Clinically relevant *ex vivo* fatty acid profiles from a lipid model for colorectal adenocarcinoma

By

AMANDA NEL

Submitted in fulfillment of the requirements for the degree

Magister in Medical Science (M.Med.Sc)

In the Faculty of Health Sciences
At the University of the Free State
Bloemfontein
South Africa

November 2007

MODERATOR: PROF LOUISE LOUW

CO-MODERATOR: PROF PN BADENHORST

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following people:

Prof L Louw, a superior teacher and mentor who inspired me a lot, for guidance and advice, and for the many hours she spent in reviewing this thesis;

Prof PN Badenhorst, for his approval to submit this thesis;

Dr C Pohl, who introduced me to "fatty acids in the laboratory" and who guided me through the experimental work;

Mr C van Rooyen, Division of Biostatistics, UFS, for the statistical analyses;

Prof RS du Toit and clinicians, Surgery Department, for assistance with the collection of the biopsies;

The Medical Research Council for financial support;

My husband Pieter and children, Beatri, Paul and Jacques for their wonderful love, continued tolerance and unconditional support during the preparation of this thesis.

Thanks and praise above all, to our God for His unconditional love and grace. To Him be the Glory.

DEO GLORIA

DECLARATION

I hereby declare that the work submitted here for the Magister of Medical Science is my own independent work. Where help was sought, it has been acknowledged. I further declare that this work has not previously been submitted at any other university for the purpose of obtaining a degree. In addition, copyright of this dissertation is hereby ceded in favour of the University of the Free State.

_____ _____

Amanda Nel Date

TABLE OF CONTENTS

CHAPTER 3: BACKGROUND

CHAPTER 4: METHODOLOGY

CHAPTER 5: RESULTS

CHAPTER 6: DISCUSSION

CHAPTER 7: CONCLUSION

CHAPTER 1
INTRODUCTION

1.1 STUDY APPROACH

DIETARY PREDICAMENT: Colorectal cancer (CRC) is one of the most common causes of cancer deaths in industrialized Western countries (Ballinger and Anggiansah, 2007). The most important factors in the etiology of CRC appears to be environmental and among them food plays a prominent role (Gunter and Leitzmann, 2005; Kuriki *et al*., 2006). The Western diet consumed today, high in processed foods and relatively low in fruit and vegetables, has been described as pro-inflammatory and linked to the development of many cancers (Marques-Vidal *et al*., 2006). It is believed that foods available to our ancestors before agriculture practices, allowed the establishment of a genetic pattern that is insufficient for this Western diet. The assumption that this genetic pattern could not be adjusted, led to the conclusion that modern man is now more susceptible to diseases of our time (Mulcahy *et al*., 2003). Today, the typical Western diet is hallmarked by excessive linoleic acid (LA) intake and a deficiency of omega-3 polyunsaturated fatty acids (n-3 PUFAs) (Okajuma, 1998). Anthropological, epidemiological and molecular studies indicated that human beings evolved on a diet with a ratio of n-6 PUFAs:n-3 PUFAs of approximately 1:1, whereas in Western diets the ratio is approximately 15:1 (Simopoulos, 2006). Dietary intakes that do not meet daily recommendations may be a predicament and environmental factors that interfere with lipid metabolism are a predisposing factor of CRC.

CONSTRUCTION OF A LIPID MODEL: The challenge we face today is identification of the mechanisms through which dietary factors perturb fundamental fatty acid (FA) pathways in cancer cells. An integral part of the identification process is to establish a lipid model consisting of FA profiles for the cancer entity under investigation, primarily for assessment of the cancer and secondary to serve as a sound foundation for clinical intervention. In the case of adenomatous CRC valuable research information exists, but relevant FA profiles are lacking. Of particular significance is that FAs are ligands for peroxisome proliferator activated receptors (PPARs) and vigorous research in the field of molecular biology contributed to our understanding of lipid driven cell proliferation and

apoptotic signaling pathways involved in colorectal carcinogenesis. Valuable information on PPAR family members allow the coupling of specific FAs to PPARγ, associated with inhibition of cell proliferation, PPARα, associated with induction of apoptosis and PPARβ/δ, associated with apoptotic resistance (Zuo *et al*., 2006; Martinasso *et al*., 2007). Thus, by establishing clinically relevant FA profiles for CRC cells, prominent FA role-players can be identified which drive proliferation and apoptotic signaling pathways, both steps on which cancer therapy is based.

COLORECTAL CANCER THERAPEUTIC REGIME: CRC is hallmarked by chronic inflammation and without doubt this can be ascribed to arachidonic acid (AA) that plays a central role during carcinogenesis (Aggarwal *et al*., 2006). For more than a decade CRC research was dominated by the pivotal role of cyclooxygenase-2 (COX-2) activity (Furstenburger *et al*., 2006), a bevy of factors in command of it's over-expression and pharmacological intervention for the down-regulation thereof (Arber *et al*., 2006). Interestingly, the previously concern expressed for the side-effects of COX-2 inhibitors (Andersohn *et al*., 2006) can be replaced by non-toxic FA chemotherapeutic agents that can modulate membrane FA composition and effectively down-regulate the AA cascade, including COX-2 expression (Das, 2005). Recently, immunonutrition gained prominence in cancer management (Philpott and Ferguson, 2005) and the immunocompetence of CRC patients can be improved by restoring membrane FA compositions of lipid rafts and the proliferation of T lymphocytes (Matsuda *et al*., 2006; Li *et al*., 2006). Different adjuvant FA therapeutic strategies for clinical intervention in the management of CRC patients are debated in this dissertation. Concurrent therapy with a COX-2 inhibitor and doxosahexaenoic acid (DHA) is currently evaluated and the outcome of clinical trials is still awaited. It is a personal opinion that causes of CRC rather than enhanced COX-2 expression as a consequence should be addressed and all the options, expounded in context in this study, need to be explored. Depending on etiological causes, there are those FAs advocated to: eliminate bacteria and viruses or ameliorate bacterial or viral infections; protect against smoke particles and oxidative stress, regulate and redirect enzymes where interference with essential fatty acid metabolism (EFAM) occurs, modulate membrane FA compositions and FA metabolism to prevent carcinogenesis; and regulate nuclear factor-kappa Beta (NF-κβ) and Th1 and Th2 cytokine subsets to improve immunocompetence (Larsson *et al*., 2004; O'Shea *et al*., 2004; Das *et al*., 2007). For these reasons, different adjuvant FA therapies can be included in the therapeutical regime followed for CRC, apart from the necessity to follow a healthy regimen (lifestyle),

hallmarked by good nutrition with limitation on those environmental factors that might interfere with lipid metabolism.

1.2 LITERATURE OVERVIEW

OVERALL: An attempt to assess CRC and propose adjuvant FA therapeutic strategies required insight into nutritional, biochemical, immunological and genetic research fields. A comprehensive literature study was therefore conducted and is discussed in CHAPTER 3. Applicable excerpts from the literature study are briefly outlined in this overview, supported by prominent references.

LIPID RESEARCH: For more than 3 decades dietary lipids were explored in an attempt to elucidate the role of FAs during carcinogenesis. It is generally believed that n-6 PUFAs promote carcinogenesis, while n-3 PUFAs may prevent carcinogenesis (Jones *et al*., 2003; Larsson *et al*., 2004; Roynette *et al*., 2004; Colomer *et al*., 2007). A paradigm shift in approach also revealed conjugated-linoleic acid (CLA; *t10,c12* 18:2) for its anti-carcinogenetic and immunomodulatory potential (Larsson *et al*., 2005a; Han *et al*., 2006; Colomer *et al*., 2007). An objective of FA research is to understand the metabolic pathways followed under pathological conditions and of particular interest are FAs of membrane phospholipids (PLs), since they determine membrane properties such as fluidity and flexibility and regulate the movement of ions and metabolic products across the membrane (Hulbert *et al*., 2005). Lipid gene-regulation of cellular processes is mediated by a complex array of membrane-to-nucleus signaling pathways and FAs and their various metabolites can act directly at the level of the nucleus to affect the transcription of a variety of genes or indirectly by altering other signaling pathways and thereby cause cancer (Lapillonne *et al*., 2004; Sampath and Ntambi, 2004; 2005a; Yeh *et al*., 2006). Modulation of membrane FA compositions to manipulate abnormal signaling pathways gradually became an important tool in cancer therapy and is currently receiving renewed attention.

There is a mountain of evidence in the literature regarding abnormal lipid driven signaling pathways, based on *in vitro* and *in vivo* animal model studies. With respect to colorectal carcinogenesis, prominent studies that addressed cell proliferation and apoptosis can be listed as follows: Ajuvavon and Spurlock (2005), Niki *et al*. (2005), Ohta *et al*. (2005),

Skrzydlewska *et al*. (2005), Shurequi *et al*. (2005), Van der Logt *et al*. (2005), Beppu *et al*. (2006), Boundreau *et al*. (2006), Brookes *et al*. (2006), Calder (2006), Chan (2006), Colomer and Menendez (2006), Du Toit (2006), Engelbrecht *et al.* (2006), Han *et al*. (2006), Jove *et al*. (2006), Kountourakis *et al*. (2006), Mills *et al*. (2006), Ng *et al*. (2006), Soumaraoro *et al*. (2006), Takayama *et al*. (2006), Watson (2006), Yeh *et al.* (2006), Zuo *et al*. (2006), Colomer *et al*. (2007), Courtney *et al*. (2007), Martinasso *et al*. (2007), Ponferrada *et al*. (2007), Saether *et al*. (2007), Valko *et al*. (2006 and 2007). In addition, CRC appears to be a Th2 dominant disease and in the event of down-regulation of the Th1 cytokine subset, particularly interleukin-2 (IL-2), immunodeficiency may occur that is commonly encountered in cancer patients. With respect to CRC and immune responses, prominent studies are listed as follows: Baier *et al*. (2005), Baniyash (2006), Barber (2006), Castellino *et al*. (2006), Li *et al*. (2006), Matsuda *et al*. (2006), Aggarwal *et al*. (2007).

COLORECTAL CANCER RESEARCH: Colorectal carcinogenesis is marked by prolonged inflammatory and cytokine mediated responses that eventually manifest in immunodeficiency. There is overwhelming information in the literature regarding COX-2 over-expression by factors, including saturated fatty acids (SFAs) and *trans*-fatty acids (*trans*-FAs), that eventually leads to chronic inflammation and oxidative stress-related CRC (Valko *et al*., 2004, 2006, 2007; Mills *et al*., 2005; Niki *et al*., 2005; Aggarwal *et al*., 2006; Evans *et al*., 2006; Furstenburg *et al*., 2006). AA is a major role-player during these events, characterized by the up-regulation of enzymes such as phosholipases (PLs), cyclooxygenases (COXs), lipoxygenases (LOXs) and inducible nitric oxide synthase (iNOS) (Aggarwal *et al*., 2006). Epidemiological evidence indicated that high intake of red meat rich in iron and SFAs, such as palmitic acid (PA) and stearic acid (SA), may also lead to colorectal carcinogenesis (Chao *et al*., 2005; Gunther and Leitzmann, 2005; Larsson *et al*., 2005b; Larsson and Wolk, 2006a; Craig-Schmidt, 2006; Kuriki *et al*., 2006; Seril *et al*., 2006; Valko *et al*., 2006; Kimura *et al*., 2007). Although some researchers regard iron as the principal cause of CRC, it is argued in this study that it is rather a co-factor in the multifactorial etiology of CRC.

The impact of environmental factors on essential fatty acid metabolism (EFAM) that impede LA conversion to AA via delta-6 and -5 desaturase (Δ^6d and Δ^5d) pathways with up-regulation of the fatty acid synthase (FAS) and Δ^9d pathways, and the down-regulation of LA oxidative metabolism via the 15 LOX-1 pathway with up-regulation of the COX-2

and 15-LOX-2 pathways of AA, is characteristic of colorectal carcinogenesis. Initially, possible shifts from anti-tumorigenic 15-LOX-1 and 15-LOX-2 products of LA and AA, respectively, to pro-tumorigenic 5-LOX and 12-LOX products of AA was suggested, but then it was stated that: 15-LOX-1 down-regulation, rather than a shift in the balance of LOXs, is likely the dominant alteration in LOX metabolism that contributes to colorectal tumorigenesis (polyp growths) (Shureiqi *et al*., 2005). LA conversion to certain CLA isomer products in the gut that may promote colorectal carcinogenesis (Soumaoro *et al*., 2006; Devillard *et al*., 2007) need to be further explored. The fact that virus infections, the human papillomavirus and parvovirus, may be an underlying mechanism that promotes CRC needs mentioning (Damin *et al*., 2007; Li *et al*., 2007). There also appears to be an association in the prevalence of Helicobacter pylori with some, but not all, colorectal cancer (Jones *et al*., 2007). Finally, it must be mentioned that information on PPARs provided insight into cellular over-production and anti-apoptotic pathways during CRC (Martinasso *et al*., 2006; Takayama *et al*., 2006; Zuo *et al*., 2006; Ponferrada *et al.*, 2007), whilst information on lipid rafts confirmed the impact of FAs, SFAs and eicosapentaenoic acid (EPA) on the regulation of NF-$\kappa\beta$ and immune responses in CRC (Barber *et al*., 2005; Baier *et al*., 2006; Berghella *et al*., 2006; Li *et al*., 2006; Aggarwal, 2007; Tan and Coussens, 2007).

ADJUVANT FATTY ACID THERAPY: Ample evidence exists that EPA, DHA, gamma-linolenic acid (GLA), oleic acid (OA) and CLA may have potential use for human CRC management (Field and Schley, 2004; Klaus *et al*., 2004; Larsson *et al*., 2004; Pariza, 2004; Roynette *et al*., 2004; Akihisha *et al*., 2004; Wahle *et al*., 2004; Lee *et al*., 2005; Menendez and Lupu, 2006a; Das, 2006a and 2006b; Bassaganya-Riera and Hontecillas, 2006; Beppu *et al*., 2006; Bhattacharya *et al*., 2006; Han *et al*., 2006). There is a growing body of evidence that testifies to the successful administration of these FAs in CRC management (Nakamura *et al*., 2005; Reddy *et al*., 2005; Matsuda *et al.*, 2006; Chapkin *et al*., 2007a and 2007b; Courtney *et al*., 2007; Das 2007; Read *et al*., 2007; Soel *et al*., 2007). Human trials also confirmed that FAs may actually improve the immune status of the patient and enhance the impact of chemotherapy and radiation on cancer cells (Simopoulos, 2004 and 2006; Nakamura *et al*., 2005).

1.3 MOTIVATION FOR STUDY

Among the vast evidence encountered in the literature, the longer chain *cis*-FAs that have been unequivocally linked to experimental colorectal carcinogenesis are LA, AA, PA and OA (Astorg, 2005; Bougnoux and Menanteau, 2005). During the last decade research revealed a link between other *cis*-FAs with shorter chain lengths, such as butyric acid (BA), lauric acid (LRA) and myristic acid (MA), and colorectal carcinogenesis, and also revealed the detrimental effects of *trans*-fatty acids (*trans*-FAs) on cells (Nkondjock *et al.,* 2003; Daly *et al*., 2005; King *et al*., 2005; Bhattacharya *et al*., 2006; Craig-Schmidt, 2006; Jones *et al.,* 2006; Sengupta *et al*., 2006; Tedeling *et al.,* 2007). However, the focus remains on the longer chain *cis*-FAs involved in carcinogenesis and their efficacy as therapeutic agents. Unfortunately, an investigation into *trans*-FAs fell outside the scope of this study.

Although it is in doubt whether consumption of FAs may prevent cancer risk (Geleijnse *et al*., 2006), the impact that specific FAs may have in cancer therapy is undeniable (Das 2006a, 2006b, 2007 *et al.*; Kapoor and Huang, 2006). It is advocated that adjuvant FA therapy has merit, regarding disease outcome and the general well-being of cancer patients (Colomer *et al.,* 2007). According to the literature, disturbances in the FA compositions of colon and rectal cancer received attention in several *in vitro* studies and a few *in vivo* studies (Rao *et al*., 2001; Dommels *et al.,* 2002a, 2002b, 2003; Llor *et al.,* 2003). However, a study of *ex vivo* FA profiles for CRC, as a sound basis for therapeutic approaches in the management of this cancer, is definitely required and served as motivation for this study. This *ex vivo* study on a range of FAs that include chain lengths between C12 and C24, not previously reported, is considered a valuable contribution to the literature.

1.4 PURPOSE OF STUDY

This study is an investigation into clinically relevant *ex vivo* FA profiles from a lipid model regarding total lipids (TLs), neutral lipids (NLs), phospholipids (PLs) and all the phosholipid subclasses, i.e. phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) for colorectal adenocarcinoma. A lipid model can reveal FAs role players that contribute to CRC and allow FA strategic planning for preventative actions and more effective therapeutic modalities in the management of this disease. The main questions pertaining to the

present study are the following: how do the FA profiles differ between CRC and normal colorectal cells; which conclusions can be drawn from the FA profiles of CRC cells; and to what extent may the FA profiles reflect immunodeficiency in these CRC patients? Answers to these questions may serve as a platform for the proposal of FA therapeutic options in the management of CRC, based on theoretical postulations. Therefore, the main goal of the study is to construct clinically relevant *ex vivo* FA profiles for colorectal adenocarcinoma. Aims are to identify possible FA role players responsible for CRC and to debate about their involvement in signaling pathways. The final purpose of this study is also to present a rationale for different FA therapeutic strategies (before, during and after surgery) to improve CRC outcome and the general well-being of the patient.

CHAPTER 2
COLORECTAL CANCER

2.1 INTRODUCTION

Knowledge of the descriptive epidemiology is essential to an understanding of the etiology of colorectal cancer (CRC) and the development of preventative and treatment strategies. Environmental factors play a major role in CRC risk and among them the diet has a prominent role. Lipids, implicated in the etiology of many cancers, were first linked to CRC by Wynder *et al.* (1969) and, since then, advances in molecular research contributed to our understanding of CRC. A promising therapeutic approach to reduce CRC recurrence is chemoprevention and the use of non-toxic natural and synthetic compounds or their mixtures to target molecular factors during carcinogenesis (Stack and Dubois, 2001; Akihisha, 2004; Bardon *et al*., 2005). In this regard the potential use of adjuvant FA therapy may be included in the management of CRC (Eynard, 2003).

2.2 EPIDEMIOLOGY

CRC continues to represent one of the major causes of cancer-related morbidity in all western countries with more than 945 000 new cases expected worldwide in 2006 (Yona and Arber, 2006). Dietary habits and lifestyle among environmental risks for CRC appear to play a prominent role in different countries. CRC incidence is particularly high in countries with high intake of red and processed meat which can contribute, respectively, to: enhanced AA and COX-2 activity, associated with inflammation in cancer; and trans-fatty acids, associated with oxidative stress in cancer (Slattery *et al.,* 2001; Kummerow *et al*., 2004; Norat *et al*., 2005; Larsson and Walk, 2006). In the United States an estimated 148 610 new cases and 56 290 deaths were predicted for 2007 (American Cancer Society, 2006; Martinez *et al*., 2006). Mediterranean countries have a lower CRC incidence, compared with other western countries, and this is mostly attributed to dietary habits. However, westernization of the Japanese lifestyle increased CRC incidence remarkably since the end of World War II (Koyama and Kotake, 1997; Ries *et al*., 2000). Nevertheless, Greenland Eskimo populations, eating their traditional diet compared to reference populations in the West, revealed a significantly lower CRC incidence (Norat *et*

al., 2005). Ethnic and racial differences support the concept that environmental factors may play a major role in CRC etiology. The incidence for South African white males (40.2:100 000) is lower than that of western countries, e.g. USA whites (46.5;100 000), and the incidence of South African black males (2:100 000) is comparable to those in other African countries (O'Keefe *et al*., 1999).

2.3 ETIOLOGY

Most cases of CRC are sporadic, but inflammatory bowel disease and hereditary factors may also contribute to this disease. Sporadic cases represent more than 80% and among them, environmental factors such as dietary habits and an unhealthy lifestyle play prominent roles, as previously mentioned. Among other sporadic cases, a personal or familial history is included of which the latter is the least understood. Fewer than 10% of patients have an inherited predisposition to CRC and these cases are subdivided, according to whether or not colonic polyps are a major disease manifestation. Inflammatory bowel disease contributes to less than 2% of CRC (Ponz de Leon and Roncucci 2000; Boyle and Leon, 2002; Weitz *et al*., 2005; Steele, 2006). All the risk factors and causes of CRC are summarized in TABLE 2.1. In a combined cohort study, where risk factors for colon cancer were compared with rectal cancer, it was reported that: age; gender; family history; height; body mass index; physical activity; folate intake; intake of beef, pork or lamb as a main dish; intake of processed meat; and alcohol abuse correlated significantly with colon cancer risk, whilst only age and gender were associated with rectal cancer risk (Wei *et al*., 2004).

2.3.1 The diet: red meat

The type of diet that is linked to CRC is a high-lipid, high-protein, low fiber diet (Rao *et al.,* 2001; Mathew *et al.*, 2004). High intake of red meat, containing high SFA contents (PA and SA) is considered by several researchers as the cause of CRC (Lee *et al*., 2001; Chao *et al*., 2005; Larsson *et al*., 2005b; Larsson and Wolk, 2006; Kuriki, 2006; Kimura *et al.*, 2007). The high SFA content of red meat apparently enhances COX-2 expression that contributes to prolonged inflammation and a link between inflammation, oxidative stress and cancer exists (Chao *et al*., 2005; Larsson *et al*., 2005b). There is an increasing body of evidence that indicates a relationship between cancer development and abnormal over-expression of both eicosanoid-forming enzymes (COXs and LOXs) in a wide variety

of human cancers, including CRC (Marks *et al*., 2000; Furstenberger *et al*., 2006; Soumaoro *et al*., 2006; Goossens *et al*., 2007).

TABLE 2.1 Risk factors and causes of CRC (Weitz *et al*., 2005).

A: Sporadic colorectal cancer (88-94%)

- Older age
- Male gender
- Cholecystectomy
- Ureterocolic anastomosis
- Hormonal factors: nulliparity, late age at first pregnancy, early menopause

I: Environmental factors

- Diet rich in meat and fat, and poor in fiber, folate, and calcium
- Sedentary lifestyle
- Obesity
- Diabetes mellitus
- Smoking
- Previous irradiation
- Occupational hazards (eg, asbestos exposure)
- High alcohol intake

II: Personal history of sporadic tumor

- History of colorectal polyps
- History of colorectal cancer (1.5-3% first recurrence risk in first 5 years)
- History of small bowel, endometrial, breast, or ovarian cancer

III: Familial colorectal cancer (20%)

First or second degree relatives with this cancer, criteria for hereditary colorectal cancer not fulfilled:

- One affected first-degree relative increases risk 2-3 fold
- Two of more affected first-degree relatives increase risk 4-25-fold
- Index case <45 years increases risk 3-9-fold
- Familial history of colorectal adenoma increases risk 2-fold

B: Hereditary colorectal cancer (5-10%)

- Polyposis-syndromes: familial adenomatous polyposis (FAP) Gardner's syndrome; Turcot's syndrome; attenuated adenomatous polyposis coli; flat adenoma syndrome
- Hereditary non-polyposis colorectal cancer (HNPCC)
- Hamartomatous polyposis syndromes (Peutz-Jeghers, juvenile polyposis, Cowden)

C: Colorectal cancer in inflammatory bowel disease (1-2%)

- Ulcerative colitis
- Crohn's colitis

It was also found that a high PA content in beef, as part of diacylglycerol (DAG), is a strong mitogen of adenoma cells in culture. Faecal DAG, arising from the incomplete breakdown of dietary triacylglycerol, may act as a promoter of protein kinase C (PKC) that is the target for phorbol ester tumor promoters. Phorbol esters are natural compounds that mimic the action of the lipid second messenger DAG during CRC cell signaling (Kazanietz, 2005). PA is also known for its mitogenic and anti-apoptotic potential (Scaglia and Igal, 2005; Ajuwon and Spurlock, 2005; Jove *et al*., 2006; Welters *et al*., 2006). Initially, it was suggested that CRC risk is mainly due to the haem (iron) content of red meat and is largely independent of the dietary lipid content (Sesink *et al*., 2000). Since then, epidemiological studies did indicate that a lipid-rich diet containing n-6 PUFAs may be related with the disease process (Jones *et a*l., 2003), and this implies the involvement of lipid peroxidation and cell damage. More recent studies once more suggested that iron may be the principle cause of CRC, since it is primarily responsible for the initiation of oxidative stress (Valko *et al*., 2006; Brookes *et al*., 2006). It is plausible that iron and different lipids consumed in the diet contribute to cumulative oxidative stress that can cause CRC and this will be discussed in CHAPTER 3.

2.3.2 The diet: lipids and bacteria

Numerous mechanisms whereby lipid intake may influence colon carcinogenesis have been proposed. Probably the most cited hypothesis is that high dietary lipid intakes induce the excretion of bile acids and bacteria, nuclear dehydrogenating clostridia (NDC), which act on these bile acids to produce carcinogens (Dolora *et al*., 2002). High dietary lipid intake affects the bacterial flora of the large bowel, the bowel transit time, and the amount of cellulose, amino acids and bile acids in the bowel contents (McMillan, *et al*., 2003). High protein favors the transformation of amino acids by bacteria, while low fibre reduces volatile FAs and prolongs intestinal transit so that there is more time for NDC to act on bile acids to produce carcinogens (Andoh *et al*., 2003; Wei *et al*., 2004; Sengupta *et al*., 2006). Of particular importance for this study is the influence of bacterial species on LA metabolism. A synopsis of LA metabolism by bacterial species to produce different CLA isomer products is indicated in FIGURE 2.1. The pathway by which LA is converted to different CLA isomer products is discussed under LIPIDS in CHAPTER 3.

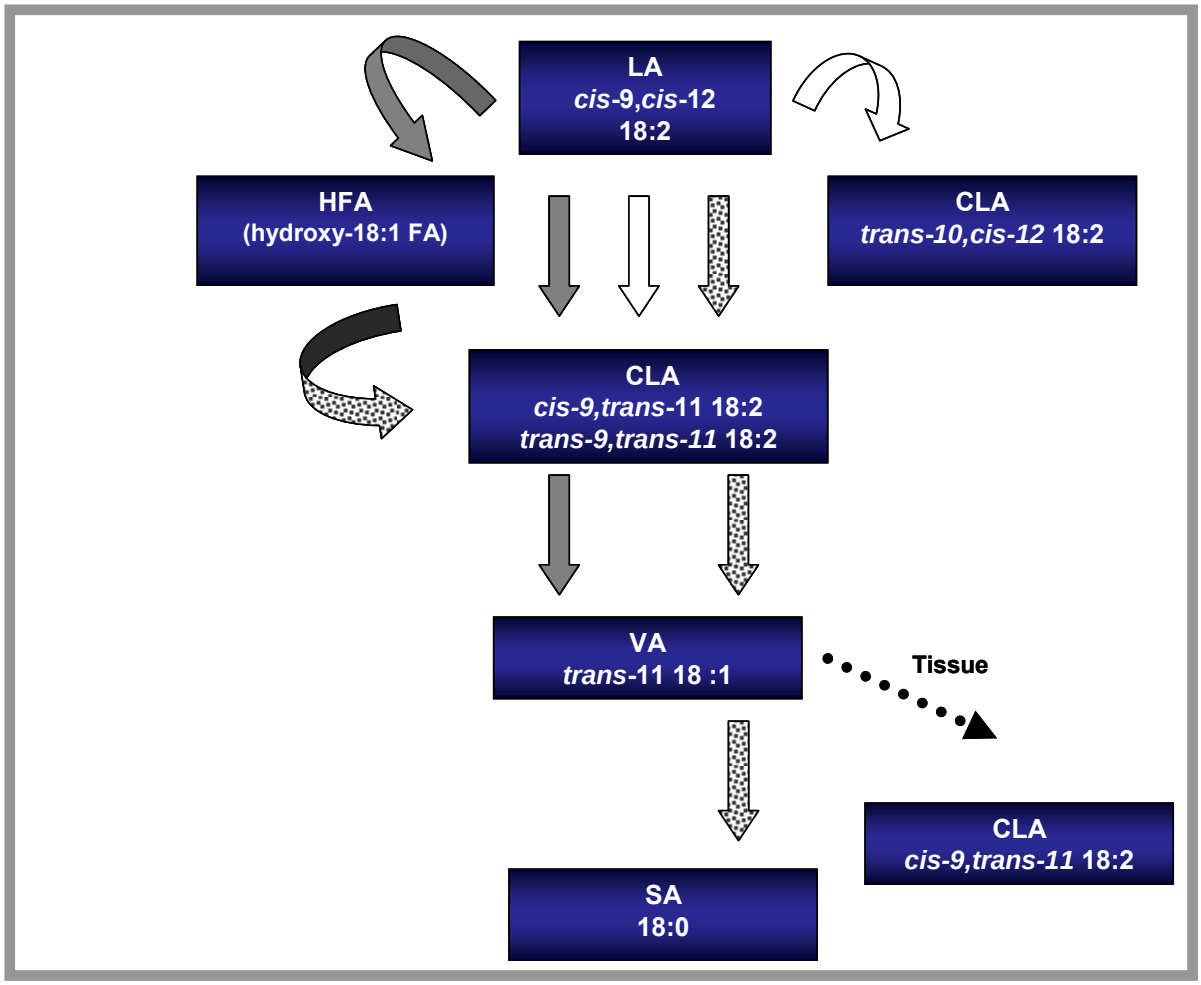

FIGURE 2.1 Proposed pathways of linoleic acid (LA) metabolism by bacterial species isolated from the human gut. The open arrows represent the bacterial activity of *Lactobacillus*, *Propionibacterium*, and *Bifidobacterium* species leading to the formation of conjugated-linoleic acid (CLA). The shaded arrows represent the bacterial activity of some *Lactobacillus*, *Propionibacterium*, and *Bifidobacterium* species and some *Clostridium*-like bacteria belonging to clusters IV (e.g., *Eubacterium siraeum*) and XIVa (e.g., *R. intestinalis* and *Roseburia faecis*) leading to the formation of hydroxy 18:1 fatty acid (HFA). The solid arrows represent the bacterial activity of *Clostridium*-like bacteria belonging to cluster XIVa leading to the formation of vaccenic acid (VA) (e.g., *Roseburia hominis* and *R. inulinivorans*). VA is a source for stearic acid (SA) and can also be converted to CLA in tissue. The dotted arrows represent activities observed in faecal microbiota for which the responsible bacterial species are still unknown (Devillard *et al*., 2007).

