Integrative model of lifestyle effects on cancer via the HbA1c biomarker

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ABSTRACT

Background: Cancer and diabetes are the second and twelfth leading global causes of death, respectively. Cancer incidence is increased in diabetics compared to non-diabetics. Common pathobiological pathways are shared by the two diseases: hyperglycaemia, hyperinsulinaemia, chronic inflammation and altered concentrations of endogenous hormones. These pathways can all directly or indirectly be linked to chronic hyperglycaemia. Lifestyle factors also affect cancer, diabetes and hyperglycaemia.

Hypothesis: Chronic hyperglycaemia is the common biological pathway linking cancer, diabetes and lifestyle factors. Chronic hyperglycaemia can be assessed by monitoring glycated haemoglobin (HbA_{1c}) levels.

Aim: The first aim is to investigate whether the link between diabetes and increased cancer risk can be explained by increasing HbA_{1c} levels.

Secondly, glycaemic and overall models of lifestyle factors should be developed and compared to determine the relative influence of lifestyle factors on blood glucose level and, subsequently, cancer risk. This could clarify whether improved glycaemic control via lifestyle factors is sufficient to significantly reduce cancer risk.

Method: Dose-response meta-analyses on cancer risk and HbA_{1c} levels were performed and the results communicated via a research article.

Statistical glycaemic and overall models were developed from published studies on colorectal cancer (CRC), lifestyle factors and HbA_{1c}, via meta-analysis. Log-linear and restricted cubic spline models were considered for studies relating CRC risk to lifestyle factors or HbA_{1c}. Linear models were considered for studies relating HbA_{1c} to lifestyle factors. Only statistically significant models were compared.

Results: Increased cancer risk with increasing HbA_{1c} levels was present for a number of cancers, with some cancer types also showing increased risk in the pre-diabetic and normal HbA_{1c} ranges.

Comparison of the glycaemic and overall models revealed that HbA_{1c} significantly affected cancer risk and was significantly affected by lifestyle factors. However, the overall effects of lifestyle factors were much stronger than their glycaemic effects (between 9% and 25% difference in risk between overall effects and glycaemic effects at the exposure levels analysed). Glycaemic and overall models for cigarette smoking and chronic stress revealed increased cancer risk with increasing exposure, but decreased cancer risk for increased dietary fibre intake. The glycaemic model for alcohol consumption

displayed decreased cancer risk, while the overall model revealed increased cancer risk, emphasising the strong effect of carcinogenic substances in alcohol.

Conclusions:
Risk for a number of cancers increased with HbA_{1c} levels in diabetic and non-diabetic persons. Cancer prevention by improved blood glucose control seems plausible.

The overall effects of lifestyle factors on cancer risk are much stronger than their glycaemic effects. Lifestyle factors alone do not provide enough reduction in blood glucose levels. Other therapeutic strategies for reducing blood glucose levels, such as pharmacotherapeutics or fasting, should be investigated. The possible harmful effects of reducing blood glucose levels, such as neuroglycopaenia, should be considered before implementation of therapeutic strategies.

Although there seems to be a strong association between HbA_{1c} and cancer risk, this does not imply causality. The possibility of residual confounding cannot be ignored, even though the most adjusted estimates were used to develop the models, where possible.

Key words:
cancer prevention; colorectal cancer; lifestyle; dose-response meta-analysis; HbA_{1c}; hyperglycaemia

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PREFACE

This dissertation is presented in the form of two research articles, preceded by a consolidating discussion. The articles are provided as Annexures A and B of this dissertation. Both articles are co-authored by Prof. L. Liebenberg. Permission was obtained from the co-author to submit the articles for the purposes of this degree.

Some repetition is inevitable between the consolidating discussion and the articles. However, more detailed background information is provided in the discussion than can be provided in the articles.

A summary of the aims and outcomes of the two articles are provided in Table 1.

Table 1: Summary of aims and outcomes of research articles.

Title (*Journal*)	Aims	Outcomes
Article 1 [1] Does cancer risk increase with HbA_{1c}, independent of diabetes? (BJC)	1. To explore whether cancer risk increases with HbA_{1c}, independent of diabetes.	a. Chronic hyperglycaemia (measured by HbA_{1c}) correlates with increased cancer risk for colorectal, pancreatic, gastric and respiratory cancers in the normal and pre-diabetic ranges. b. Chronic hyperglycaemia (measured by HbA_{1c}) correlates with increased cancer risk for colorectal, pancreatic, gastric and liver cancers in the diabetic range, and breast cancer in the upper diabetic range. c. Chronic hyperglycaemia is possibly inversely correlated with risk for prostate cancer (from borderline significant results). d. There is evidence for decreased breast cancer risk in the normal, pre-diabetic and lower diabetic range from the statistically significant model, and possible evidence for increased risk in the normal and pre-diabetic ranges from the borderline significant model. **Conclusion:** Cancer risk increases with HbA_{1c}, independently of diabetes, for a number of cancer types. **Recommendations:** Exclude data points below the reference as it complicates interpretation of the results.
Article 2 [2]	1. Develop models relating HbA_{1c} to	1. Statistically significant increasing log-linear models were developed for the

Title (*Journal*)	Aims	Outcomes
Comparison of glycaemic and overall effects of lifestyle factors on colorectal cancer risk	colorectal cancer (CRC) risk. 2. Develop models relating lifestyle factors to HbA_{1c}. 3. Develop models relating lifestyle factors to CRC risk (overall). 4. Combine statistically significant models obtained in 1 and 2 above to get glycaemic models for lifestyle factors and CRC risk, via HbA_{1c}. 5. Compare glycaemic models to statistically significant overall models.	relations between HbA_{1c} and hazard ratio (HR), odds ratio (OR) and combined risk. Relative risk (RR) was not statistically significant and decreasing. 2. Statistically significant increasing linear models were developed for the relations between HbA_{1c} and glycaemic load (GL), cigarette smoking and chronic stress. Statistically significant decreasing linear models were developed for the relations between HbA_{1c} and alcohol consumption, dietary fibre and physical exercise. 3. A statistically significant decreasing log-linear model was obtained for dietary fibre (combined). Statistically significant increasing log-linear models were obtained for alcohol consumption (HR, OR and combined), cigarette smoking (HR and combined) and chronic stress (OR). 4. Decreasing log-linear glycaemic models were obtained for alcohol consumption (HR, OR and combined), dietary fibre (HR, OR and combined) and physical exercise (HR and combined). Increasing log-linear glycaemic models were obtained for glycaemic load (HR, OR and combined), chronic stress (HR, OR and combined) and cigarette smoking (HR, OR and combined). 5. A comparison could only be done on the statistically significant models – alcohol consumption (HR, OR and combined), dietary fibre (combined), chronic stress (OR) and cigarette smoking (HR and combined). a. Alcohol consumption decreased for glycaemic model, but increased for overall model; alcohol consumption decreases HbA_{1c}, but increases cancer risk overall. b. The decrease in risk as a result of dietary fibre is higher in the overall

Title (*Journal*)	Aims	Outcomes
		model (probably caused by mechanisms that decrease exposure of the colorectal system to carcinogens and the short chain fatty acids caused by fermentation of the fibre, which may have a protective effect), but the glycaemic model also has a decreasing effect as a result of lowering the HbA_{1c}. c. The increase in risk as a result of chronic stress in the overall model is higher than that in the glycaemic model. Different stress measures were used: job strain in the glycaemic model and perceived stress in the overall model. This could have affected the results. Chronic inflammation caused by the stress response could potentially also be responsible for the difference between the overall and glycaemic models. d. The increase in risk as a result of cigarette smoking is higher than that caused by the glycaemic model. The increase in blood glucose concentration caused by smoking is significant, but other mechanisms, such as the potentially carcinogenic chemicals contained in tobacco smoke and released during the burning of tobacco also has a strong effect on increasing risk. **Conclusions:** An opportunity for therapeutic intervention exists to decrease CRC risk by reducing HbA_{1c}, but additional therapeutic measures over and above lifestyle factors should be considered, as the glycaemic effects of lifestyle factors alone do not have a big impact

Title (*Journal*)	Aims	Outcomes
		on reduction of CRC risk. The potential of residual confounding cannot be excluded, although care was taken to include studies that account for the most confounding factors. **Recommendations:** 1. More dose-response studies should be performed to assess the associations between lifestyle factors, HbA_{1c} and CRC risk. 2. A unifying model, such as equivalent teaspoons sugar, should be used to convert the lifestyle factors to a single measure so that the glycaemic effects of the lifestyle factors can be compared on the same scale.

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ABBREVIATIONS

ADP	Adenosine diphosphate
AGE	Advanced glycation end products
Akt	Protein kinase B
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate-activated protein kinase
ATP	Adenosine triphospate
AUC	Area under the curve
CI	Confidence interval
CHO	Carbohydrate
CpG	Cytosine-phosphate-guanine
CRC	Colorectal cancer
CRCED	Centre for Research and Continued Engineering Development, North-West University
CRP	C-reactive protein
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
EEG	Electroencephalogram
FPG	Fasting plasma glucose
GI	Glycaemic index
GL	Glycaemic load
GLUT	Glucose transporter
HbA_{1c}	Glycated haemoglobin/glycosylated haemoglobin
HDAC	Histone deacetylase
HDL	High-density lipoprotein
HGF	Hepatocyte growth factor
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary-gonadal
HR	Hazard ratio
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor-binding protein
II	Insulin index
IL	Interleukin
LDL	Low-density lipoprotein
MET	Metabolic equivalent
MiRNA	Micro ribonucleic acid
mRNA	Messenger ribonucleic acid

NAD	Nicotinamide adenine dinucleotide
OGTT	Oral glucose tolerance test
OR	Odds ratio
RCS	Restricted cubic spline
ROS	Reactive oxygen species
RR	Relative risk/risk ratio
SCFA	Short-chain fatty acid
SD	Standard deviation
SE	Standard error
SHBG	Sex hormone-binding globulin
SNS	Sympathetic nervous system
STS	Short-term starvation
TCA	Tricarboxylic acid
TNF-α	Tumour necrosis factor alpha
VEGF	Vascular endothelial growth factor
VLDL	Very low-density lipoprotein

GLOSSARY

Adiponectin: A cytokine secreted by fat cells (adipocytes) [3]. Adiponectin could be related to cancer risk reduction, because of its anti-diabetic, anti-angiogenic and anti-inflammatory properties [3].

Adipose tissue: Connective tissue storing fat, mainly triglyceride [4].

Anaerobic respiration: Process whereby carbohydrate and other substrates are metabolised to produce ATP, without the use of oxygen [5].

Aerobic respiration: Process whereby carbohydrate and other substrates are metabolised to produce ATP, with the use of oxygen [5]

Angiogenesis: Growth of new blood vessels from existing blood vessels [6].

Antioxidant: A substance which prevents or significantly delays oxidation of a substrate [7].

Apoptosis: Programmed cell death [8].

Beta value: Summary slope estimate obtained from meta-analysis or trend estimation.

Biomarker: A biomarker is a molecule found in bodily fluids or tissues [9] that can be measured and used as a marker of a biological process, condition or disease [9], [10]. It can also be used to determine how well a therapeutic intervention works [9], [10].

Carcinogen: Any influence which leads to the conversion of a normal cell into a cancer cell [4], e.g. alcohol or tobacco smoke.

Chemiosmosis: The process whereby some membranes form ATP using a hydrogen ion gradient [5].

Colorectal: Relating to the colon and rectum (parts of the large intestine) [11].

Cortisol: A hormone, related to long-term stress, secreted by the adrenal cortex [5]**.**

Covariance: The covariance of two variables indicates whether they vary independently from each other. If the covariance of two variables is not zero, the two variables are not independent [12].

Cytokine: Protein secreted by cells that regulate immune function [13], for instance interleukin-6 (IL-6).

Diabetes: In this dissertation, *diabetes* refers to *diabetes mellitus*, a chronic disease characterised by high blood glucose levels. Four types exist: type 1 diabetes (caused when the pancreatic β-cells are destroyed, leading to insulin deficiency), type 2 diabetes (insulin resistance), gestational diabetes (during pregnancy) and diabetes caused by other specific causes (for instance chemically-induced during certain treatments) [14].

Dose-response: The relationship between a certain outcome (such as risk) and the exposure (or dose) producing that outcome [15].

Endogenous hormones: Hormones are "chemical messenger" molecules that are secreted into the blood and regulate physiological activity in other parts of the body [4], [5]. *Endogenous* refers to the fact that the hormones are produced naturally, inside the body, and not from outside (*exogenous*) sources.

Epidemiology: Study of the incidence and control of disease [11].

Epigenetics: Changes to the function of genes, independent of the DNA sequence [16]. These changes are hereditary (can be passed on to following generations) [16].

Ethanol: Ethyl alcohol; agent in alcoholic beverages that causes intoxication [11].

Gluconeogenesis: Production of glucose from non-carbohydrate sources (e.g. lactate) [17].

Haemodialysis: The process of removing unwanted molecules from blood, outside of the body (extracorporeal), by passing the blood through a semi-permeable membrane [18].

Heterogeneity: Variation [15].

Glutamine: Amino acid involved in cell metabolism and growth [19].

Glycated haemoglobin (HbA_{1c}): Glycated haemoglobin (HbA_{1c}) is a haemoglobin (respiratory pigment in red blood cells that contains iron [5]) produced during the condensation of glucose with haemoglobin A (the major haemoglobin in humans over the age of 6 months) [20].

Glycogen: Glucose is stored in the liver in a polysaccharide form, known as glycogen [5].

Glycogenolysis: Metabolism of glycogen to glucose [4].

Hepatic: "Relating to the liver." [11]

Hyperglycaemia: Elevated blood glucose level.

Hyperinsulinaemia: Elevated insulin level.

Incidence: Number of new cases of a disease that are diagnosed in a certain period (for instance a year) [21]. Incidence is also known as morbidity.

Insulin resistance: "a state where there is reduced biological effect for any given concentration of insulin." [22]

Insulin sensitivity: "the ability of insulin to exert its physiological effect on glucose, lipid and protein metabolism and to regulate cellular growth and differentiation and vascular function." [22]

Lipoprotein: A protein that binds with and transports lipids (fat) in the blood [11].

Macrophage: A macrophage is a phagocytic cell which removes cellular debris [23].

Meta-analysis: Combining results from two or more studies using statistical methods [15].

Metabolic equivalent (MET): The energy expenditure (or cost) of an activity is measured in metabolic equivalents (METs) [24]. One MET is equal to the amount of oxygen that is consumed when a person is at rest [24].

Metformin: Diabetes drug that reduces blood glucose levels [25].

Mitogen: A factor that activates mitosis (cell division) [11].

Mortality: Death.

Neoplasm: A neoplasm is a tumour, which can be benign or malignant [4].

Nicotinamide adenine dinucleotide: Biological molecule involved in energy metabolism, DNA transcription and repair [26].

Oncogene: A gene that causes cancer [5].

Oxidative phosphorylation: Occurs in the electron-transport system in the cell's mitochondrion during cell metabolism, and requires oxygen [5]. Energy in the form of ATP is released during this process [5].

Oxidative stress: Disruption in the equilibrium between the production of reactive oxygen species and anti-oxidants [7]. This can lead to tissue damage [7].

Pathobiology: Study of the biological mechanisms that cause disease [27].

Pathogenic: The capability of causing disease [28].

Phagocyte: A cell that consumes particles from the environment that surrounds it [4].

Pharmacotherapeutics/pharmacotherapy: The use of drugs for treatment [11].

Pre-diabetic: A person with an HbA_{1c} measurement in the range between 5.7% to 6.4%, and who are at a relatively high risk of developing diabetes [14].

Prevalence: The number of persons in a population diagnosed with a disease during a specified time in the past and who are still alive at the end of the year for which the prevalence statistics are provided [29].

Reactive oxygen species (ROS): Reactive oxygen species (free radicals) are by-products of the metabolism of oxygen by the mitochondrion [30].

Secretagogue: A substance that stimulates secretion (release of another substance) [11].

Substrate level phosphorylation: Energy in the form of ATP is produced by transferring a high-energy phosphate to ADP [5].

Warburg effect: Also known as aerobic glycolysis in cancer tissues. This phenomenon is observed across several tumour types and often occurs in parallel with marked increase in glucose uptake and consumption, regardless of oxygen availability [31], [32].

CHAPTER 1 INTRODUCTION

1.1 Introduction

This chapter provides the background to the research work that is performed in this study. This includes the background and motivation for the study, problem statement, aims, benefits and contributions of the study, the scope of the study, as well as the chapter layout for the dissertation.

1.2 Background

1.2.1 Cancer and diabetes statistics

Cancer is the 2nd leading cause of death worldwide [33]. In 2008, approximately 7.6 million deaths worldwide (approximately 13% of all deaths) occurred as a result of cancer [29]. Approximately 12.7 million new diagnoses of cancer were made in 2008. It is estimated that, by 2030, the annual incidence of new cancer cases will have risen to 21.4 million [34], and the annual cancer mortality worldwide will have risen to 11.5 million [35].

Colorectal cancer (CRC) is the third most common cancer diagnosed globally, contributing to more than 9% of new cancer cases [36]. In 2000, there were approximately 944,717 new CRC cases worldwide [37], and in 2002 slightly more than 1 million new cases [36]. Approximately 394,000 deaths occur annually as a result of CRC which causes the 4th most cancer deaths worldwide [36]. More than 63% of incident CRC cases occur in developed countries, likely as a result of the Western lifestyle [36].

Diabetes is the 12th leading cause of death worldwide [33]. In 2008, 1.3 million deaths worldwide were caused by diabetes [34]. It was estimated that the worldwide prevalence of diabetes in 2008 was approximately 10% for adults older than 25 years [34] and that the prevalence of adults living with diabetes had doubled between 1980 and 2008 [38]. The increase in diabetes prevalence is mainly (70%) caused by aging and population increase, but can also be attributed partly to unhealthy dietary habits and a sedentary lifestyle, caused by a shift towards a more Western lifestyle, and resulting in obesity [38].

1.2.2 Relationship between diabetes and cancer

It has been found that diabetics have an increased risk for some cancer types compared to non-diabetics [33], [39]. The associations between diabetes and cancer, ranked from the strongest to the weakest, are for endometrial (relative risk, RR = 2.1), liver (RR = 2.01), pancreatic (RR = 1.94) [33], [39], kidney (RR = 1.42), oesophageal (RR = 1.30), colorectal (RR = 1.26), breast (RR = 1.25) and bladder (RR = 1.24) [33] cancer, and leukaemia (RR = 1.22) [39]. Lung cancer is a cancer type for which diabetes does not seem to increase risk [33].

Contrarily, prostate cancer seems to be reduced in diabetics, compared with non-diabetics (RR = 0.84 [40]) [33], [39], [41]. Two hypotheses exist surrounding the protective effect of type 2 diabetes on prostate cancer. The first is that there is a reduction of androgen levels (such as testosterone) in diabetic men; as raised androgen levels are known to increase prostate cancer risk, the decrease in androgen levels results in a decrease in risk [40]. Levels of testosterone are inversely correlated with blood glucose levels [41], but are, contrarily, positively correlated with diabetes duration [42], [43]. Kasper *et al.* [43] hypothesised that, despite the positive correlation between testosterone levels and diabetes duration, the bioavailability of testosterone could be declining as the ratio of testosterone to sex hormone-binding globulin (SHBG) reduces over time. The second hypothesis surrounding the protective effect of diabetes on prostate cancer suggests that this may be due to a protective gene that is shared between diabetes and prostate cancer [40].

From the above discussion it is apparent that site-specific cancer risks should be investigated as the associations applicable to one cancer site might not be applicable for cancer at another site [33], [39].

Not only does diabetes increase incidence of certain cancers, but mortality rates in some cancer patients with comorbid diabetes are also higher [33], [39]. This might be as a result of the "Warburg effect" (which is discussed in more detail in Chapter 2), according to which certain cancer cells are more dependent on glycolysis, a less effective method of producing energy than oxidative phosphorylation [18].

1.2.3 Possible common pathobiologic pathways between diabetes and cancer

Diabetes and cancer share a number of possible risk factors [33], [39]: modifiable (such as obesity and lifestyle factors) and non-modifiable (such as age, gender and race/ethnicity). Some biological pathways are also shared: hyperglycaemia, hyperinsulinaemia, chronic inflammation [33] and altered concentrations of endogenous hormones [39]. These biological pathways can all be linked directly or indirectly to hyperglycaemia (more detail in Chapter 2). Certain lifestyle factors can also be linked to hyperglycaemia (more detail in Chapter 3).

1.2.4 Lifestyle factors

Lifestyle factors such as cigarette smoking, unhealthy diets, heavy alcohol consumption and a sedentary lifestyle increase the risk of cancer incidence and mortality considerably [38]. By avoiding unhealthy lifestyles such as smoking, and adopting a healthy diet and regular physical activity, at least 40% of cancers and 80% of type 2 diabetes, heart disease and stroke could be circumvented [38].

CRC is strongly affected by dietary factors; it is estimated that 70% of CRC cases could have been avoided by adopting healthier dietary habits [36]. It has been stated that excess body weight and a

sedentary lifestyle contribute to approximately ¼ to ⅓ of CRC cases [36]. Cigarette smoking causes approximately 12% of CRC deaths [36].

Under the global burden of disease risk factor ranking for 2010 [44], smoking ranked second, alcohol use third, high fasting plasma glucose seventh, physical inactivity 10th and low dietary fibre intake 24th as lifestyle factors causing a high burden of disease. High fasting plasma glucose accounted for 3.4 million deaths in 2010 [44]. Low and no physical activity was responsible for 3.2 million deaths [44]. 6.3 million deaths could be accounted for by smoking and second hand smoking [44]. It is estimated that 71% of lung cancer [34] (as well as trachea and bronchus cancer [33]) and 10% of cardiovascular disease (CVD) cases are caused by tobacco smoking. Alcohol use contributed to 4.9 million deaths in 2010 [44].

It is shown in Chapter 3 that lifestyle factors can be linked to changes in blood glucose levels (either decrease, for instance when a person exercises physically, or increase, when a person eats carbohydrate-rich food).

1.2.5 Hyperglycaemia and glycated haemoglobin (HbA_{1c})

Hyperglycaemia is a possible direct and indirect risk factor for cancer incidence and mortality. Hyperglycaemia is also the major hallmark of diabetes, and can be used to diagnose the condition. Diabetes diagnosis can be performed using the fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), a random plasma glucose measurement (with glucose level ≥ 200 mg/dl in conjunction with hyperglycaemic symptoms) or via glycated haemoglobin (HbA_{1c}) measurements [14].

The biomarker that will be used to assess hyperglycaemia in this dissertation is HbA_{1c}. HbA_{1c} is a marker of the average blood glucose concentration for a prolonged period preceding the test, typically 6 weeks to 3 months - the lifespan of the erythrocytes that contain the haemoglobin required for the test [39], [45], [46]. The advantages of using HbA_{1c} compared to FPG or OGTT are that HbA_{1c} is not sensitive to daily variations [39], such as stress, illness [14] or recent meals [47] and is more reliable when repeated measurements are performed [45]. It is also more convenient as it is not necessary to fast before testing HbA_{1c} [14], [45], [47].

Disadvantages of HbA_{1c} include the higher cost of performing the test, as well as restricted accessibility in some developing countries [14]. Further to this, some individuals may not have a complete correlation between mean blood glucose and HbA_{1c}, and levels may vary according to race/ethnicity [14]. Despite these disadvantages, HbA_{1c} may provide a significant indication of the average blood glucose level over the previous 6 weeks to 3 months, and therefore chronic hyperglycaemia over an extended time.

HbA_{1c} could possibly also be used as an indirect marker of hyperinsulinaemia [48] or average blood insulin levels [49] in non-diabetics, as elevated blood glucose induces elevation of insulin levels to counteract the blood glucose concentration. This secretagogue association may, however, be affected by insulin resistance (in type 2 diabetes) and diminished insulin production (as a result of pancreatic β-cell depletion in type 1 diabetes) [49]. A more sensitive indicator of average insulin is required to evaluate the effect of hyperinsulinaemia on cancer risk [49]. This is, however, beyond the scope of this dissertation.

Several methods exist for measuring and referencing HbA_{1c}. These include the National Glycohemoglobin Standardization Program (NGSP) in the United States (which uses the Diabetes Control and Complications Trial, DCCT, reference method [50]), the Mono-S standard in Sweden, the Japan Diabetes Society (JDS) reference in Japan and the newer International Federation of Clinical Chemistry (IFCC) standard. With the publication of the DCCT in 1993, large variability existed in the results obtained by different laboratories [50]. The implementation of the NGSP, JDS and Mono-S standards have reduced this variation, and allowed better comparability between results obtained from different laboratories using the same reference standard [50]. The IFCC published a reference method for pure HbA_{1c} measurement in 2002 [51], compared to the other methods which are based on more than one glycated haemoglobin [52]. There are linear relationships between the IFCC method and the NGSP, Mono-S and JDS methods, and comparisons are, therefore, possible between results obtained from the IFCC method, and results from the other three methods [50]. However, the existence of a number of different standards could cause confusion in the medical community and in patients receiving treatment, especially if results are reported using the same units. The IFCC results are also much lower (approximately 1.3% to 1.9%) than the NGSP results [50]. To help overcome the confusion between IFCC and NGSP results, the IFCC in 2007 recommended that HbA_{1c} measured using the IFCC reference standard be reported in mmol HbA_{1c} per mol Hb (mmol/mol), instead of the % units used by the NGSP standard [53].

Journal articles do not always indicate which reference method was used. In this dissertation all HbA_{1c} values were assumed to be referenced to the NGSP standard. It must be noted that this could have caused an incorrect estimation and comparison of values and could be considered a confounding factor.

Diabetes can be diagnosed if the HbA_{1c} value (using the NGSP reference) is $\geq 6.5\%$ [14]. Pre-diabetes is deemed to exist if HbA_{1c} levels are in the range of 5.7% to 6.4% [14]. In this range the risk of developing subsequent diabetes is high. Hypoglycaemia is deemed to exist if an HbA_{1c} level below approximately 4.07% [14] (3.9 mmol/l) exists. Normal glycaemia is, therefore, between approximately 4% and 5.7%.

1.2.6 Hypothesis

Based on the preceding discussion and the expanded discussions in Chapters 2 and 3 it is hypothesised that chronic hyperglycaemia can be used as a common biological pathway linking diabetes, cancer and lifestyle factors.

Evidence of an increased risk for cancer as a result of hyperglycaemia (measured using HbA_{1c}), independent of diabetes, will be investigated. This might provide insight into whether people with a slightly elevated, but normal glycaemic range, or pre-diabetes, also have an increased risk for cancer, or whether the increased cancer risk is only seen for diabetics. This could provide evidence for further advantages of increasing glycaemic control.

Further to this, models will be developed that link the glycaemic effects of lifestyle factors to cancer risk and these will be compared to the overall effects of lifestyle factors on cancer risk to test the hypothesis of hyperglycaemia as the common link. The models will be developed specifically for CRC as this type of cancer is known to be closely linked to a Western lifestyle, and a large amount of data is available. However, these models can be easily expanded to other cancer types if enough published data is available.

1.3 Motivation for study

1.3.1 Related work

Two previous research projects focused on related work.

Espach [54] investigated the effects of lifestyle factors on breast cancer, coronary heart disease (CHD) and inflammation, using the equivalent teaspoons sugar (—*ets*) concept. This unit was originally developed to quantify the blood glucose effects on carbohydrate intake. The unit was subsequently expanded to include models for alcohol intake, stress [55], [56], exercise, dietary fibre intake and smoking [54]. The unit is used to compare the effects of different lifestyles on the same scale.

Espach [54] found that blood glucose levels (in the form of —*ets*) were increased by stress, smoking and excessive food intake and that this subsequently resulted in increased risk for breast cancer, CHD and inflammation. It was found that dietary fibre intake, moderate alcohol consumption and low to moderate intensity physical exercise decreased blood glucose levels. Decreased blood glucose resulted in decreased systemic inflammation, and decreased risk for breast cancer and CHD. The effects of smoking and alcohol consumption on breast cancer risk, and the effect of smoking on inflammation, were not shown. It was indicated that smoking had an insignificantly small effect on breast cancer risk and that the moderate alcohol consumption data for breast cancer was inconsistent.

Further investigation into the methods that were used by Espach revealed some assumptions that were made to perform the work. Risk data from all studies for a particular lifestyle factor were summed to get a single linear trend. This method is limited, as the following factors were not accounted for:

1. Odds ratio (OR) and hazard ratio (HR) values were considered to be estimates of relative risk/risk ratio (RR) and, therefore, all were compared on the same scale without transformation. This method is commonly used in published literature, under an assumption that is called *the rare disease assumption*. This assumption is based on the fact that the difference between the OR and RR is not large when the incidence of the disease in the study population is lower than approximately 10% [57]. This is a convenient assumption, but not accurate, as the OR overestimates the RR. It would be more accurate to consider the three types of risks separately or transform them to a common measure. However, it is possible that enough information on HR, OR and RR may not be available to develop separate models and that it may be required to combine the risks to be able to compare the results with published studies.
2. The risks within a study are not independent, as all risks depend on a common reference group per study and therefore the covariance matrix will not be zero [58], [59]. If independence is assumed, the standard error (SE) of the calculated slope for the study will be underestimated, leading to overestimation of the study's weight [59].
3. The reference groups for all studies are not necessarily at zero exposure, producing an intercept term in the model.
4. There is heterogeneity between studies which must be accounted for.
5. Not all studies carry the same weight or importance.
6. The trend will not necessarily be linear. It is mentioned that this assumption was made to simplify the interpretation of results and that a higher order polynomial would have provided a better fit to the data [54]. Investigation of nonlinear trends might be beneficial.
7. The blood glucose effects of the lifestyle factors were determined by converting them to —ėts, but the RRs for the original lifestyle factors were still used. This method assumes that the increase or decrease in risk depends solely on the increase or decrease in blood glucose. This method may be incorrect as other factors (for instance hormonal regulation or carcinogenic substances in cigarette smoke or alcohol) may play a role in increasing or decreasing the risk; the assumption may, therefore, be overestimating the risk of the glycaemic effect.

A study by Laubscher [60] investigated the relationship between blood glucose and CVD. First he attempted this by simulating a person with type 2 diabetes consuming different amounts of carbohydrates (between 25 and 45 —ėts per day) using the Diabetic Toolbox simulation software. He calculated the mean blood glucose values per day using this software and converted the mean blood glucose to HbA_{1c}.

He then compared the data to a published study on HbA_{1c} and CVD risk. He found that the increase in CVD risk was far less than expected.

Laubscher [60] proposed the following possible reasons for this observation:

1. "The CVD risk factors obtained from Kay Thee Khaw cohort study can be too conservative in the sense that the HbA_{1c} percentage can play a greater role in the risk factors of CVD." [60]
2. "Mean blood glucose levels are not affected in such a way as to increase the risk of CVD significantly due to *ets* intake." [60]
3. "Mean blood glucose levels are not accurate predictors of CVD risk. It is possible that the effect that mean blood glucose levels have on the risk factors, like blood lipids and blood viscosity, will be a better indication of the risk of CHD." [60]

Laubscher [60] proceeded with a second study to link —ets (from carbohydrate intake, exercise energy expenditure and stress) to CVD risk using data from published trials. He found that an increase in carbohydrate intake caused an increase in CVD risk, an increase in stress caused an increase in CVD risk and an increase in exercise caused a decrease in CVD risk.

The first study by Laubscher [60] identified the discrepancy between the glycaemic effects and the overall effects of the lifestyle factors, but did not further investigate this discrepancy. He also recommended that the link between —ets and other metabolic diseases (such as cancer and stroke) be investigated.

1.3.2 Systems biology and the engineering approach

Although the engineering and medical/biological fields both involve problem solving, the approaches used differ [61].

Engineers typically use mathematics to construct models and test their validity with different data sets [61]. In developing these models engineers are likely to make assumptions in terms of system behaviour. These assumptions may not be the best representation of real world behaviour, but reduce the complexity of the model [62]. These models also take into account the ranges or states in which the model is valid. They provide a high-level or systems level approach, rather than focusing on the details of each component of the system being modelled; focusing on function, rather than form. This approach is limited as the assumptions that are made, might reduce the model's validity in practice.

In the medical/biological fields, output is more difficult to predict and heterogeneous [63], and statistical models are usually derived from empirical studies [61]. There is more reliance on in-depth knowledge of

the components of the system, and the approach is aimed at understanding the system as a sum of its components (a reductionist approach).

The approach used in the medical/biological fields has been responsible for a great number of breakthroughs, including the initial mapping of the human genome [64]. However, this approach has some limitations:

1. The functions of individual components are known, but not the interaction between components [64].
2. The system is viewed as the sum of its components; however, biological systems are more than just the sum of their individual components [64].
3. This approach leads to treatment or therapeutic solutions which are specific to a disease, but may not be transferable to other strains or forms of diseases.

With the great amount of information available on a molecular and gene level, one would expect more breakthroughs in cancer research [63], but the limitations of the reductionist approach could be delaying progress. Therapies at a gene level possibly overcomplicates the solution and results in a vast number of combinations which must be accounted for. A large number of cancer types at different sites are not all affected by the same gene mutations and, subsequently, therapy at a gene level will differ.

A systems level approach (such as systems biology) is required to understand the system as a whole – and as more than just a sum of its components [64]. This will hopefully lead to identification of a possible common cause for different cancers and lead to better understanding on how to control it. It should be acknowledged that curing cancer might not be the solution; controlling cancer could be a more viable goal [63].

Engineers could contribute to this process by applying their knowledge of physics and mathematics to modelling biological systems, and perhaps finding simpler solutions than the ones currently available. A hierarchical approach [62] incorporating both crude engineering models and detailed medical/biological knowledge for validation/verification of the practical applicability of the models could be used synergistically to obtain a solution.

In this study, the focus is on a common biological pathway between cancer and diabetes – chronic hyperglycaemia. It is hoped that this common pathway could also shed more light on the commonalities between different cancer types, and lead to simpler therapeutics for cancer prevention and control, by focusing on cancer cell metabolism.

1.3.3 Problem statement

From the preceding discussion in the Background section, it is clear that cancer incidence (morbidity) and mortality is rising and that the risk for cancer incidence is increased in the diabetic population. The avoidance of harmful lifestyles and adoption of healthy ones could reduce the burden of cancer and diabetes considerably. It is hypothesised that the increased risk is caused by the common pathway of chronic hyperglycaemia linking cancer and diabetes, and that HbA_{1c}, as a biomarker for chronic hyperglycaemia, can be used to establish cancer risk. The effects that lifestyle factors have on blood glucose levels (measured using HbA_{1c}), and the effect that HbA_{1c} in turn has on cancer risk, will also be investigated. Methods to expand on the work done by Espach [54] and Laubscher [60] and address the limitations in estimating disease risk as a result of the glycaemic effects of lifestyle factors are be addressed in Chapter 5.

The following problems are addressed in this study:

1. Can HbA_{1c}, as a biomarker for hyperglycaemia, predict increased cancer risk in diabetics and non-diabetics, independent of diabetes?
2. Models are developed that quantify the glycaemic effects of lifestyle factors on cancer risk, accounting for the limitations and expanding on the work of Espach [54] and Laubscher [60].

1.4 Aims, benefits and contributions

The aims of the study are to address the following issues and, in doing so, expand on work done by Espach [54] and Laubscher [60]:

1. Investigate whether cancer risk increases with increasing HbA_{1c}, independent of diabetes.
2. Combine results from published studies on the association of HbA_{1c} with different RR, OR and HR values, taking into account that different studies may have different importance/weighting, HbA_{1c} reference groups, and may be heterogeneous. CRC will be studied as a large number of published studies are available on this type of cancer and it is known to be associated to lifestyle factors. The same method can be used to develop models for other types of cancer.
3. Combine results from different studies on the effects of lifestyle factors on HbA_{1c}.
4. Combine the results of 2 and 3 to establish models of the glycaemic effects of lifestyle factors (via HbA_{1c}) on CRC risk.
5. Use the method in 2 to combine results from different studies on the association of several lifestyle factors with different RR, OR and HR values, taking into account that different studies may have different importance/weighting, lifestyle factor reference groups, and may be heterogeneous.

6. Compare the relative contribution of the glycaemic effects of lifestyle factors to CRC risk via HbA_{1c} (models obtained in step 4) to the overall effect of the lifestyle factors on CRC risk (results obtained in step 5) to determine the relative contribution of the glycaemic effects.

The following people or groups will potentially benefit from the research:

1. Cancer researchers:
   a. The development of the models will ensure that researchers can quantify the glycaemic effects of lifestyle interventions on cancer.
   b. The outcome of the proposed research will show researchers whether increased cancer risk is already present in non-diabetics with somewhat elevated blood glucose, or in diabetics with good glucose control.
   c. Researchers will be informed whether it is warranted to update recommendations for stricter glycaemic control in persons with diabetes and pre-diabetes to reduce the risk for cancer incidence.
2. General public:
   a. The general public will better appreciate what effects lifestyle interventions can have on their blood glucose levels and how these interventions could protect against cancer incidence.
   b. The general public will better appreciate whether there is already increased cancer risk if they are not diabetic and whether there is a good reason to implement stricter glycaemic control if they are diabetic or pre-diabetic.
3. North-West University (CRCED):
   a. The journal articles produced as output of this study will further the research output of the CRCED and provide further exposure of the CRCED in the biomedical field.

1.5 Scope of study

The study will focus on the issues discussed in paragraphs 1.2 and 1.3. The focus will be on cancer incidence and the possibility of preventing cancer by implementing certain lifestyle interventions. Cancer mortality may be mentioned in some instances, but the aim is not to assess the effects of lifestyle factors on cancer mortality, but rather on cancer morbidity. Although related work on the matter has focused on the development and use of the equivalent teaspoons sugar (*ets*) models, these models will not form part of this study.

1.6 Layout of dissertation

The main results from this dissertation will be communicated via two research articles (currently in review), available as Annexures A and B. The rest of the dissertation will provide more detailed

background information on the subject matter covered by the articles, as well as some additional results which could not be accommodated in the articles.

The structure of the dissertation is as follows:

1. Chapter 1: Background and introduction, including an overview on cancer and diabetes statistics, the pathogenic relationship between diabetes and cancers, common pathways between the diseases, lifestyle factors, and chronic hyperglycaemia. This chapter also provides a review of two previous related studies and the possibilities of expanding on these studies, as well as the problem statement, aims, benefits and contributions and an overview of the scope and layout of the dissertation.
2. Chapter 2: Provides background information on cancers, energy metabolism of normal and cancer cells, the risk factors for development of cancers that can be related to blood glucose and potential treatment options for cancers relating to reduction of systemic blood glucose levels.
3. Chapter 3: Provides information on the links between various lifestyle factors (i.e. excessive carbohydrate intake, chronic stress, cigarette smoking, dietary fibre intake, alcohol consumption and physical exercise) and blood glucose levels.
4. Chapter 4: Provides information on the search strategy used for data collection, the criteria used for inclusion and data extraction, as well as references to the tables of data that were collected.
5. Chapter 5: Elucidates the statistical methodology employed for the combination/meta-analysis of studies to develop the models, as well as the validation and verification of the software.
6. Chapter 6: Provides and contextualises the results.
7. Chapter 7: Provides conclusions on the work performed and makes recommendations for future work.
8. Annexure A: Article 1 - "Does cancer risk increase with HbA_{1c}, independent of diabetes?" This article investigates quantitatively and qualitatively using data from published studies whether there is evidence of increased cancer risk for several types of cancer with increasing HbA_{1c} level, independent of diabetes status.
9. Annexure B: Article 2 - "Comparison of glycaemic and overall effects of lifestyle factors on colorectal cancer risk". This article provides the results from the developed models on the glycaemic effects of several lifestyle factors on CRC risk, via the HbA_{1c} biomarker, and compares these effects to the overall contributions from the lifestyle factors.
10. Annexure C: Tables of data that were collected during the search for articles.
11. Annexure D: Tables of results that complement the results contained in Chapter 6.

1.7 Conclusion

This chapter provided the background information and motivation for the current study. The aims for the study, which serve as guidelines for the rest of the study, were discussed. The scope of the study and layout of the dissertation were also provided.

Hyperglycaemia (measured by HbA_{1c}) will be investigated as the common factor linking diabetes, cancer and lifestyle factors, and models will be developed to investigate the effects of HbA_{1c} on lifestyle factors and cancer. The models will specifically be developed for CRC, but could potentially be expanded to other cancer types. The following chapter will provide insight into the development of cancer, risk factors for cancer that can be linked to hyperglycaemia, as well as potential therapeutic options linked to control of hyperglycaemia.

CHAPTER 2 CANCER

2.1 Introduction

Cancer is a chronic disease that presents itself as a cellular growth disorder in various different forms and organs. The main view on the development of cancer is that it is caused by mutations of cell cycle regulating genes [5], including mutations to genes encoding enzymes involved in the DNA repair system, proto-oncogenes (proteins stimulating the cell cycle), tumour-suppressor genes (proteins which inhibit the cell cycle) and telomeres.

Proto-oncogenes and tumour-suppressor genes regulate the cell cycle [5] by stimulation and inhibition of cell growth. When proto-oncogenes mutate, they can become oncogenes, which cause uncontrolled growth [5]. An example of an oncogene is the *ras* gene family [5]. When tumour-suppressor genes mutate, they can cause uncontrolled growth, as growth inhibition (apoptosis, programmed cell death) is not active [5]. An example of a tumour-suppressor gene is *p53* [5].

Telomerase (an enzyme in cancer cells) inhibits shortening of telomeres [5]. Telomeres are DNA segments occurring at the ends of chromosomes which shorten prior to cell division [5]. The shortening of the telomeres signal the cell to stop dividing after a number of replications [5]. If the telomeres do not shorten, cells will continue to divide and tumours will grow [5].

Mutation of genes can be caused by carcinogens such as chemical substances (tobacco smoke, asbestos), alcohol, ultraviolet light, ionising radiation and certain types of bacterial, viral or parasitic infections (for example human papilloma virus and hepatitis B).

Risk for some types of cancer is increased in some people as the result of genetic factors, as well as environmental exposure [65]. Another proposed view on the causation of cancer is that of impaired mitochondrion function, and, therefore, impaired energy metabolism, which is discussed later in this chapter.

Epigenetic changes can also cause many cancers to develop and progress [16]. These changes include DNA methylation, histone modification and microRNA (MiRNA). DNA methylation involves adding a methyl group to the cytosine nucleotide in DNA [66]. CpG (cytosine linked to guanine via phosphate) islands are sites where the majority of CpG groups are not methylated [66]. Methylation of these sites controls gene expression; hypermethylation of tumour suppressor genes can inactivate them [66]. Histone modification includes methylation, acetylation and phosphorylation of core histone N-termini [67]. These modifications lead to dysregulated oncogene and tumour-suppressor gene expression, and affect the stability of the genome [67]. MiRNA regulates the expression of mRNA [67] and can degrade

or suppress mRNA expression [66]. MiRNA dysregulates the expression of oncogenes and tumour-suppressor genes [67].

There is evidence that environmental factors (alcohol, tobacco, obesity, physical activity, energy restriction and intake of dietary fibre) could affect epigenetic changes [66]. There is emerging evidence that changes in energy metabolism and intermediary metabolites can also cause epigenetic changes [16]. The metabolism of dietary fibre produces butyrate, a type of short-chain fatty acid (SCFA), which inhibits histone deacetylases (HDAC) [16]. This inhibition stimulates apoptotic and anti-proliferative genes, which can help in cancer control [16]. It is not yet evident, however, if the fermentation of dietary fibre can produce a high enough concentration of butyrate to produce this action [16]. The ketone body β-hydroxybutyrate produced during fasting, exercise or ketogenic diets, acts in a similar way than butyrate [16]. Ketogenic diets and fasting are emerging therapeutic strategies for cancer control. These therapeutic measures also affect glucose metabolism and are discussed in section 2.6.

Epigenetics can influence diseases, such as diabetes, where macrovascular complications could still affect diabetic patients with good long-term glycaemic control, after several years of being diagnosed with the disease. This phenomenon is called "glycaemic memory", and it is thought that chronic inflammation might be partially responsible [16].

Some studies on the relation between nutritional factors and hereditary epigenetic factors have been performed, but more mechanistic studies are required [16]. These preliminary studies suggest that epigenetic changes caused by nutritional factors are hereditary [16]. Understanding the links between environmental, nutritional or metabolic factors and epigenetic changes, could lead to new therapies [16], especially since epigenetic changes are reversible [67].

The process of cancer development and growth (carcinogenesis) is as follows [5]:

1. Cells mutate as a result of carcinogens and more cells start to grow. These cells typically form a tumour.
2. The tumour is initially localised ("cancer in situ").
3. The cancer cells then invade blood and lymph vessels and spread to secondary locations.
4. Secondary tumours a distance away from the primary tumour, also known as metastases, occur.

Hanahan and Weinberg originally proposed the following six hallmarks of most cancers that distinguish cancer cells from normal cells [5], [6], [65]:

1. Cancer cells sustain and induce growth signalling.
2. Cancer cells are insensitive to signals that impede growth.
3. Cancer cells resist programmed cell death (apoptosis).

4. Cancer cells can replicate without limit compared to normal cells which can only enter the cell cycle approximately fifty times [5]. This might be as a result of telomerase expressed in cancer cells.
5. Cancer cells can create their own blood supply by angiogenesis.
6. Cancer cells invade other tissues via a process called metastasis.

Two additional emerging hallmarks were subsequently identified [6], [65]:

1. Cancer cells can change energy metabolism. There is proof that mitochondria in tumour cells are abnormal in function and structure, and can, therefore, not produce normal energy levels [31]. This typically leads to the "Warburg effect" (discussed in more detail in section 2.3). It is proposed in [31] that the mitochondrial dysfunction could actually be the cause of cancer. If that were true, strategies to prevent the dysfunction can be employed to prevent cancer. This could include avoiding substances that promote systemic inflammation (such as cigarette smoking, obesity and excessive alcohol consumption).
2. Cancer cells can avoid immune surveillance.

It is hoped that one of the outcomes of this dissertation will be exploitation of the characteristic change in energy metabolism (specifically glucose metabolism) by reducing the amount of blood glucose available to cancer cells. The latter could be achieved by the implementation of beneficial lifestyle changes and the reduction of harmful lifestyles.

To understand the change in glucose metabolism in cancer cells, an understanding of the normal glucose metabolism in human cells is necessary. This is briefly outlined in section 2.2. Thereafter, the altered glucose metabolism in cancer cells is discussed (section 2.3). The risk factors relating to cancer incidence (via hyperglycaemia) are discussed in section 2.4. Section 2.5 presents background on the epidemiology of CRC (for which models will be developed). Section 2.6 presents potential treatment/therapeutic options for controlling cancer incidence and mortality by limiting glucose availability.

