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# Dietary Factors, Type 2 Diabetes and Diabetic Retinopathy

Courtney Dow

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# Dietary factors, type 2 diabetes and diabetic retinopathy

University Paris-Saclay Doctoral Thesis  
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Doctoral School n°570 Public Health (EDSP)  
Doctoral Specialization: Epidemiology

Thesis presented and defended in Villejuif, the 12<sup>th</sup> of October, by

**Courtney DOW**

Jury Composition:

<b>Marie Aline Charles</b> Director of Research, INSERM (Paris)	President
<b>Vincent Rigalleau</b> Professor-Practitioner, Université de Bordeaux (Bordeaux)	Reporter
<b>Jean-Pierre Riveline</b> Professor-Practitioner, INSERM/Paris 7 (Paris)	Reporter
<b>Matthias Schulze</b> Director of Research-Professor, DIFE/University of Potsdam (Potsdam)	Examiner
<b>Bénédicte Stengel</b> Director of Research, Université Paris Sud (Orsay)	Examiner
<b>Guy Fagherazzi</b> Epidemiologist INSERM (Villejuif)	Thesis Director

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# ABSTRACT

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**Background:** Type 2 diabetes (T2D) presents a significant health burden that is associated with many complications, such as diabetic retinopathy (DR), that further burden people with diabetes. Modifiable risk factors, such as the diet, have been identified for both T2D and DR; yet certain aspects of the role of the diet remain unclear.

**Objectives:** The main objectives of this thesis were therefore to examine the role and impact of the diet, and in particular, the consumption of fatty acids (FAs), and other modifiable behaviours on the risk of T2D and to summarize, interpret and analyze the relationship between the diet and DR using data from both the E3N and AusDiab cohort studies.

**Results:** The results suggest that the role of FAs on the risk of T2D and DR may differ between and within subgroups, and by individual polyunsaturated fatty acids (PUFAs). The findings also suggest that strongly adhering to national dietary guidelines is not associated with the development of T2D, but strongly adhering to other recommendations for healthy behaviours (for waist circumference, physical activity and smoking) is strongly inversely associated with T2D. Modifiable behaviour could have prevented more than half of the cases of T2D.

**Conclusions:** This work underlines the importance and the complexity of the role of the diet in the development of T2D and DR. It also illustrates the impact of healthy behaviour in the etiology of T2D and confirms that T2D is largely preventable. Efforts should focus on the modification of multiple healthy behaviours in populations, and promote diets that are moderate and widely varied.

**Keywords:** type 2 diabetes, retinopathy, diet, nutrition, fatty acids, lifestyle, cohort, epidemiology

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# RESUME

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**Contexte:** Le diabète de type 2 (DT2) constitue une pathologie majeure, au lourd fardeau, associée à de nombreuses complications, comme la rétinopathie diabétique (RD). Des facteurs modifiables, comme l'alimentation, ont déjà été identifiés pour le DT2 et la RD mais certains aspects de leurs rôles restent à préciser.

**Objectifs:** Les objectifs de cette thèse étaient d'examiner le rôle de l'alimentation, en particulier la consommation d'acides gras (AGs), et des autres facteurs modifiables liés au mode de vie sur le risque de DT2 et de synthétiser, interpréter et analyser la relation entre l'alimentation et la RD.

**Résultats :** Les résultats suggèrent que le rôle des AGs sur le risque de DT2 et de la RD pourrait être différent selon leur type, et même varier au sein d'un groupe comme les AG polyinsaturés (AGPI). Les résultats suggèrent aussi qu'une forte adhésion aux recommandations alimentaires n'est pas associée avec le développement d'un DT2, mais en revanche une forte adhérence aux autres recommandations de santé (concernant le tour de taille, l'activité physique et le statut tabagique) est fortement associée avec un moindre risque de DT2. On a montré qu'avoir un mode de vie sain aurait pu empêcher la survenue de plus de la moitié des cas de DT2.

**Conclusions:** Cette thèse a permis de préciser l'importance et la complexité du rôle de l'alimentation dans le développement du DT2 et de la RD. Elle montre aussi l'impact des comportements sains dans la pathologie de DT2 et confirme que le DT2 est en grande partie, une maladie évitable. Les efforts devraient se focaliser sur la modification des comportements de santé à la fois dans la population générale et atteinte de DT2 et notamment encourager une alimentation modérée et variée.

**Mots clés:** diabète de type 2, rétinopathie, alimentation, nutrition, acides gras, mode de vie, cohorte, épidémiologie

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# SCIENTIFIC PRODUCTION

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## ARTICLES ACCEPTED FOR PUBLICATION

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## SCIENTIFIC COMMUNICATION

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**Dow C**, Mancini M, Rajaobelina K, Boutron-Ruault MC, Balkau B, Bonnet F, Fagherazzi G. Diet and diabetic retinopathy: a systematic review. *French-Speaking Diabetes Society Conference*; Poster presentation. Nantes, March 2018.

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## SUPERVISION

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## LIST OF ABBREVIATIONS

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25(OH)D = 25-hydroxyvitamin D  
AA = arachidonic acid (22:4n6)  
AHEI = Alternative Healthy Eating Index  
ALA =  $\alpha$ -linolenic acid (18:3n3)  
AGEs = advanced glycation endproducts  
ALE = advanced lipoxidation endproducts  
BM = basement membrane  
BMI = body mass index  
BRB = blood-retinal barrier  
CESP = *Centre de Recherche en Epidémiologie et Santé des Populations*  
CNIL = *Commission Nationale Informatique et Liberté*  
DASH = Dietary Approaches to Stop Hypertension  
DED = diabetic eye disease  
DHA = docosahexaenoic acid (22:6n3)  
DME = diabetic macular edema  
DNL = de novo lipogenesis  
DPA = docosapentaenoic acid (22:5n3)  
EPA = eicosapentaenoic acid (20:5n3)  
DR = diabetic retinopathy  
EPIC = European Prospective Investigation into Cancer and Nutrition  
FA = fatty acids  
FFA = free fatty acids  
FFQ = food frequency questionnaire  
GDM = gestational diabetes  
HbA<sub>1c</sub> = glycated hemoglobin  
HEI = Healthy Eating Index  
HPA = hypothalamic pituitary adrenal  
HR = hazard ratio  
INCA<sub>3</sub> = third national study of food consumption  
NPDR = non-proliferative diabetic retinopathy  
LA = linoleic acid (18 :2n6)  
MGEN = *Mutuelle Générale de l'Éducation Nationale*  
MUFA = monounsaturated fatty acid  
OR = odds ratio  
N<sub>3</sub>-PUFA = omega-3 polyunsaturated fatty acid  
N<sub>6</sub>-PUFA = omega-6 polyunsaturated fatty acid  
PAF = population attributable fraction  
PDR = proliferative diabetic retinopathy  
PUFA = polyunsaturated fatty acid  
RCT = randomized controlled trial  
ROS = reactive oxygen species  
SFA = saturated fatty acid  
T<sub>1</sub>D = type 1 diabetes  
T<sub>2</sub>D = type 2 diabetes  
TFA = trans unsaturated fatty acid  
VEGF = vascular endothelial growth factor  
VLSFA = very long chain saturated fatty acids  
WHR = waist to hip ratio

---

# INTRODUCTION

---

Diabetes mellitus is a chronic disease that affects an estimated 425 million adults worldwide<sup>1</sup>. It is characterized by an insulin deficiency that results in hyperglycemia, and can lead to other long-term complications and premature death<sup>2,3</sup>. Type 2 diabetes (T2D) represents 90-95% of diabetes, and though it is largely preventable, the prevalence of T2D continues to rise<sup>2</sup>. When badly managed, complications such as diabetic retinopathy (DR), which causes vision loss or blindness if not treated, arise as well, adding to the already heavy burden on people with diabetes<sup>4</sup>. Due to the continued rise in the prevalence of diabetes, DR has become a leading cause of blindness in the working-age population of the Western world<sup>5</sup>. Modifiable factors, such as the diet, play a critical role in the development and management of both T2D and its complications, yet the roles of certain aspects of the diet have not yet been examined clearly. For this reason, my doctoral thesis investigates the *role of dietary factors in the development of type 2 diabetes and diabetic retinopathy*, using both French and Australian data.

This introduction will be divided into four sections, with the first three sections highlighting the state of available scientific knowledge related to themes of my thesis, and the last outlining the specific objectives. Firstly, this introduction will focus on diabetes, in particular, the more prevalent type 2 diabetes, where a brief summary of its pathophysiology, epidemiology, and risk factors will be presented. Next, the pathophysiology, epidemiology, and risk factors of diabetic retinopathy will be addressed. The third section will outline the role of the diet in the development of T2D and retinopathy, with an emphasis on fatty acids, an essential component of our diet, and finally, the fourth section will address the specific objectives of this thesis.

# 1. DIABETES MELLITUS

---

## 1.1 DEFINITION

---

There are three main types of diabetes mellitus:

### 1.1.1 TYPE 1 DIABETES (T1D)

Caused by an autoimmune response, the body attacks the insulin-producing cells of the pancreas, resulting in a failure to produce adequate insulin<sup>1</sup>. The origins of the autoimmune response are currently not well understood, but both genetic and environmental factors, including infections, the diet, the psychosocial environment and perinatal conditions are believed to contribute<sup>6</sup>. T1D most frequently occurs early in life, in childhood or adolescence, and represents 5-10% of diabetes<sup>1,2</sup>.



### 1.1.2 TYPE 2 DIABETES (T2D)

Type 2 diabetes occurs when the body loses its ability to respond to insulin (insulin resistance) and to adequately produce insulin<sup>1,3</sup>. It accounts for 90-95% of diabetes cases and most commonly develops in adulthood<sup>2</sup>. As T2D is the focus of this thesis, its underlying pathophysiology will be discussed in detail in [Section 1.2](#) and the risk factors will be discussed in [Section 1.4](#).



### 1.1.3 GESTATIONAL DIABETES (GDM)

Gestational diabetes is defined by the World Health Organization as “hyperglycemia that is first recognized during pregnancy”, and thus includes both women with pre-existing diabetes discovered at pregnancy and those who develop diabetes during pregnancy<sup>7</sup>. For those who develop diabetes during pregnancy, it is normally diagnosed in the second trimester and disappears in the postpartum period<sup>3,8</sup>. GDM births increase the risk of pregnancy and delivery complications such as preeclampsia and fetal macrosomia<sup>3</sup>.

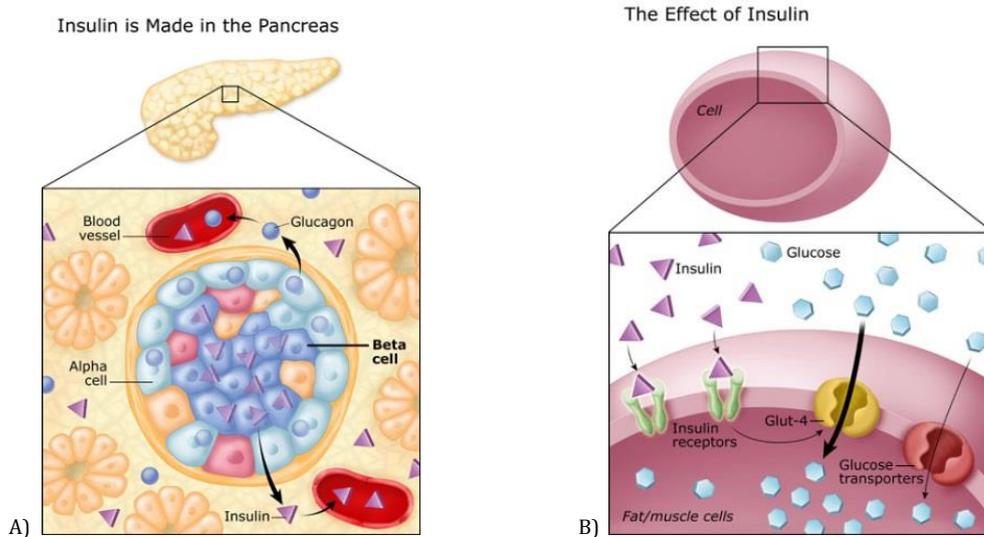


## 1.2 PATHOPHYSIOLOGY OF TYPE 2 DIABETES

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### 1.2.1 INSULIN

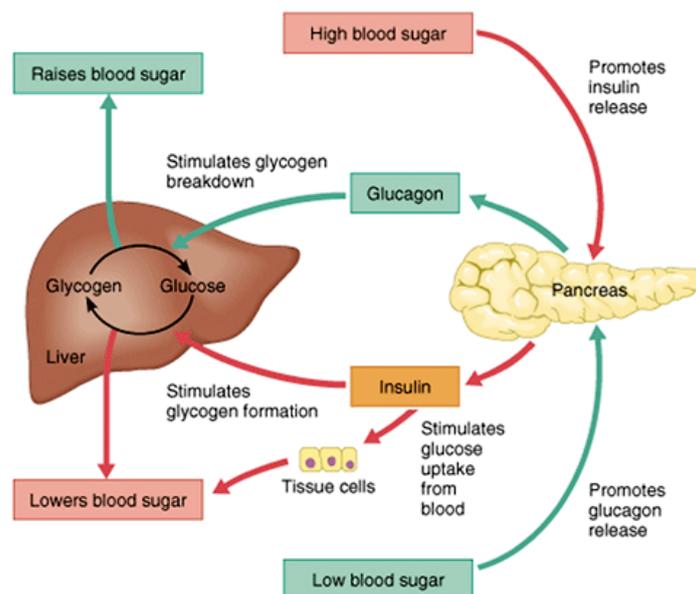
Insulin is a key player in the development of diabetes. Insulin is a hormone produced in the  $\beta$  cells of the islets of Langerhans in the pancreas<sup>9</sup> (**Figure 1a**). Together with the hormone glucagon, which is produced by the  $\alpha$  cells of the pancreas, blood glucose levels are regulated<sup>9,10</sup>. Elevated glucose stimulates insulin release from the pancreas, which activates insulin receptors<sup>10</sup>. Insulin receptors are found in the cell membranes of insulin-sensitive tissues (muscle, adipose, liver). After insulin binds to its receptor, it initiates a cascade of events that, in muscle and adipose tissue, allows the primary glucose transporter (*GLUT4*), to uptake glucose into the cell<sup>11</sup> (**Figure 1b**).



**FIGURE 1. A) INSULIN PRODUCTION IN THE PANCREAS B) INSULIN RECEPTORS AND GLUT4 TRANSPORTERS ON THE CELL MEMBRANES OF FAT/MUSCLE CELLS<sup>10</sup>**

### 1.2.2 THE FEEDBACK LOOP

In healthy individuals, between meals, insulin levels are low and glucagon is released, signalling the liver to provide energy to the body by: (1) breaking down stored glycogen to glucose in a process called *glycogenolysis*; (2) converting proteins, lipids or other substrates into glucose in a process called *gluconeogenesis*; and (3) when glycogen stores are low, to produce *ketones* from fatty acids<sup>10</sup> (**Figure 2**). After eating, glucagon levels decrease, insulin levels increase and simultaneously decrease the breakdown of fat from adipose tissue and glucose production in the liver, promote transport of glucose into tissues, and enhance the synthesis and storage of fatty acids, proteins and glycogen<sup>9,12,13</sup>.



**FIGURE 2. FEEDBACK LOOP CONTROLLING BLOOD GLUCOSE LEVELS<sup>12</sup>**

However, individuals with chronic hyperglycemia may develop *insulin resistance* (impaired action of insulin) (Figure 3) and/or  $\beta$  cell dysfunction that lead to impaired insulin secretion<sup>14</sup>. As cells become unresponsive to the effects of insulin, glucose transport into cells decreases and blood glucose levels increase. In addition, desperate for energy, cells further signal the liver to increase glucose production. To compensate,  $\beta$  cells may increase their mass and/or insulin secretion<sup>15</sup>. However, as glycemic control worsens, the cells are not able to cope and  $\beta$  cell mass and insulin secretion begins to substantially decrease<sup>15</sup>.

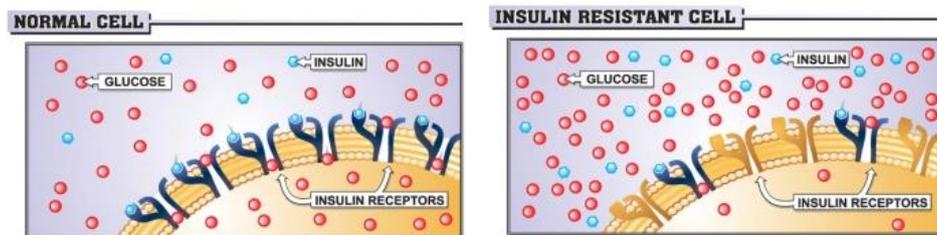


FIGURE 3. INSULIN RESISTANCE AT THE CELLULAR LEVEL<sup>16</sup>

### 1.2.3 BETA CELL DYSFUNCTION AND INSULIN RESISTANCE

Several theories have been proposed for the  $\beta$  cell dysfunction and insulin resistance associated with T2D, including: glucotoxicity, lipotoxicity, inflammation and oxidative stress.

**Glucotoxicity:** chronic hyperglycemia may lead to diminished insulin secretory granules from the  $\beta$  cells, decreasing the amount of insulin available for new glucose responses and inducing  $\beta$  cell death<sup>15,17</sup>.

**Lipotoxicity:** chronically increased levels of free fatty acids (FFA) may increase the basal rate of insulin secretion and/or affect the synthesis or conversion of proinsulin to insulin, leading to depleted insulin stores<sup>15,18,19</sup>. Lipotoxicity has also been observed to induce  $\beta$  cell apoptosis<sup>17</sup>.

**Inflammation:** associated with obesity and high levels of glucose and FFA, the insulin-sensitive tissues get stressed and release pro-inflammatory cytokines and chemokines<sup>17,20</sup> (Figure 4). The increased levels of pro-inflammatory signals causes the recruitment of immune cells that further contribute to inflammation and promote it in other tissues, such as the pancreatic islets, leading to  $\beta$  cell apoptosis<sup>17,20</sup>.

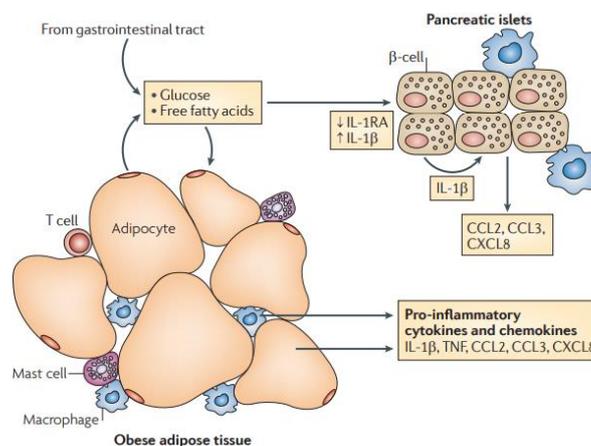


FIGURE 4. THE DEVELOPMENT OF INFLAMMATION IN DIABETES<sup>17</sup>

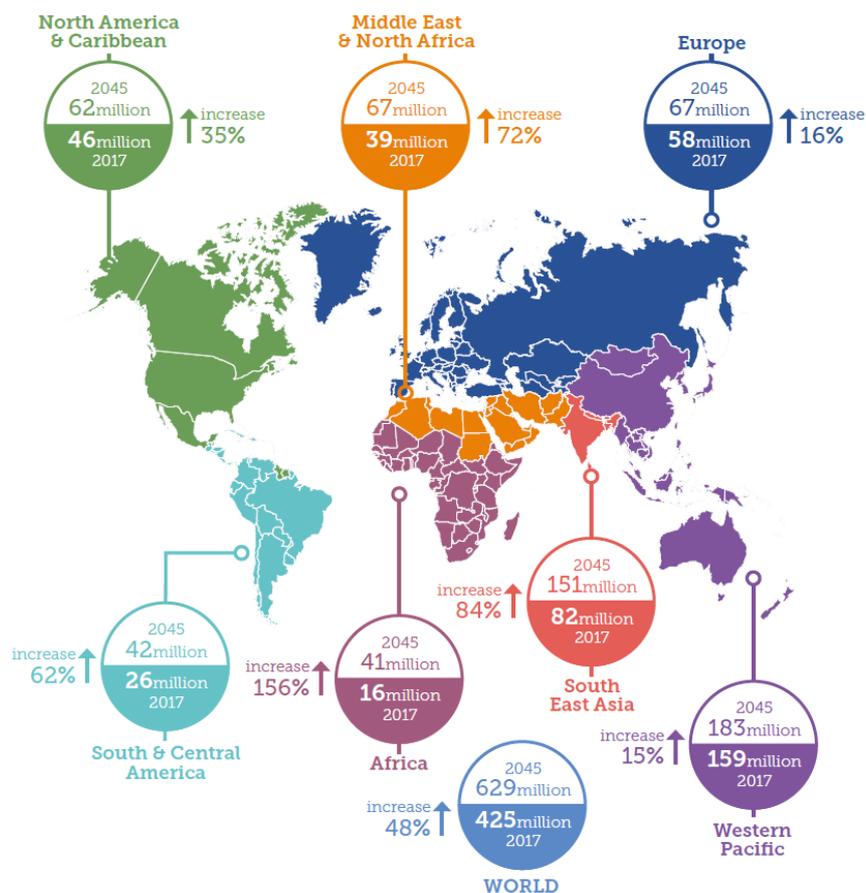
*Oxidative stress*: induced by hyperglycemia and causes generation of *reactive oxygen species* (ROS) and initiates *endoplasmic reticulum stress*<sup>17,20</sup>.

- ROS has been linked to reduced energy production, DNA damage, and the formation of *advanced glycation end products* (AGEs; see [Section 2.2.4](#))<sup>20</sup>. In addition,  $\beta$ -cells have low levels of antioxidant enzymes, making them particularly vulnerable to the effects of ROS<sup>17,20</sup>.
- The endoplasmic reticulum is a cell organelle responsible for the synthesis, modification and transport of proteins and lipids<sup>21,22</sup>. Endoplasmic reticulum stressors include hypoxia, nutrient (glucose) deprivation, and increases in protein synthesis and cause an accumulation of unfolded proteins in the endoplasmic reticulum<sup>21</sup>. This accumulation causes endoplasmic reticulum stress, to which cells respond by initiating the unfolded protein response in an attempt to maintain homeostasis<sup>21,22</sup>. However, if homeostasis is not met or is beyond the capacities of the cell, the cell undergoes apoptosis<sup>20,21</sup>.

### 1.3 DESCRIPTIVE EPIDEMIOLOGY OF DIABETES

#### 1.3.1 THE GLOBAL BURDEN

The International Diabetes Federation estimated the prevalence of diabetes to be 8.8% [7.2-11.3%] in 2017, equivalent to 425 million people worldwide<sup>1</sup> ([Figure 5](#)). This prevalence is expected to rise by more than 45% by the year 2045, to 9.9% [7.5-12.7%]<sup>1</sup>.



**FIGURE 5.** NUMBER OF PEOPLE WITH DIABETES WORLDWIDE AND PER REGION IN 2017 AND 2045 (20-79 YEARS)<sup>1</sup>



## 1.4 RISK FACTORS FOR TYPE 2 DIABETES

The risk of developing T2D is associated with both modifiable and non-modifiable risk factors; however, this section will focus on the modifiable risk factors. Individually and in combination, modifiable risk factors have been shown to predict the occurrence of T2D, and their management could prevent over three quarters of T2D cases<sup>27-31</sup>. However, the effect of each modifiable risk factor varies with the population of interest.

### 1.4.1 MODIFIABLE FACTORS

*Overweight/obesity* is one of the most important factors in the development of T2D. Meta-analyses of cohort studies suggest that overweight increases the risk of T2D by three times, whereas obesity may increase the risk of T2D by more than 7x<sup>32</sup>. In addition, lifestyle intervention programs suggest that weight loss is the most important factor in preventing or delaying T2D onset. In people with pre-diabetes, the Diabetes Prevention Program observed an average 16% reduction in T2D risk per kilogram of weight loss<sup>33</sup>.



Independently of body weight, the level of *physical activity* is also an important factor in T2D prevention<sup>34,35</sup>. Compared to inactive people, those performing the recommended amount of physical activity (150 min/week) were found to have a 26% reduction in the risk of T2D, with increasing risk reductions with more physical activity and higher intensities<sup>34,35</sup>. Eliminating physical inactivity could prevent between 5.2%-13% of diabetes cases<sup>36</sup>.

Along with overweight/obesity and physical activity, the *diet* is one of the main contributing factors to T2D. However, it will be discussed in further detail in [Section 3.0](#).



*Hypertension* is regarded as an independent risk factor for T2D, with meta-analyses estimating greater than 50% increased risk with 20 mmHg increases in systolic blood pressure or 10 mmHg increases in diastolic blood pressure<sup>37</sup>. Like T2D, hypertension is also associated with inflammatory markers and endothelial dysfunction, which can lead to insulin resistance, creating a common ground for the development of both hypertension and T2D<sup>38</sup>.

A meta-analysis of over 25 cohort studies has reported a pooled relative risk of 1.44 [1.31-1.58] for *current smokers* compared to non-smokers<sup>39</sup>. Diabetes risk was higher in heavy smokers ( $\geq 20$  cigarettes/day) than light smokers and active smokers compared to former smokers<sup>39</sup>. Smoking has been shown to induce insulin resistance and have detrimental effects on glucose control<sup>40</sup>.



Some studies have found *stress* or *psychological distress* to be a risk factor for T2D<sup>41-43</sup>. However, one meta-analysis found no overall association between work-related stress and T2D until stratification, where a positive association between stress and T2D risk was observed only in women<sup>44</sup>. Apart from the effect that stress may have on behavioural risk factors, stress may induce a chronic inflammatory response that can lead to increased adiposity, insulin resistance and hypertension<sup>45</sup>.

Both *sleep quality* and *sleep duration* have been associated with T2D. A U-shaped relationship between sleep duration and T2D suggests 7-8h/day to be the most beneficial for T2D prevention<sup>46</sup>. For those with difficulty initiating or maintaining sleep, relative risks estimate more than a 50% increased risk of T2D<sup>47</sup>. Sleep deficiency has been linked to glucose intolerance, insulin resistance and reduced insulin responses<sup>47</sup>.



### 1.4.2 NON-MODIFIABLE FACTORS

T2D is more frequently diagnosed in *older people* ( $\geq 45$  years) than younger<sup>48</sup>, and in many countries, more frequently diagnosed in *men* than women<sup>49</sup>. *Family history* of T2D is another strong predictor, with 2.4x increased risk of T2D and more than 5x increased risk if both parents have a history of T2D<sup>50</sup>.



Separately from family history, *genetic factors* appear to have an influence on the development of T2D, as the discordance rate between monozygotic twins (5.1%) appears lower than that for dizygotic twins (8.0%)<sup>51</sup> and several genetic variants have been associated with T2D<sup>52</sup>.

Women who have had *gestational diabetes* are at 7x the risk of developing T2D compared to women with a normoglycemic pregnancy<sup>53</sup>. In addition, the children of these women have 8x the risk of developing T2D or pre-diabetes between 19-27 years old and are at increased risk of being born overweight<sup>3,54</sup>. *High birth weight* ( $\geq 4000$  g vs. 2500-4000 g) is another risk factor for T2D later in life, as is *low birth weight* ( $< 2500$  g).



T2D prevalence may vary by *race* or *ethnicity*. In many studies, a higher prevalence of T2D is observed in African Americans compared to non-Hispanic white Americans, as well as Hispanic Americans<sup>55</sup>. However, when diabetes prevalence is compared among African Americans and non-Hispanic whites of similar socioeconomic status, or after controlling for socioeconomic factors, often the associations disappear<sup>56,57</sup>. Conversely, T2D appears more predominant among Asians than whites and may be due to development of T2D at lower body mass index (BMI)<sup>58</sup>.

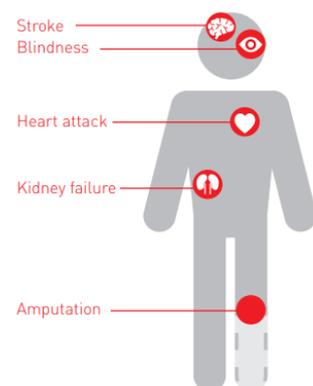
### 1.4.3 ENVIRONMENTAL FACTORS

The surrounding environment can play an influence in the development of T2D. Studies have observed higher T2D risk with increasing levels of exposure to *air pollution*, such as nitrogen dioxide and particulate matter  $< 2.5\mu\text{m}$  in diameter<sup>59</sup>. It is believed air pollutants act by triggering insulin resistance, adipose inflammation or endothelial dysfunction<sup>59</sup>. *Noise* may also increase T2D risk, likely through its association with hypertension, increased stress and increased cortisol levels<sup>59</sup>. In addition, exposure to *persistent organic pollutants* has been linked with T2D development<sup>60</sup>. Experiments show that some persistent organic pollutants decrease glucose uptake by cells, decrease insulin production and can act as endocrine disrupting chemicals<sup>60</sup>.



## 1.5 COMPLICATIONS OF DIABETES

Complications for individuals with diabetes include: cardiovascular disease (CVD), stroke, kidney damage (nephropathy), nerve damage (neuropathy), and eye disease (including retinopathy)<sup>3,3</sup>, which will be further detailed in [Section 2 \(Figure 8\)](#). Data suggest that individuals with diabetes have a 2-3x higher rate of CVD than those without diabetes and that up to 80% of end-stage renal disease is due to diabetes, hypertension, or a combination of both<sup>3</sup>. Nerve damage caused by diabetes increases rates of amputation by 10-20x in people with diabetes, while eye disease is the leading cause of vision loss in working-age adults<sup>3</sup>.



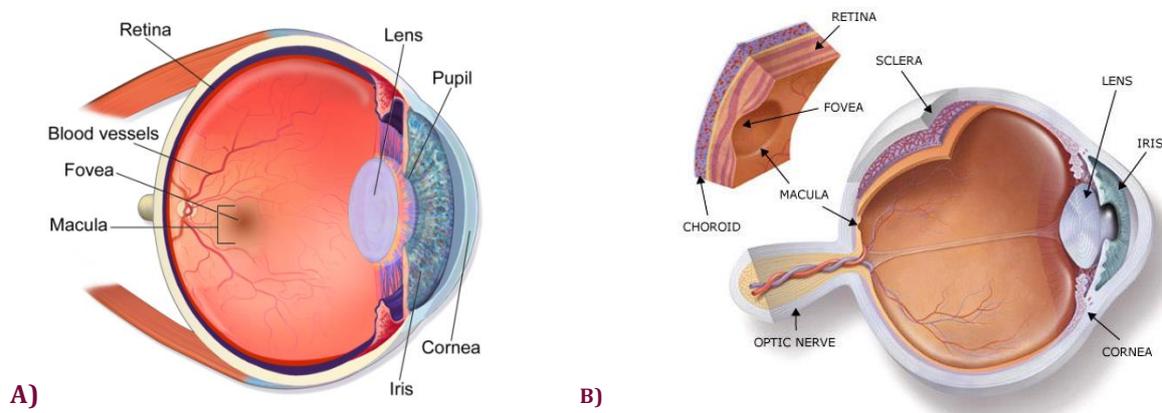
**FIGURE 8.** COMPLICATIONS OF DIABETES<sup>3</sup>

## 2. DIABETIC RETINOPATHY

### 2.1 DEFINITION

#### 2.1.1 THE RETINA

The wall of the eye is composed of three layers: the fibrous tunic, the vascular tunic and the retina<sup>61</sup>. The *retina* is the innermost layer of the eye, considered an outgrowth of the brain, where light-sensitive cells convert light to the electrical activity that is responsible for our perception of vision<sup>61</sup> (**Figure 9a**). The *macula* is found in the center of the retina and is responsible for high-resolution, colour vision<sup>61</sup>. One of the major sources of blood for the retina comes from the many blood vessels of the *choroid*, the layer of the vascular tunic in contact with the retina<sup>61</sup> (**Figure 9b**).



**FIGURE 9. A) ANATOMY OF THE EYE<sup>62</sup>; B) ANATOMY OF THE EYE SHOWING LAYERS<sup>63</sup>**

#### 2.1.2 DEFINITION OF DIABETIC RETINOPATHY

Diabetic retinopathy (DR) is a microvascular complication of diabetes mellitus, believed to be principally caused by chronic hyperglycemia, in which the retinal capillaries are damaged due to changes in blood flow, leading to leakage and blockage<sup>4</sup>. At first, DR presents with few or mild symptoms, but if left untreated, it could lead to vision loss or blindness<sup>4</sup> (**Figure 10**).



**FIGURE 10. NORMAL VISION (LEFT); ADVANCED DIABETIC RETINOPATHY (RIGHT)<sup>64</sup>**

#### 2.1.3 DEFINITION OF MACULAR EDEMA

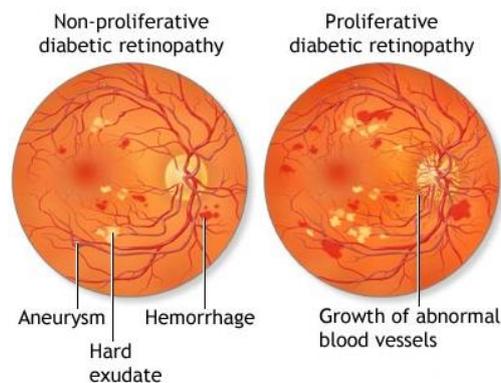
At any time during the progression of DR, diabetic macular edema (DME) can occur as an additional microvascular complication of diabetes mellitus<sup>65</sup>. DME is caused by a fluid build-up in the macula, causing swelling and thickening that results in vision loss<sup>66</sup>.

### 2.1.4 STAGES OF RETINOPATHY

There are two major stages of DR: non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR).

#### 1. *Non-proliferative diabetic retinopathy (NPDR):*

- NPDR is the early stage of DR where microvascular complications are limited to the retina<sup>4</sup>. It starts in the retinal capillary, where there is a loss of pericytes and endothelial cells, two important cell constituents of the blood vessels<sup>65,67</sup>. This causes microaneurysms (weak spots in the blood vessel walls that generate an outward bulging) and capillary microangiopathy (a disease of the blood vessels), resulting in vascular leakage and leading to capillary occlusion that causes retinal ischemia<sup>68</sup>.
- NPDR is clinically characterized by microaneurysms, retinal hemorrhages, capillary microangiopathy and *hard exudates* (lipid and lipoprotein deposits that can cause inflammation)<sup>4,68,69</sup> (**Figure 11**).



**FIGURE 11.** RETINAL PHOTOGRAPHIC REPRESENTATION OF THE CLINICAL SIGNS OF NON-PROLIFERATIVE AND PROLIFERATIVE DIABETIC RETINOPATHY<sup>70</sup>

#### 2. *Proliferative diabetic retinopathy (PDR):*

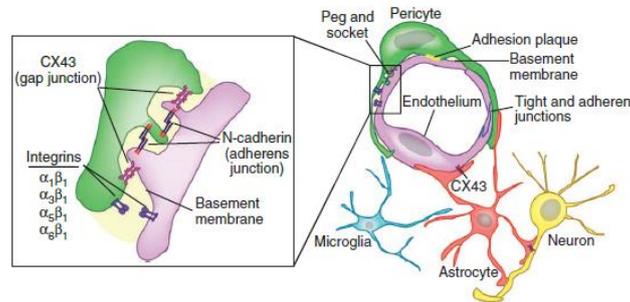
- Due to retinal ischemia, new blood vessels grow as an attempt to restore the blood supply (*neovascularization*)<sup>4</sup>. However, the new vessels are fragile and can leak, causing vitreous hemorrhage and *retinal detachment*<sup>68</sup>. The new blood vessel growth may also cause *neovascular glaucoma*, a blinding disease often observed in cases of DR<sup>71</sup>. At any point during the progression of DR, DME may also occur, due to capillary leakage and microaneurysms, but it is more likely to happen as DR worsens<sup>64,65</sup>.

## 2.2 PATHOPHYSIOLOGY OF DIABETIC RETINOPATHY

### 2.2.1 THE RETINAL CAPILLARY

Within the retinal capillary lies a single layer of *endothelial cells*, secured to a connective tissue sheath called the *basement membrane (BM)*<sup>72</sup> (**Figure 12**). These cells are responsible for assuring the retina receives sufficient oxygen and nutrients, detecting possible pathogens, controlling the passage of other molecules and cells into and out of the bloodstream, and the maintenance of the *blood-retinal barrier (BRB)*<sup>73</sup>. On the opposite side of the BM lies a layer of *pericytes*<sup>74</sup>. These cells are smooth muscle cells that regulate capillary diameter and blood flow, stabilize angioarchitecture, elicit endothelial cell survival functions, clear toxic cellular by-products, and play critical roles in

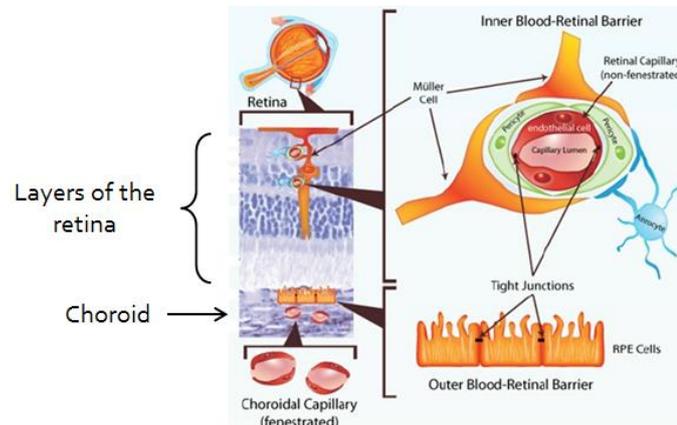
angiogenesis (new blood vessel formation)<sup>67,72,74</sup>. Pericytes are more abundant in the retina than any other part of the vasculature in the human body and their damage is believed to be the key factor in the development of DR<sup>67</sup>. Both types of cells are attached to the BM, however, in some areas the BM is absent, and pericytes and endothelial cells make direct contact through gap junctions<sup>74</sup>.



**FIGURE 12.** STRUCTURE OF THE RETINAL CAPILLARY<sup>74</sup>

### 2.2.2 THE BLOOD-RETINAL BARRIER (BRB)

The BRB controls the ion, protein and water flux into and out of the retina<sup>75</sup>. It is indispensable to maintaining the eye and is necessary for normal vision<sup>75</sup>. The BRB consists of two barriers, an inner and outer barrier<sup>75</sup> (**Figure 13**). The inner BRB is created by the fusion of adjacent endothelial cells through tight junctions, which stop the outward flow of circulating proteins and allow diffusion of molecules between the blood and the retina<sup>72,76</sup>. The outer BRB is made up of tight junctions between retinal pigment epithelial cells (which are nested in the choroid), and is important in controlling the movement of nutrients from the choroid to the sub-retinal space<sup>75,76</sup>.



**FIGURE 13.** THE BLOOD-RETINAL BARRIER (RPE: RETINAL PIGMENT EPITHELIUM)<sup>77</sup>

### 2.2.3 PROGRESSION TO DIABETIC RETINOPATHY

In early DR, hyperglycemia is directly associated with the breakdown of the inner BRB<sup>72,75</sup>. The tight junctions between endothelial cells are lost, allowing the outflow of macromolecules<sup>72</sup>. As the disease progresses, the BM of the capillaries thickens and the capillaries become stiff<sup>72,74</sup>. Pericyte loss occurs at some point, leaving empty spaces on the retinal capillary walls that endothelial cells try to correct through proliferation<sup>72</sup>. At this point, retinopathy is still not clinically detectable<sup>72</sup>. The loss of pericytes results in increased vascular permeability, which leads to vascular occlusion<sup>72</sup>. Microaneurysms develop, followed by small hemorrhages and hard exudates, and finally all cellular components are lost (acellular capillaries) and parts of the capillary become ischemic<sup>72</sup>. The microaneurysms are the first clinically detectable signs of DR<sup>72</sup>.

The retina becomes hypoxic during this stage, (pre-PDR), which eventually leads to increased levels of vascular endothelial growth factor (VEGF) and neovascularization, the defining feature of progression from NPDR to PDR<sup>72</sup>. VEGF is one of the most important players in DR development, as it activates the proliferation of endothelial cells while inhibiting apoptosis, regulating neovascularization<sup>78</sup>. However, the new capillaries are frail and usually hemorrhage, which can lead to retinal detachment and blindness, or they can grow in a way such that they cause neovascular glaucoma<sup>68,72</sup>.

#### **2.2.4 PATHOPHYSIOLOGY OF DIABETIC MACULAR EDEMA**

The thickening of the macular region is believed to be due to the breakdown of the BRB, and DME is thought to follow a similar pathophysiology as DR<sup>65,79</sup>. The increased permeability of the vessels may be the key driving force behind the development of DME, causing the leakage of proteins and lipids into the intraretinal space<sup>79</sup>. This also leads to the hydrostatic pressure, shear stress and oncotic pressure associated with DME<sup>79</sup>.

#### **2.2.5 HYPERGLYCEMIA IN RETINOPATHY**

Hyperglycemia is believed to influence the development of DR through several mechanisms:

*The Polyol Pathway:* The polyol pathway is initiated in hyperglycemia to metabolize glucose and requires an enzyme needed to replenish stores of an important antioxidant, leaving the cell vulnerable to oxidative stress<sup>72,80</sup>. In addition, fructose is a by-product of the reaction that may increase the production of AGEs<sup>80</sup>.

*Non-enzymatic Protein Glycation:* This process starts occurring naturally during embryonic development in all tissues, but occurs more rapidly in diabetes due to heightened glucose availability<sup>72,80,81</sup>. The products of non-enzymatic protein glycation are AGEs, which accumulate and alter protein structure and function in the BM and vessel walls, and also lead to inflammation, oxidative stress, and activation of VEGF<sup>72,80</sup>.

*Protein Kinase C Activation:* Hyperglycemia in endothelial cells increases protein kinase C activation, which can reduce blood flow, increase VEGF expression, vascular occlusion, and activate genes that disrupt communication between retinal cells<sup>72</sup>.

*Hexosamine Pathway:* Increased glucose metabolism results in increased levels of a glucose metabolite, which is pushed into the hexosamine pathway and leads to the increased transcription of genes that lead to vascular or capillary occlusion<sup>72</sup>.

*Reactive Oxygen Species (ROS):* Hyperglycemia also causes a build-up of ROS, which, when greater than antioxidant defenses, leads to oxidative stress by causing damage to DNA, proteins, and lipids<sup>80,82</sup>. The overproduction of ROS activates or further upregulates all of the previous pathways mentioned<sup>83</sup>.

*Hypoxia Inducible Factor:* In situations of hypoxia or inflammation, this protein complex that gets stabilized and binds to genes that cause further increases in VEGF<sup>72</sup>.

*Renin-Angiotensin-Aldosterone System:* Finally, glucose accumulation can cause secretion of renin from the kidney in the renin-angiotensin-aldosterone system, which ultimately leads to vascular inflammation and the production of ROS<sup>84-86</sup>.

## 2.3 DESCRIPTIVE EPIDEMIOLOGY OF DIABETIC RETINOPATHY

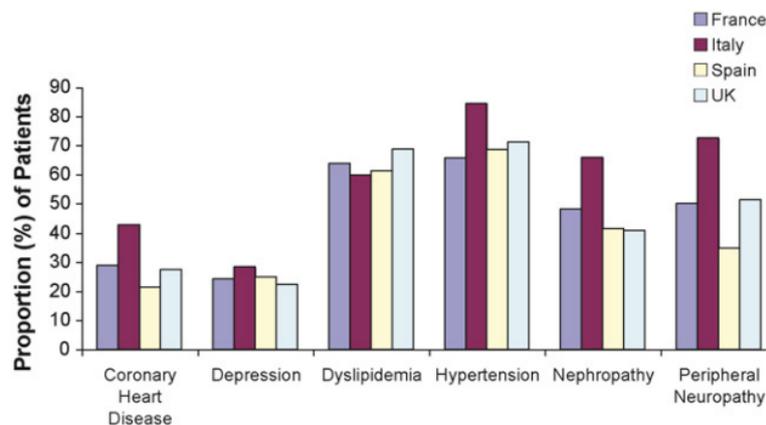
### 2.3.1 THE GLOBAL BURDEN

Retinopathy is diagnosed in more than one third (34.6%) of people with diabetes, with an estimated 10.2% of people with diabetes having vision-threatening DR<sup>4,87</sup> (**Figure 14**). As early DR is usually unnoticed, one third of people already have DR symptoms at the initial diabetes diagnosis, presenting an immediate burden on the newly diagnosed<sup>68</sup>. The prevalence of DR is expected to rise with the prevalence of diabetes and projections estimate that by 2030, more than 191 million people will be affected by DR, with ~56 million of these having a vision-threatening form<sup>88</sup>. About 80% of this rise in DR is expected to impact the poorest populations, in low and middle-income countries<sup>88</sup>.

DR has both a heavy human and economic burden. It has become a leading cause of blindness in the working-age population of the Western world, responsible for as much as 15-17% of the blindness in the US and Europe and 3-7% of the blindness in Southeast Asia and the Western Pacific<sup>5,89</sup>. It is also associated with a poorer quality of life and poorer physical, emotional and social wellbeing, and many patients report difficulty maintaining paid employment<sup>88,90,91</sup>. Estimations of annual direct and indirect costs of DR reach as high as \$3.91 billion in Germany and \$490 million in the US<sup>88</sup>. Furthermore, the incidence of DR has been reported to increase the incidence of nephropathy, cardiovascular events and all-cause mortality<sup>92,93</sup>.

### 2.3.2 RETINOPATHY IN FRANCE

The proportion of diabetes patients with DR in France has reported at 11.4% [8.8-13.9%], of which 20.8% reported PDR<sup>94</sup>. The most frequently reported comorbidity with DR in France is hypertension, followed closely by dyslipidemia<sup>94</sup> (**Figure 15**).



**FIGURE 15.** PROPORTION OF RETINOPATHY PATIENTS WITH SELECTED CO-MORBIDITIES IN THE STUDY POPULATION BY COUNTRY<sup>94</sup>

## Prevalence & Incidence

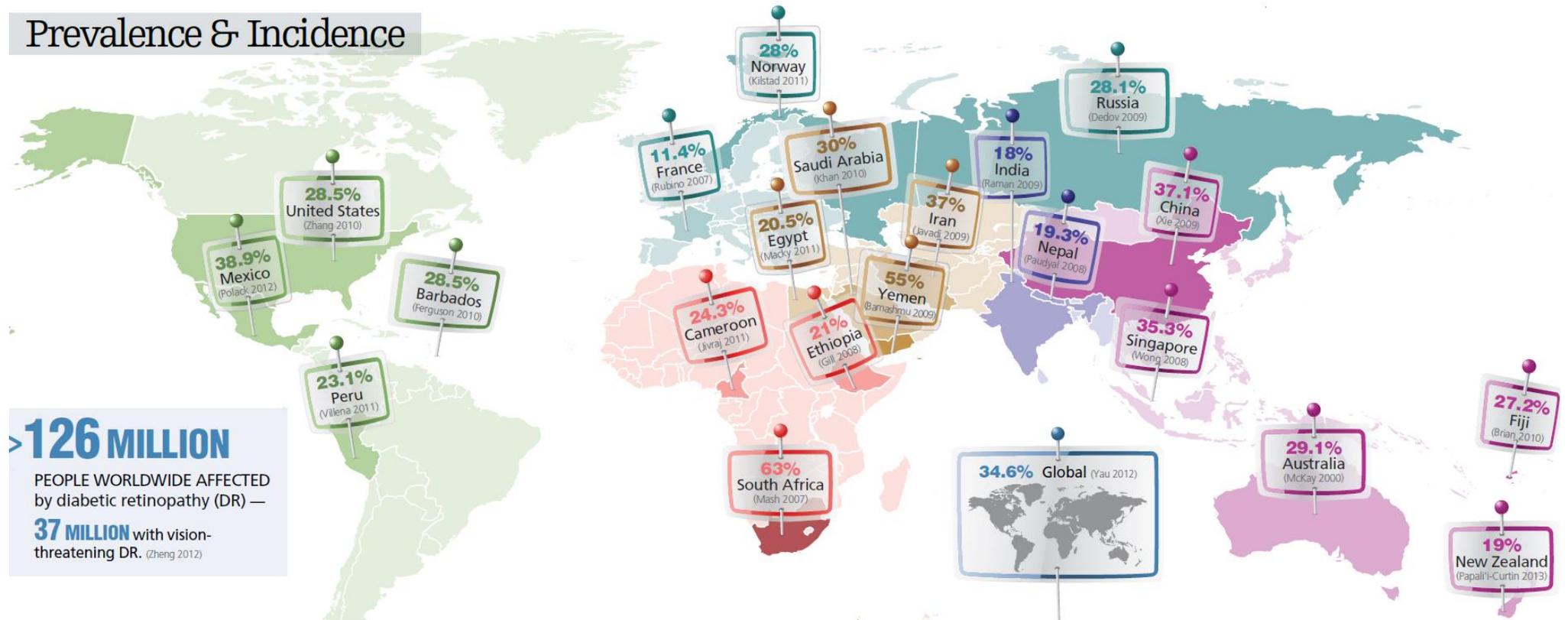


FIGURE 14. PROPORTION OF PEOPLE WITH DIABETES WITH DIABETIC RETINOPATHY OF ANY SEVERITY<sup>88</sup>

## 2.4 RISK FACTORS FOR DIABETIC RETINOPATHY

Overall, badly managed diabetes is more likely to lead to diabetic retinopathy<sup>4</sup>. Yet, there are several individual risk factors that have been consistently associated with DR, including: the duration of diabetes, hyperglycemia, hypertension and the type of diabetes<sup>4,68,95-97</sup>. Other risk factors less consistently observed include: male sex, dyslipidemia, family history and ethnicity<sup>98-100</sup>.

### 2.4.1 ESTABLISHED RISK FACTORS

As previously mentioned, *hyperglycemia* is likely the main driving force behind the mechanisms responsible for DR<sup>72,80,101</sup>. One study observed a 35% decrease in the risk of microvascular complications in people with T2D for every percentage point decrease in glycated hemoglobin (HbA<sub>1c</sub>) and the Diabetes Control and Complications Trial found that people with T1D had a 76% decreased risk of DR with intensive glucose control (median HbA<sub>1c</sub>= 7.2%) compared to conventional control (median HbA<sub>1c</sub>= 9.1%)<sup>95,102</sup>.



Along with hyperglycemia, the *duration of diabetes* is the strongest predictor for the development of DR. The incidence of DR increases with the duration of the disease, such that the 4-year incidence and 10-year incidences have been estimated at 59% and 89.3% in people with T1D, 47.4% and 79.2% in people with insulin-dependent T2D, and 34.4% and 66.9% in people with non-insulin dependent T2D, respectively<sup>95,103</sup>. The duration of diabetes may increase the risk of DR directly through the accumulation of AGEs, as levels of AGEs are associated with the onset of diabetes, or it may act indirectly through the time exposed to oxidative stress and chronic inflammation that comes with hyperglycemia<sup>72</sup>.



Tightly controlled blood pressure (<150/85 mmHg) has been shown to reduce the risk of microvascular complications in T2D by 37% and has led to a 34% decreased risk in the progression of retinopathy compared to those with less tight control (<180/105)<sup>97</sup>.

*Hypertension* may increase the risk of DR through the mechanical stretch and stress on the retinal capillary's endothelial cells, leading to endothelial dysfunction and vascular damage<sup>80</sup>. This may cause an inflammatory response with the activation of the transcription factor NF-κB<sup>80,84,85</sup>. NF-κB, (also activated by protein kinase C, ROS and the renin-angiotensin-aldosterone system), induces the expression of inflammatory markers such as cytokines and chemokines, as well as VEGF<sup>80,84,104</sup>.



Evidence suggests that people with *T1D* suffer a higher relative risk for developing DR than people with T2D, even after accounting for the duration of diabetes<sup>68,96</sup>. For example, in diabetes with a duration of diagnosis <10 years, T1D patients displayed a 38% increased adjusted-relative risk of any DR compared to T2D patients<sup>96</sup>. However, durations of disease greater than 10 years do not demonstrate significant differences in DR prevalence<sup>96</sup>.

### 2.4.2 POSSIBLE RISK FACTORS

Several studies have suggested that *men* may be more likely to develop DR than women<sup>98-100</sup>. In T1D, being male has been associated with 19% increased odds of DR, while in T2D it has been associated with 11-19% increased odds of DR<sup>98-100</sup>.



*Dyslipidemia* may affect the development of DR, but the results are inconclusive. In one study, the severity of DR was positively associated with triglyceride levels, small and medium very low density lipoprotein (VLDL) and negatively associated with high density lipoprotein (HDL) cholesterol<sup>105</sup>. However, of 5 prospective studies where lipids were measured after diagnosis of T1D or T2D, none found an association

between DR and total cholesterol or HDL cholesterol and results are contradictory with regards to triglycerides and low density lipoprotein (LDL)<sup>106</sup>.

*Genetic* components may be related to DR, as the risk of severe DR is higher in first-degree relatives of DR+ than DR- people and one gene has been associated with DR<sup>107,108</sup>. *Ethnicity* may also play a role; in the US, both non-Hispanic blacks and Mexicans with T2D were found at higher risk than non-Hispanic whites with T2D, and in the UK, those of South Asians ancestry had higher prevalence of DR than those of white European ancestry, after controlling for confounders<sup>109-111</sup>.



The risk of DR may also be affected by *smoking*, but the relationship is not clear. One study did not find an association between smoking and DR<sup>112</sup>. However, another study found that the age of onset of diabetes may modify the effect<sup>113</sup>. The true relationship may be difficult to define, as Mulhauser *et al.* found that the association changed depending on the statistical model used<sup>114</sup>. Cigarette smoking results in tissue hypoxia, and has been shown to decrease retinal blood flow and reduce the oxygen-carrying capacity of the blood<sup>115-117</sup>. Retinal hypoxia could then activate the hypoxia inducible factor pathway.

Just as the *diet* is an important factor in the development of diabetes, it may also play an important role in the development of DR and will be addressed in [Section 3](#).



Studies concerning the effects of *physical activity* on DR are scarce and those that exist are limited by small sample sizes. One review was able to identify only 5 studies in 3 populations that examined the effects of physical activity on DR, all of which were conducted on patients with T1D<sup>118</sup>. Three studies showed no association between physical activity and severe DR, DR progression, or the development of PDR, while the others noted benefits of physical activity<sup>118</sup>.

Though many studies have examined the role of *BMI* or *obesity* on the risk of DR, the results still appear inconclusive. A meta-analysis of 27 articles found that BMI was not associated with DR as a categorical variable (overweight or obese vs. normal weight) nor as a continuous variable, but there was significant heterogeneity present in the analyses<sup>119</sup>. There may be ethnic differences in the association between BMI and DR, as a positive association between BMI and DR has been observed in whites and an inverse association in Asians, or BMI may not be the best measure of obesity<sup>120</sup>. One study found inconsistent associations with BMI and the severity of DR, while abdominal obesity (defined by waist to hip ratio) was positively associated with all stages of severity<sup>120</sup>.



Finally, to my knowledge, the relationship between *environmental factors* and DR has not yet been addressed, however, the retinal microvasculature has been shown to respond to air pollution in healthy adults and this may present an important area for future investigation<sup>121,122</sup>.

## 3. THE ROLE OF THE DIET

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Studying the role of the diet in disease can be difficult, as individual diets are complex exposures with many components that vary in amount and combination. Yet, the diet has been shown to have a major impact on the risk of T2D and DR, as several studies have found substantial evidence supporting its role, both for individual components of the diet, but also for the diet as whole. A comprehensive approach, studying the effects of nutrients, food groups and dietary patterns within the same population permits a deeper understanding of the complex role of the diet in the development of T2D and DR.

### 3.1 DIETARY PATTERNS

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As people do not consume individual foods or nutrients in isolation, it is important to take into account the combinations and synergies acting between them. For this reason, epidemiological studies increasingly address the diet as a whole, using dietary patterns that can be developed *a priori* or *a posteriori*. With *a priori* dietary patterns, investigators estimate adherence to recommended intakes in order to create an index of adherence. On the other hand, *a posteriori* dietary patterns are derived using exploratory techniques that characterize the population under study<sup>123</sup>. Both approaches are useful tools to assess the role of the diet in the development of disease.

#### 3.1.1 DIETARY PATTERNS IN TYPE 2 DIABETES

The Mediterranean diet, a diet rich in fruit, vegetables, nuts, legumes, olive oil, fish, seafood and moderate alcohol consumption, has been quite consistently associated with a decreased risk of T2D<sup>123</sup>. Meta-analyses of 8 studies have estimated that the highest quintile of adherence to the Mediterranean diet was associated with a 13% risk reduction of T2D<sup>123</sup>.

Other *a priori* established diets, such as the Dietary Approaches to Stop Hypertension (DASH) diet or the Healthy Eating Index (HEI), which are recommended by the United States Department of Agriculture, or the Alternative Healthy Eating Index (AHEI), derived from food and nutrients predictive of chronic disease, have also been shown beneficial for T2D prevention<sup>123</sup>.

Similar to the Mediterranean diet, the DASH diet is rich in fruit, vegetables, and whole grains. It is also rich in low-fat dairy and limits sugar-sweetened foods, red meat and added fats<sup>124</sup>. Recent meta-analyses have associated it with an 18% decreased risk of incident T2D<sup>123</sup>. Other versions of the DASH diet, more abundant in unsaturated fats or protein have also been tested with regards to cardiovascular disease<sup>124</sup>.

DASH DIET 



The HEI, on the other hand, is composed of 10 components (grains, vegetables, fruit, milk, meat, total fat, saturated fat, cholesterol, sodium, and variety) and scored from 0-100<sup>125</sup>. The AHEI, a modified version of the HEI, is composed of 9 components (fruit, vegetables, nuts and soy protein, white meat to red meat ratio, cereal fibre, trans fat, polyunsaturated-to-saturated fat ratio, duration of multivitamin use and alcohol use) and is scored from 2.5-87.5<sup>125</sup>. More nutrient dense foods lead to higher scores on the HEI or AHEI indexes. Both of the indexes have been associated with reduced risk of T2D, ranging from reductions of 5-21%<sup>123</sup>.

Several cohort studies also support plant-based diets in T2D prevention<sup>126</sup>. Step-wise decreases in the prevalence of T2D have been observed for semi-vegetarians (6.1%), pesco-vegetarians (4.8%), lacto-ovo vegetarians (3.2%), and vegans (2.9%) compared to non-vegetarians (7.6%)<sup>126</sup>. After adjustment for BMI, estimates range from a 49-62% reduction in the odds of T2D<sup>126</sup>. Results from 4.1 million years of follow-up in the Nurses' Health Study, the Nurses' Health Study 2 and the Health Professionals Follow-up Study also suggest that those most adherent to a healthful plant-based dietary index had a 34% decreased risk of developing T2D compared to those who were least adherent, independent of other T2D risk factors<sup>126</sup>.

*A posteriori* derived dietary patterns, such as "prudent" dietary patterns, have also been shown beneficial for T2D prevention while "Western" or "unhealthy" style dietary patterns (characterized by a high consumption of refined grains, red and processed meat, fried foods, eggs, high-fat dairy, and high sugar drinks), have been quite consistently associated with an increased risk of T2D<sup>123</sup>.

### 3.1.2 DIETARY PATTERNS IN RETINOPATHY

The role of the dietary pattern in retinopathy has been much less investigated than in diabetes. Only one study can be identified, and this study investigated the role of the Mediterranean diet, which they found conferred a risk reduction in DR<sup>127</sup>.

### 3.1.3 DIETARY PATTERNS IN FRANCE

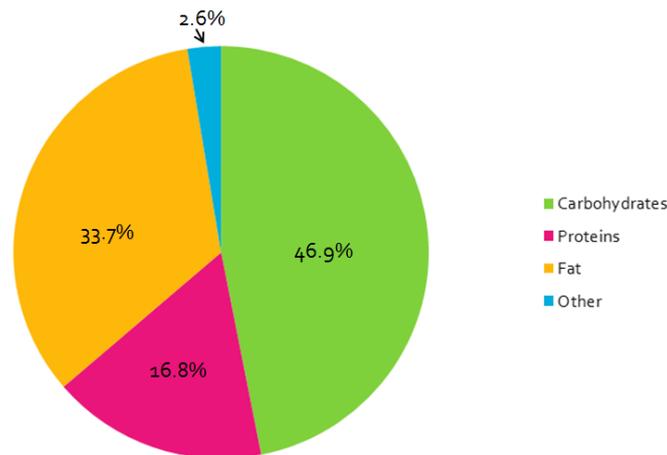
With the goal to increase the nutritional state of the population, the French Ministry of Health established the *Programme national nutrition santé* (National nutritional health program), and with it, a score to evaluate adherence to these dietary guidelines<sup>128</sup>. Adherence to this score with respect to T2D has not been analyzed, however, strong adherence has been negatively associated with cardiovascular risk factors, the prevalence of metabolic syndrome, and adiposity markers<sup>128</sup>.



