Dietary determinants, inflammation, and type 2 diabetes:

insights from observational studies

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Thesis

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Abstract

Background

Incidence of type 2 diabetes has rapidly increased during the last decades. It is a chronic disease caused by impaired insulin action and insulin secretion. Potentially, the majority of the new cases are due to changes in lifestyle, including unfavourable changes in diet. Lifestyle interventions promoting a healthy diet and physical activity indeed showed that diet has a role in the development of type 2 diabetes. However, firm conclusions about the role of most dietary factors and their association with type 2 diabetes cannot be drawn yet.

Evidence for an association between a dietary factor and type 2 diabetes is strengthened when a potential pathway is elucidated through which a dietary factor can be linked to type 2 diabetes. Chronic low-grade inflammation may be one of these pathways. Elevated concentrations of C-reactive protein (CRP) and pro-inflammatory cytokines, like TNF-α and IL-6, have been associated with a higher risk of type 2 diabetes, at least through a connection with overweight and abdominal obesity. Whether chronic low-grade inflammation is an intermediate in the association between dietary factors and risk of type 2 diabetes is not often studied so far.

Objectives

The first objective of this thesis was to study the role of selected dietary factors, i.e., fatty acids, fish, tea, meat, glycemic index (GI), and glycemic load (GL), on the development of type 2 diabetes in observational studies. The second objective was to study the extent to which chronic low-grade inflammation is a pathway through which diet can affect the processes leading to type 2 diabetes.

Methods

Data were used from several ongoing prospective cohort studies, i.e., CODAM study, Rotterdam study, EPIC-InterAct study, and Hoorn study. In these studies, information about diet was collected with food frequency questionnaires.

As a reflection of dietary fatty acid composition, the association between serum fatty acids in cholesteryl esters and glucose metabolism status was studied cross-sectionally in the CODAM study (n= 471). The prospective associations between fish (i.e., total, lean, fatty), meat (i.e., unprocessed red meat, processed red meat, poultry), GI, and GL and risk of type 2 diabetes were studied in the Rotterdam study (n= ≈4,400; $n_{\text{incident cases}}$= ≈460). The EPIC-InterAct case-cohort study was used to investigate the prospective association between intake of tea and risk of type 2 diabetes ($n_{\text{subcohort}}$= 16,154; $n_{\text{incident cases}}$= 11,541; eight European countries).

To investigate the second objective, the mediating role of CRP in the association between meat, GI, or GL and risk of type 2 diabetes was studied. Furthermore, the cross-sectional associations between a literature-based index that reflects the inflammatory potential of the diet, the Adapted Dietary Inflammatory Index (ADII), and markers of glucose metabolism were investigated in CODAM and Hoorn studies (n= 1,034). In the Rotterdam study, a dietary pattern that relates to CRP was constructed and related to risk of type 2 diabetes.

Results

Intake of lean fish (Relative Risk $(RR)_{\geq 23 \text{ vs. } 0 \text{ g/day}}$= 1.30 [95%Confindence Interval (95%CI) 1.01, 1.68]) and intake of processed meat ($RR_{>30 \text{ vs. } 0 \text{ g/day}}$= 1.73 [95%CI 1.16, 2.57]) were associated with a higher risk of type 2 diabetes. The intake of tea was associated with a lower risk of type 2 diabetes ($RR_{\geq 4 \text{ vs. } 0 \text{ cups/day}}$= 0.84 [95%CI 0.81, 1.00]). No statistically significant associations were

observed for the other dietary factors, i.e., proportions of saturated, mono-unsaturated, trans, and poly-unsaturated fatty acids in cholesteryl esters, intake of fatty fish, intake of red meat, intake of poultry, GI, and GL. Our findings showed that the mediating role of CRP in the association between intake of meat, GI, or GL and risk of type 2 diabetes was small. However, the total dietary inflammatory potential of the diet, as estimated by ADII and a pro-inflammatory dietary pattern, were associated with insulin resistance or risk of type 2 diabetes, respectively.

Conclusion

The findings in this thesis together with results from other studies indicate that high intake of tea and low intake of processed meat can help lower the risk of type 2 diabetes. The findings also suggest that some diets can affect the development of type 2 diabetes through harmful effects on chronic low-grade inflammation. Which combinations of dietary factors cause the pro-inflammatory properties of these diets remains to be determined.

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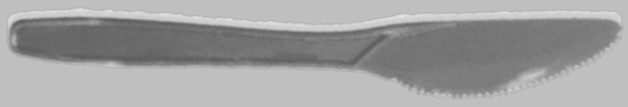
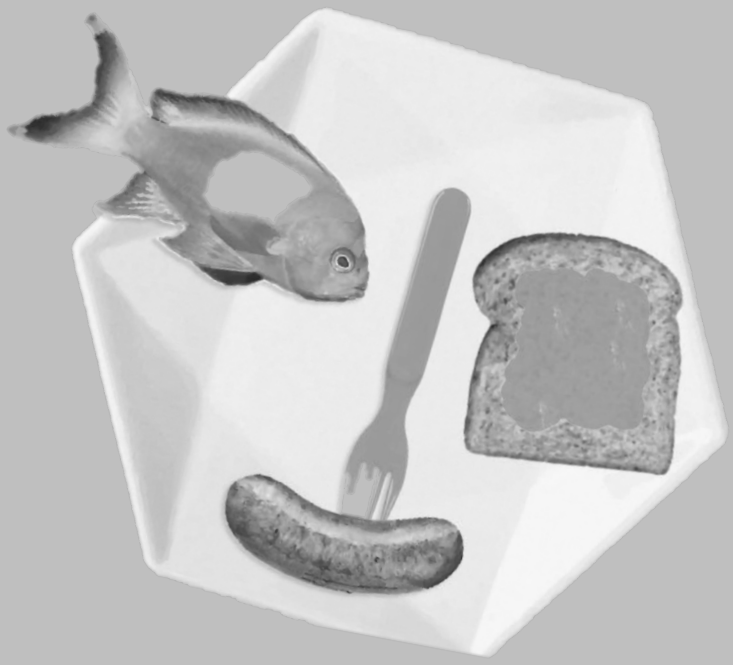
CHAPTER 1



General introduction



GJ van Woudenbergh

Outline of the introduction

This chapter introduces the context of this thesis in four parts. In *part I*, the clinical perspective and features of type 2 diabetes are described. *Part II* elaborates on the risk factors of type 2 diabetes. *Part III* highlights the dietary factors that are studied in this thesis. *Part VI* provides the objectives and outline of this thesis.

PART I: TYPE 2 DIABETES

I.1 Clinical perspective of diabetes mellitus

Diabetes mellitus was already recognized as a disease since ancient times. A papyrus document dated from 1550 BC already contained descriptions for the treatment of excessive urination.[1] Excessive urination, unusual thirst, and extreme hunger are all clinical symptoms of diabetes.[2] Aretaeus (130-200 AC) was the first who used the term diabetes in conjunction with these symptoms. His description of diabetes began with the words: *"Diabetes is a wonderful affection, not very frequent among men,……"*.[1] Nowadays, diabetes is much more frequent than at that time.

In 2000, the global prevalence of diabetes was around 171 million (2.8%).[3] This number is expected to increase until 366 million (4.4%) in 2030. In the Netherlands the prevalence of diabetes was shown to be over 800.000 in 2011 (4.8% of the total population).[4] If current trends prevail, this number is expected to increase until 1.32 million in 2025 (7.9% of the total population).[5] These prevalence numbers do not discriminate between types of diabetes, of which type 1 and type 2 are the most well-known. Type 2 diabetes counts for 90% of all diabetes cases worldwide, which indicates that prevalence numbers mainly reflect this type of diabetes.[6] Type 2 diabetes is associated with severe micro-vascular complications, like loss of vision, and macro-vascular complications, like myocardial infarction and stroke, resulting in a life-expectancy of 5 to 10 years shorter compared with persons from the general population.[7]

The prevalence of type 2 diabetes is increasing worldwide.

I.2 Features of type 2 diabetes

Glucose is an important source of energy and is used in various biosynthetic reactions in the body. Glucose concentration normally varies between 4.0 and 7.0 mmol/L and is maintained at tight control by the body.[8] In type 2 diabetes this tight control is lost, resulting in an abnormal glucose concentration. Therefore, fasting plasma glucose (FPG) concentration can be measured or an oral glucose tolerance test (OGTT) can be performed to identify persons with type 2 diabetes and persons with early glucose concentration abnormalities, i.e., with impaired fasting glucose (IFG) or with impaired glucose tolerance (IGT). Measuring FPG or performing an OGTT is especially important to identify persons who have glucose abnormalities, but do not present any clinical symptoms of type 2 diabetes, e.g., excessive urination, unusual thirst, and extreme hunger, yet.[2] An OGTT includes at least one measurement of glucose concentration over an overnight fast and one measurement of glucose concentration two hours after exposure to a 75-gram glucose drink. After measuring FPG or/and 2-hour glucose concentrations, the widely accepted classification system of the World Health Organisation (WHO) can be used to classify persons as having IFG, IGT, or type 2 diabetes (**Table 1.1**).[9] A person is classified as having type 2 diabetes when venous FPG is ≥7.0 mmol/L or 2-hour glucose concentration is ≥11.1 mmol/L.

Table 1.1 Criteria for the diagnosis of abnormal glucose concentration according to the World Health Organisation 2006 report[9]

Type 2 Diabetes		
Fasting glucose (mmol/L)[a]	OR	≥7.0
2-hour glucose (mmol/L)[a,b]		≥11.1
Impaired Glucose Tolerance (IGT)		
Fasting glucose (mmol/L)[a]	AND	<7.0
2-hour glucose (mmol/L)[a,b]		≥7.8-<11.1
Impaired Fasting Glucose (IFG)		
Fasting glucose (mmol/L)[a]	AND[c]	≥6.1-<7.0
2-hour glucose (mmol/L)[a,b]		<7.8

[a] Venous plasma glucose concentration.

[b] Measured two hours after exposure to a 75-gram oral glucose load.

[c] If only fasting glucose concentration is measured, IGT cannot be excluded.

The abnormal glucose concentration in persons with type 2 diabetes results from impaired insulin *action* due to insulin resistance of peripheral tissues, like liver and skeletal tissue, and impaired insulin *secretion* due to beta-cell dysfunction (**Figure 1.1**).

In a state of impaired insulin *action*, a normal or elevated insulin concentration produces an impaired biological response.[10] This includes a diminished ability of the cells to take up glucose from the blood stream. In persons with normal glucose metabolism, a release of insulin by the pancreas into the blood stream follows when blood glucose concentration increase. When released, insulin can bind on cell membranes to the insulin receptor at distance targets. Subsequently, the intra-cellular insulin-signalling cascade starts.[8] The cellular substrates of the insulin receptor (IRS1, IRS2, IRS3, IRS4, Cbl, APS, isoforms of Shc, Gab-1, $p60^{dok}$) are involved in the de-phosphorylation and phosphorylation of proteins, resulting in altered gene transcription rates, changes in activity of proteins, and translocation of GLUT glucose transporters to the cell-membrane.[8] As a result, uptake of glucose, fatty acids, and amino acids is stimulated and the degradation and release into the circulation of those nutrients are inhibited.[8] So, impaired insulin action results in changes in glucose, fat, and protein metabolism and could be due to defects in the insulin receptor, signal transduction, expression of GLUT-transporters, functional activity of the GLUT-transporters, and translocation of GLUT-transporters to the cell membrane.[11] To quantify impaired insulin action, a hyperinsulinemic euglycemic glucose clamp can be used as direct measure of steady state glucose homeostasis after an insulin stimulus.[12] As a clamp is time and labour intensive, surrogate measures of insulin resistance, like QUICKI, HOMA2-IR, and Matsuda index, can be used to estimate insulin resistance using fasting insulin concentrations and FPG and/or 2-hour glucose concentrations.[12]

In state of impaired insulin *secretion*, insulin secretion pattern is altered, the beta-cell mass of the Islets of Langerhans where insulin is synthesized is reduced, or both.[13] Altered insulin secretion pattern involves among others a disrupted pulsatile insulin release pattern and a decreased cleavage of pro-insulin to insulin.[13] Oxidative stress plays a major role in insulin secretion pattern alteration and beta-cell death.[13] In a state of oxidative stress, there is an imbalance between production and destruction of reactive oxidant species.[14] Eventually, this leads to cellular damage and cell death. Oxidative stress can be caused by high exposure to

glucose and free fatty acids.[15] Both can also contribute to impaired insulin secretion by other mechanisms, e.g., influencing gene expression involved in beta-cell death regulation.[13] As such, prolonged exposure to glucose and free fatty acids are detrimental for beta-cell functioning.

In general, the body initially compensates impaired insulin action by increasing insulin release to maintain glucose concentration at tight control. Deterioration of impaired insulin *action* and impaired insulin *secretion*, however, eventually results in an abnormally high glucose concentration, i.e., hyperglycemia.

Hyperglycemia often occurs in concert with dyslipidemia, i.e., high cholesterol, high triglycerides, and low high-density lipoprotein (HDL)-cholesterol concentrations, and hyperinsulinemia, i.e., high insulin concentration. Besides several other effects, hyperglycemia, dyslipidemia, and hyperinsulinemia may also cause chronic low-grade inflammation (**Figure 1.1**).[15] In this thesis, the role of chronic low-grade inflammation in the association between diet and type 2 diabetes is addressed. Therefore, a separate paragraph on the role of chronic low-grade inflammation in type 2 diabetes follows here.

The main feature of type 2 diabetes is an abnormal glucose concentration, resulting from impaired insulin action due to insulin resistance of peripheral tissues and impaired insulin secretion due to beta-cell dysfunction.

I.3 Chronic low-grade inflammation and type 2 diabetes

Inflammation can be classified as acute, chronic high-grade, or chronic low-grade inflammation.[17] Acute inflammation is essential for survival, because it initiates pathogen killing, initiates tissue repair processes, and helps to restore homeostasis after infection or tissue damage.[18] Generally, acute inflammatory responses are short-term responses. When the inflammatory processes fail to regulate themselves, host tissue becomes damaged. This is known as chronic inflammation that can be subdivided in chronic high-grade inflammation and chronic low-grade inflammation. It is classified as chronic high-grade inflammation when disturbed inflammatory processes lead to overt clinical manifestations as for example in rheumatoid arthritis.[17] When clinical manifestations are minimal or absent, it is classified as chronic low-grade inflammation.[17] Chronic low-grade inflammation is characterized by slightly elevated blood concentrations of acute-phase proteins, cytokines, and mediators with endothelial activation capacity that are involved in acute inflammation as well.[17]

It is likely that dysfunction of adipose tissue is a major contributor to chronic low-grade inflammation.[19] Adipose tissue dysfunction is characterized by a reduced capacity to store dietary lipids and an impaired endogenous lipolysis, leading to lipid overflow and ectopic fat accumulation, which has been linked to the development of insulin resistance.[20] Adipose tissue is not only a depot for lipid storage, but also has endocrine functions including secretion of pro-inflammatory and anti-inflammatory markers. Therefore, deviation from normal adipose tissue functioning plays a central role in the development of chronic low-grade inflammation. A larger size of mature adipocytes, as observed in persons with overweight, relate among others to an higher secretion of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) and a lower secretion of the anti-inflammatory adipokine adiponectin.[21] Besides secretion of cytokines by the adipocytes themselves, macrophages that infiltrate the obese adipose tissue can also secrete cytokines.[22] Being secreted, these pro-inflammatory cytokines can have autocrine and paracrine effects at the site of the adipose tissue.[23] Furthermore, these cytokines can be transported via the blood stream to act on distant targets, like the skeletal muscle and liver.

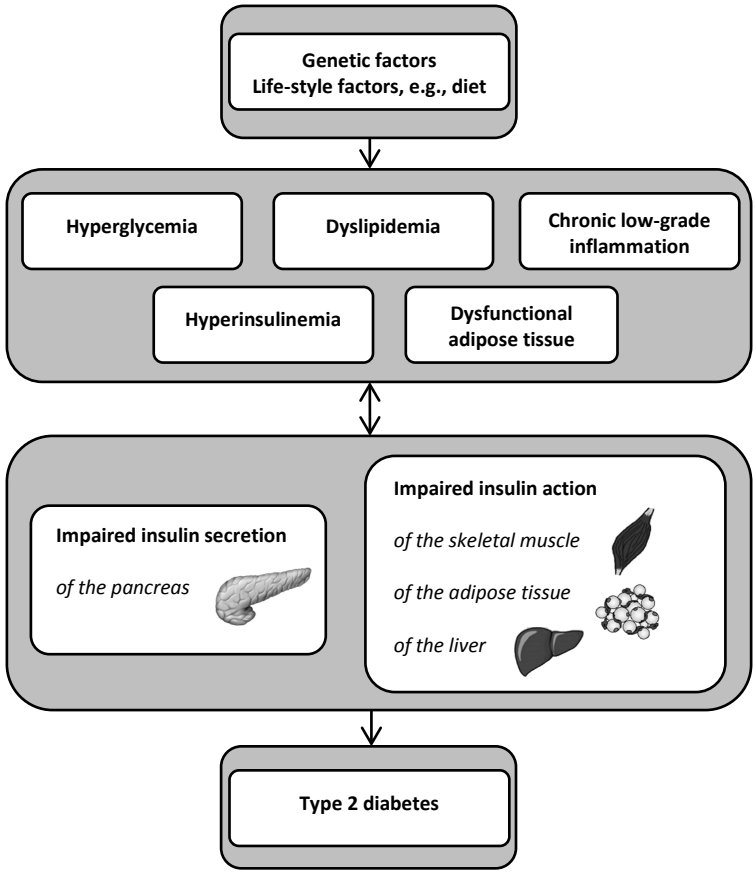


Figure 1.1 Overview of the features of type 2 diabetes (pictures reprinted with permission[16])

Besides adipose tissue, hyperglycemia itself can contribute to chronic-low grade inflammation. Hyperglycemia can stimulate the production of reactive oxygen species, that, in turn, stimulate production of pro-inflammatory cytokines, like TNF-α and IL-6.[24] Insulin, however, could counterbalance the pro-inflammatory effect of glucose by suppressing the production of the pro-inflammatory cytokines and by activating the production of anti-inflammatory cytokines, like interleukin-4 and interleukin-10.[24]

Several mechanisms have been proposed through which inflammation markers can contribute to insulin resistance. For example, TNF-α can affect the insulin signalling cascade by phosphorylation of the insulin receptor, insulin receptor substrate, and glucose transporter, can suppress expression of genes encoding for adiponectin, and can increase the expression of genes encoding for IL-6.[25] TNF-α and IL-6 also enhance oxidative stress by stimulation NF-kB or NADPH oxidase.[24] NF-kB causes a transcriptional response of genes involved in inflammatory processes.[26] A high concentration of IL-6 stimulates the production of acute-phase protein C-reactive protein (CRP) in the liver.[27] CRP is a non-specific inflammation marker that may contribute to insulin resistance by increasing phosphorylation of IRS[26] and by increasing the synthesis of cytokines like TNF-α and IL-6.[28]

In line with the proposed mechanisms, several prospective studies observed associations

between slightly elevated concentrations of the inflammation markers CRP, TNF-α, and IL-6 and type 2 diabetes.[29-33] Furthermore, some prospective cohort studies showed that participants with a polymorphism associated with higher CRP, TNF-α, or IL-6 concentration had a higher risk of type 2 diabetes.[34-37]

Taken together, this suggests that chronic low-grade inflammation may precede the development of type 2 diabetes.

Type 2 diabetes is accompanied with dysfunctional inflammatory processes.

PART II: RISK FACTORS OF TYPE 2 DIABETES

Type 2 diabetes is a multi-factorial disease and caused by genetic as well as lifestyle factors. Several genetic variants that are associated with higher risk type 2 diabetes have been identified, but to date their overall contribution to the development of the disease appear to be modest (5-10%).[38] The mechanisms of action of these genes are related to beta-cell function, beta-cell development, or interaction with lifestyle factors, but most of the mechanisms are still unknown.[39]

In 2003 the World Health Organisation (WHO) and the Food and Agriculture organisation (FAO) published a report in which the strength of evidence on lifestyle factors and risk of type 2 diabetes was described (**Table 1.2**).[40]

The risk factors overweight, physical inactivity, and maternal diabetes appeared to be the major risk factors for type 2 diabetes.[41] Body mass index (BMI) is often used in epidemiological studies to define the degree of overweight: a BMI 25-≤30 kg/m^2 is defined as being overweight and a BMI ≥30 kg/m^2 is defined as being obese.[42] The risk of type 2 diabetes is generally three times higher when being overweight and seven times higher when being obese compared with persons with a normal weight.[43] Besides maintaining a normal weight, physical activity also lowers risk of type 2 diabetes independent of body weight.[44, 45] Women with gestational diabetes have a seven times higher risk for developing type 2 diabetes compared with women without gestational diabetes.[46] Although not included in the WHO/FAO table, we also considered current smoking as a modifiable risk factor because a meta-analysis on prospective cohort studies showed that current smoking was associated with a 44% higher risk of type 2 diabetes compared with non-smokers.[47]

For dietary factors, the evidence was considered less convincing in the WHO/FAO report. Dietary factors for which the evidence was regarded as 'probable' or 'possible' include a low

Table 1.2 Strength of evidence on lifestyle factors and risk of developing type 2 diabetes (adapted from FAO/WHO report, 2003)[40]

Evidence	Decreased risk	Increased risk
Convincing	Physical activity	Physical inactivity
	Voluntary weight loss	Overweight and obesity
	in overweight and obese people	Abdominal obesity
		Maternal diabetes
Probable	Non-starch polysaccharides	Saturated fats
Possible	*n3* poly-unsaturated fatty acids	Total fat intake
	Low glycaemia index foods	Trans fatty acids
	Exclusive breastfeeding	
Insufficient	Moderate alcohol	Excess alcohol
	Chromium	
	Magnesium	
	Vitamin E	

intake of non-starch polysaccharides, high intake of saturated fatty acids, high intake of trans fatty acids, high intake of total fat, low intake *n3* poly-unsaturated fatty acids, and a high glycemic index of the diet (**Table 1.2**).[41] The strength of evidence was considered 'insufficient' for alcohol, chromium, magnesium, and vitamin E. The strength of evidence for other dietary factors, e.g., intake of food groups, was not reported.

Considering the lifestyle factors listed by the WHO/FAO, lifestyle interventions promoting weight loss, physical activity, and a healthy diet should reduce the risk of developing type 2 diabetes. Indeed, six out of the seven human intervention studies among persons with impaired glucose tolerance showed that a lifestyle intervention reduced the risk of type 2 diabetes by up to 60%.[48, 49] In the Diabetes Prevention Program, an intensive lifestyle intervention even appeared to be more effective than the insulin-sensitizer drug metformin after 10-years of follow-up.[50, 51]

Lifestyle changes, including changes in diet, are important in the prevention of type 2 diabetes. In order to strengthen the evidence-base for dietary risk factors for type 2 diabetes, the main objective of this thesis is to study the association between selected dietary factors and type 2 diabetes.

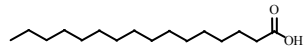
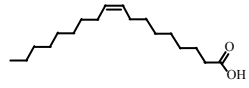
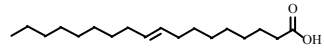
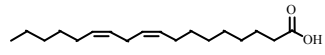
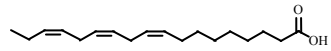
PART III: DIETARY FACTORS STUDIED IN THIS THESIS AND TYPE 2 DIABETES

The following paragraphs elaborate on the selected dietary factors, i.e., fatty acids, fish, tea, meat, glycemic index (GI), and glycemic load (GL), that were studied. Where appropriate it is explained why the mediating role of chronic low-grade inflammation was considered. Besides the individual dietary factors, the dietary patterns, as measures of the total diet, that were studied, are also elaborated on.

III.1 Fatty acids and type 2 diabetes

Fatty acids in the diet can be classified as saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), and poly-unsaturated fatty acids (PUFA) (**Table 1.2**). This classification is based on the number of double bonds in the carbon backbone: SFA contain no double bond, MUFA

Table 1.2 Characteristics of fatty acids

Type	Bond	Example	
SFA	No bond		Palmitic (C16:0)
MUFA	1 bond in cis configuration		Oleic (C18:*1n9c*)
TFA	≥1 bond in trans configuration		Elaidic (C18:*1n9t*)
PUFA			
n6 PUFA	>1 bond; 1st double bond at 6th carbon atom		Linoleic (C18:2*n6*)
n3 PUFA	>1 bond ; 1st double bond at 3rd carbon atom		Alpha-linolenic (C18:3*n3*)

Abbreviations: SFA=saturated fatty acids; MUFA=mono-unsaturated fatty acids; TFA=trans fatty acids; PUFA=poly-unsaturated fatty acids

contain one double bond, PUFA contain more than one double bonds.[52] Trans fatty acids (TFA) are either MUFA or PUFA that contain a hydrogen that are oriented opposite to the other hydrogen at the double bond.[52] PUFA can be further classified as *n3* and *n6* PUFA.

Several fatty acids can be synthesized within the body from their fatty acid family precursor after ingestion (**Figure 1.2**). The insertion of a double bond is catalysed by desaturases and the lengthening of the carbon backbone with two carbons at a time is catalysed by elongases.[52] Fatty acids in the body, therefore, reflect dietary intake as well as endogenous synthesis. Fatty acids are present in the body in free triglycerides, in cholesteryl esters, in phospholipids, in erythrocytes, and in adipose tissue. All compartments are related to another time-frame of dietary fat intake: fatty acids present in triglycerides relate to intake over the past few hours, fatty acids present in cholesteryl esters and phospholipids relate to intake over the last week, fatty acids present in erythrocytes relate to intake over the last month, and fatty acids present in adipocytes relate to the intake over one to two years.[53] As self-reported intake of fat is prone to underreporting especially in overweight persons[54], measuring fatty acids in the body compartments is an alternative to self-reported measures of fat intake. Adipose tissue is the right compartment to measure objectively long-term intake of fatty acids, because of the slow turnover of fatty acids in adipose tissue.[55] Under the assumption that short-term extreme changes in diet did not happen, circulating fatty acids in cholesteryl esters or phospholipids can also be used.[55] These circulating fatty acids are most often used in observational studies, because of its accessibility.[55] In general, the highest correlation between intake of fatty acids and circulating fatty acids in the blood have been observed for PUFA (ranged from 0.20 up to 0.50), whereas no correlation is observed for MUFA.[56-61]

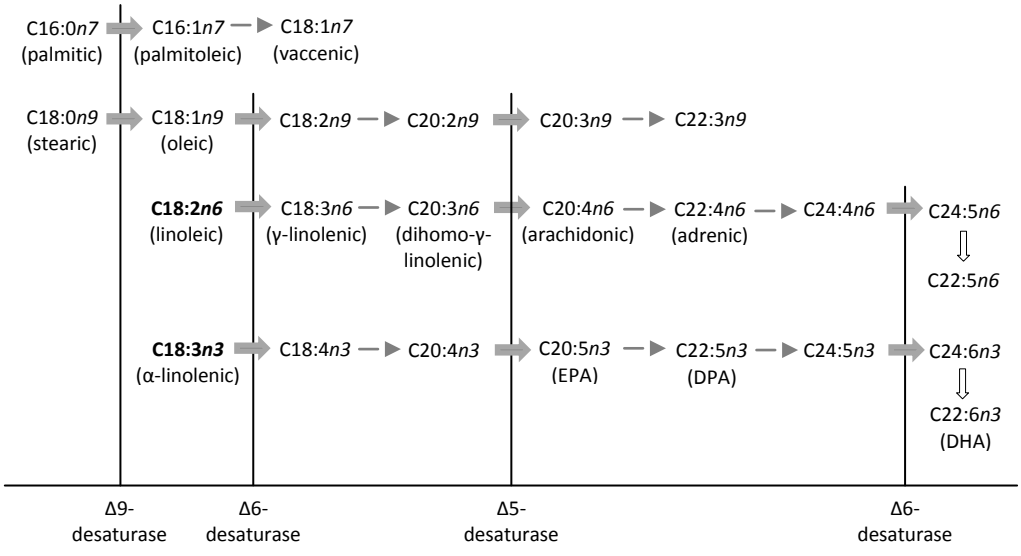


Figure 1.2 This schematic figure shows the fatty acid synthesis in the body (adapted from Warensjo *et al*.[85]). ⇒ represents desaturation; —▶ represents chain elongation; ⇩ represents beta-oxidation. Fatty acids in bold font are essential fatty acids that cannot be synthesized by the body itself. Abbreviations: EPA=eicopentaenoic acid; DPA=docosapentaenoic acid; DHA=docosahexaenoic acid.

Observational studies suggest that the type of fat, rather than total fat, is important with regard to diabetes risk.[62] Total fat, however, may be associated with the development of obesity, and as such indirectly contributes to the development of type 2 diabetes.[63] Concerning type of fat, high intake of SFA and TFA may adversely be associated with type 2 diabetes, whereas high intake of PUFA may be beneficial.[62, 64] Potential mechanisms by which fat can impact glucose metabolism include alteration of cell membrane functioning, regulation of gene expression, regulation of enzyme involved in glucose metabolism, and influencing inflammation status.[64] The effects of fatty acids types on these mechanisms could be opposite, e.g., *n3* PUFA have anti-inflammatory properties, whereas SFA have pro-inflammatory properties.[64, 65]

In line with the findings from self-reported intake of fat, investigations into the association of fatty acids in cholesteryl esters or phospholipids with type 2 diabetes suggest that a high proportion of palmitic acid (C16:0) and a low proportion of linoleic acid (C18:2*n6*) are associated adversely with type 2 diabetes.[64] An association between some other fatty acids and desaturase activities with type 2 diabetes may exist, but the evidence for an association is limited or contradictory.[66-84]

As self-reported intake of fat is prone to underreporting, fatty acid profiles in cholesteryl esters or phospholipids can be used as alternative. Although several studies already investigated the association between circulating fatty acids profiles and type 2 diabetes, too few studies are available to draw firm conclusions for most fatty acids. Therefore, the fatty acid profile in cholesteryl esters was examined in relation to glucose metabolism status (chapter 2).

III.2 Fish intake and type 2 diabetes

Fish can be classified as lean fish (e.g., cod, plaice) and fatty fish (e.g., herring, mackerel, eel). Lean fish has a high liver triglyceride content, whereas fatty fish mainly stores fat as triglycerides in the muscle. In the Netherlands, total fish consumption was estimated to be on average 70 gram per week in 1998, which was below the recommended intake of about 200 to 300 gram per week.[86, 87]

The first speculation about a potential association between intake of fish and type 2 diabetes resulted from the observations that Arctic populations with a very high intake of fish and other marine food products had a lower incidence of type 2 diabetes.[88, 89] The first prospective study which investigated the association between intake of fish and risk of type 2 diabetes supported an inverse association: eating fish was associated with a 53% lower risk of IGT or type 2 diabetes compared with participants who did not eat fish.[90] A subsequent prospective study showed that increased intake of fish was associated with lower 2-hour glucose concentration after 20 years of follow-up in men participating in the Finnish and Dutch cohorts of the Seven Countries study.[91] In contrast, in the Nurses' Health Study II women who ate at least two portions of fish per week did not have a lower risk of type 2 diabetes compared with women who ate less than one portion of fish per week.[92] Cross-sectional studies emerging at the same time showed either inverse[93, 94], no[95, 96], or positive associations[97] between habitual intake of fish and glycemic status.

Taken together, evidence from observational studies is inconsistent. This may be due to the underlying type of fish consumed, as the proposed active components in term of diabetes risk can differ among types of fish. Of the active components, *n3* PUFA and vitamin D likely have anti-diabetic properties, selenium and protein may have anti- and pro-diabetic properties, and contaminants have pro-diabetic properties. For each of these components, potential mechanisms by which these components may affect risk of type 2 diabetes are described below.

Eicosapentaenoic acid & docosahexaenoic acid present in fish

Of the *n3* PUFA provided by fish intake, eicosapentaenoic acid (EPA, C20:5*n3*) and docosahexaenoic acid (DHA, C22:6*n3*) are the most abundant ones. These fatty acids are characterized by several double bonds in their fatty acid chain, of which the first is located at the third carbon atom from the methyl end of the carbon chain (**Table 1.2**). EPA and DHA are considered semi-essential fatty acids, because the body can synthesize only small amounts of the *n3* PUFA alpha-linoleic acid to EPA and even smaller amounts are further transformed to DHA.[98] If beneficial in terms of diabetes risk, therefore, sufficient intake of EPA and DHA is important. As could be expected from the name, fatty fish is a richer source of EPA and DHA than lean fish.[99] For example, mackerel contains about 1.8-5.2 gram *n3* PUFA per 100 gram and herring about 1.2-3.1 gram *n3* PUFA per 100 gram, while the lean fishes cod and plaice provide about 0.2 gram *n3* PUFA per 100 gram.[99] So, the mechanisms by which EPA and DHA may affect diabetes risk are likely to be more abundant for fatty fish than for lean fish.

What are these mechanisms? First, EPA and DHA have anti-inflammatory properties.[65] Second, EPA and DHA stimulate production and secretion of adiponectin and leptin that both can improve insulin sensitivity.[65] Third, EPA and DHA regulate the activation of genes related to fatty acid oxidation and fatty acid synthesis.[65] Hepatic insulin sensitivity could be preserved by increasing fatty acid oxidation and fatty acid synthesis, although this oxidation may also increase gluconeogenesis from glycerol in the liver.[100] Fourth, a higher quantity of EPA and DHA in the phospholipid cell membranes bilayer could increase insulin sensitivity.[101] So, in principal, EPA and DHA could have a beneficial effect on the development of type 2 diabetes. However, a beneficial effect of EPA and DHA may be less than expected from the four pathways described, as EPA and DHA may also increase glucose concentrations by lowering glucose utilization and increasing glucagon-stimulated C-peptide.[102]

Vitamin D present in fish

Fish also contributes to the intake of vitamin D. As vitamin D is a fat-soluble vitamin, especially fatty fish is a rich source of this vitamin. For example, raw herring contains about 19 µg vitamin D per 100 gram and smoked mackerel about 8 µg vitamin D per 100 gram.[103] The vitamin D content of the lean fishes cod and plaice is not higher than 2 µg per 100 gram.[103] Thus, as for EPA and DHA, the mechanisms by which vitamin D may affect diabetes risk are likely to be more abundant for fatty fish than for lean fish.

What potential mechanisms are involved? First, vitamin D may have a beneficial effect on insulin secretion by a variety of mechanisms, including regulation of calcium homeostasis.[104] Second, vitamin D may have a beneficial effect on insulin resistance, among others through stimulating the expression of the insulin receptor.[104] Third, vitamin D may express anti-inflammatory properties as among others vitamin D can down-regulate the activation of NF-kB.[104]

Thus, a potential beneficial effect of fish may attributable to the vitamin D content.

Selenium present in fish

The selenium content of fish varies from about 20 up to about 45 µg per 100 gram.[103] No obvious difference in selenium content is observed between fatty fish and lean fish. For example, raw herring contains about 31 µg selenium per 100 gram and cod about 36 µg per 100 gram.[103]

The role of selenium in the development of type 2 diabetes is controversial. A prospective study including 623 French men showed that plasma selenium concentration was associated with lower risk of developing IFG or type 2 diabetes after nine years of follow-up[105], whereas it was positively associated with FPG concentration in another prospective study after 7.5 years of

follow-up.[106] Case-control and cross-sectional studies showed that a high toenail or serum selenium concentration was associated with lower prevalence of diabetes[107-110] In a randomized, double-blind, placebo-controlled intervention study, which included 1,202 dermatology patients, selenium supplementation increased risk of diabetes.[111] In another randomized, double-blind, placebo-controlled intervention study, which included 35,533 American men, selenium supplementation tended to increase risk of diabetes.[112]

Thus, whether selenium is associated with either a higher or lower risk of type 2 diabetes is not clear. A potential inverse effect could be ascribed to the anti-oxidant capacity or anti-diabetic insulin-like actions of selenium, whereas the adverse effect could be ascribed to pro-diabetic activities of the selenoproteins, like glutathione peroxidases x1 (GPx1) and selenoprotein P1 (SEPP1).[113, 114]

Protein present in fish

Fish also contributes to the intake of protein. In general, no obvious difference in protein content between fatty fish and lean fish is observed, e.g., herring contains about 17 gram protein per 100 gram and cod about 21 gram protein per 100 gram.[103] Fish protein is a source of essential amino acids of which leucine and lysine are the most abundant ones.[115]

Potential mechanisms behind the role of protein in the development of type 2 diabetes are contradictory. On one hand, hyperglycemia can be prevented as protein may stimulate insulin secretion.[116] Furthermore, an animal study that compared the quality of protein, i.e., cod protein versus casein protein, showed that cod protein may stimulate glucose uptake in the muscle at least by improving the translocation of the glucose transporters.[116] On the other hand, protein may impair insulin action by inhibiting glucose transport.[117] Furthermore, protein can be used to produce glucose, leading to hyperglycemia without efficient secretion of insulin.

Besides the effect of total protein, potential mechanisms by which the specific abundant amino acids leucine and lysine may affect the development of type 2 diabetes have also been suggested. Leucine might stimulate the translocation of the glucose transporters to the cell membrane and glycogen synthesis in the muscle, but leucine might impair glucose uptake as well.[116] Lysine may inhibit the binding of glucose with an aldehyde group or ketone group to a free amino group, thereby limiting the formation advanced glycation end products (AGEs).[118] As AGEs have pro-inflammatory properties[119, 120], lysine might be beneficial.

Thus, whether dietary protein is associated either inversely or adversely with the development of type 2 diabetes is not clear.

Contaminants present in fish

Fish can contain contaminants, like p,p'-Dichloro-Diphenyl-Trichloroethane (p,p'-DDE), polychlorinated biphenyls (PCB's), and mercury.

Persistent organic pollutants, like p,p'-DDE and PCB's, are man-made components that can accumulate in adipose tissue of fish. Estimations about the PCB's concentration in fish differ between publications, e.g., farmed salmon contains about 25-30 ng/g PCB's and tuna about 26-70 ng/g.[121, 122] The results from prospective studies on PCB's and p,p'-DDE and the development of type 2 diabetes are inconsistent: two observed an adverse association for p,p'-DDE blood concentrations, but no association for PCB's blood concentrations[123, 124], whereas two other studies observed an adverse association for PCB's, but no association for p,p'-DDE.[125, 126] These persistent organic pollutants may affect the development of type 2 diabetes by disrupting beta-cell function.[127]

Fish is an important source of mercury[128], although dependent on the type of fish, e.g.,

tuna 0.24 µg/g, cod 0.10 µg/g, herring <0.05 µg/g.[121] Mercury exposure was associated with higher risk of type 2 diabetes in a recent cohort study.[129] As mercury may cause beta-cell dysfunction through oxidative pathways, this may explain the higher risk of type 2 diabetes.[128]

Thus, contaminants present in fish show pro-diabetic properties.

Observational studies on intake of fish and the development of type 2 diabetes are inconsistent. This may be due to the underlying type of fish, as the active compounds differ between categories of fish. Active compounds showed anti-diabetic properties, pro-diabetic properties, or both. Therefore, the association between intake of total, lean, and fatty fish and risk of type 2 diabetes was studied (chapter 3).

III.3 Tea intake and type 2 diabetes

According to a legend, tea was discovered by the Chinese emperor Shen Nung in 2,737 BC.[130] He was drinking boiled water, when some leaves from the *Camellia Sinensis* plant were blown into this cup. He did not throw the water away, but decided to drink the water without removing the leaves: tea was discovered.

Nowadays, several types of tea are on the market, i.e., green, white, oolong, black, and herbal teas. The categorization as green, white, oolong, and black depends on the oxidation and fermentation processes of the leaves of the plant *Camellia Sinensis.* Herbal teas, e.g., red bush tea, are all teas that are not derived from the *Camellia Sinensis* plant. Due to the different oxidation processes and different sources, types of tea have a different flavonoid content, flavonoids being a group of polyphenols.[131] Catechins, theaflavins, and thearubigins are the most prominent flavonoids in tea. These flavonoids, predominately epigallocatechin gallate (EGCG), have been shown to slow down carbohydrate digestion, to inhibit carbohydrate absorption by competitively binding with the sodium-glucose transporter-1 (SGLT-1), to increase glucose uptake in muscle and fat cells by changes in GLUT-4 expression, to enhance insulin secretion, and to protect beta-cells from free-radical damage.[132, 133]

All these pathways can affect glucose concentrations and, therefore, intake of tea is proposed to have a beneficial effect on development of type 2 diabetes. Indeed, two meta-analyses on prospective studies support a beneficial effect of drinking at least 3 cups of tea per day.[134, 135] Most results from long-term randomized human intervention studies that focussed on the effect of drinking tea on markers of glucose metabolism, however, did not favour a beneficial effect of tea.[136-142] Among others, the absence of an association in human intervention studies may be due to the dose provided, as a potential non-linear association between intake of tea and type 2 diabetes was suggested by one of the meta-analyses of prospective cohort studies.[135] In this meta-analysis, drinking at least 4 cups of tea per day was associated with a 20% lower risk, whereas drinking >0-<1 or ≥1–3 cups per day did not lower the risk of diabetes compared with non-tea drinkers.[135]

Prospective studies suggest a beneficial effect of drinking tea on risk of type 2 diabetes. As a beneficial of tea may be restricted to people with high tea consumption, a potential non-linear association between intake of tea and risk of type 2 diabetes was studied (chapter 4).

III.4 Meat intake, inflammation, and type 2 diabetes

Based on differences in food composition, meat can be classified as red meat (e.g., steak, pork fricandeau, lamb chop, bacon, sausage) and poultry (e.g., chicken, turkey). The heme iron content is the main dietary component that differs between red meat and poultry. Iron in the

body can cause oxidative stress, may contribute to insulin resistance of the adipose tissue and liver, and may increase free fatty acid oxidation in the muscle.[143] As such, risk of type 2 diabetes could be different between red meat and poultry. Prospective studies that are available suggest indeed that poultry is not or even inversely, associated with risk of type 2 diabetes[92, 144-148], whereas especially processed red meat was related to a higher risk of type 2 diabetes.[149-151] Red meat is further subdivided in processed red meat, e.g., bacon, sausage, and unprocessed red meat, e.g., steak, pork fricandeau, lamb, chop. Salt, nitrite, nitrosamines, and advanced glycation end products (AGEs) are the most obvious dietary components that differ between processed red meat and unprocessed red meat.[152] High intake of salt, nitrite, nitrosamines, and AGEs may adversely affect the development of type 2 diabetes.[152]

These adverse effects may be partly mediated by chronic low-grade inflammation, because salt, nitrites, nitrosamines, and AGEs may have pro-inflammatory properties. In vitro studies showed that salt can stimulated cytokine synthesis.[153-155] Nitrites can be reduced to nitric oxide ($NO^{\cdot}$) that may act as pro-inflammatory agent.[156, 157] The irreversible yellow-brown AGE structures can generate reactive oxygen species, leading to inflammatory responses.[119, 120] These AGE structures results from a cascade of non-enzymatic reactions caused by the binding of glucose with an aldehyde group or ketone group to a free amino group, lipid, or nucleic acid.[158]

Prospective studies support an adverse effect of eating processed meat on the development of type 2 diabetes. As components present in processed meat could enhance inflammation markers, chronic low-grade inflammation may be a potential mediator. Therefore, the extent to which CRP, as measure of chronic low-grade inflammation, mediated the association between intake of meat and risk of type 2 diabetes was studied (chapter 5).

III.5 Glycemic index and glycemic load of the diet, inflammation, and type 2 diabetes

The GI and GL are two concepts that relate to the capacity of foods to raise blood glucose levels after ingestion. The GI expresses the influence of a portion of a food product containing 10-50 gram digestible carbohydrates on blood glucose concentrations over the next two hours after consumption relative to the effect of glucose.[159] The higher the GI of a food is, the faster and larger the increase in glucose concentration after consumption. The GL expresses the influence of a portion size eaten on blood glucose concentration.[160] For example, a watermelon has a high GI (about 75), but as the total carbohydrate content of a normal portion is low, the GL of watermelon is about 5.

After the introduction of the concept of GI by Jenkins *et al.*[159], numerous studies addressed the effect of GI and GL on the development of diabetes, because the GI and GL directly affect glucose concentrations. A review of seven prospective cohort studies mainly from the United States of America (USA) showed that a high GI diet was associated with a 20% and a high GL diet with a 16% higher risk of type 2 diabetes compared with a low GI or GL diet, respectively.[161] A meta-regression of human intervention studies also suggests adverse effects of a high GI diet on markers of glucose metabolism.[162] These results support the classification of a high GI diet as a 'possible' risk factor for type diabetes by the WHO/FAO.[40]

Direct and indirect pathways may explain this higher risk. High GI and GL diets cause greater fluctuations in blood glucose concentrations and consequently request a high insulin demand during the early postprandial stage.[163] Exhaustion of the beta-cells may follow, leading to impaired insulin secretion at the long run.[15] A rapid increase of insulin during the early postprandial stage results in hypoglycemia at the late postprandial stage. Consequently, fat oxidation is stimulated to meet energy requirements. Free fatty acids concentration increases due

to fat oxidation, which contributes to beta-cell dysfunction and impaired insulin action. Hyperglycemia itself may also contribute to impaired insulin action.[163] So, high GI and GL diets may directly affect the development of type 2 diabetes by inducing postprandial hyperglycemia.

It has been suggested that high GI and GL diets may indirectly affect the development of type 2 diabetes by amongst others their effect on body weight and chronic low-grade inflammation.[15] A meta-analysis of four randomized controlled intervention studies showed that body mass reduction was larger in a low GI diet than in a high GI or other diet.[164] In the DIet, Obesity and GENES (DIOGENES) project, weight regain after weight loss was lower in participants following a low GI diet than in participants following a high GI diet.[165] However, the underlying mechanisms are not clear, as postprandial glucose itself may not affect appetite and body weight maintenance.[15] A high GI or GL may have pro-inflammatory properties, because postprandial hyperglycemia may result in overproduction of free-radical molecules and release of cytokines.[166, 167] However, the results from cross-sectional studies on the association between GI or GL and markers of inflammation are inconsistent so far.[168-176]

Prospective studies, which were primarily conducted in the USA, showed that a high GI or GL diet is associated with a higher risk of type 2 diabetes. As the range of GI and GL may vary between continents, the association between GI or GL and risk of type 2 diabetes was studied in a Dutch population (chapter 6). The potential mediating role of chronic low-grade inflammation was also considered.

