folic acid), and vitamin E. Limited results were available in the literature even for non-cirrhotic patients. Manganese (Mn) is an essential antioxidant nutrient which is cell protective and a constituent of metalloenzymes or enzyme activators [44]. Three experimental studies have showed that Mn can play an antitumoral role as a cofactor of antioxidant enzymes such as manganese superoxide dismutase. It can block signaling for reactive oxygen species (which play an important role in tumor metastasis) and therefore inhibit tumorigenesis [45].

The role of diet in the prevention or the progression of cancer is now well recognized, but whether one selected food or nutrient can have an effect on a specific type of cancer remains a subject of controversy. The World Cancer and Research Fund/American Institute of Cancer Research (WCRF/AICR) have been compiling a growing database of evidence relating to cancer prevention and diet since 1997 [42]. The available evidence concerning the association between diet and liver cancer is still sparse, and the WCRF/AICR did not include the limited studies in cirrhotic patients. Among the significant results we observed in the present study, it is interesting to note that some dietary factors have been previously identified as risk or protective factors for liver cancer [43] but also for other types of cancer, particularly colorectal cancer. Fruit and fiber, for example, are foods which are low-energy but rich in bioactive compounds. Calcium is able to bind to unconjugated bile acids and free fatty acids, or to reduce cancer cell proliferation and promote cell differentiation through different cell-signaling pathways. Among factors which increase the risk of both cancers, red and processed meats are high in animal fats and heme iron (generating free radicals, N-nitroso compounds and oxidative damage), and may have carcinogenic potential. The heterocyclic amines and polycyclic aromatic hydrocarbons formed during prolonged exposure to heat, grilling or charbroiling have also been suggested as an explanation, and the nitrites and nitrates in processed meat are probable human carcinogens. The mechanisms of all these nutrients are still not clearly understood and more studies are needed to confirm preliminary findings.

To our knowledge, this is the first study to find an inverse association between potassium and HCC risk. A low potassium level may increase an individual's likelihood of developing insulin resistance and diabetes, and tumor growth and HCC risk have been linked to hyperinsulinemia and insulin resistance [46]. Folate (vitamin B9) can alter the relationship

between alcohol and some types of cancer. It plays an important role in DNA methylation and replication during cell regeneration. It also has an antioxidant effect which could limit liver damage and the risk of liver cancer [47]. Two studies have revealed the significantly association between serum folate levels and liver damage, tumor progression and development of HCC in a high risk population [48,49]. Vitamin E is an essential antioxidant, and supplementation could improve liver enzymes and decrease markers of steatosis and inflammation in liver biopsy of patients suffering from NASH [50]. Vitamin E can prevent lipid peroxidation as well as oxidative DNA damage in the cell lines of IICC [51]. Vitamin E supplementation has shown beneficial effects on hepatocarcinogenesis in mice. Unfortunately, the few human studies have reported discouraging results [26].

Our study has some limitations. First, the number of participants who responded to the diet history questionnaire reflects the challenge of such an extensive nutritional questionnaire. It may have limited the power and the representativeness of our study results, as well as the possibility of analyses stratification. Secondly, the diet history questionnaire was self-reported for the previous year and was not repeated during follow-up to detect any change in current diet. On the contrary, the strengths of our study are the use of a standardized protocol in several university hospitals and the coverage of six regions in the North-East of France. We also took into account several potential confounders, including socio-demographic, medical and biological data.

To our knowledge, few studies have analyzed the factors leading from cirrhosis to HCC. Cirrhotic patients could potentially be encouraged to adopt a particular diet, thus benefitting from preventive intervention. The perspective of our study is to contribute to the development of nutritional recommendations that would optimize the well-being of cirrhotic patients and minimize their risk of developing HCC.

In conclusion, we found significant differences between cirrhotic patients with or without HCC in terms of dietary intake of carbonated beverages, cereals, processed meat, fiber, manganese, potassium, B9, vitamin E and sodium. These results must be confirmed in larger prospective studies. Such findings could help doctors to achieve two important goals: to find a suitable and protective diet to delay the onset of HCC in cirrhotic patients, and to improve the nutritional status and quality of life of patients with HCC.

### Acknowledgment

We thank the members of hospital medical teams and the Clinical Investigation Center team for their contribution to recruitment, data collection and biological analyses. We also thank the CIRCE Study Group, which includes 1) CIRCE Coordination France: Bronowicki J.P., Di Martino V., Doffoel M., Hillon P. (CIRCE coordinator), Thieffin G., CIRCE Coordination China: Wen H., He F.P., Lu X.M., Hillon P., Vuitton D., 2) CIRCE Scientific Board: Faivre J. (President), Cercueil J.P., Cottet V., Delmas D., Duvillard L., Guennegues M., Guéant J.L., Habersetzer F., Latruffe N., Manfait M., Oudet P., Sockalingum G., 3) CIRCE pharmacologists: Sgro C., Trechot P., Valnet-Rabier M.B., Trenque T., Tebacher-Alt M.

We thank Di Martino V., Raabe J.J. and Habersetzer F. for their participation and Busson A. for statistical assistance. We thank the staff of the Centre Ferdinand Cabanne (University Hospital of Dijon, France) for their assistance in handling and storing biological samples and the E3N team (INSERM UMR1018) for their help with the diet questionnaire. This work was supported by a French Government grant managed by the French National Research Agency under the program "Investissements d'Avenir" [reference ANR-11-LABX-0021], and also by grants from the "Conseil Régional de Bourgogne", the "Fonds Européen de Développement Régional" (FEDER), the French National Cancer Institute, the "Ligue Nationale contre le Cancer", the "Canceropole Grand-Est" and the "Fondation de France". The authors declare no conflict of interest.

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## ***Deuxième objectif de thèse***

Analyse des profils de consommation alimentaire définis *a priori* et *a posteriori* ce qui permet une approche plus intégrée de l'alimentation.

Etudier les liens entre le risque de maladie et chaque aliment pris isolément permet d'identifier des facteurs alimentaires à surveiller mais ne tient pas compte des habitudes alimentaires dans leur globalité. Dans cette partie, plusieurs scores et méthodes ont été appliqués pour analyser les profils de consommation de notre population. Ces différents scores ont été développés pour mesurer le degré de mise en pratique des recommandations nutritionnelles. Le but de cette partie était de montrer l'impact du suivi des recommandations alimentaires sur le risque de développer un CHC dans notre population de patients cirrhotiques.

Dans un premier temps, trois scores ont été testés et analysés : deux versions du Healthy Eating Index (HEI), un score qui analyse la qualité des régimes alimentaires suivis selon les recommandations américaines. L'Alternate Healthy Eating Index (AHEI), une alternative du HEI qui est basée sur les aliments et les nutriments qui influencent le risque des maladies étudiées.

Dans un deuxième temps, utilisation de la méthode des analyses en composante principale pour analyser les profils de consommations alimentaires définis *a posteriori*.



## **Introduction**

Le HEI est une mesure de la qualité du régime alimentaire suivi, indépendamment de la quantité des aliments consommés, qui peut être utilisé pour évaluer la conformité aux recommandations nutritionnelles américaines et surveiller éventuellement les modifications des habitudes alimentaires. Le HEI est un score alimentaire qui a été créé, en premier lieu, en 1995 comme un outil pour évaluer le taux de suivi des recommandations alimentaires aux États-Unis (Dietary Guidelines for Americans: DGA). En 2005, la structure du HEI a été révisée et mise à jour par les chercheurs de l'institut national du cancer (National Cancer Institute: NCI) en collaboration avec le département de l'agriculture des États-Unis (United States Department of Agriculture : USDA). Il y a déjà 3 versions de ce score, renouvelées tous les 5 ans selon l'évolution de la recherche en épidémiologie nutritionnelle et des recommandations du guide alimentaire des américains. HEI-2015 est la version la plus récente en termes de conformité avec les recommandations diététiques pour les américains version 2015-2020 (53,54). Il a été démontré que le HEI peut prédire le risque de maladies chroniques chez les américains. Cependant, l'association du score du HEI avec le risque de CHC a été évaluée dans très peu d'études (55). Il nous a semblé intéressant d'appliquer le score du HEI sur une population française cirrhotique afin d'évaluer l'effet de la qualité nutritionnelle globale du régime alimentaire suivi sur le risque de développer un CHC.

L'AHEI est une alternative du HEI, conçu en 2002 pour élaborer des recommandations alimentaires spécifiques à différentes maladies chroniques. Selon plusieurs études, le score de l'AHEI a été associé à des maladies comme le diabète, les maladies cardio-vasculaires et certains types de cancer ainsi que le taux de mortalité de toutes causes (56–58).

## **Matériels et méthodes**

### **Données alimentaires**

Notre base de données initiale manquait certains composants ainsi deux autres bases de données ont été utilisées: Le FPID (Food Patterns Equivalents Ingredient Database) est une base de données qui comprend les 37 composants des profils alimentaires américains pour 100 grammes de chaque ingrédient. Le FPED (Food Patterns Equivalents Database) est une base de donnée qui convertit les aliments et les boissons en 37 composants de profils alimentaires américains (59,60). Les aliments consommés ont été transformés en différentes unité de mesure pour être équivalent aux unités des profils alimentaires : en équivalent de tasse pour les fruits, légumes et produits laitiers ; en équivalent once pour les graines et les protéines ; en équivalent cuillère à café pour le sucre ; en équivalent de gramme pour les huiles et graisses et enfin le nombre de verre d'alcool pour les intégrer dans ses bases de données.

### **Calcul du score du HEI et de l'AHEI**

Le calcul du score du HEI se base sur la somme de différents composants alimentaires selon la version adoptée. En général, le score du HEI varie entre 0 et 100, l'augmentation du score signifie que le régime alimentaire suivi s'approche des recommandations nutritionnelles. Le calcul de chaque composant se base sur une densité équivalente à 1 000 calories, à l'exception des acides gras, qui représentent un rapport acides gras insaturés/saturés. Chaque composant se calcul selon le nombre de portions consommées pour un apport énergétique donné. Si la personne a consommé le nombre recommandé de portions d'un composant, elle obtiendra la note de 10 ; cependant, lorsqu'une personne ne consomme aucune portion, elle obtiendra la note de 0. Le score, compris entre 0 et 10, est calculé proportionnellement en fonction de la

quantité consommée. Pour le HEI 2015, les calories des boissons d'alcool sont comprises dans les calories totales du dénominateur (61).

Le score de l'AHEI suit le même principe de calcul que le score du HEI sauf que les chercheurs ont choisi les composants selon leur association avec les maladies chroniques et certains cancers. La sélection et les critères de calcul des variables du score de l'AHEI-2010 sont fixés à priori et décrits en détail dans l'annexe 3. Le score total de l'AHEI varie entre 0 et 110 et chaque composant a un score qui varie entre 0 et 10. On note que les scores qui s'approchent de 10 pour les composants ou de 110 pour le score total indiquent de meilleurs choix alimentaires (56).

Dans notre étude, on s'est intéressé à calculer le HEI 2010, le HEI 2015 et l'AHEI 2010 (annexe 1,2, 3) qui seront développés séparément:

### **HEI 2010**

Le score du HEI 2010 suit les recommandations américaines 2010-2015. Le HEI 2010 (annexe 1) se compose de 12 items : 9 composants à privilégier (meilleur score pour une meilleure consommation): Total des fruits (5 points); fruits complets (sauf les jus de fruits) (5 points); total des végétaux (5 points); légumes et légumineuses (5 points); graines et céréales complètes (10 points); produits laitiers (tous les produits laitiers inclus les boissons à base de soja) (10 points); total des protéines (5 points); protéines végétales et fruits de mer (5 points); acides gras : rapport des acides gras mono- et polyinsaturés sur les acides gras saturés (10 points). Les 3 autres composants à consommer avec modération (meilleur score pour une faible consommation) : graines et céréales raffinées (10 points); sodium (10 points) et calories vides (graisses solides, alcool et sucres ajoutés) (20 points) (54,61).

Les recommandations américaines de l'édition 2010 étaient principalement les suivantes :

- Augmenter l'activité physique

- Réduire le surpoids et l'obésité
- Maintenir l'équilibre des calories
- Consommer des aliments et des boissons riches en nutriments
- Remplacer les graisses saturées par des graisses mono- ou polyinsaturées.
- Limiter les céréales raffinées, les sucres ajoutés, les graisses saturées, les acides gras-trans, le cholestérol et le sodium
- Consommer de l'alcool avec modération (62).

Ces recommandations s'alignent avec les recommandations du programme français de nutrition PNNS 2011-2015 (Programme Nationale Nutrition Santé) qui a fixé des objectifs similaires: réduire l'obésité et le surpoids dans la population, augmenter l'activité physique et diminuer la sédentarité, améliorer les choix alimentaires en augmentant la consommation de fruits et légumes et diminuer la consommation de sel (63).

### **HEI 2015**

Le score du HEI 2015 est le plus récent et il suit les recommandations américaines 2015-2020. Le HEI 2015 (annexe 2) se compose de 13 items : 9 composants à privilégier (meilleur score pour une meilleure consommation) : Total des fruits (5 points); fruits complets (sauf les jus de fruits) (5 points); total des végétaux (5 points); légumes et légumineuses (5 points); graines et céréales complètes (10 points); produits laitiers (tous les produits laitiers inclus les boissons à base de soja) (10 points); total des protéines (5 points); protéines végétales et fruits de mer (5 points); acides gras : rapport des acides gras mono- et polyinsaturés sur les acides gras saturés (10 points). Les 4 autres composants à consommer avec modération (meilleur score pour une faible consommation) : graines et céréales raffinées (10 points); sodium (10 points), sucres ajoutés (10 points) et graisses saturées (10 points) (54,61).

Les recommandations américaines de l'édition 2015 étaient principalement les suivantes :

- Suivre un régime alimentaire sain tout au long de la vie
- Choisir une variété d'aliments denses en nutriments
- Limiter les calories provenant des sucres ajoutés et des graisses saturées
- Réduire l'apport en sodium
- Adopter des choix alimentaires (aliments et boissons) plus sains
- Augmenter l'activité physique (64).

Ces recommandations sont conformes au programme français de nutrition PNNS 2015-2020 : améliorer les choix alimentaires tout en minimisant les sucres ajoutés, le sodium et les graisses saturées, prévenir les maladies chroniques et encourager les personnes à pratiquer une activité physique (65).

### **AHEI 2010**

L'AHEI est constitué initialement de 9 composants : végétaux, fruits, fruits oléagineux et protéine de soja, rapport viandes blanches/viandes rouges, fibres des céréales, acides gras trans, rapport AGPI/AGS, durée d'utilisation des multivitamines et alcool. Dans cette étude, le choix des composants alimentaires est identique aux choix de l'article « Alternative Dietary Indices Both Strongly Predict Risk of Chronic Disease » (56) à l'exception du groupe d'alcool, et des acides gras à longues chaînes (les omégas) qui ont été remplacés par le groupe du café/thé/chicorée et le groupe des poissons respectivement vu l'effet protecteur du café et des poissons sur le cancer du foie. Selon le rapport du WCRF et certaines études, une consommation supérieure à 2 tasses de café par jour (1 tasse est équivalente à 250 ml) peut avoir un effet sur le risque de développer un CHC (27,42,66). De plus, selon ce rapport, une consommation équivalente à 20g/jour de poissons peut diminuer le risque de CHC (28,67–69). Ainsi l'AHEI se compose de 11 items : 7 composants à privilégier (meilleur score pour une meilleure consommation): total des fruits (10 points); total des végétaux (10 points);

graines et céréales complètes (10 points); noix et légumineuses (10 points); poissons (10 points); acides gras polyinsaturés (10 points) et café (10 points). Les 4 autres composants à consommer avec modération (meilleur score pour une faible consommation): sucre des boissons sucrées et jus de fruits (10 points); viande rouge et charcuterie (10 points); sodium (10 points) et les acides gras trans (10 points).

### **Analyses statistiques**

Les mêmes variables que dans le premier article ont été utilisées dans cette analyse sauf pour l'étiologie de la cirrhose qui a été regroupée en 3 sous groupes au lieu de 4 : étiologie alcoolique sans virus, étiologie virale (avec et sans consommation d'alcool) et autres étiologies. Les variables continues étaient exprimées en médiane alors que les variables qualitatives en pourcentage. Les comparaisons univariées entre les cas et les témoins étaient effectuées avec le test de  $\chi^2$  (ou le test exact de Fisher lorsque les conditions n'étaient pas vérifiées) pour les variables qualitatives et le test de Student (ou le test de Wilcoxon lorsque les variables n'avaient pas une distribution normale) pour les variables continues. Les modèles logistiques multivariés ont été appliqués pour le score total du HEI et de l'AHEI ainsi que pour chacun de leur composant après tertilisation (selon la distribution des témoins) dans le but d'estimer leur association avec le risque de CHC chez les patients cirrhotiques. Les Odd Ratio et 95% de l'intervalle de confiance ont été calculés en utilisant le plus bas tertile comme catégorie de référence et exprimés ainsi ( $OR_{T2vsT1}$  and  $OR_{T3vsT1}$ ). La P de tendance a été estimée en utilisant les valeurs médianes de chaque tertile permettant de tenir compte des distances inégales entre les tertiles. Pour prendre en compte les facteurs de confusion potentiels, les modèles étaient construits selon la procédure en pas à pas descendante. Au début, toutes les variables dont le degré de significativité est inférieur à 0,20

lors de l'analyse univariée étaient incluses dans le modèle initial de régression logistique multiple. Les variables associées au risque de CHC déjà mentionnées dans la littérature ainsi que les variables associées avec un  $p < 0,10$  étaient maintenues dans le modèle. Tous les modèles étaient stratifiés par le centre.

## **Résultats**

Les résultats du HEI 2010 n'ont pas montré de différences significatives dans les modèles multivariés alors que le HEI2015 a montré des différences significatives en analyse univariée, de meilleurs résultats ont été montrés en comparaison avec le HEI 2010. A noter que les moyennes de consommation des différents composants se rapprochaient entre les cas et les témoins.

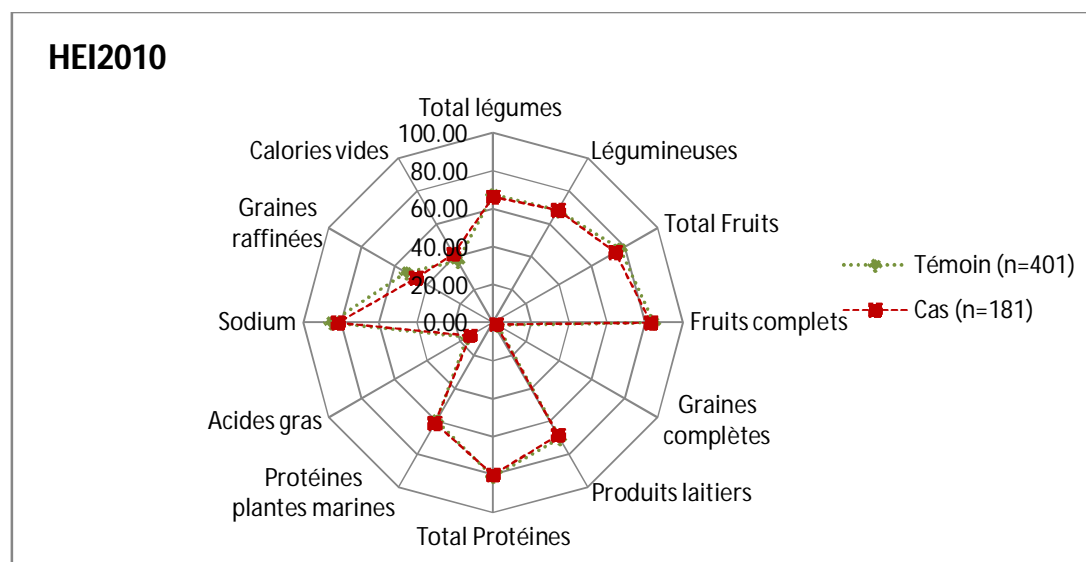
### **HEI 2010**

Les résultats des scores du HEI-2010 des 582 participants varient entre 19,4 et 86,0 avec une moyenne pour la population totale de  $53,2 \pm 11,13$ . La table 2 représente les moyennes du score du HEI-2010 ainsi que ses différents composants dans la population totale, les cas et les témoins. En comparant les cas et les témoins, la moyenne du score total du HEI-2010 était de  $53,5 \pm 11,11$  chez les témoins et de  $52,5 \pm 11,17$  chez les cas. La figure 15 représente la moyenne de consommation de chaque sous groupe du HEI chez les témoins et chez les cas, les résultats sont presque superposés. Mais on peut remarquer que les scores du sodium, de protéines, des fruits s'approchent du score maximal indiquant des consommations compatibles aux recommandations alors que les scores des acides gras et de graines complètes étaient presque nulle chez les cas et les témoins indiquant des consommations qui s'éloignent des recommandations.

**Table 2 : Moyenne ( $\pm$  SD) des scores des différents composants du score du HEI 2010 chez les cas et les témoins et la population totale.**

Score du HEI 2010	Population totale (n=582)	Témoins (n=401)	Cas (n=181)
HEI-2010 total (100pts)	53,2 $\pm$ 11,13	53,5 $\pm$ 11,11	52,5 $\pm$ 11,17
HEI-2010 total des légumes (5pts)	3,4 $\pm$ 1,44	3,4 $\pm$ 1,45	3,3 $\pm$ 1,43
HEI-2010 légumineuses (5pts)	3,4 $\pm$ 1,76	3,4 $\pm$ 1,75	3,4 $\pm$ 1,83
HEI-2010 total des fruits (5pts)	3,8 $\pm$ 1,61	3,9 $\pm$ 1,56	3,7 $\pm$ 1,70
HEI-2010 fruits complets (5pts)	4,2 $\pm$ 1,48	4,2 $\pm$ 1,43	4,2 $\pm$ 1,58
HEI-2010 graines complètes (10pts)	0,2 $\pm$ 0,93	0,2 $\pm$ 0,99	0,2 $\pm$ 0,80
HEI-2010 produits laitiers (10pts)	7,0 $\pm$ 2,68	7,0 $\pm$ 2,68	6,8 $\pm$ 2,69
HEI-2010 total des protéines (5pts)	4,1 $\pm$ 1,17	4,1 $\pm$ 1,22	4,0 $\pm$ 1,15
HEI-2010 protéines des plantes marines (5pts)	3,0 $\pm$ 1,64	3,0 $\pm$ 1,68	3,1 $\pm$ 1,54
HEI-2010 acides gras (10pts)	1,5 $\pm$ 2,35	1,6 $\pm$ 2,39	1,4 $\pm$ 2,21
HEI-2010 sodium (10pts)	8,4 $\pm$ 2,36	8,5 $\pm$ 2,18	8,2 $\pm$ 2,67
HEI-2010 graines raffinées (10pts)	5,0 $\pm$ 3,69	5,3 $\pm$ 3,64	4,7 $\pm$ 3,78
HEI-2010 calories vides (20pts)	9,2 $\pm$ 5,75	8,9 $\pm$ 5,68	9,6 $\pm$ 5,88

**Figure 15: Représentation graphique de la distribution de la moyenne des scores de chaque sous groupe du HEI 2010 chez les témoins et les cas**





**Table 3 : Comparaison entre les cas et les témoins des moyennes des scores de chaque composant du score du HEI 2010.**

Score du HEI 2010	Témoins (n=401)	Cas (n=181)	P valeur*
HEI-2010 total (100pts)	53,5 ± 11,11	52,5 ± 11,17	0,439
HEI-2010 total des légumes (5pts)	3,4 ± 1,45	3,3 ± 1,43	0,614
HEI-2010 légumineuses (5pts)	3,4 ± 1,75	3,4 ± 1,83	0,982
HEI-2010 total des fruits (5pts)	3,9 ± 1,56	3,7 ± 1,70	0,278
HEI-2010 fruits complets (5pts)	4,2 ± 1,43	4,2 ± 1,58	0,795
HEI-2010 graines complètes (10pts)	0,2 ± 0,99	0,2 ± 0,80	0,092
HEI-2010 produits laitiers (10pts)	7,0 ± 2,68	6,8 ± 2,69	0,386
HEI-2010 total des protéines (5pts)	4,1 ± 1,22	4,0 ± 1,15	0,344
HEI-2010 protéines des plantes marines (5pts)	3,0 ± 1,68	3,1 ± 1,54	0,699
HEI-2010 acides gras (10pts)	1,6 ± 2,39	1,4 ± 2,21	0,239
HEI-2010 sodium (10pts)	8,6 ± 2,18	8,2 ± 2,67	0,193
HEI-2010 graines raffinées (10pts)	5,3 ± 3,64	4,7 ± 3,78	0,073
HEI-2010 calories vides (20pts)	8,9 ± 5,68	9,6 ± 5,88	0,159

\*P valeur calculée à partir du test de wilcoxon.

La table 4 montre les caractéristiques descriptives des participants selon les différents tertiles du HEI-2010. Comparés aux patients du premier tertile (tertile 1), ceux du dernier tertile (tertile3 : participants ayant les meilleures scores) étaient des anciens fumeurs ou n'ayant jamais fumé, non buveurs d'alcool ou buveurs non abusifs, atteints de cirrhose de cause virale ou autre.

**Table 4 : Caractéristiques des participants selon les tertiles du score du HEI 2010**

Variables	HEI tertile 1	HEI tertile 2	HEI tertile 3	P valeur
	(n=193) (19,37-49,10)	(n=192) (49,10-57,17)	(n=197) (57,17-86,01)	
	n (%)	n (%)	n (%)	
<b>Age, années</b>				0,317
< 60,36	105 (54,4)	93 (48,4)	93 (47,2)	
≥ 60,36	88 (45,6)	99 (51,6)	104 (52,8)	
<b>Sexe</b>				0,339
Homme	147 (76,2)	139 (72,4)	137 (69,5)	
<b>Niveau d'éducation</b>				0,183
≤12 ans d'études	172 (89,6)	160 (83,3)	167 (84,8)	
Plus de 12 ans d'études ou diplôme des grandes écoles/universitaire	20 (10,4)	32 (16,7)	30 (15,2)	
<b>Activité physique professionnelle<sup>1</sup></b>				0,738
Aucune ou faible	138 (72,3)	142 (74,7)	148 (75,5)	
Modérée	34 (17,8)	26 (13,7)	31 (15,8)	
Importante	19 (9,9)	22 (11,6)	17 (8,7)	
<b>Activité physique de loisir</b>				0,641
Aucune ou faible	98 (50,8)	86 (44,8)	88 (44,7)	
Modérée	40 (20,7)	49 (25,5)	45 (22,8)	
Importante	55 (28,5)	57 (29,7)	64 (32,5)	
<b>Indice de Masse Corporelle</b>				0,422
Maigre ou normal	73 (37,8)	63 (32,8)	61 (31,0)	
Surpoids	75 (38,9)	75 (39,1)	74 (37,5)	
Obèse	45 (23,3)	54 (28,1)	62 (31,5)	
<b>Consommation tabac</b>				0,0004
Jamais	35 (18,1)	54 (28,1)	59 (30,0)	
Actuelle	81 (42,0)	61 (31,8)	43 (21,8)	
Ancienne	77 (39,9)	77 (40,1)	95 (48,2)	
<b>Consommation d'alcool</b>				0,008
Jamais	6 (3,1)	11 (5,7)	17 (8,6)	
Actuelle	46 (23,8)	64 (33,3)	68 (34,5)	
Ancienne	141 (73,1)	117 (61,0)	112 (56,9)	
<b>Variable d'intérêt</b>				0,948
Témoins	132 (68,4)	134 (69,8)	135(68,5)	
Cas	61 (31,6)	58 (30,2)	62 (31,5)	
<b>Ancienneté de la cirrhose, années</b>				0,321
<2	96 (49,7)	88 (45,8)	83 (42,1)	
≥2	97 (50,3)	104 (54,2)	114 (57,9)	
<b>Etiologie de la cirrhose</b>				0,008
Infection virale associée ou non avec l'abus d'alcool	46 (23,8)	65 (33,9)	66 (33,5)	
abus d'alcool	127 (65,8)	93 (48,4)	102 (51,8)	
Autres	20 (10,4)	34 (17,7)	29 (14,7)	
<b>Diabètes<sup>2</sup></b>	61 (32,3)	77 (40,7)	78 (40,0)	0,169
<b>Virus de l'hépatite B chronique</b>	5 (2,6)	14 (7,3)	12 (6,1)	0,102
<b>Virus de l'hépatite C chronique</b>	41 (21,2)	53 (27,6)	57 (28,9)	0,182
<b>Score du Child-Pugh<sup>3</sup></b>				0,509
A	103 (55,7)	117 (62,6)	112 (57,4)	
B	55 (29,7)	51 (27,3)	62 (31,8)	
C	27 (14,6)	19 (10,1)	21 (10,8)	
<b>Energie de source non alcoolique</b>				0,953
< 2230	62 (32,1)	58 (30,2)	67 (34,0)	
[2230-3160]	67 (34,7)	70 (36,5)	68 (34,5)	
≥ 3160	64 (33,2)	64 (33,3)	62 (31,5)	

Comparaison univariée en utilisant le test de chi2 pour les variables qualitative et le test de Student pour les variables continues. Les valeurs sont présentées en n (%) ou médiane [IQR].

<sup>1</sup>5 DM, <sup>2</sup>9DM, <sup>3</sup>15DM

Les modèles multivariés de régressions logistiques appliqués sur le score du HEI 2010 pour comparer les cas et les témoins sont représentés dans la table 5. Mais, après ajustement, en considérant le HEI en continu et en tertile, les résultats ne sont pas significatifs mais très proche de la significativité quand le HEI est considéré comme une variable continue.

**Table 5 : OR et 95% CI du score du HEI 2010 en continu et en tertile**

	HEI 2010 en continu		HEI 2010 en tertile		P de tendance
	OR [95% CI]	P valeur	Tertile 2	Tertile 3	
			(49,10-57,17)	(57,17-86,01)	
		OR [95% CI]	OR [95% CI]		
Cas/témoins	181/401		62/133	55/135	
Modèle univarié*	0,99 [0,97-1,01]	0,195	0,89 [0,57-1,40]	0,90 [0,58-1,39]	0,621
Modèle multivarié**	0,98 [0,96-1,00]	0,051	0,76 [0,45-1,27]	0,72 [0,43-1,19]	0,200

\*Stratifié par centre

\*\*Modèle de régression logistique ajusté sur : âge, sexe, énergie totale de source non alcoolique, ancienneté de la cirrhose, score du Child-Pugh, diabète, étiologie de la cirrhose, activité physique professionnelle et stratifié par centre

## HEI 2015

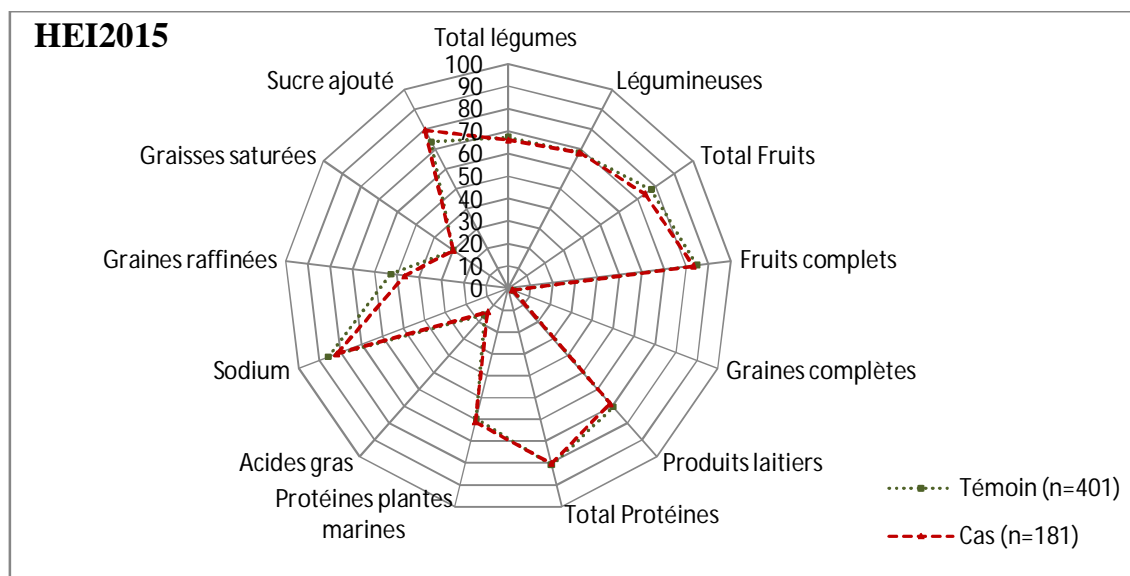
Les analyses statistiques du HEI 2015 ont montrées de meilleurs résultats en comparaison avec le HEI 2010 vu que le dernier composant (calories vides) a été catégorisé en graisses saturées et sucre ajouté mais les moyennes des scores sont restées très proches entre les cas et les témoins. Les résultats du score HEI-2015 des 582 participants varient entre 26,5 et 85,5 avec une moyenne de  $54,6 \pm 9,79$ . La table 6 représente les moyennes du score du HEI-2015 ainsi que ses différents composants dans la population totale, les cas et les témoins. On remarque que les moyennes se rapprochent énormément entre les cas et les témoins. Mais, comme on a remarqué avec le HEI 2010, les scores du sodium, les protéines, fruits et sucre ajouté ont des scores qui s'approchent des scores maximum montrant un suivi des recommandations concernant ces catégories. Alors que les scores d'acides gras et de graines complètes sont presque nuls chez les cas et les témoins. En comparant les cas et les témoins,

la moyenne du score total du HEI-2015 était de  $55,0 \pm 9,91$  chez les témoins et de  $53,9 \pm 9,48$  chez les cas.

**Table 6 : Moyenne ( $\pm$  SD) des scores des différents composants du HEI 2015 chez les cas et les témoins et la population totale.**

Score du HEI 2015	Population totale (n=582)	Témoins (n=401)	Cas (n=181)
HEI-2015 total (100pts)	$54,6 \pm 9,79$	$55,0 \pm 9,91$	$53,9 \pm 9,48$
HEI-2015 total des légumes (5pts)	$3,3 \pm 1,44$	$3,4 \pm 1,45$	$3,3 \pm 1,43$
HEI-2015 légumineuses (5pts)	$3,4 \pm 1,78$	$3,4 \pm 1,75$	$3,4 \pm 1,83$
HEI-2015 total des fruits (5pts)	$3,8 \pm 1,61$	$3,9 \pm 1,56$	$3,7 \pm 1,70$
HEI-2015 fruits complets (5pts)	$4,2 \pm 1,48$	$4,2 \pm 1,43$	$4,2 \pm 1,58$
HEI-2015 graines complètes (10pts)	$0,2 \pm 0,93$	$0,2 \pm 0,99$	$0,2 \pm 0,80$
HEI-2015 produits laitiers (10pts)	$7,0 \pm 2,68$	$7,0 \pm 2,68$	$6,8 \pm 2,69$
HEI-2015 total des protéines (5pts)	$4,0 \pm 1,19$	$4,1 \pm 1,22$	$4,0 \pm 1,15$
HEI-2015 protéines des plantes marines (5pts)	$3,0 \pm 1,64$	$3,0 \pm 1,68$	$3,1 \pm 1,54$
HEI-2015 acides gras (10pts)	$1,5 \pm 2,33$	$1,6 \pm 2,39$	$1,4 \pm 2,21$
HEI-2015 sodium (10pts)	$8,4 \pm 2,35$	$8,6 \pm 2,18$	$8,2 \pm 2,67$
HEI-2015 graines raffinées (10pts)	$5,1 \pm 3,69$	$5,3 \pm 3,64$	$4,7 \pm 3,78$
HEI-2015 acides gras saturés (10pts)	$3,0 \pm 3,38$	$3,0 \pm 3,41$	$3,0 \pm 3,33$
HEI-2015 sucre ajouté (10pts)	$7,6 \pm 2,80$	$7,4 \pm 2,79$	$8,0 \pm 2,79$

**Figure 16 : représentation graphique de la distribution de la moyenne des scores de chaque sous groupe du HEI 2015 chez les témoins et les cas**



La table 7 montre la moyenne ( $\pm$ SD) des scores des différents composants du HEI 2015. Les différences entre les 2 groupes étaient minimes et non significatives. Pour le sucre ajouté, les témoins consommaient significativement plus de sucre que les cas ; le score chez les cas s'approche des standards d'un score maximal.

**Table 7 : Comparaison entre les moyennes ( $\pm$ SD) des scores des composants du score du HEI 2015 entre les cas et les témoins.**

Score du HEI 2015	Témoins (n=401)	Cas (n=181)	P valeur*
HEI-2015 total (100pts)	55,0 $\pm$ 9,91	53,9 $\pm$ 9,48	0,286
HEI-2015 total des légumes (5pts)	3,4 $\pm$ 1,45	3,3 $\pm$ 1,43	0,614
HEI-2015 légumineuses (5pts)	3,4 $\pm$ 1,75	3,4 $\pm$ 1,83	0,982
HEI-2015 total des fruits (5pts)	3,9 $\pm$ 1,56	3,7 $\pm$ 1,70	0,278
HEI-2015 fruits complets (5pts)	4,2 $\pm$ 1,43	4,2 $\pm$ 1,58	0,795
HEI-2015 graines complètes (10pts)	0,2 $\pm$ 0,99	0,2 $\pm$ 0,80	0,092
HEI-2015 produits laitiers (10pts)	7,0 $\pm$ 2,68	6,8 $\pm$ 2,69	0,387
HEI-2015 total des protéines (5pts)	4,1 $\pm$ 1,22	4,0 $\pm$ 1,15	0,344
HEI-2015 protéines des plantes marines (5pts)	3,0 $\pm$ 1,68	3,1 $\pm$ 1,54	0,690
HEI-2015 acides gras (10pts)	1,6 $\pm$ 2,39	1,4 $\pm$ 2,21	0,240
HEI-2015 sodium (10pts)	8,6 $\pm$ 2,18	8,2 $\pm$ 2,67	0,193
HEI-2015 graines raffinées (10pts)	5,3 $\pm$ 3,64	4,7 $\pm$ 3,78	0,073
HEI-2015 acides gras saturés (10pts)	3,0 $\pm$ 3,41	3,0 $\pm$ 3,33	0,924
HEI-2015 sucre ajouté (10pts)	7,4 $\pm$ 2,79	8,0 $\pm$ 2,79	0,002

\*P valeur calculée en appliquant le test de wilcoxon.

La table 8 montre les caractéristiques descriptives des participants selon les différents tertiles du HEI-2015. Comparés aux patients du premier tertile (tertile 1), ceux du dernier tertile (tertile 3 : participants ayant les meilleures scores) étaient plus souvent obèses et non-fumeurs ou anciens fumeurs.

**Table 8 : Caractéristiques des participants selon les tertiles du score du HEI 2015**

Variables	HEI tertile 1	HEI tertile 2	HEI tertile 3	P valeur
	(n=197) (26,52-50,85)	(n=195) (50,85-59,22)	(n=190) (59,22-85,47)	
	n (%)	n (%)	n (%)	
<b>Age, années</b>				0,801
< 60,36	102 (51,8)	97 (49,7)	92 (48,4)	
≥ 60,36	95 (48,2)	98 (50,3)	98 (51,6)	
<b>Sexe</b>				0,326
Homme	149 (75,6)	143 (73,3)	131 (68,9)	
<b>Niveau d'éducation</b>				0,359
≤12 ans d'études	174 (88,8)	165 (84,6)	160 (84,2)	
Plus de 12 ans d'études ou diplôme des grandes écoles/universitaire	22 (11,2)	30 (15,4)	30 (15,8)	
<b>Activité physique professionnelle<sup>1</sup></b>				0,899
Aucune ou faible	146 (74,5)	142 (74,0)	140 (74,1)	
Modérée	33 (16,8)	28 (14,6)	30 (15,9)	
Importante	17 (8,7)	22 (11,4)	19 (10,0)	
<b>Activité physique de loisir</b>				0,429
Aucune ou faible	89 (45,2)	100 (51,3)	83 (43,7)	
Modérée	42 (21,3)	44 (22,6)	48 (25,3)	
Importante	66 (33,5)	51 (26,1)	59 (31,0)	
<b>Indice de Masse Corporelle</b>				0,021
Maigre ou normal	77 (39,1)	60 (30,8)	60 (31,6)	
Surpoids	75 (38,1)	86 (44,1)	63 (33,2)	
Obèse	45 (22,8)	49 (25,1)	67 (35,2)	
<b>Consommation tabac</b>				0,019
Jamais	37 (18,8)	56 (28,7)	55 (28,9)	
Actuelle	77 (39,1)	60 (30,8)	48 (25,3)	
Ancienne	83 (42,1)	79 (40,5)	87 (45,8)	
<b>Consommation d'alcool</b>				0,210
Jamais	6 (3,1)	12 (6,2)	16 (8,4)	
Actuelle	58 (29,4)	63 (32,3)	57 (30,0)	
Ancienne	133 (67,5)	120 (61,5)	117 (61,6)	
<b>Variable d'intérêt</b>				0,729
Témoins	133 (67,5)	133 (68,2)	135 (71,1)	
Cas	64 (32,5)	62 (31,8)	55 (28,9)	
<b>Ancienneté de la cirrhose, années</b>				0,957
<2	89 (45,2)	91 (46,7)	87 (45,8)	
≥2	108 (54,8)	104 (53,3)	103 (54,2)	
<b>Etiologie de la cirrhose</b>				0,265
Infection virale associée ou non avec l'abus d'alcool	49 (24,9)	62 (31,8)	66 (34,7)	
abus d'alcool	119 (60,4)	103 (52,8)	100 (52,6)	
Autres	29 (14,7)	30 (15,4)	24 (12,6)	
<b>Diabètes<sup>2</sup></b>	69 (36,1)	72 (37,3)	75 (39,7)	0,767
<b>Virus de l'hépatite B chronique</b>	7 (3,5)	10 (5,1)	14 (7,4)	0,239
<b>Virus de l'hépatite C chronique</b>	43 (21,8)	53 (27,2)	55 (28,9)	0,249
<b>Score du Child-Pugh<sup>3</sup></b>				0,463
A	117 (61,3)	112 (58,9)	103 (55,4)	
B	57 (29,8)	56 (29,5)	55 (29,6)	
C	17 (8,9)	22 (11,6)	28 (15,0)	
<b>Energie de source non alcoolique</b>				0,090
< 2230	55 (27,9)	59 (30,3)	73 (38,4)	
[2230-3160[	68 (34,5)	69 (35,4)	68 (35,8)	
≥ 3160	74 (37,6)	67 (34,3)	49 (25,8)	

<sup>1</sup>1 DM ; <sup>2</sup>5 DM ; <sup>3</sup>9 DM ; <sup>4</sup>1DM

Après ajustement, la comparaison des score du HEI 2015 entre les cas et les témoins a montré une association inverse et significative en considérant le HEI en continu (OR=0,98; 95% CI = 0,96-1,00; p-value=0,048) et en tertiles, les résultats étaient presque significatifs (OR<sub>T3vsT1</sub>=0,61; 95% CI = 0,36-1,04 ; p-trend=0,068) indiquant qu'une meilleure catégorisation des composants du score ainsi qu'une meilleure qualité du régime alimentaire suivi (tertile 3) diminue le risque de développer un CHC chez les témoins par rapport aux cas (table 9).