2.3.3 The diet: different fatty acids

Apparently, different FAs may either prevent or stimulate colorectal carcinogenesis. A high content of fermentable cellulose leads to high levels of volatile short chain fatty acids (SCFAs) (particularly butyrate) which appear to be protective, since it lowers intestinal proliferation that is associated with a decreased colon cancer risk (Dolora *et al*., 2002; Hinnebusch *et al*., 2002; Menzel *et al*., 2002; Andoh *et al*., 2003; McMillan *et al*., 2003; Comalada *et al.,* 2006). SCFAs, such as butyrate (4:0), are produced in the colon through fermentation of dietary fiber. The ratio of butyrate to SCFA production was reduced in patients with colonic cancer, compared with healthy control subjects (Clausen *et al.,* 1991; Lagergren *et al*., 2001). There is mounting evidence based on animal studies that butyrate elicits effects that include enzyme induction, NF-κβ inhibition and binding of potential carcinogens in the colon (Daly *et al*., 2005; Tedeling *et al*., 2007). Therefore, it seems feasible that low colonic butyrate concentrations, associated with low-fiber diets, may contribute to a higher risk for colon cancer (Niba and Niba, 2003; Nguyen *et al*., 2006). However, in humans the relationship between luminal butyrate exposure and CRC has been examined only indirectly by measuring faecal butyrate concentrations with contradictory results (Sengupta *et al*., 2006). It has been proposed that among the long chain fatty acids (LCFAs), LRA (12:0) and MA (14:0) may be associated with prevention or risk of CRC (Nkondjock *et al.,* 2003). Other LCFAs are further discussed in CHAPTER 3.

2.3.4 The diet: other factors

Direct evidence from animal studies and indirect evidence from human studies suggested that high insulin concentrations increase the risk of CRC (Nilsen and Vatten, 2001). By decreasing the content of PUFAs or increasing the content of SFAs within cell membranes it was demonstrated that membrane fluidity and the number and activity of insulin receptors can be decreased (Giovannucci, 1995). Evidence also suggested that aspirin-like drugs, post-menopausal hormones and micronutrient supplements, such as folic acid (folate), calcium (Ca), selenium (Se), and vitamin E, may also help to prevent CRC (Wu *et al.,* 2002; Chlebowski *et al.,* 2004). What may be of particular significance in the case of CRC is that zinc (Zn) and magnesium (Mg) are necessary co-factors for normal delta 6-desaturase (Δ^6d) activities and that Se is reported to increase interleukin-2 (IL-2) production that improves anti-viral resistance. Zn supplementation is associated with decreased oxidative stress that prevents tumorigenesis and increased maturation of lymphocytes that improves immune function (Prasad and Kucuk, 2002; Kidd *et al*., 2003;

Kahmann *et al*., 2006). Interestingly, Zn deficiency is more widespread than is often assumed and although present in a large variety of food, its levels are low in most foods except for seafood and certain meat types. Also of interest is that Zn deficiency correlated with enhanced COX-2 activity and that Zn supplementation can down-regulate COX-2 activity, according to findings with a rat model study by Fong *et al*. (2005).

2.4 BIOLOGY

Normal colorectal mucosa cells can undergo hyperproliferation and transform to neoplastic growth. A colorectal polyp growth (adenoma) is considered premalignant and during this stage complete cure is obtainable by surgical removal. However, once the polyp shows severe cellular abnormalities and becomes invasive, it is considered malignant (adenocarcinoma) and there is an ongoing search for optimal treatment. Colorectal histology, multistage carcinogenesis, CRC pathology and the molecular concepts of gatekeeper and caretaker pathways, as well as the wingless type pathway involved in intestinal cell renewal are briefly outlined in this section. An overview of molecular factors involved in cell signaling is also given.

2.4.1 Cell biology

2.4.1.1 Normal histology

The normal colorectal mucosa is constituted by three main elements: epithelium, lamina propria and muscularis mucosae. Colon epithelial cells are arranged in crypts and cell to cell communication is vital for individual cell survival. Colon epithelial cells originate at the bottom of the crypt and migrate upwards in a crypt column, while they undergo several in transit divisions. In general, cells cease dividing two-thirds of the way up the crypt column and become fully differentiated. This is accompanied by the development of a well formed microvillous brush border. Toward the top of the crypt they may undergo apoptosis and be exfoliated in the faecal stream (Lynch, 2002). Limitation on the proliferation potential appears to depend on integration of external signals from the contents of the intestinal lumen with genetic pathways that are activated to generate intracellular and intercellular cell growth signals. Cellular differentiation and apoptosis are important in maintaining the integrity and function of the intestinal mucosa. Apoptosis is an essential component of cell number regulation and a crucial mechanism to prevent damaged or mutated cells from surviving and dividing. An increase in the number of mutated cells, may contribute to carcinogenesis (Ponz de Leon and Di Gregoria, 2001; Augenlicht *et al*., 2002). A recent

study by Courtney *et al.* in 2007 demonstrated that EPA (n-3 PUFA) reduced crypt cell proliferation and increased apoptosis in normal colonic mucosa, in subjects with a history of colorectal adenomas (polyps). Therefore, the beneficial therapeutic use of EPA to normalize intestinal cells should not be underestimated.

2.4.1.2 Multistage carcinogenesis

Multistage carcinogenesis is a three-stage process that consists of initiation, promotion and progression (Bertram, 2000; Young *et al.*, 2003). Initiation is the interaction of tissue with carcinogens and the production of cells that are the precursors of the future tumor. Promotion involves the clonal expansion of initiated cells and facilitates the expression of the initiated phenotype. Progression is the acquisition of additional genetic changes that manifest in malignant cells. Initiation alone does not lead to the development of cancer and promotion is reversible, whilst progression is irreversible. The primary step in colorectal carcinogenesis is disruption of mechanisms that regulate epithelial renewal due to interactions between the epithelial crypt cells and carcinogens. The second step is characterized by a neoplastic clonal expansion of crypt cells, as a result of mutations in genes responsible for DNA repair and mutations in genes controlling the cell cycle (proto-oncogenes and tumor suppressor genes). The mutant form of a proto-oncogene is an oncogene that is responsible for normal cell division. A single mutation in an oncogene is usually sufficient to induce cancer. Tumor suppressor genes suppress normal cell division and are mutated (inactive) in cancer. Mutations associated with cancer can be small-scale changes (the substitution of a single nucleotide) or large-scale abnormalities (chromosome rearrangements, gain or loss of chromosomes, or even the integration of viral DNA or RNA) (Yuspa, 2000). CRC is a multistep process and it is the accumulation of multiple genetic mutations rather than their sequence that determines the biological behavior of the tumor (Fearon and Vogelstein, 1990; Gatenby and Vincent, 2003).

2.4.1.3 Pathology

When normal mechanisms that regulate epithelial renewal are disrupted, intraepithelial neoplasia spreads to multiple sites of the colonic mucosa and gives rise to polyps that remain preinvasive and premalignant at this stage (Roynette *et al.,* 2004). Most human CRCs are thought to arise from benign adenomatous polyps that may eventually transform to adenocarcinoma. This hypothesis is supported by pathologic, epidemiologic, and observational clinical data (Ponz de Leon and Roncucci, 2000; Weitz *et al.*, 2005). Mostly, CRC malignancies (95%) are well to moderately differentiated adenocarcinomas

and in a few cases (10-20%) a mucinous component may be present (Ponz de Leon and Di Gregoria, 2001; Roynette *et al.,* 2004). The macroscopic appearance of an adenocarcinoma of the large bowel is demonstrated in FIGURE 2.1. The time interval for a polyp to evolve into carcinoma is a process that, on average, takes 10 to 12 years (Underwood, 2004). The carcinoma consistently elicits an inflammatory and desmoplastic (growth of fibrous tissue) reaction, that is particularly prominent at the edge of the tumor. Most of the inflammatory cells are T lymphocytes, B lymphocytes, plasma cells and histiocytes (Cotran *et al*., 2004).

Tumor staging is the clinical or pathological assessment of the extent of tumor spread. The staging systems used to define the extent of disease at diagnosis are the Duke's classification, the staging system described by Astler and Coller which represents a modification of classification proposed by Dukes and Kirklin and the TNM staging system of the American Joint Committee on Cancer (AJCC) (Dukes, 1932; Astler and Coller, 1954; Beets-Tan *et al*., 2005*)*. Almost seventy years after its original description, Duke's staging is still commonly used to assess the prognosis and, to some degree, determine the treatment of patients with CRC. In 1986, Hutter and Sobin proposed an Universal Staging System for Cancer of the Colon and Rectum, and demonstrated that the TNM (tumour/node/metastasis) system could easily be adapted in order to correspond to the Duke's stages into four main categories (A, B, C and D). The TNM staging system of the American Joint Committee on Cancer (AJCC) and the International Union against Cancer (IUC) is the standard for CRC staging, recommended by the College of American Pathologists (Compton and Greene, 2004). For the purpose of this study CRC biopsies (mostly T2 and T3), without consideration of other clinical paramaters, were used.

2.4.2 Molecular biology

2.4.2.1 Gatekeeper and caretaker pathways

Transformation of normal colonic epithelium into carcinomas requires mutations of genes, such as the adenomatous polyposis coli (APC), beta-catenin, k-ras and p53 genes (Ponz de Leon and Di Gregoria, 2001; Lynch and Hoops, 2002). The most critical gene in the early development of CRC appears to be the APC tumor suppressor gene. Traditionally, colorectal carcinogenesis is explained by two pathways: gatekeeper and caretaker pathways. The gatekeeper pathway regulates growth and mutations of APC tumor

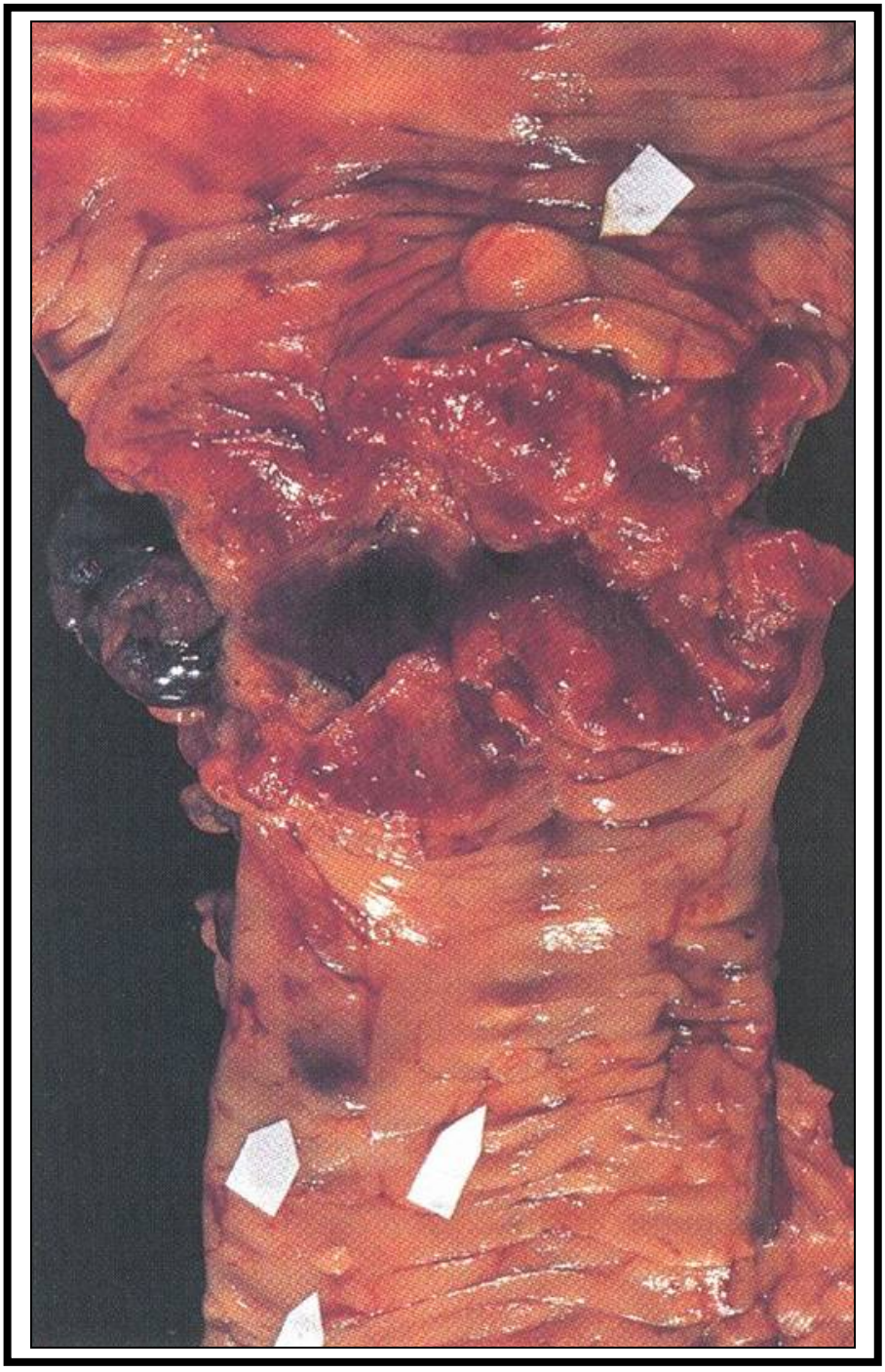

FIGURE 2.2 Carcinoma of the descending colon. The arrows indicate mucosal polyps (Cotran *et al*., 2004).

suppressor genes and initiates the process of neoplastic transformation. The gatekeeper pathway is responsible for about 85% of sporadic CRCs and is the mechanism of carcinogenesis in patients with familial adenomatous polyps (FAP). The caretaker pathway is characterized by mutations or epigenetic changes of genes that maintain genetic stability (e.g. mismatch repair genes) (Weitz *et al*., 2005).

2.4.2.2 Embryonic wingless pathway

The Wingless (Wnt) pathway is an evolutionarily conserved signal transduction pathway that is necessary for embryonic development and it controls cell proliferation and body patterning throughout development (Willert and Jones, 2006). The Wnt pathway also plays a central role in supporting intestinal epithelial renewal, an important fact, since CRC is thought to originate in the expansion of colonic crypt cells (van Es *et al*., 2003). Recent reports showed that one of the bioactive products of COX-2, PGE_2, activates components of the canonical Wnt signaling system (Buchanan and DuBois, 2006). The normal APC protein appears to prevent the accumulation of cytosolic and nuclear β-catenin by mediating its phosphorylation and resultant degradation. A mutation in the APC gene under abnormal circumstances results in accumulation of β-catenin in the cytoplasm and it enters the nucleus to activate genes that stimulate cell proliferation. Wnt signaling during normal development has the same effect, but it does so by down-regulating the complex of proteins that phosphorylates β-catenin. The decreased phosphorylated β-catenin is shunted to the proteasome where it is degraded. Loss of functional APC results in β-catenin accumulation that binds and activates the transcription factor T-cell factor-4 (Tcf-4) in the nucleus (Giles *et al*., 2003). It is proposed that β-catenin/Tcf-4 acts as a switch**,** controlling proliferation versus differentiation in the intestinal crypt epithelial cells. Activation of this Wnt pathway prevents the cells from either entering G1 arrest or undergoing terminal differentiation and induces resistance to apoptosis. The end result is cellular proliferation (van Es, *et al*., 2003). Watson (2006) also reported on this link between the mutated genes (APC tumor suppressor and β-catenin) and the Wnt signaling pathway among most sporadic CRCs.

2.4.2.3 Molecular factors involved in signaling pathways

A synopsis of factors (FAs, eicosanoids, growth factors, cytokines, enzymes, signaling proteins, oxidative stress, second messengers and tumor-derived factors) that can be responsible for CRC are summarized in FIGURE 2.2. Prominent factors that apply to this study are discussed in CHAPTER 3.

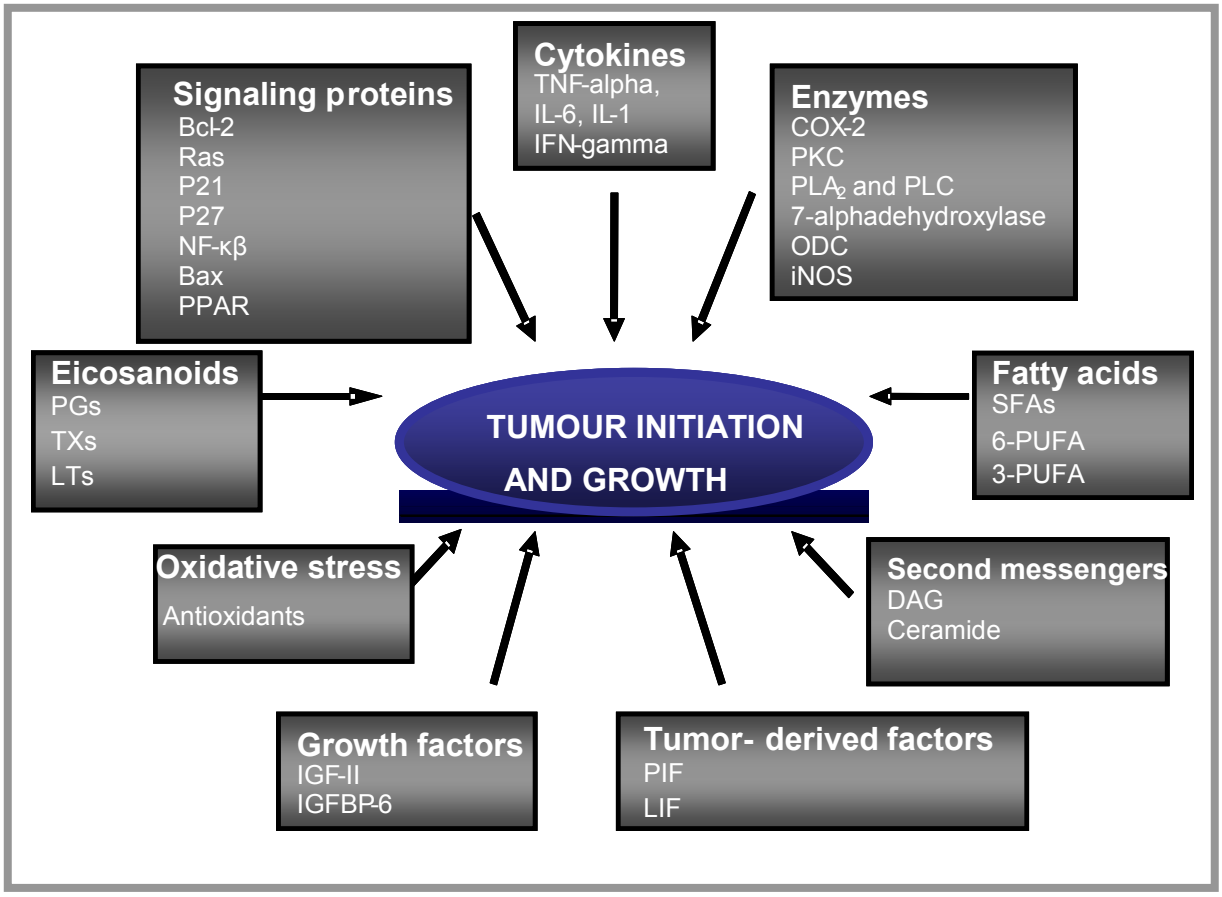

FIGURE 2.3 Factors involved in colorectal carcinogenesis (Roynette *et al.*, 2004). ***Abbreviations:*** *COX-2, cyclooxygenase-2; DAG, diacylglycerol; iNOS, inducible nitric oxide synthase; IGF, insulin growth factor; IGFBP, insulin growth factor binding protein; IL, interleukin; IFN, interferon; LTs, leukotrienes; LIF, lipolysis inducing factor; NFκB, nuclear factor-kappa Beta; n-3 and n-6 PUFAs, omega-3 and omega-6 polyunsaturated fatty acids; ODC, ornithine decarboxylase; PPAR, peroxisome proliferator activator receptor; PL, phospholipase; PGs, prostaglandins; PKC, protein kinase C; PIF, proteolysis inducing factor; SFAs, saturated fatty acids; TNF, tumor necrosis factor; TXs, thromboxanes.*

2.5 CURRENT THERAPIES

For CRC prevention regular screening is important to detect polyps before they can become cancerous. Surgery is the primary mode of therapy for the vast majority of CRCs and the only curative strategy remains complete surgical removal, but the overall survival rate is only 50-60%. This is mostly related to occult distant micro-metastases not detectable at the time of the first diagnosis. During the past 10 years, clinical studies helped to establish the value of adjuvant therapy for CRC. In advanced CRC disease outcome can be achieved through sequential application of combined systemic chemotherapy with drugs (bolus fluorouracil, leucovorin and capecitabine) as first line treatment, depending on the TNM classification. The aim of adjuvant chemotherapy is to prevent local recurrence or distant metastases and to prolong survival. Adjuvant radiotherapy is not recommended in colon cancer, mostly because of micro-metastasis and enhancement of oxidative stress. While colon cancer has a propensity to recur in distant sites, local recurrence is a major problem in rectal cancer after surgery. Therefore, for patients with rectal cancer (T2 and T3) adjuvant radiochemotherapy is considered the standard treatment, because it improves local control and overall survival when compared with surgery alone or combined surgery and radiation. These patients also appear to benefit from preoperative (neoadjuvant) radiation or radiochemotherapy (Martenson *et al*., 2004). Overall, treatment decisions are based on the patient's individual risk profile.

A new therapeutic direction that seems promising is manipulation of molecular tumor mechanisms with monoclonal antibodies against the epidermal growth factor receptor (EGFR) or vascular endothelial growth factor (VEGF). Several new drugs that aim to interrupt molecular pathways leading to increased proliferation, escape from apoptosis, angiogenesis and tumor metastasis (spreading) to distant sites are under development. They target growth factors, their receptors, or intracellular proteins involved in important signaling cascades. The monoclonal antibody against VEGF, bevacizumab, and the monoclonal antibody against EGFR, cetuximab, has been approved by the Food and Drug Administration (FDA) and European authorities for the treatment of metastatic CRC (Martenson *et al*., 2004; Schmiegel, 2005; Chau and Cunninghan, 2006).

Celecoxib has been approved by the FDA and concurrent therapy with celecoxib and standard CRC chemotherapy after surgery have entered randomized clinical trials in patients with FAP (Blanke *et al*., 2005; Sanborn and Blanke, 2005; Andre' *et al*., 2006; Arber *et al.,* 2006). There has been concern that selective COX-2 inhibitors may increase

the risk of cardiovascular events (Yona and Arber, 2006). For this reason the beneficial use of harmless FA therapeutic agents, correctly administered, needs consideration. Lastly, epidemiologic studies suggested that aspirin use can reduce the risk of CRC by approximately 40% to 50% (Chan *et al.,* 2004; Chan, 2006; Larsson *et al*., 2006). Therapeutic interventions that target COX-2 activity are outlined later in CHAPTER 3.

CHAPTER 3
BACKGROUND

3.1 GENERAL

3.1.1 Chapter exposition

For the purpose of this dissertation the principal concepts of dietary lipids, oxidative stress, signaling pathways and immunity are presented, since lipid driven signaling pathways contribute to CRC and immunodeficiency in these patients. Of importance for this study are those factors involved in signaling pathways that may serve as targets for clinical intervention in CRC management. Among these factors peroxisome proliferator receptors (PPARs), mitogen activated protein kinases (MAPKs), protein kinase C (PKC), nuclear factor kappa beta (NF-κB), activator protein-1 (AP-1) and oncogenes (ras, p53 and Bcl-2), as well as growth factors and cytokines are mentioned and reference is made to their roles during colorectal carcinogenesis. Already three decades ago, Meade and Mertin (1978) also revealed a role for FAs in immunity. Insight into FA gene-regulation of immune responses is considered imperative, since immunodeficiency definitely hampers the treatment of CRC patients. During carcinogenesis excessive cell proliferation and apoptotic resistance are encountered, both crucial factors on which therapy is based. Therapeutic approaches to combat CRC and to improve immunocompetence are an integral part of this study.

3.1.2 Cellular over-production and apoptosis

GENERAL: Cellular replication is composed of several distinct phases. G_1 is an initial growth phase that leads to DNA synthesis (S phase), followed by a gap phase (G_2), and finally by mitosis (M phase). Two important families of regulatory molecules promote progression through the cell cycle, the cyclins and the cyclin-dependent kinases. Cyclins D and A, respectively, are key proteins involved in facilitating entry of the cells into the cell cycle and progression through the S phase. Normal cells progress through the cell cycle after stimulation of these regulatory molecules by exogenous agents, such as growth factors, hormones, or cytokines. Cancerous cells, however, appear to lose their dependency on these external signals and often progress, unregulated, through many cell cycles. Cell death can occur by way of necrosis or apoptosis. Necrosis generally results

from an insult of toxic reactions and triggers inflammation. Apoptosis describes the distinct energy-requiring process of programmed cell death, characterized by DNA- and nuclear membrane fragmentation. Apoptosis, a normal cell clearance process during embryology, homeostasis, immunity and cell damage, can be induced under stress conditions, such as: genotoxic stress, i.e. DNA damage induced by carcinogens, oxidative free radicals and irradiation; oncogenic stress, i.e. aberrant activation of growth factor-signaling cascades; and non-genotoxic stress, i.e. hypoxia. Oxidative free radicals that cause oxidative stress and cell damage have been proposed to play a key role in the development of CRC (Valko *et al.*, 2004, 2006; and 2007).

RESEARCH: It was reported that DHA, EPA and CLA supplementation can arrest the growth of colon cancer cell lines in different phases of the cell cycle, and this growth arrest correlated with a down-regulation of cyclin protein expression in some instances (Chen and Istfan, 2000; Boudreau *et al.,* 2001; Cheng *et al.,* 2003; Bhattacharya *et al.,* 2006). Other studies with different tumor cell types also revealed that: EPA supplementation can modulate cyclin expression and arrest cell cycle progression in human leukemic K-562 cells (Chiu *et al.,* 2001); fish oil fed to rats can prolong DNA replication time of an implanted mammary tumor cell line, supporting the hypothesis that n–3 PUFAs may slow down progression through the S phase (Boudreau *et al.,* 2001; Llor *et al.,* 2003); CLA fed to rats can modify cell cycle proteins by up-regulating p53 expression involved in monitoring the quality of DNA after the G_1 phase and, if DNA is damaged, it will block entry of the cell into the S phase by altering the expression of genes to arrest growth / reduce cell proliferation (Field and Schley, 2004; Wahle *et al.,* 2004; Bhattacharya *et al.,* 2006).

3.2 LIPIDS

Lipid modulation of membrane FA compositions to normalize cell function, inhibit carcinogenesis or improve disease outcome requires insight into FA structure, nomenclature and metabolism. A summary of applicable research done over the years on modulation of membrane FA compositions is included.

3.2.1 Lipid classification

Among the total lipid (TL) content of a cell, neutral lipids (NLs) include monoglycerides, diglycerides and triglycerides, as well as free fatty acids. Among membrane lipids, the phosholipids (PLs) are the most important and they are divided into phosphatidylinositol

(PI), phosphatidylethanolamine (PE), phosphatidylcholine (PC), and phosphatidylserine (PS) subclasses. Membrane PLs play an important role in membrane-to-nucleus gene-regulation and, thus, colorectal carcinogenesis (Yeh *et al.,* 2006). Each class or subclass consists of a fatty acid (FA) composition or a FA profile, characteristic of normal or abnormal cells. Upon stimulation, FAs are removed from their membrane lipid stores by the action of various lipases and phospholipases (PLAs). The resulting free fatty acids (FFAs) may act as regulators or can be metabolized to biologically active compounds (Das, 2006a), discussed under FA metabolism.

3.2.2 Membrane phosholipids

Membrane PLs have a number of vital functions in the human body. Changes in tumor cell membrane PL FA compositions may result from changes in the metabolism of FAs during various stages of carcinogenesis (Jones *et al.,* 2003; Shim *et al.,* 2005). Research revealed that FAs released from membrane PLs by cellular PLAs or available to the cell from the extracellular environment are:

- modulators of membrane fluidity and cellular interaction (Murray *et al*., 2002; Bull *et al*., 2003; Yu *et al*., 2003a; Hulbert *et al*., 2005; Das, 2006b);
- cellular signaling molecules (Litman *et al*., 2001; Jump, 2002; Schaffer, 2002; Bull *et al*., 2003; Yu *et al*., 2003a; Yeh *et al*., 2006);
- secondary messengers involved in the transduction of external signals (Marks *et al*., 2000; Tapiero *et al*., 2002);
- messengers for PKC, that induces signal transduction and cell regulation (Mirnikjoo *et al*., 2001; Wansheng *et al.,* 2003);
- substrates for the generation of free radicals (Nigam and Schewe, 2000; Davydov and Bozhkov, 2003; Cho *et al.,* 2003; Baek and Eling 2006; Valko *et al.,* 2006 and 2007);
- regulators of gene-expression (Jump and Clarke 1999; Grimaldi, 2001; Duplus and Forest, 2002; Cheng *et al*., 2003; Yeh *et al.*, 2006; Vermeulen *et al.,* 2006);
- ligands for transcription factors that control cellular metabolic gene-expression (Schaffer, 2002; Takayama *et al.,* 2006);
- important role players in the regulation of the immune system by acting as precursors for the synthesis of eicosanoids (Marks *et al*., 2000; Tapiero *et al*., 2002; Kew *et al*., 2004; Guy, 2005; Chapkin *et al.,* 2007a and b).

3.2.3 Fatty acid structure

The simplest lipids are carboxylic acids and they are generally known as fatty acids (FAs). The structure of a FA consists of a chain of hydrocarbon molecules and a carboxylic acid moiety is attached at one end of the structure. Depending on the chain length, FAs are referred to as short-chain fatty acids (SCFAs) when the chain length is 2 to 4 carbon atoms, medium-chain fatty acids (MCFAs) when the chain length is between 6 and 10 carbon atoms and long-chain fatty acids (LCFAs) when the chain length is more than 12 carbon atoms (Fatty acids, 2007). FAs can be classified as saturated fatty acids (SFAs) with no double bonds, monounsaturated fatty acids (MUFAs) with a double bond and polyunsaturated fatty acids (PUFAs) with 2 or more double bonds.

3.2.4 Fatty acid nomenclature

In the designation of a FA structure the numerical notation indicates the number of carbon atoms followed by the number of double bonds and an "omega" designation (n or ω) refers to the position of the first double bond from the methyl terminus. Therefore, EPA (20:5 n-3 or 20:5 ω-3) refers to a 20-carbon PUFA, containing 5 double bonds with the first double bond located at the third bond position from the methyl terminus (Jump*,* 2002). The n-3 and n-6 PUFAs are also referred to as the ω-3 and ω-6 PUFAs (Rose and Connolly, 1999). Another system (not widely in use) to assign abbreviation to FAs come from the number of carbon atoms, followed by the number of sites of unsaturation, e.g. palmitic acid (PA) is a 16-carbon FA with no unsaturation and is designated by 16:0. The site of unsaturation in a FA is indicated by the symbol Δ and the number of the first carbon of the double bond, e.g. palmitoleic acid (PoA) is a 16-carbon FA with one site of unsaturation between carbons 9 and 10, and is designated by $16{:}1^{\Delta 9}$. This method is an easy identification of the chemical structure of the FA. Desaturation of FAs involves a process that requires molecular oxygen, NADH, and cytochrome B_5. The most common desaturation reactions involve the placement of a double bond between carbons 9 and 10 in the conversion of PA to PLA and the conversion of SA to OA (Smith *et al.,* 2005). Some biologically important FAs are summarized in TABLE 3.1.