III.6 Dietary patterns, inflammation, and type 2 diabetes

The above sections III.1 to III.5 concern individual dietary factors. As foods are not consumed individually, but with others, a dietary pattern approach can be used to assess how the overall diet can affect the development of type 2 diabetes. A dietary pattern approach can be either hypothesis-driven, exploratory, or of hybrid form.[177]

Hypothesis-driven dietary patterns are obtained using pre-defined calculation rules, like the healthy eating index[178, 179], the Dutch healthy eating index[180], the diet quality index[181], the recommended food score[182], the Mediterranean diet score[183], and the dietary inflammatory index (DII)[184]. Of these a hypothesis-driven dietary patterns, the DII was especially designed to measure the inflammatory potential of the diet. The DII relies on the results of 929 published studies that examined the association between dietary components and markers of inflammation. Based on these results, the inflammatory potential of 42 dietary components was assessed. Summing the multiplications of the inflammatory potential of each dietary component with the intake, results in the DII score. If the DII would be associated with the development of type 2 diabetes, this suggests that diet can affect, at least in part, the processes leading to type 2 diabetes through its effect on chronic low-grade inflammation. Whether the DII reflects indeed the inflammatory potential of the diet, however, is not well established, because the DII was studied only once, in the original publication.[184] In this USA investigation, a five-point increase in the DII was associated with a 24% lower risk of an elevated CRP concentration.[184] It is not known yet whether this DII also applies in other populations and whether the DII is associated with the development of type 2 diabetes.

Exploratory dietary patterns are not hypothesis-driven, but data-driven, i.e., completely based on data of the population under investigation. Factor analysis, principle component analysis, and cluster analysis are methods used to identify such dietary patterns.[185] Factor analysis and principle component analysis are statistical techniques that can be used to group intake of dietary components into dietary patterns based on the underlying correlation structure of the

dietary components. Cluster analysis is a statistical technique that can be used to cluster participants based on similarities in their food intake. Dietary patterns that are constructed by these methods, do not necessary explain a high proportion of variation in an intermediate, e.g., inflammation, in the association between diet and type 2 diabetes. A dietary pattern that explained as much variation in inflammation is of interest, when studying whether diet affects, at least in part, risk of type 2 diabetes through its effect on chronic low-grade inflammation. Such a dietary pattern can be obtained by using a hybrid approach. This approach combines a priori information about underlying pathways with the data of the population under investigation. In the Nurses' Health study a dietary pattern that explained as much variation in inflammation as possible was constructed using reduced rank regression as statistical technique.[186] Women with the highest pro-inflammatory dietary pattern had a tree times higher risk to developed type 2 diabetes compared with women with the lowest pro-inflammatory score.[186] The results are not yet confirmed by other studies.

Evidence of an association between dietary patterns that reflect the inflammatory potential of the diet and type 2 diabetes is limited. Therefore, the association between a dietary inflammatory index or a dietary inflammatory pattern and type 2 diabetes was investigated (chapter 7, 8). As such, the extent to which chronic low-grade inflammation is a pathway through which diet can affect the processes leading to type 2 diabetes can be further elucidated.

PART IV: OBJECTIVES AND OUTLINE OF THIS THESIS

The prevalence of type 2 diabetes is rapidly growing all over the world. Effective strategies for primary prevention, therefore, are warranted. Guidelines to prevent type 2 diabetes should be evidence based and should focus on modifiable risk factors, like overweight, smoking, and diet.

As diet can play an important role in the development of type 2 diabetes, the main objective of this thesis is to investigate the role of selected dietary factors, i.e., fatty acids, fish, tea, meat, GI, GL, on the development of type 2 diabetes. Furthermore, the extent to which chronic low-grade inflammation is a pathway through which diet can affect the processes leading to type 2 diabetes is studied (**Figure 1.3**).

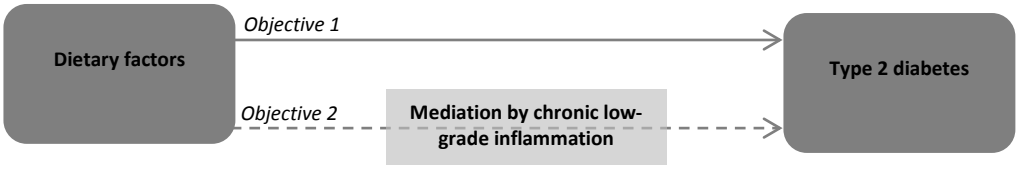


Figure 1.3 Figure representing the two objectives of this thesis.
The straight line represents the association between dietary factors and type 2 diabetes (objective 1). The striped line represents the inflammatory pathway through which diet may affect the processes leading to type 2 diabetes (objective 2).

An overview of the content of **chapters 2 to 8** is given in **table 1.3** and described below.

In **chapter 2**, the association between proportions of circulating fatty acids and glucose metabolism status was investigated cross-sectionally in Dutch persons participating in the CODAM study (*n*= 471). In **chapter 3, 5, 6**, the prospective associations between fish (i.e., total, lean, fatty),

meat (i.e., unprocessed red meat, processed red meat, poultry), GI, or GL and risk of type 2 diabetes were studied in the Rotterdam study (*n*= ≈4,400; $n_{\text{incident cases}}$= ≈460). In **chapter 4**, the association between intake of tea and risk of type 2 diabetes was investigated in persons from eight European countries participating in the EPIC-InterAct case-cohort study ($n_{\text{subcohort}}$= 16,154; $n_{\text{incident cases}}$= 11,541).

To investigate the second objective, the mediating role of CRP, as measure of chronic low-grade inflammation, in the association between meat, GI, or GL and risk of type 2 diabetes was studied in **chapters 5 and 6.** In **chapter 7**, we addressed whether a dietary inflammatory index was associated with markers of glucose metabolism in the CODAM and Hoorn studies (*n*= 1,034). In **chapter 8**, a hybrid approach was used to derive a dietary inflammatory pattern. Subsequently, this pattern was studied in relation to risk of type 2 diabetes in the Rotterdam study. In **chapter 9** the main conclusions were described and put into a broader perspective.

Table 1.3 Overview of the content of the chapters of this thesis

Chapter	Design	Study population	*n* (T2D cases)	Exposure	Outcome	
Individual dietary factor approach						
2	CS	CODAM study Dutch high risk population aged 60 (SD 7)	471 (77)	Circulating cholesteryl FA Desaturase activity	Glucose metabolism status and markers	
3	P	Rotterdam study General Dutch population aged 63 (SD 8)	4,472 (463)	Total fish Lean fish Fatty fish	Incidence T2DM	
4	P	EPIC-InterAct study Populations form 8 European countries aged 52 (SD 9)	26,039 (11,541)	Tea	Incidence T2DM	
Individual dietary factor approach and the potential mediation by chronic low-grade inflammation						*Mediation by*
5	P	Rotterdam study General Dutch population aged 67 (SD 8)	4,366 (456)	Unprocessed red meat Processed red meat Poultry	Incidence T2DM	CRP
6	P	Rotterdam study General Dutch population aged 67 (SD 8)	4,366 (456)	Glycemic index Glycemic load	Incidence T2DM	CRP
Dietary pattern approach						*Mediation by*
7	CS	CODAM and Hoorn studies Dutch high risk populations aged 64 (SD 9)	1,024 (234)	Adapted dietary inflammatory index	Glucose metabolism markers	Summary score for chronic low-grade inflammation
8	P	Rotterdam study General Dutch population aged 67 (SD 8)	4,366 (456)	Dietary inflammatory pattern	Incidence T2DM	CRP

Abbreviations: CS=cross-sectional design; P=prospective design; SD=standard deviation; T2D=type 2 diabetes; FA=fatty acids; CRP=C-reactive protein

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Abstract

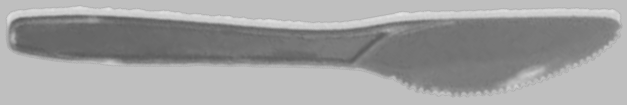
Objective: To investigate whether serum proportions of cholesteryl fatty acids and desaturase activities are associated with glucose metabolism status and insulin resistance.

Methods: Data were obtained from a cross-sectional study among 471 Dutch participants aged ≥40 years. Individual fatty acids in serum cholesteryl esters were determined and endogenous conversions by desaturases were estimated from product-to-precursor ratios. Proportions of fatty acids were compared among participants with normal glucose metabolism, impaired glucose metabolism, and newly diagnosed type 2 diabetes. Partial Spearman correlation coefficients between fatty acids and homeostasis model assessment for insulin resistance (HOMA-IR) were calculated. Adjustments were made for lifestyle and nutritional factors.

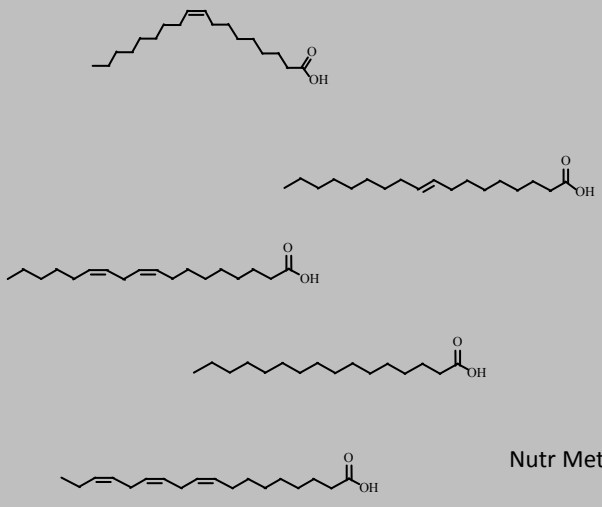
Results: The proportions of total saturated, mono-unsaturated, trans, and poly-unsaturated fatty acids did not differ significantly between groups, but several individual fatty acids did; the proportions of C18:0 and C20:3*n6* were higher, whereas those of C18:1*n7* and C20:4*n6* were lower in participants with type 2 diabetes compared with those with normal glucose metabolism. Activity of Δ5-desaturase, that is, ratio of C20:4*n6* to C20:3*n6*, was lower (p= <0.01) in participants with type 2 diabetes (7.4) than with normal glucose metabolism (8.4). HOMA-IR was correlated positively with Δ9-desaturase activity (r= 0.11, p= <0.01) and inversely with Δ5-desaturase activity (r= -0.21, p= <0.01).

Conclusion: The observed lower Δ5-desaturase activity in participants with type 2 diabetes and its inverse association with HOMA-IR suggest that changes in fatty-acid metabolism may play a role in the aetiology of type 2 diabetes.

CHAPTER 2



Comparison of fatty acid proportions in serum cholesteryl esters among people with different glucose metabolism: the CODAM study



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Introduction

Fatty acids, measured as components of cholesteryl esters or phospholipids present in plasma or serum, reflect intake of dietary fat over the last few weeks.[1] As such, they are markers of intake and may be preferred to self-reported intake assessed with a questionnaire, which is prone to underreporting of intake.[2] In addition, plasma or serum fatty acid profiles reflect endogenous conversion of ingested fatty acids by desaturation, elongation, or both.

The association between the proportion of individual fatty acids within fatty acid profiles and type 2 diabetes and related markers have been investigated in several cross-sectional[3-16] and longitudinal studies[17-21]. In general, these studies suggest that a high proportion of C16:0 (palmitic) and a low proportion of C18:2*n6* (linoleic) are associated with type 2 diabetes. Some of these studies also suggest that higher endogenous enzymatic conversion of fatty acids by Δ9-desaturase and lower conversion by Δ5-desaturase are associated with the onset of type 2 diabetes.

Considering the entire fatty acid profile, however, observed associations are inconclusive, and information on the association between proportions of trans fatty acids (TFA) and type 2 diabetes is sparse.[6, 20] Proportions of TFA are of interest because they may increase inflammatory cytokines that could affect the processes leading to type 2 diabetes.[22, 23] Furthermore, observational studies have rarely taken dietary factors into account, although proportions of fatty acids depend, at least partly, on the composition of diet.

We examined, therefore, whether proportions of fatty acids in serum cholesteryl esters and estimated activity of desaturases and elongases were associated with glucose metabolism status and insulin resistance, taking dietary factors into account.

Methods

Population for analysis

To establish the Dutch Cohort study on Diabetes and Atherosclerosis Maastricht (CODAM), Caucasian men and women, 40 through 70 years of age with a high risk for type 2 diabetes, were selected from an existing population-based study in the Netherlands. Participants were at high risk, because they had either a body mass index (BMI) >25 kg/m^2, a positive family history of type 2 diabetes, a postprandial blood glucose >6.0 mmol/L, glucosuria, or prescribed anti-hypertensive medication. Each participant, who was willing to participate (*n*= 2,820, response rate= 46%), underwent an oral glucose tolerance test (OGTT) using capillary blood samples. Participants diagnosed by the OGTT as having an impaired glucose metabolism (IGM) or type 2 diabetes and a random selection of participants with normal glucose metabolism (NGM) were invited for a second OGTT using venous blood samples. Of these participants, 508 were eventually included in the CODAM study. The local Medical Ethical Committee of Maastricht University approved the study protocol. Participants gave their written informed consent before they entered the study. For the current analysis participants with missing values for serum cholesteryl fatty acids (*n*= 30), physical activity (*n*= 5), or family history of diabetes (*n*= 2) were excluded, resulting in 471 participants.

Glucose and insulin

Glucose metabolism status was determined using a 75-gram OGTT. The World Health Organisation (WHO) 1999 criteria were used to define three groups: NGM, IGM, and type 2 diabetes. IGM was defined as participants who had either impaired fasting glucose or impaired glucose tolerance. Fasting plasma glucose and 2-hour glucose concentrations were measured using standard enzymatic methods (Glucose HK125, ABX Diagnostics, Montpellier, France).

Hemoglobin A1c (HbA1c) was measured using ion-exchange high-performance liquid chromatography (HPLC) (Bio-Rad, Veenendaal, the Netherlands). Fasting plasma insulin concentration was measured using a two-sided immunoradiometric test with use of paired monoclonal antibodies (Medgenix Diagnostics, Fleurus, Belgium). An updated computer model, which was based on formulas introduced by Matthews *et al*. in 1985, was used to calculate insulin resistance (HOMA2-IR) (http://www.dtu.ox.ac.uk).

Fatty acid profile

A venous blood sample was collected after an overnight fast. Serum and plasma were separated after centrifugation and stored at –80 °C until analysis. Gas chromatography was used to quantify proportions of fatty acids in serum cholesteryl esters. After deproteination and chloroform extraction, cholesteryl esters were separated from the lipid fraction by aminopropyl solid-phase column (Bond-Elut NH2 200 mg, Varian Associates). Subsequently, esters were simultaneously hydrolysed and methylated. The fatty-acid-methyl esters were separated on a 100x0.25mm ID wall coated open tubular columns (WCOT) fused silica capillary column using gas chromatograph-3900 (Varian Associates). Identification and quantification of peaks was done using Galaxie software. The amount of each fatty acid was expressed as percentage of the total area under the curve.

A total of 32 individual fatty acids were identified that together explained 95.7% of the total area under the curve. Activity of desaturases and elongases were estimated as product-to-precursor ratios of individual fatty acids as follows: Δ9-desaturase= C16:1*n7*/C16:0, Δ6-desaturase= C18:3*n6*/C18:2*n6*, Δ5-desaturase= C20:4*n6*/C20:3*n6*, and elongase= C22:5*n3*/C20:5*n3* or C20:3*n6*/C18:3*n6*. Additional individual fatty acids C14:1, C16:1*n9*t, C18:2*n6*t, C20:2, C20:1*n9*t, C20:1*n9*, C22:1*n9*, C24:1, C22:2, C22:4*n6*, C20:3*n3*, and C20:4*n3* were included in the total sum of mono-unsaturated fatty acids (MUFA), TFA, or poly-unsaturated fatty acids (PUFA), but were not investigated separately due to their small contribution (<0.10%), rarity of occurrence (*n*= <30%), or both, in this population.

Other factors

Height (cm) and body weight (kg) were measured with the participants wearing light clothing and no shoes. Subsequently, BMI was calculated as weight divided by the square of height (kg/m^2).

A self-administered questionnaire was used to obtain information on demographics and lifestyle variables, for example, smoking habits and family history of type 2 diabetes. Physical activity was measured with a validated Short Questionnaire to Assess Health-enhancing Physical Activity (SQUASH).[24] This questionnaire measured duration and intensity of different activities (min per week*intensity). Dietary intake was estimated with a validated food-frequency questionnaire (FFQ).[25] Total cholesterol was measured in fasting serum samples by enzymatic methods (Roche Diagnostics, Mannheim, Germany).

Statistics

Descriptive data were expressed as a mean (standard deviation (SD)), a median (p25-p75), or a percentage, where appropriate.

All analyses were adjusted for age (years), sex, physical activity (min per week*intensity), smoking (current, former, or never), and intake of total energy (kJ/day), alcohol (0-10 or >10 g/day), and fibre (g/day). BMI (kg/m^2) was examined as potential intermediate between proportions of fatty acids and glucose metabolism status.

First, adjusted means (analysis of covariance (ANCOVA)) of cholesteryl fatty acids and activity of desaturases and elongases were compared among three groups: NGM (*n*= 279), IGM (*n*= 115), and newly diagnosed diabetes (*n*= 77). Furthermore, we studied whether the association between desaturases and glucose metabolism status was modified by intake of total saturated fatty fat (SFA) (median split, 14.6 energy-%) and PUFA (median split, 7.2 energy-%), because modification by intake of fat has been suggested before.[26]

Second, we obtained partial Spearman correlation coefficients between proportions of fatty acids, estimated enzyme activities, and HOMA-IR and its components, fasting insulin (pmol/L) and fasting glucose (mmol/L).

Analyses were carried out using the Statistical Analysis Software (SAS) version 9.1. A two-sided *p*-value less than 0.05 was considered as statistically significant for all analysis.

Results

All markers of glucose and insulin metabolism increased from NGM to IGM to type 2 diabetes (*p*= <0.01) (**Table 2.1**). Participants with type 2 diabetes were more likely to have a family

Table 2.1 Characteristics of participants by glucose metabolism status (n_{total}= 471)[a]

	Normal glucose metabolism (*n*= 279)	Impaired glucose metabolism (*n*= 115)	Newly diagnosed diabetes (*n*= 77)
Age (years)	58.8 (7.4)	59.8 (6.7)	60.2 (6.1)
Sex (% men)	59.5	59.1	67.5
Body mass index (kg/m^2)	27.7 (3.9)	28.9 (4.4)	30.2 (4.6)
Family history of diabetes (%)	35.5	46.1	54.6
Total cholesterol (mmol/L)	5.2 (0.92)	5.3 (0.94)	5.5 (1.2)
Smoking (% current)	20.4	19.1	19.5
Physical activity level (hours/day)[b]	4.7 (2.7-7.1)	3.7 (2.0-6.3)	4.5 (2.4-5.7)
Diet prescription (%)	5.4	20.9	18.2
Glucose and insulin metabolism			
HOMA-IR[b,c]	1.0 (0.78-1.3)	1.3 (0.88-1.9)	1.7 (1.1-2.6)
Fasting plasma insulin (pmol/L)[b,c]	52.0 (41.0-70.0)	66.5 (45.0-97.0)	86.5 (55.0-128)
Fasting plasma glucose (mmol/L)[c]	5.3 (0.38)	5.9 (0.52)	7.3 (1.1)
2-hour glucose (mmol/L)[b]	5.7 (4.6-6.6)	8.8 (7.8-9.9)	12.9 (11.2-15.4)
HbA1c (%)[c]	5.6 (0.42)	5.8 (0.44)	6.6 (0.78)
Dietary intake			
Total energy (kJ/day)	9581 (2815)	8938 (2819)	9166 (2489)
Protein (en-%)	15 (2)	16 (2)	16 (2)
Carbohydrates (en-%)	45 (6)	44 (7)	43 (7)
Fat (en-%)			
Total	36 (5)	36 (6)	36 (6)
SFA	15 (3)	15 (3)	15 (3)
MUFA	12 (2)	13 (3)	12 (2)
TFA	1 (0.4)	1 (0.5)	1 (0.4)
PUFA	7 (2)	7 (2)	8 (2)
Alcohol (g/day)[b]	9 (2-24)	9 (1-23)	16 (3-30)
Fibre (g/day)	26 (7)	24 (8)	24 (6)

Abbreviations: HOMA-IR=homeostasis model assessment of insulin resistance; HbA1c=hemoglobin A1c; en-%=percent of total energy intake; SFA=saturated fatty acids; MUFA=mono-unsaturated fatty acids; TFA=trans fatty acids; PUFA=poly-unsaturated fatty acids

[a] Values were expressed as mean (standard deviation) or percentages unless otherwise indicated.

[b] Expressed as median (p25-p75), because of their skewed distribution.

[c] *n*= 464, 465, 470, 455, respectively.

history of type 2 diabetes and to have a higher BMI than participants with IGM and NGM.

C18:2*n6* (≈50%), C18:1*n9* (≈16%), and C16:0 (≈11%) were the main fatty acids in cholesteryl esters (**Table 2.2**). A non-significant higher proportion of total SFA (p= 0.08) was observed in participants with type 2 diabetes (13.2%) than in participants with NGM (12.8%). This was mainly due to the higher proportion of C18:0 (p= 0.02) (**Table 2.2**).

The proportions of total MUFA, TFA, and PUFA did not differ significantly among the three

Table 2.2 Adjusted mean proportion [95% confidence interval] of serum cholesteryl fatty acids and enzymatic conversion activities by glucose metabolism status (n_{total}= 471)[a]

	Normal glucose metabolism (n= 279)	Impaired glucose metabolism (n= 115)	Newly diagnosed diabetes (n= 77)
Total SFA	12.8 [12.6, 13.0]	12.9 [12.5, 13.2]	13.2 [12.8, 13.6]
C14:0	0.77 [0.74, 0.79]	0.78 [0.74, 0.82]	0.80 [0.75, 0.85]
C15:0	0.21 [0.20, 0.21]	0.20 [0.19, 0.21]	0.21 [0.19, 0.22]
C16:0	10.8 [10.6, 10.9]	10.8 [10.7, 11.0]	10.9 [10.6, 11.1]
C17:0	0.10 [0.10, 0.11]	0.10 [0.09, 0.10]	0.11 [0.10, 0.12][c]
C18:0	0.94 [0.84, 1.05]	0.95 [0.80, 1.11]	1.19 [1.01, 1.38][b,c]
Total MUFA	20.6 [20.2, 20.9]	20.7 [20.1, 21.2]	20.4 [19.8, 21.1]
C16:1*n7*	2.77 [2.63, 2.90]	2.89 [2.69, 3.10]	2.91 [2.66, 3.16]
C16:1*n9*	0.50 [0.49, 0.51]	0.50 [0.48, 0.51]	0.50 [0.48, 0.52]
C18:1*n7*	1.07 [1.05, 1.10]	1.05 [1.00, 1.09]	0.97 [0.92, 1.03][b,c]
C18:1*n9*	16.1 [15.9, 16.4]	16.2 [15.8, 16.5]	16.0 [15.5, 16.4]
Total TFA	0.57 [0.54, 0.60]	0.52 [0.47, 0.57]	0.55 [0.49, 0.61]
C16:1*n7*t	0.20 [0.19, 0.21]	0.19 [0.17, 0.20]	0.21 [0.19, 0.23]
C18:1*n9*t	0.26 [0.24, 0.28]	0.25 [0.22, 0.28]	0.24 [0.20, 0.27]
Total PUFA	61.7 [61.2, 62.2]	61.8 [61.0, 62.5]	61.3 [60.3, 62.2]
C16:2*n4*	0.19 [0.18, 0.20]	0.16 [0.15, 0.18][d]	0.18 [0.16, 0.20]
Total *n6* PUFA	58.8 [58.3, 59.3]	59.0 [58.2, 59.8]	58.3 [57.3, 59.3]
C18:2*n6*	50.5 [49.9, 51.1]	50.4 [49.5, 51.3]	50.3 [49.2, 51.4]
C18:3*n6*	1.00 [0.96, 1.05]	1.05 [0.99, 1.12]	1.02 [0.94, 1.10]
C20:3*n6*	0.80 [0.78, 0.82]	0.85 [0.82, 0.88][d]	0.86 [0.82, 0.90][b]
C20:4*n6*	6.48 [6.31, 6.66]	6.65 [6.38, 6.92]	6.11 [5.79, 6.44][b,c]
Total *n3* PUFA	2.52 [2.42, 2.61]	2.45 [2.31, 2.58]	2.64 [2.47, 2.81]
C18:3*n3*	0.59 [0.57, 0.61]	0.55 [0.52, 0.58][d]	0.59 [0.56, 0.63]
C20:5*n3*	0.95 [0.88, 1.02]	0.93 [0.82, 1.03]	1.03 [0.90, 1.16]
C22:5*n3*	0.35 [0.33, 0.38]	0.35 [0.31, 0.39]	0.37 [0.32, 0.42]
C22:6*n3*	0.60 [0.58, 0.62]	0.60 [0.57, 0.63]	0.62 [0.58, 0.65]
Desaturases			
Δ9 (C16:1*n7*/C16:0)	0.26 [0.24, 0.27]	0.27 [0.25, 0.28]	0.27 [0.25, 0.29]
Δ6 (C18:3*n6*/C18:2*n6*)	0.020 [0.019, 0.021]	0.021 [0.020, 0.023]	0.021 [0.019, 0.022]
Δ5 (C20:4*n6*/C20:3*n6*)	8.41 [8.10, 8.72]	8.00 [7.54, 8.46]	7.36 [6.80, 7.93][b]
Elongases			
C20:3*n6*/C18:3*n6*	0.89 [0.85, 0.93]	0.90 [0.84, 0.96]	0.92 [0.84, 1.00]
C22:5*n3*/C20:5*n3*	0.49 [0.44, 0.54]	0.45 [0.37, 0.52]	0.49 [0.40, 0.58]

Abbreviations: SFA=saturated fatty acids; MUFA=mono-unsaturated fatty acids; TFA=trans fatty acids; PUFA=poly-unsaturated fatty acids

[a] Mean proportions are adjusted for age, sex, smoking status, total physical activity, family history of diabetes, and intakes of energy, alcohol, and fibre.

[b] Newly diagnosed diabetes vs. normal (p= <0.05); [c] Newly diagnosed vs. impaired (p= <0.05); [d] Impaired vs. normal (p= <0.05)

groups, but several individual MUFA and PUFA did (**Table 2.2**). The proportion of C18:1*n7* was lower in participants with type 2 diabetes (0.97%) compared with NGM (1.07%) or with IGM (1.05%). In participants with type 2 diabetes (0.86%) and IGM (0.85%), the proportion of C20:3*n6* was higher than in those with NGM (0.80%). Between participants with IGM and NGM, the proportions of C16:2*n4* and C18:3*n3* also differed. Between participants with type 2 diabetes and IGM, it was the proportion of C20:4*n6* that also differed.

We did not observe different activity of Δ9-desaturase, Δ6-desaturase, or elongases among the three groups, but activity of Δ5-desaturase was lower (p= <0.01) in participants with type 2 diabetes (7.4) compared with NGM (8.4) (**Table 2.2**). The directions of the observed associations did not change after inclusion of BMI into the model (data not shown).

Our investigation into potential modification by intake of fat showed that the activity of Δ6-desaturase was not affected by intake of PUFA. Activity of Δ9-desaturase, however, was higher (p= <0.01) in participants with type 2 diabetes (0.30) than with NGM (0.25) within the low SFA intake group (<14.6 energy-%; n= 236), but not within the high SFA intake group (n= 235). The lower activity of Δ5-desaturase in participants with type 2 diabetes in the total group was also observed for participants, who had a low PUFA intake (≤7.2 energy-%; n= 237; 6.9 type 2 diabetes vs. 8.2 NGM; p= <0.01), but not for those with a high PUFA intake (7.7 type 2 diabetes vs. 8.5 NGM; p= 0.08).

Considering the total sum of types of fatty acids and estimated enzyme activities, we observed positive correlations between the proportion of Δ9-desaturase and elongase C20:3*n6*/C18:3*n6* and HOMA-IR, whereas we observed a negative correlation between Δ5-desaturase and HOMA-IR (**Table 2.3**). Correlations between proportions of fatty acids and plasma insulin or fasting plasma glucose concentration were in line with these of HOMA-IR, except the correlation between Δ9-desaturase or elongase C20:3*n6*/C18:3*n6* and fasting plasma glucose (**Table 2.3**).

Table 2.3 Partial Spearman correlation coefficients between proportions of cholesteryl fatty acids and HOMA-IR, insulin, and glucose[a]

	HOMA-IR (n= 464)	Plasma insulin (pmol/L) (n= 465)	Plasma glucose (mmol/L) (n= 470)
Total SFA	0.09	0.08	0.06
Total MUFA	0.02	0.02	0.06
Total TFA	-0.02	-0.02	-0.02
Total PUFA	-0.04	-0.04	-0.03
Total *n6* PUFA	-0.03	-0.03	-0.02
Total *n3* PUFA	-0.06	-0.06	-0.04
Desaturases			
Δ9 (C16:1*n7*/C16:0)	0.11[b]	0.11[b]	0.07
Δ6 (C18:3*n6*/C18:2*n6*)	0.01	0.01	0.06
Δ5 (C20:4*n6*/C20:3*n6*)	-0.21[c]	-0.21[c]	-0.12[b]
Elongases			
C20:3*n6*/C18:3*n6*	0.12[c]	0.13[c]	0.03
C22:5*n3*/C20:5*n3*	-0.05	-0.05	-0.08

Abbreviations: HOMA-IR=homeostasis model assessment of insulin resistance; SFA=saturated fatty acids; MUFA=mono-unsaturated fatty acids; TFA=trans fatty acids; PUFA=poly-unsaturated fatty acids

[a] Coefficients adjusted for age, sex, smoking status, total physical activity, family history of diabetes, intakes of energy, alcohol, and fibre.

[b] p= <0.05

[c] p= <0.01

Discussion

In our cross-sectional study, the proportions of total SFA, MUFA, TFA, and PUFA were not associated with the presence of type 2 diabetes. Estimated activity of Δ5-desaturase was lower in participants with type 2 diabetes than with NGM. Accordingly, HOMA-IR and its individual markers were associated with lower activity of Δ5-desaturase.

Studies comparing the total proportion of total SFA between participants with and without type 2 diabetes showed null associations and positive associations (**Figure 2.1**). The positive associations found could be the result of differences in intake of other components present in food between participants with and without type 2 diabetes or a reduced conversion of SFA-to-MUFA in persons with type 2 diabetes. We have no evidence to favour one of these explanations. If differences in the proportion of SFA were caused by differences in intake, we would have expected that our results would change after adjustment for dietary factors, but they did not. If the higher proportion of SFA originated from changes in metabolism, we would have expected a lower activity of Δ9-desaturase. Participants with type 2 diabetes, however, did not have lower estimated activity of Δ9-desaturase in our study or in general (**Figure 2.1**). By contrast, some observational studies[4, 11, 21] and our cross-sectional study showed that higher activity of Δ9-desaturase was correlated with higher fasting insulin concentrations. This finding is supported by an experiment in mice, where disruption of Δ9-desaturase improved insulin sensitivity.[27] This suggests that the conversion of SFA-to-MUFA may be higher, rather than lower, in persons with type 2 diabetes.

Of the proportions of total MUFA and individual MUFA, it is the proportion of C16:1*n7*, the product of Δ9-desaturase activity, which may be associated positively with type 2 diabetes (**Figure 2.1**). C16:1*n7* is normally not present in the diet; thus, its proportion mainly reflects the conversion of ingested C16:0 by Δ9-desaturase.[28] C18:1*n9*, however, is present in large quantities in olive oil. The proportion of C18:1*n9* within the body, therefore, is less affected by possible changes in fat metabolism associated with the development of type 2 diabetes. This might explain the absence of an association between the proportion of 18:1*n9* and type 2 diabetes (**Figure 2.1**).

To the best of our knowledge, the proportion of total TFA has only been studied twice before in relation to type 2 diabetes in observational studies.[6, 20] One study showed a negative association[20], whereas the other study and our study observed a null association[6]. In the previously published studies and in ours, however, TFA was not measured optimally, because a second column for complete separation of TFA was not used.

Neither the proportion of total *n3* PUFA nor that of *n6* PUFA was associated with type 2 diabetes (**Figure 2.1**). Given the high correlations observed between dietary and serum or plasma *n3* PUFA[29], absence of an association with the proportion of *n3* PUFA could be expected since dietary *n3* PUFA was also not clearly associated with risk of type 2 diabetes.[30-32]

Of the individual *n6* PUFA, C18:2*n6* appeared to be lower, in general, but not in our study, whereas C20:3*n6* appeared to be higher in participants with type 2 diabetes. As suggested by an animal study, insulin could trigger conversion of C18:2*n6*-to-C18:3*n6* by stimulating activity of Δ6-desaturase.[33] This might explain the lower proportion of C18:2*n6* in persons with type 2 diabetes, who could have higher fasting insulin concentrations when beta-cell function is not completely lost. Estimated activity of Δ6-desaturase, however, was generally not higher in persons with type 2 diabetes than in persons without diabetes (**Figure 2.1**). A lower proportion of C18:2*n6* could also be due to lower intake of this essential fatty acid[34], which has been associated with higher risk of type 2 diabetes.[35]

The higher proportion of C20:3*n6* in persons with type 2 diabetes may be due to lower activity of Δ5-desaturase, which is based on the ratio between C20:4*n6* and C20:3*n6*. As in our

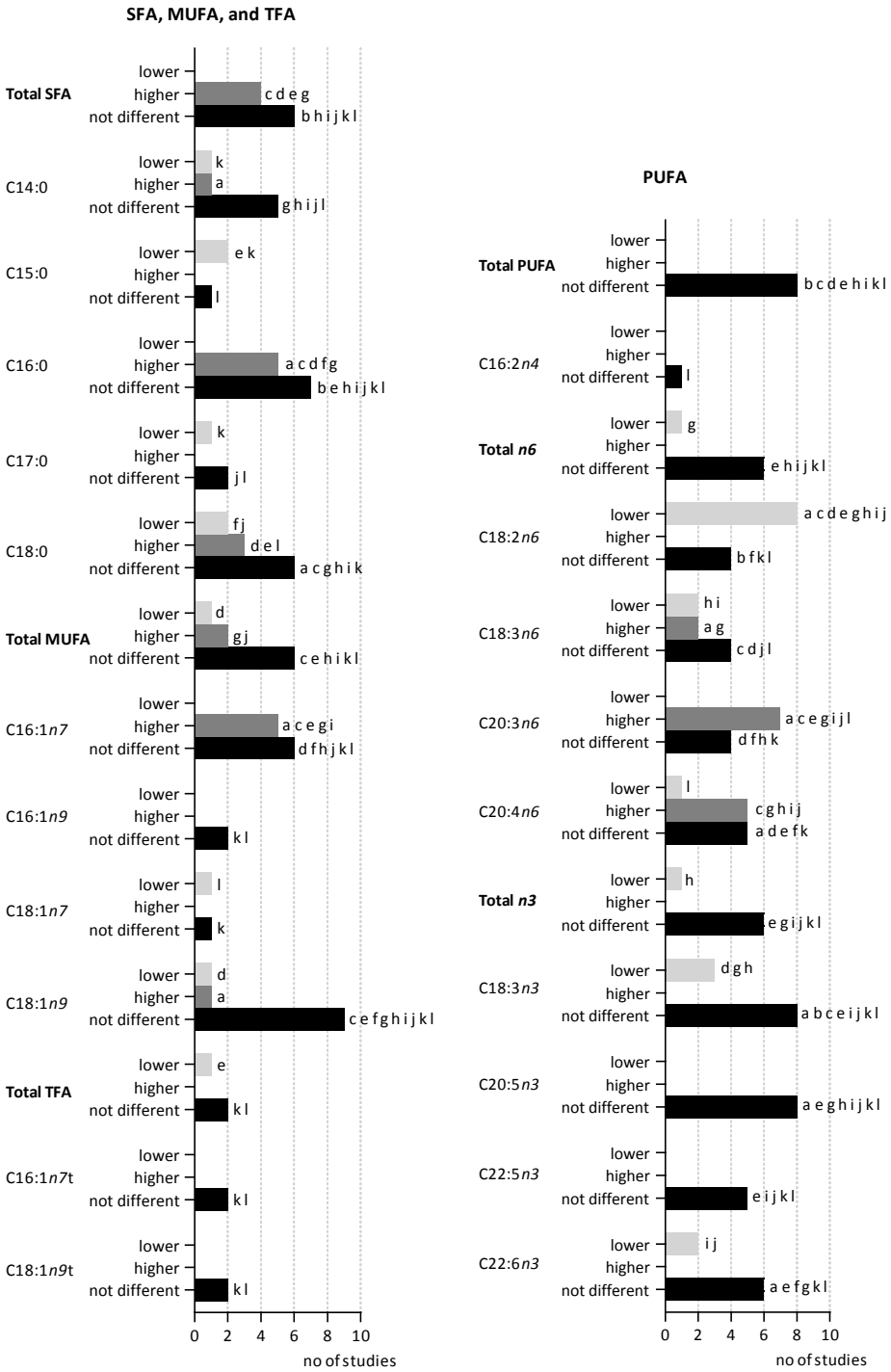
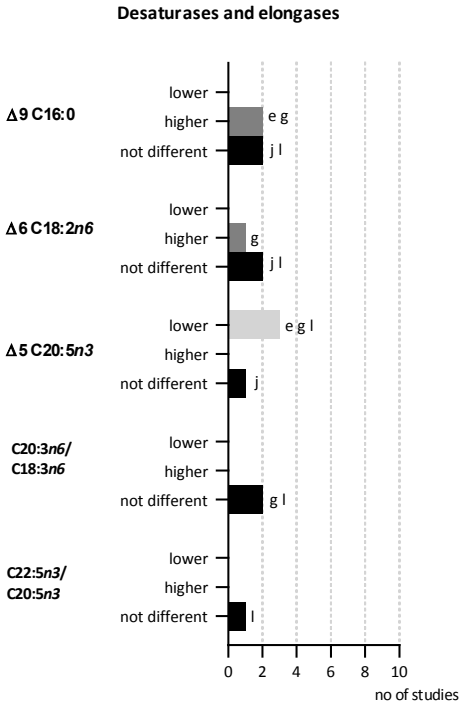


Figure 2.1 Association between type 2 diabetes (DM) and proportions of fatty acids measured as components of cholesteryl esters (CE) or phospholipids (PL) present in blood *(continues on next page)*



Cohort studies
a) Vessby[17], *n*= 1,828, DM vs. NGM, CE
b) Laaksonen[18], *n*= 895, IFG+DM vs. NGM, n/a
c) Wang[19], *n*= 2,909, DM vs. non-DM, CE
d) Wang[19], *n*= 2,909, DM vs. non-DM, PL
e) Hodge[20], *n*= 3,737, DM vs. non-DM, PL
Cross-sectional studies
f) Faas[3], *n*= 10, DM vs. non-DM, n/a
g) Salomaa[4], *n*= 520, DM vs. IGM vs. NGM, PL
h) Pelikanova[7], *n*= 35, DM vs. non-DM, CE
i) Pelikanova[7], *n*= 35, DM vs. non-DM, PL
j) Pelikanova[5], *n*= 45, newly diagnosed DM vs. NGM, PL
k) Rodriguez[6], *n*= 26, newly diagnosed DM vs. non-DM, PL
l) Van Woudenbergh, *n*=356, newly diagnosed DM vs. NGM, CE

Figure 2.1 (*continued*) Association between type 2 diabetes (DM) and proportions of fatty acids measured as components of cholesteryl esters (CE) or phospholipids (PL) present in blood

study, others also showed that lower activity of Δ5-desaturase was associated with higher fasting insulin concentrations[4, 11] and insulin resistance (HOMA-IR)[12]. Furthermore, genetic variation of the FADS1 gene, which codes for Δ5-desaturase, was shown to be associated with fasting glucose concentrations in a large genome-wide association study.[36]

The observed lower activity of Δ5-desaturase in participants with type 2 diabetes could partly be due to concomitant higher BMI. Persons with type 2 diabetes are more likely to have a higher BMI and low activity of Δ5-desaturase was observed in persons who are obese.[37] In our study, participants with type 2 diabetes were indeed more likely to be obese and had a lower activity of Δ5-desaturase than NGM. When we took BMI into account, however, the association did not change.

Our investigation into a potential interaction between activity of desaturases and intake of fat showed that, in line with observations in a human intervention study[26], activity of Δ9-desaturase was modified. By contrast, however, in our study, the association between activity of Δ6-desaturase and type 2 diabetes was not, whereas that of the activity of Δ5-desaturase was modified by intake of PUFA. The reason for this difference is, at present, unclear. Change in hepatic expression of Δ5-desaturase, however, was not observed in mice fed a high-fat diet compared with a low-fat diet, whereas the expression of Δ9-desaturase was suppressed.[38]

The major strength of this study was that information on serum cholesteryl fatty acids and diet was available. Hence, we were able to adjust for dietary factors and to study possible effect modification by fat intake. Adjustment for energy, fibre, and alcohol hardly influenced mean proportions, which may suggest that the associations between proportions of fatty acids and

glucose metabolism status is not affected by these dietary factors. Residual confounding, however, cannot be excluded completely, although the relative validity of the FFQ used was considered good.[25] Furthermore, although our study was of cross-sectional nature, it is not likely that diabetes status affected dietary intake as we included newly diagnosed diabetes cases.

A limitation of our study, as of all earlier studies[4, 5, 11-13, 20, 21], may be the use of a ratio of individual fatty acids to estimate activity of desaturases and elongases. It is not possible, therefore, to draw conclusions about actual enzyme activity within the body, but estimated activity gave at least some indication about these processes. That our study population included a NGM group with a high risk profile may limit our results. It is likely that variation in proportions of fatty acids would have been larger, if our NGM group was selected randomly from a general population.

In conclusion, the observed lower estimated activity of Δ5-desaturase in participants with type 2 diabetes and its inverse association with HOMA-IR suggest that changes in fatty acid metabolism play a role in the etiology of type 2 diabetes.

Acknowledgements

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Authors' responsibilities were as follows: GJvW prepared the data for analyses, performed the analysis, drafted the manuscript; AK and EJMF contributed to the interpretation of the data; CJvdK, MMvG, CDS, EEB, and EJMF participated in the design and coordination of the study; AK, CJvdK, MMvG, CDS, EEB, and EJMF critically revised the manuscript. None of the authors had a conflict of interest.

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Abstract

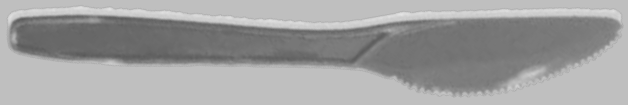
Objective: To investigate the relation between intake of total fish, type of fish (lean or fatty), and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and risk of type 2 diabetes in a population-based cohort.

Methods: The analysis included 4,472 Dutch participants aged ≥55 years without diabetes at baseline. Dietary intake was assessed with a semi-quantitative food frequency questionnaire. Hazard ratios (HR) with 95% confidence intervals (95%CIs) were used to examine risk associations adjusted for age, sex, lifestyle, and dietary factors.

Results: After 15 years of follow-up, 463 participants developed type 2 diabetes. Median intake of fish, mainly lean fish (81%), was 10 g/day. Total fish intake was associated with higher risk of type 2 diabetes; the HR was 1.32 [95%CI 1.02, 1.70] in the highest total fish group (≥28 g/day) compared with that for non-fish eaters (p_{trend}= 0.04). Correspondingly, intake of lean fish tended to be associated with higher risk of type 2 diabetes ($HR_{\geq 23\ g/day\ vs.\ 0}$= 1.30 [95%CI 1.01, 1.68]; p_{trend}= 0.06), but fatty fish was not. No association was observed between intake of EPA&DHA and type 2 diabetes ($HR_{\geq 149\ vs.\ <49\ \ mg/day}$= 1.22 [95%CI 0.97, 1.53]). With additional adjustment for intake of selenium, cholesterol, and vitamin D this HR decreased to 1.05 ([95%CI 0.80, 1.38]; p_{trend}= 0.77).

Conclusion: The findings do not support a beneficial effect of total fish, type of fish, or EPA&DHA intake on the risk of type 2 diabetes. Alternatively, other dietary components, such as selenium and unmeasured contaminants present in fish, might explain our results.

CHAPTER 3



Eating fish and risk of type 2 diabetes:

a population-based, prospective follow-up study

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Introduction

Potential benefits of intake of fish on the development of type 2 diabetes could be attributed to its high content of dietary *n3* poly-unsaturated fatty acids (PUFA), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Higher EPA and DHA quantities in the phospholipid cell membranes could increase insulin sensitivity.[1] EPA&DHA supplementation increased insulin sensitivity in animal models and in some human studies.[2] Results of prospective studies on intake of long-chain *n3* PUFA and risk of type 2 diabetes, however, did not show a relation.[3, 4] Apart from EPA and DHA, other components within fish, such as selenium and vitamin D, could also be related to type 2 diabetes. Vitamin D could be negatively and selenium could be positively associated with type 2 diabetes.[5, 6]

Results of studies that investigated the association between intake of fish and risk of type 2 diabetes are inconclusive. An ecological study reported that high fish may lower the risk of type 2 diabetes in populations with a high prevalence of obesity.[7] Cross-sectional studies reported inverse[8, 9], no[10, 11], or positive associations[12] between habitual intake of fish and glycemic status. Prospective evidence suggested that intake of fish is either inversely[13, 14] or not associated[15] with the risk of type 2 diabetes.

Taken together, the effects of fish intake and EPA&DHA intake on the development of type 2 diabetes are ambiguous. Furthermore, studies conducted in this field did not report associations between different types of fish and risk of type 2 diabetes. EPA and DHA are mainly present in fatty fish, which might indicate that it is also important to pay attention to the type of fish that is eaten instead of total fish intake alone.

Therefore, we investigated the relation between intake of total fish, type of fish (lean or fatty), and EPA&DHA, and risk of type 2 diabetes in a population of men and women aged ≥55 years. We hypothesized that intake of fish and especially intake of fatty fish is related to a lower risk of type 2 diabetes.