Several independent *a posteriori* dietary patterns have been identified in the French population based on data from the Second National Study of Food Consumption (INCA2)<sup>129</sup>. The pattern "Small eater" was the most prevalent in the population (23.0%) and was characterized by the consumption of all foods but in lower quantities than the overall population<sup>129</sup>. The second most prevalent pattern was a "Traditional" dietary pattern, and represented 16.5% of the population<sup>129</sup>. This pattern was characterized by a high consumption of wine, cheese, bread products with wheat flour, red and processed meat, coffee, grains and nuts, and cakes and pastries<sup>129</sup>. A "Sweet and processed" pattern was next most prevalent (13.5%), characterized by a high consumption of sweetened products, followed by a "Mediterranean" (13.0%), and a "Health Conscious" pattern (12.6%) that was associated with intake of low-fat or light foods, soups, fruit, tea, and cakes and pastries<sup>129</sup>.

#### *The Average Adult French Diet*

Data from the Third National Study of Food Consumption (INCA3) suggests that macronutrient intakes as a percent of energy are in the range of those recommended<sup>130</sup> (**Figure 16**). According to INCA3, the largest contributors to the average adult French diet are fruit (11.1%), vegetables (11.1%), bread (9.7%), soup (8.5%), and yogurts and *fromage blanc* (6.5%)<sup>131</sup>. Water was the most heavily consumed beverage, followed by hot beverages such as coffee and tea<sup>131</sup>. More than half (~55%) of energy intake came from cereal products, dairy products, meat, fish, eggs and fruit and vegetables, however, pastries, cakes, biscuits, cookies, sandwiches, pizzas, pies, and tarts made up more than 40% of energy intake<sup>131</sup>.



**FIGURE 16.** MAJOR CONTRIBUTIONS TO ENERGY INTAKE (WITHOUT ALCOHOL) IN THE AVERAGE ADULT FRENCH DIET BASED ON DATA FROM THE THIRD NATIONAL STUDY OF FOOD CONSUMPTION<sup>131</sup>

### 3.1.4 DIETARY PATTERNS IN AUSTRALIA

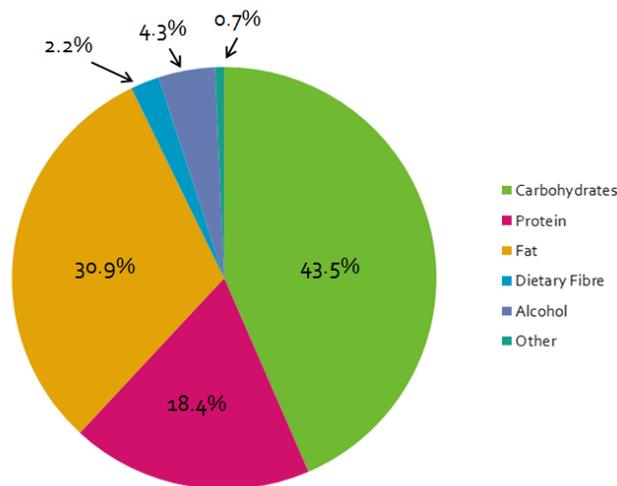
Based on the available scientific evidence, the National Health and Medical Research Council of Australia has developed the Australian Dietary Guidelines to promote the health and wellbeing of the population<sup>132</sup>. Strong adherence to previous versions of these guidelines is inconclusive with regards to T2D prevention, as strong adherence to one *a priori* score did not show an association and the other showed a decreased risk of T2D in a cohort of women followed for 6 years<sup>133</sup>.



*A posteriori* developed dietary patterns in an Australian cohort of women have identified “prudent” and “Western” dietary patterns<sup>134</sup>. The “Western” dietary pattern was characterized by a high consumption of high fat dairy, red and processed meat, white bread, snacks, take-out food, potatoes with fat, poultry, and soft drinks and a low consumption of low and medium fat dairy, high fibre bread, stalk and leafy vegetables and tea and water<sup>134</sup>. This diet was associated with an increased risk of T2D<sup>134</sup>.

#### *The Average Adult Australian Diet*

According to the Australian Health Survey (AHS), most of the energy in the average adult Australian diet comes from carbohydrates<sup>135</sup> (Figure 17). The results of the 2014-2015 Australian Health Survey found that slightly more than half of Australians were meeting the recommended minimum number of servings of fruit per day<sup>25,135</sup>. In contrast, only 7.0% of Australians were meeting recommendations for vegetable intake, with potatoes contributing the largest to the vegetable group<sup>25,135</sup>. White bread made up more than half of the bread consumed and dairy was widely consumed, with milk consumed twice as often as cheese<sup>135</sup>. Almost three-quarters of the population consumed meat regularly, with chicken as the most popular, followed by beef<sup>135</sup>. Over one third of total energy came from “discretionary foods”, foods of little nutritional value and high in saturated fats, sugars, salt and/or alcohol<sup>135</sup>. The highest contribution to discretionary foods was cakes, muffins, scones and similar desserts<sup>135</sup>. Besides water, the most popular beverage was coffee, and soft drinks and flavoured mineral waters were consumed by almost a third of the population<sup>135</sup>.



**FIGURE 17.** MAJOR CONTRIBUTIONS TO ENERGY INTAKE IN THE AVERAGE ADULT AUSTRALIAN DIET BASED ON DATA FROM THE AUSTRALIAN HEALTH SURVEY 2011-2012<sup>135</sup>

## 3.2 FOODS

Examining the dietary pattern as a whole can provide an important global overview, however, it is still important to analyze the effects of the individual constituents of the diet, in order to identify the most beneficial or harmful components and provide evidence to support new guidelines.

### 3.2.1 FOODS IN TYPE 2 DIABETES

Concerning individual food groups, for most groups, the literature is not entirely consistent with regards to T2D. Though meta-analyses suggest an inverse relationship between high fruit consumption and T2D risk, the results are not consistently statistically significant<sup>136-138</sup>. Likewise, total vegetable intake is not associated with T2D in meta-analyses, though several vegetable groups may be beneficial<sup>136-138</sup>. The evidence regarding grain and dairy consumption is also unclear, though studies strongly suggest benefits of whole grains as opposed to refined grains and some evidence suggests protective effects of low-fat dairy<sup>139-141</sup>. Meta-analyses have not observed significant associations between nuts, legumes, fish, eggs and T2D risk, neither between high vs. low intake groups nor with non-linear dose-response relationships<sup>139,142,143</sup>. However, they have detected significant heterogeneity by geographic location with regards to fish and nut intake, such that inverse relationships are observed in Asian areas, but not other areas<sup>139,142,143</sup>. On the other hand, the evidence strongly supports detrimental effects of high intakes of red meat, processed meat and sugar-sweetened beverages on the development of T2D<sup>139</sup>.

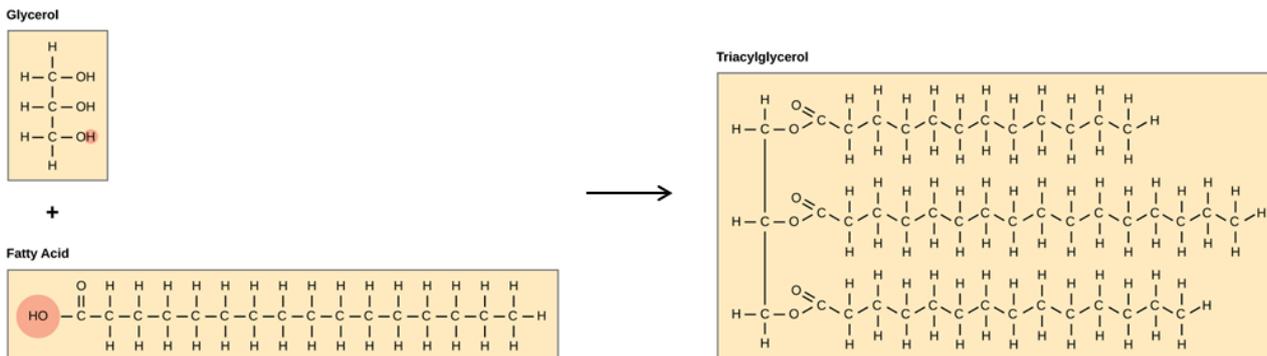
### 3.2.2 FOODS IN RETINOPATHY

The few studies that exist on the association between foods and retinopathy support the role of fruit, vegetables and fish in the prevention of DR<sup>144-147</sup>. The effects of others foods or food groups do not appear to have been investigated yet.

## 3.3 FATTY ACIDS

### 3.3.1 DEFINITION

Fatty acids (FA) are the main component of fat, one of the three main macronutrients of the diet. Fat is essential to our diet, as it is required for normal growth and development as a source of energy, for the absorption of certain vitamins, to provide cushion for the organs, and to maintain the cell membrane<sup>148,149</sup>. A fat molecule is composed of a glycerol backbone and three FA tails, which can vary<sup>150</sup> (**Figure 18**). Many FAs are found in the form of fat molecules (triglycerides or triacylglycerol), but many are also free in the body as FFA<sup>149,150</sup>.

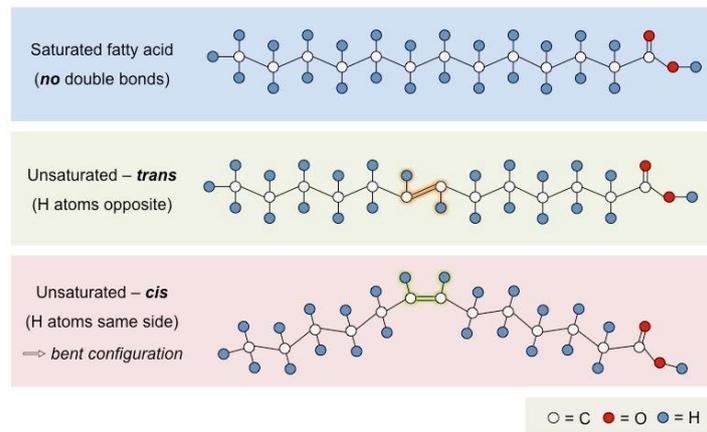


**FIGURE 18.** TRIACYLGLYCEROL/TRIGLYCERIDE IS FORMED FROM THE BOND BETWEEN A GLYCEROL BACKBONE AND THREE FATTY ACIDS<sup>150</sup>

### 3.3.2 TYPES OF FATTY ACIDS

As pictured in **Figure 18**, FAs are comprised of a long hydrocarbon chain attached to a carboxyl group (COOH)<sup>151</sup>. The chains can differ in their length from short chains ( $\leq 6$  carbons), to medium chains (7-12 carbons), to long chains (13-19 carbons), to very long chains ( $\geq 20$  carbons)<sup>152,153</sup>. Within the hydrocarbon chain, there are either single or double bonds between neighbouring carbons<sup>149-151</sup>. FAs with only single bonds are *saturated fatty acids (SFA)*, as they are saturated with hydrogen<sup>149,150</sup> (**Figure 19**). FAs that contain a double bond are said to be unsaturated; those with a single double bond are *monounsaturated fatty acids (MUFA)* and those with multiple double bonds are *polyunsaturated fatty acids (PUFA)*<sup>149-151</sup>. The PUFA group can be further subdivided into omega-3 and omega-6 fatty acids (n-3 PUFA and n-6 PUFA)<sup>151</sup>.

The double bonds that make up unsaturated FA can be either in a *cis* or *trans* configuration; in the *cis* configuration, the hydrogens of the bond are on the same side, creating a kink or bend in the FA<sup>149,150</sup>. When the hydrogens associated with the bond are on opposite sides, this is considered a *trans* configuration and a *trans unsaturated fatty acid (TFA)*<sup>149,150</sup>. TFA are not common in nature but are produced artificially through industrial processing (hydrogenation)<sup>150</sup>.



**FIGURE 19.** TYPES OF FATTY ACID CONFIGURATIONS; TOP (SATURATED), MIDDLE (UNSATURATED TRANS), BOTTOM (UNSATURATED CIS)<sup>154</sup>

### 3.3.3 ESSENTIAL FATTY ACIDS

*Essential fatty acids* are required for health but cannot be metabolized by the body and therefore must be obtained from the diet<sup>149,155</sup>. There are two FAs considered essential:  $\alpha$ -linolenic acid (ALA), an omega-3, and linoleic acid (LA), an omega-6<sup>149,155</sup>.

### 3.3.4 THE ROLE OF FATTY ACIDS IN METABOLISM

FAs have several roles in metabolism: (1) they are important sources of energy and energy storage; (2) they act as precursors to eicosanoids, important signalling molecules for cellular functions such as platelet aggregability and cell growth; (3) they play a large role in cell membrane fluidity, as they are incorporated into membrane phospholipids; (4) they act as substrates and interact with different enzymes and receptors; (5) they may undergo lipid peroxidation, producing products acting as signalling molecules; (6) they may be added to proteins, which is important for the folding of some proteins or anchoring of others in membranes; and finally, (7) they may act as signalling molecules to alter gene transcription<sup>151</sup>.

### 3.3.5 THE ROLE OF FATTY ACIDS IN DIABETES AND RETINOPATHY

The evidence concerning the role of FA in the development of T2D and DR is limited and controversial. Concerning T2D, some studies have found no association between total fat intake and incidence of T2D<sup>156-158</sup>, yet there is evidence to suggest that FA groups, and even individual FAs within these groups, can have different effects on T2D risk<sup>156-160</sup>. Meta-analyses also suggest that the effects of high n<sub>3</sub>-PUFA intake differ by geographical region, with high intake associated with an increased risk of T2D in Western countries, no association in European countries, and a decreased risk in Asian countries<sup>161-163</sup>.

With regards to DR, the available literature is much more sparse, but neither total fat, TFA, total SFA, nor individual SFA intake have been observed to have an association with retinopathy<sup>164,165</sup>. On the other hand, the evidence concerning the role of MUFA and PUFA intake is not in agreement; of the identified three studies, one study did not find any significant associations while the others found high MUFA and/or PUFA intake to appear beneficial in DR prevention<sup>147,164,165</sup>.

## 4. SUMMARY AND OBJECTIVES

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Although type 2 diabetes is largely preventable, it presents a significant public health burden that is only predicted to increase in the near future<sup>1</sup>. It is associated with many other complications that further burden people with diabetes, such as diabetic retinopathy, which is diagnosed in over one third of people with diabetes and currently represents the greatest threat to blindness in the working-age population of developed countries<sup>4,5</sup>. Modifiable risk factors, such as the diet, have been identified for both T2D and DR, yet certain aspects of the role of the diet remain unclear.

One of these aspects is the role of fatty acids. Though fats are responsible for a large part of energy intake and have strong metabolic effects, their role in the development of T2D has not been established, as the current evidence is sparse and not in agreement. Studies suggest the effects of FAs may differ by geographical region, and within and between FA groups, but some FAs are almost always studied in their groups and the effects of individual FAs in T2D has been scarcely addressed.

Therefore, the **first objective** of this thesis was to examine the associations between the primary dietary FA intakes and the incidence of T2D, with particular focus on the relationship between n-3 PUFAs, n-6 PUFAs and T2D in the *French E3N cohort study*.

Currently, very few studies have evaluated the role of the diet or its components on the risk of DR, and those that have present discordant results.

Thus, the **secondary objective** of this thesis was to identify, summarize and interpret the literature on the association between the diet or dietary intake of foods, nutrients, or food groups, and the risk of DR in a systematic review, and the **third objective** of this thesis was to analyze the association between the primary dietary FA intakes and the development of DR in a *sub-study of the French E3N cohort*.

Countries establish dietary guidelines based on the current available scientific evidence to create national recommendations aimed at improving overall health and well-being through proper nutrition. However, the effect of adherence to these dietary guidelines for the prevention of T2D is not yet clear, and its magnitude and impact may vary across countries and by interactions with other risk factors. Estimating the impact of adherence to the dietary guidelines and other modifiable risk factors could provide invaluable information to public health decision makers, yet data are scarce and often concern individual risk factors.

For this reason, the **fourth objective** of this thesis was to quantify the relationship between strong adherence to individual components of the dietary guidelines, the dietary guidelines as a whole, and the risk of T2D, and in addition, to determine the population-level impact by estimating the preventable proportion of T2D with strong adherence to the dietary guidelines separately and concurrently with other modifiable risk factors in the *Australian AusDiab cohort*.

# MATERIALS AND METHODS

This section will detail the materials and methods used to achieve the objectives of this thesis. It will firstly describe the French E3N cohort study, which was the basis for the analyses on fatty acid intakes, T2D and DR, followed by a description of the AusDiab cohort study, in which the effects of following dietary recommendations on the risk of T2D were analyzed. Finally, it will detail the procedure used to complete the systematic review of the literature on the diet and DR.

## 1. THE E3N COHORT

### 1.1 OVERVIEW

#### 1.1.1 E3N

E3N, the *Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Éducation Nationale (MGEN)*, is a prospective French cohort study of 98 995 women insured by the MGEN, a national health insurance plan that primarily covers those in the education system<sup>166</sup>. E3N started in 1990 and recruited volunteers born between 1925 and 1950 throughout mainland France, with the primary goal to investigate lifestyle, nutritional, hormonal, and genetic factors associated with cancer and other non-communicable diseases in women. At the time of its initiation, E3N was the largest epidemiological cohort study in France, and today rests the largest study on women's health in France.



The cohort was initiated by Dr. Françoise Clavel-Chapelon and was approved by the *Commission Nationale Informatique et Liberté (CNIL)*, the French regulatory body that ensures data privacy laws are applied to the collection, storage and use of personal data. E3N is managed by the "Health across Generations" team of the Centre for Research in Epidemiology and Population Health (CESP, INSERM UMR 1018) located at the Gustave Roussy Cancer Institute in Villejuif, France.

#### 1.1.2 EPIC

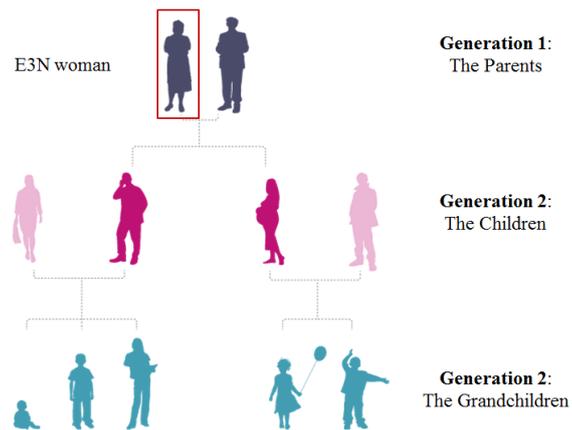
In 1993, E3N became the French constituent of the large European Prospective Investigation into Cancer and Nutrition (EPIC) study, a multi-centre prospective cohort coordinated by the International Agency for Research on Cancer to investigate the relationship between the diet, lifestyle, nutritional and metabolic characteristics on cancer and other chronic diseases<sup>167,168</sup>. Today, EPIC includes 23 centres across 10 European countries and more than 521 000 men and women (Figure 20).



FIGURE 20. LOCATION OF EPIC CENTRES AND PARTICIPATING COUNTRIES<sup>159</sup>

### 1.1.3 E4N

E4N (*Etude Epidémiologique auprès des Enfants des femmes E3N*) is an ongoing extension of E3N that includes the family members of E3N participants and will be comprised of 3 generations<sup>169</sup>. The first generation will consist of the E3N women and the fathers of their children, the second of their children, and the third of their grandchildren (**Figure 21**). This family cohort will allow the study of lifestyle, family environment, and genetics on health and offer the possibility to explore gene-environment interactions. Currently, about 17 500 fathers have been recruited since 2014 and the recruitment of children (generation 2) and grandchildren (generation 3) started in 2018.



**FIGURE 21.** GENERATIONS OF THE E4N COHORT STUDY<sup>169</sup>

## 1.2 DATA COLLECTION

### 1.2.1 RECRUITMENT AND FOLLOW-UP

Of 500 000 women initially invited and mailed a questionnaire to participate in the study, about 100 000 agreed to participate<sup>166</sup>. With respect to age and region of residence, the respondents and the non-respondents presented similar characteristics. Since then, self-administered questionnaires (Q1-Q11) have been sent to participants every 2-3 years (**Figure 22**) and the average participation rate has remained high (83% for the 11 questionnaire cycles) and the loss to follow-up since 1990 has remained low (3%; women who either: never answered a questionnaire, withdrew from the study, or whose contact information was lost).

### 1.2.2 OVERVIEW OF DATA COLLECTED

The questionnaires collected anthropometric, lifestyle, and health status data and allowed for the update of longitudinal data by repeating identical questions to update information on health status and factors such as menopause, smoking, or weight throughout follow-up.

A biological bank was also created from blood samples collected between 1994 and 1999 from approximately 25 000 participants (participation rate ~40%). Between 2009 and 2011, about 44 775 saliva samples were further collected from women who hadn't given blood samples (participation rate ~69%).

In addition, data are available from the MGEN, which updates the vital status and address of participants every 3 months, and since January 1<sup>st</sup>, 2004, has also provided data on drug reimbursements.



## E3N Follow-up

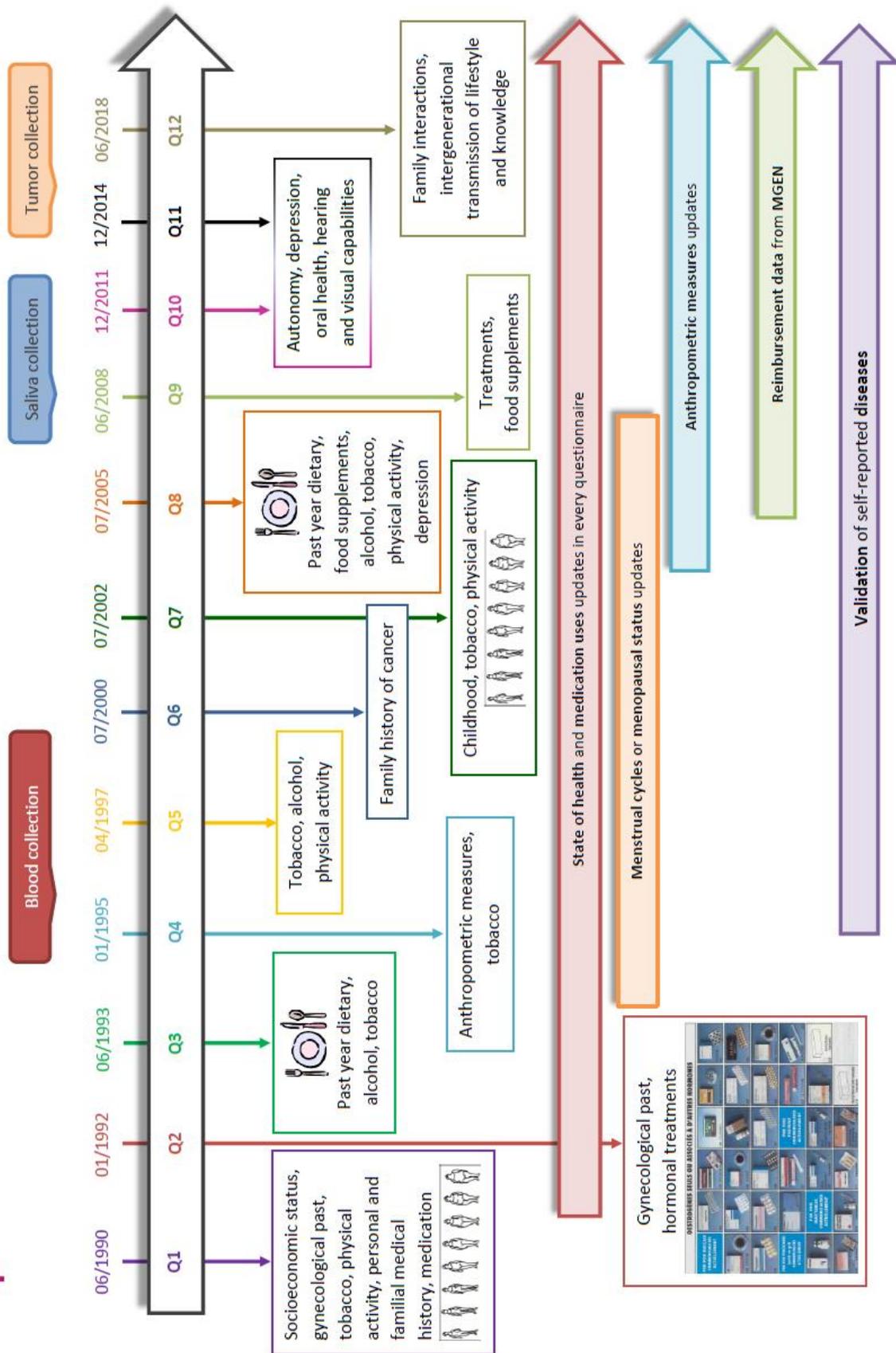


FIGURE 22. CALENDAR OF SELF-ADMINISTERED QUESTIONNAIRES IN THE E3N COHORT STUDY

### 1.2.3 THE DIETARY QUESTIONNAIRE

Dietary data was collected twice in E3N, once in 1993 and again in 2005, using a validated 208-item food frequency questionnaire<sup>170</sup>. The first food frequency questionnaire (FFQ) was sent to 93 055 women and had a response rate of 80% and the second was sent to 93 121 women and had a response rate of 77%. The FFQ was designed to assess the habitual diet of the previous year. It took food and drink consumption throughout the day into account, keeping in mind French meal patterns, and included questions regarding each meal occasion, as well as snacks and appetizers.

The questionnaire was divided into two parts: a quantitative and a qualitative section. The quantitative section described the habitual frequency and portion sizes consumed, based on 66 food types and items grouped by 8 meal occasions (breakfast, morning snack, appetizer before lunch, lunch, afternoon snack, appetizer before dinner, dinner, after-dinner snack) (Figure 23). To quantify the frequency of consumption, 11 frequency categories were used: never or less than once per month, 1, 2, or 3 times per month, or 1-7 times per week and the quantity consumed was estimated using standard units (such as number of eggs, tablespoons), or portion sizes that were indicated with the aid of a photo booklet.

Food Items	Combien de fois ? Cochez la case correspondante														Inscrivez ici vos initiales : (pour vérification)		
	Jamais							OU							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	Type de verre :	Nombre :					
<b>Réveil, Petit Déjeuner</b>																	
Eau : Robinet <input type="checkbox"/> Bouteille <input type="checkbox"/>																Type de verre : photo page 33	Nombre :
Jus de fruits (Fruits pressés ou jus de fruits du commerce)																Type de verre : photo page 33	Nombre :
Chicorie : Au lait <input type="checkbox"/> Nature <input type="checkbox"/>																Type de bol ou de tasse : photo page 2	Nombre :
Café : Au lait <input type="checkbox"/> Normal <input type="checkbox"/> Moulu <input type="checkbox"/>																Type de bol ou de tasse : photo page 2	Nombre :
Nature <input type="checkbox"/> Décaféiné <input type="checkbox"/> Soluble <input type="checkbox"/>																	

FIGURE 23. EXTRACT FROM THE QUANTITATIVE SECTION OF THE FFQ USED IN E3N

The second section allowed a more detailed description of each food consumed from each of the 6 food groups (cooked vegetables, salads, fruit, meat, fish, and dairy) and the frequency of its consumption (never, 1-3 times/month or 1-7 times/week). Detailed data were also obtained on the method of cooking, fats and sugar used. This section allowed the data from the first section to be weighed by that from the second and to calculate the average daily consumption of 208 types of foods and drinks.

### 1.2.4 FATTY ACID INTAKE

FA consumption was estimated using the database of food nutritional composition published by ANSES (the French Agency for Food, Environmental, and Occupational Health & Safety) and the results from the FFQ. For each woman, intakes of the following principal FA groups were obtained: total saturated fatty acids (SFAs), total monounsaturated fatty acids (MUFAs), total polyunsaturated fatty acids (PUFAs), (including total omega-3 polyunsaturated fatty acids (n-3 PUFAs) and total omega-6 polyunsaturated fatty acids (n-6 PUFAs)), and total trans unsaturated fatty acids (TFAs), in g/day. In addition, the consumptions of individual n-6 PUFAs: linoleic acid (LA; 18:2n6) and arachidonic acid (AA; 20:4n6), and individual n-3 PUFAs:  $\alpha$ -linolenic acid (ALA; 18:3n3), eicosapentaenoic acid (EPA; 20:5n3), docosapentaenoic acid (DPA; 22:5n3), and docosahexaenoic acid (DHA; 22:6n3) were also estimated. However, only the FA intakes at Q3 were available for analyses.



### 1.2.5 ASSESSMENT OF TYPE 2 DIABETES

Cases of T2D were first identified either in follow-up questionnaires (with declaration of at least one of the following: (1) T2D, (2) a self-reported, physician-recommended diabetic diet, (3) use of diabetic medication, or (4) hospitalization due to diabetes) or they were identified as receiving diabetic drug reimbursements from health insurance records at least once between January 2004 and March 2012<sup>171</sup>. Those who were identified in both manners were considered validated. In 2012, all women (n=7649) were mailed a diabetes-specific questionnaire which included questions on the circumstances of the diagnosis (year, symptoms, biological exams, etc.), management (diabetic diet, physical activity, medications), and results of their most recent concentrations of fasting glucose and HbA<sub>1c</sub>. Cases were validated if one of the following was met: (1) fasting plasma glucose  $\geq 7.0$  mmol/L, (2) random glucose  $\geq 11.1$  mmol/L at diagnosis, (3) report of diabetic medication use, or (4) last values of fasting glucose or HbA<sub>1c</sub> concentrations  $\geq 7.0$  mmol/L or  $\geq 7\%$ , respectively. Consequently, *5238 cases of T2D* could be validated in E3N.



In 2014, women with validated cases of T2D were invited to answer the *AfterDiab* questionnaire, which inquired about their diabetes (diagnosis, results from their last exams), treatment, diabetes-related complications (heart, digestive system, kidneys, eyes, feet, and teeth) and their quality of life (sleep, state of health, living with diabetes). A total of *3473 women* responded to this questionnaire and were then included in the E3N-AfterDiab study, which is also part of the EPIC sub-study devoted to diabetes: Interaction of genetic and lifestyle factors on the incidence of type 2 diabetes (InterAct).

### 1.2.6 ASSESSMENT OF RETINOPATHY

As diabetic macular edema (DME) is considered a further complication of DR<sup>1</sup>, potential cases of DR were first identified in the AfterDiab questionnaire with the declaration of "yes" to at least one of the following: (1) retinopathy, (2) DME, (3) the loss of vision in at least one eye, or (4) laser treatment. Validation questionnaires were then mailed to the potential cases (n=889), inquiring about a diagnosis of DR, DME, or laser treatment and requesting a copy of the most recent ophthalmological assessment. 659 validation questionnaires were returned, for a response rate of 74.1%. Cases of DR were considered validated if one of the following was met: "yes" response to retinopathy in both questionnaires and/or confirmation through ophthalmological assessment records and/or "yes" to retinopathy in the validation questionnaire and/or declaration of DR in AfterDiab and declaration of either laser treatment or DR eye injections in the validation questionnaire. Otherwise, respondents, non-respondents and the rest of the AfterDiab population were classified as non-cases. Cases of DME were validated in the same manner. In total, *77 cases* were identified, containing 60 cases of DR and 35 cases of DME.



## 1.3 STUDY POPULATIONS

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### 1.3.1 FATTY ACID INTAKE AND TYPE 2 DIABETES

From the 98 995 women in the cohort, women were excluded from analyses who did not complete the dietary questionnaire (n=24 473), had prevalent diabetes (n=859), did not complete any questionnaires after the dietary questionnaire (n=890), or who had extreme values for the ratio between energy intake and required energy (ie. the 1<sup>st</sup> and 99<sup>th</sup> percentiles of the distribution in the population) (n=1439). Thus, the analyses corresponding to the *first objective* of this thesis included *71 334 women*, of whom *2610 had a validated T2D* diagnosis during follow-up (June 1993-December 2011).



N= 71 334  
T2D= 2610

### 1.3.2 FATTY ACID INTAKE AND RETINOPATHY

From the 3473 women in the AfterDiab sub-study, women were excluded who did not respond to the dietary questionnaire sent in 1993 (n=816), who did not have a complete date of diagnosis of T2D (n=43), who had their T2D diagnosed prior to the dietary questionnaire (n=595), and who had extreme values for the ratio between energy intake and required energy (n=55), leaving 1964 women in the initial study population. After the exclusion of women who declared they had type 1 diabetes (n=6) in the DR validation questionnaire and women who responded both “yes” and “no” to DR or DME in the validation questionnaire (n=9), the final study population corresponding to the *third objective* of this thesis consisted of **1949 women** with **77 cases** of DR or DME (which, for the purposes of this thesis will be defined as diabetic eye disease (DED) and were analyzed together in order to increase statistical power).



**N= 1949**  
**DED= 77**

## 1.4 DATA MANAGEMENT

### 1.4.1 TREATMENT OF MISSING DATA

For time-independent variables with <5% of values missing during follow-up, missing values were imputed with the median of the study population (quantitative variables) or the mode (qualitative variables). In the case of ≥5% of missing values, a “missing” category was created. For time-dependent variables presenting with a missing value during follow-up, the value was imputed by the preceding available value. If the missing value was the baseline value, it was imputed with the first available value.

### 1.4.2 FACTORS OF ADJUSTMENT

**Table 1** displays the factors of adjustment included in the analyses. The values of the variables used were those obtained from the third questionnaire (Q3) in 1993. However, for the analyses on FA intake and DED, the value or response reported in the questionnaire closest to the date of diagnosis of T2D was used for BMI, exercise, and smoking status.

**TABLE 1.** FACTORS OF ADJUSTMENT FOR ANALYSES WITH FATTY ACIDS

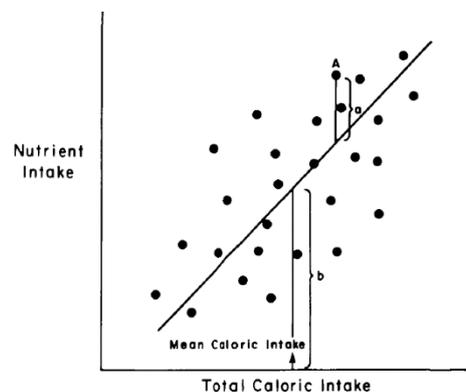
Confounding Factor	FAs and T2D	FAs and DED
Age at diagnosis of T2D	N/A	years
Alcohol consumption	grams of ethanol per 100 g/day	N/A
Body mass index	<20; 20-25; 25-30; or >30 kg/m <sup>2</sup>	<25; 25-30; ≥30 kg/m <sup>2</sup>
Daily energy intake	kcal/day	kcal/day
Duration of diabetes	N/A	years
Family history of diabetes	no; yes	no; yes; unknown
Glycated hemoglobin	N/A	normal; elevated, ≥7%; unknown
Hypertension	no; yes	normal; hypertensive
Hypercholesterolemia treatment	no; yes	no; yes; unknown
Level of education	undergraduate; postgraduate	less than high school diploma; high school + 2 years of university; ≥2 years of university
Physical activity	metabolic equivalents (MET)-hours/week	<12; 12-24; 24-44 or ≥44 MET-hours/week
Other FAs	g/day	g/day
Smoking status	non-smoker; current/former	non-smoker; ex-smoker; current
T2D treatment	N/A	no treatment or diet only; only oral hypoglycemia agents; insulin/GLP-1 and oral hypoglycemic agents

### 1.4.3 TREATMENT OF FATTY ACID DATA

Most nutrient intakes are positively correlated with total energy intake, even nutrients without caloric value such as vitamins and minerals. This is due to the fact that larger, more active and less metabolically efficient people tend to eat more in general. It is therefore important in nutritional epidemiology to analyze nutrient intakes in relation to total caloric intakes, unless the nutrient affects an organ system uncorrelated with body size or if exercise doesn't affect its metabolism. In this situation, it may be most relevant to analyze the absolute nutrient intakes<sup>172</sup>.

Otherwise, nutrient intakes should be adjusted for total energy to determine whether the observed effect is due to the nutrient itself rather than energy intake. To achieve this, Willett and Stampfer proposed four analytic approaches: the nutrient density method, the standard multivariate method, the energy decomposition method and the energy-adjusted (residual) method. In this thesis, the energy-adjusted method was employed<sup>173</sup>.

With this method, the energy-adjusted nutrient intakes (in this case, FA intake) are calculated from the residuals of the regression model in which total caloric intake is the independent variable and the absolute nutrient intake is the dependent variable (Figure 24). A constant is often added to the residuals, as they include negative values and have a mean of 0<sup>172</sup>.



**FIGURE 24.** ENERGY-ADJUSTED NUTRIENT INTAKE = A + B, WHERE A = RESIDUAL FOR SUBJECT A FROM REGRESSION MODEL AND B = THE EXPECTED NUTRIENT INTAKE FOR A PERSON WITH MEAN CALORIC INTAKE<sup>173</sup>

In this case, FA intakes were log transformed before computing the residuals and the constant added was the predicted nutrient intake for the mean energy intake of the study population. Energy-adjusted FA intakes were then grouped into tertile groups of intake.

### 1.5 DESCRIPTIVE ANALYSIS

The individual baseline characteristics of the population were described for both overall study populations, and by outcome (case of T2D vs. non-case; case of DED vs. non-case). The descriptive analyses were carried out using the averages and standard deviations for continuous variables and frequency distributions (numbers and percentages) for categorical variables.

## 1.6 COX MULTIVARIABLE REGRESSION

### 1.6.1 INTRODUCTION

A Cox proportional hazards model was used to investigate the relationship between FA consumption and the risk of T2D (Objective 1). Cox models are one of the models most frequently used for survival analysis or risk analysis in the biomedical field, due to the fact that they are a particularly robust model. The Cox model is a semi-parametric model that allows the estimation of the risk of an event occurring (T2D) due to an exposure (intake of FAs) while controlling for potential confounding factors. Cox models are often preferred to logistic regression models (discussed in [Section 1.7](#)) when survival time information is available and there is censoring, as it utilizes more information<sup>174</sup>.

### 1.6.2 FORMULA

This semi-parametric model calculates the instantaneous rate (in this case, for diabetes) at time  $t$  for an individual (in this case, a woman) with a given set of explanatory variables ( $\mathbf{X}$ ):

$$h(t, \mathbf{X}) = h_0(t) \cdot \exp\left(\sum_{i=1}^p \beta_i X_i\right)$$

The hazard at time  $t$  is the product of (1)  $h_0(t)$ ; the baseline hazard function and (2)  $\exp(\sum_{i=1}^p \beta_i X_i)$ ; the exponential  $e$  to the linear sum of  $\beta_i X_i$ , where the sum is over the  $p$  explanatory  $X$  variables<sup>174</sup>. If all  $X$ 's are 0, the formula reduces to only the baseline hazard function, which is an unspecified function and gives the property that makes the Cox model a semi-parametric model and a reason for its popularity<sup>174,175</sup>.

### 1.6.3 THE HAZARD RATIO

The hazard ratio (HR) is “the hazard for one individual divided by the hazard for a different individual”, where the two individuals differ by their values for the set of predictors (the  $X$ 's)<sup>174</sup>:

$$HR = \frac{\hat{h}(t, \mathbf{X}^*)}{\hat{h}(t, \mathbf{X})}$$

Where  $\mathbf{X}^*$  represents the set of predictors for one person and  $\mathbf{X}$  the predictors for a second person and  $\beta$  represents the model coefficient. After the Cox model formula is substituted for  $\hat{h}$  in the above formula, the formula simplifies to:

$$HR = \exp\left[\sum_{i=1}^p \hat{\beta}_i (X_i^* - X_i)\right]$$

### 1.6.4 PROPORTIONAL HAZARDS ASSUMPTION

This assumption requires that the HR is constant over time between categories of a predictor. Consequently, proportionality must always be verified and if needed, corrected. All the variables in the analyses between FA intake and T2D were found to satisfy the proportional hazards assumption.

### 1.6.5 TIMESCALE

The Cox model's timescale with its original development was the “time on study” timescale, which begins at the start of the observation period or the beginning of exposure to a risk factor and ends

with the end of the observation period or exposure to a risk factor. However, age is a strong determinant of the risk of disease, especially for chronic diseases, so it is often more appropriate to use age as the timescale in cohort studies rather than the time on study<sup>176</sup>. This is due to the fact that in cohort studies, the start of exposure to a risk factor and the start of the observation period are rarely the same as most individuals are at risk of developing the disease since birth but are observed only at inclusion in the study<sup>176</sup>. Using age as the timescale allows the start of exposure to be the birthdate and the start of observation the age of inclusion in the study and is recommended for survival analysis in cohort studies<sup>176</sup>.

With simulations on E3N, Thiébaud *et al.* found that using the time-on-study as the timescale rather than age as the timescale introduced bias in the results<sup>176</sup>. For this reason, age was used as the timescale for the analyses on FA intake and the risk of T2D and the time at entry was the age at the start of follow-up and the exit time was the age when participants were either: diagnosed with diabetes, died, lost to follow-up or censored at the end of the follow-up period, whichever occurred first.

### 1.6.6 STATISTICAL MODELLING

Cox models were used to estimate the HRs and 95% CIs of the relationship between FA intake and T2D risk. The univariate model included only the FA in question and was followed by *Model 1*, which was adjusted for daily energy intake, alcohol consumption, and non-dietary variables: level of education, smoking status, hypertension, hypercholesterolaemia treatment, family history of diabetes, and physical activity (see [Table 1](#) for variable modalities). *Model 2* additionally adjusted for all types of FAs besides the one in question, allowing for the examination of the independent effects of specific FA subtypes. Finally, *Model 3* was adjusted for BMI.

In addition, a stratification by BMI (<25kg/m<sup>2</sup> and ≥25kg/m<sup>2</sup>) was performed to determine whether the effects of FAs varied between overweight and non-overweight women, as the biological mechanisms leading to T2D development may be different between these two groups. Models were adjusted in the same fashion as the analyses in the whole population, first for non-dietary variables, alcohol consumption, and energy intake, and then subsequently for other FA groups.

## 1.7 LOGISTIC REGRESSION

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### 1.7.1 INTRODUCTION

Due to the fact that survival time data was not available in the analysis of FA intake and DED, logistic regression models were used (Objective 3). Logistic regression is a popular model used to model the logit-transformed probability as a relationship between a binary outcome (here, a case of DED or a non-case) and predictor variable(s)<sup>177,178</sup>.

### 1.7.2 FORMULA

When modelling with logistic regression, the relationship between the outcome  $y$  (here, DED) and variable  $x$  (here, FA intake) follows a logistic function:

$$P(y|x) = \frac{e^{\alpha+\beta x}}{1 + e^{\alpha+\beta x}}$$

Where  $P(y|x)$  is the probability of DED for a given value of FA intake and is limited to a value between 0 and 1,  $\alpha$  represents the intercept, and  $\beta$  the regression coefficient. However, for

convenience and to facilitate interpretation, this equation is translated into the logarithmic of the odds using a logit transformation:

$$\text{logit}(P) = \log\left(\frac{P}{(1-P)}\right) = \alpha + \beta x$$

where  $P$  is short for  $P(y|x)$ . Rather than modelling a probability that has a restricted range, which can be challenging, this logit transformation allows the modelling of the log odds, which range from 0 to positive infinity. The logit transformation is also one of the easiest transformations to understand and interpret<sup>177,178</sup>.

### 1.7.3 ODDS RATIO

The odds ratio (OR) is the measure of association generated by a logistic regression model. It represents the odds an outcome (DED) will occur given a particular exposure (high FA intake) compared to the odds it will occur without the exposure (low FA intake)<sup>179</sup>. The odds ratio comparing two subjects, with an explanatory variable  $x$  with 2 levels can be represented with the following equation<sup>180</sup>:

$$OR = \frac{P_2/(1-P_2)}{P_1/(1-P_1)} = e^{\beta(x_2-x_1)}$$

### 1.7.4 STATISTICAL MODELLING

Logistic regression models were used to estimate the ORs and 95% CIs of DED for each FA tertile group and each individual n-3 and n-6 PUFA. To test for linear trends, logistic regression models using the median of each tertile group of intake were run. The univariate models contained only the FA in question, and the adjusted model was adjusted for the age at diagnosis of T2D, the duration of T2D, daily energy intake, BMI group, exercise group, smoking status, T2D treatment, hypercholesterolemia treatment, hypertension, family history of T2D, level of education and HbA1c (see [Table 1](#) for variable modalities).

## 1.8 RESTRICTED CUBIC SPLINE REGRESSION

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### 1.8.1 INTRODUCTION

For analyses on FA intake and DED, FA intake was also analyzed using restricted cubic spline regression (Objective 3). Restricted cubic spline regression is a useful tool to use when one or more explanatory variables are continuous as it provides an easy way to create, test and model non-linear relationships in regression models<sup>181</sup>.

When modelling continuous predictors, there are many options available: (1) assume a linear relationship; (2) dichotomization or categorization; (3) use the predictor as a continuous variable with transformations; (4) fractional polynomials; or (5) restricted cubic splines. The assumption of linearity with method 1 may misrepresent the relationship, while method 2, though useful from a decision-making point of view, doesn't allow a smooth relationship between the predictor and outcome as it forces it into each category. In addition, for small datasets, categorization can cause problems as the number of categories increase. Transformations are not very flexible; however, fractional polynomials and cubic spline regression provide flexible functions<sup>181</sup>.

### 1.8.2 DEFINITION AND STATISTICAL MODELLING

Splines are created by dividing the range of the values of the predictor (in this case, FA intake in g/day) by a certain number of knots (in this case, three) at chosen positions (here, the 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> percentiles). Between each knot, separate polynomials are fitted. These polynomials are usually of degree 3 (creating *cubic splines*, which is here the case), as degree 3 provides enough flexibility to fit the data without sacrificing as many degrees of freedom as higher order splines. The splines are said to be *restricted* because they are constrained to be linear at the two tail ends, providing a better fit to the data and decreasing the degrees of freedom (Figure 25)<sup>181</sup>.

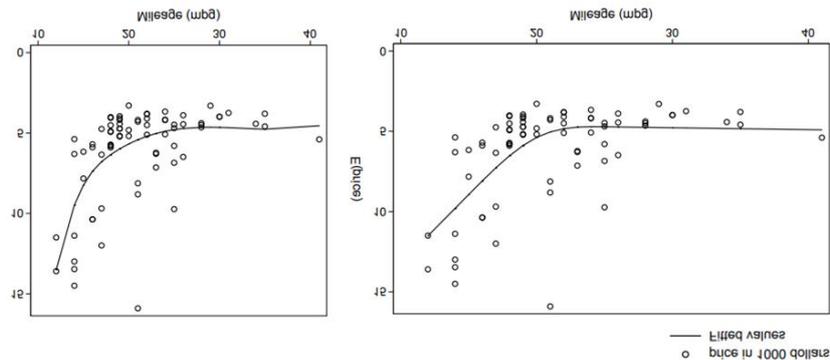


FIGURE 25. (LEFT) CUBIC SPINE; (RIGHT) RESTRICTED CUBIC SPLINE<sup>182</sup>

## 1.9 SENSITIVITY ANALYSES AND SOFTWARE

### 1.9.1 SENSITIVITY ANALYSES

For the first objective of this thesis (FA intake and T2D), in order to test for potential reverse-causation, analyses were performed on a subpopulation excluding women who developed diabetes in the first 5 years after inclusion in the study. Individual n-3 and n-6 PUFA models were also adjusted for meat consumption, to account for residual confounding related to a major source of the FAs, as it has already been reported in E3N that a high intake of processed red meat was associated to an increased T2D risk<sup>183</sup>. Scores adhering to two dietary patterns, “Mediterranean/Prudent” (characterized by a high diet quality, with a high consumption of fruit, vegetables, seafood, olive oil and sunflower oil), and “Western”, (characterized by a lesser dietary quality with a high consumption of meat, French fries, rice, pasta, potatoes, alcohol, butter, eggs, etc. were also additionally included in the sensitivity models. These dietary quality scores were derived using principal component analysis and have been previously described in published work from E3N<sup>184</sup>.

Concerning the analyses on FA intake and DED (Objective 3), sensitivity analyses were run excluding women who were determined potential cases of DED and sent a validation questionnaire but who did not respond (n=98). Models were also run to determine whether there was an association between the primary food sources of each FA group or FA and DED. In addition, sensitivity analyses were conducted for all FA models, adjusting either for their main food source or simultaneously adjusting for the consumption of other FAs, which is important, as groups and individual FAs are never ingested in isolation and therefore influence the metabolism of each other<sup>185</sup>.

### 1.9.2 SOFTWARE USED

All the statistical analyses corresponding to the first and third objectives were performed on SAS 9.2 software (SAS Institute Inc.). All statistical tests were 2-sided and considered significant at  $p < 0.05$ .

## 2. THE AUSDIAB COHORT

### 2.1 OVERVIEW

The Australian Diabetes, Obesity and Lifestyle Study (AusDiab) is a longitudinal, population-based national survey of 11 247 Australian adults aged 25 or older<sup>186</sup>. It was initiated in 1999 and had two follow-up periods in the following 12 years. Volunteers were recruited from the six states and Northern Territory of Australia with the primary goal being to determine the prevalence of diabetes, obesity and other cardiovascular disease risk factors in Australia<sup>187</sup>. AusDiab is the biggest longitudinal population-based study on diabetes, pre-diabetes, heart disease and kidney disease in Australia.



The study was launched by the National Diabetes Strategy and founded by Professors Paul Zimmet and Timothy Welborn. It was approved by the Baker IDI Heart and Diabetes Institute Ethics Committee and written informed consent was acquired from all participants. AusDiab is coordinated by the Baker IDI Heart and Diabetes Institute<sup>188</sup>.

### 2.2 DATA COLLECTION

#### 2.2.1 RECRUITMENT AND FOLLOW-UP

AusDiab's baseline survey was conducted between May 1999 and December 2000 using a stratified cluster sample. The eligible participants were  $\geq 25$  years of age and residing at their address for  $\geq 6$  months. The baseline survey consisted of an initial household interview and was followed by biomedical exams. Of the 20 347 eligible people, 55% completed both the household interview and the baseline biomedical exam, resulting in an initial study population of 11 247, mostly (>85%) of Australian, New Zealand or British background.

In 2004-2005, eligible participants were invited for the first round of follow-up. Ineligible participants were those who were deceased, had moved overseas or into a high care nursing facility, and those with a terminal illness (Figure 26). Of 10 788 eligible participants, 6537 completed the biomedical tests. A second follow-up survey was conducted in 2011-2012, where 10 337 participants were eligible to participate. Of those eligible, 4764 completed the biomedical exams.

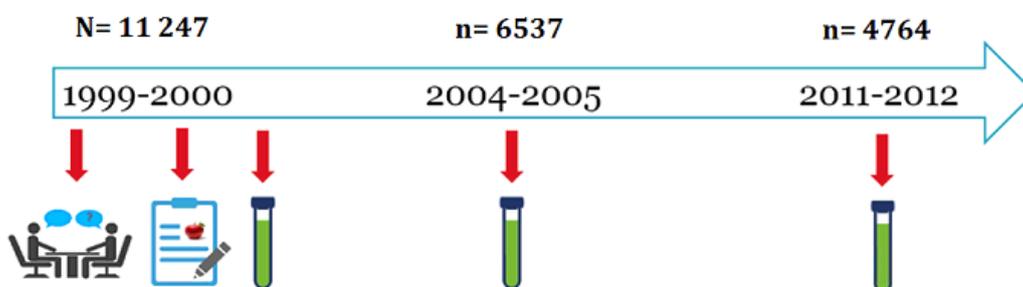


FIGURE 26. CALENDAR OF DATA COLLECTION AND RESPONDANTS IN THE AUSDIAB COHORT STUDY

### 2.2.2 OVERVIEW OF DATA COLLECTED

The questionnaires were administered by trained interviewers and collected information on health and social factors, including education, total household income, smoking status, physical activity, and family history of diabetes. Leisure-time physical activity was measured using the Active Australia questionnaire, which has been previously validated<sup>189,190</sup>. The physical exam was conducted at a test site where height, weight; waist circumference, blood pressure and blood were collected from participants<sup>187</sup>.

### 2.2.3 THE DIETARY QUESTIONNAIRE

Dietary intake was assessed using a self-administered, validated, FFQ developed by the Cancer Council of Victoria<sup>191,192</sup>. This semi-quantitative FFQ included 80 items and assessed the habitual intake of foods and beverages over the previous 12 months. In some cases, photos of serving sizes were given so participants could indicate if they had more or less of a given food per day or per week (ie. casseroles and potatoes) (Figure 27). 10 frequency categories were included in the FFQ, ranging from “never” to “three or more times per day”. The average daily intake of each item in grams was subsequently computed.



Times You Have Eaten CONTINUED		N E V E R	less than once	1 to 3 times	1 time	2 times	3 to 4 times	5 to 6 times	1 time	2 times	3 or more times
			per month			per week			per day		
VEGETABLES (INCLUDING FRESH, FROZEN AND TINNED)											
Potatoes roasted or fried (include hot chips)		<input type="checkbox"/>									
Potatoes cooked without fat		<input type="checkbox"/>									
Tomato sauce, tomato paste or fresh or tinned		<input type="checkbox"/>									
Pepper		<input type="checkbox"/>									
11. When you ate potato, did you usually eat: <input type="radio"/> I never ate potato											
		<input type="checkbox"/>									
		Less than A	A	Between A & B	B	Between B & C	C	More than C			

FIGURE 27. EXTRACT FROM THE FOOD FREQUENCY QUESTIONNAIRE USED IN THE AUSDIAB COHORT STUDY

### 2.2.4 ASSESSMENT OF TYPE 2 DIABETES

All participants but those who were currently taking treatment for diabetes or who were pregnant underwent a standard 75g oral glucose tolerance test at the biomedical exams and diabetes was classified as fasting plasma glucose  $\geq 7.0$  mmol/L, 2hour plasma glucose  $\geq 11.0$  mmol/L or current treatment with insulin or oral hypoglycemic agents (WHO classification 1999)<sup>60</sup>.

## 2.3 STUDY POPULATION

Of the 11 247 in the initial study population, participants excluded from analyses were those with prevalent or unknown diabetes status at baseline (n=1112), who did not participate in either follow-up (n=3757), who did not complete the dietary questionnaire (n=86), and those with extreme values for the ratio between energy intake and required energy (ie. the 1<sup>st</sup> and 99<sup>th</sup> percentiles of the distribution in the population) (n=50). This left 6242 participants with 376 cases of T2D in the analyses on the relationship between the adherence to dietary guidelines or to the guidelines for healthy behaviours and the risk of T2D (fourth objective).



N= 11 247  
T2D= 376

## 2.4 DATA MANAGEMENT

### 2.4.1 TREATMENT OF MISSING DATA

If <5% of values were missing, the median of the study population (for quantitative variables) or the mode of the study population (for qualitative variables) was imputed. If ≥5% of values were missing, a missing category was created.

### 2.4.2 FACTORS OF ADJUSTMENT

The models were adjusted for: sex, high triglycerides (no; yes: ≥2.0 mmol/L), low HDL (no; yes: <1.0 mmol/L), family history of diabetes (no vs. yes), physical activity (sedentary: 0 mins/wk; insufficient: 1-150 mins/wk; sufficient: >150 mins/wk), smoking status (current smoker; ex-smoker; non-smoker), energy intake (kJ/day), hypertension (no vs. yes: ≥140-90) and waist circumference group (low weight-related health risk: <94 cm for men, <80 cm for women; increased weight-related health risk: 94-102 cm for men, 80-88 cm for women; high weight-related health risk: ≥102 cm for men, ≥88 cm for women), and the level of education (secondary school/trade school/technician's certificate or less vs. Bachelor's degree, post graduate degree, nursing or teaching qualification).

### 2.4.3 CREATING THE DIETARY INDEX OF ADHERENCE

An index of adherence to the Australian Dietary Guidelines was created based on the 2013 recommendations set forth by the National Health and Medical Research Council, which specifies the recommended number of servings per day by sex and age for each of the five food groups ("Fruit"; "Vegetables": vegetables and legumes/beans; "Grains": grain (cereal) foods; "Dairy": milk, yogurt, cheese and/or alternatives; "Proteins": lean meats and poultry, fish, eggs, tofu, nuts/seeds, and legumes/beans)<sup>132</sup>, as well as "no more than two standard drinks on any day" for men and women (standard drink = 10g of ethanol)<sup>193</sup> (Figure 28). The dietary index was therefore determined by adherence to recommendations of *six components*: Fruit, Vegetables, Grains, Dairy, Proteins and Alcohol. As the category "legumes/beans" appeared twice in the five food groups, green beans, bean sprouts, and peas were considered as "Vegetables" whereas baked beans and other beans were considered as "Proteins", due to their higher protein content.



FIGURE 28. AUSTRALIAN GUIDE TO HEALTHY EATING, 2013<sup>132</sup>

Serving sizes were calculated by taking the amount consumed per day of each food reported in the dietary questionnaire and dividing it by the quantity outlined by the National Health and Medical Research Council as that which constitutes a serving for the particular food item (**Annex 2**). For example, a serving of cooked lean red meat (beef, lamb, veal, pork, goat, and kangaroo) is reported as 65g, whereas a serving of cooked lean poultry such as chicken or turkey is 80g and a serving of cooked fish filet is 100g (**Figure 29**). The numbers of servings for each food group were then summed to obtain a final quantity for each food group for each individual in servings/day. For example, for Proteins, it was the sum of the number of servings of cooked lean red meat, cooked lean poultry, cooked fish filet, eggs, cooked or canned legumes/beans, tofu, etc.



**FIGURE 29.** WHAT IS A SERVING OF "PROTEINS"?<sup>129</sup>

For each age group (25-50, 51-70, ≥70 years), the adherence to the dietary guidelines was determined. Those within a half serving range were considered strong adherers. For example, 6 servings of grains are recommended for men ages 19-70. A man consuming 5.5-6.5 servings of grains was considered a strong adherer, a man consuming 2.75-5.5 servings of grains an intermediate adherer, a man consuming <2.75 a weak adherer and a man consuming ≥6.5 servings of grains was considered as "exceeding the limits" (**Table 2**). Concerning alcohol, no alcohol intake was considered as "strong adherence", <1 standard drink (10g ethanol) was considered as "intermediate adherence", 1-2 standard drinks as "weak adherence" and ≥2 standard drinks as "exceeding the limits". The final variable for adherence to the dietary guidelines was determined by the total number of components to which the individual was considered to have strong adherence and ranged from 0-6 by intervals of 1, with 6 representing the strongest adherence.