TABLE 3.1 Chemical structures of some biological important fatty acids (Bartsch *et al.,* 1999; Larsson *et al.,* 2004)

NUMERICAL ABBREVIATION	COMMON NAME	STRUCTURE
SATURATED FATTY ACIDS		
4:0	**Butyric acid**	**$CH(CH_2)_2COOH$**
12:0	**Lauric acid**	**$CH_3(CH_2)_{10}COOH$**
14:0	**Myristic acid**	**$CH_3(CH_2)_{12}COOH$**
16:0	**Palmitic acid**	**$CH_3(CH_2)_{14}$ COOH**
18:0	**Stearic acid**	**$CH_3(CH_2)_{16}COOH$**
MONOUNSATURATED FATTY ACIDS		
16:1	**Palmitoleic acid**	**$CH_3(CH_2)_5C=C(CH_2)_7COOH$**
18:1	**Oleic acid**	**$CH_3(CH_2)_7C= C(CH_2)_7COOH$**
UNSATURATED FATTY ACIDS		
18:2 n-6	**Linoleic acid**	**$CH_3(CH_2)_4C=CCH_2C= C(CH_2)_7COOH$**
18:3 n-3	**Alpha-linolenic acid**	**$CH_3CH_2CH=CHCH_2CH= CHCH_2CH=CH(CH_2)COOH$**
18:3 n-6	**Gamma-linolenic acid**	**$CH_3(CH_2)C=CCH_2C=CCH_2C= C(CH_2)_7COOH$**
20:3	**Dihomo-gamma-linolenic acid**	**$CH_3(CH_2)_4C=CCH_2C=CCH_2C= C(CH_2)_6COOH$**
20:4	**Arachidonic acid**	**$CH_3(CH_2)_3$ $(CH_2C=C)_4$ $(CH_2)_3COOH$**
20:5	**Eicosapentaenoic acid**	**$CH_3CH_2CH=(CCH_2CH)_4= CH(CH_2)_3COOH$**
22:6	**Docosahexaenoic acid**	**$CH_3CH_2CH=(CCH_2CH)_5= CH(CH_2)_2COOH$**

3.2.5 Individual fatty acids

Acetate, propionate and butyrate are major SCFAs that arise in the large bowel during bacterial fermentation of dietary fiber and starch (Topping and Clifton, 2001; Pool-Zobel *et al*., 2002; Andoh *et al*., 2003; Tedeling *et al.,* 2007). Ingestion of non-digestible carbohydrates such as resistant starch and subsequent SCFA production are associated with induction of the enzyme glutathione transferase, a chemopreventative enzyme (Wollowski *et al*., 2001). Since lower intestinal proliferation is associated with a decreased colon cancer risk, it was suggested that treatment diets that increase SCFA cell contents might be beneficial for the colon mucosa (Dolara *et al*., 2002; Lupton, 2004). LCFAs under discussion in this study are: lauric acid (LRA; 12:0); myristic acid (MA; 14:0); palmitic acid (PA; 16:0); palmitoleic acid (PoA; 16:1n-7); stearic acid (SA; 18:0); oleic acid (OA; 18:1n-9); linoleic acid (LA; 18:2n-6); gamma-linolenic acid (GLA; 18: 3n-6); alpha-linolenic acid (ALA; 18:3n-3); dihomo-gamma-linolenic acid (DGLA; 20:3n-6); arachidonic acid (AA; 20:4n6); eicosapentaenoic acid (EPA; 20:5n-3); and docosahexaenoic acid (DHA; 22:6n-3) (also see FIGURE 3.1). The focus is on those LCFAs responsible for CRC and those with beneficial therapeutic use in the management of CRC patients.

3.2.6 Fatty acid series or families

FA series are named according to the position of the first double bond from the methyl terminus of the hydrocarbon chain of the molecule, e.g. in the case of the n-6 or n-3 PUFA series the first double bonds are between C6 and C7, and C3 and C4, respectively (Diggle, 2002). The n-6 and n-3 PUFA series participate in essential fatty acid metabolism (EFAM), whilst the n-7 and n-9 FA series represent the n-7 and n-9 FA pathways that are followed in the case of essential fatty acid deficiency (EFAD) or interference with EFAM.

3.2.7 Fatty acid isomers

The biological important FA isomers are those occurring in the *cis*-configuration. Those in the *trans*-configuration are either consumed in the diet (ruminal food products) or metabolized in the body (e.g. vaccenic acid), or can be the result of food processing and commercial cooking and frying (Craig-Schmidt, 2006). The designation *cis* means that the hydrogens are on the same side of the double bond and the acyl chains on the other side, whilst in *trans*-FAs, the acyl chains are on opposite sides of the double bond. *Trans*-isomers are pro-inflammatory and may cause carcinogenesis in those diets where *trans*-FAs are consumed or metabolized in excess (Slattery *et al*., 2001; King *et al*., 2005).

However, different CLA products can be converted from LA by bacterial species in the human gut (e.g. hydrogenation). In the case of the latter, the possibility exists that the formation of different CLA products (consisting of different isomer compositions) under the influence of bacterial species in the human gut, identified by Devillard *et al.* (2007), may contribute to the pathogenesis of CRC. To what extent harmful CLA products may have a direct effect on colonocytes in the human gut is not yet fully clarified and deserves more attention. Nevertheless, the uptake of CLA produced by bacterial activation in the gut appears to be minimal and CLA intake by ruminal products optimal (Wahle *et al*., 2004).

3.2.7.1 Conjugated linoleic acid

Of particular importance to this study is conjugated linoleic acid (CLA) that consists of *cis (c)-* and *trans (t)-* isomers. CLA comprises a family of positional and stereoisomers of LA, also referred to as isomers of octadecadienoic acid. Conjugated double bonds in either *c-* or *t-* configurations are predominantly present in position 8 and 10, 9 and 11, 10 and 12, or 11 and 13 (Bhattacharya *et al.,* 2006). In the human gut LA (*c*9,*c*12-18:2) is converted by different bacterial activities to CLA products (*c*9,*t*11-18:2; *t*9,*t*11-18:2 and *t*10,*c*12-18:2) or via the hydroxyl-18:1 fatty acid (HFA) to *c*9,*t*11-18:2 and *t*9,*t*11-18:2. Summarized, LA (*c*9*,c*12-18:2) is metabolized in the human colon via CLA (mainly *c*9,*t*11-18:2) to vaccenic acid (VA) (*t*11-18:1), and then to stearic acid (SA) (18:0). The uptake of CLA formed in the intestine seems to be minor (Kamlage *et al.,* 1999). Ewaschuk *et al.* (2006) suggested that the amount of LA available for CLA formation in the colon may vary and is dependant upon the amount ingested and the efficacy of absorption in the small intestine. However, local effects on gut tissue might be anticipated and research studies did confirm that specific CLA products have anti-proliferative and anti-inflammatory effects on colonocytes (Kemp *et al.,* 2003; Bassaganya-Riera *et al.,* 2004; Bhattacharya, *et al.,* 2006). Thus, provision of CLA products in the intestinal lumen can be considered beneficial, particularly under inflammatory conditions. Currently, a commercial product of CLA (Clarinol^{TM}G-80, that consists of *c*9,*t*11-18:2 and *t*10,*c*12 18:2) is available and more clinical trials are awaited for proper evaluation. Nevertheless, VA (*t*11-18:1) is also converted in the human body to beneficial CLA (*c*9,*t*11-18:2). In ruminant animals similar biohydrogenation processes occur in the rumen and CLA (*c*9,*t*11-18:2), the most abundant natural and beneficial product present in dairy products, and VA (*t*11-18:1), the major *trans*-MUFA present in the fat of ruminant food products, are formed (Wahle *et al*., 2004). Other studies confirmed that CLA arises along the ruminal pathway and reaches full saturation into SA (Kris-Etherton *et al.,* 2000; Devillard *et al.,* 2007). However, low pH may inhibit the biohydrogenation of VA to SA, leading to accumulation of VA in tissues. Humans,

therefore, obtain CLA directly from the consumption of ruminal food products or it is produced in the gut from LA and in tissues from VA.

3.2.8 Dietary fatty acid sources

The major SFAs in the diet are PA, followed by SA, MA and LRA. PA is particularly abundant in palm oil, butter, milk, cheese and meat (beef). SA is found predominantly in cocoa butter (used in chocolate) and in fats from cattle and sheep. MA is found in palm kernel oil and the principal dietary source of LRA is coconut oil (Araujo de Vizcarrondo *et al.,* 1998; Nkondjock *et al.,* 2003). The major MUFAs in human diets are OA and PoA. OA is present in all edible fats and oils, but olive, canola and rapeseed oils are particularly rich sources. PMA is found in the oil of the macadamia nut (Larsson *et al*., 2004). The major PUFAs are LA and ALA. LA is mostly found in vegetable seeds and oils (safflower, soybeans, corn and sunflower oil). Linseed oil, rapeseed, walnuts and dark green leafy plants are rich in ALA (Bartsch *et al*., 1999). GLA is found in evening primrose oil and borage oil. Dietary LA is considered to be the main source of endogenous AA, but beef- and pork meat are also exogenous AA sources (Bartsch *et al*., 1999; Rose and Connolly, 1999). AA is arguably the most important PUFA in normal and diseased tissues (Brash, 2001). In comparison to n-6 PUFAs, n-3 PUFAs are consumed at much lower levels, mostly in the form of ALA (Kris-Etherton *et al*., 2000). Deep cold-water fatty fish (such as salmon, tuna, herring, mackerel and anchovy) are rich sources of EPA and DHA. The marine food chain is based on n-3 PUFAs, which are present in plankton and algae on which fish feed (Rose and Connolly, 1999; Roynette *et al.,* 2004).

Trans-MUFAs are produced during industrial hydrogenation of polyunsaturated vegetable oils (Craig-Schmidt, 2006). Major sources of *trans*-unsaturated FAs are hard margarines and partially hydrogenated fats, such as frying fats used in industrial food preparation and in fast-food restaurants. The major dietary sources of CLA are foods derived from ruminal food products, such as milk, yoghurt, cheese, butter and meat (Larsson *et al.,* 2005a).

3.2.9 Fatty acid metabolism

For unknown reasons, FA synthesis stops in practically all cells when the chain is 16 carbon atoms long. Only small amounts of 12- and 14-carbon FAs are formed, and none with more that 16 carbons. FA synthase (FAS) contributes to the *de novo* synthesis of these longer-chain FAs. Glucose is converted to acetyl-CoA by glycolysis and a portion of the acetyl-CoA is carboxylated to malonyl-CoA. PA, the predominant product of FAS, is

synthesized *de novo* from the substrates acetyl-CoA, malonyl-CoA, and NADPH (Kuhajda, 2000; Shah *et al.,* 2006). SA is either obtained in the diet or synthesized by the elongation of PA. Along with PA, SA is the major substrate for the enzyme stearoyl-CoA desaturase, which catalyzes the conversion of SA to OA, the preferred substrate of the synthesis of triglycerides and other complex lipids (Ntambi, 2004; Sampath and Ntambi, 2005a and 2005b). Furthermore, humans do not possess the Δ12- and Δ15-desaturase (Δ^{12}d and Δ^{15}d) enzymes of plants that is required for LA and ALA synthesis. Therefore, these two PUFAs must be consumed in the diet in order to maintain adequate body pools and they serve as substrates for the n-6 and n-3 PUFA pathways (Gurr, 1996), known as EFAM. LA and ALA are metabolized to AA and EPA, respectively, by a series of Δ6- and Δ5-desaturase (Δ^{6}d and Δ^{5}d) and elongase activities. Following a series of steps (including elongation, desaturation and β-oxidation) DHA is produced from EPA. The n-6 and n-3 PUFA series are not interconvertable and compete for metabolic enzymes, i.e. desaturases and elongases, common to both pathways (Hansen-Petrik *et al*., 2000). However, these enzymes have a greater affinity for n-3 PUFAs and they are therefore preferentially metabolized (Rose and Connolly 1999; Simopoulos, 2002). Interference with Δ^{6}d and Δ^{5}d activities by environmental factors and endogenous *trans*-FAs, as well as SFAs were previously reported to prevent the conversion of LA and ALA to their important biologically metabolites (Horrobin, 1993). However, in the case of EFAD, often encountered in malnourished persons or inflammatory diseases, the n-7 and n-9 FA metabolic pathways are followed (Saether *et al.,* 2007). The n-3 and n–6 FA pathways and the n-7 and n–9 FA pathways are illustrated in FIGURES 3.1(a) and 3.1(b).

3.2.9.1 Cyclooxygenases and lipoxygenases

Eicosanoids (biologically potent, short-lived and hormone like lipids) are crucial molecules in the regulation of cell growth, inflammation and immune responses. FAs that are precursors for eicosanoid metabolism are DGLA, AA and EPA. These FAs are converted by cyclooxygenases (COXs) or lipoxygenases (LOXs) to eicosanoids that consist of prostanoids, leukotrienes (LTs) and lipoxins (LXs). Among the prostanoids the prostaglandins (PGs), prostacyclins (PCs) and thromboxanes (TXs) are included (Larsson *et al.,* 2004). COX metabolism gives rise to the prostanoids (PGs and TXs) and LOX metabolism gives rise to LT's, hydroxyl FAs and lipoxins (Larson *et al*., 2004).

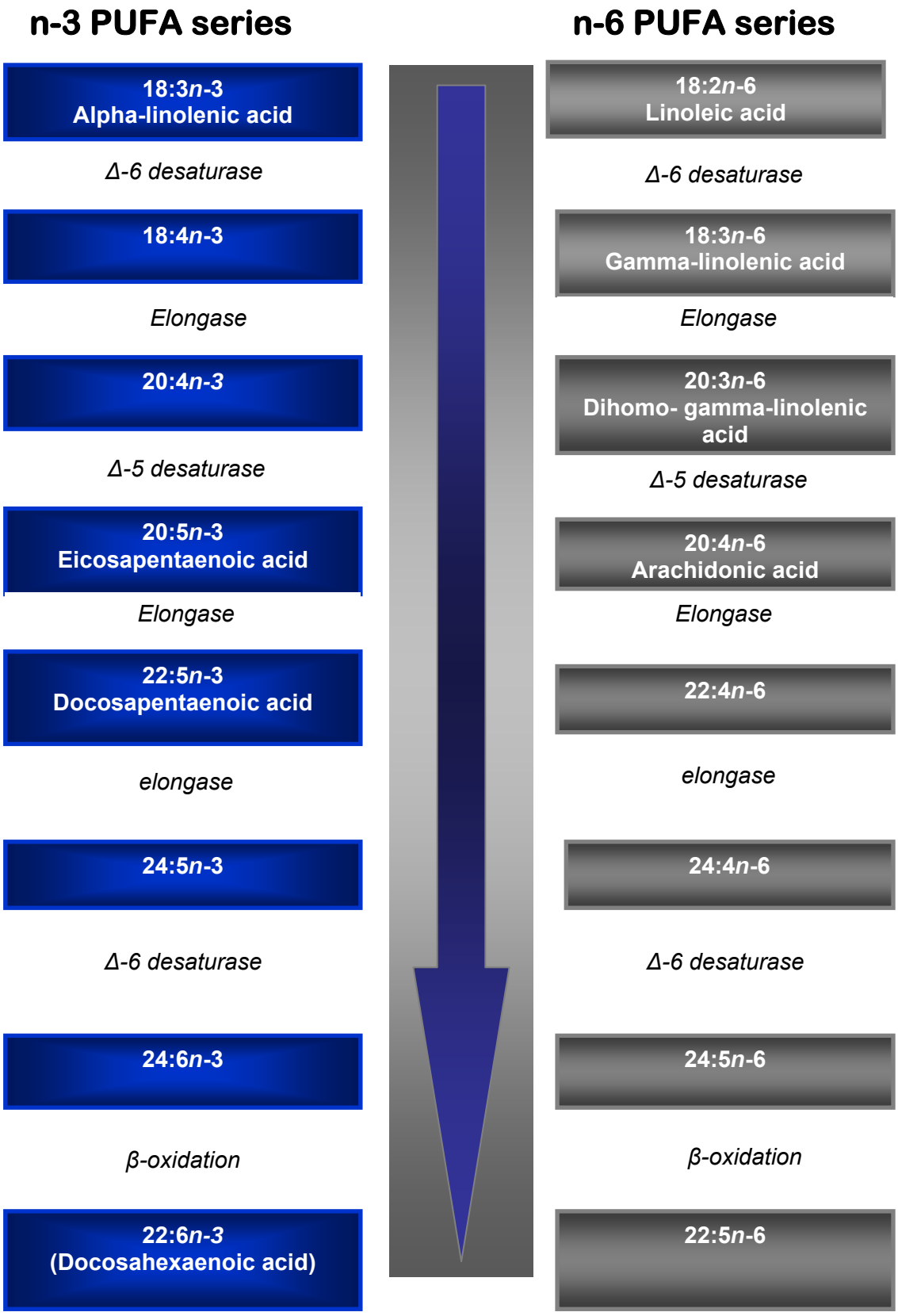

FIGURE 3.1(a) Elongation and desaturation of n-3 and n-6 fatty acids Kelley (2001).

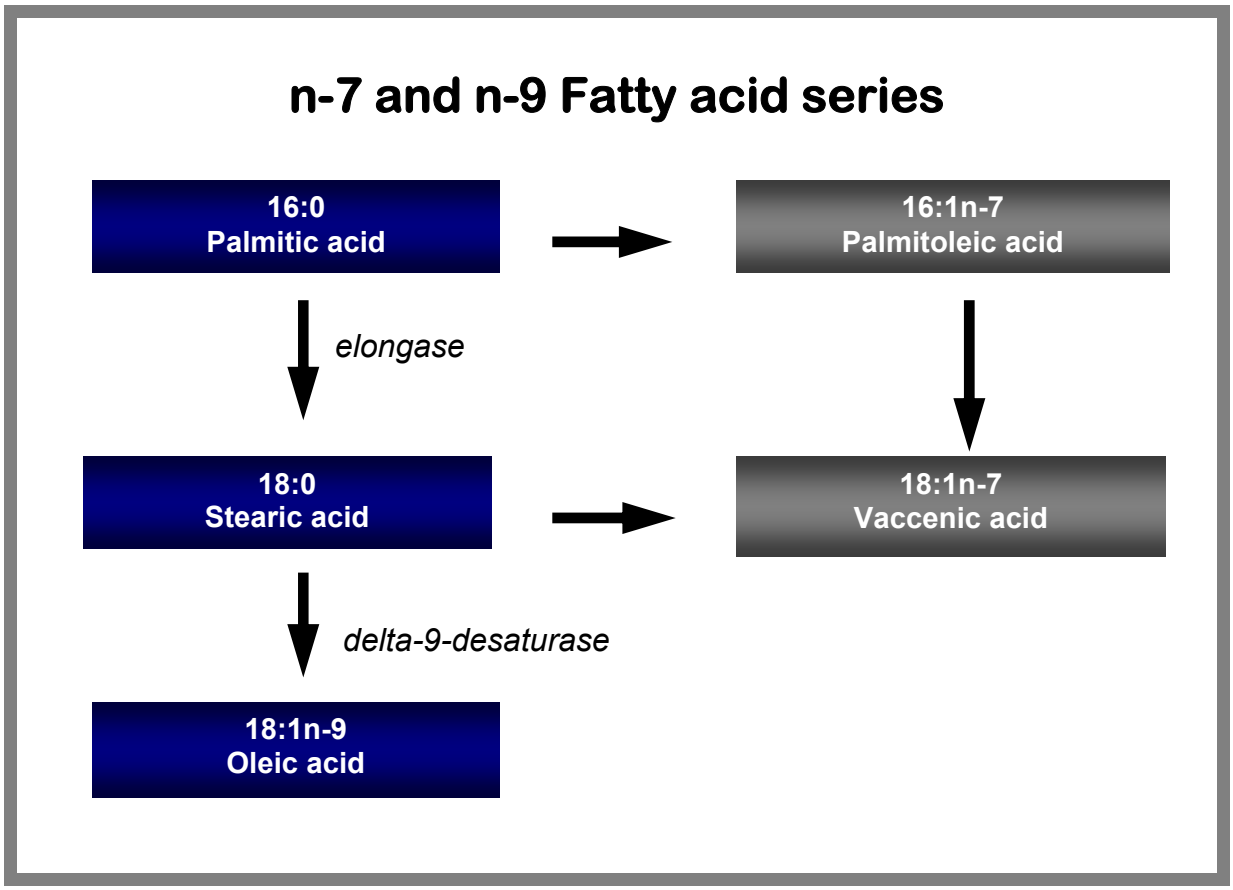

FIGURE 3.1(b) The n-7 and n-9 FA metabolic pathways.

COX enzymes are responsible for the oxidation of AA and EPA. There are three forms of COXs (COX-1, COX-2 and COX-3) described in the literature (Sano *et al*, 1995; Smith *et al*., 2000; Kawai *et al.,* 2002; Dommels *et al*., 2003; Rao *et al*., 2004; Hersh *et al.,* 2005). Of relevance to this study is COX-2 that undergoes rapid induction in response to a variety of stimuli, including mitogens, cytokines, and growth factors (Furstenberger *et al.,* 2006). Lipoxygenases (5-, 12- and 15-LOX) are lipid peroxidizing enzymes that act upon LA, AA and EPA to produce eicosanoids. FIGURES 3.2(a), 3.2(b) and 3.2(c) illustrate eicosanoid metabolism from LA, AA and EPA.

LINOLEIC ACID METABOLITES: Linoleic acid is converted to GLA by Δ^6d and GLA, in turn, to DGLA by elongases. DGLA is the substrate for conversion to AA by Δ^5d, and is also the substrate for the PG_1 series and TXs (Kapoor and Huang, 2006). During EFAM the conversion of LA to GLA by the key enzyme Δ^6d is a rate-limiting process. For this reason GLA is then often supplemented in the case of inflammatory diseases or conditions marked by EFAD to effectively provide anti-inflammatory PGE_1 metabolites (Das,2006a and 2007).

ARACHIDONIC ACID METABOLITES: AA is preferentially incorporated into the Sn-2 position of membrane PLs, where it contributes to maintenance of normal structure and membrane fluidity. This membrane bound AA is released primarily by cytosolic PLA_2 to provide a substrate for the production of a large group of eicosanoids (Nigam and Schewe, 2000; Taketo and Sonoshita, 2002). AA is the substrate for two classes of enzymes: COX, also named prostaglandin synthase (PGS), that produces the prostanoids (PGs and TXs); and the LOXs, which catalyze the biosynthesis of the hydroxyeicosatetraenoic acids (HETEs) and LTs (Tapiero et al., 2002). These eicosanoids behave as “local hormones” and are chemical transmitters for a variety of intercellular and intracellular signals. PGs catalyze two sequential reactions: first, the COX activity of the enzyme converts AA to PGG_2, and then the peroxidase activity reduces PGG_2 to PGH_2. The PGH_2 produced from AA is then converted to various prostanoids. AA is a precursor of the prostanoid-2 series with 2 double bonds and the LT4-series with 4 double bonds (Funk, 2001). Without a source for AA or compounds which can be converted into AA, synthesis of PGs by a COX enzyme would not be compromised, and this would seriously affects many normal metabolic processes (Jones *et al.,* 2003). Of particular importance in this study is PGE_2, a pro-inflammatory metabolite of AA (Shao *et al.,* 2003; Shao *et al.,* 2004; Castellone *et al.,* 2005).

EICOSAPENTAENOIC ACID METABOLITES: EPA is a precursor of the prostanoid-3 series with 3 double bonds and LTs of the 5-series with 5 double bonds. This n-3 PUFA is converted by COX to PGG_3 and, in turn, to PGH_3 that is then further metabolized to PGE_3, PGI_3, and TXA_3. These pathways correspond to those by which AA is converted to the prostanoid-2 series, and EPA competes effectively with the n-6 PUFA series for available COX activity (Rose and Connolly, 1999). Of particular importance to this study is that PGE_3 is an anti-inflammatory metabolite and EPA is usually supplemented together with GLA to provide sufficient anti-inflammatory metabolites.

SUMMARY OF LIPOXYGENASE END PRODUCTS: A summary of all the LOX end products of LA, AA and EPA is as follows: when the 15-LOX enzyme acts upon LA, 9-and 13-hydroxyoctadecadienoic acids (13-HODEs) are produced, whereas the 15-LOX enzyme acts upon AA to form 15-hydroxyeicosatetraenoic acid (15-HETE) products and EPA to form lipoxins (LXs); when the 12-LOX enzyme acts on AA and EPA, 12-hydroxyeicosatetraenoic acid (12-HETE) and 12-hydroxyeicosapentaenoic acid (12-HEPE) are produced, respectively; when the 5-LOX enzyme acts on AA and EPA, 5-hydroxyeicosatetraenoic acid (5-HETE), LTA_4 and LTB_4 are produced in the case of AA, and 5-hydroxyeicosapentaenoic acid (5-HEPE), LTB_5 and LTE_5 are produced in the case of EPA. The lipoxygenase end products are also illustrated in FIGURES 3.2(a), 3.2(b) and 3.2 (c). The peroxidase-catalyzed conversion of PGG_2 to PGH_2, and the conversion of hydroperoxy fatty acids to hydroxyl fatty acids, both yield oxygen free radicals (Hwang and Rhee, 1999).

3.2.10 Membrane fatty acid modulation

GENERAL APPROACH: Supplementation studies to modulate membrane FA compositions contributed tremendously to our understanding of membrane remodeling and membrane functioning. As previously mentioned, It is known that there is competition between n-6 and n-3 PUFAs for their metabolic conversion via desaturase and elongase enzymes, which are common to both pathways (Hansen-Petrik et al, 2000). It is known that apart from the diet (SFAs and *trans*-FAs), environmental influences (alcohol, smoke particles, bacterial and viral infections) may influence EFAM and n-7 and n-9 alternative metabolic pathways are then followed (Ewaschuk *et al.,* 2006). It is also known that the latter pathways are followed in cancer cells, since FAS and SOD (Δ^6d) activities are up-regulated (Menendez *et al.,* 2005a; Saether *et al.,* 2007).

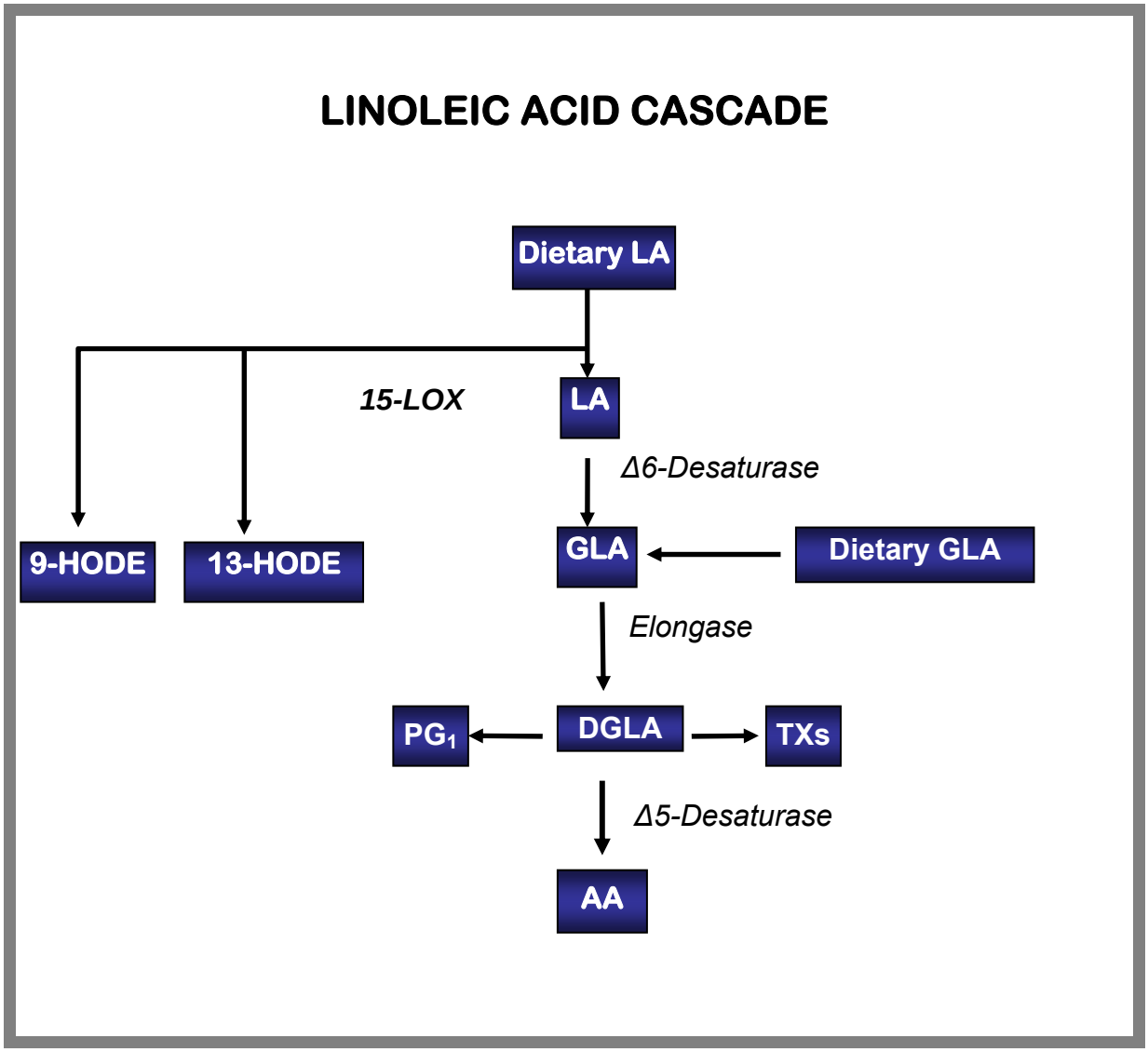

FIGURE 3.2(a) Overview of linoleic acid metabolism into eicosanoids.

Abbreviations: *AA, arachidonic acid (20:4n-6); DGLA, dihomo-gamma-linolenic acid (20:3n-6); GLA, gamma-linolenic acid (18:3n-6); HODE, hydroxyoctadecadienoic acid; LA, linoleic acid; LOX, lipoxygenase; PG_1, prostaglandin 1-series; TX, thromboxane* (Larsson *et al*., 2004).