**Table 9 : OR et 95% CI du score du HEI 2015 en continu et en tertile**

	HEI 2015 en continu		HEI 2015 en tertile		P de tendance
	OR [95% CI]	P valeur	Tertile 2	Tertile 3	
			(50,85-59,22) OR [95% CI]	(59,22-85,47) OR [95% CI]	
Cas/témoins	181/401		62/133	55/135	
Modèle univarié*	0,99 [0,97-1,01]	0,204	0,94 [0,61-1,45]	0,83 [0,53-1,30]	0,423
Modèle multivarié**	0,98 [0,96-1,00]	0,048	0,83 [0,50-1,37]	0,61 [0,36-1,04]	0,068

\*Stratifié par centre

\*\*Modèle de régression logistique ajusté sur : âge, sexe, énergie totale de source non alcoolique, ancienneté de la cirrhose, score du Child-Pugh, diabète, étiologie de la cirrhose, activité physique professionnelle et stratifié par centre

## AHEI 2010

Les analyses statistiques de l'AHEI 2010 ont montrées des résultats significatifs car le choix des composants était basé sur l'impact de ces derniers sur la maladie étudiée à l'inverse du HEI 2015 ou les composants sont choisis selon les recommandations nutritionnelles de la population générale. Les résultats du score de l'AHEI des 582 participants varient entre 13,1 et 83,9 avec une moyenne pour la population de  $50,7 \pm 11,3$ . En comparant les cas et les témoins, la moyenne du score total de l'AHEI-2010 était de  $50,8 \pm 11,5$  chez les témoins et de  $50,3 \pm 10,7$  chez les cas.

La table 10 présente la médiane de consommation du score de l'AHEI 2010 et de ses différents composants. Les témoins consommaient significativement plus de boissons sucrées

et jus que les cas (en moyenne, la consommation de boissons sucrées était faible contrairement au jus de fruit qui était plus consommé chez les témoins en comparaison avec les cas).

**Table 10: Comparaison des médianes des composants du score de l’AHEI entre les cas et les témoins**

Score de l’AHEI	Témoins (n=401)	Cas (n=181)	P valeur*
AHEI 2010 total (110pts)	52,0 (21,2-81,0)	51,4 (13,1-83,9)	0,564
AHEI-Total des légumes (10pts)	1,9 (0,0-8,9)	2,0 (0,0-8,5)	0,812
AHEI-Total des fruits (10pts)	4,9 (0,0-10,0)	4,5 (0,0-10,0)	0,558
AHEI-Total des graines complètes (10pts)	0,0 (0,0-10,0)	0,0 (0,0-8,3)	0,096
AHEI-Légumineuses (10pts)	2,7 (0,0-10,0)	2,9 (0,0-10,0)	0,863
AHEI-Poissons (10pts)	10,0 (0,0-10,0)	10,0 (0,0-10,0)	0,631
AHEI-Café (10pts)	6,4 (0,0-10,0)	6,0 (0,0-10,0)	0,388
AHEI-Acides gras Polyinsaturés (10pts)	4,6 (0,0-10,0)	4,0 (0,0-10,0)	0,105
AHEI-Sodium (10pts)	4,7 (0,0-10,0)	5,1 (0,0-10,0)	0,577
AHEI-Viandes rouges (10pts)	1,1 (0,0-10,0)	0,5 (0,0-10,0)	0,331
AHEI-Graisses Trans (10pts)	9,9 (0,0-10,0)	9,8 (0,0-10,0)	0,732
AHEI-Boissons sucrées et jus de fruit (10pts)	5,6 (0,0-10,0)	8,8 (0,0-10,0)	0,038

\*comparaison des médianes de consommation de chaque composant de l’AHEI 2010 entre les cas et les témoins en appliquant le test de wilcoxon.

La table 11 montre les moyennes du score de l’AHEI ainsi que ses différents composants dans la population totale, les cas et les témoins.



**Table 11: Moyenne ( $\pm$  SD) des scores de chaque catégorie chez les cas, les témoins et la population totale**

Score de l'AHEI	Population (n=582)	Témoins (n=401)	Cas (n=181)
AHEI 2010 total (110pts)	50,7 $\pm$ 11,3	50,8 $\pm$ 11,5	50,3 $\pm$ 10,7
AHEI-Total des légumes (10pts)	2,3 $\pm$ 1,6	2,3 $\pm$ 1,7	2,2 $\pm$ 1,5
AHEI-Total des fruits (10pts)	5,1 $\pm$ 3,4	5,2 $\pm$ 3,4	4,9 $\pm$ 3,3
AHEI-Total des graines complètes (10pts)	0,3 $\pm$ 1,3	0,4 $\pm$ 1,4	0,3 $\pm$ 1,0
AHEI-Légumineuses (10pts)	3,9 $\pm$ 3,4	3,9 $\pm$ 3,5	3,7 $\pm$ 3,2
AHEI-Poissons (10pts)	7,4 $\pm$ 3,6	7,3 $\pm$ 3,6	7,5 $\pm$ 3,4
AHEI-Café (10pts)	5,6 $\pm$ 4,4	5,7 $\pm$ 4,4	5,3 $\pm$ 4,4
AHEI-Acides gras Polyinsaturés (10pts)	4,7 $\pm$ 2,9	4,8 $\pm$ 2,9	4,4 $\pm$ 2,9
AHEI-Sodium (10pts)	4,6 $\pm$ 3,4	4,5 $\pm$ 3,4	4,7 $\pm$ 3,3
AHEI-Viandes rouges (10pts)	2,6 $\pm$ 3,2	2,7 $\pm$ 3,2	2,4 $\pm$ 3,1
AHEI-Graisses Trans (10pts)	8,9 $\pm$ 2,0	8,9 $\pm$ 1,9	8,8 $\pm$ 2,1
AHEI-Boissons sucrées et jus de fruit (10pts)	5,4 $\pm$ 4,5	5,2 $\pm$ 4,5	6,0 $\pm$ 4,5

La table 12 montre les caractéristiques descriptives des participants selon les différents tertiles de l'AHEI. Comparés aux patients du premier tertile (tertile1), ceux du dernier tertile (tertile3 : participants ayant les meilleures scores) étaient plus âgés et anciens fumeurs.

**Table 12: Caractéristiques des participants selon les tertiles du score de l'AHEI**

Variables	AHEI tertile 1	AHEI tertile 2	AHEI tertile 3	P valeur
	(n=195) (13,1-46,0)	(n=197) (46,0-56,2)	(n=190) (56,2-83,9)	
	n (%)	n (%)	n (%)	
<b>Age, années</b>	59,9 [52,1-65,5]	59,8 [53,5-66,4]	61,7 [55,9-68,3]	0,027
<b>Sexe</b>				0,119
Homme	134 (68,7)	141 (71,6)	148 (77,9)	
<b>Niveau d'éducation</b>				0,702
≤12 ans d'études	169 (87,1)	170 (86,3)	160 (84,2)	
Plus de 12 ans d'études ou diplôme des grandes écoles/universitaire	25 (12,9)	27 (13,7)	30 (15,8)	
<b>Activité physique professionnelle<sup>1</sup></b>				0,758
Aucune ou faible	145 (75,1)	140 (71,4)	143 (76,1)	
Modérée	31 (16,1)	32 (16,3)	28 (14,9)	
Importante	17 (8,8)	24 (12,2)	17 (9,0)	
<b>Activité physique de loisir</b>				0,413
Aucune ou faible	102 (52,3)	86 (43,7)	84 (44,2)	
Modérée	42 (21,5)	46 (23,4)	46 (24,2)	
Importante	51 (26,2)	65 (33,0)	60 (31,6)	
<b>Indice de Masse Corporelle</b>				0,383
Maigre ou normal	68 (34,9)	74 (37,6)	55 (28,9)	
Surpoids	78 (40,0)	70 (35,5)	76 (40,0)	
Obèse	49 (25,1)	53 (26,9)	59 (31,1)	
<b>Consommation tabac</b>				0,003
Jamais	56 (28,7)	42 (21,3)	50 (26,3)	
Actuelle	75 (38,5)	65 (33,0)	45 (23,7)	
Ancienne	64 (32,8)	90 (45,7)	95 (50,0)	
<b>Consommation d'alcool</b>				0,704
Jamais	8 (4,1)	14 (7,1)	12 (6,3)	
Actuelle	62 (31,8)	56 (28,4)	60 (31,6)	
Ancienne	125 (64,1)	127 (64,5)	118 (62,1)	
<b>Variable d'intérêt</b>				0,729
Témoins	133 (68,2)	133 (67,5)	135 (71,1)	
Cas	62 (31,8)	64 (32,5)	55 (28,9)	
<b>Ancienneté de la cirrhose, années</b>				0,970
<2	91 (46,7)	90 (45,7)	86 (45,5)	
≥2	104 (53,3)	107 (54,3)	103 (54,5)	
<b>Etiologie de la cirrhose</b>				0,680
Infection virale associée ou non avec l'abus d'alcool	57 (29,2)	65 (33)	55 (28,9)	
abus d'alcool	111 (56,9)	108 (54,8)	103 (54,2)	
Autres	27 (13,8)	24 (12,2)	32 (16,8)	
<b>Diabètes<sup>2</sup></b>	64 (34,0)	70 (35,9)	82 (43,2)	0,153
<b>Virus de l'hépatite B chronique</b>	9 (4,6)	15 (7,6)	7 (3,7)	0,200
<b>Virus de l'hépatite C chronique</b>	49 (25,1)	51 (25,9)	51 (26,8)	0,929
<b>Score du Child-Pugh<sup>3</sup></b>				0,967
A	111 (58,4)	108 (57,1)	113 (60,1)	
B	58 (30,5)	57 (30,2)	53 (28,2)	
C	21 (11,1)	24 (12,7)	22 (11,7)	
<b>Energie de source non alcoolique</b>				0,131
≥2230	64 (32,8)	60 (30,5)	70 (36,8)	
<2230 et >3160	69 (35,4)	58 (29,4)	67 (35,3)	
≤3160	62 (31,8)	79 (40,1)	53 (27,9)	

Comparaison univariée en utilisant le test de  $\chi^2$  pour les variables qualitative et le test de Student pour les variables continues. Les valeurs sont présentées en n (%) ou médiane [IQR].

<sup>1</sup>5DM, <sup>2</sup>9DM, <sup>3</sup>15DM

Après ajustement, la comparaison des score de l’AHEI entre les cas et les témoins a montré une différence inverse et significative en considérant l’AHEI en continu (OR=0,95; 95% CI = 0,91-0,98; p-valeur=0,001) et en tertile (OR<sub>T3vsT1</sub>=0,55; 95% CI = 0,33-0,91 ; p-tendance=0,025) indiquant qu’une meilleure qualité du régime alimentaire suivi (tertile 3) diminue le risque de développer un CHC chez les témoins par rapport aux cas. De plus, les analyses de sensibilité ont montré une association inverse et significative avec l’AHEI en continu chez les patients atteints de cirrhose d’étiologie virale (table 13).

**Table 13: OR et 95% CI du score de l’AHEI 2010 en continu et en tertile**

	AHEI score				
	Continuuous		In tertile		P tendance
	OR [95% CI]	P valeur	Tertile 2 (46,0/56,2) OR [95% CI]	Tertile 3 (56,2/83,9) OR [95% CI]	
Cases/controls	181/401		55/134	61/134	
Crude model	0,99 [0,98-1,01]	0,576	1,15 [0,74-1,78]	0,87 [0,56-1,36]	0,589
Model 1*	0,95 [0,91-0,98]	0,001	0,98 [0,60-1,61]	0,55 [0,33-0,91]	0,025

\*Stratifié par centre

\*\*Modèle de régression logistique ajusté sur : âge, sexe, ancienneté de la cirrhose, score du Child-Pugh, diabète, étiologie de la cirrhose et stratifié par centre

## Discussion

Ces différents scores suivent le même principe et ont pour but commun d’analyser la qualité des régimes alimentaires suivis. En général, les scores du HEI ne montraient pas de différences significatives entre l’alimentation des témoins et celle des cas car les recommandations suivis dans les différents composants sont pour la population générale et non pour une population atteinte de cirrhose qui devrait suivre des recommandations spécifiques. Par contre, le choix des composants du score de l’AHEI a été basé sur l’association des groupes alimentaires avec des maladies chroniques et certains cancers. Ces résultats viennent soutenir l’hypothèse que les choix alimentaires sont des moyens de prévention intéressants pour diminuer le risque de développer un cancer.

Les résultats de l'AHEI et du HEI 2015 illustrent mieux la qualité et les régimes alimentaires suivis par notre population vu que leurs composants représentent mieux les groupes alimentaires qui ont un impact sur le développement du CHC.

Les scores du HEI montrent des associations inverses et non significatives avec le risque de développer un CHC. Les études ont mis en évidence qu'une meilleure alimentation basée sur des recommandations nutritionnelles internationales diminue le risque de CHC mais en comparant des patients atteints du cancer avec des patients sains (55). Les résultats de ces scores étaient encourageants en les testant avec plusieurs types de cancers, maladies chroniques et même avec le taux de mortalité (70–73).

Par contre, un meilleur score de l'AHEI a montré une diminution significative du risque du CHC car le choix des composants était spécifique à cette population et basé sur les résultats des études précédentes concernant les aliments protecteurs ou nocifs sur l'évolution de la cirrhose ou le développement du CHC.

Certains composants de ces scores doivent être privilégiés vu leur richesse en certains nutriments qui ont montré un effet protecteur sur le développement de certains cancers. Parmi ces composants la consommation de fruits, légumes, légumineuses et céréales complètes. Tous ces aliments sont riches en fibres, vitamines, minéraux et certains antioxydants. Ainsi, il faut privilégier les fruits et légumes de saison et remplacer les céréales raffinés par des céréales complètes. Les études ont montré que les légumes et en moindre effet les fruits peuvent diminuer le risque car ce sont des aliments pauvres en énergie mais riches en fibres, antioxydants et certains composants bioactifs (31,44,46). Le fait de privilégier les céréales complètes à la place des céréales raffinées augmente davantage la quantité de fibres consommée et diminue le risque d'obésité qui est un facteur de risque important pour la NASH, la NAFLD et par suite le risque de développer un CHC (17,18).

Il a été démontré dans plusieurs études que la consommation élevée de viandes rouges et de charcuteries augmentent le risque de plusieurs cancers entre autre le cancer du foie. Par contre la consommation de poissons et de fruits mer, qui sont moins riches en graisses et fer que la viande rouge mais riches en oméga 3, diminue le risque de CHC (28,31). Ainsi il vaut mieux préciser le type de protéines à privilégier ainsi que la quantité à consommer comme c'est le cas dans l'AHEI (28,31,56).

Les graisses saturées, les acides gras trans et dans une moindre mesure les acides gras polyinsaturés de type oméga 6 sont considérés comme des aliments cancérigènes si elles sont consommées en grandes quantités (74–77). Ainsi il est important de préciser les limites et les proportions à consommer de chaque type de graisse pour mieux analyser les risques.

Le sucre ajouté et les boissons sucrées, en considérant les boissons gazeuses et les jus de fruits, sont associés à un risque élevé d'obésité (33) ainsi qu'au risque de développer un CHC (32,35). Les boissons sucrées contiennent une forte teneur en sucres ajoutés, elles assurent une faible satiété sans compenser l'énergie totale nécessaire pour un bon fonctionnement du corps. De plus, les jus de fruits ont été associés au risque de diabète (78). Le diabète, l'obésité sont deux facteurs de risque du NAFLD et augmentent le risque de CHC indépendamment de la cirrhose (3,26).

Les produits laitiers sont une source importante de calcium, vitamine D, lipides et protéines. La revue de la littérature a montré des résultats controversés concernant la relation entre les produits laitiers et le risque de CHC. Des études montrent une association inverse entre la consommation de lait et de certains produits laitiers et le risque de CHC mais, à l'inverse, l'importance des produits laitiers et du calcium dans la diminution de certains cancers digestifs comme le cancer du colon (70). D'autres études ont montré que le calcium, la vitamine D, les lipides et les protéines de source laitière étaient associés à un risque accru de

CHC, alors que les mêmes nutriments provenant de sources non laitières présentaient des associations inverses ou nulles (30).

### **Conclusion**

En résumé, nos résultats ont montré une association inverse mais non significative avec l'HEI mais significatif avec l'AHEI entre le suivi des recommandations nutritionnelles et le risque de développer un CHC. Les résultats des autres études qui se sont servies de ces scores suggèrent que suivre les recommandations est important pour lutter contre le cancer. Mais, comme notre population est atteinte de cirrhose, le choix des composants et des limites de consommations doivent être spécifiques selon l'avancement de la maladie ou le stade du cancer pour que les résultats soient plus efficaces. Ainsi, il est important de bien choisir les composants du score de l'AHEI selon leurs effets sur les maladies ou cancer étudiés. Ces résultats doivent être confirmés dans des études prospectives et plus larges.

Deuxième partie : Profil de consommation alimentaire, score *a posteriori*.

Application de la méthode des analyses en composante principale

*Guilloteau Adrien, Rizk Maud, Binquet Christine, Boutron-Ruault Marie-Christine, Jooste Valérie, Bonithon-Kopp Claire, Thieffin Gérard, Barraud Hélène, Bronowicki Jean-Pierre, Richou Carine, Di Martino Vincent, Doffoel Michel, Jouve Jean-Louis, Hillon Patrick, Cottet Vanessa. Dietary patterns and risk of hepatocellular carcinoma in cirrhotic patients: a case-control study. (Manuscrit soumis)*

Cet article a été initié par Adrien Guilloteau et Vanessa Cottet. J'ai participé à cet article en tant que co-auteurs. J'ai remis en perspective les résultats d'Adrien et les miens, j'ai assisté à l'interprétation des résultats et la relecture de l'article. Cet article a été soumis dans la revue Clinical Nutrition.

### **Introduction**

Etudier l'alimentation des patients permet d'identifier des aliments à privilégier ou à limiter. Néanmoins, nous ne les consommons pas isolément. De telles méthodes ne permettent pas de tenir compte de l'alimentation dans sa globalité ou de tenir compte des interactions entre nutriments. Cet article a pour but de déterminer la relation entre certains profils de consommations alimentaires définis *a posteriori* et le risque de CHC dans notre population d'étude. L'utilisation de score *a posteriori* permet par des méthodes statistiques d'identifier des profils de consommation alimentaire propre à la population étudiée.

### **Méthodes**

Toujours dans notre étude cas-témoins, CiRCE, incluant 592 patients qui ont complété le questionnaire alimentaire, les patients présentant des valeurs extrêmes d'apports énergétiques ont été exclus de l'analyse finale (n = 10) ainsi que les patients ayant des données cliniques incomplètes (n=20). Ainsi, cette étude a inclus 562 patients dont 177 patients cirrhotiques avec un CHC récemment diagnostiqué (cas) et 385 patients cirrhotiques sans CHC (témoins). Les habitudes alimentaires ont été déterminées par analyse en composantes principales dans le groupe témoin et le score de chaque patient a été classé en tertiles (adhérence faible, moyenne et forte). Nous avons également effectué des analyses spécifiques chez certains sous-groupes de l'étiologie de cirrhose : les patients cirrhotiques atteints d'hépatite virale et chez les sujets atteints de cirrhose alcoolique/métabolique.

## **Résultats**

Les résultats ont montré quatre profils dans la population globale : le profil «Diversifié» a inclus la consommation de charcuteries, légumes, féculents, pizza et frites. Le profil «Snacks et sucreries» est caractérisé par une consommation élevée de sucre, sandwich et frites. Le profil «Prudent» a inclus la consommation d'aliments sains comme les légumes, les fruits, les poissons, l'huile d'olive, les produits allégés et peu de consommation d'alcool. Enfin, le profil «Viande» qui réunit les grands consommateurs de viande et de graisses animales. Tous ces profils constituaient un mélange d'aliments qui augmentent le risque de CHC ainsi que ceux qui protègent et diminuent le risque. Les analyses de régression logistique n'ont pas montré d'associations significatives entre les différents profils et le risque de CHC. Dans les analyses de sensibilité en fonction de l'étiologie de la cirrhose, les profils retrouvés étaient proches de ceux de la population globale.

## **Conclusion**

Cette étude a pu déterminer 4 profils alimentaires qui sont spécifiques à notre population cirrhotique. C'est la première étude à analyser les relations entre les profils alimentaires et le CHC dans une population cirrhotique occidentale. Aucun profil alimentaire n'a été associé à un risque plus élevé de CHC. Néanmoins, nos résultats sont compatibles avec un risque élevé de CHC associé à un profil de grands consommateurs de viande. Ces résultats concernant le profil « viande » viennent compléter les résultats de l'article sur les groupes alimentaires où la charcuterie et sodium (des composants essentiels de ce profil) ont montré une association positive et significative avec le risque de CHC. Des études complémentaires sont nécessaires pour confirmer nos résultats, en particulier chez des patients cirrhotiques.



## **Dietary patterns and risk of hepatocellular carcinoma in cirrhotic patients: a case-control study**

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### **Highlights:**

Study conducted in occidental cirrhotic patients with various etiology

First study on relations between dietary patterns and HCC risk in such population

Four patterns found: “Diversified”, “Snacks & sweets”, “Prudent”, and “Meat”.  
After adjustment, no pattern was significantly associated with cancer risk

**Key-words:**

Liver cancer, diet, meat, principal component analysis, occidental population

**List of abbreviations:**

HCC –Hepatocellular carcinoma

OR – Odds-ratio

PCA – Principal component analysis

CI – Confidence interval

GNRI/NRI -(Geriatric) Nutritional Risk Index

**Financial support:**

This work was supported by a French Government grant managed by the French National Research Agency under the program “Investissements d’Avenir”[reference ANR-11-LABX-0021], and also by grants from the “Conseil Régional de Bourgogne”, the “Fonds Européen de Développement Régional” (FEDER), the French National Cancer Institute, the “Ligue Nationale contre le Cancer”, the “Canceropole Grand-Est” and the “Fondation de France”. The first author was financed by a fund provided by the Regional Health Agency.

**Abstract:**

Background & Aims: Hepatocellular carcinoma (HCC) is the most common type of liver cancer and the second cause of death by cancer worldwide. It occurs in 80% of cases after cirrhosis with various etiologies. Diet has been little investigated among cirrhotic patients although it is a modifiable factor with preventive potential. Methods: To explore relationships between dietary patterns and HCC risk, we conducted a multicenter case-control study in 562 patients in France, including 177 cirrhotic patients with newly diagnosed HCC (cases) and 385 cirrhotic patients without HCC (controls). Dietary patterns were determined by principal component analysis among the control group and the score for each patient was categorized in tertiles (weak, medium and strong adherence). We also conducted specific analyses among viral cirrhotic patients and among subjects with alcoholic/metabolic cirrhosis. Results: We found four patterns in the overall population: “Diversified”, “Snacks & sweets”, “Prudent”, and “Meat”, all including both food groups suspected to increase HCC risk as well as suggested protective foods. Unconditional logistic regression did not show significant associations between the patterns and HCC risk. In specific analyses according to cirrhosis etiology, we identified patterns close to that found in the overall population. Conclusion: This is the first study to analyze relationships between dietary patterns and HCC in an occidental cirrhotic population. No dietary patterns were associated with higher HCC risk overall. Nevertheless, our findings are consistent with an increased risk of HCC with higher meat consumption, which deserves further investigation, especially in such a population of cirrhotic patients characterized by impaired metabolism.

## **Introduction:**

Liver cancer is one of the most common cancers worldwide with 782,000 new cases reported in 2012(1). It accounts for 9% of world cancer mortality with 746,000 deaths (1). In France, the incidence is one of the highest in Europe, with age-standardized rates of 16.6 and 3.6 per 100,000 for men and women, respectively, in France versus 10.0 and 3.3 for Europe (2). In Europe, 90% of primary liver cancers are hepatocellular carcinomas (HCC) and 80% of HCC occur in the setting of cirrhosis, whatever its cause (3). There is no cure for cirrhosis, except transplantation, which is offered only to patients with terminal liver failure because of the scarcity of organs. Thus, it is of utmost interest to identify new factors of HCC among these patients and to learn about the physiopathology of the disease. Viral hepatitis, alcoholism, hemochromatosis, and non-alcoholic steato-hepatitis are well-established risk factors for HCC.

To date, nutritional factors have been poorly investigated (4). It has been suggested that some foods, such as fruit, vegetables, fish and white meat, have a protective effect (5–11), whereas red and processed meat seems to be associated with an elevated risk of HCC (9,11,12). Strong evidence exists only for the relationship between liver carcinogenesis and coffee consumption, with an odds-ratio of 0.5 for high versus low consumption in a meta-analysis of 16 studies (13). Overall, results are not sufficiently consistent across studies to consider that relationships between specific foods and HCC as convincing (14).

People are exposed to a wide variety of foods with complex combinations of nutrients and synergies. Dietary patterns are therefore more suited to capture the overall effects of dietary exposure, including the cumulative effects of multiple foods and nutrients. They avoid issues of confounding, of interactions between nutrients and of collinearity. Furthermore, the effects of a single nutrient or food item may be too small to detect, while the analyses of a large number of nutrients or food items may produce statistically significant associations simply by chance. The dietary pattern approach can also help in the drafting of public health recommendations, because global nutritional advice could be easier to implement than the traditional approach by portions or grams of nutrient/food per day.

The dietary pattern approach has often been used in cancer research, but only three publications have concerned HCC. These support a reduced HCC risk associated with strong adherence to the “Vegetable-based” pattern (15), or to the Mediterranean Diet Score (16,17). However, one of these studies was performed in Asia where diet and HCC risk factors are somewhat different. The two others (in the US and in Italy and Greece) used an approach by score and focused on the Mediterranean Diet, which may not be relevant to all populations. None of these studies were specific to the cirrhotic population. In this context, our aim was to explore the relationship between dietary patterns and HCC risk in a French population of cirrhotic patients with different etiologies of cirrhosis.

## **Patients and Methods**

### **Study sample**

The multicenter case-control study CiRCE (“Cirrhose et Risque de Carcinome hépatocellulaire dans le grand Est”) was designed to identify the environmental, nutritional and metabolic risk factors of developing HCC among cirrhotic patients. Patients (controls and cases) were recruited in six hospitals of the North-East of France (Besançon, Dijon, Metz, Nancy, Reims, and Strasbourg) between June 2008 and December 2012. Included patients were at least 35 years old and had cirrhosis, either histologically confirmed through liver biopsy or, in the absence of biopsy, established on typical clinical, morphological and biological data. Cases were cirrhotic patients followed in the participant centers with HCC histologically confirmed, according to criteria defined by the European Association for Study of the Liver (18). Controls were cirrhotic patients without HCC at inclusion, and were not matched with cases. The absence of HCC was assessed through high-quality imaging examinations (abdominal ultrasound, computerized tomography scan or magnetic resonance imaging) and alpha-fetoprotein below 100 ng/mL during the two months preceding inclusion. Patients were excluded if they had progressive extrahepatic cancer, primary liver cancer other than HCC, HCC without cirrhosis, acute alcoholic hepatitis, human immunodeficiency virus infection, or severe diseases not compatible with inclusion in the study. All of the patients provided informed consent at entry in the study. The study protocol was approved by the local ethics committee (East I, France) and by the French legal authorities.

### **Data collection**

In all centers, the investigators collected data on factors likely to be involved in liver carcinogenesis using a standardized form. These included individual characteristics (gender, age, alcohol consumption, smoking status, and physical activity), clinical data (patient and family medical history, VHB/VHC status, and tumor characteristics) and metabolic characteristics (anthropometric measurements, dyslipidemia, and diabetes mellitus). We also conducted a thorough survey of current and past medication records.

Dietary data were collected through a diet history questionnaire. As it was a self-administered questionnaire, no systematic instructions and help were given. The questionnaire assessed quantitative and qualitative food consumption over the year before inclusion (or HCC diagnosis for cases) and took into account seasonal variations. It detailed all meals and snacks consumed in a day. It covered 208 food items the food portions and the frequency of consumption (11 possibilities, from never to seven per week). It was accompanied by a booklet of photographs to help patients assess portion sizes. Both the questionnaire and booklet have been validated and were tested for reproducibility after one year (19,20). Finally, food portions and the frequency of consumption were converted into daily gram intake, total energy intake (Kcal) and nutrient consumption with a diet composition table. This table was based on the French CIQUAL table (2008), a program from the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). Patients with extreme values

(first and ninety-ninth percentiles) for the ratio between their energy intake and energy requirement were excluded from the final analyses. For nutritional status, patients were categorized as having “no”, “moderate” or “severe” malnutrition by a hierarchical algorithm using the (Geriatric) Nutritional Risk Index (GNRI/NRI), loss of weight in 6 months, albumin level, and BMI. If at least one of these tests indicated severe malnutrition based on international norms, patients were categorized as having “severe malnutrition”. Among the remaining patients, if at least one of the tests indicated malnutrition, patients were categorized as “moderate malnutrition”.

### **Dietary patterns**

The 208 items were summarized in 54 food groups listed in Appendix 1 (Supplementary file). Dietary patterns based on these predefined food groups were generated using principal component analysis (PCA) in the control group. PCA builds up components (=patterns), which are combinations of the 54 food groups, each weighted by a coefficient defined by the PCA process. These coefficients, called factor loadings, are interpreted as correlation coefficients. A value near to 1 indicates a strong influence of the food item on the value of the pattern. To interpret the patterns, we considered foods with at least moderate influence, thus with a factor loading below -0.25 or over 0.25. We used the “Varimax” transformation for easier interpretation (21). To determine the number of components to retain, we considered 1) eigenvalues (proxy of the explained variance) greater than 1.25, 2) the scree plot test, and 3) the interpretability of the patterns (22). The labeling was primarily descriptive and based on our interpretation of the pattern structure regarding foods strongly associated with the pattern (Table 2). For each patient, we calculated a score for each pattern by summing up observed daily intake of each food group, weighted by the food group factor loading. The factor score measures the degree of adherence of a patient’s diet to the given pattern.

### **Statistical analyses**

Because of a large proportion of both non-drinkers and very heavy drinkers, alcohol intake was analyzed in three categories (non-drinkers, <20g/day, more than 20g/day). Educational level was considered in two groups: <12 years of schooling and up to 12 years or high-school diploma. Severity of liver cirrhosis was assessed using the Child-Pugh score, which ranges between 5 and 15, based on total bilirubin level, serum albumin, prothrombin time, ascites and hepatic encephalopathy. The cirrhosis etiology was defined by clinicians during clinical interviews and was considered in three categories: alcohol abuse, viral, and others. Other etiologies were mainly metabolic (Non-alcoholic steato-hepatitis), but also included auto-immune (primary biliary cirrhosis) and idiopathic causes.

Continuous variables were expressed as medians and inter-quartile ranges; categorical variables were described as percentages. Univariate comparisons between cases and controls were performed using the chi-square test (Fisher’s exact test if conditions were not met), and

the Student's test (in case of non-normal variable, the Kruskal-Wallis test). Unconditional logistic regression models were performed for each pattern score in tertiles to estimate associations with the risk of hepatocellular carcinoma among cirrhotic patients. Results were expressed as odds ratios and 95% confidence intervals. To account for potential confounders, we constructed models with a forward stepwise logistic regression that included all variables with a significance level below 0.20 in unadjusted analyses, as well as variables reported to be associated with HCC risk in the literature. All models were stratified on study center and additionally adjusted for age, gender, time between diagnosis of cirrhosis and inclusion in the study and total energy intake. We assessed the log-linearity of the relationship with HCC for all continuous variables using graphical methods (splines, lowess plot). In case of departure from linearity, variables were included as tertiles (time since the diagnosis of cirrhosis and alcohol consumption) P values for linear trends were estimated using the median values within each tertile of a dietary pattern, thereby taking the unequal distances between tertiles into account. Potential interactions either suggested in the literature or related to plausible underlying mechanisms (particularly with sex and cirrhosis etiology) were tested. The only interaction term found, between cirrhosis etiology and the Child-Pugh score, was introduced in the final models concerning the overall population.

In addition, we conducted specific analyses in the following subpopulations: men, patients with cirrhosis of a viral origin, and patients with alcohol- or metabolic-related cirrhosis. First, we determined dietary patterns in these specific populations by PCA; then performed analyses using the same strategy as that in the overall population. A *p* value below 0.05 was considered statistically significant. We used SAS v 9.3 (SAS Institute Inc., Cary, North Carolina) and R v3.1.2 (R-Core Team 2014, Vienna) software.

## Results

Among the 1,179 subjects included in the CiRCE study, 592 completed the dietary questionnaire. After exclusion of subjects with extreme energy intake ( $n=10$ ) or incomplete clinical data ( $n=20$ ), the study sample comprised 562 cirrhotic patients: 177 cases with HCC and 385 controls (Appendix 2). The sample size provided a power of 80% to detect adjusted odds ratios of at least 1.85. Compared with the 562 included patients, non-included subjects were slightly older, and more frequently presented an alcoholic etiology of cirrhosis and more severe liver disease (Appendix 3). The characteristics of the study population are summarized in table 1. Compared with controls, cases were older than controls, were more frequently men or heavy drinkers, and had more recent and less severe cirrhosis. Specific food-group consumptions (coffee, red meat, fish, fruit, raw and cooked vegetables) were not associated with HCC risk in univariate analysis, except for processed meat ( $p=0.040$ ) (Table 1). Indeed, the odds-ratio for processed meat consumption was 1.52 (95% CI: 0.99-2.35) for the third tertile (over 25g/day) with the first as the reference ( $p$  for trend = 0.026). Notably, coffee was not significantly associated with HCC risk: the odds ratio was 1.09 (95% CI: 0.70-1.70) for the third tertile (400ml/day or more), with the first as the reference ( $p$  for trend = 0.15).

In the overall population, four dietary patterns were retained and accounted for 22% of the total variance (Table 2). The first pattern was named “Diversified” because it was mainly positively correlated with processed meat, vegetables, starchy foods, pizza, and French fries. The second pattern was characterized by high-sugar intake and snacking foods like sandwiches and French fries. Thus, it was labeled “Snacks & sweets”. The third pattern corresponded to the most cautious consumptions: high contribution of vegetables, fruit, fish, olive oil, “low fat” products, and an inverse correlation with beer and wine; it was named “Prudent”. The fourth pattern was mainly positively correlated with meat and animal fats. It was labeled “Meat”. Relationships between baseline characteristics and dietary patterns are presented in table 3. Patients with strong adherence to the “Diversified” pattern were more frequently men and had a more recent diagnosis of cirrhosis, higher alcohol consumption and higher overall nutrient intake (particularly lipids and proteins). A strong adherence to the “Snack& sweets” pattern was associated with lower alcohol intake, younger age, higher energy intakes and more frequently cirrhosis of alcoholic origin. The “Prudent” diet was significantly associated with careful behavior: less smoking and lower alcohol consumption. Strong adherents to this diet were more frequently women, older and with a less severe cirrhosis or a longer time since the diagnosis of cirrhosis. High adherence to the “Meat” pattern was associated with a younger age and higher protein intake. In multivariate analyses, none of the dietary patterns were significantly associated with HCC risk in cirrhotic patients (Table 3). Unexpectedly, strong adherence to the “Meat” pattern was not significantly associated with an increased risk of HCC ( $OR_{\text{tertile3 vs. tertile1}}: 1.45; 95\% \text{ CI: } 0.86\text{-}2.47; p \text{ for linear trend}=0.17$ ).

We performed specific analyses in three sub-groups: men ( $n=409$ ), patients with cirrhosis mainly related to alcoholic or metabolic etiologies ( $n=362$ ), and patients with viral cirrhosis only ( $n=165$ ). When we restricted the population to men (which represented 72.8% of the overall population), patterns and their associations with HCC status were very close to those in the overall population (data not shown). In patients with alcoholic/metabolic cirrhosis, three dietary patterns were retained (Appendix 4). The first pattern was labeled “Junk food” due to the positive correlations with pizza, French fries, pasta, pulses, sweets, sugar, cakes, ham, processed and red meat. The second pattern was very close to the Mediterranean Diet, often described in the literature. It was mainly characterized by high consumptions of vegetables, pulses, pasta, fish, shellfish, olive oil and low-fat dairy products. The third pattern, named “Meat”, was quite similar to the “Meat” pattern found in the overall population with a high correlation with meat, animal fat, and pasta. However in this subpopulation, the pattern did not correlate with fish, but correlated with beer and processed meat consumption. None of these patterns was associated with HCC risk in alcoholic/metabolic cirrhosis patients. In patients with viral cirrhosis, the three dietary patterns derived from PCA were somewhat different from those in the overall population (Appendix 5). The first pattern was named “Diversified”, it was highly correlated with vegetables, fish, potatoes, and was similar to the “Diversified” pattern in the overall population. The second pattern was named “Snacks& sweets” (ham, cakes, soft drinks, sandwiches and dairy products). The third pattern “Meat” was similar to the “Meat” pattern in the alcohol- and metabolic-related cirrhotic patients, with the addition of positive correlation with snack food and coffee. Strong adherence to the

“Diversified” pattern was significantly associated with an increased risk of HCC (OR<sub>tertile3 vs. tertile1</sub>: 2.82; 95% CI: 1.02-8.20; p for trend = 0.03). No other statistically significant association was found between dietary patterns and HCC risk among patients with viral cirrhosis. Models for the specific analyses are presented in the supplementary data (Appendix 6).

## Discussion

This is the first study to analyse dietary habits among cirrhotic patients in a European population, while taking into account the cirrhosis etiology. The observed dietary patterns were quite similar in the overall population, in patients with viral cirrhosis and in patients with alcohol/metabolic cirrhosis. None of these patterns were significantly associated with HCC risk with the exception of a positive association between HCC risk and the “Diversified” pattern in patients with viral cirrhosis.

Among the ten patterns retained from the main and secondary analyses, we identified dietary patterns close to others previously described in the literature. The “Diversified” pattern was quite similar to the “Pork and potatoes” pattern in European cohorts (23) and a “Mixed” pattern in a British prospective study (24). It had some resemblance to the “Western” diet largely described in the literature (14,25,26), but with higher consumption of vegetables and no contribution of red meat and alcoholic beverages. These differences toward healthier dietary habits may have reduced the positive association expected with HCC risk.

The pattern labeled “Prudent”, seemed to be a more cautious approach to food consumption and to health with reduced alcohol consumption and not smoking. Such patterns, close to dietary recommendations, are the most homogeneous in the literature. A variation of this pattern, the often-described Mediterranean Diet is habitually associated with decreased cardio-vascular and cancer risk as well as a longer lifespan (27). However, such protective potential remains to be shown in a cirrhotic population marked by impaired metabolism.

In the three subpopulations, PCA retained a “Meat” pattern, which could be specific to our population (Northern France, and/or cirrhotic patients). There is some evidence in the literature that high red meat consumption could increase the risk of HCC (9,12), but the associations never reached statistical significance in our study. Our “Meat” pattern was not strongly correlated with processed meat (whereas it was the only food group associated with HCC in univariate analyses), but more with poultry and to a lesser extent with fish. As the consumption of poultry and fish has been associated with a lower risk of HCC (11), they could have played a role in diminishing the impact of red meat on HCC development. As well as the “Meat” pattern, the “Junk food” pattern found in the alcoholic/metabolic cirrhosis subpopulation was in part characterized by food considered protective against HCC: fish, and shellfish (5–8,11), coffee(13).

Overall, the dietary patterns found by our *a posteriori* approach highlighted some ambivalent consumption habits in our population. This is one advantage of this approach, which does not



use a score qualifying a theoretical diet like the *a priori* Mediterranean Diet Score but may better reflect actual dietary behaviors in specific populations. In addition, the consumption of expensive foods (like meat and fish) was certainly associated with a high socio-economic status, which is often linked to better overall health. Thus, other behaviors, not taken into account, may have attenuated the association between HCC and the “Meat” dietary pattern.

