LIPID METABOLISM IN COLORECTAL CANCER: ALTERATIONS, THERAPEUTIC OPPORTUNITIES, AND OUTLOOK ON MOLECULAR DIAGNOSTICS

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This thesis is dedicated to my mother: her fight against injustice goes on. May my work inspire others to investigate and develop possibilities for battling this cruel illness.
1 Abstract

Colorectal cancer (CRC) is one of the most prevalent and deadliest cancers worldwide, which has prompted scientists to search of better screening methods and adequate therapies for this disease. Lipidomics, the study of lipid profiles in cells, tissues and other organisms, is an under-investigated research field, but has already showed promising results in many types of cancers, including CRC. This literature review tries to comprehensively resume all alterations in lipid metabolism that are linked with CRC. There are four major lipid groups to be discussed: free fatty acids, glycerolipids (including triglycerides and glycerophospholipids), sphingolipids and sterol lipids. The changing distribution of lipid bodies and lipoproteins in CRC will also be mentioned. Firstly, free fatty acids in tumour samples showed up-regulation of de novo synthesis-related enzymes (for instance ACLY and FASN) and significant alterations in fatty acid distribution, shifting towards more desaturated, pro-inflammatory ω-6 fatty acids. Regarding glycerolipids, triglycerides were mainly down-regulated and phosphatidylcholine was more augmented in CRC tissue samples, while lysophosphatidylcholines was shown up-regulated in plasma of CRC patients. Ceramide is the major component in sphingolipid metabolism and due to its anti-proliferative, pro-apoptotic properties CRC cells try to evade pathways that lead to the generation of this metabolite. Cholesterol de novo synthesis and uptake from lipoproteins was enhanced in these neoplastic cells, which was also highlighted by their higher expression of LDL and HDL cellular receptors. Lastly, intracellular lipid droplet augmentation has already been associated with malignancy and indeed was present in colon cancer cell lines. These findings indicate that lipidomics could provide many opportunities in targeted therapy and prognostic or screening biomarkers in CRC, although more intensive research is necessary if lipid metabolism is to be integrated in our therapeutic and screening policy, not only for CRC but also other types of cancers.
2 Introduction

Colorectal cancer still remains a major health topic: last year’s data show that the yearly incidence amounts to almost 2 million people with more than 600 000 deaths throughout the globe. These data vary in different countries, depending on age, sex, hereditary factors and risk-factors concerning lifestyle-issues such as alcohol consumption, smoking, red meat intake, obesity, diabetes and inflammatory bowel diseases (IBD). (1)

The current treatment strategy and prognosis are mainly determined by the clinicopathological staging system TNM. Based on the Dukes staging system, it provides information concerning the local tumor growth, lymph nodal invasion and metastasis of the colorectal neoplasm. (2) Today, surgery still remains the main curative option for non-metastatic colorectal cancer, with adjuvant or neo-adjuvant therapy (using radiotherapy, chemotherapy or radio-chemotherapy) conserved for higher stages or high-risk patients. (1)

Metabolomics is a new research field, attempting to profile intra- and extracellular metabolites (sugars, amino acids, nucleotides and lipids) to acquire new biomarkers for diagnosis and prognosis, and to gain better insight in the fundamental steps involved in carcinogenesis. Metabolomics has shown promising results in CRC staging and metastasis detection and some minor results were achieved when pharmaco-metabolomics were used in cancer treatment. (3) Lipidomics is a branch of metabolomics that examines the lipid pathways and networks by quantitatively defining lipid profiles in organelles, cells and organisms and has taken some huge steps forward regarding applications and further analytical technologies. Mass spectrometry remains the cornerstone for investigating these lipidomes. (4)

Lipids are complex but unique molecules with an irreplaceable role in cell structure maintenance, energy provision, as signaling. (5) According to the classification by Fahy et al., these lipid molecules are commonly divided into eight main groups: free fatty acids, sphingolipids, glycerolipids, phosphoglycerolipids, sterols, prenols, saccharolipids and polyketides. (6) The first five main groups contain bioactive (signaling) lipid molecules such as fatty acids, diacylglycerol, ceramide, sphingosine, lysosphatic acid that interfere in the regulation or (de)activation of different signaling lipid pathways.
Prenol lipids, saccharolipids and polyketides however are mainly of bacterial, fungal and plant origin and will therefore not be discussed in this thesis. (5)

There is substantial evidence that neoplasm cells show alterations in their lipid metabolism. (7) These alterations can cause changes in cell membrane structures, energy homeostasis and cell signaling with defects in gene expression, protein distribution and cell functioning, including the processes of apoptosis, autophagy, necrosis, proliferation, differentiation, growth, drug resistance and chemotherapy response. (5) (7)

By comprehending this phenomenon of lipid alteration, we could obtain vital information about the pathogenesis in CRC, create new biomarkers for screening or diagnosing CRC in earlier stages, and suggest new molecular targets for anticancer therapy. (8)

In this review, we will describe current used approaches for lipid profiling and detection of lipid deformities in CRC with an eye to biomarker discovery and potential treatment options. Most importantly, we will give a brief description of the metabolic pathways of each lipid subgroup and its function in the colorectal tissue, and the abnormalities in these pathways that could lead to the development of CRC.
3 Method

The related articles were acquired via the PubMed database by querying results with different MeSH-terms. The most useful MeSH-terms were ‘colorectal neoplasms’, ‘neoplasm staging’ and ‘lipids/metabolism’.

The review by Huang et al. contains a division of those lipids in different categories: fatty acids, sterols, sphingolipids, glycerolipids and glycerophospholipids (5) and these terms were subsequently used for further investigation in combination with the above noted MeSH terms. This method allowed enough reviews to be retrieved to obtain a sufficient background on these topics.

However, a more profound analysis was necessary, so the investigation was expanded by utilizing Google Scholar and Google as additional search engines. When a sufficient amount of relevant articles were acquired, the exploration was continued by using specialized terms in these search engines to deepen the investigation on certain topics such as ‘epoxygenases’, ‘lipoxygenases’, ‘cyclooxygenases’, ‘eicosanoids’, ‘glycerophosphate acyltransferase’, ‘sphingomyeline’, and ‘ceramide’ all coupled with ‘colorectal cancer’.

Lastly, the snowball effect method was used based on certain articles of interest, in which the references included in these articles led to additional articles of interest. The results were filtered by restricting the retrieved articles to those written in English.
4 Results

4.1 Colorectal cancer

4.1.1 Epidemiology
CRC is one of the most diagnosed cancers and causes the majority of cancer-related deaths in the Western world, with a global incidence of two million people, more than half a million deaths per year and a cumulative lifetime risk of developing CRC of approximately 5%.(9)
In recent years, more and more risk factors have been discovered including age (with a peak incidence at 70 years), male sex, family history, obesity, diabetes, IBD, alcohol consumption, tobacco use, red or processed meat consumption, low fruit and vegetable intake and even intestinal bacterial infections (e.g. Helicobacter pylorum). (1) A high variability in incidence exists between countries, though these incidence rates are highest in Western European countries, Australia and New Zealand. This suggests that a Western life style may be a significant risk factor in CRC development. It is important to note that the increasing prevalence can also be due to improved screening procedures, which allow detection of more asymptomatic patients, combined with the beneficial progression in treatment, which expands the patients’ survival.(10)

4.1.2 Prevention and screening
Knowing that CRC has such a large impact on global health, prevention is an essential part of this medical topic. Primary prevention is the most effective strategy in avoiding CRC and can be maintained by reducing the adjustable risk factors and promoting a healthy lifestyle. (11) Primary prevention by intake of certain drugs such as NSAID or hormone therapies however, has important side effects and still lacks fundamental evidence.

Secondary prevention comprises early detection of CRC, which can be obtained by screening with faecal occult blood tests, colonoscopy or sigmoidoscopy and even virtual colonoscopy by using CT imaging. This latter technique has been proposed as a screening technique, though exposition to radiation remains an important consequence, while the cost-effectiveness of this procedure is doubted, especially for primary prevention.

Options for tertiary prevention of surviving CRC patients have not been fully explored yet. Physical exercises, cessation of smoking and long term intake of aspirin or NSAIDs have been recommended, but more investigation remains necessary. (10)
4.1.3 Classification method
Officially, there are two classification methods that are utilized for staging CRC: Dukes stadium and the TNM system. Biopsy samples and imaging techniques (ultrasonography, (PET-) CT, MRI) are necessary for determining which stage belongs to a specific patient. Because the Dukes staging classification received the connotation of being out-dated and confusing, tumors are expected to be categorized according to the latest version of the UICC TNM-system since 2003. (2)(12) Table 1 (see appendix) provides an overview of the Dukes and TNM classification systems, and describes the different stages according to the findings of imaging techniques and histopathological investigation. Categorizing the stage of the patient’s CRC is important for determining the prognosis and type of therapy this patient should receive. (11)

4.1.4 Treatment

4.1.4.1 Non-metastatic colorectal cancer
As mentioned above, surgery remains the imperative curative option in non-metastatic CRC. Colon surgery is performed by removing the tumor and its corresponding lymph nodes with a clear resection margin. Treatment of rectal cancer is executed by total mesorectal excision. These procedures were initially performed using open surgery, but laparoscopy has proven to be equally efficient with a lower mortality, morbidity and shorter hospitalization, although it is technically more difficult for the surgeon.

Locoregional recurrence is a risk factor after surgery and is associated with a poorer prognosis. Due to the anatomical complexity of the pelvis, rectal cancer has a higher risk of recurrence compared with colon cancer. Avoiding recurrence can be accomplished by resection using wide margins and, if necessary, combined with pre-operative and/or post-operative chemotherapy, radiotherapy or chemo-radiotherapy. Adjuvant chemotherapy can also be advised when there is an increased risk for distant metastasis after resection. The decision whether surgery with or without (neo-) adjuvant therapy should be applied depends on the CRC stage, together with the patient’s own preference. (1) (8) (11)

4.1.4.2 Metastatic colorectal cancer
The treatment policy for CRC patients with distant metastasis varies from case to case. Surgery of liver or lung metastases can be offered if these metastases seem resectable, but when irresectable, palliative chemotherapy should be the standard. Alongside traditional
chemotherapeutics, more and more new drugs can be utilized as a form of personalized therapy. Examples are Bevacizumab and Aflibercept, targeting VEGF (vascular endothelial growth factor) or Cetuximab and Panitumumab, targeting EGF (epidermal growth factor). (1)(11)

4.2 Metabolomics in colorectal cancer

4.2.1 Current and upcoming ‘omics’ approaches for biomarkers

Over the past years, genomics and proteomics have been the center of investigation for carcinogenesis. This has provided an abundance of information that led to the development of new insights, new therapeutic targets and strategies, and nucleotide- or protein-based biomarkers. (13) Some of the most prevalently used and investigated biomarkers are described in this section.

