Gastrointestinal manifestations in patients with diabetes mellitus

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PROEFSCHRIFT

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General introduction

Sander de Kort

Diabetes mellitus

Epidemiology

The worldwide epidemic of obesity results in a continually increasing prevalence of diabetes mellitus. It was estimated that around 415 million people were living with diabetes mellitus worldwide in 2015 and that we can expect another 227 million patients by 2040. Especially type 2 diabetes mellitus (T2DM) plays a major part in the rapidly increasing prevalence. In high-income countries ~90% of adults with diabetes mellitus have T2DM. The 2015 prevalence of diabetes among the general population in the Netherlands was 7.9% with diabetes expenses of more than 6,350 euros per diabetic patient per year and yearly around 7,600 diabetes related deaths[1].

Pathophysiology of type 2 diabetes mellitus

Type 1 diabetes mellitus is characterized by the inability to produce the glucose regulating hormone "insulin" due to auto-immune destruction of pancreatic β-cells. Patients with T2DM still produce insulin and secrete this hormone in response to nutrient ingestion. A defect in pancreatic β-cells, resulting in an inappropriate response to serum glucose levels, and an impaired sensitivity to insulin have resulted in the concept that T2DM is a disease of "insulin resistance"[2]. An interesting concept, that may partly explain the onset of insulin resistance in T2DM patients, is that of a leaky gut. This concept is based on a presumed increased intestinal permeability that allows intraluminal content to initiate and continue a low-grade inflammatory state eventually leading to insulin resistance. Currently it is thought that T2DM results from a longstanding defect in the glucose homeostasis feedback loop[3]:

1 Insulin is released in response to β-cell stimulation and promotes uptake of glucose by insulin-sensitive tissues (e.g. liver, muscles).
2 These end-tissues signal information about their insulin-need in return.
3 In insulin resistance (e.g. in obesity) β-cells increase insulin output due to higher demand.
4 β-cells cannot meet the high demand and therefore plasma glucose concentrations rise. A β-cell dysfunction continuum develops from normal glucose to pre-diabetes to diabetes.
5 Diabetes is diagnosed by increased fasting plasma glucose levels (≥7.0 mmol/l) or by a disturbed oral glucose tolerance test (2-h plasma glucose ≥11.1 mmol/l).

Risk factors for type 2 diabetes mellitus

Much research in the past decades has focused on T2DM since the disease is highly prevalent with significant socio-economic impact. Throughout the years several risk factors have been identified that contribute to the development of T2DM. The strongest and most important risk factor is obesity or an increased body mass index (BMI). In the "Nurses' health study" an increased relative risk of 40 or higher was observed in men and women with a BMI of 31 kg/m2 or greater as compared to a BMI of 22 kg/m2 or less[4]. Reversal of obesity decreases T2DM risk and improves glucose regulation in previously established disease. Other strong risk factors (relative risk (RR) > 2) include family history[5] and eth-

nicity[6], independent of BMI. Important risk factors are related to lifestyle. Lifestyle factors are of moderate importance (RR < 2), but have the advantage that they are modifiable, and can influence familial and obesity related risk factors. Known factors are physical activity, diet, smoking, and alcohol consumption[7].

Treatment of type 2 diabetes mellitus

Lifestyle adjustments such as weight loss, an increase of physical activity and dietary modifications are of key-importance in treating (pre)diabetes[8]. Because of the difficulty in achieving weight loss and thereafter maintaining weight and adequate glucose control, consensus has been reached that pharmacological therapy should be initiated together with lifestyle intervention at the time of diagnosis, and not pharmacological therapy separately[9].
Since the first treatment with animal insulin in 1921[10], anti-diabetic drugs have been developed and can be roughly categorized according to mechanisms of action:
1 ***Insulin analogues*** mimic the effect of endogenous insulin.
2 ***Insulin sensitizers*** decrease gluconeogenesis in the liver and increase insulin sensitivity in end-tissues (thiazolidinediones, biguanides). Metformin, a biguanide, is most frequently used as first line treatment in T2DM.
3 ***Insulin secretagogues*** stimulate β-cells to increase their insulin secretion (sulfonylureas, meglitinides).
4 ***Newer/Other antidiabetic drugs*** act indirectly through the activation of gut hormones (incretins) or by delaying postprandial glucose absorption (dipeptidyl peptidase 4, α-glucosidase inhibitors).
The World Health organization developed a classification of drugs and medical products called the Anatomical Therapeutic Chemical (ATC) classification system[11]. In this system "drugs used in diabetes" is a therapeutic subgroup coded "A10" which is useful in the study of diabetes pharmacoepidemiology.

Gastrointestinal disorders in diabetes mellitus

Diabetes mellitus is associated with multiple disorders of practically every organ system. Notorious are the microvascular and macrovascular complications such as coronary heart disease, retinopathy, nephropathy as well as the diabetic foot, that also results from peripheral neuropathy[12]. Although very common in long-standing diabetes, gastrointestinal (GI) complications frequently remain unnoticed, partly due to visceral afferent neuropathy in diabetes patients[13]. Frequently occurring GI complications include:

1 ***Esophageal dysmotility and gastroesophageal reflux disease (GERD)*** due to autonomic neuropathy, remodeling of esophageal musculature, reduced lower esophageal sphincter tone and hyperglycemia. These factors result in an increased rate of transient relaxations of the lower esophageal sphincter (TLESR's)[14]. Management of GERD involves the use of proton pump inhibitors, prokinetic agents and an optimized glycemic control to reduce the amount of TLESR's[15].

2 ***Delayed gastric emptying and gastroparesis*** are considered to be relatively uncommon and often remain an unrecognized complication. The reported gastroparesis incidence is 1-5%/10 years in diabetes patients[16]. Delayed gastric

emptying may be caused by several factors such as autonomic dysfunction, tissue damage due to advanced glycation end products in diabetes, resulting in antral hypomotility, increased pyloric tone, disturbed intestinal feedback and uncoordinated antroduodenal contractions. Motility recordings may reveal functional loss of the normal interdigestive migrating motor complexes (MMC), reduced antral contractions and poor meal accommodation in diabetic patients[17]. These entities can explain symptoms like nausea, early satiety, stasis and vomiting and contribute to difficulties in the management of diabetes. Delayed gastric emptying contributes to poor glycemic control and poor glycemic control may prove to be an indication that the patient has delayed gastric emptying. Early recognition of this complication is of key importance as poor glycemic regulation is able to exacerbate the gastroparesis.

3 ***Enteropathy*** with diarrhea, constipation or fecal incontinence may result from neuropathy, from motility disorders, bacterial overgrowth but also from accelerated intestinal transport[18]. More importantly, extrinsic factors such as polypharmacy or common psychological disorders such as anxiety and depression may contribute to symptomatology and should be taken into account[19]. Knowledge, recognition, diagnosis and treatment of GI complications are all relevant to management of symptoms, to improve quality of life and optimize glucose regulation. A co-occurrence of diabetes mellitus type 1 and celiac disease has been reported based on shared autoimmunity and genetic background. No association has been observed between celiac disease and type 2 diabetes mellitus.

4 ***Nonalcoholic fatty liver disease (NAFLD)***, an umbrella term covering a spectrum of (non-alcoholic) liver diseases, characterized by an abnormal intrahepatic fat content, ranging from simple hepatic steatosis to steatohepatitis (NASH), chronic hepatitis and liver cirrhosis. A meta-analysis provided data on the prevalence of NAFLD among the population. Based on ultrasonography, nearly 25% of the Northern Europe population has typical features of NAFLD [20]. In T2DM patients the prevalence of NAFLD was estimated to be 2-3x higher, up to 65%[21]. It is well known that NAFLD may progress to NASH, liver cirrhosis and hepatocellular carcinoma. Apart from liver diseases, NAFLD is an independent risk factor in T2DM patients that is associated with a two-fold increased risk of cardiovascular events[22]. The cornerstones of treatment of NAFLD and NASH consist of modifications in diet and lifestyle together with weight reduction. Up to now, no drugs are available with proven efficacy in terms of beneficial changes in liver histology[18].

5 ***The association of diabetes with (GI-tract) cancer***. In the past decades extensive research has been conducted with regard to diabetes and an increased cancer risk at various sites in the gastrointestinal tract. Several meta-analyses have been published, more recently also an essential umbrella review of all these meta-analyses[23]. In this umbrella review significantly increased hazard ratios (HR) of the following cancer subtypes in T2DM patients were reported, with robust supporting evidence: cholangiocarcinoma (HR 1.97, 95% CI 1.11-3.49) and colorectal cancer (CRC) (HR 1.27, 95% CI 1.07-1.52). The association of T2DM with cancer of the gall bladder (HR 1.52, 95% CI 0.99-2.33), hepatocellular

carcinoma (HCC) (HR 2.31, 95% CI 0.66-8.02) and pancreatic carcinoma (HR 1.95, 95% CI 0.87-4.34) appears strong, but the probability of biased results is high. Small gall bladder cases numbers (n = 1,821), publication bias with small study effects in HCC incidence meta-analysis (significant egger's p value) and high heterogeneity as indicated by I^2 values higher than 95% within meta-analyses of pancreas and HCC all provide uncertainty about the true effects. Gastric cancer (HR 1.09, 95% CI 0.72-1.65) and esophageal cancer risks (HR 1.29, 95% CI 0.86-1.95) are small and not significantly increased in diabetic patients, probably due to small numbers in studies. Challenges in these diabetes-cancer association studies lie in the confounding effect of multiple shared risk factors, the small effect size and the corresponding need for large case and sample size studies. With respect to CRC, inconsistencies remain on sex-specific and subsite-specific associations. Such information is crucial for CRC screening programs and for developing CRC preventive strategies. It has been documented that surveillance endoscopy is less effective in reducing CRC mortality of proximally located compared to distally located CRC[24].

Colorectal cancer

Epidemiology: worldwide and in the Netherlands

In the year 2012, approximately 1,361,000 CRC cases have been diagnosed worldwide[25]. CRC is the third most common cancer in men and the second in women encompassing 10% and 9.2% of the total cancers respectively. Globally, the age-standardized mortality rate was 8.3 per 100,000 person years (PY). Data from the Netherlands are in line with other high-income countries and show one of the highest age-specific incidence rates (43.2/100,000 PY) and mortality rates (13.4/100,000 PY) worldwide. It should be taken into account that the rates are stabilizing due to increased use of colonoscopy and polypectomy and are expected to decrease in countries where population based CRC screening has been implemented[26,27].

Pathophysiology of colorectal cancer

The carcinogenesis of colorectal cancer is one of the most thoroughly studied topics in oncology research. It is thought that tumors evolve from benign, pre-malignant lesions to malignant tumors by acquiring series of mutations over time. Starting with a "gatekeeping" mutation, most often in the **APC** gene, colonic epithelial cells acquire a growth advantage compared to neighboring cells resulting in a slow growing adenoma. In the years to follow, other occurring so-called "driver" mutations such as **KRAS** and **TP53** trigger clonal expansion and eventually generate a malignant tumor that invades the underlying basement membrane. This process of accumulating mutations is slow and takes years to develop. On average the gatekeeping mutation occurs through the 3rd to 5th decade of life with carcinoma developing on average 20 years later. This mechanism of chromosomal instability carcinogenesis is one of three, often overlapping, proposed mechanisms and constitutes most of the sporadic tumors[28]. The other two known mechanisms are 1) the "CpG island methylator phenotype", which is characterized by promotor hypermethylation of various

tumor suppressor genes, and 2) Microsatellite instability which involves inactivating mutations of DNA mismatch repair genes and can lead to cancer development as these genes are responsible for correcting DNA replication errors. Cancer genome research harbors opportunities for tool development targeted on the prevention and treatment of CRC, eventually reducing CRC morbidity and mortality.

The link between diabetes and colorectal adenoma and cancer development
The association between T2DM and CRC may be based on shared risk factors. Epidemiological data, however, support a role for increased levels of circulating endogenous insulin as a cancer promoting hormone[29]. Insulin not only has metabolic, but also mitogenic effects. Through its own "insulin receptor (IR)" and the "insulin-like growth factor receptor (IGF-R)", in high doses, insulin is able to activate the MAPK pathway (mitogen-activated protein kinases) that promotes cell growth[30]. This theory suggests that anti-diabetic medication, altering the systemic levels of insulin, may beneficially influence CRC risk in T2DM. For instance, metformin, an insulin sensitizer, is considered to lower cancer risk in T2DM patients. It should be acknowledged that published data up to now, are conflicting (e.g. different comparators) and possibly are also biased[31–33]. Other theories on the relation between T2DM and CRC are based on cell damage through a chronic pro-inflammatory state or on chronic hyperglycemia and advanced glycation end products. In addition, altered bowel transit time due to enteropathy may increase mucosal exposure time to carcinogenic substances such as bile acids[34,35].

The role of genetic variation, diabetes and colorectal cancer development
In the previous paragraph, the insulin-IGF pathway with MAPK pathway activation has been proposed as route that links diabetes to colorectal cancer. An association of CRC with an increased genetic susceptibility for high insulin levels or diabetes and obesity development could add to the role of this pathway in CRC carcinogenesis in diabetic patients. Genetic variation between individuals exists in approximately 1% of the DNA. DNA is built out of two sugar-phosphate strands both lined with 4 different (A, T, C, and G) nucleotide bases forming pairs with nucleotides on the opposing strand creating a double helix. An alteration in a single base-pair is the most common type of genetic variation and is called a single nucleotide polymorphism (SNP). The impact of a SNP on phenotype, health and disease is often unclear. A number of genome wide association studies (GWAS) have analyzed multiple SNP's and associated them with diseases or metabolic traits, such as CRC or the insulin-IGF metabolism[36].

Colorectal cancer screening and surveillance

The role of diabetes mellitus and other colorectal cancer risk factors

It is obvious that not one single risk factor accounts for all or most CRC cases. It is generally accepted that a substantial part of the colorectal cancer risk can be ascribed to genotypic and phenotype factors[37]. The following factors have been established in the literature: increased age, male sex, family history of CRC, history of inflammatory bowel disease, smoking, excessive alcohol consumption,

high red and processed meat consumption, obesity and type 2 diabetes mellitus[38]. Environmental factors play a major role in sporadic CRC and often co-occur or interact. Currently, in the Netherlands, individuals with hereditary and familial CRC, longstanding inflammatory bowel disease (IBD) or IBD with associated diagnosis of primary sclerosing cholangitis are enrolled in colonoscopy surveillance programs[39]. While CRC appears to be more prevalent among T2DM patients, this has not resulted in recommendations with respect to intensifying the CRC screening program in T2DM patients.

A large cohort study from the USA has clearly shown that colonoscopic screening with removal of colorectal polyps significantly reduced CRC mortality with 53%[40]. In January 2014 population based CRC screening has been implemented in the Netherlands. This program focuses on the average risk population aged 55-75 years. In 2015 a total of 848,761 individuals participated in the screening program that is based on fecal occult blood testing (FOBT). When immunochemical testing (FIT) was positive, participants were referred for colonoscopy. In 2015 a total of 42,465 colonoscopies were performed. The 2015 screening resulted in 3,692 individuals (8.7%) diagnosed with CRC and 20,536 (48.5%) diagnosed with advanced adenoma with a detection rate of 28.5 per 1,000 individuals screened[41]. The CRC screening program is associated with a shift towards a more favorable tumor stage at the time of diagnosis[41]. Especially T2DM patients may benefit from implementation of population based CRC screening. On the one hand because of the assumed increased risk to develop CRC in diabetic patients and on the other hand because it has been shown that T2DM cancer patients are treated less aggressively and have worse prognosis compared to CRC patients without diabetes[42].

An important part in endoscopic polyp screening and surveillance is the registration of colorectal polyp (histopathologic) characteristics that are associated with metachronous occurrence of CRC. Known endoscopic characteristics that define high-risk individuals are presence of multiple adenomas (e.g. 3 or more), proximally located adenomas, large adenomas (> 9 mm) and adenomas with a predominant villous aspect. Individuals with high risk findings are offered a more intensive endoscopic surveillance program.

In pursuit of better and more tailored prevention strategies we need to identify subgroups at higher CRC risk, such as patients with T2DM. Therefore, we need more detailed information on endoscopic features and CRC subtypes that occur in T2DM patients. Identification of T2DM patients at higher risk through the presence of pathways that link T2DM to CRC, will further help in T2DM tailored CRC screening and surveillance.

Aims of this thesis

The overall aim of the research described in this thesis is to investigate the association between type 2 diabetes mellitus and the GI system, on gastrointestinal manifestations of T2DM, and on carcinogenesis with focus on colorectal

cancer. The research goal can be divided in two parts. In the first part, the aim is to examine the effects of T2DM on gastrointestinal symptoms and complaints and also to explore in more detail the role of the gastrointestinal barrier in diabetes patients through a review of the current literature. We hypothesize that GI symptoms are more prevalent in T2DM patients. We also hypothesize that intestinal barrier function is altered in T2DM and that barrier dysfunction may underlie the development of diabetes and of diabetic complications. In the second part of this thesis, research is focused on the risk of colorectal cancer in type 2 diabetic patients. The aim is to elucidate the hypothesized increased CRC risk in diabetic patients in a Dutch population. We hypothesize that the increased CRC risk could vary across colon subsites, patient ages and genetic profiles. Moreover, we theorized that the prevalence of pre-cursor CRC lesions was also increased in T2DM patients. By focusing on subsite of colonic neoplasms and the age of CRC diagnosis, we try to identify T2DM patients that may benefit from tailored prevention strategies.

Thesis outline

In chapter 2 the association between GI symptoms and psychosocial factors in diabetic patients is described. In chapter 3 the mechanisms involved in changes in intestinal barrier function and the role of an impaired barrier function in the etiology of diabetes mellitus and diabetic complications are reviewed. In chapter 4 and 5 the association between T2DM, genetic variation in the IGF pathway and colorectal cancer is analyzed. In chapter 6 the endoscopic phenotype and histopathology of colorectal polyps in T2DM patients are examined. CRC risk in T2DM patients with attention to age of diagnosis is reported in chapter 7. Finally, chapter 8 provides a general discussion of the findings of this thesis. Based on these findings we define relevant research questions to be answered in the coming years.

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Part I

Prevalence of gastrointestinal symptoms and the barrier (dys) function in diabetic patients

2

Gastrointestinal symptoms in diabetes mellitus and their relation to anxiety and depression

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Summary

Background
Prevalence of gastrointestinal (GI) symptoms is increased in patients with diabetes mellitus. In general, GI symptoms are influenced by psychological factors such as anxiety and depression, but little is known about this association in diabetic patients. Aim: We tested the hypothesis that anxiety and depression have major impact on GI symptoms in diabetic patients.

Methods
280 diabetic patients and 355 non-diabetic, age and sex matched controls were studied by validated questionnaires: (1) PAGI-SYM and GSRS for common GI symptoms and (2) HADS for anxiety and depression. Data were compared using logistic regression analysis.

Results
Patients with diabetes scored significantly ($p < 0.05$) higher on the symptoms diarrhea (OR 1.64, 95% CI 1.05–2.56), early satiety (OR 2.50, 95% CI 1.39–4.49) and bloating (OR 1.58, 95% CI 1.03–2.43), but not on other symptoms. Prevalence of anxiety and depression (HADS scores ≥ 8) in diabetics and controls was respectively 27.5% and 20.6% for anxiety ($p < 0.05$), and 19.6% and 13.4% for depression ($p < 0.05$). After adjusting for anxiety and depression only the GI symptom ''early satiety'' remained significantly more prevalent in the patients with diabetes.

Conclusions
The prevalence of the gastrointestinal symptoms diarrhea, bloating and early satiety, and of anxiety and depression is significantly increased in our cohort of predominantly patients with longstanding type 2 diabetes mellitus compared to controls. When adjusted for anxiety and depression, only the gastrointestinal symptom ''early satiety'' remained more prevalent in these diabetic patients, pointing to a somatic based origin. Thus, in our diabetic population psychological factors to a large extent are associated with gastrointestinal symptoms and should be taken into account when considering treatment of the gastrointestinal symptoms.

Introduction

Gastrointestinal complaints are commonly reported by diabetic patients. Previous studies indicate that about 70 to 75% of diabetic patients have at least one gastrointestinal symptom[1-3]. The gastrointestinal disturbances in diabetes may result from various factors such as autonomic neuropathy, micro- and macroangiopathy, altered visceral motor or sensory function but also from psychiatric comorbidity[2,4]. Glucose control and gastrointestinal symptoms are closely linked. On the one hand hyperglycemia is known to impair gastric and small intestinal motility, possibly through vagal-cholinergic neural inhibition or by altering serum osmolality and gastrointestinal peptide secretion[5]. On the other hand, gastrointestinal motility disorders such as diabetic gastroparesis may give rise to postprandial glycemic dysregulation. Thus, gastrointestinal disorders resulting from diabetes may negatively influence diabetic control, diabetic complications, and eventually also survival[6]. Gastrointestinal symptoms also negatively affect health related quality of life in diabetes, especially in type 2 diabetes[7].
Previous studies have pointed to an association between anxiety and depression with gastrointestinal symptoms in diabetic patients. Diabetic patients with anxiety and depression had a twofold higher prevalence of gastrointestinal symptoms[2,8]. Based on the Hospital Anxiety and Depression Scale (HADS), high levels of anxiety and depressive symptoms were present in respectively 32% and 22% of a mixed type 1 and 2 diabetic population[9]. Aim of the present study was (1) to determine the prevalence of gastrointestinal symptoms and symptoms of anxiety and depression in our population of diabetic patients, having predominantly longstanding type 2 diabetes mellitus, mainly treated with insulin versus a non-diabetic control population, and (2) to explore in detail the possible association between gastrointestinal symptoms with anxiety and depression. Our hypothesis was that psychological factors such as anxiety and depression have major impact on gastrointestinal symptoms in patients with diabetes. Therefore, our objective was to discriminate gastrointestinal symptoms predominantly influenced by psychological factors from gastrointestinal symptoms associated with diabetes mellitus only.

Materials and Methods

A cross sectional design was used to compare the prevalence of clinically relevant gastrointestinal complaints between a diabetic and a non-diabetic population. Recruitment of diabetic patients was performed from September 1 to October 15, 2009. In this six weeks period, a total of 331 consecutive diabetic patients, visiting the outpatient diabetic clinic of the Maastricht University Medical Center (MUMC), that serves as single diabetic care center for a local population of about 200.000 inhabitants, were asked to participate. These patients are treated in the context of a disease management program, and are predominantly patients with longstanding type 2 diabetes on insulin therapy. We extracted the data on diabetes related complications and co-morbidities out of the patient files. In our department of endocrinology a standard form is used for reporting these issues during each consultation. Patients were informed about the study by letter a

week before their regular appointment. Upon agreement to participate, patients filled out a questionnaire containing 1) validated symptom scoring lists concerning gastrointestinal complaints: the "Gastrointestinal Symptom Rating Scale" (GSRS) and the "Patient Assessment of Upper Gastrointestinal Symptoms" (PAGI-SYM), and 2) the "Hospital Anxiety and Depression Scale" (HADS) to objectify symptoms of anxiety and depression. Meanwhile, a total of 1084 age and sex matched controls, at random derived from the municipal database from residents of the city of Maastricht were also sent these questionnaires. Data collection ended five weeks after sending the questionnaires. A total of 355 (32.7%) non-diabetic controls returned a completed questionnaire.

Participants

Inclusion criterion for cases was the presence of diabetes mellitus type 1 or 2 in patients aged 18 years or older. Exclusion criteria were the presence of transient gestational diabetes and language restriction (such as aphasia or foreigners who have not mastered the Dutch language). The only exclusion criterion for controls returning questionnaires was the reported presence of diabetes mellitus.

Variables

Primary outcomes were the total scores, sub-scores and symptom-scores on the GSRS and PAGI-SYM. The HADS score and diabetic complications were considered as secondary outcome measures. The following data were extracted from electronic patient files: the presence of retinopathy, nephropathy (microalbuminuria), macrovascular complications (CVA, coronary disease, and peripheral vascular disease) and neurological complications (diabetic polyneuropathy, small fiber neuropathy and autonomic neuropathy). Body weights, length, type of diabetes, HbA1c and medication use were also extracted from electronic patient files. Subjects with diabetes were asked about frequency of hypoglycemic events and hypoglycemic events in which the help of a third person was necessary, in order to evaluate their glucose regulation over time. None of the patients was on GLP-1 therapy, as this treatment was about to become reimbursed when the study was executed.

Questionnaires

GSRS

The GSRS consists of a total of 15 items to evaluate common gastrointestinal symptoms. Each item can be answered on a 7-point Likert scale that ranges from "no discomfort" to "very severe discomfort". The higher the score is, the more severe the symptom. Subscores for indigestion, diarrhea, constipation, abdominal pain and reflux can be calculated by taking the mean of coupled items. The total score is calculated by taking the mean of all five subscores. The GSRS has been reported to have good reliability and validity for countries in Europe[10,11].

PAGI-SYM

The PAGI-SYM consists of a total of 20 items and was developed to evaluate common upper gastrointestinal symptoms in patients with GERD, gastroparesis and dyspepsia. Each item can be scored on a 6-point Likert scale that ranges

from "no complaints at all" to "very severe complaints". The higher the score is, the more severe the symptom. The following six subscores can be calculated using the mean of coupled items; nausea and vomiting, early satiety, bloating, upper abdominal pain, lower abdominal pain, and heartburn and regurgitation. The total score is calculated by taking the mean of the subscores. The PAGI-SYM has been reported to have good reliability and validity in the Netherlands[12,13].

HADS

The HADS questionnaire is a self-reporting scale for anxiety and depression. The HADS contains 14 items, 7 items on depression and 7 items on anxiety. Patients score to the extend they agree with each statement on a 4-point scale, ranging from 0 to 3. A score of 8 or above is considered abnormal. The HADS questionnaire has been reported to have good reliability and validity in the Netherlands[14]. On all questionnaires the complaints and symptoms were limited to their presence during the past week.

Quantitative variables

In the analysis, a score rated "2 or above" on subscores and total scores of the PAGI-SYM was considered to be clinically significant. These cut-off points were based on previous studies with GERD, dyspepsia and gastroparesis[13,15]. For the GSRS a cut-off point of "3 or above" was used and considered clinically significant[16,17] and for the HADS questionnaire a cut-off point of "8 or above"[14].

Sample size

Power calculation for the study was based on the prevalence of one specific symptom, "abdominal pain or discomfort", reported in both of the following studies. The prevalence of abdominal pain or discomfort was 11% in a population of 8000 controls without diabetes as reported by Bytzer et al.[18], and 21% in a Dutch population of patients suffering from diabetes, as reported previously by Samsom et al.[19] Assuming a power of 90% and a two-tailed p-value of 0.05 a population consisting of 270 patients with diabetes and 270 controls was calculated.

Statistical analyses

Differences in characteristics between patients with diabetes and controls were determined with chi-square or Fisher's exact tests for categorical variables. The Student's t-test and the Mann-Whitney U test were used for continuous variables. To identify differences in symptom complexes between patients with diabetes and controls and to correct for age, sex and the known confounders anxiety and depression, odds ratios (OR) and 95% confidence intervals (CI) were derived using logistic regression analysis. Also, to uncover other potential confounders, we performed a subanalysis on the following characteristics: BMI, alcohol consumption (>10/week), the presence of gastrointestinal disease, a history of gastrointestinal surgery, use of opioids, NSAID's and statins. When a potential confounder induced at least a 10% change in calculated odds ratio, the characteristic was included in the final model. This was the case for BMI and the use of statins. The same method of calculation was used to identify differences in symptom complexes between well regulated and poor regulated patients with diabetes, using

HbA1c and hypoglycemic events as independent variables. Calculations were performed for the group of diabetes patients as a whole (type 1 and type 2) versus controls, and also separately for the group of diabetes patients type 2 versus controls.

Results

A total of 331 patients with diabetes were asked to participate of which 51 patients declined. Reasons for non-participation were: no interest (n=38), lack of time (n=8) and language barrier (n=5). A total of 388 of 1084 (35.8%) controls returned their completed questionnaires; control subjects with diabetes mellitus (n=33) were excluded from participation, resulting in 355 (32.7%) subjects who were included. Baseline characteristics of both groups were similar with respect to age and gender (Table 1). A significant difference in BMI between both groups was observed. The diabetes group consisted of type 1 (n= 81, 28.9%) and type 2 (n= 199, 71.1%) patients. Most patients (91.4%) were on insulin therapy; the median of used insulin units per day was 54 U (quartile ranges 32-80 U). The average regulation of their diabetes was moderate with a mean HbA1c of 8.0% (SD 1.4%). A total of 31.4% reported the occurrence of a hypoglycemic events (serum glucose <4 mmol/L) at least once a week. The percentage that reported a hypoglycemic event at least once a week of which help of a third person was needed amounted 2.9%. Diabetic complications are reported in Table 2. The median duration of diabetes was 15 years (quartile ranges 7-23 years).

The prevalence of gastrointestinal symptoms was not significantly different among both types of diabetes patients and controls apart from "diarrhea" (17.9% vs. 11.7%, p = 0.030), "early satiety" (12.5% vs. 5.4%, p = 0.002), and "bloating" (19.6% vs. 13.3%, p = 0.038), see Table 3A. The percentage of subjects with scores on HADS anxiety "8 or above" was significantly higher in diabetics vs. controls (27.5% vs. 20.6%, p = 0.048); this was also true for depression "8 or above" (19.6% vs. 13.4%, p = 0.039).
Of the diabetic patients 46.4% had one or more clinically significant GI symptom score on the GSRS and/or PAGI-SYM questionnaires in the past week, compared to 38.4% in the non-diabetic control population (p = 0.048). The percentages with one or more clinically significant symptom scores in the past week for GSRS were 41.9% and 36.4% (p = 0.168), and for PAGI-SYM were 29.5% and 20.6% (p = 0.011), in respectively diabetic patients and controls.
Table 4A shows the extracted Odd's ratio's after logistic regression analysis. The following symptom scores in diabetic patients were significantly different from the controls: GSRS diarrhea (OR 1.64 (CI 1.05-2.56), p = 0.031), PAGI-SYM early satiety (OR 2.50 (CI 1.39-4.49), p = 0.002), and PAGI-SYM bloating (OR 1.58 (CI 1.03-2.43), p = 0.037), after correction for age and gender. The symptom "upper abdominal pain" was not significantly different (p = 0,072).
When the independent variables "HADS anxiety and depression scores" were included in the logistic regression model, only the symptom score 'PAGI-SYM early satiety' remained significantly different from controls (OR 2.27 (CI 1.21-4.24), p = 0.011). When the potential confounders "BMI" and "use of statins" were added to

the final model an Odds ratio of 2.77 (CI 1.30-5.90, p = 0.008) was derived for 'PAGI-SYM early satiety'. Other GI symptom scores in patients with diabetes were not significantly different from controls after adjustment for anxiety and depression, BMI and use of statins.