Methods

Population for analysis

The current study was conducted within the Rotterdam study, an ongoing prospective population-based study, which has been described in detail elsewhere.[16] In short, 7,983 inhabitants who resided in the district Ommoord of Rotterdam, the Netherlands, and were aged ≥55 years agreed to participate (response rate 78%). Our study population consisted of 4,472 participants, because participants without (*n*= 2,339) or with unreliable (*n*= 209) dietary data, those with known or newly diagnosed diabetes at baseline (*n*= 516), and those who had not sufficient clinical or anthropometric data (*n*= 447) were excluded. The Medical Ethics Committee of Erasmus Medical Centre (Rotterdam, the Netherlands) approved the study. All participants gave informed consent.

Baseline information

Baseline information on current health status was obtained by a questionnaire and clinical examinations between 1990 and 1993. Anthropometric information was obtained during a visit to the research centre. BMI was calculated from height and weight (weight in kilograms divided by the square of height in meters). Waist circumference (cm) was measured at the level midway between the lower rib margin and the iliac crest with participants in standing position. Blood pressure was measured at the right brachial artery with a random-zero sphygmomanometer with the participant in a sitting position. The mean of two consecutive measurements was used.

Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or use of blood pressure-lowering medication.

Blood samples were used to determine serum total cholesterol by an automated enzymatic procedure using CHOD-PAP reagent (Roche Diagnostics). High-density lipoprotein (HDL) cholesterol was measured with HDL cholesterol assay (Roche Diagnostics) using polyethylene glycol-modified enzymes and dextran sulphate. A history of coronary heart diseases (CHD) was defined as a self-reported myocardial infarction or angina pectoris with hospital admission. A family history of type 2 diabetes was defined as having a parent, sibling, or both with type 2 diabetes.

For a subsample of the population (*n*= 2,424), the physical activity level was measured with a physical activity questionnaire (Longitudinal Aging Study Amsterdam (LASA) Physical Activity Questionnaire) between 1997 and 2000.[17] Body weight, hours of different activities, and the corresponding MET score were used to calculate energy expenditure (kcal/day).

Dietary intake

Dietary assessment comprised a self-administered questionnaire followed by a structured interview with a trained dietician at the research centre. Participants had to mark the foods and drinks they had consumed at least twice a month in the preceding year. Subsequently, the dietician obtained accurate information on the amount of food eaten using a validated 170-food item semi-quantitative food frequency questionnaire.[18] Food intake data were converted to energy and nutrient intake using a Dutch Food Composition table (1993). For the intake of EPA and DHA and trans fatty acids a later version was used (2006). The amounts of energy from total fat, saturated fat, trans fatty acids, carbohydrates, and protein were calculated as a percentage of total energy intake (energy percent).

Total fish intake (g/day) was divided into four categories: no fish intake and approximate tertiles of fish consumers. The variables lean fish (i.e., plaice, stockfish, cod, fish fingers, perch, pike, octopus, pollack, tuna, and sole) and fatty fish (i.e., mackerel, herring, eel, and salmon) were categorized in the same way. Shell fish intake (i.e., mussels and shrimps) was dichotomized. Participants were categorized as fried fish eaters when they ate pollack or cod. Intake of EPA&DHA (mg/day) was divided into tertiles.

Follow-up information

Participants were continuously monitored for major events using the information from general practitioners and pharmacy databases. Information on vital status was obtained regularly from the municipal health authorities in Rotterdam. With this information follow-up data could be censored at time of death for 1,337 (30%) participants.

Incident diabetes cases were defined according to the American Diabetes Association 1997 criteria and the World Health Organisation 1999 criteria (fasting plasma glucose level ≥7.0 mmol/L and/or random plasma glucose level ≥11.1 mmol/L and/or use of anti-diabetes medication and/or treatment by diet) and cases had to be registered by a general practitioner as having type 2 diabetes. Follow-up data were available until July 2005.

Statistical analysis

Baseline characteristics across categories of total fish intake were expressed as mean (standard deviation (SD)), median (p25-p75), or a percentage. Hazard ratios (HR) and 95% confidence intervals (95%CI) were calculated to investigate the association between intake of (1) total fish, (2) lean fish, (3) fatty fish, and (4) EPA&DHA and incident type 2 diabetes. Non-fish

consumers, non-consumers of lean or fatty fish (irrespective of their other fish consumption), and low intake of EPA&DHA were considered as the reference group, respectively.

To evaluate whether the risk of type 2 diabetes differed among the intake categories we performed Cox proportional hazard analyses. In the crude model no adjustments were made. In the first model adjustments were made for age (years), sex, smoking (never, former, current), and level of education (low [primary education], intermediate [lower vocational, secondary general, or vocational education], high [higher vocational education or university]). The second model was additionally adjusted for dietary factors, i.e., intake of energy (kcal/day), trans fatty acids (g/day), fibre (g/day), and alcohol (no, low [>0–3 g/day], medium [≥3–14 g/day], or high [≥14 g/day]. For intake of lean fish as exposure, intake of fatty fish (grams per day) was included as a confounder in model 2 and vice versa. To investigate whether other components present in fish confounded the association between intake of EPA&DHA and type 2 diabetes, only for this association model 2 was additionally adjusted for intake of selenium (μg/day), vitamin D (μg/day), and cholesterol (mg/day). Other potential confounders including family history of diabetes, medically prescribed diet, and intakes of saturated fatty acids, mono-unsaturated fatty acids, linoleic acid, α-linolenic acid, fruit, vegetables, coffee, and meat were examined, but did not affect the results. Potential effect modification by sex was investigated.

In additional analysis, the potential intermediates, i.e., BMI (kilograms divided by meters squared), waist circumference (cm), total cholesterol (mmol/L), HDL cholesterol (mmol/L), and hypertension (no or yes), were investigated. A linear test for trend across categories was performed based on the median values of each category. All statistical analyses were performed using the statistical program SAS 9.1 for Windows. For all analyses a two-sided *p*-value <0.05 was considered statistically significant.

Results

The study population consisted of 4,472 participants with an average age of 67.2 (SD 7.7) years at baseline. Median follow-up time was 12.4 years during which 463 (10%) incident cases of type 2 diabetes were diagnosed. Mean age of diabetes onset was 73.9 (SD 6.9) years. Fifteen participants (0.3%) used fish oil capsules and 475 (11%) were on a prescribed diet.

Zero fish intake was reported by 29% of the population (**Table 3.1**). Median fish intake in the total population was 10 grams per day. The fish consumed consisted on average of 81% lean fish, 18% fatty fish, and 0.9% shellfish. Of the non-consumers of lean fish, 88% were also non-consumers of fatty fish and 43% of the non-consumers of fatty fish were also non-consumers of lean fish. Lean fish consumers ate 19 (SD 15) grams per day, fatty fish consumers ate 9.1 (SD 12) grams per day, and shell fish consumers ate 4.8 (SD 5) grams per day on average of lean fish, fatty fish, and shell fish, respectively. Median intake of EPA&DHA was 89 (interquartile range 35-187) milligrams per day. Spearman correlation between intake of total fish and EPA&DHA was 0.87 (*p*= <0.01). Participants with higher intake of total fish were younger, had higher total cholesterol levels, and were more likely to drink alcohol (**Table 3.1**). Intake of trans fatty acids was lower in these participants, whereas the intake of fibre, cholesterol, selenium, and vitamin D was higher. Intake of fish contributed 13, 12, and 5% to the total intake of selenium, vitamin D, and cholesterol, respectively.

In contrast to our hypothesis, we observed a positive association for total fish intake and diabetes risk (**Table 3.2**). The HR for participants in the highest group of total fish intake compared with that for the non-fish eaters was 1.32 ([95%CI 1.02, 1.70]; p_{trend}= 0.04) when adjusted for lifestyle and dietary factors (model 2). When further adjusted for intake of fried fish (no or yes), the association for intake of total fish was borderline significant (HR in the highest group: 1.26

Table 3.1 Baseline characteristics of 4,472 Dutch adults across categories of total fish intake[a]

	Fish intake (g/day)			
	No	**Low (>0-12)**	**Moderate (≥12-<28)**	**High (≥28)**
	(*n*= 1,314)	**(*n*= 1,061)**	**(*n*= 1,007)**	**(*n*= 1,090)**
Age (years)	67.8 (8.1)	67.6 (7.5)	66.8 (7.5)	66.6 (7.4)
Follow up (years)	10.9 (3.7)	10.8 (3.6)	11.0 (3.4)	10.8 (3.6)
Sex (% men)	38.6	41.9	41.1	42.9
Body mass index (kg/m^2)	26.1 (3.5)	26.3 (3.7)	26.4 (3.8)	26.2 (3.4)
Waist circumference (cm)	89.1 (11.0)	90.1 (11.0)	90.0 (10.9)	89.8 (11.0)
Family history of diabetes (%)	28.2	27.6	26.9	27.9
History of CHD (%)	11.7	12.7	13.5	10.9
Hypertension (%)	29.8	32.0	32.7	31.0
Cholesterol (mmol/L)				
Total	6.6 (1.3)	6.7 (1.1)	6.7 (1.2)	6.8 (1.2)
HDL	1.4 (0.36)	1.4 (0.35)	1.4 (0.36)	1.4 (0.40)
Smokers (%)				
Current	21.8	24.6	22.2	24.0
Former	40.6	43.4	43.1	46.2
Never	37.6	32.1	34.7	29.8
Educational level (%)				
Low	35.5	37.5	31.6	30.9
Intermediate	54.9	51.7	54.9	53.2
High	9.6	10.8	13.5	15.9
Diet prescription (%)	9.6	11.1	11.1	10.9
Dietary intake				
Energy (kcal/day)	1,962 (485)	1,953 (503)	1,989 (524)	2,025 (521)
Protein (en-%)	17 (3)	17 (3)	17 (3)	18 (3)
Carbohydrates (en-%)	45 (7)	44 (7)	44 (7)	43 (7)
Total fat (en-%)	37 (6)	37 (6)	37 (6)	36 (6)
SFA en-%)	15 (3)	15 (3)	14 (3)	14 (3)
TFA (en-%)	1 (0.5)	1 (0.5)	1 (0.4)	1 (0.5)
EPA&DHA (mg/day)[b]	25 (13-41)	64 (40-95)	132 (100-188)	245 (182-374)
Fibre (g/day)	17 (5)	17 (5)	17 (5)	17 (5)
Cholesterol (mg/day)	225 (81)	226 (80)	233 (80)	251 (86)
Selenium (µg/day)	27 (7)	30 (8)	33 (8)	42 (11)
Vitamin D (µg/day)	2 (1)	2 (1)	2 (1)	3 (2)
Alcohol (%)				
No	23.8	20.4	15.4	16.8
Low	29.5	30.5	29.7	24.3
Moderate	26.1	24.4	26.8	26.0
High	20.6	24.7	28.1	32.9
Fish (g/day)				
Lean	0	5 (3)	15 (6)	34 (16)
Fatty	0	1 (2)	3 (5)	8 (14)
Shell	0	0.08 (0.5)	0.2 (1)	0.3 (2)

Abbreviations: CHD=coronary heart disease; HDL=high-density lipoprotein; en-%=percent of total energy; SFA=saturated fatty acids; TFA=trans fatty acids; EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid

[a] Values were expressed as mean (standard deviation) or percentages unless otherwise indicated.

[b] Value expressed as median (p25-p75), because of skewed distribution.

([95%CI 0.97, 1.64]; p_{trend}= 0.06).

When analyses were stratified for types of fish, intake of lean fish tended to be associated with a higher risk (HR= 1.30 [95%CI 1.01, 1.68]; p_{trend}= 0.06), whereas fatty fish intake did not (HR= 0.99 [95%CI 0.71, 1.38]). In none of the models, statistically significant associations were found for

intake of shellfish (Model 2, $HR_{yes\ vs.\ no\ shellfish}$ = 1.04 [95%CI 0.61, 1.77] (data not shown)).

Furthermore, no associations were observed for intake of EPA&DHA (**Table 3.3**). The HR was 1.22 [95%CI 0.97, 1.53] for the highest level of EPA&DHA intake compared with the lowest. Additional adjustments for intake of selenium, vitamin D, and cholesterol lowered the HR to 1.05 [95%CI 0.80, 1.38].

In a subsample (*n*= 2,424) energy expenditure was added to model 2, but the HRs did not change appreciably (data not shown). Furthermore, when BMI and waist circumference were taken into account in addition to model 2, the HRs did not alter substantially ($HR_{highest\ total\ fish\ intake\ vs.\ 0}$= 1.29 [95%CI 1.00, 1.67]). The other potential intermediates, i.e., total cholesterol, HDL cholesterol, and hypertension, did not change any of the HRs either. Sex did not modify the observed relations (*men*: $HR_{highest\ total\ fish\ intake\ vs.\ 0}$= 1.38 [95%CI 0.94, 2.02], *women*: $HR_{highest\ total\ fish\ intake\ vs.\ 0}$= 1.26 [95%CI 0.90, 1.78]). In all analyses exclusion of either participants with CHD at baseline (*n*= 544), participants who consumed fish oil capsules (*n*= 15), or participants who did not eat fish nor meat (*n*= 18) did not change the results.

Table 3.2 Hazard ratios and 95% confidence intervals for incident type 2 diabetes by fish intake categories in 4,472 Dutch adults

	Median	*n* (cases)	PY	Crude HR [95%CI]	Model 1[a] HR [95%CI]	Model 2[b] HR [95%CI]
Total fish (g/day)						
No	0	1,314 (121)	14,267	1 (ref)	1 (ref)	1 (ref)
Low (>0-<12)	6.6	1,061 (112)	11,492	1.15 [0.89,1.49]	1.14 [0.88,1.47]	1.15 [0.89,1.48]
Moderate (≥12-<28)	17.5	1,007 (107)	11,073	1.13 [0.87,1.47]	1.14 [0.88,1.48]	1.19 [0.92,1.54]
High (≥28)	35.6	1,090 (123)	11,819	1.22 [0.95,1.56]	1.23 [0.96,1.58]	1.32 [1.02,1.70]
p for trend				*0.18*	*0.14*	*0.04*
Lean fish (g/day)						
No	0	1,488 (139)	16,119	1 (ref)	1 (ref)	1 (ref)
Low (>0-10)	6.5	992 (110)	10,769	1.18 [0.92,1.52]	1.17 [0.90,1.51]	1.15 [0.89,1.49]
Moderate (≥10-23)	14.3	992 (99)	10,974	1.03 [0.80,1.34]	1.05 [0.81,1.37]	1.07 [0.82,1.40]
High (≥23)	30.6	1,000 (115)	10,788	1.22 [0.96,1.57]	1.24 [0.96,1.60]	1.30 [1.01,1.68]
p for trend				*0.19*	*0.16*	*0.06*
Fatty fish (g/day)						
No	0	3,087 (313)	33,586	1 (ref)	1 (ref)	1 (ref)
Low (>0-3)	1.6	461 (51)	5,124	1.05 [0.78,1.42]	1.01 [0.74,1.36]	1.04 [0.77,1.42]
Moderate (≥3-7)	5.3	499 (57)	5,329	1.14 [0.86,1.51]	1.07 [0.80,1.43]	1.11 [0.83,1.49]
High (≥7)	15.7	425 (42)	4,612	0.97 [0.70,1.34]	0.92 [0.66,1.28]	0.99 [0.71,1.38]
p for trend				*0.98*	*0.70*	*0.93*

Abbreviations: PY=person-years; HR=hazard ratio; 95%CI=95% confidence interval

[a] Model adjusted for age, sex, smoking, and education level. Fish categories were mutually adjusted.

[b] Model 1 with additional adjustments for intakes of energy, alcohol, trans fatty acids, and fibre.

Discussion

The results of this prospective study in older Dutch men and women with a low habitual level of fish intake do not support the hypothesis that intake of fish could protect against risk of type 2 diabetes. On the contrary, we observed that intake of total fish was associated with a higher risk of type 2 diabetes. This result was mainly due to intake of lean fish, which accounted for 81% of total fish intake. Intake of fatty fish and EPA&DHA were not related to risk of type 2 diabetes.

In this study, it is unlikely that the association was obscured because of misclassification of diabetes incidence. Onset of diabetes was monitored continuously through general practitioners and follow-up visits. The extensive information on potential confounders, which minimized the possibility of residual confounding, also strengthened our results. Information about physical activity was available for only a subsample of the population. Adjustment for energy expenditure did not affect the HR, through which it is unlikely that confounding by physical activity explained our results. Another strength of our study was the large reference group, which enabled us to show an association, if one would have existed. Within fish eaters, however, the contrast of fish intake appeared to be small. Total fish intake is rather low (≈10 g/day) in this population, which limited the possibility of studying the effects of high fish intake on risk of type 2 diabetes. Furthermore, the investigation into the effect of fatty fish intake might have been restricted because of the high intake of lean fish relatively to fatty fish. We cannot rule out potential misclassification of fish intake due to changes in intake of fish during follow-up. However, participants with type 2 diabetes or CHD at baseline who were likely to change their diet as a consequence of their disease were excluded or did not change the results, respectively.

In contrast with our findings, two earlier cohort studies showed protective effects of fish intake.[13, 14] The study of Feskens *et al.*[13], that showed an odds ratio of 0.47 for fish eaters compared with non-fish eaters, was smaller (59 cases) and had a shorter follow-up period (4 years) than our study. In the Dutch and Finnish cohorts of the Seven Countries Study, which used 2-hour blood glucose levels instead of type 2 diabetes risk, a change in fish intake was also associated with a lower risk (*beta*= -0.18).[14] In the Nurses' Health Study II an association between fish intake and risk of type 2 diabetes was not found ($HR_{\geq 2 \text{ portions/week vs. } <1 \text{ portion/week}}$= 1.04).[15] Differences in range, type, and preparation of fish might explain in part the observed differences

Table 3.3 Hazard ratios and 95% confidence intervals for incident type 2 diabetes by tertiles of EPA&DHA intake in 4,472 Dutch adults

	Median	*n* (cases)	PY	Crude HR [95%CI]	Model 1[a] HR [95%CI]	Model 2[b] HR [95%CI]	Model 3[c] HR [95%CI]
EPA&DHA (mg/day)							
Low (<49.1)	23.8	1,490 (142)	16,085	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Moderate (≥49.1-<149.4)	89.4	1,491 (158)	16,303	1.09 [0.87,1.37]	1.10 [0.88,1.38]	1.13 [0.90,1.42]	1.06 [0.84,1.34]
High (≥149.4)	236.8	1,491 (163)	16,263	1.12 [0.89,1.40]	1.13 [0.90,1.42]	1.22 [0.97,1.53]	1.05 [0.80,1.38]
p for trend				*0.38*	*0.33*	*0.11*	*0.77*

Abbreviations: PY=person-years; HR=hazard ratio; 95%CI=95% confidence interval; EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid

[a] Model adjusted for age, sex, smoking, and education level.

[b] Model 1 with additional adjustments for intakes of energy, alcohol, trans fatty acids, and fibre.

[c] Model 2 with additional adjustments for intakes of selenium, vitamin D, and cholesterol.

in risk estimates among studies.

Concerning *n3* PUFA, in line with our study two other prospective studies also showed no association. In the Health Professionals Follow-up Study[3], a HR of 1.01 (upper versus lower quintile) was observed and the Iowa Women's Health Study[4] showed a HR of 1.11 between the upper and lower quintiles of long-chain *n3* PUFA intake and diabetes risk.

The different findings between the intakes of fish and EPA&DHA might be partly explained by the intake of deep fried fish that is generally lean fish. Deep-fat frying can affect the potential benefits of fish by lowering the EPA&DHA content.[19] Indeed, although detailed information on the preparation method was not available, after additional adjustment for intake of fried fish, the HR attenuated for the highest intake category of total fish in our cohort.

Furthermore, the potential beneficial effect of EPA&DHA intake could be counteracted by intake of total cholesterol, which was associated with intake of fish in our study. Elevated cholesterol levels may impair pancreatic beta-cell function and insulin secretion.[20] It should be noted, however, that when additional adjustments were made for intake of cholesterol, selenium, and vitamin D, the HR for the highest intake group of EPA&DHA compared with the lowest group was especially attenuated after adjustment for intake of selenium. Plasma selenium levels increase with increasing intake of fish[21] and may be associated with a higher risk of type 2 diabetes.[22] Selenium supplementation increased diabetes risk in a trial among 1,202 dermatology patients[6] and tended to increase diabetes risk in the Selenium and Vitamin E Cancer Prevention Trial (SELECT) including 35,533 men.[23] An adverse effect of selenium on diabetes risk might therefore explain the higher risk in the highest total fish and EPA&DHA intake groups.

Finally, particularly at high exposure levels, it may be that the potential beneficial effects of EPA and DHA were counteracted by ingestion of contaminated fish, especially lean freshwater fish. Mice models showed that elevated blood mercury decreased plasma insulin and elevated blood glucose levels.[24] Serum concentrations of persistent organic pollutants were strongly related with diabetes prevalence in a cross-sectional study.[25] Unfortunately, we did not have information available on intake of contaminants in the current study.

In summary, the findings of this prospective study do not support a protective effect of intake of total fish, type of fish, nor EPA&DHA on the development of type 2 diabetes. Intake of total fish even appeared to be associated with higher risk of type 2 diabetes in this study. Dietary components and contaminants present in fish should be studied extensively when the potential role of fish in the development of type 2 diabetes is examined further. At this point, given the conflicting results on intake of fish and risk of type 2 diabetes, we think it is too early to give recommendations regarding intake of fish in relation to type 2 diabetes.

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Abstract

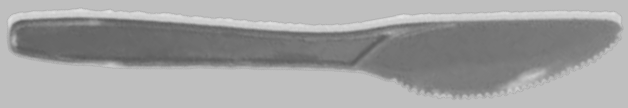
Objective: To investigate the association between tea consumption and incidence of type 2 diabetes in a European population.

Methods: The EPIC-InterAct case-cohort study was conducted in 26 centers in 8 European countries and consists of a total of 12,403 incident type 2 diabetes cases and a stratified subcohort of 16,835 individuals from a total cohort of 340,234 participants with 3.99 million person-years of follow-up. Country-specific hazard ratios (HR) for incidence of type 2 diabetes were obtained after adjustment for lifestyle and dietary factors using a Cox regression adapted for a case-cohort design. Subsequently, country-specific HR were combined using a random effects meta-analysis. Tea consumption was studied as categorical variable (0, >0-<1, 1-<4, ≥4 cups/day). The dose-response of the association was further explored by restricted cubic spline regression.

Results: Country specific medians of tea consumption ranged from 0 cups/day in Spain to 4 cups/day in United Kingdom. Tea consumption was associated inversely with incidence of type 2 diabetes; the HR was 0.84 [95% confidence interval (95%CI) 0.71, 1.00] when participants who drank ≥4 cups of tea per day were compared with non-drinkers ($p_{\text{linear trend}}$= 0.04). Incidence of type 2 diabetes already tended to be lower with tea consumption of 1-<4 cups/day (HR= 0.93 [95%CI 0.81, 1.05]). Spline regression did not suggest a non-linear association ($p_{\text{non-linearity}}$= 0.20).

Conclusion: A linear inverse association was observed between tea consumption and incidence of type 2 diabetes. People who drink at least 4 cups of tea per day may have a 16% lower risk of developing type 2 diabetes than non-tea drinkers.

CHAPTER 4



Tea consumption and incidence of type 2 diabetes in Europe: the EPIC-InterAct case-cohort study

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Introduction

Increasing our understanding of modifiable lifestyle factors associated with the development of type 2 diabetes is important, as the prevalence of diabetes is increasing rapidly.[1] Obesity is a major risk factor for the development of type 2 diabetes[2], but dietary factors may also play a role. One dietary factor of interest is tea consumption. Tea consumption may lower the risk of type 2 diabetes by influencing glucose digestion, glucose uptake, and by protecting beta-cells from free-radical damage.[3-5] This beneficial effect may be due to the polyphenols present in tea.

A meta-analysis, including nine cohort studies, reported that drinking at least 4 cups of tea per day was associated with a 20% lower risk, whereas drinking >0-<1 or 1-3 cups per day did not lower the risk of diabetes compared with non-tea drinkers.[6] In line with this, no association was observed when tea consumption was studied as continuous variable. This may indicate that the protective effect of tea is restricted to people with high tea consumption, although a potential biological mechanism has not been described yet.

Studies in which tea consumption is low, therefore, may not observe an association between tea consumption and risk of type 2 diabetes. This is supported by three out of the four additional cohort studies, which were published after the meta-analysis.[7-10] Two of those studies, in which the highest tea consumption category was relatively low, at least 3 cups per day[7] or at least 2 cups per day[8, 9], did not observe an association between tea consumption and risk of diabetes, whereas one study, in which the highest tea consumption category was more than 5 cups per day, observed a substantial lower risk.[10] In contrast, one study did not observe an association, even though the highest category of tea consumption included participants who drank at least 4 cups per day.[9] So, to date it is unclear whether or how tea consumption is associated with risk of type 2 diabetes.

Therefore, we investigated the association between tea consumption and incidence of type 2 diabetes in European citizens who were part of the EPIC-InterAct study. The size of the study is comparable with the meta-analyses reported to date on this topic[6, 11] and provides the opportunity to explore a potential non-linear association between tea consumption and risk of type 2 diabetes across European countries.

Methods

Ethics statement

The study complied with the Declaration of Helsinki. The Internal Review Board of the International Agency for Research on Cancer and the Institutional Review Board of all centres, i.e., France, Heidelberg, Potsdam, Copenhagen, Aarhus, Asturias, Granada, San Sebastian, Murcia, Navarro, Cambridge, Oxford, Imperial, Florence, Milan, Ragusa, Turin, Naples, Bilthoven, Utrecht, Malmö, and Umeä, approved the EPIC study. Written consent was obtained from each EPIC participant at enrolment into the study.

Study population

The EPIC-InterAct study is a case-cohort study embedded in the EPIC study. The EPIC study is a prospective study conducted in 10 European countries.[12] Eight countries also participated in the EPIC-InterAct study (Spain, Italy, Sweden, France, Denmark, Germany, The Netherlands, and United Kingdom), with a total of 26 centres. The rationale and design of the EPIC-InterAct study has been described in detail elsewhere.[13] In short, a centre-stratified random sample of 16,835 participants, aged 20-79 years, was taken as sub-cohort. Subsequently, a number of 548 participants with prevalent diabetes and 133 with unknown diabetes status were excluded,

resulting in 16,154 participants. After verification of all eligible EPIC participants for diabetes incidence, 12,403 verified cases were obtained of which 778 belongs to the sub-cohort.

Population for analysis

Our analysis included 26,039 participants of the 27,779 participants included in the EPIC-InterAct study, because we excluded in consecutive order 117 participants without dietary data, 619 with unreliable food intake data (top and bottom 1% of the distribution of energy intake to basal energy requirement assessed by WHO/FAO/UNU equation including weight and height)[14], and 955 with missing information about potential confounders which were included in the final model (289 without physical activity measurements, 134 without information on smoking status, 367 without information on educational status, and 165 without information on body mass index (BMI)). Furthermore, since few participants from Spain (*n*= 39) and Italy (*n*= 10) drank ≥4 cups of tea per day, country-specific hazard ratios (HR) comparing ≥4 vs. 0 cups per day could not be obtained in Spain and Italy. Therefore, those 49 participants were also excluded. The final sample included 11,541 cases and 15,277 sub-cohort members, including 729 cases in the sub-cohort.

Dietary intake including tea consumption

Dietary intake over the last 12 months was assessed by country-specific or centre-specific semi-quantitative or quantitative dietary questionnaires, validated within each country.[12] More information about the questionnaires can be found elsewhere.[12] All questionnaires included at least one question about consumption of tea. Each centre converted the information about tea consumption into grams per day. For the analysis, tea consumption in grams per day was divided by 125 to be able to calculate HR by cups per day. In line with the meta-analysis by Jing *et al.*[6], the frequency of tea consumption was divided into 4 categories: 0, >0-<1, 1-<4, ≥4 cups per day.

Diabetes incidence

A pragmatic, high sensitivity approach for case ascertainment was used in order to identify all potential incident type 2 diabetes cases and excluding all individuals with prevalent diabetes.[13] Briefly, ascertainment of incident diabetes involved a review of the existing EPIC datasets at each centre using multiple sources of evidence including self-report, hospital admissions, linkage to primary care registers, linkage to secondary care registers, linkage to drug registers, and mortality data. Cases in Denmark and Sweden were not ascertained by self-report, but via diabetes and pharmaceutical registers. Hence, all ascertained cases were considered to be verified. To increase the ability to exclude false negatives for countries other than those from Denmark and Sweden, we sought further evidence for all cases with information on incident type 2 diabetes from less than 2 independent sources which have been described in detail elsewhere.[13] Follow-up was censored at the date of diagnosis, the 31st of December 2007, or the date of death, whichever occurred first.

Non-dietary covariates

Socio-demographic and lifestyle information, e.g., age, sex, education level, smoking status, and physical activity during work and leisure time[15], was obtained with questionnaires at baseline.

Questionnaires were also used to obtain information about diseases of the participant and his family, i.e., history of angina pectoris (not in The Netherlands, Sweden, and one centre in Germany), history of myocardial infarction, history of stroke (not in one centre in Sweden), presence and/or treatment for hypertension, presence and/or treatment of hyperlipidemia (not in

one centre in Sweden), and family history of type 2 diabetes (not in Spain, Italy, one centre in Germany, and one centre in the United Kingdom).

Information about height and weight was obtained using a standard protocol during a visit at the research centre at baseline for all participants, except in France and in some of the participants from one centre in the United Kingdom. Self-reported or corrected height and weight were used in those centres without measured height and weight.[12]

Statistical analysis

The association between tea consumption (categorized as 0, >0-<1, 1-<4, ≥4 cups/day) and risk of type 2 diabetes was examined by country using modified Cox proportional hazard models with age as underlying time scale. The models were modified for the case-cohort design according to the Prentice method.[16] In order to adjust for time to follow-up, age at recruitment (1-year categories) was included as stratum variable. Summary HR and 95% confidence intervals (95%CI) were obtained by pooling country-specific HR using random effects meta-analyses and visualized in forest plots. Between country heterogeneity was assessed by I^2 statistic, i.e., the percentage of variation in the HR attributable to between country heterogeneity.[17]

To obtain adjusted country-specific HR, four Cox models were constructed. Variables included in these models were considered main potential confounders in the association between tea consumption and risk of type 2 diabetes based upon literature. Model 1 included, in addition to tea consumption, four other covariates: sex, smoking status (never, former, current), physical activity level (inactive, moderately inactive, moderately active, and active), and education level (lowest, secondary, and highest). Model 2 was similar to model 1 with additional adjustments for energy and intake of seven nutrients: protein (energy-%), carbohydrates (energy-%), saturated fatty acids (energy-%), mono-unsaturated fatty acids (energy-%), poly-unsaturated fatty acids (energy-%), alcohol (0, >0-6, >6-12, >12-24, and >24 g/day), and fibre (g/day). Model 3 was similar to model 2 with additional adjustment for intake of drinks: coffee (g/day), juices (g/day), soft-drinks (g/day), and milk (g/day). Model 4 was similar to model 3 with additional adjustment for BMI (kg/m^2).

After investigating the association between tea consumption as a categorical variable and risk of type 2 diabetes, the dose-response of the association was further explored by studying linear trends across categories, by restricted cubic spline regression, and by studying consumption of tea (cups per day) as a continuous variable. To test for linear trends across categories, the median value of each category of tea consumption was modelled as a continuous variable. The restricted cubic spline regression was performed using SAS Macro RCS, which was also based on the modified Cox proportional hazard regression. The knots were located at 1, 4, 7 cups per day and non-tea drinkers were used as reference group. This analysis was adjusted as described for model 4.

Potential effect modification was investigated by including an interaction term between tea categories and sex or tea categories and BMI categories into the models and by studying the association between tea consumption and risk of type 2 diabetes by sex (*men*: *n*= 11,249; *women*: *n*= 15,030) and by BMI (*normal*: BMI <25.0 kg/m^2: *n*= 8,267; *overweight*: BMI ≥25.0-<30.0 kg/m^2: *n*= 10,840; *obese*: BMI ≥30.0 kg/m^2: *n*= 6,932).

To investigate the robustness of the associations, sensitivity analyses were performed by excluding one by one participants for each of the following diseases at baseline: a history of stroke (*n*= 261), a history of angina pectoris (*n*= 521), a history of a myocardial infarction (*n*= 545), hypertension (*n*= 5,682), and hyperlipidemia (*n*= 4,362). Furthermore, participants with a family history of diabetes (*n*= 2,928) and who developed type 2 diabetes within 2 years (*n*= 955) were

excluded in the sensitivity analyses. We excluded these participants in sensitivity analysis, because they may have changed their diet recently. In centres which did not obtain information about disease history, participants were considered as not having the disease at baseline.

Analyses were carried out using the statistical software program SAS version 9.2, except for the random effects meta-analyses which were conducted in STATA 11.0. A two-sided *p*-value

Table 4.1 Characteristics of the sub-cohort of the EPIC-InterAct study by categories of tea consumption (*n*= 15,227)[a]

	Tea intake (cups/day)			
	None	**>0-<1**	**1-<4**	**≥4**
	(*n*= 5,458)	**(*n*= 4,032)**	**(*n*= 3,444)**	**(*n*= 2,293)**
Age (years)	51.7 (8.6)	51.4 (9.1)	53.0 (9.7)	54.7 (8.8)
Sex (% men)	40.5	41.3	32.7	33.6
Body mass index (kg/m^2)	27.0 (4.3)	25.8 (4.0)	25.5 (4.0)	25.0 (3.8)
Country (%)				
Spain	83.0	7.5	9.5	0
Italy	43.1	44.2	12.8	0
Sweden	33.6	30.5	28.2	7.7
France	36.7	21.1	27.4	15.1
Denmark	17.3	38.8	9.8	34.1
Germany	6.1	39.6	36.5	17.8
Netherlands	8.3	18.6	42.1	31.0
United Kingdom	5.2	8.9	39.0	46.9
Smoking (% current)	31.9	27.5	19.6	18.8
Education level (% high)	13.7	21.7	25.3	28.8
Physical activity (% inactive)	31.6	20.1	20.4	15.6
Hypertension (%)	17.5	19.1	20.3	17.4
Hyperlipidaemia (%)[b]	17.9	15.8	17.0	10.3
Family history of diabetes (%)[c]	13.9	17.1	16.9	17.9
Stroke (%)[d]	0.7	0.9	1.1	0.7
Angina pectoris (%)[e]	1.3	2.4	3.1	2.4
Myocardial infarct (%)	1.1	1.4	1.6	1.7
Dietary intake				
Total energy (kcal/day)	2188 (662)	2140 (645)	2067 (591)	2124 (603)
Protein (en-%)	18 (3)	16 (3)	17 (3)	17 (3)
Carbohydrates (en-%)	42 (7)	45 (7)	45 (7)	46 (7)
Fat (en-%)				
Total	35 (6)	35 (6)	35 (6)	34 (6)
Saturated fatty acids	12 (4)	14 (3)	14 (3)	14 (3)
Mono-unsaturated fatty acids	15 (4)	13 (3)	12 (3)	11 (2)
Poly-unsaturated fatty acids	6 (2)	5 (2)	6 (2)	6 (2)
Fibre (g/day)	23 (8)	22 (7)	22 (7)	25 (8)
Alcohol (% >24 g/day)	22.6	19.9	15.1	15.5
Coffee (g/day)	154 (60-400)	400 (130-700)	363 (125-525)	375 (86-500)
Soft drinks (g/day)	0 (0-29)	16 (0-86)	14 (0- 90)	16 (0-90)
Juices (g/day)	1 (0-25)	22 (3-94)	40 (4-120)	29 (3-100)
Milk (g/day)	180 (61-300)	138 (25-271)	150 (25-295)	193 (36-387)

[a] Values were expressed as mean (standard deviation), median (p25-p75), or percentage.
[b] Based on *n*= 13,345, because information about hyperlipidemia was not collected in one centre of Sweden.
[c] Based on *n*= 8,802, because information about family history of diabetes was not collected in Italy, Spain, one centre of Germany, and one centre of the United Kingdom.
[d] Based on *n*= 14,262, because information about a history of stroke was not collected in one centre of Sweden.
[e] Based on *n*= 10,168, because information about a history of angina was not collected in The Netherlands, Sweden, and one centre of Germany.

≤0.05 was considered as statistically significant for all analyses.

Results

Overall, 64% of this European study population reported that they drank tea. The highest median total tea consumption was observed in the United Kingdom (3.8 (interquartile range (IQR) 3.8-6.8) cups per day); the lowest in Spain (0.0 (IQR 0-0) cups per day) (**Figure 4.1**). The median total tea consumption among drinkers was 1.2 (IQR 0.3-3.7) cups per day. In general, participants with higher tea consumption had a lower BMI, had a higher level of education, and smoked less (**Table 4.1**). Intake of carbohydrates and saturated fatty acids was higher, whereas intake of mono-saturated fatty acids was lower across tea categories. Tea drinkers drank less alcohol, but more coffee, soft drinks, and juices than non-tea drinkers.

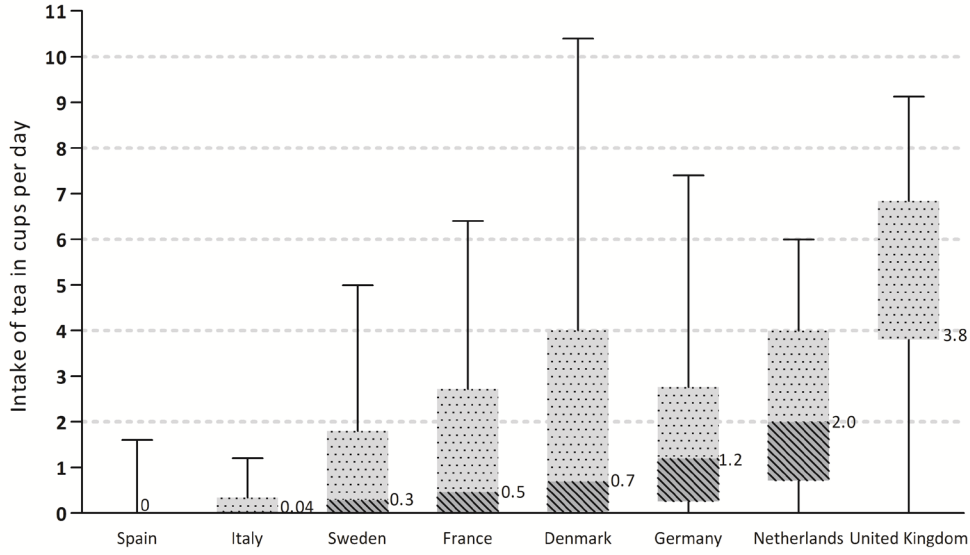

Figure 4.1 Intake of tea based on data from a food frequency questionnaire in the sub-cohort of the EPIC-InterAct study by country (*n*= 15,227).
Bar represents median (p25-p75); error line represent p5 till p95.

When tea consumption was divided into categories and country-specific HR were combined, the crude analysis showed that, for all categories, participants who drank tea had a lower risk of developing type 2 diabetes compared with non-tea drinkers (**Table 4.2**). The risk estimates for type 2 diabetes in all categories of tea consumption were attenuated slightly in model 1. Additional adjustment for the intake of nutrients (model 2) and drinks (model 3) did not affect the risk estimates. Adjustment for BMI (model 4), however, attenuated the risk estimates further, but risk of type 2 diabetes was still 16% lower in participants drinking at least 4 cups of tea per day compared with non-tea drinkers ($HR_{\geq 4\ cups/day\ vs.\ 0}$= 0.84 [95%CI 0.71, 1.00]; $p_{linear\ trend}$= 0.04). Risk of type 2 diabetes already tended to be lower among participants drinking 1 to 4 cups per day compared with non-tea drinkers (Model 4, $HR_{1-<4\ cups/day\ vs.\ 0}$= 0.93 [95%CI 0.81, 1.05]) (**Table 4.2; Figure 4.2**). No evidence of between country heterogeneity was observed in any category of tea consumption (**Figure 4.2**).

The association was further explored by performing spline regression and by studying tea

consumption as continuous variable. Cubic spline regression confirmed that risk of type 2 diabetes may be lower with higher intake of tea, although it suggests that the risk may level off after 5 cups per day ($p_{\text{non-linearity}}$= 0.20; **Figure 4.3**). Since the risk reduction may level off, the association between tea consumption on a continuous scale and type 2 diabetes was restricted to participants drinking 5 cups per day or less (*n*= 23,778). This analysis suggested that 1 cup of tea per day was associated with a 3.1% lower risk, which was nearly statistically significant (Model 4, HR 0.97 [95%CI 0.94, 1.00], *p*= 0.06).

Stratified analyses showed that effect modification was not observed for sex (Model 4, $p_{\text{interaction}}$= 0.14, *men*: $HR_{\geq 4 \text{ cups/day vs. } 0}$= 0.89 [95%CI 0.71, 1.10], *women*: $HR_{\geq 4 \text{ cups/day vs. } 0}$= 0.82 [95%CI 0.58, 1.16]) and for BMI (Model 4, $p_{\text{interaction}}$= 0.26, *normal weight*: $HR_{\geq 4 \text{ cups/day vs. } 0}$= 0.79 [95%CI 0.52, 1.19], *overweight*: $HR_{\geq 4 \text{ cups/day vs. } 0}$= 0.87 [95%CI 0.63, 1.20], *obese*: $HR_{\geq 4 \text{ cups/day vs. } 0}$= 0.79 [95%CI 0.59, 1.05]).

None of the sensitivity analyses changed the results substantially (data not shown).

Table 4.2 Hazard ratios and 95% confidence intervals for incident type 2 diabetes by categories of tea consumption (*n*= 26,039)[a]

	Median	*n* (cases)	Crude HR [95%CI]	Model 1[b] HR [95%CI]	Model 2[c] HR [95%CI]	Model 3[d] HR [95%CI]	Model 4[e] HR [95%CI]
Tea (cups/day)							
0	0	9,499 (4,389)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
>0-<1	0.2	7,060 (3,197)	0.89 [0.80, 0.99]	0.93 [0.81, 1.07]	0.96 [0.84, 1.10]	0.97 [0.85, 1.10]	1.03 [0.91, 1.16]
1-<4	2.0	5,751 (2,437)	0.77 [0.66, 0.90]	0.83 [0.69, 0.99]	0.85 [0.71, 1.01]	0.84 [0.72, 0.98]	0.93 [0.81, 1.05]
≥4	6.8	3,729 (1,518)	0.63 [0.50, 0.80]	0.68 [0.52, 0.90]	0.72 [0.53, 0.96]	0.70 [0.54, 0.90]	0.84 [0.71, 1.00]
p for trend			*<0.01*	*<0.01*	*<0.01*	*<0.01*	*0.04*

Abbreviations: HR=hazard ratio; 95%CI=95% confidence interval

[a] HR and 95%CI were derived from a modified Cox proportional hazard model by age at baseline and are based on pooled estimates from country specific analyses using a random effects meta-analysis.

[b] Model adjusted for sex, smoking, physical activity, and education level.

[c] Model 1 with additional adjustments for intakes of energy, protein, carbohydrates, saturated fatty acids, mono-unsaturated fatty acids, poly-unsaturated fatty acids, alcohol, and fibre.

[d] Model 2 with additional adjustments for intakes of coffee, juices, soft drinks, and milk.

[e] Model 3 with additional adjustments for body mass index.

Discussion

In this large European population, a linear inverse association was observed between tea consumption and incidence of type 2 diabetes. This significant linear association and the spline regression suggests that a threshold of drinking at least 4 cups of tea per day to lower risk of type 2 diabetes does not appear to exist.

Strengths of our analyses included the ability to study the association between tea consumption and risk of type 2 diabetes in populations from eight European countries, resulting in a larger variation of tea consumption than when these countries were analysed separately. Our results were also strengthened by the standardized protocol to verify cases of diabetes among countries and to process information about lifestyle and dietary factors. As such, consumption of tea was converted into grams per day for all countries, through which we were able to standardize cup size among countries in the analysis.

Our observation that drinking tea was associated inversely with risk of type 2 diabetes,

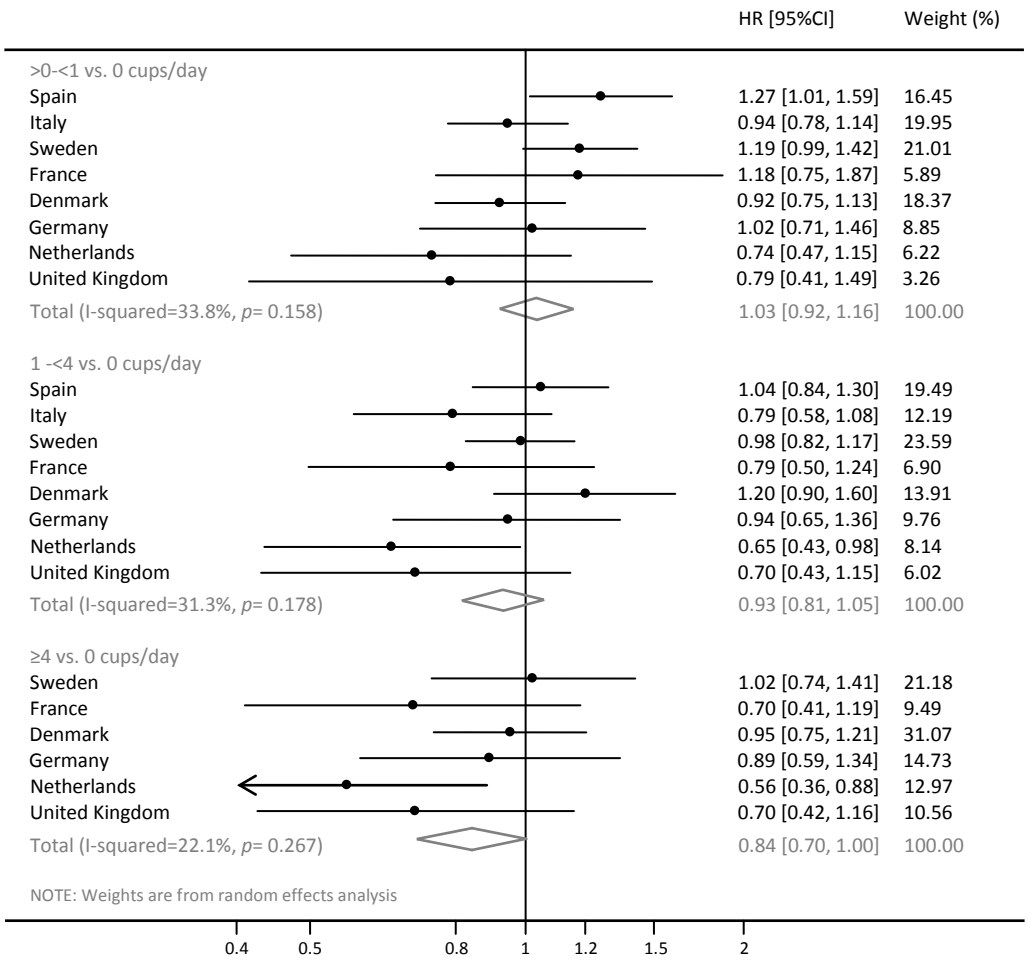

Figure 4.2 Association between intake of tea as a categorical variable (>0-<1 vs. 0, 1-<4 vs. 0, ≥4 vs. 0 cups/day) based on data from a food frequency questionnaire and incident type 2 diabetes (*n*= 26,039) Country-specific hazard ratios (HR) and 95% confidence intervals (95%CI) were pooled using random effects meta-analyses. HR were adjusted for sex, smoking, physical activity, education level, intakes of energy, protein, carbohydrates, saturated fatty acids, mono-unsaturated fatty acids, poly-unsaturated fatty acids, alcohol, fibre, coffee, juices, soft drinks, milk, and body mass index.

was in line with the observation of two published meta-analyses equalling the power of our study.[6, 11] We observed a protective association on risk of type 2 diabetes with habitual tea consumption of at least 4 cups per day, Jing *et al.*[6] also with at least 4 cups per day, and Huxley *et al.*[11] with at least 3-4 cups per day. In line with the meta-analysis by Jing *et al.*, we observed that tea drinkers who drank <1 cups per day had a similar risk of type 2 diabetes as non-tea drinkers. Risk of type 2 diabetes, however, already tended to be lower with 1-<4 cups of tea per day in our analysis.