Micro-satellite Instability (MSI), a short repetitive DNA nucleotide sequence involved in DNA repair after replication, is currently one of the most used biomarkers. The MSI status is divided in 3 different categories: MSI high (>30%), MSI low (10-30%) or MSS (microsatellite stable). It still remains unclear if the MSI status can be interpreted as a positive or negative factor for the prognosis. Studies have showed that the MSI status is associated with some clinicopathological variables and patients with a high MSI status have a higher general five year survival rate. (8) (14)

CIMP or hypermethylation of the CpG promoter island, is a well-known transcriptional silencer of DNA repair genes and tumor suppressor genes. It is often correlated with MSI and CIN and CRCs are therefore increasingly divided according to their CIMP status: CIMP-high, CIMP-low and CIMP-negative. (8)

CIN or Chromosomal instability is a characteristic found in almost 60-80% of all CRCs. It depicts the chromosome alterations, either structural or numerical, in CRC cells and is associated with poor prognosis and moderately differentiated types of cancer cells. (8)(12)

KRAS is a proto-oncogen that, when mutated, continuously activates the MAPK pathway and PI3K/Akt, both of which promote carcinogenesis by cellular proliferation. (8) It is present in 30-50% of all CRCs but still hasn’t been considered as an essential predictive biomarker. (14)
BRAF is an inhibitor of the RAS/MAPK intracellular signaling pathway, encoding a serine/threonine kinase. Mutations of this gene are correlated with the early stages of colorectal cancer development. Moreover, KRAS and BRAF mutations have both been associated with poor response on EPGF-inhibitors. (12) (14)

VEGF, vascular endothelial growth factor, is a pro-angiogenic factor in the development of CRC and has been proposed as a molecular biomarker for lymph node metastasis. (8)

LOH or Loss Of Heterozygosity, especially located in chromosome 18q, is able to inactivate tumor-suppressor genes and has a certain prognostic value, though this potential biomarker needs further investigation. (8)

Interestingly there is also a metabolomics based biomarker under development: a prediction model using the metabolites 2-hydroxy butyrate, aspartic acid, kynurenine and cystamine has already been tested and proposed as an early detection method for CRC stage 1 and 2. (15)

4.2.2 Lipidomics and its analytical technology

In recent years, lipidomics has been introduced as a new chapter in metabolomics, representing the analysis of lipid metabolism in organelles, cells, tissues and organisms, in health and disease. (6) (13) Lipids embody essential roles in human physiological and pathological processes such as apoptosis, proliferation and carcinogenesis, and although they are extremely complex, more and more scientists are investing time in the development of new techniques for separation and identification of these lipid molecules. (16) The first step in lipid analysis comprises extraction of lipid molecules. This can be accomplished by using a mixture of methanol, chloroform and water (the Blight and Dyer-method). Many more techniques for lipid extraction from samples are available but will not be further discussed here. (16)

Due to its high reproducibility, selectivity and sensitivity for detecting different lipid components in body fluids or tissue samples, mass spectrometry (MS) is the most widespread standard procedure for identification of lipid molecules. MS can be combined with separation techniques such as capillary electrophoresis (CE), gas chromatography (GC) or in most cases, high performance liquid chromatography (HP-LC). (3)(6)
HP-LC separates the different lipid components according to their chemical or physical properties by introducing the lipids/liquid to a high pressurized monolithic silica tube or column, called the stationary phase. A compound solvent, dubbed the mobile phase, is then pushed over the column at high pressure, and this compound solvent is differentially mixed from hydrophilic and hydrophobic primary solvents. Lipids first adhere to the column, but dissolve into the compound solvent mixture and thus elute from the column when the hydrophobicity is sufficiently high. This system thus effectively allows the separation of lipids with different physiochemical properties over time.

Once the separated components have left the column, these lipids are in the MS instrument. Initially, ionization of the lipids is performed, usually via electrospray ionization (ESI), which introduces a charge on the lipid molecules, creating lipid ions. Next, separation according to the mass-over-charge ratio (m/z) is performed by bringing the ionized molecules into a mass analyzer, typically the rapidly oscillating magnetic field of a quadrupole for lipids. The m/z-separated ionized lipid molecules then impact an electron multiplier that serves as a detector, allowing a spectrum to be created of the number of ion impacts per m/z. (17)

4.3 Lipid metabolism and colorectal cancer:

Lipids are apolar, hydrophobic molecules with an essential role in the thermal isolation of the human body and the emulsification of food in the intestines using bile acids. Most importantly for this thesis, they are also an alternative energy supply for cells, provide structural integrity for cellular membranes, and are essential signaling components, be it as second messengers or as hormones.

Cellular lipid metabolism contains complex processes and includes uptake, synthesis, degradation and transport. Every lipid can be regulated by different pathways in different tissues or cells, and activated or inhibited by physiological, pathological or therapeutic circumstances.

Bioactive lipids transduct signals to regulate lipid metabolism using different pathways:

- G-protein coupled receptors
- Tyrosine kinases
- Integrin signaling
- Ion channel signaling
- pH changes
- Oxidative stress
Cancer cells gain defects or alterations in their lipid metabolism, causing changes different cellular processes such as proliferation, differentiation, motility and growth. (5) This could be induced by the over-expression of essential lipid enzymes, altering the cell’s functions and enhancing lipid biosynthesis. Excessive lipogenic enzyme function is correlated with enhanced transcription of lipogenic genes, for instance by the transcription-regulator SREBP (sterol regulating element-binding protein) by growth factors or by growth signaling pathways. Epidermal growth factor, keratinocyte growth factor, erBb-receptors and Her2/neu receptor tyrosine kinase have already been identified, while the PI3K/Akt pathway is activated in multiple types of cancer and plays a central role in the expression of lipogenic enzymes. (18)

According to the Lipid Classification and Nomenclature Committee, lipids are divided in eight main groups: free fatty acids, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides. (5) However, in this thesis, a slight modification in this classification method will be applied. Because mono-, di- and triacylglyceroles as well as glycerophospholipids all originate from a glycerol-backbone and undergo a common biosynthetic pathway (until phosphatic acid). ‘Glycerolipids’ and ‘glycerophospholipids’ will therefore be unified as one term in this thesis: glycerolipids. In addition, lipoproteins could also be included as unique lipid entities. (19) These different lipid classes will be discussed in more detail in the subsequent sections, with a specific focus on their pathway, and any alterations thereof in CRC.

4.3.1 Free Fatty Acids

4.3.1.1 Pathway

The FA biosynthesis usually begins with the provision of acetyl-groups by citrate, generated in the Krebs-cycle. Subsequently, citrate is converted into acetyl-CoA and oxaleacetate by ATP citrate-lyase (ACLY). Acetyl-CoA carboxylase (ACC) is the rate-limiting step of fatty acid-biosynthesis, converting acetyl-CoA into malonyl-CoA. Acetyl and malonyl groups are attached together by fatty acid synthase (FASN). Eventually this generates the substrate palmitic acid (16 C) due to repeated condensations of acetyl-groups. Desaturation is an optional step in the fatty acid biosynthesis, taking place in the endoplasmatic reticulum and executed by SCD (stearoyl-CoA desaturase). This introduces a double bond in the saturated...
fatty acids, making it a mono-unsaturated fatty acid. Palmitic acid is the main saturated fatty acid and can be converted into other saturated or unsaturated fatty acids such as stearic acids, oleic acid and more. (20)

It is important to notice that the pathway described above represents the production of non-essential fatty acids only. The essential fatty acids cannot be produced by humans and other mammals, and can only be obtained through the diet. (21)

The eicosanoids are derived from 20-carbon essential fatty acids: prostanoids, leukotrienes and epoxygenases are produced from ω-6 arachidonic acid (AA), while resolvins and protectins are obtained from ω-3 PUFA (poly-unsaturated fatty acids), including eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). (22)

AA is an essential fatty acid and a key metabolite for the production of eicosanoids via the COX, LOX and CYT P450 (EPOX) pathways. The grand majority of eicosanoids acquire their AA from membrane glycerophospholipids by activation of PLA2, which releases the substrate at the sn2-position of the glycerol-backbone. Another fraction of the AA is obtained via linoleic acid (LA): firstly, γ-linoleic-CoA (GLA) can be obtained by diet or by the slow reaction mechanism of LA-CoA with a ∆6-desaturase. When going through the microsomal elongation pathway, GLA-CoA is exchanged for dihomo-γ-linoleoyl-CoA (DGLA) which is converted into AA by a ∆5-desaturase. (23)

AA can eventually be metabolized through three different pathways for generating eicosanoids: cyclooxygenase (COX)-pathway providing prostanoids, lipoxygenases, leukotrienes and P450 epoxygenase supplying hydroxyeicosatetraenoic acid (HETE), epoxygenes and DHA epoxygenase supplying hydroperoxyeicosatetraenoic acid (HPETE). (22) An overview of the eicosanoid pathways and its products can be seen in the figure below.
Fig. 1: overview of arachidonic acid metabolism
As mentioned above, a small portion of the eicosanoids are derived from the ω-3 PUFA, EPA and DHA, obtained by the α-linolenic acid-metabolism or by diet, and may as well go through the three oxidative pathways in the same way as arachidonic acid. This creates variant series of (benign) prostaglandins, leukotriens, resolvins, protectins and other bioactive lipid mediators as depicted in the figure below. The red marked compounds represent moderately pro-inflammatory products while the green marked compounds represent anti-inflammatory and anti-tumorigenic products. (24)

![Diagram of ω-3 fatty acids metabolism](image)

**Fig.2: ω-3 fatty acids metabolism**

### 4.3.1.2 Known alterations in colorectal cancer

When manufacturing new structural components, healthy cells commonly rely on dietary circulating fatty acids or exogenous intake of carbons, which can be converted into FA’s by liver or adipose tissue. With the exception of some metabolically active tissues such as fetal lung tissue, hepatocytes, adipocytes, lactating breasts and the cycling endometrium, *de novo* lipogenesis-pathway is reduced in most healthy cells. (18) (25)

Cancer cells require sufficient molecular building blocks as a response for their enhanced proliferation and growth capabilities. (20) In these cases, ‘de novo’ synthesis of FA’s is essential for building lipid membranes and signaling molecules, although some tumors are able to capture FA’s from their environment (e.g. using FABP4). Enhanced influx of carbons, introduced as glucose or glutamine, supports the anabolic pathways for building these new fatty acids. Consequently, blocking FA *de novo* synthesis by limiting its supplies, enhancing
FA degradation, pushing FA’s into storage, and/or downsizing FA release from storage could offer new therapeutic strategies in cancer therapy. (18) (26) (27)