2.2 Glucose metabolism in normal cells

Two methods of energy metabolism are available to cells: aerobic respiration (in the presence of oxygen) and anaerobic respiration (in the absence of oxygen) [68]. Aerobic respiration is the preferred method as it produces the greatest amount of adenosine triphosphate (ATP) molecules. If cells are deprived of oxygen (hypoxia), they will enter a state of anaerobic respiration.

2.2.1 Aerobic respiration

Energy in the form of ATP is produced during the respiration process. During aerobic respiration 36 ATPs are generated from one glucose molecule [68]. The aerobic respiration process can be divided into four processes: glycolysis, pyruvate oxidation, the Krebs cycle and the electron-transport system.

I. Glycolysis (substrate level phosphorylation)

Glycolysis takes place in the cell's cytoplasm. Carbohydrates are made up of monosaccharaides (glucose, fructose and galactose) [68]. Fructose and galactose can be converted to glucose in the liver [69]. During the process of glycolysis, glucose is converted to pyruvic acid (pyruvate), with the release of energy in the form of ATP. The reaction is described by equation (1) [68].

$$glucose\ (C_6H_{12}O_6) + 2\ ATP + 2\ NAD^+ \rightarrow 4\ ATP + 2\ NADH + 2\ pyruvic\ acid \quad (1)$$

A net of 2 ATPs results from this process → 2 ATPs are required to perform the process and 4 ATPs are formed during the process. The pyruvic acid is transferred to the cell's mitochondrion for further processing in the Krebs cycle. NAD^+ transports some of the hydrogen and electrons released during the process to the electron-transport system in the form of NADH.

II. Pyruvate oxidation (substrate level phosphorylation)

The pyruvic acid created during glycolysis is transformed to acetyl coenzyme A (acetyl-CoA) [68]. This is performed in the mitochondrion of the cell and the reaction is described by equation (2) [68].

$$pyruvic\ acid + NAD^+ + Coenzyme\ A\ (CoA) \rightarrow CO_2 + NADH + acetyl\text{–}CoA \quad (2)$$

The acetyl-CoA will now enter the Krebs cycle.

III. Krebs cycle

The Krebs cycle (also called the tricarboxylic acid, TCA, cycle or citric acid cycle) is performed in the cell's mitochondrion [68]. One ATP is formed per acetyl-CoA molecule. The Krebs cycle runs twice, once for each pyruvic acid molecule (and subsequently each acetyl-CoA molecule) formed during glycolysis. Some of the hydrogen and electrons are transported to the electron-transport system in the form of NADH and $FADH_2$. The reaction is described by equation (3) [68].

$$acetyl\text{–}CoA + ADP + 3\ NAD + FAD \rightarrow CoA + ATP + 2\ CO_2 + 3\ NADH + FADH_2 \quad (3)$$

IV. Electron-transport system and chemiosmosis (oxidative phosphorylation)

The electron-transport system produces 32 ATPs in the cell's mitochondrion. Oxygen required for this process is obtained from the environment. The reaction is described by equation (4) [68].

$$32\ ADP + 10\ NADH + 2\ FADH_2 + 6\ O_2 \rightarrow 32\ ATP + 10\ NAD^+ + 2\ FAD + 12\ H_2O \quad (4)$$

2.2.2 Anaerobic respiration

In the absence of oxygen, certain cells can undergo anaerobic respiration. The anaerobic respiration process consists of glycolysis and lactic acid formation. When oxygen becomes available, lactic acid can be converted back to glucose. An example of when anaerobic respiration takes place is during intense physical exercise when insufficient oxygen is available to the cells.

I. Glycolysis

The glycolysis process described in section 2.2.1(I) is followed. A net of 2 ATPs is generated per glucose molecule. As oxygen is not available, the pyruvate (pyruvic acid) will not be oxidised and will not enter the Krebs cycle. The pyruvate formed during glycolysis is converted to lactic acid (lactate) [68] with the help of NADH. NAD^+ is formed. This process reduces the NADH levels (required for oxidative phosphorylation) and increases NAD^+ levels, which are essential for glycolysis to continue [68], [69].

II. Cori cycle

The process by which lactic acid can be reused is called the Cori cycle [70]. Lactic acid diffuses out of the cell, into the bloodstream and travels via the bloodstream to the liver. In the liver, the excess lactic acid is transformed to pyruvic acid, which can be converted to glucose. The glucose diffuses back to the bloodstream. ATP is required to convert the pyruvic acid to glucose. The process of converting lactic acid to glucose is called gluconeogenesis.

2.3 Glucose metabolism in cancer cells ("Warburg effect")

The "Warburg effect", named after its discoverer, German physiologist Otto Warburg, is the effect where glycolysis is increased in certain cancer cells, while oxidative phosphorylation is decreased [18], [69]. The "Warburg effect" is present whether oxygen is available or not [18], [31]. This is as a result of overexpression of glycolytic enzymes in most cancers [31], [32], [69], [71], as well as of glucose transporters [71], [72] in cancer cells, caused by genetic and physiological effects. In most tumour cells, HIF-1α (hypoxia-inducible factor-1α) is also elevated (in the presence or absence of hypoxia) which could lead to aerobic glycolysis [31].

The genetic effect on cancer metabolism is as a result of DNA mutations. The physiological effect is by way of the characteristic hypoxic environment of most tumours, where oxygen is not available to continue with the Krebs cycle and oxidative phosphorylation. The tumour environment becomes hypoxic as the tumour outgrows its blood supply [71]. Interestingly, Wu *et al.* [73] demonstrated that nutrient deprivation with Hank's buffered salt solution (HBSS) also caused the "Warburg effect". It would, therefore, seem that the "Warburg effect" is induced when the cell environment is not favourable for normal cell metabolism. This increases cancer cell proliferation even in adverse conditions [73].

Epigenetic changes could possibly also induce the "Warburg effect". FBP1 (fructose-1,6-biphosphatase-1) decreases glycolysis [74]. It has been shown that hypermethylation (and, therefore, inactivation) of FBP1 in gastric cancer patients led to a poor outcome. It is not clear whether these changes are also present in other cancer types [74].

It can, therefore, be seen that most cancer cells are dependent on large amounts of glucose (up to approximately 70% of the cell's ATP supply [71]) to fuel their energy needs. Glycolysis is an inefficient way to produce energy, as it delivers only a net of 2 ATPs per glucose molecule, compared to 32 ATPs delivered by oxidative phosphorylation. Consequently, more glucose molecules are needed during glycolysis to produce the same amount of energy than that produced by glycolysis plus oxidative phosphorylation.

Other cancer cell fuels include glutamine (at least 10% of the cell's ATP supply; provides energy via substrate level phosphorylation in the Krebs cycle [31]), *de novo* produced fatty acids (approximately 11%) and possibly lactate [71]. Data on lactate as an energy source is inconclusive, but better oxygenated subpopulations of cancer cells which use the lactate produced by neighbouring hypoxic cancer cells as fuel during the Krebs cycle, have been found [6]. Glutamine metabolism is dependent on glucose availability, thus implying that glucose is at the top of the metabolic hierarchy [75].

The increased glycolytic activity of cancer cells is used to detect tumours using FDG-PET (2-[^{18}F]-2-deoxy-D-glucose positron emission tomography) imaging [71]. FDG is a glucose analogue which cannot be metabolised and accumulates in the tissue proportionally to the rate of glucose usage. The standardised uptake values (SUVs) of FDG in tumours are much larger compared to most tissues in the body. The brain, however, has SUV values in the range of tumour SUVs. Any potential glucose-deprivation therapy must therefore take into account the minimum glucose requirement of the brain (approximately 2 mmol/l [71]).

2.4 Risk factors

Risk factors for cancer that can be related to chronic hyperglycaemia are discussed next. Figure 1 provides an overview of how hyperglycaemia can be related to cancer incidence and mortality, directly and indirectly. These links are investigated in more detail in the following sections.

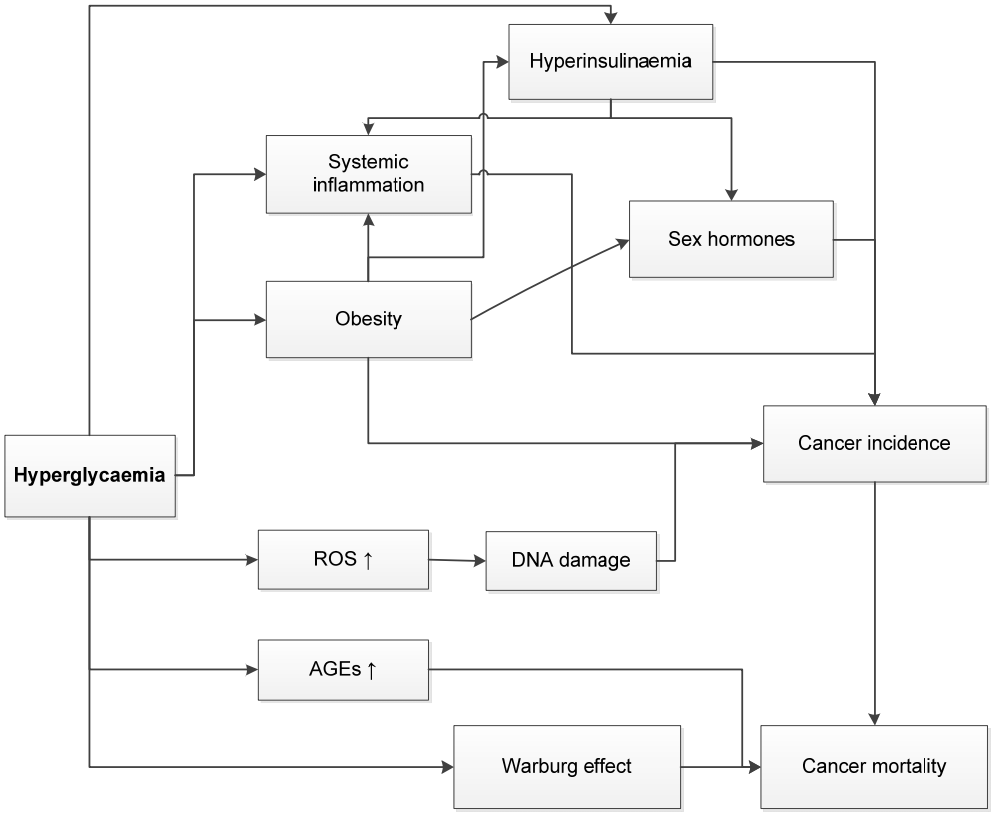

Figure 1: Diagram showing the link between hyperglycaemia and cancer incidence and mortality. (ROS – reactive oxygen species; AGEs – advanced glycation end products)

2.4.1 Hyperglycaemia

Hyperglycaemia can be linked to risk in certain types of cancer via the following mechanisms:

1. Chronic hyperglycaemia causes a rise in production of reactive oxygen species (ROS) [3], [39] which can cause oxidative stress [76] if ROS are not utilised in the cell [39]. Oxidative stress can induce DNA damage [39]. Hyperglycaemia causes increased formation of advanced glycation end products (AGE) [39], [76] which serves to promote growth of tumours in certain cancer types [39], inhibits apoptosis [3] and is pro-inflammatory [76].
2. As a result of the change in glucose metabolism in a large number of cancers (as discussed in section 2.3), increased supply of glucose may be required by tumour cells to grow [33], [39], [46], [77].

3. Increases in blood glucose stimulate secretion of insulin via the pancreas [76]. It is shown in section 2.4.2 that hyperinsulinaemia is a risk factor for cancer incidence.
4. Chronic hyperglycaemia can increase systemic inflammation [76]. It will be shown in section 2.4.3 that chronic systemic inflammation is a risk factor for cancer incidence.
5. Hyperglycaemia can be used as a marker for obesity [46], as high blood glucose levels result from increased carbohydrate intake and decreased physical activity, which results in obesity. It is shown in section 2.4.5 that obesity is a risk factor for cancer incidence.
6. Hyperglycaemia can be linked to sex hormone levels via hyperinsulinaemia and obesity.

2.4.2 Hyperinsulinaemia

Insulin is secreted by the pancreatic β-cells in response to increased blood glucose levels [76]. Insulin acts to reduce blood glucose levels by increasing uptake of blood glucose into tissue.

When diets with high glycaemic index (GI) or glycaemic load (GL) are consumed, blood glucose, and subsequently insulin, levels are increased [78]. This can eventually lead to impaired glucose tolerance, hyperinsulinaemia and insulin resistance [78] (where cells are resistant to insulin action leading to impaired uptake of blood glucose into cells [79]). Elevated insulin levels have been shown to increase the risk for pancreatic, breast, colorectal [78] and endometrial cancers [39].

Insulin can be linked to cancer risk via the following mechanisms:

1. Hyperinsulinaemia stimulates insulin receptors [46], [80], [81] and reduces insulin-like growth factor-binding proteins (IGFBP-1 and IFGBP-2), which increases insulin-like growth factor-1 (IGF-1) availability [46], [78]–[80] and can facilitate migration of cancer cells [39].
2. Hyperinsulinaemia can increase growth of cancer [82] and impede apoptosis [39], [76], [78] as it has mitogenic (stimulating mitosis, cell division) effects on cells [78].
3. IGF-1 causes proliferation and growth of cancer cells [82], [83] and impedes apoptosis [39], [78], [80], [84], [85]. IGF-1 can also control angiogenesis, as shown for lung cancer [86].
4. Insulin and IGF-1 can increase sex hormone production and decrease sex hormone-binding globulin (SHBG) production [83], [87], [88].
5. Insulin stimulates glycolysis, which is required for energy production in the majority of cancers [31].
6. Cancer cells are more sensitive to insulin action and for this reason more glucose is stored in cancer cells than normal cells [71]. This stored glucose acts as the fuel for energy production in cancer cells.
7. Insulin can activate systemic inflammation [87].
8. Chronic hyperinsulinaemia can lead to insulin resistance, inducing higher glucose and insulin levels.

2.4.3 Systemic inflammation

Hyperglycaemia increases activation of inflammatory pathways (for instance IL-6) and increases inflammation via the production of AGEs [76]. It is estimated that at least 80% of cancer incidence can be reduced by reducing exposure to chronic inflammation and damage to cell mitochondria [31].

Systemic inflammation can be linked to cancer risk via the following mechanisms:

1. Inflammatory cells produce ROS, which increase mutation of cancer cells via oxidative stress [6], [89]. Damage to cells via inflammation causes damage to the mitochondrion of the cell [31], which can further influence cell metabolism.
2. Pro-inflammatory cytokines (such as IL-6, TNF-α and C-reactive protein, CRP) can be associated with a higher risk for cancer [83]. IL-6 and TNF-α increase insulin resistance [90]. IL-6 may also activate increased growth of cancer cells [90]. CRP production is regulated by IL-6, and is associated with increased estradiol (a form of oestrogen) levels [90].

2.4.4 Sex hormones

Several sex hormones can be linked to risk for some cancer types. This includes breast, ovarian and endometrial cancers [91].

Sex hormone availability is influenced by other cancer risk factors, including hyperinsulinaemia [33] and obesity [90], which are in turn influenced by hyperglycaemia.

Sex hormones can be linked to cancer risk via the following mechanisms:

1. Oestrogen can increase the growth in the number of cancer cells [83], [89] and increase mutation of normal cells [90]. Longer exposure to oestrogen (for instance women who had an early menarche, a late menopause or no children) is a risk factor for breast [89], [90], ovarian and type I endometrial cancer [91].
2. Oestrogen can be linked to insulin resistance [90], a known risk factor for cancer.
3. Androgens, such as testosterone and androstenedione, can directly increase growth and proliferation of cells [90]. Androgens can also be converted to oestrogen via aromatase in adipose tissue [83], [90], [91].

2.4.5 Obesity

Obesity is linked to risk for a number of cancers, including liver, colorectal, pancreas, postmenopausal breast, oesophageal, kidney and endometrial cancers [33], [92], [93].

Obesity can be linked to cancer risk via the following mechanisms:

1. Increased intake of food (especially food with a large amount of calories) and decreased physical activity can cause obesity [92]. These lifestyle factors can also be linked to increased blood glucose levels. Hyperglycaemia may, therefore, act as a marker for obesity [46].
2. Obesity increases production of oestrogen [39], [83] and testosterone [94]. It was shown in section 2.4.4 that oestrogen is a risk factor for some types of cancer.
3. Obesity can cause insulin resistance [39], leading to hyperinsulinaemia [91]. Weight loss in individuals improves insulin levels [95].
4. Obesity is related to increased body fat (adipose tissue). Adipokines/cytokines (such as leptin, adiponectin, IL-6 and TNF-α) are secreted by adipose tissue [94].
    a. Leptin regulates energy homeostasis by increasing metabolism and decreasing appetite [92]. Leptin can assist angiogenesis [39] and growth of cancer cells [90], and decreases apoptosis [39].
    b. Increased IL-6 and TNF-α levels decrease adiponectin levels [94]. Adiponectin has positive properties: it increases insulin sensitivity, increases apoptosis [39], while decreasing angiogenesis [3], [39], [92], as well as being anti-diabetic and anti-inflammatory [3], [39], [90]. It also decreases blood glucose and insulin levels [3].
5. Obesity causes chronic inflammation [92] via pro-inflammatory cytokines like TNF-α and IL-6, which are secreted by adipose tissue. These cytokines increase insulin resistance [90]. IL-6 increases survival, invasion and increase of cancer cells, and suppresses the immunity of normal cells against tumours [33].
6. During obesity, oxygen-deprivation results and causes hypoxia. This up-regulates HIF-1α, IL-6 and leptin, while decreasing adiponectin [92].
7. Adipose tissue is responsible for the secretion of growth factors, including HGF (hepatocyte growth factor), VEGF (vascular endothelial growth factor) and IGF [91], which promote tumour cell growth and angiogenesis [92].

2.5 Epidemiology of colorectal cancer (CRC)

The models in this study will be derived for colorectal cancer (CRC). As discussed in Chapter 1, CRC is a highly incident cancer and is more common in developed countries with a Western lifestyle than in developing countries.

Between 67% and 90% of CRC is preceded by adenomatous polyps in the wall of the bowel [37]. Large polyps, those which appear villous (lined with villi – protrusions) and those containing abnormal (dysplastic) cells have the highest likelihood of progressing to cancer [37]. Genetic mutation of mucosal

cells and genes regulating the cell cycle causes abnormal cell growth [37]. Epigenetic modifications also affect CRC [67].

Non-modifiable risk factors for CRC include increased age (especially above 40 years), personal history of adenomatous polyps or inflammatory bowel disease, family history of adenomatous polyps or CRC, and certain inherited genes (approximately 5% to 10% of CRC cases) [36]. Modifiable factors which may increase CRC risk include dietary factors, such as consumption of large amounts of fat and meat, decreased dietary fibre intake, a sedentary lifestyle, obesity, smoking and alcohol consumption [36].

A recent meta-analysis on CRC risk factors [96] found that the following factors were significantly associated with CRC risk (increasing CRC risk, except when stated otherwise): inflammatory bowel disease, history of CRC in a close (first-degree) relative, body mass index (BMI), physical activity (which decreased CRC risk), cigarette smoking, consumption of red meat, and consumption of fruit and vegetables (which decreased CRC risk). The highest risks were present for the non-modifiable risk factors, with moderately increased risk for the modifiable factors [96]. The increased CRC risk as a result of alcohol and processed meat consumption was not statistically significant [96].

The preceding discussion confirms that prevention of CRC through modification of lifestyle factors is possible. It is clear that not all risk factors for CRC can be linked to hyperglycaemia. However, this study will focus on those lifestyle factors which can be linked to hyperglycaemia and potentially lead to strategies for prevention of CRC via blood glucose reduction (i.e. cigarette smoking, physical activity/exercise, alcohol consumption and dietary fibre intake). Two risk factors (chronic stress and excessive carbohydrate intake) which have not been identified in the studies mentioned in the preceding discussion, but could potentially be linked to hyperglycaemia, as well as risk for other cancer types, are also investigated. The following section discusses potential therapeutic options that could be employed to reduce blood glucose, and subsequently cancer risk.

2.6 Potential treatment/therapeutic options

Potential therapeutic options that have been proposed for cancer control via blood glucose regulation include the following [71]:

1. Lifestyle intervention.
2. Pharmacotherapeutics (“drugs”).
3. Isolated limb haemodialysis.
4. Single-zone haemodialysis.
5. Two-zone haemodialysis.

Each of these options is briefly discussed in the following sections.

2.6.1 Lifestyle intervention

Lifestyle factors (such as physical exercise, excessive carbohydrate intake, prolonged stress, cigarette smoking, dietary fibre intake and alcohol consumption) have an influence on blood glucose levels and concomitantly on cancer risk. This is discussed in more detail in Chapter 3.

Other lifestyle-related strategies that can be employed to reduce blood glucose levels include dietary energy restriction strategies such as calorie restriction and short-term starvation (STS).

Subjects on a calorie restriction diet are typically fed 20% to 40% fewer calories than a comparison group on an unrestricted diet [97]. Calorie restriction counters angiogenesis [77], stimulates apoptosis [77] and is anti-inflammatory [77], [97]. In addition, it reduces insulin, IGF-1 and blood glucose levels [25], [97], which are all known cancer risk factors.

The ketogenic diet is a type of calorie restriction diet. It is a high-fat diet, with restricted carbohydrate and protein intake [77]. A 4:1 ratio of fat to carbohydrate + protein is considered effective in kerbing cancer proliferation [77]. It results in the production of ketone bodies from fatty acid metabolism in the liver. Due to genetic and mitochondrial abnormalities many cancer cells cannot use ketone bodies for energy [77], whereas normal cells can. This diet reduces glucose and glutamine availability. This diet is also advantageous as a result of the increased number of ketone bodies, which can act against inflammation [31].

A further type of calorie restriction diet involves the restriction of carbohydrates only (which may or may not induce ketogenesis), as proposed by Fine *et al.* [72]. This type of diet primarily targets concomitant insulin inhibition, by reducing the blood glucose level, and, therefore, the amount of glucose available as energy fuel to cancer cells. This leads to reduced insulin levels with resultant reduced mitogenic effects.

STS or fasting is a therapeutic option where a subject is exposed to cycles of no caloric intake for several days (for instance 60 hours), followed by cycles of eating at liberty [98]. This therapy causes a decrease in IGF-1 and blood glucose, protects normal cells against high doses of chemotherapy and also plays a role in sensitising cancer cells to the action of chemotherapy drugs [98], [99]. STS seems to be more effective in reducing IGF-1 [99] and blood glucose levels [98] than calorie restriction.

It must be noted that commitment is required from the patient to remain on an STS or calorie restricted diet [100]. These diets may also cause weight loss, which could be harmful in cachectic cancer patients. The haemodialysis methods described in sections 2.6.3, 2.6.4 and 2.6.5 may reduce the mental

commitment required from patients, as well as the treatment time, and with potentially stronger cancer cell killing effects.

2.6.2 Pharmacotherapeutics

Pharmacologic suppression of blood glucose levels can be achieved using anti-diabetic drugs such as metformin, whereas suppression of glutamine can be achieved via glutamine inhibitors such as phenyl acetate [31], [71].

Metformin suppresses liver gluconeogenesis [91], [101], [102] and disrupts glycolysis [102]. It enhances insulin sensitivity [91], which results in decreased insulin levels [101], [103], and reduces chronic inflammation [102]. Metformin additionally reduces glucose uptake by decreasing phosphorylation of Akt which is required for glucose uptake via GLUT1 and GLUT4 glucose transporters [25]. Metformin also indirectly activates adenosine monophosphate-activated protein kinase (AMPK), by inhibiting mitochondrial complex I [25]. This increases the ratio of adenosine monophosphate (AMP) to ATP, and subsequently activates AMPK [25]. AMPK hinders progression of the cell cycle and the synthesis of proteins, thereby decreasing cancer cell division and growth [25], [102]. Glucose-lowering medications such as injected insulin [104] and sulfonylureas have been found to increase cancer risk [103], possibly by increasing insulin levels [105].

Pharmacologic suppression of blood glucose demand can be achieved with the use of psychological stress modulators such as benzodiazepines or β-blockers, which reduce the effects of stress hormones and, subsequently, also reduces blood glucose [71].

Anti-glycolytic drugs can also be combined with dietary energy restriction (as discussed in section 2.6.1) to reduce toxicity and enhance efficacy of treatment [31].

Glutamine restriction can be achieved using a drug such as phenylacetate, which binds to glutamine [31]. The resultant phenylacetylglutamine can be expelled through urination [106].

2.6.3 Isolated limb nutrient-haemodialysis

For cancer that is localised in a limb, haemodialysis may be used to control blood glucose or glutamine levels in that limb [71], [107]. Additionally, neutralisation of the acidic tumour environment may be performed by the addition of carbonate to the dialysate [71]. Depriving the tumours of glucose increases their sensitivity to radiotherapy and chemotherapeutics, possibly reducing the required dose of chemotherapy or radiotherapy to safer levels [107].

2.6.4 Single-zone nutrient-haemodialysis

For metastatic tumours or tumours that are hard to reach, whole-body haemodialysis via the brachial artery (in the upper arm) for the removal of glucose and glutamine may be a viable option [71]. With this method, the whole body, including the brain, will be affected by the treatment. Therefore, the minimum glucose requirement of the brain (typically 2 mmol/l, patient-specific) must be taken into account. Minimum glutamine levels to the brain must also be accounted for, and should not fall lower than typically 0.3 mmol/l (patient-specific) if the brain glucose levels are controlled at 2 mmol/l.

To lower the brain glucose requirement further, drugs which modulate stress, such as β-blockers or benzodiazepine, can be administered. Another strategy which could potentially be employed to lower the brain glucose requirement is the administration of a diet which promotes the increase of ketone bodies (such as the ketogenic diet discussed in section 2.6.1). The brain should be able to function with a glucose level of 0.45 mmol/l in the presence of ketone bodies [71].

This single-zone haemodialysis process will not be able to remove all the glucose and glutamine from the body, as minimum requirements of brain and heart must be met (as evaluated by electroencephalogram, EEG, and electrocardiogram, ECG, monitoring during the deprivation therapy). The glucose/glutamine haemodialysis strategy will, therefore, need to be repeated on a regular basis to control tumour proliferation, rather than completely eradicate them [71]. The frequency with which such a treatment needs to be repeated should be determined, and will be patient-specific.

2.6.5 Two-zone nutrient-haemodialysis

To be able to lower the body glucose and glutamine levels to 0 mmol/l the brain glucose and glutamine requirement must be controlled separately. To achieve this, a transcatheter arterial approach could be followed to supply the brain's glucose and glutamine requirement [71] separate from that of the rest of the body. It must be noted that this approach carries risks of blood vessel damage and subsequent haemorrhage. More information is provided in [18].

2.7 Conclusion

Carcinogenesis and the hallmarks of cancer were briefly discussed. Altered energy metabolism was highlighted as one of the hallmarks of cancer. Differences between normal and cancer cell metabolism were subsequently elucidated. Most cancer cells require more glucose than normal cells to proliferate, as cancer cells switch to glycolysis ("Warburg effect"), which is less efficient in producing energy for cell growth than the oxidative phosphorylation process employed by normal cells. The links of hyperglycaemia with hyperinsulinaemia, systemic inflammation, sex hormones and obesity, and concomitantly with cancer risk were discussed. The epidemiology of colorectal cancer (CRC) was

detailed. Potential therapeutic strategies for blood glucose regulation were highlighted: lifestyle factors, pharmacotherapeutics and three haemodialysis strategies. The effects of lifestyle factors on blood glucose control are discussed in more detail in the following chapter.

CHAPTER 3 LIFESTYLE FACTORS

3.1 Introduction

Chapter 1 shows that lifestyle factors could have a significant impact on the development [83] and prevention of cancer and diabetes. In this chapter, the focus is on the possible links between lifestyle factors, blood glucose and cancers.

3.2 Excessive carbohydrate intake

Carbohydrate (CHO) intake can be linked to blood glucose levels using the Glycaemic Index (GI) concept. The GI compares the blood glucose response produced by a test food (containing 50 g of available CHO) with the blood glucose response produced by 50 g of glucose [108]. The GI of a food can be determined using equation (5) [108]. Glucose, as the reference food, will have a GI of 100.

$$GI = \frac{\sum_{x=1}^{n} F_x / \bar{G}_x \times 100}{n} \tag{5}$$

where

$GI = glycaemic\ index$

$F_x = area\ under\ the\ curve\ (AUC)\ of\ glycaemic\ response\ for\ subject\ x\ for\ 50\ g\ of\ available\ CHO\ from\ the\ test\ food$

$\bar{G}_x = mean\ AUC\ for\ subject\ x\ from\ two\ or\ three\ 50\ g\ glucose\ tests$

$n = number\ of\ subjects\ (\geq 10)$

Sometimes white bread is also used as a reference food [109]. A GI value referenced to white bread can be converted to a GI value referenced to glucose using equation (6) [110].

$$GI_{referenced\ to\ glucose} = GI_{referenced\ to\ white\ bread} \times 0.7 \tag{6}$$

GI is a property of the food and contributes to the glycaemic response, but cannot be seen as equivalent to the glycaemic response, as other factors, such as the amount of fat, protein and CHO ingested, also affect the glycaemic response [108]. A recent article by one of the co-developers of the GI concept [108] aimed to address some criticisms that have surfaced in the published literature regarding the GI concept. Some of the criticisms and the author's comments to the criticisms were as follows [108]:

1. It was stated that the methodology used to measure and calculate GI is not standardised, accurate or precise and contains flaws. The author responded to this criticism:

a. An International Standards Organization (ISO) standard describing the GI methodology was published in 2010, but it is not always used correctly.
b. Different GI values for the same food are provided in the International GI tables. Some of the variation could be due to methodological errors, but it could also be due to variations in the food (for instance different varieties of potatoes).
c. The GI of a food is affected by processing and the conditions in which it is grown.
d. There is day-to-day variability within and between subjects. The GI methodology has been improved to address this by setting stricter requirements: the mean of more than two tests must be used for the glycaemic response elicited by the reference food; the coefficient of variation for the reference food must be less than 30% and the AUC of the glycaemic response must be calculated from the mean of two fasting blood glucose samples. Another improvement to the method is the requirement for the GI to be determined from the responses of ten or more test subjects. It is, therefore, possible that the GI method based on the newer ISO standard could perform better as improvements were made to the methodology.

2. It was said that GI values do not represent a meaningful food property as there is too much variability in the values (based on a standard deviation of 33 for the GI of potato).
    a. The author responded that the standard deviation of 33 was based on subject variability, not the variability of the GI values. The standard deviation for the variability of the GI values was approximately 9. Although there is day-to-day variation in subjects, the above methods in 1.d is aimed at reducing the impact of this.
3. It was stated that GI was limited as some healthy foods (such as whole grains) have a high GI, while some unhealthy foods have a low GI; one can, therefore, not discriminate healthy from unhealthy foods based on the GI.
    a. The author investigated a number of foods and found that there was quite a large variation in GI values, because of differing processing methods, composition and variety. Some healthy foods may have high GI and some unhealthy foods may have low GI, but there is variability. One also needs to take into account the quantity of the CHO in a food (e.g. some high GI foods only contain a small amount of CHO, and some low GI foods contain a large amount of CHO).
4. There is a conception that fibre and whole grain present better markers of carbohydrate quality.
    a. Whole grain products and low-GI diets work together and affect different risk factors for disease (for instance, whole grains reduce systolic blood pressure, and possibly LDL cholesterol, while low-GI diets reduces body weight and inflammation, while having a beneficial effect on blood lipids and possibly insulin sensitivity).
    b. Fibre comes in different forms, including viscous and non-viscous fibres. Viscous fibre decreases postprandial glucose and affects serum cholesterol, while non-viscous fibres add to the faecal bulk, thereby decreasing the travel time of the stool and the time for

exposure to carcinogens. Diets high in viscous fibres are, therefore, related to the GI by its impact on health via reduction of blood glucose.

From the above it is clear that there are some concerns with regard to the GI concept, although the author has addressed a number of these in [108]. There is day-to-day variability within and between subjects, but using the improved methods such as those stated in 1.d above is aimed at addressing these concerns. Further refinements may be required [108]. Different studies use different GI tables and, therefore, different GI values [108], and the GI values for some food types might not match the GI for other varieties of the same food type. The GI value that a researcher uses for a study (if obtained from a table) might, therefore, not match the actual GI of the food used in the study. The ideal would be for GI testing to be done (according to the specified ISO standard) on the specific foods used in a trial.

If the correct methodologies are followed, the GI could be an even more useful tool for determining the glycaemic response elicited by a test food. There may still be inaccuracies between different trials, but at present it should be possible to at least perform a comparison between low and high GI foods, as the GI method can differentiate with reasonable confidence between low and high GI foods [108], even if the absolute values of the GI of the foods are not exact.

In addition to the GI concept, the glycaemic load (GL) concept was developed to take into account the quantity of CHO (mass in grams), as well as the quality of the CHO (GI) [110]. The glycaemic response to a typical portion of food can, therefore, be determined. The GL can be calculated using equation (7).

$$GL = \frac{GI \times m_{carbohydrates}}{100} \tag{7}$$

where

$$GI = glycaemic\ index$$
$$m_{carbohydrates} = mass\ of\ CHO[g]$$

CHO ingestion increases blood glucose, and subsequently induces insulin secretion [111] in humans with functioning pancreatic β-cells. Insulin induces the absorption of glucose from the blood into the muscle, liver and fat, and reduces efflux of glucose from the liver to the blood [111].

It was shown in Chapter 2 that insulin is a risk factor for cancer. Lowering the GI or GL of a diet, by eating foods with a lower GI and restricting the amount of carbohydrates ingested [72], will lower blood glucose levels and consequently also insulin secretion [78]. According to a study performed by Holt *et al.* [112] on 38 foods, glycaemic response accounted for only approximately 23% of changes in the insulin response; other foods (such as protein or fat) which do not necessarily increase the glycaemic response

increases the insulin response [112]; however, this accounted for only 10% of the changes in insulin response [112]. 67% of the variability in insulin response in these tests could not be explained [112].

It must be noted that hyperinsulinaemia may contribute more to the development of cancer than hyperglycaemia [81]. For this reason, it may be more accurate to use the insulin index (II) [112] of foods as a measure of postprandial insulin response and measure cancer risk in this way [81]; however, only a small number of studies have been performed on insulin index and cancer risk. A search on Scopus for articles with "insulin index" AND "cancer" in the title, abstract and keywords produced only 6 results. A similar search on ScienceDirect produced no results.

Excessive CHO intake can, therefore, increase the risk of cancer, and mainly via the following mechanisms:

1. Diets with high GI and GL cause higher blood glucose levels and consequently higher insulin levels. Hyperinsulinaemia is a cancer risk factor. High blood glucose levels can also directly contribute to cancer mortality by providing fuel for cancer cells to grow.
2. Diets with high GI and GL can lead to increases in weight and body fat, thus leading to obesity, which is considered a risk factor for some types of cancers.

3.3 Chronic stress

Chronic stress can increase the risk of cancer via the following mechanisms:

1. Chronic stress causes an increased production of cortisol [113]. Cortisol acts by stimulating gluconeogenesis and glycogenolysis in the liver [55]. This leads to increased production of blood glucose.
2. Cortisol can lead to immune suppression [114], [115] and DNA damage [116], [117], which in turn can lead to carcinogenesis.
3. Chronic stress may cause obesity and insulin resistance through an abnormal cortisol rhythm, or indirectly via changes in dietary and exercise patterns [114].
4. Epinephrine and norepinephrine are hormones that are released during stressful situations and cause increased heart rate, increased blood pressure and glucose release from energy stores into the blood [115].
5. Stress can increase angiogenesis by increasing cytokines such as IL-6 and IL-8 [115].
6. Stress can promote cancer cell adhesion, invasion and survival via various mechanisms [115].

Stress may potentially have protective effects on certain types of cancer via the following mechanisms:

1. Stress can cause decreased synthesis of sex hormones, such as oestrogen and testosterone, as stress affects the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis which in turn inhibits the hypothalamic-pituitary-gonadal (HPG) axis responsible for synthesis of sex hormones [114].

3.4 Cigarette smoking

Cigarette smoking can increase the risk of cancer via the following mechanisms:

1. Smoking increases blood glucose concentrations in the short term and might also have an influence on insulin sensitivity [118] as a result of increased catecholamine and cortisol levels [119].
2. The nicotine in cigarettes causes an increase in secretion of cortisol, which stimulates gluconeogenesis and glycogenolysis in the liver, leading to increased blood glucose levels [119].
3. Nicotine has an effect on inflammation and adiponectin levels, which in turn may increase blood glucose concentrations [119].
4. More than 70 components of cigarette smoke are considered carcinogens [120].

Cigarette smoking may be protective against certain types of cancer as it decreases oestrogen levels [121]. However, this may lead to accumulation of adipose tissue, and in turn, insulin resistance [119].

3.5 Dietary fibre intake

Dietary fibre intake is proposed to reduce cancer risk by the following mechanisms:

1. Dietary fibre ingested with a carbohydrate-containing meal decreases the glycaemic response to that meal and hence lowers insulin levels [122], by slowing down the absorption of glucose in the small intestine [85], [122].
2. Dietary fibre lowers circulating oestrogen levels [109], [123] by reducing reabsorption of oestrogen in the bile and by increasing excretion of oestrogen via faeces [124], [125].
3. Dietary fibre increases insulin sensitivity by being fermented to short-chain fatty acids (SCFAs); this also influences lipid synthesis [126].
4. Dietary fibre decreases IGFs [124], [127] by increasing levels of IGF binding proteins, such as IGFBP-3 [109].
5. Dietary fibre has a lower energy density and may therefore increase weight loss [126].
6. Dietary fibre has an effect on the inflammatory response by regulating gut microflora populations [126], [128].

7. Dietary fibre decreases the time that food travels in the gastrointestinal tract, which results in shorter exposure to carcinogens [127]. Dietary fibre also dilutes potential carcinogens [127]. This may have an effect on lowering the risk of colorectal cancer [127].
8. Non-starch polysaccharide, a form of dietary fibre, has a stimulating effect on natural killer cells (which suppress tumour growth) [127]. It also acts as an antioxidant.

3.6 Alcohol consumption

Alcohol consumption has been shown to increase cancer risk, even in small amounts [129]. This is in contrast to cardiovascular risk, which is actually reduced by moderate alcohol consumption [95]. Cancers affected by alcohol use are liver, breast, colorectal, pharynx, larynx, mouth and oesophageal cancers [130]. There is also a possible link with pancreatic cancer risk [130].

Alcohol consumption suppresses hepatic gluconeogenesis, possibly by up to 45% [131]. The mechanism proposed for this action is based on the metabolism of alcohol by alcohol dehydrogenase to acetaldehyde [131]. This is then metabolised to acetate by aldehyde dehydrogenase [131]. The acetate depletes nicotinamide adenine dinucleotide in the liver, which is required for gluconeogenesis [131]. Despite the reduced production of glucose and, therefore, decreased blood glucose levels, alcohol consumption increases cancer risk.

The proposed mechanisms by which alcohol consumption can increase cancer risk are:

1. Alcohol contains ethanol, which is considered a carcinogen [132].
2. Alcohol increases oestrogen levels [80], [91], [123], [130], [132].
3. Alcohol may act as a folate antagonist and hence decrease folate levels in serum [95], [123].
4. Alcohol metabolises to acetaldehyde, a possible carcinogen [65], [80], which can damage DNA [130].
5. Alcohol allows better transport of carcinogens across cell membranes and acts as a solvent for certain carcinogens, such as tobacco [80]. This might explain why simultaneous alcohol and tobacco use synergistically increases the risk of larynx, mouth and oesophageal cancers [130].
6. Alcohol increases ROS production and decreases repair of DNA, which can lead to carcinogenesis [80], [132].
7. Excess alcoholic intake can contribute to weight gain [130].
8. Heavy alcohol consumption can cause chronic pancreatitis, a risk factor for pancreatic cancer [133].

Some alcoholic beverages are proposed to have anti-carcinogenic effects:

1. Beer is proposed to have a number of anti-carcinogenic effects. It acts as an antioxidant which blocks free radicals via the phenolic compounds contained therein; however, the availability of these compounds in beer is very low [132]. Beer also inhibits angiogenesis [134] via the xanthohumol contained therein [132]. Beer contains some dietary fibre, which may contribute to decreasing cancer risk [134]. Beer, however, contains toxic amines, which can increase risk of certain cancers [134]. Beer has a relatively high GI (e.g. 74 for an ordinary German beer [135], which is slightly higher than the GI for white bread) which could be causally linked to cancer. However, its carbohydrate-content is relatively low, resulting in a lower GL than might be expected.
2. Wine is also proposed to have anti-carcinogenic effects, mainly via the anti-oxidative properties of resveratrol [132].

3.7 Physical exercise

Evidence from a review done in 2010 indicates that physical exercise has a convincing beneficial effect on colon cancer, a probable beneficial effect on breast and endometrial cancer and a possible beneficial effect on prostate, ovary and lung cancer [94]. There is substantial evidence of no effect on rectal cancer [94].

During exercise, absorption of glucose from the blood to the muscles is increased independently of insulin [111]. GLUT4 glucose transporter is translocated to the muscle plasma membrane to allow increased uptake of glucose from the blood [111]. Other mechanisms involved are glucose phosphorylation and increased blood flow in the muscle [111]. The liver produces glucose in an attempt to control blood glucose levels [111]. During moderate exercise, insulin levels decrease [136] to sensitise the liver to glucagon action [111].

A number of mechanisms are proposed by which cancer risk might be reduced by exercise [83], although the evidence is not conclusive on all effects [94]:

1. Exercise reduces body weight [137] and body fat.
2. Exercise reduces sex hormone levels [94], [136], [138]. Exercise also increases SHBG levels [94], which further reduce the levels of bioavailable sex hormones.
3. Exercise reduces insulin resistance and modulates factors related to insulin, such as IGF-1, C-peptide, insulin and glucose levels [94].
4. Exercise possibly increases adiponectin levels by reducing IL-6 and TNF-α (which reduces secretion of adiponectin) [94] and possibly also reduces leptin [94]. Leptin is a breast cancer risk factor as it stimulates mitogens, which in turn stimulate cell division. Leptin can also promote insulin resistance, which causes a rise in oestrogen levels.

5. Exercise has an effect on inflammation and the immune system [138]. Moderate exercise can activate natural killer cells [94] and activity in macrophages that eliminate tumour cells. Exercise may reduce levels of inflammatory markers such as CRP, IL-6 and TNF-α, directly and via weight loss [94].
6. Exercise may reduce oxidative stress and damage [94], [138], [139].
7. Exercise decreases the time that food travels in the gastrointestinal tract, which results in shorter exposure to carcinogens, and also lowers concentration of faecal bile acid [139]. This may have an effect on reducing colon cancer risk.
8. Exercise may also have a positive effect on lipid profiles - lowering triglycerides, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol, and increasing high-density lipoprotein (HDL) cholesterol [140].

The effect of exercise on biochemical biomarkers in persons with established cancer is mixed. A review on exercise in persons with cancer [138] revealed that IGF-1 and IGFBP-3 changed significantly as a result of exercise in most reviewed studies, but glucose and insulin levels were not significantly changed. One study revealed significantly lower C-peptide levels, while another found that insulin levels decreased borderline significantly as a result of exercise [138]. Another study found that insulin levels remained stable in the exercise group, in contrast to increasing in the control group [137]. A review [138] revealed some inconsistent results with regard to the effects of exercise on immunity and inflammation in cancer patients. However, four studies were reviewed that found decreased CRP levels as a result of exercise [138]. Markers of oxidative damage were found to be variable between studies, with one study indicating an increase in F2-isoprostanes, while another study found reduced excretion of 8-oxo-dG in cancer patients participating in moderate-intensity exercise [138]. In terms of sex hormone levels, three studies revealed no change in prostate-specific antigen or testosterone in men with prostate cancer as a result of exercise [138]. In a review on physical activity in breast cancer patients [137], leptin, cortisol and adiponectin were not affected by exercise, whereas serotonin was.

3.8 Conclusion

This chapter examined the relationships between lifestyle factors, blood glucose and cancer. It was shown that excessive carbohydrate intake, chronic stress and cigarette smoking can increase blood glucose levels. Dietary fibre intake, alcohol consumption and physical exercise can reduce blood glucose levels. It was found that excessive carbohydrate consumption, cigarette smoking and alcohol consumption could increase the risk for some cancers via several mechanisms. The blood-glucose lowering effect of alcohol, therefore, does not seem to be strong enough to protect against cancer risk, when compared to its carcinogenic properties. Dietary fibre intake and physical exercise could protect against certain cancer types, via a number of proposed mechanisms. Chronic stress could potentially have adverse and protective effects on cancer, the latter via the reduction of several sex hormones.

The following chapters provide more detail on the data that was collected and the methods that were used to examine the above relationships quantitatively.

CHAPTER 4 DATA COLLECTION

4.1 Introduction

In this chapter the search strategy, criteria for inclusion and data extraction, as well as references to the tables of data that were collected, are provided. The tables with data are provided in Annexure C. The following information was extracted from [1] and [2]. Scientific journal databases such as ScienceDirect and Scopus were used to identify articles. Only studies using English as medium, were included [1], [2].

4.2 Search strategy

4.2.1 Article 1: "Does cancer risk increase with HbA_{1c}, independent of diabetes?"

Literature searches were performed to identify articles which relate HbA_{1c} to cancer incidence and mortality risk [1]. Combinations of the following search terms were used [1]: ("cancer" OR "malignancy" OR "tumor" OR "tumour" OR "neoplasm" OR "neoplasia") AND ("hemoglobin a1c" OR "hba1c" OR "glycated hemoglobin" OR "glycosylated hemoglobin" OR "a1c" OR "haemoglobin a1c" OR "glycated haemoglobin" OR "glycosylated haemoglobin" OR "glycohemoglobin a" OR "glycohaemoglobin a"). Additional articles were identified by scanning the reference lists of articles identified during the searches.

4.2.2 Article 2: "Comparison of glycaemic and overall effects of lifestyle factors on colorectal cancer risk"

Articles which relate HbA_{1c} to risk of CRC incidence were identified [2]. Articles were identified using combinations of the following search terms: "colorectal cancer" AND "a1c", "glycated hemoglobin", "glycosylated hemoglobin", "glycated haemoglobin", "glycosylated haemoglobin", "hba1c" [2]. Additional articles were identified by scanning the reference lists of articles identified during the searches. Articles that contained information on CRC risk that were identified during a similar search on "cancer" and the above-mentioned keywords were also included [2].

Articles that relate HbA_{1c}, as well as risk for CRC, to lifestyle factors (excessive carbohydrate intake, dietary fibre intake, cigarette smoking, alcohol consumption, chronic stress and physical exercise) were identified using combinations of the following search terms: "colorectal cancer" OR "a1c" OR "glycated hemoglobin" OR "glycosylated hemoglobin" OR "glycated haemoglobin" OR "glycosylated haemoglobin" OR "hba1c" AND "alcohol", "dietary fibre", "dietary fiber", "fiber", "fibre", "glycaemic load", "glycemic load", "stress", "smoking", "exercise", "physical activity". With the exception of studies stratifying by gender, stratified data (for instance by gene polymorphisms) were not included [2].