A large proportion of these cirrhotic patients were malnourished (65%), even though the median protein and energy intake were within the ESPEN recommendations specific to liver diseases (35-40 kcal/kg/day – 1.2-1.5 g/kg/day of proteins) (28). The majority of parameters used to define the nutritional status (NRI/GNRI, albumin, and BMI) were measured at inclusion while the diet history questionnaire assessed the intakes during the previous year. It is possible that the malnutrition may have worsened during the period just before the diagnosis of HCC and inclusion in this study. Cirrhosis is characterized by disturbed metabolism, which can explain malnutrition beyond dietary restrictions and anorexia: impaired intestinal absorption, altered nutrient and glycogen synthesis, increased protein catabolism and lipolysis, and high levels of pro-inflammatory cytokines resulting in a hypermetabolic state. In addition, we cannot exclude an overestimation of dietary intakes by the self-administered questionnaire.

No significant relationships between patterns and HCC were detected, apart from the “Diversified” pattern that we found in the viral cirrhosis subpopulation, which is difficult to explain. Inverse causality may be one explanation: patients with a high risk of HCC or with early-stage cancer could have applied recommendations of clinicians to adopt a more careful diet. Compliance with a prescribed diet may be higher in this subset than in the alcoholic/metabolic cirrhosis subset and could explain differences. Such variations in compliance according to the etiology of the cirrhosis have previously been observed regarding surveillance programs of HCC screening (29). An over-estimated odds-ratio for the “Diversified” pattern may be due to non-adjustment for all healthy behaviors, which probably correlated with this dietary pattern. Finally, we cannot rule out that this association was found by chance.

Three previous studies have also analyzed the relationships between dietary patterns and HCC risk. Zhang *et al* identified three patterns in the Shanghai studies using a factor analysis (“Vegetable-based”, “Fruit-based” and “Meat-based”), which explained 79% of the variance (15). The important difference in the percentage of explained variance compared with our study may be due to less diversity in the foods available (recruitment for their study began in 1997, when incomes in China were much lower than those in Europe), but also due to a more stereotypical dietary intake in the Shanghai area. The difference could also be due to a less detailed questionnaire (with around eighty food items) and to the use of only thirteen groups in factor analysis. In contrast, explained variance was between 20% and 30% in other European studies with equivalent questionnaires and number of food groups (25). Another important difference is the large predominance of viral cirrhosis in China. In patients with a history of chronic liver disease, Zhang *et al*. found a lower risk of HCC with strong adherence to the “Vegetable-based” pattern, but no association with the “Fruit-based” or “Meat-based” patterns. Differences between patterns (our “Mediterranean” and “Prudent” patterns were not

only characterized by vegetables alone) and between methodology (design or confounders taken into account) may explain the differences between the results. The two other studies that analyzed the relationships between dietary patterns and HCC risk used an *a priori* approach with the Mediterranean Diet Score (MDScore) (16), adapted to the US population for one of them (17). In these studies, the authors found a significantly lower risk of HCC with the highest MDScore compared with the lowest. Our “Prudent” and “Mediterranean” patterns, defined by an *a posteriori* approach, thus closer to the actual diets in the study samples, differed from their *a priori* MDScore by a lower correlation with wine and whole-grain foods.

Some weaknesses of this study could have affected the results. The major one was the small sample size; only half of the inclusion sample had exploitable dietary data, which reflects the difficulty of answering this extensive nutritional questionnaire. However, differences between respondents and non-respondents were almost similar in cases to those in controls in stratified analyses; thus it is unlikely that this could have markedly affected the results. Dietary habits are closely associated with other behaviors, environmental factors and social determinants that could influence cancer risk. Unfortunately, it is difficult to identify and assess such factors with sufficient precision in epidemiologic studies. The unexpected decrease in HCC risk in patients with a high Child-Pugh score, observed in our study, is certainly a consequence of the hospital-based recruitment. All HCC cases (with low or high Child-Pugh score) are diagnosed in hospital but only the most serious cirrhotic patients (with high Child-Pugh score) are followed in hospital. Last, although this was a multicenter study involving hospitals in the north-eastern quarter of France and included various populations, diets in cirrhotic patients may have presented too little variability to ensure good discrimination between risk groups.

One of the advantages of this study is the choice of a study population entirely composed of cirrhotic patients. Thus, compared with results from previous studies, which included heterogeneous controls with or without liver disease, our results concerned this high-risk group of HCC patients, who had specific habits and behaviors. Another strength is the probable absence of misclassification between cases and controls due to the confirmation of HCC diagnosis according to recommendations of the European Association for the Study of the Liver (EASL) (18) and the confirmation of HCC absence by high-quality imaging examinations. Contrary to studies based on food frequency questionnaires, our dietary evaluation was based on a qualitative and quantitative diet history questionnaire. However, we could not rule out temporal or memory bias since the assessment could have been simultaneous to the HCC diagnosis. Although alcohol consumption did not correlate strongly with any dietary patterns in our study, we systematically adjusted the models for this variable to limit the influence of alcohol in the relationship between dietary patterns and HCC. Finally, one major strength of our study was the use of an *a posteriori* dietary pattern approach, which considers diet in all its complex relationships and potential interactions. The dietary pattern approach is particularly advantageous when planning nutritional preventive counseling strategies. Having a diet that complies with health recommendations may have a beneficial effect on liver carcinogenesis through several possible pathways. Among these, chronic inflammation and oxidative stress have been associated with hepatic carcinogenesis (3,30,31).

To conclude, our study is the first to use a global approach by *a posteriori* patterns to explore relationships between HCC risk and diet in an occidental cirrhotic population. Some patterns seemed specific to our population, thus underlining that previous results from healthy populations should be extrapolated with caution in ill populations with impaired metabolism, like cirrhotic patients. Larger prospective studies are needed to confirm our exploratory results, but should be conducted in cirrhotic populations. Such studies may benefit from complementary qualitative approaches to target subpopulations who may be receptive to dietary recommendations.

### **Acknowledgments**

We thank the members of the CiRCE Study Group for their contribution to the recruitment, data collection and biological analyses. We thank Pr Raabe for his participation. We thank the staff of the Centre Ferdinand Cabanne (University Hospital of Dijon, F) for their assistance in handling and storing biological samples. We thank the E3N team (INSERM UMR1018) for their help with dietary questionnaire. We also thank Mr. Bastable for the review.

**Table 1:** Baseline characteristics of study cirrhotic patients by CHC status (CIRCE study – n=562)

Variables	All (n = 562)	Cases (with HCC at inclusion) (n = 177)	Controls (without HCC at inclusion) (n = 385)	p
Age (years)	60.4 (13.0)	64.4 (11.8)	59.0 (12.4)	< 0.001
Men (%)	72.8	86.4	66.5	< 0.001
Time since the diagnosis of cirrhosis (years)	2.4 (1.0 - 6.1)	1.3 (0.8 - 4.8)	2.8 (1.2 - 6.3)	< 0.001
Alcohol daily intake				
Non-drinkers (%)	43.6	40.1	45.2	0.044
< 20g/day (%)	24.0	20.3	25.7	
≥ 20g/day (%)	33.4	39.6	29.1	
History of cancer (%) (except liver cancer)	5.3	3.4	6.2	0.16
Child-Pugh score (15 points)	6 (5 - 8)	6 (5 - 7)	6 (5 - 8)	0.006
Cirrhosis etiology				
Alcoholic (%)	57.1	54.8	58.2	0.75
Viral (%)	29.4	31.0	28.6	
Metabolic and other(%)	13.5	14.1	13.2	
High school diploma (Yes - %)	22.1	19.8	23.1	0.37
Smoking status				
Never (%)	34.9	33.9	35.3	0.06
Current (%)	29.2	31.6	28.1	
Former (%)	35.9	34.5	36.6	
Physical activity				
None (%)	36.1	35.0	36.6	0.72
Little or Moderate (%)	54.3	56.5	53.2	
Intense (%)	9.6	8.5	10.1	
Malnutrition				
None (%)	34.9	33.9	35.3	0.68
Moderate (%)	29.2	31.6	28.1	
Severe (%)	35.9	34.5	36.6	
Energy (kCal/d)	2888 (2116 - 3844)	2905 (1952 - 3852)	2872 (2142 - 3814)	0.85
Energy intake per kg (kCal/kg/d)	37.0 (25.7 - 51.0)	36.3 (25.1 - 50.5)	37.4 (26.4 - 51.3)	0.53
Protein intake per kg (g/kg/d)	1.28 (0.92 - 1.76)	1.29 (0.91 - 1.74)	1.28 (0.93 - 1.78)	0.68
Total fat (grams/d)	113.6 (77.5 - 162.1)	114.3 (79.2 - 168.7)	112.3 (77.2 - 160.6)	0.86
Dietary intake in grams per day*				
Coffee	253.3 (36.0 - 506.1)	270.0 (100.0 - 500.0)	253.3 (20.0 - 515.0)	0.71
Fruit	267.5 (104.3 - 478.0)	242.9 (102.4 - 469.3)	272.5 (106.3 - 480.0)	0.40
Raw vegetables	65.1 (31.5 - 113.1)	70.6 (29.0 - 114.5)	64.1 (32.9 - 112.1)	0.69
Cooked vegetables	74.1 (26.7 - 139.0)	70.0 (24.5 - 133.3)	76.2 (28.1 - 143.7)	0.36
Fish	11.6 (4.4 - 27.2)	11.6 (4.3 - 23.2)	11.6 (5.8 - 29.0)	0.36
Red meat	32.0 (5.8 - 63.4)	30.4 (0.0 - 63)	33.0 (6.9 - 64.1)	0.28
Processed meat	15.3 (4.0 - 33.3)	18.7 (5.3 - 40.0)	13.3 (3.7 - 30.7)	0.040

Note: continuous variables are expressed as medians (interquartile interval) / categorical variables are expressed as percentages

\*: All intakes are per day.

**Table 2:** Dietary patterns derived from principal components analysis: factor loading for food groups (CiRCEstudy – n=562)

Food items*	Diversified	Snacks & Sweets	Prudent	Meat
Crisp bread			0.36	
Bread		0.25		
Cakes	0.26	0.50		
Croissant-like /Danish pastries		0.40		
Appetizers		0.28		
Biscuits		0.35		
Chocolate		0.37		
Sugar, marmalade, honey		0.52		
Sweets		0.45		
Dairy-based sweet puddings		0.46		
High-fat dairy products		0.42		
Low-fat dairy products			0.44	
Cheese		0.28		
Sunflower oil	0.42			
Olive oil	0.25		0.42	
Other vegetable oils	0.48			-0.25
Animal fats				0.64
Salad dressing-light			0.39	
Fruit			0.46	
Raw vegetables	0.53		0.53	
Cooked vegetables	0.54		0.48	
Pulses	0.54			
Potatoes	0.52			
Rice, pasta, semolina	0.51			0.25
Pizza, pies	0.34			
French fries	0.40	0.36		
Sandwiches		0.26		
Fish	0.30		0.38	0.29
Canned fish	0.37			
Shellfishes	0.46			
Ham	0.49			
Offal	0.33			
Processed meat	0.34	0.26		
Red meat				0.82
Poultry				0.82
Soft drinks-regular		0.42		
Juices		0.47		
Mineral water		0.28	0.37	
Tap water	0.26			
Coffee	0.27			
Tea			0.33	
Beer and cider			-0.32	
Wine			-0.28	

\*Eleven food groups and items are not present due to factor loadings for each pattern < 0.25: breakfast cereals, butter, chicory, hot chocolate, eggs, high-alcohol beverages, margarine, mayonnaise, soup, salad dressing-regular and soft drinks-diet.

Note: factor loadings can be interpreted as correlation coefficients: the absolute value indicates the importance of the food group in the dietary pattern. For example, “-0.26” for “other vegetable oil” in the meat pattern means that a person who highly adhere to this pattern, is unlikely to eat “other vegetable oil”.

**Table 3:** Baseline characteristics of study cirrhotic patients by tertiles of dietary patterns (CIRCE study – n=562)

Tertiles	Diversified			p	Snacks &Sweets			p	Prudent			p	Meat			p
	1	2	3		1	2	3		1	2	3		1	2	3	
Adhesion	Weak	Moderate	Strong		Weak	Moderate	Strong		Weak	Moderate	Strong		Weak	Moderate	Strong	
Sample size	185	191	186		185	192	185		185	192	185		185	191	186	
Age (years)	60.8 (12.4)	61.2 (11.7)	59.5 (13.7)	0.24	62.1 (12.8)	62.2 (12.6)	57.5 (12.8)	< 0.01	59.0 (12.8)	61.3 (13.2)	60.8 (13.6)	0.06	62.1 (10.3)	60.9 (13.8)	57.8 (13.8)	< 0.01
Men(%)	63.24	73.82	81.18	< 0.01	70.27	76.56	71.35	0.34	85.41	69.27	63.78	< 0.01	70.27	72.25	75.81	0.48
Time since the diagnosis of cirrhosis (years)	2.8 (1.3 - 6.5)	2.0 (1.0 - 5.9)	2.2 (0.9 - 5.5)	0.04	2.3 (1.1 - 6.1)	2.4 (0.9 - 6.4)	2.4 (1.1 - 5.3)	0.97	1.9 (0.8 - 4.9)	2.5 (1.1 - 5.9)	2.8 (1.2 - 7.3)	< 0.01	2.3 (1.0 - 5.8)	2.4 (1.1 - 5.7)	2.7 (1.0 - 6.5)	0.70
Alcohol intake																
non-drinkers (%)	55.14	38.74	37.10		42.16	40.63	48.11		25.41	53.13	51.89		44.86	41.36	44.62	
<20g/day (%)	21.08	27.75	23.12	< 0.01	26.49	23.44	22.16	0.51	21.08	20.83	30.27	< 0.01	24.86	25.65	21.51	0.83
≥20g/day (%)	23.78	33.51	39.78		31.35	35.94	29.73		53.51	26.04	17.84		30.27	32.98	33.87	
Child-Pugh score	6 (5 - 9)	6 (5 - 8)	6 (5 - 8)	0.13	6 (5 - 8)	6 (5 - 8)	6 (5 - 8)	0.37	6 (5 - 8)	6 (5 - 8)	6 (5 - 8)	0.03	6 (5 - 8)	6 (5 - 8)	6 (5 - 8.75)	0.61
Cirrhosis etiology																
Alcoholic (%)	57.84	54.97	58.60		55.14	55.21	61.08		68.11	53.13	50.27		57.84	57.59	55.91	
Viral (%)	32.97	30.37	24.73	0.16	25.41	30.73	31.89	0.01	23.78	32.29	31.89	< 0.01	31.35	26.70	30.11	0.64
Metabolic and other (%)	9.19	14.66	16.67		19.46	14.06	7.03		8.11	14.58	17.84		10.81	15.71	13.98	
Malnutrition																
None (%)	28.11	35.60	40.86		31.89	36.98	35.68		28.11	43.23	32.97		35.68	31.94	37.10	
Moderate (%)	31.35	31.41	24.73	0.09	28.65	28.13	30.81	0.73	34.59	21.88	31.35	0.02	27.03	29.84	30.65	0.68
Severe (%)	40.54	32.98	34.41		39.46	34.90	33.51		37.30	34.90	35.68		37.30	38.22	32.26	
High school diploma (Yes%)	24.32	20.94	20.97	0.66	20.54	21.88	23.79	0.75	21.62	23.44	21.08	0.85	17.84	23.04	25.27	0.20
Smoking status																
Never (%)	27.57	24.61	25.27		26.49	26.04	24.86		20.54	26.04	30.81		29.73	24.61	23.12	
Current (%)	31.89	27.75	33.87	0.57	30.81	24.48	38.38	0.05	42.70	29.69	21.08	< 0.01	29.73	28.80	34.95	0.42

<b>Former (%)</b>	40.54	47.64	40.86		42.70	49.48	36.76		36.76	44.27	48.11		40.54	46.60	41.94	
<b>Physical activity</b>																
<b>None (%)</b>	41.62	34.55	32.26		33.51	41.15	33.51		36.76	36.98	34.59		42.16	30.37	36.02	
<b>Little or moderate (%)</b>	48.65	57.07	56.99	0.33	58.92	49.48	54.59	0.25	54.59	52.60	55.68	0.96	49.19	60.21	53.23	0.18
<b>Intense (%)</b>	9.73	8.38	10.75		7.57	9.38	11.89		8.65	10.42	9.73		8.65	9.42	10.75	
<b>Energy intake (kCal)</b>	2101 (1490 - 2854)	2763 (2279 - 3475)	3833 (3029 - 4895)	<b>&lt; 0.01</b>	2199 (1583 - 2939)	2712 (2136 - 3427)	3902 (3037 - 5118)	<b>&lt; 0.01</b>	2905 (1941 - 4032)	2609 (1975 - 3426)	3056 (2258 - 4204)	<b>&lt; 0.01</b>	2865 (2017 - 3701)	2729 (1932 - 3554)	3028 (2337 - 4019)	<b>0.03</b>
<b>Energy intake per kg (kCal/kg)</b>	25.34 (18.95 - 36.82)	36.55 (27.16 - 46.64)	50.53 (37.32 - 63.36)	<b>&lt; 0.01</b>	26.85 (20.13 - 38.26)	34.37 (25.26 - 46.58)	50.47 (38.08 - 65.30)	<b>&lt; 0.01</b>	37.14 (24.46 - 53.35)	33.43 (24.23 - 44.04)	40.09 (28.19 - 56.07)	<b>&lt; 0.01</b>	36.13 (25.12 - 52.47)	36.55 (23.70 - 50.33)	38.50 (26.74 - 50.85)	0.47
<b>Glucides intake (grams)</b>	238.9 (179.3 - 329.0)	287.2 (223.6 - 353.4)	383.8 (281.2 - 516.7)	<b>&lt; 0.01</b>	213.7 (164.6 - 279.1)	273.9 (223.2 - 344.2)	423.8 (340.0 - 591.3)	<b>&lt; 0.01</b>	259.9 (202.5 - 356.0)	284.4 (213.2 - 384.5)	343.7 (247.5 - 508.9)	<b>&lt; 0.01</b>	301.6 (215.8 - 434.0)	287.2 (212.7 - 376.8)	295.3 (221.6 - 398.8)	0.28
<b>Proteins intake (grams)</b>	71.6 (52.6 - 95.8)	97.6 (81.9 - 117)	138.3 (113.0 - 170.8)	<b>&lt; 0.01</b>	84.1 (60.6 - 111.3)	96.4 (73.8 - 135.2)	123.4 (97.5 - 162.9)	<b>&lt; 0.01</b>	96.5 (71.6 - 129.4)	94.3 (67.4 - 123.5)	113.4 (85.1 - 147.0)	<b>&lt; 0.01</b>	87.0 (62.9 - 125.2)	97.1 (78.4 - 122.3)	116.7 (89.7 - 147.7)	<b>&lt; 0.01</b>
<b>Protein intake per kg (g/kg)</b>	0.91 (0.62 - 1.27)	1.26 (1.00 - 1.67)	1.76 (1.31 - 2.21)	<b>&lt; 0.01</b>	1.07 (0.73 - 1.53)	1.23 (0.88 - 1.73)	1.56 (1.23 - 2.12)	<b>&lt; 0.01</b>	1.22 (0.83 - 1.73)	1.21 (0.85 - 1.67)	1.53 (1.10 - 1.91)	<b>&lt; 0.01</b>	1.14 (0.74 - 1.67)	1.23 (0.92 - 1.82)	1.49 (1.09 - 1.86)	<b>&lt; 0.01</b>
<b>Lipids intake (grams)</b>	74.0 (44.2 - 105.0)	111.7 (81.5 - 146.1)	168.6 (127.5 - 219.0)	<b>&lt; 0.01</b>	89.7 (55.9 - 130.7)	110.0 (76.9 - 150.0)	147.0 (108.0 - 206.7)	<b>&lt; 0.01</b>	114.0 (75.2 - 160.7)	106.3 (73.0 - 148.2)	120.9 (85.8 - 173.5)	<b>&lt; 0.01</b>	108.0 (75.2 - 172.3)	111.9 (77.4 - 147.8)	120.8 (82.4 - 164.9)	0.11

Note: continuous variables are expressed as medians (interquartile interval) / categorical variables are expressed as percentages

**Table 4:** Risk of hepatocellular carcinoma associated with dietary pattern scores among cirrhotic patients (CIRCE study – n=562)

<b>Pattern</b>	<b>Odd-Ratio</b>	<b>95% Confidence interval</b>	<b>P for linear trend</b>
<b>Diversified</b>			
- Tertile 1	1	.	p = 0.40
- Tertile 2	0.748	[0.435 - 1.280]	
- Tertile 3	1.189	[0.655 - 2.166]	
<b>Snacks &amp; sweets</b>			
- Tertile 1	1	.	p = 0.34
- Tertile 2	0.864	[0.515 - 1.445]	
- Tertile 3	0.736	[0.391 - 1.373]	
<b>Prudent</b>			
- Tertile 1	1	.	p = 0.72
- Tertile 2	0.949	[0.555 - 1.624]	
- Tertile 3	0.901	[0.510 - 1.591]	
<b>Meat</b>			
- Tertile 1	1	.	p = 0.17
- Tertile 2	1.146	[0.684 - 1.922]	
- Tertile 3	1.449	[0.858 - 2.466]	

Note: unconditional regression models were adjusted for: age (as a continuous variable), gender, center, energy intake (kCal), time since the diagnosis of cirrhosis (in tertiles), Child-Pugh score (as a continuous variable), cirrhosis etiology, interaction between cirrhosis etiology and Child-Pugh score, and alcohol consumption (in categories: non –drinkers, <20g/day, ≥20g/day). For this table, tertiles are calculated on the adherence score in the overall population.



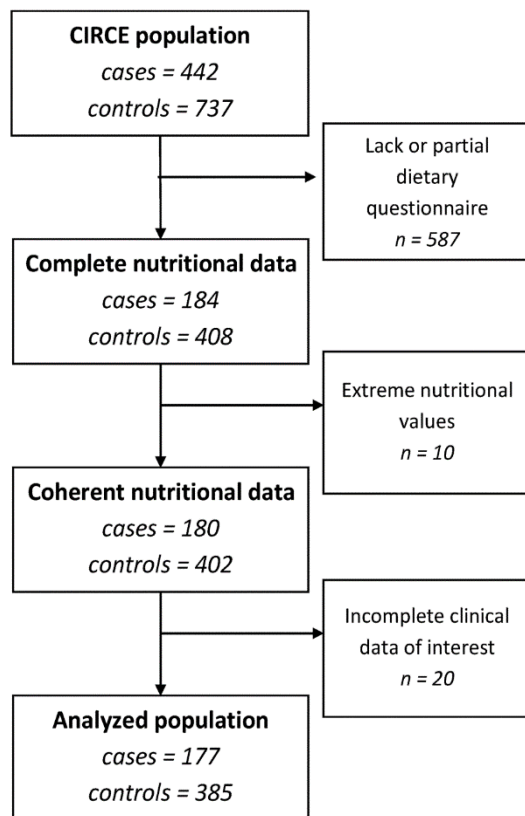
**Appendix 1:** The 54 food groups and food items introduced in the principal component analysis (CIRCE study – n=562)

<b>Food group</b>	<b>Details</b>
Animal fats <sup>1</sup>	Goose, duck fat
Appetizers	Savory biscuits, olives and nuts
Biscuits	Sweet biscuits such as cookies and chocolate-coated biscuits.
Bread	White bread, toast and whole meal bread
Breakfast cereals	Sweetened and unsweetened cereals
Butter, cream <sup>1</sup>	Salted and unsalted butter, cream
Cakes	Cakes, sweet pies
Canned fish	Canned fish: anchovies, sardines, tuna.
Cheese	All sorts except for cottage cheese and yogurt
Chicory	As a hot drink (substitute for coffee)
Chocolate	Chocolates, chocolate bars
Coffee	Espresso, instant, from coffee machines, etc.
Cooked vegetables	Cooked vegetables
Crisp bread	Manufactured rusks
Croissant-like / Danish pastries	Breakfast pastries such as croissants
Dairy-based sweet puddings	Cream or milk desserts, rice or semolina puddings, ice cream
Eggs	Hard-boiled, omelette, etc.
Fish	Fresh or deep- frozen fish
French fries	Homemade, deep-frozen, fast food
Fruits	All fresh, preserved fruits and canned fruits except nuts, olives and juices
Ham <sup>2</sup>	Cured and cooked ham
High-alcohol beverages	Spirits, vodka, gin, whisky, aniseed beverages, and cocktails
High-fat dairy products	Full-fat milk, full-fat yogurt and cottage cheese
Hot chocolate	Mostly milk plus sweetened cocoa powder
Juices	Homemade or commercial pure fruit juice
Low-alcohol beverages	Beer and cider
Low-fat dairy products	Half-fat and semi-skimmed milk, Low-fat yogurt
Margarine <sup>1</sup>	For spread and home cooking
Mayonnaise	Homemade or manufactured
Mineral water	Bottled mineral water, spring water (plain or sparkling)
Offal	Liver, kidney, tongue, etc.
Olive oil <sup>1</sup>	Added for cooking and dressings
Other vegetable oil <sup>1</sup>	Added for cooking and dressing, except olive oil and sunflower oil
Pizza, pies	Pizza, savory tarts and pies
Potatoes	Potatoes, except French fries
Poultry	Chicken, turkey, duck, goose and rabbit
Processed meat <sup>2</sup>	All except ham (sausages, pâté, etc.)
Pulses	Dried peas, lentils
Raw vegetables	Raw vegetables
Red meat	Pork, beef, veal, mutton, lamb
Rice, pasta, semolina	Rice, pasta, wheat or corn semolina

Salad dressing-light	Low-fat manufactured salad dressing
Salad dressing-regular	Manufactured salad dressing
Sandwiches	Including hamburgers
Shellfishes	Shrimps, mussels, etc.
Soft drinks-diet	Soda and fruit beverages (except pure fruit juice) with artificial sweeteners
Soft drinks-regular	Soda and fruit beverages (except pure fruit juice)
Soup	Soups and broths (homemade or commercial)
Sugar, marmalade, honey	Added sugar, honey; home-made and commercial jam and marmalade
Sunflower oil	Added for cooking and dressings
Sweets	Sweets, assorted candy, caramels, toffee, gums, liquorice
Tap water	Tap water
Tea	As a hot drink
Wine	Wine (red and white), champagne

<sup>1</sup> Various types of seasoning and cooking fats were studied separately because of large regional differences, as well as differences in perception as healthy or unhealthy.

<sup>2</sup> Ham was studied separately from other processed meats, as it is often part of a low-energy diet, unlike other processed meats such as sausage or pâté.



**Appendix2** : Flow-chart

**Appendix 3:** Differences between respondents and non-respondents (CIRCE study – n=562)

Variable	All (n = 1179)	Non-analyzed (n = 617)*	Analyzed (n = 562)	p	
Age (years)	Median (IQR)	61.1 (14.5)	61.8 (16.4)	60.4 (13.0)	<b>0.02</b>
Gender	Men (%)	74.7	76.5	72.8	0.16
Time since the diagnosis of cirrhosis (years) **	Median (IQR)	2.2 (0.9 - 5.8)	2.0 (0.8 - 5.3)	2.4 (1.0 - 6.1)	<b>0.03</b>
BMI (kg/m <sup>2</sup> )**	Median (IQR)	28.0 (24.6 – 32.0)	27.6 (24.5 - 31.9)	28.3 (24.8 - 32.0)	0.33
Cancer antecedent (except liver cancer) **	Yes (%)	5.3	5.2	5.3	1.0
Cannabis consumption	No (%)	90.3	90.4	90.2	0.11
	Yes (%)	9.0	8.4	9.6	
	Not declared (%)	0.7	1.1	0.2	
Cirrhosis etiology**	Alcoholic (%)	61.1	64.8	57.1	<b>0.03</b>
	Viral (%)	26.4	23.8	29.4	
	Metabolic and other (%)	12.4	11.4	13.5	
Child-Pugh score **	Median (IQR)	6 (5 - 8)	7 (5 - 9)	6 (5 - 8)	< <b>0.001</b>
HCC (% cases)	Yes (%)	37.5	42.9	31.5	< <b>0.001</b>
High school diploma**	Yes (%)	18.7	15.5	22.1	<b>0.005</b>
Physical activity	None (%)	40.4	44.2	36.1	<b>0.01</b>
	Little or moderate (%)	50.0	46.2	54.3	
	Intense (%)	9.6	9.6	9.6	
Smoking status	Never (%)	24.5	23.3	25.8	<b>0.03</b>
	Current (%)	35	38.6	31.1	
	Former (%)	40.5	38.1	43.1	

\* Due to missing questionnaire, extreme consumption, or missing clinical data

\*\* All missing data were in the non-analyzed group: Child-Pugh (22), cirrhosis etiology (3), history of cancer (1), high school diploma (6), time since the diagnosis of (2), BMI (3)

Note: Only age had a normal distribution, thus it was expressed as means (standard deviation) / other continuous variables are expressed as medians (interquartile interval) / categorical variables are expressed as percentages

**Appendix 4:** Dietary pattern loadings in alcoholic/metabolic cirrhosis subpopulation (n=362)

<b>Food items*</b>	<b>Junk foods</b>	<b>Mediterranean</b>	<b>Meat</b>
Cakes	0.60		
Croissant-like / Danish pastries	0.30		-0.25
Appetizers	0.30		
Biscuits	0.33		
Chocolate	0.37		
Sugar, marmalade, honey	0.53		-0.26
Sweets	0.42		
Dairy-based sweet puddings	0.44		
High-fat dairy products	0.27		-0.32
Low-fat dairy products		0.40	
Cheese	0.34		
Margarine	0.33		
Sunflower oil		0.37	
Olive oil		0.49	
Other vegetable oil		0.40	
Animal fats			0.48
Salad dressing-light		0.38	
Fruits			-0.32
Raw vegetables		0.79	
Cooked vegetables		0.75	
Pulses	0.34	0.31	
Potatoes	0.29	0.37	
Soup		0.27	
Rice, pasta, semolina	0.42	0.27	0.25
Pizza, pies	0.44		
French fries	0.58		
Sandwiches	0.30		
Eggs	0.36		
Fish	0.27	0.40	
Shellfishes	0.32	0.29	
Ham	0.38		
Offal		0.29	
Processed meat	0.43		0.25
Red meat	0.28		0.71
Poultry			0.69
Soft drinks-diet		0.26	
Soft drinks-regular	0.30		
Juices	0.39		-0.27
Mineral water			-0.30
Tap water	0.36		
Coffee	0.35		
Beer and cider			0.31

\*Twelve food groups and items are not present due to factor loadings for each pattern < 0.25: crisp bread, bread, breakfast cereals, butter and cream, mayonnaise, salad dressing-regular, canned fish, hot chocolate, chicory, tea, high-alcohol beverages, and wine

**Appendix 5:** Dietary patterns loadings in viral cirrhosis subpopulation (n=165)

<b>Food items*</b>	<b>Diversified</b>	<b>Snacks&amp; Sweets</b>	<b>Meat</b>
Bread		0.26	
Cakes		0.44	
Croissant-like / Danish pastries		0.29	0.31
Appetizers			0.31
Sugar, marmalade, honey		0.52	
Sweets		0.43	
Dairy-based sweet puddings		0.35	
High-fat dairy products		0.44	
Low-fat dairy products		0.37	
Cheese			0.34
Butter, cream	0.39	0.28	
Sunflower oil	0.32		0.26
Olive oil	0.40	-0.28	
Other vegetable oil	0.58		
Animal fats			0.44
Mayonnaise		0.27	
Salad dressing-light	0.26		
Fruits		0.28	
Raw vegetables	0.58		
Cooked vegetables	0.69		
Pulses	0.55		
Potatoes	0.63		
Soup	0.35		
Rice, pasta, semolina	0.56		0.43
Pizza, pies	0.32		0.40
French fries	0.25		0.36
Sandwiches		0.41	0.40
Eggs	0.33	0.26	
Fish	0.39		
Canned fish	0.34		0.35
Shellfishes	0.59		
Ham	0.34	0.45	
Offal	0.27		0.26
Processed meat		0.43	
Red meat			0.66
Poultry and rabbit			0.69
Soft drinks-regular		0.63	
Juices		0.52	
Mineral water		0.40	
Coffee			0.40
Beer and cider	0.36	0.25	

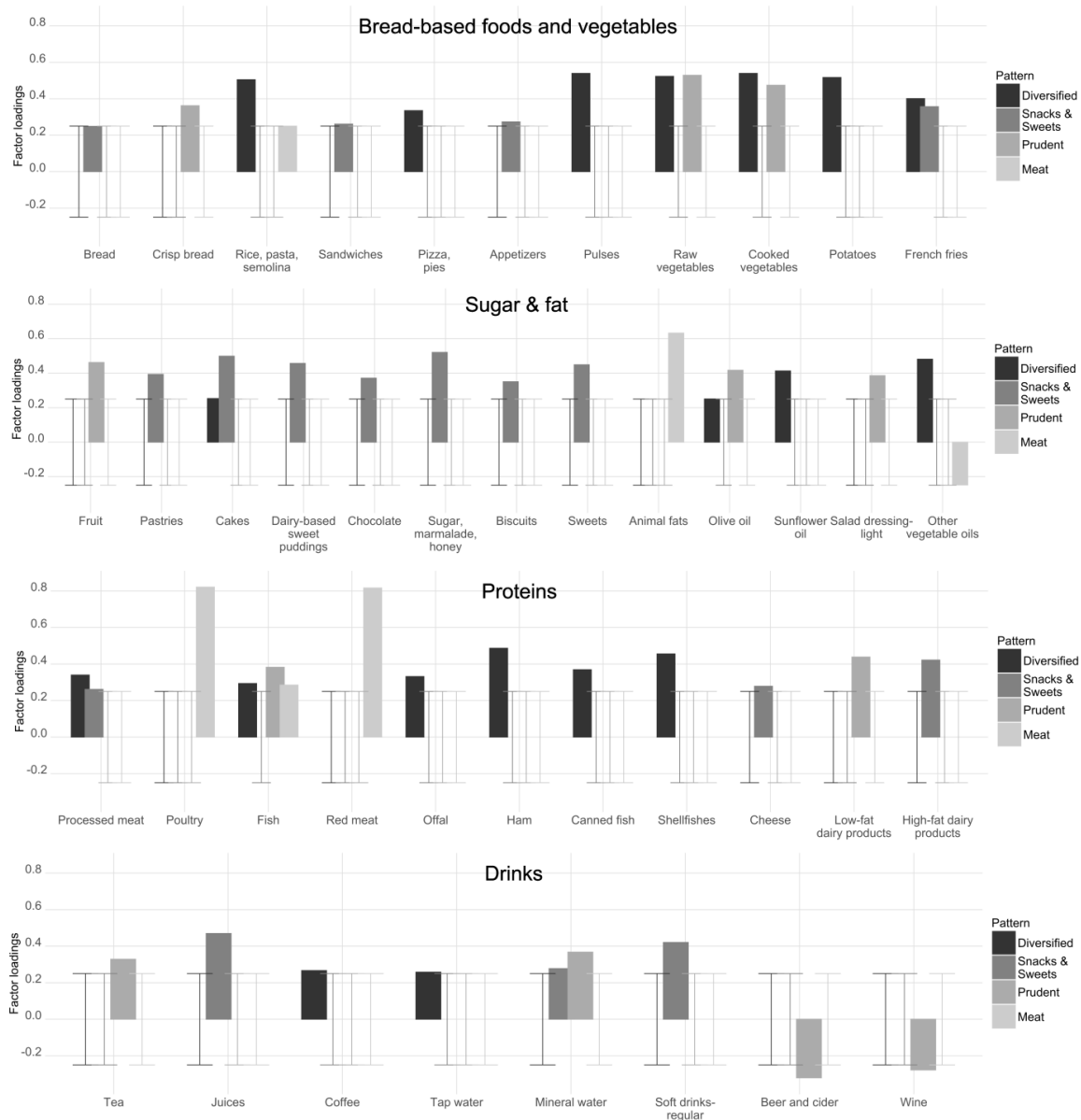
\*Thirteen food groups and items are not present due to factor loadings for each pattern < 0.25: biscuits, breakfast cereals, chocolate, salad dressing-regular, soft drinks-diet, tap water, hot chocolate, chicory, tea, high-alcohol beverages, wine, and margarine.

**Appendix 6:** Risk of hepatocellular carcinoma associated with dietary pattern scores among cirrhotic patients according to cirrhosis etiology

Population	Variable	OR	Confidence interval	p	Linear trend
Alcoholic / metabolic cirrhosis*	<b>Junk foods</b>				
	Tertile 1	1	.	.	p = 0.67
	Tertile 2	1.092	[0.568 - 2.103]	0.789	
	Tertile 3	1.166	[0.578 - 2.360]	0.666	
	<b>Mediterranean</b>				
	Tertile 1	1	.	.	p = 0.80
	Tertile 2	1.245	[0.641 - 2.427]	0.516	
	Tertile 3	1.109	[0.572 - 2.155]	0.757	
	<b>Meat</b>				
Tertile 1	1	.	.	p = 0.20	
Tertile 2	1.405	[0.738 - 2.692]	0.300		
Tertile 3	1.548	[0.797 - 3.032]	0.198		
Viral cirrhosis**	<b>Diversified</b>				
	Tertile 1	1	.	.	<b>p = 0.03</b>
	Tertile 2	0.901	[0.319 - 2.530]	0.843	
	Tertile 3	2.820	[1.023 - 8.199]	<b>0.049</b>	
	<b>Snacks&amp; sweets</b>				
	Tertile 1	1	.	.	p = 0.24
	Tertile 2	0.395	[0.133 - 1.109]	0.084	
	Tertile 3	1.812	[0.635 - 5.409]	0.272	
	<b>Meat</b>				
Tertile 1	1	.	.	p = 0.23	
Tertile 2	1.924	[0.707 - 5.392]	0.203		
Tertile 3	2.012	[0.706 - 5.936]	0.194		

\*unconditional regression models were adjusted for: age (in tertiles), gender, center, time since the diagnosis of cirrhosis (in tertiles), Child-Pugh score (as a continuous variable)

\*\*unconditional regression models were adjusted for: center, time since the diagnosis of cirrhosis (in natural cubic splines with 2 knots), type of virus (HBV or HCV), and alcohol consumption (in categories: non-drinkers, <20g/day, ≥20g/day)



**Appendix 4:** Factor loadings by food groups and patterns.

*Note: food group with no factor loadings superior to 0.25 or inferior to -0.25 were excluded. As factor loadings value between -0.25 and 0.25 means that corresponding food groups do not influence patterns, they are only represented by an error bar.*

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### ***Troisième objectif de thèse***

Utilisation du Diet Inflammatory Index conçu pour estimer l'effet inflammatoire de l'alimentation chez des patients atteints de cirrhose.

Le nouveau Diet Inflammatory Index (DII) est un score développé par Shivappa N. *et al.* qui se calcule en se basant sur la consommation alimentaire de chaque patient. La répétition d'épisodes inflammatoires suite à des agressions de plusieurs origines (virales, alcooliques, cirrhotiques) sont des causes majeures de la cirrhose et de la carcinogenèse hépatique. Les aliments contiennent des substances pro-et anti-inflammatoires. Les Dr Hébert et Shivappa ont développé un score basé sur la consommation alimentaire de chaque individu et permettant d'évaluer le potentiel inflammatoire de l'alimentation. Ce score est basé sur la revue de la littérature de 1943 articles publiés entre 1950 et 2010, il prend en compte l'effet de 45 aliments et nutriments sur la CRP et cinq cytokines : IL-4, IL-1 $\beta$ , IL-6, IL-10 et TNF- $\alpha$  qui augmentent en cas de réponse immunitaire. L'indice inflammatoire d'un régime alimentaire et le risque de CHC chez des patients cirrhotiques a encore été peu exploré. Ainsi, cette étude vise à calculer le potentiel inflammatoire de l'alimentation grâce au DII puis analyser l'impact de la consommation de ces différents aliments sur le risque de développer un CHC chez notre population atteinte de cirrhose. Cet article a été soumis dans la revue American Journal of Clinical Nutrition.

## **Association between Dietary Inflammatory Index and the risk of hepatocellular carcinoma in a French cirrhotic population**

**Maud Rizk, Thomas Mouillot, Brigitte Bernard-Chabert, Jean-Pierre Bronowicki, Carine Richou, Michel Doffoel, Jean-Louis Jouve, Cyril Boisson, James R. Hebert, Nitin Shivappa, Marie-Christine Boutron-Ruault, Mona Diab Asaf, Patrick Hillon, Vanessa Cottet.**

### **Méthodes**

Cette étude porte à nouveau sur 181 cas (avec CHC) et 401 témoins (sans CHC). Le calcul du DII tel qu'il a été conçu par ses auteurs nécessite les données quantitatives de 45 composants alimentaires mais dans notre étude, les scores du DII ont été calculés sur la base de 36 composants dont la consommation était évaluée par le questionnaire type histoire alimentaire. Un score DII positif implique un régime alimentaire pro-inflammatoire alors qu'un score DII négatif implique un régime alimentaire anti-inflammatoire. Compte tenu de la pathologie, un second score DII a été calculé en enlevant le composant alcool et en considérant l'alcool comme une variable d'ajustement. Les Odds Ratio (OR) et leurs intervalles de confiance (CI) à 95% étaient obtenus par régression logistique multivariée avec un ajustement sur : âge, sexe, énergie totale de source non alcoolique, ancienneté de la cirrhose, score du Child-Pugh, diabète, étiologie de la cirrhose et stratifié par centre.