4.3.1.3 Alterations in free fatty acid synthesis pathways and distribution

4.3.1.3.1 Enzymes and other proteins

The simplest way to obstruct synthesis is the down-regulation of the conversion of carbohydrates to citrate in the Krebs cycle, blocking the mitochondrial citrate carrier (CIC or protein SLC25A1) or inhibiting the enzymes that take part in the enhanced de novo lipogenesis in neoplastic cells. (20) The mitochondrial citrate carrier is an essential transporter in de novo FA synthesis: citrate exits the mitochondria and is cleaved into oxaloacetate and two acetyl-CoA molecules. Some studies reported that these citrate carriers are up-regulated in colon cancer. (28)

ACLY is a homotetrameric cytosolic enzyme that converts mitochondrial citrate into acetyl-CoA, and thus may provide essential substrates for the FA synthesis pathway and the mevalonate-pathway. A significant increase in ACLY expression and activity has been shown in colonic cancer cells. (25) Another study noted that ACLY was up-regulated in CRC compared to normal tissue, as well in chemo-resistant CRC cells compared to chemo-naive variants. In addition, activation of the PI3K/Akt-pathway, a signal-transduction pathway that promotes cell survival and growth as a response to extracellular factors, is correlated with ACLY activity: enhanced glucose uptake and metabolism leads to upregulated PI3/Akt which phosphorylates and triggers ACLY, increasing FA synthesis and being partially responsible for carcinogenesis. (29) (30)

These findings suggest that inhibition of ACLY may be introduced as a new therapy for targeting cancer cells. RNAi (RNA interference), or pharmacological inhibitors could be functional for down-regulating ACLY, resulting in a growth arrest of those tumor cells. (25)

Experiments with the citrate analogues (+) and (-) 2,2-difluorocitrate, on rat livers resulted in a reduction of ACLY-activity. This outcome was also reached with (-) hydroxycitrate in HepG2cells (a cell-line of polar human hepatocytes), although a restricted membrane transport and necessity of high concentrations of this citrate analogue were profound limitations. Radicicol, an antifungal macrolide, also inhibits the enzyme in rat liver cells, whereas SB-204990 restricts proliferation in lung cancer cells. Also, combining the ACLY
inhibitors with a statin seems to have profound anti-tumorigenic effects. These findings are still somewhat controversial and further investigation is desirable. (25)

As in many cancers, fatty acid synthase (FASN) is over-expressed in CRC, with the metastatic variant taking account for the highest expression and correlating with a poor prognosis. The theory behind this relationship remains unclear. (31) FASN knockdown neutralizes CRC cells by restraining the cells’ energy metabolism and with it, the mTOR-pathway, a serine/threonine kinase member of the PI3K pathway that enhances expression of different lipogenic enzymes. (32)

It seems that the clinical stage of CRC is significantly correlated with the serum FASN of the patient: concentrations were higher in stage 3 and 4 than in stage 1 and 2. (33) Another study suggested that the FASN serum level is associated with the TNM-stage: patients with a tumor extent T1 and T2 without lymph node metastasis nor distant metastasis have a significantly lower FASN serum than patients with a T3 or T4 tumor extent and lymph node metastasis or distant metastasis. Furthermore, the disease-free interval and the five-year overall survival rate was smaller for patients with an elevated serum FASN level. This suggests that FASN could be a potential biomarker, informing us about the clinical state of the tumor and patient prognosis. (34)

FASN could also be a potential anti-angiogenetic target: knockdown of this enzyme decreases the microvascular density and inhibits release of VEGF-A. This stabilization in secretion of anti- and pro-angiogenetic factors, could inhibit the tumor vascularisation and by consequence, reduce the probability of metastasis. (31) Inhibitors of FASN, cerulenin, orlistat and C75, induce apoptosis in several cancer cell lines, including colorectal cancer, and could be a new effective treatment strategy. (35)

Acetyl-CoA carboxyl (ACC) has two subtypes in the human body, both having different metabolic functions: ACCα and ACCβ. (20) TOFA(5-tetradecyloxy-2-furoic acid), an allosteric inhibitor of ACCα and a cytotoxic agent, disturbs de novo lipogenesis, induces apoptosis in cells, and thus may be of use as a therapeutic in colon cancer. (36)

Stearoyl COA-desaturase (SCD) has a clear role in the lipid metabolism and growth pathways of cancer cells: by increasing lipogenesis through inhibition of FA oxidation and alteration of several intracellular pathways, activation of Akt-pathway and deactivation of AMPK-
pathways, it creates a benevolent environment for cell survival and proliferation. Inhibition is known to cause apoptosis in many cancer types, including colonic tissue, and could be a promising new therapeutic target in cancer interventions. (20) Although a higher expression and activity of SCD1 were found in some colonic carcinoma tissues, there is still insufficient evidence regarding this enzyme in CRC. (37) One study measured the SCD1-activity by calculating the 16:1 n-7/16:0 ratio, which showed a lower mean activity in colorectal cancer than in normal mucosa. The SCD2 activity on the other hand, measured by the 18:1 n-9/18:0 ratio, showed an augmentation of this enzyme in CRC tissue. (38)

Despite the proof that blockage of lipogenic enzymes could reduce tumor growth in colorectal cancer cells in vitro, more research has to be performed to examine if this could be a long term strategy in CRC treatment in vivo, especially because more evidence has been revealed that an alternative mitochondrial pathway for de novo lipogenesis in mammalian cells exists. (39)

The CPT (carnitine palmitoyl transferase) 1-transporter, transferring the long FA chains into the mitochondria where β-oxidation takes place, seems to be significantly decreased in colorectal and breast carcinoma. (40) CPT-1 participates during the metabolic transformation (solid) cancer cells undergo when faced with metabolic stress. Continuous tumor growth deprives cancer cells from their oxygen and nutrient (glucose) supply, forcing them to search for an alternative, anaerobic form of energy. Apparently, cancer cells up-regulate their CPT1C-gene, leading to promoted FFA-oxidation and generation of ATP as energy source. Indeed, experiments on colorectal cancer xenografts showed a decrease in tumor growth when CPT1C was depleted. (41)

4.3.1.3.2 Fatty acid distribution
FA distribution of healthy and CRC tissues illustrated that there is a definite correlation between the colorectal cancer stage and FA profiles. Both samples contained non-essential palmitic acid, stearic acid, oleic acid, and the essential linolic acid as the most profuse FA. (38)

CRC tissue showed a shift in FA distribution with a higher rate of saturated FA. While a 50% increase of stearic acid was noted, the amount of myristic and palmytic acid remained relatively stable in CRC. On the contrary, the mono-unsaturated FA palmitoleic and oleic acid were respectively 50 and 20% lower in CRC than in normal mucosa. (38)
The ratio in \( \omega-6/ \omega-3 \) PUFA was increased in CRC with the total \( \omega-3 \) PUFA being decreased compared to normal samples. (21) Dihomo-\( \gamma \) linolic acid (DGLA) and AA showed an augmentation of respectively 34% and 38%. (38) One study proved that linolic acid levels were downsized: this finding combined with the elevated values of AA could indicate that there is a higher turnover into this pro-inflammatory product. (21) This hypothesis was contradicted by another study, where linolic acid levels were slightly elevated together with arachidonic acid. Furthermore, AA/LA, AA/DGLA, and AA/ -total \( \omega-6 \) PUFA ratios were higher, thus indicating that desaturase-enzymes may be up-regulated in CRC. (38)

The concentrations of EPA and DHA, described as percentages of total FA concentration, were respectively 88% and 37% decreased in CRC samples compared to healthy samples. These two products are anti-inflammatory and decrease the levels of colorectal biomarkers. (38)

The relationship between FA distribution and clinicopathological stages seems to remain unclear. When comparing the saturated FA and mono unsaturated fatty acids (MUFA), there were no significant differences between colorectal tissues with Duke B and C stages. Only palmitoyl acid and 18:1 \( \omega-9 \) were almost half the amount in the C-stadium than in the B-stadium. Concerning the PUFA, the ratio \( \omega-6/ \omega-3 \) was strongly increased in the Dukes C stadium compared with the B stadium. AA levels in stage C were six times higher than stage B, but the linoleic acid level was reduced by almost 66% in stage C compared with stage B. Another study observed no differences in SFA, MUFA and PUFA between the clinicopathological stages (I, II and III-IV) or lymph node metastasis. (21).

\( \Omega-3 \) fatty acids (EPA and DHA) suppress AA-derived eicosanoid production by replacing the membrane bound-AA by \( \omega-3 \) PUFA, competing with desaturases and elongases and inhibiting formation of LA to AA. Altogether this makes AA less available and consequently, suppresses AA-derived eicosanoid production. (42) These \( \omega-3 \) PUFA replace pro-inflammatory substrates in the COX-2-pathway while EPA acts as the preferred substrate for the LOX-pathway, blocking pro-angiogenetic and inflammatory prostaglandins or leukotrienes. (28)(42) In addition, EPA and DHA seem to have a protective effect against CRC by enhancing the caspase-dependent apoptosis pathway through down-regulation of two regulatory elements, FLIP and XIAP. Another encouraging finding is that EPA and DHA do not trigger apoptosis in healthy colorectal cells, which could make these potential and effective adjuvants with other chemotherapeutic agents. (44)
These results suggest that the ω-6 PUFA, AA in particular, promote carcinogenesis in colorectal tissue and that ω-3 PUFA, DHA and EPA, attempt to restrain it.

4.3.1.4 Alterations in eicosanoid metabolism and distribution

4.3.1.4.1 COX-pathway

There are 3 variants of COX-enzymes: COX-1, COX-2 and COX-3. COX-1 is a house-keeper enzyme of gastric mucosa, renal bloodflow and platelet activation that remains stable under physiological and pathological circumstances. COX-2 has an unmistakable role in inflammation and other pathophysiological processes. COX-3 is a variant of COX-2 with a currently unclear function.