4.3 Criteria for inclusion and data extraction

4.3.1 Article 1: "Does cancer risk increase with HbA_{1c}, independent of diabetes?"

The following inclusion criteria (for including studies in the quantitative analysis) were used (from [1]):

1. The trends adjusted for the most confounders were analysed [96], where enough information was available on that trend.
2. Only studies reporting RR, OR and HR as risk measures were analysed.
3. Studies had to provide data on a specific cancer type, not cancer in general.
4. If more than one study used the same or a portion of the same study population, only the newest study or the study with the most information was included.
5. Only studies providing three or more exposure levels were included.
6. Except for three studies which explicitly stated that they used the Swedish Mono-S HbA_{1c} reference method, all studies were assumed to have used the NGSP reference method. For the above-mentioned three studies, conversion to the NGSP reference was performed using equation (8).

$$\text{NGSP}\,[\%] = \frac{\text{Mono}-\text{S} + 0.8925}{0.9718} \tag{8}$$

The following data were extracted from the identified articles (from [1]:

1. Reference details.
2. Total number of persons per HbA_{1c} level for RR and OR.
3. Number of controls per HbA_{1c} level for OR.
4. Number of non-cases per HbA_{1c} level for RR.
5. Number of person-years per HbA_{1c} level for HR.
6. Number of cases per HbA_{1c} level for OR, RR and HR.
7. Gender.
8. Cancer site.
9. Risk measure (RR, OR or HR).
10. HbA_{1c} level or range.
11. Risk per HbA_{1c} range.
12. 95% CIs.
13. Two study authors provided additional information so that the studies could be included in the analysis.

4.3.2 Article 2: "Comparison of glycaemic and overall effects of lifestyle factors on colorectal cancer risk"

The following inclusion criteria were used (from [2]):

1. The trend adjusted for the most confounders were analysed [96].
2. Only studies reporting RR, OR and HR as risk measures were analysed.
3. Only articles that specifically refer to colorectal cancer (not colon or rectal cancer separately) were included.
4. Studies relating to colorectal cancer precursors (neoplasms, adenomas or adenomatous polyps) were not included.
5. Studies which assessed cancer stage, grade or mortality risk were excluded.
6. If more than one study used the same or a portion of the same study population, only the newest study or the study with the most information was included.
7. Only studies providing three or more exposure levels were included in the analysis of CRC risk.

Only journal articles quantifying lifestyle factors in the following units, or units that can be transformed to these units were included [2], [54]:

1. Glycaemic load per day (GL/day).
2. Grams of dietary fibre intake per day (g/day).
3. Number of cigarettes per day (cigarettes/day).
4. Grams of ethanol (alcohol) per day (g/day).
5. No, low, medium and high levels of chronic stress, job strain or associated measures.
6. Metabolic equivalents (METs), MET-hours per day (MET-h/day) or kcal of exercise.

The following data were extracted from the identified articles (from [2]):

1. Reference details.
2. Risk measure (RR, OR or HR).
3. Total number of persons per exposure level for RR and OR.
4. Number of controls per exposure level for OR.
5. Number of non-cases per exposure level for RR.
6. Number of person-years per exposure level for HR.
7. Number of cases per exposure level for OR, RR and HR.
8. Gender.
9. 95% CIs (or standard error/standard deviation for studies relating lifestyle factors to HbA_{1c}).
10. Risk per exposure range.
11. Exposure range.

12. Additional comments regarding the study or patients, for instance the reference food in articles relating to GL.

4.4 Data collected

4.4.1 Article 1: "Does cancer risk increase with HbA_{1c}, independent of diabetes?"

Due to the amount of data that was extracted, a table of the data is not included in this dissertation. The collected data was stored in comma separated variable (CSV) files for processing using the *R* software.

4.4.2 Article 2: "Comparison of glycaemic and overall effects of lifestyle factors on colorectal cancer risk"

Table 5 in Annexure C shows the data that was collected relating HbA_{1c} levels to risk of CRC incidence (from [2]).

Table 6 in Annexure C shows the data that was collected relating HbA_{1c} levels to lifestyle factors (from [2]). Equation (9) was used to compute the standard error (SE) for each study if the 95% CIs were available. $z_{\alpha/2}$ equals 1.96 for a 95% CI [58]. CI_{upper} is the upper limit of the 95% confidence interval and CI_{lower} is the lower limit of the 95% CI.

$$SE = \frac{\left[\ln(CI_{upper}) - \ln(CI_{lower})\right]}{2 \times z_{\alpha/2}} \tag{9}$$

Equation (10) was used to compute the SE if only the standard deviation (*SD*) and population size (*n*) was available.

$$SE = \frac{\mathrm{SD}}{\sqrt{n}} \tag{10}$$

Table 7 in Annexure C shows the data that was collected relating lifestyle factors to risk of CRC incidence (from [2]). This is required for comparison purposes.

4.5 Conclusion

This chapter provided the search strategy, criteria for inclusion and references to the tables of data that were extracted from the journal articles. The actual data that was extracted is provided in Annexure C. The methods to transform and combine this data to develop useful models are described in the following chapter.

CHAPTER 5 STATISTICAL BACKGROUND

5.1 Introduction

To address the limitations and expand on the work in Espach [54] and Laubscher [60] as described in Chapter 1, understanding of the statistical background used to develop the models is required. The statistical background and methods used to calculate the models are provided in this chapter. Validation and verification of the most important parts of the statistical software is also performed by comparing the values obtained using the software to those obtained in published studies.

5.2 Relative risk, odds ratio and hazard ratio

Three different risk measures are commonly used to measure the increase or decrease in risk for a disease given a certain treatment or exposure. These measures are relative risk (RR), also called risk ratio, odds ratio (OR) and hazard ratio (HR).

The RR (cumulative incidence data) is the ratio of the risk in the exposed (experimental) group to the risk in the control group [15]. RR (without adjusting for confounders) can be calculated from a 2x2 table (such as Table 2) using equation (11).

Table 2: 2x2 table for computing RRs and ORs [15].

	Event	**Non-event**	**Total**
Exposed group	A	C	A + C
Control group	B	D	B + D

$$RR = \frac{\frac{A}{(A+C)}}{\frac{B}{(B+D)}} \tag{11}$$

The OR (case-control data) is the ratio of the odds of an event occurring in the group that is exposed to the treatment or disease compared to the odds of that event in the control group [15]. The OR (without adjusting for confounders) can be calculated from Table 2 using equation (12).

$$OR = \frac{\frac{A}{(A+C)}}{\frac{C}{(A+C)}} \div \frac{\frac{B}{(B+D)}}{\frac{D}{(B+D)}} = \frac{\left(\frac{A}{C}\right)}{\left(\frac{B}{D}\right)} = \frac{AD}{BC} \tag{12}$$

If ORs are misinterpreted as RRs, the RR will be overestimated when above 1 and underestimated when below 1 [57]. This effect is not as significant when the incidence of the disease is below 10% in the study population [57]. This is called *the rare disease assumption*. However, as the incidence of the disease increases above 10% and especially when the OR is higher than 2.5 or lower than 0.5 [57], the differences between ORs and RRs become significant.

An approach that was considered to convert the OR to a RR was using equation (13) [141]. $p_{unexposed}$ is the risk of the event occurring in the unexposed group ($p_{unexposed} = \frac{B}{(B+D)}$). This approach was not followed as confounders might influence the results [141] and additional information was required to perform the conversion. It was also found that only a small number of ORs were greater than 2.5 or less than 0.5. A greater number of 95% CIs, however, still contained ORs greater than 2.5 or less than 0.5.

$$RR = \frac{OR}{\left(1 - p_{unexposed}\right) + p_{unexposed} \times OR} \tag{13}$$

The HR (incidence-rate data) measures instantaneous risk (and can, therefore, change over time) [15]. If a Cox proportional hazards model is used to estimate the HR, it is assumed that the HR does not change over time [142]. The HR provides the RR over the study duration and takes into account the number of events as well as the timing of the events, while the RR only takes into account the cumulative incidence of the events at the end of the study [143].

ORs, HRs and RRs are not the same and will provide numerically different values. To combine these risk measures can overestimate the risk. RRs, ORs and HRs will be analysed separately to assess the risk. However, for better comparison with published data (as a number of studies use the rare disease assumption to combine the risk) models of the combined risk will also be developed.

5.3 Combining dose-response effects of different studies

5.3.1 CRC risk and lifestyle factors or HbA_{1c}

When studies with different exposures and design are combined, several things must be kept in mind:

1. The risks within a study are not independent, as all risks use a common reference group per study [58], [59].
2. The reference group for each study is not necessarily at zero exposure.
3. Studies have different designs and test subjects - heterogeneity between studies must, therefore, be accounted for.
4. Some studies are more important or carry a greater weight than others.
5. The trend might have a different form than linear.

To resolve the first issue, the method of Greenland and Longnecker or Hamling must be followed to obtain the within-study covariance matrices [144]. Orsini *et al.* [144] have corrected errors regarding the computation of variances that were present in the original article by Greenland and Longnecker. The Greenland and Longnecker method is cited more than that of Hamling [144]; however, the Greenland and Longnecker method assumes that the correlation matrices for the unadjusted and adjusted risk measures are about the same, whereas the Hamling method accounts more clearly for confounding [144]. As the most adjusted risk measures were included in the meta-analyses and confounding could, therefore, have played a role, the Hamling method was preferred.

To address the second issue and set the exposure in the reference group for each study to zero, the exposure in the referent group must be subtracted from all the exposures in that study [96], thereby estimating a trend that intersects the origin. Referent exposure subtracted models will in effect be delta exposure models.

To address the third issue, heterogeneity between studies must be incorporated. Heterogeneity between studies exists as a result of many factors, including the variety of characteristics of subjects used in different studies, as well as different methods that are used to complete studies [145]. To incorporate the heterogeneity, a random-effects model can be used. This model assumes that the effects estimated by different studies follow a certain statistical distribution and are, therefore, not identical [15].

To assess the heterogeneity of the models, a chi-squared test using the chi-squared statistic (Q) must be performed [15]. If the *p*-value of the chi-squared (X^2) test is smaller than 0.1, significant heterogeneity is presented [15]. To determine the impact of the heterogeneity on the model (measure the inconsistency between studies), an I^2 statistic can be calculated using equation (14) [15].

$$I^2 = \frac{Q - df}{Q} \times 100\% \tag{14}$$

where

$$I^2 = measure\ of\ inconsistency$$
$$Q = chi - squared\ statistic$$
$$df = degrees\ of\ freedom\ (number\ of\ studies - 1)$$

Some guidelines on the interpretation of I^2 (in conjunction with the interpretation of the *p*-value for the chi-squared test) are [15]:

1. 0% to 40% - might not be significantly heterogeneous
2. 30% to 60% - may be moderately heterogeneous
3. 50% to 90% - may be substantially heterogeneous

4. 75% to 100% - considerably heterogeneous

To solve the fourth problem, the studies included in the meta-analysis must be weighted, as not all studies have the same importance. To do this, a weighting matrix that is dependent on the variances and co-variances per study must be calculated [58].

The fifth issue can be addressed by investigating linear and nonlinear trends to get the best fit for the data. Risk data is usually transformed using the natural logarithm of the risk, *ln(risk)*, as it has better statistical properties than the untransformed risk [59]. Orsini *et al.* [144] proposed methods to obtain linear and nonlinear dose-response relations of the natural logarithm-transformed risks. A linear model of the transformed risk (log-linear model of the untransformed risk) will correspond to the form shown in equation (15).

$$\text{Risk} = e^{\beta \times \text{exposure}} \tag{15}$$

β is the slope, estimating the rate of change of the natural logarithm of the risk as a result of a change in the exposure [59]. The risk measure will depend on the original data (either RR, OR or HR).

The nonlinear relation uses a restricted cubic spline (RCS) approach [144]. This model works with piecewise cubic polynomial functions between knots, and linear functions below the first and above the last knot [146]. Knots indicate places where a change in direction of the plot is possible. The first and second derivatives of the function at the knot positions are continuous [147]. The RCS model is given by equations (16) to (18) [148], [149]. The b_0 coefficient will be 0, as no intercept model is used in this case.

$$f(X) = b_0 + b_1 X_1 + b_2 X_2 + \cdots + b_{k-1} X_{k-1} \tag{16}$$

where

$$b_0 = 0 \text{ (no intercept, referent exposure subtracted models)}$$
$$b_0 \dots, b_{k-1} = spline\ coefficients$$
$$k = number\ of\ knots$$
$$X = X_1 = exposure$$

$$X_i = \frac{(X - knot_{i-1})_+^3 - \frac{(knot_k - knot_{i-1})(X - knot_{k-1})_+^3 - (knot_{k-1} - knot_{i-1})(X - knot_k)_+^3}{(knot_k - knot_{k-1})}}{(knot_k - knot_1)^2} \tag{17}$$

where

$$i = 2, \dots, k-1$$
$$k = number\ of\ knots$$

$$(u)_+ = \begin{cases} u, for\ u > 0 \\ 0, for\ u \leq 0 \end{cases}, where\ u\ is\ the\ function\ contained\ in\ brackets$$

$$X = X_1 = exposure$$

$$Risk = e^{f(X)} \tag{18}$$

For the purposes of this study an RCS model with three knots (k = 3) will be chosen. Knots are located at the 10th, 50th and 90th percentiles of the data [96], [149].

The nonlinearity can be assessed by a hypothesis test. The null hypothesis to be tested is that the coefficients of the splines $(b_2, \ldots, b_{k-1})$) is zero [96]. Therefore, if the *p*-value for nonlinearity is > 0.05 the relation can be assumed to be linear [96]. In this study, only the coefficient of the second spline will need to be assessed for nonlinearity as only three knots are used.

To incorporate the changes required to address issues 1 to 5 and perform the dose-response meta-analysis, several options were available. All of the below modules are available free of charge. Orsini *et al.* [58], [144] created a module for Stata, called *GLST* (generalised least squares for trend estimation), which can perform random-effects and fixed-effects meta-analysis, using the Greenland and Longnecker or Hamling methods to calculate the covariance matrices. A software module, *dosresmeta*, has been developed for the open-source statistical software R [150]. The module was developed by Alessio Crippa and is the R-equivalent of the *GLST* Stata module created by Orsini *et al.* [58], [144]. A SAS module,%*metadose*, with similar functionality, was created by Li and Spiegelman [144].

The preferred choice was the *dosresmeta* module, as the statistical software, R, required is available free of charge, while license fees are required for SAS and Stata.

5.3.2 Lifestyle factors and HbA_{1c}

To combine studies relating HbA_{1c} to lifestyle factors, a slightly different approach was followed. The slope and SE of each study included in the meta-analysis were obtained using the inverse-variance weighting method [59]. The overall dose-response estimate was then calculated by combining the slopes of each study using a random-effects meta-analysis. In one case the between-study variance (τ^2) for the random-effects analysis was found to be negative [2]. In this case the model was reduced to a fixed-effect model. A Microsoft Excel spread sheet was developed to perform the analysis and calculate the required *p*-values. The results of the analysis (except for the *p*-values) were validated using the Excel spread sheet developed by Neyeloff *et al.* [151]. Only linear models were evaluated.

The data was prepared by subtracting the exposure (lifestyle factor dose) in the referent category from the exposures in each category. The values of the dependent variable (HbA_{1c}) were transformed to a delta HbA_{1c} level by subtracting the HbA_{1c} value in the referent category from the HbA_{1c} values in each category. This resulted in an exposure and HbA_{1c} of 0 in the referent category for each study.

5.3.3 Development of the glycaemic models and comparison with overall models

The glycaemic models were created by combining the models created using CRC risk and HbA_{1c} to those created using lifestyle factors and HbA_{1c}. The range for which the combined models are valid was calculated from the intersection of the models [2]. Only statistically significant models were combined. These models could then be compared to the statistically significant models obtained using lifestyle factors and CRC risk directly.

5.4 Assigning a single exposure for a range of exposures

When a range of exposures are provided per dose-response category, a single value needs to be selected to represent that range. The method for assigning a single value can, however, potentially affect the outcome [152]. Method 1 in Hartemink *et al.* [152] was selected. Other possibilities that could possibly have been explored included fitting the dose response to a statistical distribution if that distribution fits the data (such as a gamma distribution, if there is a linear dose-response relationship) or adjusting for a non-linear dose-response relationship using an iterative method [152]. Method 1 was, however, chosen as it simplifies the solution and is a commonly-used method.

Method 1 works as follows. Where a range of exposure values is provided the midpoint (median or average) is selected to represent that range. Where an open-ended range is specified the exposure value for the lower open-ended range can be calculated by subtracting half of the width of the second lowest range from the specified upper value for the bottom open-ended range [152]. The exposure for the upper open-ended range is calculated by adding the width of the second highest range to the specified bottom value of the upper open-ended range [152]. If the calculation would result in a negative value for the lowest exposure category a value of 0 was chosen to represent this category. If mean or median values for ranges were specified in a study these values were used. An example of this method provides clarification: If three ranges of exposures are specified (e.g. < 10, 10 to 15, and > 15), the exposure for the bottom range is $10 - ½ \times (15 - 10) = 7.5$. The dose for the middle range is $½ \times (15 + 10) = 12.5$. The dose for the top range is $15 + (15 - 10) = 20$.

5.5 Validation and verification of statistical software used

The verification and validation for the most important parts of the statistical software that were used to perform the calculations are shown in Table 3. As not all published studies presented results on the same factors or in the same way, different components were compared. The percentage errors between the expected and observed values were calculated using Equation (19).

$$\% \, error = \frac{|observed - expected|}{expected} \times 100 \tag{19}$$

It is clear that the percentage error in each case is small (less than 1%), and, therefore, the expected and observed values are close (with the expected values identical to the observed values when rounded to three decimal digits). The software can, therefore, be used to perform the calculations.

Table 3: Verification/validation of statistical software used.

Component (software module)	**Expected values (Published values)**	**Observed values (Software)**	**Percentage (%) error**
Random-effects meta-analysis – log-linear: CRC risk and HbA_{1c} CRC risk and lifestyle factors (*dosresmeta*, *R*) Compared to [144]	**RR for 12 g of alcohol per day:** 1.08 **95% CI for 12 g of alcohol per day:** (1.05, 1.11)	**RR for 12 g of alcohol per day:** 1.079647 **95% CI for 12 g of alcohol per day:** (1.045735, 1.114659)	**RR for 12 g of alcohol per day:** 0.033% **95% CI for 12 g of alcohol per day:** (0.406%, 0.420%)
Trend per study: HbA_{1c} and lifestyle factors (Author's own Microsoft Excel	**Beta:** 0.0459	**Beta:** 0.045845939	**Beta:** 0.118%

Component (software module)	Expected values (Published values)	Observed values (Software)	Percentage (%) error
spread sheet) Compared to [59]	**SE:** 0.0156	**SE:** 0.015551322	**SE:** 0.312038462%
Meta-analysis: HbA_{1c} and lifestyle factors (Author's own Microsoft Excel spread sheet) Compared to [151]	**Beta:** 0.196427619 **SE:** 0.004741741	**Beta:** 0.196427619 **SE:** 0.004741741	**Beta:** 0% **SE:** 0%

5.6 Conclusion

This chapter provided the statistical background and the methods used in the development of the models. Validation and verification of the software used to implement the models were also provided in this chapter.

The following chapter provides the results and a discussion of the results from the models developed using the methodologies described in this chapter.

CHAPTER 6 RESULTS AND DISCUSSION

6.1 Introduction

The consolidated results are presented in this chapter. Only the salient results from the two journal articles are repeated here. Some additional graphs are included to better contextualise the findings.

6.2 Does cancer risk increase with HbA_{1c}, independent of diabetes?

Selected results obtained from the study on the effects of HbA_{1c} on cancer risk are presented in the following sections [1]. Results for CRC are discussed in detail in section 6.2.1 as those will be expanded on in the subsequent sections. A summary of the statistically significant or borderline significant results for all cancer types investigated is provided in section 6.2.2.

6.2.1 CRC risk and HbA_{1c}

The results presented here are taken from article 1 [1]. Figures 2 and 3 show the statistically significant or borderline significant models obtained from studies relating HbA_{1c} levels to CRC incidence or mortality risk.

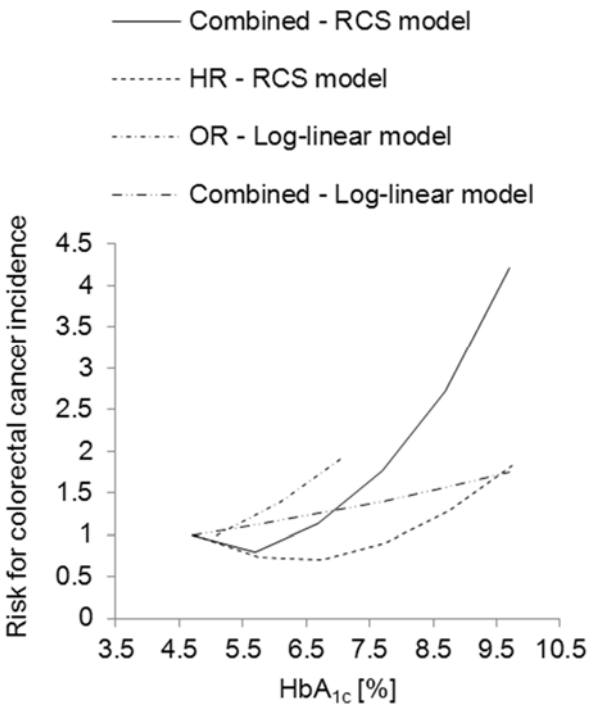

Figure 2: Relationship between risk for colorectal cancer (CRC) incidence and HbA_{1c} level.

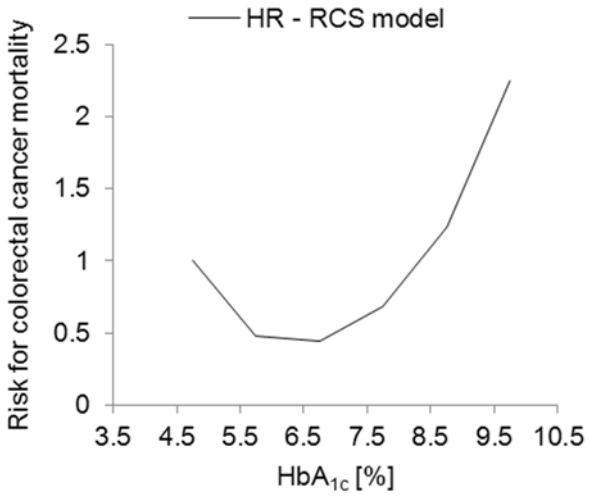

Figure 3: Relationship between risk for colorectal cancer (CRC) mortality and HbA_{1c} level.

1. **Figure 2:** There is evidence of increased risk in the pre-diabetic and normal HbA_{1c} ranges from the OR and combined log-linear models. There is evidence of decreased risk in the pre-diabetic, normal and the lower portion of the diabetic ranges from the RCS models. The risk increases sharply in the diabetic HbA_{1c} range for all models. The models were obtained by including all data points (also those below the reference level). The study by Joshu *et al.* [46] found high risks below the reference level.
2. **Figure 3:** Only one study [46], with datasets for both men and women, was included in the mortality model. This model showed increased risk in the upper diabetic range, but decreased risk in the pre-diabetic, normal and lower diabetic range. It must be noted that these studies included data points below the reference with higher risks than the reference. As the model is referenced to the lowest data point in both studies, the model starts at a risk of 1 and decreases. However, it must be noted that the risk is relative to a higher reference level at the first data point, which does not mean that there is no risk at the lowest HbA_{1c} level and a decreased risk at the pre-diabetic levels, but rather a higher risk at the lowest data point and a lower risk in the pre-diabetic range.

The above provides evidence for increased CRC risk with increasing HbA_{1c}, but it is clear that some of the evidence is contradictory. The results from one study [46] showed increased risk below the reference HbA_{1c} level (< 5%). The authors of [46] speculated that low glycaemic levels may indicate poor health or undetected cancer, but could not find evidence of this using reverse causation; they indicated that more research was required into those with low HbA_{1c} levels. The low glycaemic level results may be due to some confounding factors affecting that specific study population or study design. This could also indicate that there are risks involved in lowering the HbA_{1c} levels too much. This must be kept in mind

when designing treatment strategies. Including the data points below the reference complicates interpretation of the results, as results are rescaled to be referenced to the lowest data point in the studies. This issue is addressed in article 2 [2], and the results are provided in sections 6.3 to 6.7.

6.2.2 Summarised discussion

Cancer sites should be investigated separately as different trends are observed at different sites [1]. It is possible that some cancer sites or types are affected more by increases in HbA_{1c} than others.

The study provided evidence for [1]:

- increased cancer risk in the normal and pre-diabetic ranges for colorectal, pancreatic, gastric and respiratory cancers.
- higher risk for cancer incidence in the diabetic range for colorectal, pancreatic, gastric and liver cancers and, in the upper diabetic range, for breast cancer.
- decreased risk for breast cancer incidence in the normal and pre-diabetic range.

The study also provided possible evidence (from borderline significant results) for [1]:

- decreasing prostate cancer incidence risk in the diabetic, pre-diabetic and normal ranges.
- increased breast cancer incidence risk in the diabetic, pre-diabetic and normal ranges.

6.3 Effects of HbA_{1c} on CRC risk

To assess the relationship between HbA_{1c} and CRC risk quantitatively dose-response meta-analysis methods were used. The models that were developed for the relation between CRC risk and a change (Δ) in HbA_{1c} are shown in Table 8 in Annexure D (adapted from [2]). The following discussion emanates from article 2 [2].

Eight studies on the relation of HbA_{1c} with CRC risk were found. Two studies [49], [153] were excluded as the study populations or a portion of the study populations that were used for these studies were already included in two other studies [154], [155]. Only the newest studies [154], [155] were included.

One study by Joshu *et al.* [46] investigated the relation between HR for CRC and HbA_{1c}. No statistically significant relationship was found (p = 0.1312). Two data points were present below the referent category. This could have influenced the results as the lowest category was not the referent. It was also not possible to rescale the study so that the lowest category became the referent category, as the 95% CIs for the referent category were not provided. In a further analysis the data points below the reference

were, therefore, excluded. This resulted in an increasing, log-linear model ($p = 0.0389$). This final model was used to develop the glycaemic models for comparison purposes.

Three studies [156], [154], [157] investigated the relation between OR for CRC and HbA_{1c}. The meta-analysis of these studies resulted in an increasing log-linear dose-response relationship ($p = 0.0050$).

Two studies [158], [155] examined the relation between RR for CRC and HbA_{1c}. Neither the log-linear ($p = 0.3859$) nor nonlinear ($p = 0.8576$) models were statistically significant.

None of the models showed significant heterogeneity ($p = 0.8299$, $I^2 = 1\%$ for HR; $p = 0.9121$, $I^2 = 1\%$ for HR excluding data points below the reference; $p = 0.3110$, $I^2 = 16.1\%$ for OR; $p = 0.7731$, $I^2 = 1\%$ for RR).

Combining all risks under the rare disease assumption resulted in a statistically significant log-linear trend ($p = 0.0134$). Exclusion of the data points below the reference for the HR studies resulted in an increasing log-linear trend ($p = 0.0020$). No significant heterogeneity was identified in either case ($p = 0.1633$, $I^2 = 33.2\%$ for combined risk; $p = 0.2517$, $I^2 = 22.3\%$ for combined risk excluding data points below reference). The model with excluded data points was used to develop the glycaemic model for comparison purposes.

The final statistically significant models that were used to create the glycaemic models are presented in Figure 4.

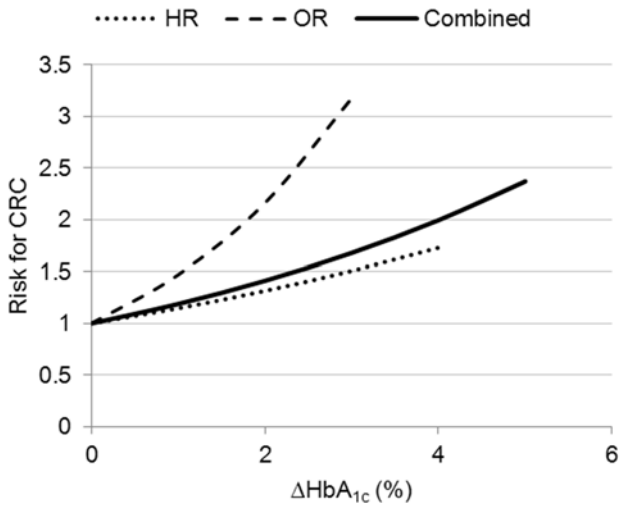

Figure 4: Effects of changes in HbA_{1c} on CRC risk.

6.4 Effects of lifestyle factors on HbA_{1c}

The models of the effects of changes in lifestyle factors on HbA_{1c} are shown in Table 9 in Annexure D (adapted from [2]). The discussions in the subsequent subsections follow from article 2 [2].

6.4.1 Excessive carbohydrate intake (glycaemic load)

Two studies [159], [160] examined the relation between changes in carbohydrate intake, measured as glycaemic load, and changes in HbA_{1c}. The between-study variance (τ^2) in the random-effect model was found to be negative. A fixed-effect model was, therefore, used. The results presented a statistically significant increasing linear trend ($p = 0.0035$), with no evidence of significant heterogeneity ($p = 0.5773$, $I^2 = 0\%$). The association was weakened and became non-significant ($p = 0.2695$ using a random-effects model) when a third (statistically non-significant) study by Van Aerde *et al.* [161] was included. The heterogeneity was, however, not significantly altered, when comparing the model obtained via a fixed-effect method (excluding the study) to the model obtained using a random-effect method (including the study). The statistically significant model (excluding [161]) was used to develop the glycaemic model. The results are shown in Figure 5.

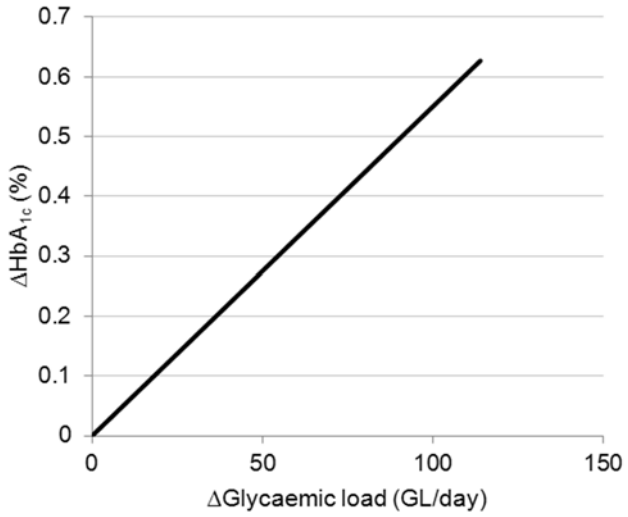

Figure 5: Relationship between changes in HbA_{1c} and changes in glycaemic load (GL units per day).

6.4.2 Chronic stress

The increasing linear model that was obtained from the results of two studies [162], [163] was not statistically significant ($p = 0.5425$). No significant evidence of heterogeneity was found ($p = 0.3173$, $I^2 = 0\%$). After the study by Feldman *et al.* [162], which was adjusted for sex and age only, was excluded, the increasing linear trend became statistically significant ($p = 0.0000$). This final model was used to develop the glycaemic models. The results are shown in Figure 6.

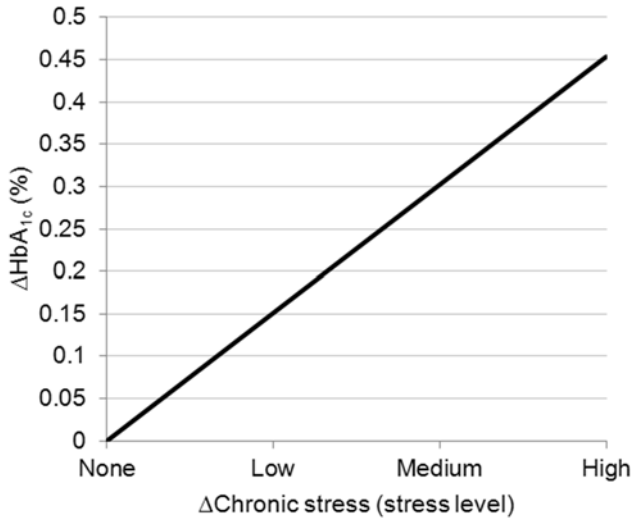

Figure 6: Relationship between changes in HbA_{1c} and changes in chronic stress levels.

6.4.3 Cigarette smoking

Three studies [118], [164], [165] examined the relationship between changes in the number of cigarettes smoked per day and changes in HbA_{1c}. The linear trend was increasing and statistically significant ($p = 0.0005$), with no significant evidence of heterogeneity ($p = 0.5238$, $I^2 = 0\%$). The results are shown in Figure 7.

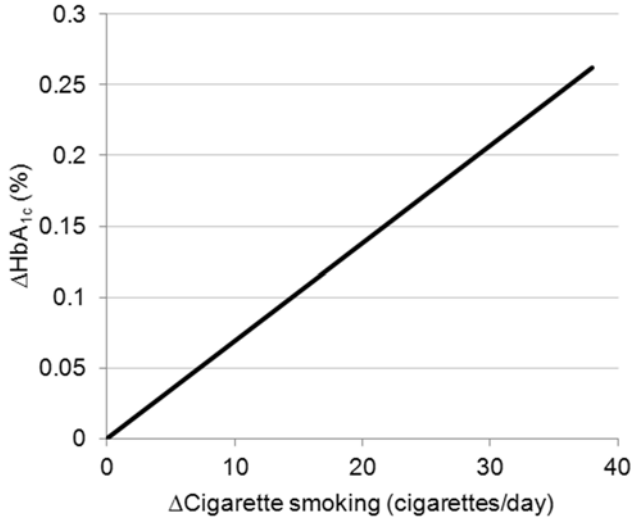

Figure 7: Relationship between change in HbA_{1c} and change in number of cigarettes smoked per day.

6.4.4 Dietary fibre intake

Three studies [166], [167], [168] investigated the relation between changes in dietary fibre intake and changes in HbA_{1c}. A statistically non-significant increasing trend ($p = 0.0952$), with some evidence of heterogeneity ($p = 0.0522$, $I^2 = 66.1\%$) was found. Excluding the age- and sex-adjusted study of Ikeda *et al.* [166] resulted in a statistically significant decreasing linear model ($p = 0.0002$), with no significant evidence of heterogeneity ($p = 0.3173$, $I^2 = 0\%$). The statistically significant model (excluding [166]) was used to develop the glycaemic model. The results are shown in Figure 8.

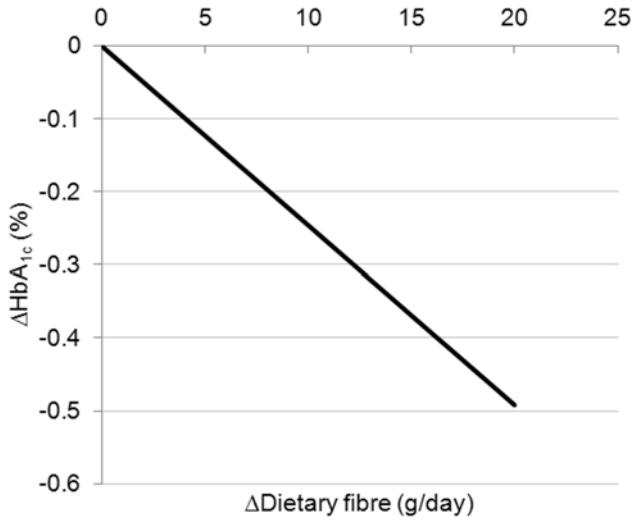

Figure 8: Relationship between change in HbA_{1c} and change in dietary fibre intake (grams of fibre per day).

6.4.5 Alcohol consumption

Five studies analysed the relationship between changes in HbA_{1c} and changes in alcohol. The meta-analysis of these studies resulted in a statistically significant decreasing linear trend ($p = 0.0000$), with no evidence of significant heterogeneity ($p = 0.4914$, $I^2 = 0\%$). The results are shown in Figure 9.

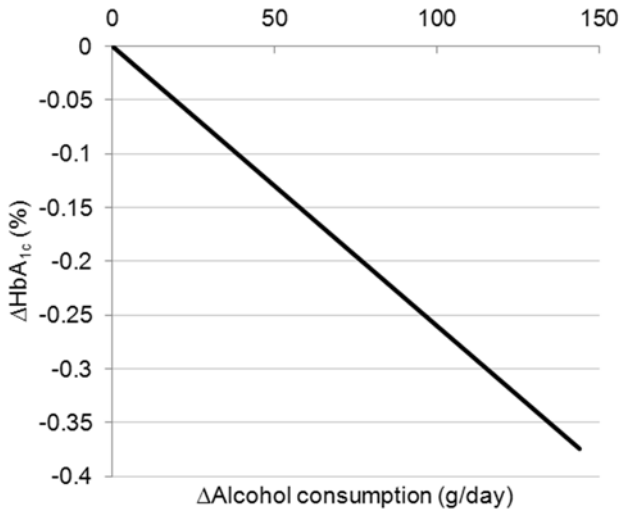

Figure 9: Relationship between change in HbA_{1c} and change in alcohol consumption (grams of ethanol per day).

6.4.6 Physical exercise

One study [169] with two datasets, one for moderate and one for intense activity, was analysed. The resulting decreasing linear trend was statistically significant ($p = 0.0002$), with no significant evidence of heterogeneity ($p = 0.3173$, $I^2 = 0$). The results are shown in Figure 10.

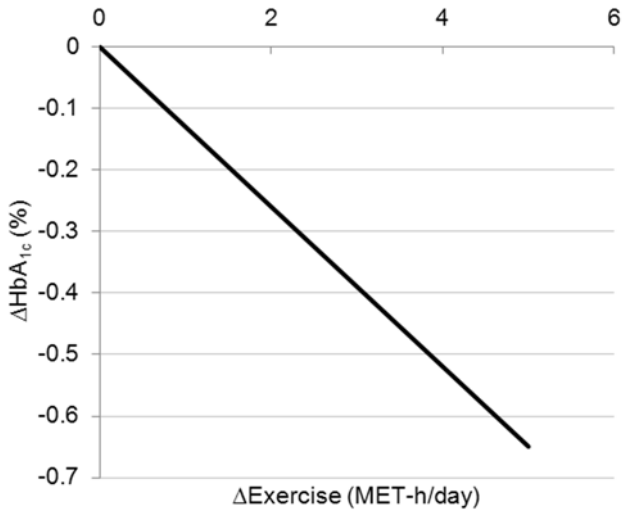

Figure 10: Relationship between HbA_{1c} and change in exercise (metabolic equivalent hours per day).

6.5 Glycaemic effects of lifestyle factors on CRC risk

The results from the combination of the statistically significant HbA_{1c} and lifestyle factor models with the statistically significant CRC risk and HbA_{1c} models (taking into account the range of values for which the intersection of the models are valid) are shown in Table 10 in Annexure D. No models for RR could be developed as the RR and HbA_{1c} models were not statistically significant. The results from the RR studies were, however, included in the combined models.

6.5.1 Excessive carbohydrate intake (glycaemic load)

Increasing log-linear models for the relation between changes in GL intake and CRC risk were obtained. The results are shown in Figure 11.

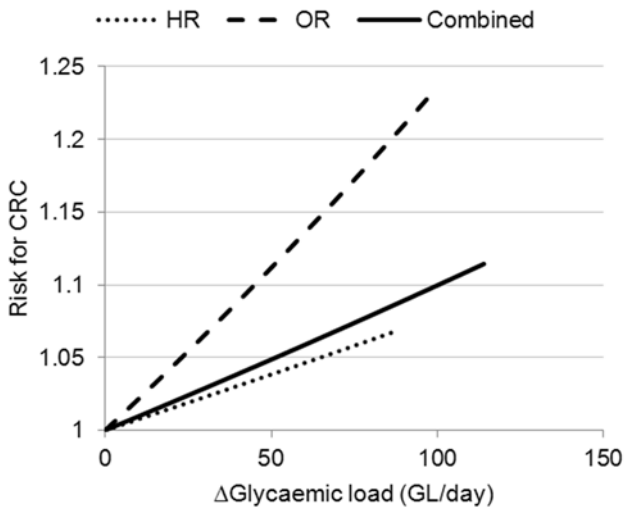

Figure 11: Glycaemic models of relationship between CRC risk and changes in glycaemic load (GL units per day).

6.5.2 Chronic stress

Increasing log-linear models for the relations between changes in stress levels and CRC risk were obtained. The results are shown in Figure 12.

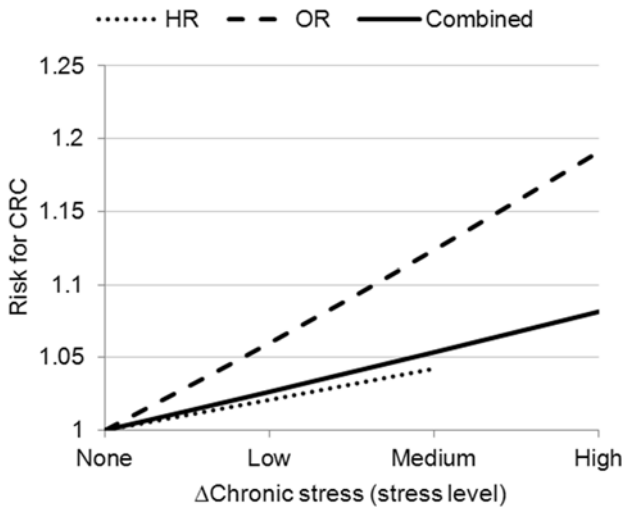

Figure 12: Glycaemic models of relationship between CRC risk and changes in stress levels.

6.5.3 Cigarette smoking

Increasing log-linear models for the relations between changes in number of cigarettes smoked per day and CRC risk were obtained. The results are shown in Figure 13.

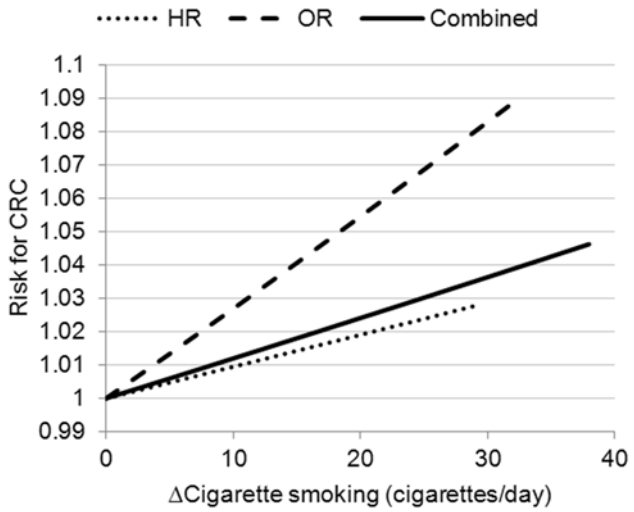

Figure 13: Glycaemic models of relationship between CRC risk and changes in number of cigarettes smoked per day.

6.5.4 Dietary fibre intake

Decreasing log-linear trends were found for the relations between changes in dietary fibre intake and CRC risk. The results are shown in Figure 14.

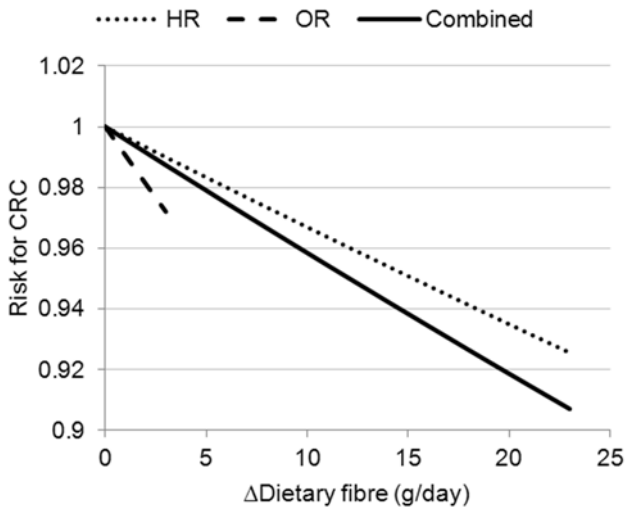

Figure 14: Glycaemic models of relationship between CRC risk and changes in dietary fibre intake (grams of fibre per day).

6.5.5 Alcohol consumption

Decreasing log-linear trends were found for the relations between changes in dietary fibre intake and CRC risk. The results are shown in Figure 15.

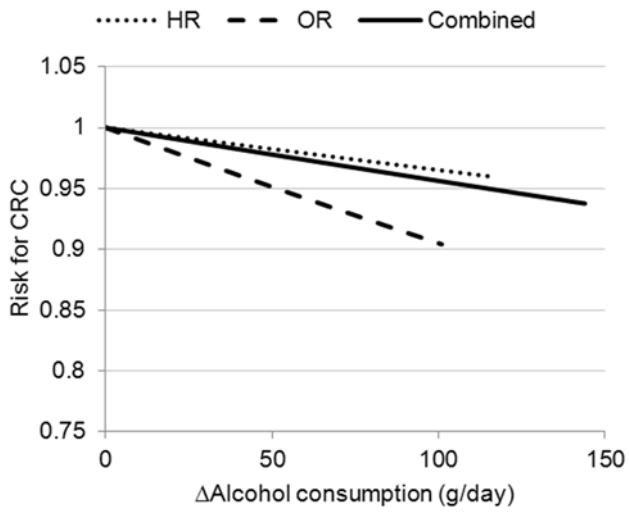

Figure 15: Glycaemic models of relationship between CRC risk and changes in alcohol consumption (grams of ethanol per day).

6.5.6 Physical exercise

Decreasing log-linear models for the relations between changes in exercise and CRC risk were obtained. The results are shown in Figure 16.

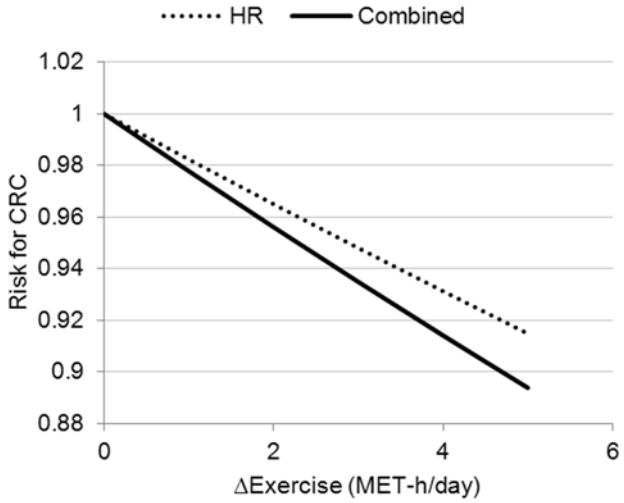

Figure 16: Glycaemic models of relationship between CRC risk and changes in exercise energy expenditure (metabolic equivalent-hours per day).