### **Résultats**

Les scores du DII variaient entre -4,77 to 7,59 avec une valeur moyenne de  $1,03 \pm 2,87$  chez les 181 cas et  $0,83 \pm 2,87$  chez les 401 témoins, indiquant un régime pro-inflammatoire dans les deux groupes. Les cas étaient plus âgés que les témoins (64.4 ans vs 59 ans,  $p < 0.001$ ), plus souvent de sexe masculin ( $p < 0.001$ ), diabétiques ( $p < 0.001$ ), moins actif ( $p = 0,005$ ) et souffrant d'hépatite B ( $p = 0,006$ ) que les témoins. L'ancienneté de la cirrhose chez les cas était inférieure à celle des

témoins (médiane respective 1 an [0-5] et 2 ans [1-6]) ( $p < 0.001$ ). En comparaison avec les patients ayant un faible score de DII, les patients ayant un score élevé de DII consommaient moins d'énergie, fruits, légumes, poissons et fruits de mer, lipides, AGMI, oméga 3, oméga 6, AGPI, vitamines A, C, E, B1, acide folique, cuivre, manganèse, potassium et flavonoïdes. Contrairement, ces patients consommaient plus de céréales surtout céréales raffinés, alcool, café, protéine, sodium, phosphore et zinc. Après ajustement, une association significative était mise en évidence entre une alimentation pro-inflammatoire (DII positif) et le risque de développer un CHC, en considérant le DII en continue (OR= 1,14[1,02-1,27],  $p = 0,021$ ) ou en tertiles (OR<sub>t3vst1</sub>= 2,33 [1,16-4,67],  $p$ -trend= 0,021). Des résultats similaires ont été retrouvés avec le score DII sans alcool en continue (OR=1,15 [1,03-1,28],  $p=0,013$ ) ou en tertiles (OR=2,35 [1,18-4,68],  $p$ -trend=0,015). Le score DII était également associé à des biomarqueurs plasmatiques de l'inflammation, tels que la CRP et l'IL-6.

### **Conclusion et perspectives**

La présente étude a montré que le potentiel inflammatoire du régime alimentaire pouvait influencer le risque de CHC chez des patients atteints de cirrhose. Les résultats obtenus ont été validés par une association positive avec des marqueurs biologiques. Nos résultats devront être confirmés en prospectif et dans de plus larges études.

## **Association between the dietary inflammatory index and the risk of hepatocellular carcinoma in a French cirrhotic population**

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Data described in the manuscript, code book, and analytic code will not be made available because all data of this multicentric study are the property of the academic sponsor of the study.

### **Sources of support:**

This work was supported by a French Government grant managed by the French National Research Agency under the program “Investissements d’Avenir” [reference ANR-11-LABX-0021], and also by grants from the “Conseil Régional de Bourgogne”, the “Fonds Européen de Développement Régional” (FEDER), the French National Cancer Institute, the “Ligue Nationale contre le Cancer”, the “Canceropole Grand-Est” and the “Fondation de France”.

### **Abbreviations**

HCC: Hepatocellular Carcinoma

DII: Dietary Inflammatory Index

CiRCE: Cirrhosis and Risk of Hepatocellular Carcinoma in the East

OR: Odds ratios

CI: Confidence Intervals

HBV: Hepatitis B virus

HCV: Hepatitis B virus

IL-1 $\beta$ : Interleukin-1 $\beta$

TNF- $\alpha$ : Tumor Necrosis Factor- alfa

CRP: C-Reactive Protein

PUFA: Polyunsaturated Fatty Acids

BMI: Body Mass Index

### **Clinical Trial Registry**

This study was registered in [clinicaltrials.gov](http://clinicaltrials.gov) as NCT01798173

1 **Abstract**

2 **Background:** Hepatocellular carcinoma (HCC) is recognized as an inflammation-related  
3 cancer. However, the relation between inflammation deriving from the diet and HCC risk  
4 among cirrhotic patients has not yet been investigated.

5 **Objective:** This study aimed to examine the inflammatory potential of diet evaluated by the  
6 dietary inflammatory index (DII<sup>®</sup>) and its association with HCC risk among cirrhotic patients.

7 **Design:** Clinical and dietary data were collected from the French case-control study CiRCE  
8 (Cirrhosis and Risk of hepatocellular Carcinoma in the East), which included 401 cirrhotic  
9 patients without HCC (controls) and 181 cirrhotic patients with HCC (cases) recruited between  
10 2008 and 2012 in six French university hospitals. DII scores (36 food items and nutrients) were  
11 assessed using a validated self-administered diet history questionnaire. Odds ratios (OR) and 95%  
12 confidence intervals (CI) were estimated with logistic regression models (adjusted for age,  
13 gender, time from cirrhosis diagnosis, etiology of cirrhosis, Child-Pugh score, and diabetes).

14 **Results:** The DII scores ranged between -4.77 to +7.59 with a mean value of  $1.03 \pm 2.87$  among  
15 cases and  $0.83 \pm 2.87$  among controls, indicating that both groups were following a pro-  
16 inflammatory diet. After multi-variable adjustment, there was a positive association between  
17 HCC risk and the DII score when considered as a continuous variable ( $OR_{\text{continuous}}=1.14$  [1.02-  
18 1.27],  $p\text{-value}=0.021$ ) or categorized in tertiles ( $OR_{T3vsT1}=2.33$ [1.16-4.67],  $p\text{-trend}=0.021$ ).  
19 Similar results were found when alcohol was omitted from the DII calculation but considered as a  
20 co-variate:  $OR_{\text{continuous}}=1.15$  [1.03-1.28],  $p\text{-value}=0.013$ , and  $OR_{T3vsT1}=2.35$  [1.18-4.68],  $p\text{-}$   
21  $\text{trend}=0.015$ . A positive correlation was observed between the DII scores and two inflammatory  
22 biomarkers (CRP, IL-6) among controls.

23 **Conclusion:** The present study reported an association between a pro-inflammatory DII score  
24 and the risk of HCC in cirrhotic patients. Correlations between the scores and biological  
25 parameters support this result. It should be confirmed in larger prospective studies, and could lead  
26 to nutritional prevention in cirrhotics.

27 **Keywords:** Case-control study, hepatocellular carcinoma, cirrhosis, DII scores, pro-inflammatory  
28 diet.



## 29 **Introduction**

30 Hepatocellular carcinoma (HCC) is considered an inflammation-related cancer. It occurs in the  
31 setting of chronic liver inflammation that share cirrhosis as an endpoint in 80 to 90% of patients  
32 with HCC(1). Worldwide, HCC is the fifth most frequently diagnosed cancer in men and the  
33 ninth in women. The estimated number of new cases of liver cancer in 2018 was 596 574 in men  
34 and 244 506 in women, while the estimated number of deaths was 548 375 and 233 256  
35 respectively according to GLOBOCAN 2018(2). HCC incidence has increased over the past  
36 decades as a result of infections with hepatitis B (HBV) or C (HCV) viruses, consumption of  
37 food contaminated with aflatoxin, obesity, the metabolic syndrome, and alcoholic liver  
38 disease(3,4).

39 Progression from the status of fibrosis to cirrhosis, and finally to HCC involves a complex,  
40 chronic, non-resolving inflammation, and persistent hepatic injuries(5). Epidemiological evidence  
41 confirms that chronic inflammation plays a double role in cancer proliferation. It affects the  
42 initiation, progression, and proliferation of cancer cells, and it decreases the efficacy of the  
43 immune system. The tumor micro-environment is composed of several types of immune cells  
44 (mainly macrophages), cytokines (e.g. interleukin-1 $\beta$  (IL-1 $\beta$ ), Tumor Necrosis Factor-alfa (TNF-  
45  $\alpha$ )), and reactive mediators. Once the inflammatory cascade is activated, inflammatory signals are  
46 continuously emitted and, in the presence of reactive oxygen species, the injured tissue is altered,  
47 in turn favoring the transformation of premalignant cells into cancer cells(6,7).

48 The diet has an influence on the development of diseases through its influence on immune  
49 responses(8). The Dietary Inflammatory Index (DII®) score was designed to evaluate the  
50 inflammatory potential of an individual's overall diet. It is based on a review of the literature

51 examining the association between various dietary components and six known inflammatory  
52 markers: C-reactive protein (CRP), IL-4, IL-1 $\beta$ , IL-6, IL-10, and TNF- $\alpha$ (9).

53 Several studies have reported positive associations between the DII score and cardiovascular  
54 diseases, the metabolic syndrome, or cancers such as lymphoma, and prostate, breast, ovarian,  
55 gastric, pancreatic, and colorectal cancers(10–16).The DII score and its association with HCC  
56 have been examined in only two articles (17,18). They both compared HCC in patients with and  
57 without liver disease and found that a pro-inflammatory diet (higher DII score) was associated  
58 with the risk of HCC especially among men, in patients not infected by hepatitis B or C(17),  
59 patients with lower education levels, smokers, and non-drinkers of alcohol (18). However, the  
60 possible relationship between exposure to an inflammatory diet and HCC risk in cirrhotic patients  
61 has not yet been investigated. Patients with cirrhosis certainly have specific dietary consumption  
62 profiles and have a high risk of developing cancer. Accordingly, the present study aimed to  
63 examine the inflammatory potential of diet (assessed by the DII score) in a high-risk population,  
64 and to investigate the association between the DII score and the HCC risk in cirrhotic patients.

65

## 66 **Subjects and Methods**

### 67 **Study sample**

68 The French multicenter case-control study CiRCE, “Cirrhosis and Risk of Hepatocellular  
69 Carcinoma in the East”, was conducted between June 2008 and December 2012 in six university  
70 hospitals in north-eastern France (Besancon, Dijon, Metz, Nancy, Reims, and Strasbourg). The  
71 characteristics of this population have been previously reported (19,20). Patients were at least 35  
72 years old and had cirrhosis, either histologically proven or, in the absence of a liver biopsy,  
73 established from clinical, morphological, and biological data. Cases were cirrhotic patients with

74 HCC that was newly diagnosed in one of the participating hospitals through histology,  
75 echography, scanner, magnetic resonance imaging (MRI), or alpha-fetoprotein (AFP) according  
76 to criteria defined by the European Association for the Study of the Liver, European Organization  
77 for Research and Treatment of Cancer (EASL-EORTC)(21). Controls were cirrhotic patients  
78 without HCC at inclusion. The absence of HCC was assessed through high-quality imaging  
79 examinations (abdominal ultrasound, computerized tomography or MRI), and AFP below  
80 100ng/mL within the two months preceding inclusion. Patients were excluded if they had  
81 progressive extrahepatic cancer, primary liver cancer other than HCC, HCC without cirrhosis,  
82 acute alcoholic hepatitis, human immunodeficiency virus infection, or other severe diseases  
83 resulting in an estimated life expectancy of less than one year. For the present study, only  
84 participants who completed the dietary questionnaire were included (n=592) (20). Patients with  
85 extreme values (1<sup>st</sup> and 99<sup>th</sup> percentiles) for the ratio between their energy intake and energy  
86 requirement (EIER) were excluded (n=10). EIER and basal metabolic rate were calculated based  
87 on height, weight, physical activity, and occupational physical activity. Thus, the final analyses  
88 included 401 cirrhotic patients without HCC (controls) and 181 cirrhotic patients with HCC  
89 (cases). All patients read and signed an informed consent form before inclusion. The study  
90 protocol was approved by the local ethics committee (Comité de Protection des Personnes Est I)  
91 and by the French legal authorities (Commission Nationale de l'Informatique et des Libertés).

92

### 93 **Biological analyses**

94 An overnight-fasting blood sample was taken from both HCC cases and controls (Results for  
95 CRP and the other biomarkers were available for 554 and 560 patients respectively) and aliquots  
96 were separated immediately by centrifugation and stored at -80 °C until centralized analysis in  
97 the coordinating centre (CRB-Dijon). Serum CRP was measured using conventional laboratory

98 techniques. Plasma levels of five other cytokines were assayed using a commercially available  
99 enzyme-linked immunosorbent assay (ELISA) kit (Quantikine; R&D Systems Inc., Minneapolis,  
100 MN, USA) according to the manufacturer's instructions. All samples were assayed in duplicate.  
101 The intra- and inter-assay coefficients of variation were below 5% for all measurements. All  
102 samples were analysed by trained staff blinded to the case-control status. Control and case  
103 samples were mixed in batches of <50 samples.

104

### 105 **Data collection**

106 At inclusion, every patient underwent a clinical examination, blood tests, and anthropometric  
107 measurements by physicians and qualified investigators. They completed a standardized  
108 questionnaire concerning clinical and sociodemographic data, family history of cancer and of  
109 diabetes, smoking status, and physical activity. They had to fill-in a self-administered validated  
110 diet history questionnaire(22,23). It evaluated quantitative and qualitative food consumption  
111 according to the patients' eating habits for the year before inclusion, and allowed for seasonal  
112 variations. It recorded all meals and snacks throughout the day and was accompanied by a  
113 booklet of photographs to help patients assess portion sizes for 208 food items. Food portions and  
114 the frequency of consumption (11 possibilities, from never to 7 times per week) were converted  
115 into daily intake in grams, total energy intake, alcohol, and the breakdown for 23 nutrients  
116 (protein, carbohydrate, fat, vitamins, minerals, etc) using the French CIQUAL composition table  
117 (version 2008 and 2017)(24); an online database for the polyphenol contents in foods was also  
118 used to provide details about polyphenol intake(25). Trans-fatty acids were assessed from  
119 different sources including the USDA Standard Release database and manufacturer data(26,27).

120

### 121 **Dietary Inflammatory Index**

122 The Dietary Inflammatory Index was the first score to evaluate the inflammatory potential of an  
123 individual's diet. The development and validation of the DII have been described elsewhere(9).  
124 Briefly, it is based on a wide-ranging literature review of 1943 articles published between 1950  
125 and 2010, and it takes into consideration the effect of 45 dietary components on six well-known  
126 inflammatory biomarkers (IL-1 $\beta$ , IL-4, IL-6, IL-10, TNF- $\alpha$  and CRP) that increase in case of non-  
127 specific immune response. The 45 dietary components were scored according to whether they  
128 increased (+1), decreased (-1), or had no effect (0) on inflammation. The final DII score for each  
129 participant was calculated by summing up all the calculated scores for each food component. A  
130 high DII score indicates a pro-inflammatory diet and conversely a low DII score denotes an anti-  
131 inflammatory diet. Only intake from foods, and not from supplements, was used in the DII  
132 calculations(9). In the present study, 36 components of the 45 were available to calculate the DII  
133 score: carbohydrates, proteins, total fat, saturated fatty acids, monounsaturated fatty acids  
134 (MUFA), polyunsaturated fatty acids (PUFA),  $\omega$ -3 fatty acids,  $\omega$ -6 fatty acids, alcohol, dietary  
135 fiber, cholesterol, vitamins A, B-6, B-12, C, D, and E, thiamin, riboflavin, niacin, iron,  
136 magnesium, zinc, selenium, folic acid,  $\beta$ -carotene, onion, green/black tea, caffeine, trans-fatty  
137 acids, isoflavones, flavan-3-ol, flavones, flavonols, flavanones, and anthocyanidins. Food  
138 components not included were: energy, eugenol, garlic, ginger, saffron, turmeric, pepper,  
139 thyme/oregano and rosemary.

140

#### 141 **Statistical analysis**

142 Occupational physical activity and leisure time physical activity were categorized as: inactive  
143 (<1h/week), moderately active (1h-3h30/week), and active (>3h30/week). Body mass index  
144 (BMI) was calculated as body weight in kilograms divided by the square of height in meters, and  
145 respondents were considered as: underweight/normal weight, overweight, or obese (respectively:

146  $\leq 25$  kg/m<sup>2</sup>,  $>25$  and  $<30$  kg/m<sup>2</sup>,  $\geq 30$  kg/m<sup>2</sup>). Time from diagnosis of cirrhosis was  
147 dichotomized according to the median:  $< 2$  or  $\geq 2$  years. The underlying cause of cirrhosis was  
148 divided into 3 categories which were determined according to medical records: alcohol abuse  
149 (alone), viral infection (associated or not with alcohol abuse), and other causes (autoimmune  
150 hepatitis, hemochromatosis, Wilson's disease, rare viral liver infections, parasitic infection, toxic  
151 chemicals, and some medications). Smoking status was categorized as: never, current, or former  
152 (former smoker was considered as having quit smoking for a period  $>6$  months before inclusion).  
153 For alcohol status, consumption was considered as abusive for more than 2 glasses/day for  
154 women and 3 glasses/day for men). Two groups were established for educational level:  $\leq 12$  years  
155 of schooling or over 12 years and high-school diploma. BMI missing data (2 patients) were  
156 handled by imputation with linear regression method.

157 The DII was analyzed both as a continuous variable and by tertiles of exposure computed among  
158 controls. Continuous variables were expressed as medians with interquartile ranges or as means  
159 with standard deviations, and qualitative variables as percentages. Associations between DII and  
160 individual characteristics of cases and controls, demographic, and clinical data were examined  
161 using the Chi-square test for qualitative variables (Fisher's exact test if conditions were not met)  
162 and the Anova test (or Kruskal-Wallis test in case of non-normal variables) or Student's test  
163 (Wilcoxon test, in case of non-normal variables) for continuous variables. A nonparametric test  
164 for trend (Cuzick's test) was used to assess the association with the biomarkers across DII score  
165 tertiles.

166 All covariates associated with DII or HCC risks ( $P$ -value  $\leq 0.20$ ) as well as potential confounding  
167 factors identified in the literature were retained for backward selection. To explore the  
168 relationships between DII and HCC risk, an unconditional logistic regression model was used to  
169 estimate odds ratios (OR) and 95% confidence intervals (95% CI). The linear trend was estimated

170 using the median values within each tertile of the DII score, thereby taking the unequal distances  
171 between tertiles into account.

172 Interactions between the DII and age, gender, and cirrhosis etiology were evaluated in separate  
173 analyses by including interaction terms in the final model.

174 All p-values were based on two-tailed statistical tests and a p-value of <0.05 was considered  
175 statistically significant. Analyses were performed with SAS® software, version 9.4 (SAS  
176 Institute Inc., Cary, NC).

177

## 178 **Results**

179 The present analyses included 582 cirrhotic patients, of whom 181 had HCC at inclusion and 401  
180 no HCC. HCC patients were older than controls, with median ages of 64.4 years and 59.0 years,  
181 respectively. HCC cases were more often male, diabetic, classified A on the Child-Pugh score,  
182 less active, and infected with HBV. The median time since cirrhosis diagnosis was 1 year [0-5]  
183 for HCC cases and 2 years [1-6] for controls (p<0.001) (**Table1**). The DII value for the whole  
184 population ranged from -4.77 to +7.59 with a mean value of  $0.90 \pm 2.87$ . The mean DII value was  
185  $1.03 \pm 2.87$  among cases, and  $0.83 \pm 2.87$  among controls, indicating that both groups were  
186 following a pro-inflammatory diet, though cases had slightly higher DII scores.

Table 1. Baseline socio-demographic, clinical and medical characteristics of the 582 cirrhotic patients from the CiRCE study.

Variables, n (%)	Cirrhotic Controls n=401 <sup>1</sup>	HCC Cases n=181 <sup>1</sup>	p-value <sup>2</sup>
<b>Age, years<sup>3</sup></b>	59.0 [51.8-64.4]	64.4 [58.2-70.1]	<0.001
<b>Gender</b>			<0.001
Male	267 (66.6)	156 (86.2)	
<b>Education level</b>			0.092
≤12 years of schooling	337 (84.3)	162 (89.5)	
> 12 years or high school diploma	63 (15.7)	19 (10.5)	
<b>Occupational physical activity</b>			0.005
Inactive	285 (71.4)	143 (80.3)	
Moderately active	76 (19.1)	15 (8.4)	
Active	38 (9.5)	20 (11.3)	
<b>Leisure-time physical activity</b>			0.472
Inactive	192 (47.9)	80 (44.2)	
Moderately active	94 (23.4)	40 (22.1)	
Active	115 (28.7)	61 (33.7)	
<b>BMI,</b>			0.187
Underweight/normal weight (≤25 kg/m <sup>2</sup> )	138 (34.4)	59 (32.6)	
Overweight (>25 and <30 kg/m <sup>2</sup> )	161 (40.2)	63 (34.8)	
Obese (≥ 30 kg/m <sup>2</sup> )	102 (25.4)	59 (32.6)	
<b>Smoking status</b>			0.057
Never	112 (27.9)	36 (19.9)	
Current	129 (32.2)	56 (30.9)	
Former	160 (39.9)	89 (49.2)	
<b>Alcohol status</b>			0.112
Never	28 (7.0)	6 (3.3)	
Non-abusive	127 (31.7)	51 (28.2)	
Abusive	246 (61.3)	124 (68.5)	
<b>DII score</b>			0.410
-4.77- -0.80	133 (72.7)	50 (27.3)	
-0.80- 2.19	134 (67.0)	66 (33.0)	
2.19-7.59	134 (67.3)	65 (32.7)	
<b>Time since cirrhosis diagnosis</b>			<0.001
< 2 years	160 (39.9)	107 (59.4)	
≥ 2 years	241 (60.1)	73 (40.6)	
<b>Cirrhosis etiology</b>			0.193
Virus infection associated with alcohol abuse	122 (30.4)	55 (30.4)	
Alcohol abuse	215 (53.6)	107 (59.1)	
Other <sup>4</sup>	64 (16.0)	19 (10.5)	
<b>Chronic C Hepatitis</b>	109 (27.2)	42 (23.2)	0.311
<b>Chronic B Hepatitis</b>	16 (4.0)	15 (8.3)	0.033
<b>Diabetic status</b>	128 (32.5)	88 (49.2)	<0.001
<b>Child-Pugh Score</b>			0.006
A	210 (54.1)	122 (68.1)	
B	126 (32.5)	42 (23.5)	
C	52 (13.4)	15 (8.4)	

<sup>1</sup>Total number may differ due to missing data: Education level (1 missing); Occupational physical activity (5 missing); cirrhosis diagnosis (1 missing); Hepatitis B virus (1 missing); Diabetic status (9 missing); Child-Pugh score (15 missing)

<sup>2</sup>P-values were derived from the Chi-square test

<sup>3</sup>Values are median [IQR], p-value was obtained using the Wilcoxon test

<sup>4</sup>Other: autoimmune hepatitis, hemochromatosis, Wilson's disease, rare viral liver infections, parasitic infection, toxic chemicals, and some medications



188 Characteristics of the participants were presented across tertiles for the DII score (**Table2**).  
189 Patients in the third tertile were older than the other tertiles. Differences were minor and non-  
190 significant, and borderline for cirrhosis etiology, HCV and Child-Pugh.

Table 2. Baseline population characteristics by energy-adjusted DII tertiles, the CiRCE study.

Characteristics, n(%)	DII score			p-value <sup>2</sup>
	DII tertile 1 (-4.77/-0.80) n=183 <sup>1</sup>	DII tertile 2 (-0.80/2.19) n=200 <sup>1</sup>	DII tertile 3 (2.19/7.59) n=199 <sup>1</sup>	
<b>Gender</b>				0.636
Male	134 (73.2)	149 (74.5)	140 (70.4)	
<b>Age, years<sup>3</sup></b>	58.9 [51.5-65.9]	60.4 [54.2-67.6]	62.2 [55.6-66.7]	0.021
<b>Education level</b>				0.388
≤12 years of schooling	153 (83.6)	176 (88.4)	170 (85.4)	
> 12 years or high school diploma	30 (16.4)	23 (11.6)	29 (14.6)	
<b>Occupational Physical activity</b>				0.091
Inactive	126 (69.2)	145 (73.2)	157 (79.7)	
Moderate active	37 (20.3)	28 (14.2)	26 (13.2)	
Active	19 (10.5)	25 (12.6)	14 (7.1)	
<b>Leisure-Time Physical activity</b>				0.763
Inactive	80 (43.7)	93 (46.5)	99 (49.8)	
Moderate active	47 (25.7)	44 (22.0)	43 (21.6)	
Active	56 (30.6)	63 (31.5)	57 (28.6)	
<b>BMI</b>				0.434
Underweight/normal weight (≤25 kg/m <sup>2</sup> )	53 (29.0)	68 (34.0)	76 (38.2)	
Overweight (>25 and <30 kg/m <sup>2</sup> )	77 (42.1)	75 (37.5)	72 (36.2)	
Obese (≥ 30 kg/m <sup>2</sup> )	53 (28.9)	57 (28.5)	51 (25.6)	
<b>Medical History</b>				
<b>Time since cirrhosis diagnosis,</b>				0.728
<2 years	80 (44.0)	96 (48.0)	91 (45.7)	
≥2 years	102 (56.0)	104 (52.0)	108 (54.3)	
<b>Cirrhosis etiology</b>				0.057
Virus Infection (with or without alcohol abuse)	65 (35.5)	58 (29.0)	54 (27.1)	
Alcohol	96 (52.5)	104 (52.0)	122 (61.3)	
Other <sup>4</sup>	22 (12.0)	38 (19.0)	23 (11.6)	
<b>Diabetes</b>	63 (34.8)	74 (37.0)	79 (41.2)	0.437
<b>Chronic B hepatitis</b>	7 (3.8)	12 (6.0)	12 (6.0)	0.547
<b>Chronic C hepatitis</b>	59 (32.2)	48 (24.0)	44 (22.1)	0.058
<b>Smoking status</b>				0.708
Never	48 (26.2)	53 (26.5)	47 (23.6)	
Current	55 (30.1)	59 (29.5)	71 (35.7)	
Former	80 (43.7)	88 (44.0)	81 (40.7)	
<b>Alcohol status</b>				0.260
Never	10 (5.5)	14 (7.0)	10 (5.0)	
Non-abusive	58 (31.7)	69 (34.5)	51 (25.6)	
Abusive	115 (62.8)	117 (58.5)	138 (69.3)	
<b>Child-Pugh Score</b>				0.060
A	96 (53.9)	128 (65.3)	108 (56.0)	
B	64 (36.0)	44 (22.5)	60 (31.1)	
C	18 (10.1)	24 (12.2)	25 (12.9)	

<sup>1</sup>Total number may differ due to missing data: Education level (1 missing); Occupational physical activity (5 missing); cirrhosis diagnosis (1 missing); Hepatitis B virus (1 missing); Diabetic status (9 missing); Child-Pugh score (15 missing)

<sup>2</sup>P-values were derived from the Chi-square test

<sup>3</sup>Values are median [IQR], p-value was obtained using the kruskal Wallis test

<sup>4</sup>Other: autoimmune hepatitis, hemochromatosis, Wilson's disease, rare viral liver infections, parasitic infection, toxic chemicals, and some medications

191 **Table 3** presents the consumption of food groups and nutrients in medians with their IQR, and p-  
192 value from Kruskal-Wallis test when the conditions were not met for the Anova test. However,  
193 due to a large number of non-consumers, we also presented means and SD for some food groups  
194 with a particular distribution. Compared to participants in the first DII tertile, patients in the third  
195 tertile had lower intakes of energy, fruit, vegetables, fish and seafood, dietary fiber, lipids,  
196 MUFAs, omega-3 and omega-6, PUFAs, vitamins A, C, E, and B1, folic acid, copper,  
197 manganese, potassium, and flavonoids. Inversely, a higher DII score was associated with higher  
198 intakes of cereals (mainly refined grains), alcohol, coffee, proteins, sodium, phosphorus, and zinc.  
199 When analyzing different patterns of alcohol consumption according to DII tertiles, patients in  
200 the third tertile were mainly former or current drinkers. Among current drinkers, heavy drinkers  
201 mostly belonged to the third tertile.

Table 3. Consumption of food groups and micronutrients by DII tertiles calculated for 1000 kcal of daily energy intake from nonalcoholic sources

	DII score			P-value <sup>2</sup>
	Tertile 1 n=183	Tertile 2 n=200	Tertile 3 n=199	
Food groups <sup>1</sup>				
Energy (kcal/day)	3851.49 [3067.35-5182.57]	2685.70 [2160.27-3188.75]	1830.67 [1398.17-2386.03]	<0.001
Alcohol (g/day)	9.95 [0.00-130.37]	2.15 [0.00-153.72]	0.00 [0.00-241.11]	0.696
(mean±SD)	116.34 ± 208.56	139.60 ± 320.32	205.75 ± 419.77	0.023
Added sugar (g/day)	6.14 [1.51-12.40]	4.90 [0.04-13.42]	3.19 [0.00-13.88]	0.057
Desserts (g/day)	44.26 [18.26-79.47]	34.62 [13.19-70.45]	33.32 [8.77-77.45]	0.144
Carbonated beverages (ml/day)	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.147
(mean±SD)	19.66 ± 64.68	14.86 ± 72.02	18.84 ± 74.97	0.774
Coffee (ml/day)	70.80 [17.01-139.07]	106.22 [18.63-207.02]	115.30 [12.53-237.73]	0.004
All cereals (g/day)	47.20 [30.76-65.84]	61.09 [38.38-86.68]	75.95 [41.68-102.38]	<0.001
Dairy products (g/day)	90.14 [50.39-131.61]	94.41 [47.81-155.59]	85.58 [48.11-166.86]	0.505
Total meat (g/day)	24.11 [11.79-41.21]	24.26 [11.18-44.49]	22.15 [5.82-46.98]	0.425
Processed meat (g/day)	15.91 [8.61-23.89]	13.59 [7.35-22.93]	16.78 [5.61-29.93]	0.381
Red meat (g/day)	13.07 [5.24-21.99]	13.47 [4.07-28.05]	8.06 [0.00-28.05]	0.067
Fish and seafood (g/day)	10.37 [5.19-17.68]	7.90 [3.94-13.46]	6.63 [2.65-15.89]	0.002
Eggs (g/day)	8.73 [4.48-14.39]	9.84 [4.72-16.99]	9.27 [3.10-22.12]	0.457
Fruits (g/day)	104.52 [57.46-179.63]	87.26 [35.42-174.67]	62.42 [10.39-140.99]	<0.001
Vegetables (g/day)	94.33 [58.48-153.77]	99.08 [59.12-157.52]	75.87 [33.64-161.83]	0.029
Protein (g/day)	36.07 [32.58-40.29]	38.44 [33.83-43.37]	38.31 [32.71-44.33]	0.005
Carbohydrate (g/day)	112.52 [97.09-126.74]	114.47 [101.30-128.66]	116.27 [98.20-139.15]	0.071
Fat (g/day)	45.18 [38.74-51.39]	42.91 [36.48-48.57]	40.90 [33.35-48.61]	<0.001
Saturated fat (g/day)	17.64 [14.76-21.07]	17.19 [13.62-20.97]	17.06 [13.51-22.77]	0.361
Monounsaturated fat (g/day)	15.04 [13.18-17.75]	14.58 [12.39-16.71]	13.48 [10.59-16.01]	<0.001
Trans-fat (g/day)	0.70 [0.44-1.07]	0.64 [0.27-1.24]	0.58 [0.17-1.20]	0.185
Polyunsaturated fat (g/day)	7.46 [5.76-9.65]	6.88 [5.01-9.14]	5.24 [4.03-7.14]	<0.001
N-3 PUFA (g/day)	0.62 [0.47-0.87]	0.53 [0.37-0.70]	0.43 [0.31-0.55]	<0.001
N-6 PUFA (g/day)	6.11 [4.61-7.95]	5.33 [3.79-7.47]	4.42 [2.94-6.63]	<0.001
Fiber (g/day)	10.03 [7.93-11.76]	9.35 [7.59-11.35]	7.86 [6.33-9.82]	<0.001
Cholesterol (mg/day)	160.63 [129.55-201.71]	169.08 [131.83-205.16]	176.85 [132.76-228.35]	0.149
Micronutrients <sup>1</sup>				
Vitamin B1 (mg/day)	0.58 [0.51-0.67]	0.58 [0.50-0.67]	0.54 [0.46-0.65]	0.007
Vitamin B2 (mg/day)	0.86 [0.75-1.08]	0.93 [0.76-1.11]	0.91 [0.77-1.10]	0.379
Vitamin B3 (mg/day)	7.71 [6.44-9.74]	8.14 [6.24-10.40]	7.24 [5.92-9.85]	0.057
Vitamin B5 (mg/day)	2.53 [2.21-2.98]	2.66 [2.33-3.03]	2.62 [2.21-3.19]	0.288
Vitamin B6 (mg/day)	0.77 [0.69-0.92]	0.77 [0.66-0.88]	0.74 [0.60-0.89]	0.059
Vitamin B9 (µg/day)	169.03 [145.04-204.32]	165.27 [136.18-192.89]	144.55 [117.56-177.46]	<0.001
Vitamin B12 (µg/day)	2.79 [1.90-4.49]	2.69 [1.79-3.99]	2.56 [1.68-4.01]	0.239
Vitamin D (µg/day)	1.27 [1.01-1.63]	1.19 [0.95-1.53]	1.20 [0.84-1.63]	0.198
Vitamin C (mg/day)	67.81 [49.42-97.35]	58.79 [36.04-88.71]	40.39 [24.73-57.38]	<0.001
Vitamin A (µg/day)	438.83 [273.06-693.85]	398.11 [226.64-639.44]	348.77 [234.10-624.47]	0.042
Vitamin E (mg/day)	6.83 [5.30-9.61]	6.10 [4.46-8.08]	4.59 [3.30-6.28]	<0.001
Calcium (mg/day)	408.02 [330.05-491.60]	422.02 [350.87-512.97]	418.66 [308.72-533.85]	0.411
Copper (mg/day)	0.78 [0.66-0.97]	0.75 [0.64-0.96]	0.68 [0.60-0.93]	0.003

Iron (mg/day)	6.23 [5.30-7.78]	6.44 [5.17-7.58]	6.04 [4.92-8.14]	0.727
Selenium (µg/day)	24.14 [21.14-29.04]	25.07 [21.68-29.76]	25.79 [21.34-32.29]	0.066
Magnesium (mg/day)	134.21 [120.38-157.45]	138.62 [121.98-161.97]	133.80 [113.56-157.45]	0.095
Manganese (mg/day)	2.49 [1.95-3.24]	2.15 [1.64-2.82]	2.07 [1.52-2.71]	<0.001
Sodium (mg/day)	1112.66 [954.14-1283.16]	1203.55 [1050.31-1411.39]	1253.46 [1068.92-1528.18]	<0.001
Phosphorus (mg/day)	567.98 [510.29-635.28]	590.23 [527.93-669.02]	596.27 [508.54-691.90]	0.046
Potassium (mg/day)	1568.40 [1348.26-1875.31]	1577.54 [1337.25-1883.49]	1407.53 [1162.92-1781.30]	<0.001
Iodine (mg/day)	56.14 [47.72-68.43]	59.24 [48.83-71.23]	58.16 [47.68-74.19]	0.364
Zinc (mg/day)	4.37 [3.75-4.84]	4.48 [3.96-5.18]	4.50 [3.88-5.49]	0.023
Total flavonoids (mg/day)	118.30 [68.07-215.51]	103.84 [40.87-221.42]	62.72 [12.95-217.67]	<0.001
Flavanol (mg/day)	77.15 [39.82-139.30]	66.45 [23.18-159.45]	39.99 [5.44-137.06]	<0.001
Flavone (mg/day)	0.00 [0.00-0.07]	0.00 [0.00-0.02]	0.00 [0.00-0.00]	0.003
(mean±SD)	0.94 ± 5.93	0.35 ± 2.68	0.32 ± 3.09	0.252
Flavanol (mg/day)	15.22 [7.50-34.93]	8.71 [4.33-19.96]	5.28 [1.33-12.10]	<0.001
Flavanone (mg/day)	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.786
(mean±SD)	0.12 ± 1.14	0.42 ± 5.48	0.40 ± 2.59	0.669
Isoflavone (mg/day)	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	<0.001
(mean±SD)	0.40 ± 1.27	0.84 ± 4.41	0.12 ± 1.10	0.032
Anthocyanide (mg/day)	6.25 [2.14-14.98]	5.31 [1.01-14.93]	1.58 [0.00-8.88]	<0.001

<sup>1</sup>Values are presented as median [IQR] (or mean ±SD for some food groups with a particular distribution)

<sup>2</sup>P-values were derived from kruskal Wallis test (or Anova test)

202 **Table 4** shows the ORs and 95% CI for the association between HCC status and the DII score.  
203 When the DII was considered as a continuous variable, there was a positive association between  
204 increasing DII and HCC risk in the multivariate logistic regression model (adjusted OR=1.14  
205 [1.02-1.27], p-value=0.021 per one-unit increment of the DII score, corresponding to  
206 approximately 8% of its maximum range). When the DII score was categorized in tertiles, HCC  
207 risk was associated with the DII score (OR<sub>t3vst1</sub>=2.33 [1.16-4.67], p-trend=0.021).  
208 No interaction term was statistically significant.

Table 4. Association between the DII score and HCC risk (n=582 patients)

	continuous DII score	p value	DII score in tertiles		p-trend
			Tertile 2 (-0.80/2.19)	Tertile 3 (2.19/7.59)	
N= Cases/controls	181/401		66/134	65/134	
Crude model	1.04 [0.98-1.11]	0.191	1.41 [0.90-2.21]	1.43 [0.91-2.24]	0.139
Adjusted model <sup>1</sup>	1.14 [1.02-1.27]	0.021	1.75 [0.96-3.20]	2.33 [1.16-4.67]	0.021

Odds ratios were estimated using unconditional logistic regression models, stratified by center. Regressions were adjusted for age, gender, total energy from nonalcoholic sources, cirrhosis diagnosis, Child-Pugh score, diabetes and etiology of cirrhosis.

209  
 210 The association between DII score and plasmatic inflammatory biomarkers is difficult to interpret  
 211 among cases with HCC during this phase. Among controls, there was a positive correlation  
 212 observed between DII score and two plasmatic inflammatory biomarkers. The Spearman  
 213 correlation coefficient was 0.12 (p-value=0.021) with CRP, and 0.14 (p-value=0.004) with IL-6.  
 214 **Table 5** presents the medians of cytokines across DII tertiles among controls. The values were  
 215 often undetected for some inflammatory biomarkers, so the median was calculated only among  
 216 patients with detected values. The percentage of undetected values was not significantly different  
 217 across tertiles. The value of CRP and IL-6 significantly increased across the DII score tertiles (p-  
 218 trend= 0.017 and 0.0006 respectively).

219 Table 5 The medians of plasmatic inflammatory biomarkers across DII score tertile among  
 220 controls

Inflammatory markers	DII score			p-value*	p-trend**
	Tertile 1	Tertile2	Tertile3		
	n=133	n=134	n=134		
<b>CRP</b>	6.15 [3.79-11.55]	6.63 [3.88-12.85]	7.76 [4.22-17.56]	0.052	0.017
<b>IL-6</b>	3.01 [1.48-7.06]	3.44 [1.72-7.73]	5.68 [1.86-12.38]	0.016	0.006
<b>IL-1-β</b>	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.902	0.934
<b>Detected values of IL-1-β (n-%)<sup>1</sup> median [IQR]</b>	16 (12.0) 1.40 [0.98-3.41]	18 (13.4) 3.04 [1.40-3.53]	17 (12.7) 1.40 [1.18-3.41]	0.265	0.824
<b>IL-4</b>	0.00 [0.00-0.00]	0.00 [0.00-1.40]	0.00 [0.00-7.00]	0.234	0.229
<b>Detected values of IL-4 (n-%)<sup>1</sup> median [IQR]</b>	25 (18.8) 22.07 [8.84-29.46]	34 (25.4) 22.07 [13.04-29.46]	36 (26.9) 22.07 [14.31-29.80]	0.853	0.578
<b>TNF-α</b>	0.00 [0.00-1.53]	0.26 [0.00-1.80]	0.03 [0.00-2.42]	0.350	0.334
<b>Detected values of TNF-α (n-%)<sup>1</sup> median [IQR]</b>	62 (46.6) 1.60 [0.86-3.16]	73 (54.5) 1.60 [1.06-3.57]	70 (52.2) 2.32 [0.34-3.76]	0.829	0.629
<b>IL-10</b>	0.61 [0.00-1.78]	0.80 [0.00-1.86]	0.67 [0.00-1.72]	0.912	0.812

Values were presented as medians [IQR]

P-values were derived from \*Kruskal Wallis test, \*\*Cuzick's test

<sup>1</sup>The values were often undetected for some inflammatory biomarkers, so the median was calculated only among patients with detected values.

221

222 **A modified DII score**

223 In our population, alcohol was the main cause of cirrhosis in 55.3% of patients, and 63.6% were

224 heavy drinkers. Considering alcohol as an anti-inflammatory component in the DII score did not

225 seem reasonable. Thus, the DII score not including alcohol was recalculated for the 582 cirrhotic

226 patients. Consequently, 35 out of 45 components initially recommended were used. The new

227 modified DII score, calculated without the alcohol component, ranged from -5.05 to +7.75 with a

228 mean value of  $0.82 \pm 2.86$  for the whole population. The mean of the modified DII score was

229  $1.00 \pm 2.85$  among cases, and  $0.74 \pm 2.86$  among controls, still indicating a pro-inflammatory diet

230 in both groups.

231 **Table 6** presents the consumption of food groups and nutrients in medians with IQR, and the p-  
232 values from Kruskal-Wallis tests when the conditions were not met for Anova tests. However,  
233 due to a large number of non-consumers, we also presented means and SD for some food groups  
234 with unusual distributions. When compared to participants with a lower DII score, the results of  
235 the patients with a higher DII were similar to the initial results. However, there was a difference  
236 in sugar intake since the patients in the third tertile consumed less than patients in the first tertile.