Due to the large proportion of type 2 diabetes patients in our study population a subanalysis was done. The prevalence of clinical significant symptoms was compared between type 1 and type 2 diabetes patients and type 1 and controls. Analysis showed no significant differences in prevalence of gastro-intestinal symptoms (data not shown). The analysis between type 2 diabetes patients and controls demonstrated similar differences as found between the whole group of type 1 and 2 diabetes patients and controls, as shown in table 3B. A second logistic regression analysis for the type 2 diabetes patients was done. The results are shown in table 4B. After correction for anxiety and depression scores, the symptoms GSRS "diarrhea" (1.85 (CI 1.10-3.11) p = 0,021) and PAGI-SYM "early satiety" (3.26 (CI 1.59-6.70) p = 0,001) remain statistical significant different.

To elucidate the known role of BMI in diarrhea, the symptom score was put through a separate logistic regression model. The symptom "diarrhea" was corrected for age, sex and BMI in diabetic patients vs. controls, resulting in a statistically insignificant odds ratio of 1.34 (CI 0.83-2.19), p = 0,234 suggesting the role of BMI as important confounder in the development of this specific symptom besides psychological factors. For the same reason we performed a subanalysis on the influence of metformin on the diarrhea subscore. When patients using metformin were excluded from the analysis the prevalence of the symptom diarrhea was 13.0% in patients with diabetes vs. 11.7% in controls (p=0.669). From the 17.9% diabetic patients who reported diarrhea, 56% was on metformin therapy. In the subgroup of metformin-using diabetics, 25.5% had a clinical relevant diarrhea subscore vs. 13.0% in non-metformin using diabetic patients (p=0.008), pointing to an influence of metformin on the symptom "diarrhea".

Table 1 Characteristics of the 280 patients with diabetes mellitus and 355 age and sex-matched controls

	Patients with diabetes	Controls	p-value
Baseline characteristics			
Sex (% men)	61.1%	61.9%	0.870
Mean age (years)	58.4y	58.6y	0.888
BMI	29.8 kg/m^2	25.9 kg/m^2	<0.001
Origin (% Caucasian)	96.1%	97.2%	0.507
Gastrointestinal disease present *	9.6%	6.5%	0.181
History of Abdominal Surgery **	30.0%	24.8%	0.151
Smoking			
% Smoking now	23.2%	19.7%	0.329
% Smoking now >10 cigarettes/day	38.5%	55.7%	0.058
% Never smoked	34.3%	34.4%	1.000
Alcohol			
No alcohol intake	51.8%	23.4%	<0.001
1-5 Units a week	26.8%	38.9%	0.002
6-15 Units a week	15.4%	27.6%	<0.000
16-30 Units a week	3.6%	8.5%	0.013
More than 30 units a week	2.5%	1.7%	0.576
Medication			
PPI/H2- antagonist	17.1%	14.4%	0.379
NSAID's	2.9%	5.6%	0.119
Opioid	5.0%	3.7%	0.434
Statin	66.1%	17.8%	<0.001
ACE-inhibitor	35.0%	5.1%	<0.001
Beta-antagonist	34.3%	15.7%	<0.001
Aspirin	27.1%	14.2%	<0.001
Metformin	39.3%	0%	<0.001

*acid related disorder (reflux disease), pancreatic disorder, hepatobiliary disorder, inflammatory bowel disease, coeliac disease, motility disorders.
** appendectomy, cholecystectomy, hysterectomy, caesarean section, bowel resection, nephrectomy, pancreaticoduodenectomy.

Table 2 Type of diabetes, complications and glucose regulation in the 280 patients with diabetes mellitus.

Type of diabetes mellitus	**%**
Type 1	28.9%
Type 2	71.1%
Complications	
Retinopathy	23.3%
Nephropathy	25.4%
Foot ulcer	2.5%
Neuropathy	42.7%
Autonomic neuropathy	13.3%
Polyneuropathy	38.0%
Small fibre neuropathy	3.2%
Macroangiopathy	38.0%
Coronary artery disease	25.2%
Peripheral arterial disease	12.9%
CVA	7.6%
Hypertension (treated for)	56.6%
Glucose regulation	
Hypoglycaemic events	
Never	26.4%
>1/year	15.0%
>1/month	27.1%
>1/week	31.4%
Hypoglycemic events; help of third person	
Never	86.8%
>1/year	7.9%
>1/month	2.5%
>1/week	2.9%
Mean HbA1c in %	8.0% (SD 1.4%)

CVA: cerebrovascular accident; SD: standard deviation

Table 3A Prevalence of clinically relevant gastrointestinal symptoms (GSRS score of ≥ 3, PAGISYM score ≥ 2), and symptoms of anxiety and depression (HADS scores ≥ 8), in patients with diabetes mellitus (type 1 and 2) versus controls.

	patients with diabetes (%)	controls (%)	p-value
Total score GSRS	9.7	8.4	0.577
Indigestion	24.7	24.6	1.000
Diarrhea	17.9	11.7	0.030
Constipation	16.1	14.6	0.656
Abdominal pain	15.1	10.3	0.069
Reflux	8.6	10.8	0.420
Total score PAGI-SYM	4.3	3.5	0.678
Nausea and vomiting	4.3	2.6	0.267
Early satiety	12.5	5.4	0.002
Bloating	19.6	13.3	0.038
Upper abdominal pain	10.4	6.3	0.077
Lower abdominal pain	14.0	12.1	0.551
Heartburn and regurgitation	2.9	4.0	0.516
HADS			
Anxiety	27.5	20.6	0.048
Depression	19.6	13.4	0.039

GSRS: Gastrointestinal Symptom Rating Scale; PAGI-SYM: Patient Assessment of Gastrointestinal Disorders Symptom Severity Index; HADS: Hospital Anxiety and Depression Scale

Table 3B Prevalence of clinically relevant gastrointestinal symptoms (GSRS score of ≥ 3, PAGISYM score ≥ 2), and symptoms of anxiety and depression (HADS scores ≥ 8), in patients with diabetes mellitus type 2 versus controls.

	Patients with type 2 diabetes (%)	Controls (%)	p-value
Total score GSRS			
Indigestion	23.2	24.6	0.756
Diarrhea	20.7	11.7	0.006
Constipation	17.1	14.6	0.462
Abdominal pain	15.7	10.3	0.077
Reflux	9.5	10.8	0.771
Total score PAGI-SYM			
Nausea and vomiting	5.0	2.6	0.147
Early satiety	13.6	5.4	0.001
Bloating	20.1	13.3	0.039
Upper abdominal pain	11.1	6.3	0.051
Lower abdominal pain	14.6	12.1	0.430
Heartburn and regurgitation	3.5	4.0	1.000
HADS			
Anxiety	27.6	20.6	0.073
Depression	23.6	13.4	0.003

GSRS: Gastrointestinal Symptom Rating Scale; PAGI-SYM: Patient Assessment of Gastrointestinal Disorders Symptom Severity Index; HADS: Hospital Anxiety and Depression Scale

Table 4A Adjusted odds ratio's and confidence intervals of clinically significant scores on GSRS, PAGI-SYM and HADS in patients with diabetes mellitus (type 1 and 2) versus controls.

Questionnaire Score	adjusted OR (95% CI)*	p-value	adjusted OR (95% CI)**	p-value	adjusted OR (95% CI)***	p-value
Total score GSRS	1.16 (CI 0.67-2.01)	0.607	0.80 (CI 0.43-1.52)	0.503	0.74 (CI 0.35-1.59)	0.441
Indigestion	0.99 (CI 0.68-1.43)	0.951	0.86 (CI 0.57-1.28)	0.449	0.86 (CI 0.54-1.39)	0.549
Diarrhea	1.64 (CI 1.05-2.56)	0.031	1.47 (CI 0.92-2.35)	0.108	1.20 (CI 0.68-2.11)	0.533
Constipation	1.10 (CI 0.71-1.72)	0.661	0.88 (CI 0.54-1.43)	0.602	0.87 (CI 0.49-1.56)	0.641
Abdominal pain	1.55 (CI 0.96-2.51)	0.076	1.30 (CI 0.77-2.19)	0.336	1.48 (CI 0.80-2.77)	0.216
Reflux	0.77 (CI 0.45-1.32)	0.336	0.67 (CI 0.38-1.18)	0.161	0.72 (CI 0.37-1.40)	0.329
Total score PAGI-SYM	1.23 (CI 0.54-2.80)	0.631	0.90 (CI 0.35-2.27)	0.819	1.07 (CI 0.34-3.33)	0.905
Nausea and vomiting	1.68 (CI 0.69-4.05)	0.250	1.36 (CI 0.54-3.41)	0.514	1.75 (CI 0.59-5.24)	0.315
Early satiety	2.50 (CI 1.39-4.49)	0.002	2.27 (CI 1.21-4.24)	0.011	2.77 (CI 1.30-5.90)	0.008
Bloating	1.58 (CI 1.03-2.43)	0.037	1.44 (CI 0.92-2.27)	0.112	1.36 (CI 0.80-2.34)	0.259
Upper abdominal pain	1.72 (CI 0.95-3.09)	0.072	1.47 (CI 0.79-2.74)	0.228	1.94 (CI 0.94-4.03)	0.075
Lower abdominal pain	1.16 (CI 0.72-1.85)	0.543	0.96 (CI 0.57-1.60)	0.860	1.11 (CI 0.61-2.02)	0.736
Heartburn	0.69 (CI 0.28-1.67)	0.409	0.46 (CI 0.17-1.23)	0.121	0.55 (CI 0.18-1.63)	0.278
HADS						
Anxiety	1.45 (CI 1.00-2.11)	0.049				
Depression	1.58 (CI 1.03-2.42)	0.036				
One or more clinical significant symptoms	1.37 (CI 0.99-1.91)	0.059	1.34 (CI 0.93-1.93)	0.113	1.15 (CI 0.74-1.78)	0.530

OR: odds ratio's; CI: confidence intervals; GSRS: Gastrointestinal Symptom Rating Scale; PAGI-SYM: Patient Assessment of Gastrointestinal Disorders Symptom Severity Index; HADS: Hospital Anxiety and Depression Scale
* corrected for age and sex; ** corrected for age, sex and HADS anxiety and depression; *** corrected for age, sex and HADS anxiety and depression, statin use and BMI.

Table 4B Adjusted odds ratio's and confidence intervals of clinically significant scores on GSRS, PAGI-SYM and HADS in patients with diabetes mellitus type 2 versus controls.

Questionnaire Score	adjusted OR (95% CI)*	p-value	adjusted OR (95% CI)**	p-value	adjusted OR (95% CI)***	p-value
Total score GSRS	1.41 (CI 0.75-2.65)	0.280	0.86 (CI 0.42-1.79)	0.695	0.75 (CI 0.29-1.92)	0.542
Indigestion	1.08 (CI 0.70-1.66)	0.726	0.90 (CI 0.57-1.44)	0.670	0.94 (CI 0.51-1.72)	0.832
Diarrhea	2.17 (CI 1.33-3.56)	0.002	1.85 (CI 1.10-3.11)	0.021	1.44 (CI 0.72-2.88)	0.297
Constipation	1.09 (CI 0.67-1.78)	0.730	0.84 (CI 0.49-1.43)	0.512	0.82 (CI 0.41-1.65)	0.585
Abdominal pain	2.07 (CI 1.19-3.60)	0.010	1.61 (CI 0.88-2.94)	0.122	2.01 (CI 0.91-2.45)	0.086
Reflux	0.99 (CI 0.55-1.81)	0.491	0.80 (CI 0.43-1.51)	0.491	0.93 (CI 0.41-2.11)	0.860
Total score PAGI-SYM	1.67 (CI 0.65-4.27)	0.286	0.95 (CI 0.33-2.71)	0.920	1.11 (CI 0.23-5.35)	0.900
Nausea and vomiting	2.77 (CI 1.03-7.45)	0.044	1.92 (CI 0.68-5.38)	0.217	4.02 (CI 1.08-15.0)	0.039
Early satiety	4.06 (CI 2.05-8.05)	0.000	3.26 (CI 1.59-6.70)	0.001	4.71 (CI 1.78-12.5)	0.002
Bloating	1.95 (CI 1.20-3.18)	0.007	1.66 (CI 0.99-2.77)	0.055	1.55 (CI 0.78-3.05)	0.209
Upper abdominal pain	2.55 (CI 1.30-5.00)	0.006	1.94 (CI 0.95-3.94)	0.068	3.51 (CI 1.35-9.17)	0.010
Lower abdominal pain	1.40 (CI 0.83-2.38)	0.210	1.08 (CI 0.60-1.94)	0.792	1.48 (CI 0.69-3.19)	0.315
Heartburn	1.11 (CI 0.42-2.89)	0.839	0.66 (CI 0.23-1.92)	0.449	1.09 (CI 0.29-4.07)	0.897
HADS						
Anxiety	1.78 (CI 1.16-2.72)	0.009				
Depression	1.98 (CI 1.25-3.15)	0.004				
One or more clinical significant symptoms	1.70 (CI 1.17-2.48)	0.006	1.60 (CI 1.05-2.42)	0.028	1.49 (CI 0.86-2.58)	0.153

OR: odds ratio's; CI: confidence intervals; GSRS: Gastrointestinal Symptom Rating Scale; PAGI-SYM: Patient Assessment of Gastrointestinal Disorders Symptom Severity Index; HADS: Hospital Anxiety and Depression Scale
* corrected for age and sex; ** corrected for age, sex and HADS anxiety and depression; *** corrected for age, sex and HADS anxiety and depression, statin use and BMI.

Discussion

We observed an increased prevalence of the GI symptoms diarrhea, early satiety and bloating in our population of diabetic patients having predominantly long-standing type 2 diabetes mellitus, mainly treated with insulin versus controls. In a subanalysis we showed that metformin use and BMI partly explains the observed difference in incidence of the symptom of diarrhea between diabetic patients and controls.

An increased prevalence of GI symptoms in patients with diabetes has previously been reported by others[3,18,20]. Bytzer et al. performed their study in a community setting with a large study population and showed an increased prevalence of diarrhea or constipation (15.6%), bloating (12.3%), and early satiety (5.4%)[18]. In our diabetic population the prevalence of these symptoms was considerably higher with percentages of respectively 17.9% for diarrhea, 16.1% for constipation, 19.6% for bloating and 12.5% for early satiety. The differences in results between the study of Bytzer et al. and our data may result from differences in setting, in the definition of a clinically significant symptom and in methodology. Ko et al.[3] reported that patients with type 2 diabetes scored significantly higher for the symptoms diarrhea, epigastric fullness, early satiety, and abdominal pain compared to controls. In a third study by Ricci et al.[20] only the prevalence of early satiety and bloating appeared significantly higher in patients with diabetes. On the other hand several other studies such as those by Janatuinen et al. and Maleki et al.[21, 22] conclude that occurrence of GI-symptoms in a general diabetic population is not different from a middle aged general control population. The diabetic population we studied predominantly consists of diabetic patients with longer disease duration and a more complicated course of their diabetes. For that reason they were referred to our tertiary center. In our opinion, the difference in gastrointestinal symptom prevalence among studies depends on the specific diabetes population.

After correction for anxiety and depression, early satiety (consisting of four items: sensation of fullness, early postprandial satiety, decrease of appetite and not being able to finish a normal meal portion) was the only subscore of which the prevalence remained significantly different from controls. This finding indicates that patients with diabetes have early satiety complaints more frequent than controls, independently of anxiety or depression, suggesting a somatic based origin of "early satiety". Thus, the symptom "early satiety" may be an indicator of gastrointestinal pathology in patients with diabetes. For example Kojecky et al. found a significant positive correlation between the duration of gastric emptying and the symptom early satiety[23]. Early satiety in dyspeptic patients refers to pathophysiological changes such as impaired postprandial accommodation of the stomach, visceral hypersensitivity or changes in intragastric meal distribution. Indeed, in diabetic patients sensory-motor dysfunction of the stomach has repeatedly been demonstrated with either impaired gastric accommodation, gastric hypersensitivity or both[24-26]. The other GI scores and symptoms were related to anxiety and depression rather than to the presence of diabetes. Anxiety and depression symptoms were more frequent in patients with diabetes versus controls. This has also been acknowledged for other chronic diseases[27,28]. When treating diabetic patients these psychological factors should be taken into account be-

cause to a large extent, they contribute to the generation and impact of GI symptoms. Quan et al. observed that a reduction in depression scores during follow-up in diabetic patients was associated with a marked reduction in gastrointestinal symptoms[29]. These findings point to the relevance of recognizing and treating anxiety and depression in diabetic patients.
Talley et al.[8] found a prevalence of an anxiety and depression score of 11 or above (using HADS questionnaire) in respectively 14.5% and 7.6% of a population consisting of clinical and community based patients. Our study is in accordance with these results showing a prevalence of respectively 13.9% and 13.2% for anxiety and depression using the cut-off point of 11 or above. The prevalence of anxiety and depression scores of 8 or above in our controls was respectively 20.6% and 13.4% and is in line with data from other studies[30].
Our study is not a population-based survey, which is a limitation. Due to the design of this study the control population was not tested for glucose intolerance, therefore early diabetes could not be excluded. However, in the case early diabetes had been excluded in all controls, the differences in symptom score between the group having diabetes mellitus and the controls would have expected to be even more pronounced.
Our diabetes population consists of patients with longstanding diabetes. Also the majority of patients have insulin-treated type 2 diabetes. The mean HbA1c of 8.0% points to relatively poor glucose regulation and may contribute to an increased prevalence of gastrointestinal symptoms. However, in a subanalysis no significant relation was observed between the prevalence of clinically significant gastrointestinal symptom scores and HbA1c value or hypoglycaemic events. The diabetes clinic of the Maastricht University Medical Centre is part of a disease management program; more than 90% of the patients are treated by GP's and only patients with severe disease and usually multiple complications are treated in the diabetes clinic, probably correlating with longer duration of diabetes and more intense exposure to glucose imbalances.
From the non-diabetic controls, almost 33% returned a completed questionnaire. This lower response rate may have affected the results. We cannot exclude that controls with GI complaints would be more inclined to answering the questionnaires than those without complaints. On the other hand, the responders did not differ with respect to sex, age, origin, presence of gastrointestinal disease or history of abdominal surgery from controls. Therefore we do not believe that the responders consist of a selected subgroup.

In conclusion, we have observed a significantly increased prevalence of the gastrointestinal symptoms diarrhea, bloating and early satiety, and of anxiety and depression in patients with predominantly longstanding type 2 diabetes mellitus on insulin therapy, treated in an outpatient clinic, compared to controls. When adjusted for anxiety and depression, only the gastrointestinal symptom "early satiety" remained more prevalent in these diabetic patients, suggesting a somatic based origin. Thus, in our specific diabetic population, psychological factors to a large extent seem to affect gastrointestinal symptoms and should be taken into account when considering treatment of these symptoms. Further investigation, in a more prospective designed study, is needed to further clarify the relationship between gastrointestinal symptoms and psychological factors in these patients with diabetes mellitus.

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Leaky gut and diabetes mellitus: What is the link?

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Summary

Diabetes mellitus (DM) is a chronic disease requiring lifelong medical attention. With hundreds of millions suffering worldwide, and a rapidly rising incidence, diabetes mellitus poses a great burden on health care systems. Recent studies investigating the underlying mechanisms involved in disease development in diabetes point to the role of the dysregulation of the intestinal barrier. Via alterations in the intestinal permeability, intestinal barrier function becomes compromised whereby access of infectious agents and dietary antigens to mucosal immune elements is facilitated, which may eventually lead to immune reactions with damage to pancreatic beta cells and can lead to increased cytokine production with consequent insulin resistance. Understanding the factors regulating the intestinal barrier function will provide important insight into the interactions between luminal antigens and immune response elements. This review analyzes recent advances in the mechanistic understanding of the role of the intestinal epithelial barrier function in the development of type 1 and type 2 diabetes. Given our current knowledge, we may assume that reinforcing the intestinal barrier can offer and open new therapeutic horizons in the treatment of type 1 and type 2 diabetes.

Introduction

According to the reports of the World Health Organisation (WHO), globally an estimated 220 million people are suffering from diabetes mellitus. Without further actions or interventions, this number is likely to double by the year 2030. In the past decades , the prevalence of both type 1 and type 2 diabetes mellitus has dramatically increased, resulting from changes in diet, reduced physical activities and exposure to certain environmental factors described in the "hygiene" and "overload" hypotheses[1]. Certainly, as type 1 and type 2 diabetes are multifactorial diseases, genetic factors consisting of multiple susceptibility genes as well as environmental influences contribute to disease development. In a number of countries, type 2 diabetes mellitus has become the most prevalent type of diabetes in children.[2] The dramatic rise in prevalence will have impact on the socio-economic perspective of the population. For instance, the WHO estimates that in the coming five years, China will lose over 300 billion dollars income due to heart disease, stroke and diabetes[3]

Diabetes affects the gut: there is ample evidence that diabetes mellitus affects gastrointestinal morphology and function. Conversely, *the gut affects diabetes*: several recent publications provide evidence that an altered bowel function contributes to the pathogenesis of diabetes mellitus. In this respect, the intestinal barrier is particularly relevant with focus on intestinal permeability (IP), immune response and intestinal microbiota. Intestinal barrier function is compromised in various gastrointestinal disorders such as inflammatory bowel disease, celiac disease, NASH / NAFLD and irritable bowel disease, but also in autoimmune and systemic diseases.[4] This review explores the mechanisms involved in changes in intestinal barrier function and their role in the aetiology of diabetes mellitus. Secondly, approaches and strategies will be discussed that help to enhance barrier function in diabetes mellitus.

Introduction Intestinal barrier function and the role of tight junctions

Along the gastrointestinal tract, an adjacent layer of cells separates the internal body systems from the external environment. This separation exists to ensure protection from a wide range of environmental pathogens entering the lumen, thereby preventing infection, inflammation and alteration of normal body functions. Besides the tight lining of epithelial cells, other products such as mucus, immunoglobulins and other anti-microbial agents are important in maintaining a proper barrier function. The absorptive functions of the small intestine are regulated through two mechanisms. The first is transcellular transportation across the enterocyte brush border, usually facilitated by transport carriers or by means of passive diffusion. The second path is movement through paracellular spaces, not mediated by carriers and thus based solely on passive diffusion of molecules. The movement of substances across paracellular spaces is under influence of several factors such as 1) the concentration gradient across the

barrier 2) the surface area of the epithelium 3) the time available for diffusion/ permeation and 4) the intrinsic permeability properties of the barrier itself.

Several recent reports have reviewed the structure and function of tight junctions,[4,5] which appear to have a principal role in regulating paracellular transport across the intestinal epithelium. In brief, the junctions between adjacent epithelial cells consist of the more luminally situated *tight junctions* and the basally situated *adherens junctions*. Tight junction proteins form fibrils that cross the membrane to interact with neighbouring cells. The fibrils between two cells consist of at least two proteins, namely *occludin* and members of the *claudin family*. These two proteins are indirectly linked with *actin filaments* through the intracellular scaffold proteins *ZO-1, ZO-2 and ZO-3*. It is thought that contraction of these cytoskeletal actin filaments regulates paracellular permeability.

Tight junctions are considered to be dynamic structures. Epithelial tight junctions open and close in response to various stimuli such as dietary products, humoral or neuronal signals, inflammatory mediators and mast cell products, and pathological impact of viral or bacterial agents. An important physiological mechanism in the intestinal wall, through which various bacterial agents operate, is the upregulation of *zonulin*[6] secreted in the lumen from cells in the lamina propria. Mechanistically, zonulin binds to the zonulin receptor on intestinal epithelial cells and induces rearrangement of the cytoskeleton, downregulation of ZO-1 and occludin, and disruption of TJ complex integrity, increasing epithelial permeability.[7]

Intestinal permeability

When evaluating intestinal permeability, researchers are particularly interested in the regulatory mechanisms and properties concerning the intrinsic permeability of the gut barrier. To measure the barrier function, different sets of probes have been employed such as monosaccharides (mannitol, L-rhamnose), disaccharides (lactulose, sucralose), polyethylene glycol, and non-degraded radiolabeled chelates (^{51}Cr-EDTA). The probes share specific characteristics: they are small-sized, water-soluble, not degraded or metabolized in the gut lumen, non-toxic, in total excreted by the kidney and can therefore easily be detected in urine samples. Measurements using a single molecule (such as ^{51}Cr EDTA) may be influenced by inter-individual differences not related to permeability such as intestinal transit or urinary excretion. Thus far, human intestinal permeability has been measured by urinary excretion of two probes of different sizes but similar transit and uptake processes, calculating the excretion ratio of a monosaccharide and a disaccharide such as mannitol and lactulose, respectively. These probes differ in manner of transport, i.e. paracellular or transcellular. In this way two routes of uptake are compared. The most widely accepted method of measuring IP in the small intestine is the lactulose/mannitol or lactulose/ rhamnose urine excretion test in humans. In the healthy small bowel, the permeability for larger sugars such as lactulose is much lower than for smaller sugars such as mannitol or rhamnose. Lactulose and other larger molecules pass through the intercellular spaces which are regulated by intercellular tight junc-

tions. Under pathological conditions (such as mucosal inflammation) the permeability for the larger sugars increases, whereas the permeability of the smaller sugars remains stable or decreases.[8] This results in an increased urinary excretion ratio of large to small sugars.

An increased intestinal permeability, often referred to as a 'leaky gut,' has been proposed to be associated with several disorders, including intestinal and liver diseases, autoimmune disorders and also type 1 and type 2 diabetes. Although an altered intestinal barrier function can be a consequence of disease exacerbation, clinical evidence suggests that it might be a primary causative factor predisposing to disease development.[4] For example, healthy first-degree relatives of patients with IBD and celiac disease have increased intestinal permeability.[7] Although the diseases associated with increased permeability do differ in terms of pathogenesis and clinical presentation, there seems to be a common denominator: an altered barrier function is believed to facilitate increased exposure to antigens that can trigger immune reaction and autoimmune destruction and alterations of normal body function.

The gut affects diabetes: diabetes mellitus type 1 and intestinal barrier

Intestinal permeability and microscopic alterations

In diabetic patients, increased intestinal permeability was first reported in 1986 by Mooradian et al.[9] The authors measured urinary secretion of lactulose and rhamnose in diabetic patients and controls. Although lactulose and rhamnose excretion both were significantly increased in diabetic patients, the ratio of percent urinary excretion for lactulose/rhamnose did not differ significantly compared to healthy subjects. However, this study did not take into account the possible bias of diseases with increased IP associated with diabetes such as celiac disease. Later on, additional studies have been published with more standardized test methods (lactulose/mannitol (L/M) ratio), in better defined diabetic populations. A significantly increased L/M ratio was observed in diabetic patients in comparison to controls, but no significant correlation was found with duration of disease or mean HbA1c values. These findings have been confirmed in other studies.[10,11] Prediabetic subjects had the greatest increase, suggesting that increased intestinal permeability precedes the onset of clinical diabetes. Ultrastructural examination of duodenum from diabetic patients revealed altered TJ structure and an increase in the paracellular space between epithelial cells and cells from healthy control subjects.[12] However, no differences in histological pattern (e.g. villus properties and lymphocyte infiltration) were found in studied patients that would confirm results of a previous study in biobreed diabetes-prone rats, an inbred line in which autoimmune diabetes spontaneously develops when weaned onto a normal diet.[13] On the other hand, in that study, the percentage of goblet cells was higher in diabetes-prone rats compared to diabetes-resistant rats. Goblet cells produce mucus warding possible pathogens, suggesting the presence of inflammatory response before the onset of diabetes.[14]

Zonulin and tight junction proteins

The acute phase of celiac disease is characterized by a gliadin-triggered zonulin pathway.[15,16] Experimental data indicate that the protein zonulin appears also to play an important role in the pathogenesis of diabetes mellitus through modulation of intestinal permeability. It is also noteworthy that when celiac disease is seen together with type 1 diabetes, most commonly diabetes has developed first.[17] Watts et al.[13] found that zonulin expression is increased in diabetic-prone rats and correlated this with increased permeability. Upon administration of a zonulin antagonist, the cumulative incidence of diabetes type 1 was significantly reduced in the diabetic-prone rats.

Sapone et al.[11] observed a large population 339 diabetic type 1 children and their relatives. Zonulin serum levels were significantly increased not only in diabetic patients but also in their relatives. In the diabetic patients, lactulose, but not mannitol urinary excretion was increased pointing to an increased paracellular IP. A strong positive correlation between the L/M ratio and zonulin serum levels was found. Also, a trend towards changes in gene expression of the tight junction proteins claudin-1, claudin-2 and myosin IXB was suggested in a small studied subgroup.[11] Studies in biobreed rats have pointed out that the increased permeability detected in pre-diabetic rats is related to decreased expression of claudin-1 and occludin.[14,18] Two groups observed increased intraluminal zonulin levels already in a pre-diabetic stadium, suggesting that increased permeability precedes diabetes mellitus type 1.[11,13] Bosi et al.[19] observed no differences in enteropathy, measured by the lactulose/mannitol test, between pre-clinical and longstanding diabetes, suggesting that the duration of diabetes does not further influence intestinal permeability and an increased IP precedes, rather than is caused by diabetes mellitus type 1.