It is well well-known that COX-2 expression is augmented in CRC: 50% of adenoma and 85% of adenocarcinoma had elevated values. (45)

The prostaglandin PGE2 seems to be a key factor in colorectal tumorigenesis, promoting cell growth, angiogenesis (via VEGF) and tumor survival. (31) PGE2 was strongly up-regulated in metastatic tumors, thus enhancing invasion and urine samples with elevated PGE2 values gave an increased risk in colorectal cancer development. (22) (46) It is the most dominant prostaglandin present in CRC tissue. (45) Lung, head and neck and breast cancer also had an elevated quantity of PGE2 and were all associated with poor prognosis. (28,29) PGE2 prostaglandins are degraded by 15-PGDH, which if knocked out in murine subject animals, induced a 7.6 times increase in risk for developing colon tumor with a doubling of PGE2 values. Transgenic mice deficient in prostaglandin target receptors EP (1-4), display a significant reduction in colon cancer incidence. (22)

Prostacycline- or PGI2-values appear to be decreased in CRC tissue, meaning that this prostaglandin may not be essential in colorectal carcinogenesis, though a study showed that addition of PGI2 analogues had a protective effect against metastasis. (22) It may have a role in tumor progression due to the fact that it activates PPARδ, thus accelerating intestinal tumor growth in mice subjects. Further investigation will hopefully clarify the specific role of PGI2. (45)

Although prostanoid receptors DP2 gradually stop being expressed during the adenoma-carcinoma sequence, PGD2s function still remains unclear. Inhibition of CRC cell proliferation has been observed when treated with PGD2, but its metabolites (PGJ2 and 15dPGJ2) induced cancer cell proliferation and survival. (22) One proposed hypothesis is that 15dPGJ2 may inhibit tumor cell growth through binding of the PPARγ-receptor. (47) Another
theory is that over-expression of PGD-synthase could lead to a metabolic shift in which less PGE2 is produced and PGH2 is predominantly converted into PGD2, suppressing tumor growth. More investigation about PGD2 and its effects in CRC and other cancer cells seems necessary. (45)

Prostaglandin F2α has no known role in carcinogenesis of CRC and will not be further discussed. (48)

TxA2 is derived from the COX-1 and COX-2 pathway and contributes to tumor growth and angiogenesis promotion. (45) Targeting COX-1 with aspirin proved to have an anti-carcinogenic effect. Furthermore, a direct addition of TxA2 after deletion of tromboxane synthase erased cell arrest in CRC and promoted proliferation. The tromboxane synthase seems to gain some expression in CRC tissue compared to normal mucosa, with TxB2 metabolites and their urinary excretion being significantly raised in these patients. (22) Also, a TxA2 synthase-inhibitor was shown to be able to block liver metastasis in CRC. (45)

NSAIDs have proven their effects in blocking inflammation, but could also offer anti-carcinogenic effects thus reducing the risk of colon cancer and other prevalent solid tumors (breast, prostate, lung) via COX-2 inhibition. (47) A quantity of studies confirmed that intake of aspirin or other NSAID on a regular basis during a 10-15 years period gives a relative risk reduction of 40-50% for the development of colorectal adenoma. (45) As mentioned before, the question thus arises if NSAIDs or aspirins could be part of the colorectal cancer prevention strategy, albeit in combination with proper exercise, healthy diet and avoidance of other carcinogenic risk factors. (49)

4.3.1.4.2 LOX-pathway

CRC patients often gain enhanced activity of 5-LOX and 12-LOX. With 5-LOX being over-expressed in human tubular adenoma, villous adenoma and colorectal adenocarcinoma, it may be associated with chronic inflammation and carcinogenesis. 12-LOX over-expression could be more of an incentive for metastasis, while also participating in angiogenesis and cancer cell proliferation. (42) Eicosanoid profiling illustrated that not only PGE2 and AA, but also 12-HETE was significantly altered in CRC tissue compared to normal mucosa, though without any correlation with Dukes stadium for this metabolite. (48) Even though 15-LOX-2 didn’t show any significant alterations in expression, a 125-patient prospective study found down-regulated 13-S-HODE (a 15-LOX-1 product from linoleic acid) concentrations when going through the mucosa-adenoma-carcinoma sequence of the colon. (22) Indeed, 13-HODE
showed significantly augmented values in normal colorectal mucosa compared to colorectal adenoma. (50) 13-S-HODE has a tumor-suppressive role, inducing cell cycle arrest in cancer cells and restoring apoptosis via PPAR-δ activation. (51) Other LOX products effects are also induced via the superficial G-protein cell receptors or activation of the PPAR family. (22)

Both COX-2 and LOX-5 have pro-carcinogenic effects and blocking these pathways could enhance a shift of free AA to another, producing more pro-tumorigenic metabolites. Dual blockade of both enzymes could provide as a new chemo-preventive strategy: it has already been found safe and effective as treatment in osteoporosis. (42) An inverse relation between 15-LOX-1 and COX-2 has also been noted: this was confirmed in a study where metabolism of LA shifted towards the COX-2 and away from the 15-LOX-1 pathway when progressing through the adenoma-carcinoma sequence: while 96% of low-grade adenomas expressed the 15-LOX-1 gene, this was only the case for 43% of carcinoma-in-adenoma lesion subjects. Regarding COX-2, 2% of low-grade adenoma expressed the gene, whereas this was 71% for the carcinoma-in-adenoma and even 92% of the advanced carcinomas. (52) Furthermore, products that up-regulate 15-LOX-1 and down-regulate COX-2, such as sulforaphane or Honokiol, inhibit intestinal polyp formation and gastric carcinogenesis, respectively. Development of new chemotherapeutic agents and using these metabolites as molecular biomarkers could become valuable in colorectal cancer screening, prevention or treatment. (46)

4.3.1.4.3 CYT P450 epoxygenase pathway

The explicit role of this pathway in cancer development has not been cleared up yet, though CYP2J2 and other CYP enzymes seem to be over-expressed in a variety of cancer cell lines, including colon cancer.(42) It is supposed that that this pathway metabolizes free AA, inhibiting ceramide production and apoptosis (see 4.3.3.2), but also gaining 14-,15-EET, inhibitors of apoptosis via the PI3K-Akt pathway. (53) Though there is little known about the role of EETs in CRC progression, patients with these types of solid tumors have elevated levels of these compounds in blood and urine. Treatment of endothelial cells with 14-,15-EET induced tumor growth and metastasis promotion. On the other hand, inhibitors of epoxygenases or EET-antagonists were capable of containing tumor development in some cancer types in rat subjects (for example glioblastoma), thus prolonging the animals’ survival. (54) Whether these findings could be similar for CRC in humans needs to be further investigated.
As mentioned, DHA is in competition with AA for being processed along the epoxygenase pathway. This generates epoxydocosapentaenoic acids (EDPs), mediators with anti-angiogenic and anti-tumorigenic effects. In mice, administration of 19,20-EDP blocked VEGF- and FGF2–induced angiogenesis, restrained primary tumor growth by 50-90% and gave a 70% reduction of lung metastasis foci and weight. The question arises if DHA or other ω-3 PUFA can be administered to CRC patients with high epoxygenase expression profiles to reduce EET levels and increase EDP levels in patients, thus contributing to a better prognosis. (24)

4.3.2 Glycerolipids

4.3.2.1 Pathway

The triglycerides (TG) and glycerophospholipids are glycerolipids that share a common biosynthetic pathway: the glycerophosphate pathway. (5) Both products can be obtained via the intermediate phosphatidic acid (PA), though all products have a completely differing FA composition. (55)

TG are the first type of glycerolipids, consisting of a glycerol backbone and three esterified FA’s, all different in length and saturation level. TG can be stored in lipoproteins (e.g. LDL, HDL chylomicrons…) as well as in lipid droplets, present within every cell-type, but most commonly found in adipocytes. (55)(56)

Glycerophospholipids are also built of a glycerol backbone with two esterified FA at the sn1- and sn2-position but with a polar head group attached to position sn3. These are the cell membrane’s most abundant building blocks, for which the composition varies from each type of cell, organelle, inner or outer membrane. Each composition in turn shows dissimilarities in cellular functions such as vesicular transport, membrane viscosity and signal transduction. Due to their FA remodeling system (see further), glycerophospholipids are an extremely important source of lipid mediators such as lysophospholipids and saturated or unsaturated FA’s, which can be converted into eicosanoids and other lipids. (57)

The glycerophospholipids are classified according to the structure of their polar head groups. (58) Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) or
cardiolipin are the major classes. These phospholipids can be further subclassified according to the type of chemical bond with the FA at the sn1-position: diacyl, alkylacyl and alkenylacyl. (55) (56) The heterogenity of glycerophospholipids is further increased due to the different FA-groups at the sn1 and sn2-position: while saturated FA’s are more common on the sn1-position, unsaturated FA’s are mainly present on the sn2-position. (55)

In tissues other than adipocytes, synthesis of PA commences with the glycerol-backbone, which is activated by phosphorylation at position C3. The glycerol-3-phosphate is incorporated with a FA-CoA via the Glycerol-3-Phosphate acyltransferase (GPAT), converting it into lysophosphatic acid (LPA). This is followed by 1-acylglycerol-3-phosphate acyltransferase (AGPAT), creating phosphatic acid (PA), the key intermediate in triglyceride and glycerophospholipid synthesis. This metabolite can also be produced via a different pathway that is mainly used in adipocytes, which are lacking the glycerol-kinase enzyme. (56) Consequently, 1-alkyl LPA is gained through use of dihydroxyaceton-phosphate (DHAP), a metabolite produced during glycolysis, by acylation with DHAP-acyltransferase (DHAPAT) and reduction by DHAP oxidoreductase. Eventually, AGPAT becomes involved in the process and leads to the formation of 1-alkyl PA. (55) Lastly, PA can also be obtained through phosphorylation of DAG, using the DAG-kinase enzyme. (58) The mitochondrial GPAT is more likely to utilize saturated FA’s, while the microsomal GPAT generally uses unsaturated FA’s. (59) There also seems to be a correlation between the type of mitochondrial GPAT and AGPAT and the selection of fatty acids as substrates, although the mechanism behind this remains uncertain. (60)

When PA follows the TAG pathway, PA-phosphatase (PAP) removes the phosphate group, gaining 1,2-diacylglycerol (1,2-DAG). Eventually, the last fatty acid group is esterified by DAG acyltransferase (DGAT), recovering TAG. (60) Though this pathway is commonly present in liver and adipose tissue, intestines use the mono-acyl glycerol pathway in gaining TAG, where absorbed dietary MAG can be sequentially converted into TAG by MGAT and DGAT. (55)
Homeostasis of glycerophospholipids is extremely complex and the mechanisms concerning de novo synthesis, degradation, fatty acid remodeling and interorganelle transport are not yet fully understood. \cite{58} Briefly summarized, glycerophospholipids can be gained via two different pathways. Initially, after dephosphorilation of PA by PAP, DAG is converted into PC and PE: this is the CDP-choline and CDP-ethanolamine pathway, also referred to as the Kennedy pathway. The CDP-DAG pathway on the other hand, produces PI, PS and PG through the reaction of PA with inositol, serine and glycerol respectively, while being aided by CDP-DAG-synthase. \cite{5,55,61} Furthermore, glycerophospholipids are able to convert themselves into another type of phospholipid: PG can be irreversibly converted into cardiolipin, while PS can alter into PE and vice versa. \cite{61} A more detailed description of the synthesis of glycerophospholipids is comprehensively described in a review about glycerophospholipid homeostasis in mammalian cells. \cite{58}