**TABLE 2.** AUSTRALIAN DIETARY GUIDELINES (SERVINGS/DAY) AND CRITERIA USED TO CREATE THE INDEX OF ADHERENCE TO THE AUSTRALIAN DIETARY<sup>a</sup>

	Fruit	Vegetables	Grains	Proteins	Dairy	Alcohol (General)	Alcohol (T2D)
<b>Men</b>							
19-50							
Recommended	2	6	6	3	2.5	0	0.5-1
Strong Adherence	1.5-2.5	5.5-6.5	5.5-6.5	2.5-3.5	2.0-3.0	0	0.5-1
Partial Adherence	0.75-1.5	2.75-5.5	2.75-5.5	1.25-2.5	1.0-2.0	<1	<0.5
Weak Adherence	<0.75	<2.75	<2.75	<1.25	<1.0	1-2	1-2
Exceeding the Limits	≥2.5	≥6.5	≥6.5	≥3.5	≥3.0	≥2	≥2
51-70							
Recommended	2	5.5	6	2.5	2.5	0	0.5-1
Strong Adherence	1.5-2.5	5.0-6.0	5.5-6.5	2.0-3.0	2.0-3.0	0	0.5-1
Partial Adherence	0.75-1.5	2.5-5.0	2.75-5.5	1.0-2.0	1.0-2.0	<1	<0.5
Weak Adherence	<0.75	<2.5	<2.75	<1.0	<1.0	1-2	1-2
Exceeding the Limits	≥2.5	≥6.0	≥6.5	≥3.0	≥3.0	≥2	≥2
≥70							
Recommended	2	5	4.5	2.5	3.5	0	0.5-1
Strong Adherence	1.5-2.5	4.5-5.5	4.0-5.0	2.0-3.0	3.0-4.0	0	0.5-1
Partial Adherence	0.75-1.5	2.25-4.5	2.0-4.0	1.0-2.0	1.5-3.0	<1	<0.5
Weak Adherence	<0.75	<2.25	<2.0	<1.0	<1.5	1-2	1-2
Exceeding the Limits	≥2.5	≥5.5	≥5.0	≥3.0	≥4.0	≥2	≥2
<b>Women</b>							
19-50							
Recommended	2	5	6	2.5	2.5	0	0.5-1
Strong Adherence	1.5-2.5	4.5-5.5	5.5-6.5	2.0-3.0	2.0-3.0	0	0.5-1
Partial Adherence	0.75-1.5	2.25-4.5	2.75-5.5	1.0-2.0	1.0-2.0	<1	<0.5
Weak Adherence	<0.75	<2.25	<2.75	<1.0	<1.0	1-2	1-2
Exceeding the Limits	≥2.5	≥5.5	≥6.5	≥3.0	≥3.0	≥2	≥2
51-70							
Recommended	2	5	4	2	4	0	0.5-1
Strong Adherence	1.5-2.5	4.5-5.5	3.5-4.5	1.5-2.5	3.5-4.5	0	0.5-1
Partial Adherence	0.75-1.5	2.25-4.5	1.75-3.5	0.75-1.5	1.75-3.5	<1	<0.5
Weak Adherence	<0.75	<2.25	<1.75	<0.75	<1.75	1-2	1-2
Exceeding the Limits	≥2.5	≥5.5	≥4.5	≥2.5	≥4.5	≥2	≥2
≥70							
Recommended	2	5	3	2	4	0	0.5-1
Strong Adherence	1.5-2.5	4.5-5.5	2.5-3.5	1.5-2.5	3.5-4.5	0	0.5-1
Partial Adherence	0.75-1.5	2.25-4.5	1.25-2.5	0.75-1.5	1.75-3.5	<1	<0.5
Weak Adherence	<0.75	<2.25	<1.25	<0.75	<1.75	1-2	1-2
Exceeding the Limits	≥2.5	≥5.5	≥3.5	≥2.5	≥4.5	≥2	≥2

\*Standard drinks/day (10g ethanol)

<sup>a</sup>Serving sizes were determined based off the 2013 Australian Dietary Guidelines; a summary can be found in [Annex 3](#)<sup>132</sup>

#### 2.4.4 CREATING THE INDEX OF ADHERENCE TO HEALTHY BEHAVIOURS

A healthy behaviour index was created based off an index developed by Dartois *et al.*<sup>194</sup>. Four lifestyle behaviours comprised our *a priori* index: tobacco smoking, level of recreational physical activity, waist circumference, and the adherence to dietary guidelines. Individuals were assigned scores based on their adherence to recommendations set by either the Australian Department of Health or the World Health Organization<sup>193,195-198</sup>. A score was assigned for adherence to each individual characteristic of the index, with a score of 1 was assigned to those considered to demonstrate strong adherence to, 0.5 to those showing partial adherence, and 0 to those showing weak adherence. For example, individuals received a score of one if they were non-smokers, had sufficient recreational physical activity ( $\geq 150$  mins/week), a low weight-related health risk ( $< 94$  cm for men and  $< 80$  cm for women), or high adherence to the Australian dietary guidelines (strong adherence to the recommendations for  $\geq 3$  components). The criteria used to rank each individual can be found in **Table 3**. The final score was obtained by summing the score from each lifestyle behaviour to obtain a score that ranged from 0-4 by intervals of 0.5, with 4 representing the adherence to all recommendations.

**TABLE 3.** CRITERIA FOR CREATION OF THE HEALTHY BEHAVIOUR INDEX BASED ON FOUR LIFESTYLE CHARACTERISTICS

Behaviour	Weak Adherence (0)	Partial Adherence (0.5)	Strong Adherence (1)
Tobacco Smoking	Current smoker	Former smoker	Non-smoker
Recreational Physical Activity (mins/week)	None	1-150	$\geq 150$
BMI (kg/m <sup>2</sup> )*	$\geq 30$	25-30	$< 25$
Waist circumference (cm)			
Men	$\geq 102$	94-102	$< 94$
Women	$\geq 88$	80-88	$< 80$
Adherence to Australian dietary guidelines	Index score = 0	Index score 1-2	Index score $\geq 3$

\*BMI was substituted for waist circumference in the sensitivity analyses

#### 2.5 DESCRIPTIVE ANALYSIS

The individual baseline characteristics of the population were described for the overall study population, and by group of adherence to the healthy behaviour index (weak adherence; partial adherence; strong adherence). The descriptive analyses were carried out using the averages and standard deviations for continuous variables and frequency distributions (numbers and percentages) for categorical variables.

## 2.6 COX MULTIVARIABLE REGRESSION IN AUSDIAB

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Cox proportional hazards models with age as the timescale were used to estimate HR and 95% CI of T2D risk for each component of the dietary index, for each characteristic of the healthy behaviour index, and for the healthy behaviour index itself. The time at entry was the date of the baseline physical exam and the exit time for those who did not develop T2D was the most recent date they attended a follow-up. For those who developed T2D between the baseline and the 2004-2005 follow-up, the exit time taken a randomly generated date between these two dates, and for those who developed T2D between the 2004-2005 and 2011-2012 follow-ups, the exit time was a randomly generated date between these two dates.

In the analyses between adherence to components of the dietary guidelines and T2D, *Model 1* included only the exposure in question and was adjusted for sex. *Model 2* additionally adjusted for education, smoking status, recreational physical activity, high triglycerides, low HDL cholesterol, family history of diabetes, energy intake, and hypertension. Finally, *Model 3* additionally adjusted for waist circumference.

## 2.7 CUBIC SPLINE REGRESSION IN AUSDIAB

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Restricted cubic spline regression with 3 knots was also performed to visualize the potential non-linear relationship between the number of servings per day of each food group or the number of standard drinks per day and the risk of T2D. The splines were adjusted for the same factors as the Cox proportional hazards models.

## 2.8 POPULATION ATTRIBUTABLE FRACTIONS

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### 2.8.1 INTRODUCTION

The population attributable fraction (PAF) is a population-specific estimate that takes into account both the prevalence of the risk factor in the population and the magnitude of its association. It therefore provides an estimate of the contribution of a risk factor (such as weak adherence to the recommendations for healthy behaviours) to death or disease (in this case, T2D) and the proportional reduction in the population that would occur if exposure to the risk factor was ideal (in this case, strong adherence to recommendations concerning healthy behaviours)<sup>199</sup>.

The PAF is usually expressed as a percent, but because most diseases are multifactorial and individual risk factors can interact, the PAFs for individual risk factors can add up to more than 100%.

### 2.8.2 FORMULA

The formula for the PAF is as follows:

$$\frac{(P(E) \times (RR - 1))}{((P(E) \times (RR - 1)) + 1)}$$

where  $P(E)$  is the proportion of the population exposed to the factor and  $RR$  is the relative risk of T2D associated with the factor<sup>200</sup>.

### 2.8.3 STATISTICAL MODELLING

The PAFs were computed to determine the proportion of T2D cases that could have been avoided with full adherence to the recommendations, if all other risk factors had remained unchanged.

Under the assumption of a causal relationship and no change in the participant's characteristics over time, PAFs were calculated for full adherence to recommendations for each component of the dietary index, each characteristic of the healthy behaviour index (a score of 1), and the full healthy behaviour index (a score  $\geq 3.5$ ). Using methods developed by Dartois *et al.* and Spiegelman *et al.*, point estimates and 95% CIs were computed with simultaneous adjustments for potential confounders<sup>194,201</sup>.

## 2.9 SENSITIVITY ANALYSES AND SOFTWARE

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### 2.9.1 SENSITIVITY ANALYSES

As a moderate consumption of alcohol may be beneficial in preventing diabetes<sup>202</sup>, the substitution of moderate alcohol consumption (0.5-1.0 standard drinks per day; standard drink = 10g ethanol) was tested as beneficial and individuals with moderate alcohol consumption were considered as full adherers to the recommendations. BMI was also substituted for waist circumference, where a normal BMI was defined as  $< 25 \text{ kg/m}^2$ , overweight as  $25\text{-}30 \text{ kg/m}^2$  and obese as  $\geq 30 \text{ kg/m}^2$ .

Because the prevalence estimates in AusDiab may not be fully representative of the population, PAFs were also estimated using updated prevalence estimates from the 2014-2015 Australian National Health Survey and the unadjusted hazard ratios obtained for this cohort.

### 2.9.2 SOFTWARE USED

All analyses were conducted using STATA version 12, excluding the PAFs which were estimated using SAS version 9.3.

### 3. SYSTEMATIC REVIEW OF THE LITERATURE

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In line with Objective 2 of this thesis, a systematic review of the literature was conducted to identify, summarize, and interpret the literature on the association between the diet or dietary intake of foods, nutrients, or food groups, and the risk of diabetic retinopathy or diabetic macular edema.

#### 3.1 INCLUSION CRITERIA

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Using the PRISMA Checklist for systematic reviews and meta-analyses, all studies published in peer-reviewed journals in the English language that evaluated the association between dietary intakes of foods (individual food items or broader food groups such as fruit or vegetables), macronutrients (carbohydrates, protein, fat, fibre), micronutrients (minerals, vitamins), dietary supplements (as vitamins or minerals) or dietary patterns (a posteriori or a priori) and their association with the development of DR or DME were included. DR included retinopathy at all stages of development (non-proliferative and proliferative; ICD 10: E10.3, E11.3) with or without macular edema (ICD 10: H35.8). Diabetic retinopathy and macular edema could be evaluated through: ophthalmological assessment, retinal photographs graded against standard photographs, mydriatic or non-mydriatic fundus photography, review of medical records for retinopathy diagnosis or review of medical records for laser photocoagulation treatment for retinopathy.

Eligible study designs included randomized controlled trials (RCTs), and cohort, case-control, cross-sectional, and ecological studies. All publication years and publication statuses were considered. Studies evaluating the association between dietary intake or dietary patterns and other health outcomes were excluded, as well as studies conducted on laboratory animals and studies assessing the progression or severity of diabetic retinopathy.

#### 3.2 SEARCH STRATEGY

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Searches were conducted in both PubMed and Web of Science in November 2015 and last updated in October 2017. The search strategy employed for the Pubmed search was based on the protocol developed in a systematic review by Schwingshackl *et al.* to identify food intake and risk of chronic disease<sup>203</sup> and modified to include other search terms we defined as pertinent. The search strategy is available in **Annexes 3 and 4**.



#### 3.3 STUDY SELECTION

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The titles and abstracts of articles identified in the searches were screened by a single researcher (Courtney Dow, CD) and checked by a second (Guy Fagherazzi, GF). The full texts of all potentially eligible articles from each database were obtained and examined by a researcher (CD) to determine if they fit the eligibility criteria. In those fitting the criteria, reference lists were verified for further relevant studies.

### 3.4 DATA EXTRACTION

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Data extraction was performed on full texts by a researcher (CD) for the following characteristics: first author's surname, year of publication, study design, country of study, year of study, sample size, and number of cases, as well as the potential restrictions on age and sex of included participants, type of diabetes mellitus, duration of follow-up, exposure of interest, exposure assessment, retinopathy assessment, potential confounders controlled for and primary findings (the most adjusted risk estimates, hazard ratios, risk ratios, or odds ratios with their 95% confidence intervals) and this extraction was checked by a second researcher (GF).



### 3.5 ASSESSMENT OF THE QUALITY OF INCLUDED STUDIES

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#### 3.5.1 RANDOMIZED CONTROLLED TRIALS

To assess the quality of the randomized controlled trials (RCTs) included in this systematic review, the Cochrane Risk of Bias Tool for Randomized Controlled Trials was used. This tool classifies RCTs according to their risk of bias by seven criteria: random sequence generation, allocation concealment, selective reporting, other bias, blinding of participants and personnel, blinding of outcome assessment, and incomplete outcome data. RCTs are considered to be good quality if all criteria are met<sup>204</sup>.



#### 3.5.2 OBSERVATIONAL STUDIES

As the Cochrane Handbook identifies the Newcastle-Ottawa Scale as one of the most useful tools to assess the methodological quality or risk of bias in non-randomized studies, this scale was used to assess the quality of the observational studies<sup>205</sup>. This scale assesses cohort and case-control studies in three areas: selection (four stars), comparability (two stars), and outcome (three stars), for a total score of 9 stars. Studies were considered good quality if they had 7 or more stars, medium quality if they had 5 or 6 stars and low quality with 4 or fewer stars. A modified version of the scale was implemented to assess the cross-sectional studies<sup>206</sup>. This version allots five stars to selection, 2 to comparability and 3 to outcome, for a maximum possible score of 10 stars. Cross-sectional studies were considered good quality with 8-10 stars, medium quality with 6-7 stars and low quality with 5 or fewer stars.

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# RESULTS AND DISCUSSION

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This section will detail the results and the conclusions drawn pertaining to the four objectives of this thesis. It will first present the results of the analyses on FA intake and the risk of T2D (Objective 1), followed by the results of the systematic review on the diet and DR (Objective 2). This will be followed by the results on FA intake and DED (Objective 3) and lastly by the results on adherence to dietary guidelines and the risk of T2D, and population attributable fractions (Objective 4).

## 1. OBJECTIVE ONE: FATTY ACID INTAKE AND RISK OF TYPE 2 DIABETES

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### 1.1 BASELINE CHARACTERISTICS

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In total, 2610 cases of incident T2D were diagnosed during 1 158 131 person-years between June 1993 and December 2011. The incidence of T2D was 225 per 100 000 person-years. Median follow-up was 18 years (ranging from 29 days to 18 years) for non-cases, and 11 years (ranging from 18 days to 18 years) for cases. The mean age of the study participants was  $52.9 \pm 6.7$  (Table 4). The majority (66%) of women had a healthy BMI ( $20\text{-}24.9\text{ kg/m}^2$ ), while 19.5% had an excess weight, including 3.3% obese women. Hypertension was declared by 37.1% of women, and hypercholesterolemia by 7.1%. 11.1% of women reported a family history of diabetes. Average daily energy intake was  $2131 \pm 544$  Kcal/day and average physical activity was  $49.4 \pm 50.5$  MET-h/wk. Mean ( $\pm$  SD) FA group consumptions were:  $36.4 \pm 9.9$  (SFAs),  $31.4 \pm 9.9$  (MUFAs),  $1.6 \pm 0.7$  (TFAs),  $14.2 \pm 5.4$  (PUFAs),  $1.5 \pm 0.5$  (n-3 PUFAs), and  $12.6 \pm 5.0$  (n-6 PUFAs) g/day.

### 1.2 MAIN FATTY ACID GROUPS

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#### 1.2.1 SATURATED FATTY ACIDS

SFA consumption above the upper tertile was associated with an increased risk of T2D in the univariate analysis (HR=1.13 [1.02-1.24]), and in the multivariable model adjusted for non-dietary factors, energy intake and alcohol (Model 1: HR=1.17 [1.06-1.28]) (Table 5) when compared to the first tertile group. Further adjustment for other FA groups slightly decreased the association, rendering it non-significant in Model 2 (HR=1.13 [0.97-1.31]).

#### 1.2.2 MONOUNSATURATED FATTY ACIDS

MUFA consumption above the first tertile was associated with a higher risk of T2D in the unadjusted model and Model 1 (third tertile group: HR=1.15 [1.04-1.27], HR=1.32 [1.20-1.46], respectively). Additional adjustment for FA groups in Model 2 decreased the strength of the association for the second tertile group (HR=1.10 [0.99-1.22]), whereas the association in the third tertile group became statistically non-significant after adjustment for BMI (Model 3: HR=1.06 [0.95-1.19]).

#### 1.2.3 POLYUNSATURATED FATTY ACIDS

PUFA intake was associated with a 29% increased risk of T2D in women above the third tertile of intake after adjustment for other types of FAs in Model 2 (HR=1.29 [1.17-1.43]). However, there was no longer an association after adjusting for BMI (Model 3: HR=1.06 [0.96-1.17]). Positive associations were observed for both n-3 and n-6 PUFAs in Model 1, but after adjusting for other FAs and BMI, only the association above the third tertile of n-3 PUFA intake remained (HR=1.26 [1.13-1.41]).

**TABLE 4.** BASELINE CHARACTERISTICS OF THE STUDY POPULATION (E3N COHORT DATA; N = 71 334 WOMEN)

Variable	Study		Non-cases		Incident	
	Population (n=71 334)	SD or (%)	(n=68 724)	SD or (%)	T2D (n=2610)	SD or (%)
Age (y)	52.9	6.7	52.8	6.7	54.4	6.7
BMI (kg/m <sup>2</sup> ) [n (%)]						
<20	10 307	(14.5)	10239	(14.9)	68	(2.6)
20-24.9	47 101	(66.0)	46073	(67.0)	1028	(39.4)
25-30	11 554	(16.2)	10572	(15.4)	982	(37.6)
>30	2372	(3.3)	1840	(2.7)	532	(20.4)
Birth cohort [n (%)]						
Before 1930	5437	(7.6)	5186	(7.6)	251	(9.6)
1930-1934	9175	(12.9)	8702	(12.7)	473	(18.1)
1935-1939	13 530	(19.0)	12991	(18.9)	539	(20.7)
1940-1944	17 142	(24.0)	16494	(24.0)	648	(24.8)
After 1944	26 050	(36.5)	25351	(36.9)	699	(26.8)
Education [n (%)]						
Undergraduate	7913	(11.1)	7493	(10.9)	420	(16.1)
>Undergraduate	63 421	(88.9)	61231	(89.1)	2190	(83.9)
Smoking status [n (%)]						
Never	38 504	(54.0)	37115	(54.0)	1389	(53.2)
Current/former	32 830	(46.0)	31609	(46.0)	1221	(46.8)
Hypertension [n (%)]						
No	44 857	(62.9)	43773	(63.7)	1084	(41.5)
Yes	26 477	(37.1)	24951	(36.3)	1526	(58.5)
Hypercholesterolaemia treatment [n (%)]						
No	66 297	(92.9)	64085	(93.3)	2212	(3.1)
Yes	5037	(7.1)	4639	(6.8)	398	(15.3)
Family history of T2D [n (%)]						
No	63 432	(88.9)	61451	(89.4)	1981	(75.9)
Yes	7902	(11.1)	7273	(10.6)	629	(24.1)
Physical activity (MET- h/wk)	49.4	50.5	49.3	50.1	50.0	59.2
Alcohol consumption (g of ethanol per 100g/day)	11.6	13.9	11.6	13.8	12.1	15.9
Daily energy intake (kcal/day)	2130.6	543.7	2128.8	541.6	2178.5	593.1
Average consumption (g/day)						
SFA	36.4	13.0	36.4	12.9	37.6	14.1
MUFA	31.3	9.9	31.3	9.8	32.6	10.8
TFA	1.6	0.7	1.6	0.7	1.6	0.7
PUFA	14.2	5.3	14.1	5.3	15.2	5.9
n-3 PUFA	1.5	0.5	1.5	0.5	1.6	0.6
n-6 PUFA	12.6	5.0	12.6	5.0	13.6	5.6

\*BMI, body mass index; MET, metabolic equivalents; MUFA, monounsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, trans unsaturated fatty acids

### 1.2.4 TRANS UNSATURATED FATTY ACIDS

The second tertile group of TFA intake was associated with a lower T2D risk in the univariate analysis (HR=0.87 [0.79-0.96]) and in Model 2 (HR=0.88 [0.78-0.98]) after adjustment for other FAs, but not Model 1 (HR=0.93 [0.85-1.02]). After adjustment for BMI, high TFA intake was no longer associated with T2D (HR=0.90 [0.80-1.00]).

**TABLE 5.** COX PROPORTIONAL HAZARDS RATIOS [95% CI] FOR THE RISK OF INCIDENT TYPE 2 DIABETES BY FATTY ACID CONSUMPTION (G/DAY) IN THE E3N STUDY COHORT (N=71 334 WOMEN)

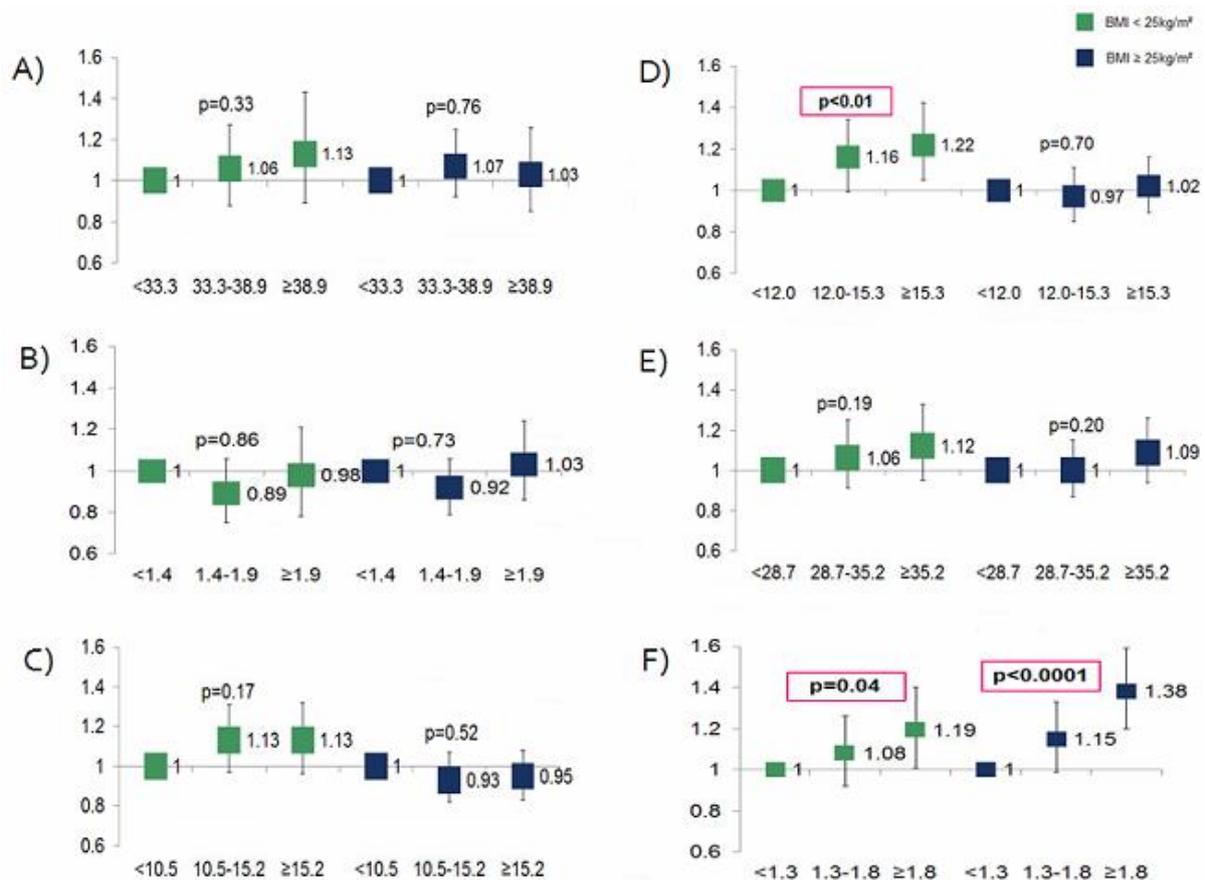
Variable (g/day)	No. of cases/ noncases	Univariate	Model 1	Model 2	Model 3
<b>SFA</b>					
<33.3	843/22 698	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
33.3-38.9	847/22 692	1.04 [0.95-1.14]	1.10 [1.00-1.21]	1.11 [0.99-1.25]	1.06 [0.95-1.19]
≥ 38.9	920/23 334	<b>1.13 [1.02-1.24]</b>	<b>1.17 [1.06-1.28]</b>	1.13 [0.97-1.31]	1.07 [0.92-1.25]
P for trend		<b>0.01</b>	<b>0.001</b>	0.14	0.40
<b>MUFA</b>					
<28.7	785/22 755	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
28.7-33.3	839/22 702	<b>1.11 [1.01-1.23]</b>	<b>1.15 [1.04-1.27]</b>	1.10 [0.99-1.22]	1.01 [0.91-1.12]
≥ 33.3	986/23 267	<b>1.31 [1.19-1.44]</b>	<b>1.32 [1.20-1.46]</b>	<b>1.23 [1.10-1.38]</b>	1.06 [0.95-1.19]
P for trend		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.001</b>	0.25
<b>PUFA</b>					
<12.0	720/22 821	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
12.0-15.3	835/22 704	<b>1.17 [1.06-1.29]</b>	<b>1.14 [1.03-1.26]</b>	<b>1.13 [1.02-1.25]</b>	1.03 [0.93-1.14]
≥ 15.3	1055/23 199	<b>1.46 [1.33-1.61]</b>	<b>1.34 [1.21-1.47]</b>	<b>1.29 [1.17-1.43]</b>	1.06 [0.96-1.17]
P for trend		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.24
<b>n-6 PUFA</b>					
<10.5	734/22 807	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
10.5-13.7	840/22 700	<b>1.15 [1.04-1.27]</b>	<b>1.13 [1.02-1.25]</b>	1.07 [0.96-1.18]	1.01 [0.91-1.12]
≥ 13.7	1036/23 217	<b>1.41 [1.28-1.55]</b>	<b>1.29 [1.17-1.42]</b>	<b>1.15 [1.04-1.27]</b>	1.00 [0.90-1.10]
P for trend		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.01</b>	0.94
<b>n-3 PUFA</b>					
<1.3	665/22 876	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
1.3-1.6	819/22 720	<b>1.25 [1.13-1.38]</b>	<b>1.23 [1.11-1.37]</b>	<b>1.18 [1.06-1.31]</b>	1.10 [0.99-1.22]
≥ 1.6	1126/23 128	<b>1.68 [1.52-1.84]</b>	<b>1.60 [1.45-1.76]</b>	<b>1.47 [1.32-1.64]</b>	<b>1.26 [1.13-1.41]</b>
P for trend		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
<b>TFA</b>					
<1.4	920/22 621	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
1.4-1.7	794/22 745	<b>0.87 [0.79-0.96]</b>	0.93 [0.85-1.02]	<b>0.88 [0.78-0.98]</b>	0.90 [0.80-1.00]
≥ 1.7	896/23 358	0.98 [0.89-1.07]	1.03 [0.94-1.13]	0.93 [0.81-1.07]	1.02 [0.89-1.17]
P for trend		<b>&lt;0.01</b>	0.79	0.25	0.85

\*Model 1: adjusted for daily energy intake, alcohol consumption and non-dietary factors: level of education, family history of diabetes, physical activity, hypertension, hypercholesterolaemia treatment, and smoking status; Model 2: Model 1 + tertile groups of remaining fatty acid groups; Model 3: Model 2 + BMI

†MUFA, monounsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, trans unsaturated fatty acids

### 1.3 STRATIFICATION BY BMI

After stratification by BMI, in the fully adjusted models, neither SFAs, nor MUFAs nor TFAs were associated with T2D risk in either BMI stratum (**Figure 30**). Regarding total PUFAs, a high intake was associated with increased T2D risk in non-overweight women (HR=1.22 [1.05-1.42]), but not in overweight women (HR=1.02 [0.90-1.16]). N-6 PUFAs were not associated with T2D risk in either BMI stratum (HR=1.13 [0.96-1.32] and HR=0.95 [0.83-1.08]; non-overweight and overweight women, respectively), while high n-3 PUFA consumption was associated with an increased risk of T2D in both strata in fully adjusted models (HR=1.19 [1.01-1.40] and HR=1.38 [1.20-1.59]; non-overweight and overweight women, respectively).



**FIGURE 30.** BMI STRATIFIED COX PROPORTIONAL HAZARD RATIOS [95% CI] OF TYPE 2 DIABETES BY TERTILE GROUP OF FATTY ACID CONSUMPTION (G/DAY). **A)** SATURATED FATTY ACIDS; **B)** MONOUNSATURATED FATTY ACIDS; **C)** TRANSUNSATURATED FATTY ACIDS; **D)** POLYUNSATURATED FATTY ACIDS; **E)** OMEGA-6 POLYUNSATURATED FATTY ACIDS; **F)** OMEGA-3 POLYUNSATURATED FATTY ACIDS<sup>b</sup>

<sup>b</sup> Models adjusted for daily energy intake, alcohol consumption, level of education, family history of diabetes, physical activity, hypertension, hypercholesterolaemia treatment, smoking status and tertile groups of remaining fatty acid groups

**TABLE 6.** COX PROPORTIONAL HAZARDS RATIOS [95% CI] OF THE RISK OF INCIDENT TYPE 2 DIABETES BY OMEGA-3 AND OMEGA-6 POLYUNSATURATED FATTY ACID CONSUMPTION (G/DAY) WITH BMI STRATIFICATION IN THE E3N STUDY COHORT (N=71 334)

Variable (g/day)	No. of cases/non-cases	n = 71 334	No. of cases/non-cases	BMI <25kg/m <sup>2</sup> (n=57 408)	No. of cases/non-cases	BMI ≥ 25kg/m <sup>2</sup> (n=13 926)
<b>ALA</b>						
<0.90	757/22784	1 (Reference)	358/19213	1 (Reference)	399/3571	1 (Reference)
0.90-1.14	843/22696	1.00 [0.90-1.12]	365/18649	0.93 [0.79-1.09]	478/4047	1.08 [0.94-1.25]
≥ 1.14	1010/23244	1.03 [0.92-1.15]	373/18450	0.90 [0.75-1.07]	637/4794	<b>1.17 [1.01-1.36]</b>
P for trend		0.62		0.24		<b>0.04</b>
<b>EPA</b>						
<0.09	711/22830	1 (Reference)	329/19275	1 (Reference)	382/3555	1 (Reference)
0.09-0.20	823/22716	0.88 [0.73-1.06]	344/18694	0.86 [0.65-1.14]	479/4022	0.87 [0.67-1.12]
≥ 0.20	1076/23178	0.88 [0.67-1.15]	423/18343	0.79 [0.53-1.19]	653/4835	0.93 [0.65-1.32]
P for trend		0.51		0.32		0.96
<b>DPA</b>						
<0.05	610/22931	1 (Reference)	307/19912	1 (Reference)	303/3019	1 (Reference)
0.05-0.08	805/22734	<b>1.15 [1.02-1.30]</b>	364/18723	<b>1.23 [1.04-1.47]</b>	441/4011	1.15 [0.98-1.36]
≥ 0.08	1195/23059	<b>1.41 [1.23-1.63]</b>	425/17677	<b>1.45 [1.17-1.80]</b>	770/5382	<b>1.54 [1.27-1.85]</b>
P for trend		<b>&lt;0.0001</b>		<b>&lt;0.01</b>		<b>&lt;0.0001</b>
<b>DHA</b>						
<0.19	702/22839	1 (Reference)	327/19243	1 (Reference)	375/3596	1 (Reference)
0.19-0.38	839/22701	1.15 [0.95-1.38]	344/18743	1.06 [0.80-1.41]	495/3958	1.23 [0.96-1.58]
≥ 0.38	1069/23184	1.11 [0.85-1.44]	425/18326	1.24 [0.83-1.86]	644/4858	1.01 [0.71-1.43]
P for trend		0.65		0.27		0.62
<b>LA</b>						
<10.3	740/22801	1 (Reference)	330/19348	1 (Reference)	410/3453	1 (Reference)
10.3-13.5	838/22702	0.98 [0.89-1.08]	382/18764	1.10 [0.94-1.28]	456/3938	0.90 [0.78-1.03]
≥ 13.5	1032/23221	0.97 [0.87-1.07]	384/18200	1.08 [0.92-1.26]	648/5021	0.91 [0.80-1.04]
P for trend		0.52		0.39		0.25
<b>AA</b>						
<0.19	579/22961	1 (Reference)	327/20068	1 (Reference)	252/2893	1 (Reference)
0.19-0.25	750/22790	1.11 [0.99-1.24]	329/18869	1.05 [0.90-1.23]	421/3921	<b>1.28 [1.09-1.50]</b>
≥ 0.25	1281/22973	<b>1.49 [1.33-1.66]</b>	440/17375	<b>1.50 [1.28-1.76]</b>	841/5598	<b>1.74 [1.49-2.03]</b>
P for trend		<b>&lt;0.0001</b>		<b>&lt;0.0001</b>		<b>&lt;0.0001</b>

\*AA, arachidonic acid (20:4n6); ALA, α-linolenic acid (18:3n3); DHA, docosahexaenoic acid (22:6n3); DPA, docosapentaenoic acid (22:5n3); EPA, eicosapentaenoic acid (20:5n3); LA, linoleic acid (18:2n6)

## 1.4 INDIVIDUAL POLYUNSATURATED FATTY ACIDS

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### 1.4.1 OMEGA-6S

LA was not associated with T2D risk in the whole population (HR=0.97 [0.87-1.07]) or either BMI stratum (**Table 6**). Conversely, a high consumption of AA was associated with an almost 50% increased risk of T2D (HR=1.49 [1.33-1.66]). Within BMI strata, AA intake above the third tertile was associated with T2D risk in non-overweight women (HR=1.50 [1.28-1.76]) and intake above the second tertile was associated with T2D risk in overweight women (HR=1.74 [1.49-2.03]).

### 1.4.2 OMEGA-3S

In the full population, neither ALA intake, nor intake of long-chain n-3 PUFAs (EPA or DHA) were associated with T2D in the fully adjusted models (HR=1.03 [0.92-1.15]; HR=0.88 [0.67-1.15]; HR=1.11 [0.85-1.44], respectively). However, DPA intake above the second tertile was associated with an increased risk of T2D (HR=1.41 [1.23-1.63]). There was no association with EPA or DHA in models stratified on BMI. However, high ALA intake was associated with an increased T2D risk in women with excess weight (HR=1.17 [1.01-1.36]) for the highest tertile group, but not in the normal-weight women (HR=0.90 [0.75-1.07]). High DPA intake was associated with an increased risk of T2D in both BMI strata (HR=1.45 [1.17-1.80] and HR=1.54 [1.27-1.85], respectively).

## 1.5 SENSITIVITY ANALYSIS

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In a sensitivity analysis excluding the 388 women who developed diabetes during the first 5 years of follow-up, thus on a population of 70 946 women with 2222 incident cases of diabetes, the associations were similar to those in the full analysis (**Table 7**).

Meat was the food group that contributed most to both AA and DPA intakes (42.7% and 31.3%, respectively). Dietary ALA primarily came from dairy products (11.9%), followed by fats (8.2%), olives (7.0%), and meat, processed meats, and offal (6.9%). After additional adjustment for total meat consumption, including processed meats and offal, ALA intake was no longer associated with T2D risk (HR=1.03 [0.92-1.15]) in the overall study population (**Table 8**), while high intakes of DPA and AA remained associated with T2D risk (HR=1.21 [1.03-1.42] and HR=1.40 [1.23-1.59], respectively). After stratification by BMI, high ALA and high DPA intakes were associated with T2D risk in women with BMI $\geq$ 25kg/m<sup>2</sup> (HR=1.17 [1.01-1.35]; HR=1.33 [1.08-1.64]; ALA and DPA, respectively), while high AA intake increased T2D risk in the non-overweight strata (HR=1.31 [1.09-1.59]) and all intakes above the reference tertile group increased T2D risk in the overweight BMI strata (HR=1.68 [1.41-2.00], third tertile group compared to first tertile group).

Adjustment for the overall dietary pattern, using scores adhering to “Mediterranean/Prudent” or “Western” dietary patterns yielded similar results, with the exception of a modest increase in risk for high TFA intake (*results not shown*).

**TABLE 7.** SENSITIVITY ANALYSIS OF THE RISK OF TYPE 2 DIABETES BY FATTY ACID CONSUMPTION, EXCLUSION OF 388 WOMEN WHO DEVELOPED DIABETES IN THE 5 YEARS AFTER STUDY INCLUSION (HAZARD RATIOS [95%CI]) (N=70 946)

Variable (g/day)	No. of cases/non-cases	Univariate	Model 1	Model 2	Model 3
<b>SFA</b>					
<33.3	717/22698	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
33.3-38.9	721/22692	1.04 [0.94-1.15]	1.10 [0.99-1.22]	1.11 [0.98-1.26]	1.07 [0.94-1.21]
≥ 38.9	784/23334	<b>1.13 [1.02-1.25]</b>	<b>1.18 [1.06-1.30]</b>	1.13 [0.96-1.34]	1.08 [0.92-1.28]
P for trend		<b>0.02</b>	<b>&lt;0.01</b>	0.15	0.35
<b>MUFA</b>					
<28.7	671/22755	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
28.7-33.3	706/22702	1.10 [0.99-1.22]	<b>1.13 [1.02-1.26]</b>	<b>1.08 [0.96-1.21]</b>	1.00 [0.89-1.12]
≥ 33.3	845/23267	<b>1.31 [1.18-1.45]</b>	<b>1.33 [1.20-1.48]</b>	<b>1.24 [1.10-1.39]</b>	1.07 [0.95-1.21]
P for trend		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.001</b>	0.21
<b>PUFA</b>					
<12.0	607/22821	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
12.0-15.3	718/22704	<b>1.19 [1.07-1.32]</b>	<b>1.17 [1.05-1.30]</b>	<b>1.15 [1.03-1.29]</b>	1.06 [0.95-1.18]
≥ 15.3	897/23199	<b>1.47 [1.33-1.64]</b>	<b>1.36 [1.22-1.51]</b>	<b>1.31 [1.18-1.46]</b>	1.09 [0.98-1.21]
P for trend		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.13
<b>n-6 PUFA</b>					
<10.5	618/22807	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
10.5-13.7	727/22700	<b>1.18 [1.06-1.32]</b>	<b>1.16 [1.04-1.29]</b>	1.10 [0.99-1.23]	1.05 [0.94-1.17]
≥ 13.7	877/23217	<b>1.42 [1.28-1.57]</b>	<b>1.31 [1.18-1.45]</b>	<b>1.18 [1.06-1.32]</b>	1.03 [0.92-1.15]
P for trend		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.01</b>	0.72
<b>n-3 PUFA</b>					
< 1.3	583/22876	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
1.3-1.6	694/22720	<b>1.21 [1.08-1.35]</b>	<b>1.19 [1.07-1.33]</b>	<b>1.13 [1.01-1.27]</b>	1.06 [0.95-1.19]
≥ 1.6	945/23128	<b>1.61 [1.45-1.78]</b>	<b>1.54 [1.39-1.71]</b>	<b>1.40 [1.24-1.57]</b>	<b>1.21 [1.08-1.36]</b>
P for trend		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.001</b>
<b>TFA</b>					
< 1.4	782/22621	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
1.4-1.7	680/22745	<b>0.88 [0.79-0.98]</b>	0.93 [0.84-1.04]	<b>0.88 [0.78-0.99]</b>	0.90 [0.80-1.01]
≥ 1.7	760/23358	<b>0.88 [0.79-0.98]</b>	0.93 [0.84-1.04]	<b>0.88 [0.78-0.99]</b>	0.90 [0.80-1.01]
P for trend		0.71	0.53	0.34	0.88

\*Model 1: adjusted for daily energy intake, alcohol consumption and non-dietary factors: level of education, family history of diabetes, physical activity, hypertension, hypercholesterolaemia, and smoking status; Model 2: Model 1 + tertile groups of remaining fatty acid groups; Model 3: Model 2 + BMI

†MUFA, monounsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, transunsaturated fatty acids

**TABLE 8.** SENSITIVITY ANALYSES: COX PROPORTIONAL HAZARDS RATIOS [95% CI] OF THE RISK OF INCIDENT TYPE 2 DIABETES BY OMEGA-3 AND OMEGA-6 FATTY ACID CONSUMPTION (G/DAY) AFTER ADJUSTMENT FOR MEAT CONSUMPTION (G/DAY) IN THE E3N STUDY COHORT (N=71 334) WITH BMI STRATIFICATION

Variable (g/day)	No. of cases/non-cases	n = 71 334	No. of cases/non-cases	BMI <25kg/m <sup>2</sup> (n=57 408)	No. of cases/non-cases	BMI ≥ 25kg/m <sup>2</sup> (n=13 926)
<b>ALA</b>						
<0.90	757/22784	1 (Reference)	358/19213	1 (Reference)	399/3571	1 (Reference)
0.90-1.14	843/22696	1.00 [0.90-1.12]	365/18649	0.93 [0.79-1.09]	478/4047	1.08 [0.93-1.24]
≥ 1.14	1010/23244	1.03 [0.92-1.15]	373/18450	0.90 [0.76-1.08]	637/4794	<b>1.17 [1.01-1.35]</b>
P for trend		0.60		0.28		<b>0.04</b>
<b>DPA</b>						
<0.05	610/22931	1 (Reference)	307/19912	1 (Reference)	303/3019	1 (Reference)
0.05-0.08	805/22734	1.06 [0.93-1.21]	364/18723	1.09 [0.90-1.31]	441/4011	1.07 [0.90-1.27]
≥ 0.08	1195/23059	<b>1.21 [1.03-1.42]</b>	425/17677	1.16 [0.91-1.48]	770/5382	<b>1.33 [1.08-1.64]</b>
P for trend		<b>0.01</b>		0.26		<b>&lt;0.01</b>
<b>AA</b>						
<0.19	579/22961	1 (Reference)	327/20068	1 (Reference)	252/2893	1 (Reference)
0.19-0.25	750/22790	1.08 [0.96-1.22]	329/18869	0.98 [0.83-1.16]	421/3921	<b>1.27 [1.07-1.50]</b>
≥ 0.25	1281/22973	<b>1.40 [1.23-1.59]</b>	440/17375	<b>1.31 [1.09-1.59]</b>	841/5598	<b>1.68 [1.41-2.00]</b>
P for trend		<b>&lt;0.0001</b>		<b>&lt;0.01</b>		<b>&lt;0.0001</b>

\*Models adjusted for daily energy intake, alcohol consumption level of education, family history of diabetes, physical activity, hypertension, hypercholesterolaemia, and smoking status and tertile groups of remaining fatty acids

†AA, arachidonic acid (20:4n6); ALA, α-linolenic acid (18:3n3); DPA, docosapentaenoic acid (22:5n3)

## 1.6 DISCUSSION

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In this study, high total n-3 PUFA intake was associated with T2D risk, whatever the BMI. Yet, within the n-3 PUFAs, only *DPA* and *ALA*, (ALA in overweight women only), were associated with increased T2D risk. Total n-6 PUFA intake was not associated with T2D risk, but *AA* intake was associated with increased T2D risk. The other groups of FAs (SFAs, MUFAs, TFAs) were not associated with the risk of T2D.

### 1.6.1 SATURATED FATTY ACIDS

Similar to our study, other studies have also found no association between high total SFA consumption and T2D, whether total SFA consumption was measured with biomarkers<sup>207</sup> or estimated with dietary questionnaires<sup>157,158,208,209</sup>. But, several studies have reported high levels of SFA biomarkers associated with T2D risk<sup>210,211</sup>.



However, recent evidence suggests that SFAs may not have homogeneous effects on T2D risk. Plasma phospholipid measures of very long chain SFAs (VLSFA) and odd chain SFAs both individually and in combination, have been observed to be associated to decreased T2D risk, while even chain SFAs were observed associated with increased T2D risk both individually and combined<sup>153,160,207</sup>.

Even chain SFAs, besides their dietary sources, are also endogenous products of *de novo lipogenesis* (DNL), a process that converts excess carbohydrates and alcohol into SFAs and MUFAs<sup>212</sup>. Studies suggest that their circulating levels are only weakly correlated with dietary intake<sup>211</sup>. On the other hand, odd chain FAs have been shown to be good markers of dairy fat intake and are more likely to be representative of dietary intake<sup>160</sup>. The effects of the dietary intake of even and odd chain SFAs on T2D should be evaluated in future studies, as the SFA group may not have homogeneous effects.

### 1.6.2 MONOUNSATURATED FATTY ACIDS

The findings that MUFA intake was not associated with T2D is in agreement with other studies both using biomarkers<sup>207</sup> and dietary estimates<sup>157,158</sup>. Yet, some studies have found extra virgin olive oil, an important source of MUFAs<sup>213</sup>, appear beneficial for T2D risk<sup>214,215</sup>. However, in this cohort, dairy and meat intake were the primary sources of MUFAs (13.4% and 11.9%, respectively) and prospective studies suggest either an inverse or no association of dairy intake with T2D risk<sup>216,217</sup> and a positive association of meat intake with T2D risk<sup>183,217</sup>, which could explain the null association observed.



### 1.6.3 POLYUNSATURATED FATTY ACIDS

The present findings contrast other studies where PUFA intake was associated with decreased T2D risk<sup>157,158</sup>. But, the PUFAs may be a group likely to have different effects on T2D risk and could explain why some clinical trials find no association between n-3 PUFA intake and cardiovascular disease<sup>218</sup>. The results support this hypothesis, as individual FAs within the n-3 and n-6 PUFA groups had differing effects on T2D risk.

Few studies have examined the associations of individual n-3 PUFAs on T2D risk, yet available evidence concerning the dietary intake of ALA contradicts the results of these analyses and suggests no association with T2D risk<sup>161,163,219</sup>. However, we observed ALA to be associated with an increased risk in overweight women only and not in the overall population with BMI adjustment. When adjusting for BMI and waist-to-hip ratio (WHR), Hodge *et al.* also did not observe an association with ALA intake<sup>219</sup>. But, without adjustment for BMI and WHR, they observed almost a 50% increased risk of T2D associated with the highest quintile of ALA intake. BMI may be a mediator or effect modifier in the relationship and thus may account for the differences between studies. In addition,

ALA may be differentially associated with T2D in populations, with a decreased risk in Asian countries but no association in Western countries<sup>161</sup>.

The marine n-3 PUFAs (EPA, DPA and DHA) are often grouped together and thus rarely analyzed on their own. However, an Australian case-cohort study looked at each individually and did not find any associations with T2D risk<sup>219</sup>. But, average intakes of DPA were lower in their population than in the E3N cohort (0.03 g/day ± 0.03 (SD) and 0.07 g/day ± 0.03, respectively)<sup>219</sup> and the main sources of DPA may have been different - one study found that fish and meat provided nearly equal contributions to the consumption of DPA (50.4% and 49.2%, respectively) in Australians<sup>220</sup> and in E3N, meat contributed to 31.3% of intake and seafood to 26.6%. Furthermore, in meta-analyses stratified by sex, intake of marine n-3 PUFAs has not been associated with T2D risk in the whole population or in men, but was associated with an increased T2D risk in women<sup>162</sup>. Since the Australian case-cohort differed in the amounts and dietary sources of DPA and included both men and women, this could explain the discrepancies with our findings.



To our knowledge, only the Australian case-cohort by Hodge *et al.* examined the relationship between the dietary intake of individual n-6 PUFAs and T2D and found no association with any of the n-6 PUFAs<sup>219</sup>. Studies using plasma measurements of n-6 PUFAs have also reported no association with AA, and have mixed results concerning LA, as one found no association and two reported an inverse association with T2D<sup>207,208,219</sup>. However, average AA intakes appear much lower in the Australian case-cohort than in E3N (0.04 g/day ± 0.03 and 0.22 g/day ± 0.09, respectively), and meat may contribute to more than the majority of AA intake (70.2%) in Australians<sup>220</sup>, whereas in E3N, meat contributed to 42.7% of AA intake and could account for the observed differences in results.

#### 1.6.4 TRANS FATTY ACIDS

Total dietary intake of TFAs was not associated with incident T2D, supporting the findings of other prospective studies<sup>158,208,221</sup> and contrasting with one study<sup>157</sup>. Residual dietary confounding could explain the inconsistency in results, as the mentioned cohorts all found significant associations with TFAs until adjustment for other dietary factors<sup>158,208,221</sup> or, TFAs may also be a heterogeneous group. Plasma measurements of two TFAs have been associated with an increased T2D risk<sup>221</sup>, while evidence from a prospective cohort suggests that trans-palmitoleate (trans-16:1n-7), a naturally-occurring dairy TFA, is associated with decreased T2D risk<sup>222</sup>.



#### 1.6.5 POTENTIAL BIOLOGICAL PATHWAYS

Chronic inflammation may be the basis for the development of T2D and appears related to diet-induced inflammation<sup>223</sup>. Insulin resistance appears to start in the *hypothalamus*, where excess calories and high fat diets, (especially SFA), cause inflammation and have been linked to a pathway that leads to the inhibition of the normal signalling pathways for insulin and the hormone leptin, needed for satiety and usually resulting in increased appetite<sup>223</sup> (Figure 31). This eventually leads to resistance to leptin and insulin signalling<sup>223,224</sup>.

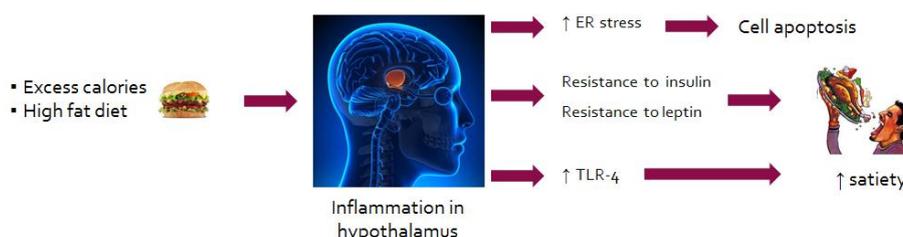
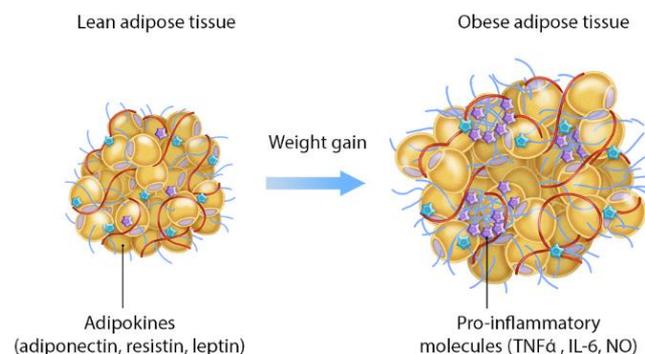


FIGURE 31. HIGH FAT DIETS OR EXCESSIVE CALORIC INTAKE LEAD TO ER STRESS AND INCREASED SATIETY

High fat diets have also been associated with increases in ER stress and the increased expression of TLR-4 receptors that damage neurons needed for satiety<sup>223</sup>. In addition, high levels of FFA (especially palmitic acid) sensed at the blood-brain-barrier have been observed to activate the hypothalamic pituitary adrenal (HPA) axis, which increases levels of cortisol and increases insulin resistance<sup>223</sup>. On the other hand, if the blood-brain-barrier senses FFAs such as oleic acid, hormones are released to increase satiety<sup>223</sup>. In animal models, hypothalamic inflammation can be seen as early as 24h after starting a high fat diet and this inflammation precedes any weight gain in the adipose tissue<sup>223</sup>. This suggests the hypothalamus could be the starting point for insulin resistance.

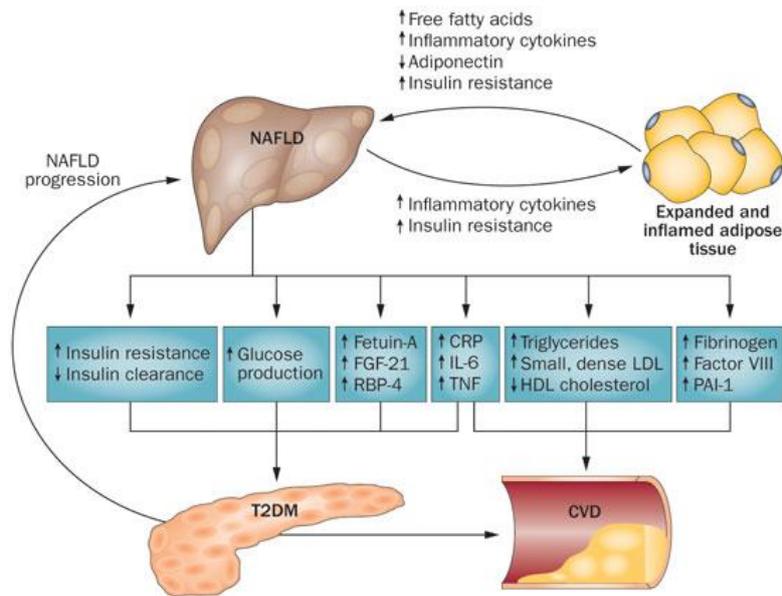
Decreased satiety often leads to increased dietary intake<sup>223</sup>. The excess fat calories (including those from carbohydrates converted to fat in the liver) are most effectively stored in the *adipose tissue*<sup>223</sup>. Once the adipose tissue gets overwhelmed, the fat cells begin to expand, causing hypoxia and inflammation in fat cell, and then insulin resistance in the fat cell<sup>223</sup> (**Figure 32**). Inflammation also causes macrophage migration into adipose tissue and increases in inflammatory cytokines such as TNF $\alpha$  to further increase insulin resistance and down-regulates the PPAR $\gamma$  gene-transcription factor needed to generate new, healthy fat cells<sup>223</sup>. On the other hand, PPAR $\gamma$  activity is increased by n-3 PUFAs and polyphenols<sup>223</sup>. Once insulin resistance and inflammation develop in the adipose tissue, increased levels of lipolysis cause higher levels of FFAs to enter circulation and are taken up by the liver and skeletal muscle, inducing lipotoxicity<sup>223</sup>. The inflammation seen in adipose tissue is not as rapid as that in the hypothalamus and is usually seen around 12-14 weeks<sup>223</sup>.



**FIGURE 32.** OBESE ADIPOSE TISSUE AND INFLAMMATION<sup>225</sup>

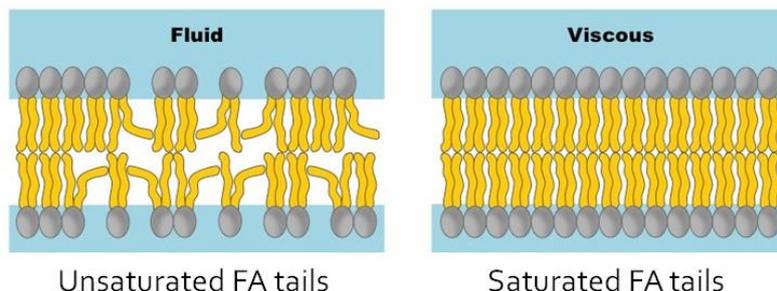
The *liver* can't safely store excess fat, and this leads to a buildup of fat deposits (non-alcoholic fatty liver disease)<sup>223</sup>. Macrophages are activated that lead to the release of cytokines that decrease the ability of insulin to reduce glucose production in the liver<sup>223</sup>. In addition, the rapid buildup of FA in the liver overwhelms its capacity to convert FA to TG and causes increased formation of diacylglycerol, which inhibits insulin signalling, promotes ER stress and can activate the protein kinase C pathway (PKC pathway mentioned in [Section 2.2.4](#))<sup>223</sup>.

In the *skeletal muscle*, the key area of glucose uptake, cytokines from other organs appear to have a role in generating insulin resistance<sup>223</sup>. SFA can also increase signalling of TLR-4 receptors and decrease FA oxidation of lipids, as well as increase the synthesis of ceramides, lipid molecules that have demonstrated a key role in the pathogenesis of diabetes and its complications<sup>223,226</sup>. Levels of FA oxidation can be increased in skeletal muscle with exercise<sup>223</sup>. These mechanisms may all act to increase inflammatory agents the pancreas is exposed to<sup>223</sup> (**Figure 33**).



**FIGURE 33.** EFFECTS OF ADIPOSE INFLAMMATION ON THE LIVER AND PANCREAS<sup>227</sup>

Another mechanism through which FAs are believed to affect the development of T2D is through their effects on *cell membranes*. The FA tails of the phospholipid bilayer can differ in both length and saturation (with double bonds reducing their ability to pack closely together), thus the FA composition of the cell membrane affects its fluidity (**Figure 34**), membrane protein incorporation, enzyme activities, and receptor functions<sup>228</sup>. As recent studies have demonstrated that the FA composition of the membrane is at least partially determined by the diet, this may have important health implications<sup>229,230</sup>. Participants randomly assigned to a diet with a high proportion of SFAs later showed higher proportions of SFAs in skeletal muscle phospholipids, compared to individuals with a high-proportion of MUFAs in their diet<sup>229</sup>. Increasing ratios of SFAs to PUFAs (*decreased fluidity*) in the skeletal muscle cell membrane has been observed to reduce insulin sensitivity and decrease glucose effectiveness through the GLUT<sub>4</sub> transporter in humans, which could theoretically lead to T2D<sup>231,232</sup>. However, both the proportion and absolute amount of FAs can affect plasma concentrations, so both may be important in determining skeletal muscle phospholipid composition as well, and could explain why we did not observe a similar relationship with SFAs in our study<sup>230</sup>. In addition, as the food preparation methods could alter the FA content differentially by FA group and type of food, this offers another theory to the discrepancy in our results with the biological studies<sup>233</sup>.



**FIGURE 34.** STRUCTURE OF THE CELL MEMBRANE<sup>234</sup>

FAs may also exert their effects through gene expression, as they act as signalling molecules of genes coding for metabolic enzymes<sup>235</sup>. Studies have shown that persistent exposure of pancreatic

islet cells to high levels of FFAs prevented transcription of acetyl-CoA carboxylase, an enzyme required for glucose-stimulated insulin release from the pancreas<sup>235</sup>.

A few studies have suggested that AA could stimulate insulin secretion<sup>236,237</sup> and suggested that the plasma concentration of AA could be a key determinant of insulin resistance<sup>238</sup>. By-products of AA have also been shown to be toxic to pancreatic  $\beta$ -cells<sup>223</sup>. But, the relationship between AA and insulin is not yet clear, as studies also suggest that in the case of diabetes, insulin may activate enzymes responsible for AA synthesis<sup>239</sup>. In addition, though AA and its products are classically viewed as proinflammatory agents in the literature<sup>223</sup>, it has also been observed to produce anti-inflammatory eicosanoids<sup>240</sup>. Pure DPA has been difficult to isolate and was not readily accessible, thus there is no literature on the biological relationship between DPA and T2D<sup>241</sup>. However, ALA has been observed to have anti-inflammatory effects, though much less potent than those of long-chain PUFAs<sup>240</sup>, and is suggested to activate PPAR $\gamma$ <sup>242</sup>. Further investigation into the biological effects of ALA is necessary.

### 1.6.6 STRENGTHS AND LIMITATIONS

The potential limitations should be considered. The dietary estimates are based on a single questionnaire at baseline, thus misclassification of exposure is possible. However, as the study is prospective, any effects are likely to be non-differential and would lead to an attenuation of the true association. T2D cases were carefully identified and validated, yet it is possible there was an under-ascertainment of T2D cases, especially in asymptomatic women. Finally, we were not able to adjust for contaminants such as mercury (found in fish) and persistent organic pollutants (major sources of which are fish, meat and dairy products) that have been associated with incident T2D in some studies<sup>243-245</sup>. However, epidemiological evidence on persistent organic pollutants has been considered inconsistent enough that studies suggest that the potential hazards of the low levels of exposure present in seafood do not outweigh the benefits of seafood consumption<sup>246</sup>. In addition, meta-analyses in East Asian countries where fish consumption is high demonstrate a decreased risk of T2D associated with high fish consumption<sup>142,247</sup>. Yet, interpretation of the findings should be cautious in the absence of adjustment for contaminants.

The strengths of this study include the prospective design that minimizes recall and selection biases and the large study population. The high rate of follow-up allowed the validation of many cases of incident T2D and the dietary questionnaire was validated and specific for this French population. Extensive information on potential confounders was collected, minimizing residual confounding. We also controlled for other FA groups besides the one in question in our analyses. This is important, as the fate of FAs depends heavily on the overall FA profile of the diet, likely due to competition between FAs for enzymes<sup>185</sup>.