6.6 Full effects of lifestyle factors on CRC risk

The overall models of the full effects of lifestyle factors on CRC risk are shown in Table 11 in Annexure D (from [2]).

6.6.1 Excessive carbohydrate intake

No statistically significant trend ($p = 0.9092$) was obtained from six studies [170]–[175]. Heterogeneity was significant ($p = 0.0520$, $I^2 = 51.9\%$).

6.6.2 Chronic stress

Two studies presented results on the relation between changes in chronic stress and CRC risk; one study presented results for HR [114], while the other study presented results for OR [176]. No studies reported results for RR. There was a statistically non-significant decreased risk for HR ($p = 0.5611$), while there was a statistically significant increased risk for OR ($p = 0.0428$). Combining all studies resulted in no statistically significant trends. Both the HR and combined models showed significant evidence of heterogeneity ($p = 0.0503$, $I^2 = 73.9\%$ for HR; $p = 0.0137$, $I^2 = 76.7\%$ for combined data), while this was not present in the OR model. The statistically significant OR model is shown in Figure 17.

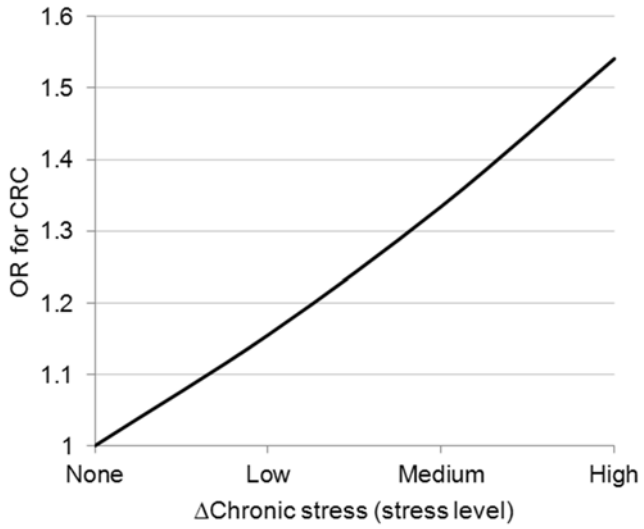

Figure 17: Relationship between CRC risk and change in stress level.

6.6.3 Cigarette smoking

Nineteen studies were analysed. Four studies showed results for HR, fourteen for OR, and one for RR. The increasing trend was only statistically significant for HR (p = 0.0186). The combination of all studies resulted in a statistically significant increasing trend (p = 0.0174). All models, except for RR, showed significant evidence of heterogeneity (p = 0.0206, I^2 = 69.3% for HR; p = 0.0001, I^2 = 66.3% for OR; p = 0.0000, I^2 = 67.2% for combined data). The statistically significant results are shown in Figure 18.

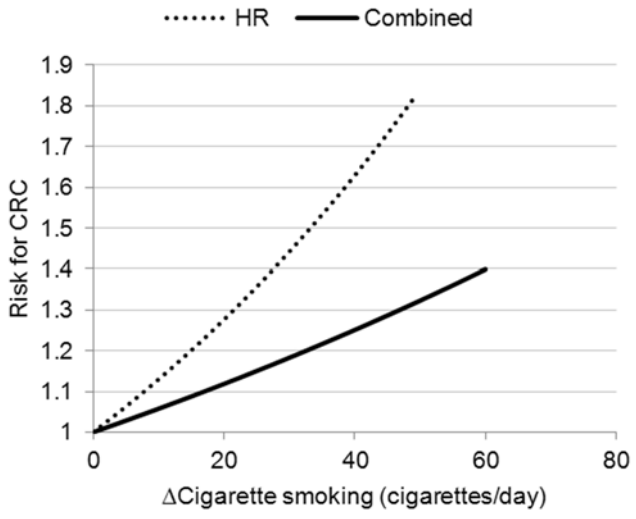

Figure 18: Relationship between CRC risk and change in number of cigarettes smoked per day.

6.6.4 Dietary fibre intake

Nine studies were analysed. Five studies showed results for HR and four studies for OR; no studies reported on RR. The decreasing trend was only statistically significant for HR ($p = 0.0445$). This model did not show evidence of significant heterogeneity ($p = 0.1290$, $I^2 = 39.4\%$), in contrast to the OR model where significant heterogeneity was observed ($p = 0.0003$, $I^2 = 84.1\%$). When the data points below the reference in the HR model were excluded the model became statistically non-significant ($p = 0.0949$), and showed some heterogeneity ($p = 0.0937$, $I^2 = 44.6\%$).

Combination of all studies resulted in a nonlinear trend ($p = 0.0479$ for nonlinearity), with significant evidence of heterogeneity ($p = 0.0024$, $I^2 = 53\%$). Excluding the data points below the reference in the HR data resulted in a log-linear decreasing trend ($p = 0.0068$), but the results were still significantly heterogeneous ($p = 0.0001$, $I^2 = 72.1\%$). The statistically significant results (excluding the data points below the reference level) are shown in Figure 19.

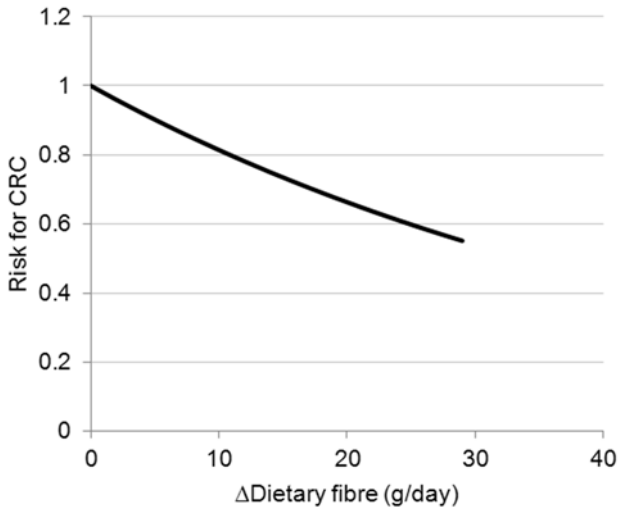

Figure 19: Overall model of relationship between CRC risk and changes in dietary fibre intake (grams of fibre per day).

6.6.5 Alcohol consumption

Twenty-three studies were analysed. Eight studies showed results for HR, 15 studies for OR and two studies for RR. Both the HR and OR models were statistically significant and showed increasing log-linear trends ($p = 0.0000$ for HR; $p = 0.0412$ for OR). There was no statistically significant trend for RR data ($p = 0.5187$). Significant evidence of heterogeneity was present in both the HR and OR models ($p = 0.0640$, $I^2 = 47.6\%$ for HR; $p = 0.0000$, $I^2 = 98.5\%$ for OR), with no evidence of heterogeneity for RR data ($p = 0.3837$, $I^2 = 1\%$). When the data points below the reference were excluded in both the OR and

HR models, the models changed only slightly (p = 0.0000 for HR; p = 0.0326 for OR) and were still significantly heterogeneous (p = 0.0730, I^2 = 46% for HR; p = 0.0000, I^2 = 98.5% for OR).

Combination of all risk measures resulted in a statistically significant log-linear model (p = 0.0001), with significant heterogeneity (p = 0.0000, I^2 = 82%). Exclusion of the data points below the reference resulted in a log-linear increasing model (p = 0.0031) which still presented significant heterogeneity (p = 0.0000, I^2 = 97.5%). The nonlinear term was borderline significant in this case (p = 0.0529 for nonlinearity). The statistically significant results (excluding the data points below the reference level) are shown in Figure 20.

Figure 20: Overall models of relationship between CRC risk and changes in alcohol consumption (grams of ethanol per day).

6.6.6 Physical exercise

Analysis of three studies produced no statistically significant trends (p = 0.3597 for HR, p = 0.1235 for OR and p = 0.5509 for RR), although there seems to be evidence of decreasing risk as the MET-h/day increased. Combination of all risks produced no statistically significant trends (p = 0.1485). The RR and combined models showed some evidence of heterogeneity (p = 0.0676, I^2 = 70.1% for RR; p = 0.0969, I^2 = 49.1% for combined data). No statistically significant models were available for further comparison.

6.7 Comparison of glycaemic and overall models

Comparison of the glycaemic and overall models were only possible for seven models as only those models were statistically significant and were defined for overlapping ranges – three for alcohol (HR, OR and combined risk), one for dietary fibre (combined risk), one for chronic stress (OR) and two for

cigarette smoking (HR and combined risk). The overall models had a greater effect than the glycaemic models on increasing or decreasing CRC risk.

In the case of alcohol consumption, the direction of the association for the overall models (increasing) was different to those of the glycaemic models (decreasing) as shown in Figure 21.

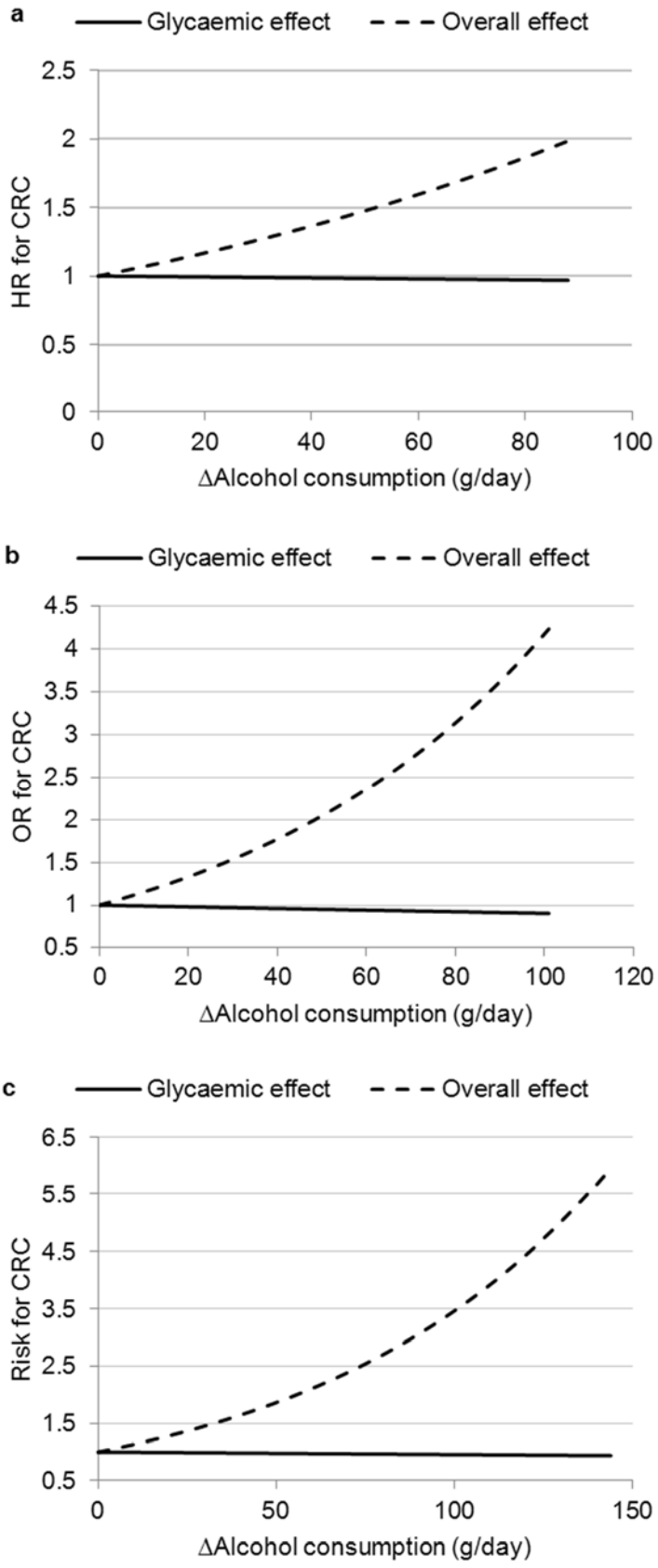

Figure 21: Comparison between glycaemic and overall models showing relations between changes in alcohol consumption and risk for CRC (a shows HR, b shows OR and c shows the combined risk).

The direction of the association for the overall and glycaemic models was the same and decreased for dietary fibre intake (Figure 22).

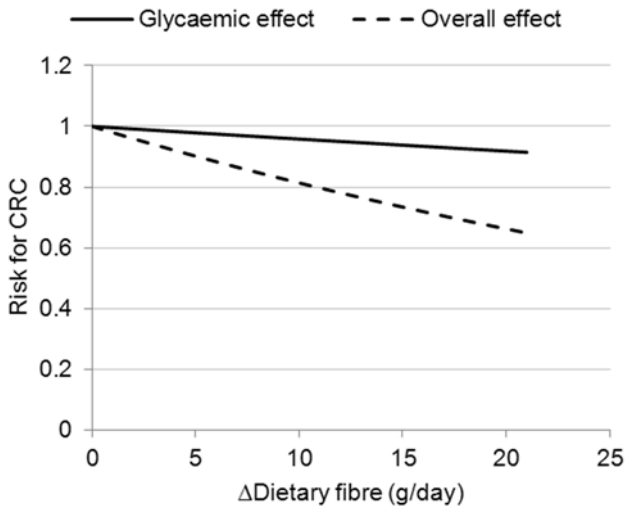

Figure 22: Comparison between glycaemic and overall models showing relations between changes in dietary fibre intake and risk for CRC (combined model).

The direction of association was the same (increasing) for both the glycaemic and overall models for chronic stress (Figure 23).

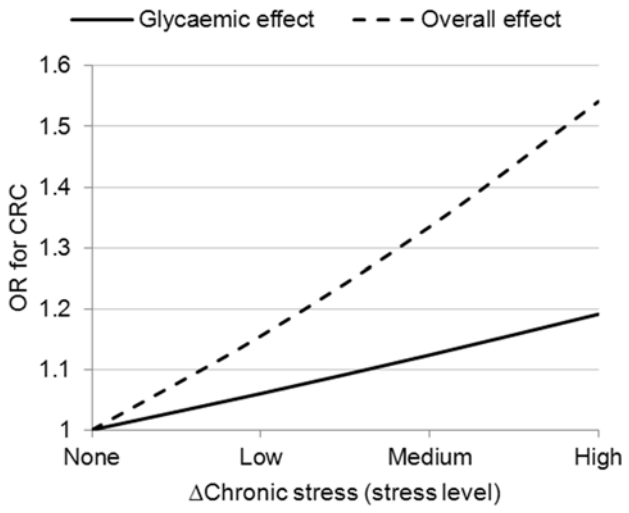

Figure 23: Comparison between glycaemic and overall models showing relations between changes chronic stress levels and OR for CRC.

The risk for CRC increased for both the glycaemic and overall models with increased number of cigarettes smoked (Figure 24).

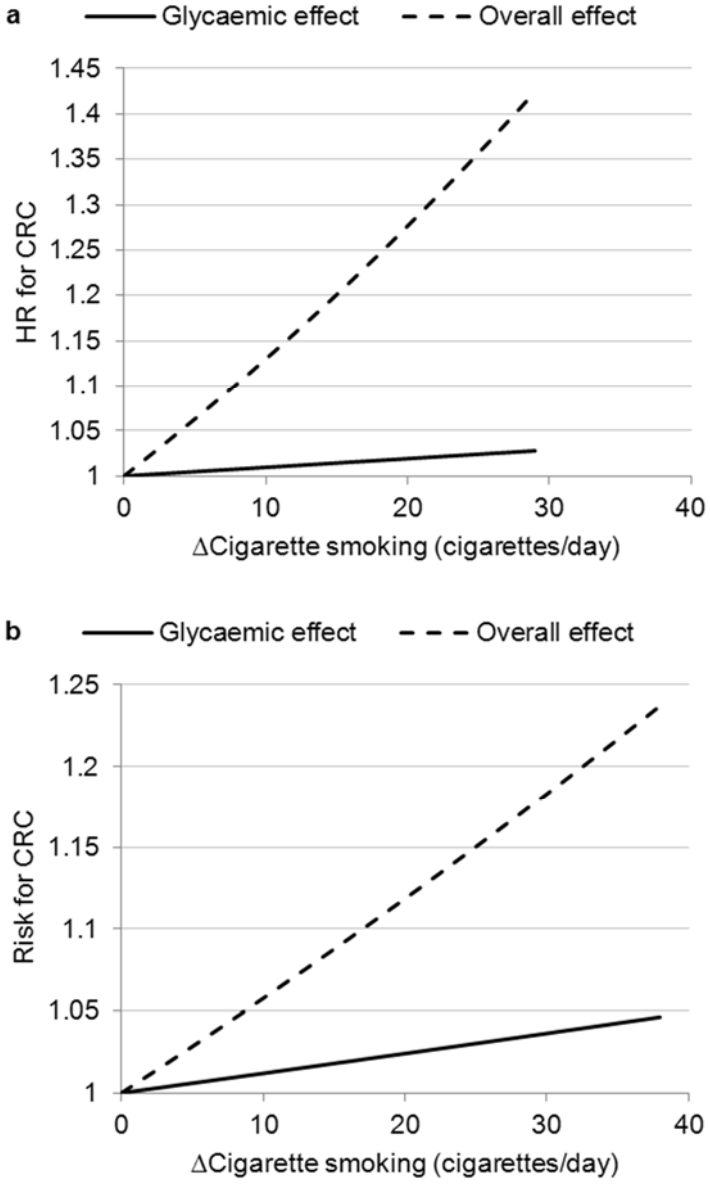

Figure 24: Comparison between glycaemic and overall models showing relations between changes in number of cigarettes smoked and risk for CRC (a shows HR, b shows the combined risk).

6.8 Discussion

6.8.1 Effects of HbA_{1c} on CRC risk

The final statistically significant models for the relation of CRC risk with HbA_{1c} showed increases in CRC risk as HbA_{1c} increased. This resulted in increases in risk per each 1% increase in HbA_{1c} of HR = 1.15 (95% CI = 1.01 to 1.31), OR = 1.47 (95% CI = 1.12 to 1.92) and combined risk = 1.19 (95% CI =

1.07 to 1.33). The HR and combined models show somewhat lower risks than the results obtained by Khaw *et al.* [153] of RR = 1.3 (95% CI = 1.04 to 1.61) per each 1% increase in HbA_{1c}. HR, OR and combined models predict higher risk than that obtained by Rinaldi *et al.* [154] of OR = 1.1 (95% CI = 1.01 to 1.19) for each 10% increase in HbA_{1c}. This 10% increase was approximately equal to the increase in HbA_{1c} level in the highest quintile (> 6.1%) compared to that in the lowest quintile (≤ 5.4%), and refers to fractional increase, rather than percentage unit increase (therefore, not an increase from an HbA_{1c} level of 5.4% to 15.4%, but rather an increase from 5.4% to 5.4% x 1.1 = 5.94%). The HR and combined models are valid in the normal, pre-diabetic and diabetic ranges, while the OR model is only valid in the normal and pre-diabetic ranges. This provides quantitative evidence that risk is already increased in the normal and pre-diabetic HbA_{1c} ranges.

6.8.2 Effects of lifestyle factors on HbA_{1c}

Statistically significant evidence was provided for increased HbA_{1c} with increases in the number of cigarettes smoked per day, increasing stress level and increased carbohydrate intake in the form of GL (by eating a greater amount of carbohydrates, eating carbohydrates with a higher GI or by the combination of the two). Statistically significant evidence was provided for decreased HbA_{1c} with increased alcohol consumption, dietary fibre intake and physical exercise in the form of MET-h/day (by increasing the time spent exercising, the type of exercise or intensity of the exercise, or a combination).

6.8.3 Glycaemic effects of lifestyle factors on CRC risk

The glycaemic models have the same direction of association (increasing or decreasing) as the corresponding lifestyle factor and HbA_{1c} models. Evidence was provided for increased CRC risk with increased GL, stress level and number of cigarettes smoked per day. Evidence was provided for decreased CRC risk with increased alcohol consumption, dietary fibre intake and exercise.

6.8.4 Full effects of lifestyle factors on CRC risk

The overall models of the full effects of lifestyle factors on CRC risk showed no statistically significant associations between GL or exercise and CRC risk. There were statistically significant increasing risks with increased alcohol consumption, chronic stress and cigarette smoking. There were statistically significant decreased risks with increased dietary fibre intake.

6.8.5 Comparison of glycaemic and overall models

A comparison between the glycaemic and overall models for alcohol consumption revealed that the direction of association differed between the overall and glycaemic models. The overall models showed an increase in risk as alcohol consumption increased, whereas the glycaemic model showed a decrease in risk with an increase in alcohol consumption. The contrasting results could be explained by several

factors. The decrease in HbA_{1c} (and subsequently in risk in the glycaemic model) could be caused by suppressed gluconeogenesis in the liver as a result of increased alcohol consumption, possibly by up to 45% [131]. It is hypothesised that this action is caused by the depletion of nicotinamide adenine dinucleotide (which is required for gluconeogenesis), as a result of the metabolism of the alcohol to acetate [131]. The increased risk seen in the overall model is likely caused by the carcinogenic effects of the ethanol contained in alcohol [132], and by its metabolism to acetaldehyde, which is a possible carcinogen [65], [80] which can cause damage to DNA [130]. Excess alcoholic intake may also indirectly affect CRC risk by increasing weight gain [130] and in turn obesity.

The overall alcohol consumption models in the current study showed significant heterogeneity. The combined risk model showed an increase of 1.36 for an increased alcoholic intake of 25 g (approximately two standard drinks) per day. When the data points below the reference were not excluded, a log-linear model resulted, with a risk of 1.2 for an increase of 25 g per day. A study by Fedirko *et al.* [177] found a second-order fractional polynomial dose-response relationship with $RR = exp(0.006992 \times alcohol - 0.00001 \times alcohol^2)$, corresponding to a more modest RR of 1.18 for an increase of 25 g per day. The Fedirko *et al.* study also showed significant heterogeneity. A meta-analysis on CRC mortality [178] in the US did not find any increase in CRC mortality for men, when consuming between 0 g and 39.99 g of alcohol per day; this study did, however, found an increased RR = 1.16 in women consuming between 20 g and 39.99 g of alcohol per day.

Both the glycaemic and overall models showed a decreased risk with increasing dietary fibre intake. In the overall combined model the risk for a 10 g per day increase in dietary fibre intake was approximately 0.81, whereas that in the glycaemic model was approximately 0.96 (approximately 16% lower risk in the overall model compared to the glycaemic model). The overall model was significantly heterogeneous. A meta-analysis by Aune *et al.* [179], which did not present significant heterogeneity, revealed a more modest RR of 0.9 (95% CI = 0.86 to 0.94) for a 10 g per day increase in dietary fibre intake. This analysis was performed based on sixteen studies, compared to the nine studies investigated in the overall model in the current study. When the overall (and Aune *et al.*) and glycaemic models were compared, it became clear that, although the decrease in risk as a result of a decrease in HbA_{1c} was significant, as seen in the glycaemic model, there were also other factors contributing to the protective effect of increased dietary fibre intake on CRC. Mechanisms which may be involved include those that decrease the amount of exposure of the colorectal system to carcinogens, such as the dilution of carcinogens in faeces as well as the reduced time it takes for faeces to travel in the digestive tract, as a result of an increase in dietary fibre intake [179].

The overall and glycaemic cigarette smoking models showed increased CRC risk with increasing number of cigarettes smoked per day. The effects seen in the overall models are, however, much stronger than those seen in the glycaemic models, as can be illustrated when comparing the risks when increasing

smoking by 20 cigarettes (one pack) per day (between 10% and 25% higher risk compared to the glycaemic model). The risk associated with an increase of 20 cigarettes per day was approximately 1.28 in the overall HR model, but only 1.02 in the glycaemic HR model (25% higher risk in overall compared to glycaemic model). Comparison of the combined risk model revealed that the risk in the overall model was approximately 1.12, compared to a risk of 1.02 in the glycaemic model (10% higher risk in overall than glycaemic model). A meta-analysis by Liang *et al.* [180] found an increased RR = 1.175 for an increase of 20 cigarettes per day and 1.38 for an increase of 40 cigarettes per day ($p < 0.0001$). This was based on eleven studies and showed no significant heterogeneity, in contrast to the significant heterogeneity in the overall model in the current study. A meta-analysis by Tsoi *et al.* [181] revealed a RR = 1.02 (95% CI = 0.96 to 1.08) for less than 20 cigarettes per day (based on 13 studies), and RR = 1.31 (95% CI = 1.10 to 1.54) for 20 or more cigarettes smoked per day (based on 10 studies). The results in the current overall models are, therefore, more modest than those in [180] and [181]. The increase in HbA_{1c} caused by cigarette smoking only slightly increased CRC risk (2% increase in risk per 20 cigarettes per day increase in smoking). The main carcinogenic effects of cigarette smoking could likely be attributed to a number of possible carcinogenic chemicals present in tobacco smoke [182], [183], which could be responsible for DNA damage when ingested or present in the circulation [182].

Increased CRC risk with increased stress levels was found for both the glycaemic and overall OR models. A one level increase in stress resulted in OR = 1.16 in the overall model, but only OR = 1.06 in the glycaemic model (9% higher risk in overall model compared to glycaemic model). Only one study was included in each model. A study which indicated increased HbA_{1c} with increases in stress level [184], was excluded, as sufficient SE information was not available. A study be Heikkilä *et al.* [185], comparing job strain to no job strain, showed a statistically non-significant HR = 1.16 (95% CI = 0.90 to 1.48) for a pooled analysis of 12 studies. The authors of this study concluded that it was not likely that job strain was a major risk factor for CRC, but also conceded that other types of stress could be related to cancer risk [185]. Increased HbA_{1c} does seem to play a role in increasing CRC risk. The increase in HbA_{1c} results when gluconeogenesis and glycogenolysis is stimulated [55] by cortisol, which is released as part of the stress response [113]. A review by Hansen *et al.* [186] supports these findings, as they described increases in HbA_{1c} compared to a number of occupational stressors (based on seven studies). The difference between the glycaemic and overall models could likely be explained by chronic inflammation caused by the stress response [185]. The difference in stress measures and the ways that stress levels are defined could also have contributed; for instance, the glycaemic model was derived from job strain data, whereas the overall model was based on perceived stress data. Measuring and scoring stress objectively and including more studies or participants would improve the models.

No statistically significant overall models for CRC risk and exercise were obtained. The models did indicate a statistically non-significant decrease in CRC risk with increases in exercise. The risk for each 4 MET-h/day increase in exercise in the overall combined model was approximately 0.95 (95% CI = 0.88

to 1.02), while amounting to approximately 0.90 in the combined risk glycaemic model. The glycaemic models were developed from data covering only a small portion of the diabetic HbA_{1c} range, which could potentially have affected the outcome. No consistent association between rectal cancer risk and exercise was obtained when published studies were examined, but there was a strong inverse relationship with colon cancer [187]–[189]. No dose-response meta-analysis of CRC risk and exercise was found in the published literature, and, therefore, no direct comparison could be made with statistically significant data. A meta-analysis on colon cancer mortality risk in the US [178] found an increased RR = 1.8 for sedentary versus highly active (≥ 1600 MET-min/week) individuals, suggesting that increased physical activity can decrease colon cancer mortality considerably.

The overall models of GL and CRC risk found no statistically significant associations. There was a statistically non-significant increased risk of approximately 1.0055 for every 50 units increase in GL per day, with significant heterogeneity. The risk estimated by the glycaemic model was approximately 1.049 per 50 units increase in GL. The risks in the glycaemic HR and OR models were 1.038 and 1.112, respectively. Aune *et al.* [190] found, based on twelve studies, a statistically non-significant increase in RR = 1.01 for every 50 units increase in GL per day, with significant heterogeneity, corresponding well to the results obtained for the overall model in the current study. When one study was excluded in [190], a RR = 1 with reduced heterogeneity was found, but the result was still statistically non-significant. Two meta-analyses comparing the highest with the lowest GL categories found increased risk, although only significant in [191]. Gnagnarella *et al.* [191] obtained a RR = 1.26 (95% CI = 1.11 to 1.44) with significant heterogeneity, based on eight studies. Mulholland *et al.* [192] found a RR = 1.17 (95% CI = 0.98 to 1.39) with significant heterogeneity, based on nine studies.

It is probable that the increase in CRC risk as a result of increases in GL could well be mediated in full by the increase in HbA_{1c}, presented by the glycaemic model, as the GL concept is closely linked to blood glucose levels, and there does not seem to be many other contributing factors. Obesity could perhaps also play a role in increasing the overall risk. It is difficult to conclude why the values presented by the glycaemic model are higher than that presented by the overall model, as no overall statistically significant dose-response model was available for comparison.

6.9 Conclusion

Article 1 [1] provided evidence of increased risk for several cancer types with increasing HbA_{1c} levels. The results for CRC presented in article 2 [2] confirmed this. From these results it could be concluded that at least some portion of the link between diabetes and CRC risk is caused by the increased HbA_{1c} levels associated with diabetes. However, it was also shown that the risk is already increased in the normal and pre-diabetic ranges for a number of cancers, including CRC. This provides evidence for a possible therapeutic window, where cancer risk could be decreased by decreasing HbA_{1c} levels (thus,

improving glycaemic control) in the diabetic and non-diabetic population. To achieve this, appropriate lifestyle or therapeutic interventions, such as the ones discussed in section 2.6, could be used. One study showed an increased risk below the reference level at 5% for CRC mortality and incidence. This could indicate possible risks when lowering the HbA_{1c} by too much and must be taken into account when implementing therapeutic interventions.

Article 2 [2] examined the relationship between lifestyle factors, HbA_{1c} and CRC risk in more detail. It was shown that HbA_{1c} levels increase with increased carbohydrate intake (measured by GL), increased stress levels and increased cigarette smoking. This also increased CRC risk. It was shown that increasing the amount of exercise energy expenditure, dietary fibre intake and alcohol consumption could reduce HbA_{1c} levels, and subsequently the risk of diabetes. This led to decreased CRC risk, except in the case of alcohol consumption, which increased CRC risk in the overall model. Alcohol consumption is, therefore, not a viable lifestyle factor to reduce HbA_{1c}, and subsequently diabetes risk, as it increases CRC risk.

Comparison of the glycaemic and overall models revealed that the full effects of the lifestyle factors are stronger (approximately 16% lower risk for dietary fibre intake increase of 10 g per day, approximately 9% higher risk for stress increase of one level and approximately 10% to 25% higher risk for cigarette smoking increase of one pack per day in the overall compared to the glycaemic models) than that of the glycaemic effects. However, the glycaemic effects are still significant. Implementing beneficial lifestyle factors (e.g. exercise and additional dietary fibre with meals) or reducing harmful lifestyle factors (e.g. cigarette smoking) alone does not have a large impact on reducing CRC risk via HbA_{1c} (for example, only a 4% decrease in risk for a 10 g increase in fibre intake per day). The use of additional therapeutic strategies for decreasing HbA_{1c} (i.e. pharmacotherapeutics, fasting, etc.) could be beneficial for the reduction of CRC risk. However, the possible harmful effects of a reduction in HbA_{1c} levels should be considered and a medical doctor consulted, before implementation. These harmful effects include the incidence of neuroglycopaenia, whereby the brain does not get a sufficient supply of glucose. Neuroglycopaenia causes deterioration in cognitive function and can induce seizures or coma in extreme circumstances [193].

The possibility of residual confounding cannot be excluded, although care was taken to reduce this as much as possible by including those estimates adjusted for the most confounding factors. All studies, however, do not adjust for the same factors, which could affect the results. It is possible that some studies used HbA_{1c} reference methods other than the NGSP (such as IFCC, Mono-S or JDS methods). This could have affected the obtained relationships between HbA_{1c} and lifestyle factors, as well as HbA_{1c} and cancer. This was only taken into account for article 1 [1], but not for article 2 [2]. As the glycaemic models were obtained by indirectly linking lifestyle factors and cancer risk via HbA_{1c}, these errors in HbA_{1c} values could be compounded in the glycaemic models. The use of slightly different methods of obtaining the

slopes between lifestyle factors and HbA_{1c} (inverse-variance weighting), and HbA_{1c} and cancer risk (generalised least squares), could have also caused additional confounding.

CHAPTER 7 CONCLUSION

7.1 Introduction

This chapter provides a perspective on the work, consolidation of the work performed during the study, recommendations for future research and conclusions.

7.2 Perspective on work

In this study, an integrative approach was followed, combining statistical data from empirical medical studies with a mathematical approach. This approach indirectly linked lifestyle factors and cancer risk via HbA_{1c}. This systematic view was taken at a high level (macroscopic, focussing on lifestyle factors and CRC risk), as well as a low level (microscopic, focussing on the chemical pathway of HbA_{1c}).

Some of the complexities involved in cancer morbidity were elucidated using this approach. The importance of using a combination of therapeutic strategies was highlighted. Adopting certain lifestyles might be beneficial in preventing cancer incidence, but other interventions (such as pharmacotherapeutics) might be similarly or more important in preventing and controlling cancer.

The author, a qualified electronics engineer, could apply this knowledge to biological principles in order to investigate the hypotheses that were generated. The author is also cognisant of the fact that the developed models might not be applicable outside the ranges in which they were developed and that this could potentially mean that treatment outside of these ranges could have no effect or be harmful (e.g. hypoglycaemia resulting from too strict glycaemic control). The insights from the models led to the suggestion of using cancer control or therapeutic processes which are more familiar to engineers (i.e. glucose-haemodialysis via a machine).

Existing knowledge obtained from empirical medical studies was integrated in a consolidating, systematic way to make predictions and deductions. This is the traditional engineering approach - applying theories, laws, and observations to design new concepts. It is hoped that the statistical models developed in this study will contribute to successful biomedical application, such as the proposed treatment of cancer patients via glucose-haemodialysis.

7.3 Consolidation of work done

The literature study revealed that the annual number of new cancer cases is expected to increase by approximately 8.7 million between 2008 and 2030. The annual cancer mortality is expected to increase by approximately 3.9 million between 2008 and 2030. It was also shown that diabetes prevalence in adults had doubled between 1980 and 2008.

It was shown that cancer incidence is increased in people with diabetes. Several risk factors are shared between diabetes and cancer and could explain the high co-occurrence of cancer in diabetic patients. The risk factors include modifiable factors (for instance obesity and lifestyle factors) and non-modifiable factors (for instance age and gender). Several biological pathways are also shared between diabetes and cancer: hyperglycaemia, hyperinsulinaemia, chronic inflammation and altered concentrations of endogenous hormones. It was hypothesised that the common factor linking the modifiable factors to the biological pathways could be hyperglycaemia (high blood glucose) as all of these factors are affected by changes in blood glucose levels, directly or indirectly, and in turn affect the risk for diabetes and cancer. Hyperglycaemia is also the hallmark of diabetes and can be used to diagnose the disease. The links of hyperglycaemia with the biological pathways were discussed in Chapter 2, while the links of hyperglycaemia with lifestyle factors were discussed in Chapter 3.

The metabolic biomarker that was used to evaluate chronic hyperglycaemia is glycated haemoglobin (HbA_{1c}). It provides a measure of the average blood glucose concentration in the preceding 6 weeks to 3 months. Data from published studies on the relationships of HbA_{1c} with cancer risk, lifestyle factors with HbA_{1c} and cancer risk with HbA_{1c} was investigated. As a large number of published studies were available on colorectal cancer (CRC) this cancer type was investigated. It is also a cancer type which is known to be associated with lifestyle factors.

Some issues for further investigation were identified from two previous studies. Espach [54], in an investigation into the effects of lifestyle factors on breast cancer, coronary heart disease (CHD) and inflammation, summed all studies on a particular lifestyle factor and disease combination to get a single linear trend. Some factors were not accounted for:

1. All risk measures (OR, HR and RR) were assumed to be estimates of RR and, therefore, combined on one scale, without transformation. This method is convenient and, as it is commonly used in literature under an assumption called *the rare disease assumption*, also necessary to compare results with published meta-analyses. It must be considered that this may overestimate the risk or the degree to which the treatment is beneficial. A better estimate would be to develop separate models for HR, OR and RR, or to transform all of these to a common measure before combining.
2. As all the risks within a study are dependent on a common reference group per study, the SE would be estimated incorrectly, and subsequently the weight of the study would be overestimated if the covariance matrix is assumed to be zero, as was the case for the Espach study.
3. When the reference groups are not all set to zero exposure, an intercept term is produced, which could lead to a biased estimate and complicates the models.

4. As studies differ in their design and participants, heterogeneity between studies exists. This must be accounted for.
5. Not all studies carry the same weight or importance.
6. Espach indicated that the assumption of a linear term was performed to simplify the interpretation of data, but that a higher order polynomial could have provided a better fit.
7. Espach transformed the blood glucose effects of the lifestyle factors to equivalent teaspoons sugar (—ets), but still used the full RRs for the overall effects of the lifestyle factors. This method, therefore, assumes that the full effects of the risk are mediated solely by changes in blood glucose levels. This may not be correct as other factors could play a role (and even a more important role) in carcinogenesis. This was recognised by Espach in the conclusion of the study.

Laubscher [60], in an investigation into the relationship between blood glucose and CVD, found that the increase in CVD risk as a result of an increase in HbA_{1c} was far less than expected. Two of the reasons that he proposed for this observation provided grounds for further investigation in the current study: he proposed that mean blood glucose levels might not be affected significantly by —ets intake, or that mean blood glucose does not predict CVD risk accurately and that other indirect effects of blood glucose might be better risk indicators. He identified a discrepancy between the glycaemic effects and the overall effects of lifestyle factors, but did not investigate the discrepancy further. The current study investigates the discrepancy further and also builds on one of the recommendations for further work made in the study by investigating the link between blood glucose and cancer.

Table 4 provides a summary of the aims of the study as described in section 1.3, the methods used and the outcomes of the study (i.e. how the aims were accomplished and the problems were addressed).

Table 4: Summary of aims, methods and outcomes of the current study.

No.	Aims	Methods used	Outcomes
1.	Investigate whether cancer risk increases with increasing HbA_{1c}, independent of diabetes.	a. Dose-response meta-analysis of published studies on HbA_{1c} and cancer risk.	Based on statistically significant results: a. Cancer risk was increased in the normal and pre-diabetic ranges for colorectal, pancreatic, gastric and respiratory cancers. b. Cancer risk was increased in the diabetic range for colorectal, pancreatic, gastric and liver cancers and, in the upper diabetic range, for breast cancer. c. Risk was decreased for breast cancer in the normal and pre-diabetic ranges. Based on borderline significant results: a. Prostate cancer incidence risk is possibly decreased in the diabetic, pre-diabetic and normal ranges. b. Breast cancer incidence risk is possibly increased in the diabetic, pre-diabetic and normal ranges. **Conclusions:** a. Cancer risk increases with HbA_{1c}, independent of diabetes, for a number of cancer types. b. This addresses the first problem in section 1.2.2. c. The results are communicated via research article 1 [1].

No.	Aims	Methods used	Outcomes
			Limitations: The inclusion of data points below the reference complicated the interpretation of results.
2.	Combine results from published studies on the association of HbA_{1c} with different RR, OR and HR values for CRC, taking into account that different studies may have different importance/weight, HbA_{1c} reference groups and be heterogeneous.	a. Results from published studies were combined using meta-analysis with the *dosresmeta* module in R. b. Models were developed for RR, OR and HR data. c. Heterogeneity was incorporated using random-effect methods. d. Heterogeneity was assessed using the probability of the X^2-test and I^2-statistic. e. The weights of different studies were determined using the variances and co-variances of the studies. f. The reference exposure per study was subtracted from each exposure level. g. Nonlinearity was assessed using RCS.	a. Obtained statistically significant increasing log-linear models for HR, OR and combined risk. b. RR model decreased and was not statistically significant.
3.	Combine results from different studies on the effects of lifestyle factors	a. The slope and SE for the estimate of each study was obtained using the inverse-variance weighting method (own Excel spread sheet).	a. Obtained statistically significant increasing linear models for GL, chronic stress and cigarette smoking. b. Obtained statistically significant decreasing linear models for alcohol consumption, dietary fibre and exercise.

No.	Aims	Methods used	Outcomes
	on HbA_{1c}.	b. The overall dose-response estimate was obtained by combining the slopes of each study using a random-effects meta-analysis (own Excel spread sheet validated by comparison with the Excel spread sheet developed by Neyeloff *et al.* [151]). c. In one case the between-study variance (τ^2) for the random-effects analysis was found to be negative and that model was reduced to a fixed-effect model. d. Exposures were reference level subtracted. e. HbA_{1c} levels were transformed to delta HbA_{1c} values by subtracting the HbA_{1c} value in the referent category from the HbA_{1c} values in each category. f. Only linear models were considered. g. Heterogeneity was assessed using the probability of the X^2-test and I^2-statistic.	
4.	Combine the results of 2 and 3 to establish models of the glycaemic effects of lifestyle factors (via	a. Statistically significant CRC risk and HbA_{1c} models were combined with lifestyle factor and HbA_{1c} models by substituting the lifestyle models into the CRC risk models and adjusting	a. Obtained decreasing log-linear glycaemic HR, OR and combined models for dietary fibre and alcohol consumption. b. Obtained decreasing log-linear glycaemic HR and combined models for exercise.

No.	Aims	Methods used	Outcomes
	HbA_{1c}) on CRC risk.	the ranges of validity according to the intersection of the models.	c. Obtained increasing log-linear glycaemic HR, OR and combined models for GL, cigarette smoking and stress.
5.	Use the method in 2 to combine results from different studies on the association of several lifestyle factors with different RR, OR and HR values, taking into account that different studies may have different importance/weight, lifestyle factor reference groups and may be heterogeneous.	a. Same method as in 2. b. Compared full effects with published literature.	a. Obtained a statistically significant decreasing log-linear model for dietary fibre intake (combined). b. Obtained statistically significant increasing log-linear models for alcohol (HR, OR and combined), smoking (HR and combined) and stress (OR). c. Combined and OR alcohol models predicted much higher values than Fedirko *et al.* [177]. Fedirko *et al.* model was closer to the HR model for alcohol or combined model when data points below reference were not excluded. Both overall and Fedirko *et al.* models were heterogeneous. d. Combined overall fibre model predicted reduced values compared to Aune *et al.* [179]. Aune *et al.* model was not significantly heterogeneous and included more studies, while overall fibre model was heterogeneous. e. Combined overall smoking model predicted slightly lower values than Liang *et al.* [180] and Tsoi *et al.* [181]. The Liang *et al.* model was based on a smaller number of studies, but showed no significant heterogeneity, in contrast to the significant heterogeneity observed in the overall model in the current study. HR smoking model predicted higher values than Liang *et al.* [180], but close to Tsoi *et al.* [181]. f. No statistically significant dose-response data available for comparison of stress. g. No statistically significant dose-response data available for comparison of

No.	Aims	Methods used	Outcomes
			exercise. h. Statistically non-significant GL data compared well with Aune *et al.* [190].
6.	Compare the relative contribution of the glycaemic effects of lifestyle factors to CRC risk via HbA_{1c} (models obtained in step 4) to the full effect of the lifestyle factors on CRC risk (results obtained in step 5) to determine the relative contribution of the glycaemic effects.	a. Compare graphically using plots. b. Compare selected values: 25 g of alcohol/day, 20 cigarettes/day and 10 g of fibre/day.	a. Alcohol consumption (HR, OR and combined), dietary fibre (combined), chronic stress (OR) and cigarette smoking (HR and combined) models were compared. b. Obtained decreasing glycaemic models for alcohol consumption, but increasing overall models; likely caused by the carcinogenic effects of ethanol and acetaldehyde being much stronger than the HbA_{1c}-lowering effects (caused by suppressed gluconeogenesis) of alcohol. c. Obtained decreasing overall and glycaemic models for dietary fibre. The overall model had a more protective effect than the glycaemic model (approximately 16% less risk in overall model compared to glycaemic model for a 10 g per day increase in dietary fibre intake); likely caused by the additional mechanisms that decrease exposure to carcinogens and the protective effect of SCFAs. d. Obtained increasing overall and glycaemic models for stress. The overall model had a stronger effect on CRC risk (approximately 9% increase in risk for a one level increase in stress) than the glycaemic model. Results were based on only one study per model. Stress measures were different (job strain and perceived stress) and could have affected the results. The difference could possibly also be attributed to chronic inflammation caused by the stress response.

No.	Aims	Methods used	Outcomes
			e. Obtained increasing overall and glycaemic models for cigarette smoking. Overall models had a stronger effect (approximately 10% to 25% increase in risk for an increase of 20 cigarettes per day) than glycaemic models to increase CRC risk; likely caused by the additional mechanisms of potentially carcinogenic chemicals contained in tobacco smoke. **Conclusions:** a. Glycaemic models for smoking, alcohol, chronic stress and dietary fibre could be compared to overall models. b. Overall models, and published data for full models, showed a stronger effect than glycaemic models (between 9% and 25% increase or decrease), as expected. c. The evidence for increased CRC risk with increasing HbA_{1c} levels in step1 above was confirmed by the results of step 2. Further analysis was done by excluding data points below the reference. d. Evidence was provided for a possible therapeutic window for the reduction of CRC risk by the reduction of HbA_{1c} levels. e. It was shown that the glycaemic effects of lifestyle factors alone do not have a strong effect. It could, therefore, be beneficial to supplement good lifestyle habits with additional therapeutic strategies such as pharmacotherapeutics and fasting, but not without considering the possible risks of such strategies. f. This addresses the second problem in section 1.2.2.

No.	Aims	Methods used	Outcomes
			g. The results are communicated via research article 2 [2]. **Limitations:** a. It was not possible to compare exercise models as they were not statistically significant. As colon cancer risk reduction is closely linked to increased physical activity, and rectal cancer is not associated with physical activity, it might be worthwhile to investigate these cancer types separately for physical exercise. b. It was not possible to compare GL models as they were not statistically significant. It is possible that GL between studies may vary as the GI values for the same type of food differs between GI tables, and sometimes incorrect methodologies are used to determine the GI. However, following the discussion on GI in Chapter 3, one should at least be able to determine the clinical value of low GI (and GL) diets in lowering disease risk by lowering blood glucose, even if the absolute values are not exactly comparable. c. Data points below the reference levels were excluded. d. Some studies were not included as sufficient information was not available to include them in the meta-analysis. e. Three studies were excluded from the final models, because of inconsistencies. f. Only log-linear and RCS models were assessed for the CRC risk models. Other models could perhaps have provided a better fit to some of the

No.	Aims	Methods used	Outcomes
			datasets. g. Only linear models were assessed for the lifestyle factors/HbA_{1c} models. This, however, does not seem to have affected the outcome, as statistically significant models could be derived for all lifestyle factors. h. No transformation of RR, OR or HR data was done when these risks were combined. This could have overestimated the risk. However, the results from published studies for comparison are likely also combined without transformation, as nothing to the contrary was stated in the studies. i. It was not possible to create models for HR, OR and RR for every factor or combination, as studies were not available for all of these factors. j. Although care was taken to use estimates adjusted for the highest number of confounding variables, residual confounding could still have impacted on the results. k. The type of HbA_{1c} reference method was not considered (all studies were assumed to have used the NGSP reference method). This could have affected the models. These errors could have been compounded in the glycaemic models which indirectly linked lifestyle factors to cancer risk.