Luminal antigens trigger immune reactions through increased IP

As mentioned earlier, increased intestinal permeability is observed not only in patients who have developed type 1 diabetes but already in its pre-clinical stadium. A subclinical inflammation, found in young diabetic patients and characterized by increased IL-4, TNF-α and IFN-γ is possibly involved in compromising the integrity of epithelial barrier leading to increased IP of the gut.[20-23] Whether a subclinical inflammation precedes or is caused by increased IP has to be further investigated. Nevertheless, increased IP makes the host more viable and prone to immune reactions against antigens from dietary (cow milk substances like bovine insulin[24] or wheat gliadins), viral or bacterial origin. These agents can activate humoral responses and translocate to lymphoid tissue surrounding the pancreas where they may trigger autoimmune reactions against insulin producing beta-cells (see figure 1). For example, Richardson et al. found an increased prevalence of enteroviral pathogens expressed on the beta-cells of diabetic tissue compared to controls at young age,[25] suggesting that an enterovirus might precipitate autoimmunity. Furthermore, Oikarinen et al. pointed out that an ongoing enteroviral infection in diabetic patients may contribute to an increased permeability.[26]

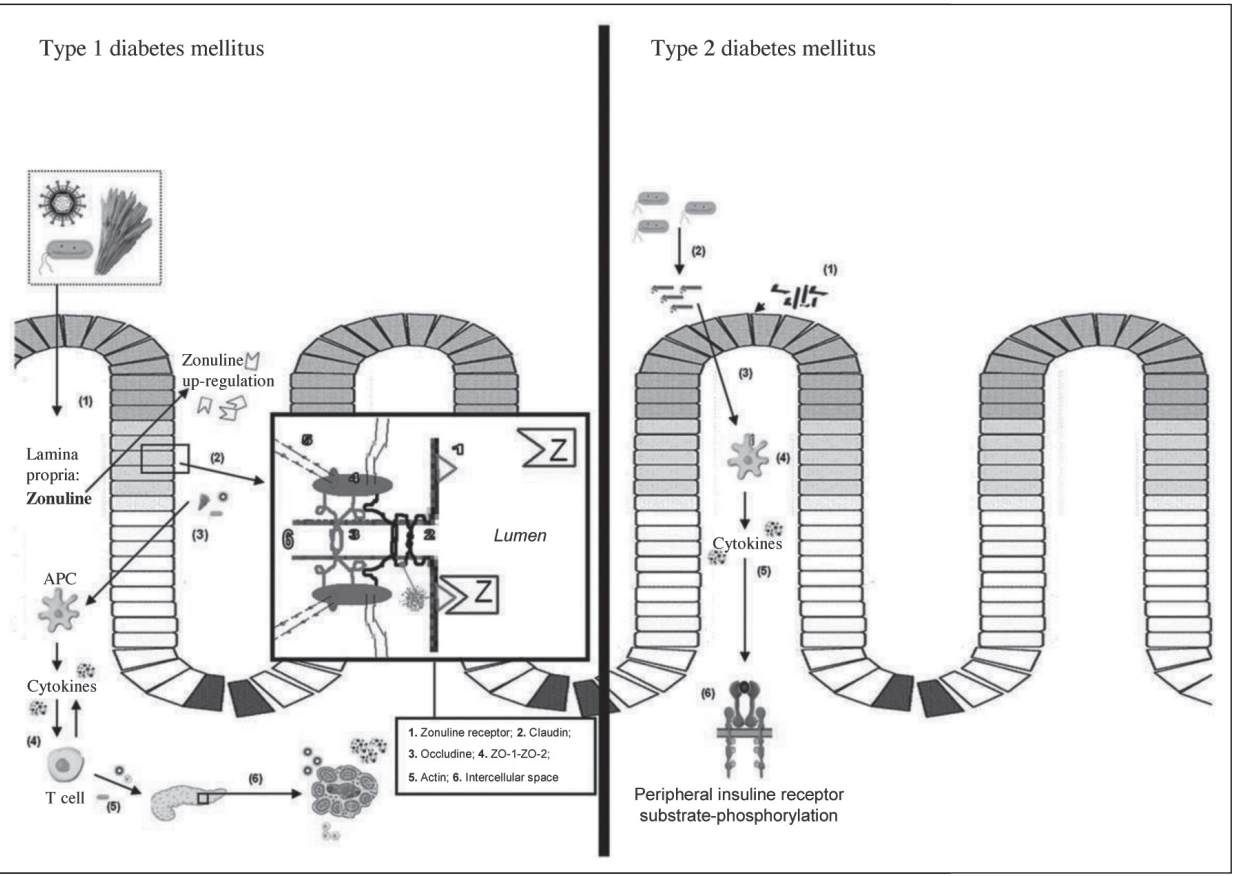

Figure 1 Type 1 diabetes mellitus: (1) Viral infection/microbiota/gliadin trigger subclinical inflammation and zonuline pathway; (2) Phosphorilation of tight junction proteinsÐLeaky gut; (3) Transportation of luminal antigens; (4) T-cells activation; (5) Pancreatic lymphnodes (viral transport, activation local T cells, activating autoimmune beta cells and CD8+ cells); (6) Pancreatic isle inflammation and beta-cell destruction because of viral damage, autoimmune reaction and cytokine-mediated destruction. Type 2 diabetes mellitus: (1) Fatty acids increase intestinal permeability; (2) Gram-negative bacteria produce lipopolysaccharide (LPS); (3) LPS passes intestinal barrier; (4) LPS attached to toll-like-4 receptor on macrophages; (5) macrophages produces cytokines; (6) phosphorilation of insuline receptor substrate and consequent insulin resistance. APC, Antigen presenting cell.

The question arises which factors truly contribute to or trigger an increase in IP. Several studies have investigated the role of microbiota in the development of type 1 diabetes. It is well-established that the presence of a balanced commensal microbiota contributes to the development of host innate and adaptive immune systems and establishes antigen tolerance. For example, the "polysaccharide A" produced by *B. fragilis* has been proven to activate IL-10 producing T-cells thereby protecting against *H. hepaticus*-induced colitis in non-diabetic mice.[27,28] This theory of the protective characteristics of IL-10 appears to be of importance in preventing type 1 diabetes in non-obese diabetic (NOD)-mice.[29] Furthermore, one study found that oral antibiotics, especially in combination with a hydrolysed casein diet, decreased the incidence of diabetes type 1 in diabetogenic mice. The reduction in gut microbiota, induced by antibiotics, suggests an important role of microbiota in development of diabetes[30]. A recent study conducted by Lee et al.[31] showed that the toxin "espF" (known as toxin released by *E. coli*) from wild type *C. rodentium* increases intestinal permeability in NOD-mice, followed by an increased count of *C. rodentium* as well as increased activity and proliferation of diabetogenic CD8+ cells in pancreatic lymph nodes. These phenomena point to an accelerated development of "insulitis" (inflammation of the islets of Langerhans), i.e. a pre-stadium of diabetes type 1. Thus, alterations in intestinal microbiota may either have pro- and/or anti-diabetic effects depending on microbial composition and on metabolic activity.

The gut affects diabetes: diabetes mellitus type 2, obesity and intestinal barrier function

Type 2 diabetes mellitus is characterized by obesity and peripheral insulin resistance. Several pathophysiological mechanisms have been postulated to explain the observed association between obesity and insulin resistance, and eventually type 2 diabetes: endocrine (fatty acids, adipokines), neuronal, cellular metabolic (oxidative stress, mitochondrial dysfunction) and inflammatory factors.[32] Here we focus on the role of intestinal permeability, microbiota and inflammation in the pathogenesis of diabetes type 2.
An increased systemic production of pro-inflammatory cytokines (interleukins, TNF-α) and C-reactive protein has been observed in obesity and in type 2 diabetes mellitus. These cytokines are known to induce peripheral insulin resistance by activating JNK1 and NF-κB, which results in serine phosphorylation of insulin receptor substrate-1 and insulin resistance.[33] On the other hand, increased intestinal permeability may give rise to inflammatory type responses, possibly contributing to the development of insulin resistance. The following paragraphs reflect on possible mechanisms through which the intestinal barrier and microbiota contribute to the development of diabetes mellitus type 2.

Increased intestinal permeability and lipopolysaccharides
Recent studies have investigated the relationship between dietary composition, intestinal microbiota, epithelial integrity and obesity, which has extensively been reviewed recently by Diamant et al.[34] An important role for the gut microbiota and more specifically for lipopolysaccharide (LPS) as a trigger molecule

has been postulated in the onset of metabolic disorders associated with obesity and type 2 diabetes. The study by Creely et al. revealed a significant increase in plasma LPS in type 2 diabetic patients compared to controls.[35] High-fat and high-caloric diets have been shown to favor the colonization of the intestine with Gram negative microbiota, leading to increased plasma LPS levels (metabolic endotoxemia), whereas the quantity of *Bacteroidetes spp.* decreases and that of *Firmicutes spp.* increases.[36] An increase in the proportion of *Bacteroidetes spp.* in the gut is associated with weight loss in human obese subjects,[37] possibly due to low-fat and low-caloric diet. Increased plasma LPS levels induce production of pro-inflammatory cytokines and may lead to insulin resistance. LPS is transported from the intestinal lumen towards the target tissue by a mechanism facilitated by chylomicrons synthesized from the intestinal epithelial cells in response to a high-fat diet.[38] LPS then binds to toll-like-4 receptors (TLR-4) on macrophages and triggers secretion of pro-inflammatory cytokines (see figure 1). Furthermore, high-fat diet strongly increases intestinal permeability, probably mediated by reducing the expression of ZO-1 and occludin, favoring translocation of LPS through the intestinal wall.[39]
Although these findings support a role of increased epithelial permeability, altered intestinal microbiota and subsequent metabolic endotoxemia as causal factors in diabetes type 2, Secondulfo et al.[12] failed to find an increase in IP in type 2 diabetic patients compared to healthy controls. More human studies are needed in order to identify the exact mechanisms involved in alterations of intestinal barrier function in type 2 diabetes.

Microbiota, pre- and probiotics and intestinal barrier function
Colonization of the intestine with *Bifidobacteria spp.* has been shown to reduce luminal endotoxin formation.[40] Prebiotics, defined as non-digestible food ingredients that can beneficially affect intestinal microbiota, have also been shown to favor colonization with *Bifidobacteria*. These changes were accompanied by an enhanced intestinal barrier function, which correlated with the increased expression of tight junction proteins ZO-1 and occludin.[40] Prebiotic modulation of the intestine appears to increase villus length and crypt depth and leads to thicker mucosal layer in the jejunum and in the colon.[41] This improved tight junction integrity may result in decreased translocation of bacterial endotoxin resulting in decreased production of pro-inflammatory cytokines. Small chain fatty acids, such as butyrate, have a role in enhancing barrier function. Butyrate, which is produced by bacterial fermentation of undigested dietary carbohydrates, acts as an energy substrate for colonocytes and has trophic effects on the mucosa. Butyrate has been shown to prevent diet-induced obesity and insulin resistance, by promoting energy expenditure and inducing mitochondrial function in mice.[42] Furthermore, butyrate has anti-inflammatory properties by inhibiting NF-κB and interferon-γ production.[43] Also, supplementation with prebiotics to obese humans resulted in weight loss and meal-related suppression of the orexigenic hormone ghrelin and induction of the satiety hormone PYY.[44]

Another mechanism contributing to the beneficial effects of prebiotics on barrier function is via secretion of GLP-2. GLP-2 is an enteroendocrine peptide re-

leased from the intestinal L-cells in response to luminal nutrients. GLP-2 is known to induce satiety in animals. This has been confirmed for the human situation.[45] GLP-2 is also involved in modulation of intestinal permeability.[45-48] Cani et al. observed that prebiotics induce secretion of GLP-2 and GLP-1.[38] Higher endogenous GLP-2 release is associated with an improved barrier function leading to decreased LPS concentrations and a blunted inflammatory response. Accordingly, GLP-2 antagonists decrease the expression of tight junction proteins. GLP-2 also increases the rate of crypt proliferation, villus elongation and reduced apoptosis, contributing to an enhanced barrier function.[49] These findings suggest that specific changes in gut microbiota may improve intestinal permeability and inflammatory state via GLP-2 and/or butyrate-dependent mechanisms.

On the other hand, gut microbiota may promote obesity by increasing the capacity of the host to extract energy from ingested food.[50] For instance, indigestible food particles are converted by microbiota to digestible substrates increasing energy extraction from meals. It remains to be established whether small changes in caloric extraction rate truly can lead to significant differences in body weight.[51] One should realize that even the slightest disturbances in energy balance can affect a person's body weight when this process continues for several years.[51] Obesity can occur by altering peripheral metabolism of fat. For instance, in germ-free mice, a 40% lower body fat content compared to control (colonized) mice fed the same chow composition was observed. Remarkably, the daily consumption of control mice was 30% lower than that of germ-free mice. Moreover, lean mice colonized with microbiota from obese mice showed an increase in their body weight. It was postulated that microbiota suppress the "Fasting-Induced Adipose Factor (FIAF)" protein, thereby decreasing fatty acid oxidation in muscle and increasing storage of triglycerides in adipose tissue.[52]

Diabetes affects the gut: increased intestinal permeability as consequence of diabetes?

Patients with diabetes mellitus develop morphological and functional alterations and finally also complications throughout the GI-tract.[53] These alterations include changes in gastrointestinal motility as consequence of diabetic autonomic neuropathy. In the esophagus, abnormal peristalsis with abnormally oriented or double peaked contractions and impaired lower sphincter tone reflect diabetic changes.[54] Gastroparesis is seen in 5% to 12% of diabetic patients, related to neuropathy especially impaired vagal control, chronic hyperglycemia and to damage of interstitial cells of Cajal (ICC). Intestinal enteropathy occurs frequently in diabetic patients and is manifested by complaints as constipation and diarrhea.[53] Several studies have shown that DM causes morphological alterations in the small intestine,[54] including increase in mucosal surface area, intestinal weight and number of goblet cells per villus . Among these changes, the most convincing observations to parallel rodent data are the depletion of ICCs in the intestine of patients with longstanding diabetes.[55] Disturbances in intestinal motor function can lead to stasis with development of bacterial overgrowth thereby possibly disturbing the intestinal barrier and affecting permeability.[56]

Damci et al.[57] reported higher urinary ^{51}Cr-EDTA excretions (increased IP) in diabetic patients with neuropathy compared to diabetic controls. Thus, neuropathy may directly (hyperglycemia) or indirectly (e.g. bacterial overgrowth) alter intestinal permeability.

In patients with diabetes mellitus type 1, IP is frequently increased not only in clinical manifest disease but already in the pre-clinical phase of the disease. As mentioned earlier, this increase in IP is probably associated with upregulation of zonulin and does not correlate with the duration of disease or the HbA1c levels. Furthermore, increased serum zonulin levels were found in 70% of pre-diabetic relatives, who were classified based on the presence of positive autoantibodies in the absence of clinical disease.[11] In support of the human data, biobreed diabetes-prone rats have been found to have increased intestinal permeability associated with decreased expression of the TJ protein claudin-1 before the onset of insulitis and clinical diabetes. In support of this, a recent study examined barrier function in diabetic patients at various stages of disease progression. Intestinal permeability was increased in all diabetic groups; however, pre-diabetic subjects had the most pronounced increase, suggesting that increased intestinal permeability precedes the onset of clinical diabetes.[14,18] These studies support the postulated causative role for IP in the pathogenesis of type 1 diabetes, rather than considering it to be an epiphenomenon. As for type 2 diabetes, studies in rodents have highlighted the key role from metabolic endotoxemia as a result from increased intestinal permeability. However, further human studies are necessary to confirm findings from rodent studies.

Therapeutic implications

Although type 1 and 2 diabetes clearly differ with respect to pathophysiological mechanisms and clinical presentation, they possibly share an important initiating organ in common: the gut. In future preventive or therapeutic interventions in diabetic and pre-diabetic patients, reinforcement of the intestinal barrier may become a major goal to achieve. There are several routes through which an intervention on gut barrier can be established:1) by altering exposure to nutrients (antigens, especially at young age) 2) by alterations in microbiota composition (pre-, pro- and antibiotics) 3) by modification of gut-barrier proteins and other regulatory proteins 4) by restraining the inflammation responsible for insulin-producing beta-cell depletion or insulin resistance.

Dietary manipulation

Recent studies have underlined the pivotal role of a high-caloric and high-fat diet in the alteration of microbiota in type 2 diabetes. Insulin sensitivity is enhanced by losing weight, through a decrease in caloric intake and by an increase in caloric expenditure. A low-fat diet enables redistribution of commensal microbiota and favors colonization of *Bacteroides spp.*, followed by decrease of low grade inflammation and improvement of insulin resistance.

In type 1 diabetes, several food products are considered to be involved in the development of autoimmune reactions, among them gluten and dietary cow insu-

lin. Mice fed with a wheat-containing diet showed a higher incidence of diabetes, signs of small intestinal enteropathy and increased pro-inflammatory cytokines levels.[58] An early study showed a substantially lower diabetes incidence in NOD-mice on a gluten-free diet.[59] Surprisingly, a more recent study pointed out that a gluten-free diet as well as a gluten-enriched diet prevented the development of diabetes in NOD-mice.[60] This could be explained by the fact that gliadin stimulates innate immune responses and that prolonged exposure to a gluten enriched diet can induce tolerance or unresponsiveness to gluten. Also, diet can induce changes in the composition of microbiota and the mucosal immune system which may also result in prevention of diabetes development. Tiittanen et al.[24] concluded that higher concentrations of insulin in breast milk created more tolerance towards bovine insulin, a dietary substance known to induce specific insulin immune response in infants, thus resulting in lower insulin IgG antibodies. On the contrary, high levels of breast milk insulin were associated with beta-cell autoimmunity in a subgroup, suggesting that tolerance is not induced in children prone to beta-cell autoimmunity. Meanwhile, dietary interventions (the use of hydrolyzed casein and delayed exposure to cow milk proteins) have been shown to decrease the development of auto-antibodies associated with the increased risk of diabetes type 1.[61]

Attenuation of the intestinal barrier

As discussed earlier, several proteins and products positively affect intestinal barrier function, among which are GLP-2 agonists, butyrate, anti-inflammatory products, pre-, pro- and antibiotics. In the study by Hadjiyanni et al., administration of GLP-2 agonist (Teduglutide) in non-obese diabetic prone mice (NOD) in the weaning phase strengthened the gut barrier by increasing small bowel weight and length and by reducing intestinal permeability.[62] Unfortunately, no significant reduction in incidence of diabetes was found.
The effects of butyrate on the development of diabetes and survival rate were investigated by Li et al.[63]. Several groups of diabetes-prone rats were followed, but no significant difference was found between the butyrate-treated vs control group.

Valladares et al. administered the dominant commensal *Lactobacillus johnsonii*, derived from diabetes-resistant biobreed rats to diabetes-prone rats in the post-weaning phase. In this study, a non-significant decrease in progression and incidence of type 1 diabetes was observed, combined with an increase in goblet cells in intestinal epithelial mucosa, a decrease in interferon-Ð production and increased claudin expression. Taken collectively, these effects contribute to a fortification of the intestinal barrier.[64]

Brugman et al. administered co-trimoxazol in combination with either hydrolyzed casein or conventional plant-based diet in biobreed rats. The combination of hydrolyzed casein diet and the anti-biotic resulted in protection to develop diabetes type 1. The protective role of antibiotic doxycycline was also found in NOD-mice. These results suggest that microbial composition is of importance in development of diabetes type 1 in genetically susceptible rodents.[30,65] In addition, in obese mice, the administration of norfloxacin and ampicilline ameliorates glucose tolerance and reduces hepatic steatosis. The authors related a

reduction in TNF-α expression and plasma LPS-levels to a treatment-induced decrease in caecal bacteria.[66] Studies have also shown that an increase in *Bifidobacteria* by means of prebiotics clearly seems effective in reducing intestinal LPS levels and improving barrier function.[67,68] Therefore, based on data in animal models, pre- and probiotics may have therapeutic potentials in diabetes mellitus by favoring a beneficial microbial composition and reinforcing the intestinal barrier. However, as not all dietary interventions have been proven to be successful in reducing the incidence of diabetes, further studies will be necessary to elucidate the underlying mechanisms of potential beneficial actions.

Conclusion

The intestinal epithelial cells form a selective barrier and ensure the regulation of the trafficking of macromolecules between the environment and the host. Alteration in this barrier function can have profound effects on the interactions between the mucosal immune system and luminal contents, including dietary antigens and microbial products. Increased permeability can therefore contribute to systemic malfunctioning and disease development. Clinical and experimental evidence suggests that both type 1 and type 2 diabetes are associated with an increased intestinal permeability. Whether intestinal epithelial barrier function is a primary causative factor in the predisposition to disease development and to which extent it contributes to the pathogenesis of diabetes remains further to be elucidated. However, recent animal studies have identified a number of plausible mechanisms that could account for an increased exposure of luminal contents to immunoreactive host cells contributing to insulin deficiency or resistance. This increased exposure to luminal antigens can possibly result in an autoimmune destruction of pancreatic beta-cells as has been proposed in type 1 diabetes, or in an increased peripheral insulin resistance as a consequence of increased cytokine production as has been postulated for type 2 diabetes. Therefore, reinforcing intestinal barrier function may become an important objective to help prevent or counteract pathophysiological mechanisms in diabetic patients. Interventions with butyrate, prebiotics, antibiotics and GLP-2 agonists have been employed with varying success in animal studies. In humans, it is presently unclear whether oral administration of specific dietary supplements is in fact able to modify intestinal barrier function to a clinically significant degree as human studies are largely lacking. Furthermore, it is unknown whether observations of beneficial effects of dietary interventions made in rodents can be extrapolated to humans for clinical intervention purposes. Although scientific knowledge on the role of the intestinal barrier in diabetes has increased exponentially over the past decades, confirming evidence in human studies is still needed. Nevertheless, we believe that there is sufficient evidence to provide a basis for further investigating a possible pathogenetic role of an impaired intestinal barrier function in the development of both type 1 and type 2 diabetes mellitus. A more complete understanding of the molecular pathways involved in the regulation of intestinal barrier will indeed have important clinical implications by potentially opening new horizons in the treatment and prevention of diabetes mellitus and related metabolic disorders.

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Part II

Diabetes mellitus
and colorectal cancer

4

Diabetes mellitus type 2 and subsite-specific colorectal cancer risk in men and women: results from the Netherlands Cohort Study on Diet and Cancer

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Summary

Type 2 diabetes mellitus (T2DM) is associated with an increased risk of colorectal cancer (CRC), however studies differentiating between subsites of CRC are limited. We investigated how DM was associated with subsite-specific CRC risk in men and women. The Netherlands Cohort Study on diet and cancer is a prospective study among 120,852 men and women aged 55-69 years old at baseline in 1986. Information on DM, anthropometric, dietary, and lifestyle factors was self-reported at baseline. T2DM was defined as diagnosis of DM after 30 years of age. Incident CRC cases were identified by record linkage with the Netherlands cancer registry and the Dutch pathology registry. After 17.3 years of follow-up, 1,735 incident male CRC cases and 1,321 female CRC cases were available for analyses. Subsite-specific hazard ratios for CRC were estimated in case-cohort analyses using Cox regression. At baseline, 3.1% of subcohort members reported T2DM of whom 80% were diagnosed after 50 years of age. Multivariable-adjusted models showed that the risk of proximal colon cancer was significantly increased in women with T2DM versus women without T2DM (HR=1.80, 95% CI: 1.10-2.94). There was no association between T2DM and the risk of overall CRC, distal colon cancer, and rectal cancer in women. In men, T2DM was not associated with overall CRC (HR=0.98, 95% CI: 0.64-1.50), nor with risk at any subsite. This prospective study showed an increased proximal colon cancer risk in women with T2DM as compared with non-T2DM women.

Introduction

Colorectal cancer (CRC) is an important health problem around the world. Evidence regarding risk factors for CRC has accumulated considerably in the past decades. Type 2 diabetes mellitus (T2DM), which shares several risk factors with CRC such as an increased BMI, abdominal fat, low levels of physical activity and a Western diet (e.g. high consumption of red and processed meat)[1,2], has been associated with an increased risk of CRC[3].The main hypothesis linking T2DM to CRC relates to an altered regulation of growth factors; i.e. in diabetic patients hyperinsulinemia occurs due to insulin resistance, which may stimulate the growth of colorectal cells both directly and indirectly by increasing the bioavailability of insulin-like growth factor (IGF)[4,5]. This hypothesis has been supported by several nested case-control studies in which high levels of insulin, IGF-1 and C-peptide (a measure for endogenous insulin production) were associated with an increased risk of CRC[6–8]. Despite numerous studies on the association between T2DM and CRC, inconsistencies remain regarding sex-specific and subsite-specific risks and regarding a potential influence of the age at onset and duration of T2DM on risk. The latter is important to take into account because variation in intrinsic insulin production over the course of the disease renders the relationship between T2DM and CRC time-dependent and complex. In the present prospective study with long follow-up, we aimed to elucidate the association between T2DM and the sex- and subsite-specific risk of CRC. We also report on this association after short and long follow-up and we modelled the age at onset of T2DM and duration of T2DM in relation to sex- and subsite-specific CRC risks.

Materials and Methods

The Netherlands Cohort Study on diet and cancer (NLCS) was initiated in 1986[9]. The study protocol was approved by the Medical Ethics Committee of the Maastricht University and TNO Nutrition Zeist, the Netherlands. The design is briefly explained below and a more detailed description of the design of the NLCS is available elsewhere[9]. For reasons of efficiency, a case-cohort approach is used for processing and analyzing data within the NLCS. This approach entails that incident cancer cases are enumerated from the entire cohort, while a random subcohort (n=5000), selected immediately after baseline, is followed for vital status to estimate the accumulated person-time at risk but also to estimate the exposure within the entire cohort. The NLCS includes 58,279 men and 62,573 women who were between 55-69 years old at baseline. Each participant completed a self-administered questionnaire with questions on dietary habits, lifestyle, chronic conditions, and demographic factors at baseline. The questionnaire consisted of 11 pages, of which only the first page was scanned and processed digitally for each cohort participant. The remaining pages had to be key-entered manually, which was done for cancer cases and subcohort members for reasons of efficiency. Processing occurred blinded with respect to case/(sub)cohort status in order to minimize observer bias in coding and interpretation of the data. Incident CRC cases are identified by record linkage to the Netherlands Cancer Registry and the Dutch national pathology registry (PALGA) (>96% completeness).

Follow-up of vital status is performed via linkage to the Central Bureau of Genealogy and the municipal registries (~100% completeness). NLCS participants reporting a history of cancer at baseline other than non-melanoma skin cancer were excluded from follow-up, leaving a total of 4,774 subcohort members. After 17.3 years of follow-up 3,056 CRC cases were available for analyses. Among these, 1,044 were proximal colon cancer cases (International classification of diseases for Oncology first edition codes 153.0, 153.1, 153.4, 153.5, and 153.6), 970 distal colon cancer cases (codes 153.2, 153.3, and 153.7), and 678 rectal cancer cases (code 154.1). The remaining 364 colon cancers could not be classified according to colon localization or were located in the rectosigmoid.

Case-cohort versus full cohort analyses

Primarily, analyses were performed using the case-cohort approach. Complete questionnaire data for cases and subcohort members were available for 17.3 years follow-up (as stated above). In addition, information on chronic conditions (e.g. DM status) and demographics (e.g. age, gender) from the first page of the baseline questionnaire was available for the whole cohort for 20.3 years of follow-up, enabling full cohort analyses for 20.3 years of follow-up. Full cohort data for 20.3 years of follow-up were available at the time of this study due to the digital processing of the first page of the questionnaire. Although the unavailability of the remaining pages meant limited possibilities for adjustment for potential confounders in the full cohort analysis, the advantage in the full cohort analysis was a gain in power, because a higher number of incident CRC cases had occurred after 20.3 years follow-up (n=4,593). Any gain in power is important considering that the prevalence of diabetes is low in the NLCS. In total, after exclusion of prevalent cancer cases other than those with non-melanoma skin cancer, the full cohort includes 4,292 participants who reported T2DM at baseline (3.7%) and 110,211 participants who did not (96.3%). Analyses in which T2DM patients were subdivided into smaller groups, i.e. according to the duration of T2DM or the age at diagnosis, were performed using full cohort data only because of power considerations.

Assessment of DM status and relevant confounders

DM status and age at diagnosis (classified in 5-year age groups) were derived from the first page of the baseline questionnaire based on the question: "Has a physician ever diagnosed you with diabetes mellitus and what was your age at that time?" Participants could indicate age categories ranging from "younger than 30 years of age", and then 5-year age categories, e.g. "30 to 34 years" and "35 to 39 years", up until the category "65 to 69 years". Individuals who reported to have been diagnosed with DM after age 30 years old were classified as T2DM. This cut-off point was based on epidemiological data on DM prevalence and previous literature[10–12]. Individuals with a diagnosis of DM before the age of 30 years (n=11) were regarded as non-T2DM in this study. Duration of DM was calculated by subtracting the median age at diagnosis of DM from the age at baseline.

The covariates used in the analyses were also derived from the baseline questionnaire. These covariates included: age, gender, body mass index (BMI), trouser/skirt size, non-occupational physical activity, family history of CRC (yes or no),

smoking, intake of alcohol and dietary habits (total meat, fruit and vegetables, fish, sweets, added sugar, saturated fats, fiber intake, and energy). BMI (kg/m^2) was calculated using self-reported weight and height. Trouser or skirt size was reported from individuals' clothing label. Trouser/skirt size has been shown to correlate with hip and waist measurements in a subset of weight-stable individuals and was associated with endometrial and renal cell cancer risk in a manner as would be expected for waist circumference, rendering trouser/skirt size as a good proxy for waist circumference[13]. Non-occupational physical activity was a sum measure of several activities: daily walking/cycling (in minutes per day), weekly recreational walking/cycling, weekly engagement in gardening/odd jobs and weekly participation in sports/gymnastics (categories: never, 1, 1–2 and >2 hours/week). Non-occupational physical activity was classified as low (<30 minutes/day), low-intermediate (>30-60 minutes/day), high-intermediate (>60-90 minutes/day) and high (>90minutes/day). Dietary and alcohol intake were assessed using a semi-quantitative food-frequency questionnaire (FFQ), which was part of the baseline questionnaire and which included 150 food items. Nutrient intake was calculated using the Dutch food composition table 1986-1987[14]. The FFQ was found to rank individuals adequately according to dietary intake as compared to a 9-day dietary record[15] and was shown a good indicator of intake for at least five years[16].

Statistical analyses

We performed sex- and subsite-specific analyses using Stata (version 12, Statacorp, College Station, TX, USA). Baseline characteristics were compared between diabetic subcohort members and non-diabetic subcohort members with student's t-test, Mann-Whitney-U, fisher's exact test and chi^2-tests, where appropriate. Age- and multivariable-adjusted hazard ratios (HR) for CRC were calculated using Cox regression. Confounders were selected a priori on the basis of literature and checked for their potential to change hazard ratios by more than 10% using a backward procedure. Although none changed hazard ratios by more than 10%, all were included in multivariable-adjusted models.

For all analyses, the proportional hazards assumption was tested using the scaled Schoenfeld residuals and by visual inspection of the -log-log transformed hazard curves. Additionally, analyses were repeated by splitting up the follow-up time at half point in two equal periods. To account for the additional variance introduced in case-cohort analyses, standard errors were estimated using the robust option[17]. Statistical significance was tested at the 0.05 level using two-sided tests. In the analyses, individuals reporting a diagnosis of DM before 30 years of age were included in the non-exposed group. To assess the impact of the potential presence of type 1 DM patients in the unexposed population, a sensitivity analysis was done excluding individuals reporting diagnosis of DM before the age of 30 years (n=11).