Newly synthesized glycerophospholipids have an altered fatty acid composition compared to mature phospholipids. \cite{61} All glycerophospholipids are submitted to fatty acid remodeling, also called the Land’s cycle, thus creating a deacylation-reacylation reaction between phospholipids and lysophospholipids. Deacylation is catalyzed by PLA1 or PLA2, depending on whether it takes place at the sn1 or sn2 positions respectively. Lysophospholipid acyl transferase (LPAT) substitutes the original FA by another FA on the glycerol backbone and
are divided in two protein families according to their substrate specificity: AGPAT (also mentioned in the glycerophosphate pathway) and membrane bound O-acyltransferase (MBOAT). (55)

Fig. 4: Fatty acid remodeling in glycerophospholipids; adapted from (55)

Degradation is another important step in the glycerophospholipid homeostasis process and it is maintained via different lysosomal and non-lysosomal phospholipases. Alongside PLA1 and PLA2, PLC and PLD may also participate in the glycerophospholipid homeostasis. How the coordination between biosynthesis and degradation of glycerophospholipids is realized, still remains unclear. (58)

Fig. 5: Action points of phospholipases on the glycerol backbone; adapted from (5)
4.3.2.2 Glycerolipids and colorectal cancer

4.3.2.2.1 Alterations in the glycerophosphate pathway

When starting with the first step in the glycerophosphate pathway, an enhanced uptake of glycerol in HCT-15 colon cancer cells was observed. (62) Glycerol, an important metabolite in gluconeogenesis, oxidation and lipogenesis, is transported via a specific Na⁺-dependent carrier. This glycerol transporter may be a new target for drug development by reducing the rate of the glycerophospholipid pathway, though more research is necessary on this subject. (62) (63)

While GPAT seems to deliver no contribution to the carcinogenesis process, some evidence indicated that AGPAT is over-expressed in several human cancers. AGPAT2 inhibition induces growth arrest, necrosis or apoptosis and blocks RAS/RAF/Erk and PI3kinase/Akt pathways. In addition, the expression of AGPAT11/LPCAT2 and AGPAT9/LPCAT1 were found to be up-regulated in CRC tissue in comparison with normal mucosa. (64) This could imply that there is a particular role for PA, being involved in cancer cell progression by amplifying the Ras signal and activating the pro-survival MAPK and PI3K/Akt pathways and thus the survival of tumors. (61)

LPA is an essential intermediate, influencing many pathological processes such as fibrosis, inflammation, asthma, atherosclerosis and cancer. LPA is produced after Lyso-PLC reacts with Lysophospholipase D or when PA is deacylated by PLA1 or PLA2. The effects of LPA are maintained by LPA receptors, which are coupled with specific G-proteins for regulating downstream receptors. (65) Although LPA is a mitogen capable of enhancing proliferation in CRC via interference with the APC/β-catenine pathway, this specific pathway is mutated in the majority of tumor cells. There are hypotheses that LPA compensates this by over-expression of Krüppel-like factor, a transcription factor that enhances intestinal crypt cell proliferation. (66) Either way, the proliferation effects of LPA in an affected colon cancer cell line depends on the type of LPA receptor. For instance, proliferation of DLD1 colon cancer cells is promoted by LPA1, while HCT116 cells require LPA2 and LPA3. Moreover, activation of LPA2 would deliver anti-apoptotic signals to colon cancer cells. (67) LPA2 also showed elevated levels in CRC, while LPA1 receptors were down-regulated in colorectal adenocarcinoma compared with normal colonic mucosa. The fact that these receptors are aberrantly expressed in CRC and with LPA demonstrated to have proliferating, anti-apoptotic
effects could mean these play a certain role in the development of this disease. (68) Lastly, cyclic Phosphatidic acid (cPA) is a structural analogue of LPA but instead, it has anti-proliferative effects on DLD1 colon cancer cells. It inhibits cyclin D expression, blocks the PI3K pathway and may have some potential as a therapeutic inhibitor of CRC progression. (69)

4.3.2.2 Glycerophospholipids

The fatty acid remodeling enzymes, LPCAT, are able catalyze alterations in the colorectal lipid profile, consequently contributing to cell malignancy. (67) Phosphatidyl choline (PC) is an important structural element in cell membranes and plays a key role in cell cycle regulation, proliferation and apoptosis by providing lipid second messengers. (27) Augmented values of PC (16/0:16/1) were observed in CRC samples with more advanced stages. Moreover, the ratio of PC (16/0:16/1)/LPC (16/0:16/1) was increased in colon cancer cell membranes, implying an up-regulated activity of LPCAT4. Yet it still remains unclear if other factors also play a role in this PC increase. (70)

Distribution of choline containing phospholipids in plasma were compared between healthy subjects, individuals with adenomatous polyps and CRC patients. A detailed description of these data can be read in Li, S. et al. Shortly summarized, LPC plasma levels were gradually decreased through the colorectal adenoma-carcinoma sequence, with the lowest concentrations present in healthy individuals. Instead of colonoscopies, different species of LPC could be utilized as new detection biomarkers for CRC. These could even be combined with other lipid metabolites that are typically decreased in patient plasma such as sphingomyeline or sphingophosphocholine. (71)

PI molecules are best known for their roles in the PI3K-Akt/mTOR pathways: PI3Ks are important kinases in gaining inactive PIP, PIP2 and active PIP3, which act as second messengers and transduct signals from trans-membrane receptors to the cytosol. Since these interfere in different cellular processes, failing of this pathway may result in continuous activation with up-regulated angiogenesis, proliferation and cell survival in tumors due to enhanced lipid metabolism and protein synthesis. Deletion of or mutations in the PTEN tumor suppressor gene, which codes for a phosphatase that degenerates PIP3 was observed in CRC tissues. Methods for inhibition of PI3K or conversion of active PIP3 to PIP2 or PIP, could be valuable as new therapies in many different cancers, including CRC. Recent years, different
small molecule PI3K inhibitors have already been tested in clinical trials, with promising results, but more research still remains necessary. (72) (73) (74)

4.3.2.2.3 Phospholipases

Findings suggest that PLA’s may be important regulators of cell growth. Glycerophospholipids are known to be catabolized by PLA’s into FFA and lysolipids. Using PC as example, these metabolites has potential proliferative effects on the one hand, but can also re-enhance PC synthesis on the other hand by integrating these catabolized products again in the CDP-choline pathway. This mechanism could be similar for the catabolism of other types of glycerophospholipids. (27)

PS-PLA1 is a phospholipase that acts specifically on PS (phosphatidylserine) to produce lysophosphatidylserine (LPS). Expression of PS-PLA1 was significantly higher in CRC samples with increased tumor size, deeper invasion and presence of hematogenous metastasis. Higher PS-PLA1 levels were also correlated with a decrease in disease-free survival. (75)

PLA2 is essential for releasing FFA at the sn2 position of glycerophospholipids. This includes AA, a metabolite of the COX-1 and, more important, COX-2 pathways. As described earlier, this is the source of pro-tumorigenic prostaglandins, for example PGE2. Defects in PLA2 could therefore be a protective factor in colorectal tumorigenesis. Cytoplasmic PLA2 was already showed to be augmented in almost 50% of all types of colon cancer cell lines and indeed, COX-2 expression was significantly correlated with cPLA2 expression. cPLA2 expression could enhance colon cancer development, although possibly in a less determining way in comparison to COX-2, which is over-expressed in 80% of all colon cancer cell lines. (76) In addition, it seems that (groupIIA) PLA2 positive cancer cells are correlated with a lower disease free survival and patients live significantly shorter than those with PLA2 negative tumors. Furthermore, PLA2 is most abundantly expressed in stage II colorectal cancer and the colorectal tumor’s position showed some correlation with the PLA2 expression: almost 70% of right-sided tumors were completely negative for PLA2 while this was the case for only 42% of the left sided tumors. (77)

Platelet activating factor (PAF) does not only play a role in coagulation and immunology, but is also suspected to take part in the carcinogenesis of colorectal and other tissue. PAF is generated from a membrane-bound glycerophosphocholine by PLA2, gaining Lyso-PAF
which is followed by acetylation of the Lyso-group. Lastly, PAF can be catabolised by acetylhydrolase (AHA). Remarkably, a worsening prognosis correlates with the amount of catabolic products and enzymes of this pathway that are present in CRC tissue: T1-T4N0M0 had up-regulated activity of PLA2 and AHA with elevated tissue levels of LysoPAF and PAF, TxN1M0 CRC showed the same profile but lacks elevation of PAF, while distant metastasis (TxNxM1) CRC only showed an augmentation of AHA levels. (78) Furthermore, plasma levels of sPLA2 and AHA were significantly, though modestly, increased in patients with CRC. (79) These results imply that PAF de novo synthesis is increased in CRC, maybe causing amplification of angiogenesis by VEGF production. Much still remains unclear however, and the question thus arises if these findings are relevant. In particular, information about this topic has apparently not evolved since 2003, which may indicate that further investigation has not been successful, or that PAF has no interesting prospective in CRC. (78)(79)

PLCδ1, a variant of phospholipase C, had a significantly reduced expression in CRC cell lines. The data indicated that this enzyme inhibits tumor development, motility and invasiveness. (80)

Phospholipase D degrades PC into PA and choline, while being aided by cofactor PIP2. phospholipase D has a dual function: it provides structural integrity to (intra)cellular membranes but also has a signaling function via PA and protein-protein interactions with GTPases, kinases and phosphatases. Over-expression of phospholipase D has been found in a variety of tumor types including CRC and is directly associated with angiogenesis, tumor survival, cell migration and metastasis. Small molecules that are capable of inhibiting PLD could be a promising new strategy in many types of cancers. (81)

4.3.2.2.4 Triglycerids

Serum and tissue concentrations of triglycerides have also been intensively investigated in healthy and CRC patients. When serum and tissue lipid levels were compared between normal and malignant colorectal tissue, tissue triglycerides were significantly down-regulated in cancerous tissue but no significant correlation was noted for serum triglycerides. The clinical TNM stages of colorectal cancer did show a correlation for both tissue as serum triglycerides, where a progression in reduced triglycerides levels was noted in stage 3 and 4. Triglyceride levels were lower in all patients with lymph node metastasis than those without lymph node
metastasis. Furthermore, a dose-dependent positive association between adenoma occurrence and triglyceride levels, with the distal colonic adenoma having the highest significance, suggests a location dependent mechanism of lipid metabolism in colon cancer development. (82) (83)

4.3.3 Sphingolipids

4.3.3.1 Pathway

Sphingolipids are composed of a sphingoid base backbone, most commonly sphingosine, sphinganine or dihydrosphingosine, connected to a FFA via an amide-bond and a head group (hydrogen, choline, serine, ethanolamine…) via an oxygen-bond. Depending on the type of head group, the sphingolipids are divided in three main groups: sphingomyeline (SM), ceramide and glycosphingolipids. (84)

![Fig. 6: overview of sphingolipid metabolism](image)

Ceramide is the central molecule in the sphingolipid metabolism. De novo synthesis commences with palmitoyl-CoA and serine, followed by the intermediate products sphinganine and dihydroceramide and eventually, synthesis of ceramide. (85) Ceramide can be converted into ceramide-1-P, SM, glycerosphingolipids and sphingosine. A phosphate-
group can be added to this latter product, creating sphingosine-1-phosphate (S1P). Up to this point, all mentioned steps can also be reversed for generating ceramide. Lastly, S1P-lyase irreversibly divides S1P into ethanolamine-phosphate and palmitaldehyde.