## 1.7 CONCLUSION

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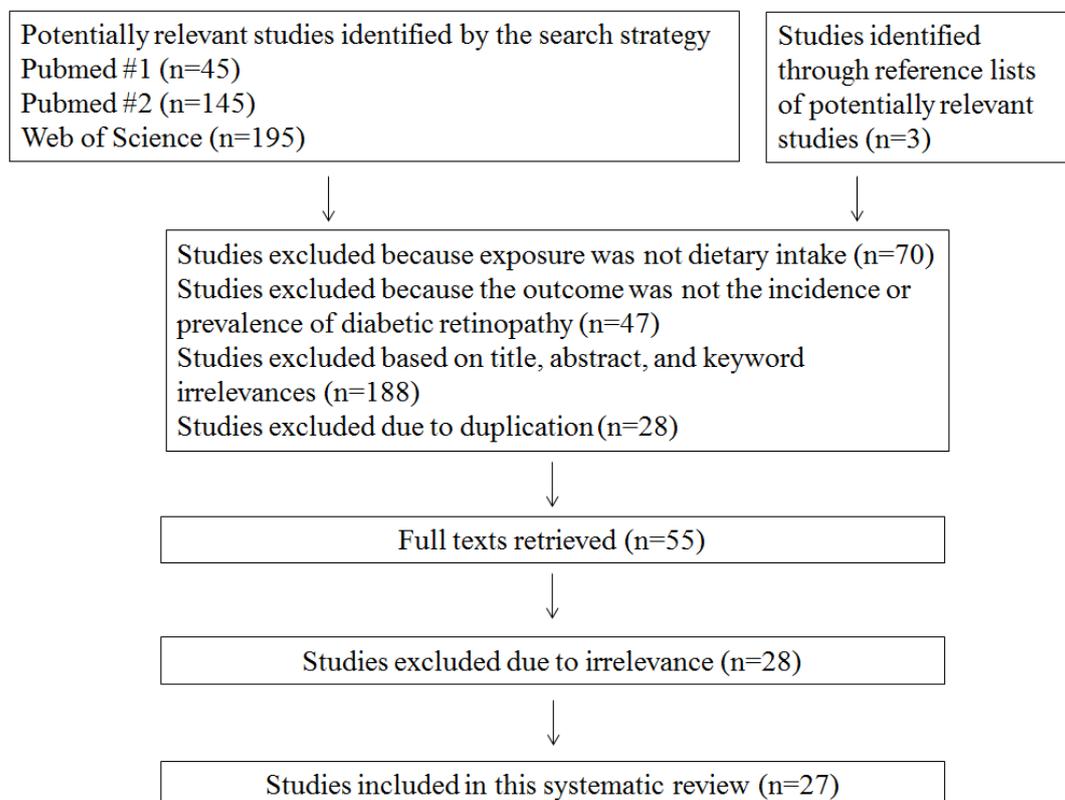
Despite the large body of evidence suggesting that PUFAs, and specifically n-3 PUFAs, are beneficial for cardiovascular health<sup>248</sup>, based on our results we could not recommend a high consumption of either as beneficial for diabetes prevention, as the effects of PUFAs appear heterogeneous within the group and even within subgroups. High AA and DPA intakes, and high ALA intake in overweight women were associated with an increased risk of incident T2D, independently of meat consumption, the primary food group contributor. The association could be even more pronounced in the general population, as our population is relatively homogeneous. Further investigation into the associations of individual dietary n-3 and n-6 PUFA intake, especially in European populations, men, and populations with greater variability in the socioeconomic profiles with the risk of T2D is warranted.

## 2. OBJECTIVE TWO: SYSTEMATIC REVIEW ON DIET AND DIABETIC RETINOPATHY

### 2.1 INCLUDED STUDIES

#### 2.1.1 RELEVANT STUDIES

The flow diagram for the process used for identifying and selecting relevant studies for this systematic analysis is depicted in **Figure 35**. A total of 385 potentially relevant studies were identified using the search parameters previously mentioned. Of these, 45 studies were identified using the Pubmed Mesh Terms, 145 from the Pubmed search of food items, and 195 from the Web of Science search. After reviewing the titles, abstracts, and if necessary, the full texts of all potentially relevant articles, 27 studies were identified for inclusion in this systematic review, of which 3 were identified through the reference lists of potentially relevant studies. Only one study on macular edema was identified<sup>249</sup>.



**FIGURE 35.** FLOW DIAGRAM OF THE SELECTION PROCESS FOR STUDIES INCLUDED IN THIS SYSTEMATIC REVIEW

#### 2.1.2 CHARACTERISTICS OF INCLUDED STUDIES

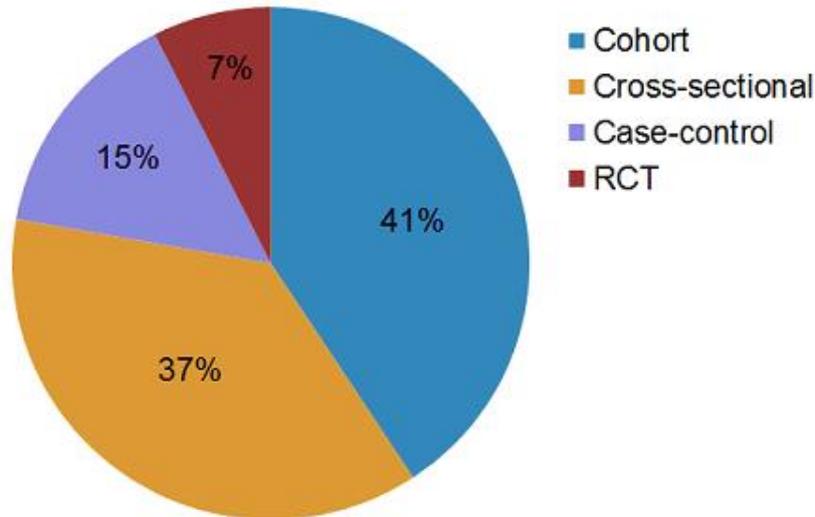
A summary of information extracted from the included studies can be found in **Table 9**. The majority were prospective studies (n=13)<sup>127,144,145,147,249-257</sup>, of which 6 were RCTs or nested case-control studies within RCTs<sup>127,147,249,252,254,256</sup> (**Figure 36**). One study, though prospective, collected some information cross-sectionally<sup>144</sup>. The remainder of the studies were cross-sectional (n=10)<sup>146,165,258-265</sup> or case-control studies (n=4)<sup>164,266-268</sup>. Most studies were conducted in Europe (n=12)<sup>127,147,164,249,250,252,254,256,258,259,264,266</sup>, with six in the US<sup>144,146,257,261,263,268</sup>, two in Australia<sup>165,262</sup>, six in Asia<sup>145,251,255,260,265,267</sup> and one throughout four continents<sup>253</sup>.

**TABLE 9.** GENERAL CHARACTERISTICS OF THE STUDIES INCLUDED AND THEIR SAMPLE POPULATIONS

Study	Publication Year	Country	Year of Conduct	Study Design	Sample Size	Age	Recruitment	Exposure	Outcome Assessment
Alcubierre et al. <sup>164</sup>	2016	Spain	2010-2013	Case-control	294	40-75	Participants of the Department of Ophthalmology's screening and treatment program for diabetic retinopathy	Intake of macronutrients (carbohydrates, protein, fat, fibre)	Ophthalmologist assessment
Beulens et al. <sup>258</sup>	2008	16 European countries	1989-1991	Cross-sectional	1528	15-60	EURODIAB Prospective Complications cohort	Alcohol consumption	Retinal photographs
Diaz-Lopez et al. <sup>127</sup>	2015	Spain	2003-2009	Prospective study nested in RCT	3614	55-80	PREDIMED (Prevención con Dieta Mediterránea) clinical trial	Mediterranean diet, enriched with either olive oil or nuts	Ophthalmologist assessment
Engelen et al. <sup>259</sup>	2014	16 European countries	1989-1991	Cross-sectional	1880	15-60	EURODIAB Prospective Complications cohort	Sodium and potassium intake	Retinal photographs
Ganesan et al. <sup>260</sup>	2016	India	N/A	Cross-sectional	1261	≥40	Sankara Nethralaya-Diabetic Retinopathy Epidemiology and Molecular Genetic Study 1	Fibre intake	Retinal photographs
Giuffre et al. <sup>266</sup>	2004	Italy	N/A	Case-control	1019	>40	Random recruitment in Casteldaccia	Risk factors for retinopathy	Retinal photographs
Horikawa et al. <sup>251</sup>	2014	Japan	1995-2003	Cohort	978	40-70	JDCS (Japan Diabetes Complications Study)	Sodium intake	Ophthalmologist assessment
Horikawa et al. <sup>255</sup>	2017	Japan	1995-2003	Cohort	936	40-70	JDCS (Japan Diabetes Complications Study)	Proportions of carbohydrate intake	Ophthalmologist assessment
Howard-Williams et al. <sup>252</sup>	1985	UK	1973-1982	RCT	149	<66	Radcliffe Infirmary Diabetic Clinic	Low-carb diet or modified fat diet	Ophthalmologist assessment
Lee et al. <sup>253</sup>	2010	14 countries	N/A	Cohort	1239	55-81	AdRem (Action in Diabetes and Vascular Disease: Preterax and Diamicon MR Controlled Evaluation (ADVANCE) Retinal Measurement) study	Alcohol consumption	Retinal photographs
Ma et al. <sup>267</sup>	2015	China	2013	Case-control	200	>18	Hospital of Xiang Cheng District, Suzhou	Green tea consumption	Ophthalmologist assessment
Mahoney et al. <sup>146</sup>	2014	US	2003-2006	Cross-sectional	155	≥40	NHANES 2003-2006 (National Health and Nutrition Examination Survey)	High flavonoid fruit and vegetable consumption index score (HFVC)	Retinal imaging exam

*Continued on following page*

Mayer-Davis et al. <sup>261</sup>	1998	US	1984-1990	Cross-sectional	387	20-74	San Luis Valley Diabetes Study	Antioxidant intake (vitamins C, E, and $\beta$ -carotene)	Retinal photographs
Martín-Merino et al. <sup>256</sup>	2016	UK	2000-2007	Nested case-control	17130	All ages	THIN (The Health Improvement Network) database	Risk factors for retinopathy	Computerized patient profiles
Martín-Merino et al. <sup>249</sup>	2017	UK	2000-2007	Nested case-control	2405	All ages	THIN (The Health Improvement Network) database	Risk factors for diabetic macular edema	Computerized patient profiles
McKay et al. <sup>262</sup>	2000	Australia	1992-1996	Cross-sectional	239	>40	VIP (Visual Impairment Project)	Risk factors for retinopathy	Ophthalmologist assessment
Millen et al. <sup>263</sup>	2003	US	1988-1994	Cross-sectional	930	$\geq 40$	NHANES III (The Third National Health and Nutrition Examination Survey)	Duration of vitamin C and E supplement use	Retinal photographs
Millen et al. <sup>257</sup>	2004	US	1987-1995	Cohort	1353	45-64	ARIC (Atherosclerosis Risk in Communities Study)	Vitamin C and E intake	Retinal photographs
Millen et al. <sup>244</sup>	2016	US	1987-1995	Cohort/Cross-sectional	1339	45-65	ARIC (Atherosclerosis Risk in Communities Study)	Dietary intake of vitamin D; vitamin D or fish oil supplement use	Retinal photographs
Roig-Revert et al. <sup>254</sup>	2015	Spain	2013-2014	RCT	208	25-80	10 centers	Supplements with antioxidants/n3-PUFA	Fundus exam and retinograph
Sahli et al. <sup>268</sup>	2016	US	1993-1995	Case-control	1430	45-65	ARIC (Atherosclerosis Risk in Communities Study)	Lutein intake	Retinal photographs
Sasaki et al. <sup>265</sup>	2015	Australia	2009-2010	Cross-sectional	379	>18	DMP (Diabetes Management Project)	Fatty acid intake	Retinal photographs
Sala-Vila et al. <sup>247</sup>	2016	Spain	2003-2009	Prospective study nested in RCT	3482	55-80	PREDIMED (Prevención con Dieta Mediterránea) clinical trial	Meeting the dietary long chain n3-PUFA recommended intake (500mg/day)	Review of medical records
Segato et al. <sup>264</sup>	1991	Italy	N/A	Cross-sectional	1321	N/A	Diabetic clinics in the Veneto region	Risk factors for retinopathy	Ophthalmologist assessment
Tanaka et al. <sup>245</sup>	2013	Japan	1995-2004	Cohort	978	40-70	JDCS (Japan Diabetes Complications Study)	Fruit intake	Ophthalmologist assessment
Yang et al. <sup>265</sup>	2012	Korea	2008-2009	Cross-sectional	998	>19	KNHANES (Korea National Health and Nutrition Examination Survey)	Risk factors for retinopathy	Retinal photographs
Young et al. <sup>250</sup>	1984	Scotland	N/A	Cohort	296	20-59	Male clinic population	Risk factors for retinopathy	Ophthalmoscopy



**FIGURE 36.** STUDY DESIGNS INCLUDED IN THE SYSTEMATIC REVIEW BY PROPORTION

## 2.2 QUALITY OF INCLUDED STUDIES

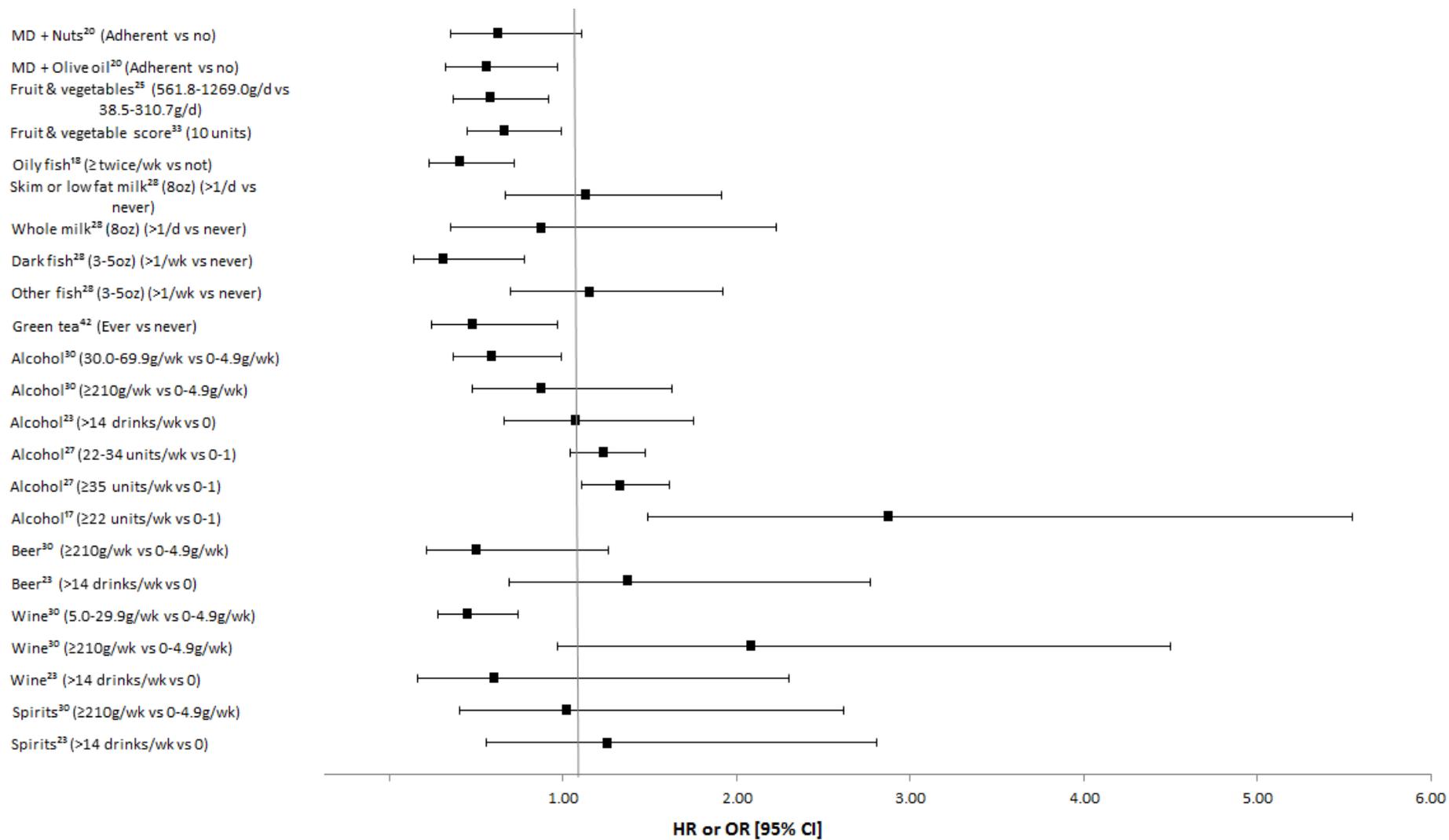
Both of the RCTs were determined to be of poor quality due to the lack of information presented in the papers<sup>252,254</sup>. Neither study elaborated on the method of randomization, allocation concealment, blinding of the participants, or blinding of the outcome assessment. In total, 14 studies were determined to be of good quality, 7 of medium quality and 4 of low quality. Most of the studies that were not considered high quality lost one or two stars due to failure to control for important confounding factors, such as the duration of diabetes<sup>127,146,257,259,262,264</sup>, diabetes treatment<sup>144,146,165,249,250,253,256,258-260,262,263,268</sup> or the level of glycated hemoglobin<sup>147,164,259,262,264,266</sup>. Many of the cross-sectional studies also did not provide adequate descriptions of the response rate or characteristics of the responders and non-responders<sup>144,146,259-264</sup>, so the majority (75%) of studies considered low quality were cross-sectional studies. Two of the studies considered to be of low quality also did not provide an adequate description of method of assessment of the exposure<sup>264,265</sup>.

## 2.3 RESULTS

### 2.3.1 DIETARY PATTERNS

Only one study evaluated the association between the diet as a whole and DR<sup>127</sup> (**Figure 37**). Diaz-Lopez *et al.* investigated the *Mediterranean diet*, enriched with either extra virgin olive oil or nuts and compared it with a low-fat diet in a prospective study spanning 6 years in over 3600 participants<sup>127</sup>. The Mediterranean diet enriched with olive oil was associated with more than a 40% decreased risk of retinopathy<sup>127</sup>. In addition, those in the highest quintile of adherence to the Mediterranean diet enriched with olive oil had more than a 60% decreased risk of retinopathy compared to those with the lowest adherence<sup>127</sup>. The Mediterranean diet enriched with nuts was associated with a 37% decreased, though non-statistically significant reduction in the risk of retinopathy<sup>127</sup>.





**FIGURE 37.** ODD RATIOS OR HAZARDS RATIOS AND 95% CONFIDENCE INTERVALS OF THE OUTCOMES OF STUDIES EXAMINING THE RELATIONSHIP OF DIETS, FOODS OR FOOD GROUPS WITH DIABETIC RETINOPATHY OR MACULAR EDEMA

### 2.3.2 FOOD GROUPS

Other studies have examined the intake of food groups (Figure 37). A Japanese cohort study of people with T2D found that high *fruit* consumption ( $\geq 173.2$ g per day, ie. a large apple or two bananas), was associated with more than a 50% decreased risk of incident DR, compared with those consuming less than 53.2g of fruit per day<sup>145</sup>. In addition, a cross-sectional study in the US associated a high flavonoid *fruit and vegetable* consumption index score with reduced odds of retinopathy<sup>146</sup>.



 *Milk* intake has also been analyzed; neither whole milk nor skim/low fat milk was associated with DR in a sample of over 1350 people with T2D<sup>144</sup>. On the other hand, *fish* may be beneficial in avoiding development of DR, intake of oily fish at least twice a week (versus less than twice a week), was associated with an almost 60% decreased risk of retinopathy<sup>147</sup>. Another study found that 85-141g of dark fish, (salmon, mackerel, swordfish, sardines, bluefish), per week versus never was associated with almost 70% decreased odds of DR<sup>144</sup>. However, 85-141g of "other fish", (cod, perch, catfish), per week was not associated with retinopathy<sup>144</sup>.

### 2.3.3 BEVERAGES

Ten studies looked at the intake of beverages, which included alcohol and green tea<sup>250,253,256,258,262,264-267</sup> (Figure 37). Seven studies had the goal of identifying risk factors for DR, which included *alcohol* intake<sup>249,250,256,262,264-266</sup>, while the primary goal of two studies was to examine the association between alcohol consumption and DR<sup>253,258</sup>. Four of these, one cohort and 3 cross-sectional studies, did not find an association between alcohol intake and the presence of retinopathy<sup>253,262,264,265</sup>, whether it be defined as "heavy alcohol drinking ( $\geq 4$ x alcoholic drinks/week)"<sup>265</sup> or ">14 standard drinks (1.5oz)"<sup>253</sup>. Giuffre *et al.*, in a case-control study, observed that the duration of daily alcohol consumption, (20 years or more), was not associated with DR<sup>266</sup>, while another study, a cohort study, found heavy alcohol consumption (>10 pints or equivalent/week) to be predictive of retinopathy<sup>250</sup>. In the UK, heavy alcohol consumption was associated with both DR and DME in two nested case-control studies<sup>249,256</sup>. Those consuming  $\geq 22$  units (1 unit = ~8g of ethanol) per week (compared to those consuming  $\leq 1$  unit) had almost three times the odds of DME<sup>249</sup>, and those and the heaviest drinkers ( $\geq 35$  units/week) had 1.3 times the odds of retinopathy<sup>256</sup>. However, a cross-sectional study conducted over 16 European countries detected an inverse association between drinking frequency and the odds of PDR, and a U-shaped association between alcohol consumption and PDR<sup>258</sup>. But, when examining alcohol types separately, only wine was associated with PDR in a U-shaped fashion, and beer and spirit consumption were not associated<sup>258</sup>.



 In a Chinese case-control study, with sex and age-matched diabetic controls without DR, the authors found that regular (every week for at least one year) *Chinese green tea* consumption was associated with decreased odds of retinopathy in women, but not men (OR=0.32 [0.13-0.75], OR=0.79 [0.30-2.06]; women and men, respectively)<sup>267</sup>.

### 2.3.4 MACRONUTRIENTS

Seven studies looked at the intake of macronutrients, including carbohydrates<sup>164,255</sup>, fatty acids<sup>147,164,165,252</sup>, fibre<sup>145,259</sup>, and protein<sup>164</sup>. Neither the intake of *carbohydrates* nor the proportion of carbohydrates as total energy, nor *protein* was associated with DR in either a cohort study or a case-control study<sup>164,255</sup>.

With regard to fats, a cross-sectional and a case-control study both found that *total fat* intake was not associated with the odds of DR<sup>164,165</sup>, however, there are conflicting results concerning individual

fatty acid groups (**Figure 38**). In Alcubierre *et al.*'s case-control study, *total SFA* consumption and *individual SFA* consumption (*palmitic* or *stearic acid*) was not associated with retinopathy risk<sup>164</sup>, while Sasaki *et al.*'s cross-sectional study of participants with well-controlled T1D or T2D (HbA1c <7.0%) noted higher odds of DR with increasing intake of SFA after stratification on diabetes control<sup>165</sup>. Results concerning *MUFA* intake are also mixed. Here, Sasaki *et al.* did not find an association with retinopathy, whereas Alcubierre *et al.* observed higher intake of MUFA and oleic acid to be associated with decreased odds of DR<sup>164,165</sup>. Alcubierre *et al.* did not observe an association between retinopathy and *TFA* intake, nor with *PUFA* intake, including *n-3 PUFA* and *n-6 PUFA*<sup>164</sup>. On the other hand, Sasaki *et al.* noted an inverse association of PUFA intake with DR, though only in people with well-controlled diabetes (HbA1c <7.0%)<sup>165</sup>, and one prospective study found that participants meeting the dietary recommendations for long-chain n-3 PUFA intake for cardiovascular prevention (500mg/day) had lower risk of retinopathy<sup>147</sup>. A Spanish RCT also demonstrated less onset of DR in people supplemented with antioxidants and n-3 PUFA<sup>254</sup>. In 1985, Howard-Williams *et al.* conducted a RCT where they randomly assigned participants to either a low-carb diet or a modified fat diet, rich in *linoleic acid* (LA)<sup>252</sup>. Compliers of the modified fat diet had DR less often than compliers of the low-carb diet and non-compliers, though the difference was not statistically significant<sup>252</sup>. Howard-Williams *et al.* also observed a greater frequency of DR in people with poorly controlled diabetes (HbA1c >8%) with low levels of linoleic acid (<50%) in cholesterol ester<sup>252</sup>. In contrast, Alcubierre *et al.* did not observe the intake of linoleic acid to be associated with DR<sup>164</sup>.

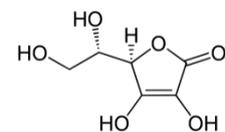


Concerning *fibres*, no association was observed in two studies but one Indian study showed beneficial effects of high fibre intake<sup>145,164,260</sup>. This cross-sectional study including T2D participants from the general population in India, demonstrated that a low fibre score was associated with up to a 41% increased odds of retinopathy<sup>260</sup>.

### 2.3.5 MICRONUTRIENTS

Seven studies examined the associations between intakes of micronutrients and retinopathy risk<sup>144,145,251,257,259,261,268</sup> (**Figure 39**). In two studies, a Japanese cohort of individuals with T2D, and a cross-sectional study across 16 European countries of individuals with T1D, neither *potassium* intake nor *sodium* intake was associated with risk of DR<sup>145,251,259</sup>. *Carotenoid* intake and DR were investigated by three studies<sup>145,261,268</sup>. The first was a case-control study of diabetic people enrolled in the Atherosclerosis Risk in Communities Study (ARIC). They did not observe an association between lutein intake and DR<sup>268</sup>. The other two studies looked at *β-carotene* intake and it was not associated with DR in one cross-sectional study of Hispanic and non-Hispanic white Americans<sup>261</sup>, but was associated with decreased risk of retinopathy in a Japanese cohort<sup>145</sup>.

Results concerning *vitamin C* are also not in agreement - a Japanese cohort study found high vitamin C intake (4<sup>th</sup> quartile) associated with a 40% decreased risk of DR<sup>145</sup>, while two cross-sectional studies did not show any association with DR<sup>257,261</sup>, with the exception of increased odds of DR in the 9<sup>th</sup> decile (but not 10<sup>th</sup>) of vitamin C intake in one study<sup>261</sup>. *Vitamin E* intake was not associated with DR in two studies, Millen *et al.*'s American cross-sectional study and Tanaka *et al.*'s Japanese cohort<sup>145,257</sup>, but was associated with a higher risk of DR in another American cross-sectional study, but only in people with diabetes not treated with insulin<sup>261</sup>. When Millen *et al.* stratified by treatment type, they did not observe any association in people exclusively treated with insulin or diet, but saw increased odds of DR among those taking oral hypoglycemic agents in the highest quartile of vitamin E intake<sup>257</sup>. In analyses stratified by glycemic control, those with poor glycemic control (defined as serum glucose ≥140mg/dL) and in the highest quartile of vitamin E intake, had more than doubled odds of DR compared to those in the lowest quartile group of intake, while no associations were observed for

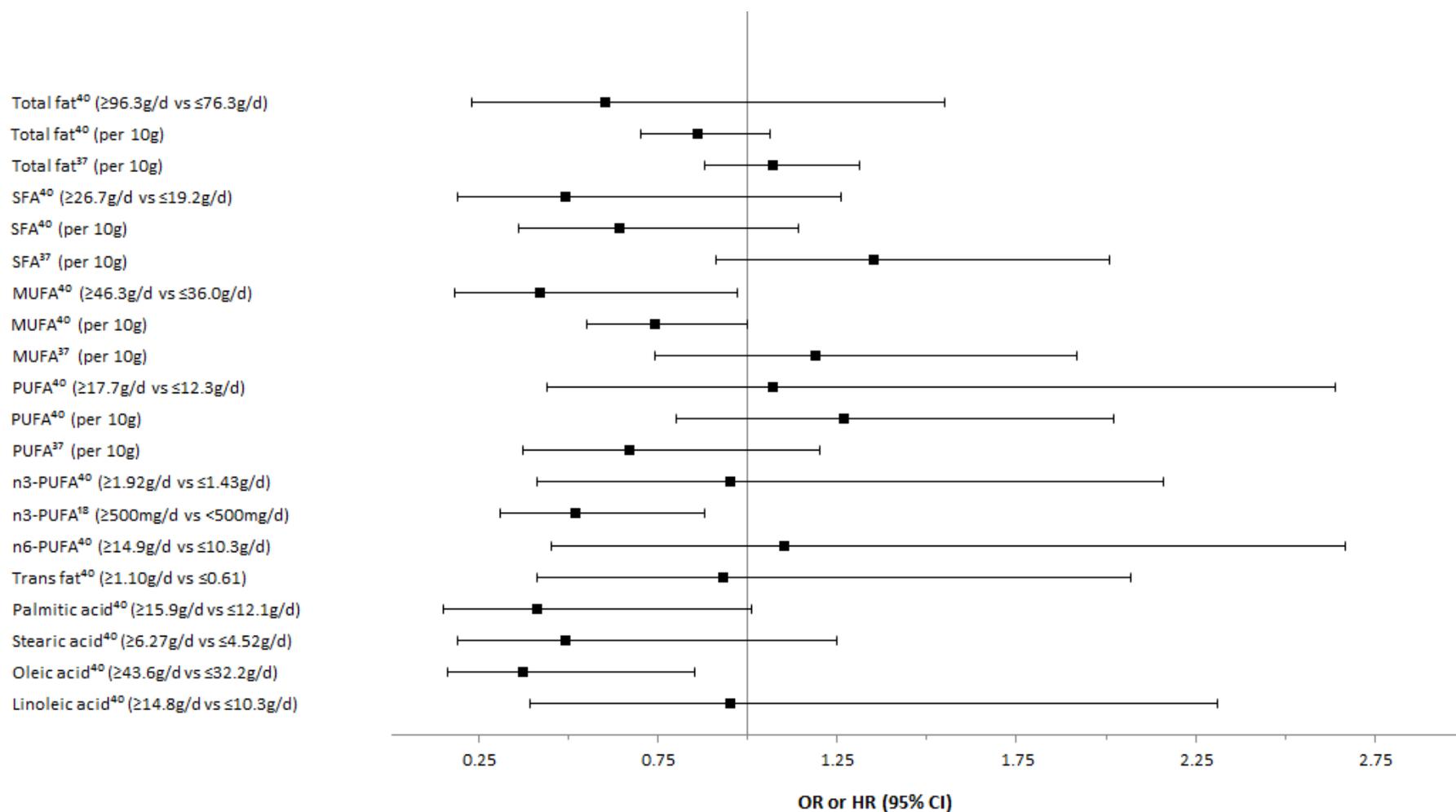


those with good glycemic control<sup>257</sup>. Tanaka *et al.* also tested for an interaction with HbA1c (<9% vs ≥9%), and vitamin E intake and found no association with DR risk. Dietary intake of *vitamin D* was also not associated with retinopathy in a large prospective study of over 1300 Americans<sup>144</sup>.

### 2.3.6 DIETARY SUPPLEMENTS

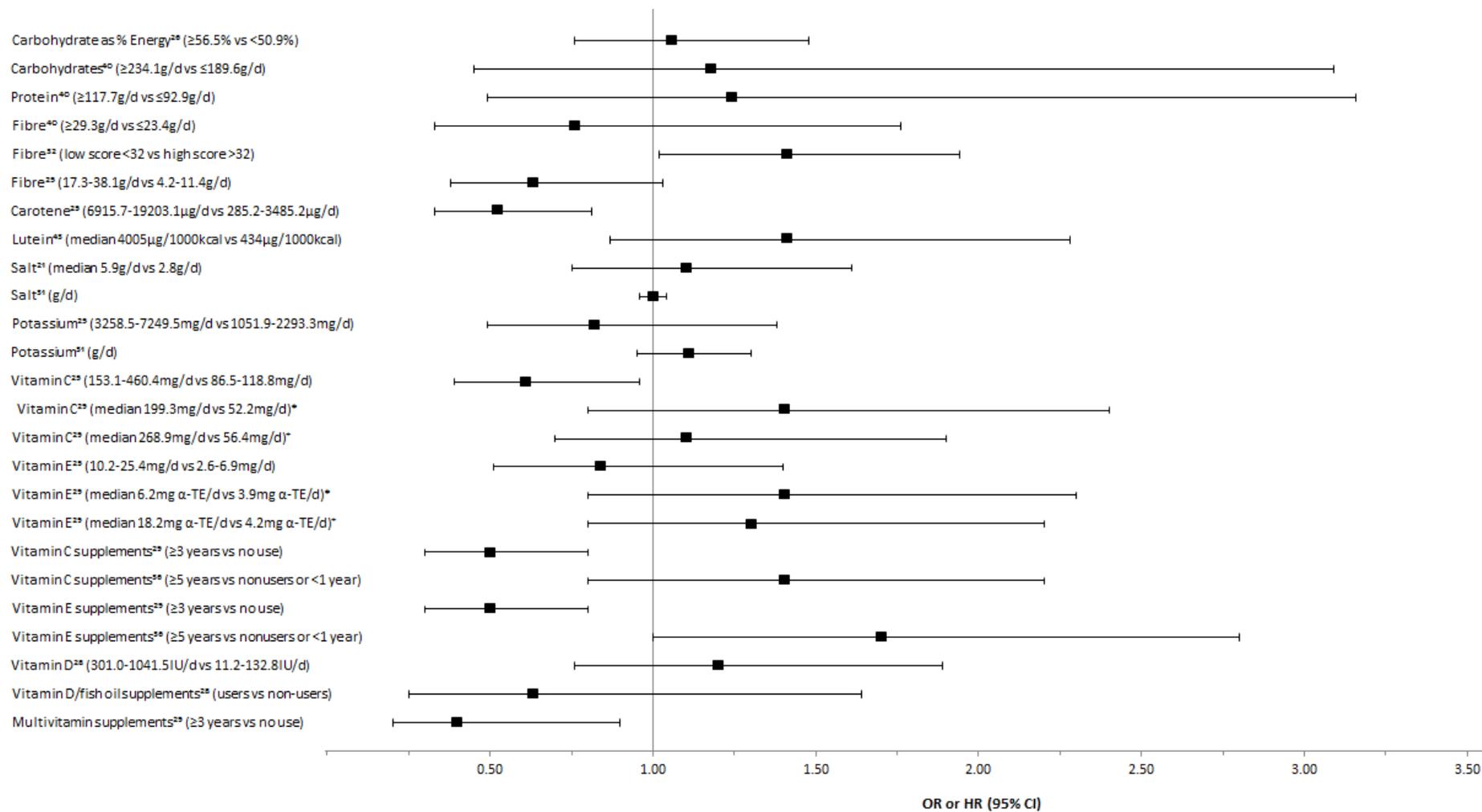
Four studies analyzed the association between dietary supplements and DR<sup>144,254,257,263</sup> (**Figure 39**). In a prospective study of participants of the ARIC, Millen *et al.* found no impact on the probability of DR when looking at *vitamin C and E* intake from food alone or food and supplements combined<sup>257</sup>. Yet, an interaction with race was detected with vitamin E; high intakes of vitamin E from food alone, or food and supplements combined, were associated with increased prevalence of DR in Caucasians, but not in African Americans<sup>257</sup>. Decreased odds of DR were noted in users (≥3 years) of vitamin C, E, and *multivitamin supplements* compared to non-users<sup>257</sup>. But, Millen *et al.*, in a cross-sectional analysis of NHANES III participants, did not find any association between long-time users of vitamin C or E supplements (≥5 years vs <1 year) and the presence of retinopathy<sup>263</sup>. Use of *vitamin D and fish oil supplements*, compared to non-use, was not associated with the odds of DR in the ARIC<sup>144</sup>. Regarding *n-3 PUFA supplements*, in an RCT where people with diabetes were randomly assigned to n-3 PUFA supplements for 18 months, those taking the supplements had lesser risk of DR<sup>254</sup>.





**FIGURE 38.** ODDS RATIOS OR HAZARD RATIOS AND 95% CONFIDENCE INTERVALS OF THE OUTCOMES OF STUDIES EXAMINING THE DIETARY INTAKE OF FATTY ACIDS AND THEIR ASSOCIATION WITH DIABETIC RETINOPATHY<sup>c</sup>

<sup>c</sup>SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n3-PUFA, omega-3 polyunsaturated fatty acids; n6-PUFA, omega-6 polyunsaturated fatty acids



**FIGURE 39.** ODDS RATIOS OR HAZARD RATIOS AND 95% CONFIDENCE INTERVALS OF THE OUTCOMES OF STUDIES EXAMINING DIETARY INTAKE OF MACRONUTRIENTS OR MICRONUTRIENTS AND THEIR ASSOCIATION WITH DIABETIC RETINOPATHY (\*FROM FOOD ONLY; \*FROM FOOD AND SUPPLEMENTS)

## 2.4 DISCUSSION

Out of 27 studies identified that explored the relationship between the diet and diabetic retinopathy or macular edema, the majority were prospective or nested within prospective studies and only one considered the diet as a whole. Two studies examined the effects of fruit and vegetable intake, two looked at fish intake, and one looked at milk intake, while others evaluated the intakes of macronutrients (carbohydrates, fatty acids, fibre, and protein), micronutrients (salt, potassium, lutein, carotene, and vitamins C, D and E), alcohol, green tea and dietary supplements of vitamins C, D, E or multivitamins.

### 2.4.1 THE MEDITERRANEAN DIET

The only study that examined the role of a dietary pattern, a post-hoc analysis of an RCT, found that the Mediterranean diet enriched with olive oil was associated with a decreased risk of incident DR compared to the low-fat control diet, with higher adherence conferring a larger risk reduction<sup>127</sup>. Through factor analysis, a cross-sectional study in Australia also identified a Mediterranean dietary pattern as protective against diabetic retinopathy<sup>269</sup>. The Mediterranean diet is a diet rich in fruit, vegetables, whole grains, plant proteins, fish, and low-fat dairy products (Figure 40) and has been associated with reduced risk of T2D<sup>270</sup> and improved glycemic control, body weight and cardiovascular risk factors in people with T2D<sup>271</sup>. The Mediterranean diet supplemented with mixed nuts was surprisingly not associated with reduced DR risk in the post-hoc analysis, though many of the nutrients or compounds found in nuts, and nuts themselves, have been associated with prevention of T2D<sup>272</sup>. But, within the same intervention groups, the glycemic loads and the glycemic indices of both Mediterranean diet groups showed favourable significant associations compared to the control group<sup>273</sup>. As glycemic control is the most important factor and is consistently associated with the development of DR, it is possible that the Mediterranean diet could be advantageous in the prevention of retinopathy through its beneficial effects on glycemic control<sup>72,80,101</sup> or perhaps through its effects on lipid profiles. In the same Spanish study, both Mediterranean diet interventions (supplemented with olive oil or nuts) improved high-density lipoprotein functions<sup>274</sup>.

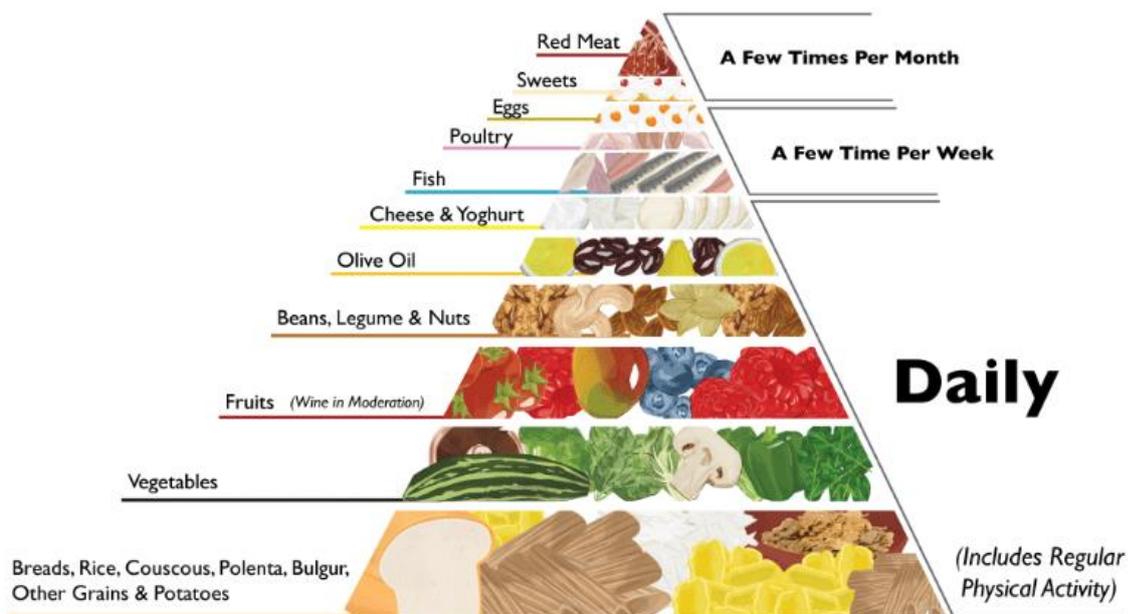


FIGURE 40. MEDITERRANEAN DIET FOOD PYRAMID<sup>275</sup>

#### 2.4.2 FRUIT, VEGETABLE AND FISH CONSUMPTION

Fruit, vegetables and fish play vital roles in the composition of the Mediterranean diet. In agreement with the studies on the Mediterranean diet, high fruit, vegetable and oily fish intake have been observed to confer strong protective effects on the development of retinopathy<sup>144-147</sup>. Evidence also suggests that higher fruit and vegetable consumption, or plant-based diets have a protective role in the development of T2D<sup>139</sup>, though the evidence concerning fish and T2D seems to be less clear<sup>276</sup>. Fish may exert its protective effects through its omega-3 or vitamin D content and fruit and vegetables may exert their protective effects through their antioxidant content, vitamins C and E, carotenoids or polyphenols. Trials have found that antioxidant supplementation was associated with reductions in ROS and slowed the progression of DR in subjects with non-proliferative DR<sup>277,278</sup>, and some polyphenols may also inhibit the onset of retinopathy<sup>279</sup>. However, the literature concerning the role of vitamins C and E in the development of DR is not consistent.

#### 2.4.3 MICRONUTRIENT CONSUMPTION

Vitamin C intake was associated with decreased risk of DR in one prospective study<sup>145</sup>, however, this inverse association was not reported in two cross-sectional studies<sup>257,261</sup>. Vitamin C supplement use could suggest that vitamin C aids in DR prevention, as users were found to have decreased odds of DR compared to non-users<sup>257</sup>, but the duration of supplement use may not be a factor<sup>263</sup>. The benefits of vitamin C in DR are supported by five cross-sectional, hospital-based studies in people with diabetes, which have consistently reported lower serum vitamin C levels in those with DR than in those without DR<sup>280</sup>. A cross-sectional study among NHANES III participants also observed an inverse association between serum vitamin C and DR, though not statistically significant, and only after the exclusion of vitamin C supplement users<sup>263</sup>.



Overall, studies did not find an association between vitamin E and DR<sup>145,257,261</sup>. Yet, vitamin E demonstrated positive associations with the odds of DR in people not treated with insulin<sup>261</sup>, those taking oral hypoglycemic agents<sup>257</sup>, and those with poor glucose control in a cross-sectional<sup>257</sup>, but not a cohort study<sup>145</sup>. However, the participants of this cohort had well controlled HbA1c, BMI, triglycerides and systolic blood pressure and thus may have lacked the heterogeneity for demonstrating the interaction<sup>145</sup>. In contrast, vitamin E supplement use has demonstrated an inverse association with DR<sup>257</sup>, though the duration of use does not seem to play a role<sup>263</sup>. Cross-sectional, hospital-based studies examining vitamin E concentrations are inconsistent with their results<sup>145</sup>. A systematic review found that two studies showed no association between vitamin E levels in people with diabetes with vs. without retinopathy, but one study reported higher vitamin E levels in people with diabetes with DR than in people with diabetes without DR<sup>280</sup>.

Neither vitamin D intake, nor vitamin D or fish oil supplements were associated with retinopathy in a cross-sectional study<sup>144</sup>. However, those who were vitamin D deficient (25-hydroxyvitamin D (25(OH)D) <75nmol) had 61% higher odds of DR in this study<sup>144</sup> and 27% higher odds in meta-analyses of 14 other observational studies (25(OH)D <20ng/mL)<sup>281</sup>.



With regards to carotenoids, lutein intake was not significantly associated with retinopathy<sup>268</sup>. But, in a prospective study, serum concentrations of lutein were lower in people with diabetes with non-proliferative DR compared to people with diabetes without DR<sup>282</sup>. In addition, a cross-sectional study suggested beneficial effects of high combined lutein/zeaxanthin and lycopene plasma concentration towards DR risk<sup>283</sup>. Concerning carotene, higher  $\beta$ -carotene intake was associated with a decreased risk in one study and had no association with DR in another<sup>145,261</sup>. But, after stratification of participants treated by insulin vs. not treated by insulin, a positive association was observed between  $\beta$ -carotene intake and severe DR in those treated with insulin<sup>261</sup>. Plasma concentrations suggest the strongest predictor of DR could be the ratio of the plasma

concentrations of non-provitamin A carotenoids (lutein, zeaxanthin, lycopene) to provitamin A carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin)<sup>283</sup>.

The literature addressing vitamins C, D, E and carotenoid intake is not entirely consistent. It suggests either a positive or no effect of vitamin C intake and a negative, or no effect of vitamin E intake, with the exception of vitamin E supplements, which may be beneficial. The favourable effects of these vitamins could partially explain the inverse association high fruit and vegetable consumption has with the risk of DR, though this hypothesis is neither supported nor refuted by biomarkers of vitamin C and E. Though oily fish intake showed favourable associations on DR outcomes, its effects may not act through its vitamin D content, as neither intake nor supplements were shown to have an association with DR. Results concerning carotenoid intake are similarly varying, with plasma concentrations offering no clarification on the subject. However, results from studies using plasma biomarkers should be interpreted with caution with concerns to dietary intake. Only moderate correlations ( $<0.5$ ) have been observed between plasma measures of vitamin C, E, and carotenoids and estimated dietary intake<sup>284,285</sup>. 25(OH)D may also not be the optimal indicator of dietary vitamin D intake, as it explained only 1% of the between person variation in serum concentrations of 25(OH)D, which reflects all sources of vitamin D<sup>144</sup>.



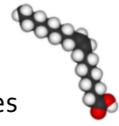
Other discrepancies in the results could be due to the fact that some studies did not adjust for important confounders that could influence the relationship such as the duration of diabetes<sup>257</sup> or diabetes treatment<sup>144,263,268</sup>, or it could be attributed to the fact that five studies were conducted in Western populations<sup>144,257,261,263,268</sup>, while the last was in a Japanese population<sup>145</sup>. The sources, quantities ingested, and methods of food preparation of foods containing the micronutrients in question may all vary due to cultural differences. Indeed, favourable effects of vitamin C and  $\beta$ -carotene were both observed in the Japanese cohort, but not in any of the Western studies. Inconsistencies may also be due to ethnicity; the ARIC population was approximately a third African-American, with the remainder being Caucasian American<sup>144,257,268</sup>, while Mayer-Davis *et al.*'s population was 65% Hispanic and 35% non-Hispanic Caucasians<sup>261</sup>, and the NHANES III study population was more than two thirds non-Hispanic white, with the majority of the remainder being non-Hispanic black and a minority being Mexican American (5-10%)<sup>263</sup>. Ethnicity and BMI have been found to interact to influence the development of diabetes<sup>286</sup>, and ethnic differences in risk of developing macrovascular and some microvascular complications of diabetes have also been reported<sup>287</sup>. In addition, these studies, with the exception of the Japanese study, were not prospective in design, hindering the ability to infer temporality. One study may have also have been restricted as their data were based on a single 24h recall, which may not be representative of long-term intake<sup>261</sup> and the Japanese study may have been limited by the lack of variation in their population of type 2 diabetics with well-controlled diabetes and BMIs<sup>145</sup>. For these reasons, there is an urgent need for prospective studies to evaluate the associations of the intakes of these important micronutrients and the risk of DR.

Studies on other micronutrients and retinopathy risk are less controversial, but also less numerous. Potassium intake was not associated with DR risk<sup>145,259</sup>, but serum potassium has been inversely associated with fasting glucose<sup>288</sup>, insulin sensitivity<sup>289</sup>, and DR risk<sup>288,290,291</sup>. However, other studies have not found an association between serum or dietary potassium and incident T2D<sup>289-291</sup>. It is possible that potassium intake may be influenced by race, as one study uncovered an interaction with race which suggested lower intakes of potassium beneficial in Caucasians and detrimental in African-Americans for T2D risk, though the association was not linear<sup>291</sup>. The relationship between potassium and DR remains unclear for the moment. Within the same study populations, salt intake was also not associated with DR<sup>251,259</sup>. High intakes of salt have been found to have detrimental effects with regards to cardiovascular disease<sup>292</sup>, but its role in the development of T2D remains

unclear. Urinary sodium excretion has been associated with end-stage renal disease, microalbuminuria, and all-cause mortality in those with T1D<sup>259,293</sup>, but the association in individuals with T2D has been less studied and warrants attention.

#### 2.4.4 MACRONUTRIENT CONSUMPTION

Neither total fat<sup>164,165</sup>, trans fat<sup>164</sup>, total SFA<sup>164,165</sup>, nor individual SFA intakes<sup>164</sup> were associated with DR, until one study stratified by glycemic control, and observed increased odds of DR with increased SFA intake among participants with well-controlled diabetes (HbA1c <7.0%)<sup>165</sup>. It is possible that the effects of SFA differ by diabetes control, or that the effects of SFA vary by type (even chain vs. odd chain or very-long-chain), making it more accurate to stratify analyses<sup>160</sup>. Sasaki *et al.* did not observe effects of MUFA with retinopathy<sup>165</sup>, while Alcubierre *et al.* observed MUFA and oleic acid to be inversely associated with odds of DR<sup>164</sup>. But, MUFA intake in Sasaki *et al.*'s Australian population may not have been strong enough to detect an association, as the median intake (23.6 [19.9,26.3], 23.5 [19.3, 25.3], 23.7 [20.0, 26.7]; median intake [interquartile range] g/day for all participants, those with well-controlled (HbA1c <7%) diabetes, and those with poorly controlled diabetes, respectively) appears much lower than the average intake in Alcubierre *et al.*'s Spanish population (43.6 [12.8], 39.9 [14.2]; mean [standard deviation] g/day for participants without DR and with DR, respectively)<sup>164,165</sup>. Some evidence suggests PUFA intake to be beneficial in preventing DR<sup>147,165,254</sup>, though one study did not observe any associations<sup>164</sup>. Supplementation with n3-PUFA has been shown to decrease the number of retinal acellular capillaries associated with diabetes and inflammatory markers in the retina of diabetic animal models<sup>294,295</sup>, but it may be necessary to examine the effects of PUFAs separately, as their effects may differ within the group, and even within the subgroups<sup>296</sup>.



Regarding fibre intake, two studies did not find an association with DR, but an Indian study observed an inverse relationship<sup>145,164,260</sup>. The observed differences could be due to the cultural and ethnic differences in the populations, or perhaps because the Japanese cohort was part of a randomized trial originally initiated to assess the effectiveness of lifestyle intervention on diabetic complications, this population may have had more access to information that could help them better manage their diabetes. Fibre intake has been shown to improve glycemic control in people with T2D, including Japanese people with T2D, presenting another important possibility for further investigation<sup>297,298</sup>.



#### 2.4.5 BEVERAGE CONSUMPTION

Green tea was associated with DR prevention<sup>267</sup> and has also been found to be associated with decreased risk of developing T2D<sup>299</sup>, lower fasting blood glucose levels<sup>300,301</sup>, and reduced serum insulin concentrations<sup>301</sup>. Results concerning alcohol are much more inconsistent. The majority of studies did not find an association between alcohol intake and DR<sup>253,262,264-266</sup>, while three observed a positive association with DR or DME<sup>249,250,256</sup>, and one a U-shaped distribution with DR<sup>258</sup>. But, heavy drinking is also associated with decreased diet quality<sup>302,303</sup> which could explain the observed association, as the diet was not taken into account during the analyses. In addition, in one study, beer and spirit consumption was not associated with the risk of proliferative DR, but wine consumption was associated with DR in a U-shaped fashion<sup>258</sup>. As moderate wine consumption has been associated with decreased incidence of cardiovascular disease, diabetes, hypertension and some types of cancer (colon, basal cells, ovarian and prostate carcinoma), it seems necessary to separately examine the effects of different types of alcohol<sup>304</sup>. However, a cohort study across 14 countries did not note differential associations between alcohol consumption by type (beer, wine or spirits) and DR risk, but, the authors cite a lack of power due to a small number of cases of DR as a possible reason for this<sup>253</sup>.

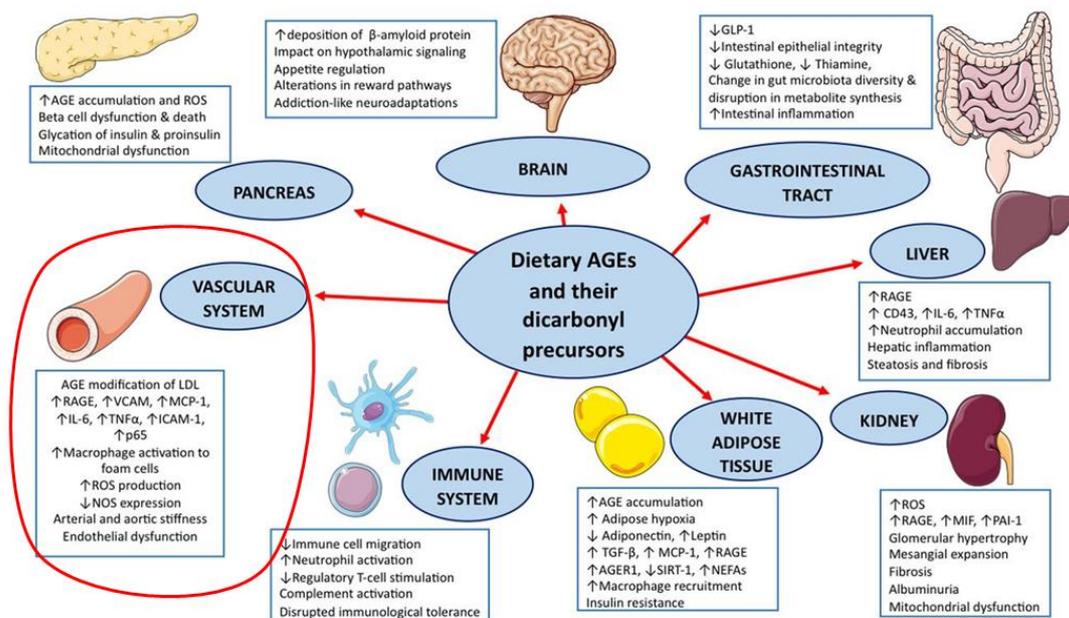


### 2.4.5 BIOLOGICAL MECHANISMS

As badly managed diabetes is more likely to lead to DR<sup>4</sup>, intake of foods or nutrients that improve glycemic control are likely to be beneficial in the prevention of DR. Studies have shown that hyperglycemia, along with the duration of diabetes, act as the strongest predictors for DR<sup>72,80,101</sup>. Hyperglycemia may act to influence the development of retinopathy through several pathways: the polyol pathway, non-enzymatic protein glycation, activation of protein kinase C, activation of the hexosamine pathway, build-up of ROS, induction of hypoxia-inducible factor and activation of the renin-angiotensin-aldosterone system<sup>72,80,101</sup> (See [Introduction Section 2.2.4](#)).

Besides having effects on glycemic control, the diet may affect DR development because the diet is an exogenous source of AGEs. AGEs are a product of non-enzymatic protein glycation, a reaction that starts occurring naturally during embryonic development in all tissues<sup>72,80</sup>. Throughout our lives, AGEs accumulate slowly in our bodies and can cause destruction, as they are able to alter protein structure and function in places including the BM and vessel walls<sup>72,80</sup> (**Figure 41**). They can also interact with cell surface receptors and lead to inflammation and oxidative stress<sup>72,80</sup>. Their levels in the serum, skin and cornea are associated with the onset or severity of DR and there is also evidence of their implication in almost all diabetic complications, other chronic diseases (including cardiovascular disease and Alzheimer's), and aging<sup>72,80,305,306</sup>.

AGEs are present in many foods, with animal-derived foods high in fat and protein being the largest contributors<sup>307</sup>. Uribarri *et al.*, after analyzing the levels of two well-known AGEs, found that the highest amounts were found in beef and cheese, followed by poultry, pork, fish, and eggs. Though fruit and vegetables contribute less to the consumption of dietary AGEs, fruit still has high amounts of fructose, which is susceptible to react with proteins and form AGEs<sup>308</sup>. The method of food preparation also has an effect on the levels of AGEs, as longer exposure to higher temperatures and low moisture were found to increase AGE formation compared to shorter heating times, lower temperatures and high moisture<sup>307</sup>. A significant portion of the Western diet includes meats and dairy products, which are often exposed to high temperatures. In addition, high fructose corn syrup is frequently added to drinks and baked goods, explaining why the Mediterranean diet could be beneficial compared to a Western diet and offering dietary AGE restriction as a potentially critical target in preventing DR<sup>308,309</sup>.



**FIGURE 41.** DIETARY AGEs AND THEIR EFFECTS ON THE BODY<sup>310</sup>

Fruit and vegetables may exert protective effects through their polyphenol content, as polyphenols have been shown to improve glucose homeostasis and insulin resistance, as well as to reduce inflammation<sup>279</sup>; alternatively, they could act through other antioxidants, such as vitamins C, E, or carotenoids, which have been observed to reduce neovascularization, restore retinal blood flow, and scavenge free radicals<sup>311</sup>. Vitamins C and E have been shown to suppress VEGF production in animal models and decrease AGE accumulation; in addition, vitamin C may also decrease protein kinase C activation<sup>280</sup>, prevent glucose-driven apoptosis of pericytes<sup>312</sup>, and reduce oxidative stress in human retinal pigment epithelium<sup>313</sup>. Diabetic animal models have also demonstrated favourable effects of long-term antioxidant administration. Compared to the diabetic controls fed standard diets, diabetic rats fed diets supplemented with vitamins C and E, or with an antioxidant mixture, (including vitamins C, E, and  $\beta$ -carotene), developed significantly fewer acellular capillaries and pericyte ghosts, suggesting antioxidant supplementation may be efficient in inhibiting the development of retinopathy<sup>314</sup>.

Fatty acids may affect DR through several pathways. Firstly, just as the accumulation of glucose increases flux through the protein kinase C pathway, so does the accumulation of long-chain fatty acids<sup>81</sup>. Secondly, the retina is an extremely oxidative environment rich in PUFAs, and an accumulation of lipids can result in lipid peroxidation and in advanced lipoxidation end products (ALEs), which are very similar to AGEs and can accumulate in tissues<sup>80,81,309</sup>. Both ALEs and AGEs exert part of their influence through the receptor for AGEs, which activates a sustained response of the proinflammatory transcription factor NF- $\kappa$ B, and will also diminish antioxidant defenses, depress the immune system, impair DNA repair, and increase both the build-up of toxins in tissues and the rate of infection<sup>308,309</sup>. Evidence suggests that lipids may be as important as carbohydrates in the chemical modification of protein and accumulation of AGEs/ALEs<sup>308</sup>.

Ethanol has been reported to be associated with decreases in glutathione levels<sup>315</sup>, an important antioxidant whose depletion is also a result of increased flux through the polyol pathway<sup>72,80</sup>. Ethanol may also increase lipid peroxidation and the generation of free radicals<sup>315,316</sup>. On the other hand, green tea contains polyphenols that may scavenge ROS and exert anti-inflammatory effects<sup>317</sup>.

## 2.5 CONCLUSION

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The diet is an important factor in the development of diabetes and is suspected to play a role in the development of retinopathy, yet relevant studies are limited. Many aspects of the diet have not yet been addressed and there remains an urgent need for prospective studies. Given that retinopathy affects a third of individuals with diabetes, it is important to further explore the role of the diet, in order to prevent complications in individuals already burdened with a chronic disease. A Mediterranean diet, as well its key components such as fruit, vegetables and fish, is believed to be beneficial in the prevention of diabetes and likely in the prevention of diabetic retinopathy, offering a promising path of dietary intervention that necessitates immediate attention.

### 3. OBJECTIVE THREE: FATTY ACID INTAKE AND DIABETIC EYE DISEASE

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#### 3.1 BASELINE CHARACTERISTICS

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The characteristics of the E3N AfterDiab participants are presented in **Table 10**. The average age at diagnosis of T2D was 64.1 years and the average duration of diabetes was 9.9 years. Most women had more than a high school diploma, were overweight or obese, and were non-smokers. More than half of women reported HbA<sub>1c</sub> in the normal range (<7%), and more than 80% treated their T2D with oral hypoglycemic agents. About three quarters of the study population was not hypertensive and not taking treatment for hypercholesterolemia. Women with DED had a longer duration of diabetes ( $p<0.05$ ), were more likely to report a normal HbA<sub>1c</sub>, and were more often treating their T2D with only oral hypoglycemic agents than women without DED.