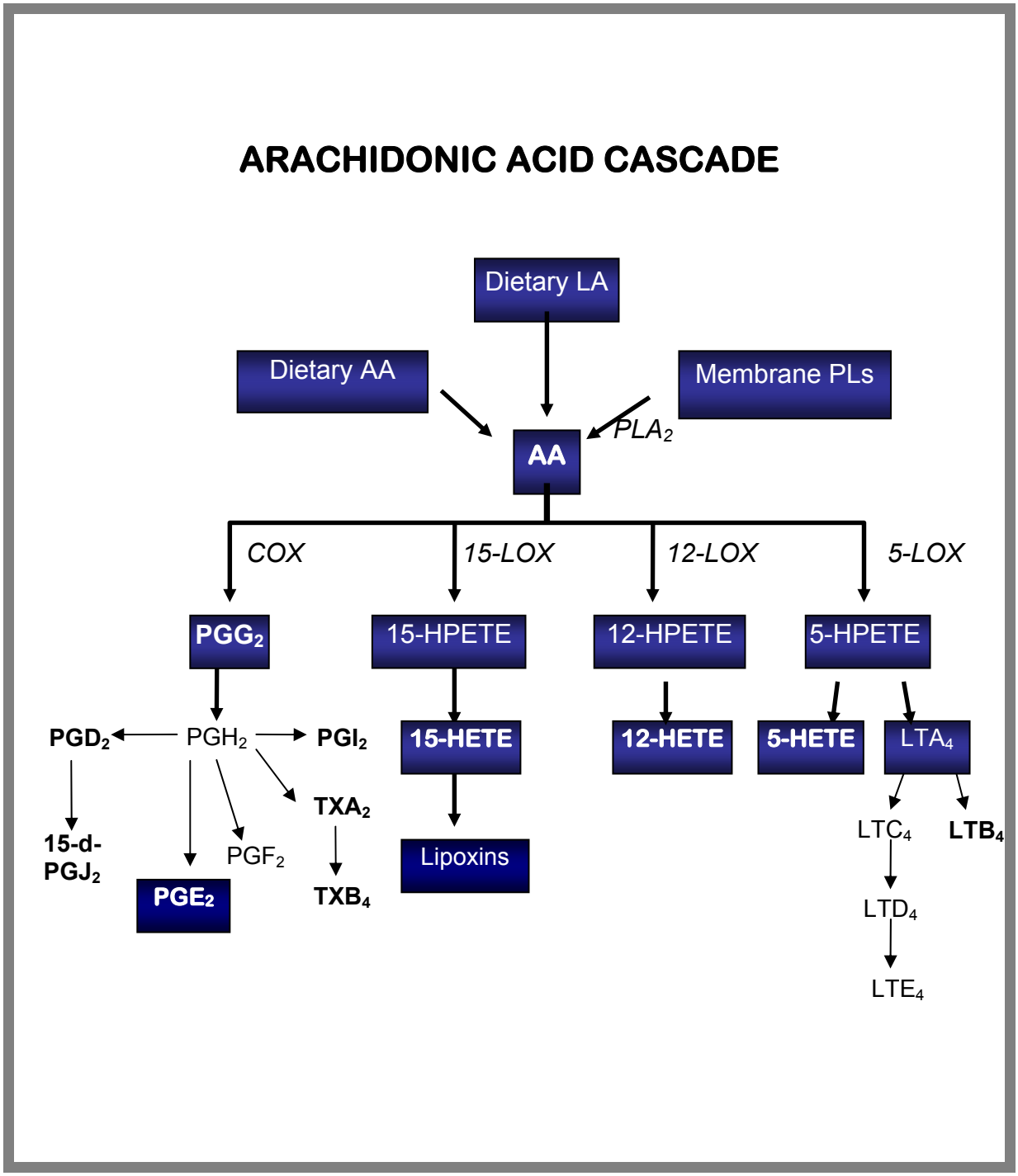

FIGURE 3.2(b) Overview of arachidonic acid metabolism into eicosanoids.

Abbreviations: *AA, arachidonic acid; COX, cyclooxygenase; 15-HETE, 15-hydroxyeicosatetraenoic acid; 12-HETE, 12-hydroxyeicosatetraenoic acid; 5-HETE, 5-hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LA, linoleic acid; LOX, lipoxygenase; LT, leukotriene; PLs, phospholipids; PLA_2, phospholipase A_2; PG, prostaglandin; TX, thromboxane* (Larsson *et al*., 2004).

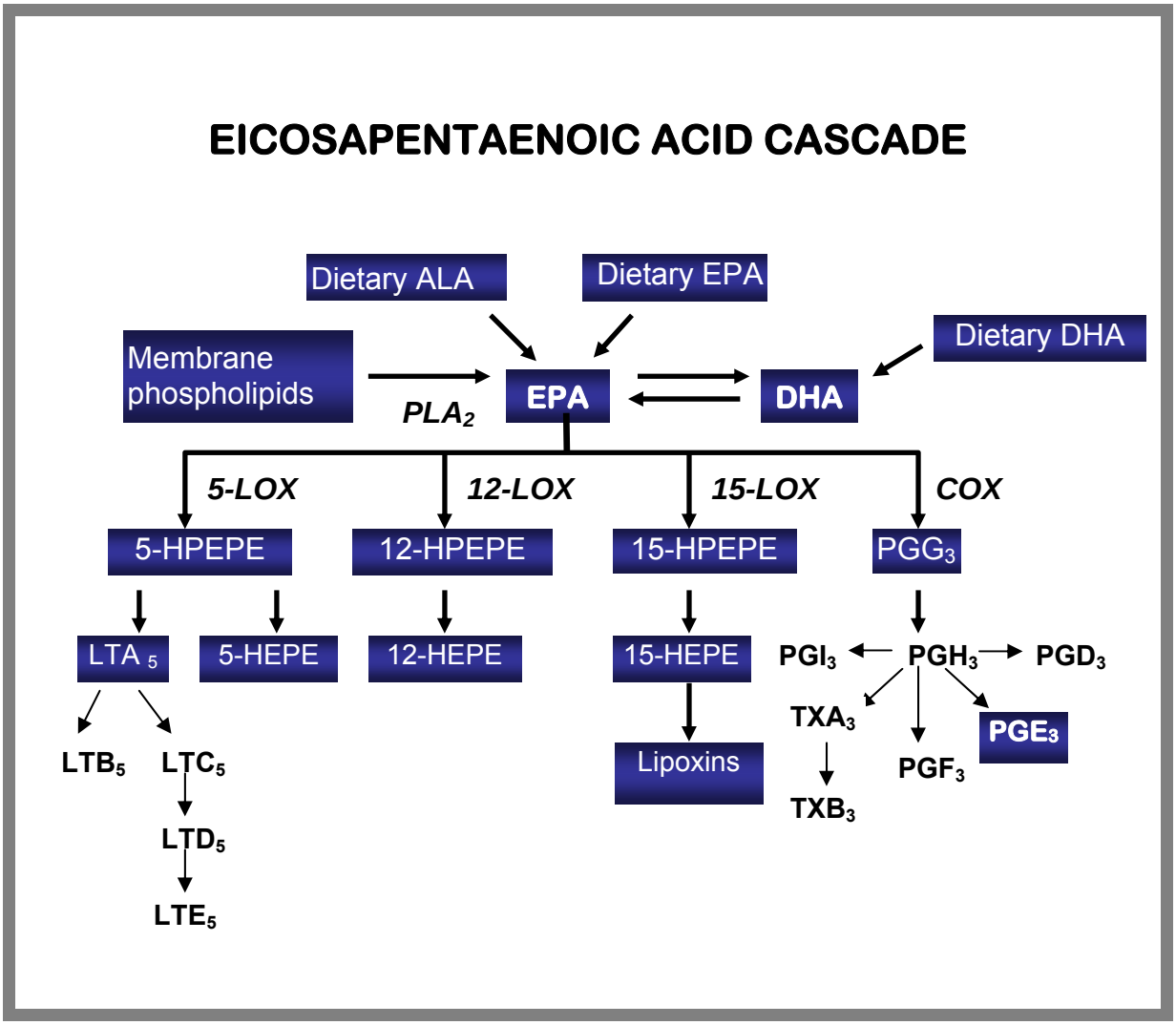

FIGURE 3.2(c) Overview of eicosapentaenoic acid metabolism into eicosanoids. ***Abbreviations:*** *ALA, alpha-linolenic acid; AA, arachidonic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; HPEPE, hydroperoxyeicosapentaenoic acid; HODE, hydroxyoctadecadienoic acid; LT, leukotriene; LOX, lipoxygenase; PLA$_2$, phospholipase A$_2$; PG, prostaglandin; TX, thromboxane* (Larsson *et al.*, 2004).

MECHANISMS INVOLVED: Initially, *in vitro* supplementation studies demonstrated the cytotoxic effects of n-6 PUFAs on cancer cells (Horrobin 1994; Jones *et al.,* 2003; Larsson *et al.,* 2004). Thereafter, cell line, animal and human studies emphasized the beneficial use of n-3 PUFAs in health and disease (Llor *et al.,* 2003; Jho *et al*., 2004; Larsson *et al.,* 2004). Currently, CLA is widely explored with *in vitro* and *in vivo* supplementation studies in search of its optimal beneficial use in health and disease (Lee *et al.,* 2005; Bhattacharya *et al.,* 2006; Soel *et al.,* 2007). All the mechanisms involved are not yet fully unraveled; however, basic explanations that rest upon cell line, animal model and human studies are given. In the case of n-3 PUFAs their incorporation into membrane PLs can reduce desaturation and elongation of LA to AA and shift eicosanoid biosynthesis in favor of EPA-derived PGE_3 that is anti-inflammatory (Calviello *et al*., 1999; Rose and Connolly 1999; Crawford *et al.,* 2000; Petrik *et al.,* 2000; Rao *et al.,* 2001; Belury *et al.* 2002; de Pablo *et al.,* 2002; Dommels, 2002a and 2002b; Simopoulos, 2002; Bull *et al.,* 2003; Llor *et al*., 2003; Larsson *et al.,* 2004; Wahle *et al.,* 2004; Lee *et al.,* 2005; Yeh *et al.,* 2006). Interestingly, the EPA metabolite, namely 5-HEPE, has even a more inhibitory effect on carcinogenesis than EPA (Vang *et al.,* 2005; Yeh *et al.,* 2006). Modulation of membrane FA compositions with CLA has the potential to inhibit carcinogenesis by down-regulation of the AA cascade (Belury *et al.,* 2002; O'Shea *et al*., 2004; Lee *et al.,* 2005), whilst GLA has the potential to down-regulate FAS and SCD (Δ^9d) activities during carcinogenesis (Menendez *et al*., 2004a).

3.3 OXIDATIVE EVENTS

The cumulative production of oxygen-free radicals, reactive oxygen species (ROS) along with nitric oxide (NO•) during chronic inflammation, and other free radicals cause oxidative stress with consequent cell damage that is common for many types of cancer. CRC is hallmarked by an oxidative burst and excessive oxidative stress and all the radicals and mechanisms involved in cell damage are accordingly discussed.

3.3.1 Radicals

GENERAL: Under normal conditions free radicals perform useful functions in the body and scavenging pathogens is one of them. Free radicals can be produced during stress conditions (e.g. radiation), carcinogen exposure and by tumor promoters (phorbol esters) (Babbs *et al.,* 1990). Seen in perspective, a free radical can be defined as a molecule or a fraction of a molecule containing one or more unpaired electrons. In writing, the

unpaired electron and radical nature are indicated with a heavy superscript dot. A common process in biological systems by which free radicals are formed is by electron transfer that requires energy. The most important free radicals are radical derivates of oxygen. Oxygen free radicals are not the only important free radicals in biochemistry, although they are the initial species formed. Other free radicals of importance are the wide range of carbon-centered radicals that arise from the attack of an oxidizing radical on a biological molecule such as a lipid, nucleic acid, carbohydrate or protein. Although hydrogen peroxide (H_2O_2) is an important compound in free radical biochemistry, it is not a free radical and falls into the category of reactive oxygen species (ROS). The latter not only includes oxygen free radicals, but also non-radical oxygen derivates that are involved in oxygen radical production. Hydrogen peroxide is an important compound in free radical formation, since it can easily break down, particularly in the presence of transition metal irons, to produce the most reactive and damaging of the oxygen free radicals, the hydroxyl radical. Cells of the immune system produce ROS (superoxide anion) and NO• during an oxidative burst, triggered during inflammatory processes. In turn, oxidants can both promote and inhibit the initiation of lipid peroxidation (Athar, 2002; Sander *et al.,* 2004a). Alone, it acts as a potent inhibitor of the lipid peroxidation chain but the superoxide anion and NO• may react together to produce a much more oxidatively active molecule (peroxynitrite anion), a potent oxidizing agent that can cause DNA fragmentation and lipid oxidation (Hogg and Kalyanaraman, 1999; Dröge, 2002; Baniyash, 2006; Valko *et al.,* 2007).

BIOCHEMISTRY: The biochemistry of oxygen-free radical formation is well described and illustrated in the literature (Valko *et al.,* 2006 and 2007). Key players in the biochemistry of oxygen free radicals (apart from oxygen itself) are superoxide, hydrogen peroxide, transition metal ions, and the hydroxyl radical. The production of superoxide radicals occurs mostly within the mitochondria of a cell, where the mitochondrial electron transport chain is the main source of ATP in the mammalian cell. During energy transduction a small number of electrons “leak” to oxygen prematurely, with consequent superoxide anion radical formation that has been implicated in the pathophysiology of a variety of diseases (Dröge *et al*. 2002; Marnett, 2002; Valko *et al.,* 2006 and 2007). Superoxide radicals, formed on both sides of mitochondrial inner membranes, are efficiently detoxified by superoxide dismutase (SOD) that works in conjunction with hydrogen peroxide (H_2O_2)-removing enzymes. One of the physiological functions of H_2O_2 is the activation of redox sensitive transcription factors, such as NFκβ and AP-1, which subsequently allows the transcription of specific genes (Shah and Channon, 2004). The

activity of PKC seems to play a crucial role in this activation (Gate *et al.,* 1999). *In vivo,* under stress conditions, an excess of superoxide releases iron (Fe^{2+}) from iron-containing molecules. The released Fe^{2+} can participate in the Fenton reaction with H_2O_2, generating the highly reactive hydroxyl radical. The superoxide radical can also participate in the Haber-Weiss reaction, which combines a Fenton reaction and the reduction of Fe^{3+} by superoxide, yielding Fe^{2+} and oxygen. It is known that metal-induced generation of oxygen radicals results in the attack of not only DNA in the cell nucleus but also PUFAs in the cell membrane, which are extremely sensitive to oxidation (Valko *et al.,* 2007).

3.3.2 Lipid peroxidation

BIOCHEMICAL PROCESS: The overall process of lipid peroxidation by which carbon-centered free radicals attack lipids to form peroxyl, hydroperoxide and malondialdehyde is also well described and illustrated in the literature (Hogg and Kalyanaraman, 1999; Valko *et al.*, 2006). Lipid peroxidation is initiated by free radicals, which capture a hydrogen atom from a methylene carbon in the polyalkyl chain of the FA to give a pentadienyl carbon-centered lipid radical. Excessive generation of the hydroxyl radical close to cellular membranes enables it to attack PL side chains, as well as FFAs, producing hydroperoxides (Nigam and Schewe, 2000; Niki *et al.,* 2005). Accumulation of these agents in a membrane reduces its fluidity and permeability, causing it to collapse. In addition, lipid hydroperoxides can decompose to yield a range of highly cytotoxic aldehydes, which can further perpetuate the tissue damage (Niki *et al.,* 2005). It was suggested that this damage might be an essential prerequisite to enable carcinogens to initiate the neoplastic process (Davydov and Bozhkov, 2003). The naturally occurring PUFAs are more susceptible to hydrogen abstraction by oxidizing radicals than are the fully SFAs (Hogg and Kalyanaraman, 1999). Once formed, peroxyl radicals can be rearranged with the final product of the peroxidation process, being malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). MDA was found to be mutagenic in bacterial and mammalian cell lines and carcinogenic in a rat experimental study, whilst HNE is weakly mutagenic but appears to be the major toxic product of lipid peroxidation (Nigam and Schewe, 2000; Niki *et al.,* 2005).

3.3.3 Antioxidant defenses

In humans, exposure to free radicals has led to the development of a series of defense mechanisms. The effect of ROS and reactive nitrogen species (RNS) is balanced by enzymatic- and non-enzymatic antioxidants (Gate *et al.,* 1999; McCall and Frei, 1999;

Valko *et al.,* 2004). Enzymatic antioxidant defenses include enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GP) and catalyse (CAT) (Valko *et al.,* 2007). Among non-enzymatic antioxidants ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids, and others antioxidants are included. Mitochondrial-superoxide dismutase (Mn-SOD) is one of the most effective antioxidant enzymes that have anti-tumor activity. Interestingly, a marked over-expression of Mn-SOD has been detected in cancers of the gastrointestinal tract, however, the general statement of Mn-SOD as a tumor-suppressor protein is far from clear (Valko *et al.,* 2006). In addition, protein and DNA repair enzymes are also considered to be part of the antioxidant system. According to Oberley (2002), proteins that sequester metals are important in modulating the cell redox state. It is also known that deprivation of certain trace elements, Cu, Mn, Zn, Se or vitamins such as riboflavin, may lead to the inactivation of antioxidant enzymes and to increased oxidative stress associated with diseases (Fong, *et al.,* 2005). Antioxidant therapy in cancer patients should be used with caution, since excessively reduced free radicals can prevent apoptosis and, thereby, promote carcinogenesis (Valko *et al*., 2007).

3.3.4 Cell damage

Radicals have an affinity for PUFAs and among them LA and AA are excellent sources for lipid oxidation. Available information on these two FAs is given, with special reference to CRC.

ABUNDANT LA: Linoleates are abundant lipids *in vivo* and their free radical-mediated oxidation contributes to oxidative stress. Although previous *in vitro* studies indicated that 12-LOX is involved in cancer cell proliferation and survival (Ding *et al.,* 2003), it was revealed that 15-LOX is associated with attenuated colorectal cell proliferation in mice experimental studies (Nixon *et al.,* 2004). Of interest is that lipid peroxidation of LA by 15-LOX produces 9- and 13-HODE metabolites, activators and ligands of PPARγ that induces apoptosis (Niki *et al.,* 2005). It was recently reported that enhanced 15-LOX expression in human CRC has anti-tumorigenic activity (Shureqei *et al.,* 2005; Baek *et al.,* 2006). However, it appears that over-expression of PPARs in favor of PPARδ may prevent apoptosis of oxidative damaged CRC cells, based on *in vitro* research findings of Zuo *et al*. (2006).

ENHANCED AA METABOLISM: CRC is associated with chronic inflammation and the involvement of oxidants (ROS and NO•) appear to be the common denominator of DNA

damage, mutations and altered gene-expression, all key role players in the process of colorectal carcinogenesis (Valko *et al*., 2006). Studies confirmed that enhanced inducible nitric oxide synthase (iNOS) activity occurs during *in vivo* human colon carcinogenesis (Lagares-Garcia *et al*., 2001; Ohta *et al*., 2005). This may directly impair the activity of caspases, p53 and DNA repair enzymes, and indirectly stimulate COX-2 expression (Jaiswal *et al*., 2000; Lala and Chakraborty, 2001; Liu *et al.,* 2003; Ohta *et al.,* 2005). Enhancement of AA and COX-2 expression during chronic inflammation contributes to oxidative stress during CRC (Bartch and Nair, 2002; Aggarwal *et al*., 2006).

3.3.5 Oxidative stress-related cancer

Oxidative stress plays a major role in carcinogenesis and the production of ROS and NO• not only cause direct damage to DNA, but also exert indirect effects such as de-regulation of cell proliferation and apoptosis (Hensley, 2000; Okada, 2002; Becuwe *et al.,* 2003; Klaunig and Kamendulis, 2004). The effects of ROS on cell proliferation occur at low or transient concentrations, whilst high concentrations of ROS cause apoptosis. ROS (superoxide and hydrogen peroxide) are released by neutrophils (the major component of phagocytic cells in the human bloodstream) upon encountering pathogens. During tumor initiation, promotion and progression of other cells of the immune system (macrophages, leucocytes and lymphocytes) are also involved. RNS are generated by specific nitric oxide synthase (sNOS), an enzyme that metabolizes arginine to citrulline with the formation of NO• during the process (Valko, *et al.,* 2007). Under circumstances of cumulative oxidative stress, the oxidative burst is responsible for excessive oxidants with detrimental effects on cells, since key components of cell regulatory systems (AP-1, NF-κ B, and p53) are effected (Hussain *et al.,* 2003; Turpaev and Litvinov, 2004; Ohta *et al*., 2005). It was suggested that an increase in oxidative stress could occur as a consequence of the up-regulation of enzymes such as PLA_2, iNOS, LOX and COX-2, (with the exeption of 15-LOX-1) that have been detected in many organs in early and late stages of carcinogenesis including CRC (Bartsch and Nair, 2002; Van der Logt *et al.,* 2005; Aggarwal *et al*., 2006). This leads to increased release of AA, faster AA oxygenation and increased ROS and NO• production that triggers lipid peroxidation of PUFAs that causes cell damage.

It was observed that oxygen radical production and lipid peroxidation products increased with clinical staging of CRC and the levels of non-enzymatic levels of antioxidants (e.g. vitamins C and E and glutathione decreased with progression of CRC (Kondo *et al*., 1999;

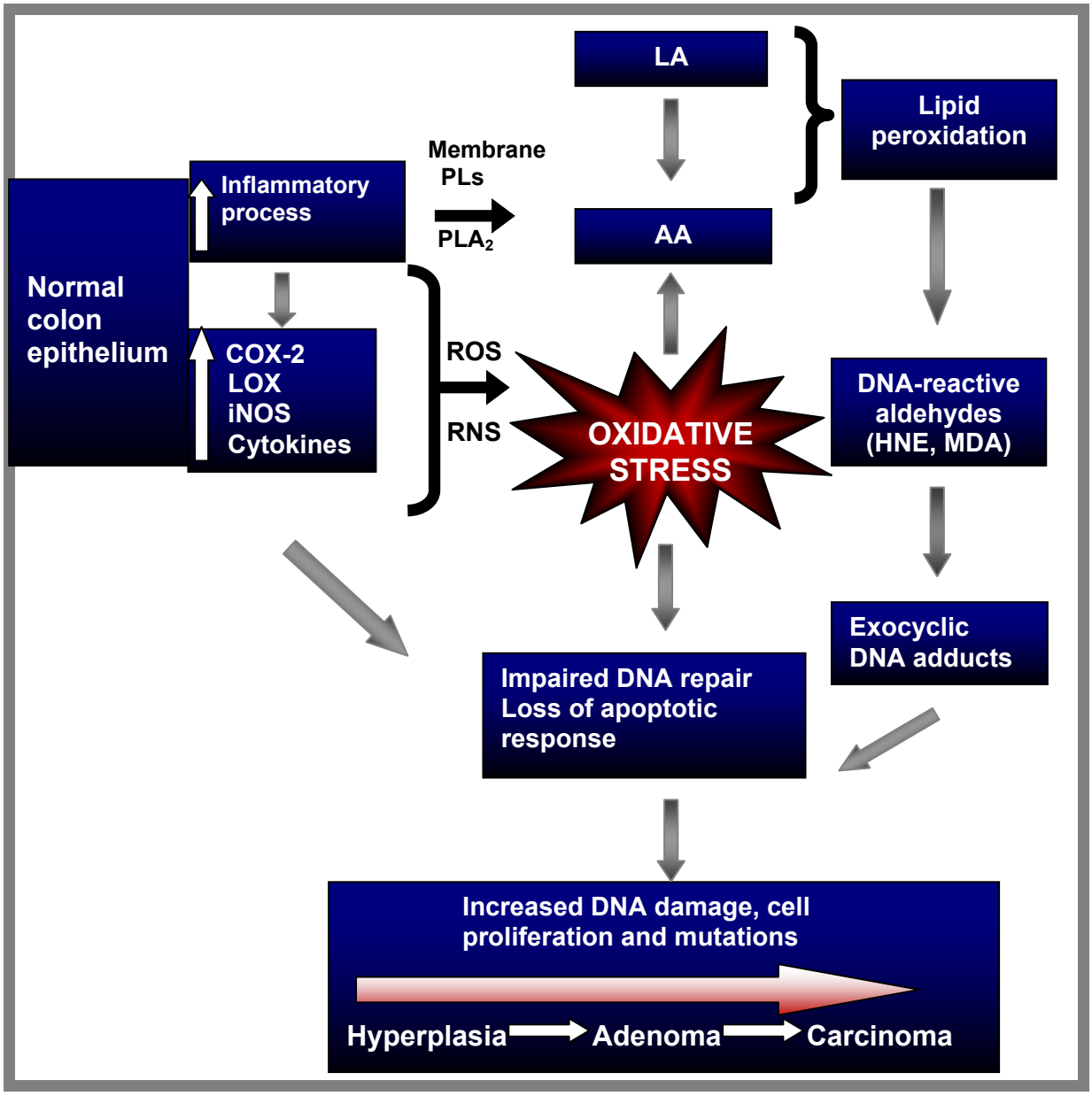

FIGURE 3.3 Hypothetical scheme linking linoleic acid (LA) and arachidonic acid (AA) metabolism, persistent oxidative stress and DNA damage to multistage colorectal carcinogenesis. ***Description:*** *In initiated or preneoplastic cells serum phospholipase A_2 (PLA_2), cyclooxygenase (COX), lipoxygenase (LOX) and inducible nitric oxide synthase (iNOS) are often over-expressed. This leads to increased release of AA, faster AA oxygenation and higher levels of reactive oxygen (ROS) and nitrogen species (RNS). These can cause DNA damage and trigger lipid peroxidation of polyunsaturated fatty acids in a self-perpetuating process, leading to various forms of exocyclic DNA base and protein modifications via trans-4-hydro-2-nonenal (HNE) and malondialdehyde (MDA) (Bartsch and Nair, 2002).*

Skrzydlewska *et al.,* 2003 and 2005). Because of the link between iron and oxidative stress, iron is considered a principal determinant of CRC in highly developed, meat-eating countries (Nelson, 2001; Lee *et al.,* 2004; Brookes *et al.,* 2006; Seril *et al.*, 2006). CRC is marked by excessive oxidative stress (Aggarwal *et al.,* 2006; Valko *et al*., 2006) and the pivotal role of oxidative stress during colorectal carcinogenesis is illustrated in FIGURE 3.3.

3.3.6 Prevention of oxidative stress

MEMBRANE FA MODULATION: PUFAs are more sensitive to lipid peroxidation than are MUFAs or SFAs (Fernandes *et al.,* 1996). An increase in PUFA intake can increase oxidative stress that, if not counterbalanced by antioxidant nutrients, can cause lipid peroxidation (Bartsh and Nair 2002). It was suggested that incorporation of n-3 PUFAs into the membrane can increase requirements for antioxidants, such as tocopherol, that protects against lipid peroxidation (De Pablo and De Cienfuegos, 2000; Chapkin *et al.,* 2007a). However, studies also suggested that lipid peroxidation caused by increased levels of n-3 PUFAs in tumor cell membranes can induce apoptosis by selective tumor cell detachment from the substratum (Johnson, 2002; Larsson *et al*., 2004). It was demonstrated with mice studies that EPA, DHA and CLA supplementation suppressed inflammation and, thus, the over-production of free radicals and carcinogenesis (Colquhoun and Schumacher, 2001; Grimm *et al.,* 2002). It was also demonstrated that DHA can decrease iNOS expression and consequently NO• production in a Caco-2 cell line study (Narayanan *et al.*, 2003). Of particular importance is the apoptotic potential of DHA that was demonstrated *in vitro* with mouse colonocytes (Ng *et al.,* 2005). Of further interest is the fact that both olives and olive oil contain peroxidation-resistant and anti-oncogenetic OA, as well as substantial amounts of compounds deemed to be anti-cancer agents (Bartoli *et al.,* 2000; Owen *et al.,* 2004; Schwartz *et* al., 2004; Menendez *et al*., 2005b; Colomer and Menendez, 2006; Esrich *et al*., 2006 and 2007).

3.4 SIGNALING PATHWAYS

Regulation of all the critical phases of cell growth is mediated by a complex array of signaling pathways, precisely coordinated by different factors. Since FAs can modulate the composition of membrane PLs, they can modify the activities of these factors involved in membrane-to-nucleus signaling pathways. During cell signaling growth factors and FAs are, respectively, first and second messengers. Since cytokines are involved during cell

growth they are mentioned in this section but, because of their major roles during immune responses, they are discussed under the immune system.

3.4.1 Factors involved during cell signaling

Reference is made to growth factors, cytokines and several other transmission and transcription factors during normal cell signaling, as well as oncogenes during abnormal cell signaling. Basic concepts (definitions and functions) and research findings in support of the roles of these factors during cell signaling are given, based on *in vitro* and *in vivo* animal and human studies. These research findings provide a sound foundation for clinical intervention with FA therapeutic agents that is addressed under THERAPEUTIC APPROACHES (section 3.6)

3.4.1.1 Growth factors

GENERAL: Normal cells proliferate in response to an array of growth factors that serve as first messengers in signaling pathways. Among these growth factors are the epidermal growth factor (EGF), fibroblast growth factor (FGF), tumor growth factor alpha (TGF-α), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF). The lipophobic character of most of these growth factors prevents them from passing through the plasma membrane of the cell. Therefore, they bind to receptors located on the surface of their target cells. The activated receptor firstly interacts with another protein such as tyrosine kinase, a GTP-binding protein (G-protein), depending on the nature of the receptor (Silverthorn *et al.,* 2004). After binding to appropriate trans-membrane receptors, growth factors exert their proliferative action by inducing a cascade of responses that mostly involve phosphorylation events. Tumor cells have found mechanisms to enable constant activation of these proliferative signals. These mechanisms differ from cancer to cancer entity depending upon cell type, and within a specific tumor type by pure chance, but the end result is continued mitogenic stimulation. Over-expression of growth factor receptors is a consequence of gene amplification, i.e., an increased copy number, usually as a result of end-to-end replication of this gene at the same chromosome location (Nebert, 2002). FAs and eicosanoids are second messengers in signaling pathways, since they are lipophylic and can cross membranes to interact with intercellular receptors.

RESEARCH: Of interest is that among heregulins (HRGs), members of the ErbB family (ErB1,-2,-3, and -4) of receptor tyrosine kinases bind to growth factor receptors and ErbB2

and ErbB3 have been implicated in the development of CRC (Cho *et al.,* 2003). The EGF is commonly up-regulated in cancer, including CRC (Kountourakis *et al.,* 2006). FA therapeutic agents with the potential to down-regulate COX-2 may also down-regulate the epidermal growth factor receptor (EGFR), since a link between PGE_2, EGFR and COX-2 was demonstrated (Chun and Surh, 2004; Wu *et al.,* 2006). The insulin growth factor (IGF- II) promotes cell proliferation, however, its binding protein (IGFBP-6) can sequester and limit the action of IGF-II. It was found that treatment of Caco-2 cells with DHA or EPA increased IGFBP-6 secretion and lowered IGF-II activity and, thereby, inhibited proliferative growth signals (Kim *et al.,* 2000).

3.4.1.2 Cytokines and chemokines

GENERAL: Cytokines and chemokines are secreted proteins that regulate cell growth, as well as immune responses. They can be divided into several groups, which include interferons (IFN-α, β and γ), tumor necrosis factor (TNF-α and β), interleukins (IL-1 to IL-21), transforming growth factors (TGFs), and the hematopoietic colony-stimulating factors (CSF). Pro-inflammatory cytokines serve as autocrine and paracrine growth factors for several cancers, and high levels of these cytokines may correlate with a poor prognosis and increased production of angiogenic factors during tumor growth (Angelo and Kurzrock, 2007). See immune system (section 3.5.3.1) for further information on cytokines.