It is important to notice that SM is commonly obtained by release from the cell membrane due to sphingomyelinase (SMase). (86)

Glycosphingolipids are an extremely complex subgroup and it falls beyond the reach of this thesis to characterize these. Briefly summarized, ceramide can be converted into galactosylceramide or glucosylceramide, which in their turn can be elongated with other saccharides such as mannose, fucose, galactose, N-acetylgalactosamine and N-acetyleneuraminic acid. The complexity of this process is illustrated in the figure below, which represents the synthesis of glycosphingolipids in the brain. (87)
4.3.3.2 Sphingolipids in colorectal cancer

Sphingolipids are abundant in the gut system, with twice the amount located in the small intestine compared to the colon. (84) Not only are these compounds crucial structural components in cell membranes, but they also provide important bioactive metabolites for intracellular signaling and regulation of countless cellular processes including apoptosis, growth, differentiation, proliferation, angiogenesis, cell adhesion, migration, inflammation and lymphocyte traffic. (43) Even though ceramide, sphingosine and S1P are considered the most important bioactive sphingolipids, other metabolites also have their role. (89) An important remark is that sphingolipids are quite restricted in traveling to other cellular compartments. Therefore, effects of sphingolipids will largely take place within the compartment where they are normally settled. (90)

As sphingolipids encompass many cellular functions, it is not improbable that alterations in their metabolism could trigger processes that promote colorectal carcinogenesis or that grant them resistance against certain (chemo)therapies. (90) Discrepancies in CRC concerning the metabolism of every sphingolipid subgroup will be described in the next paragraphs with some suggestions for therapy or screening opportunities. (86)

Regarding the SMnases, there are three subtypes of this enzyme: neutral SMase, alkaline SMase and acidic SMase. A 1997 study showed distinct alterations in activity or presence of these subtypes in CRC patients, who were ordered according to their Dukes stadium (A, B or C). Alkaline SMase and neutral SMase had a decrease of respectively 75% and 50% in CRC tissue compared with normal colorectal mucosa. Both enzymes were significantly lower in stage B and C than in stage A. Acid SMase on the other hand, had no significant activities in
CRC. An important issue is that this study contained only 18 subjects, so these results should be interpreted cautiously. (91)

Introducing 1,2-dimethylhydrazine, a DNA-methylating carcinogenic agent, to rat colon mucosa gave a significant SM increase and SMase decrease, just before the stage when colonic adenoma developed. (86) (88) This strongly suggested that a reduction in hydrolysis of SM prior to the malignancy process takes place. (9)(88) In addition, Dillehay et al. discovered that a SM-rich diet introduced to mice, who were again treated with 1,2-dimethylhydrazine, restricted formation of aberrant crypts (pre-stadium lesions of colon cancer). However, colon cancer incidence was not significantly reduced in these mice. (92) Plasma levels of SM were also decreased in patients with adenomatous polyposis (AP) compared with healthy subjects and CRC patients, meaning that this could be a useful biomarker for detecting AP. (71) Altogether, these results point out that SM could play its part in the early stages of colorectal cancer development. (86) (93) (94)

Ceramide is a bioactive molecule that inhibits cell proliferation and activates apoptosis by modification of different molecules and pathways (AKT, Bcl-2, PKCa…) (88) It can undergo glycolysis, hydrolysis or phosphorylation, which determines whether this sphingolipid metabolite will obtain apoptotic or mitogenic capacities. Additionally, an abundant quantity of ceramide is generated when the colorectal cancer cells are stressed by radiotherapy, chemotherapy, hypoxia or nutrient deprivation which activates apoptotic pathways. (95)

Investigation of primary and metastatic colon cancer in human subjects, pointed out that ceramide levels were less than half the amount found in normal colon mucosa. (93) Ceramidase, which also is divided in a neutral, acid and alkaline subtype, is an important regulator of sphingosine and S1P. However, the ceramidase activity did not appear altered in CRC patients, implying that low ceramide levels are predominantly caused by reduced SMase activity. (91) This theory however, still requires more evidence because other factors may be responsible for the changes in SM and ceramide levels such as alterations in de novo sphingolipid synthesis, ceramide glycolysation and other processes. (86)

Here again, administration of ceramide analogues, C2 and C6, or inhibitors of ceramidase, D-MAPP and B13, induced cell death in human CRC cell lines (SW403). In particular B13 had astounding effects when introduced to mice, who had their liver injected with human colon
cancer cells. 70% of the animals with the SW403 and 100% of the animals with the LoVo cell line remained tumor-free after treatment with B13. (93) Furthermore, treatment of CRC with chemotherapy combined with an adjuvant that blocked de novo ceramide synthesis (Fumonisin B1) or GlcCer production (1-phenyl-2-palmitoylamino-3-morpholino-1-propanol or PPMP) was observed. Fumonisin B1 as adjuvant significantly decreased the rate of CRC cell apoptosis and increased the GlcCer levels in comparison with chemotherapy alone. PPMP administration on the other hand doubled the ceramide concentrations and increased the cell death ratio by 88%. (96) All of these molecules that interfere with the ceramide metabolism by augmenting ceramide concentrations, may prove useful as adjuvant chemotherapeutic agents against colorectal cancer. (97)

Breakdown of ceramide by ceramidase offers sphingosine, a sphingoid base. Literature claimed this molecule is quantitatively restricted in comparison to its phosphorylated variant, S1P. Sphingosine has been put forward as a signaling molecule with pro-apoptotic and tumor-suppressive properties by regulating Protein Kinase C-isomorfs and acidic nuclear phosphoproteins. (98) When sphingosine and sphinganine were added to a human colon cancer cell line, this induced cell arrest at G2/M and augmented the rate of apoptosis in the neoplastic cells. This effect was similar for C2-ceramide, a short chain ceramide analogue, but didn’t emerge for C2-dihydroceramide, a short chain dihydroceramide analogue, indicating that the effects have something to do with the 4,5-trans double bond in ceramide, which is absent in dihydroceramide. (99)

S1P is more likely to be a pro-carcinogenic factor, with mitogenic and pro-angiogenic properties, inhibiting apoptosis and promoting maturation of colorectal neoplasm cells via S1P-receptors. (86) It neutralizes the pro-apoptotic effects of ceramide and sphingosine when added to tumor cells under stress reactions. (98) S1P also appears to be the missing connection between colorectal inflammation and CRC development: by continuously activating NF-kB and STAT-3, it provides proliferative and survival advantages to colorectal cells. Moreover, STAT-3 again activates the S1P-R, creating a vicious circle in the promotion of cell clonal expansion. (100) New therapeutic strategies with specific anti-S1P antibodies already appear quite promising in different tumor lineages, including CRC. (101)
Deficiency of sphingosine-kinase-1 (SphK1) in mice inhibits colon polyp formation and furthermore, is generally over-expressed in human colon cancer. (100)

Observation of SphK in colorectal tissue showed no expression in normal mucosa, negative or moderately positive expression in adenoma, but positive expression in 89% of adenocarcinoma and significantly higher expression in all metastatic adenocarcinoma. There was, however, no correlation between SphK-expression and tumor invasion. (89)

Furthermore, S1P-lyase and Sphingosine-Posphatase were under-expressed in human colon cancer cell lines, thus blocking S1P catabolism and apoptosis. The fact that S1P-lyase was under-expressed could even suggest that sphingolipids obtained by diet, are not metabolized in colon cancer. (102)

The S1P-pathway and the COX-2 pathway have been shown to share a connection: SphK shutdown decreased COX-2 and PGE2 production, while S1P enhances COX-2 expression and PGE2 production. (88) Then again, ceramide kinase and ceramide-1-P are necessary for translocation and activation of cPLA2, which provides substrates (arachidonic acid) for the COX-2 inflammatory and pro-carcinogenic pathway. Both these sphingolipid metabolites significantly augment PGE2-values via two different but completely coordinated and synergistic pathways than when given separately and in the same quantity. (103) Interestingly, tissue samples of colorectal adenocarcinoma and their metastatic variants with an elevated COX-2 expression were also positive for SphK, while a positive expression of SphK was not always linked to positive COX-2 expression. (89)

As previously mentioned, glycosphingolipids are extremely diverse, although changes in the glycolysation pattern of glycolipids is a familiar phenomenon in a large quantity of cancers, including CRC. (104) As an illustration: sialidation is often over-expressed in colon cancer cells. Indeed, human Neu3 activity is elevated in neoplastic colons, blocking programmed cell death and even modulating cell differentiation. (105) These findings are partially confirmed by the augmentation of lactosylceramide (2 to 5 times higher than in normal mucosa) and a sialidase product in colon cancer cells. (106) Another study revealed that glycosphingolipids in CRC obtained an increased fucosylation, a decreased glycan acetylation, sulfation and ganglioside disialylation. (104) Furthermore, the higher the Dukes stage or metastatic potential of the patient’s CRC, the higher the sulfogalactosylceramide levels. (107) Lastly, chemosensitizers combined with GCS-inhibitors induced cytotoxicity and therefore, apoptosis in colon cancer. (97) Sphingolipid glycolysation could lead us to new insights on colorectal
carcinogenesis, new strategies for therapy or new biomarkers for screening. However, a real pattern in aberrant glycolysis has not actually been determined yet, because these differ in every type of cancer and make use of different glycan-groups. (104)

### 4.3.4 Sterols

#### 4.3.4.1 Pathway
Cholesterol is an extremely important molecule in the human body: it is an essential component in cell membranes, in which the majority is non-esterified cholesterol, and intracellular membranes, where the greater part consists of esterified cholesterol. Furthermore, it is a precursor of bile acids, Vitamin D and steroid hormones. (108)

![Chemical structure of cholesterol](image)

**Fig. 8: chemical structure of cholesterol**

Cholesterol is a polycyclic structure containing a total of 27 carbon atoms. The hydroxyl-group on C3 can be replaced by an ester-bond. Both cholesterol forms are transported in lipoprotein-particles (chylomicrons, VLDL, LDL, IDL and HDL) due to its lack of solubility in water or plasma. Cholesterol can be obtained through diet, but is mainly *de novo* synthesized. Cholesterol biosynthesis is a complex process: aided by the mevalonate pathway, it commences with condensation of acetyl-CoA and acetoacetyl-CoA, producing HMG-CoA. Eventually, this product is reduced to mevalonate by HMGCoA-reductase, the rate-limiting step of cholesterol biosynthesis. (7) Afterwards, two kinase reactions sequentially take place, directly followed by a decarboxylation, forming isopentenylpyrophosphate (IPP). This latter product can be converted into dimethylallylpyrophosphate (DMPP) by isomerisation. Condensation of IPP and DMPP produces geranylpyrophosphate (GPP), which sequentially undergoes a condensation reaction with IPP, gaining farnesylpyrophosphate (FPP). Both these condensations are sustained by
geranylpyrophosphate-synthase (GPPs) and farnesylpyrophosphate-synthase (FPPs). Squalene is synthesized in the next step, derived from FPP. With help of the squalene-epoxidase and lanosterol-synthase, it is converted into lanosterol. From here on, 19 different reactions aided by nine different enzymes, are necessary to gain the final product, cholesterol. (108)

In addition, cholesterol can be esterified with a long FFA by acyl-coenzyme cholesterol acyltransferase (ACAT), gaining cholesteryl esters. This is the main form in which cholesterol can be stored in cells, and transported through the blood via lipoproteins.