Together with the results of the spline regression, our results, therefore, do not support that the protective effect of tea consumption is restricted to participants reporting the highest intake of tea. Therefore, even a smaller amount may lower risk of type 2 diabetes.

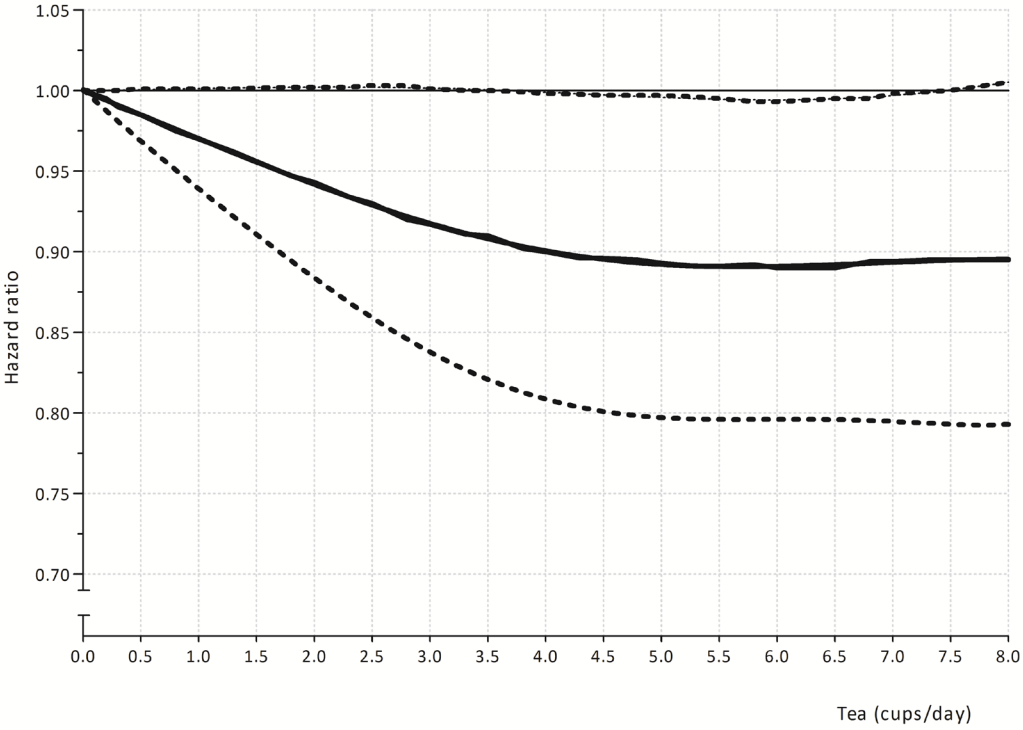

Figure 4.3 Association between intake of tea based on data from a food frequency questionnaire and risk of type 2 diabetes obtained by spline regression with 3 knots (1, 4, 7 cups/day) and 0 cups/day as reference.

Dotted lines represent 95% confidence intervals. $p_{\text{non-linearity}} = 0.20$. Hazard ratios were adjusted for sex, smoking, physical activity, education level, intakes of energy, protein, carbohydrates, saturated fatty acids, mono-unsaturated fatty acids, poly-unsaturated fatty acids, alcohol, fibre, coffee, juices, soft drinks, milk, and body mass index.

Our spline regression suggests that the risk reduction levelled off at around 5 cups per day. Potential mechanisms, however, explaining the observed plateau around 5 cups per day are not established. Furthermore, we could not study country-specific associations between a very high tea consumption (>7 cups per day) and risk of type 2 diabetes in a sufficient number of participants in our analysis. We already had to exclude Spain and Italy from the highest tea category, because of small numbers in this category. The biological mechanism underlying a beneficial effect of tea, however, is unlikely to differ between Northern and Southern European countries.

The flavonoids present in tea are of importance, because the beneficial effect of tea on risk of type 2 diabetes may be attributable to these components.[4, 5] Catechins, theaflavins, and thearubigins are the most prominent flavonoids in tea. These flavonoids, predominately epigallocatechin gallate (EGCG), have been shown to slow down carbohydrate digestion, to inhibit carbohydrate absorption by competitively binding with the sodium-glucose transporter-1 (SGLT-1), to increase glucose uptake in muscle and fat cells by changes in GLUT-4 expression, to enhance insulin secretion, and to protect beta-cells from free-radical damage.[4, 5] All these pathways can affect glucose concentrations and, thereby, could explain a beneficial effect of tea consumption on risk of type 2 diabetes. Most randomized controlled trials investigating the association

between long-term tea consumption and markers of glucose of insulin metabolism, however, showed no associations.[18-23]

Studies investigating the association between tea consumption and risk of type 2 diabetes may differ by several factors, such as the type of tea consumed, the preparation method used, the cup size used, and the sample size under investigation. The main type of tea consumed may result in discrepancies among studies and among countries in our study, because types of tea differ in chemical composition. Herbal teas may contain less anti-oxidants than black and green tea, because herbal teas, in contrast to black or green tea, are not derived from the *Camellia Sinensis* plant.[24] The difference in anti-oxidant capacity may indicate that the beneficial effect of drinking tea is stronger for black or green tea than for herbal tea. It is likely that tea consumption in our study mainly reflects the intake of black tea, because at the time of dietary data collection in this study, green tea and herbal tea were not as popular as black tea in most countries. Unfortunately, we could not investigate whether the type of tea consumed affects risk of type 2 diabetes differently, because detailed information about type of tea was not collected in most countries.

The preparation method used may also result in discrepancies. The preparation method, including brewing time and substances added, can influence the amount of flavonoids present in a cup of tea[25, 26] and, consequently, the association between tea consumption and risk of type 2 diabetes. Studies, therefore, might find an association at 1-<4 cups of tea per day if the brewing time is long in general, whereas other studies might not find an association if the brewing time is short. Since information about brewing time was lacking in all published studies, including ours, it was not possible to adjust for brewing time. As brewing time was not reported in our study, non-differential misclassification of actual amount of tea consumption could also have occurred within countries. Besides the short brewing time, adding milk may also lower the bioavailability of flavonoids due to the interaction between milk proteins and flavonoids present in tea.[27] Five out of six trials, however, showed that adding milk to tea did not affect the bioavailability of tea flavonoids or anti-oxidant capacity after consumption.[26, 28-32] Furthermore, the beneficial effect of tea consumption on risk of type 2 diabetes may be counterbalanced by the addition of sugar. Since we did not observe heterogeneity among countries in our study, we do not think that differences in preparation methods among countries have influenced our results.

In our study, tea consumption was associated with a healthier lifestyle, e.g., people who drank tea were more physically active and smoked less than who did not drink tea. This may indicate that the inverse association between tea consumption and risk of type 2 diabetes reflects a healthier lifestyle rather than tea consumption itself. In our analysis, however, we tried to disentangle the effect of a healthier lifestyle from tea consumption by adjusting the HR for a range of lifestyle and dietary factors, and by excluding people with chronic diseases at baseline in sensitivity analyses. Inclusion of lifestyle and dietary factors except BMI, however, did not change the risk estimates much. This might indicate that the effect of a healthier lifestyle could not be adequately adjusted for due to measurement error in these factors. Even though inclusion of BMI into the model attenuated the association between tea consumption and risk of type 2 diabetes, residual confounding by BMI might also be present. As BMI could also be considered as intermediate[33], however, caution should be taken when interpreting the model including BMI.

Furthermore, the inclusion of only clinical cases of diabetes rather than undiagnosed cases of diabetes might have limited our results if the prevalence of undiagnosed cases of diabetes differed substantially among tea categories. We have no indication, however, that this differential misclassification in the outcome was likely.

In conclusion, we observed a linear inverse association between tea consumption and incidence of type 2 diabetes. People who drink at least 4 cups of tea per day may have a 16%

lower risk of developing type 2 diabetes than non-tea drinkers. Whether consumption of all types of tea is associated similarly with reduction in risk of type 2 diabetes and whether this association is causal should be further investigated.

Acknowledgments

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Abstract

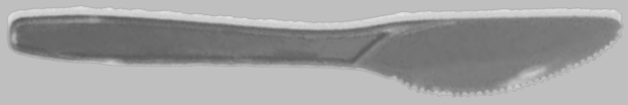
Objective: To investigate whether intake of different types of meat is associated with circulating C-reactive protein (CRP) and risk of type 2 diabetes in a prospective cohort study.

Methods: Our analysis included 4,366 Dutch participants who did not have diabetes at baseline. During a median follow-up period of 12.4 years, 456 diabetes cases were confirmed. Intake of red meat, processed meat, and poultry was derived from a food-frequency questionnaire and their association with serum high-sensitivity CRP was examined cross-sectionally using linear regression models. Their association with risk of type 2 diabetes was examined using multivariate Cox proportional hazards models, including age, sex, family history of diabetes, and lifestyle and dietary factors.

Results: An increment of 50 gram of processed meat was associated with increased CRP concentration ($beta_{\text{processed meat}} = 0.12$; $p = 0.01$), whereas intake of red meat and poultry were not. When comparing the highest to the lowest category of meat intake with respect to diabetes incidence, the adjusted hazard ratios were as follows: for red meat 1.42 [95% confidence interval (95%CI) 1.06, 1.91], for processed meat 1.87 [95%CI 1.26, 2.78], and for poultry 0.95 [95%CI 0.74, 1.22]. Additional analysis showed that the associations were not affected appreciably after inclusion of CRP into the model. After adjustment for BMI, however, the association for red meat attenuated to 1.18 [95%CI 0.88, 1.59].

Conclusion: Intake of processed meat is associated with higher risk of type 2 diabetes. It appears unlikely that CRP mediates this association.

CHAPTER 5



Meat consumption and its association with C-reactive protein and risk of type 2 diabetes: the Rotterdam study

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Introduction

Since the prevalence of type 2 diabetes has increased rapidly over the last decades, investigations into the effect of dietary and other lifestyle factors on type 2 diabetes have become important.[1] One of the dietary factors of interest is meat. Three meta-analyses of prospective cohort studies showed that intake of processed meat is associated with a higher risk of type 2 diabetes.[2-4] For red meat, two of these meta-analyses observed an adverse association[2, 4], whereas one did not[3]. For poultry, no data from meta-analyses were available. Results from six prospective studies on poultry, however, showed that it is not likely that poultry is associated with a higher risk of type 2 diabetes; three studies observed an inverse association[5-7], whereas three did not observe an association[8-10].

Intake of red meat and processed meat may increase risk of type 2 diabetes by mechanisms that increase circulating pro-inflammatory markers. Positive associations have been observed between red meat or processed meat and the pro-inflammatory blood marker C-reactive protein (CRP), which in turn has been associated with higher risk of type 2 diabetes.[11-13] The positive association between intake of meat and CRP might be explained by several biological pathways. The binding capacity of iron in the body could be exceeded by the intake of meat, which contains high amounts of heme iron. Free iron can increase oxidative stress, thereby acting as pro-inflammatory agent.[14] Advanced glycation end products (AGEs), which occur naturally in meat and are formed through heat processing[15], may also have pro-inflammatory actions[16]. Thus, the observed positive associations between intake of red meat and processed meat and CRP and CRP and risk of type 2 diabetes, may indicate that CRP mediates the association between intake of meat, especially red and processed meat, and risk of type 2 diabetes.

Therefore, we investigated whether intake of red meat, processed meat, and poultry was associated with CRP and risk of type 2 diabetes in a Dutch population.

Methods

Population for analysis

The current analysis was conducted within the Rotterdam study. The Rotterdam study is a population-based prospective cohort study among inhabitants of Ommoord, a district of the city of Rotterdam, The Netherlands.[17] In 1990, all inhabitants of this district who were aged ≥55 years were invited for participation (*n*= 10,215). Of the 7,983 responders (78%), 2,339 did not fill out a dietary questionnaire, 209 did not provide sufficient dietary data, 516 had type 2 diabetes at baseline, 448 had not sufficient data on CRP, and 105 had not sufficient information on follow-up time or other covariates (**Figure 5.1**). Hence, 4,366 participants were included in the current analysis.

Compared with participants who were included in the analysis, participants who were excluded tended to be older (74.6 (SD 10) vs. 67.3 (SD 8), *p*= <0.01), smoked less (22% vs. 23%, *p*= <0.01), and were less likely to be men (37% vs. 40%, *p*= 0.01), whereas BMI does not appear to be different (26.3 (standard deviation (SD) 4) vs. 26.3 (SD 4), *p*= 0.51). The association between CRP and risk of type 2 diabetes was studied in 4,092 participants because we excluded participants with missing data on waist circumference (*n*= 253), systolic and diastolic blood pressure (*n*= 17), and high-density lipoprotein (HDL) cholesterol (*n*= 4) (**Figure 5.1**). The Medical Ethics Committee of Erasmus Medical Centre approved the study. All participants gave informed consent.

Meat intake and other dietary covariates

Dietary assessment was performed at baseline (1990-1993) and comprised two steps: first, participants had to mark the foods and drinks they had consumed at least twice a month in the

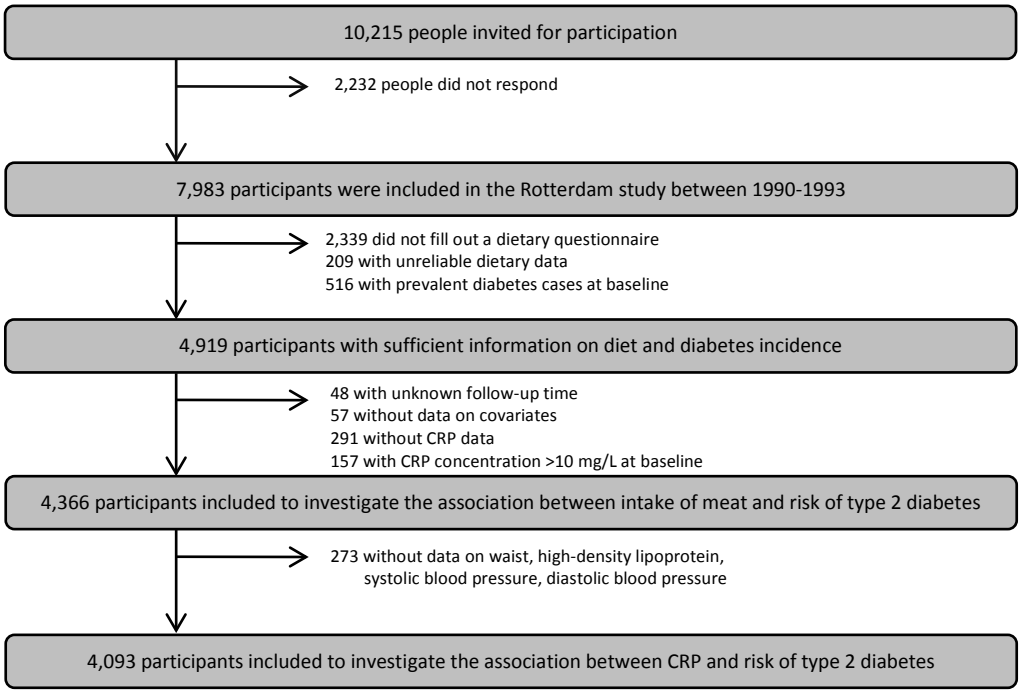

Figure 5.1 Flow diagram for inclusion of participants to investigate whether the intake of meat is associated with serum C-reactive protein (CRP) and with risk of type 2 diabetes

preceding year on a self-administered questionnaire at home; and second, at the research centre, a trained dietician obtained accurate information on the amount of foods and drinks indicated on the questionnaire using a semi-quantitative food frequency questionnaire.[18] This food frequency questionnaire comprised 170 food items in 13 food groups and additional questions about prescribed diets.

The food items included the intake of meat products, through which the intake of red meat, processed meat, and poultry could be calculated in grams per day. Total meat included red meat (e.g., beefsteak, pork fricandeau), processed meat (e.g., sausage, cold cuts), and poultry (i.e., chicken). Processed meat included meats that are preserved by smoking, curing, salting, or addition of preservatives. For the analysis, types of meat were adjusted for energy according to the residual method.

Intake of all food items was converted into total intake of energy and nutrients using the Dutch Food Composition table 1993 (NEVO). Intake of fibre was derived from the next version of this table (NEVO 1996), because data on fibre were not sufficient in 1993.

C-reactive protein

Non-fasting serum samples were collected at the research centre at baseline. These samples were immediately put on ice and processed within 30 minutes. High-sensitivity CRP was measured using a rate near-infrared particle immunoassay (Immage Immunochemistry System, Beckman Coulter, Fullerton, CA). The procedure has been described in more detail elsewhere.[19] CRP concentrations >10 mg/L at baseline were excluded because these higher concentrations reflect rather acute than chronic inflammation.

Diabetes prevalence and incidence

Participants were considered a prevalent diabetes case when they used anti- diabetes medication or had a non-fasting or post-load glucose concentration of ≥11.1 mmol/L.[20]

During follow-up, information from general practitioners, pharmacies' databases, and follow-up examinations in 1993-1995, 1997-1999, 2002-2004 was used to identify cases of diabetes. Participants were considered incident diabetes case when they were registered by a general practitioner as having type 2 diabetes and had at least one of the following four criteria: plasma glucose concentration ≥7.0 mmol/L, random plasma glucose concentration ≥11.1 mmol/L, anti-diabetes medication, and/or treatment by diet. Diabetes cases were monitored until July 2005.

Non-dietary covariates

General information (e.g., smoking status, education level, family history of type 2 diabetes) was obtained with a questionnaire at baseline. A family history of type 2 diabetes was defined as having a parent, sibling, or both with type 2 diabetes. Information on energy expenditure (kcal/day) was obtained with a physical activity questionnaire (Longitudinal Aging Study Amsterdam Physical Activity Questionnaire) during follow-up from 1997 to 2000 for 3,244 participants of our study population.[21] Consequently, energy expenditure could be used as measure of physical activity in those participants.

Information on cardiovascular risk factors of each participant was obtained by clinical examinations during a visit at the research centre at baseline. Height and weight were measured and BMI (kg/m²) was calculated. Waist circumference (cm) was measured at the level midway between the lower rib margin and the iliac crest with the participant in standing position. Blood pressure was measured twice at the right brachial artery with a random-zero sphygmomanometer with the participant in a sitting position. The mean of two consecutive measurements was used. Hypertension was defined as a systolic blood pressure ≥140 mmHg, and/or diastolic blood pressure ≥90 mmHg, and/or use of blood pressure-lowering medication.

Serum total cholesterol was determined in blood samples with an automated enzymatic procedure using Roche CHOD-PAP reagent agent. HDL cholesterol was measured with Roche HDL cholesterol essay using polyethylene glycol-modified enzymes and dextran sulphate.

Data analysis

Descriptive data were expressed as a mean (standard deviation (SD), a median (p25-p75), or a percentage by the lowest and highest category of types of meat intake.

The association between intake of energy-adjusted types of meat per 50 gram increase and $\log_e$(CRP) was investigated using linear regression models. CRP was transformed logarithmically to achieve a symmetric distribution. Adjustments were made for age (years), sex, family history of diabetes (yes or no), diet prescription (yes or no), smoking (current, former or never), intake of energy (kcal/day), intake of energy-adjusted carbohydrates (g/day), intake of energy-adjusted poly-unsaturated fatty acids (g/day), intake of alcohol (0, >0-10, >10-20, or >20 g/day), intake of energy-adjusted fibre (g/day), intake of energy-adjusted milk products (g/day), intake of energy-adjusted cheese (g/day), intake of soya (consumers or non-consumers), intake of fish (non-consumers and approximate tertiles), and intake of tea (g/day).

The association between CRP and risk of type 2 diabetes, as shown previously in the Rotterdam study (*n*= 5,901)[19], was verified in our subpopulation of the Rotterdam study (*n*= 4,093; n_{cases}= 423; *median follow-up time*= 11.0 years). Adjustments were made for age (years), sex, BMI (kg/m^2), waist circumference (cm), HDL cholesterol (mmol/L), systolic blood pressure (mmHg), and

diastolic blood pressure (mmHg).

After obtaining the associations between intake of meat and CRP and CRP and risk of type 2 diabetes, we could further study the potential mediating effect of CRP on the association between energy-adjusted types of meat and risk of type 2 diabetes (*n*= 4,366; n_{cases}= 456; *median follow-up time*= 12.4 years).

Intake of red meat was divided into quartiles based on the population distributions of intake. As processed meat and poultry was not used by a considerable number of participants, intake of processed meat and poultry were divided into four categories: non-consumers and approximate tertiles based on the population distributions of intake. After testing the proportional hazards assumption, Cox proportional hazards models were used to calculate hazard ratios (HR) and 95% confidence intervals (95%CIs). The HR expressed the risk relative to the lowest category. The crude model included the intake of meat as independent variable, but was not adjusted for any covariate. In addition to intake of meat, model 1 included five other covariates as follows: age (years), sex, family history of diabetes (yes or no), diet prescription (yes or no), and smoking (current, former, or never). Model 2 was similar to model 1 with additional adjustment for intake of five dietary factors and five food products as follows: energy (kcal/day), energy-adjusted carbohydrates (g/day), energy-adjusted poly-unsaturated fatty acids (g/day), alcohol (0, >0-10, >10-20, or >20 g/day), energy-adjusted fibre (g/day), energy-adjusted milk products (g/day), energy-adjusted cheese (g/day), soya (consumers or non-consumers), fish (non-consumers and approximate tertiles), tea (g/day). Categories of meat were mutually adjusted.

To investigate the potential mediating effect of CRP on the association between types of meat and type 2 diabetes, baseline $\log_e$(CRP) (mg/L) was added to model 2 as additional covariate. Additional to $\log_e$(CRP), BMI (kg/m^2) was included in second additional model.

To investigate potential effect measure modification by sex or BMI, an interaction term between types of meat and sex or BMI was included in model 2 with additional adjustment for $\log_e$(CRP) and BMI. Sensitivity analyses were performed by excluding vegetarians (*n*= 33), participants who developed type 2 diabetes within 2 years of follow-up (*n*= 437), or participants with coronary heart disease at baseline (*n*= 514), but none of them changed the interpretation of the results.

Tests for trend across categories were performed by assigning the median value for each category to each participant and modelling this variable as a continuous variable.

All statistical analyses were performed in SAS (version 9.2, SAS Institute, Cary, NC). A two-sided *p*-value ≤0.05 was considered as statistically significant for all analyses.

Results

Meat was eaten by nearly all participants (99.2%). Red meat was the main type consumed (98.7%), followed by processed meat (88.2%), and poultry (75.6%). The relative contribution of red meat to total meat was 68%, of processed meat 19%, and of poultry 13%. Mean total meat intake was 112 gram daily (SD 45). The highest category of red meat and processed meat included more men, smokers, participants with a larger BMI, and soya eaters than the lowest category (**Table 5.1**). In contrast, the highest category of poultry included fewer smokers and soya eaters than the lowest category, and the sex distribution did not differ between the highest category and lowest category of poultry (**Table 5.1**).

In line with the higher CRP concentration observed in the highest category of processed meat intake compared with the lowest (1.7 vs. 1.5 mg/L) (**Table 5.1**), linear regression analysis showed that a 50 gram higher intake of processed meat was associated with a higher CRP concentration after adjustment for dietary factors and other lifestyle factors (model 2) (**Table 5.2**).

Table 5.1 Baseline characteristics of Dutch adults aged ≥55 years by the lowest and highest category of energy-adjusted intake of meat

	Red meat (g/day)		Processed meat (g/day)		Poultry (g/day)	
	(≤53.6)	(>97.7)	(0)	(>29.8)	(0)	(>18.0)
	(*n*= 1,091)	(*n*= 1,091)	(*n*= 506)	(*n*= 1,287)	(*n*= 1,051)	(*n*= 1,105)
Age (years)	68.1 (7.8)	66.1 (7.2)	68.1 (7.9)	66.7 (7.5)	68.4 (7.8)	66.0 (7.3)
Sex (% men)	38.0	47.7	25.7	54.9	42.3	42.0
Body mass index (kg/m^2)	25.6 (3.6)	26.9 (3.6)	26.0 (3.8)	26.5 (3.6)	26.1 (3.5)	26.7 (3.7)
Waist circumference (cm)						
Men	92.1 (8.8)	95.5 (9.6)	95.6 (9.3)	95.6 (9.3)	94.0 (9.8)	94.7 (9.2)
Women	86.0 (11.4)	87.9 (11.6)	88.5 (11.3)	88.5 (11.3)	86.5 (11.0)	87.5 (11.8)
Smoking (% current)	18.2	28.8	27.6	27.6	27.9	21.2
Hypertension (%)	50.8	52.1	51.5	51.5	54.4	51.9
Family history of diabetes (%)	25.5	28.6	28.1	28.1	27.0	29.6
History of CHD (%)	11.6	12.1	12.4	12.4	12.5	13.2
Total cholesterol (mmol/L)	6.6 (1.2)	6.8 (1.2)	6.7 (1.2)	6.7 (1.2)	6.7 (1.2)	6.7 (1.2)
HDL cholesterol (mmol/L)						
Men	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.3 (0.4)	1.2 (0.3)	1.2 (0.3)
Women	1.5 (0.3)	1.5 (0.4)	1.5 (0.3)	1.5 (0.4)	1.5 (0.4)	1.5 (0.3)
C-reactive protein (mg/L)[a]	1.5 (0.8-3.0)	1.7 (0.8-3.2)	1.5 (0.8-2.8)	1.7 (0.9-3.1)	1.7 (0.9-3.0)	1.7 (0.8-3.1)
Diet prescription (%)	11.7	9.8	18.4	6.6	7.7	12.5
Dietary intake						
Energy (kcal/day)	2017 (544)	2029 (537)	1763 (448)	2068 (502)	1975 (530)	1982 (512)
Protein (en-%)	16 (3)	18 (3)	18 (4)	17 (3)	17 (3)	18 (3)
Carbohydrates (en-%)	47 (7)	41 (7)	47 (7)	42 (7)	45 (7)	43 (7)
Total fat (en-%)	35 (6)	38 (6)	34 (7)	38 (6)	37 (6)	36 (6)
SFA (en-%)	14 (3)	15 (3)	14 (4)	15 (3)	15(3)	14 (3)
MUFA (en-%)	11 (3)	14 (3)	11 (3)	13 (3)	12 (3)	12 (3)
TFA (en-%)	1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)
PUFA (en-%)	7 (3)	7 (3)	7 (3)	7 (3)	7 (3)	7 (3)

Table 5.1 continues on next page

Table 5.1 (*continued*) Baseline characteristics of Dutch adults aged ≥55 years by the lowest and highest category of energy-adjusted intake of meat

	Red meat (g/day)		Processed meat (g/day)		Poultry (g/day)	
	(≤53.6)	(>97.7)	(0)	(>29.8)	(0)	(>18.0)
	(*n*= 1,091)	(*n*= 1,091)	(*n*= 506)	(*n*= 1,287)	(*n*= 1,051)	(*n*= 1,105)
Cholesterol (mg/day)	218 (82)	260 (93)	194 (72)	252 (86)	223 (85)	247 (83)
Fibre (g/day)	28 (8)	26 (7)	27 (9)	27 (7)	26 (7)	27 (8)
Iron (mg/day)	12 (3)	13 (3)	11 (3)	13 (3)	12 (3)	12 (3)
Alcohol (g/day) (%)						
0	25.0	19.1	29.8	15.9	24.5	18.8
>0-10	47.6	42.0	45.3	42.7	44.2	43.4
>10-20	13.0	15.7	12.3	19.0	15.4	17.0
>20	14.4	23.3	12.7	22.5	16.0	20.8
Total meat (g/day)	65 (33)	160 (46)	71 (44)	136 (46)	102 (57)	129 (46)
Red meat (g/day)	33 (17)	124 (38)	57 (40)	82 (39)	80 (49)	77 (40)
Processed meat (g/day)[a]	15 (2-26)	20 (10-33)	0	38 (32-48)	18 (6-32)	17 (8-31)
Poultry (g/day)[a]	9 (3-19)	10 (0-21)	7 (0-20)	9 (0-18)	0	29 (21-36)
Fish (g/day)[a]	11 (0-26)	7 (0-18)	8 (0-21)	8 (0-21)	3 (0-14)	14 (2-29)
Soya consumers (g/day)	7.0	0.6	8.1	1.2	3	4
Tea (g/day)	390 (271)	329 (241)	358 (250)	349 (254)	368 (258)	346 (251)
Milk products (g/day)[b]	451 (298)	351 (249)	401 (242)	361 (253)	397 (255)	377 (260)
Cheese (g/day)	40 (29)	35 (11)	39 (34)	34 (22)	37 (25)	36 (23)

Abbreviations: en-%=percent of total energy intake; SFA=saturated fatty acids; MUFA=mono-unsaturated fatty acids; TFA=trans fatty acids; PUFA=poly-unsaturated fatty acids

[a] Variables were expressed as median (p25-p75), because of their skewed distribution. Other values are means (standard deviation) or percentages

[b] All milk products except cheese

Table 5.2 Beta-coefficients (SEE) for the association of energy-adjusted category of meat intake with $\log_e$(C-reactive protein) in 4,366 Dutch adults aged ≥55 years[a]

	Crude model beta (SEE)	Model 1[b] beta (SEE)	Model 2[c] beta (SEE)
Red meat (per 50 gram)	0.03 (0.02)	0.03 (0.02)	0.01 (0.02)
	p= 0.09	*p*= 0.13	*p*= 0.59
Processed meat (per 50 gram)	0.13 (0.04)	0.12 (0.04)	0.12 (0.04)
	p= <0.01	*p*= <0.01	*p*= 0.01
Poultry (per 50 gram)	-0.09 (0.05)	-0.04 (0.05)	-0.04 (0.05)
	p= 0.06	*p*= 0.37	*p*= 0.43

[a] Values are beta-coefficients with standard error of the estimate (SEE).

[b] Model adjusted for age, sex, smoking, diet prescription, and family history of diabetes.

[c] Model 1 with additional adjustments for intakes of energy, energy-adjusted carbohydrates, energy-adjusted poly-unsaturated fatty acids, energy-adjusted fibre, energy-adjusted milk, energy-adjusted cheese, soya, fish, alcohol, and tea. Categories of meat were mutually adjusted.

Intakes of red meat and poultry were not associated with CRP. As intake of processed meat was associated with CRP and our analysis confirmed that CRP at baseline was associated with a higher risk of type 2 diabetes ($HR_{CRP\ Q4\ vs.\ Q1}$= 1.76 [95%CI 1.27, 2.45]; p_{trend}= <0.01), we could further investigate a potential mediating effect of CRP on the association between intake of meat and risk of type 2 diabetes.

Initially, red meat ($HR_{>97.7\ vs.\ \leq53.6}$= 1.42 [95%CI 1.06, 1.91]; p_{trend}= 0.01) and processed meat ($HR_{>29.8\ vs.\ 0}$= 1.87 [95%CI 1.26, 2.78]; p_{trend}= 0.02) were associated positively with risk of type 2 diabetes (model 2) (**Table 5.3**). Intake of poultry was not associated with risk of type 2 diabetes ($HR_{>18.0\ vs.\ 0}$= 0.95 [95%CI 0.74, 1.22]; p_{trend}= 0.55) (**Table 5.3**). Additional adjustment for CRP did not change HRs appreciably. Additional adjustment for BMI, however, attenuated the association for intake of red meat ($HR_{>97.7\ vs.\ \leq53.6}$= 1.18 [95%CI 0.88, 1.59]; p_{trend}= 0.17) (**Table 5.3**). The association between intake of total meat and risk of type 2 diabetes was also not statistically significant (Model 2+$\log_e$(CRP)+BMI $HR_{\leq85.2\ vs.\ >139.1}$= 1.21 [95%CI 0.88, 1.67]; p_{trend}= 0.30). Additional adjustment by waist circumference (*n*= 4,113; n_{cases}= 427) or physical activity (*n*= 3,244; n_{cases}= 391) did not attenuate associations further. Furthermore, components of meat which may explain the observed associations (i.e., saturated fatty acids, vitamin B_{12}, and heme iron) were added to the model one by one, but inclusion of these components did not change the interpretation of the results.

On the basis of *p* for interaction, the association between intake of red meat ($p_{interaction\ sex}$= 0.29; $p_{interaction\ BMI}$= 0.83), processed meat ($p_{interaction\ sex}$= 0.26; $p_{interaction\ BMI}$= 0.49), or poultry ($p_{interaction\ sex}$= 0.07; $p_{interaction\ BMI}$= 0.93) and risk of type 2 diabetes did not differ by sex and BMI.

Discussion

In this prospective cohort study of Dutch adults, high intake of processed meat was associated with a higher risk of type 2 diabetes compared with no intake of processed meat, independently of CRP and BMI. Therefore, CRP does not appear to be an intermediate. Intakes of red meat and poultry were not associated with risk of type 2 diabetes.

Strengths of our analyses included the prospective design, the inclusion of verified cases of diabetes, and the extensive information on potential confounders, which minimized the presence of residual confounding.

The adverse association between intake of processed meat and risk of type 2 diabetes

Table 5.3 Hazard ratios and 95% confidence intervals for incident type 2 diabetes by intake categories of energy-adjusted meat intake in 4,366 Dutch adults aged ≥55 years

	Median	*n* (cases)	PY	Crude HR [95%CI]	Model 1[a] HR [95%CI]	Model 2[b] HR [95%CI]	Model 2+log$_e$CRP HR [95%CI]	Model 2+log$_e$CRP+BMI HR [95%CI]
Red (g/day)								
≤53.6	39.2	1,091 (92)	11,827	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
>53.6-≤74.6	64.6	1,092 (98)	12,087	1.03 [0.78, 1.37]	1.01 [0.76, 1.34]	0.98 [0.73, 1.31]	0.94 [0.70, 1.25]	0.93 [0.70, 1.25]
>74.6-≤97.7	84.8	1,092 (119)	12,072	1.26 [0.96, 1.66]	1.25 [0.95, 1.64]	1.14 [0.86, 1.53]	1.11 [0.83, 1.48]	1.00 [0.75, 1.34]
>97.7	117.6	1,091 (147)	11,955	1.59 [1.23, 2.07]	1.57 [1.21, 2.04]	1.42 [1.06, 1.91]	1.35 [1.01, 1.82]	1.18 [0.88, 1.59]
p for trend				*<0.01*	*<0.01*	*0.01*	*0.02*	*0.17*
Processed (g/day)								
0	0	506 (33)	5,806	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
>0-≤17.4	11.2	1,286 (128)	14,171	1.60 [1.09, 2.34]	1.61 [1.10, 2.37]	1.72 [1.16, 2.54]	1.68 [1.13, 2.48]	1.76 [1.19, 2.61]
>17.4-≤29.8	23.0	1,287 (147)	14,038	1.86 [1.27, 2.71]	1.87 [1.28, 2.73]	1.87 [1.27, 2.75]	1.81 [1.23, 2.67]	1.85 [1.26, 2.73]
>29.8	39.2	1,287 (148)	13,927	1.88 [1.29, 2.75]	1.83 [1.25, 2.69]	1.87 [1.26, 2.78]	1.79 [1.20, 2.67]	1.73 [1.16, 2.57]
p for trend				*<0.01*	*0.01*	*0.02*	*0.04*	*0.11*
Poultry (g/day)								
0	0	1,051 (125)	11,222	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
>0-≤9.1	6.3	1,105 (86)	12,235	0.62 [0.47, 0.82]	0.64 [0.49, 0.84]	0.65 [0.49, 0.86]	0.65 [0.49, 0.86]	0.67 [0.51, 0.89]
>9.1-≤18.0	13.9	1,105 (110)	12,369	0.78 [0.61, 1.01]	0.80 [0.62, 1.04]	0.79 [0.61, 1.02]	0.79 [0.61, 1.03]	0.78 [0.60, 1.02]
>18.0	27.6	1,105 (135)	12,115	0.98 [0.77, 1.25]	0.99 [0.77, 1.26]	0.95 [0.74, 1.22]	0.94 [0.73, 1.21]	0.87 [0.67, 1.12]
p for trend				*0.31*	*0.33*	*0.55*	*0.59*	*0.79*

Abbreviations: PY=person-years; HR=hazard ratio; 95%CI=95% confidence interval; CRP=C-reactive protein; BMI=body mass index

[a] Model adjusted for age, sex, smoking, diet prescription, and family history of diabetes.

[b] Model 1 with additional adjustments for intakes of energy, energy-adjusted carbohydrates, energy-adjusted poly-unsaturated fatty acids, energy-adjusted fibre, energy-adjusted milk, energy-adjusted cheese, soya, fish, alcohol, and tea. Categories of meat were mutually adjusted.

observed in the current study is in line with a meta-analysis conducted by Aune *et al.* ($RR_{highest\ vs.\ lowest\ category}$= 1.41 [95%CI 1.35, 1.60]).[2] The observed higher risk could be due to components present in processed meat, such as AGEs. AGEs, which are naturally present in meat and formed in meat through heating, have been associated with insulin resistance[22, 23] and type 1 diabetes[24] in animal models. In addition, treatment with AGE inhibitor reduced risk of type 2 diabetes in mice.[25] A six-day, randomized, cross-over intervention study, however, did not observe differences in changes in serum glucose concentration between a high- and low-AGE diet in participants with diabetes.[26] AGEs might influence risk of type 2 diabetes by its pro-inflammatory properties.[16] A crossover study showed that compared with a low-AGE diet, a high-AGE diet increased concentration of plasma CRP in participants with diabetes.[27] In line with these findings, a randomized trial showed that circulating inflammation markers increased after eating a high-AGE diet for 6 weeks compared with eating a low-AGE diet.[28] As intake of processed meat was not associated with CRP concentrations, however, our study did not find clear support for an inflammatory pathway trough which processed meat increased the risk of type 2 diabetes. In line with our findings, a cross-sectional study among children and adolescents living in the U.S., showed that the intake of processed meat was not higher in children with a CRP concentration of >3 mg/L than those children with a CRP concentration of <1 mg/L.[29]

The higher risk observed for processed meat may also be explained by additives, e.g., nitrites, as processed meat contains more additives compared with other types of meat.[30] Nitrites may be converted to nitrosamines within the food product or stomach by interaction with amines.[30] These nitrosamines are of concern in the development of diabetes. Intake of nitrosamines was associated with type 1 diabetes in children[31-34] and decreased insulin secretion in animals.[35] The role of nitrosamines in the etiology of type 2 diabetes is less clear. Low doses of streptozotocin, a nitrosamine related compound, combined with dietary-induced insulin resistance, however, resulted in metabolic conditions in mice that are similar to type 2 diabetes in humans.[36, 37]

The higher risk may also be explained by the higher content of saturated fat[38], whereas it is not likely that the higher risk is explained by a higher iron intake associated with processed meat. Processed meat does not contain more iron compared with red meat for which we did not observe a higher risk after adjusting for BMI.

Although not statistically significant, the point estimate for red meat (HR= 1.18) was in line with the point estimate observed in the meta-analysis conducted by Aune *et al.* (Relative $Risk_{highest\ vs.\ lowest\ red\ meat\ category}$= 1.21).[2] Without adjusting for BMI, however, a higher risk was also observed for red meat in our study. As additional adjustment for CRP or heme iron in our analysis did not appreciably attenuate the association for red meat, it is not likely that heme iron explained the observed association either by its direct effect on glucose metabolism or via an inflammatory pathway.[14]

The small variation in intake of poultry may explain why a linear association between intake of poultry and risk of type 2 diabetes was not found. Our risk estimate in the highest category of poultry intake in the total population after inclusion of CRP and BMI, however, was comparable with relative risks reported by studies showing inverse associations.[5-7] A potential inverse association may reflect a 'healthy diet' rather than a direct effect of consuming poultry. We were able to adjust for a range of lifestyle factors, including dietary factors, through which confounding due to a 'healthy diet' was minimized.

Another explanation for the absence of an adverse association of poultry may be its effect on CRP. Dietary patterns including high loadings on poultry have been related to lower CRP concentrations.[39-41] In the current study, however, it is not likely that CRP mediates the

association between intake of poultry and risk of type 2 diabetes as additional adjustment for CRP did not affect the risk estimates.

Some limitations of the current study should be mentioned. First, information on dietary intake was obtained once. It could be that participants changed their diet through follow-up. However, exclusion of participants who are likely to change their diet during follow-up because of previous illness did not change the results. Second, misclassification of type and amount of meat could have occurred. As measurement error from dietary assessment was unlikely to be related to diabetes endpoint, it is likely that misclassification of meat intake was rather non-differential than differential and would have attenuated observed associations overall, if present. Third, it is questionable whether BMI should be included in the model when investigating the association between intake of red meat and risk of type 2 diabetes. As BMI may reflect an unhealthy lifestyle, BMI can be a confounder and, therefore, should be included into the model. If BMI is an intermediate, however, inclusion of BMI into the model will underestimate the association. BMI could be considered as intermediate, because BMI is a major risk factor for diabetes and intake of red meat was associated with weight gain.[42] Fourth, we may have missed undiagnosed cases of diabetes, because diabetes incidence was derived from general practitioner registration. Based on the descriptive tables we can assume that undiagnosed diabetes may also have been higher in the high red meat and processed meat categories, and therefore our results may even have been attenuated toward the null.

In conclusion, intake of processed meat was associated with higher risk of type 2 diabetes. It does not appear likely that CRP mediates this association. The underlying mechanism by which processed meat may increase risk of type 2 diabetes requires further investigation.

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GJvW prepared the data for analyses, performed the analysis, and drafted the manuscript. AK, BT, and EJMF contributed to the interpretation of the results and revised the manuscript. EJGS, FJAvR, and JCMW participated in the coordination of the study and revised the manuscript. AH participated in the design and coordination of the study.

GJvW is the guarantor of this work and, as such, had full access to all the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Abstract

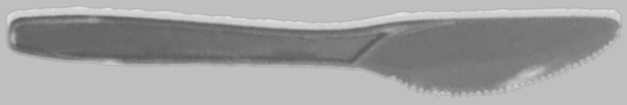
Objective: To investigate whether the glycemic index (GI) or glycemic load (GL) of a diet is associated with C-reactive protein (CRP) and risk of type 2 diabetes in a prospective study.

Methods: Our analysis included 4,366 participants who did not have diabetes at baseline. During follow-up 456 diabetes cases were confirmed. Dietary GI and GL were derived from a food-frequency questionnaire and its association with CRP was examined cross-sectionally using linear regression models. The association of GI and GL with diabetes incidence was examined using Cox proportional hazard models.

Results: GL, but not GI, was associated independently with $\log_e$CRP at baseline ($beta_{GL}$= 0.11 per 50 units; p= 0.01). When comparing the highest to the lowest tertile of GI with respect to diabetes incidence, a hazard ratio (HR) of 0.95 [95% confidence interval (95%CI) 0.75, 1.21] was found after adjustment for lifestyle and dietary factors. For GL the HR for diabetes incidence was 1.00 [95%CI 0.74, 1.36]. Additional adjustment for CRP did not change HRs.

Conclusion: Since GI was not associated with CRP and risk of type 2 diabetes, it is unlikely that a high GI diet induces the previously shown positive association between CRP and risk of type 2 diabetes by increasing CRP concentrations.

CHAPTER 6



Glycemic index and glycemic load and their association with C-reactive protein and incident type 2 diabetes

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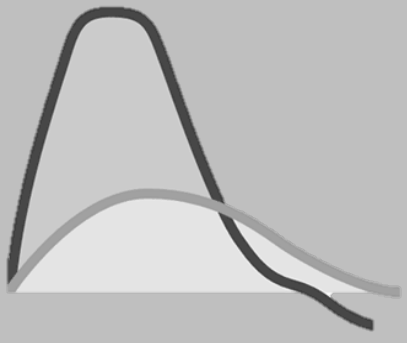

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Introduction

A growing body of evidence suggests a role of low-grade chronic inflammation in the development of type 2 diabetes. C-reactive protein (CRP) is a physiological marker of inflammation and reflects chronic low-grade inflammation when the concentration of this marker is slightly elevated over a longer period of time.[1] A meta-analysis of 16 prospective cohort studies showed that CRP was associated with a higher risk of type 2 diabetes.[2] This risk may be attributed to central adiposity.[2] It is also suggested that elements of the diet, like glycemic index (GI) and glycemic load (GL), may play a role.[3] The GI expresses the influence foods on blood glucose concentrations after consumption.[4] The GL makes allowance for the GI of a food product and the portion size eaten.[5] At least four cross-sectional studies showed a positive association of GI or GL with CRP.[6-9] GI and GL have also been related to a higher risk of type 2 diabetes in several cohort studies[10-14], but not in all[15-18]. So, GI or GL of a diet may be of importance in the development of type 2 diabetes, possibly due to its effect on CRP concentrations.