Table 6. Consumption of food groups and micronutrients by modified DII tertiles calculated for 1000 kcal of daily energy intake from nonalcoholic sources

	Modified DII score <sup>1</sup>			P-value <sup>2</sup>
	Tertile 1 n=185	Tertile 2 n=195	Tertile 3 n=202	
Food groups <sup>1</sup>				
Energy (kcal/day)	3794.01 [3058.05-5162.54]	2723.25 [2160.99-3217.27]	1835.92 [1403.98-2385.32]	<0.001
Alcohol (g/day)	4.47 [0.00-112.23]	0.52 [0.00-153.63]	11.28 [0.00-297.98]	0.294
Added sugar (g/day)	5.83 [1.12-12.21]	4.98 [0.07-13.91]	3.31 [0.00-13.45]	0.047
Desserts (g/day)	43.61 [17.86-78.40]	34.86 [14.82-71.37]	33.96 [8.78-81.32]	0.317
Carbonated beverages (ml/day)	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.146
(mean±SD)	19.93 ± 64.65	18.89 ± 89.78	14.60 ± 53.41	0.732
Coffee (ml/day)	68.46 [18.06-130.84]	112.89 [22.42-213.89]	108.57 [5.56-229.39]	0.001
All cereals (g/day)	48.00 [31.10-68.29]	60.54 [37.64-85.48]	74.79 [41.68-102.38]	<0.001
Dairy products (g/day)	90.14 [49.82-134.41]	96.48 [51.37-154.35]	86.47 [47.11-166.86]	0.496
Total meat (g/day)	23.21 [11.16-41.09]	24.87 [11.29-46.55]	22.23 [6.79-44.53]	0.316
Processed meat (g/day)	15.82 [8.13-23.51]	13.52 [7.59-22.69]	17.73 [5.93-30.73]	0.255
Red meat (g/day)	12.73 [5.07-21.99]	13.71 [3.47-28.58]	9.05 [0.00-27.28]	0.057
Fish and seafood (g/day)	10.40 [5.38-18.30]	7.87 [3.93-13.32]	6.92 [2.63-15.54]	<0.001
Eggs (g/day)	8.57 [4.43-14.34]	9.71 [4.72-16.91]	9.33 [3.28-24.06]	0.378
Fruits (g/day)	112.49 [60.45-182.71]	80.82 [37.03-167.11]	60.81 [10.39-140.99]	<0.001
Vegetables (g/day)	94.41 [59.65-154.12]	99.54 [58.96-157.83]	75.66 [34.89-154.08]	0.015
Protein (g/day)	36.07 [32.18-40.29]	38.16 [33.76-42.79]	38.81 [32.82-44.33]	0.008
Carbohydrate (g/day)	112.89 [98.45-127.06]	114.01 [99.14-128.64]	116.14 [100.41-138.29]	0.107
Fat (g/day)	44.49 [38.33-50.78]	43.17 [36.52-49.65]	41.05 [33.35-48.49]	0.002
Saturated fat (g/day)	17.38 [14.59-20.90]	17.36 [13.87-21.11]	17.12 [13.34-22.64]	0.848
Monounsaturated fat (g/day)	14.92 [13.11-17.75]	14.75 [12.36-17.05]	13.59 [10.59-15.70]	<0.001
Trans-fat (g/day)	0.68 [0.42-1.07]	0.68 [0.29-1.24]	0.57 [0.16-1.13]	0.103
Polyunsaturated fat (g/day)	7.36 [5.70-9.64]	6.95 [5.01-9.33]	5.25 [3.99-7.20]	<0.001
N-3 PUFA (g/day)	0.62 [0.47-0.87]	0.53 [0.37-0.67]	0.42 [0.32-0.55]	<0.001
N-6 PUFA (g/day)	6.09 [4.58-7.80]	5.34 [3.74-7.48]	4.40 [3.05-6.68]	<0.001
Fiber (g/day)	10.13 [8.11-11.93]	9.17 [7.45-11.15]	7.91 [6.54-9.83]	<0.001
Cholesterol (mg/day)	160.56 [125.67-201.19]	168.32 [131.85-205.83]	177.07 [133.76-229.00]	0.064
Micronutrients <sup>1</sup>				
Vitamin B1 (mg/day)	0.58 [0.51-0.67]	0.57 [0.50-0.67]	0.55 [0.47-0.65]	0.039
Vitamin B2 (mg/day)	0.86 [0.75-1.08]	0.92 [0.76-1.11]	0.92 [0.77-1.11]	0.177
Vitamin B3 (mg/day)	7.74 [6.49-9.76]	8.18 [6.22-10.21]	7.22 [5.93-9.90]	0.122
Vitamin B5 (mg/day)	2.53 [2.21-2.98]	2.62 [2.30-3.01]	2.67 [2.26-3.20]	0.197
Vitamin B6 (mg/day)	0.77 [0.69-0.92]	0.76 [0.65-0.88]	0.75 [0.61-0.89]	0.123
Vitamin B9 (µg/day)	173.36 [145.11-207.49]	160.80 [135.03-190.56]	144.92 [119.27-178.63]	<0.001
Vitamin B12 (µg/day)	2.79 [1.90-4.56]	2.67 [1.82-3.94]	2.63 [1.68-4.01]	0.212
Vitamin D (µg/day)	1.28 [1.01-1.64]	1.18 [0.95-1.54]	1.20 [0.84-1.62]	0.131
Vitamin C (mg/day)	68.87 [49.11-98.06]	57.88 [36.35-86.17]	40.46 [24.46-59.42]	<0.001
Vitamin A (µg/day)	438.83 [273.77-693.85]	386.45 [227.96-628.96]	349.49 [227.13-624.68]	0.043
Vitamin E (mg/day)	6.83 [5.39-9.50]	6.08 [4.40-8.09]	4.58 [3.33-6.28]	<0.001
Calcium (mg/day)	408.21 [330.05-487.66]	420.91 [351.60-512.72]	419.59 [315.48-533.85]	0.464
Copper (mg/day)	0.78 [0.66-0.94]	0.75 [0.63-0.96]	0.68 [0.60-0.93]	0.008

Iron (mg/day)	6.18 [5.28-7.62]	6.48 [5.16-7.70]	6.10 [5.02-8.32]	0.880
Selenium (mg/day)	24.31 [21.18-29.28]	24.93 [21.55-29.39]	26.02 [21.34-32.29]	0.080
Magnesium (mg/day)	134.21 [120.38-156.74]	138.29 [121.45-160.77]	134.42 [113.96-157.73]	0.287
Manganese (mg/day)	2.49 [1.94-3.18]	2.15 [1.65-2.81]	2.08 [1.53-2.71]	<0.001
Sodium (mg/day)	1122.82 [959.00-1283.16]	1180.57 [1033.92-1416.90]	1259.39 [1087.81-1506.79]	<0.001
Phosphorus (mg/day)	567.98 [506.81-637.36]	589.84 [528.01-666.90]	598.34 [512.08-701.74]	0.037
Potassium (mg/day)	1567.44 [1349.45-1875.31]	1564.23 [1309.20-1876.24]	1430.34 [1189.55-1796.50]	0.003
Iodine (mg/day)	56.00 [47.72-67.90]	59.20 [48.30-69.92]	59.14 [49.01-74.47]	0.145
Zinc (mg/day)	4.33 [3.72-4.85]	4.48 [4.03-5.19]	4.51 [3.89-5.48]	0.009
Total flavonoids (mg/day)	118.20 [68.07-210.02]	101.55 [40.37-222.54]	69.61 [15.30-224.51]	0.002
Flavanol (mg/day)	76.98 [39.81-137.92]	68.15 [21.93-157.84]	41.10 [7.55-144.24]	0.002
Flavone (mg/day)	0.00 [0.00-0.07]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.003
(mean±SD)	0.94 ± 5.90	0.35 ± 2.71	0.32 ± 3.07	0.257
Flavonol (mg/day)	15.22 [7.66-31.57]	8.37 [3.98-20.36]	5.51 [1.74-11.66]	<0.001
Flavanone (mg/day)	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.612
(mean±SD)	0.12 ± 1.13	0.04 ± 0.25	0.78 ± 6.00	0.079
Isoflavone (mg/day)	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	<0.001
(mean±SD)	0.41 ± 1.28	0.85 ± 4.46	0.12 ± 1.09	0.028
Anthocyanide (mg/day)	6.49 [2.26-14.33]	4.28 [0.61-14.61]	2.03 [0.00-12.57]	<0.001

<sup>1</sup>Values are presented as medians [IQR] (or mean ±SD for some food groups with a particular distribution)

<sup>2</sup>P-values were derived from the Kruskal Wallis test (or Anova tests)

237 **Table 7** shows the ORs and 95% CI for the association between HCC status and DII score.  
238 Alcohol consumption was added as an adjustment variable in the multivariate logistic regression  
239 models. When considering DII as a continuous variable, a positive association was observed with  
240 HCC risk in the multivariate logistic regression model (adjusted OR=1.15 [1.03-1.28], p-  
241 value=0.013 for a one-unit increment of the DII score, corresponding to approximately 8% of its  
242 maximum range). Additionally, when categorized in tertiles, HCC cirrhotic cases had a higher  
243 DII score than cirrhotic controls (OR<sub>t3vst1</sub>=2.35 [1.18-4.68], p-trend=0.015).

Table 7. Association between the modified DII score and HCC risk (n=582 patients)

	DII score in continuous	p-value	DII score in tertiles		p-trend
			Tertile 2 (-0.83/2.00)	Tertile 3 (2.00/7.75)	
N= Cases/controls	181/401		61/134	67/135	
Crude model	1.05 [0.99-1.12]	0.118	1.27 [0.81-2.00]	1.43 [0.91-2.23]	0.127
Adjusted model <sup>1</sup>	1.15 [1.03-1.28]	0.013	1.54 [0.85-2.80]	2.35 [1.18-4.68]	0.015

Odds ratios were estimated using unconditional logistic regression models, stratified by center. Regressions were adjusted for age, gender, total energy from nonalcoholic sources, cirrhosis diagnosis, Child-Pugh score, diabetes, etiology of cirrhosis and alcohol consumption.

244

245 The modified DII score without alcohol was also correlated with CRP and IL-6: the Spearman

246 correlation coefficient was 0.13 (p-value=0.007) with CRP, and 0.16 (p-value=0.001) with IL-6.

247 For the modified DII score, the difference between tertiles was also significant, among controls,

248 with CRP and IL-6 (p-trend= 0.017 and 0.003, respectively) (**Table 8**).

Table 8. The medians of plasmatic inflammatory biomarkers across the modified DII score tertile and among controls

Inflammatory markers	DII score			p-value*	p-trend**
	Tertile 1	Tertile2	Tertile3		
	n=132	n=134	n=135		
<b>CRP</b>	6.03 [3.79-11.48]	6.63 [3.88-13.26]	7.90 [4.20-17.56]	0.056	0.017
<b>IL-6</b>	2.88 [1.41-6.03]	3.73 [1.72-8.91]	4.85 [1.88-11.78]	0.012	0.003
<b>IL-1-β</b>	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.745	0.963
<b>Detected values of IL-1-β (n-%)<sup>1</sup></b>	16 (12.1)	19 (14.2)	16 (11.9)		
<b>median [IQR]</b>	1.40 [0.98-3.27]	2.95 [1.40-3.66]	1.40 [0.88-3.41]	0.194	0.901
<b>IL-4</b>	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-7.00]	0.469	0.400
<b>Detected value of IL-4 (n-%)<sup>1</sup></b>	28 (21.2)	30 (22.4)	37 (27.4)		
<b>median [IQR]</b>	22.07 [10.94-41.74]	22.07 [13.04-27.42]	22.07 [13.04-25.90]	0.850	0.755
<b>TNF-α</b>	0.00 [0.00-1.53]	0.26 [0.00-1.80]	0.04 [0.00-2.48]	0.283	0.274
<b>Detected value of TNF-α (n-%)<sup>1</sup></b>	61 (46.2)	73 (54.5)	71 (52.6)		
<b>median [IQR]</b>	1.60 [0.90-3.16]	1.60 [1.06-3.57]	2.42 [0.34-3.76]	0.748	0.581
<b>IL-10</b>	0.71 [0.00-1.86]	0.66 [0.00-1.86]	0.71 [0.00-1.69]	0.773	0.547

Values were presented as medians [IQR]

P-values were derived from \*Kruskal Wallis tests, \*\*Cuzick's tests

<sup>1</sup>The values were often undetected for some inflammatory biomarkers, so the median was calculated only among patients with detected values.

249 **Discussion**

250 This case-control study is the first to our knowledge to examine the relationships between the  
251 inflammatory potential of the diet, evaluated by the DII score, and HCC risk among cirrhotic  
252 patients. Our results showed that the DII score was associated with the risk of HCC. The DII  
253 score in controls was associated with plasmatic biomarkers of inflammation, including CRP and  
254 IL-6. As hypothesized, in specific populations with a liver disease such as cirrhosis, a pro-  
255 inflammatory diet does appear to increase HCC risk. In accordance with our hypothesis, an  
256 Iranian case-control study with 999 subjects(29), and the NHANES study with 20 643 US  
257 participants showed that the consumption of a pro-inflammatory diet is associated with increased  
258 non-alcoholic fatty liver disease (NAFLD) risk(30).

259 The present findings, obtained using a score that reflects some specific properties of diet,  
260 confirmed previous results obtained in the same case-control study(20). Some components of the  
261 DII score or foods associated with the score as referred in the Table 3 have been previously  
262 associated with the risk of HCC in our population. Carbonated beverages and total cereals (with  
263 potentially high levels of sugar or refined carbohydrates), processed meat, and sodium were  
264 found to be highly consumed by HCC cases, whereas the intake of dietary fiber, and some  
265 vitamins (E and B9) and minerals (manganese and potassium) were lower than in cirrhotic  
266 controls without HCC.

267 Other studies have also used the DII score to explore the consumption of nutrients and foods with  
268 anti-inflammatory values in patients with HCC, but in comparison with individuals without liver  
269 disease(31–35). Likewise, these studies found that a higher consumption of dairy products, soft  
270 drinks and juices, foods rich in saturated fat or sugar were associated with an increased risk of  
271 HCC (36–39).On the other hand, regular consumption of vegetables, dietary fiber, fish, and  
272 coffee (3-4cups/day) seemed to be associated with reduced risk of HCC(35,40–42). Vitamin D,

273 calcium, and the phenols contained in red wine, green tea, or olive oil may also have a protective  
274 role(31–34,36). Some of these nutrients and food groups are listed as anti-inflammatory foods in  
275 the DII score(9).

276 Chronic inflammation is an important factor in the development of HCC. The body's inability to  
277 regenerate normal cells and to maintain normal hepatic function during inflammation leads to  
278 cirrhosis. The immune response that is triggered in an attempt to fight against this aggression  
279 results in the secretion of pro-inflammatory cytokines such as IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 as well as  
280 the anti-inflammatory IL-10 by hepatic macrophages(5). A higher DII score (pro-inflammatory  
281 diet) has been associated with a number of inflammatory biomarkers, including CRP, TNF- $\alpha$ , and  
282 IL-6(43–45). Our study actually revealed a positive association between the DII score and CRP or  
283 IL-6 concentrations. Previous studies have compared the effect of different types of diet on the  
284 inflammatory response by measuring the variation of serum inflammatory biomarkers or by  
285 analyzing oxidative stress. The Western-type diet, defined by the consumption of red meat, high-  
286 fat dairy products, refined grains, and simple carbohydrates was associated with a pro-  
287 inflammatory status and higher levels of CRP and IL-6(43,46,47). The Mediterranean diet, rich in  
288 fruit and vegetables, fish, whole grains, and olive oil, and with moderate wine consumption, is  
289 associated with a lower level of inflammation and a reduction in oxidative stress as well as a  
290 beneficial effect on the metabolic syndrome, cardiovascular diseases, cancer risk, and  
291 NAFLD(33,48–50).

292 The inflammatory potential of the diet, as indicated by a high DII score, has been associated with  
293 higher risk of all-cause mortality, cardiovascular disease, and metabolic syndrome(51–53). In  
294 numerous studies, the DII score was positively associated with various types of cancer, including  
295 breast, prostate, lymphoma, lung, and digestive, in many regions and populations(10–  
296 12,14,16,48). In addition, several meta-analyses have summarized the current published literature

297 discussing the association between DII and cancer risk and other non-communicable  
298 diseases(51,52,54). Overall, individuals who consumed pro-inflammatory diets (higher DII score)  
299 had a 17% higher risk of developing cancer on average (depending on the study and cancer type)  
300 compared with those who consumed an anti-inflammatory diet(51).

301 However, only two studies (one in Italy and one in China) have investigated the association  
302 between DII and HCC. These studies compared HCC cases with hospitalized patients or healthy  
303 participants without any dietary modification or any medical condition related to major risk  
304 factors for HCC (controls). Both studies found a positive association between higher DII and  
305 HCC risk, with an  $OR_{T3vsT1}=2.43(95\% CI=1.27-4.68; p\text{-trend}=0.03)$  in the Italian study and  
306  $OR_{T3vsT1}= 3.22 (95\% CI= 1.30-7.98, p\text{-trend} = 0.009)$  in the Chinese study. Their results were  
307 very similar to our findings. The Italian study found that a higher DII was associated with HCC  
308 risk in male participants with no history of hepatitis B or C. In the Chinese study, a strong  
309 association between a pro-inflammatory diet and HCC was found only in male smokers with  
310 lower levels of education, and non-drinkers of alcohol (17,18). However, our study did not show  
311 any interaction or statistically significant associations in stratified analyses.

312 Our study presents some limitations. First, it has the usual limitations of a case-control design  
313 with classical associated risks of biases. Second, the limited number of participants who  
314 responded to the diet history questionnaire reflects the difficulty of asking patients to answer such  
315 an extensive nutritional questionnaire. Third, only 36 of 45 food parameters were available for  
316 the DII calculation. However, this is also often the case in the published literature. Moreover,  
317 some of the missing food parameters (eugenol, saffron, ginger and turmeric) are seldom  
318 consumed in our population and would barely have affected the final results. On the other hand,  
319 missing information on consumptions of rosemary, thyme, garlic, and pepper could have had  
320 some impact on the overall DII estimate.

321 The strengths of this study are, first, the use of a validated and reproducible diet history  
322 questionnaire which considers seasonal variations. Second, the use of a standardized protocol in  
323 six French university hospitals allowed for a comprehensive assessment of major metabolic,  
324 nutritional, and environmental risk factors for HCC among cirrhotic patients. Third, the detailed  
325 sociodemographic and clinical questionnaire filled in by qualified physicians and investigators  
326 concerning the major risk factors for HCC, made it possible to make detailed adjustments.  
327 Another one of our study's strengths lies in the measurement of inflammatory markers and DII  
328 score in the same population and confirmed the pertinence of the DII score.

329 In conclusion, the present study on the risk of HCC and the DII score in a French cirrhotic  
330 population showed that the inflammatory potential of one's diet may be associated with the risk  
331 of liver carcinogenesis. However, these results and the possibility to evaluate interventions must  
332 be confirmed in larger prospective studies. According to the World Cancer Research Fund  
333 International, increasing the consumption of vegetables and fruits that are rich in fiber and  
334 nutrients, replacing red meat by white meat, and increasing the consumption of coffee and  
335 unsaturated fatty acids (mainly n-3 and n-6 fatty acids) are good approaches for reducing the  
336 consumption of pro-inflammatory food. Taking such steps may subsequently decrease HCC risk.  
337 It would be worthwhile to carry out further investigations among cirrhotic patients to confirm the  
338 interest of an anti-inflammatory diet and to define recommendations for this specific population.



339 **Acknowledgment**

340 The authors' responsibilities were as follows: MR analyzed, interpreted data and wrote the  
341 manuscript. CB performed statistical analysis. JH and NS revised the manuscript. MDA provided  
342 critical revision of the manuscript. VC designed the research (analysis plan, interpretation of  
343 data and manuscript editing).TM, BB-C, J-PB, CR, MD, M-CB-R, J-LJ, PH and the CIRCE  
344 Study Group conducted research and provided essential materials and critical revision of the  
345 manuscript. All authors read and approved the final manuscript. We thank Suzanne Rankin from  
346 the Dijon University hospital for proofreading the manuscript.

347 We thank the staff of the Biological Resource Center Ferdinand Cabanne, Dijon University  
348 Hospital, France – (BB-0033-00044) for their assistance in handling and storing biological  
349 samples and the E3N team (INSERM UMR1018) for their help with the diet history  
350 questionnaire.

351 The authors declare no conflict of interest.

352  
353 Disclosure: Dr. James R. Hébert owns controlling interest in Connecting Health Innovations LLC  
354 (CHI), a company planning to license the right to his invention of the dietary inflammatory index  
355 (DII) from the University of South Carolina in order to develop computer and smart phone  
356 applications for patient counselling and dietary intervention in clinical settings. Dr. Nitin  
357 Shivappa is an employee of CHI.

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Focus sur un groupe alimentaire : les lipides.

Plusieurs études se sont intéressées aux rôles des différents types de lipides consommés et aux profils plasmatiques/sériques en acides gras et sur le développement des tumeurs.

Les acides gras essentiels jouent un rôle important dans les réactions métaboliques complexes. Le métabolisme des AGPI semble être une étape importante associée à la cancérogenèse. Par exemple, les régimes alimentaires riches en AG n-6 sont souvent associés à une augmentation du risque de développer un cancer. En effet, la perturbation du métabolisme et des propriétés de signalisation cellulaire de l'acide arachidonique ont été associées à la prolifération cellulaire, l'apoptose et la survie des cellules cancéreuses. Par contre, les AG n-3 peuvent avoir des effets bénéfiques en agissant à plusieurs niveaux : en tant que ligands de certains récepteurs nucléaires dans la transduction du signal, en entrant en compétition avec les AG n-6 dans la synthèse des eicosanoïdes via la voie de la cyclooxygénase-2, en renforçant l'apoptose et la différenciation cellulaire, et finalement en modifiant le statut oxydatif cellulaire via l'amélioration de la peroxydation des lipides. Les résultats discordants des études épidémiologiques concernant l'analyse des lipides pourraient être liés à des difficultés de mesure des consommations alimentaires notamment par les questionnaires alimentaires des différents types de lipides : AGS, AGMI dont les oméga 9 (n-9), AGPI parmi lesquels on distingue oméga 6 (n-6) et oméga 3 (n-3). Un profil lipidique perturbé peut entraîner des altérations de la membrane des phospholipides et du métabolisme des AGPI entraînant une modification de la distribution des phospholipides et une diminution du statut oxydatif cellulaire. Les recherches sur les cellules cancéreuses hépatiques murines et humaines montrent des similitudes dans les altérations du profil lipidique associé au développement du cancer du foie. Plusieurs différences sont observées dans les teneurs des AGPI dans ces cellules : une diminution des AGPI n-3 et du rapport

phosphatidylcholine/phosphatidylethanolamine, une augmentation de certains AG comme l'acide oléique, l'acide arachidonique, le rapport AG n-6/ n-3 et du rapport acide arachidonique/acide eicosapentanoïque. Ces différents changements en parallèle d'une augmentation du cholestérol vont induire des altérations au niveau de la structure membranaire ce qui influence la stabilité, la fluidité, la fonction de la membrane ainsi que la signalisation cellulaire et l'apoptose (74). Ainsi, il serait important de bien quantifier les différents types d'acides gras pour mieux analyser leurs rôles dans la cancérogenèse.

Deux articles ont été rédigés à ce sujet, un article a été initié et rédigé par Thomas Mouillot et Vanessa Cottet, le second par Alexia Cotte et Patrick Hillon. J'ai eu l'occasion de participer comme co-auteur dans ces deux articles. J'ai pu participer à l'interprétation des résultats et à la phase de rédaction et relecture des articles. L'article de Thomas va être soumis dans la revue *Journal of Hepatology* alors que l'article d'Alexia a été publié dans la revue *Oncotarget*.

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## **Introduction**

Le foie joue un rôle central dans le métabolisme des nutriments, en particulier des lipides. Pourtant, les liens entre consommation de lipides, métabolisme lipidique et risque de CHC en cas de cirrhose ne sont pas encore bien définis. L'objectif de ce travail était d'étudier le profil en acides gras de la membrane érythrocytaire (reflet des apports alimentaires et du métabolisme) chez des personnes atteintes de cirrhose, selon la présence ou non d'un CHC.

## **Matériel et méthodes**

La composition en AG de la membrane des érythrocytes a été déterminée par chromatographie en phase gazeuse à haute performance chez 349 cas et 550 témoins. Les AG dans ces membranes sont considérés comme des marqueurs biologiques précis et sensibles permettant d'estimer les apports nutritionnels à 3 mois. Le renouvellement des AG dans la membrane érythrocytaire se produit à des vitesses différentes, ainsi les concentrations en AG dans ces membranes reflètent les apports des jours ou des semaines qui précède le prélèvement. La composition des tissus biologiques est également affectée par les enzymes impliquées dans l'élongation ou la désaturation des acides gras. Ainsi, les activités enzymatiques de la delta-5-désaturase, la delta-6-désaturase, l'élongase 2/5 et la stéaroyl-CoA désaturase ont été estimées. En plus des échantillons sanguins, les données sociodémographiques, cliniques et médicales étaient disponibles pour chaque patient. L'association entre le risque de CHC et la concentration de différents AG et enzymes découpée en tertiles a été évaluée par régression logistique multivariée.

## Résultats

Les cas étaient plus âgés que les témoins [âge médian : 64,2 versus 58,9 ans, ( $p=0,04$ )] et plus souvent des hommes. Les résultats ont montré plusieurs différences de concentration d'AG entre les cas et les témoins. Les AGPI étaient surtout composés des oméga-6 et les AGS étaient prédominants. Une association positive était mise en évidence entre le risque de CHC et la concentration en acide linoléique (18 :2  $n-6$ ) [*Odds Ratio* (OR)=2,22 [Intervalle de Confiance (IC) à 95 % 1,43–3,43] ( $p < 0,001$ )] et en AG saturés spécifiques des produits laitiers (15 :0+17 :0) [OR=4,53 [1,97–9,63] ( $p = 0,002$ )]. À l'inverse, les concentrations en acide stéarique (18 :0) et en acide arachidonique (20 :4  $n-6$ ) étaient plus faibles chez les cas que chez les témoins (respectivement [OR=0,36 [0,23–0,56] ( $p < 0,001$ )], et [OR=0,27 [0,15–0,50] ( $p < 0,001$ )]. Enfin, certains marqueurs de la fonction enzymatique ont aussi montré des différences entre les cas et les témoins. L'activité de la  $\delta$ -6-désaturase était inversement associée au risque de CHC [OR<sub>T3vsT1</sub> = 0,36 [0,18-0,73] ( $p = 0,004$ )]. L'activité enzymatique de la  $\delta$ -6-désaturase + l'élongase 5 avait une tendance à être plus faible parmi les cas de CHC, la différence était presque significative [OR<sub>T3vsT1</sub> = 0,56 [0,31-1,01] ( $p = 0,059$ )]. L'évaluation de la  $\beta$ -oxydation peroxysomale, a montré une association positive avec le risque de CHC chez les patients cirrhotiques [OR<sub>T3vsT1</sub> = 1,46 [0,76-2,80] ( $p = 0,024$ )].

## Conclusion

Les profils en AG des membranes érythrocytaires étaient différents selon la présence ou non de CHC chez les personnes atteintes de cirrhose. Les activités enzymatiques semblaient également être perturbées. Ces résultats et les hypothèses mécanistiques devront être confirmés dans une étude prospective.

## FATTY ACID COMPOSITION OF THE ERYTHROCYTE MEMBRANE AND RISK OF HEPATOCELLULAR CARCINOMA IN CIRRHOTIC PATIENTS

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**Disclosure of Interest:** None Declared

## **ABSTRACT:**

**Introduction:** Malnutrition and disturbances in fatty acid (FA) metabolism have been reported in patients with cirrhosis. But their roles in the development of hepatocellular carcinoma (HCC) are still unclear. The use of biomarkers may be an interesting approach to explore associations between exogenous and endogenously produced FAs and the risk of cancer.

**Aims:** The objective of the study was to investigate the relationships between FA content of erythrocyte membranes and the risk of HCC in cirrhotic patients.

**Material and Methods:** The case-controlled study CiRCE (“Cirrhose et Risque de Carcinome hépatocellulaire dans le grand Est”) recruited cirrhotic patients in six hospitals of north-eastern France between 2008 and 2012. Cases were cirrhotic patients with an HCC confirmed (n=349); controls were cirrhotic patients without HCC at inclusion (n=550). The FA composition of erythrocyte membrane phospholipids was determined by high performance gas chromatography. FA concentrations were divided into tertiles. Odds ratios (ORs) for the risk of HCC with increasing concentrations of FAs were estimated by multivariate logistic regression.

**Results:** Compared with controls, HCC patients were older (median age: 58.8 versus 64.2 years,  $p < 0.001$ ) and were more often men (87% and 70% males in cases and controls, respectively,  $p < 0.001$ ). In both cases and controls, saturated fatty acids (SFAs) represented more than 39% of all erythrocyte membrane fatty acids, monounsaturated fatty acids around 14% and polyunsaturated fatty acids (PUFAs) around 46%. The results showed a significant positive association between HCC risk and C15:0+C17:0 [OR: 4.35, [95% CI: 1.97-9.63], ( $p < 0.001$ )], C20:1 n-9 [OR: 2.26[1.36-3.75] ( $p < 0.001$ )], C18:2 n-6 [OR: 2.23[1.44-3.45] ( $p < 0.001$ )], C20:2 n-6 [OR: 2.47[1.25-4.90] ( $p = 0.002$ )], but an inverse association with the sum of SFAs [OR: 0.64[0.42-0.98] ( $p = 0.045$ )], C18:0 [OR: 0.36 [0.23-0.56], ( $p < 0.001$ )], and C20:4 n-6 [OR: 0.27 [0.15-0.50] ( $p < 0.001$ )].

**Conclusions:** Patients with cirrhosis presented abnormalities in the FA composition of erythrocyte membranes. These may reflect particular dietary patterns and altered fatty acid metabolism. The fatty acid profiles differed according to the presence of HCC. These results remain to be prospectively investigated and the mechanistic hypotheses need to be confirmed.

**Keywords:** Fatty acids; Erythrocyte membrane; Hepatocellular carcinoma; Cirrhosis; Biomarkers; Arachidonic acid



## **Funds**

This work was supported by a French Government grant managed by the French National Research Agency under the program “Investissements d’Avenir” [reference ANR-11-LABX-0021], and also by grants from the “Conseil Régional de Bourgogne”, the “Fonds Européen de Développement Régional” (FEDER), the French National Cancer Institute, the “Ligue Nationale contre le Cancer”, the “Canceropole Grand-Est” and the “Fondation de France”.

## **Acknowledgements**

We thank the members of the CiRCE Study Group for their contribution to the recruitment, data collection and biological analyses. We thank the staff of the Clinical Investigation Center and the Biological Resource Center Ferdinand Cabanne of the University Hospital of Dijon (BB-0033-00044) for their assistance in handling and storing biological samples. Special thanks to Professor Laurent Brondel for his judicious advice in writing this article. We also thank Mr. Philip Bastable for the review.

## **INTRODUCTION**

The incidence of hepatocellular carcinoma (HCC) has increased dramatically in recent years and its prognosis remains very severe. Currently, HCC is the 6<sup>th</sup> most common cancer in the world (749,000 new cases per year) and the third leading cause of cancer deaths (692,000 deaths each year) (1). It accounts for more than 90% of primary liver cancers and remains a real public health problem.

In 75-80% of cases, HCC develops on a cirrhotic liver (2). As there is no cure for cirrhosis except transplantation, it is extremely important to identify new factors of HCC development and to learn about the pathophysiology of the disease. Diet is a potential preventive tool but to date, it has been poorly investigated in cirrhotic patients. Some studies using comparisons with healthy subjects have shown associations between certain dietary factors and HCC risk. It has been suggested that fruit, vegetables, fish and white meat have a protective effect (3–5), whereas red and processed meat seems to be associated with an elevated risk of HCC (6–8). Overall, the results are not sufficiently consistent across studies to consider relationships between specific foods and HCC as convincing (3,9–11). More specifically, a recent meta-analysis of 11 observational studies showed that the consumption of fish rich in Omega 3 FA reduced the risk of HCC (12). It has been suggested that a high-PUFA diet has a beneficial effect on the occurrence of HCC in subjects with a cirrhotic liver (13). Different FAs are provided by the diet but can also be synthesized by “lipogenesis” (14). FAs are an important source of metabolic energy and play a fundamental structural role through various types of lipids (phosphoglycerates, sphingolipids, etc.). They are also precursors of several intra- and extracellular messengers, especially n-3 and n-6 PUFAs (15).

Once consumed, dietary FAs are more or less absorbed and metabolized through enzymatic pathways, resulting in a variable individual exposure to exogenous and endogenously produced

FAs. Technical advances now allow sensitive and precise analyses of an increasing number of different FAs present at very low concentrations in plasma or erythrocyte phospholipids (16). These FAs can be considered biological markers of FA dietary intake and metabolism and constitute a more objective measurement of an individual's exposure to bioavailable FAs, irrespective of the source and quality of the food, than do data from questionnaires. Because FA turnover in these tissues occurs at different rates, FA concentrations in human serum or in erythrocyte membranes reflect intakes of the preceding days or weeks (17,18). FA composition of the erythrocyte membrane is a reliable, reproducible and feasible measurement in clinical practice (19). Some studies have investigated the FA profile of the erythrocyte membrane of cirrhotic patients (20–22), but never to examine the associations with the risk of HCC.

Our objective was to investigate the associations between the risk of HCC and the fatty acid composition of the erythrocyte membrane in a large cohort of cirrhotic subjects.

## **PATIENTS AND METHODS**

### **Study population**

The CiRCE study (“Cirrhose et Risque de Carcinome hépatocellulaire dans le grand Est”) is a multicentre case-controlled study which aims to assess the impact of metabolic and environmental factors (alcohol, tobacco, viruses, diet) as well as drugs on the risk of HCC among cirrhotic patients, whatever the aetiology. It is completed by the study of nutrition-related biochemical markers and polymorphisms involved in metabolism. This project relies on collaboration between gastroenterologists, epidemiologists, biostatisticians and biologists. Cirrhotic patients with or without HCC treated in the University Hospitals of Dijon, Metz, Nancy, Reims, and Strasbourg were included between June 2008 and December 2012.

For cases and controls, the diagnosis of cirrhosis whatever the aetiology was based on histological confirmation by liver biopsy, or, in the absence of biopsy, on typical clinical, morphological and biological data. Criteria for the diagnosis of HCC were those defined by the European Association for Study of the Liver (EASL) (1). The absence of HCC in controls at inclusion was assessed through high-quality imaging examinations (abdominal US, CT-scan or MRI) and Alpha-Foeto-Protein below 100 ng/ml during the 2 months preceding inclusion. Control patients who developed HCC during the 6 months following inclusion were considered cases.

Exclusion criteria were: age under 35 years old, patients with progressive extrahepatic cancer, human immunodeficiency virus infection (HIV), acute alcoholic hepatitis, major somatic or psychiatric illness not compatible with inclusion in the study, or non-HCC primary liver cancer.

At the end of December 2012, 349 cases (cirrhotic patients with HCC) and 550 controls (cirrhotic patients without HCC) who met the eligibility criteria were recruited. The sample size provided a power of 80% to detect adjusted odds ratios of at least 1.80. All patients gave written informed consent to participate in the study, which was approved in 2008 by the local ethics committee (Comité de Protection des Personnes Est: agreement n°2008/09) and by the French drug safety

agency (Agence Nationale de Sécurité des Médicaments et des Produits de Santé (ANSM): agreement n° 2008-A00023-52)

### **Biological sample collection and measurement**

In each study centre, preoperative blood samples (15 mL) were collected after overnight fasting and immediately stored at 4°C. Through a common protocol and within a maximal delay of 4 hours, samples were processed, separated into aliquots of plasma, serum, lymphocytes, and erythrocytes, and frozen separately at -80°C. Frozen samples were regularly transported to be stored at -80°C in the central biobank ("Ferdinand Cabanne" Biological Resources Centre, BB-0033-00044, Dijon University Hospital, Dijon, France).

All samples were analysed by trained staff blinded to the case or control status. Control and case samples were mixed in batches of < 50 samples. For quality control, we added, in random order, two aliquots of a standard pool to each batch of samples analysed.

Red blood cells (25 µL) were spiked with 25 µL of internal standard mix (containing 1300, 5640, 4200, 3252, 5.2, 2160, 5.4, 540, 2.6 and 2.0 ng of 14:0d3, 16:0d3, 18:0d3, 18:2d4, 20:0d3, 20:4w6d8, 22:0d3, 22:6w3d5, 24:0d4 and 26:0d4, respectively) and complex lipids were hydrolysed with potassium hydroxide 0.6M in ethanol (45 min at 56 °C). After acidification with 1.2 M hydrochloric acid (HCl), total FA were extracted with 2 ml of hexane and further derivative with PFBBr/DIPEA/acetonitrile (5/5/90 v/v/v) for 30 min at 37°C. Total FA were quantified and analysed as penta-fluorobenzyl esters by Gas Chromatography-Mass Spectrometry (Thermo-Electron Corp.) using a chemical ionisation source operating in negative mode with methane as the reagent gas. Gas chromatography peaks were integrated by using an SP 4400 integrator (Spectra Physics). FA were quantified with calibration curves obtained with authentic standards processed as samples (19). The data are expressed as percentages of total phospholipids.

### **Collection of clinical data**

In all centres, the characteristics of patients and data on factors likely to be involved in liver carcinogenesis were collected using a standardized form. These included individual characteristics, clinical data (patient and family medical history, HBV/HCV status, alpha-fetoprotein level, hepatic morphology examinations and tumour characteristics) and metabolic characteristics (anthropometric measurements, dyslipidaemia, and diabetes mellitus).

Dietary data, the lifetime history of alcohol and tobacco consumption were also recorded. A survey of current and past medication records was also conducted.

Excessive alcohol consumption was defined as more than 40g/day in men and 20g/day in women. Smoking status was classified into three categories as never smoker, former smoker, and current smoker. Leisure time physical activity (e.g. walking, biking, sports activities, do-it-yourself activities, and gardening) was defined as high if patients had an intensive physical activity (leading to sweating or breathlessness) >2h/week, low if patients had no intensive physical activity or only moderate activity (not leading to sweating or breathlessness, <1h/week, and medium in other

situations). Professional physical activity was defined in three categories according to the intensity of the work. Educational level was classified in two groups: <12 years of schooling and more than 12 years or a high-school diploma (French Baccalaureate). BMI (in  $\text{kg}\cdot\text{m}^{-2}$ ) was calculated as the weight divided by the height squared. Type 2 diabetes was defined as self-reported, a physician's diagnosis of diabetes, fasting plasma glucose  $>126$  mg/dl, or current treatment of diabetes. Patients were classified in three categories according to the major cause of cirrhosis based on the clinician's diagnosis: alcohol, virus and other aetiologies (for example, NASH, haemochromatosis ...).

In addition to individual FAs, we calculated erythrocyte membrane concentrations of total SFAs, MUFAs, and total n-3 and n-6 PUFAs. The ratio of n-6 to n-3 PUFAs and of the two essential PUFAs, 18:2n-6 to 18:3n-3, were also determined. The FA composition of biological tissues is influenced by the diet but also by enzymes involved in the elongation or desaturation of FAs. The enzymatic activities were estimated by product-to-precursor FA ratios. The ratio of 20:4n-6 to 20:3n-6 reflects delta-5-desaturase activity and 18:3n-6 to 18:2n-6 reflects delta-6-desaturase activity. The ratio of 20:3n-6 to 18:2n-6 is an indicator of combined delta-6-desaturase and elongase activity. Elongase 2/5 activity was estimated by the ratios of 22:4n-6 to 20:4n-6 and 22:5n-3 to 20:5n-3, which competes for the same enzyme. FA desaturation indexes (desaturation index for n-7 equals the ratio of 16:1n-7 to 16:0, and the desaturation index for n-9 equals the ratio of 18:1n-9 to 18:0) were calculated as an indicator of the activity of the rate-limiting enzyme stearoyl-CoA desaturase (SCD). SCD-1, which is the main isoform in humans, transforms some saturated FAs into MUFAs (23,24). The ratio of 16:1n-9 to 18:1n-9 is an indicator of the peroxisomal  $\beta$ -oxidation level.

### **Statistical methods**

Continuous variables were expressed as medians with their interquartile ranges (IQR) and categorical variables as percentages. Univariate comparisons between cases and controls were performed using chi-square tests or Fisher exact tests, when appropriate, for qualitative variables, and using Kruskal-Wallis tests for quantitative variables.

To investigate associations between HCC case/control status and erythrocyte membrane concentrations of FA, unconditional logistic regression models were used. Individual FAs, ratios or sums were divided into tertiles based on the distribution among controls. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using the lowest tertile as the reference category. Tests for linear trends across tertiles of FA were performed by assigning the median value to each tertile and modelling this as a continuous variable in separate regression models.

Multivariate models were systematically adjusted for age, gender, centre, aetiology and time from the diagnosis of cirrhosis. To select other potential confounding factors, all characteristics associated with HCC case/control status with a p-value  $< 0.20$  in the univariate analysis were tested and a p-value  $< 0.05$  was chosen to keep them in the final model. The Child-Pugh score, diabetes, history of arteritis and educational level were retained after backward selection.