3.4.1.3 Peroxisome proliferator-activated receptors

GENERAL: Peroxisome proliferator-activated receptors (PPARγ; PPARα; PPARδ/β) are FA sensitive ligand-activated transcription factors belonging to the nuclear receptor family (Feilchenfeldt *et al*., 2004). Upon activation by specific agonists and forming of dimers with certain receptors they translocate to the nucleus where they act as agonist-dependent transcription factors and regulate gene-expression by binding to specific promoter regions of target genes. Generally, FAs are considered agonists for PPARs, but a previous study demonstrated that DHA and some other PUFAs act as antagonists instead of agonists for transactivation of PPAR-regulated gene expression (Tan *et al.,* 2002; Lee and Hwang, 2002). PPARs are involved in cell proliferation and differentiation, as well as modulation of inflammatory and immune responses, and, thus, can promote or suppress carcinogenesis (Vamecq and Latruffe, 1999; Gelman *et al.*, 1999; Cunard *et al.,* 2002; Zhang *et al.* 2002; Shureiqi, *et al.,* 2003). It is believed that: PPARα can be activated by PA, OA, LA, AA, CLA and EPA; that the preferred ligands for PPARγ are LA, ALA, AA, and EPA; and that activators for PPARδ (often referred to as PPARβ) are

DGLA, EPA, AA, PA and prostaglandins (PGA_1 and PGD_3), while their preferred natural ligands are LA, ALA, AA, and EPA (Berger and Moller 2002; Nixton *et al.,* 2003; Larsson *et al*., 2004). Of particular importance for this study appears to be the fact that modulation of PPARδ/β over-expression can suppress PPARγ activity during colorectal carcinogenesis (Zuo *et al*., 2006).

RESEARCH: More than a decade ago it was demonstrated that PPARγ has anti-tumorigenic and pro-apoptotic actions in different cell types (Chang and Szabo, 2000). Research focused mostly on PPARγ and several contributions were made in the case of CRC. In 1998 it was suggested that PPARγ play a role in the promotion of CRC, since the activation of PPARγ enhanced the formation of polyps and cancer in a mouse model (Saez *et al*., 1998; Seed, 1998). Aberrant expression of PPARγ in colon cancer cells was confirmed by Du Bois (1998) and Jump and co-workers (2002), whilst the study of Bull and co-workers (2003) confirmed the important role PPARγ plays in the differentiation of intestinal cells. Recently, more advanced research shed some light on the different families of PPARs: PPARγ can act as a tumor suppressor in colon cancer (carcinoma) but colon tumors (polyps) with mutations in the APC gene appear to be exceptions, since thiazollidinediones (agonists for PPARγ) promote growth in these tumors; PPARα have anti-inflammatory and anti-tumoral properties through the inhibition of AP-1 genes that are involved in inflammation and tumor progression; and PPARδ/β over-expression can cause apoptotic resistance. The physiological and pharmacological roles of PPARδ/β are just beginning to emerge, but inconsistent reports leave conclusions controversial (Grau *et al*., 2006; Martinasso *et al*., 2007). Research studies on colon cancer cell lines revealed that up-regulation of 13-HODE (the metabolic end product of LA by 15-LOX-1 activity) can activate PPARγ by the down-regulation of PPARδ/β and, thereby, induce apoptosis and inhibit carcinogenesis (Ding *et al*., 2003; Nixon *et al*., 2004; Shureiqi *et al.,* 2005). Shureiqi and co-workers (2005) expressed the opinion that down-regulation of 15-LOX-1 activity, rather than a shift in LOX metabolism, is responsible for colorectal carcinogenesis. The study of Heslin and co-workers (2005) confirmed that 15 LOX down-regulation is an early event in the adenoma-carcinoma sequence. It was proposed that conventional NSAIDs (inhibitors of COX-2 expression) may modulate PPARγ expression by up-regulation of 15-LOX activity and down-regulation of PPARδ, to induce apoptosis and inhibit carcinogenesis (Heslin *et al*., 2005; Shureiqi *et al.,* 2005;). However, a recent *in vivo* study with mice revealed that COX-2 inhibition and PPARδ/β can be clearly dissociated (Hollingshead *et al*., 2007). Upon reviewing the literature, it is evident that

there is considerable debate whether PPARδ/β ligands potentiate or suppress colon carcinogenesis. A possible explanation for the disparity in research findings is that *in vitro* and *in vivo* circumstances are apparently not compatible, since synthetic ligands might not represent what occurs biologically with natural ligands of PPARs that are formed intracellular. Nevertheless, at this stage in time, the proposal that PPARδ/β over-expression suppresses PPARγ (and PPARα) activity and tilts the scale in favor of apoptotic resistance during colorectal carcinogenesis appears to hold strength. Of further interest is the progressive increase in PPARδ/β expression during the adenoma-carcinoma sequence, based on an immunohistochemical study with human specimens (Takayama *et al*., 2006). The opinion is expressed that the close association between PPARδ/β and the malignant morphology of CRC most probably indicate a pivotal role for PPARδ in these cancer cells. Logical explanations regarding different lipid metabolic pathways manipulated by FA ligands for PPAR gene-regulating are debated in CHAPTER 6.

3.4.1.4 Protein kinases

GENERAL: As a rule, protein kinases and regulatory GTP-ases are arranged together with other signaling proteins in cascades. They play a pivotal role in the transmission of signals from the cell surface receptors to the nucleus (Leppa *et al.,* 1998). PKC is activated by Ca, PS, DAG or phorbol esters and *cis*-PUFAs. It is thought that PKC resides in the cytosol in an inactive conformation and translocate to the plasma membrane upon cell activation, where it modifies various cellular functions through phosphorylation of target substrates (Keenan and Kelleher, 1998). The PKC families of serine-theonine kinases are important signal transducers participating in many different agonist-induced signaling cascades (Pahl, 1999; Murray *et al.,* 2004). The MAPK pathway is closely associated with cell proliferation and apoptosis and the balance between them could determine a cell's fate. Three MAPK families have been identified in mammalian cells: the extracellular regulated kinases (ERKs); the c-Jun N-terminal kinases (JNKs); and the p38 MAPKs. MAPK pathways are organized in cascades and incoming signals are transmitted from kinase- to -kinase. The cellular targets controlled by these cascades include metabolic enzymes and cytoskeletal proteins, as well as signal transduction proteins such as protein kinases and transcription factors. These pathways of interconnected signal-processing proteins serve as transducers of extracelllular signals (such as transmitted by cytokines, growth factors, and other hormones) into the cell's interior, including the nucleus (Johanson and Lapadat, 2002). The ERK family is associated with cell proliferation and survival, whilst the JNK and p38 MAPK families are

associated with stress and apoptosis (Obata *et al.,* 2000; Owuor and Kong, 2002). These pathways are widely explored and valuable contributions in regard to apoptosis in cancer cell lines are reported (Engelbrecht *et al.,* 2005, Du Toit *et al.*, 2006). However, all the mechanisms involved are not yet fully understood. Lastly, reference can be made to the phosphatidylinositol 3-kinase/protein kinase B or Akt (PI3K-Akt) signaling pathway that plays a critical role in cell growth and survival. This pathway mediates extracellular signals, such as mitogenetic growth factors and stress, and intracellular signals, such as altered tyrosine receptor kinases and Ras expression (Michi and Downward, 2005). All the downstream factors in the PI3K-Akt pathway are also not yet fully unraveled. Nevertheless, of importance is that in CRC this PI3K-Akt pathway is frequently up-regulated and apoptosis is prevented (Greenhough *et al.,* 2007).

RESEARCH: An important target of the action of n-3 PUFAs is the inhibitory effect on PKC activity *in vitro* on carcinogenesis (Murray *et al.,* 1999; Musashi *et al*., 2000; Seung *et al.,* 2001). Dietary fat and fiber can modulate the luminal concentration of several activators of PKCβII, including FFAs, secondary bile acids, and DAG. Research revealed that n-3 PUFAs can inhibit colonic PKCβII activity (a lipid-dependant protein kinase) that induces COX-2 activity and block PKCβII-mediated hyperproliferation and carcinogenesis in colonic cells of transgenic PKCβII mice (Jiang *et al*., 1996; Gokmen-Polar *et al*., 2001; Yu *et al.,* 2003b). Alone, EPA can inhibit PKCßII activity and COX-2 expression, as well as suppress TGF-ßRII expression (a potent growth inhibitor of epithelial cells) (Murray *et al.* 2002 and 2003; Wansheng *et al.* 2003; Matsuzaki *et al.,* 2006). Recently, the loss of TGF-β expression was confirmed to be commonly encountered in CRCs (Matsuzaki *et al.,* 2006). With reference to the MAPK pathways studies revealed that blocking of these pathways can suppress the growth of colon tumor cells *in vivo* (Sebolt-Leopold *et al.,* 1999). *In vitro* studies revealed that butyric acid can induce apoptosis in colon adenoma via the p38 MAPK and PKC-δ pathways (Scheppach *et al*., 1995; McMillan *et al*., 2003). Researchers also proposed that DHA can down-regulate survival-associated kinase activity by improving membrane fluidity and by down-regulating PKC activity during the ERK pathway, based on a study with CaCo2 colon cancer cells (Du Toit *et al*., 2006). Currently, concurrent therapy with DHA and Celecoxib (COX-2 inhibitor) is evaluated for colon cancer therapy.

3.4.1.5 Nuclear factor-kappa Beta

GENERAL: NF-κB is an inducible and ubiquitously expressed transcription factor for genes involved in cell growth and survival, as well as inflammation. It was discovered by Sen and Baltimore in 1986 as a factor in the nucleus of B cells that binds to the enhancer of the kappa light chain of immunoglobulin and is expressed in the cytoplasm of cells. It translocates to the nucleus when activated, where it regulates the expression of over 200 genes that control cytokine gene expression and immune responses, cell proliferation and apoptosis. The NF-κB family plays an important part in the inflammatory reaction, by controlling the expression of pro-inflammatory molecules in response to cytokines, oxidative stress, infectious agents and PKC activators (Schwartz *et al.* 1999; Webster and Perkins, 1999; Bours *et al.,* 2000a and 2000b; Hsu *et al.,* 2000; Shishodia and Aggarwal, 2004; Vermeulen *et al.,* 2006). Of paticular interest is that NF-κB mediates both proliferation and apoptosis, depending on the circumstances, and this duality is especially striking in relation to cancer. Initially, it was controversial whether NF-κB activation is a friend or foe in cancer, as pointed out by Shishodia and Aggarwal (2004) in their review article. Paradoxically, most agents (cytokines, chemotherapeutic agents and radiation), which are known to induce apoptosis, have also been shown to activate NF-κB. This indicates that NF-κB is part of the auto-defense mechanism of a cell and, thus, may mediate desensitization, chemoresistance, and radioresistance (Sishodia and Aggarwal, 2004). However, increased evidence is lining up that activation of NF-κB may cause cancer. Recent research revealed that carcinogens, as well as chemotherapeutic agents and γ-radiation, can induce NF-κB activity, and that enhanced NF-κB activity and Bcl-2 expression then contribute to cancer (Shishodia and Aggarwal, 2004; Aggarwal, *et al.,* 2006; Schottelius and Dinter, 2006, Aggarwal, 2007).

RESEARCH: NF-κB is the central mediator of the immune system and it is regulated by growth factors, eicosanoids, cytokines, oncogenes and free radicals (Boone *et al.,* 2002). Feedback mechanisms exist whereby NF-κB also stimulates growth factors, cytokines, eicosanoids and oncogenes (Shishodia and Aggarwal, 2004; Aggarwal, 2007). Because of the link that exists between inflammation and cancer, NF-κB is considered an interesting target for the development of both anti-inflammatory and anti-cancer therapeutics (Young *et al.,* 2003; Vermeulen *et al.,* 2006). NF-κB activity is usually constitutionally active in most inflammatory conditions and a key regulator of COX-2 expression (Aggarwal *et al*., 2006; Schottelius and Dinter, 2006*).* Apart from enhanced NF-κB, Bcl-2 expression that prevents apoptosis is also up-regulated in CRC (Yu *et al*., 2003a). It is reported that increased expression of RelA/ NF-κB (first identified member of

NF-κB) plays an important role in the pathogenesis of CRC, i.e. the transition from colorectal polyps with low-grade dysplasia (altered differentiation) to adenocarcinoma (Evertsson and Sun 2002; Yu *et al.,* 2003a). It was observed with *in vitro* and *in vivo* colon cancer studies that PPARγ can suppress the activity of NF-κB, a transcription factor that induces the expression of Bcl-2 that prevents apoptosis (Bocher *et al*., 2002; Chen *et al*., 2002; Cheng *et al.,* 2003; Yu *et al.* 2003a). This can be attributed to the fact that the promoter region of the human Bcl-2 gene contains a binding site for NF-κB (Chen *et al.,* 2002). The focus is therefore on successful down-regulation of NF-κB activity, as well as Bcl-2 expression and certain FA therapeutic agents appear to have such potential. The n-3 PUFAs can decrease NF-κB activity and Bcl-2 expression (Novak et al, 2003). Of particular importance for this study is that PA can stimulate NF-κB activity as demonstrated in adipocytes (Ajuwon and Spurlock, 2005). It was also observed that CLA treatment can decrease Bcl-2 expression and induce apoptosis in CRC polyps, but not in normal cells (Lee *et al.,* 2005). From the above information it is once more clear that CLA and n-3PUFAs (EPA and DHA) therapy has a place in adenomatous polyp and CRC management.

3.4.1.6 Activator protein-1

GENERAL: Activator protein-1 (AP-1) dependent gene-transcription is required for tumor promotion. AP-1 regulates a wide range of cellular processes, including cell proliferation and differentiation, as well as cell survival and death. MAPK can induce AP-1 activity through the phosphorylation of distinct substrates. Induction of AP-1 by pro-inflammatory cytokines and genotoxic stress is mostly mediated by the JNK and p38 MAPK cascades (Shaulian and Karin, 2002). An understanding of the network of dynamic interactions between AP-1 proteins and other transcription factors is far from complete. However knowledge is emerging and research revealed that PPARα has anti-inflammatory and anti-tumor properties through the inhibition of the AP-1 gene (Martinasso et al., 2007).

3.4.2 Oncogenes and abnormal cell signaling

Oncogenes are homologes of normal cellular genes and they code for proteins which are involved in cell cycle regulation. A mutational change in an oncogene leads to constitutive activation of the gene, which then results in uncontrolled cellular proliferation (Ponz de Leon and Di Gregoria, 2001). Because the normal gene function is activated, these are referred to as gain of function mutations. Genes which are said to be potentially oncogenes are known as proto-oncogenes. Identifying why the protein has become an

oncogene is one of the challenges of modern cancer therapy. Some genes suppress tumor formation and they are called tumor suppressor genes, because they normally have an inhibitory influence on the cell cycle. Once these genes are deleted or their function reduced, normal control mechanisms are no longer operative and growth proceeds unchecked. At the cellular level tumor suppressor genes act in a recessive fashion, meaning that the function of the normal protein is lost only when both copies (alleles) of the gene are inactivated by point mutations, rearrangements, or deletions (Nebert, 2002). A host of genes have been implicated in colorectal carcinogenesis. Apart from the APC oncogene as the most important oncogene involved in CRC, other oncogenes with prominent roles are ras, p53 and Bcl-2 that are briefly discussed.

3.4.2.1 Ras

GENERAL: The ras oncogene was originally identified as the transforming component of the Kirsten (K) and Harvey (H) rat Rous sarcoma virus, and three cellular variants have been identified (H-ras, K-ras, and N-ras). Ras is involved in kinase signaling pathways that control the transcription of genes, which then regulate cell proliferation and apoptosis. It was recently revealed that the ras oncogene can regulate transcriptional activation and stabilization of COX-2 expression by several mechanisms during carcinogenesis (Backlund *et al.,* 2005). The ras protein is involved in proliferation and apoptosis of colonocytes, but as a G-protein, requires membrane binding for malignant transformation.

RESEARCH: Ras mutations (that require PKC-α) are found in up to 50% of sporadic CRCs and 50% of colon adenomas (larger than 1 cm). However, 100% of aberrant crypt foci (ACF), believed to be the first intermediate between normal colonic mucosa and adenomatous polyps, and 25% of hyperplastic polyps have ras mutations, but their significance is unclear (Takayama *et al.,* 2001). Previously, *in vitro* and *in vivo* studies indicated that: n-6-PUFAs can promote colon carcinogenesis by the up-regulating of ras p21 and COX-2 expression; n-3 PUFAs may exert anti-tumor effects by suppressing ras p21 and COX-2 expression; n-3 PUFAs can suppress carcinogen induced ras activation in the colon by preventing persistent activation and chronic down-regulation of PKC isoenzymes; DHA can lower the activation of ras oncogenes in tumor cells of the mouse colon (Chapkin *et al*., 1997; Badawi *et al.,* 1998; Colett *et al*., 2001; Turner *et al*., 2002). Apparently, n-3 PUFAs decrease intracellular ras levels and, thereby, interfere with post-translational modification that is necessary for its binding to the cell membrane and subsequent activity. Although all three ras oncogenes, when mutated, have the ability to

transform normal cells, K-ras is the most frequently mutated in human CRC and it plays a pivotal role in COX-2 regulation during colorectal carcinogenesis (Backlund *et al.,* 2005).

3.4.2.2 Tumor suppressor gene p53

GENERAL: The p53 gene has been called “the guardian of the genome” and it plays a key role in protecting a cell from tumorigenesis. Previous research indicated that: p53 is a tumor suppressor gene that halt the cell cycle or initiate apoptosis if the cell is damaged (Cahill *et al.,* 1999; Carrier *et al.,* 1999); p53 can activate p21 to stop the cell cycle in the G1 phase, resulting in DNA repair or apoptosis (Lakin and Jackson, 1999); and that stress conditions can monitor p53 and direct the cell towards an appropriate response (Heerdt *et al.,* 1998; Hall and Angele, 1999). The study of Katsumata *et al*. (2003) elaborated on the activation of suppressed/inactive p53 and its interaction with other apoptotic genes or participation in pro-apoptotic signaling pathways. In this regard reference can be made to the inhibition of Bcl-2 expression or the enhancement of Bax expression by p53, and the fact that Bax, in turn, can inhibit Bcl-2 expression. In a nutshell, p53 participates in a network of functions: it is believed to couple apoptosis to mitogenetic signals and survival pathways; and many studies demonstrated mutations in p53, caused by direct action of ROS or by carcinogenic metals (Watson *et al*., 2006; Valko *et al.,* 2007). Although it was suggested that a link exists between COX-2 expression and p53 (Baek and Eling, 2006), a recent study could not confirm it (Lim *et al*., 2007).

RESEARCH: The tumor suppressor gene p53 (located on chromosome 17p) is the most commonly mutated gene in human cancer. The expression of p53 is lost in 75% of CRCs, while it is rarely lost in adenomas and aberrant crypt foci, suggesting that p53 loss represents a relatively late event in colorectal carcinogenesis (Watson, 2006). The loss of p53 expression in the majority of CRCs and the resulting resistance to apoptosis, which would normally be triggered by genome instability or by many cancer chemotherapeutic agents, make restoration of p53 an attractive target for gene therapy (Mehigan *et al.,* 2006). Recent research findings revealed that: DHA, a primary tumor suppressive n-3 PUFA, can inhibit CRC growth independent of p53 mutational status (Kato *et al*., 2007); and that no association between p53 and COX-2 expression exists (Lim *et al*., 2007), in contrast with a previous suggestion that a link does exist between COX-2 expression and p53 (Baek and Eling, 2006). FA supplementation studies confirmed the following: adding EPA, DHA or CLA to culture media induced apoptosis in human colon cancer cell lines (Wahle *et al.,* 2004; Field and Schley, 2004); and feeding DHA, EPA, CLA or a mixture of these FAs increased apoptosis in colon tumor cells of experimental rodent models

(Larsson *et al.,* 2004; Lee *et al.,* 2005). Recent research findings revealed that: DHA, a primary tumor suppressive n-3 PUFA, can inhibit CRC growth independent of p53 mutational status (Kato *et al*., 2007).

3.4.2.3 Bcl-2

GENERAL: B cell lymphoma gene-2 (Bcl-2) proteins, associated with membranes and membrane activity, is part of a complex system of signal transduction that controls apoptosis. An increased amount of Bcl-2 protein that causes apoptotic resistance has been reported in many different types of cancers (Sakuragi *et al.,* 2002; Epand *et al.,* 2004; Haddad, 2004).

RESEARCH: Enhanced expression of COX-2 and Bcl-2 during colorectal carcinogenesis is reported in the literature (Sheng *et al.,* 1998). Several *in vitro* and *in vivo* studies were conducted that illustrated the down-regulation of COX-2 and oncogenes regarding colon cancer. It was demonstrated that: n-3 PUFA intake can down-regulate Bcl-2 expression and induce apoptosis in normal human colon cells of patients with polyps (Cheng *et al*., 2003); olive oil dose-dependently can down-regulate the expression of both COX-2 and Bcl-2 in the colonic mucosa of rats (Schwartz *et al.,* 2004); CLA can decrease proliferation and induce apoptosis in the colonic mucosa of rats, and these effects may be attributed, in part, to decreased PGE_2 levels and an increased Bax/Bcl-2 ratio (Park *et al.,* 2004); and DHA can down-regulate COX-2 and Bcl-2 expressions in human colon cancer cell lines (de Pablo *et al*., 2000a, 2000b and 2002; Dommels *et al*., 2002a, 2002b and 2003; Llor *et al*., 2003).

3.4.3 Fatty acid gene-regulation

COMMENTS: The *ex vivo* study of Yeh and co-workers (2005) demonstrated that 80% of the genes involved in FA metabolism pathways were over-expressed in CRC. Lipid gene-regulation, mediated by a complex array of membrane-to-nucleus signaling pathways, is not yet fully defined in the case of CRC. Nevertheless, research revealed the role of some factors involved during signal transduction that are responsible for cellular over-production and apoptotic resistance. By correcting membrane FA compositions, a cascade of events such as: enhanced FAs and eicosanoids; growth factors (EGF); enzymes (PLA_2, COX-2, LOXs, MAPKs, PKCs); transcription factors (PPARs, NF-κB, AP-1); oncogenes (ras, p53, p21, Bcl-2) and the cytokine balance, can be regulated during signaling pathways to prevent or improve CRC after surgical intervention.

3.5 THE IMMUNE SYSTEM

Immunity is concerned with the recognition and disposal of foreign or non-self material that enters the body. Resistance to infection caused by foreign material may be inborn (innate) or adaptive (acquired). The immune system can be classified into two components consisting of innate and acquired components and both components consist of cellular and humoral elements (Playfair and Chain, 2005). However, adaptive mechanisms perform many of their functions by interaction with the innate mechanisms. Prominent role-players during innate and acquired immune responses are: toll-like receptors (TLRs), specific to innate immunity; natural killer cells (NKs) and cytokines, active during innate and acquired immunity; T lymphocytes (T cells) and B lymphocytes (B cells), associated with cellular- and humoral (antibody)- mediated responses; major histocompatibility (MHC) molecules, associated with innate and acquired immunity; and the complement system, active during innate and acquired immunity (Tosi, 2005; Tan and Coussens, 2007). There is vigorous ongoing research in the field of immunology and recent advances revealed emerging evidence of a breakdown in conventional hallmarks of the distinct innate and adaptive parts of the immune system (Borghesi and Milcarek, 2007).

3.5.1 Innate immunity

Innate immunity, the inherited resistance to infectious disease, provides the first line of defense against pathogens using pattern recognition receptors to recognize different types of microorganisms which have managed to penetrate the host (Gomez *et al.,* 2005). After binding, an army of cells are activated to fight invaders with an arsenal that includes phagocytosis, microbicidal activities and inflammatory mediators. In the case of intestinal mucosal membranes, epithelial cells can produce human β-defensins (HBD) in response to activation by bacterial products through toll-like receptors (TLRs) (Oppenheim *et al.,* 2003; Vora *et al.,* 2005; Hume and Hertzog, 2006; Tsan, 2006). HBD display antimicrobial activity against a broad range of bacteria, fungi and enveloped viruses (Oppenheim *et al.,* 2003). Granulocytes, dendritic cells (DCs), macrophages, natural killer cells (NK cells) and mast cells, express a variety (10 different types) of TLRs. TLRs were named after the *Drosophila Toll* gene (Hume and Hertzog, 2006). Broadly speaking, two major pathways are activated by TLRs. The first is associated with nuclear translocation of NF-κβ (a master switch for inflammation) that regulates a broad range of cytokines and other pro-inflammatory products, which, in turn, activate the production of other cytokines and chemokines, lipid mediators (such as PGs), and ROS (Tosi, 2005). The second leads

to activation of enzymes such as MAPK, p38 and JNK (O' Neill, 2006). TLR binding to ligands, such as liposaccharides and DNA types, activates signaling cascades and cytokine secretion and, thereby, initiates the development of specific immune responses (Chinen and Shearer, 2004). Inflammation initiated by the cells of the innate system is not always a response to infection and it could occur under sterile conditions, such as those characterizing autoimmune diseases (Baniyash, 2006).

3.5.2 Acquired immunity

In contrast to innate immunity, acquired immunity is a more specialized form of immunity based on special properties of T and B cells which lead to a specific memory and a permanently altered pattern of response when the body is challenged by antigens. Innumerable different receptors are found on T and B cells, each one sensitive to one individual molecular structure. T cells associated with cellular immunity do not have surface membrane antibodies but recognize antigens by their principal recognition tool, the T-cell receptor (TCR*)*, and other accessory adhesion molecules. B cells associated with humoral immunity synthesize antibodies which are exported to the surface membrane where they recognize antigens.

3.5.2.1 Cellular immunity

Cellular immunity describes any adaptive immune response in which antigen-specific T cells participate. T-cells recognize antigens (Ag) by the T-cell receptor (TCR). The TCR is associated with the CD3 molecule which is important for the transduction of the activation signal through the lymphocyte membrane and the whole unit is called the TCR/CD3 complex (Minguet, 2007). All mature T cells express CD4 or CD8 molecules in a mutually exclusive fashion. T-cells can be further categorized into cytotoxic T-cells, helper T-cells and suppressor T-cells. Cytotoxic T cells refer to antigen-specific MHC-restricted cytotoxic T-lymphocytes and both helper CD4 T-cells and suppressor CD8 T-cells can function as cytotoxic T-cells, depending on whether MHC class II or I is recognized, respectively (Dong and Flavell, 2001; Castellino and Germain, 2006). T-helper cells (CD4 T-cells) are essential for most cell- and antibody-mediated responses. Different types of effector/helper T cells have been recognized: Th1 cells drive the type-1 pathway associated with cell-mediated immunity, to fight intracellular pathogens and eliminate cancerous cells; Th2 cells drive the type-2 pathway associated with humoral-mediated immunity, to up-regulate antibody production and eliminate extracellular organisms; Th 17 cells, probably also effective in the protection against bacteria, play a role in the amplification of autoimmune disorders (Mosmann *et al.,* 1986; Mosmann and

Sad, 1996; Castellino and Germain, 2006). T-suppressor cells (CD8 T-cells) are less well characterized, but appear to suppress inappropriate activation of the adaptive immune system through inhibition of other classes of T-cells, particularly self-reactive T-cells (Philpott and Ferguson, 2004).

3.5.2.2 Humoral immunity

As the central component of humoral immunity, B-cells function in antibody production, antigen presentation and secretion of pro-inflammatory cytokines. B cells defend against extracellular pathogens, by synthesizing and secreting antibodies with specificity against the foreign substances. Antibodies are a heterogeneous mixture of serum globulins, all of which share the ability to bind individually to specific antigens and to perform a common biological function. All serum globulins with antibody activity are referred to as immunoglobulins (Ig). In the context of cancer development, in addition to altering local and circulating profiles of cytokines, B cells inhibit Th1-mediated anti-tumor immune responses (Tan and Coussens, 2007).

3.5.3 Other factors involved during immune responses

3.5.3.1 Cytokines

CYTOKINE FAMILIES: Cytokines, non-immunoglobulin polypeptides secreted by T-cells in response to interaction with an antigen, interact with specific cell surface receptors and may act in an autocrine or paracrine manner. They are proteins with growth, differentiation, and activation functions involved in nearly every facet of immunity and inflammation, from induction of the innate immune response to the generation of cytotoxic T-cells and the development of antibodies by the humoral system (Tosi *et al.,* 2005). Although the various cytokines and their effects are usually listed separately, it is important to remember that cytokines act in concert, in tandem, or in conflict in a given immune response. As previously mentioned, cytokines are classified as: IFN-α, β and γ, TNF-α and β; interleukins (ILs); TGFs; and CSF (Steinke and Borish, 2006).

CYTOKINE SUBSETS: Cytokine subsets derived from Th1- and Th2-cells are in principle the Th1 subset that consists of IL-2, IFN-γ, and TNF-α, associated with cell-mediated or cytotoxic responses; and the Th2 subset that consists of IL-4, IL-5, IL-6 and IL-10, associated with humoral-mediated immune response. Cytokines play a critical role in the pathogenesis of inflammation. Pro-inflammatory cytokines favor the production of inflammatory reactions and include IL-1, IL-6, IL-8, TNFα and those produced by the Th1

cells (IL-2, IFN-γ and TNF-α). Anti-inflammatory cytokines favor the production of immunoglobulin E (IgE) and activation and/or production of eosinophils and mast cells. These include IL-1 receptor antagonist, TGF-β and those produced by the Th2 cells (IL-4, IL-5, IL-6 and IL-10). An imbalance between pro- and anti-inflammatory cytokines leads to inflammatory or allergic diseases (Kelley, 2001). Although cytokine-mediated effects are an essential part of the response to trauma and infection, excessive production of pro-inflammatory cytokines, or production of cytokines in the wrong biological context, increases the risk of a wide range of diseases, including cancer (Aggarwal *et al*., 2006). Normal inflammation is self-limiting because the production of pro-inflammatory cytokines (Th1 cytokines) is followed almost immediately by production of anti-inflammatory cytokines (Th2 cytokines). Cytokines that received a lot of attention because of their fundamental importance in acute inflammation as part of defense responses are IL-1 and TNF-α (Tosi, 2005). Chronic inflammation can lead to cancer and, according to the recent study of Steinke and Borish (2006), TNF, IL-1, IL-6, IL-8 cytokines (primarily derived from mononuclear phagocytic cells) and IL-12, IL-15, IL-18, IL-23 and IL-27 chemokines play a critical role in the pathogenesis of inflammation.

Th1/Th2 BALANCE: The Th1/Th2 balance hypothesis emerged in the late 1980's. These Th1 and Th2 cells are responsible for the production of cytokine subsets as previously mentioned. However, different grouping (inclusions or exclusion among a subset) were encountered in the literature and it seems conceivable that different diseases may be associated with different groupings (Mosmann *et al.,* 1986; Steinke and Borish, 2006; Sing, 2007). Over-activation of either pattern can cause disease, and either pathway can down regulate the other. While cytokines or chemokines can re-direct Th1/Th2 responses, both responses can be regulated by T regulatory (Treg) cells as suggested by (Romagnani, 2006). However, although Th1 and Th2 were virtually anointed with the responsibility of coordinating the immune system, Kidd reported since 2003 that critical investigators are finding discrepancies in this hypothesis. A look at the Th1 and Th2 paradigm has suggested that diseases may be either Th1- or Th2-dominated (Kidd, 2003). Cancer is considered a Th2-dominated disease, since IFN-γ and other Th1 cytokines are typically lower in advanced cancer patients, while the Th2 marker IL-4 can be higher or unchanged. Under the influence of IL-4 (Th2), tumor cells apparently up-regulate IL-10 that suppresses nearby NKs. With both IL-4 and IL-10 being proven inhibitors of Th1 and promoters of Th2 activity, the recognized capability of cancerous tissue to suppress immunity is readily rationalized (Kidd, 2003; Baier, *et al.,* 2005; Osawa *et al.,* 2006).