#### 3.2 FATTY ACID CONSUMPTION

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**Table 11** shows the ORs and 95% CIs of DED by group of FA consumption. In univariate and fully adjusted analyses, neither SFA, MUFA, PUFA, n-3 PUFA nor TFA intake was significantly associated with the presence of DED, and there was no evidence of a linear relationship. Cubic spline regression did not suggest evidence of non-linear relationships either (**Figure 42**). However, n-6 PUFA intake was significantly associated with reduced odds of DED in the middle tertile group of intake (tertile 2 median 12.6 g/day; OR=0.49 [0.27-0.87]), and this association did not change with adjustment for other confounders in the fully adjusted model (tertile 2 median 12.6 g/day; OR=0.50 [0.28-0.89]). Cubic spline regression indicated a significant overall association ( $p=0.05$ ) and a significant non-linear association ( $p=0.04$ ) of n-6 PUFA intake with DED, such that moderate amounts were associated with decreased odds.

Concerning individual omega-3 and omega-6 fatty acids, a moderate EPA intake was associated with increased odds of DED in the univariate model (tertile 2 median 0.1 g/day; OR=1.80 [1.03-3.15]), but this association was attenuated after adjustment for confounders in the fully adjusted model (tertile 2 median 0.1 g/day; OR=0.76 [1.00-3.12]) (**Table 12**). There was no evidence of either a linear or non-linear association between EPA intake and DED (**Figure 43**). Moderate LA intake was associated with reduced odds of DED in the univariate model (tertile 2 median 12.4 g/day; OR=0.49 [0.27-0.86]) and this association remained similar after adjustment for confounders in the fully adjusted model (tertile 2 median 12.4 g/day; OR=0.48 [0.26-0.86]). Cubic spline regression suggested a significant non-linear association of LA intake with the presence of DED ( $p=0.04$ ), such that moderate intakes were associated with reduced odds. No relationship was observed between the tertile groups of intake of ALA, DPA, DHA, and AA with the presence of DED in univariate or fully adjusted analyses, and there was no indication of a linear relationship. However, a non-linear association of AA was associated with the presence of DED ( $p=0.01$ ), suggesting that AA was associated with increased odds of DED.

**TABLE 10.** BASELINE CHARACTERISTICS OF THE STUDY POPULATION (E3N-AFTERDIAB COHORT; N=1949 WOMEN)

Variables	Overall Population (n=1949)	SD or (n)	Cases (n=77)	SD or (n)	Non-cases (1872)	SD or (n)	P-value
Age of diabetes (years)	64.1	7.5	64.2	8.1	64.1	7.5	0.89
Duration of diabetes (years)	9.9	4.6	11.6	4.3	9.8	4.6	<0.01
Daily energy intake (kcal/day)	2320.2	681.8	2256.2	643	2322.9	683	0.40
Level of Education (% (n))							
Less than high school diploma	15.6	304	20.8	16.0	15.4	288	0.31
High school + 2 years of university	56.9	1108	49.4	38.0	57.2	1070	
≥2 years of university	27.6	537	29.9	23	27.5	514	
BMI (kg/m <sup>2</sup> ) (% (n))							
Normal (<25)	27.8	541	22.1	17	28.0	524	0.35
Overweight (25-30)	40.6	792	48.1	37	40.3	755	
Obese (≥30)	31.6	616	29.9	23	31.7	593	
Physical activity (MET-h/week) (% (n))							
Quartile 1 (<12)	25.7	500	24.7	19	25.7	481	0.71
Quartile 2 (12-24)	24.2	471	28.6	22	24.0	449	
Quartile 3 (24-44)	25.4	495	20.8	16	25.6	479	
Quartile 4 (≥44)	24.8	483	26.0	20	24.7	463	
Smoking status (% (n))							
Non-smoker	53.1	1035	48.1	37	53.3	998	0.39
Ex-smoker	39.7	773	46.8	36	39.4	737	
Current smoker	7.2	141	5.2	4	7.3	137	
Family history of diabetes (% (n))							0.37
No	59.6	1162	64.9	50	59.4	1112	
Yes	27.8	541	20.8	16	28.0	525	
Unknown	12.6	246	14.3	11	12.6	235	
HbA1c (% (n))							
Normal	54.1	1055	40.3	31	54.7	1024	0.03

*Continued on the following page*

Elevated ( $\geq 7\%$ )	28.3	552	33.8	26	28.1	526	
Unknown	17.6	342	26.0	20	17.2	322	
Diabetes treatment (% (n))							
No treatment or diet only	4.3	84	2.6	2	4.4	82	<0.0001
Only oral hypoglycemic agents	83.9	1635	71.4	55	84.4	1580	
Insulin/GLP-1 + oral hypoglycemic agents	11.8	230	26.0	20	11.2	210	
Hypertension (% (n))							
Normal	75.3	1468	83.1	64	75.0	1404	0.11
Hypertensive	24.7	481	16.9	13	25.0	468	
Hypercholesterolaemia treatment (% (n))							
No	73.4	1431	71.4	55	73.5	1376	0.20
Yes	14.4	280	10.4	8	14.5	272	
Unknown	12.2	238	18.2	14	12.0	224	
Fatty acids(g/day)							
SFA	37.9	14.6	38.5	15.3	37.9	14.3	0.70
MUFA	32.7	11.1	32.9	11.8	32.7	11.1	0.89
PUFA	15.3	6.0	14.7	7.2	15.4	6.0	0.47
n3-PUFA	1.6	0.6	1.6	0.6	1.6	0.6	0.33
n6-PUFA	13.7	5.6	13.2	6.9	13.7	5.6	0.50
TFA	1.6	0.7	1.7	0.7	1.6	0.7	0.62
ALA	1.1	0.4	1.0	0.4	1.1	0.4	0.33
EPA	0.2	0.1	0.2	0.1	0.2	0.1	0.49
DPA	0.1	0.0	0.1	0.0	0.1	0.0	0.61
DHA	0.3	0.2	0.0	0.2	0.3	0.2	0.47
LA	13.4	5.6	12.8	6.8	13.4	5.5	0.48
AA	0.3	0.1	0.3	0.1	0.3	0.1	0.47
Dairy Products	359.0	209.4	352.7	197.2	359.1	210.5	0.79

*Continued on the following page*

Meats	132.2	61.9	144.9	81.6	131.7	61.1	0.16
Fish and seafood	42.6	30.2	40.6	28.7	42.6	30.4	0.57
Sauces	17.2	9.7	17.1	11.0	17.2	9.6	0.95

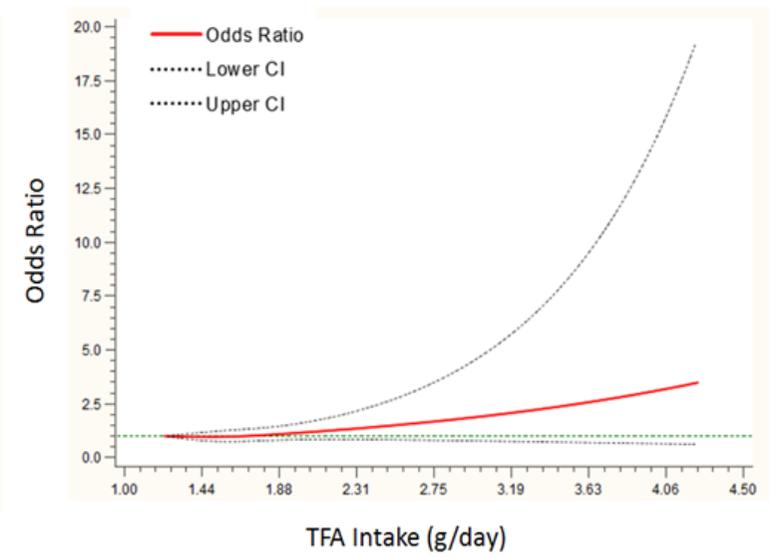
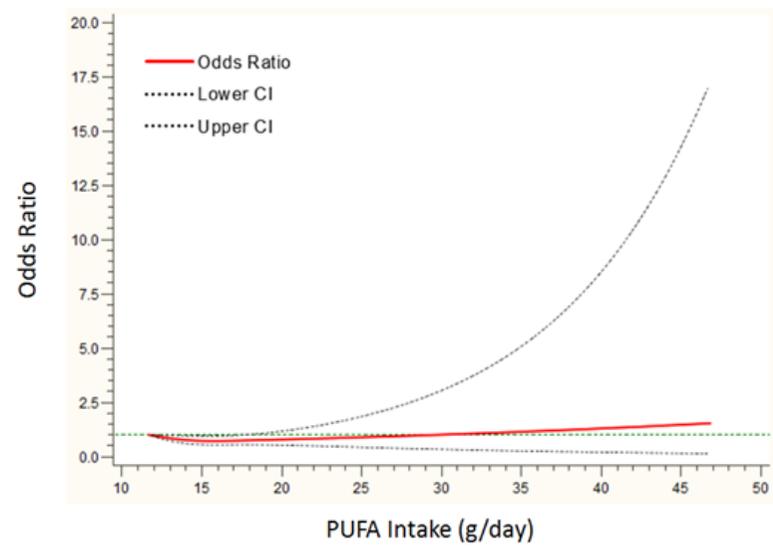
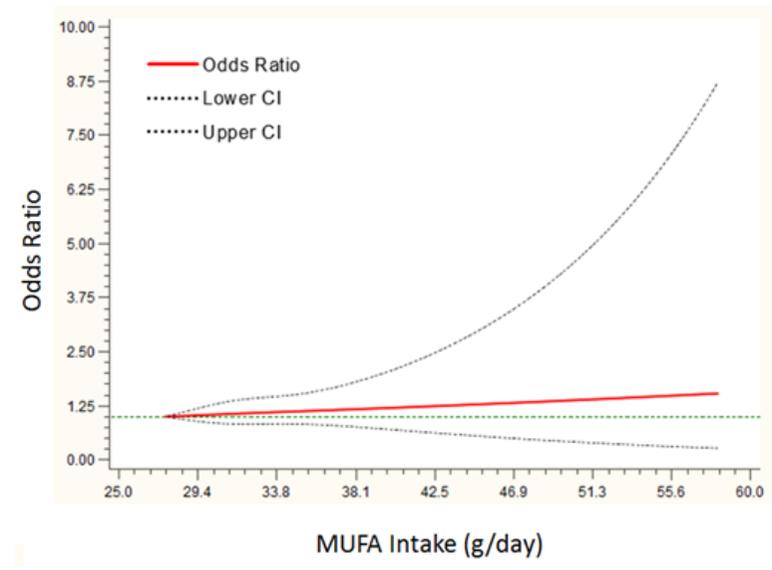
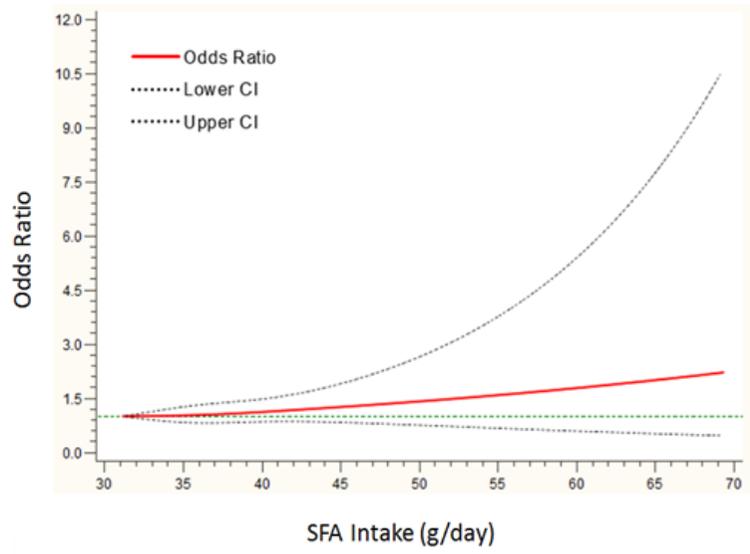
\*BMI, body mass index; DED, diabetic eye disease; HbA<sub>1c</sub>, glycated hemoglobin; MUFA, monounsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, trans unsaturated fatty acids; AA, arachidonic acid (20:4n6); ALA,  $\alpha$ -linolenic acid (18:3n3); DHA, docosahexaenoic acid (22:6n3); DPA, docosapentaenoic acid (22:5n3); EPA, eicosapentaenoic acid (20:5n3); LA, linoleic acid (18:2n6)

**TABLE 11.** ODDS RATIOS AND 95% CONFIDENCE INTERVALS OF DIABETIC EYE DISEASE BY TERTILE GROUP OF FATTY ACID CONSUMPTION (G/DAY) IN THE E3N-AFTERDIAB STUDY (N=1949 WOMEN)

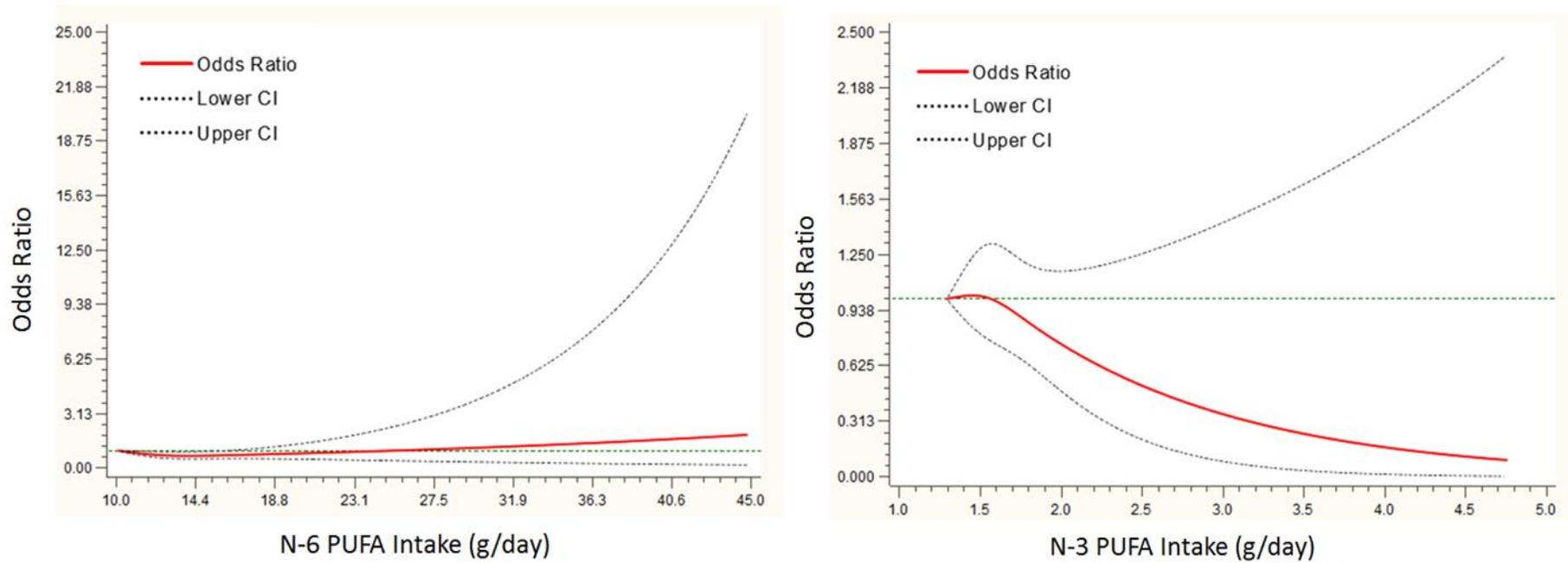
Variable (Median g/day)	Univariate	Fully Adjusted
<b>SFA</b>		
Tertile 1 (29.1)	1 (Reference)	1 (Reference)
Tertile 2 (35.7)	0.87 [0.48-1.58]	0.89 [0.48-1.63]
Tertile 3 (43.5)	1.31 [0.76-2.25]	1.45 [0.83-2.52]
P for trend	0.28	0.13
<b>MUFA</b>		
Tertile 1 (26.0)	1 (Reference)	1 (Reference)
Tertile 2 (31.2)	1.32 [0.76-2.29]	1.38 [0.78-2.43]
Tertile 3 (36.8)	1.01 [0.57-1.82]	1.18 [0.65-2.16]
P for trend	0.99	0.56
<b>PUFA</b>		
Tertile 1 (10.7)	1 (Reference)	1 (Reference)
Tertile 2 (14.3)	0.62 [0.36-1.09]	0.64 [0.36-1.13]
Tertile 3 (19.1)	0.66 [0.39-1.14]	0.67 [0.38-1.17]
P for trend	0.11	0.11
<b>n-6 PUFA</b>		
Tertile 1 (9.1)	1 (Reference)	1 (Reference)
Tertile 2 (12.6)	<b>0.49 [0.27-0.87]</b>	<b>0.50 [0.28-0.89]</b>
Tertile 3 (17.3)	0.61 [0.36-1.04]	0.61 [0.35-1.06]
P for trend	0.07	0.08
<b>n-3 PUFA</b>		
Tertile 1 (1.2)	1 (Reference)	1 (Reference)
Tertile 2 (1.5)	0.78 [0.45-1.32]	0.85 [0.49-1.47]
Tertile 3 (2.0)	0.60 [0.34-1.05]	0.61 [0.34-1.09]
P for trend	0.07	0.09
<b>TFA</b>		
Tertile 1 (1.1)	1 (Reference)	1 (Reference)
Tertile 2 (1.5)	0.84 [0.46-1.51]	0.86 [0.47-1.57]
Tertile 3 (2.0)	1.22 [0.71-2.08]	1.24 [0.72-2.16]
P for trend	0.42	0.35

\*Fully adjusted model was adjusted for age at diabetes diagnosis (years), duration of diabetes (years), daily energy intake (kcal/day), BMI group (kg/m<sup>2</sup>), exercise group (MET-h/week), smoking status, treatment for T2D, hypercholesterolemia treatment, hypertension, family history of T2D, level of education and HbA1c

†SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acids; TFA, trans unsaturated fatty acids



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**FIGURE 42.** RESTRICTED CUBIC SPLINE REGRESSION WITH 3 KNOTS OF FATTY ACID CONSUMPTION (G/DAY) AND DIABETIC EYE DISEASE IN THE E3N-AFTERDIAB STUDY (N=1949)<sup>de</sup>

<sup>d</sup>Models were adjusted for age at diabetes diagnosis (years), duration of diabetes (years), daily energy intake (kcal/day), BMI group (kg/m<sup>2</sup>), exercise group (MET-h/week), smoking status, treatment for T2D, hypercholesterolemia treatment, hypertension, family history of T2D, level of education and HbA1c

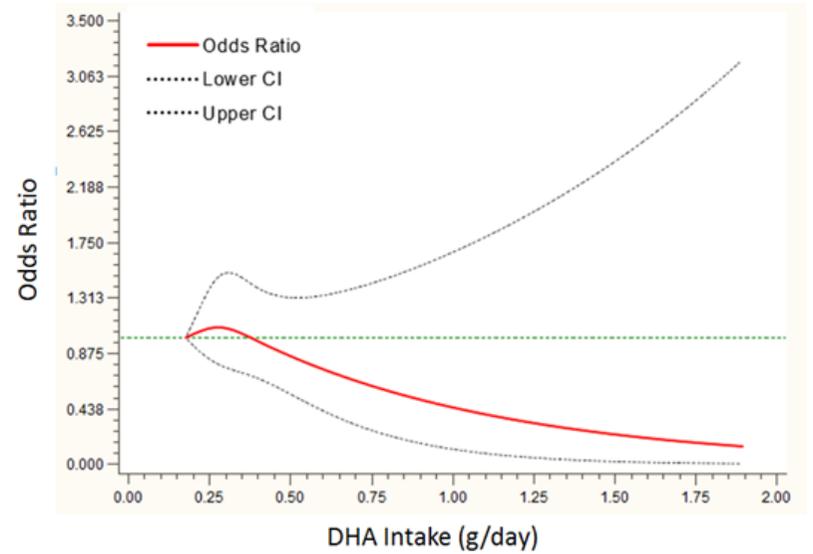
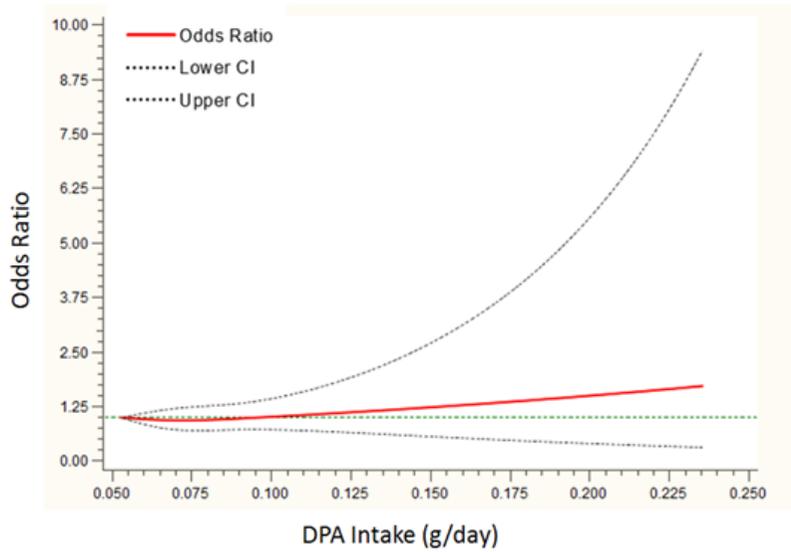
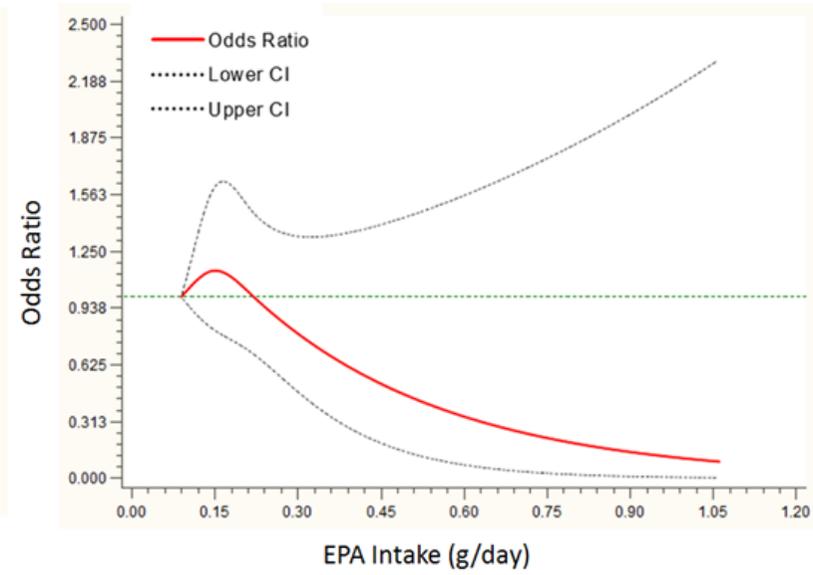
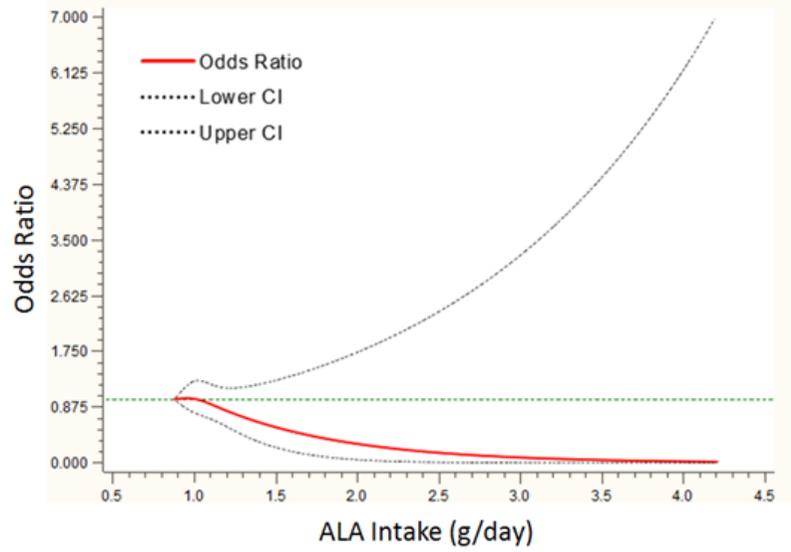
<sup>e</sup>MUFA, monounsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, trans unsaturated fatty acids

**TABLE 12.** ODDS RATIOS AND 95% CONFIDENCE INTERVALS OF DIABETIC EYE DISEASE BY TERTILE GROUP OF OMEGA-3 AND OMEGA-6 FATTY ACID CONSUMPTION (G/DAY) IN THE E3N-AFTERDIAB STUDY (N=1949 WOMEN)

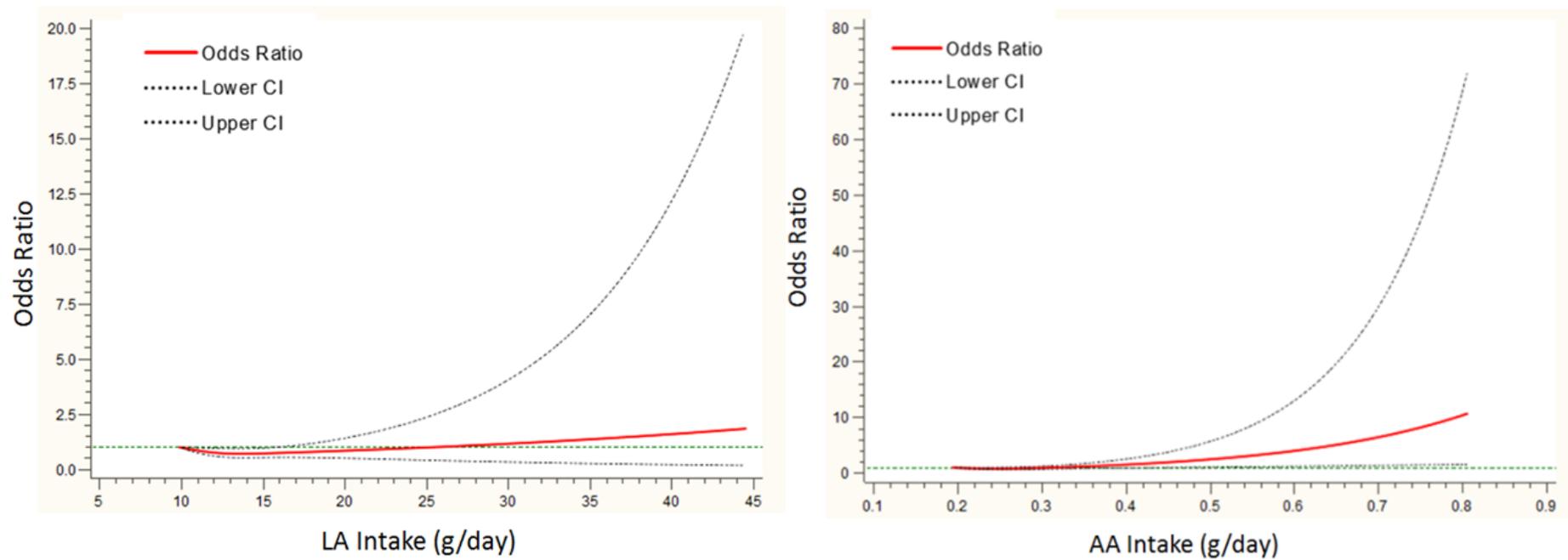
Variable (Median g/day)	Univariate	Fully Adjusted
<b>ALA</b>		
Tertile 1 (0.8)	1 (Reference)	1 (Reference)
Tertile 2 (1.0)	1.12 [0.65-1.93]	1.26 [0.72-2.20]
Tertile 3 (1.2)	0.82 [0.46-1.45]	0.91 [0.50-1.65]
P for trend	0.44	0.72
<b>EPA</b>		
Tertile 1 (0.1)	1 (Reference)	1 (Reference)
Tertile 2 (0.1)	1.80 [1.03-3.15]	0.76 [1.00-3.12]
Tertile 3 (0.3)	1.07 [0.58-1.99]	0.98 [0.52-1.83]
P for trend	0.87	0.60
<b>DPA</b>		
Tertile 1 (0.0)	1 (Reference)	1 (Reference)
Tertile 2 (0.1)	0.77 [0.42-1.38]	0.70 [0.38-1.29]
Tertile 3 (0.1)	1.17 [0.68-1.99]	1.07 [0.62-1.86]
P for trend	0.50	0.73
<b>DHA</b>		
Tertile 1 (0.1)	1 (Reference)	1 (Reference)
Tertile 2 (0.3)	1.26 [0.73-2.15]	1.25 [0.72-2.17]
Tertile 3 (0.5)	0.81 [0.45-1.46]	0.76 [0.42-1.39]
P for trend	0.30	0.21
<b>LA</b>		
Tertile 1 (8.9)	1 (Reference)	1 (Reference)
Tertile 2 (12.4)	<b>0.49 [0.27-0.86]</b>	<b>0.48 [0.26-0.86]</b>
Tertile 3 (17.0)	0.61 [0.36-1.04]	0.60 [0.35-1.04]
P for trend	0.07	0.07
<b>AA</b>		
Tertile 1 (0.2)	1 (Reference)	1 (Reference)
Tertile 2 (0.2)	0.71 [0.39-1.32]	0.71 [0.38-1.33]
Tertile 3 (0.3)	1.34 [0.79-2.27]	1.36 [0.78-2.36]
P for trend	0.22	0.25

\*Fully adjusted model was adjusted for age at diabetes diagnosis (years), duration of diabetes (years), daily energy intake (kcal/day), BMI group (kg/m<sup>2</sup>), exercise group (MET-h/week), smoking status, treatment for T2D, hypercholesterolemia treatment, hypertension, family history of T2D, level of education and HbA1c

†ALA, α-linolenic acid (18:3n3); EPA, eicosapentaenoic acid (20:5n3); DPA, docosapentaenoic acid (22:5n3); DHA, docosahexaenoic acid (22:6n3); LA, linoleic acid (18:2n6); AA, arachidonic acid (20:4n6)



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**FIGURE 43.** RESTRICTED CUBIC SPLINE REGRESSION WITH 3 KNOTS OF INDIVIDUAL OMEGA-3 AND OMEGA-6 FATTY ACID CONSUMPTION (G/DAY) AND DIABETIC EYE DISEASE IN THE E3N-AFTERDIAB STUDY (N=1949)<sup>f,g</sup>

<sup>f</sup> Models were adjusted for age at diabetes diagnosis (years), duration of diabetes (years), daily energy intake (kcal/day), BMI group (kg/m<sup>2</sup>), exercise group (MET-h/week), smoking status, treatment for T2D, hypercholesterolemia treatment, hypertension, family history of T2D, level of education and HbA1c

<sup>g</sup> AA, arachidonic acid (20:4n6); ALA,  $\alpha$ -linolenic acid (18:3n3); DHA, docosahexaenoic acid (22:6n3); DPA, docosapentaenoic acid (22:5n3); EPA, eicosapentaenoic acid (20:5n3); LA, linoleic acid (18:2n6)

### 3.3 SENSITIVITY ANALYSIS

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#### 3.3.1 EXCLUSION OF POTENTIAL CASES

After excluding the 98 women who were considered potential cases of DED, sent a validation questionnaire and who did not respond to the validation questionnaire, we observed similar results (*results not shown*). Likewise, simultaneously adjusting for the intakes of other FAs did not significantly change our results.

#### 3.3.2 PRIMARY FATTY ACID FOOD SOURCES

Dairy products were the primary source of SFA (32.1%), MUFA (16.9%), TFA (27.8%) and ALA (16.3%), while sauces were the primary source of PUFA (34.2%), n-6 PUFA (37.4%) and LA (38.1%) (**Table 13**). The primary source of n-3 PUFA (22.9%), EPA (69.6%) and DHA (66.5%) was fish and seafood and the primary source of DPA (46.5%) and AA (54.7%) was meat. Dairy products (tertile 2: 242.0-404.82 g/day, OR=1.10 [0.62-1.97]; tertile 3:  $\geq$ 404.82 g/day, OR=1.00 [0.55-1.79]), sauces (tertile 2: 12.1-19.6 g/day; OR=0.74 [0.42-1.32]; tertile 3:  $\geq$ 19.6 g/day, OR=0.82 [0.46-1.45]) and fish and seafood intake (tertile 2: 25.7-47.8 g/day, OR=1.40 [0.79-2.47]; tertile 3  $\geq$ 47.8 g/day, OR=1.10 [0.61-2.00]) did not show any relationship with the presence of DED by tertile groups of intake (**Table 14**), nor did they show any evidence of a linear or non-linear relationship (**Figure 44**) in the fully adjusted models ( $p \geq 0.05$ ).

Meat consumption, (includes meats such as chicken, beef, and rabbit, prepared sausages, *pâté*, offal, and processed meat), was not associated with DED when meat intake was classified by tertile groups of intake (tertile 2: 102.3-152.0 g/day, OR=0.83 [0.44-1.57]; tertile 3:  $\geq$ 152.0 g/day, OR=1.65 [0.91-3.00]), however, cubic spline regression suggested that a high consumption of meat was associated with increased odds of DED ( $p=0.009$ ) for consumptions above 300g/day. This association remained even after adjustment for AA intake. The relationship between LA intake and DED did not change with further adjustment for the primary source of LA, sauces, in either the analyses by tertile groups of intake (tertile 2 median 12.4 g/day; OR=0.49 [0.27-0.86]), nor by cubic spline regression ( $p=0.04$ ). On the other hand, after adjustment for the primary source of AA, meat, the association between AA and DED changed directions in the cubic spline regression, such that moderate intakes of AA showed an inverse relationship with the odds of DED ( $p=0.0109$ ).

**TABLE 13.** PRINCIPAL FOOD SOURCES OF FATTY ACIDS IN THE E3N-AFTERDIAB STUDY (N=1949)

	Principal Source	Contribution (%)
SFA	Dairy products	32.1
MUFA	Dairy products	16.9
PUFA	Sauces	34.2
n-6 PUFA	Sauces	37.4
n-3 PUFA	Fish and seafood	22.9
TFA	Dairy products	27.8
ALA	Dairy products	16.3
EPA	Fish and seafood	69.6
DPA	Meat	46.5
DHA	Fish and seafood	66.5
LA	Sauces	38.1
AA	Meat	54.7

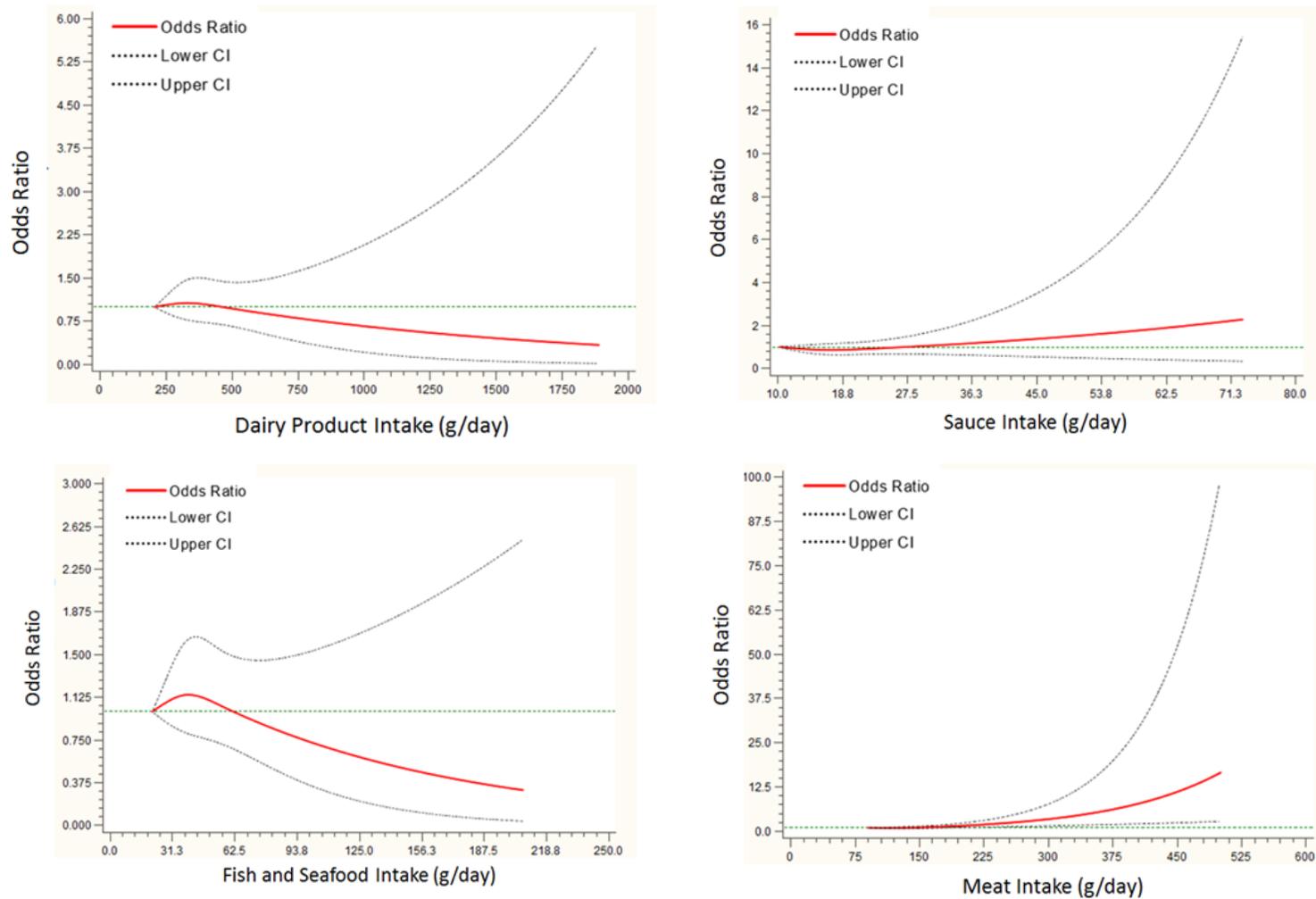
\*Dairy products include: milk, yogurt, cheese, *fromage blanc*, ice cream and creamy desserts; sauces include: home-made and store-bought vinaigrettes with various vegetable oil bases, and mayonnaise; fish and seafood includes: fresh fish, canned fish, and seafood such as mollusks, and crustaceans; meats include: meats such as chicken, beef, and rabbit, prepared sausages, *pâté*, offal, and processed meat

†SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acids; TFA, trans unsaturated fatty acids; ALA,  $\alpha$ -linolenic acid (18:3n3); EPA, eicosapentaenoic acid (20:5n3); DPA, docosapentaenoic acid (22:5n3); DHA, docosahexaenoic acid (22:6n3); LA, linoleic acid (18:2n6); AA, arachidonic acid (20:4n6)

**TABLE 14.** ODDS RATIOS AND 95% CONFIDENCE INTERVALS OF DIABETIC EYE DISEASE BY TERTILE GROUP OF CONSUMPTION FOR THE MAIN FOOD SOURCES FOR EACH FATTY ACID GROUP (G/DAY) IN THE E3N-AFTERDIAB STUDY (N=1949 WOMEN)

Variable (g/day)	Univariate	Fully Adjusted
<b>Dairy products</b>		
< 242.0	1 (Reference)	1 (Reference)
242.0 - 404.8	1.10 [0.62-1.92]	1.10 [0.62-1.97]
≥ 404.8	1.10 [0.63-1.93]	1.00 [0.55-1.79]
P for trend	0.75	0.93
<b>Fish and seafood</b>		
< 25.7	1 (Reference)	1 (Reference)
25.7 - 47.8	1.33 [0.76-2.33]	1.40 [0.79-2.47]
≥ 47.8	1.10 [0.62-1.96]	1.10 [0.61-2.00]
P for trend	0.85	0.92
<b>Meat</b>		
< 102.3	1 (Reference)	1 (Reference)
102.3 - 152.0	0.79 [0.43-1.46]	0.83 [0.44-1.57]
≥ 152.0	1.41 [0.82-2.40]	1.65 [0.91-3.00]
P for trend	0.15	0.08
<b>Sauces</b>		
< 12.1	1 (Reference)	1 (Reference)
12.1 -19.6	0.75 [0.43-1.31]	0.74 [0.42-1.32]
≥ 19.6	0.82 [0.48-1.42]	0.82 [0.46-1.45]
P for trend	0.51	0.48

\*Fully adjusted model was adjusted for age at diabetes diagnosis (years), duration of diabetes (years), daily energy intake (kcal/day), BMI group (kg/m<sup>2</sup>), exercise group (MET-h/week), smoking status, treatment for T2D, hypercholesterolemia treatment, hypertension, family history of T2D, level of education and HbA<sub>1c</sub>



**FIGURE 44.** RESTRICTED CUBIC SPLINE REGRESSION WITH 3 KNOTS OF FATTY ACID FOOD SOURCE INTAKE (G/DAY) AND DIABETIC EYE DISEASE IN THE E3N-AFTERDIAB STUDY (N=1949)<sup>h</sup>

<sup>h</sup>Models were adjusted for age at diabetes diagnosis (years), duration of diabetes (years), daily energy intake (kcal/day), BMI group (kg/m<sup>2</sup>), exercise group (MET-h/week), smoking status, treatment for T2D, hypercholesterolemia treatment, hypertension, family history of T2D, level of education and HbA1c

## 3.4 DISCUSSION

In this study, a moderate intake of n-6 PUFA (tertile 2 median 12.4 g/day) was associated with reduced odds of DED. Upon closer analysis within the n-6 PUFA group, the moderate intake of LA (tertile 2 median 12.4 g/day) was negatively associated with the odds of DED with a non-linear relationship indicated, and the intake of AA showed a non-linear, positive relationship with DED. These relationships persisted despite adjustment for confounding factors and the intakes of other FAs. The relationship with LA also persisted after adjustment for the intake of the primary source of LA, saucers. However, after adjustment for the intake of the primary source of AA, meat, the non-linear relationship between AA and DED changed directions, indicating that moderate intakes of AA were associated with reduced odds of DED. Meat intake was independently strongly associated with the odds of DED in restricted cubic spline regression.

### 3.4.1 SATURATED FATTY ACIDS

Previous studies have similarly not observed an association between total SFA intake and DR<sup>164,165</sup>. But as mentioned previously, it may be more relevant to study individual SFAs or SFAs grouped by chain length, as biomarkers of odd-chain SFAs have been linked to decreased T2D risk<sup>160,207</sup>. But, one case-control study examined the effects of individual SFA intakes on DR and 2 even-chain SFAs, palmitic and stearic acid<sup>164</sup>. No association was observed with palmitic acid and reduced odds for the middle tertile group of intake (4.53-6.26 g/day) for stearic acid were observed<sup>164</sup>. This phenomenon should be investigated in future studies, as both these SFAs have been previously linked to insulin resistance<sup>318,319</sup>, and palmitic acid has been identified as an activator of the HPA axis<sup>223</sup>.



### 3.4.2 MONOUNSATURATED FATTY ACIDS

Concerning MUFA intake, our results are in agreement with a previous cross-sectional study on DR<sup>165</sup>. But, one study observed an inverse relationship between MUFA and oleic acid intake with DR<sup>164</sup>. It is possible that the MUFA intake in our population was not high enough to detect the association found in the Spanish population, or the relationship may have been confounded by the source of MUFAs. In our study, the primary sources of MUFA intake were dairy products and meat, whereas in a Spanish population, the primary source of MUFA may more likely be olive oil, which has been shown to improve glycemic control in people with T2D<sup>320,321</sup>.

### 3.4.3 POLYUNSATURATED FATTY ACIDS

We found no evidence of a relationship between PUFA intake and DED, in agreement with another study on DR<sup>164</sup>. Nevertheless, similar to SFAs, FAs within the PUFA group have previously shown differing effects on the risk of T2D<sup>296</sup>. Though neither our study nor Alcubierre *et al.*'s study observed an association with n-3 PUFA intake, a prospective study in Spain found that participants with T2D with long chain n-3 PUFA intake  $\geq 500$  mg/day had almost a 50% decreased risk of sight-threatening DR<sup>147</sup>. Similarly, a randomized controlled trial that supplemented patients with T2D with antioxidants and n-3 PUFAs observed less onset and progression of DR in those supplemented<sup>254</sup>. However, to our knowledge, this is the first study to analyze the association between the dietary intake of individual n-3 PUFAs and DED, and we did not observe any significant associations though we previously detected an increased risk of T2D in E3N with high intakes of n-3 PUFAs that seemed to be driven by DPA intake<sup>296</sup>. N-3 PUFAs may have favourable outcomes on lipid profiles in people with T2D<sup>322,323</sup>, and may decrease the number of retinal acellular capillaries and inflammatory markers associated with diabetes<sup>294,295</sup>. As the effects of n-3 PUFAs may differ by individual FA, geographic location, or sex, it is important that future studies analyze this association<sup>162,276</sup>.



After controlling for the primary source of AA, the intake of meat, our results suggested that moderate intakes of n-6 PUFAs, LA, and AA were associated with decreased odds of DED. Only one other study looked at the intakes of n-6 PUFAs or LA, and did not detect an association<sup>164</sup>. However, this study did not use an energy adjustment method, which could have introduced an important source of bias. N-6 PUFAs have been shown to have anti-inflammatory properties on vascular endothelial cells and high LA intakes may improve insulin resistance and lower cholesterol<sup>324,325</sup>. However, other studies suggest that LA, AA and n-6 PUFAs may also have proinflammatory effects<sup>324,326</sup>. The relationship appears delicate, as eicosanoids derived from AA appear to have both proinflammatory and anti-inflammatory effects, but the overall physiological effect appears to depend on the timing of their generation, the concentrations present, the sensitivity of the target cells, and the presence of other FAs such as EPA and DHA, which have been observed to affect AA metabolism<sup>240</sup>.

#### 3.4.4 INTAKE OF MEAT

We found the intake of meat independently associated with increased odds of DED in a dose-response manner. High intakes of red and processed meat have been previously linked to T2D risk in this cohort and in meta-analyses<sup>139,183,327</sup> and have been associated with higher fasting glucose and insulin concentrations<sup>328</sup>. High meat consumption may affect the development of DED through the iron content, which may potentiate oxidative stress and destruction of the retinal pigment epithelium<sup>329,330</sup>, the dietary AGE content, as animal-derived foods high in fat and protein and foods exposed to higher temperatures are the largest contributors<sup>307,308</sup>, or increased inflammation<sup>331</sup>. Further studies are needed to confirm this observed association.



#### 3.4.5 STRENGTHS AND LIMITATIONS

There are several strengths of this study. This is the first study to prospectively analyze the association between the dietary intake of individual PUFAs and DED. This study was performed in a large, prospective cohort that minimizes recall and selection biases. We were able to use the most recent information available on confounders such as BMI or physical activity in our analyses and the dietary questionnaire was validated and specific for this French population. We also performed sensitivity analyses to determine if the observed associations between the individual FAs and DED were independent of their primary dietary sources, and were able to control for the intake of other FA groups besides the one in question, which is important, as the overall dietary FA profile may affect the fate of individual FAs<sup>185</sup>. However, there are some limitations in our analyses. The dietary intakes of FAs are based on a single questionnaire at baseline, so misclassification of exposure is a possibility. But, as dietary intake was measured before both the diagnosis of diabetes and DED, the effects are likely non-differential and would attenuate the true association. It is also possible that women significantly changed their diets after their diabetes diagnosis and we were unable to capture this change. However, symptoms of DR are present in one third of people with T2D at the initial diabetes diagnosis<sup>68</sup>, suggesting the development of DED may not necessarily be asynchronous with the development of T2D. We identified and carefully validated all cases of DED, but under-ascertainment of cases is possible, especially as DED initially presents with few or mild symptoms<sup>4</sup>. But, the possible under-ascertainment of cases would likely only attenuate our observed associations. Though we tried to control for other factors that may influence the development of DED, residual confounding cannot be completely eliminated.

### 3.5 CONCLUSION

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Our results suggest that the intake of n-6 PUFAs such as LA and AA may be beneficial in preventing the development of DED. In contrast, a high consumption of meat may be detrimental to the development of DED. As the literature concerning the roles of n-6 PUFAs and their effects on inflammation is controversial and this is the first study analyzing the association between the dietary intake of n-6 PUFAs and DED, further studies should address n-6 PUFAs. Our findings should also be confirmed with respect to meat, as its consumption seems to be increasing in many populations and could therefore provide an important avenue for the prevention of DED.

## 4. OBJECTIVE FOUR: ADHERENCE TO DIETARY GUIDELINES, TYPE 2 DIABETES RISK AND POPULATION ATTRIBUTABLE FRACTIONS

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### 4.1. BASELINE CHARACTERISTICS

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A total of 376 cases of diabetes were diagnosed during 63 086.3 person years of follow-up between May 1999 and August 2012 in the AusDiab cohort study. The incidence of diabetes was 6.0 [5.39-6.59] per 1000 person years. Median follow-up time was 11.7 years, (ranging from 2.0-13.1 years). Those most strongly adhering to the healthy behaviour index were slightly younger, more educated, more likely to live in a major city, not be disadvantaged, not have a family history of diabetes, have a normal blood pressure, a level of triglycerides (<2.0mmol/L), HDL cholesterol level ( $\geq$ 1.0mmol/L) and reported significantly higher energy intakes than those with intermediate or weak adherence to the index of healthy behaviours (**Table 15**).

### 4.2 ADHERENCE TO THE DIETARY GUIDELINES

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In the overall study population, almost one third (27.5%) of the population reported meeting dietary recommendations for fruit intake, whereas very few (1.8%) reported an adequate vegetable consumption. 14.9% reported strong adherence to dietary recommendations for grain intake, 15.4% for dairy intake and 29.1% for protein-rich food intake. Overall adherence to the dietary recommendations was determined as strong in 6.3% of the population and weak in 33.1% of the population.

Strong adherence to fruit and dairy recommendations was significantly associated with a 32% and 40% decreased risk of diabetes, respectively, after adjustment for confounders (**Table 16**). However, exceeding the limits of recommended fruit intake did not appear beneficial for T2D risk (HR=1.04 [95% CI 0.76-1.43]). Strong adherence to recommendations for vegetables, grains, protein-rich foods, and alcohol (not drinking) were not associated with diabetes risk (HR=0.83 [0.37-1.88], HR=1.08 [0.72-1.59], HR=1.10 [0.75-1.61], HR=0.86 [0.59-1.26] respectively) even after further adjustment for adherence to the guidelines for the remaining dietary components (*results not shown*). But, exceeding the limits of intakes for protein-rich foods was associated with a 56% increased risk of type 2 diabetes (HR = 1.56 [1.01-2.43]).

Cubic spline regression for the dose-response association between the number of servings/day of fruit and diabetes risk indicated a curvilinear association, where up to 3.2 servings of fruit per day was associated with a risk reduction in T2D ( $p=0.0001$  for global relationship), after adjustment for confounders (**Figure 45**). Increasing dairy servings was strongly inversely associated with a decreased risk of diabetes ( $p=0.003$ ), whereas increasing servings of protein-rich foods was associated with an increased risk of diabetes ( $p=0.03$ ). Finally, the number of standard drinks per day, servings of vegetables and grains were not significantly associated with diabetes risk ( $p=0.09$ ,  $p=0.09$ ,  $p=0.43$ , respectively).