## **RESULTS**

Table 1 gives the characteristics of the 349 cirrhotic subjects with HCC and the 550 cirrhotic control subjects (without HCC) included in the study. The most common aetiologies in the two groups were alcohol (58.8%) and viruses (29.2%) with no statistical differences between cases and controls. The average age of cases was 64.2 (58.8-71.6) and for controls was 58.8 (51.6-65.0) ( $p < 0.001$ ). Cases were more often inactive ( $p < 0.006$ ) and former smokers ( $p = 0.002$ ) than were controls. There was no significant difference between the two groups for BMI, aetiology of cirrhosis or the history of extrahepatic neoplasia. Cirrhosis had been diagnosed for less time and was significantly less severe according to the Child-Pugh score ( $p < 0.001$ ) in the control group than in cases. Cases had significantly more hypertension ( $p < 0.001$ ), diabetes mellitus ( $p < 0.001$ ) and a history of arteritis disease than did controls ( $p = 0.004$ ).

### **Fatty acid profile of the erythrocyte membrane of cases and controls**

The mean percentages of FAs of the erythrocyte membrane of the two groups are shown in Table 2. Although some differences were observed between cases and controls, globally, the polyunsaturated FA, mainly n-6 PUFAs, and saturated FAs predominated. N-6 PUFAs were composed of at least 14% of linoleic acid (18:2n-6) and around 19% of arachidonic acid (20:4n-6). N-3 PUFAs consisted mainly of at least 2% of docosapentaenoic acid (22:5n-3) and around 5% of docosahexaenoic acid (22:6n-3). Saturated FAs consisted of 23% palmitic acid (16:0) and 15% of stearic acid (18:0). The sum of pentadecanoic acid and margaric acid (15:0 + 17:0) represented around 0.6%. The monounsaturated FA of around 14% consisted mainly of 3% of palmitoleic acid (18:1n-7) and 9% of oleic acid (18:1n-9).

### **Association between the risk of hepatocellular carcinoma and the fatty acid profile of the erythrocyte membrane (Table 3)**

In multivariate analysis, the sum of saturated FAs was inversely associated with the risk of developing HCC, [odds ratio of the third tertile versus the first tertile;  $OR_{T3vsT1} = 0.64$  [95% CI: 0.42-0.98] ( $p = 0.045$ )]. In detail, only stearic acid was inversely associated with the risk of HCC [ $OR_{T3vsT1} = 0.36$  [0.237-0.56] ( $p < 0.001$ )]. In contrast, the sum of pentadecanoic acid (15:0) and margaric acid (17:0), FAs with an odd number of carbon atoms, were strongly associated with the risk of developing HCC [ $OR_{T3vsT1} = 4.35$  [1.97-9.63] ( $p < 0.001$ )]. Among mono-saturated FAs, only eicosenoic acid (20:1 n-9) was statistically associated with HCC risk [ $OR_{T3vsT1} = 2.24$  [1.35-3.73] ( $p < 0.001$ )]. For n-6 PUFAs, their sum was not associated with HCC risk. However, linoleic acid [ $OR_{T3vsT1} = 2.23$  [1.44-3.45] ( $p < 0.001$ )] and eicosadienoic acid [ $OR_{T3vsT1} = 2.47$  [1.25-4.90] ( $p = 0.002$ )] showed a positive association with the risk of HCC in univariate and multivariate analysis. In contrast, arachidonic acid was inversely associated with the risk of HCC [ $OR_{T3vsT1} = 0.27$  [0.15-0.50] ( $p < 0.001$ )]. The association between HCC risk and n-3 PUFAs did not reach the significance level after adjustment.

Finally, some markers of enzyme function showed differences between cases and controls.  $\delta$ -6-desaturase FA desaturase 1 activity estimated by the ratio of 20:4 n-6 to 20:3 n-6 was inversely associated with the risk of HCC [ $OR_{T3vsT1} = 0.36$  [0.18-0.73] ( $p = 0.004$ )]. This lower enzymatic activity combined with elongase 5 activity (evaluated by the 20:3 n-6 to 18:2 n-6 ratio) tended to

be lower among HCC cases although the difference was close to significance [ $OR_{T3vsT1}=0.56$  [0.31, 1.01] ( $p=0.059$ )]. The ratio of 16:1 n-9 to 18:1 n-9, as an evaluation of peroxisomal  $\beta$ -oxidation, showed a positive association with HCC risk among cirrhotic patients [ $OR_{T3vsT1}=1.46$  [0.76, 2.80] ( $p=0.024$ )].

## **DISCUSSION**

This case-controlled study among 899 cirrhotic patients showed differences in the fatty acid composition of the erythrocyte membrane between cases with hepatocellular carcinoma and cirrhotic controls without HCC. The sum of saturated fatty acids and, in particular, the concentrations of stearic acid (18:0) as well as PUFA, the concentration of arachidonic acid (C20:4 n-6) were lower in cases than in controls. In contrast, the sum of fatty acids with a high number of carbon atoms [pentadecanoate (15:0) plus heptadecanoate/margaric acid (17:0)], and the concentration of eicosenoic acid (20:1 n-9) or linoleic acid (18:2 n-6) were positively associated with a risk of HCC on a cirrhotic liver.

The fatty acid composition of the erythrocyte membrane appears interesting to study the medium-term changes in the composition of circulating fatty acids. The rigidity of the erythrocyte membrane is modified according to its fatty acid composition (25). The modifications alter the fluidity of the membrane and affect several cellular functions, including mediator transport, the properties of certain membrane-bound enzymes, binding to insulin and opiate receptors, phagocytosis, endocytosis, depolarization-dependent exocytosis, immunology and chemotherapeutic cytotoxicity, prostaglandin production and cell growth (14). Many studies have shown that a change in the fatty acid composition of the erythrocyte membrane is associated with an excess risk of developing neoplasia (26–28). Baro et al. suggested that patients with colorectal cancer presented smaller amounts of saturated, monounsaturated and polyunsaturated fatty acids, particularly n-6 PUFA, than did matched healthy subjects (29). Similarly, in a study of more than 900 cases with a diagnosis of prostate cancer and more than 1,000 healthy controls, Crowe et al. found significant positive associations between levels of myristic acid, alpha-linolenic acid and eicosapentaenoic acids and the risk of high-grade prostate cancer (30). Similar results suggesting an association between the risk of cancer and fatty acid composition of erythrocyte membranes have been found for colon cancer, adenoma occurrence in high-grade dysplasia and breast cancer, as well as for non-Hodgkin's lymphoma (28,31–34).

Some studies have highlighted changes in the fatty acid composition of the erythrocyte membrane of cirrhotic patients (20,25,35,36). For example, Guarini et al. showed higher levels of palmitic acid (C16:0) and saturated SFA levels, but lower levels of PUFAs in 33 cirrhotic patients than in 40 control subjects (20). Differences were found in the aetiology of cirrhosis with specific alterations in patients with alcoholic cirrhosis (24,25,45). Shiraishi et al. showed significantly lower concentrations of PUFAs, linoleic acid (18:2 n-6), arachidonic acid (20:4 n-6) and eicosapentaenoic acid (20:5 n-3) in the group of alcoholic patients than in healthy controls (21). These alterations change the fluidity of the erythrocyte membrane, thus deforming the cell and aggravating disorders of hepatic microcirculation (37).

Although some studies have investigated the fatty acid composition of the erythrocyte membrane in cirrhosis, few have investigated differences in comparison with erythrocyte FA composition in subjects with HCC (4,38,39). Among the rare studies on this subject, Qiu et al. showed that concentrations of linoleic acid (18:2 n-6), oleic acid (18:1 n-9), arachidonic acid (20:4 n-6) and palmitic acid (16:0) were associated with the risk of HCC (38). Many studies have shown the effect of consuming specific polyunsaturated fatty acids on the risk of developing HCC (6,9). In our study, the risk of HCC was not associated with the erythrocyte membrane concentration of n-3 PUFAs after adjustment but an association was found for n-6 PUFAs. PUFAs are fundamental for signalling pathways and cellular communication. The two families, n-6 and n-3, compete for their incorporation into cell membranes but also for many intracellular or membrane enzymes so as to modulate their functions (signalling, communication, inflammation...) (40). The ratio between n-3 and n-6 is an essential parameter in pathophysiological processes (41). In his study, Gao et al. showed that the consumption of n-3 PUFAs, mainly derived from the consumption of fatty fish (salmon, tuna, etc.), with quantities estimated by questionnaires, was associated with a reduction in the risk of developing liver cancer (12). These protective effects against the risk of cancer are mainly explained by the antioxidant and anti-inflammatory effects of n-3 PUFAs (42–44). N-3 PUFAs appear to decrease the production and activity of anti-inflammatory cytokine IL-10, pro-inflammatory cytokines (IL-1, IL-6, IL-12 and TNF-alpha) and prostaglandins E2 (45), whereas omega-6 PUFAs have an inverse effect (46). In our study, levels of linoleic acid were higher while levels of arachidonic acid were lower in cirrhotic subjects with HCC than in controls. Linoleic acid (18:2 n-6) is transformed by an enzymatic cascade of desaturase and elongase into arachidonic acid (20:4 n-6). Our results seem to suggest a defect in the metabolism of arachidonic acid in cirrhotic patients with HCC. In stress situations, arachidonic acid contained in the membrane is released and transformed into leukotrienes, thromboxanes or prostaglandins (47,48). However, numerous studies have demonstrated decreased plasma levels of arachidonic acid in cirrhotic patients (20,35,49,50) and especially those with HCC (38). Cabré et al. showed that a plasma deficiency of arachidonic acid was associated with a higher risk of mortality in cirrhotic subjects but only used univariate analysis (50). Some studies showed that arachidonic acid had a beneficial role in subjects with HCC (48,51,52). Indeed, several hypotheses were suggested to explain this decrease in arachidonic acid: altered synthesis from linoleic acid via gamma-linolenic acid and di-homo-gamma-linolenic acid in the cirrhotic liver (53); stimulation of the conversion of arachidonic acid to eicosanoids (54); or enhancement of the oxidation of arachidonic acid in mononuclear cells (55). According to these results, these pathways could be interesting prevention tools to limit arachidonic acid deficiency (48). However, new studies and intervention trials are still needed before systematic supplementation can be implemented in cirrhotic patients.

In our study, concentrations of pentadecanoate (15:0) plus heptadecanoate (17:0) were higher in erythrocyte membranes of patients with HCC than in those without HCC. Fatty acids containing an odd number of carbon atoms cannot be synthesized *de novo* and only derive from ruminant products (56). A positive association between serum phospholipid pentadecanoate plus heptadecanoate concentrations and dietary dairy products has been reported (57). Thus, our results could suggest that high dietary intakes of dairy products may be associated with HCC risk in cirrhotic patients.

Several limitations must be emphasized in this study. First, it was a case-controlled study with differences regarding the characteristics of recruited individuals. However, the statistical analyses were adjusted to minimize the potential impact of these differences. Moreover, the patients were all recruited from University Hospitals and were therefore not representative of the general population. Finally, a comparison with "healthy" controls would have allowed us to better understand the modifications specific to cirrhosis. Nonetheless, our study presented some strength. First, it had the advantage of including relatively large samples of well-characterized cases and controls, which allowed potential confounders to be taken into account. This is the first multicentre study involving so many subjects to investigate the fatty acid composition of the erythrocyte membrane in cirrhotic subjects with or without HCC. The results relied on centralized and blinded analyses by a specialized lipidomics laboratory on blood samples stored in optimal conditions. The use of a recent optimized method (58) allowed the more accurate measurement of PUFAs.

## **CONCLUSION**

In conclusion, we observed different fatty acid profiles of the erythrocyte membrane in cirrhotic patients according to the presence of hepatocellular carcinoma. Although it suggests a possible effect of unbalanced dietary intake and changes in fatty acid metabolism, no firm links to HCC can be made. Our hypotheses need to be confirmed in other epidemiological studies and the mechanisms involved should be explored in experimental studies.

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#### **LEGENDS for TABLES AND FIGURES:**

**TABLE 1: Baseline characteristics of cases and controls in the CIRCE study**

**Notes:** BMI, body mass index; TIA, transient ischemic attack; HBP, high blood pressure. \*Chi square tests or Fischer-exact tests were used according to the applications' condition \*\* Median (IQR). \*\*\* Beware virus infection require special attention; chronic hepatitis is not necessarily the main cirrhosis aetiology.

1: underweight or normal [ $<25$  kg/m<sup>2</sup>], overweight [25-30 kg/m<sup>2</sup>], and obese [ $\geq 30$  kg/m<sup>2</sup>];

2: systolic BP  $\geq 140$  mmHg or diastolic BP  $\geq 90$ mmHg.

**TABLE 2: Mean percentages of the fatty acids of the erythrocyte membrane at baseline in cases and controls in the CIRCE study**

**Notes:** All values are means  $\pm$  SDs. CIRCE, Cirrhose et Risque de Carcinome hépatocellulaire dans le grand Est, SFAs, Saturated Fatty Acids; MUFAs, Mono-Unsaturated Fatty Acids; PUFAs, Poly-Unsaturated Fatty Acids.

**TABLE 3: ORs (95% CIs) for the risk of hepatocellular carcinoma according to tertiles of fatty acids in the erythrocyte membrane (in percent of total fatty acids)**

**Note:** OR, odds ratio; FA, fatty acid; MUFA, mono-unsaturated fatty acid; PUFA, polyunsaturated fatty acid; ALA,  $\alpha$ -linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SCD-1, enzyme steroyl-CoA desaturase. 1: Unconditional logistic regression models were adjusted for age, study centre, gender, seniority of cirrhosis, diabetes, aetiology of cirrhosis, Child-Pugh score, history of arteritis and educational level.

**TABLE 1: Baseline characteristics of cases and controls in the CIRCE study**

Variables	Controls n=550 n (%)	Cases n=349 n (%)	pvalue*
<b>Socio-demographic characteristics</b>			
<b>Women</b>	165 (30.0)	45 (13.0)	<b>&lt;0.001</b>
<b>Age, years**</b>	58.8 (51.6-65.0)	64.2 (58.8-71.6)	<b>&lt;0.001</b>
<60.87	327 (59.4)	123 (35.2)	
≥60.87	223 (40.6)	226 (64.8)	
<b>Education level</b>			<b>0.014</b>
≤12 years of schooling	473 (86.6)	321 (92.0)	
More than 12 years or high-school diploma	73 (13.4)	28 (8.0)	
<b>Professional Physical activity</b>			<b>0.006</b>
Inactive	378 (69.4)	259 (75.3)	
Moderate active	104 (19.1)	38 (11.0)	
Active	63 (11.5)	47 (13.7)	
<b>Leisure-Time Physical activity</b>			<b>0.548</b>
Inactive	276 (50.4)	182 (52.2)	
Moderate active	117 (21.4)	64 (18.3)	
Active	155 (28.3)	103 (29.5)	
<b>BMI class<sup>1</sup></b>			<b>0.152</b>
Underweight or normal	204 (37.1)	112 (32.1)	
Overweight	204 (37.1)	128 (36.7)	
Obese	142 (24.8)	109 (31.2)	
<b>Smoking status</b>			<b>0.002</b>
Never	146 (26.5)	76 (21.8)	
Current	216 (39.3)	105 (30.1)	
Former	188 (35.2)	168 (48.1)	
<b>Alcohol status</b>			<b>0.865</b>
Never	40 (7.7)	23(7.0)	
Current	131 (25.0)	79 (24.0)	
Former	352 (67.3)	227 (69.0)	
<b>Characteristics of cirrhosis</b>			
<b>Time since cirrhosis diagnosis, years**</b>	2.0 (1.0 - 5.0)	1.0 (0.0 - 5.0)	<b>&lt;0.001</b>
<2	231 (42.0)	201 (57.9)	
≥2	319 (58.0)	146 (42.1)	
<b>Main cirrhosis aetiology***</b>			<b>0.662</b>
Virus infection with or without alcohol	156 (28.4)	102 (29.2)	
Alcohol without virus	337 (61.2)	205 (58.8)	
Other causes	57 (10.4)	42 (12.0)	
<b>Child-Pugh Score</b>			<b>&lt;0.001</b>
A	258 (48.4)	223 (64.5)	
B	177 (33.2)	87 (25.1)	
C	98 (18.4)	36 (10.4)	

<b>Diabetes</b>			<b>&lt;0.001</b>
No	284 (51.6)	157 (45.0)	
Yes	181 (32.9)	168 (48.1)	
Missing Data	85 (15.5)	24 (6.9)	
<b>Medical History</b>			
<b>History of extrahepatic cancer</b>	26 (4.9)	20 (5.7)	0.510
<b>Cardiovascular disease</b>			
<b>Stroke / TIA</b>	17 (3.1)	12 (3.5)	0.770
<b>Acute coronary syndrome</b>	29 (5.3)	21 (6.0)	0.632
<b>Arteritis</b>	49 (9.0)	53 (15.4)	<b>0.004</b>
<b>HBP<sup>2</sup></b>	184 (33.5)	167 (48.1)	<b>&lt;0.001</b>
<b>Anti-diabetic, hormonal and cholesterol-lowering treatments</b>			
<b>Fibrates</b>	24 (4.4)	21 (6.0)	0.268
<b>Statin</b>	66 (12.0)	59 (16.9)	<b>0.038</b>
<b>Anti-diabetic</b>	134 (48.2)	138 (76.7)	<b>&lt;0.001</b>

**Notes:** BMI, body mass index; TIA, transient ischemic attack; HBP, high blood pressure.

\*Chi square tests or Fischer-exact tests were used according to the conditions of application

\*\* Median (IQR).

\*\*\* N.B. virus infection requires special attention, chronic hepatitis is not necessarily the main cirrhosis aetiology.

1: underweight or normal [ $<25$  kg/m<sup>2</sup>], overweight [25-30 kg/m<sup>2</sup>], and obese [ $\geq 30$  kg/m<sup>2</sup>];

2: systolic BP  $\geq 140$  mmHg or diastolic BP  $\geq 90$ mmHg.

**TABLE 2: Mean percentages of the fatty acids of the erythrocyte membrane at baseline in cases and controls in the CIRCE study**

<b>Variables</b>	<b>Controls n=550 n (%)</b>	<b>Cases n=349 n (%)</b>
<b>SFAs</b>		
<b>Sums of SFAs</b>	39.63 $\pm$ 3.28	39.68 $\pm$ 4.95
<b>12:0</b>	0.21 $\pm$ 0.09	0.21 $\pm$ 0.10
<b>14:0</b>	0.44 $\pm$ 0.18	0.45 $\pm$ 0.18
<b>15:0</b>	0.24 $\pm$ 0.18	0.27 $\pm$ 0.20
<b>16:0</b>	23.42 $\pm$ 2.27	23.47 $\pm$ 3.06
<b>17:0</b>	0.34 $\pm$ 0.29	0.39 $\pm$ 0.31
<b>18:0</b>	14.79 $\pm$ 1.61	14.71 $\pm$ 2.17
<b>19:0</b>	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01
<b>20:0</b>	0.06 $\pm$ 0.02	0.06 $\pm$ 0.02
<b>21:0</b>	0.002 $\pm$ 0.0007	0.002 $\pm$ 0.0007
<b>22:0</b>	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01
<b>23:0</b>	0.002 $\pm$ 0.0001	0.003 $\pm$ 0.0001
<b>24:0</b>	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01
<b>26:0</b>	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01
<b>15:0 + 17:0</b>	0.58 $\pm$ 0.46	0.66 $\pm$ 0.50
<b>MUFAs</b>		
<b>Sums of MUFAs</b>	14.01 $\pm$ 4.72	14.32 $\pm$ 4.63

<b>16:1 (n-7)</b>	1.05 ± 1.05	1.09 ± 1.07
<b>16:1 (n-9)</b>	0.18 ± 0.17	0.20 ± 0.20
<b>18:1 (n-7)</b>	3.15 ± 2.24	3.26 ± 2.28
<b>18:1 (n-9)</b>	9.20 ± 2.24	9.34 ± 2.08
<b>20:1 (n-7)</b>	0.03 ± 0.02	0.03 ± 0.01
<b>20:1 (n-9)</b>	0.26 ± 0.07	0.27 ± 0.07
<b>24:1 (n-9)</b>	0.08 ± 0.03	0.09 ± 0.03
<b>PUFAs</b>		
<b>Sums of PUFAs</b>	46.36 ± 6.05	46.00 ± 6.97
<b>Sums of n-6 PUFAs</b>		
<b>18:2 (n-6)</b>	13.67 ± 2.65	14.13 ± 2.74
<b>18:3 (n-6)</b>	0.07 ± 0.06	0.07 ± 0.05
<b>20:2 (n-6)</b>	0.26 ± 0.13	0.30 ± 0.15
<b>20:3 (n-6)</b>	1.78 ± 0.78	1.82 ± 0.78
<b>20:4 (n-6)</b>	19.52 ± 5.97	18.33 ± 6.33
<b>22:2 (n-6)</b>	0.03 ± 0.01	0.03 ± 0.01
<b>22:4 (n-6)</b>	2.62 ± 0.62	2.58 ± 0.72
<b>22:5 (n-6)</b>	0.55 ± 0.19	0.54 ± 0.19
<b>Sums of n-3 PUFAs</b>		
<b>18:3 (n-3)</b>	0.07 ± 0.05	0.08 ± 0.06
<b>20:5 (n-3)</b>	0.75 ± 0.47	0.81 ± 0.52
<b>22:3 (n-3)</b>	0.06 ± 0.03	0.06 ± 0.03
<b>22:5 (n-3)</b>	2.16 ± 0.52	2.16 ± 0.57
<b>22:6 (n-3)</b>	4.73 ± 1.35	5.02 ± 1.60
<b>24:6 (n-3)</b>	0.01 ± 0.004	0.01 ± 0.004

**Notes:** All values are means ± SDs. CIRCE, Cirrhose et Risque de Carcinome hépatocellulaire dans le grand Est, SFAs, Saturated Fatty Acids; MUFAs, Mono-Unsaturated Fatty Acids; PUFAs, Poly-Unsaturated Fatty Acids.

**TABLE 3: ORs (95% CIs) for the risk of hepatocellular carcinoma according to tertiles of fatty acids in the erythrocyte membrane (in percent of total fatty acids)**

Fas (in %)	FA name	Controls n=550 n (%)	Cases n=349 n (%)	Crude OR (95% CI)	P-trend	Adjusted <sup>1</sup> OR (95% CI)	P-trend
<b>SFAs</b>							
<b>Sum of SFAs</b>					<b>0.016</b>		<b>0.046</b>
≤ 38.69		182 (33.0)	128 (36.7)	1.000 (reference)		1.000 (reference)	
38.69 - 40.04		184 (33.5)	139 (39.8)	1.074 (0.783 ; 1.473)		0.989 (0.665 ; 1.470)	
> 40.04		184 (33.5)	82 (23.5)	0.634 (0.449 ; 0.895)		0.646 (0.425 ; 0.981)	
<b>14:0</b>	Myristic acid				0.908		0.187
≤ 0.34		182 (33.0)	118 (33.8)	1.000 (reference)		1.000 (reference)	
0.34 - 0.48		184 (33.5)	110 (31.5)	0.922 (0.662 ; 1,284)		0.907 (0.596 ; 1.381)	
> 0.48		184 (33.5)	121 (34.7)	1.014 (0.732 ; 1.405)		1.316 (0.829 ; 2.089)	
<b>15:0</b>	Pentadecanoic acid				<b>0.015</b>		<b>0.005</b>
≤ 0.11		182 (33.0)	83 (23.8)	1.000 (reference)		1.000 (reference)	

0.11 - 0.27		184 (33.5)	130 (37.2)	1.549 (1.099 ; 2.184)		1.594 (0.863 ; 2.946)	
> 0.27		184 (33.5)	136 (39.0)	1.621 (1.152 ; 2.280)		2.634 (1.307 ; 5.311)	
<b>16:0</b>	<b>Palmitic acid</b>				<b>0.109</b>		<b>0.957</b>
≤ 22.54		182 (33.0)	125 (35.8)	1.000 (reference)		1.000 (reference)	
22.54 - 23.78		185 (33.7)	128 (36.7)	1.007 (0.731 ; 1.388)		0.957 (0.647 ; 1.415)	
> 23.78		183 (33.3)	96 (27.5)	0.764 (0.546 ; 1.069)		1.009 (0.665 ; 1.532)	
<b>17:0</b>	<b>Margaric acid</b>				<b>0.010</b>		<b>0.002</b>
≤ 0.11		182 (33.0)	74 (21.2)	1.000 (reference)		1.000 (reference)	
0.11 - 0.36		185 (33.7)	141 (40.4)	1.874 (1.324 ; 2.654)		2.470 (1.188 ; 5.134)	
> 0.36		183 (33.3)	134 (38.4)	1.801 (1.269 ; 2.556)		4.472 (1.890 ; 10.580)	
<b>18:0</b>	<b>Stearic acid</b>				<b>0.013</b>		<b>&lt;0.001</b>
≤ 14.10		183 (33.3)	132 (37.8)	1.000 (reference)		1.000 (reference)	
14.10 - 15.22		184 (33.4)	132 (37.8)	0.995 (0.725 ; 1.365)		0.608 (0.401 ; 0.906)	
> 15.22		183 (33.3)	85 (24.4)	0.644 (0.458 ; 0.906)		0.356 (0.227 ; 0.560)	
<b>15:0 + 17:0</b>					<b>0.007</b>		<b>0.002</b>
<0.23		183 (33.3)	74 (21.2)	1.000 (reference)		1.000 (reference)	
0.23-0.64		184 (33.4)	141 (40.4)	1.895 (1.338 ; 2.683)		2.621 (1.346 ; 5.104)	
≥ 0.64		183 (33.3)	134 (38.4)	1.811 (1.276 ; 2.569)		4.353 (1.968 ; 9.627)	
<b>cis MUFA</b>							
<b>SumMUFAs</b>					<b>0.270</b>		<b>0.079</b>
≤ 11.17		183 (33.3)	97 (27.8)	1.000 (reference)		1.000 (reference)	
11.17 - 15.58		184 (33.4)	131 (37.5)	1.343 (0.963 ; 1.874)		1.145 (0.753 ; 1.743)	
> 15.58		183 (33.3)	121 (37.7)	1.247 (0.891 ; 1.747)		1.604 (0.944 ; 2.727)	
<b>16:1 (n-7)</b>	<b>Palmitoleic acid</b>				<b>0.935</b>		<b>0.496</b>
≤ 0.45		182 (33.0)	111 (31.8)	1.000 (reference)		1.000 (reference)	
0.45 - 1.16		184 (33.5)	122 (35.0)	1.087 (0.783 ; 1.510)		0.846 (0.506 ; 1.414)	
> 1.16		184 (33.5)	116 (33.2)	1.034 (0.742 ; 1.439)		1.064 (0.599 ; 1.892)	
<b>16:1 (n-9)</b>	<b>Cis-7-</b>				<b>0.558</b>		<b>0.185</b>
≤ 0.08	<b>Hexadecenoic acid</b>	183 (33.3)	106 (30.3)	1.000 (reference)		1.000 (reference)	
0.08 - 0.18		183 (33.3)	122 (35.0)	1.151 (0.826 ; 1.603)		0.807 (0.465 ; 1.400)	
> 0.18		184 (33.4)	121 (34.7)	1.135 (0.815 ; 1.581)		1.226 (0.650 ; 2.311)	
<b>18:1 (n-7)</b>	<b>Vaccenic acid</b>				<b>0.390</b>		<b>0.095</b>
≤ 1.72		183 (33.3)	104 (29.8)	1.000 (reference)		1.000 (reference)	
1.72 - 3.86		184 (33.4)	123 (35.2)	1.176 (0.844 ; 1.639)		0.505 (0.267 ; 0.956)	
> 3.86		183 (33.3)	122 (35.0)	1.173 (0.841 ; 1.635)		1.219 (0.588 ; 2.528)	
<b>18:1 (n-9)</b>	<b>Oleic acid</b>				<b>0.689</b>		<b>0.607</b>
≤ 8.34		182 (33.0)	111 (31.8)	1.000 (reference)		1.000 (reference)	
8.34 - 10.03		184 (33.5)	118 (33.8)	1.051 (0.756 ; 1.463)		1.022 (0.692 ; 1.508)	
> 10.03		184 (33.5)	120 (34.4)	1.069 (0.769 ; 1.487)		0.897 (0.594 ; 1.355)	
<b>20:1 (n-9)</b>	<b>Eicosenoic acid</b>				<b>&lt;0.001</b>		<b>&lt;0.001</b>
≤ 0.22		183 (33.3)	87 (24.9)	1.000 (reference)		1.000 (reference)	
0.22 - 0.27		183 (33.3)	106 (30.4)	1.218 (0.859 ; 1.729)		1.283 (0.798 ; 2.062)	
> 0.27		184 (33.4)	156 (44.7)	1.783 (1.279 ; 2.487)		2.258 (1.358 ; 3.752)	
<b>n-6 PUFAs</b>							
<b>Sum of n-6 PUFAs</b>					<b>0.074</b>		<b>0.134</b>
≤ 35.87		183 (33.3)	132 (37.8)	1.000 (reference)		1.000 (reference)	
35.87 - 41.16		183 (33.3)	119 (34.1)	0.902 (0.654 ; 1.243)		0.586 (0.367 ; 0.936)	
> 41.16		184 (33.4)	98 (28.1)	0.738 (0.530 ; 1.029)		0.652 (0.369 ; 1.150)	
<b>18:2 (n-6)</b>	<b>Linoleic acid</b>				<b>0.003</b>		<b>&lt;0.001</b>
≤ 12.40		183 (33.3)	90 (25.8)	1.000 (reference)		1.000 (reference)	
12.40 - 14.65		184 (33.4)	111 (31.8)	1.227 (0.869 ; 1.732)		1.564 (1.040 ; 2.353)	



> 14.65		183 (33.3)	148 (42.4)	1.644 (1.179 ; 2.293)		2.217 (1.434 ; 3.428)	
<b>18:3 (n-6)</b>	<b><math>\gamma</math>-Linolenic acid</b>				<b>0.086</b>	<b>0.204</b>	
≤ 0.02		183 (33.3)	96 (27.5)	1.000 (reference)		1.000 (reference)	
0.02 - 0.07		183 (33.3)	120 (34.4)	1.250 (0.892 ; 1.752)		0.894 (0.561 ; 1.424)	
> 0.07		184 (33.4)	133 (38.1)	1.378 (0.988 ; 1.922)		1.224 (0.727 ; 2.062)	
<b>20:2 (n-6)</b>	<b>Eicosadienoic acid</b>				<b>&lt;0.001</b>	<b>0.002</b>	
≤ 0.17		182 (33.0)	83 (23.8)	1.000 (reference)		1.000 (reference)	
0.17 - 0.30		184 (33.5)	115 (32.9)	1.370 (0.967 ; 1.942)		1.255 (0.692 ; 2.274)	
> 0.30		184 (33.5)	151 (43.3)	1.799 (1.284 ; 2.521)		2.472 (1.247 ; 4.900)	
<b>20:3 (n-6)</b>	<b>Dihomo-<math>\gamma</math>-linoleic acid</b>				<b>0.201</b>	<b>0.449</b>	
≤ 1.22		182 (33.0)	87 (24.9)	1.000 (reference)		1.000 (reference)	
1.22 - 2.13		184 (33.5)	146 (41.8)	1.660 (1.187 ; 2.321)		1.330 (0.801 ; 2.209)	
> 2.13		184 (33.5)	116 (33.3)	1.319 (0.934 ; 1.863)		1.327 (0.728 ; 2.421)	
<b>20:4 (n-6)</b>	<b>Arachidonic acid</b>				<b>&lt;0.001</b>	<b>&lt;0.001</b>	
≤ 16.09		183 (33.3)	154 (44.1)	1.000 (reference)		1.000 (reference)	
16.09 - 21.81		184 (33.4)	114 (32.7)	0.736 (0.536 ; 1.011)		0.352 (0.221 ; 0.560)	
> 21.81		183 (33.3)	81 (23.2)	0.526 (0.375 ; 0.738)		0.272 (0.148 ; 0.500)	
<b>22:4 (n-6)</b>	<b>Adrenic acid / Docotetraenoic acid</b>				<b>0.363</b>	<b>0.073</b>	
≤ 2.44		183 (33.3)	126 (36.1)	1.000 (reference)		1.000 (reference)	
2.44 - 2.89		183 (33.3)	114 (32.7)	0.905 (0.653 ; 1.253)		0.731 (0.491 ; 1.089)	
> 2.89		184 (33.4)	109 (31.2)	0.860 (0.620 ; 1.194)		0.686 (0.454 ; 1.037)	
<b>n-3 PUFAs</b>							
<b>Sum of n-3 PUFAs</b>					<b>0.004</b>	<b>0.425</b>	
≤ 7.00		183 (33.3)	88 (25.2)	1.000 (reference)		1.000 (reference)	
7.00 - 8.72		183 (33.3)	116 (33.2)	1.318 (0.934 ; 1.860)		0.986 (0.621 ; 1.566)	
> 8.72		184 (33.4)	145 (41.6)	1.639 (1.173 ; 2.290)		0.846 (0.524 ; 1.365)	
<b>18:3 (n-3)</b>	<b>ALA</b>				<b>0.091</b>	<b>0.219</b>	
≤ 0.04		183 (33.3)	96 (27.5)	1.000 (reference)		1.000 (reference)	
0.04 - 0.07		184 (33.4)	123 (35.2)	1.274 (0.910 ; 1.784)		1.021 (0.639 ; 1.631)	
> 0.07		183 (33.3)	130 (37.3)	1.354 (0.970 ; 1.891)		1.302 (0.781 ; 2.169)	
<b>20:5 (n-3)</b>	<b>EPA</b>				<b>0.036</b>	<b>0.750</b>	
≤ 0.47		182 (33.0)	98 (28.1)	1.000 (reference)		1.000 (reference)	
0.47 - 0.84		185 (33.7)	112 (32.1)	1.124 (0.801 ; 1.579)		0.846 (0.531 ; 1.346)	
> 0.84		183 (33.3)	139 (39.8)	1.411 (1.014 ; 1.962)		1.009 (0.602 ; 1.691)	
<b>22:5 (n-3)</b>	<b>DPA</b>				<b>0.290</b>	<b>0.162</b>	
≤ 1.97		182 (33.0)	97 (27.8)	1.000 (reference)		1.000 (reference)	
1.97 - 2.43		184 (33.5)	135 (38.7)	1.377 (0.988 ; 1.918)		1.222 (0.775 ; 1.926)	
> 2.43		184 (33.5)	117 (33.5)	1.193 (0.851 ; 1.673)		0.762 (0.470 ; 1.235)	
<b>22:6 (n-3)</b>	<b>DHA</b>				<b>0.002</b>	<b>0.677</b>	
≤ 4.22		183 (33.3)	92 (26.4)	1.000 (reference)		1.000 (reference)	
4.22 - 5.22		183 (33.3)	104 (29.8)	1.130 (0.799 ; 1.600)		0.855 (0.558 ; 1.310)	
> 5.22		184 (33.4)	153 (43.8)	1.654 (1.189 ; 2.300)		0.895 (0.583 ; 1.374)	
<b>Ratio and enzymatic activity index</b>							
<b>16:1 (n-7) cis to 16:0</b>	<b>SCD-1 desaturase index n-7</b>				<b>0.945</b>	<b>0.844</b>	
≤ 0.01		183 (33.3)	112 (32.1)	1.000 (reference)		1.000 (reference)	
0.01 - 0.04		183 (33.3)	123 (35.2)	1.098 (0.791 ; 1.524)		0.757 (0.447 ; 1.279)	
> 0.04		184 (33.4)	114 (32.7)	1.012 (0.727 ; 1.410)		0.894 (0.499 ; 1.604)	
<b>18:1 (n-9) to 18:0</b>	<b>SCD-1 desaturase index n-9</b>				<b>0.125</b>	<b>0.125</b>	
≤ 0.55		183 (33.3)	98 (28.1)	1.000 (reference)		1.000 (reference)	
0.55 - 0.69		184 (33.4)	124 (35.5)	1.258 (0.900 ; 1.759)		1.327 (0.896 ; 1.966)	

> 0.69		183 (33.3)	127 (36.4)	1.296 (0.928 ; 1.809)	1.385 (0.907 ; 2.115)
<b>18:3 (n-6) to 18:2 (n-6)</b>	$\delta$ -6-desaturase			0.271	0.651
≤ 0.002		183 (33.3)	104 (29.8)	1.000 (reference)	1.000 (reference)
0.002 - 0.005		184 (33.4)	118 (33.8)	1.128 (0.808 ; 1.575)	0.821 (0.521 ; 1.296)
< 0.005		183 (33.3)	127 (36.4)	1.221 (0.878 ; 1.699)	1.004 (0.586 ; 1.723)
<b>20:4 (n-6) to 20:3 (n-6)</b>	$\delta$ -5-desaturase			<b>0.001</b>	<b>0.004</b>
≤ 7.78		183 (33.3)	128 (36.7)	1.000 (reference)	1.000 (reference)
7.78 - 18.59		184 (33.4)	143 (41.0)	1.111 (0.812 ; 1.521)	0.715 (0.443 ; 1.153)
> 18.59		183 (33.3)	78 (22.3)	0.609 (0.430 ; 0.863)	0.362 (0.181 ; 0.723)
<b>20:3 (n-6) to 18:2 (n-6)</b>	$\delta$ -6-desaturase			0.457	0.060
≤ 0.08	+ Elongase	182 (33.1)	107 (30.7)	1.000 (reference)	1.000 (reference)
0.08 - 0.16	5	184 (33.4)	141 (40.4)	1.303 (0.942 ; 1.803)	0.773 (0.484 ; 1.235)
> 0.16		184 (33.4)	101 (28.9)	0.934 (0.664 ; 1.312)	0.561 (0.308 ; 1.020)
<b>Sum of n-6 PUFAs to sum of n-3 PUFAs</b>				0.005	0.826
≤ 4.13		183 (33.3)	140 (40.1)	1.000 (reference)	1.000 (reference)
4.13 - 5.76		184 (33.4)	121 (34.7)	0.855 (0.622 ; 1.175)	1.030 [0.702 ; 1.511]
> 5.76		183 (33.3)	88 (25.2)	0.622 [0.444 ; 0.870]	0.943 [0.563 ; 1.580]
<b>18:1 (n-7) to 16:1 (n-7)</b>	Elongase 5 or 6			0.574	0.154
≤ 2.89		182 (33.1)	109 (31.2)	1.000 (reference)	1.000 (reference)
2.89 - 4.37		185 (33.6)	137 (39.3)	1.236 (0.894 ; 1.710)	1.223 (0.834 ; 1.792)
4.37		183 (33.3)	103 (29.5)	0.940 (0.670 ; 1.318)	0.774 (0.507 ; 1.181)
<b>16:1 (n-9) to 18:1 (n-9)</b>	Peroxisomal B-oxidation			0.251	<b>0.024</b>
≤ 0.01		182 (33.1)	105 (30.1)	1.000 (reference)	1.000 (reference)
0.01 - 0.02		185 (33.6)	116 (33.2)	1.087 (0.778 ; 1.518)	0.755 (0.429 ; 1.330)
> 0.02		183 (33.3)	128 (36.7)	1.212 (0.872 ; 1.686)	1.464 (0.764 ; 2.806)
<b>18:3 (n-6) to 18:2 (n-3)</b>				0.254	0.927
≤ 169.19		182 (33.1)	125 (35.8)	1.000 (reference)	1.000 (reference)
169.19-310.53		185 (33.6)	120 (34.4)	0.944 (0.683 ; 1.305)	0.661 (0.441 ; 0.992)
> 310.53		183 (33.3)	104 (29.8)	0.827 (0.594 ; 1.152)	0.951 (0.548 ; 1.650)
<b>22:4 (n-6) to 20:4 (n-6)</b>	Elongase 2/5			0.044	0.077
≤ 0.11		183 (33.3)	93 (26.7)	1.000 (reference)	1.000 (reference)
0.11 - 0.17		183 (33.3)	123 (35.2)	1.323 (0.943 ; 1.855)	1.434 (0.869 ; 2.368)
> 0.17		184 (33.4)	133 (38.1)	1.422 (1.018 ; 1.988)	1.686 (0.969 ; 2.934)
<b>22:5 (n-3) to 20:5 (n-3)</b>	Elongase 2/5			0.028	0.478
≤ 2.73		182 (33.1)	134 (38.4)	1.000 (reference)	1.000 (reference)
2.73 - 4.10		185 (33.6)	122 (35.0)	0.896 (0.651 ; 1.233)	0.819 (0.552 ; 1.215)
> 4.10		183 (33.3)	93 (26.6)	0.690 (0.494 ; 0.965)	0.820 (0.497 ; 1.352)

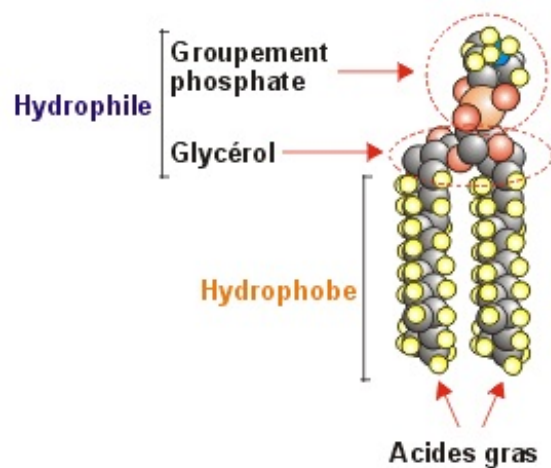
**Note:** OR, odds ratio; FA, fattyacid; MUFA, mono-unsaturated fatty acid; PUFA, polyunsaturated fatty acid; ALA,  $\alpha$ -linoleicacid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SCD-1, enzyme steroyl-CoA desaturase. 1: Unconditional logistic regression models were adjusted for age, study centre, gender, seniority of cirrhosis, diabetes, aetiology of cirrhosis, Child-Pugh score, history of arteritis and educational level.