Results

Case-cohort analyses using 17.3 years follow-up

After 17.3 years of follow-up and after exclusion of individuals with incomplete/inconsistent questionnaires and missing values on variables included in the mul-

tivariable-adjusted analysis, a total of 3,919 subcohort members, of which 122 T2DM (3.1%) and 130 CRC (3.3%) subjects, and 3,056 CRC cases, of which 92 T2DM subjects (3.0%), were available for analyses. Baseline characteristics of subcohort members are presented in **table 1**. Compared to men without T2DM, men with T2DM were older ($p<0.01$), had a significantly lower intake of "sweets" and "added sugar" ($p<0.001$ for both) and had a slightly lower but non-significant ($p=0.071$) total energy intake. Women with T2DM were significantly older ($p=0.01$), had a significantly higher BMI ($p<0.01$), and a borderline significantly larger trouser/skirt size ($p=0.052$) compared to women without T2DM. With regard to dietary factors women with T2DM on average had a higher meat intake ($p<0.01$) and a lower intake of "sweets" and "added sugar" ($p<0.001$ for both). CRC cases were older, had a higher BMI, and more often a family history of CRC compared to subcohort members[18] (data not shown).
Overall and subsite-specific associations between T2DM and CRC are presented in table 2 for men and women separately. In multivariable-adjusted models, T2DM was not associated with the risk of overall CRC in men and women (HR=0.98, 95% CI: 0.63-1.49 and HR=1.24, 95% CI: 0.83-1.86, respectively). The subsite-specific analyses showed that T2DM was associated with a statistically significant increased proximal colon cancer risk in women (HR=1.82, 95% CI: 1.11-2.98), but not men (HR=1.37, 95% CI: 0.77-2.42). No association was observed between T2DM and distal colon cancer and rectal cancer risk in both men and women. There were no essential differences in HRs between the age-adjusted and multivariable-adjusted analyses.
Patients with DM diagnosed before the age of 30 years (n=11) were included in analyses as non-type 2 DM patients. In sensitivity analyses, exclusion of these patients did not alter the results (data not shown).

Full cohort analyses using 20.3 years follow-up

For the full cohort analyses, we had available a total of 110,211 cohort members, of which 4,292 T2DM patients (3.8%), and 4,593 CRC cases after exclusion of 5,975 prevalent cancer cases (excluding non-melanoma skin cancers). Age-adjusted analyses showed a significantly increased proximal colon cancer risk in women with T2DM compared to women without T2DM (HR=1.44, 95% CI: 1.05-1.99). The results of the full cohort analyses, in which the duration of T2DM and the age at self-reported diagnosis of T2DM were modelled, are shown in Table 3. Table 3 also shows the results of analyses in the full cohort according to short and long follow-up. The majority of the patients with T2DM had been diagnosed with DM 0-10 years prior to baseline and after the age of 50 years. In men, an increased duration appeared to be associated with an increasing risk of proximal colon cancer (HR (0-10 yr.) = 0.82; HR (10-15 yr.) = 1.60; HR (>15 yr.) =1.83) although the number of cases in subgroups was small and the HRs did not reach statistical significance, nor was there a statistically significant test for trend across categories. In women, a short duration of T2DM (0-10yrs) and a self-reported diagnosis of T2DM after 50 years of age versus long T2DM duration and diagnosis of T2DM before 50 years of age were both associated with a significantly increased risk of proximal colon cancer (HR=1.89, 95% CI: 1.32-2.69 and HR=1.64, 95% CI: 1.17-2.32, respectively). In both men and women, the duration since diagnosis of T2DM and the age at diagnosis of T2DM were not associated

Table 1 Baseline characteristics of non-diabetic individuals and patients with type 2 diabetes mellitus within the subcohort of the Netherlands Cohort Study.

	Men			Women		
Characteristic	No T2DM n=1,835; 97.1%	T2DM n=55; 2.9%	p-value	No T2DM n=1,962; 96.7%	T2DM n=67; 3.3%	p-value
Age at baseline (yr.)*	61.2 (4.2)	62.9 (4.4)	0.003	61.3 (4.3)	62.7 (4.2)	0.001
BMI (kg/m2)*	24.9 (2.5)	25.1 (2.8)	0.631	25.0 (3.5)	26.4 (3.9)	0.005
Pants/Skirt size (% ≥ large size)	63	66	0.668	55	67	0.052
Age at diagnosis of T2DM (yr.)*	n/a	53.7 (7.7)		n/a	56.3 (7.5)	
Exposure time T2DM (yr.)*	n/a	9.0 (7.5)		n/a	6.8 (6.4)	
Family history of CRC (% yes)	5.5	3.0	0.363	5.9	4.5	0.795
Smoking Status (%)			0.701			0.133
Never	13	11		57	69	
Ex-smoker	52	58		21	18	
Current smoker	35	31		22	13	
Alcohol intake (%)			0.151			0.085
0g/day	14	18		32	42	
0.1-30g/day	72	60		64	58	
>30g/day	14	22		3.7	0.0	
Dietary Habits						
Total meat (g/day)*†	123 (48)	123 (41)	0.893	104 (43)	119 (49)	0.004
Fruit and vegetables (g/day)*	358 (159)	377 (152)	0.370	401 (161)	407 (168)	0.760
Fish (g/day)*	14 (17)	11 (12)	0.122	11 (14)	15 (19)	0.513
Sweets (g/day)*‡	41 (26)	30 (30)	<0.001	42 (26)	27 (22)	<0.001
Added sugar (g/day)*	31 (33)	6 (18)	<0.001	12 (20)	2 (9)	<0.001
Saturated fats (g/day)*	37 (12)	35 (13)	0.131	30 (10)	29 (9)	0.263
Fibre intake (g/day)*	29 (9)	30 (8)	0.513	25 (7)	24 (7)	0.625
Energy (kcal/day)*	2,176 (509)	2,050 (475)	0.071	1,690 (397)	1,629 (343)	0.215
Physical activity (%)			0.196			0.080
≤30 min/day	17	26		23	30	
>30 - ≤60 min/day	31	20		31	36	
>60 - ≤90 min/day	20	18		24	10	
>90 min/day	32	36		22	24	

BMI: Body Mass Index; T2DM: type 2 diabetes mellitus; CRC: colorectal cancer
* values presented as: mean (standard deviation from the mean); † consisting of the sum of pork, beef, poultry, game, and processed meat; ‡ consisting of the sum of sweet sandwich filling, cookies, cake, and candy.

with the risk of distal colon cancer and rectal cancer. Analyses according to follow-up time showed generally no essential differences in estimates between short (0-10yr) and long (10-20.3yr) follow-up. Associations in relation to proximal colon cancer in men seemed stronger after longer follow-up than shorter follow-up time, but caution is warranted in interpreting results because the number of cancer cases was small in these analyses.

Table 2 Case-cohort and full cohort analysis on the association between type 2 diabetes mellitus and colorectal cancer in the Netherlands Cohort Study.

		Case-cohort analyses (0-17.3 yr. follow-up)						Full-cohort analyses (0-20.3 yr. follow-up)‡			
Colorectal cancer (n = 3,056)								Colorectal Cancer (n = 4,593)			
	T2DM†	PY at risk	N	age-adjusted HR	95% CI	MV-adjusted HR*	95% CI	PY at risk	N	age-adjusted HR	95% CI
Men	no	26,131	1,690	1		1		844,054	2,492	1	
	yes	670	45	0.98	0.64-1.50	0.98	0.63-1.49	26,416	72	0.95	0.75-1.20
Women	no	30,593	1,274	1		1		1,002,024	1,959	1	
	yes	943	47	1.16	0.78-1.71	1.24	0.83-1.86	33,165	70	1.08	0.85-1.37
Proximal colon cancer (n = 1,044)								Proximal colon cancer (n = 1,614)			
		PY at risk	N	age-adjusted HR	95%	MV-adjusted HR*	95%	PY at risk	N	age-adjusted HR	95%
Men	no	26,131	477	1		1		844,054	730	1	
	yes	670	18	1.34	0.76-2.36	1.37	0.77-2.42	26,416	25	1.13	0.76-1.68
Women	no	30,593	523	1		1		1,002,024	820	1	
	yes	943	26	1.54	0.96-2.48	1.82	1.11-2.98	33,165	39	1.44	1.05-1.99
Distal colon cancer (n = 970)								Distal colon cancer (n = 1,421)			
		PY at risk	N	age-adjusted HR	95%	MV-adjusted HR*	95%	PY at risk	N	age-adjusted HR	95%
Men	no	26,131	562	1		1		844,054	818	1	
	yes	670	12	0.78	0.41-1.50	0.78	0.40-1.51	26,416	19	0.77	0.49-1.21
Women	no	30,593	386	1		1		1,002,024	570	1	
	yes	943	10	0.83	0.42-1.63	0.81	0.41-1.61	33,165	14	0.75	0.44-1.27
Rectal cancer (n = 678)								Rectal cancer (n = 1,013)			
		PY at risk	N	age-adjusted HR	95%	MV-adjusted HR*	95%	PY at risk	N	age-adjusted HR	95%
Men	no	26,131	432	1		1		844,054	618	1	
	yes	670	5	0.44	0.17-1.12	0.41	0.16-1.05	26,416	14	0.50	0.21-1.22
Women	no	30,593	232	1		1		1,002,024	368	1	
	yes	943	9	1.23	0.60-2.52	1.18	0.56-2.48	33,165	13	1.16	0.54-2.48

PY: person years; T2DM: type 2 diabetes mellitus: HR: hazard ratio; MV: multi-variable
* hazard ratios adjusted for age, BMI, pants/skirt size, family history of colorectal cancer, smoking status, alcohol intake, dietary habits and non-occupational physical activity, using continuous and categorical variables as defined in table 1; † T2DM (age of onset ≥30years) determined by self-report at baseline; ‡ due to limited number of patients with T2DM at baseline in case-cohort analyses additional full-cohort analyses were performed with restriction to confounder adjustment.

Table 3 Full-cohort analyses on associations between duration of type 2 diabetes mellitus, age at diagnosis of type 2 DM, follow-up time and colorectal cancer risk in the Netherland Cohort Study.

			Colorectal (n=4,593)			Proximal (n=1,614)			Distal (n=1,421)			Rectal (n=1,013)		
Duration of T2DM‡		PY at risk	N	HR*	95%	N	HR*	95%	N	HR*	95%	N	HR*	95%
Men	no T2DM	844,054	2,492	1		730	1		818	1		618	1	
	yes, 0-10 yr.	16,437	44	0.95	0.71-1.28	11	0.82	0.45-1.49	15	0.99	0.60-1.66	10	0.86	0.46-1.61
	yes, 10-15 yr.	4,385	11	0.86	0.48-1.56	6	1.60	0.72-3.57	2	0.48	0.12-1.92	1	0.32	0.04-2.24
	yes, >15 yr.	4,858	17	1.16	0.72-1.87	8	1.83	0.91-3.67	2	0.42	0.10-1.68	3	0.84	0.27-2.60
p-trend					0.923			0.117			0.145			0.313
Women	no T2DM	1,002,024	1,959	1		820	1		570	1		368	1	
	yes, 0-10 yr.	20,959	53	1.30	0.99-1.71	32	1.89	1.32-2.69	7	0.60	0.41-1.33	10	1.31	0.70-2.46
	yes, 10-15 yr.	5,005	6	0.61	0.27-1.36	3	0.73	0.24-2.28	2	0.71	0.14-2.22	1	0.55	0.08-3.88
	yes, >15 yr.	6,383	10	0.79	0.43-1.47	4	0.76	0.28-2.02	5	1.38	0.45-2.61	1	0.42	0.06-3.03
p-trend					0.794			0.353			0.773			0.573
Age at diagnosis of DM‡														
Men	no T2DM	844,054	2,492	1		730	1		818	1		618	1	
	yes, diagnosed <50yr	6,978	19	1,00	0.63-1.57	8	1.47	0.73-2.96	3	0.48	0.15-1.49	3	0.62	0.20-1.92
	yes, diagnosed ≥50yr	19,438	53	0.94	0.71-1.23	17	1.02	0.63-1.65	16	0.87	0.53-1.42	11	0.78	0.43-1.42
Women	no T2DM	1,002,024	1,959	1		820	1		570	1		368	1	
	yes, diagnosed <50yr	8,628	12	0.77	0.44-1.36	5	0.79	0.33-1.90	4	0.86	0.32-2.30	3	1.01	0.32-3.14
	yes, diagnosed ≥50yr	24,536	58	1.18	0.91-1.53	34	1.64	1.17-2.32	10	0.71	0.38-1.33	10	1.10	0.58-2.06
FU time, split at half time														
Men	0-10 yr. no T2DM	493,177	1,041	1		284	1		456	1		277	1	
	T2DM	17,110	34	0.91	0.65-1.28	9	0.87	0.45-1.70	19	1.17	0.74-1.86	5	0.50	0.21-1.22
	10-20.3 yr. no T2DM	350,878	1,451	1		446	1		634	1		341	1	
	T2DM	9,306	38	0.98	0.71-1.36	16	1.35	0.82-2.22	12	0.71	0.40-1.26	9	0.99	0.51-1.92
Women	0-10 yr. no T2DM	541,028	794	1		295	1		325	1		151	1	
	T2DM	20,142	35	1.09	0.78-1.53	19	1.55	0.97-2.47	9	0.71	0.36-1.37	7	1.16	0.54-2.48
	10-20.3 yr. no T2DM	460,996	1,165	1		525	1		400	1		217	1	
	T2DM	13,023	35	1.06	0.75-1.48	20	1.33	0.85-2.09	9	0.79	0.41-1.54	6	0.98	0.43-2.20

PY: person years; T2DM: type 2 diabetes mellitus: HR: hazard ratio; FU: Follow-up
* hazard ratios adjusted for age; † T2DM (age of onset ≥30years), T2DM status, T2DM duration and age of diagnosis determined by self-report at baseline; ‡ some counts do not add up to totals because they could not be classified on "age of diagnosis of diabetes mellitus"

Discussion

In this large population-based cohort study, we found that T2DM was associated with an 82% increased risk of proximal colon cancer but not distal colon and rectal cancer in women. An increased proximal colon cancer risk was particularly observed in women with a T2DM diagnosis after 50 years of age and in women with a short duration of T2DM (0-10 years). In men, T2DM was not significantly associated with the risk of overall CRC or CRC at any subsite.

Our results appear consistent with other large cohort studies that investigated the subsite-specific CRC risk in diabetic women. The Nurses' Health Study[19], found T2DM to be associated with an increased proximal colon cancer risk in women (HR= 1.64, 95% CI 1.04-2.60), and found no association with distal colon cancer (HR= 1.38, 95% CI 0.88-2.15) and rectal cancer risk (HR= 1.11, 95% CI 0.56-2.21) in a model adjusted for age, BMI and levels of physical activity. Participants of the Nurses' Health Study were younger compared to participants of the NCLS with a mean age of 42-45 years at baseline. DM status and other variables were reassessed biennially and the follow-up time was 18 years. In the Iowa Women's Health Study, women with T2DM as compared to non-diabetic women were also at an increased risk of proximal colon cancer (HR= 1.9, 95% CI: 1.3-2.6) but not distal colon cancer (HR= 1.1, 95% CI 0.6-1.8) and rectal cancer (HR= 0.8, 95% CI 0.4-1.6). In a cohort study conducted by Weiderpass et al.[20], standardized incidence ratios (SIRs) were calculated according to CRC subsite. In both men and women, T2DM appeared more strongly associated with proximal colon cancer than with distal colon and rectal cancer. Contrary to these results, in a cohort study by Flood et al.[21], a borderline significant positive association was observed of T2DM with cancer in the distal colon and rectum (RR 1.65 [95% CI 0.98-2.78]) and a non-significant association with proximal colon cancer (RR 1.46 [95% CI 0.85-2.49]) in women in a multivariable-adjusted model similar to our study. Despite the effort put into obtaining CRC cases and characteristics in the latter study, about 25% of CRC cases could not be classified by location, which could partly explain the differences in subsite distribution of CRC cases and partly explain the non-significant RRs due to lower power in subsite specific analyses compared to our study. In a cohort of Swedish men[12], who were between 45-79 years old at baseline, a multivariable-adjusted increased risk was observed for overall CRC (RR 1.49 [95% CI 1.14-1.96]), particularly rectal cancer (RR 1.79 [95% CI 1.18-2.73]), when comparing diabetic men to non-diabetic men; the risk of proximal and distal colon cancer appeared increased, although this was not statistically significant. It should be noted that there was a markedly higher percentage of rectal cancer cases in this Swedish cohort (43%) as compared to ours (20%) and others (27%)[22], which could partly explain the difference in results found. Other more recent studies found a modestly increased risk of CRC in diabetic individuals compared with non-diabetic individuals. Although sex- and subsite-specific risk estimates were not always presented[22-25], the authors reported that results were similar for proximal and distal cancers. Finally, in a recent meta-analysis 24 cohort studies were combined. The authors found that the risk of CRC was increased in diabetic individuals and that proximal colon cancer risk appeared higher than distal colon cancer risk, though not statistically significantly different[26] .

There are several potential explanations for the increased proximal colon cancer risk that was found in diabetic women as compared with non-diabetic women, especially those diagnosed with DM after 50 years of age, in this study. Most of these explanations apply to men as well, in which diabetes was observed to be borderline significantly associated with an increased proximal colon cancer risk. First of all, in postmenopausal women, low levels of estrogen may result in loss of the protective effect of estrogen on insulin resistance and CRC[27,28]. Secondly, high age of onset strongly coincides with a short duration of DM exposure in our cohort and could reflect the period closest to exposure to high levels of serum-insulin in which colorectal neoplasms develop. Thirdly, individuals diagnosed with DM at older age are possibly less motivated to adjust their lifestyle, resulting in an increased CRC risk compared with early diagnosed diabetic patients who may be more likely to adjust their lifestyle[29]. Fourthly, as age of onset increases, type 1 DM and latent auto-immune diabetes in adults (LADA) are less likely. Thus, selection of T2DM patients based on higher age minimizes potential misclassification of T2DM resulting in stronger associations with outcome.

The literature remains inconsistent concerning the potentially differing effects of CRC risk factors (e.g. T2DM, physical activity) on specific subsites of the colorectal tract. The biological mechanisms that explain the differences in subsite-specific associations are yet to be elucidated. Studies have indicated different responses of proximal colon cells and more distally localized cells to growth stimulating hormones such as gastrin, leptin and IGF[30,31]. For example, a pilot study in rodents showed that a 50-75% reduction in circulating IGF-1 levels resulted in a reduction in azoxymethane induced colon tumors in the proximal colon but not in the distal colon[32]. A higher sensitivity to IGF1 of proximal colon cells as compared to cells in the distal colon could partly explain the findings in this, and possibly our study.

The interpretation of results regarding the associations between the onset of T2DM and duration of exposure to T2DM on one hand and CRC risk on the other hand is complex when considering a dynamic underlying pathway that involves varying degrees of insulin resistance and hyperinsulinemia, and associated alterations in insulin-like growth factor levels. First of all, intrinsic insulin levels vary from increased levels in the pre-diabetic stage to decreased levels in well-established T2DM as pancreatic beta cells become more dysfunctional[33]. Secondly, from the time of DM diagnosis, various other factors such as medication[34–36] and changes in lifestyle (dietary changes, weight loss, increased physical activity) may influence the degree of insulin resistance, and consequently serum levels of insulin. Taking into consideration this dynamic nature of the degree of insulin resistance, a history of T2DM may be viewed as a proxy for a temporary exposure to increased insulin and IGF-levels and thus colonic cell growth promotion, which could increase the risk of CRC.

The association between T2DM and proximal colon cancer found in our cohort was present in women who were between 55-69 years old at baseline, which was similar to the age range of other cohort studies in which the same association was observed as mentioned above[11,19]. This age range largely overlaps that of the screening population in the Netherlands (55-75 years) and other international cancer screening network (INCS) countries. Since interval colon cancers, liberally defined as colon cancers that occur between two colonoscopies, are frequently

located proximally[37] and women with T2DM are less likely to take part in screening programs[38], future studies could perhaps investigate whether or not more tailored screening strategies (e.g. shortening the screening interval) are of use in women with T2DM.

Factors that contribute to the strength of this study are its prospective design, the large study population including men and women, the long period of follow-up with a high number and nearly complete follow-up of histologically confirmed colorectal cancers as well as the extensive baseline questionnaire. There are some limitations to our study. Classification of T2DM was based on self-reports, which could have resulted in some misclassification as there may have been undiagnosed diabetic patients in our cohort. However, previous studies within large cohorts have shown that the self-report of treated diabetes is an accurate proxy to use in epidemiologic studies[22,28,39]. Any misclassification is also likely to have been independent of disease, and will have most likely resulted in an attenuation of hazard ratios. Another limitation may have been that there were no repeated measurements with respect to DM and covariates during the follow-up period in the NLCS. Especially with longer follow-up time, the probability of misclassification of DM (unidentified T2DM patient in the non-T2DM population), using one baseline measurement, increases. Considering this misclassification, together with a relatively low proportion of patients with T2DM at baseline, especially in men, associations are likely to be underestimated in this study.
In conclusion, after 17.3 years of follow-up, we found a moderate increase in proximal colon cancer risk in women but not men with T2DM compared to those without T2DM. Future studies are needed to determine whether a more tailored screening strategy could be of use in this subgroup of patients.

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5

Diabetes mellitus, genetic variants in the insulin-like growth factor pathway and colorectal cancer risk

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Summary

Background
Genetic variation in the insulin-like growth factor (IGF) pathway may further increase the risk of colorectal cancer (CRC) associated with type 2 diabetes mellitus (T2DM). Joint effects of T2DM and genetic variation in the IGF pathway on CRC risk can increase mechanistic insights.

Methods
Participants from the Netherlands Cohort Study (n=120,852) completed a baseline questionnaire in 1986 when 55-69 years old (case-cohort, n subcohort=5,000, n cases = 3,441 after 16.3 years follow-up). Self-reported DM at baseline with onset at ≥ 30 years was classified as T2DM. Eighteen single nucleotide polymorphisms (SNPs) from the IGF pathway were aggregated in a genetic risk score (GRS). Cox proportional hazard ratios (HRs) for CRC were estimated according to combinations of T2DM status with GRS tertiles and categories of an *IGF1* 19-CA repeat polymorphism.

Results
Baseline T2DM prevalence was 3.1% in subcohort members and 3.8% in CRC cases. Comparison of combined categories with non-T2DM individuals in the lowest GRS tertile as reference showed that those in the highest GRS tertiles with and without T2DM had significantly increased CRC risks, particularly those with T2DM (HR=2.28, 95% CI: 1.11, 4.66). As compared to *IGF1* 19-CA wild type carriers without T2DM, carrying two *IGF1* 19-CA variant repeat alleles was associated with a significantly decreased CRC risk in those without T2DM (HR=0.76, 95% CI: 0.63-0.91). This association was absent when T2DM was present.

Conclusion
This study of joint effects indicated that the presence of unfavorable alleles in the IGF pathway may further increase the risk of CRC associated with T2DM.

Introduction

Colorectal cancer (CRC) and type 2 diabetes mellitus (T2DM) are multifactorial diseases that have several risk factors in common (e.g. high BMI, physical inactivity) and both have shown an increase in incident rates over the past decades[1,2]. T2DM has been associated with an increased CRC risk (20-40%)[3]. An interesting hypothesis underlying this association involves the insulin-like growth factor (IGF) pathway. In this hypothesis, chronic hyperinsulinemia in T2DM patients leads to increased IGF levels, which can accelerate the progression from adenoma to cancer[4]. Previous research within the Netherlands Cohort Study (NLCS) studied single nucleotide polymorphisms (SNPs) in the IGF pathway in relation to CRC risk and found that carrying more risk alleles was associated with an increased CRC risk in men, specifically an increased proximal and distal colon cancer risk but not rectal cancer risk[5]. T2DM was associated with an increased proximal colon cancer risk in women in previous published data within the NLCS[6] and this confirmed findings from other cohort studies[7]. Here, we aimed to investigate whether carrying unfavorable alleles in the IGF pathway further increased the CRC risk associated with diabetes. Genetic variants were used as markers of pathway involvement. Finding a further increased CRC risk in diabetics with unfavorable alleles in the IGF pathway would substantiate the hypothesis that diabetes or chronic hyperinsulinaemia increases CRC risk by influencing the IGF pathway.

Materials and methods

Study population and design

The NLCS includes 120,852 men and women from the Dutch population, who were 55-69 years old at baseline in 1986, when completing a self-administered questionnaire on diet and cancer[8]. Roughly 90,000 participants also provided toenail clippings when returning the baseline questionnaire. Toenail DNA is a valid DNA source for genotyping[9]. For reasons of efficiency, DNA isolation, the processing of questionnaires, and follow-up, the NLCS is characterized by a case-cohort approach. This approach entails that a random subcohort of 5,000 individuals, selected at random immediately after baseline, is followed-up for vital status to estimate the accumulated person-time at risk by record linkage to the municipal registries (>99% complete). The whole cohort is followed-up for incident cancer by record linkage to the Dutch cancer registry and the national pathology database (PALGA)[10] (>96% complete)[11]. Baseline exclusion of participants with a history of cancer (except skin cancer) left 4,774 subcohort members. After 16.3 years, there were 3,441 incident CRC cases (ICD-O 153-154). Toenail clippings were available for 3,768 of 4,774 subcohort members (78.9%) and 2,580 of 3,441 CRC cases (75.0%). Finally, after exclusion of missing inconsistent / incomplete questionnaires and missing data on diabetes and covariates, 1907 CRC cases, 565 proximal colon cancer cases (ICD-O 153.0, 153.1, 153.4, 153.5 and 153.6), 533 distal colon cancer cases (codes 153.2, 153.3 and 153.7), and 432 rectal cancer cases (code 154.1) were available for analyses with respect to the genetic risk score.

Assessment of T2DM status

T2DM status was derived from the baseline questionnaire based on the question: "Has a physician ever diagnosed you with diabetes mellitus and what was your age at that time?" Individuals who reported to have been diagnosed with DM at age 30 years or older were classified as T2DM. This cut-off point was based on epidemiological data on DM prevalence and previous literature[12]. Individuals with a diagnosis of DM before the age of 30 years were regarded as non-T2DM in this study.

Variant selection, genotyping, the genetic risk score (GRS) and the IGF1 19-CA repeat

We selected SNPs in genes encoding for factors in or regulatory to the IGF pathway and genes encoding for adiponectin, adiponectin receptors and peroxisome proliferator-activated receptor gamma, as these hormones regulate insulin resistance. Briefly, SNPs were selected through literature and had to be associated with CRC or other obesity-related cancers (e.g. breast cancer), T2DM risk, or related traits (e.g. body size or markers of insulin resistance). A full description of our gene and SNP selection strategy, including a list of the selected SNP variants and references to the literature on which selection is based, is available in the paper and Supplemental Material of Simons *et al.*[5] A total of 24 SNPs were successfully genotyped on the SEQUENOM® MassARRAY® system (Hamburg, Germany) (now known as Agena Bioscience, Hamburg, Germany) and an *IGF1* 19-CA repeat polymorphism was genotyped by PCR amplification and subsequent analysis of the PCR products' length using the 96-capillary ABI 3730xl DNA Analyzer. SNP genotypes were reproducible in 98.8% of 314 random duplicate samples. SNP call rates were 92.6% or higher, with one exception of 83.6% (rs4773082). Twenty-three out of 24 SNPs adhered to Hardy-Weinberg equilibrium (HWE).

We did not exclude the SNP that deviated from HWE (rs1342387) because one in 20 tests may be expected to be significant by chance alone. We only used samples with a sample call rate of >95% in statistical analyses, which led to the exclusion of 532 samples. We aggregated 18 SNPs into a genetic risk score (GRS), as for these SNPs the risk allele in relation to the endpoints mentioned above was unequivocal on the basis of previous studies. Which allele (minor or major allele) was the risk allele can be found in the Supplemental Material of Simons *et al.*[5] Per SNP, individuals carry 2 (homozygotes for the risk allele), 1 (heterozygotes), or 0 (homozygotes for the other allele) risk alleles. The number of risk alleles carried by an individual was summed for the 18 SNPs. The theoretical range of a GRS based on 18 SNPs is between 0-36 risk alleles. The GRS was categorized into tertiles based on the distribution in the subcohort. The *IGF1* 19-CA repeat polymorphism had a call rate of 70.7% and was reproducible in 93.6% of 314 duplicate samples. This variant was analyzed separately from the GRS, as the direction of the association with CRC remains uncertain and because it is a conceptually different variant (not a single base pair change, but a variation in the number of CA repeats). According to Rosen *et al.*[13], we distinguished between individuals homozygous for the wild-type allele (192/192 CA repeats), heterozygous individuals (192/non-192 CA repeats), and individuals carrying two variant alleles (non-192/non-192 CA repeats).

Statistical analysis

Hazard ratios (HRs) and 95% confidence intervals (CIs) for CRC overall, by sex (only CRC), and by subsite were estimated using Cox proportional hazards regression analysis for combined categories of T2DM status with tertiles of the GRS and categories of the *IGF1* 19-CA repeat polymorphism. To adjust for the additional variance introduced from sampling the subcohort from the total cohort, standard errors were estimated using the robust Huber–White sandwich estimator[14]. Exclusion of participants with inconsistent/incomplete baseline questionnaires left 2,729 subcohort members and 1,821 CRC cases which could be categorized in one of the tertiles of the GRS, and 2,042 subcohort members and 1,657 CRC cases with *IGF1* 19-CA repeat status available for analyses. Models were adjusted for potential confounders [age (years), body mass index (BMI, kg/m2), pant/skirt size as a proxy for abdominal circumference[15] (below, or equal to or above median size), first-degree family history of CRC (yes/no), smoking status (never, ex, current), alcohol intake (0, 0.1–29, ≥30 g/d), intake in g/d of total meat, vegetables, fish, sweets, added sugar, saturated fats, and fibre, total energy intake (kcal/day), and non-occupational physical activity (‹30, ›30, ›60, ›90 min/day)]. The relative excess risk due to interaction (RERI) was calculated according to Rothman *et al.*[16], though we conservatively refrained from doing so in analyses including the *IGF1* 19-CA repeat polymorphism, as the combined category at lowest risk was not clear. Corresponding 95% bias-corrected confidence intervals for the RERI were estimated by bootstrapping (n bootstrap samples = 1000)[17]. Multiplicative interactions were assessed using the Wald test. All analyses were conducted using Stata version 12 (Stata Corp., College Station, TX). Statistical significance was indicated by a P-value ‹0.05 for two-sided testing.

Results

Table 1 shows the distribution of subcohort members with and without T2DM across categories of age, sex, the GRS, the **IGF1** 19-CA repeat polymorphism, and potential confounders. Compared to subcohort members without T2DM, subcohort members with T2DM were older (p‹0.001) and had a significantly lower intake of sweets and added sugar (p‹0.001 for both).