3.5.3.2 Natural Killer cells

GENERAL: Natural killer cells (NKs) are lymphocytes, which do not belong to the T- or B-cell lineages. Under the influence of cytokines associated with innate immunity, NK cells rapidly become activated and migrate to sites of infection to kill cells harboring pathogens. Thus, NK cells limit an infection until definitive acquired immunity develops (Carayannopoulos and Yokoyama, 2004; Moretta 2006). It is known that NK cells recognize many tumor cells but not normal self cells, and, thereby, eliminate them (Diefenbach and Raulet, 2002). NK cells discriminate between normal MHC class I cells and cells that have lost the expression of MHC class I molecules as a consequence of tumor transformation (Garcia-Lora *et al*., 2003; Moretta, 2005). According to the "missing-self" hypothesis, cells that express self-MHC class I molecules are protected from NK cells, but those that lack this self-marker are eliminated by NK cells (Screpanti *et al.,* 2004; Kumar and McNerney, 2005; Sandel *et al.,* 2005). Research revealed that several cancer cell lines and cells from advanced tumors have low MHC class I expression and NK cells preferentially attack cells with reduced or absent MHC class I levels (Screpanti *et al.,* 2004).

3.5.3.3 Major histocompatibility complex classes

The ability of the immune system to differentiate self from non-self is determined largely by MHC products whose genes are on chromosome 6. Human MHC molecules are called human leukocyte antigens (HLA). The three classical MHC class I molecules (HLA-A, HLA-B and HLA-C) play essential roles in the control of natural killer-cell responses and the detection and elimination of virus-infected cells, tumor cells and transplanted allogeneic cells (Abbas *et al.,* 2007). MHC class II encode the polymorphic HLA-DR, HLA-DQ and HLA-DP proteins, which play a central role in the initiation of cellular- and humoral immune responses, but they have also been implicated as contributing factors for a variety of autoimmune disorders and they play an important role in transplant rejection (Al-Daccak *et al.,* 2004; Drozina *et al.,* 2005).

The MHC class I and class II play essential roles in connecting the innate and adaptive immune responses by virtue of their ability to present peptides to T lymphocytes (T cells) (Drozina *et al.,* 2005). B lymphocytes (B cells) can respond to soluble antigen, but T cells rarely do so and recognize antigen only when embedded within the MHC. The mechanism by which antigen is processed and associated with the MHC before it is presented to the T cells is accomplished by antigen-presenting cells (APCs). They are a

heterogeneous system of molecules found on almost all nucleated cells (MHC class I) or only on specialized APCs of the immune system, e.g. B cells, macrophages, follicular dendritic cells, Langerhans cells, and activated T cells (MHC class II) (Van den Elsen *et al.,* 2004; Van den Elsen and Rudensky, 2004). By endogenous processing, Ag is produced intracellulary (e.g. by viral infection) and undergoes degradation outside the lysosomes. The resulting peptides are transported to the rough endoplasmic reticulum (RER) by transporter proteins where they are associated with MHC class I products before transport to the cell surface (Trombetta and Mellmann, 2005). The antigens of exogenous sources which undergo endocytosis and degradation in lysosomes, are associated with MHC class II products, and are transported to the cell surface. The MHC class I and class II genes encode cell-surface glycoproteins that are involved in the binding and presentation of antigenic peptides to the T-cell receptors (TCRs) of T lymphocytes. MHC class I protein present peptides from endogenous sources to $CD8^+$ T-cells, whereas MHC class II molecules mainly present peptides from exogenous sources to CD 4^+ T cells which are the main regulators of the immune response (Van den Elsen *et al.,* 2004; Van den Elsen and Rudensky, 2004).

The immune system of the host can recognize tumor antigens by the presentation of small antigenic peptides to the receptor of cytotoxic T cells, however, cancer cells can sometimes escape these specific T cell immune responses in the course of somatic clonal evolution. Various cancer cell lines and cells from advanced tumors exhibit low MHC class I expression and it has been demonstrated that NK cells preferentially attack cells with reduced or absent MHC class I levels (Screpanti *et al.,* 2004). A majority of colorectal adenocarcinomas displayed diminished MHC class I expression, making them particularly vulnerable for NK cell-mediated killing (Sandel *et al.,* 2005). It was suggested by Dalerba *et al.* (2003) that improvement of the intra-epithelial infiltration/migration of NK cells may be an important basis for the development of an effective adjuvant NK-based immunotherapy for CRC.

3.5.3.4 Complement system

Activation of the complement system is a central event during innate immune defense following pathogenic tissue assault and is also an important element involved in humoral immunity. Reaction between antigen and antibody activates this system which consists of a group of at least thirty or more proteins in serum or at cell surfaces. Activation of the complement cascade leads to the evolution of its main effector functions: microbial opsonization, phagocyte recruitment, and bacteriolysis, the end result of which is lysis of

the target or enhanced phagocytosis by phagocytic cells (Tosi, 2005; Tan and Coussens, 2007).

3.5.4 Immunocompetence

Lipid rafts, SFAs, and Th2 dominance appear to be responsible for immunosuppression in CRC and the proposed mechanisms are discussed, based on available information.

3.5.4.1 Lipid rafts

Lipid rafts are dynamic microenvironments in the PL bilayer of plasma membranes and they serve as platforms to facilitate several functions that include membrane transport and apoptosis (Simons and Toomre, 2000; Yaqoob, 2003a and 2003b). It appears that the relative abundance of SFAs is a principle reason for the liquid-ordered state of lipid rafts (Brown and London, 1998; Simons and Toomre, 2000; Katagiri, 2001). Activation of the proteins within rafts by an extracellular ligand can result in rapid clustering, that appears to be important for signal transduction in both T and B cells (Pierce, 2002; Horejsi, 2003). The T-cell receptor clusters within lipid rafts on contact with an antigen-presenting cell and forms a contact zone where intracellular signaling is thought to be initiated. Interestingly, the role of membrane rafts in Th1 and Th2 cells appears to be markedly different, with T cell receptor activation in Th1 cells being dependent on rafts, whereas that in Th2 cells is not (Balamuth *et al.,* 2001).

3.5.4.2 Th2 dominance

Studies have shown that the Th1/Th2 balance shifts toward Th2 dominance in the CRC-bearing state (Ordemann *et al.* 2002; Matsuda *et al.,* 2006). An explanation for this shift in immunity is that the majority of CRC evolves in a chronic inflammatory background, and that their precursors (polyps) are often inflammatory in nature (Zbar, 2004; Gunter and Leitzmann, 2005; Mills *et al*., 2005). In peripheral blood of patients with CRC, proportions of Th1-cells, identified by intracellular production of IFNγ or IL-2, is markedly reduced, whereas proportions of Th2 cells producing IL-4, IL-6 and/or IL-10 is significantly elevated, as compared with proportions of Th1 and Th2 in otherwise healthy patient populations (Tan and Coussens, 2007). The study of Evans *et al.,* (2006) confirmed that enhanced COX-2 and PGE_2 reduced the activation of NK cells and cytotoxic T cells, causing inhibition of IL-2 production and down regulation of IL-2 receptor expression. Thus, COX-2 over expression leads to a down regulation of the Th1 cells and promotion of Th2 cells. This phenomenon was observed to increase with cancer progression, suggesting that it

allows the tumor to locate and expand within the host (Contasta *et al.,* 2003; Berghella *et al.,* 2006).

3.5.4.3 Immunosuppression

COMMENTS: Almost all malignancies are associated with immunosuppression (Coussens and Werb, 2002; Baier *et al.,* 2005; Baruch, 2006; Evans *et al.,* 2006). It is imperative to improve the immunocompetence of CRC patients, since immunosuppression may hamper the management of this disease. Because Th1 activation is suggested to be dependent on lipid rafts, whereas that of Th2 cells is not, Fan *et al.* (2003) speculated that modulation of lipid raft composition could have a role to play in the down regulation of Th1 responses and, therefore, in the anti-inflammatory properties of n-3 PUFAs. Altering the FA composition of membrane PLs could have dramatic effects upon immune / inflammatory cell function and cancer. Although previous studies with immunomodulatory n-3 PUFAs reported a diminishment of lymphocytes, it was recently revealed by Barber *et al.,* (2005) that EPA modulates inflammatory conditions but has no effect on lymphocytes in contrast to DHA that is a potent inhibitor of lymphocyte proliferation (Verlengia *et al*., 2004). Also, the study of Irons and Fritsche (2006) confirmed that n-3 PUFAs do not significantly impact either the kinetics or magnitude of *in vivo* antigen expansion of CD8+ T-cells. Clearly, the impact of n-3 PUFAs lies in their ability to prevent inflammatory conditions and, thereby, modulate immune responses. The contradictory *in vitro* observations with EPA supplementation on isolated T cells reported by Li *et al.* (2006) can be seen in a favorite light. Although this study and other *in vivo* animal studies (Kelley *et al.,* 1999 and 2001; Stulnnig *et al.,* 2001 and 2003; Thies *et al.,* 2001a; Calder and Grimble, 2002; Calder *et al*., 2002; Calder, 2006) reported the down-regulation of lymphocytes and IL-2 after EPA supplementation, it is argued that this effect is beneficial for the down-regulation of prolonged inflammatory and cytokine mediated responses during early carcinogenesis. Therefore, the stage of clinical intervention in cancer management is of the utmost importance.

3.6 THERAPEUTIC APPROACHES

Therapeutic approaches aim at the modulation of membrane FA composition for down-regulation of COX-2 expression and inflammation, lipid peroxidation and oxidative stress, and FAS and SCD (Δ^9d) activities to prevent or inhibit cancer. Preventative approaches

include limitation on oxidative stress caused by exogenous and endogenous factors and immunonutrition of inestimable value to improve disease outcome and the general well-being of the patient.

3.6.1 Down-regulation of COX-2

GENERAL: Enhanced AA metabolism by increased COX-2 expression with excessive pro-inflammatory PGE_2 metabolite production are characteristic of inflammation and cancer (Eberhart *et al*., 1994; Tsujii *et al.,* 1997; Sheng *et al.,* 1998; Marks *et al.,* 2000; Kojima *et al.* 2001; Rao *et al*., 2004; Tuynman *et al*., 2004; Sanborn and Blanke, 2005; Chao *et al.,* 2005; Clevers *et al*., 2006; Furstenberger *et al*., 2006). It is generally accepted that COX-2 over-expression can contribute to cancer through enhanced cell proliferation, inhibition of apoptosis and immunosuppression (Furstenberger *et al*., 2006). Valuable contributions regarding COX-2 expression and colon cancer are encountered in the literature. In summary, research revealed that: AA, COX-2 and PGE_2 down-regulation can prevent Bcl-2 over-expression and apoptotic resistance in CRC marked by chronic inflammation (Gunter and Leitzman, 2005; Mills *et al*., 2005; Aggarwal, 2006); COX-2 expression may have a prognostic role in CRC patients, since there seems to be a correlation between the level of COX-2 expression and tumor size, recurrence, and metastasis (Yamac *et al.,* 2005); COX-2 activity is regulated by ras, often mutated in CRC (Bachlund *et al*., 2005); COX-2 and PGE_2 over-expression can activate components of the Wnt signaling pathway, during which mutation of the APC tumor suppressor gene stimulates cell proliferation in colon cells (Buchanan and Du Bois, 2006); enhanced COX-2 activity can be attributed in part to high red meat intake (rich in iron, SFAs and AA), contributing to oxidative stress that increase with clinical staging of CRC (Anderson, 2006; Seril *et al*., 2006; Valko *et al*., 2006a, 2006b and 2007); enhanced COX-2 expression and PKC activity with loss of TGF-β (growth inhibitor) can contribute to CRC (Matsuzaki *et al*., 2006); inhibition of COX-2 expression can inhibit the PI3K-Akt pathway, associated with ras and apoptotic resistance in CRC (Michi and Downward, 2005; Greenhough *et al*., 2007); COX-2 over-expression regulates NF-$\kappa\beta$, mostly enhanced in CRC (Aggarwal, 2007); enhanced COX-2 and PGE_2 expression can reduce activation of NK cells and cytotoxic T cells, causing inhibition of IL-2 production and down-regulation of IL-2 receptor expression with a Th2 dominance in CRC (Berghella *et al*., 2006; Matsuda *et al*., 2006; Evans *et al*., 2007; Tan and Coussens, 2007).

DRUGS: Sir John Vane suggested 30 years ago that NSAIDs can inhibit COX-2 expression and PGE_2 production and thereby suppress carcinogenesis. Since then, several studies confirmed his viewpoint (Dempke *et al.,* 2001; Chan *et al.,* 2004; Hersh *et al.,* 2005; Larsson *et al.,* 2006). Clinical intervention with NSAIDs can significantly suppress PGE_2 levels and protect against some cancers, including CRC (Thum *et al*., 2002; Sanborn and Blanke, 2005). The use of Asprin is often a common practice in CRC management, since it can prevent inflammation or inhibit polyp growths (Larsson *et al*, 2006). It is argued that FAs can be excellent therapeutic agents for the down-regulation of COX-2 over-expression, cell proliferation and apoptotic resistance, without negative side effects (Das, 2005; 2006a and 2006b; Kapoor and Huang, 2006).

FATTY ACID AGENTS: Sufficient evidence exists that fish oil, (containing EPA and DHA), olive oil (containing OA) and CLA are considered important inhibitors of COX-2, NF-κβ and MAPK (Llor *et al*., 2003; Dommels *et al.,* 2003; Larsson *et al.,* 2004; Lee *et al.,* 2005) and a few clinical trials are already important steps towards including FA therapy in the CRC therapeutic regime (Nakamura *et al.,* 2005**;** Chapkin *et al*., 2007a and 2007b; Courtney *et al.,* 2007). The research findings of Swamy *et al*. (2004), on the HCA-7 colon cancer cell line, provided a basis for concurrent therapy with DHA and a COX-2 inhibitor (Celecoxib) to arrest cell growth and induce apoptosis. Currently, this adjuvant FA modality is administered in clinical trials and an outcome is awaited. Baek and Eling (2006) suggested that inhibition of COX-2 expression causes a shift in the AA metabolite profile from PGs to hydroxylated metabolites, such as 15-HETE and 13-HODE via 15-LOX-1, and that these metabolites stimulate phosphorylation of the p53 tumor suppressor gene. This metabolic diversion may be of value in CRC therapy; however, it will address a consequence and not a cause of CRC. Without a doubt, FAs (exogenous intake and endogenous production), together with environmental factors that interfere with lipid metabolism can be considered causes of CRC. Conventional FA agents for the down-regulation of the AA cascade and consequently also COX-2 expression are GLA to inhibit cell proliferation and to normalize cells and there seems to be renewed interest in this FA (Kapoor and Huang, 2006; Das, 2006a and 2006b). Research findings with CLA opened a new direction in FA therapy and this FA appears to be an important anti-carcinogenetic and immunomodularory therapeutic agent for CRC management, based on *in vitro* cell line and *in vivo* animal studies (O'Hagan and Menzel, 2003; Bhattacharya *et al.,* 2006). In principle those fatty acids (GLA, EPA, DHA and CLA) that can down-regulate inflammation can also down-regulate oxidative stress (Narayanan *et al.,* 2006; Larsson *et al*., 2004; O'Shea *et al*., 2004; *Das*, 2007).

3.6.2 Down-regulation of FAS activity

GENERAL: In cancer cells *de novo* FAS activity is up-regulated, despite the fact that adequate dietary FA intakes may occur, and this phenomenon represents a metabolic strategy to maintain high cell proliferation rates and survival of cancer cells (Ogino *et al.,* 2007). During up-regulated FAS activity enhanced PA is the regulator of cellular over-production and apoptotic resistance, and it serves as substrate for SA and OA productions of which the latter is usually abundant in cancer cells (Shah *et al.,* 2006). The viewpoint that over-expression of FAS is linked to tumor virulence and that FAS inhibition may be a means to limit cell proliferation, triggered renewed investigations on FAS up-regulation in cancer cells, FAS inhibition by GLA and the anti-oncogenetic potential of OA (Menendez *et al* 2004a and 2004b; Menendez *et al*. 2005a and 2005b; Menendez and Lupu, 2006; Menendez *et al*., 2006a and 2006b). Of further importance is that FAS inhibition may mainly affect synthesis of raft-associated lipids, namely SFAs, and that these lipid rafts are associated with Th1 down-regulation of the immune system (Swinnen *et al*., 2003; Yaqoob 2003a and 2003b and 2004). This implies that modulation of membrane FA composition may affect the structure and function of lipid rafts in cancer cells (membrane fluidity and transport) and, thereby, functions such as apoptosis and immune responses.

FATTY ACID AGENTS: The concept that GLA (or even ALA) in cancer tissues can provide an effective influence on the outcome of FAS over-expression may have beneficial therapeutic potential in CRC with up-regulated FAS activity (Menendez *et al*., 2004a). Possible inhibition of FAS activity by GLA in the management of CRC needs attention, especially since the recent studies of Das (2005, 2006a and 2006b) and Kapoor and Huang (2006) once more proclaimed the beneficial use of GLA in cancer. Most recently, Das (2007) once more reported on experimental findings and assets of GLA for human therapy. Of particular importance for polyp and CRC management may be the potential of GLA to up-regulate p53 and down-regulate ras and Bcl-2; activities; and to protect normal cells (and tissues) from the toxic actions of radiation (and drugs).

Of further importance is the anti-oncogenetic potential of OA that can induce apoptosis and prevent development of aberrant crypt foci and colon carcinogenesis in a rat model (Schwartz *et al.,* 2004). Enhanced OA in cancer cells is suggested to be a cellular response and negative feedback mechanism to suppress FAS activity (Menendez *et al*., 2005b). It seems feasible that combined FA therapy that includes OA may have merit in CRC management, since antioxidative OA can induce apoptosis (Owen *et al*., 2004),

apart from being an anti-oncogene regulator that can prevent mutations of the APC tumor suppressor gene responsible for colon carcinogenesis, as well as genes responsible for tumor metastasis (Menendez *et al*., 2005b; Colomer and Menendez, 2006; Schuell *et al.,* 2006). The administration of OA in combination with GLA and EPA in the management of CRC, with special reference to prevention of polyp recurrence, needs proper evaluation.

3.6.3 Immunonutrition

GENERAL: Although the immunomodulatory potential of dietary FAs is known for more than two decades, immunonutrition only recently gained prominence in disorders and diseases (Braga *et al.,* 2002; Philpott and Ferguson, 2004; Farreras *et al.,* 2005; Heyland and Dhaliwal, 2005; Zhang *et al.,* 2005 and 2006). Interestingly, the review article of Yaqoob (2004) refers to the fact that FAs have finally found a niche in immunology that was envisaged as early as 1978. Immune system modulation by dietary lipids may contribute to changes in the composition of membrane PLs, composition of lipid rafts, lipid peroxidation, gene expression, eicosanoid production or proliferation of T lymphocytes to improve the immunocompetence of CRC patients (De Pablo *et al.,* 2000, and 2002; Yaqoob, 2003b; Li *et al.,* 2006; Cury-Boaventura *et al.,* 2006). For immune surveillance (the body's ability to detect and destroy tumor cells) the cellular arm (Th1) of the immune response is important as Th1 pathways typically produce activation of cytotoxic T-lymphocytes, NKs, macrophages and monocytes, all of which can attack cancer cells and generally defend against tumors. It is believed that in patients with immunodeficiency EPA and CLA administration can not only inhibit carcinogenesis, but also have the potential to markably improve the immunocompetence of patients.

FATTY ACID AGENTS: There is ample experimental evidence for the beneficial use of CLA in therapy. In a nutshell, CLA can: prevent/inhibit carcinogenesis by altering eicosanoid signaling (O'Shea *et al*., 2004); prevent oxidative stress by suppressing COX-2 and iNOS expression (Yu, 2001; Lee *et al*., 2005; Beppu *et al*., 2006); down-regulate ErB3 and PI-3K pathways, by inhibiting cell proliferation (the G1-S phase of the cell cycle) and induce apoptosis, by down-regulation of Bcl-2 (Field and Schley, 2004; Han *et al*., 2006); and by modulating PPARs involved in gene-regulation of apoptosis and proliferation of lymphocytes and subsequent effector functions in the Th1/Th2 immune response (O'Shea *et al*., 2004). In a Swedish cohort study it was confirmed that CLA may have a protective effect and prevent human CRC (Larsson *et al*., 2004). However, a lack of clinical studies

at this stage, in time, makes it difficult to recommend CLA as therapy for CRC (Bhattacharya *et al*., 2006). The viewpoint that administration of CLA must be approached with caution and due diligence until the individual effects of the two main CLA isomers (*t*10,*c*12-18:2 and *t*9,*t*11-18:2) and their interactions have been clearly characterized, led to intensive exploring of the beneficial use of CLA. *In vitro* and *in vivo* studies performed on CRC cells confirmed that *t*10,*c*12- 18:2 alone can inhibit cell growth and induce apoptosis (Larsson *et al*., 2005a). Recently, the study of Han and co-workers (2006) revealed that CLA (*t*10,*c*2-18:2) can inhibit cell growth by inhibiting the G1-S phase of the cell cycle in human colon cancer cell lines. It was also confirmed that *t*9,*t*11-18:2 can decrease Bcl-2 expression in a colon cancer cell line and, hence, has the ability to increase sensitivity of cells to lipid peroxidation and apoptosis (Beppu *et al.,* 2006). Interestingly, the study of Soel and co-workers (2007) reported that *t*9,*c*11-18:2 can inhibit progression of CRC *in vitro* and *in vivo*, whereas *t*10, *c*12-CLA had no effect. It is generally accepted that the consumption of dietary fish oil (EPA and DHA) may prove to be an effective adjuvant therapy in CRC. EPA is particularly known to exert a profound influence on the immune system (Calder *et al.,* 2002; Yaqoob, 2003a and 2003b; Stulnig, 2003; Philpott and Ferguson, 2004; Chapkin *et al.,* 2007a and 2007b). It is postulated that by up-regulating IL-2 and improving the Th1 response, the cell-mediated immune response can be improved. In this regard it is advocated that CLA is an excellent therapeutic agent (O'Shea *et. al.,* 2004). Few clinical trials (Larsson *et al*., 2005a; Song *et al*., 2005; Courtney *et al*., 2007; Soel *et al*., 2007) have seen the light. The main concern regarding CLA therapy is currently clarification of appropriate mixtures of beneficial CLA specific isomer products to address specific conditions or diseases (Salas-Salvado *et al*., 2006; Tricon and Yaqoob, 2006). Long-term randomized clinical trails with approved CLA isomers, controlled with placebo, in large patients groups for proper evaluation are suggested. For this reason a rationale for CLA and EPA is proposed for CRC management.

CHAPTER 4
METHODOLOGY

4.1 GENERAL

The research methodology followed and all the methods for FA and statistical analyses performed are given in this chapter. Patient recruitment for this study was done over a period of two years, under difficult circumstances. Less patients were available than anticipated, most patients received treatment before surgery and some patients were too ill or unwilling to participate in the research project. Another limitation on this study was that all colorectal polyps were pathologically investigated as a precaution to detect cancer and, hence, were not available for research purposes.

4.2 STUDY GROUP

The study protocol was approved by the Ethics Committee of the Medical School, University of the Free State. Patients referred for colorectal surgery were recruited at the out-patients section of the Surgical Clinic, Universitas Hospital, Bloemfontein. All patients who participated voluntarily signed an informed consent form and received one of the original signed copies as per Good Clinical Practice. Patients were also evaluated by means of a Medical History Questionnaire to confirm that they meet all the criteria of the study protocol.

Inclusion criteria for patients to be eligible for this study:

- Histopathological confirmed adenocarcinoma, according to TNM staging (T2 and T3);
- Patients between 50 to 80 years;
- White and black patients (both gender).

Exclusion criteria for patient participation in this study:

- Diabetes mellitus patients;
- HIV positive patients;
- Patients using steroids;
- Patients taking non-steroid, anti-inflammatory drugs (NSAID);

- Patients taking nutrient supplements (n-3 PUFAs etc.);
- Previous CRC treatment (radiation and/or chemotherapy);
- Patients with familial polyposis or a medical history of colorectal polyps (adenomas);
- Patients with inflammatory bowel disease;
- Patients on antibiotics in the 6-week period before recruitment.

Ten (10) eligible per protocol patients with CRC (age range, 50-80 years) were recruited for the selection of cancer (Ca) and normal (N) biopsies, taken from the same patient. Ca and N biopsies from eight (n8) of these patients were used to meet the strict criteria of the protocol for quality research. The small patient number is a common phenomenon lately encountered in clinically-laboratory orientated research. For comparative purposes, two colorectal Ca biopsies from the central parts and two N (control) biopsies form the end parts were taken after wide resection in the case of each patient. The paired normal tissue samples were dissected at locations 10 cm away from visible tumor edges for comparison (see figure 4.1). This study ruled out the possibility of bias caused by different dietary FA compositions by comparing cancerous and non-cancerous mucosal cells in the same subject. For typing of the CRC biopsies pathologists used conventional histopathological parameters, according to the World Health Organization guidelines (Beets-Tan *et al.,* 2005) and AJCC TNM classification system.

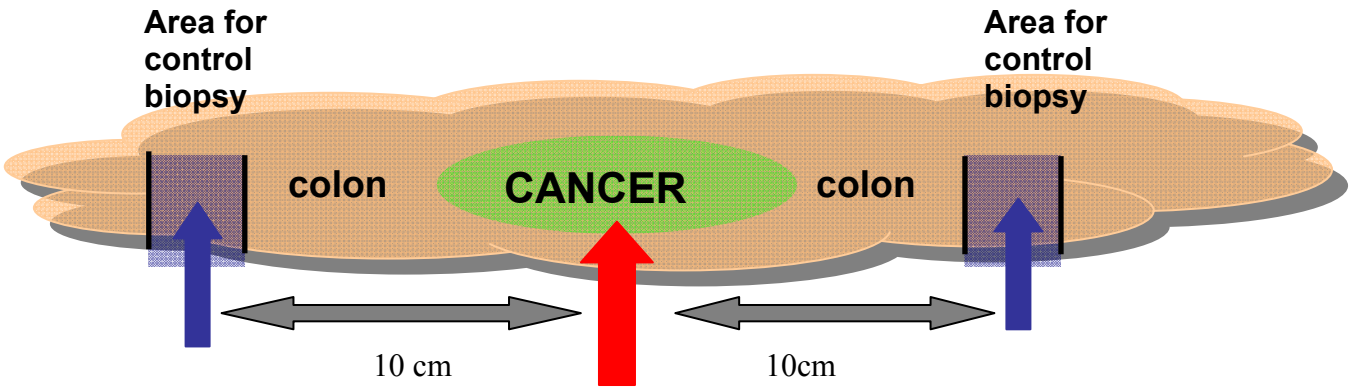

FIGURE 4.1 Areas for cancer and control biopsies.

Red arrow: area for cancer biopsies

Blue arrows:areas for control biopsies

Grey arrows: distance between biopsies.

4.3 FATTY ACID ANALYSES

4.3.1 Lipid extraction

Biopsies were placed on ice, rinsed in phosphate buffered saline (PBS) to remove all traces of blood and snap-freezed at -70º C until used. All the biochemical analyses were personally conducted. Lipid extraction was performed in the Department Basic Medical Science and lipid and FA analyses were performed at the Biolipid Technology Division, Department Biochemistry and Microbiology, University of the Free State.

Total lipid (TL) extraction was performed on frozen colorectal tissues, according to the method of Folch *et al.* (1957). This included chloroform/methanol (2:1, v/v) extractions, three washes with distilled water and final evaporation of the organic phase. A portion of the TL extraction dissolved in chloroform, was further fractionated following its application to a column (140mm x 20mm) of activated (heated overnight at 110°C) silicic acid (200 mesh) (Aldrich). Neutral lipids (NL) and phospholipids (PL) were eluted by successive application of organic solvents, according to the procedure referred to in Kock and Ratledge (1993). All the lipid fractions (TLs, NLs and PLs) were dissolved in diethyl-ether and transferred to a vial. The samples were dried in a vacuum oven at 50ºC over P_2O_5. The FA compositions of the TLs, NL, PLs and PL subclasses were determined after methylation of lipids by the addition of trimethyl sulphonium hydroxide (TMSH), according to the method of Butte (1983). Fatty acid methyl esters (FAME) were analyzed using the gas chromatographic method as described in 4.3.3

4.3.2 Phospholipid fractionation

The PLs were separated from the extracted lipids by thin-layer chromatography (TLC) on silica gel G-60 (Merck, Darmstadt, Germany). They were separated on silica gel plates with aluminium back using the one-dimensional ascending technique. The polar fraction, containing PLs, was separated using chloroform/methanol/water/acetic acid (65:43:3:1) by volume. The composition of the PLs were determined using standards of PC, PE, PI and PS. All standards were obtained from Sigma. The PL fraction was applied on a horizontal line drawn across the silica plate and then fractionated as described above. Initial visualization of fractions was facilitated by exposure of chromatograms to I_2 vapor. The fractions corresponding to the standards running on the sides of the plate were scraped off and the PL fractions dissolved in chloroform/methanol (2:1, by volume). Each fraction

was dried, weighed and stored under nitrogen gas. The FA composition associated with the different PL fractions was determined after trans-esterification by the addition of trimethyl sulphonium hydroxide, according to the method of Butte (1983). Fatty acid methyl esters (FAME) were analyzed using the gas chromatographic method as described in 4.3.3 Suitable authentic standards (Sigma, USA) were used for specific identification of experimental samples.

4.3.3 Lipid methylation

The FA compositions of the TLs, NL, PLs and PL subclasses (PC, PE, PI and PS) were determined after methylation of lipids by the addition of trimethyl sulphonium hydroxide (TMSH), according to the method of Butte (1983). FAME were analyzed using a Hewlett Packard 5890 gas chromatograph equipped with a Supelcowax 10 glass column (0.75 ID x 30m) with nitrogen as carrier gas set at flow rate of 5 $ml.min^{-1}$. The inlet temperature was set at 180ºC with the initial column temperature set at 145ºC, increasing at $3ºC.min^{-1}$ up to 225ºC, followed by a 10 min isothermal period. This period was followed by the same temperature increment to a final temperature of 240ºC. The FA peaks were detected using a flame ionization detector set at 300ºC. Peaks were identified using suitable authentic standards supplied by Sigma (USA). The individual FAs identified are indicated in table 4.1.

4.4 STATISTICAL ANALYSES

Results were expressed as a percentage of the total FAs for comparison with other studies. The descriptive statistics, i.e. medians and percentiles, were calculated for continuous data. Percentages were calculated for categorical data, i.e. cancer and normal groups for different lipid classes and phosholipid subclasses. To compare groups statistically, the paired-*t* test and signed rank test for parametric or non-parametric data were used and 95% confidence intervals (CI) for median differences were also calculated and considered to be significant at $p<0.5$. In addition, the average degree of unsaturation, the unsaturation index (UI), was derived from these results. It was calculated according to the following formula: UI = Σ (% FAs x number of double bonds of each UFA). All analyses were done at the Department of Biostatistics, University of the Free State.

