ANNEXE : Projet de thèse financé

Fatty acid intake, lipid profile and apolipoprotein levels in relation with the risk of diabetic retinopathy in the large E3N cohort study

Duration of the PhD thesis project = 36 months

A. BACKGROUND

Diabetic retinopathy (DR) is the most common complication of diabetes and the leading cause of blindness among working-age populations in the Western world¹. More than 60% of patients with type 2 diabetes (T2D) have some degree of retinopathy after 20 years of diabetes²,³. Established risk factors of DR are length of exposure and severity of hyperglycemia, hypertension, hyperlipidemia and smoking⁴. Given the rapidly escalating financial and social costs associated with diabetes care, there is an urgent need for development and implementation of studies to identify new modifiable risk factors of diabetic complications such as DR.

Fatty acids and DR

Very few data exists on the effect of fatty acids on DR. An article published in the New England Journal of Medicine suggested that consuming omega-3 long-chain polyunsaturated fatty acid (PUFAs) protects against the development of DR and age-related macular degeneration⁵. This study identified beneficial effects of dietary n-3 PUFAs on visual function in T2DM. Earlier work has already supported this hypothesis, as it has been shown that linoleic acid (n-6 PUFAs) might protect against diabetic retinopathy⁶. But, another study suggested in 1996, that dietary fish oil supplementation may be harmful for the diabetic microvasculature in the retina⁷. Based on data from a large population based study, this PhD thesis project will provide the first data in humans.

Lipid profiles, apolipoproteins and DR

There is controversy regarding the contribution of lipids in the pathogenesis of diabetic retinopathy. Those who have elevated serum LDL-cholesterol might be more likely to have retinal hard exudate⁸. Similar results were observed in several studies⁹,10,11. However, other studies have not consistently shown similar associations¹².

Some recent evidence focused on the relationship between apolipoproteins with DR showing an elevation of apolipoproteins Apo A1 and Apo B in the vitreous fluid with an increased expression in the retinal of diabetic patients¹³. A recent study suggests that serum apolipoproteins Apo B and Apo A1 are stronger biomarkers of DR than traditional lipids¹⁴. Apolipoprotein C3 (Apo C3), the second most abundant apolipoprotein circulating in human plasma, is a natural lipoprotein lipase inhibitor¹⁵ and has the potential to modulate whole-body insulin sensitivity by regulating the transfer of plasma triglycerides and nonesterified fatty acids to tissues¹⁶. But data in humans on the association between Apo C3 and DR are scarce.

B. OBJECTIVES FOR THE PHD THESIS PROJECT

We propose for the first time to quantify in the prospective E3N cohort study the associations of fatty acid intakes (MUFA, n-3 and n-6 PUFA, TFA, SFA and total fatty acids), fish/seafood consumption with DR risk (Objective 1). We will also study, from a macroscopic point of view, the relationship between the Mediterranean dietary pattern,
characterized by a large consumption of sea food and fish, and DR risk (Objective 2). Finally, we will study the interactions between serum apolipoprotein levels (Apo A1, Apo B and Apo C3), lipid profiles and dietary intakes of fatty acid on the risk of DR (Objective 3).

C. MATERIAL AND METHODS

The E3N cohort study and ascertainment of T2D in the E3N cohort

The E3N study is a prospective cohort study of 98,995 female teachers, initiated in 1990. Participants returned mailed questionnaires to update health-related information and newly diagnosed diseases every 2 to 3 years; in addition, a drug reimbursement claims database has been available since 2004, using medical insurance records (MGEN). Diabetes cases were validated by cross-checking at least two sources: self-reported diabetes in the follow-up questionnaires and/or ascertained diabetes in the specific diabetes questionnaire and/or drugs reimbursed by the insurance between 2004 and 2012. A total of 5,020 cases of T2D were validated. The team has also extensive experience in the field of the effects of fatty acids on health (on cancer and cognitive decline).

Ascertainment of DR: the E3N-AfterDiab study

A questionnaire dedicated to diabetes complications, including DR, and quality of life since the diagnosis of diabetes was sent to all people with a validated T2D. There is a long post-diagnosis follow-up (mean duration between diabetes diagnosis and the 1st January 2015 is 16 years) which is adequate to observe the incidence of complications. The following information is available. A sub-sample (N=100) of the self-reported DR cases and participants with a laser eye surgery will be validated by collecting the medical records from the participants and/or their doctors.