The genetic risk score and T2DM

CRC risks were increased across combined categories of T2DM and GRS using non-T2DM individuals in the lowest tertile of the GRS as reference (Table 2). Individuals with T2DM in the lowest GRS tertile did not differ in CRC risk (HR=1.00, 95% CI: 0.54-1.83) from those without T2DM in the lowest tertile. Non-T2DM individuals in the middle and highest GRS tertiles were at a statistically significant increased CRC risk as compared to non-T2DM individuals in the lowest GRS tertile (HRnon-T2DM in middle GRS tertile vs. non-T2DM in lowest GRS tertile=1.23, 95% CI: 1.06-1.42 and HRnon-T2DM in highest GRS tertile vs. non-T2DM in lowest GRS tertile=1.27, 95% CI: 1.09-1.49), as were T2DM individuals in the middle and highest GRS tertiles HRT2DM in middle GRS tertile vs. non-T2DM in lowest GRS tertile=1.82, 95% CI: 1.09-3.05, and HRT2DM in highest GRS tertile vs. non-T2DM in

lowest GRS tertile=2.28, 95% CI: 1.11-4.66). Sex-specific analyses showed similar results as the overall analysis, although most HRs were not statistically significant. Subsite-specific analyses showed that the results seen for CRC overall were most pronounced for proximal colon cancer (HRT2DM in highest GRS tertile vs. non-T2DM in lowest GRS tertile=3.92, 95% CI: 1.75-8.79), but less clear in relation to distal colon cancer and rectal cancer. Power was insufficient to investigate subsite-specific CRC risks in men and women separately. Stratified analyses yielded results that were not statistically significant, except when considering proximal colon cancer as endpoint and comparing T2DM with non-T2DM individuals within the highest GRS tertile (HR=3.93, 95% CI: 1.63 – 9.48). Tests for multiplicative and additive interactions (RERI) were not statistically significant.

The IGF1 19-CA repeat polymorphism and T2DM

Table 3 shows the CRC risks associated with combined categories of T2DM and the *IGF1* 19-CA repeat polymorphism. Using non-T2DM individuals homozygous for the wild type 19-CA repeat allele as reference showed that non-T2DM individuals carrying one or two variant *IGF1* 19-CA repeat alleles had (a borderline) statistically significantly decreased CRC risk (HR=0.88, 95% CI: 0.75 - 1.04 and HR=0.76, 95% CI: 0.63 - 0.91, respectively), whereas CRC risk was not significantly changed in individuals with T2DM homozygous for the wild type **IGF1** 19-CA repeat allele or those carrying one or two variant alleles (HR=1.44, 95% CI: 0.76-2.74; HR=0.73, 95% CI: 0.37-1.44; and HR=1.26, 95% CI: 0.53-2.96, respectively). Sex-specific analyses showed similar results in women and nonsignificant results in men. Subsite-specific analyses showed similar results as overall analyses. Stratified analyses showed no statistically significant associations between T2DM and CRC risk overall or by subsite, except when considering proximal colon cancer as endpoint and comparing T2DM with non-T2DM individuals within those carrying two variant IGF1 19-CA repeat alleles (HR= 3.09, 95% CI: 1.03 - 9.24). Multiplicative and additive interactions were not statistically significant.

Table 1 Baseline characteristics of subcohort members and CRC cases with and without type 2 diabetes mellitus in the Netherlands Cohort Study.

	Subcohort members			CRC Cases		
Characteristic	No T2DM (n=2,648; 97%)	T2DM (n=81; 3.1%)	p-value	No T2DM (n=1,754; 96%)	T2DM (n=67; 3.8%)	p-value
Age at baseline, mean in years (SD)	61.3 (4.2)	63.5 (4.3)	<0.001	62.0 (4.1)	63.0 (3.8)	0.03
Age at CRC diagnosis, mean in years (SD)				71.2 (5.6)	71.3 (5.6)	0.81
Gender (% male)	1,318 (50)	41 (51)	0.88	1,047 (60)	32 (48)	0.05
Genetic sum score, N (%)						
Tertile 1	988 (37)	34 (42)		557 (32)	18 (27)	
Tertile 2	991 (38)	34 (42)		697 (40)	33 (49)	
Tertile 3	669 (25)	13 (16)	0.17	500 (28)	16 (24)	0.30
IGF1 CA repeat*, N (%)						
Non 19/non 19	509 (19)	11 (14)		341 (19)	11 (17)	
19/non-19	684 (26)	24 (30)		566 (32)	14 (21)	
19/19	600 (23)	18 (22)	0.60	522 (30)	25 (37)	0.12
BMI, mean kg/m^2 ± SD	25.0 (3.1)	25.6 (3.6)	0.14	25.0 (2.9)	25.6 (3.2)	0.09
Pants/skirt size, N (%) equal or above median	1,568 (59)	53 (65)	0.26	1,086 (62)	50 (75)	0.04
Family history of CRC, N (%) yes	160 (6)	3 (4)	0.63	176 (10)	4 (6)	0.27
Smoking Status, N (%)						
Never	917 (35)	35 (43)		525 (30)	29 (43)	
Ex-smoker	1,002 (38)	30 (37)		769 (44)	28 (42)	
Current smoker	729 (27)	16 (20)	0.18	460 (26)	10 (15)	0.03
Alcohol intake, N (%)						
0g/day	610 (23)	23 (29)		342 (20)	25 (37)	
0.1-30g/day	1,801 (68)	48 (59)		1,181 (67)	37 (55)	
>30g/day	237 (9)	10 (12)	0.24	231 (13)	5 (8)	0.001
Dietary Habits						
Total meat, mean in g/day (SD)†	113 (46)	120 (46)	0.40	115 (47)	122 (41)	0.10
Vegetables, mean in g/day (SD)	381 (159)	384 (148)	0.86	376 (164)	412 (153)	0.03
Fish, mean in g/day (SD)	13 (15)	13 (17)	0.81	12 (15)	13 (13)	0.48
Sweets, mean in g/day (SD)††	42 (26)	31 (27)	<0.001	42 (26)	31 (25)	<0.001
Added sugar, mean in g/day (SD)	20 (27)	5 (17)	<0.001	21 (28)	1 (4)	<0.001
Saturated fats, mean in g/day (SD)	33 (11)	31 (11)	0.13	34 (11)	36 (16)	0.17
Fibre intake, mean in g/day (SD)	27 (8)	27 (8)	0.51	27 (8)	29 (9)	0.10
Energy, mean in kcal/day (SD)	1,922 (505)	1,823 (456)	0.06	1,976 (495)	1,928 (588)	0.51
Physical activity, N (%)						
≤30 min/day	512 (19)	21 (26)		340 (19)	19 (20)	
>30 - ≤60 min/day	860 (33)	26 (32)		533 (31)	16 (24)	
>60 - ≤90 min/day	566 (21)	12 (15)		386 (22)	18 (27)	
>90 min/day	710 (27)	22 (27)	0.34	495 (28)	14 (21)	0.14

BMI, Body mass index; T2DM, type 2 diabetes mellitus; CRC, colorectal cancer; SD standard deviation
* numbers of IGF1 19-CA repeat in baseline and analyses do not match due to exclusion of missing on the genetic sum score variable; † consists of the sum of pork, beef, poultry, game, and processed meat intakes; †† consists of the sum of intakes of sweet sandwich filling, cookies, cake, and candy

Table 2 Multivariable-adjusted hazard ratios for colorectal cancer overall and by sex and subsite for combined categories of type 2 diabetes mellitus and tertiles of genetic risk score based on SNPs in genes from the IGF pathway and for type 2 diabetics versus non-diabetics within tertiles of this genetic risk score in the Netherlands Cohort Study after 16.3 years of follow-up.

		Type 2 Diabetes Mellitus										Type 2 Diabetes Mellitus		
		No				Yes						Yes versus No		p-value for multiplicative interaction
		PY	N cases	HR†	(95% CI)	PY	N cases	HR†	(95% CI)	RERI†	(95% CI)	HR†	(95% CI)	
Colorectum	T1	14,242	587	1	(reference)	415	19	1.00	(0.54 - 1.83)			1.10	(0.59 - 2.07)	
Genetic risk score*	T2	14,176	732	1.23	(1.06 - 1.42)	446	34	1.82	(1.09 - 3.05)			1.44	(0.85 - 2.42)	
	T3	9,736	517	1.27	(1.09 - 1.49)	183	18	2.28	(1.11 - 4.66)	1.01	(-0.46 - 4.28)	1.98	(0.93 - 4.19)	0.43
Colorectum men	T1	6,821	346	1	(reference)	238	8	0.60	(0.25 - 1.46)			0.65	(0.26 - 1.62)	
Genetic risk score	T2	6,951	431	1.21	(0.99 - 1.47)	194	18	1.94	(0.92 -4.09)			1.50	(0.69 - 3.27)	
	T3	4,428	316	1.41	(1.13 - 1.75)	66	9	2.06	(0.71 - 5.95)	1.05	(-1.08 - 4.97)	1.43	(0.47 - 4.36)	0.22
Colorectum women	T1	7,421	241	1	(reference)	177	11	1.79	(0.77 - 4.17)			2.05	(0.86 - 4.92)	
Genetic risk score	T2	7,225	301	1.25	(1.00 - 1.55)	252	16	1.96	(0.94 - 4.07)			1.61	(0.76 - 3.41)	
	T3	5,308	201	1.15	(0.91 - 1.47)	118	9	2.14	(0.78 - 5.89)	0.19	(-3.04 - 5.80)	1.96	(0.66 - 5.77)	0.96
Proximal colon cancer	T1	14,242	207	1	(reference)	415	8	1.17	(0.52 - 2.65)			1.35	(0.57 - 3.21)	
Genetic risk score	T2	14,176	243	1.15	(0.93 - 1.42)	446	14	2.09	(1.07 - 4.08)			1.74	(0.88 - 3.43)	
	T3	9,736	163	1.14	(0.90 - 1.44)	183	12	3.92	(1.75 - 8.79)	2.61	(-0.47 - 7.17)	3.93	(1.63 - 9.48)	0.18
Distal colon cancer	T1	14,242	190	1	(reference)	415	5	0.79	(0.30 - 2.13)			0.78	(0.28 - 2.15)	
Genetic risk score	T2	14,176	236	1.22	(0.98 - 1.52)	446	8	1.42	(0.63 - 3.17)			1.10	(0.48 - 2.50)	
	T3	9,736	177	1.36	(1.08 - 1.72)	183	4	1.53	(0.48 - 4.90)	0.37	(-1.66 - 3.84)	1.13	(0.33 - 3.91)	0.83
Rectal cancer	T1	14,242	132	1	(reference)	415	4	0.88	(0.29 - 2.62)			0.95	(0.30 - 2.98)	
Genetic risk score	T2	14,176	167	1.24	(0.97 - 1.60)	446	7	1.72	(0.73 - 4.08)			1.40	(0.58 - 3.35)	
	T3	9,736	120	1.36	(1.03 - 1.78)	183	2	1.25	(0.27 - 5.92)	0.02	(-1.70- 6.02)	0.92	(0.18 - 4.79)	0.78

CI: confidence interval; HR: hazard ratio; PY: person-years at risk; RERI: relative excess risk due to interaction

* the range in tertiles of the (literature-based) genetic risk score was 6–14, 15–18, and 19–29 risk alleles. The theoretical maximum was 36. The distribution of subcohort members across tertiles was 988 (37.3%), 991 (37.4%), and 669 (25.3%), respectively, in individuals without type 2 diabetes mellitus and 34 (42.0%), 34 (42.0%), and 13 (16.0%), respectively, in individuals with type 2 diabetes mellitus; † hazard ratios were adjusted for age (years), body mass index (kg/m^2), pants/skirt size (below, or equal to or above median size), family history of colorectal cancer (yes/no), smoking status (never, ex, current), alcohol intake (0, 0.1-29, ≥30 g/d), intake in g/d of total meat, vegetables, fish, sweets, added sugar, saturated fats, and fiber, total energy intake (kcal/day), and non-occupational physical activity (≤30, >30, >60, >90 min/day)

Table 3 Multivariable-adjusted hazard ratios for colorectal cancer overall and by sex and subsite for combined categories of type 2 diabetes mellitus and the *IGF1* 19-CA repeat polymorphism and for type 2 diabetics versus non-diabetics within categories of the IGF1 19-CA repeat polymorphism in the Netherlands Cohort Study after 16.3 years of follow-up.

		Type 2 diabetes mellitus										Type 2 diabetes mellitus		
		No				Yes						Yes versus No		p-value for multiplicative interaction
		PY	N cases	HR†	(95% CI)	PY	N cases	HR†	(95% CI)	RERI†	(95% CI)	HR†	(95% CI)	
Colorectum	192 / 192	9,296	620	1	(reference)	280	26	1.44	(0.76 - 2.74)			1.44	(0.76 - 2.72)	
IGF1 19-CA repeat*	192 / non-192	10,950	659	0.88	(0.75 - 1.04)	304	16	0.73	(0.37 - 1.44)			0.83	(0.42 - 1.65)	
	non-192 / non-192	8,238	389	0.76	(0.63 - 0.91)	153	12	1.26	(0.53 - 2.96)	0.05	(-1.73-1.84)	1.89	(0.75 - 4.75)	0.36
Colorectum men	192 / 192	5,027	371	1	(reference)	115	10	1.05	(0.38 - 2.89)			1.01	(0.34 - 2.98)	
IGF1 19-CA repeat	192 / non-192	5,628	389	0.90	(0.73 - 1.12)	184	8	0.57	(0.23 - 1.43)			0.57	(0.23 - 1.45)	
	non-192 / non-192	3,274	234	0.98	(0.76 - 1.25)	48	5	1.57	(0.37 - 6.62)	0.54	(-2.57 - 18.4)	1.88	(0.39 - 8.94)	0.52
Colorectum women	192 / 192	4,269	249	1	(reference)	165	16	1.87	(0.83 - 4.18)			1.96	(0.84 - 4.54)	
IGF1 19-CA repeat	192 / non-192	5,322	270	0.85	0.83 - 1.08	120	8	1.22	(0.44 - 3.42)			1.07	(0.34 - 3.34)	
	non-192 / non-192	4,964	155	0.54	0.42 - 0.71	105	7	1.21	(0.41 - 3.60)	-0.20	(-3.85 - 1.75)	2.35	(0.79 - 6.97)	0.85
Proximal colon cancer	192 / 192	9,296	218	1	(reference)	280	13	1.95	(0.91 - 4.16)			1.97	(0.90 - 4.30)	
IGF1 19-CA repeat	192 / non-192	10,950	229	0.86	(0.69 - 1.07)	304	9	1.21	(0.53 - 2.74)			1.45	(0.63 - 3.34)	
	non-192 / non-192	8,238	123	0.64	(0.50 - 0.83)	153	6	1.63	(0.58 - 4.60)	0.04	(-3.00-3.07)	3.09	(1.03 - 9.24)	0.66
Distal colon cancer	192 / 192	9,296	215	1	(reference)	280	5	0.81	(0.29 - 2.27)			0.77	(0.27 - 2.16)	
IGF1 19-CA repeat	192 / non-192	10,950	209	0.82	(0.65 - 1.02)	304	3	0.41	(0.12 - 1.39)			0.51	(0.15 - 1.74)	
	non-192 / non-192	8,238	131	0.76	(0.58 - 0.96)	153	4	1.32	(0.40 - 4.36)	0.76	(-1.27-2.79)	1.95	(0.54 - 7.07)	0.34
Rectal cancer	192 / 192	9,296	126	1	(reference)	280	5	1.44	(0.51 - 4.09)			1.53	(0.54 - 4.37)	
IGF1 19-CA repeat*	192 / non-192	10,950	147	0.97	(0.74 - 1.27)	304	3	0.64	(0.18 - 2.25)			0.65	(0.18 - 2.35)	
	non-192 / non-192	8,238	91	0.90	(0.66 - 1.22)	153	2	1.07	(0.23 - 5.04)	-0.28	(-2.65-2.10)	1.30	(0.27 - 6.35)	0.64

CI: confidence interval; HR: hazard ratio; RERI: relative excess risk due to interaction; PY: person years; IGF1: insulin-like growth factor 1

* The distribution of subcohort members across categories of the *IGF1* 19-CA repeat polymorphism was 567 (28.6%), 751 (37.8%), and 667 (33.6%), respectively, in individuals without type 2 diabetes mellitus and 13 (35.0%), 24 (42.0%), and 20 (23.0%), respectively, in individuals with type 2 diabetes mellitus; † hazard ratios were adjusted for age (years), body mass index (kg/m^2), pants/skirt size (median split, Dutch sizes), family history of colorectal cancer (yes/no), smoking status (never, ex, current), alcohol intake (0, 0.1-29, ≥30 g/d), intake in g/d of total meat, vegetables, fish, sweets, added sugar, saturated fats, and fiber, total energy intake (kcal/day), and non-occupational physical activity (≤30, >30, >60, >90 min/day)

Discussion

In this long-term prospective study, we used genetic variation in the IGF pathway as time-independent markers of IGF pathway involvement. To our knowledge, this study is the first to simultaneously consider T2DM with markers of IGF pathway involvement in relation to CRC risk. Comparison of combined categories with non-T2DM in the lowest GRS tertile as reference showed that the presence of more unfavorable alleles in the IGF pathway was associated with an increased CRC risk in both the presence and absence of T2DM, with strongly increased CRC risks observed in the presence of T2DM. These data suggest a main effect of the GRS and a possible joint effect of T2DM and the GRS. As compared to non-T2DM wild type *IGF1* 19-CA repeat carriers, carrying two *IGF1* 19-CA variant repeat alleles was associated with a decreased CRC risk in the absence of T2DM, and this association was absent in the presence of T2DM. However, in all analyses, multiplicative and additive interactions were not statistically significant. We found no clear sex differences in any of the analyses performed. Subsite-specific analyses in relation to proximal colon cancer risk were most similar to overall analyses. The most recent meta-analysis has shown T2DM to be a risk factor for CRC[3], although the individual studies used showed conflicting results regarding subsite- and sex-specific CRC risks[18,19]. Using NLCS data[6], we have previously reported T2DM to be associated with an increased proximal colon cancer risk in women. In the current study, findings should be interpreted with caution as the number of CRC cases with T2DM per CRC subsite was low. However, the finding that T2DM was a statistically significant risk factor for proximal colon cancer in the presence of an accumulation of risk alleles in the IGF pathway at least shows consistency with previous findings regarding the T2DM-CRC association in the NLCS and other cohort studies[7]. Differences in embryological origin of subsites and consequently variations in susceptibility to influences of T2DM and IGF1 as a growth factor could theoretically explain why we observed associations with proximal colon cancer specifically[20].

The absence of statistically significant interactions between T2DM and a GRS based on risk alleles in genes in the IGF pathway may be due to a lack of power resulting from the low prevalence of T2DM in our study and the absence of a (strong) main effect of T2DM on CRC risk overall in individuals in the lowest GRS tertile. A prior study by Simons et al.[21] within our research group showed that a larger body size may be a CRC risk factor in men in the presence of an accumulation of unfavorable alleles in the IGF pathway. This previous study gives confidence in our present findings as both BMI and T2DM are independent CRC risk factors that both are thought to act through the IGF pathway[22].
The analyses in which we simultaneously considered T2DM and the *IGF1* 19-CA repeat polymorphism showed no risk pattern other than that *IGF1* 19-CA variant repeat carriers as compared to wild type repeat carriers in the absence of T2DM were associated with a (borderline) statistically significantly lower CRC risk. Although there is some inconsistency in the literature regarding the direction of the association between the *IGF1* 19-CA repeat polymorphism and CRC risk[23], the *IGF1* 19-CA wild type repeat status has been associated with higher IGF-1 serum levels[13] than a non-wild type repeat status, and higher IGF-1 serum levels have

been associated with an increased CRC risk[24]. For comparison, in the study by Simons et al.[21], a decreased CRC risk in women was found for *IGF1* 19-CA variant versus wild type repeat carriers regardless of BMI. Although this is not entirely consistent with the present findings, one could carefully speculate that the CRC protective effects of *IGF1* 19-CA variant repeat alleles might be negated by a metabolically unhealthy state of prolonged exposure to IGFs and other growth factors, which may be captured by the presence of T2DM but not BMI, at least not in the NLCS. The NLCS includes relatively few individuals that reported to be obese (BMI > 30 kg/m2) at baseline (9.6%), which is consistent with the relatively low prevalence of diabetes. T2DM, in this regard, as opposed to BMI, may have better or very specifically captured metabolically unhealthy individuals in our cohort.

Strengths of our study include the prospective design, limiting the chance of selection and information bias, the fact that we had information on many potential confounders, and the large number of CRC cases. In addition, the use of a GRS reduced the number of tests that had to be performed, minimizing the chance of false positive results due to multiple testing. However, the prevalence of T2DM in this cohort was low, limiting power. Another limitation of our study is that we had no repeated exposure measurements during the long follow-up period. As such, HRs may have been underestimated, considering that it is likely that some of the non-diabetic individuals were diagnosed with T2DM after our baseline measurement, as the population grew older. These individuals will have been misclassified as nondiabetic individuals in the present study, resulting in a smaller exposure contrast between the groups. Furthermore, classification of T2DM was performed on the basis of self-reports, which could have resulted in underdiagnosed T2DM in our cohort. However, previous studies within large cohorts have shown that the self-report of treated diabetes is an accurate proxy to use in epidemiologic studies[25].

To conclude, this study of joint effects indicated that the presence of unfavorable alleles in the IGF pathway may further increase the risk of CRC associated with T2DM. Genetic variation in the IGF pathway could prove useful in future studies assessing personalized CRC risk.

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6

Significantly higher rates of multiple and proximally located adenomas among patients with diabetes mellitus: a cross-sectional population-based study

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Summary

Background
Diabetes mellitus (DM) is associated with a greater risk for colorectal cancer (CRC). Objective To examine the endoscopic phenotype and histopathology of colorectal polyps in patients with vs. without DM.

Methods
We conducted a cross-sectional study of patients who underwent colonoscopy at our university hospital and who completed a questionnaire. We collected endoscopy and histopathology data regarding colorectal adenomas and serrated polyps. Cox regression analyses were used to estimate adjusted prevalence ratios (PR).

Results
We examined a total of 3,654 patients (mean age (SD): 62 (12) years, 47% males). Of them, 360 (9.9%) had DM. Overall, the prevalences of colorectal adenomas (42% vs 32%, $p<0.01$), multiple (≥ 3) adenomas (12% vs 7%, $p=0.01$) and proximal adenomas (30% vs 19%, $p<0.01$) were higher in patients with vs. without DM. Multivariable analysis showed that the prevalences of adenomas (PR 1.17, 95% CI; 1.02-1.34), multiple (PR 1.37, 95% CI; 1.00-1.86) and proximal (PR 1.37, 95% CI; 1.16-1.62) adenomas were higher in patients with vs. without DM, especially in men.

Conclusion
Patients with DM harbor more frequently multiple and proximal adenomas than those without DM. Close colonoscopic surveillance of DM patients is important to maximize the effectiveness of colonoscopic CRC prevention.

Introduction

Patients with diabetes mellitus (DM) have a 20% to 40% greater risk of developing colorectal cancer (CRC) than non-diabetic persons[1]. The gradual increase of the incidences of both DM and CRC over the past decade, with approximately 347 million patients with DM and 663 thousand CRC cases worldwide in 2008[2,3], raises concerns about the implications for CRC prevention strategies. Increased life-expectancy and shared risk factors (e.g. obesity, lack of physical activity and unfavorable dietary habits) may explain the rise in incidence rates of both diseases[4]. The exact mechanisms underlying such risk remain, however, unclear. Insulin-resistance in type 2 DM (T2DM) leads to chronic hyperinsulinism, possibly promoting the proliferation of colonic epithelial cells, either directly or indirectly, by increasing insulin-like growth factor (IGF), which, in turn, may lead to an accelerated progression from adenoma to cancer[5]. This hypothesis is supported by a few studies demonstrating an increased risk of colorectal adenomas in T2DM patients, especially in those on exogenous insulin therapy[6–8]. It has been suggested that CRC patients with DM have a worse prognosis than those without DM, in particular a greater overall mortality (20-40%) and a shorter CRC disease-free survival, perhaps due to suboptimal cancer therapy[9]. Collectively, these data highlight the need for improvements in the prevention of CRC in diabetic patients. Although studies endorse the importance of CRC screening in diabetic patients[10], little is known about the prevalence, endoscopic phenotype (e.g. location, size, shape) and histopathology of colorectal polyps in diabetic patients. Also, information on age and gender-specific variations in the prevalence of colorectal polyps in diabetic patients is sparse[11]. In view of the increasing number of CRC screening initiatives worldwide, it is important to clarify the magnitude of CRC risk in diabetic patients. Such information may provide the framework for customized screening programs, in terms of preferred test modality, age of initiation and frequency of surveillance intervals, with the goal to improve the effectiveness of CRC prevention. In a cross-sectional study, we therefore examined the prevalence and endoscopic phenotype of colorectal polyps in diabetic versus non-diabetic men and women, with special attention to the colonic subsite.

Materials and methods

Study population

In the framework of an ongoing initiative aiming to improve the quality of colonoscopy at our university hospital, we have previously trained all endoscopists on the detection, diagnosis and endoscopic resection of colorectal polyps, with emphasis on the non-polypoid colorectal lesions, as described elsewhere[12]. Special attention was paid to targets for measuring the quality of colonoscopy[13]. From February 2008 to February 2012, we have included all consecutive patients who underwent an elective colonoscopy at the endoscopy unit of our university hospital, for symptoms, screening or surveillance indications. We excluded patients aged <30 years, to preclude the inclusion of patients with DM type I. Furthermore, patients with inflammatory bowel disease, hereditary CRC syndromes (i.e. known gene mutations), those fulfilling the WHO criteria for the serrated polyposis syn-

drome, patients with a personal history of CRC, prior colonic resection or an incomplete colonoscopy were excluded. In patients who underwent more than one colonoscopy during the study period, data from the most extensive colonoscopy examination only were evaluated. Index colonoscopy data were used when a second colonoscopy was performed due to onset of new symptoms, polypectomy or post-polypectomy surveillance. In cases of incomplete first colonoscopy or poor bowel preparation requiring a second look colonoscopy, data from the second colonoscopy were used. The study was approved by the local Institutional Review Board (MEC 14-4-046).

Data collection

Clinical, endoscopic and histopathology data

Complete clinical, endoscopic and histopathology data were collected using a digital, standardized case report form. Bowel preparation was classified by the endoscopist as adequate or poor, as described previously[14]. Colorectal polyps were divided according to location into proximal (i.e. proximal from and including the splenic flexure) and distal (i.e. distal from the splenic flexure). Size of the polyps was measured endoscopically (biopsy forceps or mini-snare) and classified into diminutive (i.e. <6 mm), small (i.e. 6-9 mm) and large (i.e. >9 mm). Multiple adenomas were defined as presence of at least 3 adenomas. Non-polypoid colorectal neoplasms were defined as lesions with a height less than half of their diameter[15]. Histopathologic classification of all colorectal polyps was performed by 2 experienced GI pathologists according to the WHO classification[16]. Adenomas included tubular, tubulovillous and villous adenomas, while serrated polyps included hyperplastic polyps, sessile serrated adenomas/polyps (SSA/P) with and without dysplasia and traditional serrated adenomas. Advanced adenomas were defined as adenomas sized ≥10 mm, containing high-grade dysplasia or any villous component.

Questionnaires

A self-administered questionnaire concerning demographic features, presence of DM, smoking, body mass index (BMI), medication (i.e. use of aspirin / NSAIDs), alcohol consumption, family history of CRC, intake of vegetables and fruit was obtained from all patients, shortly after the colonoscopy. Inconsistent or missing data were verified through medical records and recorded as missing in case such information could not be retrieved. Patients who provided informed consent and completed the questionnaire were included in the multivariable analyses. Patients were classified into diabetic and non-diabetic based on self-reporting. BMI was calculated using the height and weight. A positive family history of CRC was defined as ≥1 first-degree relatives with CRC[17]. Alcohol use was categorized as no use/ sporadic use, 1-2 alcoholic consumptions per day and >2 alcoholic beverages per day. Smoking status was categorized as current, former or never and medication use was categorized as daily versus non-daily/no use. Information regarding fruit and vegetable intake was derived from the question: "Do you consume the following products (e.g. bread, fruit)? When answered by yes, the patient was asked to assess the consumption of fruit in pieces per week and the amount of vegetables in grams per day. Indications for aspirin use in this popu-

lation were primary or secondary prevention of cardiovascular events, for which standard daily doses of 100 mg were used.

Statistical analysis

Primary endpoints of this study were the prevalences of colorectal polyps, either adenomas or serrated polyps, in patients with vs. without DM. Based on previous studies[7,18], we estimated that a sample size of at least 155 diabetic women and 1350 non-diabetic women would be required to detect a 10% difference in polyp prevalence (30% vs 20%) with a power of 80% and an alpha of 0.05 in women. As polyps are more common in men than in women[18], we considered higher polyp prevalences (35% vs 25%) in the power analysis for men. We assumed that a sample size of 176 diabetic men and 1535 non-diabetic men would be sufficient to detect a 10% difference. Secondary endpoints were the characterization of the endoscopic features (e.g. location, size, shape) and histopathology of colorectal polyps in DM vs non-DM stratified by gender. Prevalence ratios (PR) of colorectal polyps in DM vs non-DM patients were calculated using cox-regression analyses with equal times of follow-up assigned to all individuals and robust variance as described previously by Barros and Hirakata[19]. Possible confounders were selected a priori, based on clinical relevance and previous literature data. Multivariable models were adjusted for age at the time of colonoscopy, gender, BMI, family history of CRC, smoking status, alcohol intake, use of NSAIDS/Aspirin, indication for colonoscopy, bowel preparation, and intake of fruit and vegetables. Individuals with missing data were excluded from the analyses. In order to compare the adenoma prevalence in the distal vs proximal colon independent of the number of polyps found on colonoscopy, sensitivity analyses were performed excluding patients with more than one adenoma found on colonoscopy. Differences in continuous variables were analyzed using Mann-Whitney-U and the independent samples t-test, differences in categorical values with the Chi-square test. Two-sided p values $\geq$0.05 were considered statistically significant and all risk ratios are presented with 95% confidence intervals (CI). Statistical analyses were conducted using Stata (version 12, Statacorp, College Station, TX, USA).

Results

Figure 1 shows the study diagram. A total of 9,363 patients with 11,067 colonoscopies were included. Of them, 6,394 (68.3%) patients returned the questionnaire, of whom 6.7% declined to fill out the questionnaire, leaving 5,771 subjects further examined in this study. Participating responders were on average 5 years older than non-responders (mean (SD) age 60 (15) years vs 55 (19), p<0.01) and had more often $\geq$1 adenoma (30% vs 23%, p<0.01), $\geq$1 serrated polyp (16% vs 11%, p<0.01) and $\geq$1 SSA/P (1.0% vs 0.7%, p=0.241).