*Alexia Karen Cotte, Vanessa Cottet, Virginie Aires, Thomas Mouillot, Maud Rizk, Sandrine Vinault, Christine Binquet, Jean-Paul Pais de Barros, Patrick Hillon and Dominique Delmas. Phospholipid profiles and hepatocellular carcinoma risk and prognosis in cirrhotic patients. Oncotarget (2019), Vol. 10, (No. 22), pp: 2161-2172, DOI: 10.18632/oncotarget.26738.*

## Introduction

Les phospholipides sont des constituants essentiels des membranes biologiques. La structure de la molécule phospholipidique est généralement constituée de deux "queues" d'acides gras hydrophobes et d'une "tête" hydrophile constituée d'un groupe phosphate. Les phospholipides diffèrent les uns des autres par la sorte d'acides gras rattachés au glycérol (figure 17). Généralement, un des deux acides gras est saturé et l'autre ne l'est pas. De nos jours, les phospholipides sont connus comme des acteurs de la progression tumorale. Leurs altérations métaboliques dans les tissus peuvent être associées à des modifications plasmatiques. Le but de cette étude était d'évaluer le profil des phospholipides plasmatiques en tant que biomarqueur du risque et facteur pronostique du CHC.

**Figure 17 : Représentation illustrée d'un phospholipide**



Source : <https://babel.cegestefoy.qc.ca/profs/gbourbonnais/pascal/fya/chimcell/notesmolecules/imagesmolecules/phospholcorel2.gif>

## **Méthodes**

Quatre-vingt-dix patients cirrhotiques avec (cas) ou sans CHC (témoins) ont été inclus dans cette étude après appariement sur le centre, l'âge, le sexe, présence d'une infection virale, l'ancienneté de la cirrhose et le score du Child-Pugh. La chromatographie en phase liquide à haute performance couplée à la spectrométrie de masse en tandem a été utilisée pour quantifier les principales espèces de huit catégories de phospholipides dans le plasma : PC, LPC, phosphatidyléthanolamine (PE), lysophosphatidyléthanolamine (LPE), sphingomyéline (SM), céramide (Cer), dihydrocéramide (DHcer) et PI.

## **Résultats**

Nos analyses ont montré des concentrations élevées de phosphatidylcholine (PC) 16:0/16:1 ( $p = 0,0180$ ), PC 16:0/16:0 ( $p = 0,0327$ ), PC 16:0/18:1 ( $p = 0,0264$ ) et de sphingomyéline (SM) 18:2/24:1 ( $p = 0,0379$ ) et des concentrations faibles de lysophosphatidylcholine 20:4 ( $p = 0,0093$ ) et de plasmalogène-phosphatidyléthanolamine (pPE) 16:0/20:4 ( $p = 0,0463$ ), pPE 18:0 /20:4 ( $p = 0,0077$ ), pPE 18:0/20:5 ( $p = 0,0163$ ), pPE 18:0/20:3 ( $p = 0,0463$ ) étaient significativement différents chez les patients atteints de CHC par rapport aux témoins cirrhotiques. Deux espèces de céramides ont été associées à une augmentation du risque de décès par le CHC. Tandis que les lysophospholipides, phosphatidylinositol polyinsaturés, certaines espèces de PC et de SM étaient associés à un faible risque de décès chez les patients atteints de CHC en 1 et/ou 3 ans.

## **Conclusion**

Cette étude a identifié les profils de phospholipides liés au risque de CHC chez les patients atteints de cirrhose hépatique ce qui nous aide à caractériser de façon plus précise les biomarqueurs potentiels du CHC se développant à partir d'une cirrhose. Malgré les difficultés et la complexité d'utiliser des marqueurs plasmatiques dans ce type de pathologie, les analyses ont montré pour la première fois le potentiel de certains phospholipides dans la prédiction de la mortalité des patients atteints de CHC.

## Phospholipid profiles and hepatocellular carcinoma risk and prognosis in cirrhotic patients

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**Keywords:** hepatocellular carcinoma; cirrhosis; phospholipids; biomarker; case-control study

**Received:** November 03, 2018

**Accepted:** February 09, 2019

**Published:** March 15, 2019

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### ABSTRACT

**Background:** Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide. Phospholipids are now well-recognised players in tumour progression. Their metabolic tissue alterations can be associated with plasmatic modifications. The aim of this study was to evaluate the potential of the plasma phospholipid profile as a risk and prognostic biomarker in HCC.

**Methods:** Ninety cirrhotic patients with (cases) or without HCC (controls) were studied after matching for inclusion centre, age, gender, virus infection, cirrhosis duration and Child-Pugh grade. High-performance liquid chromatography coupled with tandem-mass spectrometry was used to quantify the main species of seven categories of phospholipids in plasma.

**Results:** Elevated concentrations of phosphatidylcholine (PC) 16:0/16:1 ( $p=0.0180$ ), PC 16:0/16:0 ( $p=0.0327$ ), PC 16:0/18:1 ( $p=0.0264$ ) and sphingomyelin (SM) 18:2/24:1 ( $p=0.0379$ ) and low concentrations of lysophosphatidylcholine 20:4 (0.0093) and plasmalogen-phosphatidylethanolamine (pPE) 16:0/20:4 ( $p=0.0463$ ), pPE 18:0/20:4 ( $p=0.0077$ ), pPE 18:0/20:5 ( $p=0.0163$ ), pPE 18:0/20:3 ( $p=0.0463$ ) discriminated HCC patients from cirrhotic controls. Two ceramide species were associated with increased HCC risk of death while lysophospholipids, a polyunsaturated phosphatidylinositol, some PC and SM species were associated with low risk of death in HCC patients in 1 and/or 3 years.

**Conclusion:** This study identified phospholipid profiles related to HCC risk in liver cirrhotic patients and showed for the first time the potential of some phospholipids in predicting HCC patient mortality.

### INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy worldwide. Patients with early detection of HCC have a relatively good

prognosis, with a 5-year survival rate of more than 70%. Most patients with hepatocellular carcinoma are diagnosed with late-stage disease resulting in an overall 5-year survival rate of less than 20%. Its aggressiveness explains its second rank in cancer-related death [1]. In Western

countries, HCC occurs predominantly in viral, alcoholic, hemochromatosis or metabolic cirrhosis [2]. Most HCC cases are diagnosed at an advanced stage due to late diagnosis limiting treatment possibilities. Consequently, better knowledge of individual risk factors for HCC in persons with cirrhosis is the basis for disease management and for designing preventive or treatment strategies. The current issue is to provide markers making it possible to identify, among cirrhotic patients, HCC high-risk individuals who may benefit from intensive screening. Therefore, numerous studies have focused on finding new serologic or plasmatic biomarkers to combine them or not with existing diagnostic tests [3]. Among the new markers studied, lipids and particularly phospholipids retain the scientific community's attention. Alteration of tumour metabolism including lipid metabolism appears to be involved in carcinogenesis. This alteration of lipid metabolism is marked by an increase in *de novo* synthesis of all lipid classes. Two key steps in lipid synthesis that have been thoroughly described in carcinogenesis development are catalyzed by ATP citrate lyase (ACLY) and fatty acid synthase (FASN), where their inhibition could induce apoptosis and reduce tumour growth of cancer cells [4, 5]. More recently, phospholipids appear to be involved in tumour progression, through their structural function in membrane composition and their cell-signalling functions [6, 7]. In HCC, a metabolomic analysis revealed that phosphatidylcholine (PC) could be the predominant species in HCC tissue associated with overexpression of the lysophosphatidylcholine acyltransferase 1 (LPCAT1) involved in the reacylation of lysophosphatidylcholine (LPC) into PC [8]. In same manner, we have recently demonstrated that a lysophosphatidylcholine acyltransferase isoform, LPCAT2, and PC are also involved in chemoresistance in colorectal cancer [9]. Cancer development associated with phospholipid metabolism alterations also affect circulating phospholipids. Indeed, a few interventional studies in humans suggest that the plasma level of phospholipids (PLs) could serve as predictive biomarkers of progression of various cancers, such as lysophosphatidic acid (LPA) for gynaecological cancers [10, 11], lysophosphatidylcholine (LPC) for ovarian cancers [12], colorectal cancers [13] and prostate cancer where ether-phosphatidylcholine (e PC) 38:5, PC 40:3, PC 42:4 serum levels are increased [14]. This modulation of circulating phospholipid levels was also found in the serum of hepatitis B-related HCC for which Passos-Castilho et al. showed a general decrease in phosphatidylserine (PS), phosphatidylinositol (PI) and PC [15]. Patterson and colleagues showed that cirrhotic patients present higher plasma LPC concentrations compared to HCC patients [16]. Nevertheless, this study was followed by a metabolomic analysis based on the serum of hepatitis B-related cirrhotic patients and HCC patients showing an increase in two LPC species and

five PC species in HCC patients [17]. As highlighted by Patterson, numerous metabolomic studies are focused on Chinese populations. Furthermore, despite the increasing number of studies focusing on the relation between phospholipids and HCC, the small number of patients, the heterogeneity of the results and the fewer matching parameters for case-control studies led us to examine the potentiality of phospholipids to diagnose and predict the progression of HCC in cirrhotic liver, an important challenge to better knowledge of the individual risk factors for HCC.

In this paper, according to the literature [18], we studied the concentration of the major classes of circulating complex lipids of 90 well-described European patients with cirrhosis – 45 cirrhotic patients without HCC (controls) and 45 cirrhotic patients with HCC (cases) – randomised in the CiRCE study (Cirrhose et Risque de Cancer dans le Grand-Est) after matching for age, gender, virus infection, duration of cirrhosis, Child-Pugh score and inclusion centre. These matching parameters were selected considering their potential relationship to HCC incidence and/or lipid modulations [19, 20]. High-performance liquid chromatography coupled with tandem-mass spectrometry (HPLC-MS/MS) revealed for the first time on a European cohort that only some phospholipid species were elevated (phosphatidylcholine, sphingomyelin and plasmalogen-phosphatidylethanolamine), thus discriminating HCC patients from their cirrhotic controls. More importantly, we highlight that two ceramide species were associated with increased risk of death from HCC.

## RESULTS

### Patient characteristics

The median age of controls and cases was 59.9 (range, 55.7–69.4) and 62.4 (range, 58.2–71.6) years, respectively; the global sex ratio was eight males for one female in controls and cases [19, 20]. The main etiologies of cirrhosis were alcohol (51.1 and 64.4% in cases and controls, respectively, followed by hepatitis C virus (31.1 and 20.0%, respectively). In most patients liver function was normal with the Child-Pugh score between A5 and B7 (Table 1). Case patients mainly presented nodular HCC (multinodular: 51.1%, unimodular: 35.6%). No significant differences in daily alcohol consumption, smoking status, BMI, diabetes, statin and other hypolipemic treatment were observed between cases and controls (Table 1). Concerning biological characteristics, total cholesterol, LDL (low-density lipoproteins), HDL (high-density lipoproteins) and triglycerides were not statistically different between cases and controls. Gamma-glutamyl transferase (GGT) ( $p=0.0139$ ) and alpha-fetoprotein (AFP) ( $p<0.0001$ ) values were higher in cases than in controls (Table 2).

**Table 1: Demographic and clinical characteristics of the 90 cirrhotic patients with (cases) and without (controls) HCC**

	Controls n=45	Cases n=45	P-value
<b>Matching criteria (+ centre)</b>			
Age (years), median [IQR]	59.9 [55.7–69.4]	62.4 [58.2–71.6]	-
<b>Gender, n (%) :</b>			
Male	40 (88.9)	40 (88.9)	-
Female	5 (11.1)	5 (11.1)	-
Virus infection, n (%)	16 (35.6)	16 (35.6)	-
Time between cirrhosis diagnosis and inclusion in the study (years), median [IQR]	2 [1–6]	2 [0–5]	-
<b>Child-Pugh score, n (%):</b>			
A5 to B7	34 (75.6)	34 (75.6)	-
B9 to C13	11 (24.4)	11 (24.4)	-
<b>Clinical characteristics</b>			
<b>Etiology of cirrhosis, n (%):</b>			
Alcohol	23 (51.1)	29 (64.4)	
B virus	1 (2.2)	7 (15.6)	
C virus	14 (31.1)	9 (20.0)	
Others	7 (15.6)	0	
<b>Tumour characteristics, n (%):</b>			
No tumour	45	-	-
Uninodular	-	16 (35.6)	
Multinodular	-	23 (51.1)	
Diffuse and/or metastases	-	6 (13.3)	
<b>Excessive alcohol consumption, n (%):</b>			
Never	8 (17.8)	9 (20.0)	0.8674
Former	17 (37.8)	20 (44.4)	
Current	20 (44.4)	16 (35.6)	
<b>Smoking status, n (%):</b>			
Never	10 (22.2)	14 (31.1)	0.2872
Former	23 (51.1)	18 (40.0)	
Current	12 (26.7)	13 (28.9)	
BMI $\geq$ 25 kg/m <sup>2</sup> , n (%)	27 (61.4)	30 (66.7)	0.6776
Diabetes, n (%)	21 (46.7)	17 (37.8)	0.5235
<b>Treatments, n (%):</b>			
Hypolipemic	11 (24.4)	9 (20.0)	0.7905

**Table 2: Biological characteristics of the 90 cirrhotic patients with (cases) and without (controls) HCC**

Biology, median [IQR]	Controls n=45	Cases n=45	P-value
<b>Total cholesterol (g/L) [25 MD]</b>	3.79 [3.21–5.11]	4.21 [3.11–4.85]	0.4777
<b>LDL (g/L) [25 MD]</b>	2.07 [1.73–2.91]	2.30 [1.76–3.00]	0.3732
<b>HDL (g/L) [25 MD]</b>	1.11 [0.77–1.48]	1.11 [0.48–1.69]	0.7911
<b>Triglycerides (g/L) [25 MD]</b>	1.09 [0.91–1.37]	1.01 [0.88–1.47]	0.1480
<b>Anti-diabetic prescription, n (%)</b>	16 (35.6)	14 (31.1)	
<b>Glycaemia (µmol/L)</b>			
Treated group [10 MD]	7.0 [6.0–10.1]	6.7 [5.7–7.4]	-
Non-treated group [15 MD]	5.1 [4.4–5.7]	5.0 [4.4–5.7]	-
<b>Insulinaemia (µIU/mL)</b>			
Treated group [20 MD]	16.9 [7.3–20.7]	23.3 [13.3–26.8]	-
Non-treated group [36 MD]	9.8 [5.0–19.8]	11.5 [7.9–16.4]	-
<b>AFP (IU/mL) [1 MD]</b>	4 [3–7]	23 [6–157]	< 0.0001
<b>Transaminases (IU/L) [1 MD]</b>	37 [25–72]	41 [29–55]	0.8814
<b>GGT (IU/L) [2 MD]</b>	147 [66–240]	205 [120–298]	0.0139
<b>Bilirubin (µmol/L) [2 MD]</b>	18 [12–31]	22 [14–34]	0.2107

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; AFP, alpha foetoprotein; GGT, gamma-glutamyl transferase; MD, missing data.

**Distinctive plasma lipid profiles of liver cirrhosis with and without HCC**

Circulating phospholipid profiles have been previously described as modified in different manners in various cancers with some contradictory results in HCC cases [16]. To evaluate phospholipid levels in our cohort, we analysed the plasma of the 90 cirrhotic patients using the HPLC-MS/MS approach with multiple reaction monitoring (MRM). We first made qualitative analyses to identify each lipid species from the HPLC spectrum according to carbon and unsaturation numbers. Then we quantified each species using the internal standard. At first this showed no difference in the concentration of total plasma phospholipids and biliary acid concentration between cases and controls (data not shown). Nevertheless, in a second step, a comparison of each phospholipid species in each lipid category highlighted that only PC 16:0/16:1 (p=0.018), PC 16:0/16:0 (p=0.033), PC 16:0/18:1 (p=0.026) and SM 18:2/24:1 (p=0.038) concentrations were increased in cases compared to controls.

On the other hand, analyzing lysophospholipid species, which are formed by hydrolysis of phospholipids, we observed that only the LPC 20:4 (p=0.009) concentration was lower in cases than in controls (Table 3 and Supplementary Table 2).

In a similar manner, among ether-phospholipids, plasmalogen-phosphatidylethanolamine (pPE) 16:0/20:4

(p=0.046), pPE 18:0/20:4 (p=0.008), pPE 18:0/20:5 (p=0.016), pPE 18:0/20:3 (p=0.046) concentrations were also lower in cases than in controls (Table 3 and Supplementary Table 2).

Altogether, these data confirm an alteration of the qualitative and quantitative phospholipid profile in cirrhotic patients with HCC *versus* cirrhotic patients without HCC with a specificity of the levels of certain circulating species (Figure 1A).

**Relation between phospholipid profile and HCC patient survival**

In accordance with previous results, we evaluated the prognostic potential of all phospholipid species based on patient follow-up. Patients were followed up once a year for 5 years after their inclusion in the clinical study. We analysed the probability of death 1 and 3 years after the beginning of inclusion for HCC patients who had a quantity above the median for each lipid species. Table 4 summarises the univariate and multivariate analysis of 1- and 3-year mortality in HCC patients according to the median split for each phospholipid species. Phospholipid species were included in multivariate analysis when there was a trend in univariate analysis with p<0.10. In multivariate analysis, several phospholipids were associated with a low mortality rate in HCC patients at 1 and 3 years: total LPC (1 year: p=0.042; 3 years:



**Table 3: Discriminating phospholipids between HCC cases and matched controls**

Phospholipids <sup>a</sup>	Min	Max	All n=90	Controls n=45	Cases n=45	P-value
PC 16:0/16:1	4.27	113.8	27.7 [17.37–38.42]	25.39 [16.01–35.21]	29.96 [22.45–40.68]	0.018
PC 16:0/16:0	5.22	61.82	21.84 [18.38–29.01]	20.85 [16.9–24.92]	23.02 [19.77–29.5]	0.0327
PC 16:0/18:1	49.51	395.82	207.38 [186.29–240.55]	198.79 [166.19–229]	225.95 [194.24–250.62]	0.0264
LPC 20:4	1.34	12.96	4.02 [3.03–5.14]	4.3 [3.48–6.05]	3.66 [2.89–4.53]	0.0093
pPE 16:0/20:4	0.05	0.99	0.37 [0.29–0.49]	0.42 [0.3–0.55]	0.35 [0.28–0.43]	0.0463
pPE 18:0/20:4	0.04	2.03	0.75 [0.55–1.03]	0.89 [0.62–1.17]	0.63 [0.53–0.85]	0.0077
pPE 18:0/20:5	0	0.21	0.07 [0.06–0.1]	0.09 [0.06–0.11]	0.06 [0.05–0.09]	0.0163
pPE 18:0/20:3	0.01	0.31	0.1 [0.07–0.14]	0.12 [0.08–0.15]	0.09 [0.06–0.13]	0.0463
SM d18:2/24:1	6.46	38.46	22.39 [18.96–26.76]	20.81 [18.49–25.78]	23.45 [20.68–28.8]	0.0379

<sup>a</sup> Values are median [IQR] of  $\mu\text{mol/L}$  of plasma.

$p=0.022$ ); LPC 14:0 (1 year:  $p=0.024$ ; 3 years:  $p=0.015$ ); LPC 18:0 (1 year:  $p=0.049$ ; 3 years:  $p=0.016$ ); LPC 20:1 (1 year:  $p=0.008$ ; 3 years:  $p=0.033$ ); total LPE (1 year:  $p=0.017$ ; 3 years:  $p=0.008$ ); LPE 22:6 (1 year:  $p=0.030$ ; 3 years:  $p=0.001$ ); SM d19:0/24:1 (1 year:  $p=0.010$ ; 3 years:  $p=0.020$ ). PC 14:0/14:0 was only associated with a low 1-year death rate ( $p=0.038$ ) while PC 18:0/18:2 ( $p=0.015$ ); LPE 18:1 ( $p=0.008$ ); PI 38:3 ( $p=0.028$ ) were only associated with a low 3-year mortality rate (Table 4 and Figure 1B).

In the second step, we decided to include ceramides in multivariate analysis despite the absence of a trend in univariate analysis ( $p<0.600$ ). Ceramides have already been described in detail in terms of their anti-tumour function. Nevertheless, several studies started to describe their functional duality in organ injury promoting the progression of pathologies [21, 22]. In the present study, we highlighted that Cer d18:1/20:0 (1 year:  $p=0.044$ ; 3 years:  $p=0.003$ ) was associated with both high 1-year and 3-year mortality rates while Cer d18:1/24:1 ( $p=0.000$ ) was only linked with elevated 3-year mortality. Nevertheless, Cer d18:1/26:0 ( $p=0.028$ ) was associated with a low risk of death at 1 year in HCC patients (Table 4 and Figure 1B).

Collectively these data highlight that determining a specific phospholipid and ceramide species profile is necessary to predict HCC risk in liver cirrhotic patients and that some phospholipids could potentially be used to predict HCC patient mortality.

## DISCUSSION

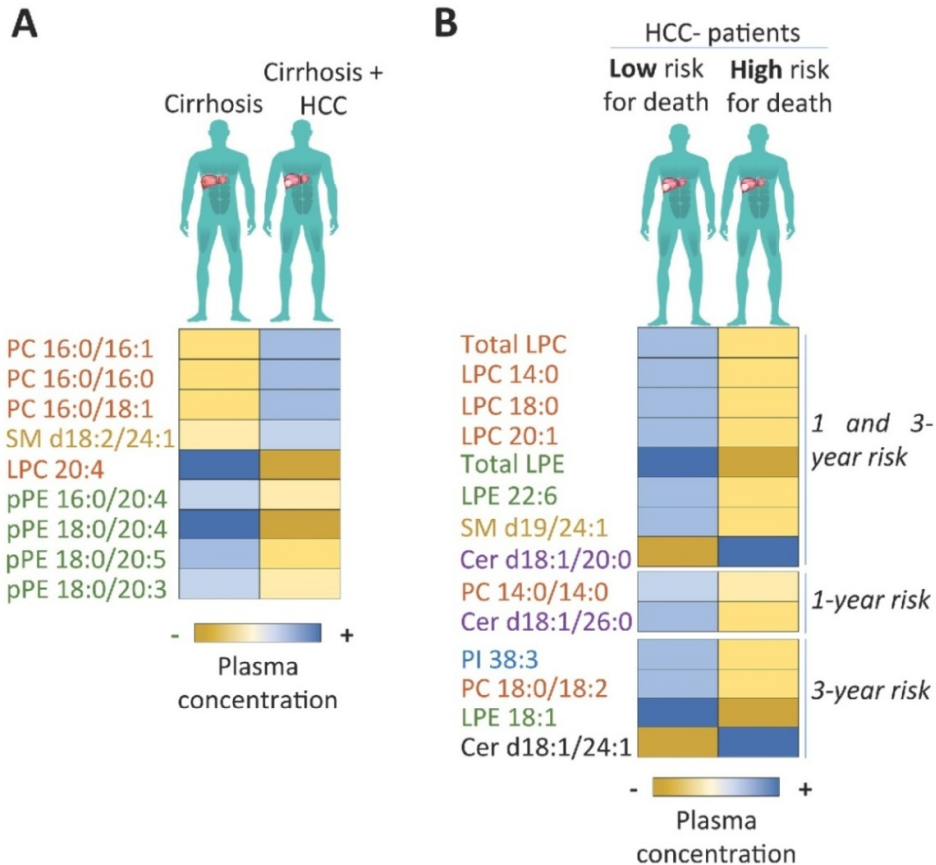
Based on a non-invasive targeted lipidomic method using HPLC-MS/MS, for the first time we have highlighted potential plasma phospholipid biomarkers of HCC risk in

90 cirrhotic patients with or without HCC in a European cohort from the CiRCE study. For this case-control study, we previously demonstrated a relationship between PNPLA3 (patatin-like phospholipase domain-containing protein 3) GG status and oxidative DNA damage, which may explain the excessive risk of cancer in patients with cirrhosis [23]. According to the literature [18] and considering that some specific phospholipid species could be associated with the progression of HCC characterised by high inflammation and oxidative status [24], we investigated the main species of eight phospholipid classes: PC, LPC, phosphatidylethanolamine (PE), lysophosphatidylethanolamine (LPE), sphingomyelin (SM), ceramide (Cer), dihydroceramide (DHcer) and PI. A matched case-control study was conducted to free our analysis of possible bias stemming from risk factors known for modulating HCC risk and/or lipid metabolism such as virus infection [20]. We found nine phospholipids discriminating presence or absence of HCC in cirrhotic patients and 14 phospholipids differentially associated with risk of death in HCC patients. Early detection of cancer and identification of new HCC prognostic factors are important to improving patient management. Phospholipidomic analysis is a promising approach in biomarker research based on alteration in membrane composition and cell signaling in HCC.

Elevated levels of saturated and monounsaturated phospholipid species have already been observed in HCC tissues. A recent study highlighted lipid alteration of HCC tissues characterised by a decrease in PE, PS, PI and ceramide compared to non-tumoural liver. PC and LPC amounts were not changed. Saturated species were increased while polyunsaturated species of these phospholipid categories were reduced [25]. A study conducted by Morita et al. using MALDI-IMS

(matrix-assisted laser desorption/ionisation-imaging mass spectrometry) revealed that PC 16:0/16:1 and PC 16:0/18:1 were significantly increased in tumours compared to non-tumour liver. These results were associated with an overexpression of LPCAT1 in tumour regions explained by the capacity of this enzyme to re-acylate specific LPC species into PC [8]. These results showing elevated PC species obtained in plasma of HCC

and cirrhotic patients complete these earlier observations. Numerous papers have also reported dysregulation of PC synthesis enzymes in HCC such as choline kinase alpha (CK $\alpha$ ) [26]. Moreover, among PC synthesis enzymes, phosphatidylethanolamine N-methyltransferase (PEMT) seems clearly downregulated during HCC [27], possibly explained by the fact that PEMT preferentially produces long-chain polyunsaturated PC [28]. A specific decrease



**Figure 1: Phospholipid profiles in cirrhotic patients depending on HCC presence and mortality risk.** (A) Phospholipid profiles according to plasma concentration of each species in cirrhotic patients with or without HCC. Blue rectangles symbolise a higher concentration of the corresponding lipid in comparison to control patients. Yellow rectangles symbolise a lower concentration of the corresponding lipid in comparison to control patients. Only phospholipids with statistical differences are represented. (B) Phospholipid profiles according to the plasma concentration of each species in HCC patients with a low or a high risk of mortality at 1 year or 3 years after their inclusion in the study. Blue rectangles symbolise a higher concentration of the corresponding lipid in comparison to the median value of this lipid in the HCC patient group. Yellow rectangles symbolise a lower concentration of the corresponding lipid in comparison to the median value of this lipid in the HCC patient group. Only phospholipids with statistical differences at 1 year or 3 years or both are represented.

**Table 4: Proportions of phospholipid species and death rates of cirrhotic patients with HCC in 1 and 3 years**

Parameter	Univariate analysis				Multivariate analysis			
	1-year mortality		3-year mortality		1-year mortality		3-year mortality	
	HR [CI]	P-value	HR [CI]	P-value	HR [CI]	P-value	HR [CI]	P-value
PC 14:0/14:0	0.393 [0.157– 0.987]	0.047	-	-	0.331 [0.117– 0.940]	0.038	-	-
PC 18:0/18:2	-	-	0.507 [0.249– 1.030]	0.061	-	-	0.318 [0.127– 0.797]	0.015
LPC	0.361 [0.144– 0.908]	0.030	0.556 [0.277– 1.115]	0.098	0.300 [0.094– 0.957]	0.042	0.313 [0.116– 0.845]	0.022
LPC 14:0	0.363 [0.145– 0.915]	0.032	0.562 [0.280– 1.128]	0.105	0.247 [0.074– 0.829]	0.024	0.367 [0.164– 0.820]	0.015
LPC 18:0	0.361 [0.144– 0.908]	0.030	0.468 [0.232– 0.944]	0.034	0.314 [0.099– 0.993]	0.049	0.299 [0.112– 0.801]	0.016
LPC 20:1	0.392 [0.156– 0.983]	0.046	0.557 [0.277– 1.120]	0.101	0.251 [0.091– 0.696]	0.008	0.434 [0.202– 0.933]	0.033
LPE	0.361 [0.143– 0.907]	0.030	0.489 [0.243– 0.987]	0.046	0.245 [0.077– 0.778]	0.017	0.301 [0.124– 0.728]	0.008
LPE 18:1	-	-	0.482 [0.239– 0.972]	0.041	-	-	0.301 [0.124– 0.732]	0.008
LPE 22:6	0.466 [0.190– 1.142]	0.095	0.485 [0.240– 0.978]	0.043	0.256 [0.077– 0.847]	0.03	0.189 [0.070– 0.512]	0.001
PI 38:3	-	-	0.539 [0.267– 1.086]	0.084	-	-	0.304 [0.105– 0.881]	0.028
SM d19:0/24:1	0.468 [0.091– 1.148]	0.097	0.452 [0.223– 0.919]	0.028	0.195 [0.056– 0.680]	0.010	0.243 [0.073– 0.803]	0.020
Cer d18:1/20:0	1.787 [0.729– 4.378]	0.204	1.206 [0.602– 2.415]	0.597	3.227 [1.033– 10.084]	0.044	5.595 [2.101– 14.162]	0.003
Cer d18:1/24:1	-	-	1.231 [0.615– 2.467]	0.557	-	-	5.454 [2.101– 14.162]	0.000
Cer d18:1/26:0	0.521 [0.213– 1.277]	0.154	-	-	0.282 [0.091– 0.874]	0.028	-	-

Death within 1 year, 20/45 (44.4%); death within 3 years, 32/45 (71.1%)

Variables of adjustment for multivariate analysis: Child-Pugh; treatment; centre; diabetes.

of these species was already reported in HCC tissues [29] due to their anti-inflammatory functions [30]. In the present study PC 16:0/22:6 and 18:0/22:6 tended to decrease in patients with HCC. LPC 20:4 downregulation also partly confirms the results obtained by Patterson et al. [16], who pointed out a decrease in LPC 14:0, LPC 18:1 and LPC 20:4 using UPLC/QTOF-MS (ultra-performance liquid chromatography / quadrupole time-of-flight mass spectrometry) in HCC patients compared to non-matched cirrhotic patients. The high level of PC can be related to low levels of LPC, since it could result in their transformation by the action of phospholipase A2 to restore PC. A diminished level of LPC is often associated with inflammation status [31]. We also observed for the first time a significant decrease in four polyunsaturated plasmalogen PEs in the plasma of HCC patients compared to the cirrhotic control group. Plasmalogens are glycerophospholipids of cell membranes containing the vinyl-ether bond at the *sn*-1 position. Their levels are particularly low in the liver due to their predominant synthesis in hepatocytes and secretion in lipoproteins [32]. They act as serum antioxidants and specifically prevent oxidation of lipoproteins [33]. These results could indicate an increase in oxidative status during HCC development, which could also be involved in the activation of inflammation pathways [34]. These observations were also confirmed by the Cox regression models. Indeed, plasmatic high concentrations of several LPC species, polyunsaturated PI, and LPE were associated with a decreased risk of death in HCC patients, thus improving the prognosis. The disruption of phospholipid species equilibrium could be associated with the progression of HCC characterised by strong inflammation and oxidative status as we previously demonstrated in this cohort [23].

The literature amply describes the involvement of ceramide in the induction of apoptosis. In case of cancer, the increase in specific ceramides could be associated with the chemotherapy response [35]. Moreover, HCC and cirrhosis can induce extensive injuries to hepatocytes, which could be reflected by hepatocyte overproduction of ceramides with ongoing apoptosis [22], while PC 14:0/14:0, PC 18:0/18:2 and SM d19:0/24:1 plasma concentrations could reflect normal hepatocyte functions. Wang and colleagues reported that cirrhotic patients and HCC patients presented lower serum concentrations of PC species compared to healthy subjects. This observation argues that liver pathology decreases hepatocyte functions [17]. Several studies have also reported an alteration of lipid metabolism by hepatitis B and C viruses manifesting as a decrease in plasma triglycerides, cholesterol and LDL. These changes were also observed in patients with HCC [36, 37]. In fact, we observed no difference in total cholesterol, HDL, LDL and triglyceride amounts between matched cases and controls. These results suggest a general impact of cirrhosis on phospholipid content,

which is also manifested in the present study by the absence of changes in the amount of total phospholipids, biliary acids, PI, PE, LPE, ceramides and DHceramides [38]. Moreover, it is intriguing that the low number and unusual species in plasma such as PC 14:0/14:0 and SM d19:0/24:1 could be easily associated with a slower progression of the disease, reinforced by the fact that saturated and monounsaturated phospholipids are often associated with cancer progression [18]. The same could be said for PC 18:0/18:2 containing 18:2 fatty acid, which is an n-6 fatty-acid mainly found in the diet. Also, it was surprising to find only one elevated SM species which had a differential level in patients with HCC correspond: SM d18:2/24:1. The variations of SM in plasma, serum or tissues differ depending on cancer types and studies [18]. SM is the major sphingolipid in blood and is associated with lipoproteins. Consequently, it is sometimes delicate to associate plasma phospholipid variation with tumour phenotype. These discrimination results could be due to an enrichment of PC 18:0/18:2 and SM d18:2/24:1 by a specific diet.

Several limitations of this study need to be mentioned. The number of patients was too small to conduct a complete study leading to pPE analysis in a Cox regression model and a real characterisation of biomarkers. These results need to be confirmed by including a greater number of patient levels. To conclude, our matched case-control study provides the opportunity to clarify the literature on phospholipids' potency in discriminating cirrhosis and HCC using a targeted HPLC/MS/MS approach. The plasma concentration variation of nine phospholipids may be useful to identify HCC risk among cirrhotic patients. Interestingly, we show the possibility of using LPC, LPE and specific ceramide species concentrations as early predictive markers of HCC prognosis.

## MATERIALS AND METHODS

### Study design and population

This exploratory investigation is an ancillary study of CiRCE (Cirrhose et Risque de Cancer dans le Grand-Est), a multicentre case-control study analyzing environmental (alcohol, tobacco, viruses), nutritional and metabolic factors involved in liver carcinogenesis among cirrhotic patients. The 90 patients studied were randomised among the 1185 CiRCE cirrhotic patients treated in the university hospitals of the French Cancéropole du Grand Est (Besançon, Dijon, Metz, Nancy, Reims, Strasbourg) who were included from November 2008 through 2012. The study was approved by the Comité de Protection des Personnes Est (agreement no. 2008/09) and by the Agence Française de Sécurité Sanitaire des Produits de Santé (agreement no. 2008-A00023-52). All patients gave written informed consent to participate in this study. They were followed up for 5 years beginning at their inclusion. All clinical

data were collected by the medical staff with the help of research assistants.

### Inclusion criteria

The diagnosis of cirrhosis, irrespective of its etiology, was based on histological confirmation by liver biopsy or, in the absence of biopsy, by a combination of clinical and biological signs of hepatocellular failure or portal hypertension and/or endoscopic features of portal hypertension and/or imaging characteristics of cirrhosis. All cases of HCC evolving in a context of cirrhotic liver were included. Criteria for the diagnosis of HCC were those defined by the European Association for the Study of the Liver. The absence of HCC in controls with liver cirrhosis at inclusion was assessed through high-quality imaging examinations (abdominal ultrasonography and/or computed tomography and/or magnetic resonance imaging) and AFP below 100 ng/mL during the 2 months preceding inclusion. Alcoholic cirrhosis was defined as alcohol consumption >14 units/week for females and >28 units/week for males or participant. The follow-up included hepatic ultrasonography and dosage of AFP every 6 months.

### Exclusion criteria

Patients under 35 years of age with progressive extrahepatic cancer, human immunodeficiency virus infection, acute alcoholic hepatitis, major somatic or psychiatric illness not compatible with inclusion in the study, or with non-HCC primary liver cancer were excluded.

### Samples

Plasma samples were collected, regardless of the cause of liver disease. Immediately after the blood test, plasma was extracted, frozen and stored at  $-80^{\circ}\text{C}$ . Plasma glucose, HDL cholesterol, LDL cholesterol, triglycerides and liver enzymes were analysed using standard procedures. For this study, 45 cirrhotic patients without HCC (controls) and 45 cirrhotic patients with HCC (cases) were matched on inclusion university hospital, age, gender, presence of virus, the time between cirrhosis diagnosis and inclusion in the study, and the Child-Pugh score.

### Lipidomic analysis

#### Extraction

All extraction procedures were performed in 10-mL glass disposable centrifuge tubes. Plasma (150  $\mu\text{L}$ ) was diluted with saline solution; 50  $\mu\text{L}$  of internal standard solution (Supplementary Table 1) prepared with a mix of chloroform ( $\text{CHCl}_3$ )/methanol ( $\text{MeOH}$ )/water ( $\text{H}_2\text{O}$ )

(60/30/4.5) was added to each sample, followed by 1.5 mL of  $\text{MeOH}$ . Samples were shaken for 2 min before the addition of 5 mL Methyl tert-butyl ether (MTBE). Then the samples were shaken for 1 h followed by the addition of 1.25 mL of  $\text{H}_2\text{O}$ . The samples were shaken for 10 min and centrifuged (10 000 g for 5 min, at  $4^{\circ}\text{C}$ ). The organic phases were transferred to new glass tubes. The older tubes were washed with 2 mL of MTBE, shaken and centrifuged. The organic phases were transferred to the first new tubes. The solvent was partially evaporated at room temperature with a Speedvac apparatus for 25 min before addition of 200  $\mu\text{L}$   $\text{MeOH}$  and a new evaporation step for 1.5 h. Dried lipids were suspended in 200  $\mu\text{L}$  of  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (60/30/4.5). The tubes were shaken for 2 min and centrifuged (10 000 g for 5 min at  $4^{\circ}\text{C}$ ). The supernatant was transferred to a vial with a 200- $\mu\text{L}$  reducer for MS analyses for LPE, PE, pPE; ceramide, dihydroceramide and PI; 2  $\mu\text{L}$  of this vial were transferred to a new vial and mixed with 98  $\mu\text{L}$  of  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (60/30/4.5) for PC, LPC and SM MS analyses.

#### LC/MS<sup>2</sup>

The samples were delivered into the electrospray ionisation (ESI)-MS ion source through a 1200 HPLC (Agilent Technologies) with an autosampler using a binary gradient of solvent A and B (Supplementary Table 1). Electrospray ionisation mass spectrometry (ESI-MS) was performed on a QQQ 6460 mass spectrometer (Agilent Technologies) in different ion modes (Supplementary Table 1).

#### Analysis

To evaluate which phospholipids could discriminate a patient with HCC from a patient without cancer, we compared the plasma values of each species for each defined phospholipid category. We chose the targeted HPLC-MS/MS approach with the multiple reaction monitoring (MRM) method to quantify specific species of these phospholipids in the plasma of the 90 cirrhotic patients. We decided to compare absolute quantification of controls and cases for future considerations in using reference values as a prognosis threshold. Concentrations of each phospholipid were determined from the ratio of the peak area of a given molecule to the peak area of the appropriate internal standard. Concentrations were calculated according to the quantity of internal standard and adjusted with the molecular weight of the corresponding species. Concentrations were expressed in micromoles per liter ( $\mu\text{mol/L}$ ) of plasma (the plasma samples were analysed in triplicate). Isotopic overlap of lipid species and data analyses for all lipid classes were corrected using MassHunter Software (Agilent). Lipid species were annotated according to the recently published proposal for shorthand notation of lipid structures that are derived from mass spectrometry [39]. In cases where fatty acid composition was not speculated, the annotation represents the total number of carbons and double bonds.

## Statistical analysis

Percentages were used to describe categorical data, and medians and interquartile range to describe continuous data. Patient characteristics were compared according to HCC status using the McNemar test (for categorical data) and the nonparametric Wilcoxon test (for continuous data) for paired samples. The association between the plasma concentration of phospholipid at inclusion and survival within 1 and 3 years in cases were assessed with Cox proportional hazards models, which estimated hazard ratios (HR) and 95% confidence intervals (CI). A p-value below 0.05 was considered significant. The statistical analyses were done using SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA).

## Abbreviations

ACLY, ATP citrate lyase; AFP, alpha fetoprotein; ATP, adenosine triphosphate; BMI, body mass index; Cer, ceramide; CK $\alpha$ , choline kinase alpha; DHCer, dihydroceramide; FASN, fatty acid synthase; GGT, gamma-glutamyl-transferases; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; HPLC-MS/MS, high performance liquid chromatography coupled with tandem mass spectrometry; LDL, low-density lipoprotein; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPCAT, lysophosphatidylcholine acyltransferase; LPE, lysophosphatidylethanolamine; LPL, lysophospholipid; MRM, multiple reaction monitoring; PC, phosphatidylcholine; pPC, plasmalogen-phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine N-methyltransferase; pPE, plasmalogen-phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipid; PNPLA3, patatin-like phospholipase domain-containing protein 3; PS, phosphatidylserine; SM, sphingomyelin; UPLC/QTOF-MS, ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry.