We investigated, therefore, whether GI or GL is associated with CRP and subsequently with risk of type 2 diabetes in an elderly Dutch population. In this population a positive association between CRP and risk of type 2 diabetes was shown previously.[19]

Methods

Population for analysis.

The Rotterdam study is a population-based prospective cohort study among inhabitants of Ommoord, a district of the city Rotterdam, The Netherlands.[20] In 1990 all inhabitants of this district who were aged ≥55 years were invited for participation (*n*= 10,215). Of the 7,983 responders (78%), 2,548 participants did not provide sufficient dietary data, 516 had type 2 diabetes at baseline, and 553 had not sufficient information on follow-up time or covariates (**Figure 6.1**). Hence, 4,366 participants were included in the current analysis. The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam, The Netherlands, approved the study. All participants gave informed consent.

Glycemic index and glycemic load

Dietary assessment at baseline comprised a self-administered questionnaire followed by a structured interview with a trained dietician at the research centre. Participants had to mark the foods and drinks they had consumed at least twice a month in the preceding year. Subsequently, the dietician obtained accurate information on the amount of food eaten using a semi-quantitative food frequency questionnaire.[21] Intake of food items was converted into total intake of energy and nutrients using the Dutch Food Composition table 1993 (NEVO). For the intake of fibre, we used the Dutch Food Composition table 1996 (NEVO). Validation of the questionnaire against 15 multiple-day food records in 80 participants showed a Pearson's correlation of 0.79 for adjusted intake of total carbohydrates.[21]

To each single food product derived from the questionnaire, GI values were assigned. These values were based on published international GI tables.[22, 23] Mean GI and GL values for each participant were calculated as follows:

$$\text{Mean GI} = \frac{\sum_{i=1}^{n} (GI_i * \text{carbohydrates}_i)}{\sum_{i=1}^{n} (\text{carbohydrates}_i)}$$

$$\text{Mean GL} = \frac{\sum_{i=1}^{n} (GI_i * \text{carbohydrates}_i)}{100}$$

GI_i is the GI value of food product i

After mean GI and GL were calculated, mean GI and GL were adjusted for energy using the residual method.[24]

C-reactive protein

Non-fasting serum blood samples were collected at baseline. In the samples, high-sensitivity CRP was measured using a rate near-infrared particle immunoassay (Immage Immuno-chemistry System, Beckman Coulter, Fullerton, CA). The procedure has been described in more detail elsewhere.[19] CRP concentration exceeding 10 mg/L at baseline were excluded from the analysis, because these higher concentrations reflect rather acute than chronic inflammation.[1]

Diabetes incidence: 1990-1993 until 2005

Participants were considered type 2 diabetes cases when they were registered by a general practitioner as having type 2 diabetes and had at least one of the following four criteria: plasma glucose concentration ≥7.0 mmol/L, random plasma glucose concentration ≥11.1 mmol/L, anti-diabetes medication, treatment by diet. Diabetes cases were monitored until July 2005.

Non-dietary covariates

General information, for example, smoking status, education level, family history of type 2 diabetes, was obtained with a questionnaire at baseline. A family history of type 2 diabetes was defined as having a parent, sibling, or both with diabetes onset between 30 and 65 years. A history of coronary heart diseases (CHD) was defined as a self-reported myocardial infarction or

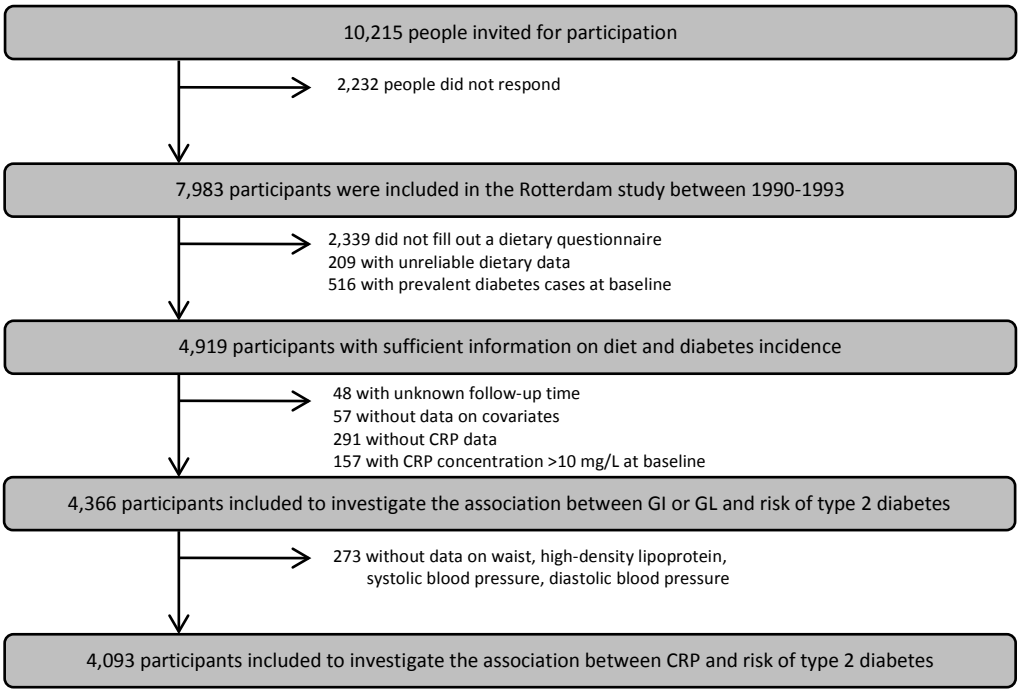

Figure 6.1 Flow diagram for inclusion of participants to investigate whether glycemic index (GI) or glycemic load (GL) is associated with serum C-reactive protein (CRP) and with risk of type 2 diabetes

angina pectoris with hospital admission. Information on energy expenditure (kcal/day) was obtained during follow-up for 3,244 participants of our study population with a physical activity questionnaire (Longitudinal Aging Study Amsterdam (LASA) Physical Activity Questionnaire).[25] Consequently, energy expenditure could be used as measure of physical activity in these participants. Information on anthropometrics was obtained during a visit at the research centre at baseline. Waist circumference was measured at the level midway between the lower rib margin and the iliac crest with the participant in standing position. Blood pressure was measured twice at the right brachial artery with a random-zero sphygmomanometer with the participant in sitting position. The mean of two consecutive measurements was used. High-density lipoprotein (HDL) cholesterol was measured with HDL cholesterol assay (Roche Diagnostics) using polyethylene glycol-modified enzymes and dextran sulphate.

Statistical analysis

Descriptive data were expressed as a mean (standard deviation (SD), a median (p25-p75), or a percentage. In order to investigate the effect of GI or GL on the association between CRP and type 2 diabetes our analysis included three steps.

Step 1. The positive association between CRP and risk of type 2 diabetes, as shown previously in the Rotterdam study (*n*= 5,901)[19], was verified in our sub-population of the same study (*n*= 4,093) (**Figure 6.1**).

Step 2. Linear regression models were used with energy-adjusted GI or GL as independent variable and CRP at baseline as dependent variable. CRP was transformed logarithmically to achieve a normal distribution. In addition to energy-adjusted GI or GL, model 1 included age (years), sex, smoking (current, former, never), and family history of diabetes (yes, no) as covariates. Model 2 was similar to model 1 with additional adjustment for intake of five dietary factors: energy (kcal/day), protein (energy-%), saturated fatty acids (energy-%), alcohol (0, >0-10, >10-20, >20 g/day), and fibre (g/day). Model 3 was similar to model 2 with additional adjustment for body mass index (BMI) (kg/m^2).

Step 3. We explored the association between energy-adjusted GI or GL and risk of type 2 diabetes using Cox proportional hazard models. Hazard ratios (HR) and 95% confidence intervals (95%CI) provided by these Cox models expressed the risk relative to the lowest tertile. Model 1, model 2, and model 3 included the same covariates as used in Step 2. To investigate the potential intermediate effect of CRP within the association of GI or GL with type 2 diabetes, an additional model was used (model 3+log$_e$(CRP)).

In steps 1 and 3, we modelled the median value of each tertile of GI or GL as continuous variable to test for linear trends across categories. To investigate potential effect measure modification, the association between GI and GL and risk of type 2 diabetes was studied separately for men and women and for participants with a low and high BMI (median split: ≤25.9 vs. >25.9 kg/m^2, respectively).

Analyses were carried out using the statistical software program SAS version 9.1. A two-sided *p*-value ≤0.05 was considered as statistically significant for all analysis.

Results

At baseline, the mean of GI was 59 (SD 3) and mean GL was 127 (SD 22). The highest tertile of GI included more smokers and more men than the lowest tertile (**Table 6.1**). Intake of polysaccharides increased, whereas intake of fibre and mono- and disaccharides decreased across tertiles of GI. Using stepwise regression, the main contributors to the variation in energy-adjusted GL appeared to be sweets (26%), fats (9%), bread (9%), alcoholic drinks (7%), and nuts (5%). The

Table 6.1 Baseline characteristics of 4,366 Dutch adults aged ≥55 years by tertiles of energy-adjusted glycemic index[a]

	Glycemic index		
	Low (<57.6) (*n*= 1,455)	**Moderate (57.6-<60.3) (*n*= 1,456)**	**High (≥60.3) (*n*= 1,455)**
Age (years)	67.3 (8.0)	67.7 (7.7)	66.9 (7.4)
Sex (% men)	26.6	39.7	54.5
Body mass index (kg/m^2)	26.5 (3.6)	26.2 (3.4)	26.0 (3.8)
C-reactive protein (mg/L)[b]	1.6 (0.8-2.9)	1.7 (0.8-3.0)	1.7 (0.8-3.1)
Anti-inflammatory medication (%)	8.0	6.6	7.7
Family history of diabetes (%)	26.5	26.8	29.7
History of CHD (%)	10.0	11.9	13.4
Smoking (% current)	15.8	20.4	32.0
Education level (% low)	33.6	33.5	35.7
Dietary intake			
Total energy (kcal/day)	1967 (555)	2005 (491)	1971 (464)
Protein (en-%)	18 (3)	17 (3)	16 (3)
Carbohydrates (en-%)	44 (7)	45 (7)	44 (8)
Mono- and disaccharides (en-%)	118 (43)	112 (40)	95.5 (40)
Polysaccharides (en-%)	95 (30)	108 (29)	114 (31)
Energy-adjusted glycemic load	119 (19)	128 (20)	133 (23)
Fibre (g/day)	27 (8)	26 (6)	25 (7)
Fat (en-%)			
Total	36 (6)	37 (6)	37 (6)
Saturated fatty acids	14 (3)	14 (3)	15 (3)
Mono-unsaturated fatty acids	12 (3)	12 (3)	13 (3)
Poly-unsaturated fatty acids	7 (3)	7 (3)	7 (3)
Alcohol drinkers (%)	79.2	82.2	79.9
Alcohol (g/day)[b,c]	14 (5-29)	6 (1-15)	3 (0.7-10)

Abbreviations: CHD=coronary heart disease; en-%=percent of total energy intake

[a] Means (standard deviation) or percentages unless otherwise indicated

[b] Values were expressed as median (p25-p75), because of their skewed distribution.

[c] Only in alcohol drinkers

main contributors to the variation in energy-adjusted GI were milk products (28%), fruit (20%), bread (13%), potatoes (5%), and cakes (2%). Median CRP concentration was 1.65 mg/L, and 1,097 (25%) participants had an elevated CRP level (>3 mg/L) at baseline.

Step 1 of our analysis included 4,093 participants of whom 423 developed type 2 diabetes during a median follow-up time of 11.0 years. The analysis confirmed that CRP at baseline was associated with a higher risk of type 2 diabetes after adjustment for age, sex, BMI, waist, systolic blood pressure, diastolic blood pressure, and HDL-cholesterol ($HR_{CRP\ Q4\ vs.\ Q1}$= 1.76 [95%CI 1.27, 2.45]; p_{trend}= <0.01). This HR was in line with HRs found when adjusted additionally for GI or GL ($HR_{CRP\ Q4\ vs.\ Q1}$= 1.76 [95%CI 1.27, 2.43]; $HR_{CRP\ Q4\ vs.\ Q1}$= 1.76 [95%CI 1.27, 2.44], respectively). The association did not differ considerably between participants with a low or high GI diet ($p_{interaction}$= 0.53) and between participants with a low or high GL diet ($p_{interaction}$= 0.99).

Step 2 of our analysis showed that after adjustment for lifestyle factors, dietary factors, and BMI, a 50 unit increase in GL was associated with a 12% higher CRP concentration at baseline (*p*= 0.01) (**Table 6.2**). No association was observed for a 10 unit increase in GI (*beta*= 0, *p*= 0.90).

Step 3 of our analysis included 4,366 participants whose median follow-up was 12.4 years. A number of 456 participants developed type 2 diabetes. When comparing the highest to the lowest tertile in this population, an adjusted HR of 0.95 [95%CI 0.75, 1.21] was found for GI

Table 6.2 Beta coefficients (SEE) for the association of energy-adjusted glycemic index or glycemic load with $\log_e$(C-reactive protein) in Dutch adults aged ≥55 years (*n*= 4,366)

	Crude beta (SEE)	Model 1[a] beta (SEE)	Model 2[b] beta (SEE)	Model 3[c] beta (SEE)
Glycemic index (per 10 units)	0.11 (0.04)	0.04 (0.04)	0.05 (0.04)	0.01 (0.04)
	p= 0.01	*p*= 0.31	*p*= 0.29	*p*= 0.90
Glycemic load (per 50 units)	-0.04 (0.03)	-0.03 (0.03)	0.09 (0.05)	0.11 (0.04)
	p= 0.25	*p*= 0.41	*p*= 0.05	*p*= 0.01

Abbreviation: SEE, standard error of the estimate

[a] Model adjusted for age, sex, smoking, and family history of diabetes.

[b] Model 1 with additional adjustments for intake of energy, alcohol, protein, saturated fatty acids, and fibre.

[c] Model 2 with additional adjustment for body mass index.

(model 3) (**Table 6.3**). For GL this adjusted $HR_{T3\ vs.\ T1}$ was 1.00 [95%CI 0.74, 1.36]. So, GI and GL were not associated with the risk of type 2 diabetes in this study. The HR found for GL was comparable with the one found for the association between intake of total carbohydrates and risk of type 2 diabetes (Model 3, $HR_{Q4\ vs.\ Q1}$= 1.04 [95%CI 0.71, 1.53]). The similar results also reflect the high correlation between intake of total carbohydrates and GL (*r*= 0.93). After adding CRP at baseline to model 3, HRs for risk of type 2 diabetes did not change considerably ($HR_{GI\ T3\ vs.\ T1}$= 0.96 [95%CI 0.75, 1.22]; $HR_{GL\ T3\ vs.\ T1}$= 0.99 [95%CI 0.73, 1.35]) (**Table 6.3**). In participants with available information on physical activity, additional adjustment for energy expenditure did also not change the HRs considerably (data not shown).

The association between GI or GL and risk of type 2 diabetes did not differ considerably between men and women (Model 3, $p_{interaction\ GI}$= 0.37; $p_{interaction\ GL}$= 0.09) and between participants with a low and high BMI (Model 3, $p_{interaction\ GI}$= 0.32; $p_{interaction\ GL}$= 0.29). Exclusion of participants with CHD at baseline (*n*= 514) did not change substantially the results (Model 3, $HR_{GI\ T3\ vs.\ T1}$= 0.93 [95%CI 0.72, 1.21]; $HR_{GL\ T3\ vs.\ T1}$= 1.03 [95%CI 0.74, 1.43]).

Discussion

In this Dutch population, GL was associated positively with CRP at baseline, but not with risk of type 2 diabetes. GI was not associated with CRP nor with risk of type 2 diabetes. A high GI diet, therefore, could not explain the positive association between CRP and risk of type 2 diabetes by increasing CRP concentrations.

We were able to study how GI and GL were associated with CRP and type 2 diabetes in a prospective cohort study with a high response rate, with a long follow-up period, with confirmed diabetes cases, and with available information on CRP concentration at baseline of a large population.

Our FFQ measured adequately intake of carbohydrates, which was correlated highly with GL, but was not designed to measure GI or GL. It could be, therefore, that food products with a very high or low GI were not taken into account. This might explain the small range in GI and GL in our study. A comparable range in GI, however, was also observed in other Dutch cohorts in whom another FFQ was used.[8, 26] In one of the cohorts even a smaller range was found when GI was based on twelve 24-hour recalls instead of on a FFQ.[26] This shows that a small range in GI may exist in the Netherlands. National data on GI values of Dutch food products, however, would have provided a more accurate measure of GI.

GL, but not GI, was associated positively with CRP at baseline in this study. Due to the high correlation between GL and intake of carbohydrates in our population, the effect of GL itself could not be separated from the effect of total carbohydrate intake. Other cross-sectional studies on the association with CRP observed either positive associations for a high GI diet[7-9] or GL diet[6] or no

Table 6.3 Hazard ratios and 95% confidence intervals for incident type 2 diabetes by tertiles of energy-adjusted glycemic index and glycemic load in Dutch adults aged ≥55 years (*n*= 4,366)

	Median	*n* (cases)	PY	Crude HR [95%CI]	Model 1[a] HR [95%CI]	Model 2[b] HR [95%CI]	Model 3[c] HR [95%CI]	Model 3+$\log_e$ CRP HR [95%CI]
Glycemic index								
Low (<57.6)	55.7	1,455 (149)	16,227	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Moderate (≥57.6-<60.3)	58.9	1,456 (141)	16,023	0.96 [0.76, 1.21]	0.91 [0.72, 1.15]	0.96 [0.76, 1.22]	0.94 [0.74, 1.19]	0.92 [0.73, 1.17]
High (≥60.3)	62.1	1,455 (166)	15,691	1.16 [0.93, 1.44]	1.02 [0.81, 1.29]	1.06 [0.83, 1.35]	0.95 [0.75, 1.21]	0.96 [0.75, 1.22]
p for trend				*0.20*	*0.84*	*0.64*	*0.71*	*0.75*
Glycemic load								
Low(<117.6)	107.1	1,455 (173)	15,825	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Moderate (≥117.6-<134.6)	126.4	1,456 (149)	16,206	0.83 [0.67, 1.03]	0.86 [0.69, 1.07]	0.92 [0.72, 1.17]	0.91 [0.71, 1.16]	0.90 [0.70, 1.15]
High (≥134.6)	146.1	1,455 (134)	15,910	0.77 [0.61, 0.96]	0.77 [0.61, 0.96]	0.98 [0.72, 1.33]	1.00 [0.74, 1.36]	0.99 [0.73, 1.35]
p for trend				*0.02*	*0.02*	*0.86*	*0.96*	*0.91*

Abbreviations: PY=person-years; HR=hazard ratio; 95%CI=95% confidence interval; CRP=C-reactive protein

[a] Model adjusted for age, sex, smoking, and family history of diabetes.

[b] Model 1 with additional adjustments for intakes of energy, alcohol, protein, saturated fatty acids, and fibre.

[c] Model 2 with additional adjustment for body mass index.

association for GI[6, 27-29] or GL[7-9, 28-30]. No associations were observed between changes in GI or GL and changes in CRP in a longitudinal study with a one-year follow-up.[27] On the contrary, one randomized controlled trial in participants with type 2 diabetes showed that reduction in CRP concentration after one year was more pronounced in a low GI diet than a high GI diet.[31] Other randomized controlled trials with a shorter duration, however, did not observe differential effects on CRP between a low GI diet and a high GI diet independently of body weight lost.[32-37] Taken these results together, it is not likely that GI affects CRP concentrations. The high within person variation in CRP, however, could have reduced the power of the statistical tests of the beta-coefficient.[38, 39] Therefore, duplicate measures of CRP should be used in new studies.

Our findings concerning the association of GI or GL with risk of type 2 diabetes are not in line with the conclusion of a meta-analysis published in 2008.[10] This meta-analysis on five cohort studies showed that high GI or GL diets were associated with a higher risk of type 2 diabetes (relative risk $(RR)_{GI}$ 1.40 [95%CI 1.23, 1.59]; RR_{GL} 1.27 [95%CI 1.12, 1.45]). After this meta-analysis, these associations were investigated additionally in eight cohort studies. These studies found associations of GI with risk of type 2 diabetes ranging from a 6% lower risk to a 50% higher risk[11, 13-18]; with two of them statistically significant[11, 13]. The associations of GL with risk of type 2 diabetes ranged from a 20% lower risk to a 41% higher risk.[11-17] Three studies reported that their findings were statistically significant in women[12, 13] or in both sexes[12-14]. Four of these newly published studies[12-15] and our study met the inclusion criteria used in the meta-analysis by Barclay *et al*..[10] Since ranges in GI do not always overlap among studies, a new pooled risk estimate would be difficult to interpret. Studies with high GI values (median of lowest category >63) observed higher risks of type 2 diabetes[5, 40, 41], whereas studies with low GI values (median of highest category <63) did not observe associations with risk of type 2 diabetes[14, 15]. Our study gives additional information about the association between lower ranges of GI values and risk of type 2 diabetes, which was lacking in the meta-analysis. Overall, this might suggest that only high GI values are associated adversely with risk of type 2 diabetes.

In conclusion, both GI and GL were not associated with risk of type 2 diabetes, although GL was associated positively with CRP concentrations. It is, therefore, unlikely that a high GI diet induces the positive association between CRP and risk of type 2 diabetes by increasing CRP concentrations.

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Abstract

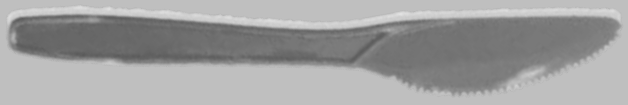
Objective: To investigate whether an adapted dietary inflammatory index (ADII) is associated with a summary score for low-grade inflammation and markers of glucose metabolism. In addition, the mediating role of inflammation in the association between ADII and markers of glucose metabolism is investigated.

Methods: We performed cross-sectional analyses of two Dutch cohort studies (*n*= 1,024). An ADII was obtained by multiplying standardized energy-adjusted intake of dietary components by literature-based dietary inflammatory weights that reflect the inflammatory potential of the components. Subsequently these multiplications were summed. Six biomarkers of inflammation were compiled in a summary score. The associations of the ADII (expressed per standard deviation (SD)) with the summary score for inflammation and markers of glucose metabolism were investigated by using multiple linear regression models. Inflammation was considered a potential mediator in the analysis with markers of glucose metabolism.

Results: A higher ADII was associated with a higher summary score for inflammation ($beta_{adjusted}$= 0.04 per SD [95%CI 0.01, 0.07]). ADII was also adversely associated with insulin resistance (HOMA-IR, $beta_{adjusted}$= 3.5% per SD [95%CI 0.6, 6.3]). This association was attenuated after inclusion of the summary score for inflammation ($beta_{adjusted+inflammation}$= 2.2% [95%CI -0.6, 5.0]. The ADII was also adversely associated with fasting glucose and 2-hour glucose concentrations, but not with HbA1c.

Conclusion: The significant mediating role of low-grade inflammation in the association between the ADII and HOMA-IR suggests that inflammation might be one of the pathways through which diet affects insulin resistance.

CHAPTER 7



The adapted dietary inflammatory index and its association with a summary score for low-grade inflammation and markers of glucose metabolism: the CODAM and Hoorn studies

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Introduction

Chronic low-grade inflammation is characterized by slightly elevated concentrations of circulating pro-inflammatory markers, including C-reactive protein (CRP), IL-6, and TNF-alpha. These markers have been associated with a higher risk of type 2 diabetes in observational studies[1, 2] and can be induced by risk factors for type 2 diabetes, such as overweight[3], physical inactivity[4], and diet[3]. Nutrients assumed to have an anti-inflammatory effect, for instance fibre and moderate amounts of ethanol[3], may be associated with a lower risk of type 2 diabetes[5, 6]. In contrast, nutrients assumed to have a pro-inflammatory effect, for instance saturated fatty acids (SFA) and trans fatty acids[3], may be associated with a higher risk of diabetes[7]. Therefore, it could be hypothesized that certain nutrients may influence risk of type 2 diabetes through its effects on chronic low-grade inflammation. Nutrients, however, are not consumed as individual components, but with others present within a certain food product.

Besides studying the effects of nutrients on inflammation and the development of type 2 diabetes, it is therefore also important to study whether the overall diet is associated with the development of type 2 diabetes through its effects on low-grade inflammation. A dietary index that reflects the quality of the diet, the alternative healthy eating index (AHEI), was associated with markers of low-grade inflammation and with risk of type 2 diabetes.[8, 9] This index, however, was not designed to reflect the inflammatory potential of the diet. To design an index that does, Cavicchia *et al.* developed dietary inflammatory weights for a number of dietary components based on a systematic review of available literature on diet and inflammation.[10] For instance, ethanol had an anti-inflammatory weight of -0.53, whereas SFA had a pro-inflammatory weight of 0.25. These weights can be used to obtain a dietary inflammatory index (DII) that reflects the inflammatory potential of the diet. Whether a DII is associated with any inflammation-related health outcomes, such as markers associated with the development of diabetes, has not been investigated yet.

The objectives of this study were to investigate whether a DII is associated with (1) a summary score for low-grade inflammation and (2) markers of glucose metabolism in a Dutch population. In addition, we investigated whether the association between a DII and markers of glucose metabolism is mediated by chronic low-grade inflammation.

Methods

Study population

The study population consisted of participants from two Dutch cohorts: the Cohort study on Diabetes and Atherosclerosis Maastricht (CODAM) and the Hoorn study.

Briefly, the CODAM study started in 1999 and is an on-going cohort study. It was designed to investigate the effects of disturbed glucose metabolism, obesity, blood lipids concentrations, lifestyle factors, and genetic factors on cardiovascular disease and mortality.[11-15] The CODAM study comprises of 574 participants, who were selected on the basis of an elevated risk for type 2 diabetes mellitus and cardiovascular diseases from a large population-based cohort (*n*= >20,000) and who had undergone a glucose metabolism screening test (*n*= 2,715).[11, 16]

Briefly, the Hoorn study started in 1989, being a sample of the general population of Hoorn, the Netherlands (*n*= 2,484).[17] The Hoorn study is a population-based cohort study designed to investigate the effect of disturbed glucose metabolism on cardiovascular disease risk factors and complications.[17] In 2000-2001 822 participants were examined again[18, 19], i.e., 648 surviving participants of the Hoorn study and an additional group of 174 participants with type 2 diabetes from the Hoorn Screening study.[20]

Both studies obtained written informed consent from all participants and were approved by the local Ethics Committees (CODAM study: Medical Ethical Review Committee of the Maastricht University Medical Centre; Hoorn study: Ethical Review Committee of the VU University Medical Centre Amsterdam).

Population for analysis

For the present investigation, data from both the baseline examination of the CODAM study and the follow-up examination of the Hoorn study were used. We combined these studies, because they had followed a similar data collection research protocol and had been used as a combined cohort in previous investigations.[21-23] From the 1,397 participants with reliable measures of food intake, obtained from a food frequency questionnaire (FFQ) (518 from CODAM; 879 from Hoorn), we excluded in consecutive order: 138 participants with missing information on glucose metabolism status or inflammation markers, 105 participants with known diabetes, 22 participants with missing information on covariates used in the analysis, and 108 with a CRP concentration higher than 10 mg/L. Participants with a CRP concentration exceeding 10 mg/L were excluded, because these higher concentrations reflect acute rather than chronic inflammation.[24] Finally, the population for analysis comprised 1,024 participants (420 from CODAM; 604 from Hoorn).

Assessment of dietary intake

In both cohorts, dietary intake was assessed by using a self-administered semi-quantitative FFQ, which had been validated in a Dutch population.[25] Intake of all food items was converted into intake of energy and nutrients, using an extended version of the Dutch Food Composition table 2001 (NEVO). The caffeine content of food items was not included in the NEVO table. Therefore, the caffeine content was estimated to be 68 milligrams per 100 ml of coffee[26], 20 milligrams per 100 ml of tea[27], and 8 milligrams per 100 ml of coke[27].

Calculation of adapted dietary inflammatory index (ADII)

Cavicchia *et al.*[10] developed literature-based dietary inflammatory weights that reflect the inflammatory potential of energy, 32 nutrients, 4 food products, 4 spices, and caffeine (**Table 7.1**). In line with Cavicchia *et al.*, we obtained a DII by multiplying the dietary inflammatory weights of the dietary components by the daily intake level. Subsequently these multiplications were summed. Details about the calculation of the DII can be found in **Table 7.1**.

Based on nutritional rationale (see below), we also obtained an ADII by multiplying the dietary inflammatory weight of 26 nutrients, 1 food product, 1 spice, and caffeine by the standardized energy-adjusted intake level. Subsequently these multiplications were summed (**Table 7.1**). Below we explain (1) why energy-adjusted intake levels were used, (2) why the intake level was standardized, and (3) why some dietary components were excluded. Other details can be found in **Table 7.1**.

1) Energy-adjusted intake

We adjusted all dietary components for energy, using the residual method in order to reduce the between-person variation in dietary intake, resulting from differences in physical activity, body size, and metabolic efficiency.[28] The ADII, therefore, is used as a measure of diet quality.

Table 7.1 Dietary components included in the dietary inflammatory index (DII) and adapted dietary inflammatory index (ADII)

Components	Unit	Inflammatory weight (IW)[a]	Components included in DII	Components included in ADII
Energy	kcal/d	0.230	included	not included[b]
Protein	g/d	-0.050	included	included
Carbohydrates	g/d	0.346	included	included
Total fat	g/d	0.323	included	not included[b]
Saturated fatty acids	g/d	0.250	included	included
Mono-unsaturated fatty acids	g/d	0.050	included	included
Trans fatty acids	g/d	0.260	not included[c]	included
n3 poly-unsaturated fatty acids	g/d	-0.384	included	included
n6 poly-unsaturated fatty acids	g/d	0.016	included	included
Cholesterol	mg/d	0.210	included	included
Fibre	g/d	-0.520	included	included
Ethanol	g/d	-0.534	included	included[d]
Wine	g/d	-0.480	included	not included[b]
Beer	g/d	-0.200	included	not included[b]
Liquor	g/d	-0.100	included	not included[b]
Caffeine	g/d	-0.035	included	included
Vitamin A	µg/d	-0.580	included	included
Beta-carotene	µg/d	-0.725	included	included
Thiamin	mg/d	-0.050	included	included
Riboflavin	mg/d	-0.160	included	included
Niacin	mg/d	-0.260	included	included
Vitamin B6	mg/d	-0.286	included	included
Folate	µg/d	-0.214	included	included
Vitamin B12	µg/d	0.090	included	included
Vitamin C	mg/d	-0.367	included	included
Vitamin D	µg/d	-0.342	included	included
Vitamin E	mg/d	-0.401	included	included
Iron	mg/d	-0.029	included	included
Magnesium	mg/d	-0.905	included	included
Selenium	mg/d	-0.021	included	included
Zinc	mg/d	-0.316	included	included
Tea	g/d	-0.552	included	included[e]

Table 7.1 continues on the next page

2) Standardized intake

To avoid that the variation in the ADII was solely driven by a few dietary components with a large range in intake, we standardized intake of all components. Standardization was done by subtracting the mean intake of the population from the individual intake and then dividing the difference by the standard deviation of the study population (*z*-score) to equilibrate the intake of all nutrients to the same unit. Therefore, it was not necessary to divide intake of vitamin A and beta-carotene by the arbitrary, data-dependent 100 and to multiply *n3* poly-unsaturated fatty acids (PUFA) and *n6* PUFA with the arbitrary, data-dependent 10 as Cavicchia *et al.* did (**Table 7.1**). We also did not divide the overall ADII by 100, because division did not improve the interpretation of the results as it improved the interpretation of the previously-published DII.[10]

3) Exclusion components

We excluded several components when calculating the ADII to avoid an over-estimation of the inflammatory effect of ethanol, fat, and energy. To reduce the impact of ethanol on the ADII, the separate anti-inflammatory effects of the alcoholic beverages beer, wine, and liquor were not taken into account. The anti-inflammatory effects of these beverages are likely to be attributable

Table 7.1 (*continued*) Dietary components included in the dietary inflammatory index (DII) and adapted dietary inflammatory index (ADII)

Components	Unit	Inflammatory weight (IW)[a]	Components included in DII	Components included in ADII
Quercetin	mg/d	-0.490	included	included
Genistein	mg/d	-0.680	not included[f]	not included[f]
Epicatechin	mg/d	-0.120	not included[f]	not included[f]
Luteolin	mg/d	-0.430	not included[f]	not included[f]
Daidzein	mg/d	-0.170	not included[f]	not included[f]
Cyanidin	mg/d	-0.130	not included[f]	not included[f]
Garlic	g/d	-0.270	included	included
Ginger	g/d	-0.180	not included[f]	not included[f]
Saffron	g/d	-0.180	not included[f]	not included[f]
Turmeric	g/d	-0.774	not included[f]	not included[f]
TOTAL DII for a participant[g]			$\frac{\sum_{i=n}(\text{intake}_i * \text{IW}_i)}{100}$	
TOTAL ADII for a participant				$\sum_{i=n}$ (energy-adjusted standardized intake$_i$ * IW$_i$)

[a] Dietary components with a positive inflammatory weight were considered pro-inflammatory. Dietary components with a negative inflammatory weight were considered anti-inflammatory.
[b] Energy was excluded in the ADII, because all macronutrients were already included. Total fat was excluded in the ADII, because all fatty acids were already included. The alcoholic beverages beer, wine, and liquor were excluded in the ADII, because intake of ethanol was already included.
[c] Trans fatty acids were not included in the previously-published DII, because intake of trans fatty acids could not be calculated in the study by Cavicchia *et al*..[10]
[d] The dietary inflammatory weight for ethanol was assumed to be zero when intake of ethanol exceeded 40 grams per day, because intake of ethanol is not likely to be anti-inflammatory when intake is higher than 40 grams per day.[29]
[e] Intake of tea was still included, because intake of epicatechin was not available.
[f] These dietary components were not taken into account in our DII and ADII calculation, because intake of these components could not be calculated from our food frequency questionnaire.
[g] The intake of *n3* poly-unsaturated fatty acids and *n6* poly-unsaturated fatty acids are multiplied by 10, because the intake is low and expressed as gram per day. The intake of vitamin A and beta-carotene is divided by 100 to equilibrate the range of intake to other micronutrients according to Cavicchia *et al*..[10]

to ethanol.[3] Energy was excluded, because it is likely that the inflammatory effect of energy is the sum of the inflammatory effects of all energy providing macronutrients. Total fat was also excluded, because it is assumed that the inflammatory effect of total fat is the sum of the inflammatory effects of all separate fatty acids.

Markers of glucose metabolism

Venous blood samples were drawn from all participants at the research centre after an overnight fast (>10 hour) to be able to measure, e.g., fasting glucose concentration, fasting insulin concentration, and hemoglobin A1c (HbA1c). Two-hour glucose concentration was determined following a standard 75 gram oral glucose tolerance test (OGTT), except in participants with established diabetes or very high fasting plasma glucose concentration (CODAM: >10 mmol/L; Hoorn: >8.0 mmol/L). Fasting and 2-hour glucose concentrations were measured in plasma by glucose hexokinase methods (CODAM study: ABX Diagnostics Glucose HK125, Montpellier, France; Hoorn study: Roche Diagnostics, Mannheim, Germany). HbA1c was analysed by ion-exchange HPLC (CODAM study and Hoorn study: Bio-rad, Veenendaal, The Netherlands). Insulin concentration was measured in plasma by a two-site immunoradiometric assay, using paired monoclonal antibodies (CODAM study and Hoorn study: Medgenix Diagnostics, Fleurus, Belgium).

Insulin resistance was estimated from fasting plasma glucose concentration and plasma insulin concentration by the homeostasis model assessment (HOMA2) calculator (www.dtu.ox.ac.uk).[30]

Markers of chronic low-grade inflammation

In both cohorts, the concentration of six biomarkers of low-grade inflammation, i.e., CRP, IL-6, IL-8, TNF-alpha, Serum Amyloid A (SAA), and soluble Intercellular Adhesion Molecule-1 (sICAM), were measured in plasma by a multi-array detection system (MDS), based on electro-chemiluminescence detection (MesoScaleDiscovery, SECTOR Imager 2400, Gaithersburg, Maryland, USA). All these measurements were performed at the Research Laboratory of the Department of Internal Medicine of the Maastricht University Medical Centre, the Netherlands (head: CGS). In the CODAM study, CRP was also measured in serum by high-sensitivity immunoturbidimetry assay (ITM) (Latex, Roche Diagnostics Netherlands BV, Almere, The Netherlands, www.roche.nl) and IL-6, SAA, sICAM were also measured in EDTA plasma by ELISA (IL-6: R&D Systems, Minneapolis, MN, USA, www.rndsystems.com; SAA and sICAM: Biosource, Invitrogen, Carlsbad, CA, USA, www.invitrogen.com). These measurements were done at the Laboratory of Toxicology, Genetics and Pathology of the National Institute for Public Health and the Environment, Bilthoven, The Netherlands. The values obtained by ITM or ELISA were calibrated on the values obtained by MDS in the CODAM study. Subsequently, the calibrated and the MDS values were averaged and used for the CODAM participants in the current analysis. The intra-assay coefficients of variation ranged from 0.6% to 6.4% and the inter-assay ones ranged from 1.9% to 17.5%. More information about the measurements can be found elsewhere.[12, 13, 31]

Calculation summary score for low-grade inflammation

A summary score for low-grade inflammation was calculated to cluster conceptually related markers of low-grade inflammation and to improve statistical efficiency. To obtain this summary score, a *z*-score for each marker of low-grade inflammation was calculated, because the markers of low-grade inflammation are expressed on different scale units. Subsequently, these *z*-scores were averaged to obtain a summary score for low-grade inflammation for each participant (summary score= (*z*-score($\log_e$CRP) + *z*-score($\log_e$IL-6) + *z*-score($\log_e$IL-8) + *z*-score(TNF-alpha) + *z*-score($\log_e$SAA) + *z*-score(sICAM)) / 6). This summary score for low-grade inflammation had been used in previous investigations.[14, 31, 32]

Covariates

In both cohorts, the participant completed a self-administered questionnaire which, among other things, included questions about (a) age, (b) sex, (c) smoking behaviour, (d) family history of diabetes in first-degree relatives, and (e) use of medication (e.g., anti-hypertensive, lipid-lowering, glucose-lowering). Based on the questions about smoking behaviour, the participant was categorized as never, former, or current smoker. Family history of diabetes was defined as a parent, a sibling, or both with diagnosed diabetes. Trained personnel measured height to the nearest centimetre and weight to the nearest 100 gram. The participant was weighed in standing position, wearing light indoor cloths and no shoes. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Waist circumference (cm) was obtained at the levels halfway between the lateral lower rib margin and the spina iliaca anterior superior. Habitual physical activity was assessed, using a validated short physical activity questionnaire (SQUASH: Short QUestionnaire to Assess Health-enhancing physical activity), which measured duration and intensity of different activities (min per week*intensity).[33]

Statistical analysis

Summary statistics were used to describe population characteristics by tertiles of the ADII. To get more insight into the contribution of the individual dietary components to the total ADII, the contribution of the different dietary components to the variation between participants in the ADII was assessed, using forward linear regression. Prior to further analysis, seven skewed variables were $\log_e$ transformed to improve their distribution towards normal (CRP, IL-6, IL-8, SAA, fasting plasma glucose, 2-hour glucose, and HOMA-IR).

First, the association between the ADII and markers of low-grade inflammation was investigated by using linear regression. Model 1 included as main independent variable the ADII (expressed per standard deviation (SD)) and the covariates age (years), sex, cohort (CODAM or Hoorn), smoking (never, former, or current), physical activity (min per week*intensity), family history of diabetes (yes, no, or missing), use of lipid-lowering medication (yes or no), having hypertension (yes or no), and intake of energy (kcal/day). Except for cohort, these covariates were included because of their association with inflammation and diabetes observed in the literature. In model 2, BMI (kg/m^2) was added to model 1, as we were also interested in the effect of ADII on inflammation independent of BMI. Waist circumference was not included as additional covariate, because inclusion did not change the conclusions and waist circumference was missing for eight participants. Effect measure modification by sex was investigated by adding an interaction term between ADII and sex to model 2, when the summary score for low-grade inflammation was studied as dependent variable.

Second, to investigate whether this study also confirmed the well-known adverse associations between low-grade inflammation and markers of glucose metabolism, the association between the summary score for low-grade inflammation and the four markers of glucose metabolism was studied. Model 1 of the linear regression model included age (years), sex, cohort (CODAM or Hoorn), smoking (never, former, or current), physical activity (min per week*intensity), family history of diabetes (yes, no, or missing), use of lipid-lowering medication (yes or no), and having hypertension (yes or no). Model 2 included BMI (kg/m^2) in addition to model 1.

Third, the association between the ADII and four markers of glucose metabolism, i.e., fasting plasma glucose, 2-hour glucose, HOMA-IR, and HbA1c, was investigated by using linear regression. Model 1 included the ADII and the other covariates as described before for model 1. To investigate the mediating role of low-grade inflammation, the summary score for low-grade inflammation was included in addition to the covariates included in model 1 (model 1+inflammation). To investigate whether the association of the ADII with markers of glucose metabolism was attributed to inflammation independent of BMI, the summary score for low-grade inflammation and BMI were simultaneously added to model 1. For this purpose we also used the multiple mediation analysis as described by Preacher and Hayes.[34] This mediation analysis provides an efficient way to quantify the independent mediating effects of low-grade inflammation and BMI (**Figure 7.1**).

All analyses were performed by using SAS statistical software package (version 9.2; SAS Institute Inc, Cary, NC). A *p*-value ≤0.05 was considered statistically significant.

Results

The mean age of the population of analysis was 64 years (standard deviation (SD) 9), 59% were participants from the Hoorn study, 55% were men, 26% had a normal weight, 18% were current smokers, and 51% had normal glucose metabolism. Compared with the CODAM study, the Hoorn study included participants with an older age (68 years (SD 7) vs. 58 years (SD 7)), more

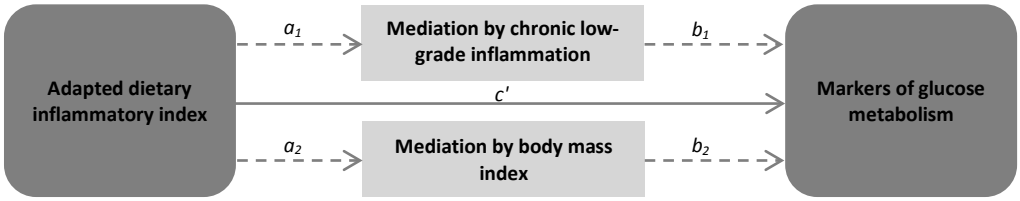

Figure 7.1 Model used in the multiple mediation analysis of the association between the ADII and markers of glucose metabolism, including fasting glucose, 2-hour glucose, HOMA-IR, and HbA1c, adapted from Preacher and Hayes.[34]

Paths *a* represent the regression coefficient of the association between the ADII and the summary score for low-grade inflammation (*path* a_1) or body mass index (BMI) (*path* a_2). Paths *b* represent the regression coefficient of the association between the summary score for low-grade inflammation (*path* b_1) or BMI (*path* b_2) and markers of glucose metabolism. In addition to other covariates, *path* b_1 is adjusted for BMI and the ADII, whereas *path* b_2 is adjusted for the summary score for low-grade inflammation and the ADII. The product of the regression coefficients of paths a and paths b represents the mediated effect of inflammation (*path* a_1**path* b_1) or BMI (*path* a_2**path* b_2). *Path c'* represents that part of the association that is not explained by low-grade inflammation or BMI. This is referred to as the direct association between the ADII and markers of glucose metabolism.

women (49% vs. 37%), and less current smokers (16% vs. 20%). The mean BMI was comparable (27 kg/m^2 (SD 4) in Hoorn; 28 kg/m^2 (SD 4) in CODAM).

ADII and its components

The ADII ranged from -12.0 to 15.7 (range: 27.7 units) (**Table 7.2**). Participants with a high ADII smoked more and were more often men compared with participants with a low ADII (**Table 7.2**). Intake of SFA, MUFA, and trans fatty acids were higher in participants with a high ADII than in participants with a low ADII. The intake of protein, *n3* PUFA, and *n6* PUFA were lower in participants with a high ADII, than in participants with a low ADII (**Supplementary Table 7.1**). The Spearman correlation between the ADII and intake of energy was low (r= 0.10, p= 0.02). Intake of magnesium explained most of the variation (34%) in the ADII, followed by intake of folate (25%), quercetin (16%), and *n3* PUFA (7%) (**Table 7.3**). Regarding the DII, which ranged from -20.7 to 6.4 (range: 27.0 units) (**Supplementary Table 7.2**), intake of tea explained most of the variation (55%) in the DII, followed by intake of SFA (17%), beer (13%), energy (6%), and wine (5%) (**Table 7.3**).

ADII and chronic low-grade inflammation

An increment of one SD in ADII (i.e., 2.9 units) was associated with a 0.04 [95%CI 0.01, 0.07] units higher summary score for low-grade inflammation in model 2 (p= 0.01) (**Table 7.4**). This association was mainly driven by four of the six markers of inflammation: CRP, IL-6, TNF-alpha, and sICAM. The original DII was not associated with the summary score for low-grade inflammation (Model 2, *beta*= -0.002 [95%CI -0.03, 0.03]) (**Table7.4**). The association between ADII and summary score for low-grade inflammation did not differ between men and women ($p_{interaction}$= 0.80).

Chronic low-grade inflammation and markers of glucose metabolism

The summary score for low-grade inflammation was associated with adverse concentration of all markers of glucose metabolism (**Table 7.5**). An increment of one unit in the summary score for low-grade inflammation was associated with a 4% [95%CI 2, 6] higher fasting glucose concentration on average, a 9% [95%CI 4, 14] higher 2-hour glucose concentration on

Table 7.2 Characteristics of the study population by tertiles of the adapted dietary inflammatory index (*n*= 1,024)[a]

	Adapted dietary inflammatory index		
	Tertile 1	**Tertile 2**	**Tertile 3**
Range	**(-12.0-<-1.3)**	**(-1.3-<1.2)**	**(1.2-<15.7)**
Median	**-2.5**	**-0.03**	**2.7**
	(*n*= 341)	**(*n*= 342)**	**(*n*= 341)**
Age (years)	65.0 (8.1)	63.9 (8.4)	64.1 (9.3)
Sex (% men)	50.7	53.8	61.6
Study (% Hoorn)	61.6	59.4	56.0
Smoking (% current)	12.3	18.1	23.5
Any physical activity (hours/day)	4.2 (2.8)	4.2 (2.9)	3.8 (3.0)
Body mass index (kg/m$^{2)}$	27.3 (3.7)	27.9 (3.8)	27.7 (4.1)
Waist circumference (cm)[3]	94.8 (10.8)	96.4 (11.4)	97.7 (12.4)
Diabetes category (% NGM)	53.7	50.0	49.9
Family history of diabetes (%)[4]	27.9	27.5	27.0
Lipid-lowering medication (%)	15.3	18.7	15.0
Anti-hypertensive medication (%)	34.3	33.9	35.5

Abbreviation: NGM, normal glucose metabolism

[a] Characteristics were expressed as mean (standard deviation) or percentages.