Clinical characteristics

A total of 3,654 (mean [SD] age 62 [12] years, 47% males) subjects were included in the final analyses, of whom 360 (9.9%) reported to have DM. Of all subjects, 1,203 individuals (33%) had $\geq$1 adenoma and 625 individuals (17%) $\geq$1 serrated polyp. Clinical characteristics of the study population subdivided according to

presence of DM are described in **Table 1**. Indications for colonoscopy were symptoms (79.8% of cases), screening (9.5%) or surveillance (10.7%). Notably, patients with DM were more likely to have a poor bowel preparation than those without DM (9% vs 6%, p=0.02). Compared with non-diabetic patients, patients with DM were older (mean age [SD] 61 [12] years vs 66 [10] years, p<0.01), had a higher mean BMI (25.7 kg/m^2 vs 28.9kg/m^2, p<0.01), and used more often NSAIDS/Aspirin daily (28% vs 44%, p<0.01). Concerning lifestyle factors, diabetic patients were more often ex-smokers (58% vs 48%, p<0.01)) than the non-diabetic patients. Approximately 74% of the diabetic patients reported never/sporadic use of alcohol compared with 58% in the non-diabetic population (p<0.01). No significant differences were found between diabetic and non-diabetic patients regarding reported intake of fruit and vegetable. In the analyzed population, the prevalence of colorectal polyps and CRC differed between men and women. Compared to women, the prevalence of colorectal adenomas (33% vs 25%, p<0.01), advanced adenomas (17% vs 11%, p<0.01) and colorectal cancer (4.1% vs 2.4%, p<0.01) was higher in men. No differences were found between men and women regarding serrated polyps or SSA/P.

Prevalence ratios of colorectal polyps in patients with vs without DM in relation to subsite, number of adenomas, and histopathology

In **Table 2** data regarding the prevalences and prevalence ratios of colorectal polyps in patients with vs without DM are presented. Overall, the prevalences of colorectal adenomas (42% vs 32%, p<0.01), multiple (≥3) adenomas (12% vs 7%, p=0.01) and proximal adenomas (30% vs 19%, p<0.01) were higher in patients with than in those without DM. No differences were found between diabetic and non-diabetic patients regarding advanced adenomas, serrated polyps and SSA/Ps. Age-adjusted analyses showed that DM was associated with an increased prevalence ratio for having ≥1 adenoma (PR 1.15, 95% CI 1.01-1.31), proximal adenomas (PR 1.37, 95% CI 1.16-1.62) and multiple adenomas (PR 1.40, 95% CI 1.03-1.90). In the multivariable adjusted model presence of DM was significantly associated with greater prevalence ratios of adenomas (PR 1.17, 95% CI: 1.02-1.34), proximal adenomas (PR 1.37, 95% CI: 1.16-1.62) and multiple adenomas (PR 1.37, 95% CI: 1.00-1.86). Because both the prevalence rates of multiple adenomas and proximal adenomas were increased in patients with DM, a sensitivity analysis was conducted excluding patients with more than 1 adenoma. The prevalence of proximal adenoma remained increased (PR 1.34 [95% CI: 0.99-1.82]) in diabetic (14%) vs non-diabetic (9%) patients without multiple adenomas. Multivariable analysis showed similar results (PR 1.43 [95% CI: 1.05-1.94]).

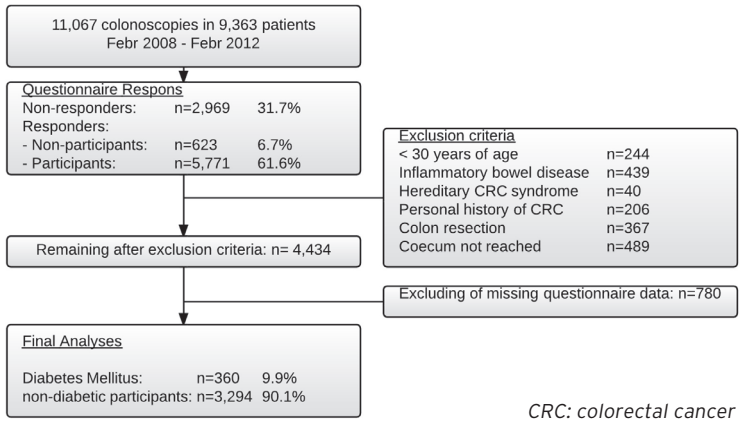
Age- and gender specific associations of DM with colorectal polyps

In **Figure 2** age group-specific distribution of the prevalences of colorectal adenomas is depicted for 442 diabetic patients and 3,511 non-diabetic individuals. For all age groups, the prevalence of ≥1 adenoma was higher in patients with vs without DM, though only statistically significant in the 65-75 years age group. Concerning patients in the pre-screening age range (45-55 years), those with DM had again a higher prevalence of adenomas than those without DM (29% vs 24%, p=0.423).

With regard to gender specific associations, **Table 2** shows that in men with DM,

the prevalences of proximal (PR 1.40, 95% CI 1.15-1.69) and multiple adenomas (PR 1.44, 95% CI 1.02-2.05) were significantly increased in multivariable adjusted models compared to men without DM. In diabetic women, the prevalences of ≥1 adenoma (PR 1.22, 95% CI 0.96-1.55), proximal adenomas (PR 1.36, 95% CI 0.97-1.90), as well as distal adenomas (PR 1.23, 95% CI: 0.91-1.67) were again higher than in non-diabetic women, though differences did not achieve statistical significance.

Figure 1 Study flowchart.



CRC: colorectal cancer

Table 1 Clinical characteristics and risk profile of the study participants stratified according to type 2 diabetes mellitus stat.

Characteristic	Total n=3654	Type 2 DM n=360 (9.9%)	No type 2 DM n=3294 (90.1%)	p-value DM vs. non DM
Mean (SD) age at colonoscopy (yr)	61.6 (11.7)	66.0 (9.8)	61.1 (11.8)	<0.01
Gender (% male)	47.4	53.9	46.6	<0.01
Mean (SD) BMI (kg/m²)	26.1 (4.2)	28.9 (5.1)	25.7 (4.0)	<0.01
Family history of CRC (% yes)	24.9	19.7	25.5	0.02
Indication for colonoscopy (%)				0.01
Symptoms	79.8	81.9	79.6	
Screening	9.5	5.3	9.9	
Surveillance	10.7	12.8	10.5	
Bowel preparation (%)				0.02
Adequate	94.2	91.4	94.5	
Poor	5.8	8.6	5.5	
Smoking status (%)				<0.01
Never	36.3	28.3	36.3	
Ex-smoker	48.3	57.8	48.3	
Current smoker	15.4	13.9	15.4	
Alcohol use (%)				<0.01
No or sporadic use	59.9	74.2	58.3	
1-2 Alcoholic consumptions a day	33.7	20.0	35.2	
> 2 Alcoholic consumptions a day	6.4	5.8	6.5	
Use of NSAID/aspirin (% yes)	29.6	43.9	28.1	<0.01
Mean (SD) vegetable intake (grams/day)	187 (81)	187 (86)	187 (80)	0.56
Mean (SD) fruit intake (pieces/week)	6.6 (3.6)	6.7 (3.6)	6.6 (3.6)	0.36

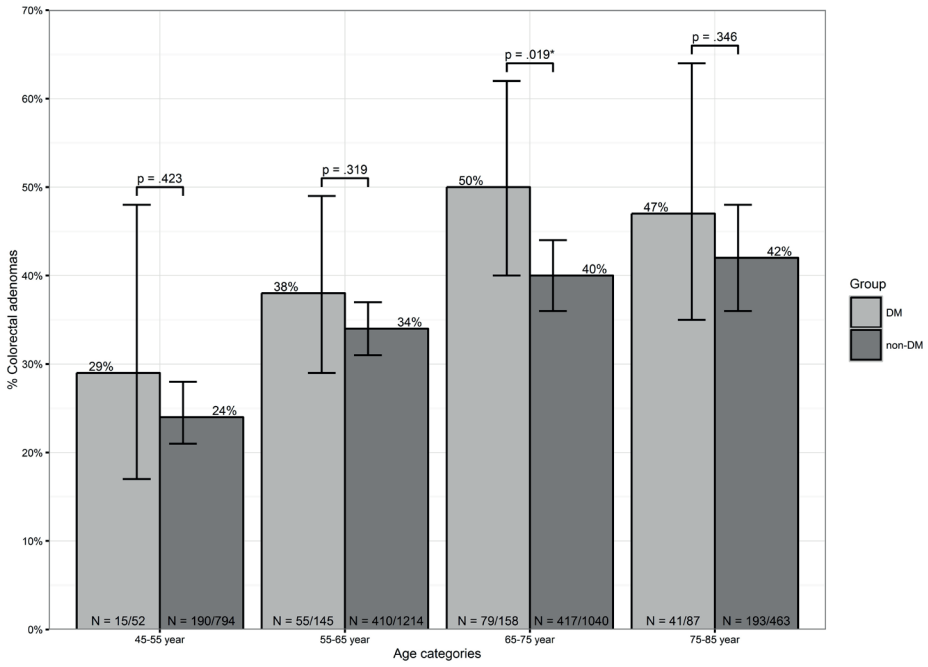
BMI: body Mass Index; DM: diabetes mellitus; CRC: colorectal cancer; NSAID: non-steroidal anti-inflammatory drugs; SD: standard deviation

Table 2 Total and gender-specific prevalence ratios of colorectal neoplasms in patients with vs. patients without diabetes mellitus.

Colorectal polyps		Total		Men		Women	
		non-DM (n=3,294)	DM (n = 360)	non-DM (n=1,536)	DM (n = 194)	non-DM (n=1,758)	DM (n = 166)
≥ 1 Adenoma	Cases (%)	1053 (32%)	150 (42%)	625 (41%)	95 (49%)	428 (24%)	55 (33%)
	PR*	1 [ref]	1.15 [1.01-1.31]	1 [ref]	1.11 [0.95-1.29]	1 [ref]	1.22 [0.96-1.55]
	PR**	1 [ref]	1.17 [1.02-1.34]	1 [ref]	1.12 [0,96-1,30]	1 [ref]	1.29 [1.02-1.65]
≥ 1 Distal adenoma†	Cases (%)	694 (21%)	91 (25%)	398 (26%)	53 (27%)	296 (17%)	38 (23%)
	PR*	1 [ref]	1.07 [0.88-1.30]	1 [ref]	0.97 [0.76-1.25]	1 [ref]	1.23 [0.91-1.67]
	PR**	1 [ref]	1.10 [0.90-1.34]	1 [ref]	0.99 [0.76-1.27]	1 [ref]	1.34 [0.98-1.82]
≥ 1 Proximal adenoma¥	Cases (%)	621 (19%)	109 (30%)	397 (26%)	76 (39%)	224 (13%)	33 (20%)
	PR*	1 [ref]	1.37 [1.16-1.62]	1 [ref]	1,37 [1.13-1.66]	1 [ref]	1,36 [0.97-1.90]
	PR**	1 [ref]	1.37 [1.16-1.62]	1 [ref]	1,40 [1.15-1.69]	1 [ref]	1,35 [0.96-1.90]
≥ 3 Multiple adenoma	Cases (%)	229 (7%)	43 (12%)	152 (10%)	32 (16%)	77 (4%)	11 (7%)
	PR*	1 [ref]	1.40 [1.03-1.90]	1 [ref]	1.44 [1.01-2.06]	1 [ref]	1.29 [0.70-2.37]
	PR**	1 [ref]	1.37 [1.00-1.86]	1 [ref]	1.44 [1.02-2.05]	1 [ref]	1.18 [0.59-2.33]
≥ 1 NP adenoma	Cases (%)	192 (6%)	32 (9%)	114 (7%)	20 (10%)	78 (4%)	12 (7%)
	PR*	1 [ref]	1.27 [0.89-1.87]	1 [ref]	1.21 [0.77-1.90]	1 [ref]	1.40 [0.77-2.56]
	PR**	1 [ref]	1.31 [0.90-1.90]	1 [ref]	1.21 [0.76-1.93]	1 [ref]	1.50 [0.81-2.79]
≥ 1 Advanced adenoma §	Cases (%)	441 (13%)	59 (16%)	250 (16%)	41 (21%)	191 (11%)	18 (11%)
	PR*	1 [ref]	1.05 [0.82-1.34]	1 [ref]	1.15 [0.86-1.55]	1 [ref]	0.87 [0.55-1.38]
	PR**	1 [ref]	1.04 [0.80-1.34]	1 [ref]	1.15 [0.84-1.56]	1 [ref]	0.89 [0.56-1.41]
≥ 1 Serrated polyp	Cases (%)	555 (17%)	70 (19%)	272 (18%)	43 (22%)	283 (16%)	27 (16%)
	PR*	1 [ref]	1.13 [0.90-1.41]	1 [ref]	1.22 [0.92-1.63]	1 [ref]	1.01 [0.70-1.45]
	PR**	1 [ref]	1.03 [0.82-1.29]	1 [ref]	1.21 [0.90-1.61]	1 [ref]	0.85 [0.59-1.24]

DM diabetes mellitus; PR prevalence ratio; NP non-polypoid; HGD high grade dysplasia
† distal from the splenic flexure; ¥ proximal from splenic flexure; § adenoma with HGD, villous component, or size ≥10mm; * PR adjusted for age; ** PR adjusted for age, colonoscopy indication, bowel preparation, BMI, family history of CRC, smoking status, alcohol use, NSAID/Aspirin use, vegetable and fruit intake

Figure 2 Age-specific prevalence of 1 colorectal adenoma, with 95% CI, in patients with and patients without diabetes mellitus.
Because only missing data on age and diabetes status were excluded, the total number of individuals is higher than that in the multivariate analysis.



CI: confidence interval; DM: diabetes mellitus

Discussion

In this population-based study of patients undergoing elective colonoscopy, we found that both diabetic men and women have a moderately increased prevalence of adenomas compared with non-diabetic subjects, in particular *multiple* and *proximally* located adenomas, which may partly explain the greater risk for developing CRC. Our findings underscore the importance of careful colonoscopic examination and close surveillance of diabetic patients, to optimize the quality and effectiveness of CRC prevention in this higher-risk subgroup.
The findings of our study are of potential relevance for the post-polypectomy surveillance of diabetic patients, as both the *multiplicity*[20] and *proximal location* of colorectal adenomas[21] are independent predictors for the development of metachronous neoplasms and are therefore incorporated in calculating the post-polypectomy surveillance intervals. Proximally located colorectal neoplasms appear to be often non-polypoid in shape[22] which partly explains why such lesions are frequently overlooked[23], especially in patients with poor bowel preparation. Indeed, in our present study, 12% of the diabetic patients had multiple, often proximally located adenomas. Of all DM cases with detected adenomas 21% had at least one nonpolypoid shaped adenoma. Of note, nearly 1 in 10 diabetic patients in our study lacked adequate bowel preparation during the colonoscopic examination. Taken together, these data emphasize the importance of high quality standards in screening and colonoscopy surveillance of diabetic patients to ensure protection against cancer.

Epidemiologic studies[24,25] previously described an increased risk for CRC in diabetic patients and a higher risk for proximally compared to distally located CRC, although data are conflicting. In the quality colonoscopy era, however, few studies examined the *endoscopic phenotype* (site, multiplicity, shape) of colorectal neoplasms in diabetic patients. An association was reported between the presence of proximal adenomas in diabetic patients and the duration of insulin use and fasting insulin levels: The study by Wong et al.[8] showed an increased risk of proximal adenomas (OR 1.8, 95% CI 1.1-2.9) in diabetic patients after at least two years of exposure to exogenous insulin compared to diabetic patients who were not exposed to exogenous insulin. Yoshida et al.[26] observed an increased risk for proximally located polyps with each 5 μU/ml increment in fasting serum insulin levels at the time of colonoscopy (OR 1.8, 95% CI 1.2-2.5). With respect to the multiplicity of adenomas detected, Cha et al.[27] found a higher prevalence of multiple adenomas (44% vs 28%) in pre-diabetic vs non-diabetic patients with adenomas. In a study by Suh et.al[28], DM appeared to be associated with multiple adenomas (OR 2.8, 95% CI 1.8-4.4), particularly in men of older age (>65 yr.).

Data regarding gender-specific adenoma- and CRC relative risk in diabetic patients are scarce. In a study of 600 postmenopausal women, Elwing et al.[7] found that diabetic women were more likely to have colorectal adenomas (OR 1.75, 95% CI 1.05-2.87) at screening colonoscopy than those without DM, in keeping with our present study. In contrast to our data, these authors found a trend towards a greater proportion of proximally located advanced adenomas in postmenopausal diabetic women. Difference in the overall proportions of advanced adenomas

between Elwing's study (6% of the non-diabetic women) and ours (11% of the non-diabetic women) may partly explain such discrepancies. Furthermore, difference in indications for colonoscopy (screening in Elwing's study versus predominantly diagnostic colonoscopy in ours) could play a role. A recent cross-sectional study by Kramer et al.[6] investigating the association of type 2 DM with colorectal neoplasia (CRN) in 1,554 participants aged 50 to 74 years, found an increased prevalence of CRN in diabetic women (PR 1.61, 95% CI 1.03-2.53), but not in men (PR 1.02, 95% CI 0.73-1.43). In our present study, we found an association of DM and the presence of ≥1 adenoma at colonoscopy in both men and women, albeit this was more pronounced in men.
The biological mechanisms underlying the increased risk for colorectal neoplasms in diabetic patients and the shift towards more proximally located lesions are unclear. It has been hypothesized that the "insulin resistance-hyperinsulinemia-IGF-axis" plays a major role. Previous nested case-control studies confirmed that increased serum levels of IGF-I and insulin are associated with the risk of adenomas, advanced adenomas and colorectal cancer in men and women[29,30].

Some features of our study need to be acknowledged. Contributing to the strength of this study, data were derived from a large population-based cohort of patients undergoing elective colonoscopy at our institution. Before the outset of this population-based cohort, both specialist registrars and consultant endoscopists were trained on the recognition, classification and endoscopic resection of colorectal neoplasms, in particular the subtle appearing nonpolypoid neoplasms.
A standardized endoscopy reporting system and comprehensive questionnaires were employed to capture clinical data and risk profiles. These data enabled us to carefully control for major confounders (e.g. BMI), albeit residual confounding due to non-measured variables cannot be excluded. As a limitation, the DM status in our study was ascertained based on self-reported questionnaire data, and, hence, the prevalence of DM could be underestimated (e.g. patients who were unaware that they had DM, such as those with glucose intolerance at the time of colonoscopy). Previous epidemiological studies pointed out, however, that self-reporting of DM is accurate[31]. Data regarding the exact subtype of DM and the age at diagnosis of DM were not available. To preclude inclusion of patients with type I DM in our study, we excluded all patients aged < 30 years. In our study, responders were older than non-responders and more likely to have colorectal polyps. A previous survey study from the Netherlands pointed out that non-response in cross-sectional population-based studies does not seem to cause bias in examined associations[32]. Nevertheless, if the prevalence of colorectal polyps was overestimated due to the older age of the responders, this was the case in both diabetic and non-diabetic patients and, hence, is unlikely to have changed the main findings of this study.
Our current data reinforce the importance of stratifying for higher-risk subgroups, such as patients with DM to increase the effectiveness of screening initiatives and colonoscopy surveillance. It remains to be shown whether such higher-risk patients would benefit from personalized cancer prevention strategies, in terms of screening test modality used, age of initiation and frequency of colonoscopy surveillance.

In conclusion, in this large population based study, we found an increased prevalence of proximal and multiple adenomas in patients with DM, which was most pronounced in men. Close colonoscopic surveillance in patients with DM with particular attention to the proximal colon, is warranted to optimize colonoscopic prevention of CRC in such higher risk subgroup.

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7

Higher risk of colorectal cancer in patients with newly diagnosed diabetes mellitus before the age of colorectal cancer screening initiation

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Summary

Type 2 diabetes mellitus (T2DM) is associated with greater risk for colorectal cancer (CRC). The age of onset of T2DM is decreasing worldwide. An increased CRC risk in young T2DM patients could be relevant for the age at which to initiate CRC screening. We report on CRC risk in T2DM patients with attention to age of diagnosis. We used pharmacy data (from 1998 to 2010) from the PHARMO Database Network linked to the Eindhoven Cancer Registry. Multivariable time-dependent Cox regression analyses were conducted to calculate hazard ratios (HR) for developing CRC comparing T2DM with non-T2DM. During 2,599,925 years of follow-up, 394 CRC cases among 41,716 diabetes patients (mean age 64.0 yr., 48% men) and 1,939 CRC cases among 325,054 non-diabetic patients (mean age 51.2 yr., 46% men) were identified. Diabetes was associated with an increased CRC risk in both men and women (HR 1.3, 95% CI 1.2–1.5), particularly in the first 6 months after T2DM diagnosis and pronounced in the proximal colon. This risk was even higher in men younger than 55 years (HR 2.0, 95% CI 1.0–3.8). T2DM was associated with a time-varying and subsite-specific increased CRC risk, which was even higher in men aged <55 years.

Introduction

Patients with type 2 diabetes mellitus (T2DM) have a moderately increased colorectal cancer (CRC) risk (20-40%) and a worse prognosis after CRC diagnosis than non-diabetic persons[1]. The incidence rates of T2DM increased over the past decades, partly because of a shift to younger age at diagnosis[2]. With approximately 415 million patients with T2DM and more than 1 million CRC cases worldwide the moderately increased CRC risk in T2DM patients has now become an issue of concern[3,4]. The underlying mechanisms explaining this association have yet to be elucidated. It has been suggested that metabolic, hormonal and inflammatory changes associated with T2DM play a role[5,6]. For example, chronic hyperinsulinemia in T2DM patients leads to increased insulin-like growth factor (IGF) levels, which, in turn, accelerate the progression from adenoma to cancer[7]. The mechanisms involved are likely complex, with some factors having a protective effect (e.g. weight loss, use of biguanides, aspirin[8,9]) and others a harmful effect (e.g. sulfonylureas[10]). Currently, the information about CRC risk in diabetic patients, including age- and sex-specific variation, tumor location and the relation with the antidiabetic medication is sparse and controversial[1,11–13]. In view of the increasing number of initiatives for CRC screening worldwide, it is important to clarify the magnitude of CRC risk in diabetic patients. In particular patients newly diagnosed with T2DM at a younger age may benefit from earlier initiation of screening. In the Netherlands, the recently initiated (January 2014) nationwide CRC screening program starts at the age of 55 years. We therefore examined the sex-specific association between T2DM and CRC, both diagnosed before and after 55 years of age with focus on the diabetes duration, type of antidiabetic medication used and the tumor location.

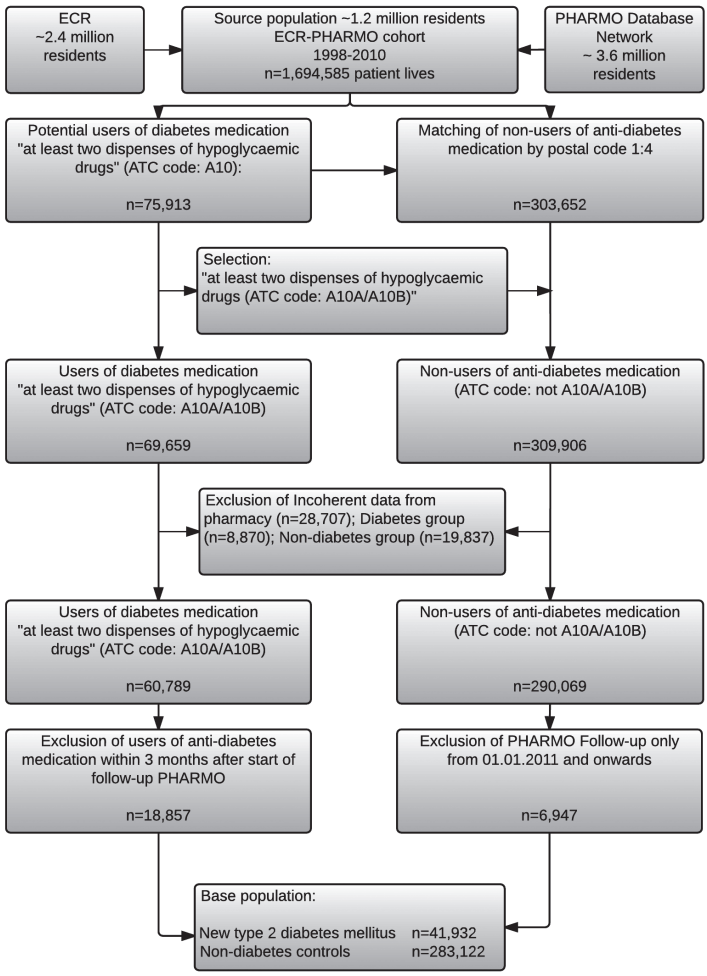
Materials and methods

Data were derived from a combined database of two Dutch research institutes (Eindhoven Cancer Registry (ECR) and the PHARMO Database Network), momentarily covering over one million individuals in the southern region of the Netherlands. Specific information on the linkage of both databases and opportunities for research have been described previously[14]. The ECR is a population-based registry maintained by the Netherlands Comprehensive Cancer Organization. The registry comprises data on all newly diagnosed cancer patients. It started in 1995 in the city of Eindhoven and gradually expanded the registration area until covering a region of 2.4 million inhabitants in the southern part of the Netherlands since 1988. Information is received and collected by trained personnel from six pathology departments, ten general hospitals and two radiotherapy institutes. The coverage of this cancer registration is over 95%[15]. The PHARMO Database Network is a population-based network of healthcare databases and combines data from different healthcare settings in the Netherlands since 1986. The data network covers a demographic region of more than 3 million inhabitants of various regions of the Netherlands. Information for this study is acquired from out-patient pharmacies and contains longitudinal drug dispensing records, including information on dispensing date. Linkage of ECR and PHARMO was per-

formed for patients diagnosed between 01-01-1998 and 31-12-2010 who were living in an overlapping registration area with nearly complete coverage. Patients were followed-up until emigration from the PHARMO-ECR catchment area; end of out-patient pharmacy data collection; end of follow-up or death, whichever occurred first.

Study population

Figure 1 Study flowchart



The study flow-chart is illustrated in **Figure 1.** To prevent inclusion of patients with type I DM and improve comparison with previous cohort studies[16,17], we included all individuals aged 30 years or older who lived in the PHARMO-ECR catchment area between 01-01-1998 and 31-12-2010. Patients with and without

diabetes were identified based on coded dispensing information according to the Anatomical Therapeutic Chemical Classification System (ATC). According to a recent Swedish study by Jansson et.al[18] about 90% of patients with diabetes are pharmacologically treated. Individuals registered with at least two dispenses of hypoglycemic drug coded A10 during follow-up (insulin and analogues and blood glucose lowering drugs) were defined as potential diabetic patients (n = 75,913). Potential diabetic patients were matched only by postal code semi-randomly at a 4:1 ratio with 303,652 persons who did not receive anti-diabetes medication during the study period, to warrant equal CRC registration coverage. Of the 75,913 potential diabetic patients (ATC code containing "A10"), 69,659 had used anti-diabetes medication (ATC code containing "A10A" or "A10B") during the study period and were defined as diabetic patients. The remaining 6,254 individuals were assigned to the non-diabetic group, as these patients were dispensed predominantly non-diabetes medication containing A10 in the ATC code (e.g. promethazine D04AA10). We excluded patients with incoherent out-patient pharmacy data (n=28,707) and patients with follow-up in the PHARMO Database Network starting from 01.01.2011 and onwards (n=6,947) as no linkage with the ECR was available at the time of this study. We defined newly diagnosed T2DM patients as patients who were dispensed antidiabetic medication for the first time after 3 months of follow-up (as recipes are commonly dispensed for ≤ 3 months). A total of 18,857 T2DM patients were dispensed antidiabetic medication within the first 3 months of follow-up and were excluded from analyses. The remaining 41,932 T2DM patients were qualified as newly diagnosed T2DM patients and were included in the analyses together with the 283,122 non-diabetic controls.

Colorectal cancer and subsite

From 01-01-1998 to 31-12-2010, 2,333 new CRC cases (in approximately 2.6 million person years of follow-up) were registered and available for analyses (ICD-10 codes C18-C20. Among these, 819 were proximal CRCs (codes C18.0-C18.5), 699 distal CRCs (codes C18.6 and C18.7), and 642 rectal cancers (C20).

Demographic features, antidiabetic medication and co-medication used

The covariates used in the analyses were derived from the PHARMO Database Network and included age at start of the study and sex. Analyses were stratified according to the type of antidiabetic drug (ADD) used: 1) *Insulin sensitizers* (biguanides "A10BA", thiazolidinediones "A10BG"), 2) *Insulin secretagogues* (sulfonylureas "A10BB", dipeptidyl peptidase-4 inhibitors "A10BH", glucagon-like peptide-1 agonists "A10BX04-07"), and 3) *insulin analogues* ("A10A"). The use of statin (ATC codes C10AA / C10BA / C10BX), aspirin (ATC codes N02BA01 / B01AC06), calcium supplement (ATC code A12AA), and vitamin D supplement (ATC codes A11CC / A11CB) were also included as potential confounders, as these medications may attenuate the CRC risk[8,19].

Statistical analyses

Adjusted hazard ratios (HR) for CRC incidence were calculated using Cox regression with T2DM as a time-dependent variable entered from the date of first dispense of any ADD. This means that the person years between the ECR-PHARMO index date and the date of first ADD dispense were added to the control

group. Each of the controls was assigned their own cohort entry date of first entry in the PHARMO database. Confounders were selected a priori on the basis of literature and checked for their potential to change HR by more than 10% using a backward procedure. Age and the use of statin changed the HR by more than 10% and were included in the multivariable-adjusted model. We also included sex distribution in the multivariate model, as male gender is an established risk indicator for CRC[20]. Statin use (at least 1 dispense of statin) was entered as a dichotomous (yes/no) time-dependent variable. Analyses were also stratified by gender and subsite to assess the impact of effect modification and to compare results with previous literature.

For the age-specific analyses a cut-off of 55 years of age was used, which is the starting age for initiation of CRC screening in our nationwide program. This resulted in a "young" group of T2DM patients (diagnosed <55 years of age) and controls (aged <55 years at cohort entry) who were followed-up until CRC diagnosis, age of 55 years, or end of follow-up.

To mitigate the potential impact of type I DM patients in this cohort on CRC risk estimates, we performed sensitivity analyses excluding DM patients using only insulin analogues during follow-up. Second sensitivity analyses were performed using all 60,789 prevalent and newly diagnosed T2DM patients to estimate potential selection bias. To account for time-varying HR, we also performed time-split analyses in three month intervals during the first year after diagnosis of diabetes according to a previously published study by Johnson et al.[21] addressing potential detection bias. In the latter analyses, follow-up time of T2DM patients prior to diagnosis of diabetes was discarded in the analyses and thus not added to the person-years of observation for non-diabetic persons.