**TABLE 15.** BASELINE CHARACTERISTICS OF THE STUDY POPULATION BY ADHERENCE GROUP TO THE HEALTHY BEHAVIOUR INDEX; AUSDIAB COHORT STUDY (N=6242)

Variables	Overall Population (n=6242)	Weak Adherence (<2.5) (n=2938)	Intermediate Adherence (2.5-3.4) (n=2568)	Strong Adherence (≥3.5) (n=736)
Age (years)	50.3 ± 12.5	51.1 ± 12.3	49.9 ± 12.6	48.4 ± 12.9
Sex (n, (%))				
Male	2813 (45.1)	1325 (45.1)	1142 (44.5)	346 (47)
Female	3429 (54.9)	1613 (54.9)	1426 (55.5)	390 (53)
Level of Education (n, (%))				
Secondary school, trade, technician's certificate or less	4128 (66.1)	2103 (71.6)	1615 (62.9)	410 (55.7)
Bachelor's degree, post-graduate, nursing or teaching qualification	2114 (33.9)	835 (28.4)	953 (37.1)	326 (44.3)
Smoking status (n, (%))				
Non-smoker	3732 (59.8)	1230 (41.9)	1786 (69.6)	716 (97.3)
Ex-smoker	1791 (28.7)	1082 (36.8)	689 (26.8)	20 (2.7)
Current smoker	719 (11.5)	626 (21.3)	93 (3.6)	0 (0)
Family history of diabetes (n, (%))				
No	5110 (81.9)	2372 (80.7)	2113 (82.3)	625 (84.9)
Yes	1132 (18.1)	566 (19.3)	455 (17.7)	111 (15.1)
Waist circumference (n, (%))				
Low weight-related health risk	2624 (42)	555 (18.9)	1370 (53.4)	699 (95)
Increased weight-related health risk	1678 (26.9)	825 (28.1)	816 (31.8)	37 (5)
High weight-related health risk	1940 (31.1)	1558 (53)	382 (14.9)	0 (0)
BMI (kg/m <sup>2</sup> ) (n, (%))				
Normal (<25)	2466 (39.5)	698 (23.8)	1234 (48.1)	534 (72.6)
Overweight (25-30)	2569 (41.2)	1319 (44.9)	1056 (41.1)	194 (26.4)
Obese (≥30)	1207 (19.3)	921 (31.4)	278 (10.8)	8 (1.1)
Recreational physical activity (n, (%))				
Sedentary (<75 mins)	941 (15.1)	1695 (57.7)	326 (12.7)	0 (0)
Insufficient (75-150 mins)	1895 (30.4)	426 (14.5)	377 (14.7)	11 (1.5)
Sufficient (≥150 mins)	3406 (54.6)	817 (27.8)	1865 (72.6)	725 (98.5)

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Hypertension (n, (%))				
Normal	4545 (72.8)	1999 (68)	1954 (76.1)	592 (80.4)
Hypertensive ( $\geq 140/90$ mmHg)	1697 (27.2)	939 (32)	614 (23.9)	144 (19.6)
Triglycerides (n, (%))				
<2.0 mmol/L	5000 (80.1)	2163 (73.6)	2170 (84.5)	667 (90.6)
$\geq 2.0$ mmol/L	1242 (19.9)	775 (26.4)	398 (15.5)	69 (9.4)
HDL cholesterol (n, (%))				
$\geq 1.0$ mmol/L	5659 (90.7)	2563 (87.2)	2387 (93)	709 (96.3)
<1.0 mmol/L	583 (9.3)	375 (12.8)	181 (7.1)	27 (3.7)
Daily energy intake (kJ/day)	8059 $\pm$ 2920	7911 $\pm$ 2926	8054 $\pm$ 2907	8670 $\pm$ 2894
Adherence to Dietary Guidelines (n, (%))				
Fruit				
Weak Adherence	1743 (27.9)	1065 (36.3)	569 (22.2)	109 (14.8)
Intermediate Adherence	1828 (29.3)	936 (31.9)	750 (29.2)	142 (19.3)
Strong Adherence	1714 (27.5)	542 (18.5)	812 (31.6)	360 (48.9)
Exceeding the Limits	957 (15.3)	395 (13.4)	437 (17)	125 (17)
Vegetables				
Weak Adherence	3744 (60)	1782 (60.7)	1538 (59.9)	424 (57.6)
Intermediate Adherence	2337 (37.4)	1090 (37.1)	962 (37.5)	285 (38.7)
Strong Adherence	109 (1.8)	40 (1.4)	45 (1.8)	24 (3.3)
Exceeding the Limits	52 (0.8)	26 (0.9)	23 (0.9)	3 (0.4)
Grains				
Weak Adherence	877 (14.1)	485 (16.5)	330 (12.9)	62 (8.4)
Intermediate Adherence	3091 (49.5)	1543 (52.5)	1254 (48.8)	294 (40)
Strong Adherence	931 (14.9)	349 (11.9)	388 (15.1)	194 (26.4)
Exceeding the Limits	1343 (21.5)	561 (19.1)	596 (23.2)	186 (25.3)
Dairy				
Weak Adherence	2050 (32.8)	1078 (36.7)	801 (31.2)	171 (23.2)
Intermediate Adherence	2939 (47.1)	1424 (48.5)	1212 (47.2)	303 (41.2)
Strong Adherence	960 (15.4)	293 (10)	440 (17.1)	227 (30.8)
Exceeding the Limits	293 (4.7)	143 (4.9)	115 (4.5)	35 (4.8)

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Protein				
Weak Adherence	736 (11.8)	352 (12)	314 (12.2)	70 (9.5)
Intermediate Adherence	2424 (38.8)	1189 (40.5)	1002 (39)	233 (31.7)
Strong Adherence	1813 (29.1)	693 (23.6)	799 (31.1)	321 (43.6)
Exceeding the Limits	1269 (20.3)	704 (24)	453 (17.6)	112 (15.2)
Alcohol				
Weak Adherence	1056 (16.9)	449 (15.3)	451 (17.6)	156 (21.2)
Intermediate Adherence	2757 (44.2)	1370 (46.6)	1091 (42.5)	296 (40.2)
Strong Adherence	772 (12.4)	302 (10.3)	344 (13.4)	126 (17.1)
Exceeding the Limits	1657 (26.6)	817 (27.8)	682 (26.6)	158 (21.5)
Overall Dietary Adherence				
Weak (0 points)	2066 (33.1)	1365 (46.5)	701 (27.3)	0 (0)
Intermediate (1-2 points)	3786 (60.7)	1505 (51.2)	1682 (65.5)	599 (81.4)
Strong ( $\geq 3$ points)	390 (6.3)	68 (2.3)	185 (7.2)	137 (18.6)

\*Values are means  $\pm$  SDs or n (% of category)

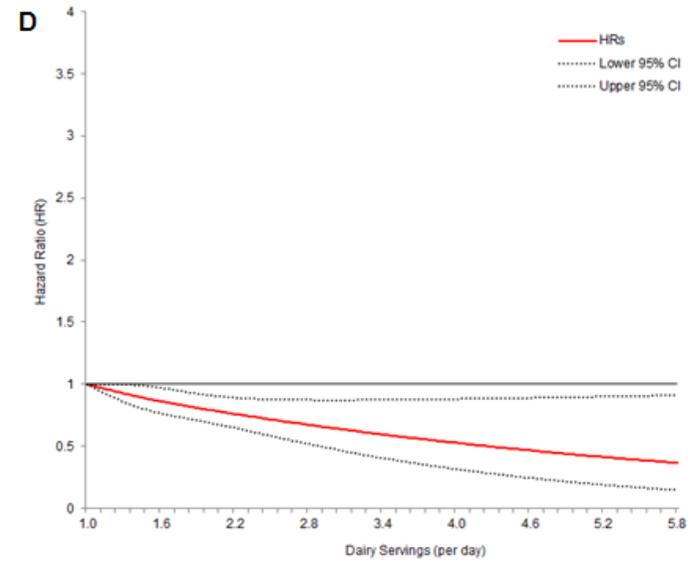
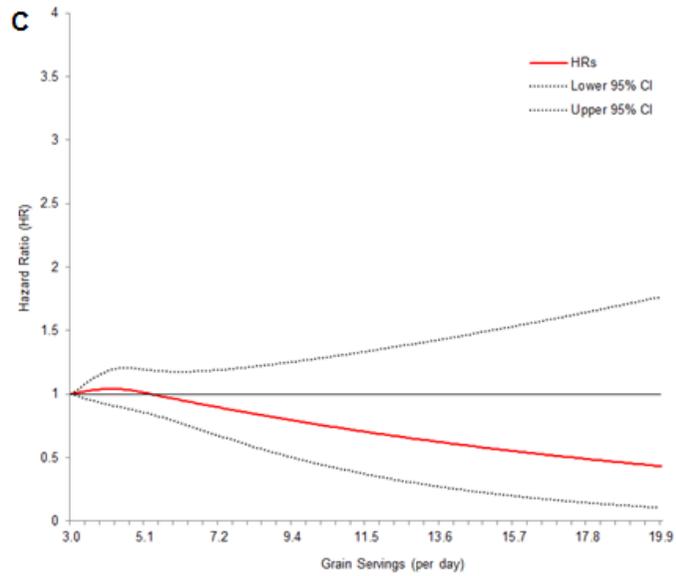
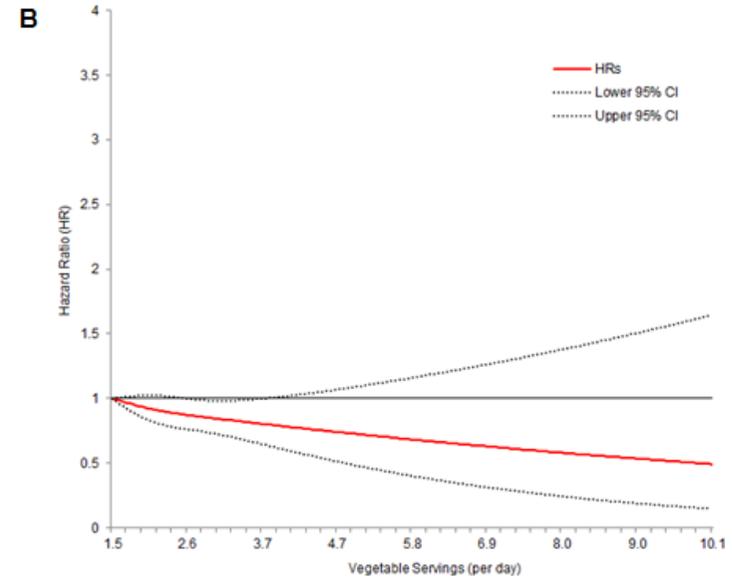
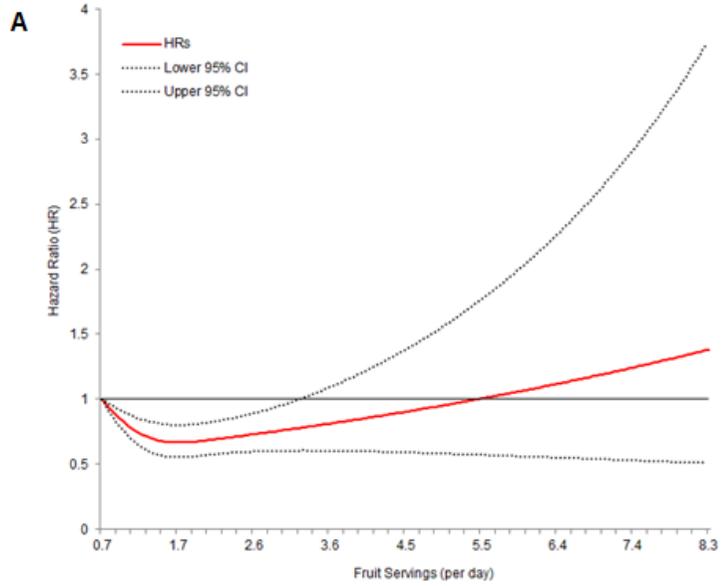
**TABLE 16.** COX PROPORTIONAL HAZARDS RATIOS [95% CI] FOR RISK OF INCIDENT TYPE 2 DIABETES BY ADHERENCE TO THE AUSTRALIAN DIETARY RECOMMENDATIONS IN THE AUSDIAB COHORT STUDY (N=6242)

	Cases/Non-Cases	Model 1	Model 2	Model 3
<b>Fruit</b>				
Weak Adherence	128/1615	REF	REF	REF
Intermediate Adherence	103/1725	<b>0.72 [0.55-0.93]</b>	0.79 [0.61-1.03]	0.81 [0.62-1.05]
Strong Adherence	80/1634	<b>0.56 [0.42-0.74]</b>	<b>0.65 [0.49-0.88]</b>	<b>0.68 [0.51-0.91]</b>
Exceeding the Limits	65/892	0.81 [0.60-1.10]	1.02 [0.74-1.41]	1.04 [0.76-1.43]
<b>Vegetables</b>				
Weak Adherence	229/3515	REF	REF	REF
Intermediate Adherence	140/2197	0.92 [0.75-1.14]	0.92 [0.74-1.15]	0.89 [0.72-1.11]
Strong Adherence	6/103	0.83 [0.37-1.87]	0.77 [0.34-1.75]	0.83 [0.37-1.88]
Exceeding the Limits	1/51	0.29 [0.05-2.07]	0.34 [0.05-2.44]	0.29 [0.04-2.10]
<b>Grains</b>				
Weak Adherence	53/854	REF	REF	REF
Intermediate Adherence	180/2911	0.87 [0.64-1.18]	0.92 [0.67-1.27]	0.96 [0.70-1.31]
Strong Adherence	64/867	0.93 [0.64-1.34]	1.03 [0.69-1.52]	1.08 [0.72-1.59]
Exceeding the Limits	79/1264	0.76 [0.53-1.08]	0.88 [0.58-1.32]	0.95 [0.63-1.42]
<b>Dairy</b>				
Weak Adherence	150/1900	REF	REF	REF
Intermediate Adherence	179/2760	0.85 [0.68-1.07]	0.90 [0.71-1.12]	0.93 [0.74-1.17]
Strong Adherence	33/927	<b>0.51 [0.34-0.74]</b>	<b>0.55 [0.37-0.82]</b>	<b>0.56 [0.38-0.84]</b>
Exceeding the Limits	14/279	0.73 [0.42-1.28]	0.81 [0.46-1.44]	0.84 [0.47-1.50]
<b>Protein</b>				
Weak Adherence	40/696	REF	REF	REF
Intermediate Adherence	120/2304	0.88 [0.61-1.26]	0.98 [0.68-1.41]	0.94 [0.65-1.35]
Strong Adherence	112/1701	1.03 [0.72-1.48]	1.21 [0.82-1.78]	1.10 [0.75-1.61]
Exceeding the Limits	104/1165	1.36 [0.94-1.97]	<b>1.85 [1.19-2.87]</b>	<b>1.56 [1.01-2.43]</b>
<b>Alcohol</b>				
Weak Adherence	53/1003	REF	REF	REF
Intermediate Adherence	164/2593	0.81 [0.60-1.10]	0.86 [0.64-1.17]	0.87 [0.65-1.18]
Strong Adherence	59/713	<b>0.63 [0.43-0.92]</b>	0.81 [0.56-1.19]	0.86 [0.59-1.26]
Exceeding the Limits	100/1557	0.72 [0.52-1.01]	0.81 [0.58-1.14]	0.84 [0.60-1.18]

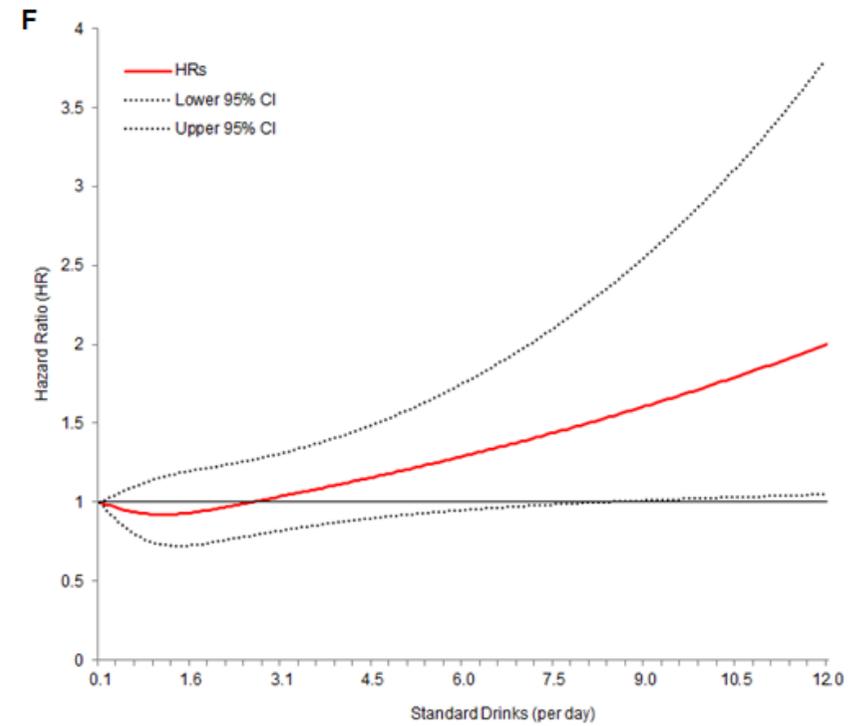
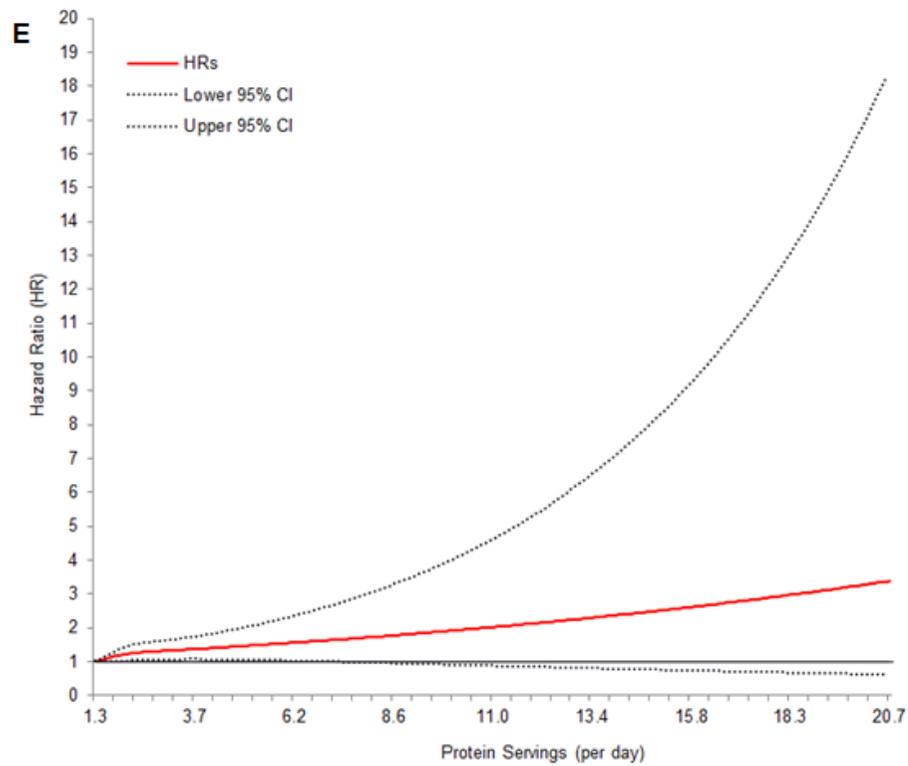
\*Model 1: sex adjusted model

†Model 2: additionally adjusted for education, smoking status, physical activity, high triglycerides, low HDL, family history of diabetes, energy intake, and hypertension

§Model 3: additionally adjusted for waist circumference



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**FIGURE 45.** CUBIC SPLINE REGRESSION MODELS BETWEEN SERVINGS OF EACH FOOD GROUP AND RISK OF TYPE 2 DIABETES IN THE AUSDIAB COHORT STUDY (N=6242); **A)** FRUIT; **B)** VEGETABLES; **C)** GRAINS; **D)** DAIRY; **E)** PROTEINS; **F)** STANDARD DRINKS

### 4.3 ADHERENCE TO THE HEALTHY BEHAVIOUR INDEX

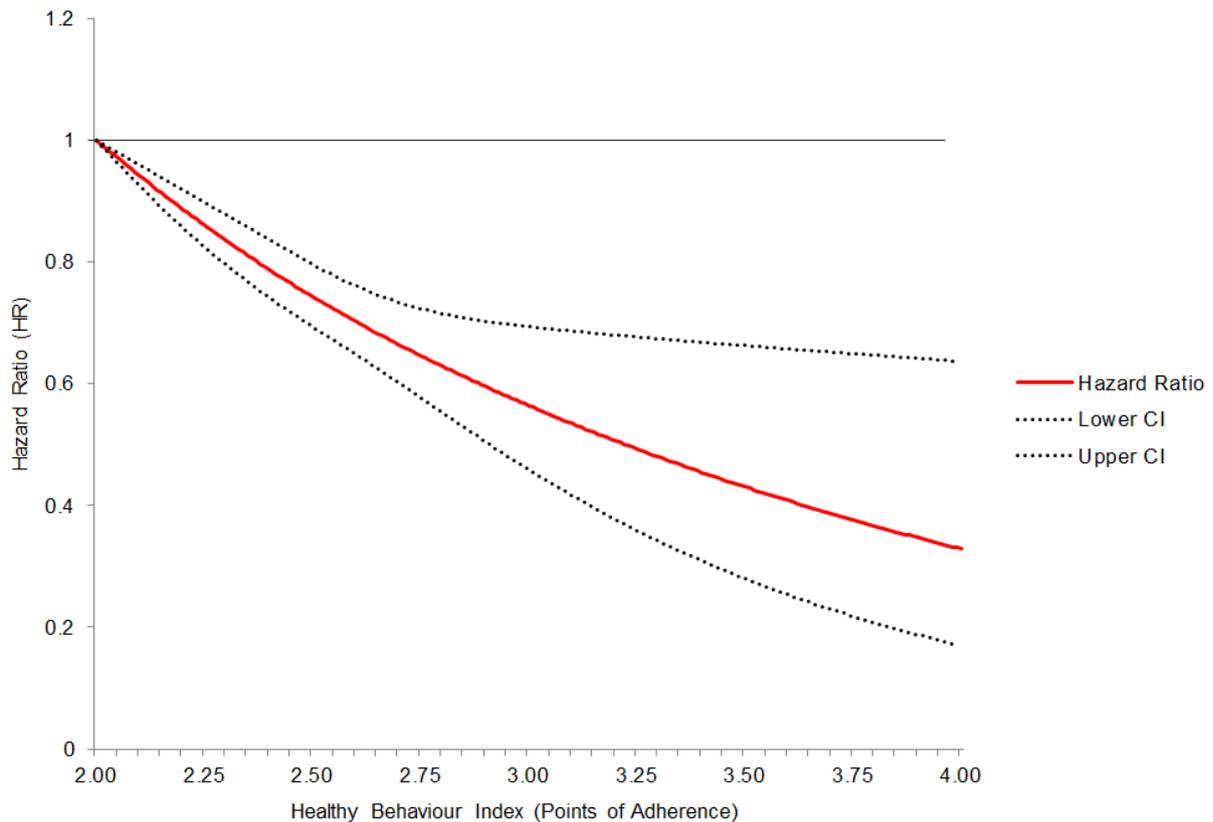
Strong adherence to the Australian dietary guidelines was not associated with diabetes risk (HR=0.64 [0.39-1.06]), though an inverse relationship was suggested (Table 17). Not smoking, performing sufficient recreational physical activity ( $\geq 150$ mins/week), and a waist circumference with low health-related risk all demonstrated strong inverse associations with diabetes risk. Strong adherence to the healthy behaviour index was associated with a 70% decreased risk of diabetes after adjustments (HR=0.30 [0.17-0.51]) and cubic spline regression suggested a significant, linear inverse relationship ( $p < 0.0001$ ) (Figure 46).

**TABLE 17.** COX PROPORTIONAL HAZARDS RATIOS [95% CI] FOR RISK OF TYPE 2 DIABETES BY ADHERENCE TO RECOMMENDATIONS FOR HEALTHY BEHAVIOURS AND BY ADHERENCE TO THE HEALTHY BEHAVIOUR INDEX IN THE AUSDIAB COHORT STUDY (N=6242)

	Cases/Non-cases	Model 1	Model 2
<b>Individual Characteristics</b>			
<b>Smoking</b>			
Score 0 (Current smoker)	59/660	REF	REF
Score 0.5 (Former smoker)	115/1676	<b>0.62 [0.45-0.86]</b>	<b>0.70 [0.51-0.97]</b>
Score 1 (Non-smoker)	202/3530	<b>0.57 [0.42-0.76]</b>	<b>0.65 [0.48-0.88]</b>
<b>Exercise</b>			
Score 0 (None)	160/1861	REF	REF
Score 0.5 (Insufficient)	49/765	0.74 [0.54-1.02]	0.82 [0.59-1.13]
Score 1 (Sufficient)	167/3240	<b>0.59 [0.47-0.73]</b>	<b>0.65 [0.52-0.80]</b>
<b>Waist circumference</b>			
Score 0 (High risk)	229/1711	REF	REF
Score 0.5 (Intermediate risk)	78/1600	<b>0.37 [0.29-0.48]</b>	<b>0.44 [0.34-0.57]</b>
Score 1 (Low risk)	69/2555	<b>0.23 [0.17-0.30]</b>	<b>0.33 [0.24-0.43]</b>
<b>Dietary adherence</b>			
Score 0 (Score 0)	130/1936	REF	REF
Score 0.5 (Score 1-2)	228/3558	0.91 [0.73-1.13]	0.93 [0.75-1.16]
Score 1 (Score $\geq 3$ )	18/372	0.68 [0.41-1.11]	0.64 [0.39-1.06]
<b>Behavioural Index</b>			
Tertile 1 (<2.5)	258/2680	REF	REF
Tertile 2 (2.5-3.5)	104/2464	<b>0.45 [0.36-0.57]</b>	<b>0.54 [0.43-0.68]</b>
Tertile 3 ( $\geq 3.5$ )	14/722	<b>0.22 [0.13-0.37]</b>	<b>0.30 [0.17-0.51]</b>
Continuous		<b>0.51 [0.45-0.58]</b>	<b>0.60 [0.52-0.67]</b>

\*Model 1: sex adjusted model

† Model 2: additionally adjusted for education, high triglycerides, low HDL cholesterol, family history of diabetes, energy intake, hypertension



**FIGURE 46.** CUBIC SPLINE REGRESSION WITH 3 KNOTS OF ADHERENCE TO THE HEALTHY BEHAVIOUR INDEX AND RISK OF T2D. CONTINUOUS LINE: HAZARD RATIO, DASHED LINES: 95% CONFIDENCE INTERVALS<sup>i</sup>

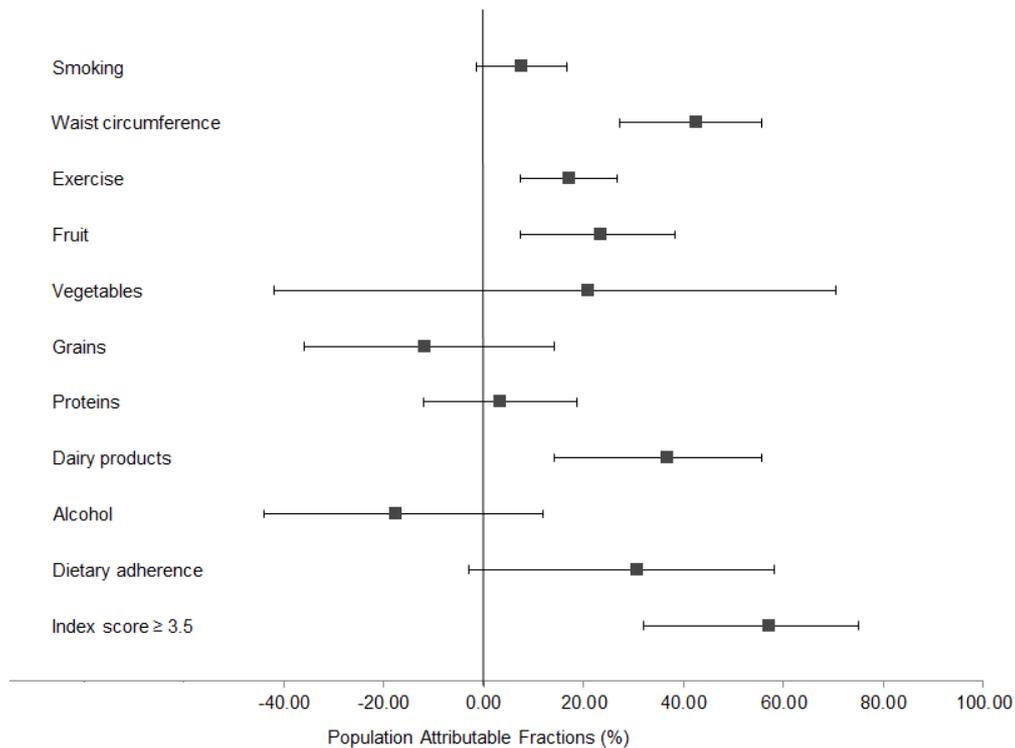
#### 4.4 POPULATION ATTRIBUTABLE FRACTIONS

The population attributable fraction was strongest for waist circumference, even after adjustment for confounders, suggesting that more than 40% of cases of T2D could be prevented if everyone had had waist circumferences below 94cm (men) or 80cm (women) (PAF=42.6% [27.5%-55.7%]) (Figure 47). The PAF for exercise demonstrated that almost 20% of new onset diabetes could have been prevented with sufficient exercise (PAF=17.3% [7.5%-26.8%]), whereas the PAF for smoking was not statistically significant (PAF=7.7% [-1.5%-16.8%]).

Following dietary guidelines for fruit consumption suggested that more than 20% of diabetes could have been prevented (PAF=23.3% [7.3%-38.2%]), and following those for dairy may have prevented 37% of cases (PAF=37.1% [14.6%-56.0%]). The PAF for adherence to the dietary recommendations (an index score  $\geq 3$ ) was not statistically significant (PAF=30.8% [-3.0%-58.1%]).

Overall, strong adherence to the healthy behaviour index indicated that almost 60% of type 2 diabetes cases could have been prevented (PAF=59.4% [36.2%-76.6%]).

<sup>i</sup>Model was adjusted for sex, high triglycerides, hypertension, low HDL, family history of diabetes, and level of education



**FIGURE 47.** POPULATION ATTRIBUTABLE FRACTIONS (%) FOR STRONG ADHERENCE TO THE COMPONENTS OF THE DIETARY INDEX AND THE HEALTHY BEHAVIOUR INDEX IN THE AUSDIAB COHORT STUDY (N=6242)

#### 4.5 SENSITIVITY ANALYSES

Moderate alcohol consumption was not associated with risk of diabetes (HR=0.89 [0.65-1.22]). However, both an intermediate and a strong overall adherence to dietary guidelines revealed an inverse association with diabetes risk, even after adjustment for confounders (HR=0.80 [0.65-0.99], HR=0.59 [0.36-0.96], respectively). But, the association between the index of healthy behaviours and diabetes did not significantly change (HR=0.32 [0.19-0.53]). The PAF for moderate alcohol consumption was not statistically significant (PAF=-18.6% [-7.2%-42.3%]), and neither the PAF for adherence to dietary guidelines nor the PAF for the index of healthy behaviours significantly changed.

The substitution of BMI for waist circumference did not materially change any of the associations observed (*results not shown*).

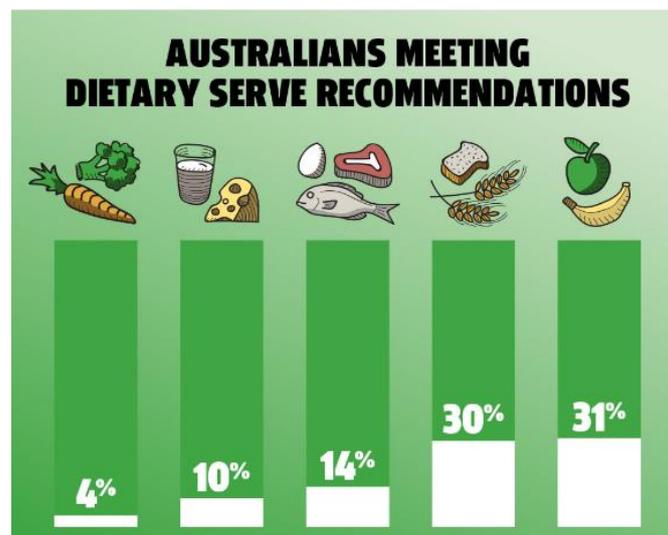
The PAFs estimated using the prevalence values from the 2014-2015 National Health Survey were similar to the PAFs estimated from the cohort. They were as follows: 4.3%, 51.1%, 19.1%, -21.6%, 21.1% and 12.8%, for smoking, overweight/obesity, insufficient recreational physical activity, alcohol consumption, and insufficient fruit and vegetable consumption, respectively.

## 4.6 DISCUSSION

Of the six components comprising the dietary index, strong adherence to the recommendations for fruit and dairy consumption suggested a 32-44% decreased risk of T2D. Exceeding the limits of recommended protein-rich food intake was associated with a 56% increased risk of T2D. Overall adherence to dietary guidelines was not associated with T2D risk when no alcohol intake was considered best. When moderate alcohol consumption (0.5-1 standard drink/day) was considered as beneficial, strong adherence to the dietary guidelines was associated with a 41% decreased risk of diabetes. Strong adherence to the dietary guidelines for fruit and dairy intake suggested that 23-37% of type 2 diabetes cases were potentially avoidable. High adherence to the index of healthy behaviours, (including dietary adherence), indicated a 70% decreased risk of T2D and that almost 60% of T2D cases could have been prevented in this population.

### 4.6.1 ADHERENCE TO DIETARY GUIDELINES FOR FOOD GROUPS

Data from the 2011-2012 Australian Health Survey found somewhat similar proportions of Australians meeting the 2013 Dietary Guidelines (Figure 48)<sup>335</sup>. However, nearly double (30% vs 14.9%, respectively) were found to meet the dietary guidelines with respect to grain intake and adherence to dairy intake was stronger in AusDiab (15.4% vs 10%, respectively)<sup>335</sup>. More people were also found to meet the recommendations for vegetable intake (4% vs 1.8%, respectively)<sup>335</sup>.



**FIGURE 48.** AUSTRALIANS MEETING THE 2013 DIETARY GUIDELINES BASED OFF DATA FROM THE 2011-2012 AUSTRALIAN HEALTH SURVEY<sup>332</sup>

Meta-analyses suggest an inverse relationship between fruit intake and T2D risk, but similar to our results, observed a curvilinear association with restricted cubic spline regression<sup>337</sup>. It is possible that the true association is curvilinear, as other studies have found moderate, but not high intakes associated with decreased risk of T2D<sup>333-335</sup> and large intakes of fructose may be detrimental. Fructose metabolism has been found to upregulate itself and increase uric acid concentrations, which induce insulin resistance<sup>336,337</sup>. The large amount of high-fructose corn syrup present in foods today may render the threshold for beneficial fruit consumption low, or the threshold for beneficial fruit consumption may be related to the intake of pesticides such as organophosphates<sup>338-340</sup> which can attenuate the incretin effect<sup>341</sup> and induce hyperglycemia through byproducts of its degradation by the gut microbiota<sup>342</sup>.





Total vegetable intake has not been associated with T2D in meta-analyses<sup>136,137</sup>, but in Asia, where vegetable consumption is high, significant inverse relationships have been observed<sup>137</sup>. In addition, the type of vegetable consumed may be important, as meta-analyses consistently report that high intakes of green, leafy vegetables may decrease T2D risk by 13-16%<sup>136-138</sup> and yellow and cruciferous vegetables have been associated with decreased T2D risk<sup>138</sup>. Vegetable intake has been negatively correlated with markers of DNA oxidation, lipid peroxidation and inflammation<sup>343-346</sup> and green, leafy vegetables are also an important source of magnesium, and which has been found to confer almost a 20% decreased risk of T2D with a 100g/d increase in intake<sup>347</sup>. Thus, we hypothesize that the low vegetable consumption, in addition to the lack of variation, (60% of the population had weak adherence), could have driven our estimates towards the null.

Strong adherence to grain intake was not associated with T2D risk, likely because risk differs by the type of grain consumed, with whole grains conferring decreased risk<sup>139,140</sup>. However, we were unable to distinguish between the two due to limits in the FFQ. Whole grains are believed to contribute protective effects through their fibre content, perhaps more strongly than the fibre contribution from fruit and vegetables<sup>348</sup> and to improve glucose metabolism by slowing the digestion and absorption of carbohydrates, likely through their food form and botanical structure rather than their fibre content or type<sup>349</sup>. In addition, whole grains are also high in antioxidant activity and suggested to have a similar level to that of fruit or vegetables on a per serving basis<sup>349</sup>. To our knowledge, the consumption of grains and T2D risk has not yet been properly assessed in the Australian population. In the Melbourne Collaborative Cohort Study, a high consumption of white bread and savoury cereal products was associated with increased T2D incidence, and "other cereal" was associated with decreased incidence<sup>335</sup>. However, the "other cereal" was not explicitly whole grains. Future studies should determine the effect of grains, and in particular, whole grains in the Australian population.



Inverse relations between dairy intake and T2D have been reported in meta-analyses<sup>141,217</sup>, though not consistently<sup>350</sup>. Grantham *et al.* have previously analyzed dairy intake in this cohort. Total dairy intake was not associated with T2D in the whole population, but was associated with decreased risk in men<sup>351</sup>. Subgroup analyses in meta-analyses have found decreased risk in women, but not in men<sup>141,217</sup>. The type of dairy consumed may be important, as pooled results suggest that low-fat dairy decreases risk of T2D by 12% per 200g/d<sup>141</sup> and high-fat dairy is not associated<sup>141,217</sup>. In this cohort, low-fat, but not full-fat milk was previously associated with a decreased risk of T2D<sup>351</sup>. Dairy intake may protect against the development of T2D through its effects on obesity<sup>352</sup>, food intake regulation<sup>353</sup>, the insulinotropic properties of whey protein, and enhanced secretion of insulinotropic amino acids and incretin hormones by lactose<sup>354</sup>. Despite the somewhat unclear effect of dairy intake on T2D in the literature, our results suggest that more than a third of the cases in this population could have been prevented with recommended dairy and/or alternatives intake.

We observed a positive dose-response relationship between protein-rich foods (lean meats and poultry, fish, eggs, tofu, nuts/seeds, and legumes/beans) and T2D risk and increased T2D with exceeding the limits of protein-rich foods, though studies do not suggest negative effects of high-protein diets<sup>355,356</sup>. However, meta-analyses do suggest high intakes of red meat increase diabetes risk<sup>139</sup>. In this cohort, red meat intake contributed more than half (53%) of the intake of protein-rich foods and was also significantly correlated with processed meat intake (0.39,  $p < 0.0001$ ) which has been strongly related to increased T2D risk<sup>139</sup>. Studies propose



benefits from replacing animal protein with plant protein such that substitution of 1% of energy from animal protein with plant protein may decrease T2D risk by almost 20%<sup>357</sup>. In addition, the method of cooking red meat may affect diabetes risk. Compared to less than once per month, broiling, barbequing, and roasting red meat  $\geq 2$ /week were each independently associated with increased risk of T2D, whereas stewing/broiling were not associated and pan-frying was inversely associated<sup>358</sup>. As these seemingly harmful methods of cooking red meat may be quite prevalent in Australia and red meat consumption represents a significant proportion of protein-rich foods, decreasing red meat intake could be beneficial in terms of diabetes risk.

#### 4.6.2 OVERALL DIETARY ADHERENCE AND TYPE 2 DIABETES

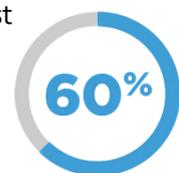
Alhazmi *et al.* have previously examined adherence to former versions of the Australian dietary recommendations and T2D<sup>133</sup>. This study was conducted in women and used two dietary indices to evaluate adherence to the dietary guidelines. The first was the Australian Recommended Food Score, and included 9 components, dichotomized based on meeting recommendations or not. The second score was the Dietary Guideline Index, which included 13 components and assigned 10 points for meeting the recommendation (ex. 2 servings of fruit per day), 5 points for one serving of fruit per day and 0 points for not consuming fruit. The Dietary Guideline Index was significantly inversely related with T2D risk (OR=0.51 [0.35-0.76]) when comparing the highest score quintile to the lowest, but the Australian Recommended Food Score was not associated (OR=0.99 [0.68-1.43]). Although adherence to some of the components was associated with T2D risk in this study, overall adherence to the dietary guidelines was not associated with diabetes, except when we considered moderate alcohol consumption as beneficial in the sensitivity analyses.

#### 4.6.3 POPULATION ATTRIBUTABLE FRACTIONS

Our estimates of the effect of excess weight in the Australian population are somewhat weaker than the effects estimated in other countries, which range from 50-77% (in the US and Finland, respectively)<sup>27,31</sup>, but are slightly stronger than the estimated effects in China (36.0% [33.5-38.4]<sup>29</sup>). We estimated that almost a fifth of incident diabetes could have been prevented with sufficient ( $\geq 150$ mins/week) recreational physical activity. Other studies have calculated the effect of eliminating physical inactivity that ranged from 5.2-13% in Canada and Finland, respectively<sup>36</sup>.

Though the PAF for total adherence to the dietary guidelines was not significant, our data suggest that strongly adhering to recommendations concerning fruit or dairy could have avoided up to 37% of incident T2D. Other studies have estimated an unhealthy diet, (defined by four components: vegetables, fruit, red meat and wheat), to contribute to the development of 26.5% [15.3-36.9] of incident type 2 diabetes<sup>29</sup>, and that 23-27% of diabetes could have been prevented with low intakes of processed red meat<sup>27</sup>.

The total combination of modifiable health-related behaviours suggests that almost 60% of the cases of T2D could have been avoided in this population. These effects appear slightly more moderate than in previous studies, as in China, Finland, and Hawaii, ideal health-related behaviours could have prevented approximately 80% of cases<sup>27,29,31</sup> and in two studies in the US, approximately 90% of cases<sup>28,30</sup>. The impact of adherence to the dietary guidelines for a single component appears large in this population, however, interventions should not focus on a single target. Studies suggest that people who adopt one healthy behaviour are likely to adopt multiple healthy behaviours, and thus multiple behaviour interventions may have the potential for a stronger impact<sup>359</sup>.



#### 4.6.4 STRENGTHS AND LIMITATIONS

Potential limitations should be considered. Dietary adherence is based on a single questionnaire at baseline in 1999, thus the data are relatively old and misclassification of exposure is possible.

However, the prospective design prevents differential bias in the collection of the dietary data, allowing the study of causation between the diet and diabetes. In addition, we estimated PAFs using the most recent prevalence data available in Australia and had similar results. Reporting bias is also a possibility, as individuals tend to over-report fruit and vegetable consumption and under-report total energy intake and protein, especially those who are overweight or obese<sup>360,361</sup>. Although we took into account the most important confounding factors, residual confounding cannot be excluded. Finally, selection bias may have occurred despite the fact that we had a large, stratified random sample of the population, and as with all volunteer study populations, a “healthy volunteer” selection bias is likely and may have attenuated our associations.

There are also several strengths; AusDiab is a large, population-based study in which glucose was objectively measured using an OGTT. To our knowledge, this is also the first study to evaluate the association between the main components of the Australian dietary guidelines and the risk of T2D, both by adherence and in a dose-response manner, and to estimate the population-level impact of adherence to these components with other modifiable behaviours. The use of an index to estimate the preventable proportion of T2D is another strength of this study, as most studies estimate PAFs for individual risk factors. But, individual risk factors often co-occur or cluster and may interact with each other, suggesting that an index could provide a more comprehensive estimate<sup>362</sup>.

#### 4.7 CONCLUSION

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Our results suggest that more than half of the cases of T2D could have been prevented with healthy behaviours in this Australian population. A healthy waist circumference could have alone prevented more than 40% of the T2D cases, and adherence to fruit or dairy intake recommendations could have prevented approximately a quarter and a third of the T2D cases. These results underline the impact that modifiable behaviour has on the risk of T2D. Vegetable consumption should be emphasized in the Australian population, but efforts should not be focused on a single component, as the greatest reduction is observed with strong adherence to recommendations for all modifiable behaviours. Interventions should focus on changing these modifiable behaviours in the population.

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# CONCLUSIONS AND FUTURE PROSPECTS

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With this work, the complex relationship between the diet, type 2 diabetes and an important microvascular complication of diabetes, diabetic retinopathy, was able to be explored.

## 1. MAIN RESULTS

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Despite studies suggesting that saturated fat may promote insulin resistance, and mounting evidence suggesting the beneficial role for n-3 PUFAs in insulin insensitivity and cardiovascular health, the findings of this thesis were not in agreement<sup>248,363</sup>. High intakes of SFAs were not related to either the risk of T2D or DED and high intakes of the n-3 PUFAs  $\alpha$ -linolenic acid and docosapentaenoic acid were associated with an increased risk of T2D in overweight women of the E3N cohort study, even after adjustment for their primary dietary source. With regards to DR, though n-3 PUFAs, and especially docosahexaenoic acid, play a significant role in the functioning of the retina, we observed no association with DED<sup>364</sup>. On the other hand, the n-6 PUFA arachidonic acid was associated with an increased risk of T2D, but decreased odds of DED. The n-6 PUFA linoleic acid was also associated with decreased odds of DED with moderate intakes.

From this, we can conclude that the effects of PUFAs may not be homogenous within the group. The conflicting results may be due to the fact that FAs such as arachidonic acid have both proinflammatory and anti-inflammatory effects, or that the metabolism of some PUFAs is affected by other PUFAs<sup>223,240,324</sup>. In addition, multiple aspects affect the conversion rate of  $\alpha$ -linolenic acid and linoleic acid to other PUFAs, and may be another factor to consider. From a public health perspective, based on these results, we could not recommend high intakes of PUFAs as beneficial for T2D risk, as intakes of arachidonic acid corresponding to around 200g of sausages/day was associated with an increased risk of T2D by 40% in the overall cohort. *A wide diet, with moderate and varied consumption seems most beneficial with respect to the present results, for both the prevention of type 2 diabetes and diabetic eye disease.*

Overall, strong adherence to the national dietary guidelines was not associated with the risk of T2D in the Australian AusDiab cohort study. But, strong adherence to the guidelines regarding dairy and fruit intake were associated with a decreased risk of T2D and may have prevented between 23-37% of T2D cases, and exceeding the limits of protein-rich foods was associated with an increased risk of T2D. *Based on these results and the existing literature, a diet with adequate fruit and dairy consumption, limiting the intake of animal-based proteins, could be recommended as ideal in the prevention of type 2 diabetes.*

Strongly adhering to the guidelines regarding modifiable behaviours may have prevented up to 60% of the incident T2D cases, underlining the importance of modifiable behaviours in the development of T2D. These results demonstrate the importance that multiple target interventions could have in public health, as the greatest reduction is observed with strong adherence to the recommendations for all modifiable behaviours. *Interventions should aim to modify multiple health behaviours for the greatest reduction in the incidence of type 2 diabetes.*

## 2. RESEARCH PERSPECTIVES

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The work of this thesis allowed a more in-depth understanding of the relationship between the diet, type 2 diabetes and retinopathy. However, there remain many areas that would be interesting to address in future studies.

### 2.1 E3N AFTERDIAB

Given the size of the E3N cohort and the Afterdiab population, the relationship between other diabetes complications and fatty acids should be addressed. It would be interesting to see whether FAs share similar associations with the development of other microvascular complications such as neuropathy and nephropathy, or macrovascular complications such as cardiovascular disease. As it is suggested that retinopathy and nephropathy may also increase the risk for each other<sup>92</sup>, it would also be interesting to determine whether the development of multiple complications are concordant with each other in this cohort.

Other aspects of the diet, such as the intake of the various food groups, different dietary patterns, and the total antioxidant capacity could also be examined, to determine whether the same kinds of patterns or food groups that may be detrimental or beneficial to the development of diabetes may also be beneficial or detrimental in the development of its complications. In particular, a Mediterranean diet, which has been inversely associated with the risk of T2D in this cohort, could be examined in relation to DR or other T2D complications.

### 2.2 E4N

A wealth of information will soon be present in E4N and would allow the invaluable opportunity to study various relationships within families that share common genetics and environments. The effect of the dietary pattern, intake of certain foods or food groups, and the intakes of FAs could be analyzed in the offspring of women with T2D to determine the concordance. In addition, the appearance of diabetic complications in the newer generations could also be determined. It would be interesting to estimate as well, the impact the diet would have in these newer generations, given that foods today may have lower nutrient compositions and higher levels of pesticides than food 20 years ago<sup>338,339,365</sup>.

### 2.3 AUSDIAB

The relationship between PUFA intake and mortality has been analyzed in the AusDiab cohort, but the relationship between PUFA, or the intake of individual FAs and T2D has not yet been analyzed. This perspective offers an opportunity to confirm or refute the findings of the first objective of this thesis in a population with a different diet, lifestyle and environment. The population would also allow the analysis in men, which E3N lacks, and may offer more variability as the sample is based off a stratified cluster sample of the general population, while E3N includes women in the MGEN.

### 2.4 POSTDOCTORAL PERSPECTIVES

I wish to continue to explore the relationship between the diet and diabetes or diabetes complications, hopefully within the setting of a postdoctoral position with the EPIC team in Potsdam, Germany. The team is currently working to collect data on diabetes complications and will have around 5000 participants reassessed by 2019/2020. I would be interested in analyzing the relationship between FA consumption and diabetes complications in this cohort, as well as the impact of the dietary pattern. They are also looking to extend their prediction models for diabetes risk into diabetic complications as well, which would provide a unique opportunity to work with a different kind of methodological model.

### 2.3 CONCLUDING REMARKS

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This thesis provided me with the valuable opportunity to work with a very large dataset in a team addressing many different thematic, from various cancers to diabetes and epigenetics. Working in such a diverse environment allowed me constant exposure to new epidemiological methods and topics, while at the same time, permitted me to refine the skills acquired throughout previous experiences. I was also afforded the chance to work with the AusDiab team in Melbourne, Australia, with a smaller, but diverse dataset on different statistical software. My knowledge in the field of diabetes and nutrition has grown extensively, through broad reviews of the literature and through my colleagues. In addition, I applied new statistical methods and models such as cubic spline regression and population attributable fractions I hadn't had the chance to previously. This opportunity was an inestimably valuable experience and I will be forever grateful to the E3N and the AusDiab teams for welcoming me, supporting me and constantly enriching my knowledge.



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# ANNEXES

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## ANNEX 1. RESUME EN FRANÇAIS

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### 1. INTRODUCTION

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Le diabète est une maladie chronique qui touche environ 425 million d'adultes dans le monde<sup>1</sup>. Il est caractérisé par une déficience en insuline qui entraîne une hyperglycémie et peut mener à différentes complications et à une mortalité prématurée<sup>2,3</sup>. Le diabète de type 2 (DT2) représente la majorité des diabètes (90-95%) et malgré le fait qu'il est en grande partie évitable, sa prévalence continue d'augmenter. Si le diabète n'est pas bien géré, des complications telles que la rétinopathie diabétique (RD), qui cause une perte de vision ou la cécité en cas d'absence de traitement, viennent s'ajouter au fardeau déjà important de la maladie diabétique<sup>4</sup>. A cause de la hausse significative dans la prévalence du diabète, la RD est devenue l'une des causes principales d'aveuglement dans la population en âge de travailler du monde occidental<sup>5</sup>. Les facteurs modifiables, comme l'alimentation, jouent un rôle critique dans le développement et le management du DT2 et de ses complications, mais les rôles des certains aspects de l'alimentation ne sont pas encore clairs. Pour cette raison, j'ai étudié pendant ma thèse le *rôle des facteurs alimentaires dans le développement de DT2 et de la RD*, en utilisant les données françaises et australiennes.

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#### 1.1 LE DIABETE

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Il y a trois principaux types de diabètes. Le *diabète de type 1 (DT1)* est causé par une réponse auto-immune où le corps attaque les cellules qui produisent l'insuline du pancréas et entraîne une incapacité à produire assez d'insuline<sup>1</sup>. Les origines de la réponse auto-immune ne sont pas connues mais les facteurs génétiques et environnementaux sont suspectés. Le DT1 survient le plus souvent pendant l'enfance et représente 5-10% des cas de diabète<sup>1,2</sup>. Le *diabète de type 2 (DT2)* survient quand le corps perd sa capacité à répondre à l'insuline (résistance à l'insuline) et à produire l'insuline<sup>3</sup>. Les origines du DT2 sont discutées plus loin dans l'introduction. Il compte pour la plupart des cas de diabète (90-95%) et survient plus souvent à l'âge adulte<sup>2</sup>. Finalement, le *diabète gestationnel* est défini par l'Organisation Mondiale de la Santé comme une hyperglycémie qui est reconnue pour la première fois pendant une grossesse<sup>7</sup>.

##### 1.1.1 PATHOPHYSIOLOGIE DE DIABETE DE TYPE 2

Des taux élevés du glucose (par exemple, après un repas), stimulent la production d'insuline par le pancréas, et l'insuline active des récepteurs insuliniques dans les tissu sensibles à l'insuline (muscle, zones adipeuses, foie) pour permettre au glucose d'entrer la cellule<sup>10,11</sup>. Mais, chez les individus avec une hyperglycémie chronique, une résistance à l'insuline peut développer et/ou entraîner des dysfonctionnements des cellules de production d'insuline<sup>14</sup>. Ceci a pour conséquence une diminution du transport de glucose dans les cellules et des taux élevés du glucose qui peuvent avoir des effets négatifs sur le corps.

### 1.1.2 EPIDEMIOLOGIE DE DIABETE

Selon la Fédération Internationale du Diabète, la prévalence mondiale de diabète était de **8.8%** [7.2-11.3%] en 2017, soit 629 millions de personnes<sup>1</sup>. Cette prévalence devrait augmenter de plus de **45%** d'ici 2045 pour s'élever à 9.9% [7.5-12.7%]<sup>1</sup>.

### 1.1.3 FACTEURS DE RISQUE DE DIABETE DE TYPE 2

Le risque de développer un diabète de type 2 est lié aux facteurs modifiables et non-modifiables. Les facteurs modifiables incluent: l'obésité ou le surpoids, le niveau d'activité physique, l'alimentation, l'hypertension, le statut tabagique, le stress, et le sommeil. Les facteurs non-modifiables incluent: l'âge, le sexe, les antécédents familiaux, les gènes, le diabète gestationnel, le poids de naissance, l'ethnicité et les facteurs environnementaux tels que la pollution et les polluants.

### 1.1.4 COMPLICATIONS DE DIABETE

Le diabète favorise la survenue de complications macro- et micro-vasculaires. Des individus avec un diabète ont 2-3 fois plus de risque de développer une maladie cardiovasculaire et 80% des maladies rénales sont dus à un diabète<sup>3</sup>. Le diabète peut aussi endommager les nerfs et augmente le taux d'amputations par 10-20 fois chez les diabétiques ; c'est aussi une cause principale de la perte de vision chez les adultes en âge de travailler<sup>3</sup>.

## 1.2 LA RETINOPATHIE DIABETIQUE

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La rétinopathie diabétique est une complication microvasculaire de diabète. On considère qu'elle est principalement causée par une hyperglycémie chronique, qui a pour conséquence un changement dans la circulation sanguine des capillaires de la rétine qui entraîne des blocages et des fuites<sup>4</sup>. Lorsque la RD n'est pas traitée, elle peut mener à une perte de vision ou la cécité<sup>4</sup>.

### 1.2.1 STADES DE RETINOPATHIE

Il y a deux stades principaux de RD ; le premier stade est la *rétinopathie non-proliférante*. Dans ce stade, il y a une perte des cellules des vaisseaux sanguins qui entraîne des obstructions et des fuites des capillaires avec pour conséquence la diminution de l'apport sanguin<sup>68</sup>. Cliniquement, ce stade est caractérisé par des anévrysmes, des hémorragies et des exudats (dépôts de lipides et lipoprotéines qui causent l'inflammation)<sup>4,68,69</sup>. Dans le deuxième stade, la *rétinopathie proliférante*, à cause de l'ischémie, il y a le développement de nouveaux vaisseaux sanguins qui peut entraîner la cécité comme ces vaisseaux sont fragiles et sujets aux ruptures<sup>68</sup>. A tout moment, un œdème maculaire peut également survenir en raison d'une accumulation de fluides dans le macula, qui se trouve au milieu de la rétine<sup>64,65</sup>.

### 1.2.2 EPIDEMIOLOGIE DE RETINOPATHIE

La rétinopathie est diagnostiquée chez plus d'un tiers des diabétiques (34.6%)<sup>4,87</sup>. On prévoit que sa prévalence augmente avec la prévalence du diabète et il est estimé qu'avant 2030, plus de 191 millions de personnes seront atteintes d'une rétinopathie diabétique<sup>88</sup>. La RD est responsable pour 15-17% de cas de cécité aux Etats-Unis et en Europe et de 3-7% et de cas en Asie du Sud-est et dans la région du Pacifique occidental<sup>5,89</sup>.

### 1.2.3 FACTEURS DE RISQUE DE RETINOPATHIE

Les facteurs de risque établis pour la rétinopathie incluent : l'hyperglycémie, la durée de diabète, l'hypertension, et le type de diabète. Les facteurs de risque possibles incluent : le sexe, la dyslipidémie, les gènes, l'ethnicité, le statut tabagique, le niveau d'activité physique, le surpoids ou obésité, et les facteurs environnementaux.

### 1.3 OBJECTIFS

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Même si le diabète est pour la plupart du temps évitable, il représente un fardeau significatif de santé publique qui est prévu d'augmenter dans le futur<sup>1</sup>. Ce fardeau est associé aux complications qui alourdissent le fardeau de la maladie chez les personnes diabétiques, comme la rétinopathie diabétique, qui est diagnostiquée chez plus d'un tiers des personnes diabétiques et qui actuellement est la plus grande cause de cécité dans les pays développés<sup>4,5</sup>. Des facteurs modifiables, comme l'alimentation, sont identifiés pour le DT2 et la RD, mais certains aspects du rôle de l'alimentation ne sont pas encore clairs.

Une de ces aspects est le rôle des acides gras. Même si les graisses constituent une grande partie de l'apport calorique et les acides gras ont des effets métaboliques forts, leur rôle dans le développement du DT2 n'est pas encore établi. Les études suggèrent que les effets des acides gras peuvent changer selon la région géographique, ou au sein ou entre les groupes d'acides gras, mais quelques acides gras ne sont presque jamais étudiés individuellement et leurs effets dans le DT2 n'est pas encore connu.

Le **premier objectif** de cette thèse était d'examiner les associations entre les groupes primaires d'acides gras et l'incidence de DT2, avec un focus particulier sur la relation entre les acides gras polyinsaturés omega-3 et 6 et le risque de DT2 dans la *cohorte française E3N*.

Actuellement, peu d'études ont évalué le rôle de l'alimentation ou ses composants sur le risque de RD et ceux qui l'ont fait ont trouvé des résultats discordants.

Le **deuxième objectif** de cette thèse était d'identifier, synthétiser, et interpréter la littérature sur l'association entre les facteurs alimentaires et le risque de RD ou œdème maculaire dans une revue systématique de la littérature, et le **troisième objectif** était d'analyser l'association entre la consommation des groupes primaires des acides gras et le développement d'une RD dans une *sous-étude de la cohorte française E3N*.

Les pays rédigent des recommandations alimentaires fondées sur les résultats de la littérature scientifique connue à ce jour avec le but d'améliorer la santé et le bien-être des populations à l'aide notamment d'une alimentation adaptée. Cependant, l'effet de l'adhésion à ces recommandations pour la prévention du DT2 n'est pas encore clair, et cet effet peut varier selon les pays et présenter des interactions avec d'autres facteurs de risque. L'estimation de l'impact d'adhésion aux recommandations portant sur l'alimentation et les autres facteurs de risque modifiables peut fournir des informations précieuses pour les dirigeants de santé publique, mais les données sont rares et souvent concernent des facteurs de risque individuels.

Pour cette raison, le **quatrième objectif** de cette thèse était de quantifier la relation entre une forte adhésion aux recommandations alimentaires et aux recommandations alimentaires et le risque de DT2. De plus, il s'agissait aussi de déterminer l'impact des recommandations alimentaires et des facteurs de risque de DT2 modifiables au niveau d'une population en estimant les fractions de risque attribuables dans la *cohorte australienne AusDiab*.

## 2. MATERIELS ET METHODES

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### 2.1 E3N

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#### 2.1.1 DESCRIPTION DE LA COHORTE

E3N (*l'Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale (MGEN)*), est une cohorte prospective de *98 995 femmes* assurées par la MGEN, dans le cadre d'un programme national de l'assurance maladie<sup>166</sup>. E3N a commencé en 1990 et a recruté des bénévoles nées entre 1925 et 1950 partout en France avec le but principal d'examiner la mode de vie et les facteurs nutritionnel, hormonaux, et génétiques associés avec le cancer et les autres maladies non-transmissibles chez les femmes. Cette étude a été approuvée par le Commission Nationale Informatique et Liberté (CNIL), et le consentement informé a été obtenu de toutes les participantes.

#### 2.1.2 COLLECTION DES DONNEES

Des auto-questionnaires (Q1-Q11) ont été envoyés aux participantes tous les 2-3 ans pour collecter les données. Le taux de participation est resté élevé : 83% pour les 11 cycles des questionnaires, et le taux de perdues de vue depuis 1990 est restée bas (3% des femmes n'ont jamais répondu à un questionnaire, ont quitté l'étude ou le contact a été perdu avec elles).

Les questionnaires ont porté sur l'anthropométrie, le mode de vie et l'état de santé. De plus, une banque biologique a été constituée avec des échantillons sanguins collectés entre 1994 et 1999 chez environ 25 000 participantes (taux de participation ~40%) et des échantillons de salive collectés entre 2009 et 2011 chez 44 775 femmes (taux de participation ~69%). De plus, les données de la MGEN sont disponibles depuis 2004 et fournissent des informations sur les remboursements des médicaments des femmes E3N.

#### 2.1.3 QUESTIONNAIRE ALIMENTAIRE

Les données alimentaires sont disponibles grâce à un questionnaire d'histoire alimentaire semi-quantitatif envoyé en 1993 et en 2005<sup>170</sup>. Environ 80% des femmes ont répondu au premier questionnaire alimentaire, soit un total de 74 552 questionnaires retournés. Le taux de réponse pour le deuxième questionnaire était de 77% et 71 412 questionnaires ont pu être analysés. Le questionnaire alimentaire permettait de décrire les habitudes alimentaires des femmes au cours de l'année passée et était composé des deux parties : une partie quantitative et une partie qualitative.

La consommation des acides gras a pu ensuite être estimée en combinant une table de composition nutritionnelle publiée par l'ANSES (l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail) et les résultats du questionnaire alimentaire.

#### 2.1.4 EVALUATION DE DIABETE DE TYPE 2

Les cas potentiels de DT2 ont été identifiés soit dans les questionnaires de suivi avec une déclaration d'une des réponses suivantes : (1) DT2 ; (2) un régime diabétique recommandé par un médecin ; (3) l'usage de médicaments anti-diabétiques ; ou (4) une hospitalisation pour un diabète ou les cas étaient identifiés comme recevant des remboursements de médicaments diabétiques<sup>171</sup>. Ceux identifiés dans les deux façons étaient considérés comme validés. Toutes les femmes ont ensuite reçu un questionnaire spécifique pour le diabète, et les cas étaient validés si : (1) glycémie plasmatique à jeun  $\geq 7.0$  mmol/L, (2) glycémie à n'importe quel moment de la journée  $\geq 11.1$  mmol/L, (3) usage des médicaments anti-diabétiques, ou (4) dernières valeurs de concentrations d'HbA1c  $\geq 7.0$  mmol/L ou  $\geq 7\%$ . Finalement, *5238 cas de diabète incidents* ont pu être validés dans E3N.

Les femmes avec des cas de DT2 validés ont été invitées à répondre à un questionnaire *AfterDiab* qui les interrogeait sur leur diabète, le traitement, les complications et leur qualité de vie. Au total, *3473 femmes* ont répondu et ont été incluses dans E3N-AfterDiab.

### 2.1.5 EVALUATION DE RETINOPATHIE

Comme l'œdème maculaire est considéré comme une complication de la RD, les cas potentiels de RD ont été dans un premier temps identifiés dans le questionnaire AfterDiab avec une réponse « oui » pour l'un des suivants : (1) rétinopathie ; (2) œdème maculaire ; (3) la perte de vision d'au moins un œil ; ou (4) traitement par laser. Des questionnaires de validation avec demande du dossier médical ont été envoyés aux cas potentiels (n=889) et 659 ont été retournés. Au total *77 personnes avec des maladies oculaires diabétiques (DED)* ont été identifiés, représentant 60 RD et 35 œdèmes maculaires.

### 2.1.6 ANALYSES STATISTIQUES

La population finale de l'étude sur les acides gras et le DT2 était de *71 334 femmes*, dont 2610 cas de DT2. Les tertiles de consommation des principaux acides gras ont été étudiés en relation avec le risque de DT2 à l'aide de modèles de Cox multivariés, ajustés sur les principaux facteurs de risque de DT2. Des hazard ratios (HR) de DT2 ainsi que leurs intervalles de confiance à 95% ([IC95%]) ont été estimés. Dans les analyses de sensibilité, les modèles ont été ajustés sur la consommation des autres acides gras ainsi que sur la source alimentaire principale de l'acide gras en question.

La population finale de l'étude entre les acides gras et les DED était de *1949 femmes*, avec 77 cas de DED. Les tertiles de consommation des principaux acides gras et leurs sources alimentaires principales ont été étudiés en relation avec la maladie oculaire diabétique avec des modèles logistiques ajustés sur les principaux facteurs de risque de RD. Des odds ratios (OR) de la DED et les intervalles de confiance à 95% ont été estimés. De plus, des modèles de régression par splines cubiques ajustés sur des facteurs de confusion ont été utilisés pour modéliser des associations potentiellement non linéaires entre les acides gras et leurs sources alimentaires principales et la DED.

## 2.2 AUSDIAB

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### 2.2.1 DESCRIPTION DE LA COHORTE

La cohorte AusDiab (Australian Diabetes, Obesity and Lifestyle Study) est une étude longitudinale basée sur la population et comprend *11 247 adultes australiens* âgés de 25 ans ou plus. Elle a commencé en 1999 et 2 collectes d'informations pendant les 12 années de suivi ont été réalisées. Les participants ont été recrutés de partout en Australie avec le but principal de déterminer la prévalence de diabète, de l'obésité et les autres facteurs de risque des maladies cardiovasculaires en Australie. L'étude est approuvée par le Baker IDI Heart and Diabetes Institute Ethics Committee et le consentement informé collecté auprès de tous les participants.

### 2.2.2 COLLECTION DES DONNEES

L'enquête principale en 1999 a collecté des données sur le mode de vie, des facteurs sociaux et les facteurs de santé et a été complétée par des examens biomédicaux. De 20 347 participants éligibles, 55% ont complété l'enquête et les examens biomédicaux pour une population initiale d'étude de 11 247. En 2004-2005 et 2011-2012, des autres enquêtes de suivi par des examens biomédicaux ont eu lieu.

### 2.2.3 QUESTIONNAIRE ALIMENTAIRE

Les données alimentaires ont été collectées avec un questionnaire de fréquence alimentaire validé et développé pour la population. Ce questionnaire permettait d'estimer la consommation moyenne, sur l'année précédente, de chaque aliment en grammes par jour.

### 2.2.4 EVALUATION DE DIABETE DE TYPE 2

Tous les participants sauf ceux qui prenaient un traitement pour le diabète ou les femmes qui étaient enceintes ont fait un test de tolérance de glucose pendant les examens biomédicaux. Le diabète était validé selon les critères de l'OMS 1999<sup>366</sup>.

### 2.2.5 CREATION DE L'INDEX D'ADHERENCE AUX RECOMMANDATIONS ALIMENTAIRES

Un index d'adhésion aux recommandations alimentaires a été construit basé sur les recommandations nationales de 2013 par âge et sexe pour *5 groupes alimentaires* principaux : (1) « fruits » ; (2) « légumes » ; (3) « céréales » ; (4) « produits laitiers » ; et (5) « protéines ». De plus, les recommandations spécifiaient, pas plus de 2 boissons alcoolisées standards par jour. L'adhésion aux 6 groupes alimentaires a été calculée et classée comme : « une forte adhésion », « une adhésion intermédiaire », « une adhésion faible » ou un « dépassement des limites ».

### 2.2.6 CREATION DE L'INDEX DE MODE DE VIE

Un index de mode de vie a été créé en donnant des scores pour une adhésion plus forte aux recommandations de l'OMS ou du Département de Santé Australien. Cet index prenait en compte *4 comportements* : le statut tabagique, le tour de taille, le niveau d'activité physique et l'adhésion aux recommandations alimentaires.