[b] Chi-square test for categorical variables; ANOVA for continuous variables.

[c] Missing for 8 participants.

[d] Missing for 122 participants.

Table 7.3 Explained inter-individual variance in the adapted dietary inflammatory index and the dietary inflammatory index by dietary components included in the index calculation (*n*=1,024)[a]

Components	**R^2**	**Model R^2**
Adapted dietary inflammatory index		
Magnesium (mg/day)	0.34	0.34
Folate (µg/day)	0.25	0.60
Quercetin (mg/day)	0.16	0.76
n3 poly-unsaturated fatty acids (g/day)	0.07	0.82
Beta-carotene (µg/day)	0.04	0.86
Ethanol (g/day)	0.04	0.89
Vitamin D (µg/day)	0.02	0.91
Other components	0.09	1.00
Dietary inflammatory index		
Tea (g/day)	0.55	0.55
Saturated fatty acids (g/day)	0.17	0.72
Beer (g/day)	0.13	0.85
Energy (kcal/day)	0.06	0.91
Wine (g/day)	0.05	0.96
Magnesium (mg/day)	0.02	0.98
Other components	0.02	1.00

[a] Forward linear regression was used to calculate the R^2 and model R^2. Components that explained more than 1% of the inter-individual variation were shown.

Table 7.4 Beta-coefficients [95% confidence intervals][a] of the association between the dietary inflammatory indexes and markers of inflammation (*n*= 1,024)

	Crude beta [95%CI]	Model 1[b] beta [95%CI]	Model 2[c] beta [95%CI]
ADII (per SD of 2.9)			
Inflammation score[d,e]	0.04 [0.01, 0.08]	0.04 [0.02, 0.07]	0.04 [0.01, 0.07]
	p= 0.01	*p*= <0.01	*p*= 0.01
C-reactive protein[e,f]	0.07 [0.02, 0.13]	0.06 [0.01, 0.12]	0.05 [-0.01, 0.10]
	p= 0.01	*p*= 0.02	*p*= 0.08
Interleukin-6[e,f]	0.06 [0.03, 0.10]	0.05 [0.01, 0.08]	0.04 [0.01, 0.08]
	p= <0.01	*p*= 0.01	*p*= 0.02
Interleukin-8[e,f]	-0.03 [-0.08, 0.01]	-0.01 [-0.03, 0.02]	-0.01 [-0.03, 0.02]
	p= 0.16	*p*= 0.64	*p*= 0.63
TNF-alpha[e]	0.15 [-0.03, 0.32]	0.17 [-0.00, 0.33]	0.16 [-0.01, 0.33]
	p= 0.10	*p*= 0.05	*p*= 0.07
Serum Amyloid A[e,f]	0.00 [-0.04, 0.04]	0.02 [-0.03, 0.06]	0.01 [-0.03, 0.05]
	p= 0.97	*p*= 0.44	*p*= 0.67
sICAM[e]	5.28 [1.81, 8.75]	4.57 [1.32, 7.81]	3.96 [0.76, 7.15]
	p= <0.01	*p*= 0.01	*p*= 0.02
DII (per SD of 2.1)			
Inflammation score[d,e]	-0.05 [-0.08, -0.01]	0.02 [-0.01, 0.05]	-0.002 [-0.03, 0.03]
	p= 0.01	*p*= 0.28	*p*= 0.90
C-reactive protein[e,f]	0.05 [-0.00, 0.11]	0.08 [0.02, 0.13]	0.03 [-0.02, 0.09]
	p= 0.06	*p*= 0.01	*p*= 0.21
Interleukin-6[e,f]	0.02 [-0.02, 0.06]	0.01 [-0.03, 0.05]	-0.003 [-0.04, 0.03]
	p= 0.29	*p*= 0.63	*p*= 0.90
Interleukin-8[e,f]	-0.16 [-0.21, -0.12]	-0.03 [-0.06, 0.00]	-0.03 [-0.06, 0.00]
	p= <0.01	*p*= 0.06	*p*= 0.05
TNF-alpha[e]	-0.23 [-0.40, -0.05]	-0.01 [-0.18, 0.17]	-0.03 [-0.20, 0.15]
	p= 0.01	*p*= 0.93	*p*= 0.75
Serum Amyloid A[e,f]	-0.02 [-0.07, 0.02]	0.03 [-0.02. 0.07]	0.01 [-0.03, 0.05]
	p= 0.30	*p*= 0.21	*p*= 0.68
sICAM[e]	-1.62 [-5.10, 1.86]	1.61[-1.77, 5.00]	-0.06 [-3.30, 3.42]
	p= 0.36	*p*= 0.35	*p*= 0.97

Abbreviations: ADII=adapted dietary inflammatory index; SD=standard deviation; TNF-alpha=tumor necrosis factor-alpha; sICAM=soluble intercellular adhesion molecule; DII=dietary inflammatory index; 95%CI=95% confidence interval

[a] The beta-coefficients and 95% CI were obtained by using linear regression.

[b] Model included age, sex, cohort, physical activity, smoking, family history of diabetes, use of lipid-lowering medication, and hypertension as covariates. The ADII was also adjusted for intake of energy.

[c] Model 1 with additional adjustment for body mass index.

[d] The summary score for low-grade inflammation was obtained by the formula: (*z*-score ($\log_e$C-reactive protein) + *z*-score($\log_e$Interleukin-6) + *z*-score($\log_e$Interleukin-8) + *z*-score(TNF-alpha) + *z*-score($\log_e$Serum Amyloid A) + *z*-score(sICAM)) / 6.

[e] Median (p25-p75) or mean (standard deviation) of the inflammation markers were as follows: inflammation score -0.1 (0.5); C-reactive protein (mg/L) 1.7 (0.97-3.4); Interleukin-6 (ng/L), 1.4 (1.1-2.9); Interleukin-8 (ng/L), 2.2 (0.7); TNF-alpha (ng/L), 7.9 (2.9); Serum Amyloid A (mg/L), 1.5 (0.97-2.4); sICAM (ug/L), 239.5 (56.7).

[f] For the analysis, these markers of inflammation were $\log_e$ transformed to improve their distribution towards normal. Therefore, when the DII or ADII was one standard deviation higher, these markers of low-grade inflammation were on average by beta*100 percent higher or lower.

Table 7.5 Beta-coefficients [95% confidence intervals][a] of the association between the summary score for low-grade inflammation and markers of glucose metabolism

	n	Crude beta [95%CI]	Model 1[c] beta [95%CI]	Model 2[d] beta [95%CI]
Inflammation score				
Fasting glucose (mmol/L)[e,f]	1,022	0.07 [0.05, 0.09] *p*= <0.01	0.05 [0.04, 0.07] *p*= <0.01	0.04 [0.02, 0.06] *p*= <0.01
2-hour glucose (mmol/L)[e,f]	907	0.12 [0.08, 0.16] *p*= <0.01	0.12 [0.07, 0.17] *p*= <0.01	0.09 [0.04, 0.14] *p*= <0.01
HOMA-IR[e,f]	1,003	0.21 [0.15, 0.26] *p*= <0.01	0.28 [0.22, 0.34] *p*= <0.01	0.16 [0.11, 0.22] *p*= <0.01
HbA1c (%)[e]	1,008	0.27 [0.21, 0.34] *p*= <0.01	0.23 [0.16, 0.31] *p*= <0.01	0.21 [0.13, 0.29] *p*= <0.01

Abbreviations: 95%CI=95% confidence intervals; HOMA-IR=homeostatis model assessment for insulin resistance; HbA1c=hemoglobin A1c

[a] The beta-coefficients and 95% CI were obtained by using linear regression.

[b] Summary score for low-grade inflammation was obtained by the formula: *z*-score ($\log_e$C-reactive protein) + *z*-score($\log_e$Interleukin-6) + *z*-score($\log_e$Interleukin-8) + *z*-score(tumor necrosis factor-alpha) + *z*-score($\log_e$Serum Amyloid A) + *z*-score(soluble Intercellular Adhesion Molecule) /6).

[c] Model 1 included age, sex, cohort, physical activity, smoking, family history of diabetes, use of lipid-lowering medication, and hypertension as covariates.

[d] Model 1 with additional adjustment for body mass index.

[e] Median (p25-p75) or mean (standard deviation) of the markers of glucose metabolism were as follows: fasting glucose (mmol/L), 5.7 (5.3-6.4); 2-hour glucose (mmol/L) 6.6 (5.3-8.6); HOMA-IR, 1.1 (0.8-1.6); HbA1c (%), 5.9 (0.6).

[f] For the analysis, these markers of glucose metabolism were $\log_e$ transformed to improve their distribution towards normal. Therefore, when the summary score for low-grade inflammation was one unit higher, these markers of glucose metabolism were on average by beta*100 percent higher.

average, 16% [95%CI 11, 22] higher HOMA-IR on average, and a 0.21% [95%CI 0.13, 0.29] higher HbA1c concentration (model 2).

ADII and markers of glucose metabolism

An increment of one SD in the ADII (i.e., 2.9 units) was associated with a 0.9% [95%CI 0.1, 1.7] higher fasting glucose concentration, a 2.3% [95%CI 0.0, 4.6] higher 2-hour glucose concentration, and with a 3.5% [95%CI 0.6, 6.3] higher HOMA-IR on average (**Table 7.6**, model 1). The ADII was not associated with HbA1c. After inclusion of the summary score for low-grade inflammation, all associations attenuated (e.g., HOMA-IR: $beta_{\text{model 1+inflammation}}$= 2.2% [95%CI -0.6, 5.0]. Additional adjustment by BMI attenuated the association further (e.g., HOMA-IR: $beta_{\text{model 1+inflammation+BMI (c')}}$= 1.4% [95%CI -1.1, 3.9] (**Figure 7.1/Table 7 6**)). When the summary score for low-grade inflammation and BMI were simultaneously added to model 1, the summary score for low-grade inflammation, but not BMI, explained a significant proportion of the association between ADII and HOMA-IR (path a_1*path b_1= 0.7% higher per SD through inflammation independent of BMI) and between ADII and 2-hour glucose (path a_1*path b_1= 0.5% higher per SD through inflammation independent of BMI) (**Figure 7.1/Table 7.6**). ADII had no direct association (*c'*) with the four markers of glucose metabolism (**Figure 7.1/Table 7.6**).

Discussion

The aims of this study were to investigate whether the inflammatory potential of the diet, as assessed with ADII, is associated with (1) the summary score for low-grade inflammation and (2) markers of glucose metabolism. We observed an adverse association between ADII and the

Table 7.6 Beta-coefficients [95% confidence intervals] of the association between the adapted dietary inflammatory index (ADII) and markers of glucose metabolism[a]

Dependent		ADII (per SD of 2.9) beta [95%CI]
Fasting glucose (mmol/L)[b,c] (*n*= 1,022)	Crude	0.008 [-0.002, 0.017] *p*= 0.10
	Total effect (model 1[d])	0.009 [0.001, 0.017] *p*= 0.03
	Model 1+BMI	0.007 [-0.001, 0.015] *p*= 0.08
	Model 1+inflammation	0.007 [-0.002, 0.0156] *p*= 0.11
	Direct effect (model 1+BMI+inflammation (c'))	0.006 [-0.002, 0.014] *p*= 0.16
	*Indirect effect through BMI (a_2*b_2)[e]*	*0.001 [0.000, 0.003]* *p*= -[g]
	*Indirect effect through inflammation (a_1*b_1)[f]*	*0.002 [0.001, 0.004]* *p*= -[g]
2-hour glucose (mmol/L)[b,c] (*n*= 907)	Crude	0.019 [-0.004, 0.042] *p*= 0.10
	Total effect (model 1[d])	0.023 [0.000, 0.046] *p*= 0.05
	Model 1+BMI	0.019 [-0.003, 0.041] *p*= 0.09
	Model 1+inflammation	0.017 [-0.006, 0.039] *p*= 0.14
	Direct effect (model 1+BMI+inflammation (c'))	0.015 [-0.007, 0.037] *p*= 0.18
	*Indirect effect through BMI (a_2*b_2)[e]*	*0.003 [-0.000, 0.008]* *p*= -[g]
	*Indirect effect through inflammation (a_1*b_1)[f]*	*0.005 [0.002, 0.010]* *p*= -[g]
HOMA-IR[b,c] (*n*= 1,003)	Crude	0.037 [0.007, 0.067] *p*= 0.02
	Total effect (model 1[d])	0.035 [0.006, 0.063] *p*= 0.02
	Model 1+BMI	0.020 [-0.005, 0.045] *p*= 0.12
	Model 1+inflammation	0.022 [-0.006, 0.050] *p*= 0.12
	Direct effect (model 1+BMI+inflammation (c'))	0.014 [-0.011, 0.039] *p*= 0.28
	*Indirect effect through BMI (a_2*b_2)[e]*	*0.014 [-0.001, 0.028]* *p*= -[g]
	*Indirect effect through inflammation (a_1*b_1)[f]*	*0.007 [0.003, 0.014]* *p*= -[g]

Table 7.6 continues on the next page

summary score for chronic low-grade inflammation, suggesting that the inflammatory potential of the diet affects markers of inflammation. The adverse association between ADII and HOMA-IR suggests that the inflammatory potential of the diet affects insulin resistance. This was supported by the mediating role of chronic low-grade inflammation in this analysis on insulin resistance.

Based on our results, it is likely that the adaptations in the DII calculation improved the

Table 7.6 (*continued*) Beta-coefficients [95% confidence intervals] of the association between the adapted dietary inflammatory index (ADII) and markers of glucose metabolism[a]

Dependent		ADII (per SD of 2.9) beta [95%CI]
HbA1c (%)[b] (*n*= 1,008)	Crude	0.010 [-0.027, 0.048] *p*= 0.05
	Total effect (model 1[d])	0.011 [-0.025, 0.046] *p*= 0.13
	Model 1+BMI	0.007 [-0.029, 0.042] *p*= 0.06
	Model 1+inflammation	0.000 [-0.035, 0.036] *p*= 0.05
	Direct effect (model 1+BMI+inflammation (c'))	-0.001 [-0.037, 0.034] *p*= 0.10
	*Indirect effect through BMI (a_2*b_2)[e]*	*0.002 [0.000, 0.008]* *p*= -[g]
	*Indirect effect through inflammation (a_1*b_1)[f]*	*0.010 [0.003, 0.019]* *p*= -[g]

Abbreviations: 95%CI=95% confidence intervals; HOMA-IR=homeostatis model assessment for insulin resistance; HbA1c=hemoglobin A1c

[a] The beta-coefficients and 95% CI were obtained by using linear regression. See **Figure 7.1** for the interpretation of c', a_1, a_2, b_1, and b_2.

[b] Median (p25-p75) or mean (standard deviation (SD)) of the markers of glucose metabolism were as follows: fasting glucose (mmol/L), 5.7 (5.3-6.4); 2-hour glucose (mmol/L) 6.6 (5.3-8.6); HOMA-IR, 1.1 (0.8-1.6); HbA1c (%), 5.9 (0.6).

[c] For the analysis, these markers of glucose metabolism were $\log_e$ transformed to improve their distribution towards normal. Therefore, when the ADII was one SD (SD= 2.9) higher, these markers of glucose metabolism were on average beta*100 percent higher or lower.

[d] Model was adjusted for age, sex, cohort, physical activity, smoking, family history of diabetes, use of lipid-lowering medication, hypertension, and intake of energy.

[e] When the ADII was one SD higher, the marker of glucose metabolism was beta*100 percent higher or lower through the effect of the ADII on BMI.

[f] When the ADII was one SD higher, the marker of glucose metabolism was beta*100 percent higher or lower through the effect of the ADII on inflammation.

[g] The multiple mediation analysis described by Preacher and Hayes did not provide *p*-values.[34]

estimation of the inflammatory potential of the diet. First, the variation in the ADII was not solely driven by the components with a large range in the intake, in contrast to the previously-published DII. Intake of tea explained most of the variation in the original DII in our study, because intake of tea ranged from 0 to 1,500 ml per day. By using the standardized intakes, the ADII is less dependent on the intake range of the components in the study under investigation. Therefore, it is likely that the results from the ADII will be more comparable between populations. Second, the ADII also avoided an over-estimation of the inflammatory effect of certain nutrients by excluding alcoholic beverages, total fat, and energy. Third, in our study ADII was associated with the summary score for low-grade inflammation, whereas the original DII was not. The previously-published DII was not associated with CRP on a continuous scale, although it was concluded that diet can affect low-grade inflammation based on the observed adverse association between the DII and elevated CRP concentration (>3 mg/L).[10]

Other diet quality scores have shown to be associated with chronic low-grade inflammation. The Alternative Healthy Eating Index (AHEI)[8], the alternate Mediterranean Diet Index (MEDI)[8], and the Mediterranean Diet Score[35] were inversely associated with CRP, IL-6, and sICAM. When examined closely, those scores have some similarities with the ADII. A low intake of

cereal fibre and a high intake of trans fat are considered unhealthy in the AHEI, which is in line with the dietary inflammatory weights for total fibre (-0.52) and trans fatty acids (0.26) in the ADII. Furthermore, AHEI gives a preference to PUFA and MEDI to MUFA over SFA. In the ADII, *n3* PUFA and MUFA are considered anti-inflammatory, whereas SFA are considered pro-inflammatory. Additionally, intake of ethanol is considered healthy in the AHEI, MEDI, and Mediterranean Diet Score, which is in line with the dietary anti-inflammatory weights for ethanol (-0.53) in the ADII. Even though the purpose of those diet quality scores was not to assess the inflammatory potential of diet, those studies do provide evidence that diet as a whole may play a role in chronic low-grade inflammation.

The summary score for low-grade inflammation explained a significant proportion of the association between the ADII and HOMA-IR, even independent of BMI. This supports the hypothesis that inflammation mediates, at least in part, the association between diet and insulin resistance. As part of an inflammatory environment, a more pro-inflammatory diet could lead to impaired action of insulin.[7]

There are a number of strengths of this study to consider. First, in addition to CRP, five other markers of inflammation were examined, providing a more thorough assessment of low-grade inflammation. Second, a validated FFQ was used to assess intake. This FFQ has been found appropriate for ranking participants for ten of the nutrients included in the ADII.[25] Third, the ADII is strengthened by its theory and literature-based instead of data-driven nature. It should be noted, however, that despite the use of a systematic approach for constructing the literature-based dietary inflammatory weights, subjective decisions were made by Cavicchia *et al*..[10]

Besides the strengths, this study has limitations as well. First, our results are limited by the cross-sectional nature of our study, which does not allow conclusions about causality. We tried to limit the possibility of reverse causation by excluding participants with known diabetes who may have changed their diet recently. Second, the external validity of our study might be low, since all participants were Caucasian and the CODAM study included participants with a high risk of impaired glucose metabolism. Including a high-risk population, however, increased the variation in the markers of glucose metabolism. Third, intake of some dietary components, which were included in the previously published DII, such as ginger and saffron, could not be calculated from our FFQ (**Table 7.1**). However, the variation in intake of those specific dietary components is expected to be low in a mostly non-vegetarian Dutch population. Fourth, despite the fact that extensive information about potential confounders was available, residual confounding might remain because potential confounders could be measured with error. Lastly, the results should be confirmed by other studies before clinical application can be considered.

In conclusion, the adverse associations between the inflammatory potential of the diet, as assessed with the ADII, with low-grade inflammation and HOMA-IR suggest that low-grade inflammation might be one of the pathways through which diet affects insulin resistance. More research is needed to verify whether the ADII is associated with low-grade inflammation in other populations and to investigate whether low-grade inflammation directly mediates the association between diet and development of diabetes.

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Authors' responsibilities were as follows: GJvW: prepared the data for analyses, performed the analysis, and drafted the manuscript; DT, EJMF: contributed to the interpretation of the data;

IF, MMvG, CJvdK, CGS, CDAS, EEB, EJMF: participated in the design and coordination of the CODAM study; CDAS, GN, and JMD: participated in the design and coordination of the Hoorn study; DT, AK, IF, MMvG, CJvdK, CGS, CDAS, MCO, GN, JMD, EEB, EJMF: critically revised the manuscript and agreed to be listed as authors. None of the authors had a conflict of interest.

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Supplementary Table 7.1 Intake of dietary components by tertiles of the adapted dietary inflammatory index (*n*= 1,024)[a]

	Adapted dietary inflammatory index		
	Tertile 1	**Tertile 2**	**Tertile 3**
Range	**(-12.0-<-1.3)**	**(-1.3-<1.2)**	**(1.2-<15.7)**
Median	**-2.5**	**-0.03**	**2.7**
	(*n*= 341)	**(*n*= 342)**	**(*n*= 341)**
Energy(kcal/day)	2079 (755)	2045 (546)	2181 (677)
Protein (g/day)	83 (24)	79 (20)	77 (23)
Carbohydrates (g/day)	232 (81)	224 (64)	242 (80)
Total fat (g/day)	80 (39)	82 (27)	89 (33)
Saturated fatty acids (g/day)	30 (12)	32 (10)	36 (15)
Mono-unsaturated fatty acids (g/day)	26 (17)	26 (9)	28 (11)
Trans fatty acids (g/day)	2 (1)	3 (1)	3 (2)
n3 poly-unsaturated fatty acids (g/day)	1 (0.7)	1 (0.5)	1 (0.5)
n6 poly-unsaturated fatty acids (g/day)	13 (7)	13 (5)	13 (5)
Cholesterol (mg/day)	215 (88)	222 (83)	231 (89)
Fibre (g/day)	27 (7)	23 (6)	21 (7)
Ethanol (g/day)	9 (1-20)	8 (2-21)	6 (0.5-21)
Wine (g/day)	14 (0-75)	10 (0-57)	3 (0-30)
Beer (g/day)	0 (0-33)	0 (0-57)	4 (0-86)
Liquor (g/day)	0 (0-3)	0 (0-7)	0 (0-7)
Caffeine (g/day)	0.3 (0.2-0.4)	0.3 (0.2-0.4)	0.3 (0.2-0.4)
Vitamin A (µg/day)	1441 (707)	1311 (665)	1189 (566)
Beta-carotene (µg/day)	3083 (1301)	2462 (963)	1897 (832)
Thiamin (mg/day)	1 (0.3)	1 (0.3)	1 (0.3)
Riboflavin (mg/day)	2 (0.5)	2 (0.5)	2 (0.5)
Niacin (mg/day)	20 (14)	18 (6)	17 (7)
Vitamin B6 (mg/day)	2 (0.6)	2 (0.4)	2 (0.5)
Folate (µg/day)	230 (64)	200 (51)	183 (54)
Vitamin B12 (µg/day)	5 (2)	4 (2)	4 (2)
Vitamin C (mg/day)	133 (45)	103 (33)	85 (34)
Vitamin D (µg/day)	3 (2)	3 (1)	3 (2)
Vitamin E (mg/day)	15 (15)	13 (5)	13 (5)
Iron (mg/day)	13 (4)	12 (3)	12 (4)
Magnesium (mg/day)	384 (162)	345 (82)	325 (93)
Selenium (mg/day)	45 (14)	43 (12)	42 (13)
Zinc (mg/day)	11 (3)	10 (3)	10 (3)
Quercetin (mg/day)	15 (6)	10 (5)	7 (4)
Tea (g/day)	375 (125-500)	125 (36-375)	125 (4-250)
Garlic (g/day)	0 (0-0.3)	0 (0-0.1)	0 (0-0.1)

[a] Data are expressed as means (standard deviation) or median (p25-p75).

Supplementary Table 7.2 Characteristics of the study population and intake of dietary components by tertiles of the dietary inflammatory index (*n*= 1,024)[a]

	Dietary inflammatory index		
	Tertile 1	**Tertile 2**	**Tertile 3**
Range	**(-20.7-<-0.86)**	**(-0.86-<0.76)**	**(0.76-<6.4)**
Median	**-2.1**	**-0.01**	**1.6**
	(*n*= 341)	**(*n*= 342)**	**(*n*= 341)**
Age (years)	66.5 (8.2)	64.2 (8.7)	62.3 (8.4)
Sex (% men)	43.7	52.6	69.8
Study (% Hoorn)	71.3	60.8	44.9
Smoking (% current)	10.6	15.5	27.9
Any physical activity (hours/day)	3.9 (2.9)	4.0 (2.8)	4.3 (3.0)
Body mass index (kg/m^2)	27.0 (3.8)	27.8 (3.8)	28.1 (3.9)
Diabetes category (% NGM)	48.4	50.9	54.3
Family history of diabetes (%)[b]	26.4	25.7	30.2
Lipid-lowering medication (%)	15.0	18.7	15.3
Anti-hypertensive medication (%)	32.3	36.6	34.9
Dietary intake			
Energy(kcal/day)	1931 (554)	1969 (669)	2406 (665)
Protein (g/day)	74 (20)	76 (22)	88 (23)
Carbohydrates (g/day)	216 (62)	216 (72)	266 (80)
Total fat (g/day)	71 (25)	79 (35)	100 (33)
Saturated fatty acids (g/day)	28 (9)	31 (11)	40 (15)
Mono-unsaturated fatty acids (g/day)	22 (9)	25 (16)	32 (11)
Trans fatty acids (g/day)	2 (1)	3 (1)	4 (2)
n3 poly-unsaturated fatty acids (g/day)	1 (0.5)	1 (1)	2 (1)
n6 poly-unsaturated fatty acids (g/day)	11 (5)	12 (6)	15 (6)
Cholesterol (mg/day)	190 (70)	213 (77)	265 (95)
Fibre (g/day)	24 (6)	22 (6)	25 (8)
Ethanol (g/day)	9 (1-26)	8 (1-20)	6 (1-17)
Wine (g/day)	14 (0-100)	10 (0-57)	3 (0-29)
Beer (g/day)	0 (0-27)	0 (0-57)	5 (0-86)
Liquor (g/day)	0.3 (0-2)	0 (0-7)	0 (0-14)
Caffeine (g/day)	0.3 (0.2-0.4)	0.3 (0.2-0.4)	0.3 (0.2-0.5)
Vitamin A (µg/day)	1180 (521)	1214 (611)	1520 (764)
Beta-carotene (µg/day)	2626 (1218)	2471 (1156)	2345 (1075)
Thiamin (mg/day)	1 (0.3)	1 (0.3)	1 (0.4)
Riboflavin (mg/day)	2 (0.5)	1 (0.5)	2 (0.5)
Niacin (mg/day)	17 (8)	18 (13)	20 (6)
Vitamin B6 (mg/day)	2 (0.6)	2 (0.5)	2 (0.5)
Folate (µg/day)	202 (60)	195 (54)	216 (62)
Vitamin B12 (µg/day)	4 (2)	4 (2)	5 (2)
Vitamin C (mg/day)	116 (46)	105 (41)	101 (40)
Vitamin D (µg/day)	3 (1.3)	3 (1)	4 (2)
Vitamin E (mg/day)	12 (4)	13 (15)	15 (6)
Iron (mg/day)	12 (3)	12 (3)	13 (4)
Magnesium (mg/day)	348 (100)	340 (154)	365 (96)
Selenium (mg/day)	40 (11)	42 (13)	48 (14)
Zinc (mg/day)	10 (3)	10 (3)	11 (3)
Quercetin (mg/day)	16 (5)	9 (4)	7 (3)
Tea (g/day)	500 (375-750)	250 (71-250)	36 (0-125)
Garlic (g/day)	0 (0-0.1)	0 (0-0.1)	0 (0-0.1)

Abbreviation: NGM=normal glucose metabolism

[a] Characteristics were expressed as mean (standard deviation), median (p25-p75), or percentages.

[b] Missing for 122 participants.

Abstract

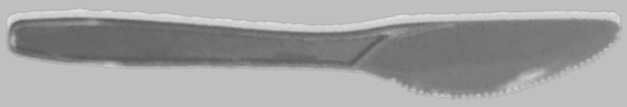
Objective: To investigate whether a dietary inflammatory pattern (DIP) is associated with risk of type 2 diabetes in a cohort study.

Methods: During a median follow-up period of 12.4 years, 456 diabetes cases were confirmed among 4,366 Dutch adults (mean age 67 years) who did not have diabetes at baseline. The intake of 42 food groups derived from a food frequency questionnaire was combined in such a way that the variation in the inflammation marker C-reactive protein (CRP) was explained as much as possible. As such, a DIP score that reflected the inflammatory potential of the diet was obtained. DIP was divided into tertiles and its association with risk of type 2 diabetes was examined using Cox proportional hazards models including major confounders as age, sex, and smoking.

Results: A higher DIP was characterized by a lower intake of whole grain products, rice, vegetable fats, cakes and cookies, fruit, and tea, and a higher intake of spirits, animal fats, processed red meat, eggs, and refined grain products. DIP was associated with a higher risk of type 2 diabetes (adjusted hazard ratio $(HR)_{T3\ vs.\ T1}$= 1.61 [95% confidence interval (95%CI) 1.27, 2.03]; p_{trend}= <0.001). Additional adjustment for $\log_e$(CRP) attenuated the association ($HR_{T3\ vs.\ T1}$= 1.38 [95%CI 1.09, 1.75]; p_{trend}= <0.01).

Conclusion: The inflammatory potential of the diet, as estimated with the DIP, was associated with risk of type 2 diabetes. This suggests that diet can increase risk of type 2 diabetes through its effect on chronic low-grade inflammation.

CHAPTER 8



Dietary inflammatory pattern and risk of type 2 diabetes: the Rotterdam study

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In preparation

Introduction

As the global prevalence of type 2 diabetes is expected to increase from 171 million in 2000 to 366 million in 2030, investigations into potentially modifiable risk factors for type 2 diabetes are important.[1, 2] Lifestyle intervention programs promoting weight loss, physical activity, and a healthy diet reduced risk of type 2 diabetes by up to 60% in persons with impaired glucose metabolism.[2, 3] The exact pathways through which these modifiable risk factors affect risk of type 2 diabetes are largely unknown.

It has been suggested that chronic low-grade inflammation could be one of these pathways. Chronic low-grade inflammation as reflected by slightly elevated concentrations of C-reactive protein (CRP) was associated with higher risk of type 2 diabetes in prospective studies[4] and a polymorphism causing a higher concentration of CRP has been associated with higher risk of type 2 diabetes as well[5, 6]. This suggests that chronic low-grade inflammation may precede the development of type 2 diabetes.

Disturbed adipose tissue functioning, as seen in persons with overweight, is considered the main cause of chronic low-grade inflammation.[7] Several dietary components can be considered as either anti-inflammatory or pro-inflammatory, because they affect adipose tissue functioning or other mechanisms related to inflammation.[8] If diet affects, in part, risk of type 2 diabetes through its effect on inflammation, it could be hypothesized that a dietary pattern favouring chronic low-grade inflammation should be associated with risk of type 2 diabetes.

A dietary pattern combines the intake of several foods or food groups into one measure taking into account that foods are eaten together. Dietary patterns can be constructed by techniques such as factor analysis or principal component analysis.[9] Using these methods, exploratory dietary patterns are derived that explain as much variation in intake of foods as possible. A recent review showed that 'meat based' or 'western' dietary patterns tended to be associated with higher concentrations of inflammation markers, whereas 'healthy' or 'vegetable and fruit' based dietary patterns tended to be associated with lower concentrations of inflammation markers.[10] Thus, it is likely that associations between these patterns and type 2 diabetes are probably to some extent explained by chronic low-grade inflammation. However, these are general dietary patterns and not per definition the most detrimental or beneficial dietary inflammatory pattern (DIP) as regards risk of type 2 diabetes. The dietary inflammatory potential is better investigated using a dietary pattern that is constructed to explain as much variation as possible in inflammation markers. With this, the extent to which diet affects the processes leading to type 2 diabetes through inflammation could be studied more optimally.

So far, the association between a dietary pattern that optimally explained variation in inflammation markers has only been studied once in relation to risk of type 2 diabetes.[11] In this prospective cohort study, the USA based Nurses' Health Study, a DIP was constructed in a subsample of the population with data on circulating inflammation markers. Subsequently, the derived DIP was applied to a larger sample. Results from the subsample and the larger sample showed that women with the highest DIP had at least a 2.5 times higher risk of type 2 diabetes compared with women with the lowest DIP.[11] As the DIP was by definition dependent on the dietary behaviour of the study population, however, these results may not be transferred to other study populations.

As it appears that available literature only refers to American women and is scarce, we constructed a DIP and investigated its association with risk of type 2 diabetes in a prospective Dutch cohort study.

Methods

Study population

The current analysis was conducted within the Rotterdam study. The Rotterdam study is a population-based prospective cohort study among inhabitants of Ommoord, a district of the city of Rotterdam, The Netherlands.[12, 13] In 1990, all inhabitants of this district who were aged ≥55 years were invited for participation (*n*= 10,215). A number of 7,983 (78%) responded. The Medical Ethics Committee of Erasmus Medical Centre approved the study. All participants gave informed consent.

Population for analysis

Of the 7,983 responders (78%), 2,339 (29%) did not fill out a dietary food frequency questionnaire, 209 did not provide sufficient dietary data, 516 had type 2 diabetes at baseline, 448 had not sufficient data on CRP, and 105 had not sufficient information on follow-up time or other covariates. Hence, 4,366 participants were included in the population for analysis (**Figure 8.1**).

Diabetes prevalence and incidence

Participants were considered a prevalent diabetes case when they used anti-diabetes medication collected by means of a questionnaire or had a non-fasting or 2-hour glucose concentration of ≥11.1 mmol/L.[14] Use of anti-diabetes medication was assessed by means of the Anatomical Therapeutical Chemical (ATC) classification index codes.

During follow-up, information from general practitioners, pharmacies' databases, and follow-up examinations in 1993-1995, 1997-1999, and 2002-2004 was used to identify cases of diabetes. Participants were considered incident diabetes case when they were registered by a general practitioner as having type 2 diabetes and had at least one of the following four criteria: fasting plasma glucose concentration ≥7.0 mmol/L, random plasma glucose concentration ≥11.1 mmol/L, anti-diabetes medication, and/or treatment by diet.[5, 15-18] Diabetes cases were recorded

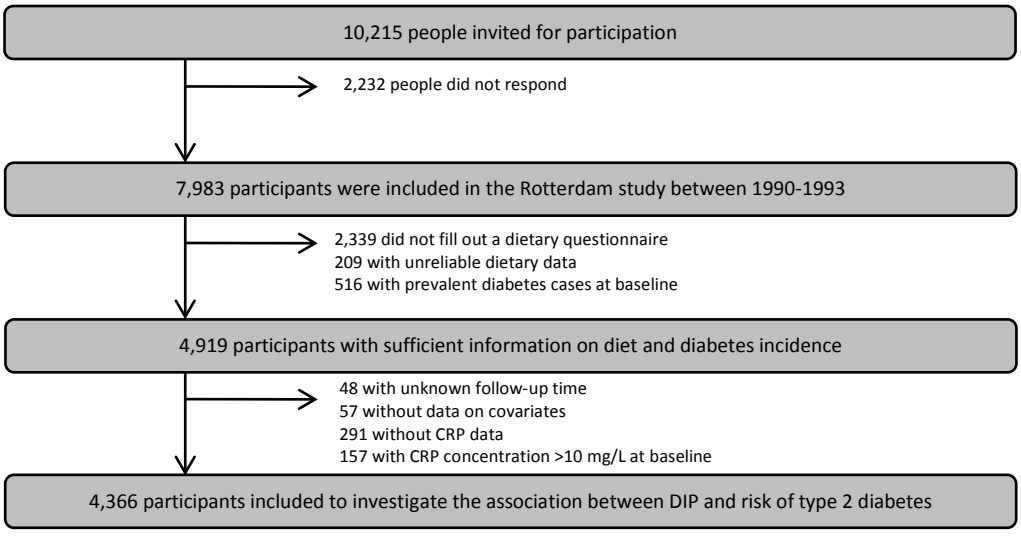

Figure 8.1 Flow diagram for inclusion of participants to investigate whether the dietary inflammatory pattern (DIP) is associated with risk of type 2 diabetes

until July 2005.[16-18]

C-reactive protein

Non-fasting serum samples were collected at the research centre at baseline. These samples were immediately put on ice and processed within 30 minutes. High-sensitivity CRP was measured using a rate near-infrared particle immunoassay (Immage Immunochemistry System, Beckman Coulter, Fullerton, California, United States). The procedure has been described in more detail elsewhere.[5] CRP concentrations >10 mg/L were excluded because these higher concentrations reflect acute rather than chronic low-grade inflammation.[19] In the Rotterdam study, serum CRP concentration was associated with a higher risk of type 2 diabetes (hazard ratio $(HR)_{\text{CRP quartile 4 vs. quartile 1}}$ = 1.76 [95% confidence interval (95%CI) 1.27, 2.45]; p_{trend} = <0.01).[5, 17, 18]

Dietary intake

Dietary assessment was performed at baseline (1990-1993) and comprised two steps: first, participants had to mark the foods and drinks they had consumed at least twice a month in the preceding year on a self-administered questionnaire at home; and second, at the research centre, a trained dietician obtained accurate information on the amount of foods and drinks indicated on the self-administered questionnaire using a semi-quantitative food frequency questionnaire.[20] This food frequency questionnaire comprised 170 food items and additional questions about prescribed diets.

Intake of all food items was converted into total intake of energy and nutrients using the Dutch Food Composition table 1993 (NEVO). Intake of fibre was derived from the next version of this table (NEVO 1996), because data on fibre were not sufficient in 1993. The relative validity of intake of nutrients ranged from 0.44 to 0.85, indicating that ranking participants was relatively good in this elderly population.[20]

Dietary inflammatory pattern

Before the actual calculation of the DIP, data had to be prepared. First, CRP was transformed logarithmically to achieve a symmetric distribution. Second, the 170 food items from the FFQ were collapsed into 42 food groups (**Supplementary Table 8.1**). To avoid that food groups with a large range of intake solely drove the variation in the DIP, the mean intake of the population was subtracted from the individual intake and subsequently the difference was divided by the standard deviation of the study population (*z*-score).

After this preparation step, the actual calculation followed. To derive a DIP score that reflected the influence of the intake of a combination of foods groups on $\log_e$(CRP) concentration, we run a linear regression model including all food groups as independent variables and $\log_e$(CRP) concentration as dependent variable. To obtain an individual DIP score, the beta-coefficient of a food group derived from this model was multiplied by the intake of the food group and summed across all food groups ($\sum$ $\text{beta-coefficient}_{\text{food group}} * \text{intake}_{\text{food group}}$). As such, the DIP score reflects predicted $\log_e$(CRP) concentration based on the intake and assigned score, i.e., beta-coefficient, of a combination of food groups (**Supplementary Table 8.1)**. A higher score implied a stronger association with CRP, and thus a more pro-inflammatory diet.

In an additional analysis, food groups were adjusted for energy according the residuals method before inclusion as independent variables in the linear regression model in order to investigate whether taken energy-adjusted intake yield different results.[21]

Non-dietary covariates

General information (e.g., smoking status, family history of type 2 diabetes) was obtained with a questionnaire at baseline. A family history of type 2 diabetes was defined as having a parent, sibling, or both with type 2 diabetes. Information on energy expenditure (kcal/day) as measure of physical activity was obtained with a validated questionnaire (Longitudinal Aging Study Amsterdam (LASA) Physical Activity Questionnaire) during follow-up from 1997 to 2000 for 3,244 participants of our population for analysis. This questionnaire includes questions about frequency and duration of bicycling, walking outside, sport activities, and house-hold activities and appeared to correlated well with a 7 day-diary (r= 0.68) and pedometer (r= 0.56).[22]

Information on cardiovascular risk factors of each participant was obtained by clinical examinations during a visit at the research centre at baseline. Height and weight were measured and body mass index (BMI) (kg/m²) was calculated. Waist circumference (cm) was measured at the level midway between the lower rib margin and the iliac crest with the participant in standing position. Blood pressure was measured twice at the right brachial artery with a random-zero sphygmomanometer with the participant in a sitting position. The mean of two consecutive measurements was used. Hypertension was defined as a systolic blood pressure ≥140 mmHg, and/or diastolic blood pressure ≥90 mmHg, and/or use of blood pressure-lowering medication. Serum total cholesterol was determined in blood samples with an automated enzymatic procedure using Roche CHOD-PAP reagent agent. HDL-cholesterol was measured with Roche HDL cholesterol essay using polyethylene glycol-modified enzymes and dextran sulphate.

Data analysis

The DIP was divided into tertiles based on the population distribution. Descriptive data were expressed as mean (standard deviation (SD)), median (p25-p75 cut-offs), or percentage according to tertiles of DIP. To determine the food groups that explained most of the variation in DIP between participants, forward linear regression was used.

The association between the DIP and $\log_e$(CRP) was investigated using linear regression models. Adjustments were made for age (years), sex, family history of diabetes (yes or no), diet prescription (yes or no), smoking (current, former, or never), and intake of energy (kcal/day). In an additional model, the association between DIP and $\log_e$(CRP) was adjusted for BMI (kg/m^2).

Cox proportional hazards models were used to calculate HR and 95%CI. The lowest tertile of the DIP was considered as the reference for the other two tertiles. The crude model included the DIP as independent variable. To obtain model 1, the crude model was extended with five covariates: age (years), sex, family history of diabetes (yes or no), diet prescription (yes or no), smoking (current, former, or never), and intake of energy (kcal/day). To investigate the potential mediating effect of CRP on the association between DIP and type 2 diabetes, $\log_e$(CRP) (mg/L) was added to model 1 as additional covariate (model 1+$\log_e$(CRP)). To investigate whether the potential mediating effect of CRP was driven by BMI (kg/m^2), BMI was added to model 1 in the second additional model (model 1+BMI). In the third additional model, $\log_e$(CRP) and BMI (kg/m^2) were added simultaneously to model 1 (model 1+$\log_e$(CRP)+BMI). As waist circumference and energy expenditure were not available in the total population for analysis, the potential confounding effect of these measures were studied in sensitivity analyses.

To study whether DIP acted via other potential pathways, model 1 with $\log_e$(CRP) and BMI was additional adjusted for total cholesterol, HDL-cholesterol, or hypertension.

To investigate potential effect measure modification by sex (*men*: n= 1,758, n_{cases}= 203; *women*: n= 2,608, n_{cases}= 253) or BMI categories (*normal weight*: n= 1,705, n_{cases}= 76; *overweight*: n= 2,055, n_{cases}= 245; *obese*: n= 606, n_{cases}= 135), an interaction term between DIP and sex or BMI

was included in model 1.

Sensitivity analyses were performed by excluding participants who developed type 2 diabetes within 2 years of follow-up (n_{cases}= 19), but the interpretation of the results did not change.

Tests for trend across categories were performed by assigning the median value for each category to each participant and modelling this variable as a continuous variable.

All statistical analyses were performed in SAS (version 9.2, SAS Institute, Cary, NC). A two-sided *p*-value ≤0.05 was considered as statistically significant for all analyses.

Results

The median DIP score was -0.01 (range -0.21 to 0.23) and explained 2.9 percent of the variation in $\log_e$(CRP) concentration. Across tertiles of DIP participants were on average older, smoked more, and had a higher BMI, waist circumference, and CRP concentration (**Table 8.1**). A higher DIP score was characterized by a lower intake of whole grain products, vegetable fats, rice, cakes and cookies, fresh fruit, and tea (all *r*≥ -0.20), and a higher intake of spirits, animal fats, processed red meat, eggs, and refined grain products (all *r*> 0.15) (**Supplementary Table 8.1**). Most of the variation in DIP was explained by intake of whole grain products (19%), followed by intake of rice (15%), processed red meat (9%), and cakes and cookies (6%).

In line with the higher CRP concentration observed in the highest tertile of the DIP compared with the lowest (2.0 vs. 1.3 mg/L), linear regression analysis showed that a one unit higher DIP score was associated with a higher $\log_e$(CRP) concentration in the crude model (*beta*= 1.00 [95%CI 0.85, 1.15], *p*= <0.01) as well as after inclusion of covariates (Model 1, *beta*= 0.83 [95%CI 0.67, 0.99], *p*= <0.01) and covariates and BMI (Model 1+BMI, *beta*= 0.66 [95%CI 0.51, 0.81], *p*= <0.01). DIP had a Spearman correlation of 0.19 with $\log_e$(CRP), 0.09 with BMI, and 0.13

Table 8.1 Characteristics of the Rotterdam study according to tertiles of the dietary inflammatory pattern (*n*= 4,366)[a]

	Dietary inflammatory pattern		
	Tertile 1 (≤-0.07) (*n*= 1,455)	**Tertile 2 (>-0.07-≤0.06) (*n*= 1,456)**	**Tertile 3 (>0.06) (*n*= 1,455)**
Age (years)	66.1 (7.3)	67.6 (7.8)	68.1 (7.9)
Sex (% men)	42.5	33.7	44.6
Body mass index (kg/m²)	25.7 (3.4)	26.5 (3.6)	26.6 (3.8)
Waist circumference (cm)[b]			
Men	92.9 (9.8)	94.0 (8.5)	95.1 (9.3)
Women	84.6 (11.1)	87.3 (10.8)	88.4 (11.1)
Cholesterol (mmol/L)			
Total	6.6 (1.1)	6.7 (1.2)	6.8 (1.2)
HDL	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
C-reactive protein (mg/L)	1.3 (0.7-2.5)	1.7 (0.8-2.9)	2.0 (1.1-3.6)
Family history of diabetes (% yes)	28.2	30.1	24.7
Hypertension (% yes)	48.5	54.5	54.8
Smokers (% current)	15.3	20.8	32.0
Physical activity (kcal/day)[c]	834 (559-1177)	847 (535-1212)	746 (453-1111)
Diet prescription (% yes)	12.0	10.2	9.4

Abbreviation: HDL=high-density lipoprotein

[a] Values are expressed as means (standard deviation), median (p25-p75) or percentages. Median was used, because of the skewed distribution.