For all analyses, the proportional hazards assumption was tested by visual inspection of the -log-log transformed hazard curves; no violation of the latter assumption was detected. Statistical significance was tested at the 0.05 level using two-sided tests. Analyses were conducted using Stata (version 12, Statacorp, College Station, TX, USA).

Results

Our study population consisted of 41,932 newly diagnosed T2DM patients and 283,122 non-diabetic controls (**Table 1**). The mean follow-up duration was 4.5 (σ 3.2) years and 7.4 (σ 4.1) years respectively. Table 1 presents both the time-dependent (used for the current analyses) and non-time-dependent (for insight into our data) baseline characteristics of the study cohort. T2DM patients were on average 12.8 years older than the non-diabetic persons (64.0 (σ 12.8) and 51.2 (σ 15.0) years respectively) (P<0.001). Of all T2DM patients, 9,413 (23%) were younger than 55 years at the first dispense of antidiabetic medication. Of all non-diabetic controls, 201,078 (62%) were younger than 55 years at the start of the study (P<0.001). The majority of T2DM patients used insulin sensitizers (82%), followed by insulin secretagogues (55%) whether or not in combination with insulin analogues (21%). Patients with T2DM more often used statins (69% vs. 16%, p<0.001) and aspirin (33% vs. 11%, p<0.001) than the non-diabetic controls. In the 41,716 patients who developed T2DM, 394 patients were diagnosed with CRC during

189,568 person-years of follow-up. Likewise, in the non-diabetic group, 1,939 patients were diagnosed with CRC during 2,410,357 person-years of follow-up. Of these 1,939 CRC cases, 216 were diagnosed with T2DM after the CRC diagnosis (date of CRC diagnosis < date of first dispense of any drug used in diabetes) and were therefore classified as CRC in the non-diabetic group. With regard to the colonic subsite, patients with T2DM more often had proximal CRC (45% vs. 33%) and less often rectal cancer (19% vs. 29%) than non-diabetic controls (overall p<0.001). In both T2DM and non-diabetic groups, 30% of the CRCs were distally located.

Table 2 shows the relative risk estimates for CRC, further stratified by sex- and subsite. After adjustment for age and statin use, T2DM was significantly associated with a higher risk of CRC in both men (HR 1.3, 95% CI: 1.1-1.5) and women (HR 1.3, 95% CI: 1.1-1.6). The association was greater for proximal CRC (men: HR 1.6, 95% CI 1.2-2.1; women: HR 1.8, 95% CI: 1.4-2.3), the effect decreasing in size as the cancer localization shifted from proximal to distal. T2DM was not significantly associated with distal colon and rectal cancer, neither in men nor in women. In sensitivity analyses, neither the exclusion of patients who used insulin only during follow-up (n=2,506) nor the inclusion of prevalent T2DM patients (n=18,857) altered the found estimates significantly (data not shown).

Table 3 shows the relative risk estimates for patients younger than 55 years at T2DM and CRC diagnosis compared to the total group. We found a stronger association between T2DM and CRC in men younger than 55 years (HR 2.0, 95% CI: 1.0-3.8 compared to HR 1.3, 95% CI: 1.1-1.5 among men in the total group), but not in women (where only 4 CRC cases were diagnosed in the younger group; HR 1.2, 95% CI 0.4-3.6 compared to HR 1.3, 95% CI: 1.1-1.6 among women in the total group).

No difference in the effect size of CRC risk was found in T2DM patients when stratifying by type of firstly dispensed antidiabetic medication. As shown in **Table 4**, the use of an insulin sensitizer (HR 1.3, 95% CI: 1.2-1.6) or an insulin secretagogue (HR 1.3, 95% CI: 1.1-1.5) as first antidiabetic medication dispensed was associated with a similar CRC risk. In total 2,003 of the 14,372 insulin secretagogue users and 25,765 insulin sensitizer users received dual (sensitizer and secretagogue) therapy at the time of T2DM diagnosis.

As shown in **Table 5**, in the first 3 months (HR 3.1, 95% CI: 1.8-5.2) as well as in the subsequent 3 months (HR 2.1, 95% CI: 1.1-3.9) after T2DM diagnosis, the risk of CRC in T2DM patients was significantly higher than in non-diabetic controls. No further increase of CRC risk was found in the following 2 three-month intervals. During the period from 6 months until the end of follow-up, the CRC risk remained increased in patients with T2DM (HR 1.3, 95% CI: 1.2-1.5), though the effect size was slightly attenuated compared to analysis in which the first 6 months of follow-up were included (HR 1.4, 95% CI: 1.2-1.6). Time split analyses performed at median half time (3.2 years in T2DM) showed similar effect sizes in the first (HR 1.3, 95% CI: 1.0-1.6) and second (HR 1.4, 95% CI: 1.2-1.6) part of the follow-up time.

Table 1 Baseline characteristics of diabetic and non-diabetic individuals within the ECR-PHARMO cohort.

Characteristic	Non time-dependent					Time-dependent*				
	No T2DM (n)	%	T2DM (n)	%	p-value	No T2DM (n)	%	T2DM (n)	%	p-value
Population	283,122	87%	41,932	13%		325,054	89%	41,716†	11%	
Mean FU time (yr. ± sd)	7.8 ± 4.1		9.2 ± 3.6		< 0.001	7.4 ± 4.1		4.5 ± 3.2		< 0.001
Mean age at inclusion (yr. ± sd)	50.0 ± 14.8		59.3 ± 13.2		< 0.001	51.2 ± 15.0		64.0 ± 12.8		< 0.001
< 55 year	185,894	66%	15,184	36%		201,078	62%	9,413	23%	
55-75 year	77,428	27%	21,892	52%		99,320	30%	23,345	56%	
>75 year	19,800	7%	4,856	12%		24,656	8%	8,958	21%	
Gender										
Men	130,124	46%	20,303	48%		150,427	46%	20,166	48%	
Women	152,998	54%	21,629	52%	< 0.001	174,627	54%	21,550	52%	< 0.001
Colorectal cancer distribution	1,723		610			1,939		394†		
proximal colon	588	34%	231	38%		642	33%	177	45%	
distal colon	503	29%	193	32%		581	30%	118	30%	
rectum	502	29%	140	23%		566	29%	76	19%	
NOS/rectosigmoid	130	8%	46	8%	0.023	150	8%	23	6%	< 0.001
DM medication (n, % yes)										
has used insulin	NA		8,908	21%		NA		8,871	21%	
has used insulin sensitizers	NA		34,208	82%		NA		34,053	82%	
has used insulin secretagogues	NA		22,89	55%	NA	NA		22,778	55%	NA
Co-medication (n, % yes)										
Statin	39,042	14%	29,13	69%	< 0.001	50,837	16%	28,964	69%	< 0.001
Aspirin	27,419	10%	13,811	33%	< 0.001	34,604	11%	13,716	33%	< 0.001
Vitamin D	2,692	1%	1,285	3%	< 0.001	3,007	1%	1,264	3%	< 0.001
Calcium supplement	6,076	2%	1,801	4%	< 0.001	6,998	2%	1,785	4%	< 0.001

FU: follow-up; NA: not applicable; NOS: not otherwise specified; SD: standard deviation; T2DM: type 2 diabetes mellitus
* Differences in (n) between non-time-dependent and time-dependent baseline values occur when T2DM patients have FU time as Non-T2DM individuals in time-dependent analyses; † In 216 T2DM patients, colorectal cancer diagnosis occurred before diagnosis of T2DM

Table 2 Gender- and subsite specific time-dependent cox-proportional hazard analyses on the association between type 2 diabetes mellitus and colorectal cancer.

	CRC Cases (T2DM / non-T2DM)	PY (T2DM / non-T2DM)	*HR (95% CI)	†HR (95% CI)
Men and Women				
Colorectal cancer	394 / 1,939	189,568 / 2,410,357	1.4 (1.3-1.6)	1.3 (1.2-1.5)
Proximal colon cancer	177 / 642	189,568 / 2,410,358	1.8 (1.5-2.2)	1.7 (1.4-2.0)
Distal colon cancer	118 / 581	189,568 / 2,410,359	1.4 (1.2-1.7)	‡1.2 (1.0-1.5)
Rectal cancer	76 / 566	189,568 / 2,410,360	1.0 (0.8-1.3)	1.0 (0.7-1.3)
Men				
Colorectal cancer	219 / 1,092	94,612 / 1,149,183	1.4 (1.2-1.7)	1.3 (1.1-1.5)
Proximal colon cancer	81 / 323	94,612 / 1,149,184	1.7 (1.3-2.2)	1.6 (1.2-2.1)
Distal colon cancer	74 / 338	94,612 / 1,149,185	1.5 (1.2-2.0)	§1.3 (1.0-1.7)
Rectal cancer	52 / 350	94,612 / 1,149,186	1.1 (0.8-1.5)	1.1 (0.8-1.5)
Women				
Colorectal cancer	175 / 847	94,956 / 1,261,174	1.5 (1.2-1.7)	1.3 (1.1-1.6)
Proximal colon cancer	96 / 319	94,956 / 1,261,175	2.0 (1.6-2.5)	1.8 (1.4-2.3)
Distal colon cancer	44 / 243	94,956 / 1,261,176	1.3 (0.9-1.8)	1.2 (0.8-1.7)
Rectal cancer	24 / 216	94,956 / 1,261,177	0.8 (0.6-1.3)	0.8 (0.5-1.2)

CRC: colorectal cancer; HR: hazard ratio; PY: person-years; T2DM: type 2 diabetes mellitus
***** adjusted for age and gender (mixed group); **†** adjusted for age, gender (mixed group) and statin use; **‡§** Not statistically significant (‡ $p = 0.052$; § $p = 0.081$)

Table 3 Gender specific analyses on the association between type 2 diabetes mellitus and colorectal cancer diagnosed before the age of 55 years compared to the total group.

	CRC Cases (T2DM / non-T2DM)	PY (T2DM / non-T2DM)	*HR (95% CI)	†HR (95% CI)
Diagnosis of T2DM and CRC < 55 years				
Men and women	17 / 215	37,658 / 1,327,066	1.8 (1.1-2.9)	‡1.7 (1.0-3.0)
Men	13 / 128	21,796 / 662,967	1.9 (1.1-3.4)	2.0 (1.0-3.8)
Women	4 / 87	15,862 / 674,099	1.4 (0.5-3.7)	1.2 (0.4-3.6)
Total group				
Men and women	394 / 1,939	189,568 / 2,410,357	1.4 (1.3-1.6)	1.3 (1.2-1.5)
Men	219 / 1,092	94,612 / 1,149,183	1.4 (1.2-1.7)	1.3 (1.1-1.5)
Women	175 / 847	94,956 / 1,261,174	1.5 (1.2-1.7)	1.3 (1.1-1.6)

CRC: colorectal cancer; HR: hazard ratio; PY: person-years; T2DM: type 2 diabetes mellitus

* adjusted for age and gender (mixed group); †adjusted for age, gender (mixed group) and statin use, ‡ not statistically significant (p = 0.052)

Table 4 Anti-diabetic medication specific analyses on the association between type 2 diabetes mellitus and colorectal cancer.

	CRC Cases (T2DM / non-T2DM)	PY (T2DM / non-T2DM)	*HR (95% CI)	†HR (95% CI)
According to 1st anti-DM prescription				
After any 1st prescription for diabetes	394 ‡ / 1939	189,568 / 2,410,357	1.4 (1.3-1.6)	1.3 (1.2-1.5)
After 1st prescription = sensitizer	191 / 1939	94,237 / 2,410,357	1.5 (1.3-1.8)	1.3 (1.2-1.6)
After 1st prescription = secretagogue	190 / 1939	85,415 / 2,410,357	1.4 (1.2-1.6)	1.3 (1.1-1.5)
After 1st prescription = insulin analogue	32 / 1939	20,541 / 2,410,357	1.3 (0.9-1.8)	1.2 (0.8-1.7)
After 1st prescription = double prescription	19 / 1939	10,655 / 2,410,357	1.3 (0.8-2.0)	1.1 (0.7-1.8)

CRC: colorectal cancer; HR: hazard ratio; PY: person-years; T2DM: type 2 diabetes mellitus

* Adjusted for age and gender (mixed group); † adjusted for age, gender (mixed group) and statin use; ‡ Counts do not add up as 19 CRC patients with T2DM start with double anti-DM medication at diagnosis of T2DM

Table 5 Type 2 diabetes mellitus and colorectal cancer risk according to different time periods after T2DM diagnosis.

	CRC Cases (T2DM / non-T2DM)	PY (T2DM / non-T2DM)	*HR (95% CI)	†HR (95% CI)
Start - end of FU	394 / 1,723	189,568 / 2,212,801	1.5 (1.4-1.7)	1.4 (1.2-1.6)
First year of FU				
Start - 3 months	33 / 38	10,277 / 70,420	3.0 (1.9-4.9)	3.1 (1.8-5.2)
3 Months - 6 months	20 / 35	9,931 / 69,489	2.0 (1.1-3.4)	2.1 (1.1-3.9)
6 Months - 9 months	12 / 40	9,593 / 68,486	1.1 (0.6-2.1)	0.9 (0.4-1.9)
9 Months - 12 months	15 / 48	9,235 / 67,503	1.1 (0.6-2.0)	1.3 (0.7-2.4)
Exclusion of the first 6 months				
6 Months - end of FU	341 / 1,650	169,360 / 2,072,892	1.5 (1.3-1.7)	1.3 (1.2-1.5)
Split at median FU time				
6 Months - 3.2 year	144 / 443	85,398 / 688,028	1.3 (1.1-1.6)	1.3 (1.0-1.6)
3.2 Year - end of FU	197 / 1,207	83,962 / 1,384,864	1.6 (1.4-1.9)	1.4 (1.2-1.6)

CRC: colorectal cancer; FU: follow-up; HR: hazard ratio; PY: person-years; T2DM: type 2 diabetes mellitus

* Adjusted for age and gender; † adjusted for age, gender and statin use

Discussion

Our study supports the role of newly diagnosed T2DM as an established risk factor for CRC. With an increased overall CRC relative risk of 30% and an increased proximal colon cancer risk of 70% found in T2DM patients compared to individuals without diabetes, our study confirms results from previous cohort studies[11,12,16,22-24]. We examined the role of T2DM as a risk factor for CRC before the age of 55 years at which in most countries population-based CRC screening starts. We found that T2DM in young patients was associated with a more pronounced increased risk of CRC diagnosis before the age of 55 years compared to the total population. After adjusting for age and statin use, we observed an increased risk for CRC diagnosis before age 55 in men with T2DM compared to non-diabetic men. In women with T2DM this risk appeared increased though statistical significance was not reached due to small numbers.

The mechanisms that underlie the association between T2DM and CRC are yet to be explored. A shared risk profile including lifestyle factors such as obesity, unfavorable diets, and low levels of physical activity that lead to metabolic abnormalities and cell proliferation (e.g. hyperinsulinemia) can partly explain the association. Hyperinsulinemia as a cancer promoting metabolic factor in T2DM patients suggests a CRC risk modifying role for anti-diabetes drugs as insulin analogues and secretagogues elevate serum insulin serum levels in contrast to insulin sensitizers that lower insulin levels. Insulin secretagogues and analogues have been associated with increased CRC risk[25] and insulin sensitizers with a decreased CRC risk[9,13,26]. However, most of the findings from conducted pharmacoepidemiological studies are possibly methodologically biased. An example is immortality bias. Immortality time refers to a period of follow-up in which death or an outcome (CRC) cannot occur due to design of the study. For instance, waiting and surviving until a first anti-diabetic prescription (e.g. metformin) is dispensed while patient is already followed-up and classified as metformin user from cohort entry. Results are biased in favor of the treatment group due to a partial survival advantage to the non-treated group[27]. In our analyses we accounted for these potential biases as much as possible and found no difference in CRC risk or effect size when stratifying according to the first type of ADD dispensed. When interpreting these findings it should be noted that ADDs could be added, stopped or switched during follow-up. Therefore, the reported increased CRC risks in our study cannot be attributed to one type of anti-diabetes drug only.

A study conducted by Johnson et al.[21] addressed the involvement of detection bias in the increased risk of CRC and other malignancies in T2DM patients. They compared T2DM with non-diabetic individuals and found an overall adjusted CRC HR of 1.2, with an initial peak in CRC risk (HR 2.8) in the first three months after diagnosis of T2DM. Exclusion of the first three months of the 10 year follow-up time resulted in a lower overall risk (HR 1.1), suggesting overestimation of the overall CRC risk estimates in T2DM patients in cohort studies. Our study confirms the initial spike in CRC risk in the first (HR 3.1) and second (HR 2.1) three months after T2DM diagnosis and a subsequent lowering of effect size of the overall CRC risk after exclusion of the first 6 months of follow-up after T2DM

diagnosis. Nevertheless, T2DM remained associated with an increased CRC risk after accounting for detection bias.
Our stratified analyses showed that a T2DM diagnosis was associated with an increased risk for CRC diagnosis before the age of 55 years in men. In women no statistically significant increased risk was observed (probably due to small numbers). To our knowledge, there are no previous studies that report on this age-specific association. Although the number of CRCs diagnosed before the age of 55 years in patients with T2DM was small in our study (13 in men and only 4 in women), our data suggest that lowering the age limit of screening initiation in patients with T2DM might be beneficial. Future studies are required to investigate the cost-effectiveness of lowering the age limit of initiation of CRC screening.

The strength in our study lies in the use of population-based outpatient pharmacy data and nearly complete and detailed cancer registration data. A very large cohort could be composed in which incident T2DM patients were selected based on anti-diabetes drug use. Additionally, a high number of histologically confirmed CRCs were identified. The methodology of this study allowed us to avoid potential biases known to occur in pharmacoepidemiological studies. However, there are also several limitations to our study. Misclassification of T2DM could have occurred as some patients remained undetected, or were diagnosed with T2DM without anti-diabetic drugs being dispensed, which entails about 10% of the total diabetes population[18]. Also no clinical laboratory data (e.g. glutamic acid decarboxylase) were available to differentiate between T1DM and T2DM. Misclassification could have resulted in slight attenuation of estimated HR.
Information regarding "over the counter" drug and supplement use (vitamin D, calcium and aspirin) was not available, resulting in underestimation of the prevalence of co-medication use. Also, lack of information regarding important confounders such as body mass index, dietary habits, smoking and physical activity could have influenced our results. On the other hand, previous prospective cohort studies showed that correction for these factors only marginally attenuated relative risks. Additionally, by adjusting for concomitant statin use we aimed to partially adjust for these confounders by proxy.

In conclusion, newly diagnosed T2DM was associated with a time-varying and subsite-specific increased CRC risk, but also with an even more pronounced increased risk of CRC diagnosis before the age of initiation of CRC screening (55 years) in men. This pronounced increased risk should be reconfirmed in future studies with more confounding information, particularly on family history. The clinical importance of such increased risk and the potential benefits and cost-effectiveness of tailoring screening strategies (e.g. lowering the age limit of CRC screening) in T2DM patients need further investigation.

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8

General discussion and future perspectives

S de Kort

The worldwide epidemic of obesity and type 2 diabetes mellitus (T2DM) is putting a huge burden on health-care systems. T2DM can lead to multiple complications in various organ systems and causes psychological and physical distress to patients. Key factors in management of such a worldwide exploding epidemic are a) early detection of the disorder through screening programs with tailored interventions and b) in case of already established disease, the early recognition of complications with interventions to delay or stop progression of complications and thereby reduce morbidity and mortality.

Summary of key findings

In the first part of this thesis we have discussed the importance of early recognition of gastrointestinal (GI) symptoms, disorders and psychological factors as well as the etiological role of the intestinal barrier (chapter 3) in T2DM patients. In chapter 2 we observed that psychological factors to a large extent are associated with GI symptoms in diabetic patients in a tertiary referral hospital.
In the second part of this thesis, we have studied in more detail the association between T2DM and the incidence of colorectal cancer (CRC), the role of T2DM in the pathogenesis of colorectal cancer and consequences for population-based colorectal cancer screening and surveillance. In chapter 4, in a large Dutch prospective cohort study, analyses indicated that T2DM is associated with an increased risk of proximal colon cancer in women, while taking self-reported anthropometric, dietary and lifestyle factors into account. Elaborating further on chapter 4, in chapter 5, analyzing the same Dutch cohort, we found that simultaneous consideration of T2DM and genetic variation in the IGF pathway could further increase CRC risk or negate protective effects, though no statistically significant interaction was demonstrated. Endoscopy and histopathology data regarding colorectal adenomas and serrated polyps from T2DM patients and controls were analyzed in chapter 6, followed by the conclusion that patients with T2DM harbor more frequently high risk (multiple and proximal) adenomas compared to those without T2DM. Finally, chapter 7 shows results from another large Dutch cohort study in which combined pharmacy and cancer registry data is used. This study showed that T2DM was associated with a time-varying and subsite-specific increased CRC risk, which was even higher in men aged <55 years.

Part I

Part I-a: the importance of early recognition of gastrointestinal (GI) symptoms, disorders and psychological factors in T2DM patients.
Several studies, among them chapter 2 of this thesis, have found evidence for an increased prevalence of gastrointestinal symptoms and symptoms of anxiety and depression in patients with diabetes mellitus compared to non-diabetic controls[1-5]. There is evidence for a dose-response relationship in diabetic patients between the reported number of GI symptoms and the severity of reported psychological complaints, suggesting possible causality[6,7]. The most common gastrointestinal symptoms reported by diabetic patients include diarrhea, constipation, early satiety and bloating. The increased anxiety and depression scores observed in diabetic patients explain a certain extent but not completely, the increased prevalence of GI symptoms in diabetic patients. We observed that the symptom "early satiety" remained to be more prevalent in diabetic patients, even after correcting for psychiatric symptoms.

Here we want to specifically address two important issues. The first is that physicians treating diabetic patients with GI complaints should be aware of the higher prevalence of concomitant psychological symptoms. Therefore these psychological factors should be screened for, more systematically and should be highlighted in the medical record. It is essential, for both physician and patient, to acknowledge the reciprocal relationship between the GI and psychological complaints, and to not confuse it with one-directional causality. In the more persistent cases, patients may benefit from an integrated medical-psychiatric approach[8]. A more integrated approach, including lifestyle interventions as described by the look AHEAD study[9], can offer beneficial effects on GI symptoms. This favorable response is probably mediated not only through weight reduction but also by paying attention to symptoms of depression and treating them appropriately[10]. In this light, paying attention to several domains of health according to the concept postulated by Huber et al.[11] "the ability to adapt and to self-manage", could perhaps improve both GI and psychological symptoms. In strive of reaching a more (cost) effective sustainable treatment, future studies incorporating several health domains via integrated approaches are therefore recommended.

The second issue is related to the difficulty in discerning functional GI complaints from severe GI (motility) disorders. This is best explained via an example: the differentiation between gastroparesis and functional dyspepsia. Gastroparesis is considered to be rare, with a cumulative incidence of 1-5%/10 years and occurs 4-5 times more often in T1DM patients than T2DM. Gastroparesis is associated with significantly impaired quality of life and much poorer glycemic control, malnutrition, and increased morbidity and mortality[12]. Unfortunately, not one upper GI symptom, nor a combination of GI symptoms or weight loss is able to adequately differentiate gastroparesis from functional dyspepsia, possibly leading to underdiagnosing of gastroparesis[13]. Nausea and vomiting are less frequently reported symptoms. Their presence is associated with more severely delayed gastric emptying, but the correlation is weak. Early satiety occurs in 85% of gastroparesis cases[14], possibly due to associated impaired postprandial accom-

modation in gastroparesis, and may reflect the increased incidence of gastroparesis in diabetes patients when compared to non-diabetic controls. Correcting hyperglycemia in diabetic patients with gastroparesis improves gastric emptying, and an improved gastric emptying in return may beneficially affect glucose regulation. The long-term efficacy of tight glucose control on symptom improvement and gastric emptying still needs to be proven[15]. Further studies are needed to develop cost-effective approaches for early detection of GI (motility) disorders in diabetic patients.

Part I-b: intestinal barrier function and diabetes mellitus.
The 3rd chapter in this thesis provided an overview of the current literature with respect to the potential role of the intestinal barrier (dys)function in the development of diabetes mellitus. The hypothesis suggests that an increased exposure to luminal gut contents can eventually lead to autoimmune destruction of pancreatic β-cells or increase insulin resistance. In the past decades several diseases (e.g. NAFLD[16]) have been linked to an impaired intestinal barrier function based on increased intestinal permeability. The evidence is predominantly based on findings from animal in-vivo studies, but has also been established in human studies. Although much scientific attention is given to the role of intestinal barrier defects in onset and development of systemic diseases, the level of evidence is still limited and the comparability between studies is difficult. In future studies, more attention should be given to uniformity in study design, methodology, and direct instead of indirect evaluation of intestinal barrier function, for instance by obtaining intestinal biopsies with histology, electron microscopy and functional evaluation of intestinal biopsies ex vivo in "Ussing" chambers. Meanwhile, randomized trials focusing on enhancing intestinal barrier function, and testing pre- and probiotics, have shown marginal but encouraging results with respect to improvement in HbA1c levels.[17]

Part II

Type 2 diabetes mellitus, colorectal cancer risk and clinical implications
As mentioned in the introduction of this thesis, multiple cohort studies have studied the association between T2DM and CRC. Meta-analyses have confirmed that T2DM increases the overall risk to develop CRC, but the risk is only slightly increased. In this thesis, two Dutch cohorts have been used to further analyze this risk and specify it in more detail according to colon subsite, time-variations and age-related factors. We found the strongest associations of T2DM with proximal (right sided) colon cancer, particularly in women and subjects with unfavorable genetic variations in the insulin-like growth factor I (IGF) pathway. Strong associations with overall CRC were found within the first 6 months after T2DM diagnosis, and in men before the age of CRC screening in the Netherlands (aged <55 years). In a cross-sectional study, chapter 6 of this thesis, we found an overall increased prevalence of CRC precursors, adenomas, in T2DM patients, preferentially located in the proximal colon. This finding is in line with the increased prevalence of CRC in the proximal colon in T2DM found in two cohort studies as described in chapters 4 and 7 of this thesis.

Part II-a: Type 2 diabetes mellitus and colorectal cancer incidence

When a trainee in Gastroenterology is asked to recite risk factors associated with colorectal cancer, T2DM will probably be the last factor mentioned or will not appear on the list of risk factors. It won't be held against them since many other risk factors have much higher impact on the development of CRC and may have more clinical relevance. With respect to published data and evidence on CRC risk in T2DM the following aspects need to be taken into account. First, numerous cohort studies and meta-analyses have been published[18-22] on this topic. In these reports no clear evidence is present for publication bias. At least ten cohort studies, correcting for smoking, BMI and physical activity could be compared with little heterogeneity ($I^2 < 10\%$) and the outcome did not differ much from studies that did not take these factors into account[22]. In our cohort studies, the minor confounding effect of the above mentioned self-reported confounders and dietary factors, based on extensive food frequency questionnaires, was also observed. Hence, we can conclude that the observed increased CRC incidence is probably not explained by known confounders or is a chance finding. Furthermore, the IGF-pathway, as described in the introduction, is a plausible biological mechanism that may underlie this association. The IGF-pathway is also the basis of the association between acromegaly and CRC. Acromegaly is a disease characterized by increased systemic growth hormone concentrations. Several studies have shown a dose-response relationship between circulating IGF levels and colorectal cancer risk[23]. Data from studies that investigated the association between duration of T2DM and CRC are conflicting. The finding that hyperinsulinemia can diminish with long-term T2DM, with lower levels relative to the prediabetes state, may help to explain this observation. The pooled relative risk (RR) of about 1.30[21] between T2DM and CRC can be considered as a small effect size. As a rule of thumb, a strong effect size indicates a higher likelihood that the association is valid or even causal. In other words, a small effect size could suggest a potentially overlooked bias or confounding. In the more recent literature data, including the study described in chapter 7, evidence suggests that the CRC incidence increment in T2DM can, at least partially, be explained by detection bias[24-27] as T2DM patients are probably more exposed to clinical investigations. On the other hand, these data show a temporal relationship as T2DM patients are at increased CRC risk already before T2DM diagnosis[26,28]. If T2DM is a causal factor of CRC, CRC risk is expected to decline when T2DM is eliminated as an exposure factor. Future studies may further establish T2DM as a risk factor by investigating the link between "cessation" of T2DM as a risk factor and change in CRC risk. Cessation of T2DM could be defined as a normal oral glucose tolerance test (OGTT) without the use of anti-diabetic medication. Trials, like the previously discussed "Look AHEAD" trial, in which intensive lifestyle interventions improved glucose regulation in T2DM patients, may prove valuable when CRC incidence could be applied as an end-point[9].

Up to this date ample evidence, including our epidemiological studies with large numbers of participants and cases, and with long follow-up time, suggest that T2DM indeed is an established risk factor for CRC, although the causality and the role of mediators remain to be elucidated.

Part II-b: Type 2 diabetes mellitus and colonoscopic surveillance

Chapter 6 of this thesis brings forth findings of potential relevance for the post-polypectomy surveillance of diabetic patients. The prevalence of adenomas, multiple adenomas, proximally located adenomas was significantly higher in diabetics compared to non-diabetics. Apart from size and histology also location and number of colorectal adenomas determine the intervals for colonoscopic surveillance. Proximally located adenomas are more frequently overlooked, especially in patients with poor bowel preparation. Indeed, several studies including our own[29,30], have reported suboptimal bowel preparation in diabetic patients. Simple adjustments or more intensified regimens for bowel preparation in T2DM patients may help to improve adenoma detection and polyp removal[31,32]. There is a definite need for high-quality standards in screening and colonoscopy surveillance of diabetic patients to ensure protection against colorectal cancer.