7.4 Recommendations for future research

The following recommendations for future research can be made:

1. To be able to quantify all of the glycaemic effects of lifestyle factors on the same scale, it is recommended that equivalent teaspoons sugar (—ets) models be developed or that the existing —ets models [54] be used to convert lifestyle factors to an equivalent scale so that the relative contributions of each lifestyle factor towards cancer risk can be compared.
2. Similar glycaemic and overall models should be developed for other cancer types.
3. Other models to fit the data should be investigated (for instance fractional polynomial models [194]).
4. More information should be obtained from authors of published trials to be able to include more studies into the meta-analyses.
5. Similar methods could be used to quantify the effects of insulin on cancer risk.
6. The glycaemic effects of certain pharmacotherapeutics (for instance metformin) or fasting as additional therapeutic measures for decreasing HbA_{1c} and, subsequently cancer risk should be investigated. The risks of implementing these measures should also be investigated and weighed against the advantages gained.

7.5 Conclusions

In conclusion, it was shown that HbA_{1c}, as a biomarker for hyperglycaemia, can predict increased risk for a number of cancer types in diabetics and non-diabetics, independent of diabetes status. The increased risk for cancer in diabetics can, therefore, at least partly be explained by the increased HbA_{1c} levels associated with diabetes. This also provides motivation to improve glycaemic control in diabetics and non-diabetics, in order to reduce cancer risk.

The developed glycaemic models were used to quantify the glycaemic effects of lifestyle factors on cancer risk. These models accounted for the limitations and expanded on the work in Espach [54] and Laubscher [60].

Comparison of glycaemic and overall models could be performed for cigarette smoking, alcohol consumption, chronic stress and dietary fibre intake. These models all showed that the effects of the overall models were stronger (between 9% and 25% increase or decrease for cigarette smoking, chronic stress and dietary fibre intake) than the glycaemic models, as expected. The statistically non-significant models for exercise and GL could not be compared, and some inconsistencies still exist.

The results show that the glycaemic effects of lifestyle factors alone do not have such a large contribution towards CRC risk reduction, as lifestyle factors do not have such a large effect on HbA_{1c} (for example, only a 4% decrease in risk for a 10 g per day increase in dietary fibre intake). However, other therapeutic strategies, such as pharmacotherapeutics or fasting, could potentially have a stronger effect on HbA_{1c}, and subsequently CRC risk. The risks of these strategies should be investigated. It is acknowledged that residual confounding factors could have affected the results.

This provides motivation for further study to improve on the models by analysing more published studies and fitting other types of models. More cancer types should also be investigated. The objectives for this study were, however, achieved.

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ANNEXURE A

Article 1

J. C. de Beer and L. Liebenberg, "Does cancer risk increase with HbA_{1c}, independent of diabetes?," *Br. J. Cancer*, in press, doi:10.1038/bjc.2014.150, 2014. [1]

A.1 Manuscript

The manuscript for article 1 [1] is provided below.

Does cancer risk increase with HbA_{1c}, independent of diabetes?

Running title: Does cancer risk increase with HbA_{1c}?

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Background: The risks for several cancer types are increased in people with diabetes. Hyperglycaemia, hyperinsulinaemia, inflammation and altered hormonal concentrations are common characteristics between the two diseases and can all be linked to hyperglycaemia.

Methods: Here, we use glycated haemoglobin (HbA_{1c}) as biomarker for chronic hyperglycaemia. We explore whether cancer risk increases with HbA_{1c}, independent of diabetes, and, therefore, if risk is already increased below the diabetic HbA_{1c} range, by analysing data from current studies linking HbA_{1c} to risk of several cancer types.

Results: The data reveal that chronic hyperglycaemia correlates with increased cancer risk for a number of cancers, except prostate cancer. Evidence is also provided that risk is already increased in the pre-diabetic and normal ranges for several cancers.

Conclusion: These results merit urgent investigation into the risks and advantages of updating recommendations for stricter glycaemic control in diabetic and non-diabetic subjects, as this could help reduce the risk of cancer incidence and mortality.

Keywords

Cancer prevention; HbA_{1c}; diabetes; cancer risk; pre-diabetes; hyperglycaemia

INTRODUCTION

People with established diabetes have an increased risk of developing certain cancer types compared to non-diabetics; the strongest associations are seen for endometrial, liver and pancreatic cancer, followed by kidney, oesophageal, colorectal, breast and bladder cancer, and leukaemia (Giovannucci *et al*, 2010; Habib and Rojna, 2013). It is, however, not clear whether hyperglycaemia, a hallmark of diabetes, correlates with increased cancer risk *independent of diabetes*. If such an association exists, the cancer risk in persons with glucose levels lower than that required to diagnose diabetes might already be increased. This would have important diagnostic and therapeutic ramifications, which we investigate here.

Research surrounding the increasing prevalence of cancer and diabetes (Giovannucci *et al*, 2010; Wagner and Brath, 2012; Mathers and Loncar, 2006; World Health Organization, 2010) has established direct pathogenetic commonalities between these two chronic diseases. These include hyperinsulinaemia, hyperglycaemia, inflammation, and altered concentrations of endogenous hormones.

The association of most cancers and diabetes with chronic inflammation (Pollak, 2012; Coussens *et al*, 2013) and the direct link between increased inflammatory signalling and high blood glucose levels in cancer models (Habib and Rojna, 2013) support the possibility that chronic hyperglycaemia may play a pivotal role in cancer risk in humans. Increased production of endogenous hormones can also be indirectly linked to hyperglycaemia via hyperinsulinaemia and obesity, and, therefore, also to diabetes and cancer risk (Montaruli *et al*, 2012; Patterson *et al*, 2013; Robien *et al*, 2013). Chronic hyperglycaemia in diabetes patients seems to be directly linked with the ubiquitous reliance of most cancer cells on high glucose flux (Hanahan and Weinberg, 2011; Mathews and Liebenberg, 2013). Onodera *et al* (2014) found that an increase in glucose uptake can activate oncogenic pathways in breast cells. This could potentially provide another pathway by which hyperglycaemia increases cancer incidence risk.

Chronic hyperglycaemia may be evaluated by measuring glycated haemoglobin (HbA_{1c}) (American Diabetes Association, 2013), a biomarker of the average blood glucose concentration for a prolonged period of time (Habib and Rojna, 2013; Travier *et al*, 2007). Importantly, HbA_{1c} may also be a good indicator of metabolic processes influencing levels of insulin (Saydah *et al*, 2003) or insulin-like growth factors, important for cancer pathogenesis (Habib and Rojna, 2013). Most studies focus on the association between cancer risk and diabetes. This approach, however, does not provide evidence for the potential causal links between the two diseases (Giovannucci *et al*, 2010).

One study (Pisani, 2008) performed a meta-analysis on epidemiological studies linking hyperglycaemia and risk for colorectal and pancreatic cancers. The researcher only assessed risk in the highest compared to the lowest categories of exposure. Another study (Johnson and Bowker, 2011) performed a meta-analysis on the impact of glycaemic control in type 2 diabetic patients and found no decrease in cancer risk with increased glycaemic control. This meta-analysis may have been influenced by confounding factors such as insulin usage, which could increase cancer risk (Pollak, 2012). Habib and Rojna (2013) reviewed some of the most important studies on the association between cancer risk and hyperglycaemia. They, however, did not attempt to consolidate the evidence per cancer type or provide a dose-response relation. No meta-analysis has yet been published that consolidates risk of different types of cancers with different HbA_{1c} ranges, including glycaemic levels lower than that associated with diabetes. If cancer risk is lower at low HbA_{1c} levels, the incidence of cancer in the diabetic and non-diabetic populations could potentially be lowered by decreasing glucose levels. This could be achieved by means of appropriate lifestyle or therapeutic interventions, and by imposing stricter recommendations for glycaemic control.

Here, we perform dose-response meta-analyses on the current published evidence regarding HbA_{1c} levels and risk of various cancers. This *post hoc* analysis attempts to establish whether cancer risk is already elevated in normal and pre-diabetic HbA_{1c} ranges. The overall goal is to

determine whether HbA_{1c} concentrations can effectively quantify cancer risk *independent of diabetes*.

MATERIALS AND METHODS

Study selection. Only English-language articles were included. Studies from 1960 to the present (date last searched - 9 January 2014) were included in the search. Articles using the following risk measures were included: relative risk (RR), odds ratio (OR) or hazard ratio (HR). For studies to be included in the quantitative meta-analysis, they had to provide risk data for at least three HbA_{1c} levels. Literature searches were performed using ScienceDirect and Scopus to identify articles that relate HbA_{1c} to the risk of cancer incidence (primary or recurrence) or mortality. More articles were identified from the reference lists of surveyed articles.

Search terms included a number of MeSH terms and were combinations of the following: ("cancer" OR "malignancy" OR "tumor" OR "tumour" OR "neoplasm" OR "neoplasia") AND ("hemoglobin a1c" OR "hba1c" OR "glycated hemoglobin" OR "glycosylated hemoglobin" OR "a1c" OR "haemoglobin a1c" OR "glycated haemoglobin" OR "glycosylated haemoglobin" OR "glycohemoglobin a" OR "glycohaemoglobin a") in the title of the study.

The trends adjusted for the most confounding variables were used, where sufficient information was available on that trend. This was done so that the effects of most of the potential confounders could be adjusted for. This may, however, have increased the heterogeneity between studies, as not all studies adjusted for the same confounders. Only studies providing data on specific cancer types were included (not studies referring to cancer in general).

It was assumed that all studies used the National Glycohemoglobin Standardization Program (NGSP) HbA_{1c} reference, unless explicitly stated otherwise in a specific study. Suitable conversion to the NGSP reference was performed for three studies (Cust *et al*, 2009; Stocks *et al*, 2007; Stocks *et al*, 2008) that used the Swedish monoS standard, using the relation NGSP [%] =

(monoS + 0.8925)/0.9718. Using the NGSP reference, "diabetes" is deemed to exist at HbA_{1c} levels greater than 6.5 % (American Diabetes Association, 2013); "pre-diabetes" if HbA_{1c} levels are between 5.7 % and 6.4 % (American Diabetes Association, 2013). The "normal glycaemic" range is taken to span from approximately 4 % to 5.7 % (American Diabetes Association, 2013).

Data extraction. The following data were extracted from the studies: reference details, number of cases per HbA_{1c} level for OR, RR and HR, number of controls per HbA_{1c} level for OR, total number of persons per HbA_{1c} level for RR, number of person-years per HbA_{1c} level for HR, gender, cancer site, whether the risk was measured in RR, OR or HR, the risk per HbA_{1c} range, HbA_{1c} range or level and the 95 % confidence intervals (CI) per HbA_{1c} level. Additional information was obtained from the authors of two studies to be able to include these studies in the quantitative analysis.

Statistical analyses. When a range of HbA_{1c} values was provided, a single value was chosen per HbA_{1c} category using method 1 in Hartemink *et al* (2006). It is acknowledged that the method that is used to select a single value per dose-response category, could potentially affect the outcome (Hartemink *et al*, 2006). The method works as follows. Where a range was specified for a dose, the midpoint of the range was selected as the point representing that range; where an open-ended range was specified, the dose for the lower open-ended range was calculated by subtracting half of the width of the second lowest range from the lowest value specified (the top value of the bottom open-ended range) (Hartemink *et al*, 2006); the dose for the upper open-ended range was calculated by adding the width of the second highest range to the highest value specified (bottom value of the upper open-ended range) (Hartemink *et al*, 2006).

Dose-response meta-analyses were performed per cancer type and for cancer incidence and mortality separately using the *dosresmeta* R package (Crippa, 2013; R Core Team, 2013). This is the R-equivalent of the *GLST* Stata-module developed by Orsini *et al* (Orsini *et al*, 2006; Orsini *et al*, 2012).

Risk estimates were transformed using the natural logarithm (*ln*). The reference HbA_{1c} level per study was subtracted from each HbA_{1c} level in the study, resulting in a model fitted through the origin (i.e. no intercept).

Linear and restricted cubic spline (RCS) models (with three knots, located at the 10^{th}, 50^{th} and 90^{th} percentiles of the data) of the natural logarithm-transformed risk estimates were developed using random-effects meta-analysis methods, to incorporate heterogeneity. If, for a certain cancer type, only a single study was available, and that study had less than six exposure levels, only a linear model was fitted, as the function that develops the RCS models requires at least six data points. The graphical displays of the statistically significant models obtained during the analyses were referenced to the lowest HbA_{1c} level for all the studies included in a model.

To assess nonlinearity, the null hypothesis that the coefficient of the second spline is zero, was tested. A significance level of $p < 0.05$ was used.

Lack of goodness of fit and heterogeneity was determined by assessing whether the p-value from the chi-squared test was smaller than 0.1. I^2 values were determined for each model.

Combined and separate models for RR, OR and HR were developed to assess whether the risk measure, and in effect the type of study, could influence the results.

RESULTS

The study selection process is illustrated in Figure 1. Thirty-six studies were identified through database searching. Thirty-two studies were identified from the reference lists of surveyed articles. Two duplicate studies were removed. The remaining 66 records were screened. Ten articles were not relevant to the study aims (for example, they did not address cancer). For six of the records, full-text articles were not available or referred to conference abstracts. Fifty full-text articles were assessed. Of these, 18 articles were excluded as they either did not provide data on a specific cancer type (9 studies), they did not provide data on cancer (2 studies) or they used glycaemic measures other than HbA_{1c} (7 studies). Thirty-two studies remained that were

relevant for the qualitative synthesis. Eighteen studies were excluded from the quantitative analysis as they did not provide information on cancer incidence or mortality, but rather on stage or grade of cancer (4 studies), they provided information on cancer pre-cursors, such as adenoma, adenomatous polyps or benign neoplasia (5 studies), they did not provide data on RR, OR or HR (2 studies), they specified less than three HbA_{1c} ranges (4 studies), the study population was already included in another study (2 studies) or enough information on the number of cases or person-years per HbA_{1c} level was not available (1 study). Fourteen studies remained that were included in the quantitative meta-analyses. Of these, thirteen studies provided data on cancer incidence only, while one study provided data on cancer incidence as well as mortality (Joshu *et al*, 2012).

The statistically significant or border-line significant models that were obtained during the dose-response analyses are shown in Figures 2 to 8. The plots are referenced to the lowest HbA_{1c} level in the studies included in each model.

Female genital cancer.

Quantitative analysis. Two studies (Miao Jonasson *et al*, 2012; Travier *et al*, 2007) on female genital cancer incidence were included in the quantitative analysis. Both used the HR as risk measure. No statistically significant models were obtained in the analysis ($p = 0.2961$ for increasing log-linear model, $p_{nonlinearity} = 0.3274$ for increasing-decreasing RCS model).

Qualitative analysis. Travier *et al* (2007) found a statistically significant (HR = 2.84, 95 % CI = 1.35 to 5.98) increased risk in the 6 % to 7 % HbA_{1c} range, compared to the reference risk in the < 6 % range. The risk was also increased in the > 7 % range, but not significantly (HR = 2.01, 95 % CI = 0.69 to 5.89). Miao Jonasson *et al* (2012) found no statistically significant associations for cancer risk in the baseline (slightly decreased HRs) or updated mean (slightly increased HRs) HbA_{1c} groups for a group of people with type 2 diabetes. Levran *et al* (1984) revealed an increased number of endometrial cancer cases with HbA_{1c} above 6.5 %, as compared

to below 6.5 %. The distribution of controls was more even across all of the HbA_{1c} ranges. They, however, did not provide risk measures for comparison. Levitan *et* al (2008) found no statistically significant trend ($p = 0.53$) for uterine or ovarian cancer mortality risk. Stevens *et al* (2012) found some evidence of increased endometrial cancer stage with increasing HbA_{1c}, but this was not statistically significant ($p = 0.07$).

Liver cancer.

Quantitative analysis. One study (Travier *et al*, 2007) was included in the quantitative analysis for liver cancer incidence. The increasing log-linear HR model obtained from this study was not statistically significant ($p = 0.5737$).

Qualitative analysis. Two studies that were excluded from the quantitative analysis as they did not provide enough HbA_{1c} levels (Kaneda *et al*, 2012; Donadon *et al*, 2010) found changes in hepatocellular carcinoma (HCC) risk as HbA_{1c} increased. Kaneda *et al* (2012) found a large risk (HR = 3.551, $p = 0.03$) for HCC recurrence as the HbA_{1c} level increased above 6.5 % for patients with diabetes. Donadon *et al* (2010) found that the OR for HCC increased by 1.508 for each percentage increase in HbA_{1c} for type 2 diabetic patients compared to controls with liver cirrhosis ($p = 0.0005$). Compared to the normal control group, the OR increased by 1.265 per 1 % increase in HbA_{1c} – this was, however, not statistically significant ($p = 0.1172$).

Pancreatic cancer.

Quantitative analysis. One study (Grote *et al*, 2011) was included in the quantitative analysis. The OR increasing log-linear model, including diabetic participants, was statistically significant ($p = 0.0049$). The increasing log-linear model, excluding diabetic participants, was also statistically significant ($p = 0.0148$).

Qualitative analysis. Another study (Levitan *et al*, 2008) found no statistically significant trends for pancreatic cancer mortality ($p = 0.40$), with decreased risk (RR = 0.86) in the lower normal

HbA_{1c} range of 4.88 % to 5.08 % and increased risk (RR = 1.36) in the upper normal range (5.09 % to 5.59 %), compared to the reference group (2.27 % to 4.87 %).

Prostate cancer.

Quantitative analysis. Four studies were included in the quantitative analysis for prostate cancer incidence (Miao Jonasson *et al*, 2012; Joshu *et al*, 2012; Stocks *et al*, 2007; Travier *et al*, 2007), while only one study (Joshu *et al*, 2012) was included in the analysis for mortality. The OR decreasing log-linear model obtained from the Stocks *et al* (2007) study was border-line statistically significant ($p = 0.0596$), while none of the other models were statistically significant ($p = 0.1114$ for the decreasing combined log-linear model, $p = 0.1783$ for the decreasing HR log-linear model, $p = 0.6502$ for the decreasing HR mortality log-linear model).

Qualitative analysis. Rusu *et al* (2011) found that HbA_{1c} was statistically significantly ($p = 0.0001$) lower in patients with prostate cancer ($HbA_{1c} = 6$ %) than in those without cancer ($HbA_{1c} = 7.1$ %) or in patients with benign prostatic hyperplasia ($HbA_{1c} = 7.4$ %). No adjustments were made for confounders in this study.

Kim *et al* (2010a) revealed statistically significant ($p = 0.001$) increased risks for higher pathological Gleason score cancer with increasing HbA_{1c} level in a population of diabetic men. Hong *et al* (2009) found a significantly higher rate of high pathological Gleason score cancers ($p = 0.005$) and extraprostatic extension ($p = 0.043$) in diabetic men with HbA_{1c} levels ≥ 6.5 % than in those with HbA_{1c} levels lower than 6.5 %.

Colorectal cancer.

Quantitative analysis. Nine studies presented data on colorectal cancer incidence. Two of these studies (Khaw *et al*, 2004; Platz *et al*, 1999) were excluded from the quantitative analysis as they provided data from the same cohorts as other studies already included. Seven studies remained that were included in the quantitative analysis (HR - Joshu *et al*, 2012; RR - Lin *et al*, 2005; OR

- Rinaldi *et al*, 2008; OR - Saydah *et al*, 2003; OR - Stocks *et al*, 2008; HR - Travier *et al*, 2007; OR - Wei *et al*, 2005). One study also presented data on colorectal cancer mortality (HR - Joshu *et al*, 2012). The colorectal cancer incidence combined RCS model ($p_{nonlinearity} = 0.0068$, $p_{heterogeneity} = 0.2046$, $I^2 = 21.4$ %) and HR RCS model ($p_{nonlinearity} = 0.0261$, $p_{heterogeneity} = 0.8494$, $I^2 = 1$ %) both showed a decreased risk at lower HbA_{1c} levels followed by an increased risk at higher HbA_{1c} levels. The combined log-linear model ($p = 0.0325$, $p_{heterogeneity} = 0.1526$, $I^2 = 33.2$ %) and OR log-linear model ($p = 0.0072$, $p_{heterogeneity} = 0.2945$, $I^2 = 18.9$ %) both showed statistically significant increasing trends. The mortality RCS HR model was also statistically significant ($p_{nonlinearity} = 0.0180$, $p_{heterogeneity} = 0.9849$, $I^2 = 1$ %), with decreasing risk at lower HbA_{1c} levels, and increasing risk at higher HbA_{1c} levels. The other models were not statistically significant ($p = 0.3089$ for increasing HR log-linear model, $p = 0.1486$ for OR RCS decreasing-increasing model, $p = 0.3942$ for decreasing RR log-linear model).

Qualitative analysis. Further to the results from the dose-response relation, Khaw *et al* (2004) found a significantly increased risk as HbA_{1c} increased in the combined ($p < 0.001$), men-only group ($p < 0.001$) and women-only group ($p = 0.03$). The risk in the women-only group was decreased in the 5 % to 5.9 % range (RR = 0.7), but increased above this range. Continuous analysis per 1 % increase in HbA_{1c} revealed an increase of RR = 1.30 for the combined group ($p = 0.02$), RR = 1.35 for the men-only group ($p = 0.02$) and RR = 1.20 for the women-only group ($p = 0.26$). The authors found that the increased risk for colorectal cancer in diabetic patients was largely due to the increase in HbA_{1c} and not due to diabetes status. Platz *et al* (1999) and Siddiqui *et al* (2008a) found a significant increase in risk for advanced-stage colorectal cancer with higher HbA_{1c} levels ($p = 0.02$ and $p = 0.002$, respectively).

The risks for colorectal cancer precursors (adenomatous and/or advanced adenomatous polyps) were significantly increased with increasing HbA_{1c} levels in diabetic and non-diabetic subjects (Siddiqui *et al*, 2008b; Kim *et al*, 2010b). Hsu *et al* (2012) found an increase of OR = 1.25 in colorectal neoplasia for each percentage increase in HbA_{1c} level for men and women ($p = 0.02$).

HbA_{1c} was not statistically significantly associated with colorectal adenoma, distal colorectal adenoma, or advanced adenoma in two studies (Wei *et al*, 2006; Yang *et al*, 2010).

Breast cancer.

Quantitative analysis. Six studies on breast cancer incidence (HR – Joshu *et al*, 2012; HR – Miao Jonasson *et al*, 2012; HR – Travier *et al*, 2007; HR – Erickson *et al*, 2011; OR – Cust *et al*, 2009; RR – Lin *et al*, 2006) and 1 study on cancer mortality (HR – Joshu *et al*, 2012) were included in the quantitative analysis. A statistically significant RCS model for combined risk ($p_{nonlinearity} = 0.0260$, $p_{heterogeneity} = 0.6457$, $I^2 = 1$ %), which showed decreased risk at lower HbA_{1c} levels and increased risk at higher HbA_{1c} levels, as well as a border-line statistically significant increasing log-linear model for HR ($p = 0.0783$, $p_{heterogeneity} = 0.6948$, $I^2 = 1$ %) was obtained. The other models were all statistically non-significant ($p = 0.2329$ for increasing combined log-linear model, $p_{nonlinearity} = 0.3118$ for HR decreasing-increasing RCS model, $p = 0.2323$ for decreasing OR log-linear model, $p_{nonlinearity} = 0.2844$ for increasing-decreasing RCS model, $p = 0.2007$ for decreasing RR log-linear model, $p = 0.1789$ for increasing HR log-linear mortality model).

Qualitative analysis. Two studies did not adjust for confounders and found contrasting results. Yadav *et al* (2012) observed that HbA_{1c} increased in pre- and postmenopausal breast cancer cases. Contrarily, Nemesure *et al* (2009) found decreased HbA_{1c} in pre- and postmenopausal breast cancer cases.

Levitan *et al* (2008) found no statistically significant linear trend for breast cancer mortality with increasing HbA_{1c} level ($p = 0.23$).

Gastric cancer.

Quantitative analysis. Two studies on gastric (stomach) cancer incidence were included in the quantitative analysis (HR - Ikeda *et al*, 2009; HR - Travier *et al*, 2007). The increasing log-

linear trend obtained from these studies was statistically significant ($p = 0.0171$, $p_{heterogeneity} = 0.9439$, $I^2 = 1$ %).

Qualitative analysis. No additional studies were available for discussion.

Respiratory cancer, including lung cancer.

Quantitative analysis. Two studies on respiratory or lung cancer incidence were included in the quantitative analysis (HR – Joshu *et al*, 2012; HR – Travier *et al*, 2007). The RCS model obtained from these studies was statistically significant ($p_{nonlinearity} = 0.0353$, $p_{heterogeneity} = 0.1537$, $I^2 = 40.1$ %), with increasing risk at lower HbA_{1c} levels, and decreasing risk at higher HbA_{1c} levels. Neither the increasing log-linear HR mortality model ($p = 0.1573$), nor the increasing-decreasing RCS HR mortality model from the Joshu *et al* (2012) study were statistically significant ($p_{nonlinearity} = 0.2996$).

Qualitative analysis. The Levitan *et al* (2008) study found increasing lung cancer mortality risk with increased HbA_{1c} level, but the linear trend was not statistically significant ($p = 0.22$).

Other cancers.

Quantitative analysis. Results from other cancer types investigated were not statistically significant. These cancer types included lymphoma and leukaemia ($p = 0.5427$ for the decreasing log-linear model), as well as melanoma ($p = 0.5514$ for the increasing log-linear model). One study (HR - Travier *et al*, 2007) presented results on each type of cancer.

Qualitative analysis. Levitan *et al* (2008) found an increase in RR for lymphoma or leukaemia mortality as HbA_{1c} increased. This was, however, not statistically significant ($p = 0.18$ for a linear trend).

DISCUSSION

Quantitative analysis. Results from the dose-response meta-analysis revealed the following statistically significant or borderline significant results:

- Increasing log-linear models for breast cancer (HR), colorectal cancer (OR and combined), gastric cancer (HR) and pancreatic cancer (OR).
- Decreasing log-linear model for prostate cancer (OR).
- Increased risk above 8.5 % for breast cancer (combined) and colorectal cancer (HR for incidence and mortality), above 6.5 % for colorectal cancer (combined) and increasing trend up to 7 % and decreasing risk above 7 % for respiratory cancer (HR).

The following relations were not statistically significant:

- Increasing log-linear models for female genital cancer (HR), liver cancer (HR), colorectal cancer (HR), breast cancer (combined and HR mortality), respiratory (HR) and melanoma (HR).
- Decreasing log-linear models for prostate cancer (combined, HR and HR mortality), colorectal (RR), breast (OR and RR) and lymphoma and leukaemia (HR).

Qualitative analysis. The qualitative analysis revealed some additional information. Cancer stage for female genital cancer was found to be non-significantly increased with increasing HbA_{1c}. Liver cancer incidence increased significantly for diabetics and in patients with HbA_{1c} above 6.5 %. A significantly higher rate of high grade prostatic tumours and extraprostatic extension was present with increasing HbA_{1c}. For colorectal cancer, advanced stage colorectal cancer incidence and pre-cursor incidence increased with increasing HbA_{1c} level. Respiratory/lung cancer, as well as lymphoma and leukaemia, mortality was non-significantly increased with increasing HbA_{1c} level.

Conclusions. Our study corroborates that cancer sites should be investigated separately, as the observed trends differ between cancer sites. Evidence is provided that indicates:

- Cancer incidence risk is already increased in the pre-diabetic and normal ranges for colorectal, gastric, pancreatic and respiratory cancers, although the results are not the same for all risk measures.
- Cancer incidence is higher at HbA_{1c} levels in the diabetic range for colorectal, gastric, pancreatic, breast and liver cancers.

There is possible evidence for:

- Decreased risk of prostate cancer incidence with increasing HbA1c level, and already in the pre-diabetic and normal ranges.
- Increased risk of breast cancer incidence in the diabetic, pre-diabetic and normal ranges.

Our study reveals that chronic hyperglycaemia, as quantified by HbA_{1c} levels, correlates with increased cancer risk in colorectal, gastric, liver and pancreatic cancers, and possibly breast cancer, while correlating with decreased prostate cancer risk. The relations for other cancer types investigated are not statistically significant. It is also clear that there is increased risk for higher cancer stage/grade and cancer pre-cursor incidence for some cancer types with increasing HbA_{1c} level.

The near-linear association of HbA_{1c} levels with risk of several cancers supports the conjecture that it might be possible to use HbA_{1c} as an independent metabolic biomarker for cancer risk in diabetic or non-diabetic persons. Significantly, the study also provides preliminary evidence for an already increased cancer risk in the normal and pre-diabetic categories for a number of cancers. The incidence of cancer in the diabetic and non-diabetic populations could, therefore, potentially be reduced by decreasing glucose levels. This could be achieved by means of appropriate lifestyle or therapeutic interventions, and by imposing stricter recommendations for glycaemic control (Krone and Ely, 2005).

The risks and advantages of updating recommendations for stricter glycaemic control (in diabetic and non-diabetic subjects) merit urgent investigation. Indications are that such stricter glycaemic control measures could help reduce the risk for cancer incidence and mortality (Krone and Ely, 2005) as long as care is taken to ensure that stricter glycaemic control does not result in hypoglycaemia, which may have other deleterious effects.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

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FIGURES

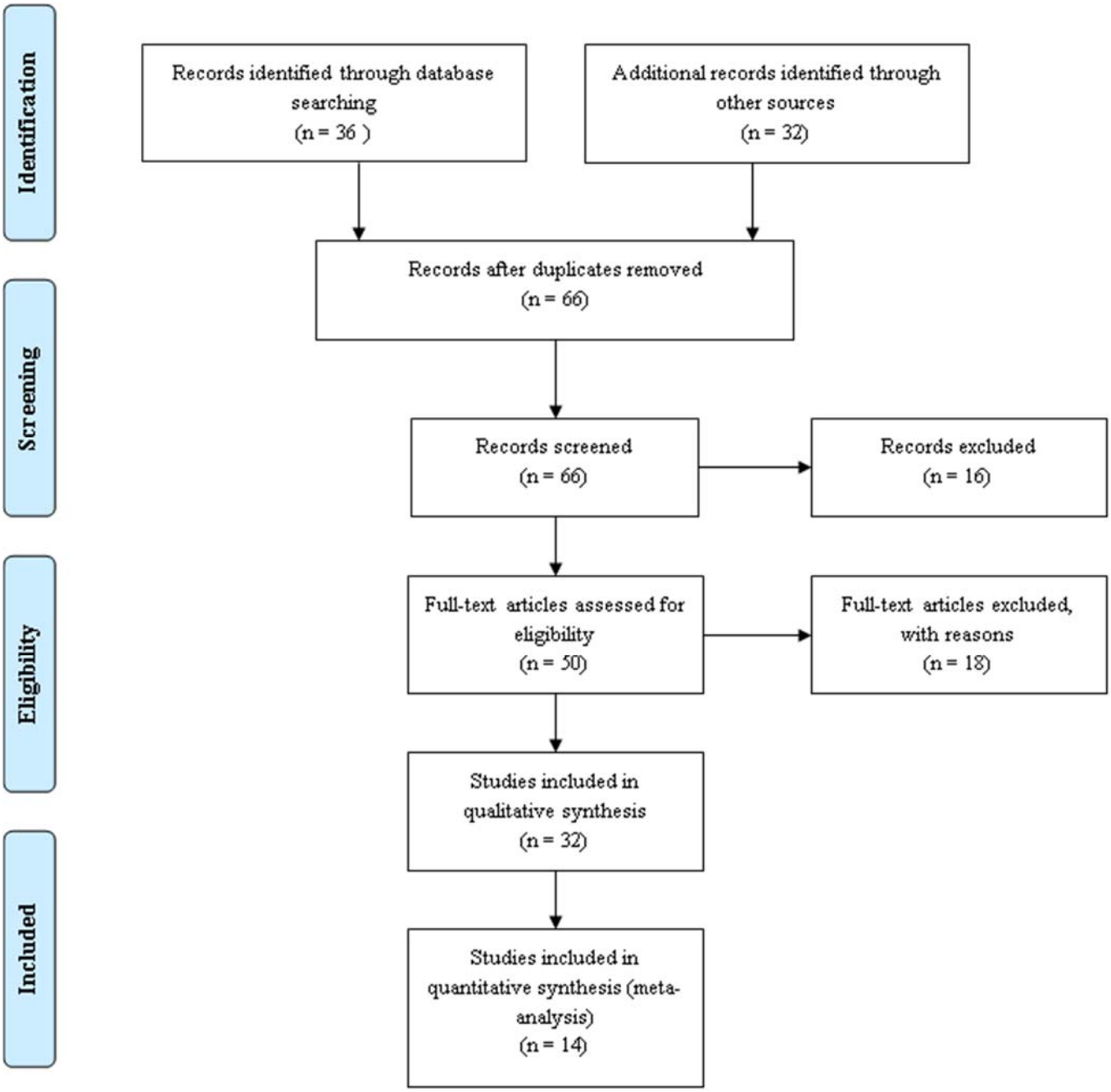

Figure 1. Flow diagram showing the study selection process (Moher *et al*, 2009).

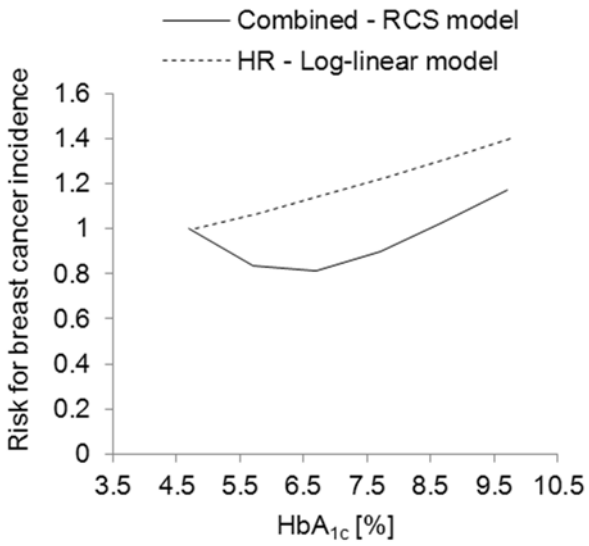

Figure 2. Relationship between risk for breast cancer incidence and HbA_{1c}.

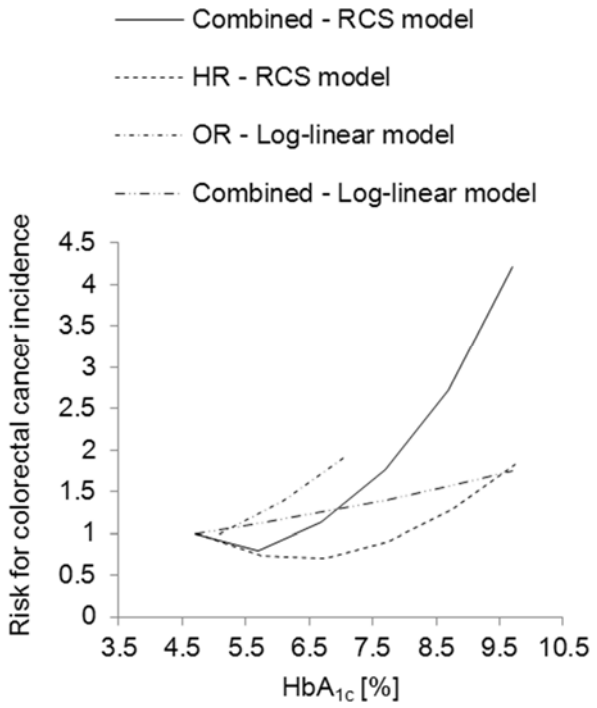

Figure 3. Relationship between risk for colorectal cancer incidence and HbA_{1c}.

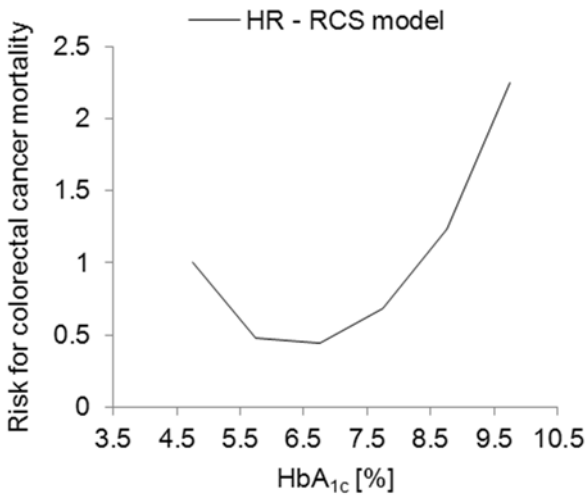

Figure 4. Relationship between risk for colorectal cancer mortality and HbA_{1c}.

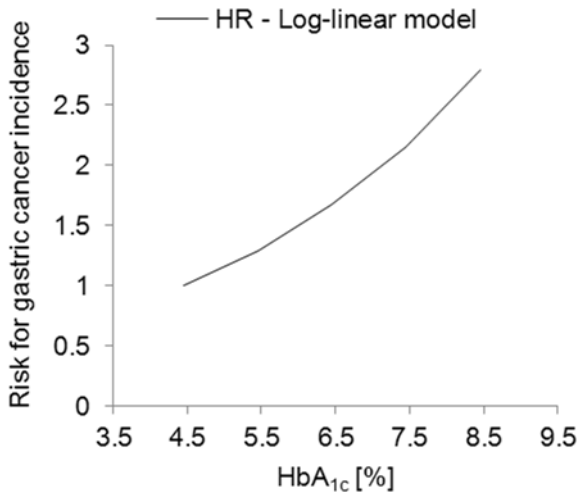

Figure 5. Relationship between risk for gastric cancer incidence and HbA_{1c}.

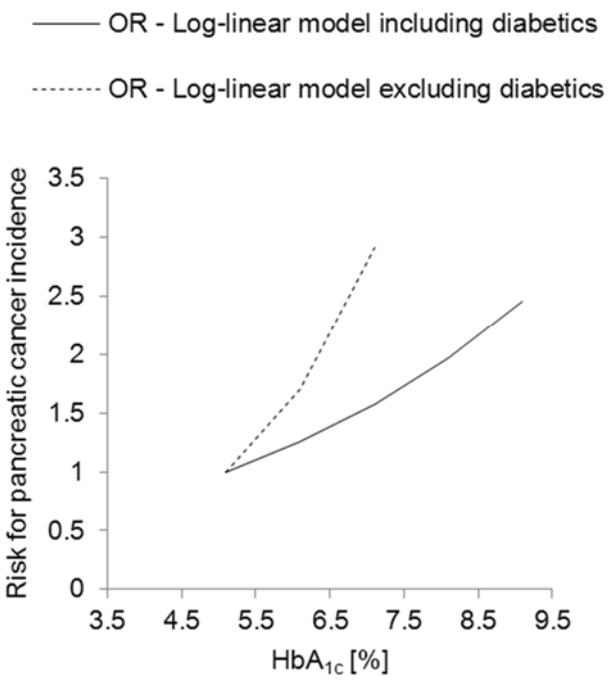

Figure 6. Relationship between risk for pancreatic cancer incidence and HbA_{1c}.

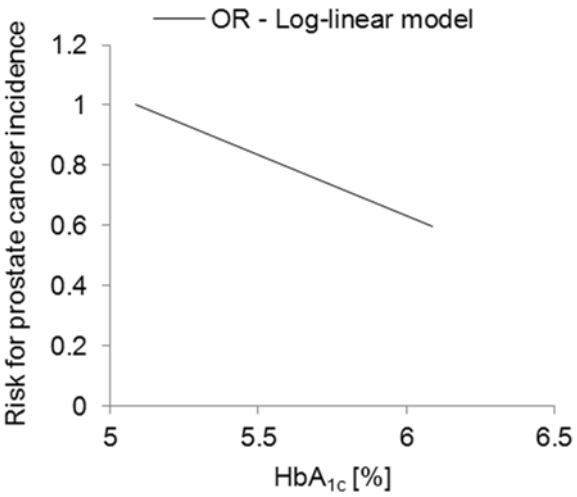

Figure 7. Relationship between risk for prostate cancer incidence and HbA_{1c}.

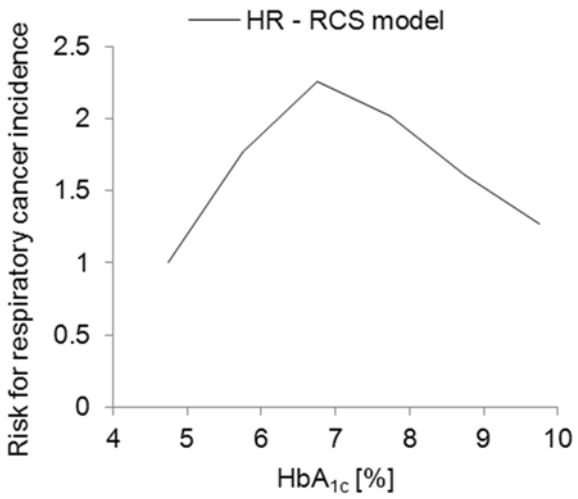

Figure 8. Relationship between risk for respiratory cancer incidence (including lung cancer) and HbA_{1c}.

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ANNEXURE B

Article 2

J.C. de Beer, and L. Liebenberg, “Comparison of glycaemic and overall effects of lifestyle factors on colorectal cancer risk”, manuscript being finalised, 2014. [2]

B.1 Manuscript

The manuscript for article 2 [2] is provided below.

Comparison of glycaemic and overall effects of lifestyle factors on colorectal cancer risk

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Abstract

Purpose To examine the extent to which colorectal cancer (CRC) risk is mediated by blood glucose levels.

Methods Models were developed using random-effect meta-analyses of published studies on glycated haemoglobin (HbA_{1c}) and CRC risk, as well as of lifestyle factors and CRC risk. Models of HbA_{1c} and lifestyle factors were developed using inverse-variance weighting. HbA_{1c} models were combined to obtain glycaemic models, which were compared to overall models.

Results Increasing HbA_{1c} generally increased CRC risk. Increases in HbA_{1c} correlated with increased glycaemic load (GL), chronic stress and an increased number of cigarettes smoked. HbA_{1c} decreased with increased alcohol consumption, physical exercise and dietary fibre intake. Overall models for cigarette smoking, chronic stress and dietary fibre showed a stronger effect than glycaemic models. Glycaemic models of alcohol predicted a decrease in risk, whereas overall models predicted an increase in risk. This inconsistency can likely be explained by the carcinogenic effects of ethanol and acetaldehyde outweighing the glucose-lowering effects (via suppressed gluconeogenesis) of alcohol. No statistically significant models for comparison of GL and physical exercise were available.

Conclusions Adopting healthy habits, such as dietary fibre intake, and avoiding unhealthy habits, such as cigarette smoking, could reduce HbA_{1c} levels, and subsequently CRC risk. Consuming alcohol could reduce HbA_{1c}, but will increase CRC risk. Reduction of HbA_{1c} could, therefore, be beneficial, but lifestyle factors alone do not provide large risk reductions. Additional therapeutic measures for decreasing HbA_{1c} could be more effective. Although estimates adjusted for confounders were used, the effects of residual confounding cannot be discounted.

Keywords

Colorectal cancer; Dose-response meta-analysis; Lifestyle; Cancer prevention; HbA_{1c}

Introduction

Colorectal cancer (CRC) contributes to more than 9% of incident cancers and is the cancer with the third most new cases globally [1]. Approximately 944,717 new CRC cases were diagnosed globally in 2000 [2]. Approximately 394,000 deaths annually can be attributed to CRC – making it the cancer causing the fourth most deaths worldwide [1]. CRC seems to be more prevalent in developed countries with a Western lifestyle, than in developing countries, with more than 63% of incident CRC cases occurring in developed countries [1]. In 2009, CRC was the 3rd leading cause of death by cancer in the United States [3], causing 51,849 deaths. Although there has been a decline in CRC deaths in the US in the past few decades (as a result of early screening and more effective treatment [2]), as well as a decrease in CRC incidence, the estimated number of deaths from CRC in 2013 in the US is still approximately 50,830, and the estimated incidence during this period is 142,820 [3].

Lifestyle factors, such as cigarette smoking, unhealthy diets, heavy alcohol consumption and a sedentary lifestyle are known to be associated with increased CRC risk [1]. A Danish study on a cohort of middle-aged men and women found that 13% of CRC cases in the cohort could possibly have been avoided if only one additional lifestyle guideline was adhered to [4]. The lifestyle factors investigated in that study were smoking, alcohol consumption, diet, physical activity and waist circumference. A recent meta-analysis [5] on published studies regarding risk factors for CRC found moderately increased CRC incidence risk for cigarette smoking, consumption of red meat, low fruit and vegetable consumption, a high BMI and low levels of physical activity. Another recent meta-analysis [6] found an increased risk for CRC in people with diabetes (RR = 1.26).

It is, therefore, apparent that there is a link between CRC risk and lifestyle factors. However, CRC can also be linked to diabetes, which in turn, is influenced by lifestyle factors. It is hypothesized that the common factor that can be used to link CRC risk, lifestyle factors and diabetes is chronic hyperglycaemia. Here, the measure that will be used to assess chronic hyperglycaemia is HbA_{1c}, as it acts as a metabolic biomarker of the mean blood glucose concentration for an extended period before the test, typically 6 weeks to 3 months [7,8]. The relevant links between lifestyle factors, hyperglycaemia and cancer risk may then be investigated.

Hyperglycaemia can be linked to risk of cancer, directly and indirectly. Chronic hyperglycaemia increases reactive oxygen species (ROS) production [7,9], leading to oxidative stress [10]. This, in turn, can damage DNA [7], which can lead to carcinogenesis. Hyperglycaemia can increase formation of advanced glycation end products (AGEs) [7,10] which promotes tumour growth in some cancers [7], as well as inhibiting apoptosis [9] and increasing inflammation [10]. The Warburg effect (which increases glycolysis in many cancers), necessitates an increased glucose supply to be able to continue growth with less efficient energy production [7,11,12,13]. Insulin is secreted

in response to increases in blood glucose [10]. Hyperglycaemia can, therefore, lead to hyperinsulinaemia (and increased insulin-like growth factor levels), which is known to increase cancer growth [14,15]. Hyperglycaemia is caused by factors such as excessive carbohydrate intake and physical inactivity, which can also lead to obesity (another known risk factor for CRC) – hyperglycaemia can, therefore, be used as an indirect marker for obesity [13].