Assessment of fatty acid intake and other dietary information in the E3N cohort

Information on diet was collected in 1993 and 2005, using a detailed 238-items validated food-frequency questionnaire. In particular, we are capable of estimating the following quantities of interest accurately:

- **Food sources of fatty acids**: Consumptions of fish (lean, fat), seafood, nuts, walnuts, seeds, soya oil (in g/day or standardized portions/day)
- **Nutrients**: Intakes of MUFA, n-3 and n-6 PUFA, TFA, SFA and total fatty acids (in g or mg/day)
- **Scores**: Dietary patterns (Mediterranean, Western) and Healthy Eating Index

The fatty acids will be adjusted for total energy intake with the use of the residual regression method and will be categorized into tertiles (and quartiles) based on distribution.

Assessment of apolipoproteins in the E3N-AfterDiab cohort
In addition to the existing data of the E3N cohort, we plan to estimate levels of the following biomarkers among all the participants with T2D and with a blood sample available (N=1590):

- **Lipid profiles:** Total, HDL-, LDL-, VLDL- cholesterol, and triglycerides
- **Apolipoproteins:** Apolipoprotein A1 (Apo A1), Apolipoprotein B (Apo B) and Apolipoprotein C3 (Apo C3)

This will allow us to design a sub-cohort analysis to study prospectively these biomarkers and their interactions with fatty acids in relation with the risk of DR. A specific funding for the assays has been requested elsewhere.

**D. DETAILED PLAN OF INVESTIGATION**

**Inclusion/Exclusion criteria**
For the study on fatty acids and DR risk, we will include all individuals of the E3N cohort with a validated incident T2D and available dietary information. **A total of 3525 women will be included.** During the follow-up, **1301 DR cases (29%) were recorded** with a median age of diagnosis of retinopathy of 67 years.

**Power analysis**
A power analysis has been performed. According to our sample size, **we will have a minimal statistical power of 1-β = 0.80 to detect a significant hazard ratio of 1.11.**

**Statistical modeling**
In addition to a large descriptive study, Cox regression models will be used to estimate hazard ratios of DR risk and 95% confidence intervals. Multivariable models will be adjusted for T2D risk factors and potential cofounders such as age, smoking status, physical activity, hypertension, hypercholesterolemia, family history of diabetes, body mass index, daily energy intake and T2D treatment. Interactions between the main exposure variables and cofactors will be tested and stratifications will be considered if the associated $P_{interaction} < 0.20$.

**Scientific valorization**
The present thesis project will lead to at least 3 scientific articles (please see the calendar below for further details) and several abstracts in international conferences (EASD and ADA annual meetings). The funding by CORDDIM Ile-de-France will be systematically acknowledged in all the related communications.

**E. PERSPECTIVES: NOVELTY AND IMPORTANCE OF THIS WORK**

This PhD thesis project is a unique opportunity to decrease the burden of DR by highlighting new modifiable risk factors. This will also be the opportunity to develop new hypotheses on the mechanisms of some fatty acids that could be tested in future clinical and biological studies, both in human and animal models. **Finally, it will help public health authorities to define new dietary guidelines regarding the consumption of fatty acids and their main food sources.**
Table 1: Schedule for the present project.

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<th>Task</th>
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<td>Selection of E3N participants with a blood sample available</td>
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<td>Validation of N=100 random self-reported diabetic retinopathy cases</td>
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<td>Writing of the scientific article 1</td>
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<td>Assays of biomarkers</td>
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<td>Data-management on biomarkers, matching with existing E3N data, and preparation of the datasets for analysis</td>
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Prevalence of diabetic retinopathy in various ethnic groups: a worldwide perspective. Sivaprasad S et al. Surv Ophta 2012


Fatty Acids and Retinopathy. Emily Y et al. NEJM 2011


Serum apolipoprotein AI and B are stronger biomarkers of diabetic retinopathy than traditional lipids. Sasongko MB et al. Diabetes Care. 2011


Increased apolipoprotein C-III levels associated with insulin resistance contribute to dyslipidemia in normoglycemic and diabetic subjects from a triethnic population. Florez H et al. Atherosclerosis. 2006


Birth weight, body silhouette over the life course, and incident diabetes in 91,453 middle-aged women from the French E3N Cohort. de Lauzon-Guillain B et al. Diabetes Care 2010