Part II-c: Type 2 diabetes mellitus and population-based CRC screening

Currently, in the Netherlands, T2DM patients are treated along the same regimen as the general population with respect to CRC screening and are offered a fecal immunochemical test (FIT) test starting at 55 years until 75 years. A Cochrane review has shown that biennially FIT testing can reduce the risk of CRC mortality by about 15%-25% before and after correction for nonattendance[33]. Considering the increased CRC risk as well as the increased mortality risk after CRC diagnosis in T2DM patients, a different approach or strategy should be considered. First, since T2DM patients probably will benefit more from a CRC diagnosis at a less advanced stage, strict adherence to the nationwide program should be encouraged. In the Netherlands, the acceptance of CRC screening among the population is high with participation rates above 70%. Previous studies on participation of T2DM patients in screening programs in other countries point to much lower participation rates in nationwide screening initiatives, possibly influenced by low socioeconomic status and education levels[34]. Socioeconomic status also appears to affect participation rates in CRC screening in the Netherlands, but to a much lesser extent than in other countries such as the USA. Second, since our results from chapter 7 suggest that the increased risk of T2DM patients for CRC already exists before the age of 55 years, more drastic measures such as reducing the age at which to start CRC screening in T2DM patients could be considered. Based on the increased prevalence of colorectal adenomas in T2DM, an earlier initiation of CRC screening may even prove to be cost-effective. In 2015, the FIT test (> 275 ng/ml) had a positive predictive value of 8.7% and 57.2% for CRC and CRC combined with advanced adenomas, respectively, with 4.3 CRCs detected per 1,000 screened. In 2015 the CRC crude incidence rate, according to the Netherlands Comprehensive Cancer Organization, was 53 and 84 per 100,000 Dutch men and women in the age categories 50-54 years and 55-59 years respectively[35]. A simple calculation (84/53) shows that the crude incidence is increased by 58% across the two age categories in 2015.

The assumption of an increased CRC incidence of 70% (chapter 7 of this thesis) in T2DM men and women aged below 55 years leads to a comparable crude incidence in age group 50-54 with T2DM as in the general population aged 55-59 years. This suggests that the FIT test could harbor the same diagnostic yield in T2DM patients aged 50-54 years as the current starting age of the screening population for CRC aged 55-59 years.
Certainly further studies are needed in T2DM to establish a solid basis before nationwide implementation is considered. A more radical approach would be to offer T2DM patients a colonoscopy instead of FIT testing at 50 or 55 years of age. Since the increased CRC risk in T2DM is only modestly increased, this measure is probably not cost-effective.
Finally, the further unraveling of genetics and gene-environment interaction could result, not only in personalized treatment, but also in a more personalized risk assessment with associated personalized CRC screening programs in both T2DM and non-T2DM individuals.

General conclusion

The continually increasing prevalence of T2DM guarantees a growing prevalence of associated multi-organ complications. In this thesis T2DM was associated with increased prevalence of gastrointestinal and psychological complaints, but also with an increased risk of CRC and colorectal adenomas, particularly located in the proximal colon. Early recognition of GI complications is of key importance in T2DM patients as most GI complications are associated with increased morbidity and mortality in T2DM patients. Future research should focus on appropriate screening modalities and their cost-effectiveness ensuring early detection and intervention of T2DM associated GI manifestations as gastroparesis or CRC.

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Summary
Samenvatting

Summary of the thesis

This thesis has focused on the association between type 2 diabetes mellitus (T2DM) and the gastrointestinal (GI) system: on functional GI manifestations of T2DM and on carcinogenesis with focus on colorectal cancer (CRC). The research goals have been divided in two parts. In the first part, the aim was to examine the effects of T2DM on GI symptoms and complaints and also to explore the role of the GI barrier in diabetic patients through a review of the current literature. In the second part of this thesis we have focused on the association between T2DM and the incidence of CRC.

In **chapter 1** a short overview of the relevant literature was given with focus on the two important research goals. Thereafter, the aims of this thesis have been described.

Chapter 2 reported on GI symptoms in diabetes mellitus (DM) and their relation to anxiety and depression. The prevalence of GI symptoms is increased in patients with DM. In general, GI symptoms are influenced by psychological factors such as anxiety and depression, but little is known about this association in diabetic patients. We tested the hypothesis that anxiety and depression have major impact on GI symptoms in diabetic patients. A group of 280 diabetic patients and 355 non-diabetic, age and sex matched controls were studied by validated questionnaires regarding common GI symptoms, anxiety and depression (HADS). Patients with DM scored significantly higher on the symptoms diarrhea (OR 1.64, 95% CI 1.05–2.56), early satiety (OR 2.50, 95% CI 1.39–4.49) and bloating (OR 1.58, 95% CI 1.03–2.43). Prevalence of anxiety and depression was significantly higher in diabetic patients compared to controls. After adjusting for anxiety and depression only the GI symptom "early satiety" remained significantly more prevalent in patients with DM and could point to a somatic-based origin. Thus, in this study psychological factors to a large extent are associated with GI symptoms in diabetic patients and should be taken into account when considering treatment of the GI symptoms.

Recent studies investigating the underlying mechanisms involved in disease development in DM point to the role of the dysregulation of the intestinal barrier. Therefore, in **chapter 3**, we reviewed the literature with respect to intestinal barrier function, dysfunction and DM. Via alterations in intestinal permeability, intestinal barrier function becomes compromised whereby access of infectious agents and dietary antigens to mucosal immune elements is facilitated, which may eventually lead to immune reactions with damage to pancreatic beta cells and can lead to increased cytokine production with consequent insulin resistance. Understanding the factors regulating the intestinal barrier function will provide important insight into the interactions between luminal antigens and immune response elements. We analyzed recent advances in the mechanistic understanding of the role of the intestinal epithelial barrier function in the development of type 1 and type 2 DM. Given our current knowledge, we may assume that reinforcing the intestinal barrier can offer and open new therapeutic horizons in the treatment of T1DM and T2DM.

Epidemiological studies showed that T2DM is associated with an increased incidence of CRC. However, studies that also differentiate between subsites of CRC are limited. In **chapter 4,** we investigated how T2DM is associated with subsite-specific CRC incidence in men and women. We used The Netherlands Cohort Study on diet and cancer (NLCS) as data source. This cohort is a prospective study among 120 852 men and women aged 55–69 years old at baseline in 1986. Information on DM, anthropometric, dietary and lifestyle factors was self-reported at baseline. T2DM was defined as the diagnosis of DM after 30 years of age. Incident CRC cases were identified by record linkage with the Netherlands Cancer Registry and the Dutch pathology registry. After 17.3 years of follow-up, 1,735 incident male CRC cases and 1,321 female CRC cases were available for analyses. At baseline, 3.1% of subcohort members reported T2DM, of whom 80% were diagnosed after 50 years of age. Multivariable-adjusted Cox models showed that the incidence of proximal colon cancer was significantly increased in women with T2DM versus women without T2DM (HR=1.80, 95% CI: 1.10–2.94). There was no association between T2DM and the incidence of overall CRC, distal colon cancer and rectal cancer in women. In men, T2DM was not associated with overall CRC or with incidence at any subsite. Thus, in this prospective study we were able to show an increased incidence of proximal colon cancer in women with T2DM compared with non-T2DM women.

In **chapter 5** we reported on the role of genetic variants in the insulin-like growth factor (IGF) pathway, T2DM and CRC risk. Genetic variation in the IGF pathway may further increase the T2DM associated CRC susceptibility. We investigate whether joint effects of T2DM and genetic variation in the IGF pathway on CRC risk can increase mechanistic insights. We used the same data source (NLCS) as described in chapter four. Additionally, eighteen single nucleotide polymorphisms (SNPs) from the IGF pathway were aggregated in a genetic risk score (GRS). Cox proportional hazard ratios (HRs) for CRC were estimated according to combinations of T2DM status with GRS tertiles and categories of an IGF1 19-CA repeat polymorphism. Baseline T2DM prevalence was 3.1% in subcohort members and 3.8% in CRC cases. Comparison of combined categories with non-T2DM individuals in the lowest GRS tertile as reference showed that those in the highest GRS tertiles with and without T2DM had significantly increased CRC risks, particularly those with T2DM (HR=2.28, 95% CI: 1.11, 4.66). As compared to IGF1 19-CA wild type carriers without T2DM, carrying two IGF1 19-CA variant repeat alleles was associated with significantly decreased CRC risk in those without T2DM (HR=0.76, 95% CI: 0.63-0.91). This association was negated when T2DM was present. We concluded that simultaneous consideration of T2DM and genetic variation in the IGF pathway, as marker of pathway involvement, influenced CRC risk in the direction as expected.

The objective of **chapter 6** was to examine the endoscopic phenotype and histopathology of colorectal polyps in patients with vs without DM. We explored in the Maastricht polyp study whether rates of colon adenomas were more prevalent among patients with DM in comparison to the general population. We conducted a cross-sectional study of patients who underwent colonoscopy at Maastricht University Medical Center and who had completed a questionnaire regarding

CRC risk factors. We collected endoscopy and histopathology data regarding colorectal adenomas and serrated polyps. A total of 3,654 patients (mean age (SD): 62 (12) years, 47% males) were examined. Of them, 360 (9.9%) had DM. Overall, the prevalence of colorectal adenomas (42% vs 32%, $p < 0.01$), multiple (≥3) adenomas (12% vs 7%, $p = 0.01$) and proximal adenomas (30% vs 19%, $p < 0.01$) was higher in patients with DM vs. patients without DM. Multivariable analysis showed that the prevalence of adenomas (PR 1.17, 95% CI; 1.02–1.34), multiple (PR 1.37, 95% CI; 1.00–1.86) and proximal (PR 1.37, 95% CI; 1.16–1.62) adenomas was higher in patients with vs. without DM, especially in men. In conclusion, patients with DM harbor more frequently multiple and proximal adenomas compared to those without DM. Close colonoscopic surveillance of DM patients is important to maximize the effectiveness of colonoscopic CRC prevention.

In **chapter 7** we investigated the incidence of CRC in patients with newly diagnosed T2DM before the age of CRC screening initiation (55 years) in the Netherlands. The age of onset of T2DM is decreasing worldwide and an increased CRC risk in young T2DM patients could be relevant for the age at which to initiate CRC screening. We used pharmacy data (from 1998 to 2010) from the PHARMO Database Network linked to the Eindhoven Cancer Registry. Multivariable time-dependent Cox regression analyses were conducted. During 2,599,925 years of follow-up, 394 CRC cases among 41,716 T2DM patients (mean age 64.0 yr., 48% men) and 1,939 CRC cases among 325,054 non-diabetic patients (mean age 51.2 yr., 46% men) were identified. T2DM was associated with an increased CRC incidence in both men and women (HR 1.3, 95% CI 1.2–1.5), particularly in the first 6 months after T2DM diagnosis and pronounced in the proximal colon. This risk was even higher in men younger than 55 years (HR 2.0, 95% CI 1.0–3.8). Our findings have relevance for population based CRC screening and the question should be answered whether colorectal screening in T2DM should be modified or intensified, for instance by starting screening at an earlier age.

Finally, in the general discussion in **chapter 8** we have integrated the observations and conclusions of the various chapters of this thesis. We reported on the increased prevalence and specificity of GI symptoms in DM patients, on intestinal barrier (dys)function, both as etiological factor and potential therapeutic pathway. We further appraised the association between CRC and T2DM with emphasis on CRC incidence, colonic polyps and surveillance and consequences for population based CRC screening.

Samenvatting van het proefschrift

Dit proefschrift heeft zich gericht op de associatie tussen type 2 diabetes mellitus (T2DM) en het gastro-intestinaal (GI) stelsel: op functionele gastro-intestinale manifestaties van T2DM met focus op colorectaal kanker (CRC). De onderzoeksdoelen zijn gesplitst in twee delen. In het eerste deel was het doel om de effecten van T2DM op GI-symptomen te onderzoeken. Tevens verkenden we de rol van de GI-barrière in T2DM patiënten in een literatuurstudie. In het tweede deel van dit proefschrift hebben we ons gericht op de associatie tussen T2DM en de incidentie van CRC.

In **hoofdstuk 1** werd een kort overzicht gegeven van de relevante literatuur met aandacht voor de twee belangrijke onderzoeksdoelen. Ook zijn daar de doelen van dit proefschrift beschreven.

Hoofdstuk 2 rapporteerde over GI-symptomen bij diabetes mellitus (DM) en hun relatie tot angst en depressie. De prevalentie van GI-symptomen is verhoogd bij patiënten met DM. In het algemeen worden GI-symptomen beïnvloed door psychologische factoren zoals angst en depressie. Er is echter weinig bekend over deze associatie bij diabetische patiënten. We testten de hypothese dat angst en depressie een grote invloed hebben op de GI-symptomen bij diabetespatiënten. Een groep van 280 diabetische patiënten en 355 niet-diabetische controlepersonen, die op leeftijd en geslacht waren gekoppeld, werden bestudeerd met gevalideerde vragenlijsten die betrekking hadden op algemene GI-symptomen, angst en depressie. Patiënten met DM scoorden significant hoger op de symptomen "diarree" (OR 1.64, 95% CI 1.05-2.56), "vroege verzadiging" (OR 2.50, 95% CI 1.39-4.49) en een opgeblazen gevoel (OF 1.58, 95% BI 1.03-2.43). De prevalentie van angst en depressie was significant hoger bij diabetische patiënten in vergelijking met de controlegroep. Na correctie voor angst en depressie bleef alleen het GI-symptoom "vroege verzadiging" significant vaker voorkomen bij patiënten met DM. Dit zou kunnen wijzen op een somatische oorsprong van de klacht. In dit onderzoek werden psychologische factoren in hoge mate geassocieerd met GI-symptomen bij diabetische patiënten. Hiermee zou rekening gehouden moeten worden bij het overwegen van de behandeling van GI-symptomen.

Recente studies naar de onderliggende mechanismen die betrokken zijn bij de ontwikkeling van DM wijzen naar het disfunctioneren van de darmbarrière. Daarom hebben we in **hoofdstuk 3** de literatuur besproken met betrekking tot de darmbarrièrefunctie, disfunctie en DM. Door veranderingen in darmdoorlaatbaarheid wordt de darmbarrièrefunctie aangetast. Hierdoor wordt de toegang van bacteriën, virussen en voedingsantigenen tot onderdelen van het immuunsysteem van het slijmvlies vergemakkelijkt. Uiteindelijk kan dit leiden tot immuunreacties met schade aan de insuline producerende bètacellen van de alvleesklier of tot insuline-resistentie. Het begrijpen van de factoren die de darmbarrièrefunctie reguleren zal inzicht geven in de interacties tussen antigenen in de darm en de onderdelen van het immuunsysteem. We analyseerden de recente vooruitgang in het mechanistische begrip van de rol van de darmbarrièrefunctie bij de ontwikkeling van type 1 diabetes mellitus (T1DM) en T2DM. Gezien onze huidige kennis kunnen we

aannemen dat versterking van de darmbarrière nieuwe therapeutische opties kan bieden bij de behandeling of preventie van T1DM en T2DM.

Epidemiologische studies hebben aangetoond dat T2DM geassocieerd is met een verhoogde incidentie van CRC. Studies die een onderscheid maken tussen de locatie van CRC in de dikke darm zijn echter beperkt. In **hoofdstuk 4** hebben we onderzocht hoe T2DM wordt geassocieerd met locatie-specifieke CRC incidentie bij mannen en vrouwen. Als bron gebruikten de Nederlandse cohortstudie over voeding en kanker (NLCS). Deze studie is prospectief opgezet en beschikt over gegevens van 120 852 mannen en vrouwen van 55-69 jaar oud bij aanvang van de studie in 1986. Informatie over DM, antropometrische-, dieet- en leefstijlfactoren was zelf-gerapporteerd. T2DM werd gedefinieerd als een diagnose van DM na het 30ste levensjaar. Incidente CRC-gevallen werden geïdentificeerd door middel van een koppeling met de Nederlandse kankerregistratie en het Nederlandse pathologie register. Na 17,3 jaar vervolgen van de studiepersonen kregen 1.735 mannen en 1.321 vrouwen de diagnose CRC. Zij werden gebruikt in de analyses. Bij baseline rapporteerde 3,1% van de subcohortleden T2DM, van wie 80% werd gediagnosticeerd met T2DM na het 50ste levensjaar. Multivariabel gecorrigeerde Cox-modellen toonden aan dat de incidentie van proximale darmkanker significant was verhoogd bij vrouwen met T2DM versus vrouwen zonder T2DM (HR = 1,80 ; 95% BI: 1,10-2,94). Er was geen verband tussen T2DM en de incidentie van alle colorectale kankers tezamen, linkszijdige colonkanker of endeldarmkanker bij vrouwen. Bij mannen was T2DM niet geassocieerd met de incidentie van colorectaal kanker noch met een specifieke locatie van CRC in de dikke darm. Concluderend waren we in staat om een verhoogde incidentie van proximale darmkanker bij vrouwen met T2DM te laten zien in vergelijking met niet-T2DM-vrouwen in deze studie.

In **hoofdstuk 5** hebben we gerapporteerd over de rol van genetische varianten in de insulineachtige groeifactor (IGF) route, T2DM en darmkankerrisico. Genetische variatie in de IGF-route kan de geassocieerde ontvankelijkheid voor darmkanker in type 2 diabetespatiënten mogelijk verder vergroten. We onderzochten of de gezamenlijke effecten van T2DM en genetische variatie in de IGF-route op CRC-risico mechanistische inzichten kunnen vergroten. We gebruikten dezelfde gegevensbron (NLCS) zoals beschreven in hoofdstuk vier. Bovendien werden achttien single nucleotide polymorfismen (SNP's) van de IGF-route samengevoegd tot een genetische risicoscore (GRS). Cox proportional hazard ratios (HRs) voor darmkanker werden geschat door combinaties van T2DM status, GRS score (in tertielen c.q. driedeling) en categorieën van een IGF1 19-CA herhalingspolymorfisme te maken. De prevalentie van T2DM was 3,1% in subcohortleden en 3,8% in de groep met darmkanker. Een vergelijking van gecombineerde categorieën met personen zonder T2DM met de laagste GRS (laagste tertiel) als referentie toonde aan dat personen met de hoogste GRS significant verhoogde CRC-risico's hadden, in het bijzonder de personen met T2DM (HR = 2,28 ; 95% BI: 1,11-4,66). Vergeleken met IGF1-19-CA wild-type dragers zonder T2DM, hadden personen zonder T2DM met twee IGF1 19-CA variant allelen een significant verlaagd CRC-risico (HR = 0,76 ; 95% BI: 0,63-0,91). Deze associatie werd teniet-gedaan wanneer er wel sprake was van T2DM. We concludeerden dat als we ge-

lijktijdig T2DM en genetische variatie in de IGF-route, als marker van IGF-betrokkenheid, laten interacteren het CRC-risico beïnvloed wordt in de verwachte richting.

Het doel van **hoofdstuk 6** was om het endoscopische fenotype en de histopathologie van colorectale poliepen bij patiënten met en zonder DM te onderzoeken. We onderzochten in het Maastrichtse polieponderzoek of darmpoliepen vaker voorkwamen bij patiënten met DM in vergelijking met de algemene populatie. Hiervoor hebben we een dwarsdoorsnede studie uitgevoerd van patiënten die een colonoscopie ondergingen bij het Universitair Medisch Centrum Maastricht. Het betrof de personen die ook een vragenlijst hadden ingevuld met betrekking tot risicofactoren voor darmkanker. We verzamelden endoscopie- en histopathologiegegevens van darmpoliepen, waaronder colorectale adenomen. Een totaal van 3.654 patiënten (gemiddelde leeftijd (SD): 62 (12) jaar, 47% mannen) werden onderzocht. Van hen had 360 (9,9%) DM. In het algemeen was de prevalentie van colorectale adenomen (42% versus 32%, p <0,01), meerdere (≥3) adenomen (12% versus 7%, p = 0,01) en rechtszijdige adenomen (30% versus 19%, p <0,01) hoger bij patiënten met DM ten opzichte van patiënten zonder DM. In multivariabele Cox-regressieanalyses berekenden we de prevalentie ratio's (PR). De prevalentie van colorectale adenomen (PR 1,17, 95% CI; 1,02-1,34), meerdere (PR 1,37, 95% CI; 1,00-1,86) en rechtszijdige (PR 1,37, 95% CI; 1,16-1,62) adenomen was hoger bij patiënten met DM dan bij personen zonder DM. Dit zagen we vooral bij mannen. We trokken de conclusie dat patiënten met DM vaker meerdere en vaker rechtszijdige colorectale adenomen hebben dan patiënten zonder DM. Nauwkeurige colonoscopiesurveillance van diabetes patiënten is nodig om effectief darmkanker te kunnen voorkomen.

In **hoofdstuk 7** hebben we de incidentie van darmkanker onderzocht bij patiënten met nieuw gediagnosticeerde T2DM vóór de leeftijd (55 jaar) waarop een inwoner van Nederland wordt opgeroepen voor het bevolkingsonderzoek naar darmkanker. De leeftijd waarop T2DM gediagnosticeerd wordt neemt wereldwijd af en een verhoogd risico op darmkanker bij jonge T2DM-patiënten kan relevant zijn voor de leeftijd waarop darmkankerscreening kan worden gestart. We gebruikten apotheekgegevens (van 1998 tot 2010) van het PHARMO-netwerk gekoppeld aan de kankerregistratie in Eindhoven. Multivariabele tijdsafhankelijke Cox-regressieanalyses werden uitgevoerd. Na 2,599,925 cumulatieve vervolgjaren van de bestudeerde personen werden 394 darmkankergevallen geïdentificeerd onder 41.716 T2DM-patiënten (gemiddelde leeftijd 64 jr., 48% mannen) en 1.939 darmkankergevallen onder 325.054 niet-diabetische patiënten (gemiddelde leeftijd 51,2 jr., 46% mannen). T2DM was geassocieerd met een verhoogde darmkankerincidentie bij zowel mannen als vrouwen (HR 1,3 ; 95% BI 1.2-1.5), vooral in de eerste 6 maanden na diagnose van T2DM en het betrof vooral kanker van de rechtszijdige dikke darm. Dit risico op CRC was zelfs hoger bij mannen jonger dan 55 jaar (HR 2,0 ; 95% CI 1,0-3,8). De bevindingen in dit hoofdstuk zijn relevant voor bevolkingsonderzoeken naar darmkanker en de vraag moet worden beantwoord of darmkankerscreening bij T2DM patiënten moet worden gewijzigd of geïntensiveerd, bijvoorbeeld door het starten van darmkankerscreening op een jongere leeftijd.

Tot slot hebben we in de algemene discussie in **hoofdstuk 8** de observaties en conclusies van de verschillende hoofdstukken van dit proefschrift geïntegreerd. We rapporteerden over de verhoogde prevalentie en specificiteit van GI-symptomen bij DM-patiënten en over de functie en disfunctie van de darmbarrière. We evalueerden de associatie tussen darmkanker en T2DM met de nadruk op darmkankerincidentie, colonpoliepen, surveillance en de gevolgen voor het bevolkingsonderzoek naar darmkanker.

Valorisation

In a document published by McKinsey in 2013[1], it is stated that by 2040, due to increasing demand and supply of health care, roughly 25% of the Netherlands' gross domestic product will be spent on its healthcare system. In pursuit of a sustainable and high quality health care system, health care managers were interviewed and bundled their thoughts in the following consensus: by 2040 the Netherlands should have the best possible health care system in the world, in term of quality and accessibility, while maintaining a fair balance between costs and returns, but most importantly the health care system emphasizes health and staying healthy. In other words, a health care system that particularly promotes effective preventive measures instead of a narrow emphasis on cure. In general, this thesis focusses on early recognition and diagnosis of gastrointestinal manifestations in patients with diabetes mellitus and provides data supporting a sustainable health care system.

Diabetes mellitus is a global health concern with a high and growing prevalence of about 9% in adults globally. Diabetes mellitus can be viewed as an economic burden with 12% of global health expenditure according to the international diabetes federation[2]. The global cost is predicted to approach 1,452 billion dollar by 2040 due to the increased prevalence of risk factors for diabetes, such as obesity, and the ageing of the world's population. Logically, diabetes mellitus is a chronic disease that needs the attention of health care managers and policymakers. In attempt to reduce diabetes incidence, numbers of trials have emerged and showed that type 2 diabetes mellitus can be prevented or even delayed through lifestyle programs or with timely start of metformin in pre-diabetic individuals[3–5]. Unfortunately, up to this date, the ideal cost-effective translation of these programs into 'real-world' settings is not known and remains a major challenge[6]. It is clear, to prevent rising health care costs, diabetes incidence needs to be reduced by means of effective primary prevention strategies. In chapter 3 of this thesis, the intestinal barrier is reviewed in relation to diabetes mellitus development. When current preventive strategies fail or need improvement, inspiration could be derived from new insights. In chapter 3 we conclude that more understanding of the intestinal barrier could have important clinical implications by potentially opening new horizons in the treatment and prevention of diabetes mellitus. For instance, future therapeutic drugs or specific diets that focus on enhancing the intestinal barrier function could be a part in the prevention strategies regarding metabolic diseases such as diabetes mellitus.

However, once diabetes mellitus is an established disease, focus must shift towards controlling the disease and preventing costly but also disabling diabetic complications such as vascular diseases, nephropathy and retinopathy. This thesis focusses on the less notorious but equally relevant gastrointestinal manifestations of diabetes mellitus. In chapter 2 of this thesis, we observed a significantly increased prevalence of the gastrointestinal symptoms diarrhea, bloating and early satiety, but also of anxiety and depression in patients with type 2 diabetes mellitus. The importance of viewing gastrointestinal and psychological symptoms simultaneously, as factors that can influence each other in either direction, is emphasized and considered relevant for physicians treating patients with diabetes. Early recognition and treatment initiation of these symptoms is key as studies

show that gastrointestinal complaints impact work productivity and is associated with absenteeism[7] and effective treatment improves productivity[8].

The major part of this thesis focusses on the association between diabetes mellitus and colorectal cancer. Colorectal cancer is the third most common cancer in men (~10%) and the second in women (~9%) with a mortality rate of 8.3 per 100,000 person years. In 2011 in the Netherlands, the costs of colorectal cancer care accumulated up to 488 million euro which contributed to ~10% of all cancer related health care costs[9]. Considering these numbers, it may be said that colorectal cancer has major impact on healthcare costs and certainly on the population's health in general. Currently, the colorectal cancer incidence rates are stabilizing and are expected to decrease in countries where population based CRC screening has been implemented.
Studies confirm the effectiveness of screening of the average risk population with colonoscopy and performing polypectomy in preventing colorectal cancer[10]. Colorectal cancer screening has recently (2014) been implicated in the Netherlands and the definite yield in terms of colorectal cancer incidence and mortality reduction has yet to be observed by the Dutch cancer registries. Since screening and surveillance programs are costly in terms of money and workforce the cost-effectiveness should be hold in account continually. One way of increasing effectiveness of colorectal screening programs is by screening those individuals at higher risk for colorectal cancer.

In this thesis, a moderate increased prevalence of colorectal adenomas (chapter 6) and incidence of colorectal cancer (chapter 4) in T2DM was observed. In chapter 7 of this thesis, particularly young individuals with type 2 diabetes mellitus appeared at higher colorectal cancer risk compared to their peers without diabetes. In chapter 7, a young diabetic patient was defined by a diagnosis of diabetes before the age of 55 years, the age at which individuals are enrolled in the colorectal cancer screening program. An earlier initiation of CRC screening in individuals with type 2 diabetes mellitus may be cost-effective. In the discussion section of this thesis, based on current colorectal cancer crude incidence rates, as reported by the Netherlands Comprehensive Cancer Organization, a simple calculation showed that the crude incidence of colorectal cancer in men and women with diabetes aged 50 to 54 years is comparable with the colorectal cancer incidence in the general population aged 55 to 59 years. This comparability in CRC incidence suggests that the FIT test, used in the current screening program, could harbor the same diagnostic yield.

Defining a high(er) risk subpopulation to screen is one way of optimizing screening strategies. Another way is to improve the effectiveness of the instruments used in the screening program. For instance, in chapter 6 of this thesis we have documented that individuals with diabetes that underwent a colonoscopy more often had an inadequate bowel preparation as compared to controls without diabetes. Paying attention to risk factors associated with an inadequate bowel preparation in pre-colonoscopy interviews followed by simple adjustments may help to improve adenoma detection and polyp removal[11]. Moreover, expensive and time-consuming repetitions of colonoscopies may be prevented.

In the future, we may be able to focus even more on the risk of the individual. For instance, personalized risk-based colorectal screening for which questionnaires and biological samples can be used to collect data from large groups of the population. This data can be used to unveil risk factors, their risk factor interactions, and categorize individuals into predefined risk categories (e.g. high risk vs. intermediate vs. low risk).
Epigenetic markers can be used to define a part of the colorectal cancer risk profile. Numerous SNP's have been reported to be associated with increased risk of developing colorectal cancer. It is very plausible that other types of biomarkers, like serum proteins of hormones of interest will be found. In chapter 5 of this thesis we observed an increased colorectal cancer risk in individuals with a genetic variation in the IGF pathway, which was more pronounced in individuals with diabetes mellitus. Studies like the one in chapter 5 could revalue phenotypic risk factors by means of genetic profiling.
In terms of screening, a political trade-off is made between benefits (e.g. life years gained), harms (e.g. colon perforation) and costs. A study has pointed out in microsimulation models that screening based on risk stratification can particularly reduce harms. As false positives outcomes are consistently lower with this method[12]. On the one hand the costs of defining risk categories in screening can be expected to increase with available biodata. On the other hand, individuals needing diagnostic investigations, such as colonoscopies, are reduced up to ~25% according to one published model[13], which in turn could reduce the screening costs.

In summary, we should pursue a sustainable future health care that particularly promotes effective preventive measures instead of adhering to a narrow emphasis on cure. This thesis can add to the knowledge needed for effective early interventions or even prevent the gastrointestinal complications associated with diabetes mellitus.

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Etcetera

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Sander

Maastricht, mei 2019,

Curriculum vitae

Sander de Kort was born on March 26th, 1986 in Tilburg, the Netherlands. After completing secondary school (Gymnasium) at the Pauluslyceum in Tilburg, he studied medicine from 2004 until 2010 at the faculty of Health, Medicine and Life Sciences at Maastricht University in Maastricht, the Netherlands. In the final year of medical school he participated in a research project on gastrointestinal complaints in patients with diabetes mellitus at the Division of Gastroenterology and Hepatology at the Maastricht University Medical Center (supervised by dr. J.W. Kruimel and prof. dr. A.A.M. Masclee). After graduation in 2010 Sander started his residency in Gastroenterology and Hepatology combined with a PhD trajectory at the VieCuri Medical Center (supervised by dr. A.J. Luik) in Venlo, the Netherlands. From January 2012 to July 2013 he was detached as a PhD student at the Division of Gastroenterology and Hepatology, the Department of Epidemiology and the school for Oncology & Developmental Biology (GROW) at Maastricht University (supervised by prof. dr. A.A.M. Masclee, prof. dr. M.L.G. Janssen-Heijnen, prof. dr. M.P. Weijenberg and dr. S. Sanduleanu). In July 2013 he resumed his residency at the Maastricht University Medical Center (supervised by prof. dr. A.A.M. Masclee) up to May 2017 and completed his residency in December 2018 at the Zuyderland Medical Center (Heerlen, the Netherlands, supervised by dr. J.C.A. Keulemans) while working on the studies described in this thesis.
Currently, Sander works as a gastroenterologist at St. Anna Hospital in Geldrop, the Netherlands.

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