Alcohol consumption suppresses hepatic gluconeogenesis via the depletion of nicotinamide adenine dinucleotide in the liver [16].

Carbohydrate intake can be linked to glycaemic response via glycaemic index (GI) and glycaemic load (GL). Diets with high GI and GL cause higher blood glucose levels and, consequently, higher insulin levels [17]. It must be noted that some criticisms on the GI concept exist. Wolever [18] aimed to address these concerns. The concern regarding day-to-day variability between subjects is addressed by the updated ISO standard methodology which places stricter requirements on the test, such as the use of the mean of ten or more subjects to calculate the GI value, and the use of the mean of two glucose tests per subject; additional refinements may be required [18].

Concerns regarding the use of different GI tables (and values) are valid, as the GI value is dependent on certain aspects such as processing, the botanical variety of the food and the conditions in which the food is grown [18]. The same type of food might, therefore, reasonably have more than one GI value. To improve the accuracy, it would be preferred that the GI for foods in a trial are tested per trial (according to the ISO standard), to ensure that the GI values that are used match the foods in that trial.

Despite these concerns, the GI is deemed to be a useful tool to determine the impact of carbohydrate-containing foods on the glycaemic response. Even though the absolute values of GI (and GL) between trials might not be exactly comparable, the concept of a low GI (and low GL) diet to reduce the glycaemic response could still be used to determine what the impact is on reduction of disease risk, as the GI method can differentiate between low and high GI foods with reasonable confidence [18].

By consuming dietary fibre with a meal containing carbohydrates, the glycaemic response to the meal can be decreased, which subsequently lowers insulin levels [19]. Dietary fibre can also decrease the contact with carcinogens by decreasing transit time through the gastrointestinal tract [20].

Physical exercise increases uptake of blood glucose to the muscles, independent of insulin [17]. GLUT4 glucose transporter, glucose phosphorylation and increased blood flow in the muscle increase the uptake of glucose from the blood [17]. Insulin levels are decreased during moderate exercise [21], and sensitizes the liver to glucagon action [17].

Cigarette smoking causes a short-term rise in blood glucose concentrations and may affect insulin sensitivity [22] via increased gluconeogenesis, glycogenolysis and glycolysis caused by increased catecholamine and cortisol levels [23]. The nicotine contained in cigarettes also affects inflammation and adiponectin levels; this may increase blood glucose concentrations [23].

Chronic stress increases cortisol production [24], which stimulates gluconeogenesis and glycogenolysis in the liver [25] and leads to increased production of blood glucose. Changes in dietary and exercise patterns, as well as an abnormal cortisol rhythm as a result of chronic stress may lead to obesity and insulin resistance [26].

In this study, models that link HbA_{1c} to CRC risk and HbA_{1c} to lifestyle factors were developed from data in published research articles and combined to observe the glycaemic effect of lifestyle factors on CRC risk. These models were then compared to models of the overall effect of lifestyle factors on CRC risk to observe what the effects of the glycaemic components of lifestyle factors compared to the overall CRC risk are.

Materials and methods

Study selection

Only English-language articles were included. Searches were performed using scientific databases such as Scopus and ScienceDirect. Only trends that were adjusted for the greatest number of confounders were included [5]. Only articles referring specifically to colorectal cancer (not colon or rectal cancer separately) were included. Studies relating to CRC precursors (neoplasms, adenomas or adenomatous polyps), as well as studies which only assessed cancer stage or mortality, were excluded.

Searches were performed to identify articles which relate HbA_{1c} to CRC risk in relative risk (RR), odds ratio (OR) or hazard ratio (HR). Combinations of the following search terms were used: "colorectal cancer" AND "a_{1c}", "glycated hemoglobin", "glycosylated hemoglobin", "glycated haemoglobin", "glycosylated haemoglobin", "hba_{1c}". The reference lists of identified articles were scanned to find more relevant articles. Relevant articles that contained information on CRC risk that were identified during a similar search on "cancer" and the above-mentioned keywords were also included.

Searches to identify articles relating HbA_{1c}, as well as risk for CRC in RR, OR and HR, to lifestyle factors, were performed. Only articles quantifying lifestyle factors in the following units, or units that could be transformed to these units, were included: glycaemic load; grams of dietary fibre intake per day; number of cigarettes per day; grams of ethanol per day; low, medium and high levels of chronic stress, job strain or associated measures; kcal,

metabolic equivalents (METs) or MET hours per day (MET-h/day) of exercise. Combinations of the following search terms were used: "colorectal cancer" or "a_{1c}" or "glycated hemoglobin" or "glycosylated hemoglobin" or "glycated haemoglobin" or "glycosylated haemoglobin" or "hba_{1c}" AND "alcohol", "dietary fibre", "dietary fiber", "fiber", "fibre", "glycaemic load", "glycemic load", "stress", "smoking", "exercise", "physical activity".

Where alcohol consumption was indicated in drinks per day or per week and no conversion factor was provided in the study, the exposure was converted to grams per day using the assumption that one drink equals 12 g of ethanol for US studies, and 9.8 g for European studies [5]. Studies stratifying data (for instance by gene polymorphisms) were not included, except for studies stratifying by gender. If sufficient details were available in these cases, both the data sets for men and women were included. The glucose reference for GL was used. If a study provided a GL referenced to white bread, it was converted to the glucose reference by multiplying by 0.7.

Data extraction

The following data entries were extracted from the articles: reference details; risk measure (RR, OR or HR); total number of persons per exposure level for RR and OR; number of controls per exposure level for OR; number of non-cases per exposure level for RR; number of person-years per exposure level for HR; number of cases per exposure level for OR, RR and HR; gender; 95% confidence intervals (CIs) or standard error (SE)/standard deviation (SD) if 95% CI was not available for studies relating lifestyle factors to HbA_{1c}; risk per exposure range; HbA_{1c} level or difference from reference HbA_{1c} level for studies relating HbA_{1c} to lifestyle factors; HbA_{1c} range or lifestyle factor range (exposure range); additional comments regarding the study or patients, for instance the reference food in articles relating to GL.

If no SE was reported, but a 95% CI was available per exposure level, the SE required for weighting of the studies in the meta-analysis was computed using equation (1). $z_{\alpha/2}$ equals 1.96 for a 95% CI [27].

$$SE = \frac{\left[\ln(CI_{upper}) - \ln(CI_{lower})\right]}{2 \times z_{\alpha/2}} \quad (1)$$

Where 95% CIs were not available, but an SD and population size (n) was reported, the SD was converted to a SE using equation (2).

$$SE = \frac{\mathrm{SD}}{\sqrt{n}} \quad (2)$$

Statistical analyses

Single values per exposure category or range were selected using method 1 in Hartemink et al. [28]. For closed ranges, the midpoint of the range was selected as the dose for that range. For the upper open-ended category, the width of the second highest range was added to the value of the upper-open ended range. For the lower open-ended range, half of the width of the second lowest range was subtracted from the value of the lower open-ended range. Where the method would result in a negative value for the lower open-ended category, a value of 0 was chosen to represent this category. If mean or median values for ranges were already provided in the article, these values were used in the analysis.

The risk measures were transformed by taking the natural logarithm (*ln*) of the RR, OR or HR, as it has better statistical properties than the untransformed risk [29]. This resulted in a risk of 0 in the reference category. To yield a model that is fitted through the origin, the exposure value in the reference category was subtracted from all exposures per study to yield an exposure of 0 in the reference category. For the association of lifestyle factors with HbA_{1c} the HbA_{1c} value in the reference category (which is the dependent variable) was subtracted from all of the HbA_{1c} levels per study to yield an HbA_{1c} of 0 in the reference category. In this way, the change in HbA_{1c} values in the lifestyle factor models could directly be compared to those in the CRC risk models.

Dose-response meta-analyses of the studies relating CRC risk to HbA_{1c} or lifestyle factors were performed using the dosresmeta package in R [30]. This module is the R-equivalent of the GLST (generalized least squares for trend estimation) module for Stata, developed by Orsini et al. [27,31]. This module is used to develop linear and non-linear (restricted cubic spline) models of the natural logarithm of the RR, OR or HR. The restricted cubic spline models use three knots (k = 3), located at the 10th, 50th and 90th percentiles of the data [5,32]. Two (number of knots minus 1) splines are generated. Only random-effects meta-analyses were used to incorporate heterogeneity.

The nonlinearity was assessed by a hypothesis test. The null hypothesis that was tested was that the coefficients of the splines from the second spline onward ($b_2, \ldots, b_{k-1}$)) were zero [5]. Therefore, for the particular case where the model had three knots, the coefficient of only the second spline was evaluated. If the *p*-value for nonlinearity for the second spline > 0.05 the linear model was used [5], otherwise the non-linear model was used. Only studies providing three or more exposure levels were included in the analysis [5]. If only a single study (with less than 6 exposures) was available for a certain model, only a linear trend was estimated, as the function used to calculate the restricted cubic spline values requires at least six values.

Dose-response meta-analyses of the studies relating HbA_{1c} to lifestyle factors were performed by first obtaining the slope and SE of each study using inverse-variance weighting [29], and then obtaining the overall dose-response estimate by combining the slopes of each study using a random-effects meta-analysis. In the case where the between-study variance for the random-effects meta-analysis was found to be negative, the model was reduced to use the fixed-effect method. The analyses were performed using a Microsoft Excel spread sheet developed by one of the authors, and the results of the meta-analysis portion were compared to the spread sheet developed by Neyeloff et al. [33]. Only linear models were assessed in this case.

Heterogeneity and lack of goodness of fit was assessed by checking whether the *p*-value derived from the chi-squared statistic was smaller than 0.1 [34].

RR, OR and HR were analysed separately. Additional analysis was performed to determine what the models would look like when the RR, OR and HR were combined under the rare disease assumption.

Results

HbA_{1c} and CRC risk

Eight studies that relate HbA_{1c} to CRC risk were collected (Table 1). Two studies [35,36] used participants from the EPIC Study cohort. Two studies [37,38] used participants from the Nurses' Health Study. Only the newest study in each case [36,38] was included in the analysis. Six studies were, therefore, included in the final meta-analysis. The resulting models are presented in Table 2.

One study [13] presented results on the relations between HR for CRC and HbA_{1c}. This study presented two sets of data, one set for women and one set for men. The increasing association was not statistically significant (p = 0.1312). When the data points below the reference for both men and women were excluded, the trend became log-linear (p = 0.0389) with an increase in risk as HbA_{1c} increased. Three studies [36,39,40] were included in the meta-analysis on the relations of OR for CRC with HbA_{1c}. An increasing log-linear dose-response relationship (p = 0.0050) was found. Two studies [38,41] on the relations of RR for CRC with HbA_{1c} were included. Both studies presented data for women only. The log-linear (p = 0.3859) and nonlinear (p = 0.8576) models were both found to be statistically non-significant.

There was no significant evidence of heterogeneity in any of the models (p = 0.8299, I^2 = 1% for HR; p = 0.9121, I^2 = 1% for HR excluding data points below the reference; p = 0.3110, I^2 = 16.1% for OR; p = 0.7731, I^2 = 1% for RR).

When all risk measures were combined under the rare disease assumption, the model had a statistically significant log-linear increasing trend ($p = 0.0134$). When the data points below the reference for the HR studies were excluded, the increasing trend remained log-linear ($p = 0.0020$). Heterogeneity was not significant in either case ($p = 0.1633$, $I^2 = 33.2\%$ for combined risk; $p = 0.2517$, $I^2 = 22.3\%$ for combined risk excluding data points below reference).

Lifestyle factors and HbA_{1c}

Fourteen studies that relate HbA_{1c} to lifestyle factors (Table 3) were collected.

Alcohol

Five studies, consisting of 7 data sets in total, were included in the analysis of HbA_{1c} and alcohol. A statistically significant decreasing linear trend was found ($p = 0.0000$). No evidence of significant heterogeneity could be found ($p = 0.4914$, $I^2 = 0\%$).

Dietary fibre

Three studies [42,43,44] provided data on the relation between HbA_{1c} and dietary fibre. The increasing trend was found to be statistically non-significant ($p = 0.0952$). When the study by Ikeda et al. [43], which was adjusted for age and sex only, was excluded from the analysis, the linear trend became statistically significant ($p = 0.0002$). Before the exclusion there was some evidence of heterogeneity ($p = 0.0522$, $I^2 = 66.1\%$), which was not present after the exclusion of [43] ($p = 0.3173$, $I^2 = 0\%$).

Physical exercise

One study [45] with two datasets was included in the analysis for exercise. A statistically significant decreasing linear trend ($p = 0.0002$) was found. No significant evidence of heterogeneity was found ($p = 0.3173$, $I^2 = 0$).

Glycaemic load (GL)

Two studies [46,47] were included in the analysis for GL. The between-study variance in the random-effect model was negative, and the fixed-effect model was, therefore, used for the final analysis. A statistically significant increasing trend was found ($p = 0.0035$). There was no evidence of significant heterogeneity ($p = 0.5773$, $I^2 = 0\%$).

The inclusion of a third study – a statistically non-significant study by Van Aerde et al. [48] – weakened the association, but also caused the model to become statistically non-significant ($p = 0.2695$ using a random-effects

model). The inclusion of this study did not significantly alter the heterogeneity of the model when comparing the fixed-effect model obtained excluding the study to the random-effect model including the study. The study was excluded from the final glycaemic models.

Cigarette smoking

Three studies [22,49,50] with four datasets in total were included in the analysis for cigarette smoking. A statistically significant increasing trend was found ($p = 0.0005$). No significant evidence of heterogeneity was found ($p = 0.5238$, $I^2 = 0\%$).

Chronic stress

Two studies relating chronic stress to HbA_{1c} were included in the initial analysis [51,52]. The resulting increasing linear trend was not statistically significant ($p = 0.5425$). No evidence of significant heterogeneity was found ($p = 0.3173$, $I^2 = 0\%$).

After exclusion of the Feldman et al. [51] study, which adjusted only for sex and age, the increasing linear trend became statistically significant ($p = 0.0000$).

Lifestyle factors and CRC risk

57 studies that relate lifestyle factors to CRC risk were collected (Table 4).

Alcohol

Twenty-three studies relating alcohol consumption to CRC risk were included. Eight studies presented results on alcohol consumption and HR for CRC, 15 studies on OR for CRC and two on RR for CRC. HR and OR data produced statistically significant increasing log-linear trends ($p = 0.0000$ for HR; $p = 0.0412$ for OR), while the trend was not significant for RR data ($p = 0.5187$). There was significant evidence of heterogeneity for the HR and OR models ($p = 0.0640$, $I^2 = 47.6\%$ for HR; $p = 0.0000$, $I^2 = 98.5\%$ for OR). There was no evidence of significant heterogeneity for the RR model ($p = 0.3837$, $I^2 = 1\%$).

Secondary analyses of the HR and OR data were performed with the exclusion of data points below the reference for each study. This resulted in only slight changes in the models. Both models were still statistically significant ($p = 0.0000$ for HR; $p = 0.0326$ for OR) and showed significant heterogeneity ($p = 0.0730$, $I^2 = 46\%$ for HR; $p = 0.0000$, $I^2 = 98.5\%$ for OR).

When combining all risk measures, the model was statistically significantly increasing, and log-linear ($p = 0.0001$). Excluding data points below the reference resulted in a log-linear increasing model ($p = 0.0031$), with almost borderline significant nonlinearity ($p = 0.0529$ for nonlinearity). Both models showed significant heterogeneity ($p = 0.0000$, $I^2 = 82\%$ for combined risk; $p = 0.0000$, $I^2 = 97.5\%$ for combined risk excluding data points below the reference).

Dietary fibre

Nine studies related dietary fibre intake to CRC risk. Five studies presented results on dietary fibre intake and HR for CRC and four studies on OR for CRC. The decreasing trend for HR was statistically significant ($p = 0.0445$), without significant heterogeneity ($p = 0.1290$, $I^2 = 39.4\%$). Secondary analysis was performed for HR by excluding the data points below the reference. This resulted in the model becoming statistically non-significant ($p = 0.0949$) and heterogeneous ($p = 0.0937$, $I^2 = 44.6\%$). The OR model was almost borderline significantly decreasing ($p = 0.0527$), but showed significant heterogeneity ($p = 0.0003$, $I^2 = 84.1\%$). No studies provided data on RR.

When all studies were combined, the trend became nonlinear ($p = 0.0479$ for nonlinearity), but there was significant evidence of heterogeneity ($p = 0.0023$, $I^2 = 53\%$). When the data points below the reference were excluded, the trend became log-linear decreasing ($p = 0.0068$), but the results were still heterogeneous ($p = 0.0001$, $I^2 = 72.1\%$).

Physical exercise

Three studies that relate exercise to CRC risk were analysed. No statistically significant trends were found ($p = 0.3597$ for HR, $p = 0.1235$ for OR and $p = 0.5509$ for RR), although the direction of the association showed that risk was decreasing as the MET-h/day increased. When all risks were combined, the trend was still non-significant ($p = 0.1485$). There was some evidence of heterogeneity for RR and for the combined estimate ($p = 0.0676$, $I^2 = 70.1\%$ for RR; $p = 0.0969$, $I^2 = 49.1\%$ for combined model).

Glycaemic load (GL)

Six studies related glycaemic load to CRC risk, all via HR. A statistically non-significant increase was found ($p = 0.9092$). There was significant evidence of heterogeneity ($p = 0.0520$, $I^2 = 51.9\%$).

Cigarette smoking

Nineteen studies were included in the meta-analysis of cigarette smoking with CRC risk. Four studies reported on smoking and HR for CRC, fourteen on OR for CRC, and one on RR for CRC. An increasing trend was found for all

studies, but it was only statistically significant for HR ($p = 0.0186$). When combining all studies, a statistically significant increasing trend ($p = 0.0174$) was found. There was significant evidence of heterogeneity for all models ($p = 0.0206$, $I^2 = 69.3\%$ for HR; $p = 0.0001$, $I^2 = 66.3\%$ for OR; $p = 0.0000$, $I^2 = 67.2\%$ for combined model), except for RR.

Chronic stress

Two studies on the relation of chronic stress with CRC risk were analysed. One study [26] reported on HR and one [53] on OR for CRC. No studies reported on RR for CRC. The association of HR for CRC and stress decreased statistically non-significantly ($p = 0.5611$), whereas that for OR increased statistically significantly ($p = 0.0428$) with increasing stress levels. When all studies were combined, the resulting increasing log-linear trend was not statistically significant ($p = 0.9740$), nor was the nonlinear relation ($p = 0.6591$ for nonlinearity). There was significant heterogeneity for both the HR and combined models ($p = 0.0503$, $I^2 = 73.9\%$ for HR; $p = 0.0137$, $I^2 = 76.7\%$ for combined model), but not for the OR model.

Comparison

Consolidated models for lifestyle factors and CRC risk, via HbA_{1c}, were obtained by substituting the statistically significant models for HbA_{1c} and lifestyle factors into the statistically significant models for CRC risk and HbA_{1c}, and adjusting the ranges for which the models are valid according to the intersection of the models. The results are shown in Table 5. Models of decreased risk were observed for alcohol consumption, dietary fibre intake and exercise. Models of increased risk were observed for GL, cigarette smoking and chronic stress.

The next step was to compare the consolidated glycaemic models to the statistically significant overall models relating lifestyle factors to CRC risk directly. Only the models that excluded the data points below the reference per study were further analysed (i.e. models where the reference value corresponded to the lowest exposure range). Seven models resulted – three for alcohol (HR, OR and combined risk), one for dietary fibre (combined risk), one for chronic stress (OR) and two for cigarette smoking (HR and combined risk).

The results of the comparison are shown in Figs. 1 to 4. In all cases, the overall effects of the lifestyle factors on CRC risk were stronger than the glycaemic effects (i.e. more protective for a decreasing model or causing more risk for an increasing model).

Discussion

For alcohol consumption, the overall effect indicated an increase in CRC risk as the amount of alcohol consumed increased. In the overall model, there was a combined risk of approximately 1.36 for an increase of 25 g per day (approximately two drinks) when the data points below the reference were excluded. Including these data points in the analysis yielded a log-linear model with a combined risk of 1.2 for an increase in alcoholic intake of 25 g per day. This is generally in agreement with the study by Fedirko et al. [54], that found a second-order fractional polynomial dose-response relationship ($0.006992 \times \text{dose} - 0.00001 \times \text{dose}^2$), which corresponds to a RR of 1.18 for a 25 g per day increase in alcohol consumption. There was significant heterogeneity in the overall model for the current study, as well as in the Fedirko et al. study [54].

In contrast, the CRC risk predicted by the glycaemic model indicated a decrease in risk as the amount of alcohol increased. This contrast can be explained by a number of factors. The decreasing trend for the glycaemic model could be caused by suppressed gluconeogenesis in the liver. Gluconeogenesis is suppressed by the consumption of alcohol, possibly by up to 45%, by metabolism of alcohol to acetaldehyde, which is metabolised to acetate by aldehyde dehydrogenase [16]. The acetate depletes nicotinamide adenine dinucleotide (which is required for gluconeogenesis) in the liver [16]. Alcohol also has a number of carcinogenic effects, which could increase CRC risk in the overall model. Alcohol contains ethanol (a known carcinogen [55]), and metabolises to acetaldehyde (which is considered a possible carcinogen [56,57], and can damage DNA [58]). Excess alcoholic intake may also add to weight gain [58] and in turn obesity, which is a known risk factor for CRC [5].

Overall, CRC risk decreased as dietary fibre intake increased. This effect was also present in the glycaemic model, but not as pronounced as in the overall model. The risk associated with a 10 g per day increase in fibre intake was approximately 0.81 in the overall model, while being approximately 0.96 in the glycaemic model. Aune et al. [59] reported a RR of 0.9 (95% CI = 0.86 to 0.94) for a 10 g per day increase in dietary fibre intake based on sixteen studies, compared to the nine studies investigated in this article. The results in [59] were not significantly heterogeneous, whereas those for the overall model in the current study was. It is, therefore, probable that the results found by Aune et al. are better for comparative purposes. A comparison between the overall models and glycaemic models indicate that, although the decrease in HbA_{1c} as a result of increased fibre intake is significant, there are also factors other than HbA_{1c} relating fibre intake to lower CRC risk. This may include mechanisms such as the lowering of the amount of time faeces travels through the digestive tract, as well as the dilution of carcinogens in faeces; all of these factors result in decreased exposure to carcinogens [59].

Both cigarette smoking models show that CRC risk increased as the number of cigarettes smoked increased. The overall effect is much stronger than the glycaemic effect caused by an increase in HbA_{1c}. For instance, the risk

associated with an increase of 20 cigarettes per day (one pack) is approximately 1.28 in the overall HR model, while only approximately 1.02 in the glycaemic HR model. In the combined risk model, the overall risk is approximately 1.12, while only approximately 1.02 in the glycaemic model. Liang et al. [60] found a RR of 1.175 for an increase of 20 cigarettes per day and 1.38 for an increase of 40 cigarettes per day ($p < 0.0001$), based on eleven studies. They did not find significant heterogeneity, which is in contrast to the significant heterogeneity observed in the overall model in the current study. Tsoi et al. [61] found a RR of 1.02 (95% CI = 0.96 to 1.08) for smoking of less than 20 cigarettes per day (based on 13 cohorts), and a RR of 1.31 (95% CI = 1.10 to 1.54) for 20 or more cigarettes smoked per day (based on 10 cohorts). It can be seen that, although cigarette smoking increases blood glucose concentration and in turn HbA_{1c}, its main carcinogenic effects are via other routes. Several potentially carcinogenic chemicals are present in tobacco smoke and released during the burning of tobacco [62,63]. These could cause DNA damage to colorectal cells when ingested or via exposure through circulation in the human body [62].

Comparison of the OR models for chronic stress revealed that CRC risk increased as stress level increased in both the glycaemic and overall models. The risk associated with a one level increase in stress resulted in a CRC risk of 1.16 in the overall OR model, while only increasing risk by 1.06 in the glycaemic model. Heikkilä et al. [64] found a statistically non-significant increased HR of 1.16 (95% CI = 0.90 to 1.48) for a pooled analysis of 12 studies comparing job strain with no job strain. They concluded that job strain was likely not an important risk factor for CRC, but also indicated that this did not mean that other types of stress could not be related to cancer risk [64]. It must be noted that only one study was included per stress model in the current study.

A study by Kawakami et al. [65] that showed an increase in HbA_{1c} with increasing stress level was excluded from the meta-analysis for HbA_{1c} and chronic stress as sufficient information on the SEs was not available. However, it seems that the relation of chronic stress and CRC risk is in part mediated by the increase in HbA_{1c}, caused by increased cortisol production as a result of the stress response [24], which stimulates gluconeogenesis and glycogenolysis in the liver [25]. Supporting this known evidence is a review by Hansen et al. [66] that found an increase in HbA_{1c} compared to a number of occupational stressors in seven studies. It is probable that the difference between the risk observed in the overall model, and that in the glycaemic model could have been as a result of chronic inflammation caused by the activation of the stress response [64] or as a result of different stress measures being evaluated (job strain in the glycaemic model and perceived stress in the overall model). The latter could also have caused different classifications for stress levels (low, medium and high). It is clear that information from more studies is needed to improve the models. An objective stress scoring system would also facilitate better comparison between studies.

All overall models between CRC risk and exercise showed a statistically non-significant decrease in CRC risk as the amount of exercise in MET-h/day increased. The risk was approximately 0.95 (95% CI = 0.88 to 1.02) for each 4 MET-h/day increase in exercise in the combined risk model. The glycaemic models indicated decreases in CRC risk as the amount of exercise increased. The association showed a decrease in CRC risk of 0.90 for each 4 MET-h/day increase in exercise in the combined risk model. It must be noted that these models were developed from data in a small portion of the diabetic HbA_{1c} range. Investigation of the published literature revealed no consistent association between rectal cancer risk and exercise, whereas there was a strong inverse relation with colon cancer [67,68,69]. Unfortunately, no dose-response meta-analysis of CRC risk and exercise for direct comparison could be found in the published literature.

The current study found a statistically non-significant increase in risk of 1.0055 (95% CI = 0.915 to 1.106) for an increase of 50 units of GL per day in the overall model, with significant heterogeneity. The glycaemic model estimated an increase in combined risk of approximately 1.049 per 50 units increase in GL, as well as a HR of 1.038 and OR of 1.112. A dose-response meta-analysis by Aune et al. [70] based on twelve studies found a statistically non-significant increase in RR for CRC with an increase in GL (RR for 50 units per day = 1.01) with significant heterogeneity. When excluding the Women's Health Study, they found a RR of 1 (still statistically non-significant), but with reduced heterogeneity.

Gnagnarella et al. [71] found a RR of 1.26 (95% CI = 1.11 to 1.44) with significant heterogeneity in eight studies, while Mulholland et al. [72] found a RR of 1.17 (95% CI = 0.98 to 1.39) with significant heterogeneity in nine studies, when comparing the highest with the lowest GL categories. As no statistically significant overall dose-response model was available for comparison it is difficult to conclude why the risk in the glycaemic model would be higher than that in the overall model. As the GL concept is closely linked to increases in blood glucose, it seems probable that the increase in CRC risk as a result of increased GL would be the same or very similar for both the overall and glycaemic models. Based on the discussion in the introduction regarding the concerns with the GI concept, it may be reasonable to focus on the effects of a low to medium GI (< 70) or low to medium GL diet (< 20) versus a high GI (≥ 70) [18] or high GL diet (≥ 20), rather than using a dose-response method based on the absolute values.

The results in the current study show that an increase in HbA_{1c} generally increases CRC risk. It provides statistically significant evidence for an increase in HbA_{1c} with increased GL, chronic stress level and increased number of cigarettes smoked. It provides statistically significant evidence for a decrease in HbA_{1c} with increased alcohol consumption, exercise and dietary fibre intake. From the results of the glycaemic models it is clear that

there is a slight increase in CRC risk with increased cigarette smoking, but that the risk in the overall model is much higher, meaning that other effects of cigarette smoking (such as the carcinogenic substances contained in tobacco smoke) have a much higher impact than the glycaemic effect. The dietary fibre glycaemic model shows that increasing dietary fibre intake reduces CRC risk in both the overall and glycaemic models, but that the effect is more prominent in the overall model. The glycaemic effect of dietary fibre on CRC risk reduction is thus significant, but does not encompass the full effect. Decreased exposure of the intestinal system to carcinogens as a result of dietary fibre may have a large effect on reducing CRC risk. The comparison of glycaemic and overall stress models show that some of the increase in CRC risk could be caused by residual factors such as chronic inflammation. It is also possible that the difference in stress measures could account for this difference. As no statistically significant dose-response data could be found to compare GL and exercise, no conclusions could be drawn with regard to the relative contributions of the glycaemic components of GL and exercise to CRC risk.

It is probable that some portion of the link between diabetes and CRC risk is a result of increased HbA_{1c} levels related to diabetes. However, an increase in HbA_{1c} is already present in the pre-diabetic HbA_{1c} range and subsequently CRC risk is already increased in non-diabetics or diabetics with good glycaemic control. It is also clear that increasing exercise energy expenditure, dietary fibre intake and alcohol consumption will reduce HbA_{1c} levels, and, concomitantly, cancer risk. However, this reduction in HbA_{1c} level does not convert to a relevant reduction in CRC risk for alcohol consumption. Alcohol consumption should, therefore, not be used as a lifestyle intervention to reduce HbA_{1c} levels, as this may increase CRC risk. Cigarette smoking cessation and decreasing chronic stress (either by therapy or pharmacologically) could also be beneficial.

The data shows that a potential therapeutic window might exist for decreasing CRC risk by reducing HbA_{1c} levels, but that the amount of reduction as a result of lifestyle factors alone is not large. Additional therapeutic measures for decreasing HbA_{1c}, and subsequently CRC risk, may be relevant.

It must be acknowledged that association does not necessarily imply causality. Although care was taken to use risk estimates and HbA_{1c} levels which account for the most confounding factors, not all studies accounted for the same confounders and residual confounding could still be possible. HbA_{1c} might also be a good marker for some other underlying factor or process which has a stronger influence on cancer risk than blood glucose.

A limitation of the current study was that publication bias was not assessed. It must be noted that a number of studies were excluded, because of lack of information required to perform the meta-analysis or because of inconsistencies. This also resulted in a smaller number of studies to perform the meta-analysis. This may have

affected the outcome. Data points lower than the reference per study were also excluded in the final models, which could have potentially affected the outcome. However, it was not possible to rescale these values to the lowest exposure, as 95% CIs were not available for the original reference group.

More dose-response studies on the association of lifestyle factors, HbA_{1c} and CRC risk are required to improve the models and provide more data for comparison. It is also suggested that a unifying model, such as the equivalent teaspoons sugar (*ets*) model, be used to convert the lifestyle factors to a single measure for direct comparison of the relative contributions of each factor's glycaemic effects [25,73].

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Table 1 Studies relating HbA_{1c} to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk (95% CI)	Exposure range (HbA_{1c},%)	Comments
RR							
[35]	4	1148	1152	Men	1	< 5%	EPIC-
	18	2616	2634		1.61 (0.54 to 4.82)	5% to 5.9%	Norfolk
	7	427	434		3.4 (0.97 to 11.92)	6% to 6.9%	Study
	2	74	76		5.19 (0.92 to 29.38)	≥ 7%	
	5	144	149		6.02 (1.47 to 24.65)	Diabetes (8.2%)	
[35]	6	1469	1475	Women	1	< 5%	EPIC-
	17	3070	3087		0.7 (0.27 to 1.82)	5% to 5.9%	Norfolk
	6	459	465		1.08 (0.33 to 3.5)	6% to 6.9%	Study
	1	60	61		1.36 (0.15 to 12.04)	≥ 7%	
	1	71	72		1.38 (0.16 to 11.76)	Diabetes (8.2%)	
[38]	41	73	114	Women	1	5.1%	Nurses'
	40	89	129		0.75 (0.43 to 1.3)	5.4%	Health
	48	87	135		0.94 (0.54 to 1.65)	5.6%	Study
	46	83	129		0.85 (0.47 to 1.51)	5.8%	
[41]	36	6743	6779	Women	1	2.3% to 4.8%	Women's
	41	6736	6777		1.02 (0.65 to 1.61)	4.8% to 5%	Health
	46	6731	6777		0.95 (0.6 to 1.5)	5% to 5.2%	Study
	45	6732	6777		0.83 (0.52 to 1.33)	≥ 5.2%	
OR							
[39]	39	87	126	C	1	< 5.38%	CLUE II
	29	86	115		0.77 (0.43 to 1.36)	5.38% to 5.54%	Cohort
	43	86	129		1.12 (0.65 to 1.91)	5.54% to 5.78%	

Table 1 Studies relating HbA_{1c} to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk (95% CI)	Exposure range (HbA_{1c},%)	Comments
	62	87	149		1.57 (0.94 to 2.6)	> 5.78%	
[37]	22	48	70	Women	1	5.2%	Nurses'
	31	60	91		1.2 (0.6 to 2.5)	5.5%	Health
	26	48	74		1.2 (0.6 to 2.7)	5.8%	Study
[36]	118	127	245	Men	1	< 5.4%	EPIC Study
	109	103	212		1.17 (0.8 to 1.69)	5.4% to 5.6%	Diabetics
	104	110	214		1.04 (0.71 to 1.53)	5.6% to 5.8%	included
	116	120	236		1.09 (0.74 to 1.6)	5.8% to 6.1%	
	114	101	215		1.25 (0.83 to 1.86)	> 6.1%	
[36]	96	91	187	Women	1	< 5.4%	EPIC Study
	84	109	193		0.74 (0.48 to 1.13)	5.4% to 5.6%	Diabetics
	85	114	199		0.71 (0.46 to 1.1)	5.6% to 5.8%	included
	118	97	215		1.13 (0.74 to 1.71)	5.8% to 6.1%	
	82	54	136		1.4 (0.87 to 2.24)	> 6.1%	
[40]	53	98	151	C	1	< 4.3%	Northern
	22	90	112		0.47 (0.26 to 0.85)	4.3% to 4.5%	Sweden
	76	114	190		1.19 (0.74 to 1.92)	4.5% to 4.7%	Health and
	69	114	183		1.17 (0.71 to 1.93)	> 4.7%	Disease
							Cohort
HR							
[13]	72	N/A	45501	Men	1	5% to 5.6% (5.38%)	ARIC Study
	16		6014		1.84 (1.07 to 3.18)	< 5% (4.75%)	
	33		16470		1.04 (0.67 to 1.6)	≥ 5.7% (6.21%)	
	18		5750		1.52 (0.88 to 2.6)	Diabetes (8.15%)	
[13]	73	N/A	60796	Women	1	5% to 5.6% (5.38%)	ARIC Study
	10		7081		1.31 (0.67 to 2.55)	< 5% (4.8%)	
	29		20064		0.99 (0.62 to 1.57)	≥ 5.7% (6.22%)	
	17		7372		1.55 (0.88 to 2.75)	Diabetes (8.6%)	

Ref = reference; CRC = colorectal cancer; RR = relative risk; OR = odds ratio; HR = hazard ratio; C = combined; N/A = not applicable; HbA_{1c} = glycated haemoglobin

Table 2 Estimated log-linear and nonlinear models of CRC risk, HbA_{1c} and lifestyle factors (referent exposure subtracted)

Risk measure	Model	*p*-value for model	*p*-value for goodness of fit
HbA_{1c} and CRC risk			
HR	$HR = exp(0.0791 \times \Delta HbA_{1c})$	0.1312	0.8299
	Range:		

Table 2 Estimated log-linear and nonlinear models of CRC risk, HbA_{1c} and lifestyle factors (referent exposure subtracted)

Risk measure	Model	*p*-value for model	*p*-value for goodness of fit
	HbA_{1c} in [4.75% to 8.6%]		
HR [a]	$HR = exp(0.1369 \times \Delta HbA_{1c})$	0.0389	0.9121
	Range:		
	HbA_{1c} in [5.38% to 8.6%]		
OR	$OR = exp(0.3853 \times \Delta HbA_{1c})$	0.0050	0.3110
	Range:		
	HbA_{1c} in [4.2% to 6.4%]		
RR	$RR = exp(-0.2201 \times \Delta HbA_{1c})$	0.3859	0.7731
	Range:		
	HbA_{1c} in [4.7% to 5.8%]		
Combined	Risk $= exp(0.1158 \times \Delta HbA_{1c})$	0.0134	0.1633
	Range:		
	HbA_{1c} in [4.2% to 8.6%]		
Combined [a]	Risk $= exp(0.1727 \times \Delta HbA_{1c})$	0.0020	0.2517
	Range:		
	HbA_{1c} in [4.2% to 8.6%]		
Lifestyle factors and CRC risk			
Alcohol consumption			
HR	$HR = exp(0.0077 \times \Delta \text{ethanol}_{\text{g/day}})$	0.0000	0.0640
	Range:		
	$\text{ethanol}_{\text{g/day}}$ in [0 to 114.9 g/day]		
HR [a]	$HR = exp(0.0078 \times \Delta \text{ethanol}_{\text{g/day}})$	0.0000	0.0730
	Range:		
	$\text{ethanol}_{\text{g/day}}$ in [0 to 114.9 g/day]		
OR	$OR = exp(0.0138 \times \Delta \text{ethanol}_{\text{g/day}})$	0.0412	0.0000
	Range:		
	$\text{ethanol}_{\text{g/day}}$ in [0 to 182.8 g/day]		
OR [a]	$OR = exp(0.0143 \times \Delta \text{ethanol}_{\text{g/day}})$	0.0326	0.0000
	Range:		
	$\text{ethanol}_{\text{g/day}}$ in [0 to 182.8 g/day]		
RR	$RR = exp(0.0040 \times \Delta \text{ethanol}_{\text{g/day}})$	0.5187	0.3837
	Range:		
	$\text{ethanol}_{\text{g/day}}$ in [0 to 35.88 g/day]		
Combined	Risk $= exp(0.0073 \times \Delta \text{ethanol}_{\text{g/day}})$	0.0001	0.0000
	Range:		

Table 2 Estimated log-linear and nonlinear models of CRC risk, HbA_{1c} and lifestyle factors (referent exposure subtracted)

Risk measure	Model	*p*-value for model	*p*-value for goodness of fit
	$\text{ethanol}_{\text{g/day}}$ in [0 to 182.8 g/day]		
Combined [a]	$\text{Risk } = exp(0.0124 \times \Delta\text{ethanol}_{\text{g/day}})$	0.0031	0.0000
	Range:		
	$\text{ethanol}_{\text{g/day}}$ in [0 to 182.8 g/day]		
Dietary fibre			
HR	$HR = exp(-0.0059 \times \Delta\text{fibre}_{\text{g/day}})$	0.0445	0.1290
	Range:		
	$\text{fibre}_{\text{g/day}}$ in [6.4 to 35 g/day]		
HR [a]	$HR = exp(-0.0060 \times \Delta\text{fibre}_{\text{g/day}})$	0.0949	0.0937
	Range:		
	$\text{fibre}_{\text{g/day}}$ in [6.4 to 35 g/day]		
OR	$OR = exp(-0.0310 \times \Delta\text{fibre}_{\text{g/day}})$	0.0527	0.0003
	Range:		
	$\text{fibre}_{\text{g/day}}$ in [8.8 to 35.2 g/day]		
Combined	$\text{Risk} = \text{e}xp(-0.0347 \times \Delta\text{fibre}_{\text{g/day}} + 0.0375 \times rcs)$	0.0479 [b]	0.0023
	Range:		
	$\text{fibre}_{\text{g/day}}$ in [6.4 to 35.2 g/day]		
Combined [a]	$Risk = exp(-0.0206 \times \Delta\text{fibre}_{\text{g/day}})$	0.0068	0.0001
	Range:		
	$\text{fibre}_{\text{g/day}}$ in [6.4 to 35.2 g/day]		
Physical exercise			
HR	$HR = exp(-0.0109 \times \Delta\text{exercise}_{\text{METh/day}})$	0.3658	0.3417
	Ranges:		
	$\text{exercise}_{\text{METh/day}}$ in [36.5 to 48.9 MET-h/day]		
OR	$OR = exp(-0.1175 \times \Delta\text{exercise}_{\text{METh/day}})$	0.1235	0.2061
	Ranges:		
	$\text{exercise}_{\text{METh/day}}$ in [0 to 5.714 MET-h/day]		
RR	$RR = exp(-0.0097 \times \Delta\text{exercise}_{\text{METh/day}})$	0.5509	0.0676
	Ranges:		
	$\text{exercise}_{\text{METh/day}}$ in [28.25 to 43.75 MET-h/day]		
Combined	$Risk = exp(-0.0131 \times \Delta\text{exercise}_{\text{METh/day}})$	0.1485	0.0969
	Range:		
	$\text{exercise}_{\text{METh/day}}$ in [0 to 48.9 MET-h/day]		

Table 2 Estimated log-linear and nonlinear models of CRC risk, HbA_{1c} and lifestyle factors (referent exposure subtracted)

Risk measure	Model	*p*-value for model	*p*-value for goodness of fit
Glycaemic load (GL)			
HR	$HR = exp(0.0001 \times \Delta \mathrm{GL}_{/\mathrm{day}})$ Range: $\mathrm{GL}_{/\mathrm{day}}$ in [46.5 to 225.9 $\mathrm{GL}_{/\mathrm{day}}$]	0.9092	0.0520
Cigarette smoking			
HR	$HR = exp(0.0122 \times \Delta \mathrm{cigarettes/day})$ Range: $\mathrm{cigarettes/day}$ in [0 to 49 $\mathrm{cigarettes/day}$]	0.0186	0.0206
OR	$OR = exp(0.0044 \times \Delta \mathrm{cigarettes/day})$ Range: $\mathrm{cigarettes/day}$ in [0 to 60 $\mathrm{cigarettes/day}$]	0.1245	0.0001
RR	$RR = exp(0.0015 \times \Delta \mathrm{cigarettes/day})$ Range: $\mathrm{cigarettes/day}$ in [0 to 30 $\mathrm{cigarettes/day}$]	0.8042	0.5270
Combined	$Risk = exp(0.0056 \times \Delta \mathrm{cigarettes/day})$ Ranges: $\mathrm{cigarettes/day}$ in [0 to 60 $\mathrm{cigarettes/day}$]	0.0174	0.0000
Chronic stress			
HR	$HR = exp(-0.0759 \times \Delta \mathrm{stress}_{\mathrm{level}})$ Range: $\mathrm{stress}_{\mathrm{level}}$ in [0 to 3 level]	0.5611	0.0503
OR	$OR = exp(0.1442 \times \Delta \mathrm{stress}_{\mathrm{level}})$ Range: $\mathrm{stress}_{\mathrm{level}}$ in [0 to 3 level]	0.0428	0.5686
Combined	$Risk = exp(0.0034 \times \Delta \mathrm{stress}_{\mathrm{level}})$ Range: $\mathrm{stress}_{\mathrm{level}}$ in [0 to 3 level]	0.9740	0.0137
Lifestyle factors and HbA_{1c}			
Alcohol consumption			
N/A	$\Delta HbA_{1c} = -0.0026 \times \Delta \mathrm{ethanol}_{\mathrm{g/day}}$ Ranges: $\mathrm{ethanol}_{\mathrm{g/day}}$ in [0 to 144 g/day] HbA_{1c} in [5% to 7%]	0.0000	0.4914
Dietary fibre			
N/A	$\Delta HbA_{1c} = 0.9123 \times \Delta \mathrm{fibre}_{\mathrm{g/day}}$	0.0952	0.0522

Table 2 Estimated log-linear and nonlinear models of CRC risk, HbA_{1c} and lifestyle factors (referent exposure subtracted)

Risk measure	Model	*p*-value for model	*p*-value for goodness of fit
	Ranges:		
	$\text{fibre}_{\text{g/day}}$ in [4.93 to 27.3 g/day]		
	HbA_{1c} in [4.7% to 8.3%]		
N/A	$\Delta HbA_{1c} = -0.0246 \times \Delta\text{fibre}_{\text{g/day}}$[c]	0.0002	0.3173
	Ranges:		
	$\text{fibre}_{\text{g/day}}$ in [4.93 to 27.3 g/day]		
	HbA_{1c} in [6.21% to 7.7%]		
Physical exercise			
N/A	$\Delta HbA_{1c} = -0.1299 \times \Delta\text{exercise}_{\text{METh/day}}$	0.0002	0.3173
	Ranges:		
	$\text{exercise}_{\text{METh/day}}$ in [0 to 4.983 MET-h/day]		
	HbA_{1c} in [7.2% to 8%]		
Glycaemic load (GL)			
N/A	$\Delta HbA_{1c} = 0.0055 \times \Delta\text{GL}_{/\text{day}}$[d]	0.0035	0.5773
	Ranges:		
	$\text{GL}_{/\text{day}}$ in [58 to 171.6 $\text{GL}_{/\text{day}}$]		
	HbA_{1c} in [5% to 6.6%]		
Cigarette smoking			
N/A	$\Delta HbA_{1c} = 0.0069 \times \Delta\text{cigarettes/day}$	0.0005	0.5238
	Ranges:		
	cigarettes/day in [0 to 38 cigarettes/day]		
	HbA_{1c} in [5.02% to 6.617%]		
Chronic stress			
N/A	$\Delta HbA_{1c} = 0.0569 \times \Delta\text{stress}_{\text{level}}$	0.5425	0.3173
	Ranges:		
	$\text{stress}_{\text{level}}$ in [0 to 3 level]		
	HbA_{1c} in [5.2% to 5.7%]		
N/A	$\Delta HbA_{1c} = 0.1512 \times \Delta\text{stress}_{\text{level}}$[e]	0.0000	0.7079
	Ranges:		
	$\text{stress}_{\text{level}}$ in [0 to 3 level]		
	HbA_{1c} in [5.2% to 5.7%]		

[a] *p*-value for coefficient of second spline

[b] Excluding data points below reference

[c] Excluding [74]

[d] Fixed-effect model

[e] Excluding [51]

Table 2 Estimated log-linear and nonlinear models of CRC risk, HbA_{1c} and lifestyle factors (referent exposure subtracted)

Risk measure	Model	*p*-value for model	*p*-value for goodness of fit

CRC = colorectal cancer; RR = relative risk; OR = odds ratio; HR = hazard ratio; g/day = grams per day; GL = glycaemic load; MET-h/day = metabolic equivalent-hours per day; HbA_{1c} = glycated haemoglobin

$$rcs = \frac{(\Delta exposure - knot_1)_+^3 - \frac{(knot_3 - knot_1)(\Delta exposure - knot_2)_+^3 - (knot_2 - knot_1)(\Delta exposure - knot_3)_+^3}{(knot_3 - knot_{2)}}}{(knot_3 - knot_1)^2}$$ [5,32]

where

$$(u)_+ = \begin{cases} u, for\ u > 0 \\ 0, for\ u \leq 0 \end{cases}, where\ u\ is\ the\ function\ contained\ in\ brackets$$