## Author contributions

A.K.C. analyzed the data and wrote the manuscript; S.V. performed statistical analyses; J.P.P.D.B. performed lipidomic analysis; V.A. and C.B. helped in setting up the project; V.C. helped in setting up the project and analysed the data; V.A., T.M., M.R., P.H., D.D. revised the manuscript; P.H. and D.D. supervised the overall project.

## ACKNOWLEDGMENTS

The authors thank Miss Linda Northrup for correcting the manuscript.

## CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

## FUNDING

This study was funded by a French Government grant managed by the French National Research Agency under the program "Investissements d'Avenir", reference ANR-11-LABX-0021 and also by the Institut National du Cancer (INCA), the French regions of Lorraine and Burgundy, the Ligue Nationale contre le Cancer (CiRCE-Nutrigenex), the Ligue Interrégionale Grand-Est contre le Cancer, the European Regional Development Fund, the Canceropole Grand-Est, and the Fondation de France.

## APPENDIX

The CiRCE Study Group includes: (1) CiRCE Coordination France: J.P. Bronowicki, V. Di Martino, M. Doffoel, P. Hillon (CiRCE coordinator), and G. Thieffin; CiRCE Coordination China: H. Wen, F.P. He, X.M. Lu, P. Hillon and D. Vuitton; (2) CiRCE Scientific Board: J. Faivre (President), J.P. Cercueil, V. Cottet, D. Delmas, P. Ducoroy, L. Duvillard, M. Guenneugues, J.L. Guéant, F. Habersetzer, N. Latruffe, M. Manfait, P. Oudet and G. Sockalingum; (3) CiRCE pharmacologists: P. Trechot, M.B. Valnet-Rabier, T. Trenque and M. Tebacher-Alt.

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## 5. Discussion

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Le carcinome hépatocellulaire qui représente 85 à 90% des tumeurs primitives du foie, est la 4<sup>ème</sup> cause de mortalité par cancer dans le monde. C'est une tumeur de mauvais pronostic pour laquelle l'efficacité du traitement, dépend beaucoup du stade où le diagnostic a été fait. Le dépistage systématique du CHC chez les patients cirrhotiques asymptomatiques reste le meilleur moyen de prévention ainsi que des mesures hygiéno-diététiques : consommer une alimentation équilibrée, pratiquer une activité physique régulière, s'abstenir de consommer de l'alcool, vacciner les enfants contre l'hépatite B, limiter la transmission du virus de l'hépatite C (l'élimination des injections inutiles, l'utilisation de matériel à usage unique, l'application du système de sécurité lors des dons de sang). En prévention secondaire, essayer de prévenir la survenue de CHC sur foie cirrhotique, notamment par la prise en charge de la cause de l'hépatopathie chronique.

Les travaux menés dans le cadre de ce travail de thèse sont basés sur la prévention par des moyens diététiques et ils ont contribué à mieux comprendre les habitudes alimentaires des malades cirrhotiques en France et essayer de diminuer l'apparition des complications et améliorer leur qualité de vie. Les études concernant les malades cirrhotiques suggèrent de plus en plus que le maintien d'un état nutritionnel approprié peut prévenir les complications. Cette population a sûrement un régime alimentaire spécifique suite aux recommandations des médecins et aux complications. Ainsi la comparaison des patients cirrhotiques atteints de CHC avec les patients cirrhotiques sans CHC a éclairé sur les différences métaboliques, nutritionnelles et sociodémographiques entre ces deux groupes.

Cette étude vient confirmer des résultats déjà mis en évidence dans la littérature mais a aussi ajouté des données à confirmer dans de plus larges études.

Ce travail a ainsi conduit à confirmer l'hypothèse sur le rôle de l'alimentation dans l'apparition du CHC chez les patients cirrhotiques. En effet, une alimentation, qui n'est pas variée et équilibrée est

nocive et ne procure pas suffisamment d'éléments protecteurs. Ces résultats sont cohérents et concordants avec des études antérieures obtenus chez des personnes atteints de CHC (29,31,35,68,77). Ainsi une alimentation riche en sucre et boissons sucrées, viandes rouges et charcuteries, graisses saturées et aliments transformés influencent négativement la fonction hépatique. Une consommation accrue de sucre et de graisse augmente rapidement le taux d'insuline dans le sang et accélère la lipogenèse et donc l'accumulation de graisse au niveau hépatique (79). Une consommation élevée de viandes rouges et de charcuteries, riches en graisses saturées, nitrite et nitrate, augmentent le risque de développer un cancer car à haute température ses dérivés se transforment en amines hétérocycliques nocives et cancérigènes (34). Une consommation élevée de sel a un effet indirect sur l'apparition du CHC. Le sodium est associé à l'hypertension mais aussi à certaines maladies métaboliques, cardiaques et fibrose hépatique qui sont des facteurs de risque du CHC (37–39,80). L'alcool est un des risques majeurs de la cirrhose et du CHC et il était consommé de façon abusive chez les cas et les témoins pour des durées moyennes de 23 ans et 19 ans respectivement. La consommation d'alcool et la cirrhose qui en résulte ont une relation de cause à effet dans le développement du CHC. La consommation chronique d'alcool aboutit à l'induction du cytochrome P450 2E1 et la génération de radicaux libres, ce qui entraîne la formation d'adduit de l'ADN et entraîne des dommages oxydatifs. L'environnement pro-inflammatoire augmente la libération des cytokines et des chimiokines, réduisant ainsi la survie des hépatocytes (8,9).

Les AG sont de plus en plus étudiés vu l'impact de certains types d'AG sur le développement de certains types de cancer. Mais les résultats concernant certains types d'acides gras sont discordantes peut être à cause de la source de ces AG ou de la quantité consommée. Les études ont montré que la composition lipidique affecte la fonction membranaire, la prolifération et la mort cellulaire et que cette composition est modifiée dans les tissus cancéreux. Une altération des concentrations de certains lipides, AG, phospholipides, lipoprotéines a été mis en évidence chez des patients atteints de différents types de cancers entre autre le cancer colorectal (75,81–83),

prostate (76) et CHC (77,84,85). Les études concernant le CHC ont montré que ce cancer est caractérisé par une réduction des céramides, des phospholipides, des AGPI et une augmentation des esters de cholestérol et de la sphingomyéline par rapport aux tissus adjacents non tumoraux. Les études ont montré que la consommation d'AGPI type oméga-3 peut réduire le risque de CHC et a des effets bénéfiques sur le développement du CHC chez des patients cirrhotiques (69,86). D'autre part, une étude a trouvé que l'acide linoléique (18: 2n-6), l'acide oléique (18: 1n-9), l'acide arachidonique (20: 4n-6) et l'acide palmitique (16:0) sont des biomarqueurs potentiels des acides gras des patients atteints de CHC (84). Une étude cas-témoin suggère que les altérations dans les profils des AG des phospholipides plasmatiques sont dues à des altérations métaboliques intrinsèques causées par le cancer lui-même, et non par le facteur alimentaire (84). Des études prospectives américaines récentes ont montré qu'une consommation élevée de graisses végétales et d'AGPI peuvent être associés à une diminution du risque de CHC. Ainsi remplacer les graisses de source animale ou produits laitiers par les graisses végétales ou remplacer les graisses saturées par les graisses polyinsaturées ou monoinsaturées pourraient réduire le risque de CHC parmi les adultes américains (88). Mais ces hypothèses doivent être testées dans des essais contrôlés randomisés. L'analyse de certains types de lipides comme les phospholipides et certains AG et le lien avec le développement du CHC chez des patients a été l'objectif de 2 articles basés sur l'étude CIRCÉ. Les lipides jouent un rôle structural dans la composition des membranes biologiques et participent au métabolisme et à la signalisation cellulaire. Ainsi les altérations qui touchent la composition des AG engendrent des modifications au niveau de certaines fonctions cellulaires (médiateurs de transport, certains enzymes membranaires et récepteurs, phagocytose, endocytose, exocytose...) ainsi que le développement, la croissance et la multiplication cellulaire (89,90).

Une faible consommation de légumes, légumineuses, fruits et céréales complètes augmente le risque de CHC car ils sont riches en fibres, vitamines, minéraux et antioxydants (31,34,91). De plus, la consommation de café diminue le risque de CHC vu que le café est riche en plusieurs

composants qui ont des effets antioxydants, antimutagènes et anti-inflammatoires. Plusieurs études (27,42,43,68) ont montré les effets protecteurs du café mais cela n'a pas été démontré dans cette étude sans doute liée à la quantité limitée de café consommé. Par ailleurs, dans notre étude, de nouvelles associations ont été observées avec certains micronutriments comme le manganèse, le potassium, les vitamines E et B9. D'autres associations ont été confirmées chez nos patients cirrhotiques alors qu'elles avaient été déjà étudiées chez des patients sains comparés à des patients atteints de CHC (28,35,68,77).

Comme le CHC est un cancer qui se développe suite à des maladies liées à des épisodes inflammatoires répétitives, il était intéressant d'explorer la relation entre le potentiel inflammatoire de l'alimentation à travers le calcul du score DII et le risque de CHC. Une association positive significative a été mise en évidence entre une alimentation pro-inflammatoire et le risque de CHC ainsi qu'avec les marqueurs inflammatoires CRP et IL-6. Ces résultats confirment des résultats précédents mais cette fois, chez des patients cirrhotiques (92,93).

Certaines forces et limites sont spécifiques à notre étude. Premièrement, le nombre de participants qui a répondu au questionnaire alimentaire reflète le défi posé par un questionnaire nutritionnel aussi complet. Cela pourrait avoir limité la puissance et la représentativité des résultats de notre étude, ainsi que la possibilité de stratifier les analyses. Deuxièmement, le questionnaire sur les antécédents alimentaires a été auto-déclaré pour l'année précédente et n'a pas été répété pendant le suivi afin de détecter tout changement dans le régime actuel. Il est possible que les cas aient déjà eu des recommandations nutritionnelles et ont répondu en fonction d'elles mais pas selon leurs habitudes de l'année antérieure.

Par ailleurs, les points forts de notre étude sont l'utilisation d'un protocole standardisé dans plusieurs hôpitaux universitaires de six régions du Nord-est de la France. Nous avons également pris en compte plusieurs facteurs de confusion potentiels, notamment des données sociodémographiques, médicales et biologiques.

## 6. Conclusion et perspectives

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À notre connaissance, peu d'études épidémiologiques ont analysé les facteurs nutritionnels menant de la cirrhose au CHC. Les patients cirrhotiques pourraient éventuellement être encouragés à adopter un régime alimentaire particulier, bénéficiant ainsi d'une intervention préventive. Les résultats de cette étude et la planification de nouvelles études pourraient contribuer à l'élaboration de recommandations nutritionnelles qui optimiseraient le bien-être des patients cirrhotiques et minimiseraient leur risque de développer un CHC. Par ailleurs, nos résultats apportent des éléments nouveaux dans l'implication de certains groupes alimentaires et des acides gras dans l'apparition des complications de la cirrhose. Cependant, compte tenu que nos études sont de type cas-témoins et le nombre de patients est limité et hétérogène, ces résultats ne permettent toutefois pas de tirer des conclusions définitives mais seulement d'émettre des hypothèses qui seront confirmées à l'aide d'études prospectives plus larges.

En conclusion, les patients cirrhotiques devraient suivre un régime alimentaire spécifique selon le stade de la maladie, les complications et les maladies chroniques dont ils souffrent. Ainsi, les régimes alimentaires doivent être personnalisés pour assurer à chaque patient ses besoins protéino-énergétiques nécessaires et éviter les aliments nocifs tout en pratiquant une activité physique régulière pour lutter contre la maladie et la malnutrition. Concernant les patients atteints de CHC, la prise en charge doit être surtout basée sur la lutte contre la malnutrition qui est fréquente chez les cirrhotiques et les patients cancéreux.

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## Annexes

### Annexe 1 : Les composants et les standards du calcul du score du HEI 2010

Component	Maximum points	Standard for maximum score	Standard for score of zero
<b>Adequacy components**</b>			
Total Fruit <sup>1</sup>	5	≥0.8 cup	No fruits
Whole Fruits <sup>2</sup>	5	≥0.4 cup	No whole fruits
Total Vegetables <sup>3</sup>	5	≥1.1 cup	No vegetables
Greens and Beans <sup>3</sup>	5	≥0.2 cup	No Dark Green Veg. or Legumes
Whole Grains	10	≥1.5 oz	No Whole Grains
Dairy <sup>4</sup>	10	≥1.3 cup	No Dairy
Total Protein Foods <sup>5</sup>	5	≥2.5 oz	No Protein Foods
Seafood & Plant Protein <sup>5,6</sup>	5	≥0.8 oz	No Seafood or Plant Proteins
Fatty Acids <sup>7</sup>	10	(PUFAs + MUFAs)/ SFAs≥2.5	(PUFAs + MUFAs)/SFAs≤1.2
<b>Moderation components***</b>			
Refined Grains	10	≤1.8 oz	≥4.3 oz
Sodium	10	≤1.1 gram	≥2.0 grams
Empty Calories <sup>8</sup>	20	≤19% of energy	≥ 50% of energy

\*Intakes between the minimum and maximum levels are scored proportionately.

\*\*Adequacy components include foods with minimum recommended intakes (higher consumption considered better quality diet).

\*\*\*Moderation components include foods with maximum recommended intakes (higher consumption considered poorer quality diet). Moderation components are thus reverse scored; with lower consumption levels awarded higher scores.

<sup>1</sup>Includes 100% fruit juice

<sup>2</sup>Includes all forms except juice

<sup>3</sup>Includes any beans and peas not counted as Total Protein Foods

<sup>4</sup>Includes all milk products, such as fluid milk, yogurt, cheese and fortified soy beverages.

<sup>5</sup>Beans and peas are included here (and not with vegetables) when the Total Protein Foods standard is otherwise not met.

<sup>6</sup>Includes seafood, nuts, seeds, soy products (other than beverages) as well as beans and peas counted as Total Protein Foods.

<sup>7</sup>Ratio of poly- and monounsaturated fatty acids (PUFAs and MUFAs) to saturated fatty acids (SFAs).

<sup>8</sup>Calories from solid fats, alcohol and added sugars; threshold for counting alcohol is >13g/1000Kcal.

## Annexe 2: Les composants et les standards du calcul du score du HEI 2015

Component	Maximum points	Standard for maximum score	Standard for score of zero
<b>Adequacy components**</b>			
Total Fruit <sup>1</sup>	5	≥0.8 cup	No fruits
Whole Fruits <sup>2</sup>	5	≥0.4 cup	No whole fruits
Total Vegetables <sup>3</sup>	5	≥1.1 cup	No vegetables
Greens and Beans <sup>3</sup>	5	≥0.2 cup	No Dark Green Veg. or Legumes
Whole Grains	10	≥1.5 oz	No Whole Grains
Dairy <sup>4</sup>	10	≥1.3 cup	No Dairy
Total Protein Foods <sup>5</sup>	5	≥2.5 oz	No Protein Foods
Seafood & Plant Protein <sup>5,6</sup>	5	≥0.8 oz	No Seafood or Plant Proteins
Fatty Acids Ratio <sup>7</sup>	10	(PUFAs + MUFAs)/ SFAs ≥2.5	(PUFAs + MUFAs)/SFAs ≤1.2
<b>Moderation components***</b>			
Refined Grains	10	≤1.8 oz	≥4.3 oz
Sodium	10	≤1.1 gram	≥2.0 grams
Added Sugars	10	≤6.5% of energy	≥26% of energy
Saturated Fat	10	≤8% of energy	≥16% of energy

\*Intakes between the minimum and maximum levels are scored proportionately.

\*\*Adequacy components include foods with minimum recommended intakes (higher consumption considered better quality diet).

\*\*\*Moderation components include foods with maximum recommended intakes (higher consumption considered poorer quality diet). Moderation components are thus reverse scored, with lower consumption levels awarded higher scores.

<sup>1</sup>Includes 100% fruit juice

<sup>2</sup>Includes all forms except juice

<sup>3</sup>Includes any beans and peas not counted as Total Protein Foods

<sup>4</sup>Includes all milk products, such as fluid milk, yogurt, cheese and fortified soy beverages.

<sup>5</sup>Beans and peas are included here (and not with vegetables) when the Total Protein Foods standard is otherwise not met.

<sup>6</sup>Includes seafood, nuts, seeds, soy products (other than beverages) as well as beans and peas counted as Total Protein Foods.

<sup>7</sup>Ratio of poly- and monounsaturated fatty acids (PUFAs and MUFAs) to saturated fatty acids (SFAs).

### Annexe 3 : Les composants et les standards du calcul du score de l'AHEI-2010<sup>1</sup>

Component	Criteria for minimum score (0)	Criteria for maximum score (10)
Vegetables, <sup>2</sup> servings/d	0	≥5
Fruit, <sup>3</sup> servings/d	0	≥4
Whole grains, <sup>4</sup> g/d		
Women	0	75
Men		90
Sugar-sweetened beverages and fruit juice, <sup>5</sup> servings/d	≥1	0
Nuts and legumes, <sup>6</sup> servings/d	0	≥1
Red/processed meat, <sup>7</sup> servings/d	≥1.5	0
<i>Trans</i> -fat, <sup>8</sup> % of energy	≥4	≤0.5
Coffee <sup>9</sup> ml/day	≤250	≥500
PUFA, <sup>10</sup> % of energy	≤2	≥10
Sodium, <sup>11</sup> mg/d	Highest decile	Lowest decile
Fish <sup>12</sup> g/day	0	≥20
Total	0	110

<sup>1</sup>Values are means ±SD unless otherwise noted. Researchers are invited to re-create and use the AHEI-2010 score in their own data. AHEI, Alternate Healthy Eating Index.

<sup>2</sup>Vegetable consumption has been associated with lower risk of cardiovascular disease (CVD) and some cancers. Green leafy vegetables in particular may lower risk of diabetes. All vegetables on the FFQ were included, except for potatoes (including French fries) because they are not associated with lower risk of chronic disease risk in epidemiologic studies and are associated with increased risk of diabetes. We considered 5 servings/d as ideal, which reflect the upper range of current dietary guidelines and are consistent with intervention studies of intermediate CVD risk factors. One serving is 0.5 cup of vegetables or 1 cup of green leafy vegetables (1 cup = 236.59 g).

<sup>3</sup>Fruit consumption has been associated with lower risk of CVD and some cancers. We included only whole fruit in our definition, because fruit juice is not associated with lower risk of CVD or cancer and may increase risk of diabetes. We considered 4 servings/d to be ideal, which is consistent with the upper range of current dietary guidelines. One serving is 1 medium piece of fruit or 0.5 cup of berries (1 cup = 236.59 g).

<sup>4</sup>Greater consumption of whole grains is associated with lower risk of CVD, diabetes, and colorectal cancer. Conversely, refined grains are not associated with lower risk and may increase risk of diabetes, coronary heart disease (CHD), and other chronic diseases. We used grams of whole grains, which accounts for the variability of the percentages of whole grain in various “whole grain” products. One serving of a 100% whole-grain product (i.e., 0.5 cup of oatmeal or brown rice) contains ~15–20 g of whole grains (per dry weight). We considered 75 g/d to be optimal (~5 servings/d) for women and 90 g/d (~6 servings/d) to be optimal for men on the basis of current guidelines for total grains.

<sup>5</sup>Intake of sugar-sweetened beverages, including soda and fruit drinks, is associated with increased risk of weight gain and obesity, CVD, and diabetes. We included intake of fruit juice in this category, given the positive association with risk of diabetes and lack of beneficial effects on CVD or cancer. The association with pancreatic cancer risk is not well established. We considered ≥1 serving/d to be the least optimal on the basis of the associations in the literature. One serving is 8 oz (1 oz = 28.35 g).

<sup>6</sup>Nuts, legumes, and vegetable protein (e.g., tofu) are important sources of protein and contain important constituents such as unsaturated fat, fiber, copper, magnesium, plant sterols, and other nutrients. Nuts and other vegetable proteins have been associated with lower risk of CVD, especially when used as a substitute for other protein sources, such as red meat. Nuts are also associated with lower risk of diabetes and weight gain, whereas their relation to cancer is inconclusive. We considered 1 serving/d to be ideal on the basis of the AHEI recommendations and the current literature. One serving is 1 oz (1 oz = 28.35 g) of nuts or 1 tablespoon (15 mL) of peanut butter.

<sup>7</sup>Consumption of red meat and processed meats is associated with greater risk of CHD (48), especially when substituted for nuts, poultry, or fish. Red meat and/or processed meats are also associated with higher risk of stroke, diabetes, and colorectal and other cancers. Less than 1 serving/mo was considered to be ideal, with an upper limit of ≥1.5 servings/d. One serving is 4 oz of unprocessed meat or 1.5 oz of processed meat (1 oz = 28.35 g).

<sup>8</sup>I chose to replace the *Trans* fatty acids group with coffee/tea group because the consumption of *Trans* fatty acids in this population was very low. Besides, studies showed an interesting beneficial effect of drinking coffee on decreasing the risk of HCC. The number of cups that should be consumed to benefit from coffee is not well defined but generally



more than 2 cups a day can lower the risk(23). More than 2 cups/day was considered to be ideal on the basis of the current literature. One cup of coffee is equivalent to 250ml.

<sup>9</sup>Compound in coffee have been shown to induce the endogenous defense system and the DNA repair capacity and to reduce the expression of genes involved in inflammation. One cup of coffee per day showed a significant decreased risk of liver cancer. The WCRF found that a higher consumption of coffee can probably protects against liver cancer. The highest score was given to individuals drinking more than 2 cups a day ( $\geq 500\text{ml/day}$ ) and the lowest score for those drinking less than 1 cup of coffee ( $\leq 250\text{ml/day}$ ).

<sup>10</sup>Replacing saturated fats with polyunsaturated fats leads to positive changes in lipid profiles, is associated with a lower risk of CHD, and may lower risk of type 2 diabetes. Furthermore, a low-fat diet had no beneficial effects on CVD risk factors, lipid profile, or blood pressure and did not reduce the risk of CVD, breast cancer, colon cancer, or total mortality. We gave the highest score to individuals with  $\geq 10\%$  of total energy intake from PUFA on the basis of current guidelines from the USDA and the AHA. PUFA does not include EPA or DHA intake.

<sup>11</sup>High sodium intake has been associated with higher blood pressure, and salt-preserved foods are associated with greater risk of stomach cancer, CVD, and total mortality. Furthermore, sodium-reduced diets significantly lowered blood pressure and CVD risk in clinical trials. Large reductions in sodium intake, to levels recommended by the USDA, may prevent a substantial number of new cases of CHD. The cutoffs for sodium were based on deciles of distribution in the population, due to lack of brand specificity in the FFQ to accurately estimate absolute intake. Values in lowest decile were  $\leq 1112$  mg/d in women and  $\leq 1612$  mg/d in men and in highest decile were  $\geq 3337$  mg/d in women and  $\geq 5271$  mg/d in men at baseline.

<sup>12</sup>One serving of fish per week, specifically of species high in long-chain (n-3) fatty acids EPA + DHA, is strongly protective against fatal cardiac arrhythmias and sudden cardiac death and may lower the incidence of other CVD. EPA + DHA were associated with lower risk of diabetes in some, but not all, studies, and the relation with cancer risk is unclear but studies showed that it decreased the formation of pre-neoplastic stages and could alter gene expression. According to WCRF, the consumption of 20g of fish/day may significantly decrease the risk of HCC..



## **VERIFICATION DES CRITERES D'INCLUSION ET DE NON INCLUSION**

### **CRITERES D'INCLUSION**(Si 1 ou plusieurs réponses NON ⇒ Ne pas inclure le patient)

	<b>Oui</b>	<b>Non</b>
Age supérieur ou égal à 35 ans.....	<input type="checkbox"/> <sup>(1)</sup>	<input type="checkbox"/> <sup>(0)</sup>
Affiliation à un régime de sécurité sociale.....	<input type="checkbox"/> <sup>(1)</sup>	<input type="checkbox"/> <sup>(0)</sup>
Atteint par une cirrhose compliquée ou non de CHC.....	<input type="checkbox"/> <sup>(1)</sup>	<input type="checkbox"/> <sup>(0)</sup>

### **CRITERES DE NON INCLUSION**(Si 1 ou plusieurs réponses OUI ⇒ Ne pas inclure le patient)

	<b>Oui</b>	<b>Non</b>
Age inférieur à 35 ans.....	<input type="checkbox"/> <sup>(1)</sup>	<input type="checkbox"/> <sup>(0)</sup>
Atteint par un cancer extra hépatique en évolution ou en cours de traitement.....	<input type="checkbox"/> <sup>(1)</sup>	<input type="checkbox"/> <sup>(0)</sup>
Atteint par un cancer du foie autre qu'un CHC.....	<input type="checkbox"/> <sup>(1)</sup>	<input type="checkbox"/> <sup>(0)</sup>
Atteint par un CHC sur foie non cirrhotique.....	<input type="checkbox"/> <sup>(1)</sup>	<input type="checkbox"/> <sup>(0)</sup>
Infecté par le VIH.....	<input type="checkbox"/> <sup>(1)</sup>	<input type="checkbox"/> <sup>(0)</sup>
Maladie somatique* ou psychiatrique grave.....	<input type="checkbox"/> <sup>(1)</sup>	<input type="checkbox"/> <sup>(0)</sup>
Sous tutelle ou sauvegarde de justice.....	<input type="checkbox"/> <sup>(1)</sup>	<input type="checkbox"/> <sup>(0)</sup>

**Consentement éclairé signé**.....  Oui<sup>(1)</sup>  
 Non<sup>(0)</sup>

Si oui, date de recueil du consentement du patient(jj/mm/aaaa) : |\_|\_| / |\_|\_| / |\_|\_|\_|\_|\_|

**Oui Non**

#### **PATIENT ELIGIBLE**

Tous les critères d'inclusion doivent être cochés OUI et tous les critères de non inclusion doivent être cochés NON

<sup>(1)</sup>  <sup>(0)</sup>

\*En cas d'hépatite alcoolique aigue sur cirrhose, le patient pourra être inclus après guérison biologique de l'hépatite (il faut attendre au moins 6 à 8 semaines avant de faire les prélèvements sanguins) !

Date de l'examen initial dans le cadre de l'étude (jj/mm/aaaa) : |\_|\_| / |\_|\_| / |\_|\_|\_|\_|

## **DIAGNOSTIC INITIAL**

### **➤ CIRRHOSE**

Année du diagnostic : |\_|\_|\_|\_|

Etiologie de la cirrhose :  Virus B<sup>(1)</sup>  Virus C<sup>(2)</sup>  Pas de virus<sup>(0)</sup>

#### **Examen(s) ayant permis de poser le diagnostic de cirrhose :**

Biopsie	<input type="checkbox"/> Oui <sup>(1)</sup>	<input type="checkbox"/> Non <sup>(0)</sup>
Si oui, préciser la date de la biopsie (mm/aaaa):  _ _  /  _ _ _ _		
Hémodynamique sus-hépatique	<input type="checkbox"/> Oui <sup>(1)</sup>	<input type="checkbox"/> Non <sup>(0)</sup>
Si oui, préciser le gradient :  _ _  mmHg		
Test biologique de fibrose	<input type="checkbox"/> Oui <sup>(1)</sup>	<input type="checkbox"/> Non <sup>(0)</sup>
Si oui, préciser : Fibrose :  _ _ (score) Activité :  _ _ (score)		
Fibroscan	<input type="checkbox"/> Oui <sup>(1)</sup>	<input type="checkbox"/> Non <sup>(0)</sup>
Si oui, préciser le score :  _ _ , _  kPa IQR :  _ _ , _		
Diagnostic clinico-biologique	<input type="checkbox"/> Oui <sup>(1)</sup>	<input type="checkbox"/> Non <sup>(0)</sup>

➤ **CHC**  Oui<sup>(1)</sup>  Non<sup>(0)</sup>

Si non, passer à l'item « Examens réalisés pour affirmer ou exclure le CHC » page 4

#### **Si oui :**

Date du diagnostic (mm/aaaa) : |\_|\_| / |\_|\_|\_|\_|

Caractéristiques du CHC :

- Uninodulaire :  Oui<sup>(1)</sup>  Non<sup>(0)</sup>

Si oui, taille du nodule : |\_|\_| cm

- Multinodulaire :  Oui<sup>(1)</sup>  Non<sup>(0)</sup>

Si oui, nombre de nodules :  ≤ 3<sup>(1)</sup>  > 3<sup>(2)</sup>

- Infiltrant diffus :  Oui<sup>(1)</sup>  Non<sup>(0)</sup>

- Métastases :  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

**Examens réalisés pour affirmer ou exclure le CHC :**

Diagnostic histologique du CHC:  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

Si oui :

Date de la biopsie (mm/aaaa) : |\_|\_|\_|\_|/|\_|\_|\_|\_|\_|\_|\_|\_|

CHC mis en évidence :  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

Echographie  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

    Si oui, CHC suspecté  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

Echographie de contraste  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

    Si oui, CHC suspecté  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

Scanner  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

    Si oui, CHC suspecté  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

IRM  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

    Si oui, CHC suspecté  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

AFP (Alfa Foëto Protéine) : |\_|\_|\_|\_|\_|\_|\_|\_|\_| UI/ml

**Spectro-IRM demandée dans le cadre de l'étude :**  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

*Uniquement dans les centres de Dijon et Strasbourg*

## EXAMEN CLINIQUE DU PATIENT

- Taille:       |\_|\_|\_|cm                                   - Tour de taille :       |\_|\_|\_|cm  
- Poids actuel :       |\_|\_|\_|kg                                   - Poids à 20 ans :       |\_|\_|\_| kg

Ancienneté de la surcharge pondérale éventuelle\* :       |\_|\_| années  NA<sup>(8)</sup>

(\*Indice de masse corporelle, IMC  $\geq$  25)

- Perte de poids dans les 6 derniers mois :  Oui<sup>(1)</sup>        Non<sup>(0)</sup>        Inconnu<sup>(9)</sup>

Si oui, préciser le poids antérieur à la perte de poids : |\_|\_|\_| kg

Ménopause :  Oui<sup>(1)</sup>        Non<sup>(0)</sup>        NA<sup>(8)</sup>

Hypertension artérielle diagnostiquée  
antérieurement :

Oui<sup>(1)</sup>        Non<sup>(0)</sup>        Inconnu<sup>(9)</sup>

Si oui, est-elle traitée :

Oui<sup>(1)</sup>        Non<sup>(0)</sup>        Inconnu<sup>(9)</sup>

## ANTECEDENTS / TERRAIN

**Antécédents de cancer extra hépatique :**        Oui<sup>(1)</sup>        Non<sup>(0)</sup>

Si oui, année du diagnostic : |\_|\_|\_|\_|

Merci de préciser la localisation : \_\_\_\_\_

(Ecrire en majuscules)

Codage CIM10 :

|\_|\_|\_|.|-|\_|

*A ne pas remplir, cadre  
réservé au CIC-EC*

### **Antécédents athéromateux :**

Accident Vasculaire Cérébral :  Oui<sup>(1)</sup>        Non<sup>(0)</sup>        Inconnu<sup>(9)</sup>

Infarctus / Angor:  Oui<sup>(1)</sup>        Non<sup>(0)</sup>        Inconnu<sup>(9)</sup>

Artérite :  Oui<sup>(1)</sup>        Non<sup>(0)</sup>        Inconnu<sup>(9)</sup>

### **Autres antécédents :**

ATCD familial de diabète :  Oui<sup>(1)</sup>        Non<sup>(0)</sup>        Inconnu<sup>(9)</sup>

Si oui, préciser:  Type I<sup>(1)</sup>        Type II<sup>(2)</sup>        Inconnu<sup>(9)</sup>

## MODE DE VIE

### Etudes

Diplôme le plus élevé obtenu :  Pas de diplôme ou certificat d'étude <sup>(1)</sup>  BEPC, CAP, BEP <sup>(2)</sup>  
 BAC <sup>(3)</sup>  BAC +2 <sup>(4)</sup>  > BAC +2 <sup>(5)</sup>

### Activité physique

Activité physique professionnelle au cours de la dernière année:

<input type="checkbox"/> Aucune ou Faible <sup>(0)</sup>	<input type="checkbox"/> Modérée <sup>(1)</sup>	<input type="checkbox"/> Importante <sup>(2)</sup>
<i>Travail sédentaire ± quelques déplacements quotidiens</i>	<i>Déplacements fréquents, port de charge de temps à autre</i>	<i>Travail physique avec port de charge</i>
<i>(Ex : Travail de bureau, Secrétaire si pas de port de charge, Caissière de supermarché, Conducteur d'engins, ...)</i>	<i>(Ex : Instituteur, Infirmière, Routier, Travail à la chaîne selon activité, ...)</i>	<i>(Ex : Maçon, Travail manutentionnaire, Travail à la chaîne selon activité, ...)</i>

Profession en clair :

(Ecrire en majuscules) \_\_\_\_\_

Activité physique de loisir au cours de la dernière année (incluant marche rapide, sports, jardinage, travaux de maçonnerie...):

<input type="checkbox"/> Aucune ou Faible <sup>(0)</sup>	<input type="checkbox"/> Modérée <sup>(1)</sup>	<input type="checkbox"/> Importante <sup>(2)</sup>
<1h/semaine	1h à 3h30/semaine	>3h30/semaine

Activité dominante en clair :

(Ecrire en majuscules) \_\_\_\_\_

### Tabac

Consommation de tabac :  Non (n'a jamais fumé) <sup>(0)</sup>  Actuelle <sup>(1)</sup>  Ancienne <sup>(2)</sup>

Si Non, passer à l'item « Alcool »

(Arrêt > 6mois)

Si consommation actuelle ou ancienne, préciser :

Quantités: - cigarettes = |\_|\_| (nb/jour)  
- cigares = |\_|\_| (nb/jour)  
- pipes = |\_|\_| (nb/jour)

Age de début : |\_|\_| ans

Age de fin si ex fumeur : |\_|\_| ans

Période(s) d'interruption(s) :  Oui <sup>(1)</sup>  Non <sup>(0)</sup>

Si oui, durée cumulée d'interruption(s) : |\_|\_|\_| mois

## **Alcool**

Consommation d'alcool :  Non (n'a jamais bu d'alcool)<sub>(0)</sub>  Oui<sub>(1)</sub>  Inconnu<sub>(9)</sub>

*Si non ou inconnu, passer à l'item « Cannabis »*

Si oui :

Consommation quotidienne :  Non<sub>(0)</sub>  Actuelle<sub>(1)</sub>  Ancienne<sub>(2)</sub>  
(Arrêt > 6mois)

Si consommation quotidienne actuelle ou passée, durée de la consommation quotidienne (années) : |\_|\_|\_|

Consommation par semaine la plus élevée : |\_|\_|\_| verres

Type de boissons alcoolisées consommées :  Vin<sub>(1)</sub>  Bière<sub>(2)</sub>  Spiritueux<sub>(3)</sub>

Consommation excessive d'alcool :  Oui<sub>(1)</sub>  Non<sub>(0)</sub>

*Une consommation excessive de boissons alcoolisées correspond plus de 21 verres/semaine (ou >3 verres/jour) pour un homme et à plus de 14 verres/semaines (ou >2 verres/jour) pour une femme. Pour référence, un verre d'alcool correspond à 10g d'alcool consommé au bar (souvent plus élevée à la maison).*

Si oui, préciser :

Durée cumulée de la consommation excessive: |\_|\_|\_| années

Consommation excessive interrompue :  Oui<sub>(1)</sub>  Non<sub>(0)</sub>

Si oui, depuis |\_|\_|\_| années

## **Cannabis**

Consommation de cannabis :  Inconnu<sub>(9)</sub>  Non (n'en a jamais consommé)<sub>(0)</sub>  Oui<sub>(1)</sub>

*Si non ou inconnu, passer à la page suivante*

Si oui, préciser :

Consommation ancienne interrompue:  Oui<sub>(1)</sub>  Non<sub>(0)</sub>

Fréquence de consommation :  Régulière<sub>(1)</sub>  Exceptionnelle<sub>(2)</sub>

Consommation actuelle :  Oui<sub>(1)</sub>  Non<sub>(0)</sub>

Fréquence de consommation :  Régulière<sub>(1)</sub>  Exceptionnelle<sub>(2)</sub>

Nombre moyen de joints par semaine de consommation : |\_|\_|\_| joints

Durée totale de la consommation : |\_|\_|\_| années

(Durée cumulée en cas d'interruptions répétées)





<b>(hormones sexuelles)</b>	Traitement hormonal substitutif		_ _	_ _ _ _
	Progestatif seul		_ _	_ _ _ _
	Anti androgènes		_ _	_ _ _ _
	Androgènes		_ _	_ _ _ _
<b>Hypolipémiants</b>	Fibrates		_ _	_ _ _ _
	Statines		_ _	_ _ _ _
<b>Antidiabétiques</b>	Sulfamides		_ _	_ _ _ _
	Biguanides		_ _	_ _ _ _
	Thiazolinediones		_ _	_ _ _ _
	Autres		_ _	_ _ _ _
	Insulines		_ _	_ _ _ _
<b>Colchicine</b> (Traitement au long cours)			_ _	_ _ _ _
<b>Médicaments amaigrissants</b>			_ _	_ _ _ _

**MÉDICAMENTS PRIS A L'INCLUSION** : photocopier l'ordonnance du traitement habituel et remplir le tableau ci-dessous (interrogatoire nécessaire pour déterminer la durée).

	Noms (DCI ou spécialité) (Ecrire en majuscules)	Durée (années)
1		_ _
2		_ _
3		_ _

4		_ _
5		_ _

Commentaires sur les traitements :

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## BILAN SANGUIN

dans les 2 mois précédant l'inclusion

### BIOLOGIE STANDARD

- Plaquettes : |\_|\_|\_|\_|\_|\_|\_|\_| /mm<sup>3</sup>
  - TP : |\_|\_|\_| %
  - INR : |\_|
  - Ferritinémie : |\_|\_|\_|\_| μg/L
  - Albuminémie: |\_|\_| g/L
  - Créatininémie : |\_|\_|\_| μmol/L
  - Glycémie: |\_|\_|,|\_| μmol/L
  - CRP: |\_|\_|\_| mg/L
  - Transaminases: |\_|\_|\_| UI/L
  - Gamma GT: |\_|\_|\_|\_| UI/L
  - Bilirubine totale: |\_|\_|\_| μmol/L
  - Cholestérol total: |\_|,|\_|\_| g/L
  - LDL : |\_|,|\_|\_| g/L
  - HDL : |\_|,|\_|\_| g/L
  - Triglycérides : |\_|,|\_|\_| g/L
  - Ig G: |\_|\_|,|\_|\_| g/L
  - Ig A : |\_|\_|,|\_|\_| g/L
  - Ig M: |\_|,|\_|\_| g/L
  - CHILD-PUGH: |\_|\_|(Score) |\_|(Lettre)
- MELD: ***Ne pas remplir: calcul automatique***

### VIROLOGIE

- |                        | Présent                                 | Absent                                  | Non recherché                           |
|------------------------|---|---|---|
| Antigène HBs :         | <input type="checkbox"/> <sub>(1)</sub> | <input type="checkbox"/> <sub>(0)</sub> | <input type="checkbox"/> <sub>(8)</sub> |
| Anticorps anti HBc :   | <input type="checkbox"/> <sub>(1)</sub> | <input type="checkbox"/> <sub>(0)</sub> | <input type="checkbox"/> <sub>(8)</sub> |
| Antigène HBe:          | <input type="checkbox"/> <sub>(1)</sub> | <input type="checkbox"/> <sub>(0)</sub> | <input type="checkbox"/> <sub>(8)</sub> |
| Anticorps anti HBe:    | <input type="checkbox"/> <sub>(1)</sub> | <input type="checkbox"/> <sub>(0)</sub> | <input type="checkbox"/> <sub>(8)</sub> |
| Anticorps anti delta : | <input type="checkbox"/> <sub>(1)</sub> | <input type="checkbox"/> <sub>(0)</sub> | <input type="checkbox"/> <sub>(8)</sub> |
| Anticorps anti VHC :   | <input type="checkbox"/> <sub>(1)</sub> | <input type="checkbox"/> <sub>(0)</sub> | <input type="checkbox"/> <sub>(8)</sub> |

Charge virale B : Recherché<sub>(1)</sub> Non recherché<sub>(0)</sub>

Si recherché, valeur de la charge virale : |\_|\_|\_|\_| |\_|\_|\_|\_| 10<sup>3</sup> UI / mL

Génotype viral B : Recherché<sub>(1)</sub>  Non recherché<sub>(0)</sub>

Si recherché, nom du génotype : |\_|

Charge virale Delta :  Recherché <sup>(1)</sup>       Non recherché <sup>(0)</sup>

Si recherché, valeur de la charge virale : |\_|\_|\_|\_|\_|\_|\_| 10<sup>3</sup> UI / mL

Charge virale C :     Recherché <sup>(1)</sup>       Non recherché <sup>(0)</sup>

Si recherché, valeur de la charge virale : |\_|\_|\_|\_|\_|\_|\_| 10<sup>3</sup> UI / mL

Génotype viral C :     Recherché <sup>(1)</sup>       Non recherché <sup>(0)</sup>

Si recherché, nom du génotype : |\_|\_|\_|

**J'atteste que les informations recueillies des pages 1 à 10 de ce cahier d'observation sont complètes et exactes.**

Fait le : |\_|\_|\_|/|\_|\_|/|\_|\_|\_|\_|

**Signature de l'investigateur :**