[b] Missing for 253 participants.

[c] Missing for 1,122 participants.

with waist circumference. Log_e(CRP) had a Spearman correlation of 0.27 with BMI and 0.29 with waist circumference. The Spearman correlation between BMI and waist circumference was 0.65.

Of the 4,366 participants, 456 developed type 2 diabetes during a median follow-up time of 12.4 years. DIP was associated with higher risk of type 2 diabetes (Model 1, $HR_{T3\ vs.\ T1}$= 1.61 [95%CI 1.27, 2.03]; p_{trend}= <0.001) (**Table 8.2**). Additional adjustment for log_e(CRP) or BMI attenuated the association to the same extent (Model 1+log_e(CRP), $HR_{T3\ vs.T1}$= 1.38 [95%CI 1.09, 1.75]; p_{trend}= 0.007; Model 1+BMI, $HR_{T3\ vs.T1}$= 1.37 [95%CI 1.08, 1.75]; p_{trend}= 0.007) (**Table 8.2**). Additional adjustment for log_e(CRP) and BMI at the same time, attenuated the association further ($HR_{T3\ vs.\ T1}$= 1.27 [95%CI 1.00, 1.62]; p_{trend}= 0.04). Inclusion of energy-adjusted food groups instead of energy-unadjusted food groups in DIP did not affect our findings (Model 1, $HR_{T3\ vs.\ T1}$= 1.61 [95%CI 1.27, 2.04]; p_{trend}= <0.01). When either whole grain products, rice, processed red meat, or cakes and cookies, that mostly contributed to the variation in DIP, were not taken into account when the DIP was calculated, the DIP was still associated with risk of type 2 diabetes (Model 1, $HR_{T3\ vs.\ T1}$= 1.56 up to 1.73).

Additional adjustment by waist circumference (n= 4,113; n_{cases}= 427) or physical activity (n= 3,244; n_{cases}= 391) did not attenuate associations between DIP and risk of type 2 diabetes (Model 1+BMI+waist circumference, $HR_{T3\ vs.\ T1}$= 1.41 [95%CI 1.10, 1.80]; Model 1+BMI+physical activity, $HR_{T3\ vs.\ T1}$= 1.41 [95%CI 1.09, 1.82]).

Inclusion of other potential intermediates, i.e., total cholesterol, HDL-cholesterol, and hypertension, did not affect the association (Model 1+BMI+CRP with total cholesterol, $HR_{T3\ vs.\ T1}$= 1.28 [95%CI 1.01, 1.62]; with HDL-cholesterol, $HR_{T3\ vs.\ T1}$= 1.33 [95%CI 1.04, 1.69]; with hypertension, $HR_{T3\ vs.\ T1}$= 1.27 [95%CI 1.00, 1.61]).

On the basis of p for interaction, the association between the DIP and risk of type 2 diabetes did not differ by sex and BMI (*men*: $HR_{T3\ vs.\ T1}$= 1.59 [95%CI 1.13, 2.25], *women* $HR_{T3\ vs.\ T1}$= 1.62 [95%CI 1.16, 2.25], $p_{interaction\ sex}$= 0.45; *normal weight*: $HR_{T3\ vs.\ T1}$= 1.45 [95%CI 0.83, 2.52], *Overweight*: $HR_{T3\ vs.\ T1}$= 1.38 [95%CI 0.99, 1.91], *Obese*: $HR_{T3\ vs.\ T1}$= 1.45 [95%CI 0.93, 2.27], $p_{interaction\ BMI}$= 0.79).

Discussion

We examined whether the inflammatory potential of the diet, as estimated by the DIP, was associated with risk of type 2 diabetes in a prospective cohort study of Dutch adults. The DIP

Table 8.2 Hazard ratios [95% confidence intervals] of the association between dietary inflammatory pattern (DIP) and risk of type 2 diabetes (n= 4,366)

	Median	*n* (cases)	PY	Crude HR [95%CI]	Model 1[a] HR [95%CI]	Model 1 +log_eCRP HR [95%CI]	Model 1 +BMI HR [95%CI]
DIP							
T1: ≤-0.07	-0.14	1,455 (122)	16,506	1 (ref)	1 (ref)	1 (ref)	1 (ref)
T2: >-0.07-≤0.06	-0.01	1,456 (153)	16,181	1.29 [1.02, 1.63]	1.25 [0.98, 1.59]	1.16 [0.91, 1.48]	1.12 [0.88, 1.43]
T3: >0.06	0.15	1,455 (181)	15,254	1.64 [1.30, 2.06]	1.61 [1.27, 2.03]	1.38 [1.09, 1.75]	1.37 [1.08, 1.75]
p for trend				*<0.001*	*<0.001*	*0.007*	*0.007*

Abbreviations: DIP=dietary inflammatory pattern; T1=tertile 1; T2=tertile 2; T3=tertile 3; PY=person-years; HR=hazard ratio; 95%CI=95% confidence interval; CRP=C-reactive protein; BMI=body mass index

[a] Model adjusted for age, sex, smoking, diet prescription, family history of diabetes, and intake of energy.

was associated with higher risk of type 2 diabetes, suggesting that diet affects in part risk of type 2 diabetes through its effect on chronic low-grade inflammation.

Consistent with our results, in two cohorts of the Nurses' Health Study DIP was also associated with a higher risk of type 2 diabetes ($HR_{Q5\ vs.\ Q1}$= 2.56 [95%CI 2.10, 3.12] and 2.93 [95%CI 2.18, 3.92]).[11] The effect estimates were much higher in the cohorts of the Nurses' Health Study compared with our study. This may be attributable to a difference in food groups that correlated to the DIP score between studies. In the Nurses' Health Study, a high DIP score was characterized by a low intake of wine, coffee, cruciferous vegetables, and yellow vegetables, and a high intake of sugar-sweetened beverages, refined grains, processed meat, diet soft drinks, and other vegetables than cruciferous or yellow. In our study, a high DIP score was characterized by a low intake in whole grain products, rice, vegetable fats, cakes and cookies, fresh fruit, and tea, and a high intake of spirits, animal fats, processed red meat, and refined grain products. This does not mean that the anti- or pro-inflammatory effect of a food product itself differed between United States of America and the Netherlands where the Nurses' Health Study and our study were conducted respectively. It indicates, however, that the contribution of food products to the inflammatory effect of the total diet depends on the underlying variation in intake of food products. For example, if tea is not much drunk, it is expected that tea will not contribute much to the DIP score, whereas tea is expected to contribute to DIP if the variation was larger as tea is generally considered to have anti-inflammatory effects.[8] This means that our DIP should best be compared with a DIP constructed in a population with a comparable variation in intake.

The higher risk estimates observed in the Nurses' Health Study compared with our risk estimate may also result from the difference in the number of inflammation markers used to obtain the DIP. The DIP in the Nurses' Health Study was based on the concentration of six inflammation markers in a subsample of the population (*n*= 1,350), of which E-selectin and CRP were most strongly correlated with their DIP. In contrast, our DIP was based on CRP concentration only, but it was measured in all 4,366 participants. As it is expected that inclusion of other inflammation markers would have resulted in a DIP more closely related to overall inflammatory status, the associations found in our study were likely underestimated.
Furthermore, differences in the magnitude of the risk estimates can also be attributable to a larger range in the DIP or differences in the food frequency questionnaires in the Nurses' Health Study compared with our study.

Although DIP was associated with CRP in our cohort, the explained variance in CRP was low (2.9%). This may suggest that DIPs that are constructed to reflect the inflammatory potential of the diet may be comparable with exploratory dietary patterns that are constructed to explain as much variance among food groups without taking into account a priori information about potential pathways. Observational studies showed that so-called 'western' dietary pattern that is generally characterized by at least a high intake of unprocessed red meat, processed red meat, and refined grains, are likely to be associated with a higher concentration of CRP and a higher risk of type 2 diabetes.[10, 23] A so-called 'healthy' dietary pattern that is generally characterized by at least a high intake of whole grain products, vegetables, and fruit, tended to be associated with a lower CRP concentration and lower risk of type 2 diabetes.[10, 23] The food groups that consistently characterize the 'western' and 'healthy' dietary patterns are also those that are evident in the DIP. Overall, both dietary pattern approaches suggest that intake of whole grain products, fruit, and vegetables are related to the anti-inflammatory properties, whereas red meat and refined grains are related to the pro-inflammatory properties of the overall diet. However, as exclusion of the main contributors from the calculation of our DIP did not affect the association with diabetes much, small or modest contributions to the total inflammatory potential of other foods than

these main contributors might be important as well.

Strengths of our analyses included the prospective design, the inclusion of verified cases of diabetes, the large number of participants with measured CRP concentration, and the extensive information on potential confounders that minimized the presence of residual confounding.

However, limitations should be considered as well. First, information on dietary intake was obtained once. If participants changed their diet through follow-up, this could have either attenuated or de-attenuated our results.

Second, CRP was measured at baseline through which a potential mediating effect of chronic low-grade inflammation could not be studied optimally. With repeated measures of CRP concentrations and other inflammation markers over time, it will be more evident whether chronic low-grade inflammation increases due to diet before diagnosis of type 2 diabetes. Furthermore, CRP was measured in non-fasting blood samples, but as the half-life of CRP is at least 15 hours, fasting CRP concentration may reflect potential acute effects of diet on CRP concentration as well.[24]

Third, as chronic low-grade inflammation is largely induced by obesity[8], it was not possible to disentangle the effect of BMI and waist circumference on chronic low-grade inflammation from its effects on other pathways leading to type 2 diabetes.

Fourth, although extensive effort was made to identify incident cases of type 2 diabetes[5], some cases without symptoms of type 2 diabetes may have been missed. If missed, our association would be rather underestimated than overestimated, because a higher DIP was likely to be associated with risk factors of type 2 diabetes.

Finally, our DIP explained 2.9% of the variation in CRP, leaving room for the DIP to act via other pathways than chronic low-grade inflammation. As our DIP was still associated with a higher risk of type 2 diabetes after adjustment for CRP, this suggests that other intermediates are involved. However, additional adjustment for total cholesterol, HDL-cholesterol, or hypertension did not change the risk estimates considerably. This may indicate that adjusting for CRP may not be enough to show the total mediating effect of chronic low-grade inflammation.

In conclusion, DIP was associated with higher risk of type 2 diabetes. Thus, it appears likely that diet can affect risk of type 2 diabetes through chronic low-grade inflammation. Before firm conclusions can be drawn, the associations should be studied in other cohorts with a comparable underlying food intake.

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GJvW prepared the data for analyses, performed the analysis, and drafted the manuscript. SSSM and EJMF contributed to the interpretation of the results and revised the manuscript. AK revised the manuscript. AH, FJAvR, EJGS, JCMW, and OHF participated in the coordination of the study and revised the manuscript. GJvW is the guarantor of this work and, as such, had full access to all the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Supplementary Table 8.1 Intake of food groups included in the calculation of the dietary inflammatory pattern (DIP) by tertiles of DIP, Spearman correlation between food groups and DIP, and beta-coefficient used to calculate an individual DIP score (*n*= 4,366)

		Dietary inflammatory pattern		
	All	**Tertile 1[a]**	**Tertile 2[a]**	**Tertile 3[a]**
		(≤-0.07)	**(>-0.07-≤0.06)**	**(>0.06)**
	(*n*= 4,366)	**(*n*= 1,455)**	**(*n*= 1,456)**	**(*n*= 1,455)**
Whole grain products (g/day)	115 (80-167)	140 (105-175)	115 (813-143)	84 (40-117)
Vegetable fats (g/day)	24 (12-36)	30 (19-42)	23 (12-34)	18 (8-30)
Rice (g/day)	5 (0-14)	10 (0-23)	5 (0-13)	0 (0-9)
Cakes and cookies (g/day)	28 (14-44)	33 (18-53)	29 (15-43)	20 (8-35)
Fresh fruit (g/day)	216 (136-297)	244 (169-331)	221 (141-297)	181 (109-266)
Tea (g/day)	375 (250-500)	375 (250-625)	375 (250-500)	250 (125-500)
Pasta (g/day)	7 (0-16)	11 (0-21)	6 (0-14)	5 (0-13)
Sandwich spreads (g/day)	12 (0-24)	16 (6-30)	12 (0-22)	10 (0-20)
Vegetarian dishes (g/day)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Raw vegetables (g/day)	42 (23-68)	49 (27-74)	41 (23-68)	37 (19-61)
Poultry (g/day)	9 (3-18)	13 (4-214)	9 (19-16)	7 (0-14)
Wine (g/day)	2 (0-34)	5 (0-50)	3 (0-35)	0 (0-21)
Low-fat cheese (g/day)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Nuts (g/day)	0 (0-5)	0 (0-9)	0 (0-4)	0 (0-4)
Low-fat dairy (g/day)	246 (96-413)	257 (116-421)	252 (113-422)	224 (66-393)
Potatoes (g/day)	118 (85-167)	128 (86-178)	112 (78-155)	118 (86-157)
Vegetable oils (g/day)	2 (0-5)	2 (0-6)	2 (0-5)	1 (0-4)
Pizza (g/day)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Lean fish (g/day)	7 (0-21)	7 (0-25)	7 (0-20)	7 (0-18)
Cooked vegetables (g/day)	155 (116-197)	160 (119-200)	156 (117-196)	147 (112-194)
Cornflakes (g/day)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Shell fish (g/day)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Legumes (g/day)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Chips (g/day)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Beer (g/day)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Coffee (g/day)	500 (313-625)	500 (313-625)	500 (375-563)	500 (250-625)
High-fat dairy (g/day)	60 (18-141)	63 (18-149)	57 (17-137)	58 (19-139)
Other fruit than fresh (g/day)	2 (0-11)	3 (0-11)	3 (0-11)	3 (0-11)
High-fat cheese (g/day)	30 (20-44)	30 (20-45)	29 (20-43)	31 (20-46)
Fruit juices (g/day)	0 (0-40)	0 (0-34)	0 (0-41)	0 (0-67)
Soup (g/day)	36 (12-71)	36 (8-71)	36 (11-71)	36 (16-82)
Confectionary (g/day)	4 (0-12)	3 (0-11)	4 (0-11)	4 (0-14)
Sugar (g/day)	6 (0-24)	5 (1-16)	5 (0-20)	9 (0-35)
Unprocessed red meat (g/day)	70 (49-94)	68 (45-91)	69 (48-91)	73 (54-99)
Fatty fish (g/day)	0 (0-2)	0 (0-1)	0 (0-16)	0 (0-4)
Soft drinks (g/day)	0 (0-25)	0 (0-0)	0 (0-13)	0 (0-50)
Water (g/day)	175 (0-349)	50 (0-349)	175 (0-349)	175 (0-524)
Spirits (g/day)	0 (0-8)	0 (0-3)	0 (0-2)	0 (0-50)
Animal fats (g/day)	9 (1-21)	6 (0-16)	9 (1-20)	12 (3-26)
Processed red meat (g/day)	18 (7-31)	15 (4-26)	17 (7-30)	22 (12-35)
Eggs (g/day)	14 (7-14)	14 (7-14)	14 (7-14)	14 (7-21)
Refined grain products (g/day)	17 (3-36)	11 (15-23)	15 (3-30)	25 (5-76)

Supplementary Table 8.1 continues on the next page

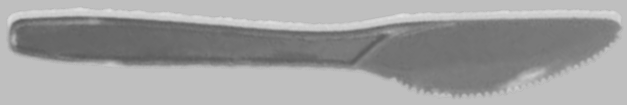
Supplementary Table 8.1 (*continued*) Intake of food groups included in the calculation of the dietary inflammatory pattern (DIP) by tertiles of DIP, Spearman correlation between food groups and DIP, and beta-coefficient used to calculate an individual DIP score (*n*= 4,366)

	Spearman correlation with DIP	Beta-coefficient[b]
Whole grain products (g/day)	-0.44	-0.063
Vegetable fats (g/day)	-0.28	-0.036
Rice (g/day)	-0.28	-0.057
Cakes and cookies (g/day)	-0.24	-0.037
Fresh fruit (g/day)	-0.23	-0.024
Tea (g/day)	-0.20	-0.027
Pasta (g/day)	-0.19	-0.019
Sandwich spreads (g/day)	-0.19	-0.002
Vegetarian dishes (g/day)	-0.18	-0.036
Raw vegetables (g/day)	-0.15	-0.013
Poultry (g/day)	-0.15	-0.023
Wine (g/day)	-0.13	-0.020
Low-fat cheese (g/day)	-0.12	0.004
Nuts (g/day)	-0.12	-0.020
Low-fat dairy (g/day)	-0.07	-0.006
Potatoes (g/day)	-0.07	-0.031
Vegetable oils (g/day)	-0.07	-0.001
Pizza (g/day)	-0.07	-0.007
Lean fish (g/day)	-0.06	-0.008
Cooked vegetables (g/day)	-0.05	0.022
Cornflakes (g/day)	-0.05	-0.005
Shell fish (g/day)	-0.03	-0.008
Legumes (g/day)	-0.03	0.008
Chips (g/day)	-0.02	-0.011
Beer (g/day)	-0.01	-0.013
Coffee (g/day)	0	-0.004
High-fat dairy (g/day)	0	-0.027
Other fruit than fresh (g/day)	0.02	0.014
High-fat cheese (g/day)	0.03	0.030
Fruit juices (g/day)	0.06	0.028
Soup (g/day)	0.06	0.008
Confectionary (g/day)	0.06	0.014
Sugar (g/day)	0.08	0.026
Unprocessed red meat (g/day)	0.09	-0.002
Fatty fish (g/day)	0.11	0.031
Soft drinks (g/day)	0.10	0.020
Water (g/day)	0.15	0.039
Spirits (g/day)	0.16	0.033
Animal fats (g/day)	0.17	0.012
Processed red meat (g/day)	0.19	0.041
Eggs (g/day)	0.23	0.034
Refined grain products (g/day)	0.24	0.025

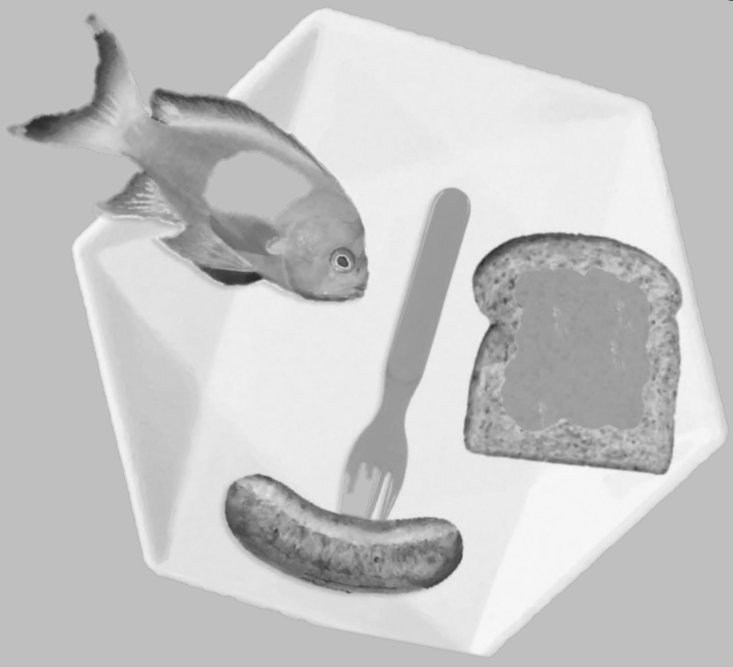
[a] Intake values are expressed as median (p25-p75).

[b] To obtain an individual dietary inflammatory pattern score, each beta-coefficient of a food group was multiplied by the intake of the food group and summed across all food groups ($\sum \text{beta-coefficient}_{\text{food group}} * \text{intake}_{\text{food group}}$).

CHAPTER 9



General discussion



GJ van Woudenbergh

Outline of the general discussion

The discussion is divided into five parts. In *part I*, the main findings are summarized. In *part II*, several methodological issues are discussed. In *part III*, our findings on the role of selected dietary factors, i.e., fatty acids, fish, tea, meat, glycemic index (GI), and glycemic load (GL), on the development of type 2 diabetes are put in a broader perspective. In *part IV*, the extent to what chronic low-grade inflammation may be one of the pathways through which diet can affect the processes leading to type 2 diabetes is addressed. In *part V*, suggestions for future research, the general conclusions and public health relevance are given.

PART I: MAIN FINDINGS

The first aim of this thesis was to study the role of selected dietary factors, i.e., fatty acids, fish, tea, meat, GI, and GL, on the development of type 2 diabetes. The second aim was to study the extent to which chronic low-grade inflammation is a pathway through which diet can affect the processes leading to type 2 diabetes. The results described in **chapters 2 to 8** are summarized in **Table 9.1**.

Our findings showed that intake of lean fish (**chapter 3**) and intake of processed meat (**chapter 5**) were associated with a higher risk of type 2 diabetes, whereas intake of tea was associated with a lower risk of type 2 diabetes (**chapter 4**). Estimated Δ5-desaturase activity was lower in participants with type 2 diabetes than in participants with a normal glucose metabolism (**chapter 2**). No statistically significant associations were observed for the other dietary factors, i.e., the proportions of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), trans fatty acids (TFA), and poly-unsaturated fatty acids (PUFA) (**chapter 2**), intake of fatty fish (**chapter 3**), intake of red meat (**chapter 5**), intake of poultry (**chapter 5**), GI (**chapter 6**), and GL (**chapter 6**).

Our findings did not show that C-reactive protein (CRP), as measure of chronic low-grade inflammation, was an important mediator of the association between intake of meat (**chapter 5**), GI (**chapter 6**), or GL (**chapter 6**) and risk of type 2 diabetes. However, the total inflammatory potential of the diet, as estimated by adapted dietary inflammatory index (ADII) and a dietary inflammatory pattern, was associated with insulin resistance (**chapter 7**) or risk of type 2 diabetes (**chapter 8**), respectively. The apparent discrepancy between the findings in **chapter 5/6** and **chapter 7/8** concerning the mediating role of chronic low-grade inflammation is discussed in part IV (pages 144-148).

PART II: OVERALL METHODOLOGICAL CONSIDERATIONS OF THE MAIN FINDINGS

When interpreting the results, it is important to consider to what extent the observed associations were affected by errors, because these errors may threaten the internal validity. The internal validity refers to the extent to which the observed association reflects the true association. In the next paragraphs, we discuss whether information error, selection error, and confounding could have affected the internal validity, and if so, how.

II.1 Information error

Errors in accessing the exposure or outcome are called information errors. The influence of information error in the exposure on the effect estimate is addressed at first. Thereafter, information error in the outcome is discussed.

Table 9.1 Overview of the main findings of this thesis

Chapter	Design	Study population	Results[a]	
Individual dietary factor approach				
2	CS	CODAM study Dutch high risk population aged 60 (SD 7), *n*= 471	Circulating cholesteryl FA: ≈ proportions of SFA, MUFA, TFA, and PUFA were not associated with T2DM Desaturase activity: ↓ Δ5-desaturase in participants with T2D compared with NGM ↓ Δ5-desaturase was also associated with ↑ HOMA-IR	
3	P	Rotterdam study General Dutch population aged 63 (SD 8), *n*= 4,472	Total $fish_{high\ vs.\ 0}$: ↑ incidence T2D (1.32 [1.02, 1.70], p_{trend}= 0.04) Lean $fish_{high\ vs.\ 0}$: ≈ incidence T2D (0.99 [0.71, 1.38], p_{trend}= 0.93) Fatty $fish_{high\ vs.\ 0}$: ↑ incidence T2D (1.30 [1.01, 1.68], p_{trend}= 0.06)	
4	P	EPIC-InterAct study Populations from 8 European countries aged 52 (SD 9), *n*= 26,039	$Tea_{\geq 4\ cups/day\ vs.\ 0}$: ↓ incidence T2DM (0.84 [0.81, 1.00], p_{trend}= 0.04)	
Individual dietary factor approach and the potential mediation by chronic low-grade inflammation				*Mediated by inflammation*
5	P	Rotterdam study General Dutch population aged 67 (SD 8), *n*= 4,366	Red $meat_{Q4\ vs.\ Q1}$: ≈ incidence T2D (1.18 [0.88, 1.59], p_{trend}= 0.17) Processed $meat_{high\ vs.\ 0}$: ↑ incidence T2D (1.73 [1.16, 2.57], p_{trend}= 0.11) $Poultry_{high\ vs.\ 0}$: ≈ incidence T2D (0.87 [0.67, 1.12], p_{trend}= 0.79)	Not likely, although processed meat was associated with CRP (*beta*= 1.13 per 50 gram; *p*= 0.01)
6	P	Rotterdam study General Dutch population aged 67 (SD 8), *n*= 4,366	Glycemic $index_{T3\ vs.\ T1}$: ≈ incidence T2D (0.95 [0.75, 1.21], p_{trend}= 0.71) Glycemic $load_{T3\ vs.\ T1}$: ≈ incidence T2D (1.00 [0.74, 1.36], p_{trend}= 0.96)	Not likely, although GL was associated with CRP (*beta*= 1.12 per 50 units, *p*= 0.01)
Dietary pattern approach				*Mediated by inflammation*
7	CS	CODAM and Hoorn studies Dutch high risk populations aged 64 (SD 9), *n*= 1,024	$ADII_{per\ SD}$: ↑ fasting plasma glucose (*beta*= 0.009, *p*= 0.03) ↑ 2-hour glucose (*beta*= 0.023, *p*= 0.05) ↑ HOMA-IR (*beta*= 0.035, *p*= 0.02) ≈ HbA1c (*beta*= 0.011, *p*= 0.13)	Likely: ADII was associated with summary score low-grade inflammation
8	P	Rotterdam study General Dutch population aged 67 (SD 8), *n*= 4,366	$DIP_{T3\ vs.\ T1}$: ↑ incidence T2D (1.61 [1.27, 2.03], p_{trend}= <0.001)	Likely: association attenuated after inclusion of CRP in the model

Abbreviations: CS=cross-sectional design; P=prospective; SD=standard deviation; FA=fatty acids; SFA=saturated fatty acids; MUFA=mono-unsaturated fatty acids; TFA=trans fatty acids; PUFA=poly-unsaturated fatty acids; T2D=type 2 diabetes; NGM=normal glucose metabolism; HOMA-IR=homeostasis model assessment for insulin resistance; vs.=versus; Q4=quartile 4; Q1=quartile 1; GI=glycemic Index; GL=glycemic load; T3=tertile 1; T1=tertile1; ADII=adapted dietary inflammatory index; HbA1c=hemoglobin Ab1c; DIP=dietary inflammatory pattern; CRP=C-reactive protein

[a] Results of prospective designs were expressed as hazard ratio [95% confidence interval]

Information error in the assessment of diet

Dietary intake was assessed with a quantitative or semi-quantitative food frequency questionnaire (FFQ) in our studies.[1-4] On these FFQs, participants indicated how often several foods and beverages were consumed and how much of it was eaten over a defined period. The collected information about frequency of consumption and portion sizes can be used to calculate habitual intake of foods, food groups, and nutrients. FFQs are often being used in large-scale observational studies, because costs and burden to the participant are lower compared with other dietary assessment methods, e.g., 24-hour recalls or food records.[5] A 24-hour recall is a dietary assessment method where the participant is asked to recall and describe every food and drink consumed over the past 24 hours. A food record is a dietary assessment method where the participant gives a detailed description of the types and amounts of food products just before eating, and records the leftovers.

In order to know whether a FFQ measures what it intended to measure, information about the validity of the FFQ should be collected. To assess the relative validity of the FFQ used in the Rotterdam study, intake of energy and nutrients assessed from the FFQ was compared with the intake assessed from 15 daily food records in 80 participants.[1] The mean Pearson's correlation coefficient between the intake of energy and nutrients derived from a FFQ and food records was 0.57.[1] The intake of protein was also validated with urinary nitrogen (r= 0.67). The relative validity of intake of food groups used in our investigations (**chapter 3, 5, 6, 8**) was not studied in the Rotterdam study. The results of the relative validity of intake of nutrients, however, indicated that the correlation coefficients for food groups are expected to be between 0.44 and 0.85. Therefore, the ranking of participants according to their intake was relatively good in this elderly population, but the absolute intakes of most nutrients derived from the FFQ were overestimated.[1] As this FFQ was used to study the associations between exposure categories and risk of type 2 diabetes, however, a reliable ranking of participants is more important than a reliable absolute intake.

The relative validity of the FFQ used in the CODAM study, the Hoorn study, and the Dutch contribution of the EPIC-InterAct study was assessed by comparing intakes of food groups, energy, and nutrients assessed from the FFQ with intakes assessed from twelve 24-hour recalls.[2, 3] The median Spearman correlation coefficient between the food group estimates based on the FFQ and 24-hour recalls were 0.61 for men and 0.53 for women.[2] The median de-attenuated Pearson's correlation coefficient between nutrient intake estimates from the FFQ and 24-hour recalls was 0.66 for men and 0.63 for women.[3] The intake of protein was also validated with urinary nitrogen (r= 0.57). The correlation coefficients were in line with results from other FFQ validation studies.[2, 3] So, ranking of participants according to their intake on population level could be considered as relatively good. The validity of absolute intakes was not reported, but it is likely that caution should be taken when interpreting absolute intakes derived from this FFQ, as a FFQ is most designed to measure relative intake and not absolute intake.[6]

Although the relative validity of the FFQs used was considered good, misclassification of the exposure could not be prevented. If misclassification was not related to the outcome, it is likely that the observed risks are closer to a null association than it would have been without any misclassification.[7] If misclassification of the exposure was related to the outcome, the observed risks could be either an underestimation or overestimation of the true effect.[7] As a prospective design was used in most of our investigations, food intake was assessed before type 2 diabetes was diagnosed. Therefore, misclassification cannot be related to the fact that participants knew that they would develop type 2 diabetes. However, high-risk groups such as obese are more likely

to misreport their intake.[8-11] Therefore, misclassification of the exposure may dependent on the development of type 2 diabetes in our investigations. If so, it is likely that net associations, if exist, are rather underestimated than overestimated. For example, it could be speculated that participants with overweight underreport their intake of processed meat, because they may selectively underreport intake of fat.[11] This means that participants with overweight are likely to be classified in lower tertiles than actually should, while having a higher risk of type 2 diabetes. This suggests that the association between intake of processed meat and risk of type 2 diabetes was underestimated to some extent.

Information error in the assessment of the outcome

The outcomes studied in this thesis are incidence of type 2 diabetes (**chapter 3, 4, 5, 6, 8**) and markers of glucose metabolism (**chapter 2, 7**). Error in the incidence of type 2 diabetes means that participants are incorrectly classified as having type 2 diabetes or non-diabetic. In order to minimize the effect of misclassification at baseline, in all studies described in this thesis effort was made to identify prevalent cases of diabetes. At best, diagnosis of type 2 diabetes should be based on two Oral Glucose Tolerance Tests (OGTT). In the Rotterdam study, the CODAM study, and the Hoorn study, a single OGTT was used to identify prevalent diabetes cases at baseline. In the EPIC-InterAct study, prevalent cases of diabetes were based on self-report of a history of diabetes, doctor-diagnosed diabetes, diabetes drug use, or evidence of diabetes after baseline with a date of diagnosis earlier than the baseline recruitment.[12] Therefore, it is likely that most prevalent diabetes cases at baseline were identified and misclassification of type 2 diabetes at baseline was minor in our studies. As a result, most prevalent cases of diabetes could be excluded from our prospective analyses (**chapter 3, 4, 5, 6, 8**). Exclusion of prevalent cases is important, because an association between dietary intake and type 2 diabetes would partly reflected how type 2 diabetes affects dietary intake if prevalent cases are still included. This is known as reverse causation. To minimize reverse causation further, participants who developed type 2 diabetes shortly after baseline were excluded in a sensitivity analysis, because these participants may have been prevalent cases at baseline. As this exclusion did not affect our results (**chapter 3, 4, 5, 6, 8**), it is not likely that reverse causation was responsible for our findings.

In order to minimize the effect of misclassification of type 2 diabetes cases during follow-up, multiple sources of evidence were used to ascertain and verify self-reported incident cases of type 2 diabetes. Therefore, it is unlikely that participants that were classified as type 2 diabetes did not have it. However, diabetes may be asymptomatic and therefore undetected for years. This suggests that without biochemical examinations during follow-up a number of incident diabetes cases is not identified. At the baseline examination of the Rotterdam study it was shown that 6.2% of the population was undiagnosed and 4.4% had diagnosed type 2 diabetes.[13] The participants of the CODAM study were derived from screening a high-risk population. In this high-risk population, 8.3% had undiagnosed type 2 diabetes.[14] In the Hoorn study, 4.8% was undiagnosed and 3.6% had diagnosed type 2 diabetes.[15] Therefore, inclusion of only diagnosed cases of diabetes rather than diagnosed *and* undiagnosed cases of diabetes might have affected our results if the prevalence of undiagnosed cases of diabetes differed substantially among intake categories.

Besides misclassification of type 2 diabetes, errors in the measurement of markers of glucose metabolism used as outcome measures in **chapters 2 and 7**, can cause errors as well. In the CODAM and Hoorn studies, markers of glucose metabolism were measured once. However, within-person variation in these markers is generally present (average within person-variation:

serum insulin, 21%; plasma glucose, 4.5%; hemoglobin A1c (HbA1c), 1.9%).[16] The measurement error caused by within-person variation in the continuous dependent variable has widened the confidence intervals of our study, resulting in less study power to detect associations.[6, 17] Our point estimates are not affected, because the within-person variation of the independent variables instead of the dependent variable affects point estimates.[6]

II.2 Selection error

Besides information error, selection issues are other phenomena that may threaten the internal validity. Selection issues will result in selection bias when the association between exposure, e.g., meat, and health outcome, e.g., type 2 diabetes, differs between those who participate and those who were theoretically eligible for the study. Selection issues include enrolment procedures and loss to follow-up. If inclusion in the study population or loss to follow-up differs by exposure category as well as health outcome, the associations become biased.

As no extensive information about participants that were not willing to participate was available, we can only speculate on the presence of selection bias due to enrolment procedures or loss to follow-up. Results may be less likely to be biased in studies with a high response rate, because the higher the response rate the more likely the exposure and health outcome distribution in the source population is retained. The response rate was 78% at baseline in the Rotterdam study[18], 60% at the examination in 2000-2001 in the Hoorn study[19], and 42% at baseline in the CODAM study. Besides non-response in the European Prospective Investigation into Cancer and nutrition (EPIC) study itself, the population of the EPIC-InterAct study may not be a representative sample of the source population because participants of the EPIC study without stored blood samples were excluded (*n*= 109,625). However, characteristics of the participants of the EPIC-InterAct study did not differ much from those of the total EPIC-study.[12] These response rates, however, do not give any quantitative information on whether selection is dependent on exposure and/or health outcome.

Speculations about the effect of selection issues on exposure or health outcome could be based on other studies that had information about participants that were not willing to participate. The results of the Stockholm Diabetes Prevention Program (SDPP) that studied the influence of non-response showed that non-response was not associated with type 2 diabetes at baseline.[20] However, incidence of type 2 diabetes was higher in non-participants than in participants. In the Study on Lifestyle intervention and Impaired glucose metabolism Maastricht (SLIM), dropout was related to a worse metabolic profile, besides a low social economic status.[21] These results suggest that loss to follow-up in our studies may be associated with a worse metabolic profile. This would have biased our results if selective loss of follow-up of diabetes cases depends on the exposure of interest. If participants with a worse metabolic profile *and* an unhealthy dietary pattern were more likely to be lost to follow-up, it could be speculated that participants who are lost to follow-up are especially those that developed type 2 diabetes and had an unhealthy dietary pattern, including the highest intake of processed meat and no intake of tea. As such, our conclusions concerning the associations between intake of tea or intake of processed meat and risk of type 2 diabetes (**chapter 4, 5**) might have been stronger.

II.3 Confounding

Besides information error and selection error, confounding is another phenomenon that can threaten the internal validity. Confounding occurs when the association between the

exposure (e.g., tea) and the outcome (e.g., risk of type 2 diabetes) is mixed with the effect of a third factor, the confounder (e.g., physical activity). A confounder in the association between intake of tea and risk of type 2 diabetes is physical activity, because it is likely that intake of tea is associated with a high physical activity level (**chapter 4**) *and* low physical activity level is a risk factor type 2 diabetes[22] *and* intake of tea does not cause a low physical activity level. If physical activity is not taken into account in the analysis, the association between intake of tea and risk of type 2 diabetes could merely reflect an inverse association between physical activity and risk of type 2 diabetes.[23]

Fortunately, in the EPIC-InterAct study information about physical activity was available (**chapter 4**) and a separate validation study showed that the questionnaire used to obtain information about physical activity was capable to rank participants appropriately according to the physical activity energy expenditure and time spent in moderate and vigorous physical activity.[24] Therefore, the association between intake of tea and risk of type 2 diabetes was not likely to be mixed with the effect of physical activity. In the Rotterdam study, information about physical activity at baseline was lacking. The associations observed with risk of type 2 diabetes for fish, meat, GI, GL, and the dietary inflammatory pattern, therefore, might have been partly confounded by physical activity. However, taken into account physical activity level by using information about physical activity obtained at follow-up did not change the results considerably. Furthermore, after taking into account physical activity together with other lifestyle factors the association between intake of processed meat and risk of type 2 diabetes was attenuated by 10% in the EPIC-InterAct study.[25] This may indicate that our association between intake of processed meat and risk of type 2 diabetes in the Rotterdam study (**chapter 5**) is overestimated by only a limited extend due to insufficient data on physical activity level.

Thus, adjusting the results for the effect of confounders is crucial. In all analyses described in this thesis, the results could be adjusted for the effect of a series of potential confounders. However, it was not possible to eliminate all confounding, because confounders can be measured with error. As a result, associations can be either underestimated or overestimated depending on the correlation between confounder and exposure, the association between confounder and outcome, the amount and direction of measurement error in the confounder, the correlation between confounders if multiple confounders are considered, the measurement error in the exposure, and the correlated measurement errors among the confounders.[26]

PART III: PUTTING THE FINDINGS FROM THE INDIVIDUAL DIETARY FACTOR APPROACH IN A BROADER PERSPECTIVE

In this part, the results reported in **chapters 2 to 6** are discussed in view of recent literature on this topic. The dietary factors are addressed in sequence of the chapters. So, first associations observed between proportions of cholesteryl ester fatty acids and prevalent type 2 diabetes are put in a broader perspective, followed by discussions on fish, tea, meat, and GI and GL.

III.1 Fatty acids and type 2 diabetes

As an alternative for self-reported intakes of fatty acids that are prone to underreporting[11], proportions of cholesteryl fatty acids were studied in relation to glucose metabolism status in **chapter 2**. The circulating proportions of SFA, MUFA, TFA, and PUFA were not associated with

type 2 diabetes. Estimated Δ5-desaturase activity was lower in participants with type 2 diabetes compared with normal glucose metabolism and was associated with lower homeostasis model assessment for insulin resistance (HOMA-IR).

Our results were already put into a broader perspective in **chapter 2** as this chapter also included a review of available evidence on the association between proportions of fatty acids in cholesteryl esters or phospholipids and type 2 diabetes. This review showed that associations with type 2 diabetes are most consistent for the proportions of linoleic acid (C18:2*n6*) and dihomo-gamma-linolenic acid (C20:3*n6*). Lower proportions of C18:2*n6* and higher proportions of C20:3*n6* have been observed in participants with type 2 diabetes or those who developed type 2 diabetes compared with non-diabetics.

After our publication, six studies were published that could be added to our review. Two study studied the total fatty acid profile[27, 28], two focused on trans-palmitoleic acid (C16:1*n7*t)[29, 30], one focused on *n3* PUFA[31], and one focused on Δ9-desaturase activity[32]. One of the studies that focused on the total fatty acid profile confirmed our conclusion on C18:2*n6* and C20:3*n6*[27], but the other study observed that these fatty acids were both higher in participants with type 2 diabetes compared with non-diabetics.[28] The two publications on C16:1*n7*t showed that a high proportion of C16:1*n7*t was associated with a lower risk of type 2 diabetes.[29, 30] The study that focused on proportions of *n3* PUFA reported that a higher proportion of alpha-linolenic acid (C18:3*n3*) was associated with a lower risk of type 2 diabetes, whereas the other *n3* fatty acids were not.[31] A meta-analysis of circulating EPA or DHA and intake of EPA&DHA did not report an effect of EPA and DHA either.[33] Of the Δ9-desaturase activities studied, only the ratio between palmitoleic acid (C16:1*n7*) and palmitic acid (C16:0) was associated with a higher risk of type 2 diabetes in the study that focused on this desaturase.[32]

After taken the results of the six additional studies into account, our conclusion remains that a higher proportion of C18:2*n6* and lower proportion of C20:3*n6* could be marked as protective. A lower proportion of C20:3*n6* observed in persons with a normal glucose metabolism compared with persons with type 2 diabetes may be due to a lower activity of Δ5-desaturase, as observed in four out of the five observational studies (**chapter 2**).[27] Evidence for associations with other fatty acids and estimated desaturase activities were not considered convincing due to mixed results or limited evidence.

Translating these findings into conclusions for intake of fatty acids, however, should be done with caution. The correlation between proportions of fatty acids and intake is not high, because proportions of fatty acids reflect intake of fatty acids as well as endogenous metabolism, the measurement of the proportions are affected by biological variation and laboratory measurement error, and one tissue fraction does not entirely reflect the fatty acid body pool.[34] Furthermore, self-reported intakes of fatty acids are prone to underreporting and nutrient tables used to calculate intake of fatty acids from intake of food groups may contain errors.[11, 35]

As the drawbacks of self-reported intakes of fatty acids could be tackled in well-designed human interventions studies, the effects of replacing fatty acids with each other in intervention studies are reflected upon. A review by Riserus *et al.* in 2009 suggests that replacing SFA for MUFA or PUFA in the diet can improve insulin sensitivity.[36] This conclusion, however, was not confirmed by the more recent results of large human intervention studies Lipgene (*n*= 417) and Risck (*n*= 548). In these trials, a differential effect of fatty acids on insulin sensitivity was not observed, but risk of the metabolic syndrome was reduced with a low-fat high-carbohydrate diet with additional *n3* PUFA compared with iso-energetic high SFA, high MUFA, or a low-fat high-carbohydrate diet in

Lipgene.[37-39]

If SFA are to be replaced by PUFA, our results suggest that the *n6* PUFA linoleic acid (C18:2*n6*) may be in favour of the *n3* PUFAs EPA and DHA (**chapter 2, 3**). These relative efficacies of linoleic acid, EPA, and DHA on the development of type 2 diabetes, however, are not elucidated. The relative efficacies may be small, as changing the ratio between *n6* and *n3* did not affect insulin sensitivity in human intervention studies.[40, 41] Furthermore, both *n6* PUFA and *n3* PUFA lower low-density lipoprotein (LDL) cholesterol, when replacing SFA.[42, 43] A differential effect may be due to differences in inflammatory properties. As precursors of eicosanoids, *n6* PUFA may express pro-inflammatory properties, whereas EPA and DHA have anti-inflammatory properties.[42, 43] Therefore, *n3* PUFA may still affect risk of type 2 diabetes through their anti-inflammatory actions and probably also through its ability to decrease circulating triglycerides concentrations.[43] In general, however, prospective studies and randomized human intervention studies do not support an effect of *n3* PUFA on risk of type 2 diabetes or insulin sensitivity, respectively.[33, 44-47] Hence, a firm conclusion about which PUFA are most beneficial in the development of type 2 diabetes cannot be drawn.

III.2 Intake of fish and type 2 diabetes

In **chapter 3**, intake of total fish and lean fish were associated with a higher rather than lower risk of type 2 diabetes. No association was observed for intake of fatty fish. Meanwhile four meta-analyses pooled our results with the other evidence on intake of total fish and type 2 diabetes from prospective studies.[33, 44, 48, 49] In these meta-analyses, different approaches were used to calculate the summary relative risk. Taken together, however, it can be concluded that intake of total fish of one serving per week (about 100 gram) was not associated with risk of type 2 diabetes, although heterogeneity among the included studies was high.

Differences in type of fish may explain part of the heterogeneity among studies. The association between categories of fish and risk of type 2 diabetes may differ due to differences in active components with pro-diabetic and/or anti-diabetic properties, e.g., EPA&DHA, vitamin D, selenium, protein, and contaminants. Underlying category of fish was not taken into account in any of the meta-analyses, because the number of studies that stratified their analyses by lean and fatty fish was low.[50, 51, chapter 3] Intake of fatty fish was not associated with risk of type 2 diabetes in the Japan Public Health Centre-based cohort and the Rotterdam study (**chapter 3**), whereas it was associated with a lower risk of type 2 diabetes in the EPIC-InterAct study.[44, 45] For intake of lean fish, a higher risk of type 2 diabetes was observed in the Rotterdam study (**chapter 3**), whereas no associations for lean fish were observed in the Japan Public Health Centre-based cohort and EPIC-InterAct study.[50, 51] A randomized single-blind human intervention study did not observe that an energy-restricted diet including either intake of lean fish, i.e. cod, or fatty fish, i.e., salmon, significantly affected HOMA-IR after eight weeks.[52] A randomized human intervention study on a farmed Atlantic salmon, observed that HOMA-IR was also not affected when different portions (180, 360, or 540 g/day) were eaten after four weeks.[53]

Taken together, the results on the association between intake of lean fish and fatty fish and risk of type 2 diabetes are heterogeneous. This heterogeneity may be explained by difference in type of fish underlying the intake of lean fish and fatty fish, preparation method, sauce dips used together with the fish, and the level of contamination. These factors may not only explain difference in associations between countries, but may also explain difference in the association within countries. In the Dutch contribution to the EPIC-InterAct study, the point estimate for total

fish pointed to an inverse association (hazard ratio (HR)= 0.86 [95% confidence interval (95%CI) 0.70, 1.07]; median intake 62 gram per day), whereas in the Rotterdam study the point estimate suggested an adverse association (**chapter 3**, HR= 1.32 [95%CI 1.02, 1.70]; median intake 70 gram per day). Both associations were mainly driven by the intake of lean fish (Dutch contribution EPIC-InterAct study, HR= 0.81 [95%CI 0.63, 1.05]; Rotterdam study, HR= 1.30 [95%CI 1.01, 1.68]). This may indicate that stratifying on type of fish is important or at least information about the contribution of type of fish to the inter-individual variation in fish should be reported.