Stress level:

0 = None

1 = Low

2 = Medium

3 = High

Table 3 Studies relating HbA_{1c} to lifestyle factors

Ref	*n*	Gender	HbA_{1c} ,% (95% CI)	SE	Exposure range	Unit	Other
Alcohol consumption							
[75]	228	Men	6.7	0.2	0	g/day	
	432		6.5	0.1	≤ 12		
	551		6.8	0.1	13 to 24		
	411		6.6	0.1	25 to 47		
	225		7	0.2	48 to 96		
	49		6.4	0.4	> 96		
[76]	72	Women	5.29	NA	0	g/day	
	144		5.24 (5.2 to 5.3)	0.0255	0.1 to < 15		
	133		5.18 (5.04 to 5.24)	0.0510	15 to < 35		
	110		5.23 (5.18 to 5.29)	0.0281	≥ 35		
[49]	982	Men	5.14 (5.03 to .5.25)	0.0561	0	g/day	
	1455		5.07 (4.96 to 5.17)	0.0536	1 to 19		
	1170		5.02 (4.92 to 5.13)	0.0536	20 to 50		
	739		5 (4.88 to 5.1)	0.0561	≥ 50		
[49]	4481	Women	5.08 (5.01 to .5.15)	0.0357	0	g/day	
	979		5.07 (5 to 5.14)	0.0357	1 to 9		
	760		5 (4.93 to 5.07)	0.0357	≥ 10		
[77]	75	Men	0	NA	0	g/day	
	55		to 0.16 (-0.4 to 0.8)	0.3061	6.6		
	141		-0.21 (-0.47 to 0.05)	0.1327	16.8		
	114		-0.11 (-0.38 to 0.16)	0.1378	30.9		
	79		-0.1 (-0.41 to 0.21)	0.1582	51.4		
[50]	220	Men	0	NA	0	units/	8 g per unit
	1482		-0.12 (-0.234 to -0.004)	0.0587	0 to 6	week	
	852		-0.1 (-0.222 to 0.022)	0.0622	7 to 13		
	637		-0.106 (-0.231 to 0.019)	0.0638	14 to 20		
	897		-0.192 (-0.314 to -0.07)	0.0622	21 to 41		
	595		-0.244 (-0.371 to -0.117)	0.0648	≥ 42		
[50]	478	Women	0	NA	0	units/	8 g per unit
	2943		-0.013 (-0.087 to 0.061)	0.0378	0 to 6	week	
	871		-0.04 (-0.128 to 0.048)	0.0449	7 to 13		
	389		-0.034 (-0.138 to 0.07)	0.0531	14 to 20		
	329		-0.113 (-0.223 to -0.003)	0.0561	21 to 41		
	75		-0.096 (-0.294 to 0.092)	0.0985	≥ 42		
Dietary fibre							
[44]	2065 in total	C	6.5 (6.32 to 6.68)	0.0918	11.4	g/day	Type 1
			6.42 (6.26 to 6.58)	0.0816	15.6		diabetics
			6.29 (6.13 to 6.45)	0.0816	20		

Table 3 Studies relating HbA_{1c} to lifestyle factors

Ref	*n*	Gender	HbA_{1c} ,% (95% CI)	SE	Exposure range	Unit	Other
			6.21 (6.04 to 6.39)	0.0893	27.3		
[43]	390	C	4.7	0.0152	10.7	g/day	Age- and
	1685		5.4	0.0073	10.7		sex-adjusted
	427		6.3	0.0145	10.9		
	101		8.3	0.0299	12.2		
[42]	308	C	7.7	0.0855	< 6.925	g/day	Type 2
	310		7.5	0.0852	6.925 to 10.915		diabetics
	308		7.4	0.1026	> 10.915		Used average of cut-offs for men and women
Physical exercise							
[45]	48	C	7.8	0.1443	0	min/	Moderate
	32		7.6	0.1945	0 to 149	week	exercise (5
	50		7.5	0.1556	150		METs) Type 1 diabetics
[45]	30	C	8	0.1826	0	min/	Intense
	69		7.8	0.1324	0 to 149	week	exercise
	31		7.2	0.1796	150		(7 METs) Type 1 diabetics
Glycaemic load (GL)							
[46]	57	C	6.2 (5.5 to 6.8)	0.3316	58	GL/	Reference
	57		6.2 (5.5 to 6.8)	0.3316	75	day	food is
	57		6.6 (6 to 7.2)	0.3061	86		glucose
	56		6.5 (5.9 to 7.1)	0.3061	100		
[47]	169	Women	5	0.1	157.665	GL/	Reference
	169		5.1	0.1	168.48	day	food is
	169		5.1	0.1	167.562		glucose
	169		5.1	0.1	171.76		Original
	169		5.2	0.1	170.665		exposure per 1000 kcal was converted to GL/day by multiplying with daily

Table 3 Studies relating HbA_{1c} to lifestyle factors

Ref	*n*	Gender	HbA_{1c} ,% (95% CI)	SE	Exposure range	Unit	Other
							energy intake and dividing by 1000.
Cigarette smoking							
[50]	4376	C	0	NA	0	cig/day	
	660		0.125 (0.06 to 0.19)	0.0332	1 to 9		
	1108		0.189 (0.136 to 0.242)	0.0270	10 to 19		
	911		0.277 (0.218 to 0.336)	0.0301	≥ 20		
[49]	1180	Men	5.02 (4.91 to 5.13)	0.0561	0	cig/day	
	436		5.07 (4.96 to 5.19)	0.0587	1 to 19		
	1015		5.11 (5 to 5.22)	0.0561	≥ 20		
[22]	918	Men	5.3	0.02	0	cig/day	
	116		5.54	0.07	< 15		
	207		5.58	0.05	≥ 15		
[22]	1894	Women	5.37	0.02	0	cig/day	
	191		5.39	0.05	< 15		
	173		5.57	0.06	≥ 15		
Chronic stress							
[51]	234 in total	C	5.38	0.04	Low	stress	
			5.36	0.032	Medium	level	
			5.3	0.034	High		
[52]	96	Men	5.2	0.0816	Active	stress	
	81		5.3	0.1	Relaxed	level	
	10		5.4	0.3795	Passive		
	23		5.7	0.2085	Strain		

Ref = reference; *n* = number of subjects; g/day = grams per day; GL = glycaemic load; MET-h/day = metabolic equivalent-hours per day; cig/day = cigarettes per day; N/A = not applicable; C = combined; HbA_{1c} = glycated haemoglobin

Table 4 Studies relating lifestyle factors to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Unit	Other
Alcohol consumption									
[78]	487	N/A	11,447	C	HR	1	0	g/day	
	652		14,375			1.06 (0.91 to 1.23)	0 to < 5		
	507		11,308			0.97 (0.82 to 1.14)	5 to < 15		
	383		7,883			1 (0.83 to 1.2)	15 to < 30		
	294		4,484			1.32 (1.06 to 1.65)	≥ 30		
[79]	19	N/A	10,761	Men	HR	1.24 (0.72 to 2.16)	0	g/week	
	39		26,627			1	0.1 to 10		
	119		59,333			1.46 (1.01 to 2.11)	10.1 to 40		
	11		8,724			0.81 (0.41 to 1.6)	40.1 to 70		
	37		21,044			1.32 (0.83 to 2.09)	70.1 to 140		
	23		10,698			1.67 (0.98 to 2.84)	≥ 140.1		
[80]	110	N/A	246,448	C	HR	0.98 (0.72 to 1.33)	0	g/day	
	433		786,239			1	0.1 to 4.9		
	444		701,946			1.05 (0.9 to 1.21)	4.9 to 14.9		
	246		358,149			1.07 (0.89 to 1.29)	15 to 29.9		
	140		168,559			1.23 (0.98 to 1.55)	30 to 59.9		
	74		64,067			1.98 (1.46 to 2.7)	> 60		
[81]	311	N/A	218,867	Men	HR	1	0	g/day	
	295		207,211			1.22 (0.92 to 1.61)	0.1 to 22.9		
	363		220,367			1.42 (1.21 to 1.66)	23 to 45.9		
	374		175,414			1.95 (1.53 to 2.49)	46 to 68.9		
	182		83,438			2.15 (1.74 to 2.64)	69 to 91.9		
	112		45,535			2.96 (2.27 to 3.86)	≥ 92		
[81]	839	N/A	884,277	Women	HR	1	0	g/day	
	97		138,327			0.93 (0.7 to 1.23)	0.1 to 22.9		
	42		38,481			1.57 (1.11 to 2.21)	≥ 23		
[82]	721	N/A	319,014	Women	HR	1	0	g/day	
	172		77,855			1.04 (0.88 to 1.24)	≤ 1.8		
	94		47,218			0.92 (0.74 to 1.15)	> 1.8 to 3.4		
	139		62,605			1.1 (0.91 to 1.33)	> 3.4 to 11		
	129		57,997			1.02 (0.83 to 1.25)	> 11		
[83]	5	N/A	8,802.4	Men	HR	1	0 to 3.2	g/week	
	13		9,098.4			2.4 (0.9 to 6.8)	3.3 to 17.2		
	13		8,758			2.5 (0.9 to 7.2)	17.3 to 48.8		
	11		8,867.5			2.2 (0.8 to 6.4)	48.9 to 115.2		
	17		8,081.5			3.5 (1.2 to 9.8)	115.5 to 2,853.1		

Table 4 Studies relating lifestyle factors to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Unit	Other
[84]	658	N/A	443,968	C	HR	1	0	drinks/	12 g/ drink
	117		77,374			0.96 (0.72 to 1.25)	< 7	week	
	70		25,177			1.84 (1.31 to 2.58)	≥ 7		
[85]	61	397	458	Men	OR	1	≤ 1	drinks/	12 g/ drink
	84	369	453			1.51 (1.05 to 2.17)	2 to 4	week	
	66	338	404			1.25 (0.85 to 1.84)	≥ 5		
[86]	73	124	197	Men	OR	1	0 to 29	g/	
	14	20	34			1.22 (0.58 to 2.58)	30 to 299	month	
	20	17	37			1.98 (0.96 to 4.09)	300 to 599		
	83	61	144			2.33 (1.47 to 3.71)	≥ 600		
[87]	429	422	851	C	OR	1	< 5	g/day	
	150	121	271			1.22 (0.88 to 1.69)	5 to 30		
	208	113	321			1.76 (1.26 to 2.46)	≥ 30		
[88]	140	294	434	C	OR	1	0	units/	9.8 g
	79	155	234			1.09 (0.78 to 1.53)	1 to 5	week	/unit
	101	140	241			1.6 (1.13 to 2.25)	6 to 13		
	164	149	313			2.57 (1.81 to 3.64)	≥ 14		
[89]	50	77	127	Men	OR	1	0	cups	21.3 g/
	33	100	133			0.51 (0.3 to 0.87)	< 1		cup
	87	147	234			0.85 (0.54 to 1.3)	1 to 1.9		
	60	47	107			1.81 (1.03 to 3.2)	2 to 2.9		
	37	24	61			2.19 (1.2 to 4.2)	≥ 3		
[90]	169	395	564	Women	OR	1	0	drinks/	12 g/ drink
	332	1,107	1,439			0.97 (0.77 to 1.23)	1 to 2	week	
	125	414	539			1.06 (0.79 to 1.42)	3 to 5		
	72	238	310			1.05 (0.74 to 1.49)	6 to 10		
	52	131	183			1.49 (1 to 2.22)	≥ 11		
[91]	68	200	268	Men	OR	1.53 (0.98 to 2.41)	0	g/day	
	39	175	214			1	> 0 to < 5		
	55	224	279			1.06 (0.66 to 1.69)	5 to < 15		
	45	188	233			1.02 (0.63 to 1.66)	15 to < 30		
	28	96	124			1.2 (0.68 to 2.12)	30 to < 45		
	31	97	128			1.24 (0.69 to 2.22)	≥ 45		
[91]	119	374	493	Women	OR	1 (0.7 to 1.42)	0	g/day	
	73	230	303			1	> 0 to < 5		
	61	219	280			0.84 (0.56 to 1.26)	5 to < 15		
	41	140	181			0.87 (0.55 to 1.37)	15 to < 30		
	12	39	51			0.9 (0.43 to 1.87)	30 to < 45		

Table 4 Studies relating lifestyle factors to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Unit	Other
	7	14	21			1.52 (0.56 to 4.1)	≥ 45		
[92]	61	63	124	Men	OR	1	0 to 9	g/day	
	39	38	77			1 (0.5 to 1.8)	10 to 39		
	25	33	58			0.7 (0.4 to 1.3)	40 to 69		
	20	12	32			1.6 (0.7 to 3.7)	≥ 70		
[93]	959	2,137	3,096	C	OR	1	0	drinks/	12 g/
	368	722	1,090			1.1 (0.93 to 1.3)	1 to 4	week	drink
	376	661	1,037			1.17 (0.99 to 1.38)	5 to 11		
	469	755	1,224			1.21 (1.03 to 1.44)	≥ 12		
[94]	382	467	849	C	OR	1	0	g/day	
	309	539	848			0.88 (0.67 to 1.17)	13.8		
	344	503	847			1.17 (0.85 to 1.61)	182.8		
[95]	108	99	207	C	OR	1	0	g/	
	131	86	217			1.21 (0.81 to 1.8)	< 400	month	
	433	487	920			0.85 (0.61 to 1.18)	> 400		
[96]	395	1,059	1,454	C	OR	1	< 1	g/day	
	256	566	822			1.15 (0.94 to 1.4)	> 1 to 11.82		
	322	565	887			1.35 (1.12 to 1.63)	> 11.82 to		
	302	568	870			1.2 (0.99 to 1.46)	22.66		
	269	565	834			1.02 (0.83 to 1.26)	> 22.66 to		
	269	567	836			0.95 (0.77 to 1.19)	34.46		
							> 34.36 to		
							51.82		
							> 51.82		
[97]	11	23	34	C	OR	1	0	g/day	
	12	24	36			1.1 (0.4 to 3.1)	1 to 20		
	18	55	73			0.7 (0.3 to 1.9)	21 to 40		
	25	30	55			2 (0.7 to 5.4)	≥ 41		
[98]	270	264	534	C	OR	1	0	drinks/	13.5 g/ drink
	241	287	528			0.9 (0.7 to 1.2)	1 to 2	day	
	60	68	128			0.8 (0.5 to 1.3)	3 to 4		
	131	98	229			1.3 (0.9 to 1.9)	≥ 5		
[99]	301	26,475	26,776	Women	RR	1	0	serving	12 g/ serving
	101	10,374	10,475			0.92 (0.73 to 1.16)	0.01 to 0.5	/day	
	52	4,617	4,669			1 (0.74 to 1.35)	0.51 to 1		
	25	2,413	2,438			0.94 (0.62 to 1.42)	1.01 to 2		
	11	895	906			1.16 (0.63 to 2.14)	> 2		
[100]	30	3,141	3,171	C	RR	1	< 1	units/	9.8 g

Table 4 Studies relating lifestyle factors to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Unit	Other
	39	4,256	4,295			1.53 (0.94 to 2.49)	1 to 7	week	/ unit
	26	3,506	3,532			1.53 (0.87 to 2.69)	> 7		
Dietary fibre									
[101]	931	N/A	1,050,093	C	HR	1	<16.4	g/day	
	918		1,050,093			0.98 (0.89 to 1.08)	16.4 to <		
	912		1,050,082			0.96 (0.86 to 1.06)	20.1		
	914		1,050,082			0.94 (0.84 to 1.05)	20.1 to <		
	842		1,050,082			0.83 (0.72 to 0.96)	23.6		
							23.6 to <		
							28.5		
							≥ 28.5		
[102]	307	N/A	241,810	Women	HR	1	< 9.9	g/day	
	348		246,971			1.16 (0.96 to 1.39)	9.9 to 13.3		
	267		248,569			0.92 (0.72 to 1.18)	13.3 to 16.7		
	289		248,736			1.1 (0.8 to 1.5)	16.7 to 21.2		
	259		245,532			1.06 (0.67 to 1.7)	≥ 21.2		
[103]	68	N/A	40,291	Men	HR	1	6.4	g/day	
	69		42,872			0.9 (0.63 to 1.3)	9.1		
	55		43,391			0.7 (0.47 to 1.1)	11.2		
	72		43,565			0.88 (0.58 to 1.3)	13.6		
	71		42,069			0.85 (0.53 to 1.4)	18.7		
[103]	34	N/A	46,443	Women	HR	1	8.3	g/day	
	27		48,077			0.61 (0.35 to 1)	11.2		
	34		49,104			0.62 (0.36 to 1.1)	13.3		
	49		50,179			0.77 (0.44 to 1.3)	15.6		
	43		49,626			0.58 (0.31 to 1.1)	20		
[104]	609	N/A	513,317	C	HR	1.18 (1.05 to 1.31)	< 10	g/day	
	1,681		1,591,322			1	10 to <15		
	2,263		1,870,758			1.02 (0.95 to 1.1)	15 to < 20		
	1,740		1,183,334			1.01 (0.92 to 1.1)	20 to < 25		
	1,001		514,142			0.99 (0.87 to 1.12)	25 to < 30		
	785		313,572			1 (0.85 to 1.17)	≥ 30		
[105]	51	N/A	30,631	Men	HR	1	6.7	g/day	
	76		31,855			1.12 (0.77 to 1.62)	9.4		
	54		32,224			0.62 (0.4 to 0.96)	11.3		
	77		32,187			0.69 (0.43 to 1.11)	13.4		
[105]	46	N/A	48,219	Women	HR	1	7.4	g/day	
	38		49,366			0.73 (0.47 to 1.14)	9.8		

Table 4 Studies relating lifestyle factors to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Unit	Other
	48		50,921			0.84 (0.54 to 1.33)	11.5		
	53		51,872			0.75 (0.46 to 1.25)	13.4		
[106]	134	399	533	C	OR	1	8.9	g/day	
	121	399	520			0.84 (0.6 to 1.18)	12.3		
	91	400	491			0.55 (0.38 to 0.81)	14.6		
	115	399	514			0.8 (0.55 to 1.17)	17.6		
	118	399	517			0.67 (0.42 to 1.05)	24.1		
[107]	172	181	353	C	OR	1	< 11.4	g/day	
	67	182	249			0.5 (0.34 to 0.73)	11.4 to 16.6		
	47	187	234			0.36 (0.24 to 0.54)	>16.6		
[94]	388	462	850	C	OR	1	15.1	g/day	
	355	493	848			0.97 (0.77 to 1.23)	23.7		
	308	539	847			0.84 (0.67 to 0.99)	35.2		
[108]	175	163	338	C	OR	1	9.2	g/day	
	152	163	315			0.87 (0.62 to 1.21)	11.7		
	178	163	341			0.95 (0.67 to 1.34)	13.7		
	153	163	316			0.81 (0.56 to 1.18)	16.2		
	158	163	321			0.87 (0.58 to 1.31)	19.8		
Physical exercise									
[109]	98	N/A	61,804	Men	HR	1	< 37.9	MET-h/	
	80		63,549			0.84 (0.62 to 1.13)	37.9 to 40.7	day	
	105		63,120			1 (0.76 to 1.33)	40.8 to 44.8		
	82		63,505			0.82 (0.6 to 1.1)	≥ 44.9		
[110]	63	56	119	Men	OR	1	0	MET-h/	
	18	11	29			2.1 (0.77 to 5.72)	< 20	week	
	9	25	34			0.3 (0.11 to 0.81)	≥ 20		
[110]	41	37	78	Women	OR	1	0	MET-h/	
	14	16	30			0.86 (0.34 to 2.16)	< 20	week	
	15	17	32			0.77 (0.31 to 1.94)	≥ 20		
[111]	84	8,177	8,261	Men	RR	1	28.25	MET-h/	
	81	7,616	7,697			0.99 (0.72 to 1.35)	33.25	day	
	64	6,011	6,075			0.85 (0.61 to 1.2)	35.25		
	61	7,748	7,809			0.69 (0.49 to 0.97)	43.75		
Glycaemic load (GL)									
[112]	26	N/A	61,084	Women	HR	1	92	GL/	Ref: gluc.
	30		61,213			1.34 (0.76 to 2.34)	106	day	
	37		61,190			1.81 (1.02 to 3.21)	117		
	32		60,976			1.63 (0.86 to 3.09)	127		

Table 4 Studies relating lifestyle factors to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Unit	Other
	49		60,872			2.85 (1.4 to 5.8)	143		
[113]	323	N/A	241,998	Women	HR	1	< 62.4	GL/	Ref: gluc.
	294		247,053			0.94 (0.79 to 1.12)	62.4 to 81.9	day	
	304		248,043			1.07 (0.88 to 1.29)	81.9 to 100.7		
	281		249,138			1.01 (0.81 to 1.27)	100.7 to		
	268		245,386			1.11 (0.82 to 1.49)	126.6		
							≥ 126.6		
[114]	152	N/A	194,565	Women	HR	1	< 164	GL/	Ref: w.b.
	168		196,016			0.98 (0.78 to 1.24)	164 to 175	day	
	156		192,505			0.85 (0.67 to 1.09)	176 to 186		
	174		192,033			0.89 (0.69 to 1.14)	187 to 199		
	220		188,305			1.06 (0.81 to 1.39)	≥ 200		
[115]	97	N/A	132,319	Women	HR	1	159.7	GL/	Ref:
	78		133,177			0.74 (0.55 to 1)	179.6	day	gluc.
	76		133,439			0.69 (0.51 to 0.93)	192.7		
	86		133,323			0.71 (0.53 to 0.96)	205.9		
	138		131,905			0.94 (0.71 to 1.24)	225.9		
[116]	104	N/A	77,304	Women	HR	1	46.5	GL/	Ref:
	98		77,414			0.93 (0.7 to 1.22)	59.8	day	gluc.
	88		77,677			0.8 (0.6 to 1.07)	67.4		
	91		77,268			0.81 (0.61 to 1.08)	74.9		
	109		76,988			0.91 (0.7 to 1.2)	89.4		
[117]	253	N/A	4,188	Men	HR	1	108.7	GL/	Ref:
	216		4,245			0.82 (0.64 to 1.04)	124.8	day	gluc.
	193		4,147			0.75 (0.58 to 0.97)	136.2		
	223		4,255			0.9 (0.7 to 1.16)	147.8		
	197		4,203			0.83 (0.64 to 1.08)	165.4		
[117]	152	N/A	4,423	Women	HR	1	82.5	GL/	Ref:
	149		4,490			0.96 (0.73 to 1.28)	94	day	gluc.
	156		4,510			1.02 (0.77 to 1.37)	101.7		
	156		4,352			1.05 (0.78 to 1.41)	107.9		
	142		4,200			1 (0.73 to 1.36)	123.6		
Cigarette smoking									
[118]	558	N/A	293,319	Women	HR	1	0	cig/	
	163		74,987			1.15 (0.95 to 1.38)	1 to 19	day	
	99		43,378			1.23 (0.97 to 1.54)	20		
	49		23,016			1.12 (0.82 to 1.54)	> 20		
[119]	126	N/A	133,387	Men	HR	1	0	cig/	

Table 4 Studies relating lifestyle factors to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Unit	Other
	13		10,013			1.32 (0.73 to 2.4)	< 20	day	
	35		17,874			2.14 (1.45 to 3.14)	≥ 20		
[120]	302	N/A	219,082	C	HR	1	0	cig/	
	68		55,787			1 (0.7 to 1.3)	1 to 10	day	
	29		21,049			1.1 (0.7 to 1.7)	11 to 20		
	6		2,179			3.1 (1.4 to 7.1)	≥ 21		
[121]	274	N/A	498,516	Women	HR	1	0	cig/	
	56		102,182			1.01 (0.75 to 1.35)	1 to 9	day	
	78		120,688			1.24 (0.96 to 1.6)	10 to 19		
	93		166,846			1.11 (0.87 to 1.41)	20 to 29		
	12		29,414			0.83 (0.46 to 1.48)	30 to 39		
	8		23,194			0.71 (0.35 to 1.43)	≥ 40		
[122]	269	457	726	Men	OR	1	0	cig/	
	93	256	349			0.6 (0.4 to 0.8)	< 15	day	
	147	393	540			0.7 (0.5 to 0.9)	15 to 24		
	74	177	251			0.7 (0.5 to 1)	≥ 25		
[122]	558	740	1,298	Women	OR	1	0	cig/	
	56	109	165			0.8 (0.5 to 1.9)	< 15	day	
	34	73	107			0.6 (0.4 to 1)	15 to 24		
	11	22	33			0.8 (0.4 to 1.8)	≥ 25		
[123]	5	57	57	C	OR	1	< 10	cig/	Assume a
	15	63	63			0.99 (0.53 to 1.82)	10 to 20	day	1:1 case to
	30	20	20			1.18 (0.49 to 2.86)	> 20		control ratio
[124]	273	845	1,118	C	OR	1	0	cig/	
	69	134	203			1.07 (0.75 to 1.52)	< 13	day	
	138	188	326			1.51 (1.1 to 2.08)	≥ 13		
[125]	282	363	645	Men	OR	1	0	cig/	
	159	175	334			0.94 (0.62 to 1.43)	1 to 10	day	
	282	275	557			1 (0.67 to 1.49)	11 to 20		
	139	140	279			0.89 (0.56 to 1.42)	21 to 30		
	247	156	403			1.51 (0.99 to 2.29)	> 30		
[125]	489	529	1,018	Women	OR	1	0	cig/	
	64	54	118			1.88 (1.02 to 3.45)	1 to 5	day	
	66	81	147			1.06 (0.58 to 1.94)	6 to 10		
	110	90	200			1.68 (0.9 to 3.15)	11 to 20		
	85	61	146			1.73 (0.87 to 3.43)	>20		
[126]	745	2,055	2,800	C	OR	1	0	packs/	20
	127	396	523			0.95 (0.74 to 1.23)	< 1	day	cigarettes

Table 4 Studies relating lifestyle factors to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Unit	Other
	252	853	1,105			0.85 (0.69 to 1.05)	1		per pack
	119	397	516			0.87 (0.67 to 1.15)	> 1		
[127]	953	2,008	2,961	C	OR	1	0	cig/	
	414	825	1,239			1.03 (0.87 to 1.24)	≤ 10	day	
	569	1,099	1,668			1.04 (0.9 to 1.21)	11 to 20		
	288	499	787			1.13 (0.93 to 1.37)	> 20		
[128]	30	160	190	C	OR	1	0	cig/	
	26	179	205			0.9 (0.5 to 1.7)	1 to 14	day	
	46	201	247			1.5 (0.9 to 2.6)	> 14		
[129]	252	292	544	C	OR	1	0	cig/	
	91	122	213			0.89 (0.63 to 1.25)	1 to 9	day	
	107	119	226			1.01 (0.71 to 1.43)	10 to 19		
	86	81	167			1.15 (0.78 to 1.71)	≥ 20		
[130]	59	180	239	Men	OR	1	0	cig/	
	65	127	192			1.3 (0.8 to 2.1)	1 to 20	day	
	29	24	53			3.2 (1.6 to 6.4)	> 20		
[131]	27	86	113	C	OR	1	0	cig/	
	21	30	51			1.2 (0.4 to 3.8)	1 to 15	day	
	38	45	83			0.8 (0.3 to 2.1)	16 to 30		
	17	14	31			2.4 (0.7 to 8.6)	≥ 31		
[132]	81	124	205	Men	OR	1	0	cig/	
	127	115	242			1.72 (1.15 to 2.59)	< 20	day	
	149	133	282			1.71 (1.15 to 2.56)	20 to 29		
	69	52	121			1.79 (1.09 to 2.95)	≥ 30		
[132]	120	146	266	Women	OR	1	0	cig/	
	107	101	208			1.16 (0.77 to 1.74)	< 20	day	
	44	42	86			1.27 (0.74 to 2.17)	20 to 29		
	5	4	9			1.91 (0.42 to 8.66)	≥ 30		
[133]	264	233	497	C	OR	1	0	cig/	
	65	57	122			1.06 (0.7 to 1.61)	1 to 10	day	
	64	68	132			0.75 (0.5 to 1.13)	≥ 11		
[92]	48	43	91	Men	OR	1	0	packs/	20 cig/ pack
	23	27	50			0.7 (0.3 to 1.4)	≤ 1	day	
	38	30	68			0.9 (0.4 to 1.8)	≥ 2		
[134]	894	1,933	2,827	C	OR	1	0	cig/	
	116	221	337			0.75 (0.58 to 0.97)	< 15	day	
	236	366	602			0.93 (0.75 to 1.16)	≥ 15		
[135]	264	29,932	30,196	C	RR	1	0	cig/	

Table 4 Studies relating lifestyle factors to CRC risk

Ref	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Unit	Other
	62	10,460	10,522			1.11 (0.82 to 1.5)	< 15	day	
	49	7,999	8,048			1.04 (0.73 to 1.48)	≥ 15		
Chronic stress									
[26]	62	N/A	33,513.51	Women	HR	1	None	stress	
	71		41,040.46			1.06 (0.75 to 1.49)	Low	level	
	22		21,359.22			0.6 (0.37 to 0.98)	Medium		
	7		6,930.69			0.52 (0.23 to 1.14)	High		
[26]	91	N/A	35,271.31	Men	HR	1	None	stress	
	48		26,519.33			0.93 (0.65 to 1.32)	Low	level	
	16		13,008.13			0.64 (0.37 to 1.1)	Medium		
	11		2,330.50			1.96 (1.03 to 3.74)	High		
[136]	304	286	590	C	OR	1	Zero score	stress	
	149	147	296			1 (0.7 to 1.3)	Low	level	
	77	54	131			1.3 (0.9 to 2)	Medium		
	39	23	62			1.7 (1 to 2.9)	High		

Ref = reference; CRC = colorectal cancer; RR = relative risk; OR = odds ratio; HR = hazard ratio; C = combined; g/day = grams per day; gluc. = glucose reference; w.b. = white bread reference; cig/day = cigarettes per day; GL = glycaemic load; MET-h/day = metabolic equivalent-hours per day; N/A = not applicable

Table 5 Estimated glycaemic models of CRC risk and lifestyle factors, via HbA_{1c} (from separate models that were statistically significant)

Risk measure	Model
Alcohol consumption	
HR [a]	$HR = -0.0004 \times \Delta\text{ethanol}_{\text{g/day}}$
	Ranges:
	$\text{ethanol}_{\text{g/day}}$ in [27.36 to 144 g/day]
	HbA_{1c} in [5.38% to 7%]
OR	$OR = -0.0010 \times \Delta\text{ethanol}_{\text{g/day}}$
	Ranges:
	$\text{ethanol}_{\text{g/day}}$ in [0 to 100.8 g/day]
	HbA_{1c} in [5% to 6.4%]
Combined [a]	$Risk = -0.0004 \times \Delta\text{ethanol}_{\text{g/day}}$
	Ranges:
	$\text{ethanol}_{\text{g/day}}$ in [0 to 144 g/day]
	HbA_{1c} in [5% to 7%]
Dietary fibre	
HR [a, b]	$HR = -0.0034 \times \Delta\text{fibre}_{\text{g/day}}$

Table 5 Estimated glycaemic models of CRC risk and lifestyle factors, via HbA_{1c} (from separate models that were statistically significant)

Risk measure	Model
	Ranges:
	$\text{fibre}_{\text{g/day}}$ in [4.93 to 27.3 g/day]
	HbA_{1c} in [6.21% to 7.7%]
OR [b]	$OR = -0.0095 \times \Delta\text{fibre}_{\text{g/day}}$
	Ranges:
	$\text{fibre}_{\text{g/day}}$ in [4.93 to 7.78 g/day]
	HbA_{1c} in [6.21% to 6.4%]
Combined [a, b]	$Risk = -0.0042 \times \Delta\text{fibre}_{\text{g/day}}$
	Ranges:
	$\text{fibre}_{\text{g/day}}$ in [4.93 to 27.3 g/day]
	HbA_{1c} in [6.21% to 7.7%]
Physical exercise	
HR [a]	$HR = -0.0178 \times \Delta\text{exercise}_{\text{METh/day}}$
	Ranges:
	$\text{exercise}_{\text{METh/day}}$ in [0 to 4.983 MET-h/day]
	HbA_{1c} in [7.2% to 8%]
Combined [a]	$Risk = -0.0244 \times \Delta\text{exercise}_{\text{METh/day}}$
	Ranges:
	$\text{exercise}_{\text{METh/day}}$ in [0 to 4.983 MET-h/day]
	HbA_{1c} in [7.2% to 8%]
Glycaemic load (GL)	
HR [a]	$HR = 0.0008 \times \Delta\text{GL}_{/\text{day}}$
	Ranges:
	$\text{GL}_{/\text{day}}$ in [84.98 to 171.6 $\text{GL}_{/\text{day}}$]
	HbA_{1c} in [5.38% to 6.6%]
OR	$OR = 0.0021 \times \Delta\text{GL}_{/\text{day}}$
	Ranges:
	$\text{GL}_{/\text{day}}$ in [58 to 157.4 $\text{GL}_{/\text{day}}$]
	HbA_{1c} in [5% to 6.4%]
Combined [a]	$Risk = 0.0010 \times \Delta\text{GL}_{/\text{day}}$
	Ranges:
	$\text{GL}_{/\text{day}}$ in [58 to 171.6 $\text{GL}_{/\text{day}}$]
	HbA_{1c} in [5% to 6.6%]
Cigarette smoking	
HR [a]	$HR = 0.0009 \times \Delta\text{cigarettes/day}$
	Ranges:
	cigarettes/day in [9 to 38 cigarettes/day]

Table 5 Estimated glycaemic models of CRC risk and lifestyle factors, via HbA_{1c} (from separate models that were statistically significant)

Risk measure	Model
	HbA_{1c} in [5.38% to 6.617%]
OR	$HR = 0.0027 \times \Delta\text{cigarettes/day}$
	Ranges:
	cigarettes/day in [0 to 32 cigarettes/day]
	HbA_{1c} in [5.02% to 6.4%]
Combined [a]	$Risk = 0.0012 \times \Delta\text{cigarettes/day}$
	Ranges:
	cigarettes/day in [0 to 38 cigarettes/day]
	HbA_{1c} in [5.02% to 6.617%]
Chronic stress	
HR [a]	$OR = exp(0.0207 \times \Delta\text{stress}_{\text{level}})$
	Range:
	$\text{stress}_{\text{level}}$ in [1 to 3 level]
	HbA_{1c} in [5.38% to 5.7%]
OR [c]	$OR = exp(0.0583 \times \Delta\text{stress}_{\text{level}})$
	Range:
	$\text{stress}_{\text{level}}$ in [0 to 3 level]
	HbA_{1c} in [5.2% to 5.7%]
Combined [a, c]	$OR = exp(0.0261 \times \Delta\text{stress}_{\text{level}})$
	Range:
	$\text{stress}_{\text{level}}$ in [0 to 3 level]
	HbA_{1c} in [5.2% to 5.7%]

[a] Excluding data points below reference

[b] Excluding [43]

[c] Excluding [51]

CRC = colorectal cancer; RR = relative risk; OR = odds ratio; HR = hazard ratio; g/day = grams per day; GL = glycaemic load; MET-h/day = metabolic equivalent-hours per day; HbA_{1c} = glycated haemoglobin

Stress level:

0 = None

1 = Low

2 = Medium

3 = High

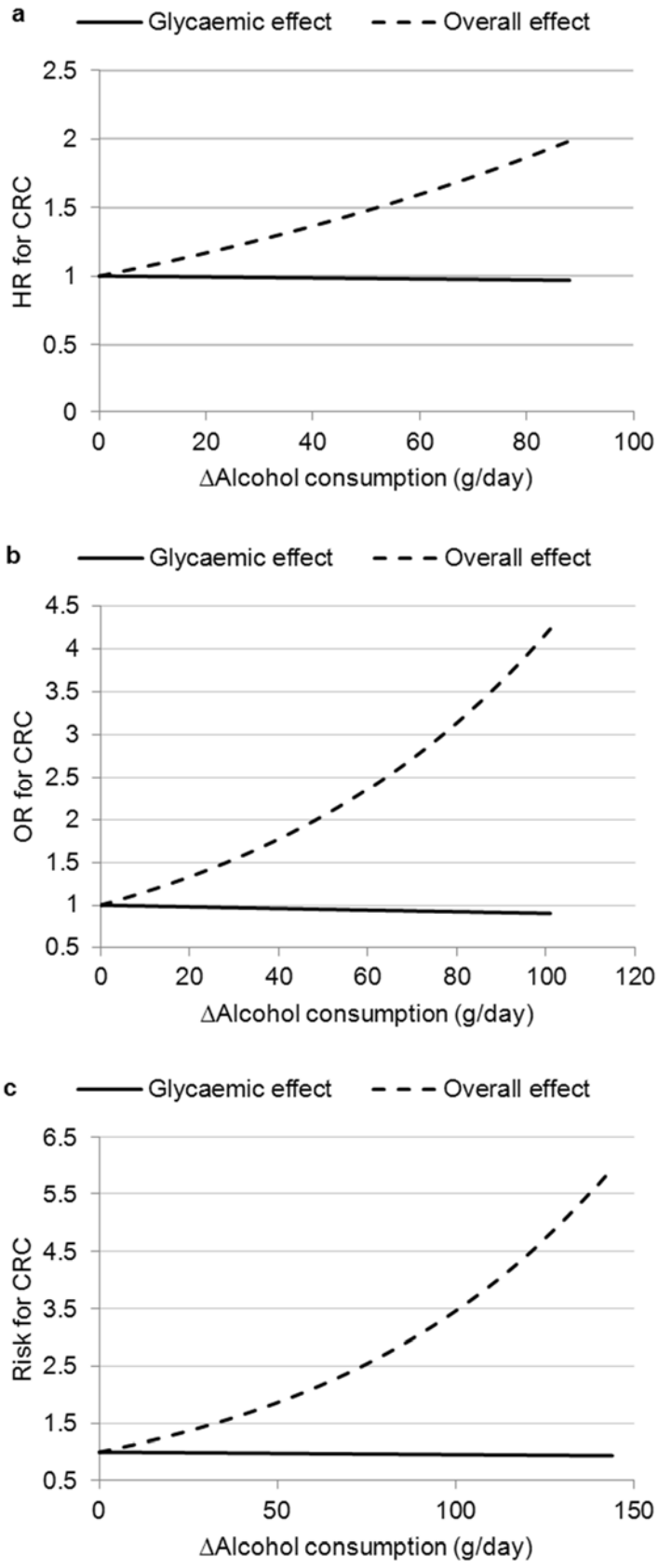

Fig. 1 Comparison of glycaemic and overall models for colorectal cancer (CRC) risk as a result of changes in daily alcohol consumption in grams per day (g/day). The solid line indicates the glycaemic

model, while the broken line indicates the overall model. **a** Hazard ratio (HR) for CRC; **b** Odds ratio (OR) for CRC; **c** Combined risk for CRC

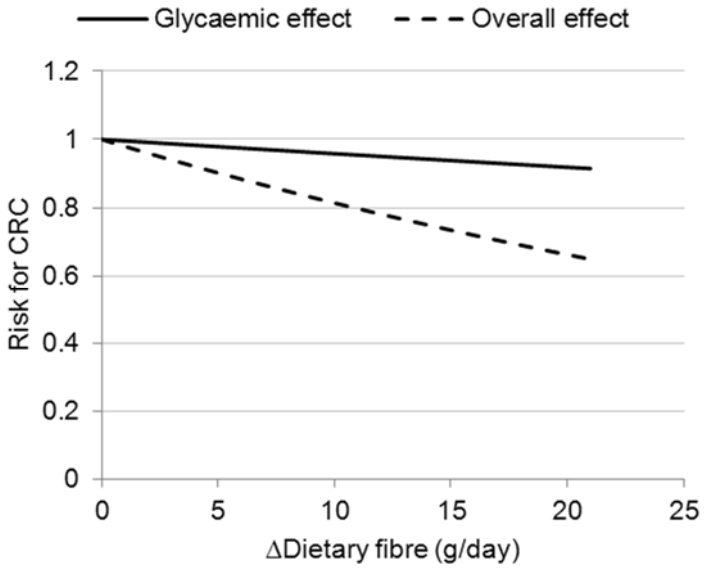

Fig. 2 Comparison of glycaemic and overall models for combined colorectal cancer (CRC) risk as a result of changes in daily dietary fibre intake in grams per day (g/day). The solid line indicates the glycaemic model, while the broken line indicates the overall model.

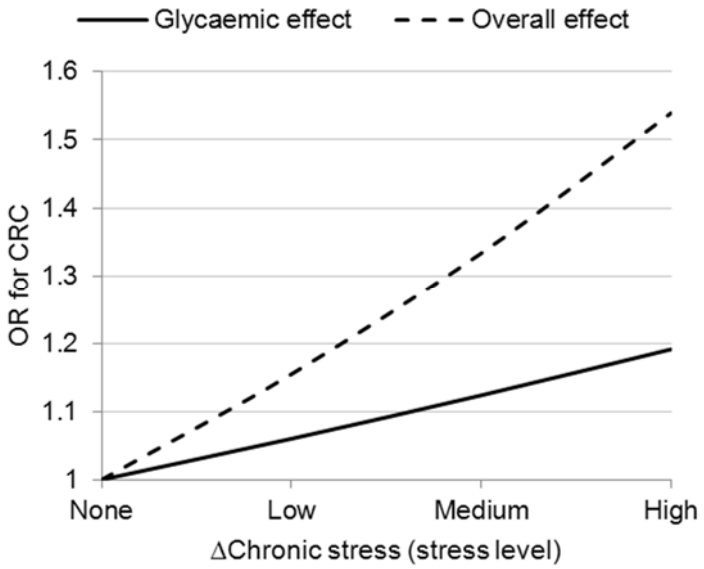

Fig. 3 Comparison of glycaemic and overall models for colorectal cancer (CRC) risk (measured as OR) as a result of changes in chronic stress levels. The solid line indicates the glycaemic model, while the broken line indicates the overall model.

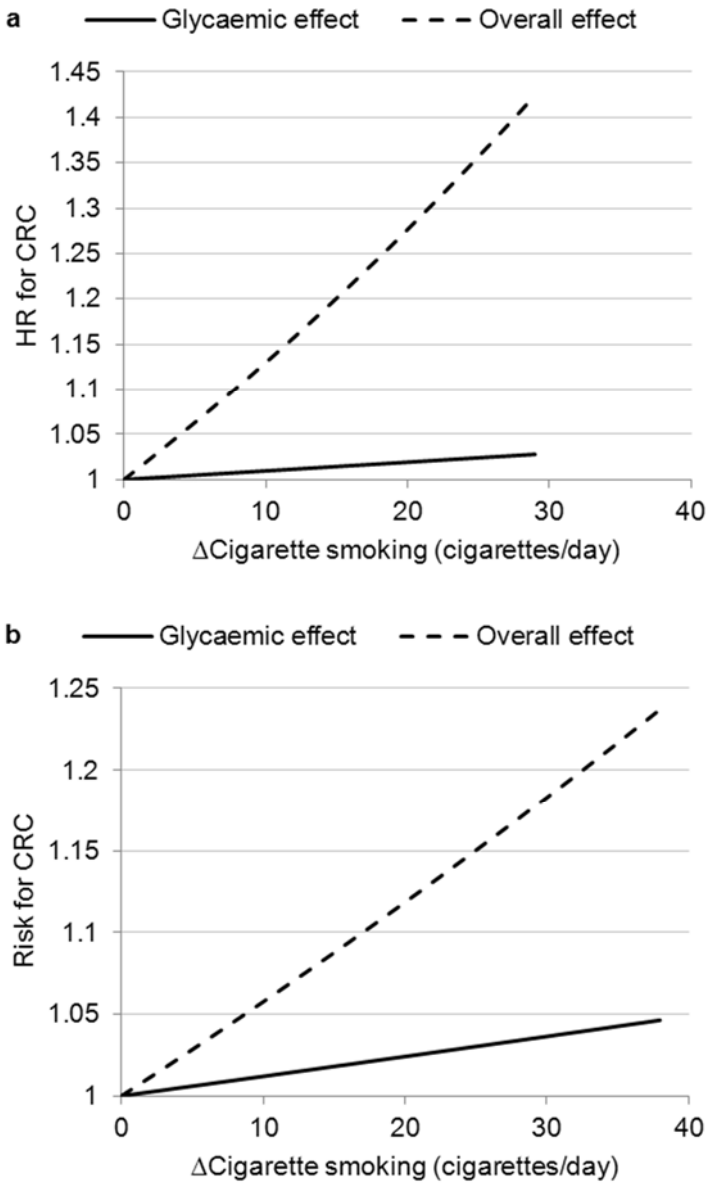

Fig. 4 Comparison of glycaemic and overall models for colorectal cancer (CRC) risk as a result of changes in the number of cigarettes smoked per day (cigarettes/day). The solid line indicates the glycaemic model, while the broken line indicates the overall model. **a** Hazard ratio (HR) for CRC; **b** Combined risk for CRC

ANNEXURE C DATA COLLECTED

Table 5: Data collected relating HbA_{1c} levels to CRC incidence risk (from [2]).