### 2.2.5 ANALYSES STATISTIQUES

La population finale de cette étude était de *6242* sujets donc *376* cas de DT2. L'adhésion aux recommandations alimentaires et mode de vie a été étudiée en relation avec le risque de DT2 à l'aide de modèles de Cox multivariés, ajustés sur les principaux facteurs de risque de DT2. Des hazard ratios (HR) de DT2 ainsi que leurs intervalles de confiance à 95% ont été estimés. En plus, les modèles de régression par splines cubiques ont été utilisés pour identifier des relations non-linéaires potentielles entre le nombre des portions de chaque groupe alimentaire et le risque d'un DT2. Des fractions attribuables (PAF) pour une forte adhésion aux recommandations ont aussi été calculées. Dans les analyses de sensibilité, une consommation modérée d'alcool au lieu d'aucun alcool était considérée comme bénéfique.

## 2.3 REVUE SYSTEMATIQUE DE LA LITTERATURE

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Une revue systématique a été menée pour identifier, synthétiser et interpréter la littérature sur l'association entre les facteurs alimentaires et la rétinopathie diabétique ou œdème maculaire.

### 2.3.1 RECHERCHE DES ARTICLES ET EVALUATION DES ARTICLES

Une recherche a été faite sur Pubmed et Web of Science sans limites de temps pour les études en anglais qui évaluaient l'association entre d'une part la consommation des aliments individuels, macronutriments, micronutriments, compléments alimentaires, et profils alimentaires et d'autre part la rétinopathie diabétique ou l'œdème maculaire. Nous avons examiné les résumés potentiels, et si nécessaire, les textes complets. La qualité des études a été évaluée en utilisant le Cochrane Risk of Bias Tool for Randomized Controlled Trials pour les essais randomisés et le Newcastle-Ottawa Scale pour les études observationnelles.

## 3. RESULTATS ET DISCUSSION

### 3.1 ACIDES GRAS ET RISQUE DE DT<sub>2</sub>

#### 3.1.1 RESULTATS

Les acides gras saturés (AGS), mono-insaturés (AGMI), poly-insaturés (AGPI), et trans insaturés (AGTI) n'ont pas été associés au risque de DT<sub>2</sub> après ajustement sur les facteurs de confusion (HR=1.07 [0.92-1.25]; HR= 1.06 [0.95-1.19]; HR=1.06 [0.96-1.17]; HR = 1.02 [0.89 -1.17], respectivement), mais dans le groupe des AGPIs, les acides gras omega-3 (n-3 AGPI), mais pas les omega-6 (n-6 AGPI) étaient associés à une augmentation de risque de DT<sub>2</sub> (HR=1.26 [1.13-1.41]; HR=1.00 [0.90-1.10], respectivement).

Après stratification par IMC, dans les modèles ajustés, les AGS, les AGMIs et les AGTIs n'ont pas été associés avec le risque de DT<sub>2</sub> dans aucune strate. Mais, une consommation élevée des AGPIs était associée avec un risque augmenté de DT<sub>2</sub> chez les femmes non en surpoids (HR=1.22 [1.05-1.42]), mais pas chez les femmes en surpoids (HR=1.02 [0.90-1.16]). Les n-6 AGPIs n'ont pas été associés avec le DT<sub>2</sub> dans aucune strate (HR=1.13 [0.96-1.32], HR=0.95 [0.83-1.08]; femmes non en surpoids, femmes en surpoids, respectivement) mais les n-3 AGPIs ont été associées à une augmentation de risque de DT<sub>2</sub> (HR=1.19 [1.01-1.40]; HR=1.38 [1.20-1.59]; femmes non en surpoids et en surpoids, respectivement).

Après analyse des AGPIs individuellement, seulement l'acide arachidonique (AA), docosapentaénoïque (DPA) et  $\alpha$ -linoléique (ALA) ont été associés au DT<sub>2</sub> (HR= 1.49 [1.33-1.66]; HR=1.41 [1.23-1.63]; HR=1.17 [1.23-1.63], respectivement), mais ALA seulement chez les femmes en surpoids, même après ajustement sur la source alimentaire principale d'AA et DPA, la viande.

#### 3.1.2 DISCUSSION

D'autres études n'ont pas non plus trouvé d'association entre les AGS et le risque de DT<sub>2</sub><sup>157,158,208,209</sup>. Mais les résultats récents suggèrent que les AGS n'ont pas d'effets homogènes sur le risque de DT<sub>2</sub>. Des mesures plasmatiques des AGS avec des chaînes très longues et des AGS avec des chaînes en nombre impair ont été associés à une diminution de risque de DT<sub>2</sub>, alors que les chaînes en nombre pair ont été associées à une augmentation de risque de DT<sub>2</sub><sup>153,160,207</sup>. Les effets de la consommation des chaînes des longueurs différentes AGS devraient être évalués dans les études futures, puisque le groupe des AGS semblent avoir des effets différents sur le risque de DT<sub>2</sub>. Nos résultats montrant que les AGMI ne sont pas associés sont en accord avec d'autres études<sup>157,158</sup>. Mais, dans notre cohorte, les produits laitiers et la viande ont été les sources principales des AGMI, alors que dans d'autres études, l'huile d'olive, une source importante des AGMIs a été trouvé bénéfique pour le DT<sub>2</sub><sup>214,215</sup>. La source des AGMIs peut être un facteur de confusion et expliquer l'association nulle qu'on a observée.

En revanche, les résultats ne sont pas en accord avec d'autres études où la consommation des AGPIs a été associée à une diminution de risque de DT<sub>2</sub><sup>157,158</sup>. Mais, tout comme les AGS, les AGPIs ont peut-être des effets différents sur le DT<sub>2</sub>. Peu d'études ont examiné les associations entre les types d'AGPIs sur le risque de DT<sub>2</sub> mais celles qui ont été menées ne suggèrent pas d'association entre la consommation d'ALA et DT<sub>2</sub><sup>161,163,219</sup>. C'est possible que l'association avec ALA diffère par région géographique ou que l'IMC, qui peut être un médiateur, ait déformé la relation, ce qui pourrait expliquer les différences entre ces études et la nôtre.

Les acides gras issus des produits de la mer (EPA, DPA, DHA) sont rarement analysés individuellement. Mais une étude cas-cohorte les a analysés et n'a pas trouvé d'association avec le risque de DT2<sup>219</sup>. Mais la consommation moyenne de DPA était plus basse dans cette étude que dans E3N, et les sources principales de DPA auraient pu confondre la relation. De plus, cette étude incluait des hommes et des différences dans les effets des acides gras peuvent exister selon le sexe et pourraient expliquer les différences dans les résultats<sup>162</sup>. Concernant l'AA, seulement une étude cas-cohorte a regardé l'association avec DT2 et ils n'a pas été trouvé d'association avec aucun n-6 AGPI<sup>219</sup>. Mais, la consommation moyenne d'AA semblait plus basse dans cette étude cas-cohorte qu'E3N, en plus, la viande aurait pu contribuer à plus de 70% à la consommation d'AA alors que la viande a contribué à seulement 42.7% à la consommation d'AA dans E3N. Ces différences peuvent expliquer les différences dans les résultats.

Les acides gras peuvent affecter le risque de DT2 par plusieurs voies. Une alimentation forte en AGS a été trouvée associée à l'inflammation et la résistance à l'insuline<sup>223</sup>. Ils peuvent aussi affecter le risque de DT2 par leurs effets sur la membrane cellulaire. Les AGS affectent la fluidité des membranes, les protéines incorporées dans la membrane, les activités des enzymes, et la fonction des récepteurs<sup>228</sup>. Comme la structure de la membrane est au moins partiellement déterminée par l'alimentation, ceci représente une autre voie où les AG peuvent affecter le développement d'un DT2<sup>229,230</sup>.

### 3.1.3 CONCLUSION

Malgré les preuves qui suggèrent que les AGPIs, et surtout les n-3 AGPIs sont bénéfiques pour la santé cardiovasculaire, avec nos résultats, on ne peut pas recommander une forte consommation des AGPIs comme bénéfique dans la prévention du DT2. Une forte consommation d'AA et DPA, et d'ALA chez les femmes en surpoids a été associée à une augmentation de risque de DT2, indépendamment de la consommation de viande. Ces associations devraient être évaluées d'autres études.

## 3.2 REVUE SYSTEMATIQUE SUR LES FACTEURS ALIMENTAIRES ET LA RETINOPATHIE

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### 3.2.2 RESULTATS

Un total de *27 études pertinentes* a été identifié et une seule étude s'est intéressée au risque de l'œdème maculaire. La plupart de ces études étaient soit prospectives ou nichées dans une étude prospective. Elles ont exploré la consommation de fruits, légumes, poissons, lait, glucides, fibres, acides gras, protéine, sel, potassium, vitamines C, D, et E, caroténoïdes, compléments alimentaires, thé vert et alcool. Il est suggéré qu'un régime alimentaire méditerranéen, la consommation de fruits, légumes, poissons et thé vert sont associées à un moindre risque de rétinopathie diabétique, bien que le niveau de preuve soit encore faible. Certains mécanismes biologiques sous-tendent la possibilité d'un mécanisme causal pour les fruits, légumes, et poissons, notamment via l'effet bénéfique antioxydant des vitamines C, D, E et caroténoïdes, mais les études ne sont pas toutes cohérentes entre elles. La consommation de glucides, protéines, sel, potassium, acides gras totales, acides gras saturés, et acides gras trans ne semblent pas associés à un sur-risque de rétinopathie diabétique.

### 3.2.2 CONCLUSION

L'alimentation semble jouer un rôle important dans le développement d'une rétinopathie diabétique mais les études sont encore limitées. De nombreux aspects de l'alimentation n'ont pas encore été abordés dans les études prospectives. Un régime méditerranéen et ses composants comme les

fruits, les légumes, et le poisson pourraient offrir des perspectives intéressantes en termes de prévention tertiaire qui nécessitent la mise en place d'études randomisées, focalisées sur ces groupes alimentaires.

### 3.3 ACIDES GRAS ET MALADIE D'ŒIL DIABÉTIQUE

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#### 3.3.1 RESULTATS

Les AGS, AGMI, AGPI, n-3 AGPI, et AGTI n'ont pas été associés au risque de DED dans notre étude. Mais, la consommation des n-6 AGPIs a été associée à une diminution des odds de DED dans le tertile moyen de consommation (tertile 2 médian 12.6 g/jour; OR=0.50 [0.28-0.89]). La régression cubique des splines a aussi indiqué une association significative non-linéaire ( $p=0.04$ ) des n-6 AGPIs avec le DED et une consommation modérée a été associée à des odds plus bas.

Concernant les AGPIs, une consommation modérée de LA a été associée à des chances réduites de DED (tertile 2, médiane 12.4 g/jour; OR=0.48 [0.26-0.86]). La régression par splines cubiques a suggéré une association non-linéaire de la consommation de LA avec DED ( $p=0.04$ ) mais aucun relation n'a été observée pour les autres AGPIs (ALA, DPA, EPA, DHA), sauf l'AA où une association positive a été notée ( $p=0.01$ ). Mais, dans les analyses de sensibilité, après ajustement sur la source principale d'AA (la viande), cette association a changé de direction pour devenir une association inverse ( $p=0.0109$ ). Par contre, la consommation de viande a été associée à une augmentation du risque de DED dans la régression par splines cubiques ( $p=0.009$ ).

#### 3.3.2 DISCUSSION

Comme dit plus haut, il est peut-être plus pertinent d'examiner les effets des AGS selon la longueur des chaînes, ce qui pourrait expliquer pourquoi on n'a pas trouvé d'association avec les AGS et le risque de DED. Une étude cas-témoin a examinée les effets des deux AGs saturés, l'acide palmitique et l'acide stéarique et la RD mais ils n'ont pas été trouvés d'association entre la RD et l'acide palmitique<sup>164</sup>. Par contre, une association inverse entre le tertile moyenne de consommation d'acide stéarique (4.53-6.26 g/jour) et la RD a été observée<sup>164</sup>. Ce phénomène devrait être exploré dans les autres études, comme ces deux AGS ont été liés à la résistance à l'insuline.

Comme nous, une autre étude transversale n'a pas trouvé d'association entre les AGMIs, ni l'acide oléique et la RD<sup>165</sup>, mais une autre étude espagnole a montré une relation inverse entre les AGMIs et l'acide oléique avec la RD<sup>164</sup>. C'est possible que la consommation des AGMI dans notre population française n'était pas assez forte de détecter l'association trouvée dans l'étude espagnole, où la relation aurait pu être confondu par la source alimentaire des AGMIs.

On n'a pas trouvé de preuve d'une relation entre les AGPIs et la DED, en accord avec une autre étude sur la RD<sup>164</sup>. Néanmoins, comme les AGS, les types d'AGPI pourraient avoir des effets différents. Une étude prospective en Espagne a trouvé que des participants avec des consommations fortes des n-3 AGPIs avaient un risque diminué de RD de presque 50%<sup>147</sup>. Cependant, à notre connaissance, cette étude est la première à analyser l'association entre la consommation alimentaire des n-3 AGPIs individus et la DED, et on n'a pas observé d'associations significatives. Mais comme les effets des n-3 AGPIs pourraient différer selon son type, la région géographique, ou le sexe, il est important que les futures études concernant les n-3 AGPIs prennent en compte ces facteurs<sup>162,276</sup>.

Après contrôle de la source alimentaire principale d'AA, la viande, nos résultats suggèrent que les consommations modérées des n-6 AGPIs, LA, et AA sont associées avec un risque diminué de DED. Seule une autre étude a évalué cette association, et il n'a pas été observé d'association avec les n-6 AGPIs ni le LA<sup>164</sup>. Mais cette étude n'a pas ajusté sur l'énergie, ce qui peut introduire un biais. Des

études biologiques suggèrent à la fois des mécanismes pro-inflammatoire et anti-inflammatoire des n-6 AGPIs, donc davantage d'études épidémiologiques sont nécessaires<sup>240,324,326</sup>.

La consommation de viande a été indépendamment associée avec un sur-risque de DED avec une relation dose-effet. Les fortes consommations de la viande rouge ont déjà été associées au risque augmenté de DT2 dans cette cohorte et dans les méta-analyses<sup>139,183,327</sup>, donnant une orientation importante pour les futures études de confirmation.

### 3.3.3 CONCLUSION

Les résultats suggèrent que la consommation des n-6 AGPIs tel que LA et AA pourraient jouer un rôle protecteur dans le développement d'une DED. En revanche, une consommation forte de viande pourrait être néfaste en ce qui concerne la DED. Comme la littérature sur le rôle des n-6 AGPIs dans les mécanismes inflammatoires est controversée et que cette étude est la première à regarder l'association entre la consommation des n-6 AGPIs et la DED, les autres études devraient s'intéresser aux types de n-6 AGPIs. Nos résultats en ce qui concerne la consommation de viande devraient aussi être confirmés d'autres études, comme sa consommation semble être en augmentation dans beaucoup de populations.

## 3.4 ADHERENCE AUX RECOMMANDATIONS ALIMENTAIRES, RISQUE DE DIABETE DE TYPE 2 ET LES FRACTION ATTRIBUABLES

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### 3.4.1 RESULTATS

Une forte adhésion aux recommandations pour les fruits et les produits laitiers a été associée à des risques diminués de DT2 de 32% et 40%, respectivement, après ajustement pour les autres facteurs de confusion. De fortes adhésion aux recommandations pour les légumes, céréales, aliments riches en protéines, et alcool (pas d'alcool) n'ont pas été associées aux risque de DT2 (HR=0.83 [0.37-1.88], HR=1.08 [0.72-1.59], HR=1.10 [0.75-1.61], HR=0.86 [0.59-1.26], respectivement). Un dépassement des limites des recommandations pour la consommation d'aliments riches en protéines a été associé à un risque augmenté de DT2 (HR = 1.56 [1.01-2.43]).

La régression par splines cubiques a indiqué une association curvilinéaire entre la consommation des fruits et le risque de DT2 ( $p=0.0001$ ). Des consommations élevées de produits laitiers ont été associés avec un risque diminué de DT2 ( $p=0.003$ ), alors que les aliments riches en protéines ont été associés à un risque augmenté de DT2 ( $p=0.03$ ). Enfin, les verres d'alcool, et les portions de légumes et de céréales n'ont pas été associés au risque de DT2 ( $p=0.09$ ,  $p=0.09$ ,  $p=0.43$ , respectivement).

Une adhésion forte aux recommandations alimentaires n'a pas été associée au risque de DT2 (HR=0.64 [0.39-1.06]). Ne pas fumer, une activité physique suffisante et un tour de taille <80 cm pour les femmes et <94 cm pour les hommes ont tous montré des associations inverse avec le risque de DT2. Une forte adhésion aux recommandations de mode de vie a globalement été associée avec un risque diminué de 70% (HR=0.30 [0.17-0.51]) et la régression par splines cubique a suggéré une relation significative, linéaire et inverse ( $p<0.0001$ ).

Les PAFs ont suggérés que 40% des cas de DT2 auraient pu être évités avec un tour de taille <80 cm pour les femmes et <94 cm pour les hommes (PAF=42.6% [27.5%-55.7%]) et presque 20% des cas avec une activité physique suffisante (PAF=17.3% [7.5%-26.8%]). Les PAFs ont suggéré que plus de 20% des cas de DT2 auraient pu être évités si toute la population adoptait les recommandations pour les fruits et 37% pour les produits laitiers (PAF=23.3% [7.3%-38.2%]; PAF=37.1% [14.6%-56.0%], respectivement). Une forte adhésion aux recommandations de l'index global de mode de vie a indiqué que presque 60% des cas de DT2 auraient pu être évités (PAF=59.4% [36.2%-76.6%]).

Les analyses de sensibilité ont trouvé un risque diminué de DT2 pour la forte adhésion aux recommandations alimentaires avec la substitution d'une consommation d'alcool modérée au lieu de ne pas consommer du tout (HR=0.59 [0.36-0.96]). Mais, le PAF n'était pas significatif (PAF=18.6% [-7.2%-42.3%]).

### 3.4.2 DISCUSSION

Les méta-analyses suggèrent une relation inverse entre la consommation des fruits et le risque d'un DT2, mais comme nous, ont observé une association curvilinéaire avec une régression par splines cubiques<sup>134</sup>. Il est possible que des fortes quantités de fructose soient néfastes; le métabolisme de fructose peut s'autoréguler positivement et augmenter les quantités d'acide urique, qui provoque la résistance à l'insuline<sup>328,329</sup>. Dans les méta-analyses la consommation totale des légumes n'a pas été associée avec le DT2<sup>133,134</sup>, mais en Asie, où la consommation des légumes est élevée, des relations significatives inverses ont été détectées<sup>134</sup>. Il se peut que la faible consommation des légumes dans notre population, et le manque de variabilité (60% de la population avait une adhérence faible) aurait pu atténuer les associations. Une forte adhésion aux recommandations pour la consommation de céréales n'a pas été associée au risque de DT2, probablement parce que le risque diffère par type de céréales consommées<sup>136,137</sup>. Mais dans une autre population australienne, une forte consommation de céréales raffinées avait été associée à un risque augmenté de DT2, alors que les autres céréales étaient associées avec une incidence diminuée. Des relations inverses entre la consommation des produits laitiers et le DT2 ont été rapporté dans des méta-analyses<sup>138,214</sup>, mais pas systématiquement<sup>342</sup>. Nos résultats suggèrent que plus d'un tiers des cas de DT2 aurait pu être évité dans cette population malgré les effets peu clairs de la consommation des produits laitiers sur le DT2. On a observé une relation positive dose-réponse entre la consommation d'aliments riches en protéines (viande maigre et volaille, poisson, œufs, tofu, noix/grains, et légumineuses) et le risque de DT2 pour les personnes qui dépassent les limites recommandées même si les études ne suggèrent pas d'effets négatifs des régimes riches en protéines<sup>347,348</sup>. Mais les méta-analyses montrent que les fortes consommations de viandes rouges augmentent le risque d'un DT2<sup>136</sup>, et dans cette cohorte, la viande rouge contribuait à plus de la moitié (53%) de la consommation des aliments riches en protéines. Une diminution de la consommation de viande rouge peut être bénéfique contre le développement d'un DT2 dans cette population.

Une autre étude par Alhazmi *et al.* a déjà examiné le risque de DT2 en lien avec l'adhésion aux recommandations alimentaires australiennes en construisant deux index<sup>130</sup>. Un index a été associé avec un risque diminué d'un DT2 (OR=0.51 [0.35-0.76]) et l'autre n'a pas été associé (OR=0.99 [0.68-1.43]).

Le fraction de risque attribuable n'a pas été significative pour l'adhésion totale aux recommandations alimentaires, mais les fractions attribuables pour les consommations des fruits et des produits laitiers ont suggérés qu'une forte adhésion aux recommandations auraient pu éviter 23% et 37% des cas de DT2. Notre estimation de 60% des cas de DT2 évitable avec une forte adhérence aux recommandations de mode de vie est plus modeste que des études antérieures, qui ont trouvés environ 80% des cas évitable (en Chine, Finlande, et Hawaii)<sup>28,30,32</sup> ou environ 90% cas évitables dans deux études américaines<sup>29,31</sup>.

### 3.4.3 CONCLUSION

Nos résultats suggèrent que plus que la moitié des cas de DT2 aurait pu été évitée avec des comportements sains dans cette population australienne. Ces résultats confirment que les facteurs modifiables ont un rôle majeur sur l'incidence DT2. Une consommation plus forte de légumes devraient être encouragée dans la population australienne, même si les efforts ne devraient pas se focaliser sur un seul comportement, puisque la réduction de risque a été montrée plus forte lorsque

plusieurs recommandations sont simultanément suivies. Les interventions devraient se focaliser sur le changement de ces comportements modifiables dans la population.

## 4. CONCLUSION

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Malgré les études qui suggèrent des effets favorables des n-3 AGPIs dans la sensibilité à l'insuline et pour la santé cardiovasculaire, les résultats de cette thèse ne vont pas dans ce sens<sup>248,363</sup>. Les effets des AGPIs ne semblent pas homogènes dans le groupe et d'un point de vue de santé publique, *basé sur ces résultats, une alimentation modérée et variés semble le plus bénéfique pour la prévention de DT2 et DED.*

Globalement, une forte adhésion aux recommandations alimentaires australiennes n'a pas été associée au risque de DT2 dans la cohorte AusDiab, mais une forte adhésion aux recommandations concernant la consommation de fruits et des produits laitiers a montré que presque un quart des cas de DT2 auraient pu être évité, et un dépassement des limites pour les aliments riches en protéines a été associé à un risque augmenté de DT2. *Ceci, suggère qu'une alimentation avec une consommation adéquate de fruits et des produits laitiers, associée à une consommation faible d'aliments riches en protéines de sources animales, pourrait être favorable dans la prévention de DT2.*

Cette thèse a permis d'approfondir la relation entre l'alimentation, le DT2 et la RD. Cependant, il reste de nombreux domaines à explorer dans les études futures. Dans l'étude E3N-Afterdiab, la relation entre les autres complications du diabète et la consommation d'acides gras pourrait être examinée. Les autres facteurs de l'alimentation, par exemple, la consommation des principaux groupes alimentaires, les profils alimentaires ou la capacité antioxydante totale de l'alimentation représentent également des pistes prometteuses.

E4N, la suite de l'étude E3N, représentera également une grande opportunité pour étudier ses associations au sein de familles qui partagent des gènes et un environnement commun. L'effet d'un profil alimentaire, la consommation de certains aliments ou de groupes alimentaires, et la consommation d'acides gras pourra par exemple être analysé chez les enfants des femmes E3N avec un DT2. De plus, les complications du diabète chez les descendants de personnes diabétiques pourraient aussi être étudiées.

A titre personnelle, j'espère continuer d'explorer la relation entre alimentation et le diabète ou des complications du diabète dans le futur, dans le cadre d'un postdoctorat dans l'équipe d'EPIC à Potsdam, en Allemagne. L'équipe en est train de collecter des données sur les complications du diabète et je souhaite analyser la relation entre la consommation d'acides gras et l'incidence des complications diabétiques dans cette cohorte, ainsi que l'impact des profils alimentaires. Ceci représentera une opportunité de poursuivre les recherches présentées dans cette thèse.

Cette thèse m'a permis de travailler avec une grande base des données dans une équipe multidisciplinaire. Avec cette opportunité, j'ai eu la chance d'apprendre de nouvelles méthodes épidémiologiques et de développer mes connaissances sur le diabète et la RD. De plus, j'ai eu l'opportunité de travailler avec l'équipe AusDiab et leurs données. Ma connaissance dans le domaine de diabète et l'alimentation est devenu plus grande grâce à cette expérience. Cette opportunité était une expérience vraiment enrichissante et je serai pour toujours reconnaissante aux équipes E3N et AusDiab de m'avoir accueillie.

## What is a serve of fruit?

A standard serve is about 150g (350kJ) or:

- 1 medium apple, banana, orange or pear
- 2 small apricots, kiwi fruits or plums
- 1 cup diced or canned fruit (no added sugar)

Or only occasionally:

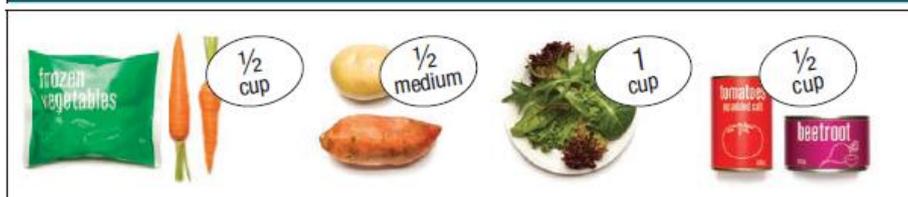
- 125ml (½ cup) fruit juice (no added sugar)
- 30g dried fruit (for example, 4 dried apricot halves, 1½ tablespoons of sultanas)



## What is a serve of vegetables?

A standard serve is about 75g (100–350kJ) or:

- ½ cup cooked green or orange vegetables (for example, broccoli, spinach, carrots or pumpkin)
- ½ cup cooked dried or canned beans, peas or lentils (preferably with no added salt)
- 1 cup green leafy or raw salad vegetables
- ½ cup sweet corn
- ½ medium potato or other starchy vegetables (sweet potato, taro or cassava)
- 1 medium tomato



## What is a serve of grain\* (cereal) food?

A standard serve is (500kJ) or:

1 slice (40g)	bread
½ medium (40g)	roll or flat bread
½ cup (75-120g)	cooked rice, pasta, noodles, barley, buckwheat, semolina, polenta, bulgur or quinoa
½ cup (120g)	cooked porridge
⅔ cup (30g)	wheat cereal flakes
¼ cup (30g)	muesli
3 (35g)	crispbreads
1 (60g)	crumpet
1 small (35g)	English muffin or scone



\*Grain (cereal) foods, mostly wholegrain and/or high cereal fibre varieties

## How much is a serve of lean meat and poultry, fish, eggs, nuts and seeds, and legumes/beans?

A standard serve is (500–600kJ):

65g	cooked lean red meats such as beef, lamb, veal, pork, goat or kangaroo (about 90-100g raw)
80g	cooked lean poultry such as chicken or turkey (100g raw)
100g	cooked fish fillet (about 115g raw) or one small can of fish
2 large (120g)	eggs
1 cup (150g)	cooked or canned legumes/beans such as lentils, chick peas or split peas (preferably with no added salt)
170g	tofu
30g	nuts, seeds, peanut or almond butter or tahini or other nut or seed paste (no added salt)*



\*Only to be used occasionally as a substitute for other foods in the group

## How much is a serve of milk\*, yoghurt\*, cheese\* and/or alternatives?

A standard serve is (500–600kJ):

- 1 cup (250ml) fresh, UHT long life, reconstituted powdered milk or buttermilk
- ½ cup (120ml) evaporated milk
- 2 slices (40g) or 4 x 3 x 2cm cube (40g) of hard cheese, such as cheddar
- ½ cup (120g) ricotta cheese
- ¾ cup (200g) yoghurt
- 1 cup (250ml) soy, rice or other cereal drink with at least 100mg of added calcium per 100ml



The following foods contain about the same amount of calcium as a serve of milk, yoghurt or cheese:

- 100g almonds with skin
- 60g sardines, canned in water
- ½ cup (100g) canned pink salmon with bones
- 100g firm tofu (check the label as calcium levels vary)

\*Choose mostly reduced fat



#### ANNEX 4. SEARCH STRATEGY EMPLOYED FOR WEB OF SCIENCE

##### *For Diabetic Retinopathy:*

#1 TITLE: (retinopathy) AND TITLE: (diabet\*)

#2 TOPIC: (dietary pattern\*) OR TOPIC: (whole grain\*) OR TOPIC: (refined grain\*) OR TOPIC: (cereal\*) OR TOPIC: (grain\*) OR TOPIC: (pasta\*) OR TOPIC: (rice\*) OR TOPIC: (potato\*) OR TOPIC: (vegetable\*) OR TOPIC: (fruit\*) OR TOPIC: (nut\*) OR TOPIC: (legume\*) OR TOPIC: (bean\*) OR TOPIC: (egg\*) OR TOPIC: (dairy) OR TOPIC: (dairies) OR TOPIC: (milk) OR TOPIC: (yogurt) OR TOPIC: (cheese) OR TOPIC: (fish) OR TOPIC: (seafood) OR TOPIC: (meat) OR TOPIC: (processed meat) OR TOPIC: (sugar sweetened beverage\*) OR TOPIC: (alcohol) TOPIC: (french fries) OR TOPIC: (pizza) OR TOPIC: (fast food) OR TOPIC: (tofu)

#3 #1 AND #2

##### *For Macular Edema:*

#1 TITLE: (macular edema) OR TITLE: (macular oedema) AND TOPIC: (diabet\*)

#2 TOPIC: (dietary pattern\*) OR TOPIC: (whole grain\*) OR TOPIC: (refined grain\*) OR TOPIC: (cereal\*) OR TOPIC: (grain\*) OR TOPIC: (pasta\*) OR TOPIC: (rice\*) OR TOPIC: (potato\*) OR TOPIC: (vegetable\*) OR TOPIC: (fruit\*) OR TOPIC: (nut\*) OR TOPIC: (legume\*) OR TOPIC: (bean\*) OR TOPIC: (egg\*) OR TOPIC: (dairy) OR TOPIC: (dairies) OR TOPIC: (milk) OR TOPIC: (yogurt) OR TOPIC: (cheese) OR TOPIC: (fish) OR TOPIC: (seafood) OR TOPIC: (meat) OR TOPIC: (processed meat) OR TOPIC: (sugar sweetened beverage\*) OR TOPIC: (alcohol) TOPIC: (french fries) OR TOPIC: (pizza) OR TOPIC: (fast food) OR TOPIC: (tofu)

#3 #1 AND #2



## Fatty acid consumption and incident type 2 diabetes: an 18-year follow-up in the female E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Éducation Nationale) prospective cohort study

Courtney Dow<sup>1,2</sup>, Marie Mangin<sup>1,2</sup>, Beverley Balkau<sup>1,2,3</sup>, Aurélie Affret<sup>1,2</sup>, Marie-Christine Boutron-Ruault<sup>1,2</sup>, Françoise Clavel-Chapelon<sup>1,2</sup>, Fabrice Bonnet<sup>1,2,4</sup> and Guy Fagherazzi<sup>1,2\*</sup>

<sup>1</sup>Gustave Roussy, F-94805, Villejuif, France

<sup>2</sup>Université Paris-Saclay, Université Paris-Sud, UVSQ, Inserm U1018, CESP, Health Across Generations Team, F-94805, Villejuif, France

<sup>3</sup>Université Versailles, F-78000, Saint Quentin, France

<sup>4</sup>CHU de Rennes, Department of Endocrinology, Diabetes and Nutrition, Hôpital Sud, 16 Boulevard de Bulgarie, F-35033, Rennes, France

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### Abstract

We evaluated the association between dietary estimates of fatty acid (FA) consumption and type 2 diabetes (T2D) risk in the French E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Éducation Nationale) cohort. In total, 71 334 women without diabetes at baseline were followed up from 1993 to 2011. Diabetes was identified using questionnaires and drug-reimbursement claims, and incident cases were validated. FA consumption in 1993 was estimated from a validated dietary questionnaire. Cox regression estimated hazard ratios (HR) and 95% CI of diabetes risk, comparing the upper tertile group with the lowest. High *n*-3 PUFA consumption was associated with T2D even after adjustment for confounders, including other FA and BMI (HR 1.26; 95% CI 1.13, 1.41; upper tertile compared with lowest). Upon stratification by overweight (BMI  $\geq 25$  kg/m<sup>2</sup>)/non-overweight, a positive association between total PUFA consumption and T2D was observed, but it was restricted to non-overweight women (HR 1.22; 95% CI 1.05, 1.42), whereas *n*-3 PUFA consumption was associated with increased T2D risk in both BMI strata (BMI  $< 25$  kg/m<sup>2</sup>: HR 1.19; 95% CI 1.01, 1.40 and BMI  $\geq 25$  kg/m<sup>2</sup>: HR 1.38; 95% CI 1.20, 1.59). Within the *n*-3 PUFA, high DPA (HR 1.41; 95% CI 1.23, 1.63) and  $\alpha$ -linolenic acid (ALA) intakes were associated with increased T2D risk, but the effects of ALA were restricted to overweight women (HR 1.17; 95% CI 1.01, 1.36). Within the *n*-6 PUFA, only arachidonic acid (AA) intake was associated with T2D risk (HR 1.49; 95% CI 1.33, 1.66). The associations with DPA and AA persisted even after adjustment of their principal source in this cohort, the consumption of meat. The effects of PUFA are heterogeneous within the FA group. Intake of DPA and AA may contribute to T2D development.

**Key words:** Fatty acids: Diabetes: *n*-3 PUFA: *n*-6 PUFA: Study cohorts: Dietary intakes: French women

The incidence of type 2 diabetes (T2D) is influenced by many modifiable risk factors, such as overweight or obesity, physical inactivity, smoking and a poor diet, whose management could prevent 83–91% of cases in women<sup>(1,2)</sup>. One important risk factor is diet; however, focus has been placed heavily on carbohydrate consumption, although fats are responsible for a large part of energy intake and also have strong metabolic effects. Fatty acids (FA), the constituents of fat, are vital sources of energy and are required for normal growth and development<sup>(3)</sup>. Nevertheless, excessive intake of some FA may have negative health effects and their consumption should be limited<sup>(4,5)</sup>.

Current evidence concerning the association between FA consumption and the risk for T2D is sparse and controversial. Some studies have found no association between total fat intake and incidence of T2D<sup>(6–8)</sup>; yet there is evidence to suggest that FA groups, and even individual FA within these groups, can have different effects on T2D risk<sup>(6–10)</sup>. Meta-analyses also suggest that the effects of *n*-3 PUFA intake differ by geographical region, with high intake associated with an increased risk for T2D in Western countries, no association in European countries and a decreased risk in Asian countries<sup>(11–13)</sup>. Moreover, *n*-3 and *n*-6 PUFA are almost always studied in their groups, and the effects of the individual PUFA rest scarcely

**Abbreviations:** AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; E3N, Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Éducation Nationale; FA, fatty acid; HR, hazard ratio; T2D, type 2 diabetes; TFA, *trans*-unsaturated fatty acids.

\* **Corresponding author:** G. Fagherazzi, fax +33 1 42 11 40 00, email guy.fagherazzi@gustaveroussy.fr



examined despite the present *n*-3 PUFA health craze. In addition, most studies take the intake of other macronutrients into account when examining FA; however, many do not control for the consumption of the remaining FA, which is important, as groups and individual FA are never ingested in isolation and influence the metabolism of each other<sup>(14)</sup>.

The objective of this study was therefore to examine the associations between the main dietary FA intakes and the incidence of T2D. In particular, we focused on the relationship between *n*-3 and *n*-6 PUFA while adjusting for the concomitant intake of other FA.

## Methods

### Study cohort

E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Éducation Nationale) is a prospective French cohort study of 98 995 females born between 1925 and 1950 and initiated in 1990. It is the French constituent of the large European Prospective Investigation into Cancer and Nutrition (EPIC), and the EPIC sub-study devoted to diabetes: interaction of genetic and lifestyle factors on the incidence of type 2 diabetes (InterAct). Data are available from mailed questionnaires that participants have returned every 2–3 years, in addition to a drug-reimbursement claims database that has been available since 2004 from the participant's medical records (Mutuelle Générale de l'Éducation Nationale). The average response rate for each of the eleven questionnaire cycles has been 83%, and to date the total loss to follow-up since 1990 has been 3% (women who either never answered a questionnaire, withdrew from the study, or whose contact information was lost). All women signed letters of informed consent, in compliance with the French National Commission for Computerized Data and Individual Freedom. From the 98 995 women in the cohort, women who did not complete the dietary questionnaire (*n* 24 473), who had pre-existing diabetes (*n* 859), did not complete any questionnaires after the dietary questionnaire (*n* 890), or who had extreme values for the ratio between energy intake and required energy (i.e. the 1st and 99th percentiles of the distribution in the population) (*n* 1439) were excluded. Thus, the analysis included 71 334 women, of whom 2610 had a validated T2D diagnosis during follow-up (June 1993–December 2011).

### Assessment of fatty acid consumption

In June 1993, a validated 208-item dietary questionnaire was sent to participants to assess the habitual diet of the previous year. Food and drink consumption throughout the day took French meal patterns into account, as questions regarding each meal occasion, as well as snacks and appetisers, were also included. Divided into two sections, the first section of the questionnaire included habitual frequency and portion size questions, based on sixty-six food types and items grouped by meals. To quantify frequency, eleven categories were used: never or less than once per month; 1, 2 or 3 times/month; or 1–7 times/week. Portion sizes were indicated with the aid of a photo booklet. The second section had a detailed qualitative description of each food

consumed from each food group and the frequency of its consumption (never; 1–3 times/month; or 1–7 times/week). After weighing the data from the first section by that from the second section, the average daily consumption of 208 types of foods and drinks was evaluated. The validity and reproducibility of the questionnaire have been previously described<sup>(15)</sup>.

FA consumption was then estimated using the database of food nutritional composition published by French Agency for Food, Environmental, and Occupational Health & Safety. For each woman, intakes of the following principal FA were obtained: total SFA, total MUFA, total PUFA (including total *n*-3 PUFA and total *n*-6 PUFA), and total *trans*-unsaturated fatty acids (TFA) (g/d). In addition, we also estimated the consumptions of *n*-6 PUFA – linoleic acid (LA; 18:2*n*-6) and arachidonic acid (AA; 20:4*n*-6) – and *n*-3 PUFA –  $\alpha$ -linolenic acid (ALA; 18:3*n*-3), EPA (20:5*n*-3), DPA (22:5*n*-3) and DHA (22:6*n*-3).

### Ascertainment of diabetes

Cases of T2D were first identified either in follow-up questionnaires (with declaration of at least one of the following: T2D; a self-reported, physician-recommended diabetic diet; use of diabetic medication; or hospitalisation due to diabetes) or they were identified as receiving diabetic drug reimbursements from health insurance records at least once between January 2004 and March 2012. Those identified through both methods were considered validated. All women were mailed a diabetes-specific questionnaire that included questions on the circumstances of the diagnosis (year, symptoms, biological exams, etc.), management (diabetic diet, physical activity, medications) and results of their most recent concentrations of fasting glucose and HbA1c. Cases were validated if one of the following was met: fasting plasma glucose  $\geq 7.0$  mmol/l; random glucose  $\geq 11.1$  mmol/l at diagnosis; report of diabetic medication use; or last values of fasting glucose or HbA1c concentrations  $\geq 7.0$  mmol/l or  $\geq 7\%$ , respectively. Prevalent and non-validated cases were excluded from analyses. Consequently, 2610 validated cases of incident diabetes and 68 724 non-cases were analysed in the present study.

### Statistical analysis

For time-independent variables with  $<5\%$  of values missing during follow-up, missing values were imputed with the median of the study population (quantitative variables) or the mode (qualitative variables). In the case of  $\geq 5\%$  of missing values, a 'missing' category was created. For time-dependent variables presenting with a missing value during follow-up, the value was imputed by the preceding provided value. If the missing value was the baseline value, it was imputed with the first available value.

Because of the high correlation between energy intake and nutrient intake, FA consumption was energy adjusted using the residual method to allow analyses independent of total energy intake<sup>(16)</sup>. Each FA group was then divided into tertile groups of consumption, with women in the first tertile group of intake systematically employed as the reference category. Cox multi-variable regression models with age as the timescale were used

to estimate hazard ratios (HR) and 95% CI of T2D risk. All variables were found to satisfy the proportional hazards assumption. The time at entry was the age at the start of follow-up and the exit time was the age when participants were diagnosed with diabetes, died, were lost to follow-up, or were censored at the end of the follow-up period, whichever occurred first. The univariate model included only the FA in question and was followed by model 1, which was adjusted for daily energy intake (kJ/d or kcal/d), alcohol consumption (grams of ethanol per 100 g/d) and non-dietary variables such as level of education (undergraduate *v.* postgraduate), smoking status (never *v.* current/former), hypertension (yes *v.* no), hypercholesterolaemia (yes *v.* no), family history of diabetes (yes *v.* no) and physical activity (metabolic equivalents (MET)-h/week). Model 2 additionally adjusted for all types of FA besides the primary considered, allowing for the examination of the independent effects of specific FA subtypes. Finally, model 3 was adjusted for BMI (<20, 20–25, 25–30 or >30 kg/m<sup>2</sup>).

In addition, stratification by BMI (<25 and ≥25 kg/m<sup>2</sup>) was performed to determine whether the effects of FA groups varied between overweight and non-overweight women. Models were adjusted in the same manner as in the analyses for the whole population, first for non-dietary variables, alcohol consumption and energy intake, and subsequently for other FA groups. All statistical analyses used SAS 9.2 software (PHREG procedure for Cox models; SAS Institute Inc.). All statistical tests were two-sided and considered significant at  $P < 0.05$ .

#### Sensitivity analyses

In order to test for potential reverse-causation, analyses were performed on a subpopulation excluding women who developed diabetes in the first 5 years after inclusion in the study. Individual *n*-3 and *n*-6 PUFA models were also adjusted for meat consumption, to account for residual confounding related to a major source of the FA, as it has already been reported in our cohort that a high intake of processed red meat was associated with an increased T2D risk<sup>(17)</sup>. Scores adhering to two dietary patterns, 'Mediterranean/Prudent' (characterised by a high diet quality, with a high consumption of fruit, vegetables, seafood, olive oil and sunflower oil) and 'Western' (characterised by a lower dietary quality, with a high consumption of meat, French fries, rice, pasta, potatoes, alcohol, butter, eggs, etc.), were also included in the models. These dietary quality scores were derived using principal component analysis and have been previously described in published work from our cohort<sup>(18)</sup>.

## Results

### Baseline characteristics

In total, 2610 cases of incident T2D were diagnosed during 1 158 131 person-years between June 1993 and December 2011. The incidence of T2D was 225/100 000 person-years. Median follow-up was 18 years (ranging from 29 d to 18 years) for non-cases and 11 years (ranging from 18 d to 18 years) for cases. Mean age of the study participants was 52.9 (SD 6.7) (Table 1). The majority (66%) of women had a healthy BMI (20–24.9 kg/m<sup>2</sup>),

whereas 19.5% had an excess weight, including 3.3% obese women. Hypertension was declared by 37.1% of women and hypercholesterolaemia by 7.1%. In total, 11.1% of women reported a family history of diabetes. The average daily energy intake was 8916 (SD 2276) kJ/d (2131 (SD 544) kcal/d) and the average physical activity was 49.4 (SD 50.5) MET-h/week. Mean FA group consumptions were as follows: 36.4 (SD 9.9) for SFA, 31.4 (SD 9.9) for MUFA, 1.6 (SD 0.7) for TFA, 14.2 (SD 5.4) for PUFA, 1.5 (SD 0.5) for *n*-3 PUFA, and 12.6 (SD 5.0) for *n*-6 PUFA g/d.

### SFA

SFA consumption above the upper tertile was associated with an increased risk for T2D in the univariate analysis (HR 1.13; 95% CI 1.02, 1.24), and in the multivariable model adjusted for non-dietary factors, energy intake and alcohol (model 1: HR 1.17; 95% CI 1.06, 1.28) (Table 2), when compared with the first tertile group. Further adjustment for other FA groups slightly decreased the association, rendering it non-significant (HR 1.13; 95% CI 0.97, 1.31).

### MUFA

MUFA consumption above the first tertile was associated with a higher risk for T2D in the unadjusted model and model 1 (third tertile group: HR 1.15; 95% CI 1.04, 1.27 and HR 1.32; 95% CI 1.20, 1.46, respectively). Additional adjustment for FA groups in model 2 decreased the strength of the association in the second tertile group (HR 1.10; 95% CI 0.99, 1.22), whereas the association in the third tertile group became statistically non-significant after adjustment for BMI (model 3: HR 1.06; 95% CI 0.95, 1.19).

### PUFA

PUFA intake was associated with a 29% increased risk for T2D in women above the third tertile of intake after adjustment for other types of FA in model 2 (HR 1.29; 95% CI 1.17, 1.43). However, there was no longer an association after adjusting for BMI (model 3: HR 1.06; 95% CI 0.96, 1.17). Positive associations were observed for both *n*-3 and *n*-6 PUFA in model 1, but after adjusting for other FA and BMI only the association above the third tertile of *n*-3 PUFA intake remained (HR 1.26; 95% CI 1.13, 1.41).

### Trans-unsaturated fatty acids

The second tertile group of TFA intake was associated with a lower T2D risk in the univariate analysis (HR 0.87; 95% CI 0.79, 0.96) and in model 2 (HR 0.88; 95% CI 0.78, 0.98), adjusted for other FA, but not in model 1 (HR 0.93; 95% CI 0.85, 1.02). After adjustment for BMI, high TFA intake showed a borderline statistically significant association with T2D (HR 0.90; 95% CI 0.80, 1.00).

### Stratification by BMI

After stratification by BMI, in the fully adjusted models, neither SFA nor MUFA nor TFA were associated with T2D risk in either BMI stratum (Fig. 1, online Supplementary Table S1). Regarding total PUFA, a high intake was associated with increased T2D

**Table 1.** Baseline characteristics of the study population (E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale) cohort data; *n* 71 334 women) (Mean values and standard deviations; numbers and percentages)

Variables	Study population ( <i>n</i> 71 334)	SD or (%)	Diabetes non-cases ( <i>n</i> 68 724)	SD or (%)	Incident diabetes cases ( <i>n</i> 2610)	SD or (%)
Age (years)	52.9	6.7	5.8	6.7	54.4	6.7
BMI (kg/m <sup>2</sup> ) ( <i>n</i> (%))						
<20	10 307	(14.5)	10 239	(14.9)	68	(2.6)
20–24.9	47 101	(66.0)	46 073	(67.0)	1028	(39.4)
25–30	11 554	(16.2)	10 572	(15.4)	982	(37.6)
>30	2372	(3.3)	1840	(2.7)	532	(20.4)
Birth cohort ( <i>n</i> (%))						
Before 1930	5437	(7.6)	5186	(7.6)	251	(9.6)
1930–1934	9175	(12.9)	8702	(12.7)	473	(18.1)
1935–1939	13 530	(19.0)	12 991	(18.9)	539	(20.7)
1940–1944	17 142	(24.0)	16 494	(24.0)	648	(24.8)
After 1944	26 050	(36.5)	25 351	(36.9)	699	(26.8)
Education ( <i>n</i> (%))						
Undergraduate	7913	(11.1)	7493	(10.9)	420	(16.1)
Graduate/postgraduate	63 421	(88.9)	61 231	(89.1)	2190	(83.9)
Smoking status ( <i>n</i> (%))						
Never	38 504	(54.0)	37 115	(54.0)	1389	(53.2)
Current/former	32 830	(46.0)	31 609	(46.0)	1221	(46.8)
Hypertension ( <i>n</i> (%))						
No	44 857	(62.9)	43 773	(63.7)	1084	(41.5)
Yes	26 477	(37.1)	24 951	(36.3)	1526	(58.5)
Hypercholesterolaemia treatment ( <i>n</i> (%))						
No	66 297	(92.9)	64 085	(93.3)	2212	(3.1)
Yes	5037	(7.1)	4639	(6.8)	398	(15.3)
Family history of T2D ( <i>n</i> (%))						
No	63 432	(88.9)	61 451	(89.4)	1981	(75.9)
Yes	7902	(11.1)	7273	(10.6)	629	(24.1)
Physical activity (MET-h/week)	49.4	50.5	49.3	50.1	50.0	59.2
Alcohol consumption (g of ethanol per 100 g/d)	11.6	13.9	11.6	13.8	12.1	15.9
Daily energy intake (kJ/d)	8914.4	2274.8	8906.8	2266.1	9114.8	2481.5
Daily energy intake (kcal/d)	2130.6	543.7	2128.8	541.6	2178.5	593.1
Average consumption (g/d)						
SFA	36.4	13.0	36.4	12.9	37.6	14.1
MUFA	31.3	9.9	31.3	9.8	32.6	10.8
TFA	1.6	0.7	1.6	0.7	1.6	0.7
PUFA	14.2	5.3	14.1	5.3	15.2	5.9
<i>n</i> -3 PUFA	1.5	0.5	1.5	0.5	1.6	0.6
<i>n</i> -6 PUFA	12.6	5.0	12.6	5.0	13.6	5.6

T2D, type 2 diabetes; MET, metabolic equivalents; TFA, *trans*-unsaturated fatty acids.

risk in non-overweight women (HR 1.22; 95% CI 1.05, 1.42), but not in overweight women (HR 1.02; 95% CI 0.90, 1.16). *n*-6 PUFA were not associated with T2D risk in either BMI stratum (HR 1.13; 95% CI 0.96, 1.32 and HR 0.95; 95% CI 0.83, 1.08, respectively, for non-overweight and overweight women), whereas high *n*-3 PUFA consumption was associated with an increased risk for T2D in both strata in fully adjusted models (HR 1.19; 95% CI 1.01, 1.40 and HR 1.38; 95% CI 1.20, 1.59, respectively, for non-overweight and overweight women).

#### *n*-6 Fatty acids

LA was not significantly associated with T2D risk in the whole population (HR 0.97; 0.87, 1.07) or in either BMI stratum (Table 3). Conversely, a high consumption of AA was associated with an almost 50% increased risk for T2D (HR 1.49; 95% CI 1.33, 1.66). Within BMI strata, AA intake above the third tertile was associated with T2D risk in non-overweight women (HR 1.50; 95% CI 1.28, 1.76) and intake above the second tertile

was associated with T2D risk in overweight women (HR 1.74; 95% CI 1.49, 2.03).

#### *n*-3 Fatty acids

In the full population, neither ALA intake nor intake of long-chain *n*-3 PUFA (EPA or DHA) was associated with T2D in the fully adjusted models (HR 1.03; 95% CI 0.92, 1.15; HR 0.88; 95% CI 0.67, 1.15; HR 1.11; 95% CI 0.85, 1.44, respectively). However, high DPA intake above the second tertile was associated with an increased risk for T2D (HR 1.41; 95% CI 1.23, 1.63). There was no association with EPA or DHA in models stratified on BMI. However, high ALA intake was associated with an increased T2D risk in women with excess weight (HR 1.17; 95% CI 1.01, 1.36) for the highest tertile group, but not in the normal-weight women (HR 0.90; 95% CI 0.75, 1.07). High DPA intake was associated with an increased risk for T2D in both BMI strata (HR 1.45; 95% CI 1.17, 1.80 and HR 1.54; 95% CI 1.27, 1.85, respectively).

**Table 2.** Cox proportional hazards ratios for the risk for incident type 2 diabetes by fatty acid consumption (g/d) in the E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale) study cohort (n 71 334 women)(Hazard ratios (HR) and 95% confidence intervals)

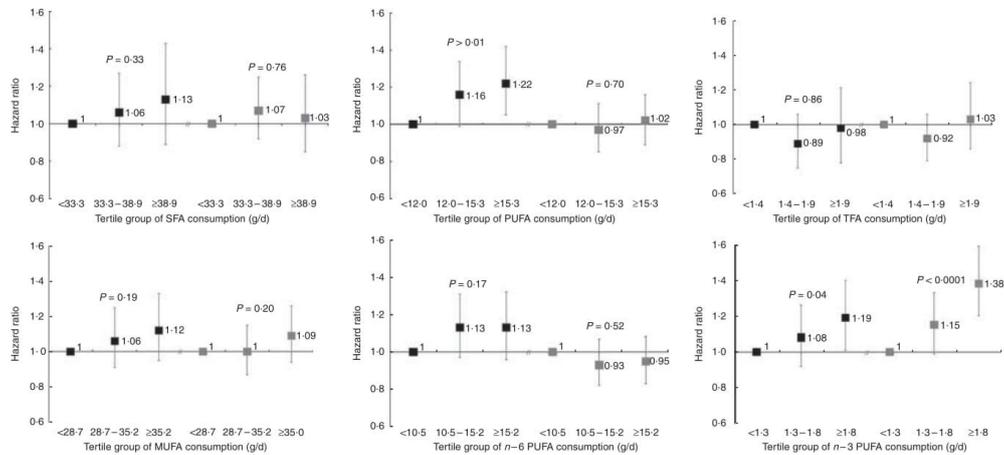
Variables (g/d)	No. of cases/ non-cases	Univariate		Model 1*		Model 2†		Model 3‡	
		HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
<b>SFA</b>									
<33.3	843/22 698	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
33.3–38.9	847/22 692	1.04	0.95, 1.14	1.10	1.00, 1.21	1.11	0.99, 1.25	1.06	0.95, 1.19
≥38.9	920/23 334	1.13	1.02, 1.24	1.17	1.06, 1.28	1.13	0.97, 1.31	1.07	0.92, 1.25
<i>P</i> <sub>trend</sub>		0.01		0.001		0.14		0.40	
<b>MUFA</b>									
<28.7	785/22 755	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
28.7–33.3	839/22 702	1.11	1.01, 1.23	1.15	1.04, 1.27	1.10	0.99, 1.22	1.01	0.91, 1.12
≥33.3	986/23 267	1.31	1.19, 1.44	1.32	1.20, 1.46	1.23	1.10, 1.38	1.06	0.95, 1.19
<i>P</i> <sub>trend</sub>		<0.0001		<0.0001		<0.001		0.25	
<b>PUFA</b>									
<12.0	720/22 821	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
12.0–15.3	835/22 704	1.17	1.06, 1.29	1.14	1.03, 1.26	1.13	1.02, 1.25	1.03	0.93, 1.14
≥15.3	1055/23 199	1.46	1.33, 1.61	1.34	1.21, 1.47	1.29	1.17, 1.43	1.06	0.96, 1.17
<i>P</i> <sub>trend</sub>		<0.0001		<0.0001		<0.0001		0.24	
<b>n-6 PUFA</b>									
<10.5	734/22 807	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
10.5–13.7	840/22 700	1.15	1.04, 1.27	1.13	1.02, 1.25	1.07	0.96, 1.18	1.01	0.91, 1.12
≥13.7	1036/23 217	1.41	1.28, 1.55	1.29	1.17, 1.42	1.15	1.04, 1.27	1.00	0.90, 1.10
<i>P</i> <sub>trend</sub>		<0.0001		<0.0001		<0.01		0.94	
<b>n-3 PUFA</b>									
<1.3	665/22 876	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
1.3–1.6	819/22 720	1.25	1.13, 1.38	1.23	1.11, 1.37	1.18	1.06, 1.31	1.10	0.99, 1.22
≥1.6	1126/23 128	1.68	1.52, 1.84	1.60	1.45, 1.76	1.47	1.32, 1.64	1.26	1.13, 1.41
<i>P</i> <sub>trend</sub>		<0.0001		<0.0001		<0.0001		<0.0001	
<b>TFA</b>									
<1.4	920/22 621	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
1.4–1.7	794/22 745	0.87	0.79, 0.96	0.93	0.85, 1.02	0.88	0.78, 0.98	0.90	0.80, 1.00
≥1.7	896/23 358	0.98	0.89, 1.07	1.03	0.94, 1.13	0.93	0.81, 1.07	1.02	0.89, 1.17
<i>P</i> <sub>trend</sub>		<0.01		0.79		0.25		0.85	

Ref., referent values; TFA, *trans*-unsaturated fatty acids.

\* Model 1: adjusted for daily energy intake, alcohol consumption and non-dietary factors such as level of education, family history of diabetes, physical activity, hypertension, hypercholesterolaemia and smoking status.

† Model 2: model 1 + tertile groups of remaining fatty acid groups.

‡ Model 3: model 2 + BMI.



**Fig. 1.** BMI-stratified Cox proportional hazard ratios (95% CI) of type 2 diabetes by tertile group of fatty acid consumption (g/d). Models adjusted for daily energy intake, alcohol consumption, level of education, family history of diabetes, physical activity, hypertension, hypercholesterolaemia, smoking status and tertile groups of remaining fatty acid groups. TFA, *trans*-unsaturated fatty acids. ■, BMI < 25 kg/m<sup>2</sup>; □, BMI ≥ 25 kg/m<sup>2</sup>.

**Table 3.** Cox proportional hazards ratios of the risk for incident type 2 diabetes by *n*-3 and *n*-6 PUFA consumption (g/d) with BMI stratification in the E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Éducation Nationale) study cohort (*n* 71 334) (Hazard ratios (HR) and 95% confidence intervals)

Variables (g/d)	No. of cases/ non-cases	<i>n</i> 71 334		No. of cases/ non-cases	BMI < 25 kg/m <sup>2</sup> ( <i>n</i> 57 408)		No. of cases/ non-cases	BMI ≥ 25 kg/m <sup>2</sup> ( <i>n</i> 13 926)	
		HR	95% CI		HR	95% CI		HR	95% CI
<b>ALA</b>									
<0.90	757/22 784	1 (Ref.)		358/19 213	1 (Ref.)		399/3571	1 (Ref.)	
0.90–1.14	843/22 696	1.00	0.90, 1.12	365/18 649	0.93	0.79, 1.09	478/4047	1.08	0.94, 1.25
≥1.14	1010/23 244	1.03	0.92, 1.15	373/18 450	0.90	0.75, 1.07	637/4794	1.17	1.01, 1.36
<i>P</i> <sub>trend</sub>			0.62			0.24			0.04
<b>EPA</b>									
<0.09	711/22 830	1 (Ref.)		329/19 275	1 (Ref.)		382/3555	1 (Ref.)	
0.09–0.20	823/22 716	0.88	0.73, 1.06	344/18 694	0.86	0.65, 1.14	479/4022	0.87	0.67, 1.12
≥0.20	1076/23 178	0.88	0.67, 1.15	423/18 343	0.79	0.53, 1.19	653/4835	0.93	0.65, 1.32
<i>P</i> <sub>trend</sub>			0.51			0.32			0.96
<b>DPA</b>									
<0.05	610/22 931	1 (Ref.)		307/19 912	1 (Ref.)		303/3019	1 (Ref.)	
0.05–0.08	805/22 734	1.15	1.02, 1.30	364/18 723	1.23	1.04, 1.47	441/4011	1.15	0.98, 1.36
≥0.08	1195/23 059	1.41	1.23, 1.63	425/17 677	1.45	1.17, 1.80	770/5382	1.54	1.27, 1.85
<i>P</i> <sub>trend</sub>			<0.0001			<0.01			<0.0001
<b>DHA</b>									
<0.19	702/22 839	1 (Ref.)		327/19 243	1 (Ref.)		375/3596	1 (Ref.)	
0.19–0.38	839/22 701	1.15	0.95, 1.38	344/18 743	1.06	0.80, 1.41	495/3958	1.23	0.96, 1.58
≥0.38	1069/23 184	1.11	0.85, 1.44	425/18 326	1.24	0.83, 1.86	644/4858	1.01	0.71, 1.43
<i>P</i> <sub>trend</sub>			0.65			0.27			0.62
<b>LA</b>									
<10.3	740/22 801	1 (Ref.)		330/19 348	1 (Ref.)		410/3453	1 (Ref.)	
10.3–13.5	838/22 702	0.98	0.89, 1.08	382/18 764	1.10	0.94, 1.28	456/3938	0.90	0.78, 1.03
≥13.5	1032/23 221	0.97	0.87, 1.07	384/18 200	1.08	0.92, 1.26	648/5021	0.91	0.80, 1.04
<i>P</i> <sub>trend</sub>			0.52			0.39			0.25
<b>AA</b>									
<0.19	579/22 961	1 (Ref.)		327/20 068	1 (Ref.)		252/2893	1 (Ref.)	
0.19–0.25	750/22 790	1.11	0.99, 1.24	329/18 869	1.05	0.90, 1.23	421/3921	1.28	1.09, 1.50
≥0.25	1281/22 973	1.49	1.33, 1.66	440/17 375	1.50	1.28, 1.76	841/5598	1.74	1.49, 2.03
<i>P</i> <sub>trend</sub>			<0.0001			<0.0001			<0.0001

ALA, α-linolenic acid (18:3*n*-3); Ref., referent values; EPA, 20:5*n*-3; DPA, 22:5*n*-3; DHA, 22:6*n*-3; LA, linoleic acid (18:2*n*-6); AA, arachidonic acid (20:4*n*-6).