III.3 Intake of tea and type 2 diabetes

In the EPIC-InterAct study, participants who drank at least 4 cups of tea per day had a 16% lower risk of type 2 diabetes compared with participants who did not drink tea (**chapter 4**). Pooling our results with seven prospective cohort studies on intake of tea and risk of type 2 diabetes confirmed that participants who drank at least 4 cups of tea per day had a lower risk of type 2 diabetes compared with participants who drank almost never or never tea (random effects meta-analysis, relative risk= 0.86 [95%CI 0.76, 0.96], I^2= 20.8, p= 0.265).[54-59, chapter 4]

Human intervention studies that investigated the effect of tea intake on markers of glucose metabolism, however, pointed to a neutral effect rather than a beneficial effect of tea.[60-69] As intake of tea also is a characteristic of a healthy lifestyle, the difference between observational studies and human intervention studies may suggest that the protective effect observed in observational studies is due to residual confounding of a generally healthier lifestyle. However, the human intervention studies performed so far, are difficult to compare, because (1) the types of tea differed (i.e., oolong tea[60, 66], green tea[61-63, 65, 67, 69], Vietnamese tea[64], and Mauritian black tea[68]), (2) the doses differed (i.e., ranging from 0.3 liter per day[69] to 1.5 liter per day[60]), (3) the duration differed (i.e., ranging from 5 days[66] to 6 months[65]), (4) the control treatment differed (i.e., nothing[62], water[60, 61, 68, 69], tea with lower concentration of polyphenols[63, 66, 67], other type of tea[64, 65]), (5) the study population differed (i.e., participants with type 2 diabetes[60, 61, 63, 64], participants with abnormal glucose metabolism[62], being overweighed breast cancer survivors[65], being healthy[66-69]), and (6) regulations concerning total dietary intake differed (i.e., ranging from fully controlled diet[66] to no restrictions[62]). This highlights the need for comparable human intervention studies.

In future human intervention studies the effect of different types of tea should be compared. Emphasize is put on comparing types of tea, because types of tea differ in composition, such as flavonoid content, as in contrast to black and green teas, herbal teas are not derived from the *Camellia Sinensis* plant, and fermentation procedures between black and green teas differ.[70] Therefore, the effect of type of tea on type 2 diabetes may differ. As the three prospective cohort studies that stratified by types of tea showed mixed results[71-73], the association between types of tea drank and risk of type 2 diabetes should also be investigated in future prospective cohort studies.

Taken together, although prospective cohort studies observed a modest beneficial effect of tea on risk of type 2 diabetes and mechanistic studies support the hypothesis that flavonoids present in tea have beneficial effects on the development of type 2 diabetes[74], the evidence could not be considered convincing as human intervention studies on intake of tea and glucose metabolism do not confirm an association yet.

III.4 Intake of meat and type 2 diabetes

In **chapter 5**, eating processed meat was associated with a higher risk of type 2 diabetes, whereas intake of red meat and poultry were not. Meanwhile our results were pooled with the other evidence on intake of meat and risk of type 2 diabetes.[75] This meta-analysis of prospective studies showed that intake of 50 gram processed meat per day was associated with a 32% higher risk, intake of 100 gram red meat per day was associated with a 13% higher risk, and intake of 100 gram poultry per day was not associated with risk of type 2 diabetes.[75] As already elaborated on in the introduction, a higher risk for processed meat compared with red meat could be explained by the higher concentration of salt, nitrate, nitrosamines, and advanced glycation end products (AGEs) in processed meat compared with red meat.

The effect of chicken[76-79] or red meat[79-81] on fasting glucose concentration, fasting insulin concentration, or both has been studied in human intervention studies. In two randomized cross-over intervention studies among participants with type 2 diabetes, fasting plasma glucose concentrations were comparable between a diet including chicken as meat source and a usual diet after a period of 4 weeks.[76, 77] A diet rich in chicken did also not affect fasting serum glucose concentrations in two parallel randomized intervention studies.[78, 79] Thus, the effects of chicken on fasting glucose concentrations in human interventions were in line with the results of prospective studies.

The results of human intervention studies with red meat pointed to a neutral effect.[79-81] Providing 26 gram per day or 160 gram per day of beef instead of the habitual amount of meat intake increased fasting serum glucose concentrations among men after a period of 6 weeks.[80] However, the increase did not differ between the low and high beef consumers. Providing an energy-restricted lacto-ovo vegetarian diet plus 250 kcal per day as beef did not change fasting serum glucose concentration, plasma insulin concentration, or HOMA-IR among women after a period of 9 weeks.[79] Providing only pork as fat source in a fully controlled diet did also not change fasting plasma glucose and insulin concentrations among women after a period of 8 weeks.[81] The number of participants in intervention studies on red meat, however, was small (at most 54 participants[79]).

Taken together, as a randomized human intervention study that compared the effects of unprocessed red meat, processed red meat, and poultry on markers of glucose metabolism is lacking, the differential effect of categories of meat on risk of type 2 diabetes observed in prospective studies is not confirmed yet by another level of evidence.

III.5 Glycemic index and glycemic load of the diet and type 2 diabetes

In **chapter 6**, a high GI or GL was not associated with risk of type 2 diabetes. In contrast to our null associations, all meta-analyses on the association between GI or GL and risk of type 2 diabetes so far showed that a high GI or GL was associated with a higher risk of type 2 diabetes.[82-85] The summary excess risks ranged from 16%[83] to 40%[82] for GI and from 20%[83] to 58%[84] for GL. These risks were primarily based on prospective cohort studies conducted in the United States of America (USA). Only four out of the 19 studies were from Europe. These four studies showed null associations with relative risks ranging from 0.87 to 1.05 for GI and 0.80 to 1.07 for GL.[86-88, chapter 6]

Several explanations can be given for the differences in relative risks between European and other study populations. Generally, the GI was lower in the European populations than in the USA populations. This suggests that rather at the higher range than at the lower range, the GI or GL of the diet have an adverse effect on the development of type 2 diabetes. Besides the lower

GI, the variation in GI and GL in European study populations was low as well. Especially when the variation is low, accurate calculation of the GI is important because small mistakes in assigning the GI values have a greater impact on the ranking than when the variation is larger.[89] Mistakes in assigning the GI values may have happened, as the GI values assigned to food products in European studies were based on the GI values derived from USA and Australian food products rather than European products. Mistakes could also be due to the subjective decisions made when assigning GI values. Probably only due to subjective decisions, researchers observed either a positive or null association between GI and risk of type 2 diabetes in the Dutch contribution of the EPIC study.[88] Furthermore, in general the tables used to assign GI values do not cover the degree of ripeness or cooking methods that both can result in different glycemic responses. So, the null associations may be ascribed to imprecise assignment of the GI values to foods, rather than to an absence of an association.

As human intervention studies also support a differential effect of a low and high GI diet on markers of glucose metabolism, it is likely that the results of several observational investigations are indeed limited due methodological constraints. A meta-regression including 45 intervention studies showed that low GI diets reduced fasting blood glucose concentration in those with fasting blood glucose concentration higher than 5 mmol/L.[90, 91] Reduction in HbA1c concentration and improvement in insulin sensitivity was also more evident in a low GI diet compared with a high GI diet.[90, 91] Effects on insulin concentrations were more pronounced in participants with an insulin concentration higher than 100 pmol/L. A more pronounced effect of low GI diets on fasting insulin concentrations was found in a meta-analysis limited to human intervention studies with a study duration of at least 6 months and conducted among participants with overweight.[92]

Furthermore, the meta-regression showed that a reduction in fasting plasma glucose is more pronounced, when the GI rather than the amount of available carbohydrates is reduced.[90, 91] This suggests that it is more important to consider the GI of the diet than the total carbohydrate intake in terms of diabetes risk. The GL combines GI and the carbohydrate content of a portion, and as such should reflect the physiological response to a diet better than the GI of the diet. However, observational studies on GL are limited, because the GL of the diet is often highly correlated with total carbohydrate intake (r= 0.43 to 0.80).[85] Especially when the correlation is high, the results of observational studies on GL may just reflect carbohydrate intake.

Taken together, methodological issues should be considered when interpreting the associations between GI or GL and risk of type 2 diabetes.

PART IV: DIET, CHRONIC LOW-GRADE INFLAMMATION, AND TYPE 2 DIABETES

This part is related to the second aim of this thesis, defined as 'to study the extent to which chronic low-grade inflammation is one of the pathways through which diet can affect the processes leading to type 2 diabetes'. The association between diet and chronic low-grade inflammation is addressed in the first paragraph, followed by a discussion about the potential mediating role of chronic low-grade inflammation in the association between diet and type 2 diabetes (**Figure 9.2**).

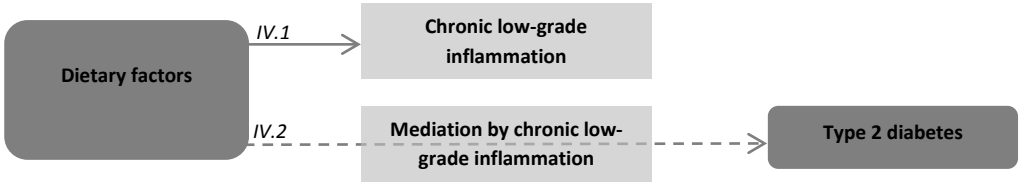


Figure 9.2 Overview of the topics described in part IV

IV.1 Diet and chronic low-grade inflammation

In this thesis, intake of processed meat and the GL of the diet were associated cross-sectionally with CRP, whereas intake of red meat, intake of poultry, and GI were not (**chapter 5, 6**). Furthermore, more pro-inflammatory diets, as estimated by the ADII and a dietary inflammatory pattern, were associated cross-sectionally with a higher summary score for chronic low-grade inflammation or CRP, respectively (**chapter 7, 8**).

A recent comprehensive review written by Calder *et al.* summarized the current evidence on several dietary components and chronic low-grade inflammation.[93] In this review, intake of five food products, i.e., whole grain food products, fruit, vegetables, alcoholic beverages, and cocoa-based foods, and five nutrients, i.e., fibre, EPA&DHA, vitamin C, vitamin E, and carotenoids, were considered anti-inflammatory when the results of observational studies and human intervention studies are taken into account (**Table 9.2**). As the Mediterranean diet and healthy dietary patterns

Table 9.2 Categorization of dietary components according their inflammatory potential based on Calder *et al*. [93]

	Total diet	Foods	Dietary components
Probable anti-inflammatory			
	Mediterranean diet[a]	Whole grain foods	Fibre
	Healthy eating patterns[b]	Total fruit[c]	EPA&DHA
		Total vegetables[c]	Vitamin C
		Alcoholic beverages	Vitamin E
		Cocoa-based foods	Carotenoids
Probable pro-inflammatory			
			Trans fatty acids
			GI/GL
			AGEs

Abbreviations: EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid; GI=glycemic index; GL=glycemic load, AGEs=advanced glycation end products

[a] Include intakes of whole-grains, vegetables, fruit, legumes, nuts, fish, low-fat dairy, moderate intake of wine, and olive oil as the main fat source.[93]

[b] Refer to healthy eating index (includes grains, vegetables, fruit, milk, meat, total fat, saturated fat, cholesterol, sodium, dietary variety), diet quality index (includes grains, vegetables, fruit, total fat, saturated fat, cholesterol, calcium, iron, dietary diversity, diet moderation), alternate healthy eating index (includes vegetables, fruit, nuts and soya, ratio white to red meat, fibre, trans fat, ratio poly-unsaturated fatty acids to saturated fatty acids, multivitamin use, alcohol), and prudent and healthy dietary patterns derived from an exploratory dietary pattern approach.

[c] Evidence for specific effects of single fruit and vegetable varieties considered not convincing.

are characterized by at least a high intake of some food products that are considered anti-inflammatory, it is not surprising that these diets were also considered to be anti-inflammatory. TFA, GI, GL, and AGEs were considered pro-inflammatory. The evidence for an effect of soya, nuts, fish, tea, coffee, milk peptides, saturated fatty acids, arachidonic acid, conjugated linoleic acids, linoleic acid, α-linolenic acid, iron, vitamin D, flavonoid subclasses or phyto-oestrogens on chronic low-grade inflammation was considered less convincing.

Intake of meat was not considered in the review. However, AGEs that are present in processed meat were considered pro-inflammatory. Furthermore, exploratory dietary patterns that are at least characterized by processed meat and red meat were associated with markers of chronic low-grade inflammation.[94] Therefore, processed meat may be considered as pro-inflammatory, which would be in line with our observation (**chapter 5**).

The conclusion of the review about GL is also in line with our observation (**chapter 6**). After the publication of the review by Calder *et al.*, a meta-analysis on three long-term human intervention studies (≥6 months) also confirmed that a low GI or GL diet decreased CRP concentration compared with high GI or GL diet in persons without type 2 diabetes.[92]

The ADII was based on the inflammatory potentials of 29 dietary factors as calculated by Cavicchia *et al.*.[95] To calculate the inflammatory potentials, the results of 929 studies on diet and markers of inflammation were taken into account. The highest pro-inflammatory weights were assigned to carbohydrates, SFA, and TFA. The highest anti-inflammatory weights were assigned to magnesium, beta-carotene, vitamin A, tea, alcohol, and fibre. Calder *et al.*[93] did not consider all these nutrients in their review, but of the ones they did, TFA also emerged as pro-inflammatory and alcohol and fibre as anti-inflammatory (**Table 9.2**). Considering the dietary components included by Calder *et al*.[93], but not by Cavicchia *et al*.[95], the ADII may be extended with an inflammatory weight for the pro-inflammatory effect of AGEs. However, an inflammatory weight for AGEs will be hardly applied in studies, because intake data on AGEs are probably not available or not validated.

The dietary inflammatory pattern derived from data on CRP of the Rotterdam study negatively correlated with intake of whole grain products, vegetable fats, rice, cakes and cookies, fresh fruit, and tea (all $r \geq -0.20$), and positively correlated with intake of spirits, animal fats, processed red meat, eggs, and refined grain products (all $r > 0.15$) (**chapter 8**). The food groups that were negatively correlated may be considered anti-inflammatory, whereas those that were positively correlated may be considered pro-inflammatory. Although intake of foods and CRP was measured at the same time, and as such a causal association cannot be established, the anti-inflammatory effects of whole grain foods, fruit, and moderate alcohol were in line with the review by Calder *et al.*.[93]

Taken together, it is likely that at least whole grain foods, fruit, and moderate intake of alcoholic drinks have anti-inflammatory properties and processed meat has pro-inflammatory properties.

IV.2 Diet, chronic low-grade inflammation, and type 2 diabetes

In **chapters 5 and 6**, CRP explained less than 5% of the associations between processed meat or GL and risk of type 2 diabetes. In **chapters 7 and 8**, 20% of the association between ADII and HOMA-IR was explained by a summary score for chronic low-grade inflammation, and 14% of the association between dietary inflammatory pattern and risk of type 2 diabetes was explained by CRP.

These results may suggest that the total diet rather than individual dietary factors should be studied with regard to diet, chronic low-grade inflammation, and type 2 diabetes. If the effects of individual dietary factors are too small to detect or cannot be disentangle from the effects of other dietary factors, studying the total diet is indeed preferred. However, approaches used to obtain an estimate of the total diet have their advantages and disadvantages. The main advantage of studying the total diet by using exploratory or hybrid dietary pattern approach is that interactions between foods and accumulative effects of foods can be taken into account. Of these approaches, exploratory dietary patterns are useful to obtain information about the underlying dietary pattern of a population.[96, 97] These exploratory dietary patterns, however, are not suitable to obtain insights into biological pathways underlying an association between diet and disease, besides that these dietary patterns may only explain a small proportion of the variance in food intake, may not be related to a health outcome, are population specific, are subjective due to subjective decisions while constructing the patterns, and cannot be used when the effect is caused by few or one dietary factor.[96, 97] As our interest lies in the chronic low-grade inflammation pathway, therefore, an exploratory dietary pattern approach was not appropriate in our analyses (**chapter 8**).

A hybrid approach was more appropriate, because a dietary pattern resulting from a hybrid approach can give information about a potential underlying pathway, because it is constructed in such a way that explains as much variation in an intermediate, e.g., inflammation, as possible. If inflammation precedes the development of type 2 diabetes, it is likely that this dietary pattern is more relevant to type 2 diabetes than an explanatory dietary pattern. Although this advantage, a hybrid approach has also limitations. First, hybrid dietary patterns are population specific as the variation in food intake and intermediates differs among populations.[96, 97] Second, subjective decisions have to be made while combining food items into food groups.[96, 97] Third, it is not possible to elucidate whether the total combination of foods or just certain foods explains the association.[97] Finally, the variation in intermediates that is explained by the dietary pattern is often low, leaving room for the dietary pattern to act via other biological pathways.[97] In our analysis, CRP was used as measure of chronic low-grade inflammation. Information about other markers of inflammation may have result in a dietary pattern that reflects the association between diet and chronic low-grade inflammation more accurately. A more accurate reflection of the inflammatory potential of the diet is probably also achieved when changes of intermediates in time are considered instead of considering only baseline measurements.

As the dietary inflammatory pattern, the ADII also reflects the inflammatory potential of the total diet. However, the ADII differs from the dietary inflammatory pattern in several aspects. First, the inflammatory potentials of the dietary factors were based on scientific evidence (ADII) rather than the correlation structure of the underlying data as used to obtain the dietary inflammatory pattern. As such, the ADII can be studied easier in other populations, although data on a wide range of dietary factors is currently needed to be able to calculate the ADII. Second, the ADII considers mainly nutrients, whereas the dietary inflammatory pattern considers foods. Therefore, errors that result from translating intakes of foods into intake of nutrients may affect the ADII, but not the dietary inflammatory pattern.

Some general aspects of the ADII should also be considered. Although the ADII was related to a summary score for chronic low-grade inflammation (**chapter 7**), its validity has not been confirmed in other studies. If validated, the number of nutrients in the score may be reduced to enhance a practical application in the future. Furthermore, the inflammatory weights assigned to

dietary factors could be improved when the strengths of the associations between dietary factors and markers of inflammation as reported in literature are taken into account as well.

Hence, given that all approaches have their own strengths and limitations, both dietary pattern approaches and individual dietary factor approaches are important to enhance our understanding of the effect of diet on chronic low-grade inflammation and type 2 diabetes.

PART V: FUTURE RESEARCH, CONCLUSION, AND PUBLIC HEALTH RELEVANCE

V.1 Future research

The findings of this thesis together with findings from others provide directions for future research. The main directions are described below.

A potential role of *n6* PUFA and the enzyme Δ5-desaturase in the development of type 2 diabetes has been suggested in this thesis (**chapter 2**). Further work needs to be done to establish whether the role of *n6* PUFA could be attributable to defects in this enzyme or to other mechanisms. Mechanisms by which a higher proportion of the trans fatty acid C16:1*n7*t causes a lower risk of type 2 diabetes, e.g., ability to suppress hepatic fat synthesis[29], should also be explored. Furthermore, the *n3* PUFA EPA and DHA should be studied in detail, as their role in the development of type 2 diabetes is in doubt. On one hand, there appeared to be no role for these PUFA, because a main source of these PUFA, i.e., fish, was not associated with the development of type 2 diabetes and these PUFA did not affect glucose concentrations in human intervention studies.[33, 44, 46, 48, 49] On the other hand, EPA and DHA may still be beneficial through their effects on circulating triglycerides and chronic low-grade inflammation.[43]

Most prospective studies that investigated the association between intake of fish and risk of type 2 diabetes did not stratify by category of fish. This is a limitation, because risk of type 2 diabetes may differ by category of fish. Observational studies that study categories of fish, therefore, are warranted. Furthermore, mechanistically oriented studies that investigate how components present in fish influence glucose metabolism can lead to a better understanding of the role of fish in the development of type 2 diabetes.

Regarding intake of tea and risk of type 2 diabetes, three main issues remain. First, data on types of tea in populations with enough variation in intake of types of tea will provide insight into a potential differential effect of types of tea on type 2 diabetes. Second, as intake of tea appears to be beneficial, it is important from a public health point of view to know which beverage can best be replaced by tea. As intake of coffee is associated with a lower risk of type 2 diabetes than tea[98], replacing coffee by tea is probably not the best option. However, this should be grounded in evidence. Third, human intervention studies that compare types of tea are needed.

Prospective studies consistently report that intake of processed meat is associated with a higher risk of type 2 diabetes. Although a consistent association is observed, universal definitions for categories of meat are not specific enough. Processed meat is often defined as meats preserved by smoking, curing, salting, or addition of chemical preservatives. The concentration of especially salt, however, is not defined. This may result in subjective categorization of meat and subsequently hampers comparisons among studies.

Of the selected dietary factors studied in this thesis, the associations between intakes of meat categories and risk of type 2 diabetes are most clear. Therefore, there is a need for a well-designed human intervention study that compares categories of meat. In such an intervention

study, participants should be exposed to a fully controlled iso-energetic diet with either red meat, processed meat, or poultry as meat source. Ideally, the outcome is incidence of type 2 diabetes and the study is well powered. This is far from realistic as resources are often limited and ethical issues play a role. Instead of incidence of type 2 diabetes, changes in HbA1c, 2-hour glucose, fasting glucose concentration, fasting insulin concentration, and results of a hyperinsulinemic euglycemic glucose clamp can be used as outcome measures. If a fully controlled diet is not possible, diet should be monitored very well in order to exclude influences of changes in diet other than meat.

Human intervention studies on the effects of low GI or GL on markers of glucose metabolism pointed to beneficial effects. The results of observational studies, especially those from Europe, however, are restricted by several methodological constraints. These include lack of European-specific GI values, a FFQ especially designed to measure GI and GL, and a lack of an objective way to assign GI and GL values to food items obtained from a FFQ.[89] The latter has been addressed in the EPIC study resulting in a flowchart to enhance objective assignment.[89] The use of GI and GL in observational studies has been put forward by this flowchart, as it will be when the other constraints are tackled.

Considering the limited amount of literature, future investigations into the ADII and dietary inflammatory patterns are required. Given the limitations of dietary scores and dietary patterns, these studies should go hand in hand with studies on individual dietary factors. Together, these studies may determine which combinations of foods have the most detrimental impact on chronic low-grade inflammation and type 2 diabetes. If determined, the number of dietary factors included in the ADII can be reduced to enlarge the possibility of a practical application of the ADII.

To enhance our knowledge about the extent to which diet can affect risk of type 2 diabetes through its effect on chronic-low grade inflammation, measurement of markers of chronic low-grade inflammation over time should preferably be used. Furthermore, the interrelation between chronic low-grade inflammation and other mediators, e.g., blood pressure, serum cholesterol, measures of body weight, should be quantified. Structural equation modelling or a multiple mediation approach is recommended to be used when studying these interrelated factors.[99-101]

An upcoming new factor, the gut microbiome, may enhance the understanding of the complex interplay between diet, inflammation, and type 2 diabetes. Diet can influence the microbiome and products of the microbiome can interact with the immune system.[102, 103] Finally, in order to establish the underlying causal pathways combining exposure with genetics, transcriptomics, proteomics, metabolomics, and intermediates is important.[104]

V.2 Conclusions and public health relevance

In this thesis, the role of selected dietary factors on the development of type 2 diabetes is investigated to enhance the scientific basis for the primary prevention of type 2 diabetes. Based on our discussion, high intake of tea and low intake of processed meat can help lower the risk of type 2 diabetes. The WHO/FAO[105], the American Diabetes Association[91], and the European Diabetes and Nutrition Study Group[92], did not include these foods in their dietary recommendations for the primary prevention of type 2 diabetes. Possibly, because nutrients rather than foods were considered. However, as communication about foods is more understandable than communication about intake of nutrients, it is worthwhile to consider

inclusion of foods into the recommendations. If considered, the evidence regarding intake of tea and processed meat should be considered as 'possible' rather than 'probable' or 'convincing', because well-designed human intervention studies on categories of tea and meat are lacking.

The evidence regarding the intake of *n3* PUFA and GI was considered 'possible' in the WHO/FAO recommendations.[105] Based on our findings and discussion, the grade of evidence concerning these dietary factors should not be upgraded.

With regard to the mediating role of chronic low-grade inflammation, the findings suggest that some diets can promote the development of type 2 diabetes through harmful effects on chronic low-grade inflammation. Which combinations of dietary factors cause the pro-inflammatory properties of these diets remains to be determined.

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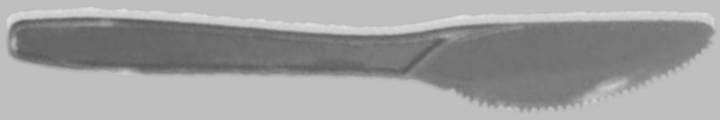
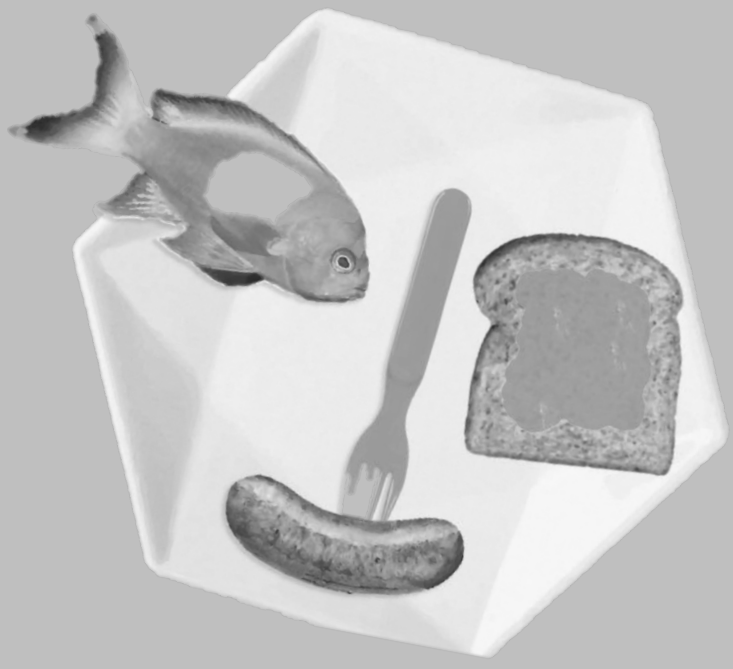
SUMMARY

(Samenvatting)





GJ van Woudenbergh

Samenvatting

Type 2 diabetes is een stofwisselingsziekte, waarbij het lichaam de bloedsuikerhuishouding niet goed meer kan regelen. Dit komt vooral doordat het lichaam niet goed op het hormoon insuline reageert of doordat het lichaam niet genoeg insuline maakt. Zonder insuline kunnen de cellen in het lichaam onvoldoende suiker uit het bloed halen en zal de suikerwaarde in het bloed te hoog blijven. Door de te hoge suikerwaarde kunnen mensen met diabetes op de lange termijn te maken krijgen met schade aan de nieren, ogen en zenuwen. Daarnaast kunnen zij vaker een beroerte en hart- en vaatziekten krijgen.

In 2011 hadden meer dan 800.000 Nederlanders diabetes. Als er niets verandert, zal dit aantal oplopen tot 1.3 miljoen in 2025 (8% van de Nederlandse bevolking). Dat het aantal mensen met type 2 diabetes stijgt, komt waarschijnlijk doordat steeds meer mensen overgewicht hebben, minder bewegen en een ongezond eten. Een te hoog lichaamsgewicht, lichamelijke inactiviteit en ongezonde voeding zijn belangrijke beïnvloedbare risicofactoren voor type 2 diabetes.

Er is nog veel onduidelijk over hoe voeding een rol speelt in het ontstaan van type 2 diabetes en welke mechanismen hierbij betrokken kunnen zijn. Daarom is het doel van dit proefschrift ten eerste om te onderzoeken of verschillende voedingsfactoren samenhangen met het ontstaan van type 2 diabetes, zoals vetzuren, vis, thee, vlees, glykemische index (GI) en glykemische lading (GL). Ten tweede is er in dit proefschrift onderzocht of voeding door de samenhang met ontstekingsstoffen het risico op type 2 diabetes kan beïnvloeden. Er zijn namelijk aanwijzingen dat voedingsfactoren gerelateerd kunnen zijn aan een verhoogd of verlaagd niveau van ontstekingsstoffen in het bloed en dat een chronische lichte verhoging van ontstekingsstoffen het risico op type 2 diabetes verhoogd. Ontstekingsstoffen zijn stoffen die normaal gesproken vrijkomen bij een ontsteking in het lichaam. Een voorbeeld van een ontstekingsstof is C-reactief proteïne (CRP).

Om bovengenoemde onderzoeken uit te voeren zijn de gegevens gebruikt van verschillende prospectieve cohortstudies. In deze studies wordt gedurende lange tijd een grote groep mensen gevolgd. Aan het begin worden allerlei gegevens van deze mensen verzameld, bijvoorbeeld over wat ze eten. Daarnaast wordt er geregistreerd wie er diabetes krijgt. Op deze manier kan onderzocht worden of voeding invloed heeft op het krijgen van type 2 diabetes.

Een bredere achtergrond van dit onderzoek is te vinden in **hoofdstuk 1**.

In **hoofdstuk 2** wordt beschreven hoe verschillende soorten vetzuren in het bloed samenhangen met het hebben van diabetes. Daarvoor werden de aan het begin verzamelde gegevens van 471 deelnemers van de CODAM Studie gebruikt. Het percentage verzadigde vetzuren, enkelvoudig onverzadigde vetzuren en meervoudig onverzadigde vetzuren in het bloed bleek niet te verschillen tussen deelnemers die type 2 diabetes hadden en deelnemers die een normale suikerhuishouding hadden. Een bepaald enzym, genaamd delta-5 desaturase, wat helpt bij het omzetten van bepaalde vetzuren in andere vetzuren in het bloed, was lager in deelnemers met type 2 diabetes vergeleken met deelnemers die een normale suikerhuishouding hadden.

In **hoofdstuk 3** staat het onderzoek beschreven naar het verband tussen het eten van vis en het krijgen van type 2 diabetes. Daarvoor werden de gegevens van 4.472 mensen gebruikt, die woonden in de Rotterdamse wijk Ommoord en deelnamen aan de Rotterdam Studie. Na gemiddeld 11 jaar bleek bij 456 deelnemers type 2 diabetes te zijn geconstateerd. Uit dit onderzoek bleek dat de groep deelnemers die veel vis aten 32% meer risico hadden op type 2 diabetes vergeleken met de groep deelnemers die geen vis aten. De viseters bleken voornamelijk magere vis te eten, zoals kabeljauw.

In **hoofdstuk 4** wordt ingegaan op het onderzoek naar het verband tussen het drinken van

thee en het krijgen van type 2 diabetes. Daarvoor werden de gegevens van 26.039 deelnemers aan de EPIC-InterAct Studie gebruikt, die werd uitgevoerd in acht Europese landen. Van deze deelnemers kregen 12.403 mensen type 2 diabetes. Deelnemers die tenminste vier kopjes thee per dag dronken, hadden 16% minder risico op het krijgen van type 2 diabetes vergeleken met deelnemers die geen thee dronken.

In **hoofdstuk 5** staat het onderzoek naar het verband tussen het eten van verschillende soorten vlees en het krijgen van type 2 diabetes. Daarvoor werden de gegevens van 4.366 deelnemers aan de Rotterdam Studie gebruikt, waarvan 456 mensen type 2 diabetes kregen. Door middel van een voedingsvragenlijst werd nagegaan hoeveel bewerkt rood vlees, onbewerkt rood vlees en gevogelte de deelnemers aten. Degenen die de grootste hoeveelheid rood bewerkt vlees aten, hadden 73% meer risico op het krijgen van type 2 diabetes vergeleken met deelnemers die geen rood bewerkt vlees aten. Onder bewerkt rood vlees vallen vleesproducten die geconserveerd zijn door middel van roken of het toevoegen van zout of andere conserveringsmiddelen, zoals worsten. Voor onbewerkt rood vlees en gevogelte werd geen verband gevonden.

In **hoofdstuk 6** wordt ingegaan op het onderzoek naar het verband tussen de GI en GL en het krijgen van type 2 diabetes onder 4.366 deelnemers van de Rotterdam Studie. De GI en GL zijn maten die aangeven wat de invloed is van een voedingsmiddel op de bloedsuikerwaarde. Er werd geen verband gevonden tussen de GI en GL en het risico op type 2 diabetes.

In de **hoofdstukken 5 en 6** is te lezen dat er geen aanwijzingen gevonden zijn dat het verband tussen vlees, GI of GL en het krijgen van type 2 diabetes verklaard kan worden door het effect van vlees, GI of GI op de ontstekingsstof CRP.

De tot hier beschreven onderzoeken richtten zich op afzonderlijke voedingsfactoren. Om het effect van de totale voeding te bestuderen is ook gebruik gemaakt van voedingspatronen. In de **hoofdstukken 7 en 8** is beschreven hoe voedingspatronen die samenhangen met hogere gehalten van ontstekingsstoffen invloed hebben op type 2 diabetes.

In **hoofdstuk 7** werden de aan het begin verzamelde gegevens van 1.024 deelnemers gebruikt aan de CODAM Studie en Hoorn Studie. Gebaseerd op gegevens uit de literatuur over het effect van verschillende voedingsstoffen op ontstekingsstoffen werd een maat voor de invloed van voeding op ontsteking berekend, de adapted dietary inflammatory index (ADII). Een hogere ADII bleek samen te hangen met een hogere waarde van verschillende bloedgehalten van de suikerhuishouding, zoals nuchtere bloedsuikerwaarde.

In **hoofdstuk 8** werden de gegevens van 4.366 deelnemers aan de Rotterdam Studie gebruikt. De groep deelnemers met een voedingspatroon dat meer samenhing met de ontstekingsstof CRP had een 61% hoger risico op type 2 diabetes vergeleken met de groep deelnemers met een voedingspatroon dat minder sterk samenhing met CRP.

In **hoofdstuk 9** worden de onderzoeken, zoals beschreven in de hoofdstukken 2 tot en met 8, bediscussieerd. Op basis van deze discussie kan worden geconcludeerd dat (1) een hoge inname van thee, en (2) een lage inname van bewerkt rood vlees van belang kan zijn in de preventie van type 2 diabetes. Daarnaast draagt een voedingspatroon dat sterker samenhangt met ontstekingsstoffen mogelijk bij aan de ontwikkeling van type 2 diabetes.

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GJ van Woudenbergh

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Opponenten

For critically reading and judging the final draft of this thesis I am grateful to the reading committee: Prof. Dr *Renger* Witkamp, Prof. Dr *Matthias* Schulze, Prof. Dr *Cees* Tack, and Dr *Ilja* Arts.

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Collega's

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September 2013

Ir. Truus Groenendijk-van Woudenbergh

ABOUT THE AUTHOR

Curriculum Vitae

List of publications

Educational training

Curriculum vitae

Geertruida Johanna (Truus) van Woudenbergh was born on 20 December 1983 in Barendrecht (NL). After she finished her secondary school education in 2003, she started with the study 'Nutrition and Health' at Wageningen University (NL). Three years later, she completed her Bachelor program with honours.

In the course of her Master program, she specialized in Epidemiology and Public Health. During the first year of her Masters, she conducted a master thesis on coffee consumption and coronary calcification at the Erasmus University Medical Centre in Rotterdam (NL) and Wageningen University (NL). This master thesis was awarded by the national Alpro Foundation Award Masters 2008 for best thesis and resulted in her first publication (*Arterioscler Thromb Vasc Bio*. 2008;28(5):1018-1023). A practical training period took place at Medical Research Council Human Nutrition Research (MRC HNR, Cambridge, UK) at the beginning of the second year of her Masters. She analysed data from the 1946 British Birth Cohort to investigate the association between intake of dairy and blood pressure. She completed another Master thesis at The Dutch Cancer Institute, Division of Psychosocial investigation and Epidemiology, Amsterdam (NL). Here, she analysed the data from a retrospective cohort conducted among BRCA1/2 mutation families to investigate potential testing bias. During her Masters, she was also appointed as treasurer of the board of a students' association. In 2008, she completed her Master program with honours.

Immediately after finishing her Masters, she started as PhD-fellow at the division of Human Nutrition at Wageningen University, which resulted in this thesis entitled 'Dietary determinants, inflammation, and type 2 diabetes: *insights from observational studies'*. Within this PhD-thesis she performed epidemiological data analyses in collaboration with partners from the Erasmus University Medical Centre, Maastricht University, VU university, and InterAct consortium. She joined several national and international conferences and courses in the field of nutrition, epidemiology, and type 2 diabetes, supervised students, organized a retreat for the division of Human Nutrition in 2009, and chaired the PhD-commission of the division.

Notably, at the European Diabetes Epidemiology Group 2010 her poster about intake of meat and type 2 diabetes was awarded a poster prize. In 2010, she also won the young investigator award at the Nutrition Diabetes Study Group in Norway. She was selected to participate in the 11th Cambridge Seminar on the Epidemiology and Public Health Aspects of Diabetes Mellitus in 2011 (Cambridge, UK). From 2010 onwards, she was appointed for one day a week as educational developer at the division of Human Nutrition (Wageningen University (NL)). She developed interactive digital learning materials on observational study designs and their validity.

Since September 2013, she has been appointed as both educational developer and postdoctoral fellow at the division of Human Nutrition. As educational developer, she is involved in the online distance-learning program of the Master Nutritional Epidemiology and Public Health.

List of publications

Publications in peer-reviewed journals

2013 **GJ van Woudenbergh**, D Theofylaktopoulou, A Kuijsten, I Ferreira, MM van Greevenbroek, CJ van der Kallen, CG Schalkwijk, CDA Stehouwer, MC Ocké, G Nijpels, JM Dekker, EE Blaak, EJM Feskens. The adapted dietary inflammatory index and its association with a summary score for low-grade inflammation and markers of glucose metabolism: the CODAM and Hoorn studies. *Am J Clin Nutr.* 2013;accepterd for publication.

D Romaguera, T Norat, PA Wark, AC Vergnaud, MB Schulze, **GJ van Woudenbergh**, D Drogan, *et al*. on behalf of the InterAct consortium. Consumption of sweet beverages and type 2 diabetes incidence in European adults: results from EPIC-InterAct. *Diabetologia*. 2013;56(7):1520-1530.

SW van den Berg, DL van der A, AM Spijkerman, **GJ van Woudenbergh**, MJ Tijhuis, *et al*. on behalf of the InterAct consortium. The association between dietary energy density and type 2 diabetes in Europe: results from the EPIC-InterAct study. *PLoS One*. 2013;8(5):e59947.

EJM Feskens, D Sluik, **GJ van Woudenbergh.** Meat consumption, diabetes, and its complications. *Curr Diab Rep*. 2013;13(2):298-306.

2012 **GJ van Woudenbergh**, A Kuijsten, B Tigcheler, EJG Sijbrands, FJA van Rooij, A Hofman, JCM Witteman, EJM Feskens. Meat consumption and its association with C-reactive protein and incident type 2 diabetes: the Rotterdam study. *Diabetes Care*. 2012;35(7):1499-1505.

GJ van Woudenbergh, A Kuijsten, D Drogan, DL van der A, D Romaguera, *et al*. on behalf of the InterAct Consortium. Tea consumption and incidence of type 2 diabetes in Europe: the EPIC-InterAct case-cohort study. *PLoS One*. 2012;7(5):e36910.

PS Patel, NG Forouhi, A Kuijsten, MB Schulze, **GJ van Woudenbergh**, *et al*. on behalf of the InterAct consortium. The prospective association between total and type of fish intake and type 2 diabetes in 8 European countries: EPIC-InterAct study. *Am J Clin Nutr.* 2012;95(6):1445-1453.

GJ van Woudenbergh, A Kuijsten, CJ van der Kallen, MM van Greevenbroek, CDA Stehouwer, EE Blaak, EJM Feskens. Comparison of fatty acid proportions in serum cholesteryl esters among people with different glucose tolerance status: the CoDAM study. *Nutr Metab Cardiovasc Dis*. 2012;22(2):133-140.

2011 **GJ van Woudenbergh**, A Kuijsten, EJG Sijbrands, A Hofman, JCM Witteman, EJM Feskens. Glycemic index and glycemic load and their association with C-reactive protein and incident type 2 diabetes. *J Nutr Metab*. 2011;623076.

2010 **GJ van Woudenbergh**, A Kuijsten, EJM Feskens. Eating fish and risk of type 2 Diabetes: a population-based, prospective follow-up study: response to Boucher and Mannan. *Diabetes Care*. 2010.

2009 **GJ van Woudenbergh**, AJ van Ballegooijen, A Kuijsten, EJG Sybrands, FJA van Rooij, JM Geleijnse, A Hofman, JCM Witteman, EJM Feskens. Eating fish and the risk of type 2 diabetes: a prospective population based follow-up study. *Diabetes Care*. 2009;32(11):2021-2026.

2008 **GJ van Woudenbergh**, R Vliegenthart, FJA van Rooij, A Hofman, M Oudkerk, JCM Witteman, JM Geleijnse. Coffee consumption and coronary calcification in elderly people: the Rotterdam coronary calcification study. *Arterioscler Thromb Vasc Bio*. 2008;28(5):1018-1023.

Published abstracts

2012 **GJ van Woudenbergh**, A Kuijsten, EJM Feskens on behalf of the InterAct consortium. Tea consumption and incidence of type 2 diabetes in Europe: the EPIC-InterAct case-cohort study. *Ned Tijdschr Diabetologie*. 2012;3:112-113.

2009 **GJ van Woudenbergh**, AJ van Ballegooijen, A kuijsten, EJG Sijbrands, FJA van Rooij, JM Geleijnse, A Hofman, JCM Witteman, EJM Feskens. Eating fish and the risk of type 2 diabetes: a population-based, prospective follow-up study. *Ned Tijdschr Diabetologie*. 2009;3:102.

GJ van Woudenbergh, A Kuijsten, EE Blaak, CJH van der Kallen, MMJ van Greevenbroek, CDA Stehouwer, EJM Feskens. Serum cholesteryl fatty acids and glucose metabolism status: the CODAM study. *Eur J Clin Nutr*. 2009;63(3):S23.

2008 JM Geleijnse, **GJ van Woudenbergh**, R Vliegenthart, JCM Witteman. Coffee consumption and coronary calcification in elderly people: the Rotterdam coronary calcification study. *Circulation*. 2008;117(11):e237, 130.

Overview of completed training activities

Discipline specific activities

Courses and workshops

4th advanced diabetes epidemiology workshop[a]	EDEG, Driebergen (NL), 2009
Nutritional and lifestyle epidemiology	VLAG, Wageningen (NL), 2009
Principles of epidemiologic data analysis	NIHES, Rotterdam (NL), 2010
EPIC workshop 'Practical approaches to deal with measurement error in dietary data'	InterAct, Cambridge (UK), 2010
Symposium 'Diabetes risk prediction models: development, evaluation, and application'	DIfE, Potsdam (DU), 2010
Masterclass 'Multilevel analysis'	VLAG, Wageningen (NL), 2011
Cambridge diabetes seminar[a]	MRC, Cambridge (UK), 2011
Statistical issues in nutritional research	Biometris, Wageningen (NL), 2012
Masterclass 'Longitudinal data analysis'	VLAG, Wageningen (NL), 2013

Conferences and meetings

Symposium 'Coffee and the metabolic syndrome'	VLAG, Wageningen (NL), 2008
Annual meeting NWO nutrition	NWO, Deurne (NL), 2008
2nd national nutrition congress[a]	Alliantie Voeding, Ede(NL), 2009
Wageningen nutritional sciences forum[b]	WUR, Arnhem (NL), 2009
Meeting InterAct consortium	InterAct, Berlin (DU), 2009
European diabetes epidemiology group conference[b]	EDEG, Wageningen (NL), 2009
Annual meeting of the Netherlands epidemiology society[b]	WEON, Amsterdam (NL), 2009
Annual meeting NWO nutrition[a]	NWO, Deurne (NL), 2009
Annual meeting NVDO[a]	NVDO, Oosterbeek (NL), 2009
Meeting InterAct consortium	InterAct, London (UK), 2010
Meeting InterAct consortium[a]	InterAct, Cambridge (UK), 2010
European diabetes epidemiology group conference[b]	EDEG, Porti Heli (Greece), 2010
Annual meeting of the Netherlands epidemiology society[b]	WEON, Nijmegen (NL), 2010
Diabetes nutrition study group conference[a]	NDSG, Oslo (Norway), 2010
Meeting InterAct consortium	InterAct, Stockholm (Sweden), 2010
Annual meeting NWO nutrition[a]	NWO, Deurne (NL), 2010
Meeting InterAct consortium[a]	InterAct, Barcelona (Spain), 2011
Meeting 'Chronic inflammation: new insights and challenges'	Federa MWD, Leiden (NL), 2011
Diabetes nutrition study group conference[a]	DNSG, Rome (Italy), 2011
Annual meeting of the Netherlands epidemiology society[a]	WEON, Rotterdam (NL), 2012
Annual meeting NVDO[a]	NVDO, Oosterbeek (NL), 2012
European diabetes epidemiology group conference[b]	EDEG, Potsdam (DU), 2013

General activities

20th edition of the PhD-week	VLAG, Bergeijk (NL), 2008
PhD competence assessment	WGS, Wageningen (NL), 2009
Techniques for writing and presenting a scientific paper	WGS, Wageningen (NL), 2009
Organizing and supervising master projects	DO, Wageningen (NL), 2010
NWO talent day ('netwerken doe je zo', 'leidinggeven voor beginners')	NWO, Utrecht (NL), 2010
NWO talent day ('media training', 'writing grant proposal')	NWO, Utrecht (NL), 2010
Effective behaviour in your professional surroundings	WGS, Wageningen (NL), 2010
Philosophy and ethics of food science and technology	VLAG, Wageningen (NL), 2011

Optional courses and activities

Preparation research proposal	HNE, Wageningen (NL), 2008-2012
Literature and discussion groups: 'Diabetes club', 'Epi-research club', 'Journal club', 'Methodology club', and 'Oldsmobiles'	HNE, Wageningen (NL), 2008-2013
Epigenetic and epigenesis	VLAG, Wageningen (NL), 2008
MSc. course 'Concepts and methods in epidemiology'	HNE, Wageningen (NL), 2008
PhD study tour Nordic countries[a,b]	HNE/VLAG, Wageningen (NL), 2009
Organizing and participating in 'Human nutrition research update'	HNE, Wageningen (NL), 2009

[a] *oral presentation given;* [b] *poster presentation given*

Colophon

The research described in this thesis was financially supported by Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition, and Health Sciences).

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