Reference	Cases	Controls/Non-cases	Total	Gender	Risk (95% CI)	Exposure range (HbA_{1c},%)	Comments
RR							
[153]	4	1,148	1,152	Men	1	< 5%	EPIC-Norfolk Study
	18	2,616	2,634		1.61 (0.54 to 4.82)	5% to 5.9%	
	7	427	434		3.4 (0.97 to 11.92)	6% to 6.9%	
	2	74	76		5.19 (0.92 to 29.38)	≥ 7%	
	5	144	149		6.02 (1.47 to 24.65)	Diabetes (8.2%)	
[153]	6	1,469	1,475	Women	1	< 5%	EPIC-Norfolk Study
	17	3,070	3,087		0.7 (0.27 to 1.82)	5% to 5.9%	
	6	459	465		1.08 (0.33 to 3.5)	6% to 6.9%	
	1	60	61		1.36 (0.15 to 12.04)	≥ 7%	
	1	71	72		1.38 (0.16 to 11.76)	Diabetes (8.2%)	
[155]	41	73	114	Women	1	5.1%	Nurses' Health Study
	40	89	129		0.75 (0.43 to 1.3)	5.4%	
	48	87	135		0.94 (0.54 to 1.65)	5.6%	
	46	83	129		0.85 (0.47 to 1.51)	5.8%	
[158]	36	6,743	6,779	Women	1	2.3% to 4.8%	Women's Health Study
	41	6,736	6,777		1.02 (0.65 to 1.61)	4.8% to 5%	
	46	6,731	6,777		0.95 (0.6 to 1.5)	5% to 5.2%	
	45	6,732	6,777		0.83 (0.52 to 1.33)	≥ 5.2%	

Reference	Cases	Controls/Non-cases	Total	Gender	Risk (95% CI)	Exposure range (HbA_{1c},%)	Comments
OR							
[156]	39 29 43 62	87 86 86 87	126 115 129 149	C	1 0.77 (0.43 to 1.36) 1.12 (0.65 to 1.91) 1.57 (0.94 to 2.6)	< 5.38% 5.38% to 5.54% 5.54% to 5.78% > 5.78%	CLUE II Cohort
[49]	22 31 26	48 60 48	70 91 74	Women	1 1.2 (0.6 to 2.5) 1.2 (0.6 to 2.7)	5.2% 5.5% 5.8%	Nurses' Health Study
[154]	118 109 104 116 114	127 103 110 120 101	245 212 214 236 215	Men	1 1.17 (0.8 to 1.69) 1.04 (0.71 to 1.53) 1.09 (0.74 to 1.6) 1.25 (0.83 to 1.86)	< 5.4% 5.4% to 5.6% 5.6% to 5.8% 5.8% to 6.1% > 6.1%	EPIC Study Diabetics included
[154]	96 84 85 118 82	91 109 114 97 54	187 193 199 215 136	Women	1 0.74 (0.48 to 1.13) 0.71 (0.46 to 1.1) 1.13 (0.74 to 1.71) 1.4 (0.87 to 2.24)	< 5.4% 5.4% to 5.6% 5.6% to 5.8% 5.8% to 6.1% > 6.1%	EPIC Study Diabetics included
[157]	53 22 76 69	98 90 114 114	151 112 190 183	C	1 0.47 (0.26 to 0.85) 1.19 (0.74 to 1.92) 1.17 (0.71 to 1.93)	< 4.3% 4.3% to 4.5% 4.5% to 4.7% > 4.7%	Northern Sweden Health and Disease Cohort

Reference	Cases	Controls/Non-cases	Total	Gender	Risk (95% CI)	Exposure range (HbA_{1c},%)	Comments
HR							
[46]	72 16 33 18	N/A	45,501 6,014 16,470 5,750	Men	1 1.84 (1.07 to 3.18) 1.04 (0.67 to 1.6) 1.52 (0.88 to 2.6)	5% to 5.6% (5.38%) < 5% (4.75%) ≥ 5.7% (6.21%) Diabetes (8.15%)	Atherosclerosis in Communities (ARIC) Study
[46]	73 10 29 17	N/A	60,796 7,081 20,064 7,372	Women	1 1.31 (0.67 to 2.55) 0.99 (0.62 to 1.57) 1.55 (0.88 to 2.75)	5% to 5.6% (5.38%) < 5% (4.8%) ≥ 5.7% (6.22%) Diabetes (8.6%)	Atherosclerosis in Communities (ARIC) Study
CRC = colorectal cancer; RR = relative risk; OR = odds ratio; HR = hazard ratio; C = combined (men and women); N/A = not applicable; HbA_{1c} = glycated haemoglobin; CI = confidence interval							

Table 6: Data collected relating HbA_{1c} levels to lifestyle factors (from [2]).

Reference	Subjects	Gender	HbA_{1c} ,% (95% CI)	Standard error (SE)	Exposure range	Exposure unit	Comments
Alcohol consumption							
[196]	228 432 551 411 225 49	Men	6.7 6.5 6.8 6.6 7 6.4	0.2 0.1 0.1 0.1 0.2 0.4	0 ≤ 12 13 to 24 25 to 47 48 to 96 > 96	g/day	

Reference	Subjects	Gender	HbA_{1c} ,% (95% CI)	Standard error (SE)	Exposure range	Exposure unit	Comments
[197]	72 144 133 110	Women	5.29 5.24 (5.2 to 5.3) 5.18 (5.04 to 5.24) 5.23 (5.18 to 5.29)	NA 0.0255 0.0510 0.0281	0 0.1 to < 15 15 to < 35 ≥ 35	g/day	
[164]	982 1,455 1,170 739	Men	5.14 (5.03 to .5.25) 5.07 (4.96 to 5.17) 5.02 (4.92 to 5.13) 5 (4.88 to 5.1)	0.0561 0.0536 0.0536 0.0561	0 1 to 19 20 to 50 ≥ 50	g/day	
[164]	4,481 979 760	Women	5.08 (5.01 to .5.15) 5.07 (5 to 5.14) 5 (4.93 to 5.07)	0.0357 0.0357 0.0357	0 1 to 9 ≥ 10	g/day	
[198]	75 55 141 114 79	Men	0 to 0.16 (-0.4 to 0.8) -0.21 (-0.47 to 0.05) -0.11 (-0.38 to 0.16) -0.1 (-0.41 to 0.21)	NA 0.3061 0.1327 0.1378 0.1582	0 6.6 16.8 30.9 51.4	g/day	
[165]	220 1,482 852 637 897 595	Men	0 -0.12 (-0.234 to -0.004) -0.1 (-0.222 to 0.022) -0.106 (-0.231 to 0.019) -0.192 (-0.314 to -0.07) -0.244 (-0.371 to -0.117)	NA 0.0587 0.0622 0.0638 0.0622 0.0648	0 0 to 6 7 to 13 14 to 20 21 to 41 ≥ 42	units/week	8 g per unit

Reference	Subjects	Gender	HbA_{1c} ,% (95% CI)	Standard error (SE)	Exposure range	Exposure unit	Comments
[165]	478 2,943 871 389 329 75	Women	0 -0.013 (-0.087 to 0.061) -0.04 (-0.128 to 0.048) -0.034 (-0.138 to 0.07) -0.113 (-0.223 to -0.003) -0.096 (-0.294 to 0.092)	NA 0.0378 0.0449 0.0531 0.0561 0.0985	0 0 to 6 7 to 13 14 to 20 21 to 41 ≥ 42	units/week	8 g per unit
Dietary fibre							
[167]	2,065 in total	C	6.5 (6.32 to 6.68) 6.42 (6.26 to 6.58) 6.29 (6.13 to 6.45) 6.21 (6.04 to 6.39)	0.0918 0.0816 0.0816 0.0893	11.4 15.6 20 27.3	g/day	Type 1 diabetics
[166]	390 1,685 427 101	C	4.7 5.4 6.3 8.3	0.0152 0.0073 0.0145 0.0299	10.7 10.7 10.9 12.2	g/day	Age- and sex-adjusted
[168]	308 310 308	C	7.7 7.5 7.4	0.0855 0.0852 0.1026	< 6.925 6.925 to 10.915 > 10.915	g/day	Type 2 diabetics Used average of cut-offs for men and women
Physical exercise							
[169]	48 32	C	7.8 7.6	0.1443 0.1945	0 0 to 149	min/week	Moderate exercise (5 METs)

Reference	Subjects	Gender	HbA_{1c} ,% (95% CI)	Standard error (SE)	Exposure range	Exposure unit	Comments
	50		7.5	0.1556	150		Type 1 diabetics
[169]	30 69 31	C	8 7.8 7.2	0.1826 0.1324 0.1796	0 0 to 149 150	min/week	Intense exercise (7 METs) Type 1 diabetics
Glycaemic load (GL)							
[159]	57 57 57 56	C	6.2 (5.5 to 6.8) 6.2 (5.5 to 6.8) 6.6 (6 to 7.2) 6.5 (5.9 to 7.1)	0.3316 0.3316 0.3061 0.3061	58 75 86 100	GL/day	Reference food is glucose
[160]	169 169 169 169 169	Women	5 5.1 5.1 5.1 5.2	0.1 0.1 0.1 0.1 0.1	157.665 168.48 167.562 171.76 170.665	GL/day	Reference food is glucose Original exposure per 1000 kcal was converted to GL/day by multiplying with daily energy intake and dividing by 1000.
Cigarette smoking							
[165]	4,376 660 1,108 911	C	0 0.125 (0.06 to 0.19) 0.189 (0.136 to 0.242) 0.277 (0.218 to 0.336)	NA 0.0332 0.0270 0.0301	0 1 to 9 10 to 19 ≥ 20	cigarettes/day	

Reference	Subjects	Gender	HbA_{1c} ,% (95% CI)	Standard error (SE)	Exposure range	Exposure unit	Comments
[164]	1,180 436 1,015	Men	5.02 (4.91 to 5.13) 5.07 (4.96 to 5.19) 5.11 (5 to 5.22)	0.0561 0.0587 0.0561	0 1 to 19 ≥ 20	cigarettes/day	
[118]	918 116 207	Men	5.3 5.54 5.58	0.02 0.07 0.05	0 < 15 ≥ 15	cigarettes/day	
[118]	1,894 191 173	Women	5.37 5.39 5.57	0.02 0.05 0.06	0 < 15 ≥ 15	cigarettes/day	
Chronic stress							
[162]	234 in total	C	5.38 5.36 5.3	0.04 0.032 0.034	Low Medium High	stress level	
[163]	96 81 10 23	Men	5.2 5.3 5.4 5.7	0.0816 0.1 0.3795 0.2085	Active Relaxed Passive Strain	stress level	
g/day = grams per day; GL = glycaemic load; MET-h/day = metabolic equivalent-hours per day; C = combined; HbA_{1c} = glycated haemoglobin; CI = confidence interval; SE = standard error							

Table 7: Data collected relating lifestyle factors to CRC incidence risk (from [2]).

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
Alcohol consumption									
[199]	487 652 507 383 294	N/A	11,447 14,375 11,308 7,883 4,484	C	HR	1 1.06 (0.91 to 1.23) 0.97 (0.82 to 1.14) 1 (0.83 to 1.2) 1.32 (1.06 to 1.65)	0 0 to < 5 5 to < 15 15 to < 30 ≥ 30	g/day	
[200]	19 39 119 11 37 23	N/A	10,761 26,627 59,333 8,724 21,044 10,698	Men	HR	1.24 (0.72 to 2.16) 1 1.46 (1.01 to 2.11) 0.81 (0.41 to 1.6) 1.32 (0.83 to 2.09) 1.67 (0.98 to 2.84)	0 0.1 to 10 10.1 to 40 40.1 to 70 70.1 to 140 ≥ 140.1	g/week	
[201]	110 433 444 246 140 74	N/A	246,448 786,239 701,946 358,149 168,559 64,067	C	HR	0.98 (0.72 to 1.33) 1 1.05 (0.9 to 1.21) 1.07 (0.89 to 1.29) 1.23 (0.98 to 1.55) 1.98 (1.46 to 2.7)	0 0.1 to 4.9 4.9 to 14.9 15 to 29.9 30 to 59.9 > 60	g/day	
[202]	311 295	N/A	218,867 207,211	Men	HR	1 1.22 (0.92 to 1.61)	0 0.1 to 22.9	g/day	

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	363 374 182 112		220,367 175,414 83,438 45,535			1.42 (1.21 to 1.66) 1.95 (1.53 to 2.49) 2.15 (1.74 to 2.64) 2.96 (2.27 to 3.86)	23 to 45.9 46 to 68.9 69 to 91.9 ≥ 92		
[202]	839 97 42	N/A	884,277 138,327 38,481	Women	HR	1 0.93 (0.7 to 1.23) 1.57 (1.11 to 2.21)	0 0.1 to 22.9 ≥ 23	g/day	
[203]	721 172 94 139 129	N/A	319,014 77,855 47,218 62,605 57,997	Women	HR	1 1.04 (0.88 to 1.24) 0.92 (0.74 to 1.15) 1.1 (0.91 to 1.33) 1.02 (0.83 to 1.25)	0 ≤ 1.8 > 1.8 to 3.4 > 3.4 to 11 > 11	g/day	
[204]	5 13 13 11 17	N/A	8,802.4 9,098.4 8,758 8,867.5 8,081.5	Men	HR	1 2.4 (0.9 to 6.8) 2.5 (0.9 to 7.2) 2.2 (0.8 to 6.4) 3.5 (1.2 to 9.8)	0 to 3.2 3.3 to 17.2 17.3 to 48.8 48.9 to 115.2 115.5 to 2,853.1	g/week	
[205]	658 117 70	N/A	443,968 77,374 25,177	C	HR	1 0.96 (0.72 to 1.25) 1.84 (1.31 to 2.58)	0 < 7 ≥ 7	drinks/ week	12 g per drink
[206]	61	397	458	Men	OR	1	≤ 1	drinks/	12 g per

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	84 66	369 338	453 404			1.51 (1.05 to 2.17) 1.25 (0.85 to 1.84)	2 to 4 ≥ 5	week	drink
[207]	73 14 20 83	124 20 17 61	197 34 37 144	Men	OR	1 1.22 (0.58 to 2.58) 1.98 (0.96 to 4.09) 2.33 (1.47 to 3.71)	0 to 29 30 to 299 300 to 599 ≥ 600	g/month	
[208]	429 150 208	422 121 113	851 271 321	C	OR	1 1.22 (0.88 to 1.69) 1.76 (1.26 to 2.46)	< 5 5 to 30 ≥ 30	g/day	
[209]	140 79 101 164	294 155 140 149	434 234 241 313	C	OR	1 1.09 (0.78 to 1.53) 1.6 (1.13 to 2.25) 2.57 (1.81 to 3.64)	0 1 to 5 6 to 13 ≥ 14	units/ week	9.8 g per unit
[210]	50 33 87 60 37	77 100 147 47 24	127 133 234 107 61	Men	OR	1 0.51 (0.3 to 0.87) 0.85 (0.54 to 1.3) 1.81 (1.03 to 3.2) 2.19 (1.2 to 4.2)	0 < 1 1 to 1.9 2 to 2.9 ≥ 3	cups	21.3 g per cup
[211]	169 332 125	395 1,107 414	564 1,439 539	Women	OR	1 0.97 (0.77 to 1.23) 1.06 (0.79 to 1.42)	0 1 to 2 3 to 5	drinks/ week	12 g per drink

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	72	238	310			1.05 (0.74 to 1.49)	6 to 10		
	52	131	183			1.49 (1 to 2.22)	≥ 11		
[212]	68	200	268	Men	OR	1.53 (0.98 to 2.41)	0	g/day	
	39	175	214			1	> 0 to < 5		
	55	224	279			1.06 (0.66 to 1.69)	5 to < 15		
	45	188	233			1.02 (0.63 to 1.66)	15 to < 30		
	28	96	124			1.2 (0.68 to 2.12)	30 to < 45		
	31	97	128			1.24 (0.69 to 2.22)	≥ 45		
[212]	119	374	493	Women	OR	1 (0.7 to 1.42)	0	g/day	
	73	230	303			1	> 0 to < 5		
	61	219	280			0.84 (0.56 to 1.26)	5 to < 15		
	41	140	181			0.87 (0.55 to 1.37)	15 to < 30		
	12	39	51			0.9 (0.43 to 1.87)	30 to < 45		
	7	14	21			1.52 (0.56 to 4.1)	≥ 45		
[213]	61	63	124	Men	OR	1	0 to 9	g/day	
	39	38	77			1 (0.5 to 1.8)	10 to 39		
	25	33	58			0.7 (0.4 to 1.3)	40 to 69		
	20	12	32			1.6 (0.7 to 3.7)	≥ 70		
[214]	959	2,137	3,096	C	OR	1	0	drinks/ week	12 g per drink
	368	722	1,090			1.1 (0.93 to 1.3)	1 to 4		
	376	661	1,037			1.17 (0.99 to 1.38)	5 to 11		

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	469	755	1,224			1.21 (1.03 to 1.44)	≥ 12		
[215]	382 309 344	467 539 503	849 848 847	C	OR	1 0.88 (0.67 to 1.17) 1.17 (0.85 to 1.61)	0 13.8 182.8	g/day	
[216]	108 131 433	99 86 487	207 217 920	C	OR	1 1.21 (0.81 to 1.8) 0.85 (0.61 to 1.18)	0 < 400 > 400	g/month	
[217]	395 256 322 302 269 269	1,059 566 565 568 565 567	1,454 822 887 870 834 836	C	OR	1 1.15 (0.94 to 1.4) 1.35 (1.12 to 1.63) 1.2 (0.99 to 1.46) 1.02 (0.83 to 1.26) 0.95 (0.77 to 1.19)	< 1 > 1 to 11.82 > 11.82 to 22.66 > 22.66 to 34.46 > 34.36 to 51.82 > 51.82	g/day	
[218]	11 12 18 25	23 24 55 30	34 36 73 55	C	OR	1 1.1 (0.4 to 3.1) 0.7 (0.3 to 1.9) 2 (0.7 to 5.4)	0 1 to 20 21 to 40 ≥ 41	g/day	
[219]	270 241 60 131	264 287 68 98	534 528 128 229	C	OR	1 0.9 (0.7 to 1.2) 0.8 (0.5 to 1.3) 1.3 (0.9 to 1.9)	0 1 to 2 3 to 4 ≥ 5	drinks/ day	13.5 g per drink

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
[220]	301 101 52 25 11	26,475 10,374 4,617 2,413 895	26,776 10,475 4,669 2,438 906	Women	RR	1 0.92 (0.73 to 1.16) 1 (0.74 to 1.35) 0.94 (0.62 to 1.42) 1.16 (0.63 to 2.14)	0 0.01 to 0.5 0.51 to 1 1.01 to 2 > 2	servings/ day	12 g per serving
[221]	30 39 26	3,141 4,256 3,506	3,171 4,295 3,532	C	RR	1 1.53 (0.94 to 2.49) 1.53 (0.87 to 2.69)	< 1 1 to 7 > 7	units/ week	9.8 g per unit
Dietary fibre									
[222]	931 918 912 914 842	N/A	1,050,093 1,050,093 1,050,082 1,050,082 1,050,082	C	HR	1 0.98 (0.89 to 1.08) 0.96 (0.86 to 1.06) 0.94 (0.84 to 1.05) 0.83 (0.72 to 0.96)	<16.4 16.4 to < 20.1 20.1 to < 23.6 23.6 to < 28.5 ≥ 28.5	g/day	
[170]	307 348 267 289 259	N/A	241,810 246,971 248,569 248,736 245,532	Women	HR	1 1.16 (0.96 to 1.39) 0.92 (0.72 to 1.18) 1.1 (0.8 to 1.5) 1.06 (0.67 to 1.7)	< 9.9 9.9 to 13.3 13.3 to 16.7 16.7 to 21.2 ≥ 21.2	g/day	
[223]	68 69	N/A	40,291 42,872	Men	HR	1 0.9 (0.63 to 1.3)	6.4 9.1	g/day	

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	55		43,391			0.7 (0.47 to 1.1)	11.2		
	72		43,565			0.88 (0.58 to 1.3)	13.6		
	71		42,069			0.85 (0.53 to 1.4)	18.7		
[223]	34	N/A	46,443	Women	HR	1	8.3	g/day	
	27		48,077			0.61 (0.35 to 1)	11.2		
	34		49,104			0.62 (0.36 to 1.1)	13.3		
	49		50,179			0.77 (0.44 to 1.3)	15.6		
	43		49,626			0.58 (0.31 to 1.1)	20		
[224]	609	N/A	513,317	C	HR	1.18 (1.05 to 1.31)	< 10	g/day	
	1,681		1,591,322			1	10 to <15		
	2,263		1,870,758			1.02 (0.95 to 1.1)	15 to < 20		
	1,740		1,183,334			1.01 (0.92 to 1.1)	20 to < 25		
	1,001		514,142			0.99 (0.87 to 1.12)	25 to < 30		
	785		313,572			1 (0.85 to 1.17)	≥ 30		
[225]	134	399	533	C	OR	1	8.9	g/day	
	121	399	520			0.84 (0.6 to 1.18)	12.3		
	91	400	491			0.55 (0.38 to 0.81)	14.6		
	115	399	514			0.8 (0.55 to 1.17)	17.6		
	118	399	517			0.67 (0.42 to 1.05)	24.1		
[226]	172	181	353	C	OR	1	< 11.4	g/day	
	67	182	249			0.5 (0.34 to 0.73)	11.4 to 16.6		

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	47	187	234			0.36 (0.24 to 0.54)	>16.6		
[215]	388 355 308	462 493 539	850 848 847	C	OR	1 0.97 (0.77 to 1.23) 0.84 (0.67 to 0.99)	15.1 23.7 35.2	g/day	
[227]	175 152 178 153 158	163 163 163 163 163	338 315 341 316 321	C	OR	1 0.87 (0.62 to 1.21) 0.95 (0.67 to 1.34) 0.81 (0.56 to 1.18) 0.87 (0.58 to 1.31)	9.2 11.7 13.7 16.2 19.8	g/day	
[228]	51 76 54 77	N/A	30,631 31,855 32,224 32,187	Men	HR	1 1.12 (0.77 to 1.62) 0.62 (0.4 to 0.96) 0.69 (0.43 to 1.11)	6.7 9.4 11.3 13.4	g/day	
[228]	46 38 48 53	N/A	48,219 49,366 50,921 51,872	Women	HR	1 0.73 (0.47 to 1.14) 0.84 (0.54 to 1.33) 0.75 (0.46 to 1.25)	7.4 9.8 11.5 13.4	g/day	
Physical exercise									
[229]	98 80 105	N/A	61,804 63,549 63,120	Men	HR	1 0.84 (0.62 to 1.13) 1 (0.76 to 1.33)	< 37.9 37.9 to 40.7 40.8 to 44.8	MET-h/day	

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	82		63,505			0.82 (0.6 to 1.1)	≥ 44.9		
[230]	63 18 9	56 11 25	119 29 34	Men	OR	1 2.1 (0.77 to 5.72) 0.3 (0.11 to 0.81)	0 < 20 ≥ 20	MET-h/ week	
[230]	41 14 15	37 16 17	78 30 32	Women	OR	1 0.86 (0.34 to 2.16) 0.77 (0.31 to 1.94)	0 < 20 ≥ 20	MET-h/ week	
[231]	84 81 64 61	8,177 7,616 6,011 7,748	8,261 7,697 6,075 7,809	Men	RR	1 0.99 (0.72 to 1.35) 0.85 (0.61 to 1.2) 0.69 (0.49 to 0.97)	28.25 33.25 35.25 43.75	MET-h/day	
[231]	53 53 45 45	9,542 9,132 8,546 7,764	9,595 9,185 8,591 7,809	Women	RR	1 1.17 (0.79 to 1.75) 0.97 (0.63 to 1.47) 1.16 (0.76 to 1.77)	28.5 33.25 35.25 43.75	MET-h/day	
Glycaemic load (GL)									
[171]	26 30 37 32 49	N/A	61,084 61,213 61,190 60,976 60,872	Women	HR	1 1.34 (0.76 to 2.34) 1.81 (1.02 to 3.21) 1.63 (0.86 to 3.09) 2.85 (1.4 to 5.8)	92 106 117 127 143	GL/day	Reference food is glucose

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
[170]	323 294 304 281 268	N/A	241,998 247,053 248,043 249,138 245,386	Women	HR	1 0.94 (0.79 to 1.12) 1.07 (0.88 to 1.29) 1.01 (0.81 to 1.27) 1.11 (0.82 to 1.49)	< 62.4 62.4 to 81.9 81.9 to 100.7 100.7 to 126.6 ≥ 126.6	GL/day	Reference food is glucose
[172]	152 168 156 174 220	N/A	194,565 196,016 192,505 192,033 188,305	Women	HR	1 0.98 (0.78 to 1.24) 0.85 (0.67 to 1.09) 0.89 (0.69 to 1.14) 1.06 (0.81 to 1.39)	< 164 164 to 175 176 to 186 187 to 199 ≥ 200	GL/day	Reference food is white bread
[173]	97 78 76 86 138	N/A	132,319 133,177 133,439 133,323 131,905	Women	HR	1 0.74 (0.55 to 1) 0.69 (0.51 to 0.93) 0.71 (0.53 to 0.96) 0.94 (0.71 to 1.24)	159.7 179.6 192.7 205.9 225.9	GL/day	Reference food is glucose
[174]	104 98 88 91 109	N/A	77,304 77,414 77,677 77,268 76,988	Women	HR	1 0.93 (0.7 to 1.22) 0.8 (0.6 to 1.07) 0.81 (0.61 to 1.08) 0.91 (0.7 to 1.2)	46.5 59.8 67.4 74.9 89.4	GL/day	Reference food is glucose
[175]	253	N/A	4,188	Men	HR	1	108.7	GL/day	Reference

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	216 193 223 197		4,245 4,147 4,255 4,203			0.82 (0.64 to 1.04) 0.75 (0.58 to 0.97) 0.9 (0.7 to 1.16) 0.83 (0.64 to 1.08)	124.8 136.2 147.8 165.4		food is glucose
[175]	152 149 156 156 142	N/A	4,423 4,490 4,510 4,352 4,200	Women	HR	1 0.96 (0.73 to 1.28) 1.02 (0.77 to 1.37) 1.05 (0.78 to 1.41) 1 (0.73 to 1.36)	82.5 94 101.7 107.9 123.6	GL/day	Reference food is glucose
Cigarette smoking									
[232]	558 163 99 49	N/A	293,319 74,987 43,378 23,016	Women	HR	1 1.15 (0.95 to 1.38) 1.23 (0.97 to 1.54) 1.12 (0.82 to 1.54)	0 1 to 19 20 > 20	cigarettes/ day	
[233]	126 13 35	N/A	133,387 10,013 17,874	Men	HR	1 1.32 (0.73 to 2.4) 2.14 (1.45 to 3.14)	0 < 20 ≥ 20	cigarettes/ day	
[234]	302 68 29 6	N/A	219,082 55,787 21,049 2,179	C	HR	1 1 (0.7 to 1.3) 1.1 (0.7 to 1.7) 3.1 (1.4 to 7.1)	0 1 to 10 11 to 20 ≥ 21	cigarettes/ day	

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
[235]	274 56 78 93 12 8	N/A	498,516 102,182 120,688 166,846 29,414 23,194	Women	HR	1 1.01 (0.75 to 1.35) 1.24 (0.96 to 1.6) 1.11 (0.87 to 1.41) 0.83 (0.46 to 1.48) 0.71 (0.35 to 1.43)	0 1 to 9 10 to 19 20 to 29 30 to 39 ≥ 40	cigarettes/ day	
[236]	269 93 147 74	457 256 393 177	726 349 540 251	Men	OR	1 0.6 (0.4 to 0.8) 0.7 (0.5 to 0.9) 0.7 (0.5 to 1)	0 < 15 15 to 24 ≥ 25	cigarettes/ day	
[236]	558 56 34 11	740 109 73 22	1,298 165 107 33	Women	OR	1 0.8 (0.5 to 1.9) 0.6 (0.4 to 1) 0.8 (0.4 to 1.8)	0 < 15 15 to 24 ≥ 25	cigarettes/ day	
[237]	5 15 30	57 63 20	57 63 20	C	OR	1 0.99 (0.53 to 1.82) 1.18 (0.49 to 2.86)	< 10 10 to 20 > 20	cigarettes/ day	Assume a 1:1 case to control ratio
[238]	273 69 138	845 134 188	1,118 203 326	C	OR	1 1.07 (0.75 to 1.52) 1.51 (1.1 to 2.08)	0 < 13 ≥ 13	cigarettes/ day	
[239]	282	363	645	Men	OR	1	0	cigarettes/	

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	159 282 139 247	175 275 140 156	334 557 279 403			0.94 (0.62 to 1.43) 1 (0.67 to 1.49) 0.89 (0.56 to 1.42) 1.51 (0.99 to 2.29)	1 to 10 11 to 20 21 to 30 > 30	day	
[239]	489 64 66 110 85	529 54 81 90 61	1,018 118 147 200 146	Women	OR	1 1.88 (1.02 to 3.45) 1.06 (0.58 to 1.94) 1.68 (0.9 to 3.15) 1.73 (0.87 to 3.43)	0 1 to 5 6 to 10 11 to 20 >20	cigarettes/ day	
[240]	745 127 252 119	2,055 396 853 397	2,800 523 1,105 516	C	OR	1 0.95 (0.74 to 1.23) 0.85 (0.69 to 1.05) 0.87 (0.67 to 1.15)	0 < 1 1 > 1	packs/day	20 cigarettes per pack
[214]	953 414 569 288	2,008 825 1,099 499	2,961 1,239 1,668 787	C	OR	1 1.03 (0.87 to 1.24) 1.04 (0.9 to 1.21) 1.13 (0.93 to 1.37)	0 ≤ 10 11 to 20 > 20	cigarettes/day	
[241]	30 26 46	160 179 201	190 205 247	C	OR	1 0.9 (0.5 to 1.7) 1.5 (0.9 to 2.6)	0 1 to 14 > 14	cigarettes/day	
[242]	252	292	544	C	OR	1	0	cigarettes/day	

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	91 107 86	122 119 81	213 226 167			0.89 (0.63 to 1.25) 1.01 (0.71 to 1.43) 1.15 (0.78 to 1.71)	1 to 9 10 to 19 ≥ 20		
[243]	59 65 29	180 127 24	239 192 53	Men	OR	1 1.3 (0.8 to 2.1) 3.2 (1.6 to 6.4)	0 1 to 20 > 20	cigarettes/day	
[218]	27 21 38 17	86 30 45 14	113 51 83 31	C	OR	1 1.2 (0.4 to 3.8) 0.8 (0.3 to 2.1) 2.4 (0.7 to 8.6)	0 1 to 15 16 to 30 ≥ 31	cigarettes/day	
[244]	81 127 149 69	124 115 133 52	205 242 282 121	Men	OR	1 1.72 (1.15 to 2.59) 1.71 (1.15 to 2.56) 1.79 (1.09 to 2.95)	0 < 20 20 to 29 ≥ 30	cigarettes/day	
[244]	120 107 44 5	146 101 42 4	266 208 86 9	Women	OR	1 1.16 (0.77 to 1.74) 1.27 (0.74 to 2.17) 1.91 (0.42 to 8.66)	0 < 20 20 to 29 ≥ 30	cigarettes/day	
[245]	264 65 64	233 57 68	497 122 132	C	OR	1 1.06 (0.7 to 1.61) 0.75 (0.5 to 1.13)	0 1 to 10 ≥ 11	cigarettes/day	

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
[213]	48 23 38	43 27 30	91 50 68	Men	OR	1 0.7 (0.3 to 1.4) 0.9 (0.4 to 1.8)	0 ≤ 1 ≥ 2	packs/day	20 cigarettes per pack
[246]	894 116 236	1,933 221 366	2,827 337 602	C	OR	1 0.75 (0.58 to 0.97) 0.93 (0.75 to 1.16)	0 < 15 ≥ 15	cigarettes/day	
[247]	264 62 49	29,932 10,460 7,999	30,196 10,522 8,048	C	RR	1 1.11 (0.82 to 1.5) 1.04 (0.73 to 1.48)	0 < 15 ≥ 15	cigarettes/day	
Chronic stress									
[114]	62 71 22 7	N/A	33,513.51 41,040.46 21,359.22 6,930.69	Women	HR	1 1.06 (0.75 to 1.49) 0.6 (0.37 to 0.98) 0.52 (0.23 to 1.14)	None Low Medium High	stress level	
[114]	91 48 16 11	N/A	35,271.31 26,519.33 13,008.13 2,330.50	Men	HR	1 0.93 (0.65 to 1.32) 0.64 (0.37 to 1.1) 1.96 (1.03 to 3.74)	None Low Medium High	stress level	
[176]	304 149 77	286 147 54	590 296 131	C	OR	1 1 (0.7 to 1.3) 1.3 (0.9 to 2)	Zero score Low Medium	stress level	

Reference	Cases	Controls/ Non-cases	Total	Gender	Risk type	Risk (95% CI)	Exposure range	Exposure unit	Comments
	39	23	62			1.7 (1 to 2.9)	High		

RR = relative risk; OR = odds ratio; HR = hazard ratio; C = combined; g/day = grams per day; GL = glycaemic load; MET-h/day = metabolic equivalent-hours per day; N/A = not applicable; CI = confidence interval

ANNEXURE D TABLES OF RESULTS

Table 8: Models of the relation between CRC risk and change in HbA_{1c} (from [2]).

Risk	Model	*p*-value for model	*p*-value for goodness of fit
HR	$HR = exp(0.0791 \times \Delta HbA_{1c})$ Range: HbA_{1c} in [4.75% to 8.6%]	0.1312	0.8299
HR [a]	$HR = exp(0.1369 \times \Delta HbA_{1c})$ Range: HbA_{1c} in [5.38% to 8.6%]	0.0389	0.9121
OR	$OR = exp(0.3853 \times \Delta HbA_{1c})$ Range: HbA_{1c} in [4.2% to 6.4%]	0.0050	0.3110
RR	$RR = exp(-0.2201 \times \Delta HbA_{1c})$ Range: HbA_{1c} in [4.7% to 5.8%]	0.3859	0.7731
Combined	Risk $= exp(0.1158 \times \Delta HbA_{1c})$ Range: HbA_{1c} in [4.2% to 8.6%]	0.0134	0.1633
Combined [a]	Risk $= exp(0.1727 \times \Delta HbA_{1c})$ Range: HbA_{1c} in [4.2% to 8.6%]	0.0020	0.2517

[a] Excluding data points below reference

RR = relative risk; OR = odds ratio; HR = hazard ratio; HbA_{1c} = glycated haemoglobin

Table 9: Models of the relation between change in HbA_{1c} and change in lifestyle factors (from [2]).

Lifestyle factor	Model	*p*-value for model	*p*-value for goodness of fit
Alcohol consumption	$\Delta HbA_{1c} = -0.0026 \times \Delta ethanol_{g/day}$ Ranges: $ethanol_{g/day}$ in [0 to 144 g/day] HbA_{1c} in [5% to 7%]	0.0000	0.4914
Dietary fibre	$\Delta HbA_{1c} = 0.9123 \times \Delta fibre_{g/day}$ Ranges: $fibre_{g/day}$ in [4.93 to 27.3 g/day] HbA_{1c} in [4.7% to 8.3%]	0.0952	0.0522
Dietary fibre [a]	$\Delta HbA_{1c} = -0.0246 \times \Delta fibre_{g/day}$ [a] Ranges: $fibre_{g/day}$ in [4.93 to 27.3 g/day] HbA_{1c} in [6.21% to 7.7%]	0.0002	0.3173
Physical exercise	$\Delta HbA_{1c} = -0.1299 \times \Delta exercise_{METh/day}$ Ranges: $exercise_{METh/day}$ in [0 to 4.983 MET-h/day] HbA_{1c} in [7.2% to 8%]	0.0002	0.3173
Glycaemic load (GL)	$\Delta HbA_{1c} = 0.0055 \times \Delta GL_{/day}$ [b] Ranges: $GL_{/day}$ in [58 to 171.6 $GL_{/day}$] HbA_{1c} in [5% to 6.6%]	0.0035	0.5773
Cigarette smoking	$\Delta HbA_{1c} = 0.0069 \times \Delta cigarettes/day$ Ranges: $cigarettes/day$ in [0 to 38 $cigarettes/day$] HbA_{1c} in [5.02% to 6.617%]	0.0005	0.5238
Chronic stress	$\Delta HbA_{1c} = 0.0569 \times \Delta stress_{level}$ Ranges: $stress_{level}$ in [0 to 3 level] HbA_{1c} in [5.2% to 5.7%]	0.5425	0.3173
Chronic stress	$\Delta HbA_{1c} = 0.1512 \times \Delta stress_{level}$ [c] Ranges: $stress_{level}$ in [0 to 3 level] HbA_{1c} in [5.2% to 5.7%]	0.0000	0.7079

Lifestyle factor	Model	*p*-value for model	*p*-value for goodness of fit

[a] Excluding [166]

[b] Fixed-effect model

[c] Excluding [162]

g/day = grams per day; GL = glycaemic load; MET-h/day = metabolic equivalent-hours per day; HbA_{1c} = glycated haemoglobin

Stress level:
0 = None
1 = Low
2 = Medium
3 = High

Table 10: Glycaemic models of the relation between changes in lifestyle factors and CRC risk via HbA_{1c} (from [2]).

Risk	Model
Alcohol consumption	
HR [a]	$HR = -0.0004 \times \Delta\text{ethanol}_{\text{g/day}}$ Ranges: $\text{ethanol}_{\text{g/day}}$ in [27.36 to 144 g/day] HbA_{1c} in [5.38% to 7%]
OR	$OR = -0.0010 \times \Delta\text{ethanol}_{\text{g/day}}$ Ranges: $\text{ethanol}_{\text{g/day}}$ in [0 to 100.8 g/day] HbA_{1c} in [5% to 6.4%]
Combined [a]	$Risk = -0.0004 \times \Delta\text{ethanol}_{\text{g/day}}$ Ranges: $\text{ethanol}_{\text{g/day}}$ in [0 to 144 g/day] HbA_{1c} in [5% to 7%]
Dietary fibre	
HR [a, b]	$HR = -0.0034 \times \Delta\text{fibre}_{\text{g/day}}$ Ranges: $\text{fibre}_{\text{g/day}}$ in [4.93 to 27.3 g/day] HbA_{1c} in [6.21% to 7.7%]

Risk	Model
OR [b]	$OR = -0.0095 \times \Delta\text{fibre}_{\text{g/day}}$ Ranges: $\text{fibre}_{\text{g/day}}$ in [4.93 to 7.78 g/day] HbA_{1c} in [6.21% to 6.4%]
Combined [a, b]	$Risk = -0.0042 \times \Delta\text{fibre}_{\text{g/day}}$ Ranges: $\text{fibre}_{\text{g/day}}$ in [4.93 to 27.3 g/day] HbA_{1c} in [6.21% to 7.7%]
Physical exercise	
HR [a]	$HR = -0.0178 \times \Delta\text{exercise}_{\text{METh/day}}$ Ranges: $\text{exercise}_{\text{METh/day}}$ in [0 to 4.983 MET-h/day] HbA_{1c} in [7.2% to 8%]
Combined [a]	$Risk = -0.0244 \times \Delta\text{exercise}_{\text{METh/day}}$ Ranges: $\text{exercise}_{\text{METh/day}}$ in [0 to 4.983 MET-h/day] HbA_{1c} in [7.2% to 8%]
Glycaemic load (GL)	
HR [a]	$HR = 0.0008 \times \Delta\text{GL}_{\text{/day}}$ Ranges: $\text{GL}_{\text{/day}}$ in [84.98 to 171.6 $\text{GL}_{\text{/day}}$] HbA_{1c} in [5.38% to 6.6%]
OR	$OR = 0.0021 \times \Delta\text{GL}_{\text{/day}}$ Ranges: $\text{GL}_{\text{/day}}$ in [58 to 157.4 $\text{GL}_{\text{/day}}$] HbA_{1c} in [5% to 6.4%]
Combined [a]	$Risk = 0.0010 \times \Delta\text{GL}_{\text{/day}}$ Ranges: $\text{GL}_{\text{/day}}$ in [58 to 171.6 $\text{GL}_{\text{/day}}$] HbA_{1c} in [5% to 6.6%]
Cigarette smoking	
HR [a]	$HR = 0.0009 \times \Delta\text{cigarettes/day}$ Ranges: cigarettes/day in [9 to 38 cigarettes/day]

Risk	Model
	HbA_{1c} in [5.38% to 6.617%]
OR	$HR = 0.0027 \times \Delta\text{cigarettes/day}$ Ranges: cigarettes/day in [0 to 32 cigarettes/day] HbA_{1c} in [5.02% to 6.4%]
Combined [a]	$Risk = 0.0012 \times \Delta\text{cigarettes/day}$ Ranges: cigarettes/day in [0 to 38 cigarettes/day] HbA_{1c} in [5.02% to 6.617%]
Chronic stress	
HR [a]	$OR = exp(0.0207 \times \Delta\text{stress}_{\text{level}})$ Range: $\text{stress}_{\text{level}}$ in [1 to 3 level] HbA_{1c} in [5.38% to 5.7%]
OR [c]	$OR = exp(0.0583 \times \Delta\text{stress}_{\text{level}})$ Range: $\text{stress}_{\text{level}}$ in [0 to 3 level] HbA_{1c} in [5.2% to 5.7%]
Combined [a, c]	$OR = exp(0.0261 \times \Delta\text{stress}_{\text{level}})$ Range: $\text{stress}_{\text{level}}$ in [0 to 3 level] HbA_{1c} in [5.2% to 5.7%]

[a] Excluding data points below reference

[b] Excluding [166]

[c] Excluding [162]

RR = relative risk; OR = odds ratio; HR = hazard ratio; g/day = grams per day; GL = glycaemic load; MET-h/day = metabolic equivalent-hours per day; HbA_{1c} = glycated haemoglobin

Stress level:

0 = None

1 = Low

2 = Medium

3 = High

Table 11: Overall models of the full effects of lifestyle factors on CRC risk (from [2]).

Risk	Model	*p*-value for model	*p*-value for goodness of fit
Alcohol consumption			
HR	$HR = exp(0.0077 \times \Delta\text{ethanol}_{\text{g/day}})$ Range: $\text{ethanol}_{\text{g/day}}$ in [0 to 114.9 g/day]	0.0000	0.0640
HR [a]	$HR = exp(0.0078 \times \Delta\text{ethanol}_{\text{g/day}})$ Range: $\text{ethanol}_{\text{g/day}}$ in [0 to 114.9 g/day]	0.0000	0.0730
OR	$OR = exp(0.0138 \times \Delta\text{ethanol}_{\text{g/day}})$ Range: $\text{ethanol}_{\text{g/day}}$ in [0 to 182.8 g/day]	0.0412	0.0000
OR [a]	$OR = exp(0.0143 \times \Delta\text{ethanol}_{\text{g/day}})$ Range: $\text{ethanol}_{\text{g/day}}$ in [0 to 182.8 g/day]	0.0326	0.0000
RR	$RR = exp(0.0040 \times \Delta\text{ethanol}_{\text{g/day}})$ Range: $\text{ethanol}_{\text{g/day}}$ in [0 to 35.88 g/day]	0.5187	0.3837
Combined	$\text{Risk} = exp(0.0073 \times \Delta\text{ethanol}_{\text{g/day}})$ Range: $\text{ethanol}_{\text{g/day}}$ in [0 to 182.8 g/day]	0.0001	0.0000
Combined [a]	$\text{Risk} = exp(0.0124 \times \Delta\text{ethanol}_{\text{g/day}})$ Range: $\text{ethanol}_{\text{g/day}}$ in [0 to 182.8 g/day]	0.0031	0.0000
Dietary fibre			
HR	$HR = exp(-0.0059 \times \Delta\text{fibre}_{\text{g/day}})$ Range: $\text{fibre}_{\text{g/day}}$ in [6.4 to 35 g/day]	0.0445	0.1290
HR [a]	$HR = exp(-0.0060 \times \Delta\text{fibre}_{\text{g/day}})$ Range: $\text{fibre}_{\text{g/day}}$ in [6.4 to 35 g/day]	0.0949	0.0937
OR	$OR = exp(-0.0310 \times \Delta\text{fibre}_{\text{g/day}})$ Range: $\text{fibre}_{\text{g/day}}$ in [8.8 to 35.2 g/day]	0.0527	0.0003

Risk	Model	*p*-value for model	*p*-value for goodness of fit
Combined	$\text{Risk} = exp(-0.0347 \times \Delta\text{fibre}_{\text{g/day}} + 0.0375 \times rcs)$ Range: $\text{fibre}_{\text{g/day}}$ in [6.4 to 35.2 g/day]	0.0479 [b]	0.0023
Combined [a]	$Risk = exp(-0.0206 \times \Delta\text{fibre}_{\text{g/day}})$ Range: $\text{fibre}_{\text{g/day}}$ in [6.4 to 35.2 g/day]	0.0068	0.0001
Physical exercise			
HR	$HR = exp(-0.0109 \times \Delta\text{exercise}_{\text{METh/day}})$ Ranges: $\text{exercise}_{\text{METh/day}}$ in [36.5 to 48.9 MET-h/day]	0.3658	0.3417
OR	$OR = exp(-0.1175 \times \Delta\text{exercise}_{\text{METh/day}})$ Ranges: $\text{exercise}_{\text{METh/day}}$ in [0 to 5.714 MET-h/day]	0.1235	0.2061
RR	$RR = exp(-0.0097 \times \Delta\text{exercise}_{\text{METh/day}})$ Ranges: $\text{exercise}_{\text{METh/day}}$ in [28.25 to 43.75 MET-h/day]	0.5509	0.0676
Combined	$Risk = exp(-0.0131 \times \Delta\text{exercise}_{\text{METh/day}})$ Range: $\text{exercise}_{\text{METh/day}}$ in [0 to 48.9 MET-h/day]	0.1485	0.0969
Glycaemic load (GL)			
HR	$HR = exp(0.0001 \times \Delta\text{GL}_{/\text{day}})$ Range: $\text{GL}_{/\text{day}}$ in [46.5 to 225.9 $\text{GL}_{/\text{day}}$]	0.9092	0.0520
Cigarette smoking			
HR	$HR = exp(0.0122 \times \Delta\text{cigarettes/day})$ Range: cigarettes/day in [0 to 49 cigarettes/day]	0.0186	0.0206
OR	$OR = exp(0.0044 \times \Delta\text{cigarettes/day})$ Range: cigarettes/day in [0 to 60 cigarettes/day]	0.1245	0.0001
RR	$RR = exp(0.0015 \times \Delta\text{cigarettes/day})$ Range: cigarettes/day in [0 to 30 cigarettes/day]	0.8042	0.5270

Risk	Model	*p*-value for model	*p*-value for goodness of fit
Combined	$Risk = exp(0.0056 \times \Delta\text{cigarettes/day})$ Ranges: cigarettes/day in [0 to 60 cigarettes/day]	0.0174	0.0000
Chronic stress			
HR	$HR = exp(-0.0759 \times \Delta\text{stress}_{\text{level}})$ Range: $\text{stress}_{\text{level}}$ in [0 to 3 level]	0.5611	0.0503
OR	$OR = exp(0.1442 \times \Delta\text{stress}_{\text{level}})$ Range: $\text{stress}_{\text{level}}$ in [0 to 3 level]	0.0428	0.5686
Combined	$Risk = exp(0.0034 \times \Delta\text{stress}_{\text{level}})$ Range: $\text{stress}_{\text{level}}$ in [0 to 3 level]	0.9740	0.0137

[a] Excluding data points below reference

[b] *p*-value for coefficient of second spline

CRC = colorectal cancer; RR = relative risk; OR = odds ratio; HR = hazard ratio; g/day = grams per day; GL = glycaemic load; MET-h/day = metabolic equivalent-hours per day; HbA_{1c} = glycated haemoglobin

$$rcs = \frac{(\Delta exposure - knot_1)_+^3 - \frac{(knot_3 - knot_1)(\Delta exposure - knot_2)_+^3 - (knot_2 - knot_1)(\Delta exposure - knot_3)_+^3}{(knot_3 - knot_{2)}}}{(knot_3 - knot_1)^2}$$ [96], [149]

where

$$(u)_+ = \begin{cases} u, for\ u > 0 \\ 0, for\ u \leq 0 \end{cases}, where\ u\ is\ the\ function\ contained\ in\ brackets$$

Stress level:

0 = None

1 = Low

2 = Medium

3 = High