Fig.9: Overview of cholesterol biosynthesis

4.3.4.2 Alterations in colorectal cancer

Cholesterol and cholesterol precursors are known to be important metabolites in carcinogenesis: cancer cells require higher concentrations during their development and further growth. Targeting this pathway could supply new therapeutic methods against CRC, although changes in this pathway may also contribute to chemotherapy resistance. (109)

Cholesterol transport also showed some discrepancies in malignant cells, but this will be described in the next section on lipoproteins.
Oxysterols are catabolic products of cholesterol, produced by cytP450-enzymes or by reactions with reactive oxygen and nitrogen. (110) They are able to connect with LXR-receptors and activate different cholesterol efflux pump and other regulatory proteins. Oxysterols are also involved in LDL-receptor degradation. (109) Some oxysterols are capable of initiating colorectal tumorigenesis by augmenting ROS/RNS activity, thus activating pro-carcinogenic proteins like COX-2 and even supporting tumor progression by promoting migration of neoplastic cells. (110) While it can therefore be stated that oxysterols have a protective influence during carcinogenesis by inhibiting cholesterol abundance in cells, the fact that some oxysterols have pro-inflammatory and pro-oxidative capacities (thus being pro-carcinogenic), contests the former theory. (109) (110)

The cellular mechanism of cholesteryl ester (CE) homeostasis remains uncertain, but findings indicate that many cancer cell lines are submitted to higher CE synthesis and increasing uptake of CE delivered by LDL and HDL. (111) In breast cancer, CE cell accumulation has already been associated with a malignant phenotype and proliferative effects, but the effects on CRC specifically have not yet been investigated. (112)

Regarding the mevalonate pathway, an elevation of HMGCoA-reductase, FPP synthase and farnesyltransferase have been noticed in different types of cancer, especially in colorectal cancer. HMGCoA-reductase is the rate limiting step in cholesterol biosynthesis, meaning this enzyme has a major impact on pathway progression. (113) Its product, mevalonate acid, is known to rapidly initiate DNA-replication, enhancing control loss of the synthesis pathway and contributing to malignant transformation. (114) Although most CRC samples have increased levels of surface LDL-receptors, HMGCoA-reductase activity in CRC was more significantly elevated in LDL-R negative colorectal tumors, as absence of LDL-R in malignant cells imply they are solely dependent on endogenous synthesis of cholesterol instead on environmental uptake. (115) Quite contradictory with the former findings, absence of LDL-R in colorectal tumors had a negative impact on patient survival, but expression of HMGCoA-reductase is more up-regulated in TNM stage I and II than in stage III and IV, thus giving patients a better prognostic value. (116) (117) It also seems that HMGCoA activity is correlated with the tumor localization in the colon: tumors on the left side of the colon had three times the enzyme activity compared with rectal tumors. (115) As the greatest risk in colon cancer is metastasis and in rectal cancer,
recurrence, these arguments feed the theory that different tumor localizations are associated with different molecular mechanisms in carcinogenesis of which the cholesterol pathway has its own unique function. (113)

Besides elevated mevalonate levels, many studies indicate that isoprenoid compounds FPP and GPP are elevated in malignancy, activating oncogenes and promoting tumorigenesis. (114)(115) Indeed, FFPs activity was enhanced in CRC tissue compared to normal mucosa.

Furthermore, FPP is the essential substrate for farnesylation, a protein isoprenylation process. These protein alterations provide them with unique capacities considering proliferation, differentiation and cell survival, properties that could be altered in neoplastic cells. An up-regulation of farnesyl transferase was noted in CRC hence confirming this theory. (114)

Inhibitors of HMGCoA-reductases, statins, are widely used for lowering cholesterol serum levels as a part of cardiovascular risk prevention but might prove themselves worthy as chemo-preventive agents in colorectal cancer. More and more evidence suggests that statin intake may also interfere in tumor proliferation, growth and metastasis. (118) Besides down-regulation of cholesterol, it reduces-PP and geranylgeranyl-PP concentrations, inhibiting prenylation of proteins and hereby restricting essential physiological functions in tumor development. (119) Although the role of simvastatin remains somewhat unclear: it showed promotion of apoptosis while reducing human cancer cell proliferation in vitro. (115) (117) Another study showed no effects in cancer cell proliferation or apoptosis, but administration inhibited cell migration and thus metastasis probability. However, geranylgeranylpyrophosphate or mevalonate addition reduced statins inhibitory effects on cell migration. (120) Conclusively, a meta-analysis reported a small but significant protective effect of statins in CRC by decreasing invasiveness, metastatic properties, and even chemosensitizing the neoplastic cells for other chemotherapeutics. (119)

Biphosphonates are commonly used for the treatment of osteoporosis, but since these are capable of targeting the FPPs in the mevalonate pathway, they could be valuable in blocking cholesterol synthesis, thus restricting further CRC development. (114) A meta-analysis concluded that oral administration of biphosphonates clearly reduces the risk of CRC, depending on the dose and duration of drug administration. (121)
Total cholesterol (TCH) and free cholesterol (FCH) were measured in patients with CRC and compared with healthy patients or benign colorectal lesions. A drop in serum TCH was observed while serum FCH was significantly elevated. Furthermore, only serum TCH concentrations were significantly and progressively reduced in CRC stage III and IV. The fact that neoplasm cells have higher need of cholesterol, could explain the drop in cholesterol serum levels due to higher uptake, while the low levels of tissue cholesterol may be caused by the cells limited cholesterol synthesis and transport capacities, while trying to keep up with the enhanced cholesterol need. This hypothesis remains vague and the mechanism behind this theory hasn’t been elucidated yet. The only conclusion that can be made is that the physiological homeostasis of cholesterol is diminished in CRC, which could offer new therapeutic targets or predictive biomarkers. One final remark is that these alterations in lipid levels could have been present before the patients illness, as they are independent risk factors in the development of CRC. (82)

Besides alterations in biosynthesis, cholesterol homeostasis can also be deregulated by deficiencies in the cholesterol efflux pumps of cells. An example is the ABCA1 efflux function in mitochondria: inhibited ABCA1 expression in colon cancer cells increases the mitochondrial cholesterol concentrations and reduces the release of cell death promoting molecules. Consequently, these tumor cells are able to have prolonged survival rates. (122)

Although cholesterol is the essential precursor molecule of Vitamin D, bile acids and sterols, alterations in their metabolism and/or the association with CRC falls beyond the reach of this thesis.

### 4.3.5 Lipoproteins and lipid droplets

Lipoproteins mainly comprise molecules that are capable of transporting lipids such as cholesterol, cholesteryl esters, TG and vitamins, to all cells. The trend to occur in a structure that is composed of a phospholipid monolayer stabilized by neutral apolipoprotein, with a central hydrophobic core within. Lipoproteins are classified into four classes according to their progressing density: chylomicrons, VLDL, IDL, LDL, and HDL. Additionally, the hydrophobic core is an ideal environment for the transportation of lipophilic agents such as certain chemotherapeutic agents, as these remain stabilized within this core and are not directly removed from the bloodstream, offering an elevated half-life for these compounds. (118)
Many types of cancers over-express HDL- and LDL-receptors on their cell surface, with serum HDL showing decreased values and augmented quantities of tissue HDL in cancer patients. Indeed, CRC patients had reduced serum HDL and LDL values while tissue HDL was increased in CRC. These values were even more highlighted when progressing through the TNM stages due to the fact that higher tumor grade is correlated with higher demand for cholesterol, and thus enhanced cholesterol transport. (82) (118) As apolipoproteins form the fundamental basis in the synthesis of lipoproteins, over-expression of these structures could be playing a role in tumor development. Apo-A1 and Apo-B may have an altered expression that could be associated with the HDL and LDL shifts seen in CRC. (82) Apolipoprotein E1 gene polymorphisms have already been correlated with higher CRC risk. (123)

Lipid droplets or lipid bodies are small, dynamic organelles, consisting of a lipid monolayer and capable of stocking all cytosolic lipids in human cells. Besides lipids, these organelles also contain a significant amount of proteins that are involved in lipid metabolism, but also other processes that could enhance carcinogenesis. Well known examples are PI3K, MAPK, cPLA2, COX, and LOX-enzymes, of which the latter three enzymes claim essential roles in the pro-inflammatory eicosanoid pathway while using AA as substrate. (124) Lipid bodies were significantly increased in colon carcinoma cell lines in comparison to normal mucosa when investigated using Raman microscopy. (125) Also, expression of COX-2 was fortified in lipid droplets of colon cancer cells, which was correlated with enhanced production of PGE2. As this is a pro-inflammatory and pro-tumorigenic factor, as described above, lipid droplets may contribute to the development of CRC and could be suggested as a new therapeutic target, while the amount of lipid droplets in CRC cells could be brought forward as a prognostic biomarker. (124)
4.4 An interpretation of previously obtained experimental data

Dr J. Foster performed data analysis for his PhD dissertation at Cambridge University, UK of comparative lipidomic profiling data of CRC samples with normal mucosa samples. His analysis not only aimed for identifying alterations of general lipid subclasses, but also of individual lipids that were organized according to their specific acyl bond.