TABLE 4.1 Summary of identified fatty acids, according to numerical abbreviations and common names

Numerical abbreviation	Common name
4:0	**Butyric acid (BA)**
12:0	**Lauric acid (LRA)**
14:0	**Myristic acid (MA)**
16:0	**Palmitic acid (PA)**
18:0	**Stearic acid (SA)**
16:1 n-7	**Palmitoleic acid (PLA)**
18:1 n-9	**Oleic Acid (OA)**
18:2 n-6	**Linoleic acid (LA)**
18:3 n-3	**Alpha-linolenic acid (ALA)**
18:3 n-6	**Gamma-linolenic acid (GLA)**
20:3 n-6	**Dihomo-gamma-linolenic acid (DGLA)**
20:4 n-6	**Arachidonic acid (AA)**
20:5 n-3	**Eicosapentaenoic acid (EPA)**
22:6 n-3	**Docosahexaenoic acid (DHA)**

CHAPTER 5
RESULTS

5.1 GENERAL

The FA analyses performed on Ca and N cells provided data that were statistically evaluated, according to FA groups (SFAs, MUFAs and PUFAs), individual FAs (see TABLE 4.1) and FA series (n-3, n-6, n-7 and n-9) in the case of lipid classes (TLs, NLs and PLs) and phospholipid subclasses (PC, PE, PI, PS).

5.2 INDIVIDUAL FATTY ACIDS

5.2.1 Total lipids (TL)

The FA values of Ca cells compared with N cells in TLs are given in Table 5.1. The TLs of Ca cells had significantly ***higher*** **14:0** (p=0.03; 95% CI for med. of differences [0.19 ; 0.93]); **16:0** (p=0.02; 95% CI for med. of differences [0.81 ; 4.23]); **18:1 n-9** (p= 0.03; 95% CI for med. of differences [0.02 ; 8.96]); **18:2 n-6** (p= 0.02; 95% CI for med. of differences [0.97 ; 3.03]), **24:0** (p= 0.03; 95%CI for med. of differences [0.08 ; 0.50] and significantly ***lower*** **12:0** (p<0,01; 95% CI for med. of differences [-1.38 ; -0.63]), **18:0** (p<0.01; 95% CI for med. of differences [-6.52 ; -0.92]), **18:3 n-6** (p=0.03; 95% CI for med. of differences [-0.18 ; 0.00]), **18:3n-3** (p=0.02; 95% CI for med. of differences [-0.22 ; 0.00]), **20:3n-6** (p= 0.02; 95 % CI for med. of differences [0.00 ; 0.65]); **20:4 n-6** (p< 0.01; 95 % CI for med. of differences [-1.31 ; -0.16]); **20:5 n-3** (p< 0.01; 95 % CI for med. of differences [-0.06 ; -0.04]); **22:6 n-3** (p<0.01; 95 % CI for med.of differences [-0.60 ; -0.34]). ***No statistical significant difference*** was observed for **16:1 n-7** (p=0.20; 95 % CI for med. of differences [-1.28 ; 7.05]).

5.2.2 Phospholipids (PL)

The FA values of Ca cells compared with N cells in PLs are given in Table 5.2. The PLs of Ca cells had significantly ***higher*** **14:0** (p=0.02; 95% CI for med. of differences [0.19 ; 0.74]); **16:0** (p<0.01; 95% CI for med. of differences [0.07 ; 11.23]); **16:1 n-7** (p=0.02; 95% CI for med. of differences [0.63 ; 6.79]); **18:1 n-9** (p<0.01; for med. of differences

[0.24 ; 9.53]); **18:2 n-6** (p=0.04; 95% CI for med. of differences [0.01 ; 3.50]), and significantly ***lower*** **12:0** (p<0.01; 95% CI for med. of differences [-1,82 ; -0.06]); **18:0** (p<0.01; 95% CI for med. of differences [-7.41 ; -1.56]); **18:3n-6** (p=0.02; 95% CI for med. of differences [-0.04; 0.00]); **18:3n-3** (p=0.01; 95% CI for med. of differences [-0.08 ; -0.02]); **20:4 n-6** (p< 0.01; 95% CI for med. of differences [-1.57 ; -0.42]); **20:5 n-3** (p<0.01; 95% CI for med. of differences [-0.05 ; -0.03]); **22:6 n-3** (p<0.01; 95 % CI for med. of differences [-0.67 ; -0.45]). ***No statistical significant difference*** was observed for **24:0** (p=0.15; 95 % CI for med. of differences [-3.86 ; 1.78]).

5.2.3 Neutral lipids (NL)

The FA values of Ca cells compared with N cells in NLs are given in Table 5.3. The NLs of Ca cells had significantly ***higher*** **14:0** (p<0.01; 95% CI for med. of differences [0.10 ; 3.72]); **16:0** (p=0.04; 95% CI for med. of differences [0.77 ; 4.92]); **16:1 n-7** (p<0.01; 95% CI for med. of differences [0.32 ; 1.82]); **18:1 n-9** (p<0.01; 95% CI for med. of differences [0.69 ; 38.86]); **18:2n-6** (p=0.03; 95 % CI for med. of differences [3.50 ; 10.54] and significantly ***lower*** **18:0** (p<0.01; 95% CI for med. of differences [-4.05 ; -0.93]); ***No statistical significant differences*** were observed for **12:0** (p=0.94; 95 % CI for med. of differences [-1.14 ; 0.85]); **24:0** (p=1.00: 95 % CI for med. of differences [-6.03 ; 2.89]); **18:3 n-6** (p=1.00; 95 % CI for med. of differences [-0.17 ; 8.81]); **18:3 n-3** (p=1.00; 95 % CI for med. of differences [-1.57 ; 0.30]); **20:4 n-6** (p=0.49; 95 % CI for med. of differences [-0.57 ; 1.44]); and **20:5 n-3** (p=0.35; 95 % CI for med. of differences [-0.65 ; 0.00]).

5.2.4 Phosphatidylcholine (PC)

The FA values of Ca cells compared with N cells in PCs are given in Table 5.4. The PC of Ca cells had significantly ***higher*** **16:0** (p<0.01; 95% CI for med. of differences [0.25 ; 18.69]); **18:1 *n*-9 (p=0.04;** 95% CI for med. of differences [5.21 ; 30.98]); **18:2 *n*-6** (p=0.02; 95% CI for med. of differences [0.07 ; 3.44]). ***No statistical significant differences*** were observed for **14:0** (p=0.69; 95% CI for med. of differences [-2.17 ; 1.84]); **18:0** (p=0.25; 95% CI for med. of differences [-12.17 ; 9.55]), **16:1 *n*-7** (p=0.10; 95% CI for med of differences [-1.39 ; 0.82]).

5.2.5 Phosphatidylethanolamine (PE)

The FA values of Ca cells compared with N cells in PEs are given in Table 5.5. The PE of Ca cells had significantly ***higher*** **16:0** (p=0.04; 95% CI for med. of differences [0.09 ;

8.01]); **18:1 *n*-9** (p=0.02; for med. of differences [0.05 ; 5.22]); **18:2 *n*-6** (p<0.01; 95% CI for med. of differences [0.11 ; 9.80]), and significantly ***lower*** **12:0** (p<0.01; 95% CI for med. of differences [-1.18 ; -0.09]); **18:0** (p=0.04; 95% CI for med. of differences [-9,67 ; -2.64]); ***No statistical significant differences*** were observed for **14:0** (p=0.47; 95 % CI for med. of differences [-0.55 ; 2.92]); **16:1 n-9** (p=0.55; 95 % CI for med. of differences [-2.08 ; 5.82]); **18:3 *n*-6** (p=0.50; 95 % CI for med. of differences [-0.02 ; 0.28]).

5.2.6 Phosphatidylinositol (PI)

The FA values of Ca cells compared with N cells in PIs given in Table 5.6. The PI of Ca cells had significantly ***higher*** **18:2 *n*-6** (P=0.03; 95% CI for med. of differences [0.75 ; 4.09)]. ***No statistical significant differences*** were observed for **12:0** (p=0.88; 95% CI for med. of differences [-1.61 ; 1.60]); **14:0** (p=0.81; 95% CI for med. of differences [-3.17 ; 1.36]); **16:0** (p=0.23; 95% CI for med. of differences [-4.74 ; 7.76]); **18:0** (p=0.31; 95% CI for med. of differences [-3.45 ; -2.32]); **16:1 *n*-7** (p=0.118; 95% CI for med. of differences [-0.72 ; 5.70]); **18:1 *n*-9** (p=0.130; for med. of differences [-3.23 ; 14.26]); **20:5 *n*-3** (p=0.251; 95 % CI for med. of differences [0.0 ; -33.19]).

5.2.7 Phosphatidylserine (PS)

The FA values of Ca cells compared with N cells in PS are given in Table 5.7. The PS of Ca cells had significantly ***higher*** **16:0** (p<0.01; 95% CI for med. of differences [0.85 ; 9.93]); **16:1 *n*-7** (p<0.01; 95% CI for med. of differences [0.17 ; 4.62]); and significantly ***lower*** **18:0** (p=0.02; 95% CI for med. of differences [-12.30 ; -1.51]). ***No statistical significant differences*** were observed for **14:0** (p=0.23; 95% CI for med. of differences [-2.68 ; 2.35]); **18:1 *n*-9** (p=0.64; for med. of differences [-2.18 ; 12.26]); **18:2 *n*-6** (p=0.74; 95% CI for med. of differences [-3.02 ; 2.64]).

5.3 FATTY ACID GROUPS

Values of FA groups (SFAs, MUFAs and PUFAs) in Ca cells and N cells in the case of TL, NL, PL and PL subclasses (PC, PI, PE and PS) are given in TABLES 5.8 - 5.14. There were no statistical significant differences between the **SFAs** values of the Ca cells compared with the N cells in the TLs, PLs, NLs, PC and PS. The **MUFA** values were significantly higher in Ca cells compared with N cells in TLs (p=0.02; 95%CI for med. of differences [1.42 ; 15.60]), in PLs (p=0.02; 95%CI for med. of differences [0.46 ; 12.59]), in NLs (p=0.01; 95% CI for med. of differences [1.30 ; 39.58]), in PC (p=0.05; 95% CI for

med. of differences [6.60 ; 29.30]), in PE (p=0.01; 95% CI for med. of differences [0.12 ; 12.75]), in PI (p=0.02; 95% CI for med. of differences [1.29 ; 19.59]) and in PS (p=0.02; 95% CI for med. of differences [0.30 ; 14.52]). There were no statistical significant differences between the **PUFA** values of the Ca cells compared with the N cells in the TLs, PLs, NLs, PC and PS, however the PUFA content was significantly higher in the Ca cells compared with N cells in PE (p=0.01 95% CI for med. of differences [0.29 ; 9.75]) and in PI (p=0.04; 95%CI for med. of differences [0.36 ; 37.28]). In all the lipid classes and phospholipid subclasses the desaturation index (ratio of MUFAs to SFAs) was significantly higher in Ca cells compared with N cells (Figure 5.5).

5.4 FATTY ACID SERIES

The n-3, n-6, n-7 and n-9 FA values are summarized in TABLES 5.15 to 5.21. The ***n*-3 FA** content of the Ca cells compared with N cells was significantly ***lower*** in the TL (p<0.01; 95% CI for med. of differences [-0.71 ; -0.51]) and in PL (p<0.01; 95% CI for med. of differences [-0.80 ;-0.53]). There were no statistical significant differences between the ***n*-6 FA** content of the Ca cells compared with the N cells in the case of TLs and PLs, but the ***n*-6 FA** content of the Ca cells compared with N cells was significantly higher in the PE (p<0.01; 95% CI for med. of differences [0.09 ; 9.80]). No statistical significant differences were observed for NLs (p=0.08; 95% CI for med. of differences [7.00 ; 38.70]), and PI (p=0.05; 95% CI for med. of differences [0.75 ; 4.09]). The ***n*-7 FA** content of cancer cells compared with N cells were significantly higher for TLs (p=0.02; 95% CI for med. of differences [0.63 ; 6.79]), NLs (p<0.01; 95% CI for med. of differences [0.32 ; 1.82]) and PS (p<0.01; 95% CI for med. of differences [0.17 ; 4.62]). The ***n*-9 FAs** content of cancer cells compared with N cells were significantly higher for TLs (p<0.01; 95% CI for med. of differences [0.11 ; 9.01]), PLs (p<0.01; 95% CI for med. of differences [0.42 ; 9.58]), NLs (p<0.01; 95% CI for med. of differences [0.69 ; 38.86]), PC (p=0.04; 95% CI for med. of differences [5.21 ; 29.16], PE (p<0.01; 95% CI for med. of differences [0.02 ; 5.93]), but no statistical significant differences were observed for PI (p=0.07; 95% CI for med. of differences [-3.23 ; 15.25]). This study showed a higher n6/n3 ratio in the Ca cells (Ca= 1.44), and N= 0,96. The double bond index was also higher in the Ca cells (TABLE 5.22).

TABLE 5.1 **TOTAL LIPID** individual fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
12:0					
Cancer	0.36	0.41	0.49	0.58	0.69
Normal	1.12	1.26	1.39	1.66	1.77
14:0					
Cancer	1.78	1.81	2.33	2.44	2.64
Normal	1.23	1.41	1.59	2.03	2.11
16:0					
Cancer	19.65	21.80	22.58	23.13	24.39
Normal	18.70	19.51	20.10	21.70	22.93
16:1 n-9					
Cancer	4.17	4.86	5.30	5.44	9.88
Normal	2.83	3.03	4.47	4.96	5.91
18:0					
Cancer	3.42	3.86	4.24	4.77	6.08
Normal	4.78	5.12	7.61	8.59	10.20
18:1 n-9					
Cancer	28.81	30.66	39.61	40.02	59.51
Normal	31.40	32.51	38.09	38.14	41.30
18:2 n-6					
Cancer	11.11	14.07	15.31	16.94	18.87
Normal	10.95	12.99	13.83	15.35	18.86
18:3 n-6					
Cancer	ND	0.19	0.26	0.37	0.45
Normal	ND	0.21	0.32	0.40	0.48
18:3 n-3					
Cancer	0.19	0.21	0.25	0.25	0.73
Normal	0.27	0.31	0.38	0.38	0.41
20:3 n-6					
Cancer	0.05	0.19	0.20	0.35	0.39
Normal	0.08	0.31	0.55	0.63	0.79
20:4 n-6					
Cancer	1.19	1.28	1.73	1.88	2.99
Normal	2.02	2.10	2.53	2.64	3.10
20:5 n-3					
Cancer	ND	ND	0.06	0.06	0.08
Normal	ND	ND	0.09	0.06	0.10
22:6 n-3					
Cancer	0.72	0.78	0.90	0.91	1.08
Normal	1.06	1.10	1.37	1.41	1.49
24:0					
Cancer	0.63	0.98	1.05	1.09	1.32
Normal	0.31	0.41	0.52	0.85	1.24

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum; ND, not detected. Results were expressed as relative percentages.

TABLE 5.2 **PHOSPHOLIPID** individual fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
12:0					
Cancer	0.62	0.66	0.80	0.87	0.96
Normal	0.93	1.10	1.79	2.24	2.78
14:0					
Cancer	1.46	1.93	2.29	2.65	3.05
Normal	1.04	1.54	1.58	1.82	2.36
16:0					
Cancer	18.49	21.39	23.44	24.53	30.42
Normal	13.21	19.19	20.59	21.39	23.73
16:1 n-9					
Cancer	2.96	3.05	5.57	5.86	9.33
Normal	1.79	2.35	2.82	4.70	4.91
18:0					
Cancer	1.11	2.98	4.31	4.60	6.08
Normal	4.97	5.64	6.44	10.64	11.47
18:1 n-9					
Cancer	28.69	30.56	40.04	41.04	43.81
Normal	25.02	27.34	33.70	37.36	41.81
18:2 n-6					
Cancer	10.48	10.72	13.58	14.89	20.06
Normal	8.27	10.14	12.83	13.54	16.94
18:3 n-6					
Cancer	0.26	0.27	0.31	0.37	0.38
Normal	0.26	0.29	0.31	0.39	0.41
18:3 n-3					
Cancer	0.28	0.29	0.39	0.44	0.77
Normal	0.32	0.34	0.40	0.49	0.85
20:3n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:4 n-6					
Cancer	1.04	1.08	1.45	1.52	1.62
Normal	2.04	2.11	2.29	2.46	2.83
20:5 n-3					
Cancer	0.01	0.02	0.04	0.05	0.08
Normal	0.04	0.06	0.08	0.11	0.13
22:6 n-3					
Cancer	0.79	0.82	0.87	0.84	0.92
Normal	1.31	1.34	1.38	1.41	1.49
24:0					
Cancer	0.32	0.65	1.23	1.78	2.09
Normal	0.21	0.31	2.38	2.53	3.86

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum; ND, not detected. Results were expressed as relative percentages.

TABLE 5.3 **NEUTRAL LIPID** individual fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
12:0					
Cancer	ND	0.39	1.04	1.37	5.4
Normal	0.50	0.54	1.14	1.41	4.55
14:0					
Cancer	2.01	2.23	2.59	3.21	3.65
Normal	1.69	1.84	2.07	2.14	2.66
16:0					
Cancer	15.33	17.79	22.40	24.06	29.87
Normal	15.72	16.06	19.91	22.60	24.95
16:1 n-9					
Cancer	2.83	2.94	3.65	4.36	5.81
Normal	2.22	2.51	2.90	3.09	4.83
18:0					
Cancer	3.07	3.32	5.71	6.19	8.89
Normal	4.37	6.72	7.73	10.09	10.85
18:1 n-9					
Cancer	28.81	30.66	39.61	40.02	59.51
Normal	18.09	20.65	28.71	36.06	40.27
18:2 n-6					
Cancer	7.28	10.10	14.11	14.98	26.94
Normal	5.25	7.09	11.42	14.82	16.43
18:3 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	0.17	ND	ND
18:3 n-3					
Cancer	ND	ND	0.27	ND	ND
Normal	ND	ND	1.57	ND	ND
20:3 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:4 n-6					
Cancer	ND	ND	0.52	ND	ND
Normal	ND	ND	0.55	ND	ND
20:5 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	0.65	ND	ND
22:6 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
24:0					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	0.90	ND	ND

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum ND, not detected. Results were expressed as relative percentages.

TABLE 5.4 PHOSPHATIDYLCHOLINE individual fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
12:0					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
14:0					
Cancer	1.84	ND	2.27	ND	2.64
Normal	1.57	1.77	2.01	2.17	257
16:0					
Cancer	19.30	22.78	25.13	25.59	31.62
Normal	12.63	12.93	19.59	20.51	24.91
16:1 n-9					
Cancer	2.12	2.16	2.78	3.16	3.85
Normal	2.90	2.99	3.07	3.34	4.79
18:0					
Cancer	8.63	10.82	12.78	15.24	30.02
Normal	17.96	18.41	20.62	22.29	23.48
18:1 n-9					
Cancer	11.92	27.79	36.21	41.19	42.47
Normal	11.49	17.13	27.90	28.78	30.39
18:2 n-6					
Cancer	5.19	5.25	7.77	10.29	12.87
Normal	3.42	5.26	6.58	6.98	10.57
18:3 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
18:3 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:3 n -6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:4 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:5 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
22:6 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
24:0					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum; ND, not detected. Results were expressed as relative percentages.

TABLE 5.5 PHOSPHATIDYLETHANOLAMINE individual fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
12:0					
Cancer	0.48	0.56	0.76	0.85	0.97
Normal	0.99	1.06	1.74	1.76	1.83
14:0					
Cancer	2.11	2.37	2.51	2.75	2.95
Normal	0.25	2.16	2.68	2.82	3.34
16:0					
Cancer	22.34	23.70	25.78	27.92	29.58
Normal	21.57	21.60	24.36	24.53	25.03
16:1 n-9					
Cancer	2.63	3.23	4.70	5.92	9.51
Normal	2.69	3.09	3.54	5.31	5.73
18:0					
Cancer	3.12	3.71	4.35	5.66	6.90
Normal	3.79	5.23	5.94	8.76	13.38
18:1 n-9					
Cancer	30.61	37.79	41.61	43.53	44.49
Normal	27.03	34.82	38.60	41.43	44.43
18:2 n-6					
Cancer	4.06	11.50	11.95	12.56	13.60
Normal	2.49	3.56	8.70	10.31	11.67
18:3 n-6					
Cancer	ND	ND	0.14	ND	ND
Normal	ND	ND	0.16	ND	ND
18:3 n-3					
Cancer	ND	ND	0.14	ND	ND
Normal	ND	ND	0.25	ND	ND
20:3 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:4 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:5 n-3					
Cancer	ND	ND	0.09	ND	ND
Normal	ND	ND	ND	ND	ND
22:6 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
24:0					
Cancer	0.25	0.46	0.74	2.38	2.96
Normal	ND	ND	ND	ND	ND

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum; ND, not detected. Results were expressed as relative percentages.

TABLE 5.6 PHOSPHATIDYLINOSITOL individual fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
12:0					
Cancer	1.06	1.41	1.71	2.23	2.56
Normal	0.96	1.56	2.07	2.14	2.67
14:0					
Cancer	ND	1.36	2.48	3.17	ND
Normal	ND	2.69	2.83	2.84	3.17
16:0					
Cancer	20.82	23.69	26.57	26.90	28.84
Normal	20.06	23.02	25.0	25.25	27.26
16:1 n-9					
Cancer	4.10	4.16	4.94	5.69	7.83
Normal	2.13	2.56	4.31	4.68	5.18
18:0					
Cancer	7.07	7.19	9.34	11.68	17.49
Normal	6.74	8.68	10.46	12.91	18.88
18:1 n-9					
Cancer	16.80	28.01	38.44	41.07	45.06
Normal	14.30	26.81	33.58	37.16	43.99
18:2 n-6					
Cancer	5.69	10.16	11.74	12.32	17.12
Normal	5.49	6.56	9.54	10.14	17.87
18:3 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
18:3 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:3 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:4 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:5 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
22:6 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
24:0					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum; ND, not detected. Results were expressed as relative percentages.

TABLE 5.7 PHOSPHATIDYLSERINE individual fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
12:0					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
14:0					
Cancer	ND	ND	ND	ND	ND
Normal	ND	2.11	2.57	2.68	ND
16:0					
Cancer	16.66	22.63	26.41	27.70	33.91
Normal	14.39	20.09	22.88	23.98	24.68
16:1 n-9					
Cancer	2.79	3.42	3.98	4.21	4.62
Normal	1.24	1.71	2.48	3.25	3.82
18:0					
Cancer	8.34	10.57	13.30	17.45	30.01
Normal	6.83	11.17	21.48	23.17	31.58
18:1 n-9					
Cancer	15.20	31.73	35.78	39.81	47.25
Normal	15.53	19.73	33.63	33.91	47.01
18:2 n-6					
Cancer	5.88	5.95	7.27	8.61	14.11
Normal	5.88	5.95	7.27	8.61	14.11
18:3 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
18:3 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:3 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:4 n-6					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
20:5 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
22:6 n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
24:0					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum; ND, not detected. Results were expressed as relative percentages.

TABLE 5.8 **TOTAL LIPID** values of fatty acid groups in cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
SATURATED FATTY ACIDS					
Cancer	29.18	31.39	32.17	32.50	32.98
Normal	28.74	31.84	33.66	34.56	34.98
MONOUNSATURATED FATTY ACIDS					
Cancer	45.62	45.99	48.04	51.36	52.82
Normal	35.80	38.76	43.01	44.61	47.46
POLYUNSATURATED FATTY ACIDS					
Cancer	16.09	17.70	19.11	21.59	23.69
Normal	15.81	18.40	19.17	21.67	23.39

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, medlian; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.9 **PHOSPHOLIPID** values of fatty acid groups in cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
SATURATED FATTY ACIDS					
Cancer	27.73	30.07	32.94	39.1	43.66
Normal	30.95	31.97	34.07	40.48	44.65
MONOUNSATURATED FATTY ACIDS					
Cancer	32.60	38.98	47.20	49.42	50.77
Normal	28.59	32.90	38.05	43.98	47.47
POLYUNSATURATED FATTY ACIDS					
Cancer	13.54	15.13	16.68	18.09	24.17
Normal	13.16	15.03	17.47	19.57	21.51

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.10 **NEUTRAL LIPID** values of fatty acid groups in cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
SATURATED FATTY ACIDS					
Cancer	22.05	29.01	32.92	38.20	43.50
Normal	30.70	32.05	33.74	36.90	39.24
MONOUNSATURATED FATTY ACIDS					
Cancer	32.26	36.02	43.80	45.75	62.45
Normal	20.60	24.30	31.68	4.58	44.58
POLYUNSATURATED FATTY ACIDS					
Cancer	7.28	12.47	14.53	17.11	35.75
Normal	5.25	7.98	13.03	15.82	17.97

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.11 PHOSPHATIDYLCHOLINE values of fatty acid groups in cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
SATURATED FATTY ACIDS					
Cancer	34.20	36.32	40.69	48.27	57.52
Normal	33.10	37.25	43.08	46.42	52.49
MONOUNSATURATED FATTY ACIDS					
Cancer	15.32	32.97	38.59	44.77	46.84
Normal	16.33	24.51	30.96	31.99	34.31
POLYUNSATURATED FATTY ACIDS					
Cancer	5.25	7.77	10.32	16.79	21.74
Normal	3.42	6.09	12.32	19.09	36.62

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.12 PHOSPHATIDYLETHANOLAMINE values of fatty acid groups in cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
SATURATED FATTY ACIDS					
Cancer	30.99	33.30	36.94	40.89	45.26
Normal	31.09	33.48	36.36	39.34	41.40
MONOUNSATURATED FATTY ACIDS					
Cancer	39.07	43.05	47.11	49.73	52.82
Normal	30.88	39.38	43.24	46.73	48.24
POLYUNSATURATED FATTY ACIDS					
Cancer	4.06	11.93	12.27	13.17	15.43
Normal	2.49	5.50	8.70	10.99	11.80

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.13 PHOSPHATIDYLINOSITOL values of fatty acid groups in cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
SATURATED FATTY ACIDS					
Cancer	36.87	38.90	40.27	41.96	50.32
Normal	35.11	37.81	39.24	43.49	48.80
MONOUNSATURATED FATTY ACIDS					
Cancer	20.96	38.27	46.69	48.41	49.99
Normal	18.29	31.60	38.06	43.90	48.81
POLYUNSATURATED FATTY ACIDS					
Cancer	5.69	10.20	11.74	18.79	46.63
Normal	5.49	8.87	9.75	10.93	17.87

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.14 PHOSPHATIDYLSERINE values of fatty acid groups in cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
SATURATED FATTY ACIDS					
Cancer	32.92	37.82	43.30	49.77	52.64
Normal	35.01	37.73	45.15	53.18	62.39
MONOUNSATURATED FATTY ACIDS					
Cancer	19.81	35.84	38.89	44.81	51.00
Normal	15.53	27.42	33.73	39.71	47.01
POLYUNSATURATED FATTY ACIDS					
Cancer	2.93	5.91	8.56	11.15	15.02
Normal	5.88	5.97	7.27	10.03	14.11

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.15 TOTAL LIPID n-3, n-6, n-7 and n-9 fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
n-3					
Cancer	1.00	1.13	1.20	1.30	1.81
Normal	1.65	1.69	1.81	1.94	2.40
n-6					
Cancer	13.19	16.03	17.27	19.84	20.30
Normal	13.98	13.24	17.29	18.50	21.57
n-7					
Cancer	4.17	4.94	5.30	5.78	9.88
Normal	2.83	3.13	4.47	5.21	5.91
n-9					
Cancer	40.62	40.90	41.61	44.54	47.46
Normal	32.04	35.11	37.88	39.97	42.06

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.16 PHOSPHOLIPID n-3, n-6, n-7 and n-9 fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
n-3					
Cancer	1.19	1.24	1.32	1.40	1.61
Normal	1.72	1.80	1.90	2.02	2.41
n-6					
Cancer	12.28	13.65	15.35	16.78	21.78
Normal	11.05	13.01	15.36	17.83	19.66
n-7					
Cancer	1.79	2.45	2.82	4.77	4.91
Normal	1.79	2.45	2.82	4.77	4.91
n-9					
Cancer	29.64	34.62	41.11	42.43	44.73
Normal	25.86	29.85	34.43	39.95	42.64

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.17 NEUTRAL LIPID n-3, n-6, n-7 and n-9 fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
n-3					
Cancer	0.00	0.00	0.00	0.22	0.60
Normal	0.00	0.00	0.00	0.65	3.14
n-6					
Cancer	1.56	22.86	28.60	32.96	71.50
Normal	10.50	15.95	24.11	31.22	32.86
n-7					
Cancer	2.83	3.18	3.65	4.54	5.81
Normal	2.22	2.22	2.90	3.70	4.83
n-9					
Cancer	28.81	32.39	39.61	40.97	59.51
Normal	18.09	21.72	28.71	36.89	40.27

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.18 PHOSPHATIDYLCHOLINE n-3, n-6, n-7 and n-9 fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
n-6					
Cancer	5.19	6.04	7.77	10.32	12.87
Normal	3.42	5.32	6.58	7.99	10.57
n-7					
Cancer	2.12	2.40	2.78	3.28	3.85
Normal	2.90	3.00	3.07	3.63	4.79
n-9					
Cancer	11.92	30.47	36.21	41.83	42.99
Normal	13.31	20.62	27.89	28.88	30.39

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.19 PHOSPHATIDYLETHANOLAMINE n-3, n-6, n-7 and n-9 fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
n-3					
Cancer	0.00	0.00	0.00	0.00	0.25
Normal	0.00	0.00	0.00	0.34	0.57
n-6					
Cancer	4.06	11.69	11.95	12.96	13.88
Normal	2.49	5.27	8.70	10.93	11.67
n-7					
Cancer	2.63	3.60	4.70	6.70	9.51
Normal	2.69	3.14	3.54	5.48	5.73
n-9					
Cancer	31.60	39.45	42.24	44.46	45.39
Normal	27.70	35.67	38.60	41.85	45.15

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.20 PHOSPHATIDYLINOSITOL in n-3, n-6, n-7 and n-9 fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
n-3					
Cancer	0.98	1.14	1.33	1.64	1.78
Normal	ND	ND	ND	ND	ND
n-6					
Cancer	5.69	10.20	11.74	12.88	17.12
Normal	5.49	7.59	9.24	10.70	17.87
n-7					
Cancer	4.10	4.32	4.94	6.30	7.83
Normal	2.13	2.82	4.31	4.75	5.18
n-9					
Cancer	16.80	33.34	39.93	43.14	45.06
Normal	14.30	29.26	34.20	38.98	43.99

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.21 PHOSPHATIDYLSERINE in n-3, n-6, n-7 and n-9 fatty acid values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
n-3					
Cancer	ND	ND	ND	ND	ND
Normal	ND	ND	ND	ND	ND
n-6					
Cancer	2.93	5.91	8.56	11.15	15.02
Normal	5.88	8.97	7.27	10.03	14.11
n-7					
Cancer	2.79	3.59	3.98	4.41	4.62
Normal	0.00	0.0	0.00	0.86	3.25
n-9					
Cancer	15.20	31.86	35.78	40.40	47.25
Normal	15.53	26.57	33.63	38.19	47.01

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum. Results were expressed as relative percentages.