### Sensitivity analysis

In a sensitivity analysis excluding the 388 women who developed diabetes during the first 5 years of follow-up, thus on a population of 70 946 women with 2222 incident cases of diabetes, the associations were similar to those in the full analysis (online Supplementary Table S2).

Meat was the food group that contributed most to both AA and DPA intakes (4.2% and 3.3%, respectively). Dietary ALA primarily came from dairy products (11.9%), followed by fats (8.2%), olives (7.0%), and meat, processed meats and offal (6.9%). After additional adjustment for total meat consumption, including processed meats and offal, ALA intake was no longer associated with T2D risk (HR 1.03; 95% CI 0.92, 1.15) (online Supplementary Table S3), whereas high intakes of DPA and AA remained associated with T2D risk (HR 1.21; 95% CI 1.03, 1.42 and HR 1.40; 95% CI 1.23, 1.59, respectively). After stratification by BMI, high DPA intake was associated with T2D risk only in women with BMI ≥25 kg/m<sup>2</sup> (HR 1.33; 95% CI 1.08, 1.64), whereas high AA intake increased T2D risk in the non-overweight strata (HR 1.31; 95% CI 1.09, 1.59) and all intakes above the reference tertile group increased T2D risk in the overweight BMI strata (HR 1.68; 95% CI 1.41, 2.00, third tertile group compared with first tertile group).

Adjustment for the overall dietary pattern using scores adhering to 'Mediterranean/Prudent' or 'Western' dietary patterns yielded similar results, with the exception of a modest increase in risk for high TFA intake (results not shown).

### Discussion

In this prospective cohort of middle-aged women, high total *n*-3 PUFA intake was associated with T2D risk, irrespective of the BMI. Yet, within the *n*-3 PUFA, only DPA and ALA (ALA in overweight women only) were associated with increased T2D risk. Total *n*-6 PUFA intake was not associated with T2D risk, but AA intake was associated with increased T2D risk. The other groups of FA (SFA, MUFA, TFA) were not associated with T2D risk.

### SFA

The absence of an association between SFA intake and T2D risk has already been reported<sup>(7,8,19–21)</sup>. However, biomarkers of very long-chain SFA and odd-chain SFA have been associated with decreased T2D risk, and even-chain SFA with increased risk, either individually or combined<sup>(10,21,22)</sup>. Biomarkers of odd-chain SFA are considered representative of dietary

intake<sup>(10)</sup>, whereas even-chain SFA are also endogenously produced<sup>(23)</sup>. It can thus be suggested that the absence of an association in our study could be due to heterogeneous effects within SFA; yet this phenomenon should also be estimated using dietary intake of SFA.

#### MUFA

Our findings that MUFA intake was not associated with incident T2D are in agreement with other studies<sup>(7,8,21)</sup>. Yet, some studies have found extra virgin olive oil, an important source of MUFA<sup>(24)</sup>, to be beneficial for T2D risk<sup>(25,26)</sup>. However, in our cohort, dairy and meat intakes are the primary sources of MUFA (13.4 and 11.9%, respectively) and prospective studies suggest either an inverse or no association of dairy intake with T2D risk<sup>(27,28)</sup> and a negative association of meat intake with T2D risk<sup>(17,28)</sup>, offering an explanation why we observed no association.

#### PUFA

Our findings contrast other findings in which PUFA intake was associated with decreased T2D risk<sup>(7,8)</sup>. However, PUFA are a group of FA likely to have different effects on T2D risk. Our results support this hypothesis as individual FA within the *n*-3 and *n*-6 PUFA groups had differing effects on T2D risk.

To our knowledge, only an Australian case-cohort study examined the relationship between the dietary intake of individual *n*-6 PUFA and T2D and found no association with any of the *n*-6 PUFA<sup>(29)</sup>. Contrary to us, studies using plasma measurements of *n*-6 PUFA reported no association with AA, and have mixed results concerning LA, as one found no association and two reported an inverse association with T2D<sup>(19,21,29)</sup>.

Few studies have examined the associations of individual *n*-3 PUFA on T2D risk; yet, available evidence concerning the dietary intake of ALA contradicts our results and suggests the absence of an association with T2D risk<sup>(11,13,29)</sup>. However, ALA may be differentially associated with T2D in populations, with a decreased risk in Asian countries but no association in Western countries<sup>(11)</sup>. The marine *n*-3 PUFA (EPA, DPA and DHA) are often grouped together and rarely analysed on their own. However, the Australian case-cohort study did look at each individually and did not find any associations with T2D risk<sup>(29)</sup>. But average intakes of DPA and AA were lower in their population than in the E3N cohort (0.03 (SD 0.03) and 0.07 (SD 0.03) g/d, respectively, for DPA and 0.04 (SD 0.03) and 0.22 (SD 0.09) g/d, respectively, for AA)<sup>(29)</sup>. In addition, although meat is the primary contributor to AA intake in the Australian population (70.2%), fish and meat provided nearly equal contributions to the consumption of DPA (50.4 and 49.2%, respectively)<sup>(30)</sup>. In meta-analyses stratified by sex, intake of marine *n*-3 PUFA was not associated with T2D risk in the whole population or in men, but was associated with an increased T2D risk in women<sup>(12)</sup>. As the Australian case-cohort differed in the amounts and dietary sources of certain PUFA and included both men and women, this could explain the discrepancies with our findings.

#### Trans-unsaturated fatty acids

Total dietary intake of TFA was not associated with incident T2D, supporting the findings of other prospective studies<sup>(8,19,31)</sup> and contrasting with one study<sup>(7)</sup>. Residual dietary confounding could explain the inconsistency in results, as the mentioned cohorts all found significant associations with TFA until adjustment for other dietary factors<sup>(8,19,31)</sup>, or TFA may also be a heterogeneous group. Plasma measurements of two TFA have been associated with an increased T2D risk<sup>(31)</sup>, while evidence from a prospective cohort suggests that *trans*-palmitoleate (*trans*-16:1*n*-7), a naturally occurring dairy TFA, is associated with decreased T2D risk<sup>(32)</sup>.

#### Potential biological pathways

One of the mechanisms through which FA are believed to affect the development of T2D is through their effects on cell membranes. The FA tails of the phospholipid (PL) bilayer can differ in both length and saturation (with double bonds reducing their ability to pack closely together); thus the FA composition of the cell membrane affects its fluidity, membrane protein incorporation, enzyme activities and receptor functions<sup>(33)</sup>. As recent studies have demonstrated that the FA composition of the membrane is at least partially determined by the diet, this may have important health implications<sup>(34,35)</sup>. Participants randomly assigned to a diet with a high proportion of SFA later showed higher proportions of SFA in skeletal muscle PL, compared with individuals consuming a high-proportion MUFA diet<sup>(34)</sup>. Increasing ratios of SFA:PUFA in the skeletal muscle cell membrane have been observed to decrease glucose effectiveness and insulin sensitivity in humans, which could theoretically lead to T2D<sup>(36,37)</sup>. However, both the proportion and absolute amount of FA can affect plasma concentrations, and thus both may be important in determining skeletal muscle PL composition as well, and could explain why we did not observe a similar relationship with SFA in our study<sup>(35)</sup>. In addition, as the food preparation methods could alter the FA content differentially by FA group and type of food, this offers another theory to the discrepancy in our results with the biological studies<sup>(38)</sup>.

FA may also exert their effects through gene expression, as they act as signalling molecules of genes coding for metabolic enzymes<sup>(39)</sup>. Studies have shown that persistent exposure of pancreatic islet cells to high levels of free FA prevented transcription of acetyl-CoA carboxylase, an enzyme required for glucose-stimulated insulin release from the pancreas<sup>(39)</sup>. A few studies have also suggested that AA could stimulate insulin secretion<sup>(40,41)</sup> and suggested that the plasma concentration of AA could be a key determinant of insulin resistance<sup>(42)</sup>. However, the relationship between AA and insulin is not yet clear, as studies also suggest that in the case of diabetes, insulin may activate enzymes responsible for AA synthesis<sup>(43)</sup>. Pure DPA has been difficult to isolate and was not readily accessible; hence, there is no literature on the biological relationship between DPA and T2D<sup>(44)</sup>.

#### Strengths and limitations

Potential limitations should be considered. The dietary estimates are based on a single questionnaire at baseline; thus,



misclassification of exposure is possible. However, as the study is prospective, any effects are likely to be non-differential and would lead to an attenuation of the true association. T2D cases were carefully identified and validated; yet it is possible there was an under-ascertainment of T2D cases, especially in asymptomatic women. With our large sample size, small deviations could lead to small *P*-values when comparing the characteristics between cases and non-cases, but these would not affect the *P*-values of the Cox models. For this reason, the mean values between cases and non-cases were used to interpret our results in Table 1 and the *P*-values were not displayed. Finally, we were not able to adjust for contaminants such as Hg (found in fish) and persistent organic pollutants (POP, the major sources of which are fish, meat and dairy products) that have been associated with incident T2D in some studies<sup>(45–47)</sup>. However, epidemiological evidence on POP has been considered inconsistent enough that studies suggest that the potential hazards of the low levels of exposure present in seafood do not outweigh the benefits of seafood consumption. In addition, meta-analyses in East Asian countries where fish consumption is high even demonstrate a decreased risk for T2D associated with a high fish consumption<sup>(48,49)</sup>. Yet, interpretation of the findings should be cautious in the absence of adjustment for contaminants. The strengths of this study include the prospective design that minimises recall and selection biases and the large study population. The high rate of follow-up allowed the validation of many cases of incident T2D, and the dietary questionnaire was validated and specific for this French population. Extensive information on potential confounders was collected, minimising residual confounding. We also controlled for other FA groups besides the one in question in our analyses. This is important, as the fate of FA depends heavily on the overall FA profile of the diet, likely due to competition between FA for enzymes<sup>(14)</sup>.

#### Conclusion

Despite the large body of evidence suggesting that PUFA, and specifically *n*-3 PUFA, are beneficial for cardiovascular health<sup>(50)</sup>, based on our results we could not recommend a high consumption of either as beneficial for diabetes prevention, as the effects of PUFA appear heterogeneous within the group and even within subgroups. High AA and DPA intakes were associated with an increased risk for incident T2D, independently of meat consumption, the primary food group contributor. The association could be even more pronounced in the general population, as our population is relatively homogeneous. Further investigation into the associations of individual dietary *n*-3 and *n*-6 PUFA intake and the risk for T2D is warranted.

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G. F., M. M. and C. D. designed the research; M. M. and C. D. analysed the data or performed the statistical analysis; C. D. wrote the paper; G. F. had primary responsibility for the final content. F. C.-C., F. B., B. B., A. A. and M.-C. B.-R. reviewed and commented on the final manuscript. All the authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

#### Supplementary materials

For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114516003883>

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## Diet and risk of diabetic retinopathy: a systematic review

Courtney Dow<sup>1,2</sup> · Francesca Mancini<sup>1,2</sup> · Kalina Rajaobelina<sup>1,2</sup> · Marie-Christine Boutron-Ruault<sup>1,2</sup> · Beverley Balkau<sup>1,2,3</sup> · Fabrice Bonnet<sup>1,2,4</sup> · Guy Fagherazzi<sup>1,2</sup>

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### Abstract

Diabetic retinopathy is a microvascular complication of diabetes that threatens all individuals with diabetes, leading to vision loss or blindness if left untreated. It is frequently associated with diabetic macular edema, which can occur at any point during the development of diabetic retinopathy. The key factors known to lead to its development include hyperglycemia, hypertension, and the duration of diabetes. Though the diet is important in the development of diabetes, its role in diabetic retinopathy has not been clearly identified. In this systematic review, we aimed to identify, summarize and interpret the literature on the association between the diet and dietary intakes of specific foods, nutrients, and food groups, and the risk of diabetic retinopathy. We searched PubMed and Web of Science for English-language studies evaluating the association between the dietary intake of individual foods, macro or micronutrients, dietary supplements, and dietary patterns and their association with retinopathy or macular edema. After reviewing potentially relevant abstracts and, when necessary, full texts, we identified 27 relevant studies. Identified studies investigated intakes of fruit, vegetables, fish, milk, carbohydrates, fibre, fat, protein, salt, potassium, vitamins C, D, and E, carotenoids, dietary supplements, green tea and alcohol. Studies suggest that adherence to the Mediterranean diet and high fruit, vegetable and fish intake may protect against the development of diabetic retinopathy, although the evidence is limited. Studies concerning other aspects of the diet are not in agreement. The role of the diet in the development of diabetic retinopathy is an area that warrants more attention.

**Keywords** Diet · Retinopathy · Diabetes · Complications

### Abbreviations

25(OH)D	25-Hydroxyvitamin D	n-3 PUFA	Omega-3 polyunsaturated fatty acids
AGE	Advanced glycation endproducts	n-6 PUFA	Omega-6 polyunsaturated fatty acids
ALE	Advanced lipoxidation endproducts	PUFA	Polyunsaturated fatty acids
BMI	Body mass index	ROS	Reactive oxygen species
HbA1c	Glycated hemoglobin	SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids	RCT	Randomized controlled trial
		VEGF	Vascular endothelial growth factor

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✉ Guy Fagherazzi  
guy.fagherazzi@gustaveroussy.fr

<sup>1</sup> Inserm U1018, Institut Gustave Roussy, CESP, 114 Rue Edouard Vaillant, 94805 Villejuif Cedex, France

<sup>2</sup> University Paris-Saclay, University Paris-Sud, Villejuif, France

<sup>3</sup> University Versailles, Saint Quentin, University Paris-Sud, Villejuif, France

<sup>4</sup> CHU Rennes, Université de Rennes 1, Rennes, France

### Introduction

Diabetic retinopathy is an important microvascular complication of diabetes mellitus, causing damage to the retinal capillaries and leading to vision loss or blindness if left untreated [1]. At any stage of its development, diabetic macular edema may also occur as a further complication and lead to substantial vision loss through a thickening of the macular region of the retina [2]. Diabetic retinopathy has become a leading cause of blindness in the working-age population of the Western world due to the increasing

incidence of diabetes and the aging of the population. It accounts for an estimated 2.4 million cases of blindness worldwide [3]; 15–17% of the blindness in the US and Europe and 3–7% of the blindness in Southeast Asia and the Western Pacific can be attributed to retinopathy [3, 4]. As diabetic retinopathy has a significant negative impact on the quality of life [5, 6], and studies suggest that it is associated with an increased risk of cardiovascular events and/or all-cause mortality [7] in addition to its threat to vision, it is crucial to understand its etiology.

Though diabetic retinopathy is present in over one third of diabetic people [1], little is known about its development. Overall, badly managed diabetes is more likely to lead to diabetic retinopathy, with the duration of diabetes, hyperglycemia, hypertension and the type of diabetes being factors consistently associated with the development of retinopathy [1, 8–11]. However, the diet is an established factor in the development of diabetes but its role in retinopathy has been much less investigated [12, 13]. For this reason, in this systematic review, we aimed to identify, summarize, and interpret the literature on the association between the diet or dietary intake of foods, nutrients, or food groups, and the risk of diabetic retinopathy or diabetic macular edema.

## Methods

### Inclusion criteria and search strategy

Using the PRISMA Checklist for systematic reviews and meta-analyses, we conducted a systematic review of all studies published in peer-reviewed journals in the English language that evaluated the association between dietary intakes of foods (individual food items or broader food groups such as fruit or vegetables), macronutrients (carbohydrates, protein, fat, fibre), micronutrients (minerals, vitamins), dietary supplements (as vitamins or minerals) or dietary patterns (a posteriori or a priori) and their association with the development of diabetic retinopathy or macular edema. Diabetic retinopathy included retinopathy at all stages of development (non-proliferative and proliferative; ICD 10: E10.3, E11.3) with or without macular edema (ICD 10: H35.8). Diabetic retinopathy and macular edema could be evaluated through: ophthalmological assessment, retinal photographs graded against standard photographs, mydriatic or non-mydriatic fundus photography, review of medical records for retinopathy diagnosis or review of medical records for laser photocoagulation treatment for retinopathy.

Eligible study designs included randomized controlled trials (RCTs), and cohort, case–control, cross-sectional, and ecological studies. All publication years and

publication statuses were considered. Studies evaluating the association between dietary intake or dietary patterns and other health outcomes were excluded, as well as studies conducted on laboratory animals and studies assessing the progression or severity of diabetic retinopathy.

Searches were conducted in both PubMed and Web of Science in November 2015 and last updated in October 2017. The search strategy employed for the Pubmed search was based on the protocol developed in a systematic review by Schwingshackl et al. [13] to identify food intake and risk of chronic disease, but was slightly modified to include other search terms we defined as pertinent. The search strategy is available in the Supplementary Material 1 and 2.

### Study selection

The titles and abstracts of articles identified in the searches were screened by a single researcher (CD) and checked by a second (GF). The full texts of all potentially eligible articles from each database were obtained and examined by a researcher (CD) to determine if they fit the eligibility criteria. In those fitting the criteria, reference lists were verified for further relevant studies.

### Data extraction

Data extraction was performed on full texts by a researcher (CD) for the following characteristics: first author's surname, year of publication, study design, country of study, year of study, sample size, and number of cases, as well as the potential restrictions on age and sex of included participants, type of diabetes mellitus, duration of follow-up, exposure of interest, exposure assessment, retinopathy assessment, potential confounders controlled for and primary findings (the most adjusted risk estimates, hazard ratios, risk ratios, or odds ratios with their 95% confidence intervals) and this extraction was checked by a second researcher (GF).

### Assessment of the quality of included studies

To assess the quality of the RCTs included in this systematic review, we used the Cochrane Risk of Bias Tool for Randomized Controlled Trials. This tool classifies randomized controlled trials according to their risk of bias by seven criteria: random sequence generation, allocation concealment, selective reporting, other bias, blinding of participants and personnel, blinding of outcome assessment, and incomplete outcome data. RCTs are considered to be good quality if all criteria are met [14]. As the Cochrane Handbook identifies the Newcastle–Ottawa Scale as one of the most useful tools to assess the methodological

quality or risk of bias in non-randomized studies, we employed this scale to assess the quality of the observational studies [15]. This scale assesses cohort and case-control studies in three areas: selection (4 stars), comparability (2 stars), and outcome (3 stars), for a total score of 9 stars. Studies were considered good quality if they had 7 or more stars, medium quality if they had 5 or 6 stars and low quality with 4 or fewer stars. A modified version of the scale was implemented to assess the cross-sectional studies [16]. This version allots five stars to selection, 2 to comparability and 3 to outcome, for a maximum possible score of 10 stars. Cross-sectional studies were considered good quality with 8–10 stars, medium quality with 6–7 stars and low quality with 5 or fewer stars.

## Results

The flow diagram for the process used for identifying and selecting relevant studies for this systematic analysis is depicted in Fig. 1. A total of 385 potentially relevant studies were identified using the search parameters previously mentioned. Of these, 45 studies were identified using the Pubmed Mesh Terms, 145 from the Pubmed search of food items, and 195 from the Web of Science search. After reviewing the titles, abstracts, and if necessary, the full texts of all potentially relevant articles, 27 studies were identified for inclusion in this systematic review, of which 3 were identified through the reference lists of potentially

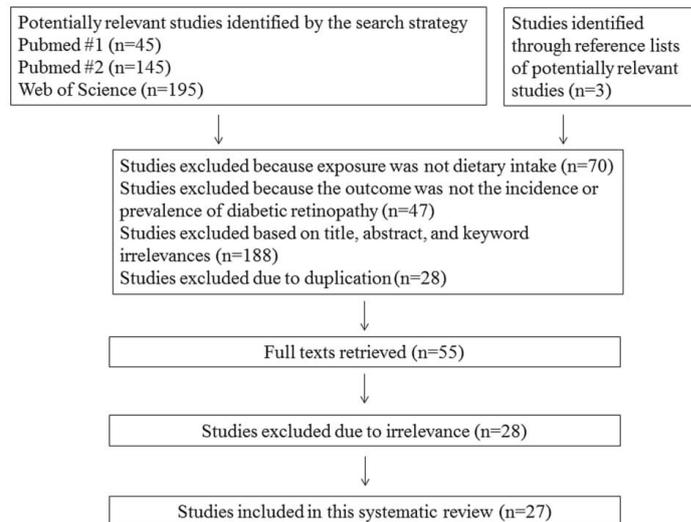
relevant studies. Only one study on macular edema was identified [17].

A summary of information extracted from the included studies can be found in Table 1.

The majority of studies were prospective ( $n = 13$ ) [17–29], of which six were RCTs or nested case-control studies within RCTs [17, 18, 20, 22, 24, 27]. One study, though prospective, collected some information cross-sectionally [28]. The remainder of the studies were cross-sectional ( $n = 10$ ) [30–39] or case-control studies ( $n = 4$ ) [40–43]. Most studies were conducted in Europe ( $n = 12$ ) [17–20, 22, 24, 27, 30, 31, 38, 40, 41], with six in the US [28, 29, 33, 34, 36, 43], two in Australia [35, 37], six in Asia [21, 25, 26, 32, 39, 42] and one throughout four continents [23].

Both of the RCTs were determined to be of poor quality due to the lack of information presented in the papers [22, 24]. Neither study elaborated on the method of randomization, allocation concealment, blinding of the participants, or blinding of the outcome assessment. In total, 14 studies were determined to be of good quality, 7 of medium quality and 4 of low quality. Most of the studies that were not considered high quality lost one or two stars due to failure to control for important confounding factors, such as the duration of diabetes [20, 29, 31, 33, 35, 38], diabetes treatment [17, 19, 23, 27, 28, 30–33, 35–37, 43] or the level of glycated hemoglobin [18, 31, 35, 38, 40, 41]. Many of the cross-sectional studies also did not provide adequate descriptions of the response rate or characteristics of the responders and non-responders [28, 31–36, 38], so the

**Fig. 1** Flow diagram of the selection process for studies included in this systematic review



**Table 1** General characteristics of the studies included and their sample populations

Study	Publication year	Country	Year of conduct	Study design	Sample size	Age	Recruitment	Exposure	Outcome assessment
Alcubierre et al. [40]	2016	Spain	2010–2013	Case–control	294	40–75	Participants of the Department of Ophthalmology's screening and treatment program for diabetic retinopathy	Intake of macronutrients (carbohydrates, protein, fat, fibre)	Ophthalmologist assessment
Beulens et al. [30]	2008	16 European countries	1989–1991	Cross-sectional	1528	15–60	EURODIAB Prospective Complications cohort	Alcohol consumption	Retinal photographs
Diaz-Lopez et al. [20]	2015	Spain	2003–2009	Prospective study nested in RCT	3614	55–80	PREDIMED (Prevención con Dieta Mediterránea) clinical trial	Mediterranean diet, enriched with either olive oil or nuts	Ophthalmologist assessment
Engelen et al. [31]	2014	16 European countries	1989–1991	Cross-sectional	1880	15–60	EURODIAB Prospective Complications cohort	Sodium and potassium intake	Retinal photographs
Ganesan et al. [32]	2016	India	N/A	Cross-sectional	1261	≥ 40	Sankara Nethralaya-Diabetic Retinopathy Epidemiology and Molecular Genetic Study 1	Fibre intake	Retinal photographs
Giuffrè et al. [41]	2004	Italy	N/A	Case–control	1019	> 40	Random recruitment in Casteldaccia	Risk factors for retinopathy	Retinal photographs
Horikawa et al. [21]	2014	Japan	1995–2003	Cohort	978	40–70	JDCS (Japan Diabetes Complications Study)	Sodium intake	Ophthalmologist assessment
Horikawa et al. [26]	2017	Japan	1995–2003	Cohort	936	40–70	JDCS (Japan Diabetes Complications Study)	Proportions of carbohydrate intake	Ophthalmologist assessment
Howard-Williams et al. [22]	1985	UK	1973–1982	RCT	149	< 66	Radcliffe Infirmary Diabetic Clinic	Low-carb diet or modified fat diet	Ophthalmologist assessment
Lee et al. [23]	2010	14 countries	N/A	Cohort	1239	55–81	AdRem (Action in Diabetes and Vascular Disease: Preterax and Diamicon MR Controlled study)	Alcohol consumption	Retinal photographs
Ma et al. [42]	2015	China	2013	Case–control	200	> 18	Hospital of Xiang Cheng District, Suzhou	Green tea consumption	Ophthalmologist assessment
Mahoney et al. [33]	2014	US	2003–2006	Cross-sectional	155	≥ 40	NHANES 2003–2006 (National Health and Nutrition Examination Survey)	High flavonoid fruit and vegetable consumption index score (HFVC)	Retinal imaging exam
Mayer-Davis et al. [34]	1998	US	1984–1990	Cross-sectional	387	20–74	San Luis Valley Diabetes Study	Antioxidant intake (vitamins C, E, and β-carotene)	Retinal photographs
Martin-Merino et al. [27]	2016	UK	2000–2007	Nested case–control	17,130	All ages	THIN (The Health Improvement Network) database	Risk factors for retinopathy	Computerized patient profiles

Table 1 continued

Study	Publication year	Country	Year of conduct	Study design	Sample size	Age	Recruitment	Exposure	Outcome assessment
Martín-Merino et al. [17]	2017	UK	2000–2007	Nested case-control	2405	All ages	THIN (The Health Improvement Network) database	Risk factors for diabetic macular edema	Computerized patient profiles
McKay et al. [35]	2000	Australia	1992–1996	Cross-sectional	239	> 40	VIP (Visual Impairment Project)	Risk factors for retinopathy	Ophthalmologist assessment
Millen et al. [36]	2003	US	1988–1994	Cross-sectional	930	≥ 40	NHANES III (The Third National Health and Nutrition Examination Survey)	Duration of vitamin C and E supplement use	Retinal photographs
Millen et al. [29]	2004	US	1987–1995	Cohort	1353	45–64	ARIC (Atherosclerosis Risk in Communities Study)	Vitamin C and E intake	Retinal photographs
Millen et al. [28]	2016	US	1987–1995	Cohort/Cross-sectional	1339	45–65	ARIC (Atherosclerosis Risk in Communities Study)	Dietary intake of vitamin D; vitamin D or fish oil supplement use	Retinal photographs
Roig-Revert et al.	2015	Spain	2013–2014	RCT	208	25–80	10 centers	Supplements with antioxidants/n3-PUFA	Fundus exam and retinograph
Sahli et al. [43]	2016	US	1993–1995	Case-control	1430	45–65	ARIC (Atherosclerosis Risk in Communities Study)	Lutein intake	Retinal photographs
Sasaki et al. [37]	2015	Australia	2009–2010	Cross-sectional	379	> 18	DMP (Diabetes Management Project)	Fatty acid intake	Retinal photographs
Sala-Vila et al. [18]	2016	Spain	2003–2009	Prospective study nested in RCT	3482	55–80	PREDIMED (Prevención con Dieta Mediterránea) clinical trial	Meeting the dietary long chain n3-PUFA recommended intake (500 mg/day)	Review of medical records
Segato et al. [38]	1991	Italy	N/A	Cross-sectional	1321	N/A	Diabetic clinics in the Veneto region	Risk factors for retinopathy	Ophthalmologist assessment
Tanaka et al. [25]	2013	Japan	1995–2004	Cohort	978	40–70	JDCS (Japan Diabetes Complications Study)	Fruit intake	Ophthalmologist assessment
Yang et al. [39]	2012	Korea	2008–2009	Cross-sectional	998	> 19	KNHANES (Korea National Health and Nutrition Examination Survey)	Risk factors for retinopathy	Retinal photographs
Young et al. [19]	1984	Scotland	N/A	Cohort	296	20–59	Male clinic population	Risk factors for retinopathy	Ophthalmoscopy

majority (75%) of studies considered low quality were cross-sectional studies. Two of the studies considered to be of low quality also did not provide an adequate description of method of assessment of the exposure [38, 39].

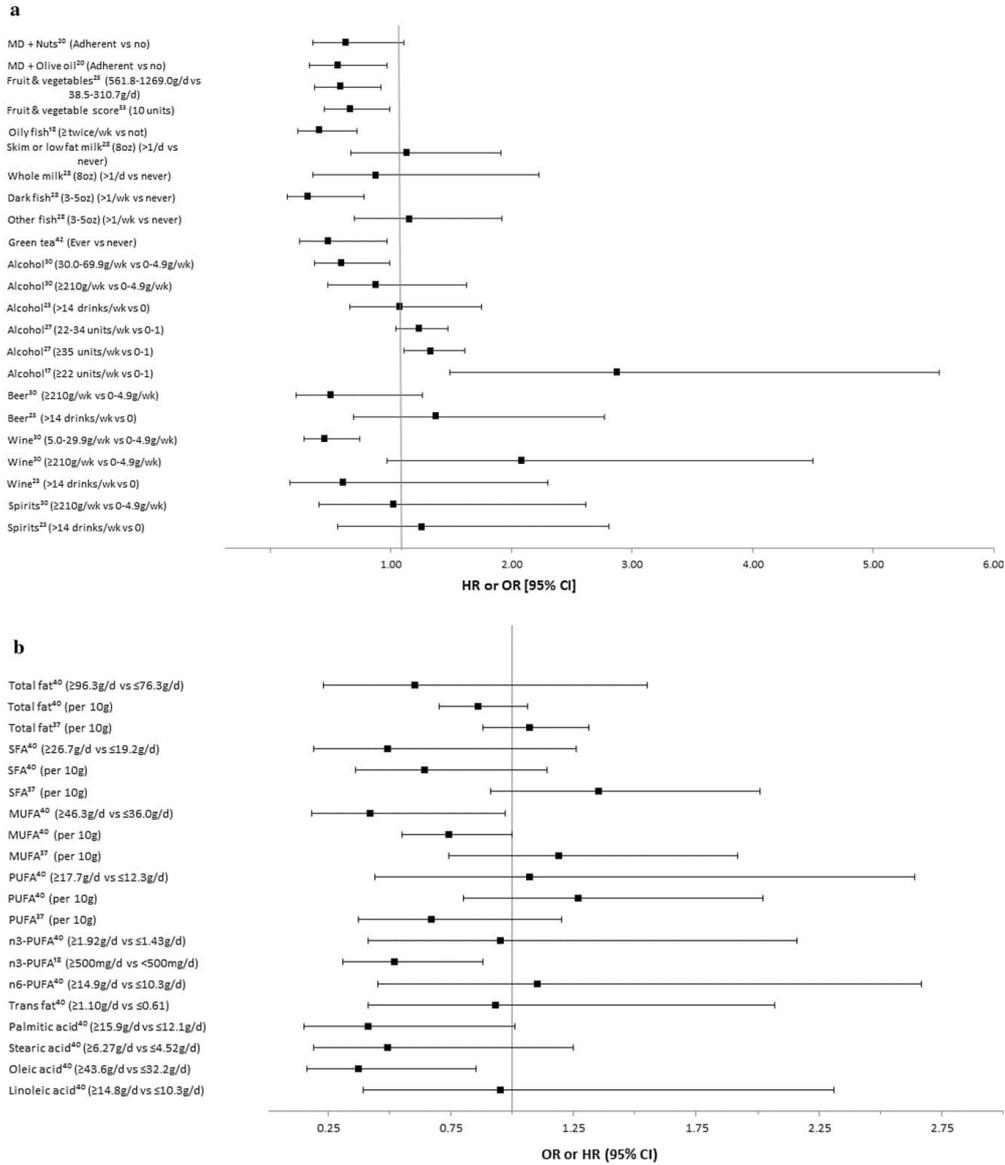
Only one study evaluated the association between the diet as a whole and diabetic retinopathy [20] (Fig. 2a). Diaz-Lopez et al. [20] investigated the Mediterranean diet, enriched with either extra virgin olive oil or nuts and compared it with a low-fat diet in a prospective study spanning 6 years, in over 3600 participants. The Mediterranean diet enriched with olive oil was associated with more than a 40% decreased risk of retinopathy [20]. In addition, those in the highest quintile of adherence to the Mediterranean diet enriched with olive oil had more than a 60% decreased risk of retinopathy compared to those with the lowest adherence [20]. The Mediterranean diet enriched with nuts was associated with a 37% decreased, though non-statistically significant reduction in the risk of retinopathy [20].

Other studies have examined the intake of food groups. A Japanese cohort study of people with type 2 diabetes found that high fruit consumption ( $\geq 173.2$  g per day, i.e. a large apple or two bananas), was associated with more than a 50% decreased risk of incident retinopathy, compared with those consuming less than 53.2 g of fruit per day [25]. In addition, a cross-sectional study in the US associated a high flavonoid fruit and vegetable consumption index score with reduced odds of retinopathy [33]. Milk intake has also been analysed; neither whole milk nor skim/low fat milk was associated with retinopathy in a sample of over 1350 people with type 2 diabetes [28]. On the other hand, fish may be beneficial in avoiding development of retinopathy, intake of oily fish at least twice a week (versus less than twice a week), was associated with an almost 60% decreased risk of retinopathy [18]. Another study found that 85–141 g of dark fish, (salmon, mackerel, swordfish, sardines, bluefish), per week versus never was associated with almost 70% decreased odds of retinopathy [28]. However, 85–141 g of “other fish”, (cod, perch, catfish), per week was not associated with retinopathy [28].

Seven studies looked at the intake of macronutrients, including carbohydrates [26, 40], fatty acids [18, 22, 37, 40], fibre [25, 31], and protein [40]. Neither the intake of carbohydrates nor the proportion of carbohydrates as total energy, nor protein was associated with diabetic retinopathy in either a cohort study or a case–control study [26, 40]. With regard to fats, a cross-sectional and a case–control study both found that total fat intake was not associated with the odds of diabetic retinopathy [37, 40], however, there are conflicting results concerning individual fatty acid groups (Fig. 2b). In Alcobierre et al.’s case–control study, total saturated fatty acid (SFA) consumption and individual SFA consumption (palmitic or stearic acid)

was not associated with retinopathy risk [40], while Sasaki et al.’s cross-sectional study of participants with well-controlled type 1 or 2 diabetes ( $\text{HbA1c} < 7.0\%$ ) noted higher odds of retinopathy with increasing intake of SFA after stratification on diabetes control [37]. Results concerning monounsaturated fatty acid (MUFA) intake are also mixed. Here, Sasaki et al. [37] did not find an association with retinopathy, whereas Alcobierre et al. [40] observed higher intake of MUFA and oleic acid to be associated with decreased odds of retinopathy. Alcobierre et al. [40] did not observe an association between retinopathy and trans-fat intake, nor with polyunsaturated fatty acid (PUFA) intake, including omega-3 PUFA (n-3 PUFA) and omega-6 PUFA (n-6 PUFA). On the other hand, Sasaki et al. [37] noted a beneficial association of PUFA intake, though only in people with well-controlled diabetes ( $\text{HbA1c} < 7.0\%$ ), and one prospective study found that participants meeting the dietary recommendations for long-chain n-3 PUFA intake for cardiovascular prevention (500 mg/day) had lower risk of retinopathy [18]. A Spanish RCT also demonstrated less onset of retinopathy in people supplemented with antioxidants and n-3 PUFA [24]. Howard-Williams et al. [22] conducted a RCT where they randomly assigned participants to either a low-carb diet or a modified fat diet, rich in linoleic acid. Compliers of the modified fat diet had retinopathy less often than compliers of the low-carb diet and non-compliers, though the difference was not statistically significant [22]. Howard-Williams et al. [22] also observed a greater frequency of diabetic retinopathy in people with poorly controlled diabetes ( $\text{HbA1c} > 8\%$ ) with low levels of linoleic acid ( $< 50\%$ ) in cholesterol ester. In contrast, Alcobierre et al. [40] did not observe the intake of linoleic acid to be associated with retinopathy. Concerning fibre, no association was observed in two studies, but one Indian study showed beneficial effects of high fibre intake [25, 32, 40]. This cross-sectional study including type 2 diabetes participants from the general population in India demonstrated that a low fibre score was associated with up to a 41% increased odds of retinopathy [32].

Seven studies examined the associations between intakes of micronutrients and retinopathy risk [21, 25, 28, 29, 31, 34, 43] (Fig. 2c). In two studies, a Japanese cohort of individuals with type 2 diabetes, and a cross-sectional study across 16 European countries of individuals with type 1 diabetes, neither potassium intake nor sodium intake was associated with risk of retinopathy [21, 25, 31]. Carotenoid intake and retinopathy were investigated by three studies [25, 34, 43]. The first was a case–control study of diabetic people enrolled in the Atherosclerosis Risk in Communities Study (ARIC). They did not observe an association between lutein intake and retinopathy [43]. The other two studies looked at  $\beta$ -



**Fig. 2 a** Odds ratios or hazards ratios and 95% confidence intervals of the outcomes of studies examining the relationship of diets, foods or food groups with diabetic retinopathy or macular edema. **b** Odds ratios or hazard ratios and 95% confidence intervals of the outcomes of studies examining the dietary intake of fatty acids and their association with diabetic retinopathy (SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty

acids; n3-PUFA, omega-3 polyunsaturated fatty acids; n6-PUFA, omega-6 polyunsaturated fatty acids). **c** Odds ratios or hazard ratios and 95% confidence intervals of the outcomes of studies examining dietary intake of macronutrients or micronutrients and their association with diabetic retinopathy (\*From food only; †From food and supplements)

macular edema [17], and the heaviest drinkers ( $\geq 35$  units/week) had 1.3 times the odds of retinopathy [27]. However, a cross-sectional study conducted over 16 European countries detected an inverse association between drinking frequency and the odds of proliferative diabetic retinopathy, and a U-shaped association between alcohol consumption and proliferative retinopathy [30]. But, when examining alcohol types separately, only wine was associated with proliferative retinopathy in a U-shaped fashion, and beer and spirit consumption were not associated [30]. In a Chinese case-control study, with sex and age-matched diabetic controls without diabetic retinopathy, the authors found that regular (every week for at least 1 year) Chinese green tea consumption was associated with decreased odds of retinopathy in women, but not men (OR 0.32 [0.13–0.75], OR 0.79 [0.30–2.06]; women and men, respectively) [42].

Four studies analysed the association between dietary supplements and diabetic retinopathy [24, 28, 29, 36]. In a prospective study of participants of the ARIC, Millen et al. [29] found no impact on the probability of retinopathy when looking at vitamin C and E intake from food alone or food and supplements combined. Yet, an interaction with race was detected with vitamin E; high intakes of vitamin E from food alone, or food and supplements combined, were associated with increased prevalence of retinopathy in Caucasians, but not in African Americans [29]. Decreased odds of retinopathy were noted in users ( $\geq 3$  years) of vitamin C, E, and multivitamin supplements compared to non-users [29]. But, Millen et al., in a cross-sectional analysis of NHANES III participants, did not find any association between long-time users of vitamin C or E supplements ( $\geq 5$  vs.  $< 1$  year) and the presence of retinopathy [36]. Use of vitamin D and fish oil supplements, compared to non-use, was not associated with the odds of retinopathy in the ARIC [28]. Regarding n-3 PUFA supplements, in an RCT where people with diabetes were randomly assigned to n-3 PUFA supplements for 18 months, those taking the supplements had lesser risk of retinopathy [24].

## Discussion

Out of 27 studies identified that explored the relationship between the diet and diabetic retinopathy or macular edema, the majority were prospective or nested within prospective studies and only one considered the diet as a whole. Two studies examined the effects of fruit and vegetable intake, two looked at fish intake, and one looked at milk intake, while others evaluated the intakes of macronutrients (carbohydrates, fatty acids, fibre, and protein), micronutrients (salt, potassium, lutein, carotene, and

vitamins C, D and E), alcohol, green tea and dietary supplements of vitamins C, D, E or multivitamins.

## The Mediterranean diet

The only study that examined the role of a dietary pattern, a post hoc analysis of an RCT, found that the Mediterranean diet enriched with olive oil was associated with a decreased risk of incident diabetic retinopathy compared to the low-fat control diet, with higher adherence conferring a larger risk reduction [20]. Through factor analysis, a cross-sectional study in Australia also identified a Mediterranean dietary pattern as protective against diabetic retinopathy [44]. The Mediterranean diet is a diet rich in fruit, vegetables, whole grains, plant proteins, fish, and low-fat dairy products and has been associated with reduced risk of type 2 diabetes [45] and improved glycemic control, body weight and cardiovascular risk factors in people with type 2 diabetes [46]. The Mediterranean diet supplemented with mixed nuts was surprisingly not associated with reduced retinopathy risk in the post hoc analysis, though many of the nutrients or compounds found in nuts, and nuts themselves, have been associated with prevention of type 2 diabetes [47]. But, within the same intervention groups, the glycemic loads and the glycemic indices of both Mediterranean diet groups showed favourable significant associations compared to the control group [48]. As glycemic control is the most important factor and is consistently associated with the development of diabetic retinopathy, it is possible that the Mediterranean diet could be advantageous in the prevention of retinopathy through its beneficial effects on glycemic control [49–51] or perhaps through its effects on lipid profiles. In the same study, both Mediterranean diet interventions (supplemented with olive oil or nuts) improved high-density lipoprotein functions [52].

## Fruit, vegetable and fish consumption

Fruit, vegetables and fish play vital roles in the composition of the Mediterranean diet. In agreement with the studies on the Mediterranean diet, high fruit, vegetable and oily fish intake have been observed to confer strong protective effects on the development of retinopathy [18, 25, 28, 33]. Evidence also suggests that higher fruit and vegetable consumption, or plant-based diets have a protective role in the development of type 2 diabetes [53], though the evidence concerning fish and type 2 diabetes seems to be less clear [54]. Fish may exert its protective effects through its omega-3 or vitamin D content and fruit and vegetables may exert their protective effects through their antioxidant content, vitamins C and E, carotenoids or polyphenols. Trials have found that antioxidant

supplementation was associated with reductions in reactive oxygen species and slowed progression of retinopathy in subjects with non-proliferative diabetic retinopathy [55, 56], and some polyphenols may also inhibit the onset of retinopathy [57]. However, the literature concerning the role of vitamins C and E in the development of retinopathy is not consistent.

### Micronutrient consumption

Vitamin C intake was associated with decreased risk of retinopathy in one prospective study [25]; however, this inverse association was not reported in two cross-sectional studies [29, 34]. Vitamin C supplement use could suggest that vitamin C aids in retinopathy prevention, as users were found to have decreased odds of retinopathy compared to non-users [29], but the duration of supplement use may not be a factor [36]. The benefits of vitamin C in retinopathy are supported by five cross-sectional, hospital-based studies in people with diabetes, which have consistently reported lower serum vitamin C levels in those with retinopathy than in those without retinopathy [58]. A cross-sectional study among NHANES III participants also observed an inverse association between serum vitamin C and retinopathy, though not statistically significant, and only after the exclusion of vitamin C supplement users [36].

Overall, studies did not find an association between vitamin E and retinopathy [25, 29, 34]. Yet, vitamin E demonstrated positive associations with the odds of retinopathy in people not treated with insulin [34], those taking oral hypoglycemic agents [29], and those with poor glucose control in a cross-sectional [29], but not cohort study [25]. However, the participants of this cohort had well controlled HbA1c, BMI, triglycerides and systolic blood pressure and thus may have lacked the heterogeneity for demonstrating the interaction [25]. In contrast, vitamin E supplement use has demonstrated an inverse association with diabetic retinopathy [29], though the duration of use does not seem to play a role [36]. Cross-sectional, hospital-based studies examining vitamin E concentrations are inconsistent with their results [25]. A systematic review found that two studies showed no association between vitamin E levels in people with diabetes with versus without retinopathy, but one study reported higher vitamin E levels in people with diabetes with retinopathy than in people with diabetes without retinopathy [58].

Neither vitamin D intake, nor vitamin D or fish oil supplements were associated with retinopathy in a cross-sectional study [28]. However, those who were vitamin D deficient (25-hydroxyvitamin D (25(OH)D) < 75 nmol

27% higher odds in meta-analyses of 14 other observational studies (25(OH)D < 20 ng/mL) [59].

With regards to carotenoids, lutein intake was not significantly associated with retinopathy [43]. But, in a prospective study, serum concentrations of lutein were lower in people with diabetes with non-proliferative diabetic retinopathy compared to people with diabetes without retinopathy [60]. In addition, a cross-sectional study suggested beneficial effects of high combined lutein/zeaxanthin and lycopene plasma concentration towards diabetic retinopathy risk [61]. Concerning carotene, higher  $\beta$ -carotene intake has been both associated with decreased risk and not associated with retinopathy [25, 34]. On the contrary, after stratification of participants treated by insulin versus not treated by insulin, a positive association was observed between  $\beta$ -carotene intake and severe retinopathy in those treated with insulin [34]. Plasma concentrations suggest the strongest predictor of retinopathy could be the ratio of the plasma concentrations of non-provitamin A carotenoids (lutein, zeaxanthin, lycopene) to provitamin A carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin) [61].

The literature addressing vitamins C, D, E and carotenoid intake is not entirely consistent. It suggests either a positive or no effect of vitamin C intake and a negative, or no effect of vitamin E intake, with the exception of vitamin E supplements, which may be beneficial. Beneficial effects of these vitamins could partially explain the inverse association high fruit and vegetable consumption has with the risk of retinopathy, though this hypothesis is neither supported nor refuted by biomarkers of vitamin C and E. Though oily fish intake showed favourable associations on retinopathy outcomes, its effects may not act through its vitamin D content, as neither intake nor supplements were shown to have an association with retinopathy. Results concerning carotenoid intake are similarly varying, with plasma concentrations offering no clarification on the subject. However, results from studies using plasma biomarkers should be interpreted with caution with concerns to dietary intake. Only moderate correlations (< 0.5) have been observed between plasma measures of vitamin C, E, and carotenoids and estimated dietary intake [62, 63]. 25(OH)D may also not be the optimal indicator of dietary vitamin D intake, as it explained only 1% of the between person variation in serum concentrations of 25(OH)D, which reflects all sources of vitamin D [28].

Other discrepancies in the results could be due to the fact that some studies did not adjust on important confounders that could influence the relationship such as the duration of diabetes [29] or diabetes treatment [28, 36, 43], or it could be attributed to the fact that five studies were conducted in Western populations [28, 29, 34, 36, 43], while the last was in a Japanese population [25]. The sources, quantities ingested, and methods of food

preparation of foods containing the micronutrients in question may all vary due to cultural differences. Indeed, beneficial effects of vitamin C and  $\beta$ -carotene were both observed in the Japanese cohort, but not in any of the Western studies. Inconsistencies may also be due to ethnicity; the ARIC population was approximately a third African-American, with the remainder being Caucasian American [28, 29, 43], while Mayer-Davis et al.'s population was 65% Hispanic and 35% non-Hispanic Caucasians [34], and the NHANES III study population was more than two thirds non-Hispanic white, with the majority of the remainder being non-Hispanic black and a minority being Mexican-American (5–10%) [36]. Ethnicity and BMI have been found to interact to influence the development of diabetes [64], and ethnic differences in risk of developing macrovascular and some microvascular complications of diabetes have also been reported [65]. In addition, these studies, with the exception of the Japanese study, were not prospective in design, hindering the ability to infer temporality. One study may have also been restricted as their data were based on a single 24 h recall, which may not be representative of long-term intake [34] and the Japanese study may have been limited by the lack of variation in their population of type 2 diabetics with well-controlled diabetes and BMIs [25]. For these reasons, there is an urgent need for prospective studies to evaluate the associations of the intakes of these important micronutrients and the risk of diabetic retinopathy.

Studies on other micronutrients and retinopathy risk are less controversial, but also less numerous. Potassium intake was not associated with diabetic retinopathy risk [25, 31], but serum potassium has been inversely associated with fasting glucose [66], insulin sensitivity [67], and retinopathy risk [66, 68, 69]. However, others have not found an association between serum or dietary potassium and incident type 2 diabetes [67–69]. It is possible that potassium intake may be influenced by race, as one study uncovered an interaction with race which suggested lower intakes of potassium beneficial in Caucasians and detrimental in African-Americans for diabetes risk, though the association was not linear [69]. The relationship between potassium and retinopathy remains unclear for the moment. Within the same study populations, salt intake was also not associated with retinopathy [21, 31]. High intakes of salt have been found to have detrimental effects with regards to cardiovascular disease [70], but its role in the development of diabetes remains unclear. Urinary sodium excretion has been associated with end-stage renal disease, microalbuminuria, and all-cause mortality in those with type 1 diabetes [31, 71], but the association in individuals with type 2 diabetes has been less studied and warrants attention.

### Macronutrient consumption

Neither total fat [37, 40], trans fat [40], total SFA [37, 40], nor individual SFA intakes [40] were associated with retinopathy, until one study stratified by glycemic control, and observed increased odds of retinopathy with increased SFA intake among participants with well-controlled diabetes ( $\text{HbA1c} < 7.0\%$ ) [37]. It is possible that the effects of SFA differ by diabetes control, or that the effects of SFA vary by type (even chain vs. odd chain or very-long-chain), making it more accurate to stratify analyses [72]. Sasaki et al. [37] did not observe effects of MUFA with retinopathy, while Alcubierre et al. [40] observed MUFA and oleic acid to be inversely associated with odds of retinopathy. But, MUFA intake in Sasaki et al.'s Australian population may not have been strong enough to detect an association, as the median intake (23.6 [19.9, 26.3], 23.5 [19.3, 25.3], 23.7 [20.0, 26.7]; median intake [interquartile range] g/day for all participants, those with well-controlled ( $\text{HbA1c} < 7\%$ ) diabetes, and those with poorly controlled diabetes, respectively) appears much lower than the average intake in Alcubierre et al.'s Spanish population (43.6 [12.8], 39.9 [14.2]; mean [standard deviation] g/day for participants without retinopathy and with retinopathy, respectively) [37, 40]. Some evidence suggests PUFA intake to be beneficial in preventing retinopathy [18, 24, 37], though one study did not observe any associations [40]. Supplementation with n3-PUFA has been shown to decrease the number of retinal acellular capillaries associated with diabetes and inflammatory markers in the retina of diabetic animal models [73, 74], but it may be necessary to examine the effects of PUFAs separately, as their effects may differ within the group, and even within the subgroups [75].

Regarding fibre intake, two studies did not find an association with retinopathy, but an Indian study observed an inverse relationship [25, 32, 40]. The observed differences could be due to the cultural and ethnic differences in the populations, or perhaps because the Japanese cohort was part of a randomized trial originally initiated to assess the effectiveness of lifestyle intervention on diabetic complications, this population may have had more access to information that could help them better manage their diabetes. Fibre intake has been shown to improve glycemic control in people with type 2 diabetes, including Japanese people with type 2 diabetes, presenting another important possibility for further investigation [76, 77].

### Beverage consumption

Green tea was observed to be beneficial for retinopathy prevention [42] and has also been found to be associated

with decreased risk of developing type 2 diabetes [78], lower fasting blood glucose levels [79, 80], and reduced serum insulin concentrations [80]. Results concerning alcohol are much more inconsistent. The majority of studies did not find an association between alcohol intake and retinopathy [23, 35, 38, 39, 41], while three observed a positive association with retinopathy or macular edema [17, 19, 27], and one a U-shaped distribution with retinopathy [30]. But, heavy drinking is also associated with decreased diet quality [81, 82] which could explain the observed association, as the diet was not taken into account during the analyses. In addition, in one study, beer and spirit consumption was not associated with the risk of proliferative diabetic retinopathy, but wine consumption was associated with retinopathy in a U-shaped fashion [30]. As moderate wine consumption has been associated with decreased incidence of cardiovascular disease, diabetes, hypertension and some types of cancer (colon, basal cells, ovarian and prostate carcinoma), it seems necessary to separately examine the effects of different types of alcohol [83]. However, the cohort study across 14 countries did not note differential associations between alcohol consumption by type (beer, wine or spirits) and retinopathy risk, but, the authors cite a lack of power due to a small number of cases of retinopathy as a possible reason for this [23].

### Biological mechanisms

As badly managed diabetes is more likely to lead to retinopathy [1], intake of foods or nutrients that improve glycemic control are likely to be beneficial in the prevention of diabetic retinopathy. Studies have shown that hyperglycemia, along with the duration of diabetes, act as the strongest predictors for retinopathy [49–51]. Hyperglycemia may act to influence the development of retinopathy through several pathways: the polyol pathway, non-enzymatic protein glycation, activation of protein kinase C, activation of the hexosamine pathway, build-up of reactive oxygen species (ROS), and induction of hypoxia-inducible factor [49–51]. One of these mechanisms, non-enzymatic protein glycation, results in accelerated accumulation of advanced glycation endproducts (AGEs), which have been implicated in the loss of the retinal capillary's pericytes, but also lead to inflammation, oxidative stress and activation of vascular endothelial growth factor (VEGF) [50, 51]. VEGF is one of the most important players in retinopathy development, as it is the key driver behind neovascularization, the defining characteristic of proliferative diabetic retinopathy [1, 84]. Activation of hypoxia-inducible factor and protein kinase C, among other consequences, also increase VEGF expression [50], whereas the hexosamine pathway has been suggested to increase levels of AGEs [50], and ROS, besides causing

oxidative stress may also upregulate the other pathways previously mentioned [85].

Besides having effects on glycemic control, the diet may affect retinopathy development as an exogenous source of AGEs. AGEs are present in many foods, with animal-derived foods high in fat and protein being the largest contributors [86]. Uribarri et al., after analyzing the levels of two well-known AGEs, found that the highest amounts were found in beef and cheese, followed by poultry, pork, fish, and eggs. Though fruit and vegetables contribute less to the consumption of dietary AGEs, fruit still has high amounts of fructose, which is susceptible to react with proteins and form AGEs [87]. The method of food preparation also has an effect on the levels of AGEs, as longer exposure to higher temperatures and low moisture were found to increase AGE formation compared to shorter heating times, lower temperatures and high moisture [86]. A significant portion of the Western diet includes meats and dairy products, which are often exposed to high temperatures. In addition, high fructose corn syrup is frequently added to drinks and baked goods, explaining why the Mediterranean diet could be beneficial compared to a Western diet and offering dietary AGE restriction as a potentially critical target in preventing diabetic retinopathy [87, 88].

Fruit and vegetables may exert protective effects through their polyphenol content, as polyphenols have been shown to improve glucose homeostasis and insulin resistance, as well as to reduce inflammation [57]; alternatively, they could act through other antioxidants, such as vitamins C, E, or carotenoids, which have been observed to reduce neovascularization, restore retinal blood flow, and scavenge free radicals [89]. Vitamins C and E have been shown to suppress VEGF production in animal models and decrease AGE accumulation; in addition, vitamin C may also decrease protein kinase C activation [58], prevent glucose-driven apoptosis of pericytes [90], and reduce oxidative stress in human retinal pigment epithelium [91]. Diabetic animal models have also demonstrated beneficial effects of long-term antioxidant administration. Compared to the diabetic controls fed standard diets, diabetic rats fed diets supplemented with vitamins C and E, or with an antioxidant mixture, (including vitamins C, E, and  $\beta$ -carotene), developed significantly fewer acellular capillaries and pericyte ghosts, suggesting antioxidant supplementation may be efficient in inhibiting the development of retinopathy [92].

Fatty acids may affect retinopathy through several pathways. Firstly, just as the accumulation of glucose increases flux through the protein kinase C pathway, so does the accumulation of long-chain fatty acids [93]. Secondly, the retina is an extremely oxidative environment rich in PUFAs, and an accumulation of lipids can result in

lipid peroxidation and in advanced lipoxidation end products (ALEs), which are very similar to AGEs and can accumulate in tissues [51, 88, 93]. Both ALEs and AGEs exert part of their influence through the receptor for AGEs, which activates a sustained response of the proinflammatory transcription factor NF- $\kappa$ B, and will also diminish antioxidant defenses, depress the immune system, impair DNA repair, and increase both the build-up of toxins in tissues and the rate of infection [87, 88]. Evidence suggests that lipids may be as important as carbohydrates in the chemical modification of protein and accumulation of AGEs/ALEs [87].

Ethanol has been reported to be associated with decreases in glutathione levels [94], an important antioxidant whose depletion is also a result of increased flux through the polyol pathway [50, 51]. Glutathione may also increase lipid peroxidation and the generation of free radicals [94, 95]. On the other hand, green tea contains polyphenols that may scavenge ROS and exert anti-inflammatory effects [96].

## Conclusion

The diet is an important factor in the development of diabetes and is suspected to play a role in the development of retinopathy, yet relevant studies are limited. Many aspects of the diet have not yet been addressed and there remains an urgent need for prospective studies. Given that retinopathy affects a third of individuals with diabetes, it is important to further explore the role of the diet, in order to prevent complications in individuals already burdened with a chronic disease. A Mediterranean diet, as well its key components such as fruit, vegetables and fish, is believed to be beneficial in the prevention of diabetes and likely in the prevention of diabetic retinopathy, offering a promising path of dietary intervention that necessitates immediate attention for the prevention of diabetic retinopathy.

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## Compliance with ethical standards

**Conflict of interest** We have no conflicts of interest to declare.

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**Titre :** Dietary factors, type 2 diabetes and diabetic retinopathy

**Mots clés :** type 2 diabetes, retinopathy, diet, nutrition, fatty acids, lifestyle, cohort, epidemiology

**Abstract :** Type 2 diabetes (T2D) presents a significant health burden that is associated with many complications, such as diabetic retinopathy (DR), that further burden people with diabetes. Modifiable risk factors, such as the diet, have been identified for both T2D and DR; yet certain aspects of the role of the diet remain unclear. The main objectives of this thesis were therefore to examine the role and impact of the diet, and in particular, the consumption of fatty acids (FAs), and other modifiable behaviours on the risk of T2D and to summarize, interpret and analyze the relationship between the diet and DR using data from both the E3N and AusDiab cohort studies. The results suggest that the role of FAs on the risk of T2D and DR may differ between and within subgroups, and by individual

polyunsaturated fatty acids (PUFAs). The findings also suggest that strongly adhering to national dietary guidelines is not associated with the development of T2D, but strongly adhering to other recommendations for healthy behaviours (for waist circumference, physical activity and smoking) is strongly inversely associated with T2D. Modifiable behaviour could have prevented more than half of the cases of T2D. This work underlines the importance and the complexity of the role of the diet in the development of T2D and DR. It also illustrates the impact of healthy behaviour in the etiology of T2D and confirms that T2D is largely preventable. Efforts should focus on the modification of multiple healthy behaviours in populations, and promote diets that are moderate and widely varied.

**Titre :** Facteurs alimentaires, diabète de type 2, et rétinopathie diabétique

**Mots clés :** diabète de type 2, rétinopathie, alimentation, nutrition, acides gras, cohorte

**Résumé :** Le diabète de type 2 (DT2) constitue une pathologie majeure, au lourd fardeau, associée à de nombreuses complications, comme la rétinopathie diabétique (RD). Des facteurs modifiables, comme l'alimentation, ont déjà été identifiés pour le DT2 et la RD mais certains aspects de leurs rôles restent à préciser. Les objectifs de cette thèse étaient d'examiner le rôle de l'alimentation, en particulier la consommation d'acides gras (AGs), et des autres facteurs modifiables liés au mode de vie sur le risque de DT2 et de synthétiser, interpréter et analyser la relation entre l'alimentation et la RD. Les résultats suggèrent que le rôle des AGs sur le risque de DT2 et de la RD pourrait être différent selon leur type, et même varier au sein d'un groupe comme les AG polyinsaturés (AGPI). Les résultats suggèrent aussi qu'une forte adhésion aux recommandations alimentaires n'est pas

associée avec le développement d'un DT2, mais en revanche une forte adhérence aux autres recommandations de santé (concernant le tour de taille, l'activité physique et le statut tabagique) est fortement associée avec un moindre risque de DT2. On a montré qu'avoir un mode de vie sain aurait pu empêcher la survenue de plus de la moitié des cas de DT2. Cette thèse a permis de préciser l'importance et la complexité du rôle de l'alimentation dans le développement du DT2 et de la RD. Elle montre aussi l'impact des comportements sains dans la pathologie de DT2 et confirme que le DT2 est en grande partie, une maladie évitable. Les efforts devraient se focaliser sur la modification des comportements de santé à la fois dans la population générale et atteinte de DT2 et notamment encourager une alimentation modérée et variée.