Generally, tumor samples showed a significant increase (P = 0.0025) in the total sum of lipid concentrations when compared to normal samples. This phenomenon repeated itself as the total amount of lipids corresponding to a specific subclass was significantly altered in CRC samples (P < 0.05) in all except three subclasses; only LPC, SM and PA did not show significant alteration. Furthermore, the concentrations of lipid substrates within these subclasses were increased in all tumor samples compared to normal mucosa, but subclasses LPA, monoacylglycerols and TG were decreased.

Glycerolipids TG, PC and PE were altered most frequently in CRC and were mainly down-regulated. These tumor samples showed a clear preference for long chain desaturated PS (phosphatidylserine) species, while short chain saturated PS species were moderately down-regulated. The total amount of all PE species concentrations was absolutely increased in tumor tissues, with evidence that some reactions shifted towards the production of PE, by using substrates PC, PS and DAG (diacylglycerol). LPC was also strongly altered in tumor tissue in comparison with normal mucosa.

Other important findings were the shifts that manifested when lipid profiles were compared between tumor and normal mucosa samples, tumors with different stages and different tumor sizes. Ceramide 16:0 levels were increased in tumor size 1 samples compared to other samples, while ceramide 18:0 levels were decreased. Additionally, PE species of patients who received radiotherapy prior to tissue sample collection were evaluated: this highlighted that PE 36:1 and PE 36:2 were reduced, while PE 38:4 was augmented in pre-radio samples in comparison with all other samples. Lastly, PI and PS values of tumor size 2 samples were put side by side with those of tumor size 3 samples. While PI 34:1 PI 36:1 and PI 36:2 were elevated, PI 38:4 was down-regulated in tumor size 3 samples compared to tumor size 2. Furthermore, tumor size 3 shifted towards lower PS 44:7 and PS 44:8 values. (126)
5 Discussion

Alterations of lipid metabolism in CRC cover an enormous quantity of information due to its extreme complexity. Lipidomics remain a somewhat under-investigated area and the findings that have been published so far are likely to reveal only the tip of the iceberg. A lot of opportunities for therapy and screening methods lay within this territory, of which some have already been mentioned above.

Regarding the FFA, ACLY and FASN have been intensively examined enzymes. Experiments with ACLY and FASN-inhibiting medicines already showed promising results, while serum FASN values could provide predictive and prognostic information. The shift in FFA distribution in CRC tissue compared to healthy colorectal cells is another interesting finding. What mechanisms drive the cancer cells in producing more 'aggressive' ω-6 FFA, AA in particular, in comparison with more ‘protective’ ω-3 FFA, EPA and DHA? Although a lot of attention has been devoted to the production of pro-inflammatory eicosanoids (e.g. PGE2, TxA2, 12-HETE) and their role in colorectal carcinogenesis, decreasing the amount of substrates used for these pathways may be of exceptional value for inhibiting further development of CRC.

Glycerolipids, and mainly glycerophospholipids, have an unmistakable role in CRC pathogenesis. In literature as well as in Foster’s thesis, TG levels were demonstrated to be altered in different CRC stages, although it is still questionable what clinical benefits could be acquired from these findings in CRC. LPA, PC and PLA2 have also been intensively examined throughout the years and showed more potential for future use. PI and moreover, PIP3, is part of a key regulator in many intracellular pathways that augment pro-tumorigenic pathways. Suppressing PI3K/Akt/mTOR pathways have already been tested with pharmaceuticals in different cancer types. This offered promising results at the beginning of the trial, but chronic treatment forced the cancer cells to adapt and use other growth-stimulating pathways, making these medicines unable to fully exterminate the cancer cells. (127) There could therefore be room for different approaches in targeting these pathways in CRC, for instance by inhibiting synthesis of PI-molecules.

Ceramide is likely to be at the epicenter of spingolipid metabolism alterations in CRC cells. All results indicate that cancer cells try to avoid pathways that favor ceramide production,
thereby evading its apoptotic and anti-proliferative capacities. Developing methods for enhancing ceramide production, or inhibit pathways that cut off ceramide supplies could be of therapeutic value.

In cholesterol metabolism, all evidence points out that *de novo* synthesis is up-regulated in CRC, together with enhanced uptake of cholesterol provided by lipoproteins LDL or HDL. Not only enhanced cholesterol synthesis, but also the increased isoprenylation activity from its intermediates FPP and GPP could be interesting targets. For decades, cholesterol metabolism has been a major topic in cardiovascular diseases while data are showing some significance in malignancy processes. This reinforces speculations that pharmaceuticals that lower cholesterol uptake or that inhibit *de novo* synthesis could also be of use in the therapy or prevention of CRC.

I conclude that there is plenty of information that suggests an essential role for lipid metabolism in CRC pathogenesis. This may lead to new opportunities in treatment and prevention of CRC, granting the patient a better prognosis due to improved and more personalized medicines and earlier detection of the tumor, preferably during curable stages. Nowadays, CRC is still classified according to the TNM or TNM clinical staging system, an instrument that is primarily focused on surgical treatment of CRC. As every CRC covers a specific, heterogeneous metabolic profile, the question arises if lipidomics (and other ‘omics’ approaches could become the new standard in adequately categorizing CRC on a molecular basis. This molecular classification could offer patients a personalized therapy-schedule, depending on the type of molecular defects their colorectal tumor acquired. Still, this thesis confirms that lipidomics is standing in its infancy, as we know so much and yet so little about the normal biological processes in mammals that interfere with lipids, let alone in pathological processes such as CRC. The majority of the literature that I have investigated mainly focused on the alterations of lipid molecules in CRC regarding absolute concentrations within a lipid sub-class. A handful of articles, including Foster’s thesis, were the only ones who attempted to examine lipid metabolism alterations in CRC by observing each individual lipid from each lipid sub-class on their quantity, distribution and composition in comparison to normal mucosa. Indeed, this is an expensive and time consuming method of investigation, but this may be the only way to fully understand the mechanisms behind CRC pathogenesis. A long road still lays ahead of us if we want to efficiently progress within this specific territory as much work remains to be done.
Colorectale darmkanker blijft één van de meest prevalent, incidentrijkste en dodelijkste maligniteiten ter wereld. Wetenschappers testen steeds nieuwe technieken te uit om efficiëntere screeningsmethoden en therapeutische benaderingen te ontwikkelen. Lipidomics is onderdeel van het netwerk van de metabolomics en bestudeert de distributie van lipiden in menselijke cellen, weefsels, maar ook andere organismen. Lipidomics blijft vooralsnog een weinig onderzocht terrein, doch heeft al veelbelovende resultaten geboekt in verscheidene soorten maligniteiten. In deze literatuurstudie werd getracht om zoveel mogelijk informatie samen te vatten die tot op heden bekend is inzake het verband tussen lipidenmetabolisme en colorectaal darmkanker. Naast de lipoproteïnen en de intracellulaire lipidendruppels, zullen de meest significante veranderingen binnen de grootste lipidengroepen worden besproken. Dit betreft de vrije vetzuren, glycerolipiden (met name de triglyceriden en de fosfoglycerolipiden), sphingolipiden en sterolen. Er werd alvast gestegen activiteit waargenomen van enzymes die de synthese van vrije vetzuren reguleren, evenals significante veranderingen in de samenstelling van vrije vetzuren in colorectale tumor specimens. Er werd alsook een gedaalde hoeveelheid triglyceriden en phosphaticylcholine glycerophospholipiden genoteerd in deze specimens. Gezien ceramide de centrale molecule is binnen het sphingolipidenmetabolisme en beschikt over pro-apoptosis en anti-proliferatieve eigenschappen, pogen colorectale maligne cellen de productie van dit specifiek metaboliet te vermijden. Een gestegen de novo synthese van cholesterol werd waargenomen, evenals een toenemen opname van cholesterol vanuit de omgeving via lipoproteïnen, wat duidelijk kon aangetoon worden gezien de gestegen cellulaire expressie van HDL- en LDL-receptoren. Tot slot is maligniteit al enige tijd geassocieerd aan een verhoogde aanwezigheid van intracellulaire lipidendruppels en dit fenomeen werd inderdaad bevestigd in colorectale darmkanker specimens. Desondanks de bovenvermelde bevindingen die aangeven dat er weldegelijk een associatie bestaat tussen colorectale darmkanker en lipidenmetabolisme en er voldoende opportunititen bestaan om nieuwe therapeutische benaderingen te ontwikkelen, staat dit gebied nog steeds in haar kinderschoenen. Heel wat onderzoek zal nog moeten verricht worden willen we het lipidenmetabolisme integreren in het huidige screenings-en therapeutisch beleid, niet alleen voor colorectale kanker maar ook voor andere soorten maligniteiten.
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## Appendix

### Dukes classification

- **Stage A**: invading submucosa
- **Stage B1**: invading into muscularis propria
- **Stage B2**: invading through muscularis propria
- **Stage B3**: equals T4N0M0
- **Stage C1**: as B1 but with lymph node invasion
- **Stage C2**: as B2 but with lymph node invasion
- **Stage C3**: as B3 but with lymph node invasion
- **Stage D**: distant metastasis

### TNM classification

- **T**=tumor
  - **T0**: no primary tumor present
  - **Tis**: tumor in situ (located intra-epithelial or invading lamina propria)
  - **T1**: tumor invading submucosa
  - **T2**: tumor invading muscularis propria
  - **T3**: tumor invading subserosa or non-peritoneal pericolic or perirectal structures.
  - **T4a**: tumor invading visceral peritoneal surface
  - **T4b**: tumor invading or adherent to surrounding organs
  - **Tx**: primary tumor cannot be identified

- **N**=lymph node invasion
  - **N0**: no lymph node metastasis
  - **N1a**: metastasis in one regional lymph node
  - **N1b**: metastasis in 2-3 regional lymph nodes
  - **N2a**: metastasis in 4-6 regional lymph nodes
  - **N2b**: metastasis in 7 or more regional lymph nodes
  - **Nx**: lymph node invasion cannot be identified

- **M**=distant metastasis
  - **M0**: no distant metastasis
M1a: distant metastasis to organ or other site
M1b: distant metastasis to multiple organs, sites or peritoneum
Mx: distant metastasis cannot be identified

**TNM disease stages**

Stage 1: T1-T2N0M0
Stage 2A: T3, N0, M0
Stage 2B: T4a, N0, M0
Stage 2C: T4b, N0, M0
Stage 3A: T1-T2, N1, M0 and T1, N2a, M0
Stage 3B: T1-T2, N2b, M0; T2-T3, N2a, M0 and T3-T4a, N1, M0
Stage 3C: T3-T4a, N2b, M0 and T4b, N1-N2, M0 and T4a, N2a, M0
Stage 4A: M1a with any T and any N
Stage 4B: M1b with any T and any N