TABLE 5.22 Double bond index values of cancer and normal cells.

	MIN	Q1	MED	Q3	MAX
Cancer	70.95	75.82	80.37	84.41	95.56
Normal	68.86	71.31	73.75	78.60	98.91

Abbreviations: CA, carcinoma; MIN, minimum; Q1, 25th percentile; MED, median; Q3, 75th percentile; MAX, maximum.

5.5 SUMMARY OF RESULTS

A summary of al the higher or lower values for Ca cells compared with N cells in the classes and subclasses are given in TABLE 5.23. Those values that are significantly higher or lower are indicated with an astrix. The TL and PL classes are marked by similarities, with the exception of: 24:0 that is not significantly higher in the PL class; 16:1 that is not significantly higher in the TL class, and 20:3n-6 that was not detectable in the PL class, whilst it was significantly lower in the TL class. Statisically higher and lower FA values of the TL class are illustrated in FIGURES 5.1 and 5.2, respectively, whilst the statistically higher and lower values of the PL class are illustrated in FIGURES 5.3 and 5.4, respectively. In all the lipid classes and phospholipids subclasses the desaturation index (ratio of MUFAs to SFAs) was significantly higher in Ca cells compared with N cells and this is illustrated in Figure 5.5.

TABLE 5.23 FATTY ACID PROFILES of all the lipid classes and phospholipid subclasses. Statistically significant results ($p<0.05$) are indicated with an astrix (*).

SATURATED FATTY ACIDS							
	TLs	PLs	NLs	PC	PS	PE	PI
12:0	↓*	↓*	↓	ND	ND	↓*	↓
14:0	↑*	↑*	↑*	↑	↓	↓	↓
16:0	↑*	↑*	↑*	↑*	↑*	↑*	↑
18:0	↓*	↓*	↓*	↓	↓*	↓*	↓
24:0	↑*	↑	ND	ND	ND	ND	ND
MONOUNSATURATED FATTY ACIDS							
	TLs	PLs	NLs	PC	PS	PE	PI
16:1	↑	↑*	↑*	↓	↑*	↑*	↑
18:1	↑*	↑*	↑*	↑*	↑	↑*	↑
POLYUNSATURATED FATTY ACIDS							
	TLs	PLs	NLs	PC	PS	PE	PI
18:2n-6	↑*	↑*	↑*	↑*	↑	↑*	↑*
18:3n-6	↓*	↓*	ND	ND	ND	↓	ND
18:3n-3	↓*	↓*	↓	ND	ND	↓	ND
20:3n-6	↓*	ND	ND	ND	ND	ND	ND
20:4n-6	↓*	↓*	↓	ND	ND	ND	ND
20:5n-3	↓*	↓*	ND	ND	ND	↓	↓
22:6n-3	↓*	↓*	ND	ND	ND	↓	ND

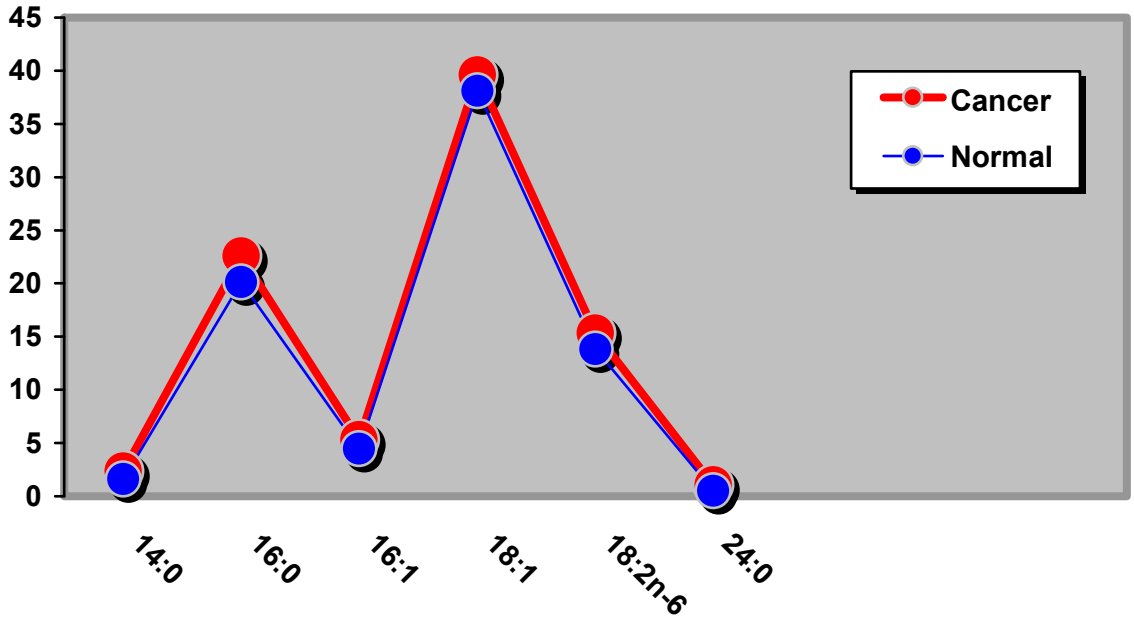

FIGURE 5.1 TOTAL LIPIDS: Significantly higher fatty acids of Ca cells compared with N cells ($p<0.05$). *Values are means expressed as relative percentages.*

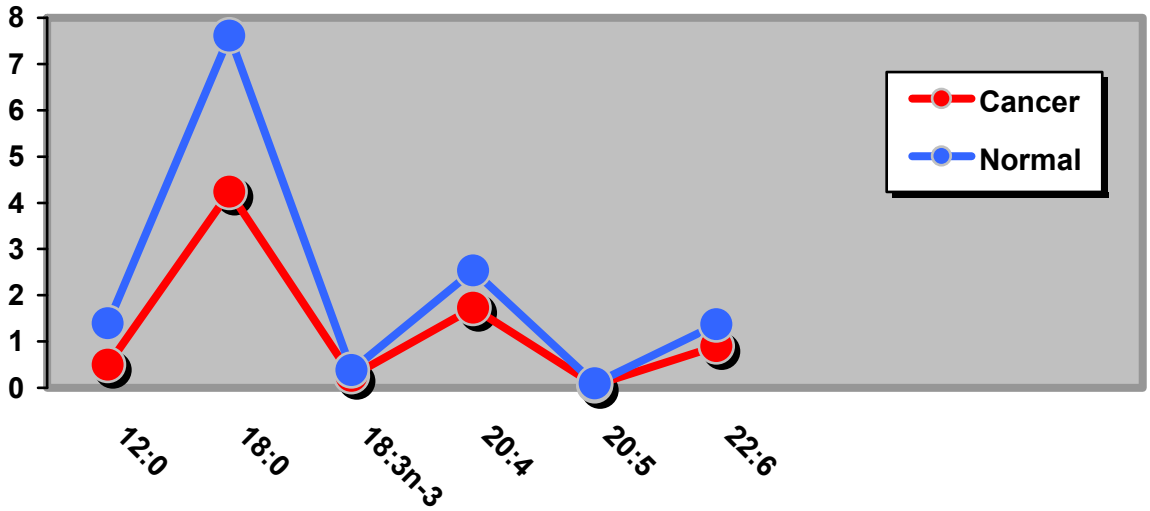

FIGURE 5.2 TOTAL LIPIDS: Significantly lower fatty acids of CA cells compared with N cells ($p<0.05$). *Values are means expressed as relative percentages.*

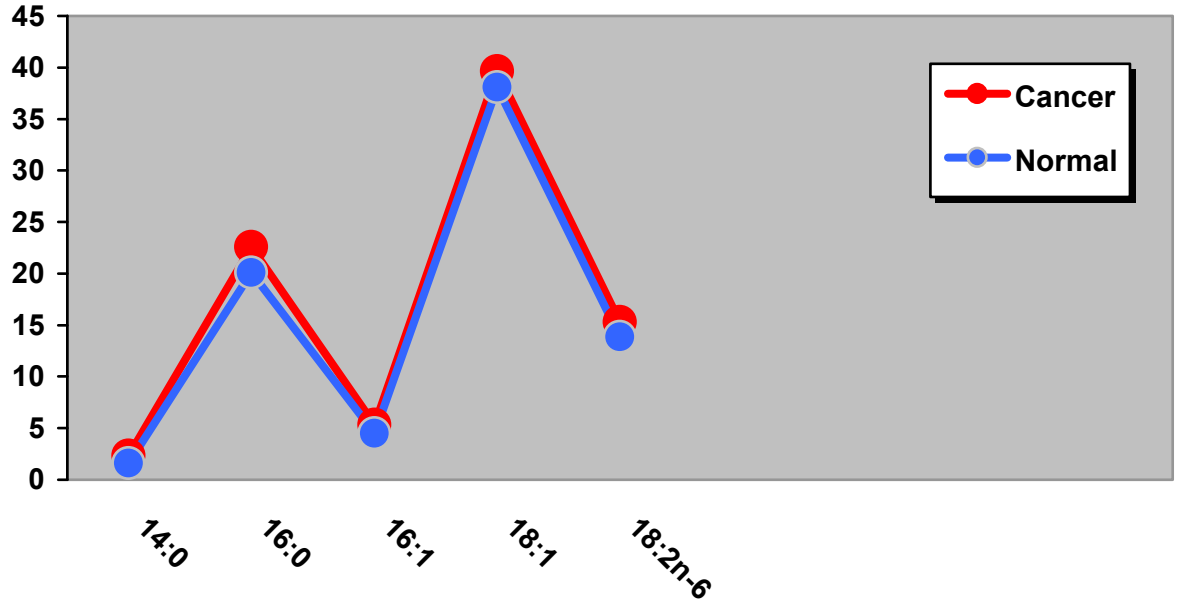

FIGURE 5.3 PHOSPHOLIPIDS: Significantly higher fatty acids of Ca cells compared with N cells ($p<0.05$). *Values are means expressed as relative percentages.*

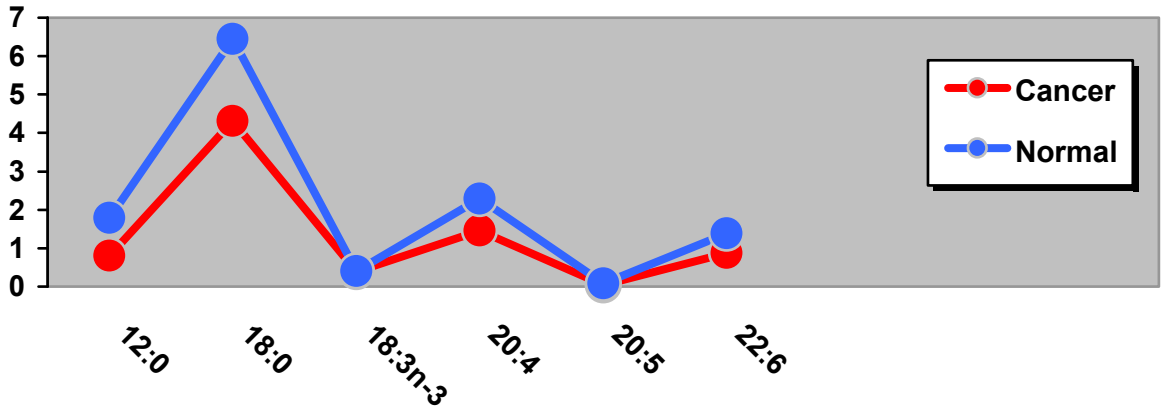

FIGURE 5.4 PHOSPHOLIPIDS: Significantly lower fatty acids of Ca cells compared with N cells ($p<0.05$). *Values are means expressed as relative percentages.*

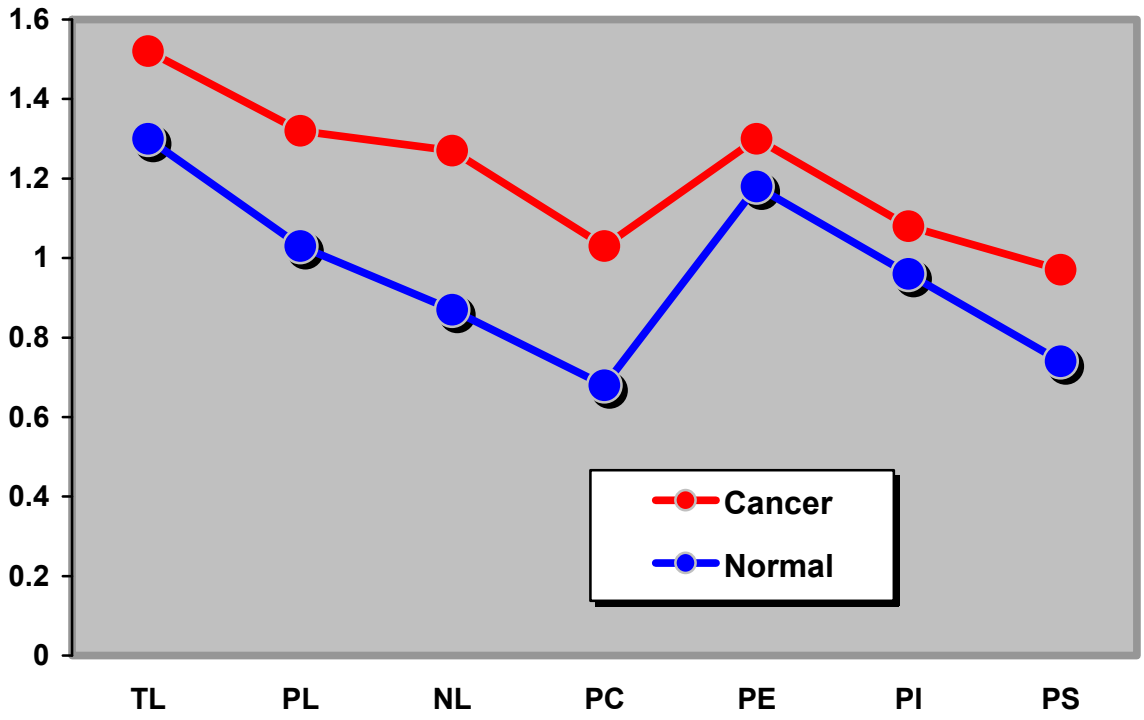

FIGURE 5.5 DESATURATION INDEX: Ratio of MUFAs to SFAs in Ca cells compared with the ratio of MUFAs to SFAs in N cells ($p<0.05$). *Results used to calculate ratios were expressed as relative percentages.*

CHAPTER 6
DISCUSSION

6.1 GENERAL

In this dissertation FA compositions of CRC cells and normal colon mucosal cells were investigated that allowed the construction of a lipid model. A lipid model for colorectal adenocarcinoma, consisting of *ex vivo* clinically relevant FA profiles for TL, NL and PL classes and PC, PE, PI and PS subclasses is presented, based on the information obtained in CHAPTER 5.

6.2 LIPID MODEL

The lipid model constructed in this study allowed the identification of important FA role-players in colorectal adenocarcinoma cells. With a few exceptions, similar results occurred in the lipid classes and subclasses and the roles of FAs representative of the PL FA profile are debated in this study, mainly for their particular importance in membrane-to nucleus cell signaling and for comparison purposes with other studies.

6.3 FATTY ACID ROLE-PLAYERS

The identification of FA role-players allowed evidence-based conclusions or suggestions regarding possible FA pathways followed during colorectal carcinogenesis and this, in turn, allowed debating of therapeutic options to propose alternative FA therapeutic strategies in search for optimal safe CRC management. Of particular interest in this study is the significantly higher myristic acid (MA; 14:0), palmitic acid (PA; 16:0), palmitoleic acid (PLA; 16:1), oleic acid (OA; 18:1) and linoleic acid (LA; 18:2), and significantly lower lauric acid (LRA; 12:0); stearic acid (SA; 18:0), gamma-linolenic acid (GLA; 18:3n-6), alpha-linolenic acid (ALA; 18:3n-3), arachidonic acid (AA; 20:4), eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) levels in the Ca cells compared with N cells. Reference is also made to the MUFA:SFA desaturation index and the n-6 PUFA:n-3 PUFA ratio where applicable.

LIPID MODEL

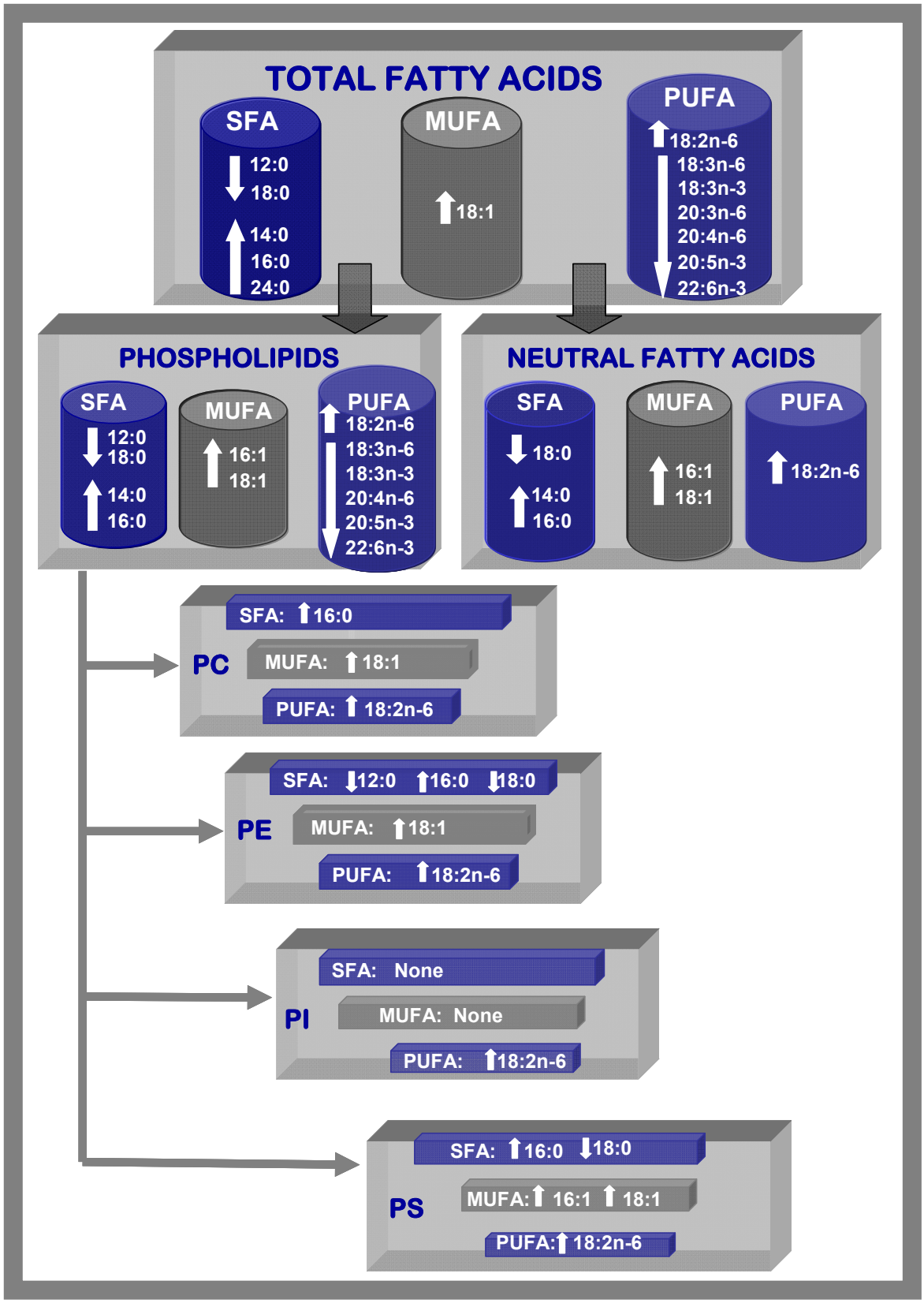

FIGURE 6.1 *Ex vivo* fatty acid profiles from a lipid model for colorectal adenocarcinoma. ***Description***: *Individual fatty acids of the lipid classes and subclasses are grouped according to saturated-, monounsaturated- and polyunsaturated fatty acids. Arrows indicate the significantly higher* (↑) *or lower* (↓) *fatty acid levels.*

6.3.1 Linoleic acid

The significantly higher LA level observed in the CRC cells is in contrast with other cancer entities (such as cervical, gastric and laryngeal cancer) previously reported (Louw *et al*., 1998a, 1998b, and 1998c; Ahn *et al.,* 2001; Louw and Claassen, 2006). Nevertheless, Shim *et al.* (2005) also observed higher LA levels in polyp and CRC cells that strengthen our finding. The presence of excessive LA in CRC cells can be attributed to the Western diet and environmental factors that interfere with EFAM. The significantly lower GLA level encountered in the CRC cells apparently testifies to interference of LA metabolism to GLA. Possibilities why the LA content in these CRC cells is significantly higher can be widely debated, based on: environmental factors, substrate availability and enzyme activities with a shift in FA metabolism during carcinogenesis; and nutritional status. Environmental factors can interfere with desaturase activities (Horrobin, 1993 and 1994) and down-regulation of Δ^6d activity was reported for aggressive breast cancer cells (Lane *et al.,* 2003), whilst both Δ^6d and Δ^5d activities were down-regulated in prostatic cancer cells (Kelavkar *et al.,* 2006). It is plausible that the CRC patients of this study may be culpable of excessive smoking and alcohol abuse, and according to the age group of these patients it is conceivable that they may be more subjected to decreased Δ^6d and Δ^5d activities. It is also feasible that these patients might have been subjected to improper diets and food preparations over decades and that excessive *trans*-FA intake may have interfered with EFAM (Craig-Schmidt, 2006; Mozaffarian, 2006). A high SFA content (exogenous intake and endogenous production) may also have interfered with EFAM, with a consequent shift in metabolism whereby different FA pathways are followed (Saether *et al*., 2007).

With respect to a shift in lipid metabolism from LA to AA that is prevalent in CRC, possible LA cell proliferation and apoptotic pathways followed may be noteworthy. High LA levels apparently initiate cellular-overproduction and the survival of CRC cells, since LA is known for its potential to stimulate cell proliferation (Larsson *et al.,* 2004). CRC cells apparently continue to survive because LA is an activator of PPARδ/β that contributes to multistage colon carcinogenesis by suppressing apoptosis (Larsson *et al*., 2004; Zuo et al., 2006; Sertzing *et al*., 2007). During oxidative metabolism of LA, the 15-LOX-1 pathway (that maintains the normal rate of apoptosis) is reported to be down-regulated during colorectal carcinogenesis and apoptosis is suppressed (Heslin *et al*., 2005; Shureiqi *et al*., 2005). The question that remains is why 15-LOX-1 activity is reported to be down-regulated in the adenoma-carcinoma sequence, compared with normal colon cells (Heslin *et al*., 2005;

Shureiqi *et al*., 2005). There appears to be sound evidence that PPARδ/β over-expression by FA ligands, particularly LA (and PA) suppresses PPARγ (and PPARα) activity in favor of apoptotic resistance and this is the reason why the CRC cells continue to survive (Grau *et al*., 2006; Zuo *et al*., 2006; Pondferrada *et al*., 2007). Regarding oxidative stress, it is possible that LA may also contribute to radical-mediated cell damage that contributes to CRC (Niki *et al.,* 2005). Lastly, the conversion of enhanced dietary LA intake to harmful CLA products by bacterial activities in the gut (Devillard *et al*., 2007) that may have direct detrimental effects on colonocytes needs mentioning and must be further explored. To conclude, CRC cells continue to survive and the diet apparently continues to supplement these cells with LA, thus, preventing diminishment of the LA source.

6.3.2 Arachidonic acid

The significantly lower AA level of the CRC cells observed in this study is in accordance with findings of other CRC studies (Jones *et al.,* 2003). During colorectal carcinogenesis enhanced AA and COX-2 expression is prevalent and enhanced MAPK activity, associated with cell proliferation, enhanced NF-κβ, associated with immunodeficiency, and enhanced PA, associated with FAS activity, are all factors that apparently contributes to the enhanced COX-2 expression (Furstenberger *et al.,* 2006) It seems logical that AA levels eventually becomes exhausted during carcinogenesis, as encountered in the CRC cells. The AA decrease observed in CRC cells might possibly stem from enhanced AA metabolism via COX- and LOX pathways, and the formation of pro-inflammatory eicosanoids and derivatives such as PGE_2, 5-HETE, 12-HETE and 15-HETE, as were recently confirmed by Soumaoro *et al*. (2006) and Goosens *et al*. (2007). AA is also very susceptible to peroxidation and together with increased ROS and NO• production during chronic inflammation reported in age-related CRC (Ohtsu *et al.,* 2005), radical-mediated cell damage contribute to CRC cells that may also reduce the AA levels. Apart from enzyme stimulation by enhanced OA that releases AA from the membrane, a feedback mechanism whereby PGE_2, released from AA by COX-2 expression, stimulates COX-2 activity (Wu *et al.,* 2005) may eventually exhaust the dietary AA source. Concurrence of 15-LOX down-regulation and COX-2 up-regulation is prevalent during colorectal carcinogenesis and the standpoint is that down-regulation of COX-2 activity may restore 15-LOX-1 activity to re-establish apoptosis, based on *in vitro, in vivo* animal and human CRC specimen studies (Heslin *et al*., 2005; Shureiqi *et al*., 2005; Yuri *et al*., 2007). It appears that switching of LA metabolism to AA by reversal of the expression of 15-LOX-1 and COX-2 can be associated with acquisition of the malignant potential of colon

neoplasia. In a recent mouse model study on colon cancer by Hollingshead and co-workers (2007) it was stated that COX-2 inhibition can be clearly dissociated from PPARδ/β activity during colon carcinogenesis. However, immunohistochemical studies on human CRC biopsies confirmed that NSAIDs can effectively decrease COX-2 expression and increase 15-LOX-1 expression, despite the disparity observed *in vivo* with mice (Yoshinaga *et al.,* 2007).

6.3.3 Palmitic acid

The significantly higher PA level in the CRC cells can be attributed to enhanced FAS (oncogenic antigen-519) activity generally observed during carcinogenesis (Menendez *et al*., 2004a). The constant high PA levels in CRC cells (exogenous dietary intake and endogenous metabolic production) can contribute to maintenance of cell proliferation, survival, oxidative stress, and immune responses. The high proliferation rate in cancerous cells requires more glucose which could lead to an increase in the production of PA that is known to sustain the fast proliferation rate of survival cells (Scaglia *et al.,* 2005). Of importance remains the fact that up-regulated FAS activity with enhanced PA production up-regulates PPARδ/β activity that suppresses apoptosis in colorectal carcinogenesis (Larsson *et al*., 2004; Shah *et al*., 2006 Takayama *et al*., 2006; Zuo *et al*., 2006). This then explains the link between up-regulated FAS activity and PA production in cancer cells and the propensity of cancer cells to thrive indefinitely. The high intake of red meat rich in SFA levels, PA (and SA) apparently contributes to oxidative stress during colorectal carcinogenesis, since it stimulates COX-2 expression and inflammation (Lee *et al*., 2001; Aggarwal *et al.,* 2006). It is plausible that PA can induce NF-κβ activity and cytokine activity (IL-6 and TNF-α expression) through PKC activation, based on studies with different cell types (Ajuwon and Spurlock, 2005; Jove *et al.,* 2006). Among SFAs, PA (and SA) may be in principle responsible for the down-regulation of the Th1 cytokine subset, especially the reported IL-2 in CRC that convincingly leads to immunodeficiency (Yaqoob, 2004).

6.3.4 Palmitoleic acid

The significantly higher PLA content observed in the CRC cells appears to be a common phenomenon in tumor and cancer cells, including CRC (Mamalakis *et al,* 2002; Pandey *et al.,* 2003; Llor *et al.,* 2003). MUFAs such as PLA is considered non-toxic and protective against the detrimental effects of PA (Hardy *et al.,* 2000; Maedler *et al.,* 2003; Mishra and

Simonson, 2005; Welters *et al.,* 2006). However, further studies are needed to elucidate the link between CRC and PLA.

6.3.5 Stearic acid

The significantly decreased SA level observed in the CRC cells can be attributed to an increase in Δ^9d (SCD) activity and OA production. Carcinogenesis is characterized by a desaturation index that is defined as the OA to SA ratio. High Δ^9d activity and increased desaturation are already evident in adenomatous polyposis, according to the study of Shim and co-workers (2005). The role of SA as an intermediate FA between PA and OA needs to be further elucidated in CRC. To what extent SA plays a role in the down-regulation of the Th1 cytokine subset and immunodeficiency is not known. The assumption that SA does play an important role in immune driven responses in CRC patients and eventually becomes exhausted needs to be confirmed or refuted.

6.3.6 Oleic acid

A significantly higher OA level observed in the CRC cells is in accordance with other studies on cancer cells (Louw *et al*., 1998a; Mamalakis *et al.,* 2002; Pandey *et al.,* 2003; Louw and Claassen, 2006). The enhanced OA is apparently the consequence of enhanced Δ^9d activity by which SA is converted to OA. It is argued that enhanced MUFAs (particularly OA) play a significant role during neoplastic cell transformation and programmed cell death (Scaglia and Igal, 2005), since Δ^9d is a regulatory enzyme in the overall *de novo* synthesis of these MUFAs. The question was raised why Δ^9d is such a highly regulated enzyme even though OA, the major product of this enzyme, is one of the most abundant FAs in the diet and is therefore readily available. Enhanced OA may be the result of a combination of many different factors, including, biosynthesis, oxidation, esterification, turn-over rate, antioxidant systems and the diet. There is evidence that OA is an important anti-oncogene-regulator during cancer, including CRC (Llor *et al*., 2003; Menendez *et al*, 2005b; Menendez and Lupu, 2006; Menendez *et al*., 2006a, 2006b). Enhanced OA, the consequence of enhanced FAS and Δ^9d activity, is a common phenomenon in all cancers. Elevated levels of cellular MUFAs, particularly OA, can activate signaling proteins with proliferative actions like the PKC system. OA has been described as a physiological activator of the classical PKC isomers α and β, and abnormally high PKC activity has been linked to tumorigenecity of neoplastic cells (Scaglia and Igal, 2005).

6.3.7 Alpha-linolenic acid

The significantly decreased ALA in the CRC cells can be attributed to insufficient dietary intake and preferential metabolism based on substrate availability, especially since the diets of the CRC patients is apparently marked by excessive LA and SFA dietary intakes. The possibility that there may be increased metabolism of the ALA substrate to provide anti-inflammatory eicosanoids of the n-3 PUFA series may be out ruled in the case of CRC, since environmental factors also interfere with the metabolism of ALA, as in the case of LA. Other possibilities for the decreased ALA content in CRC cells may be that it is also subjected to lipid peroxidation and may become diminished, and that PPARδ are also ligands for ALA and that this FA substrate may become exhausted by preventing apoptosis and sustaining cell survival, as in the case of LA.

6.3.8 Eicosapentaenoic acid and docosahexaenoic acid

Significantly decreased EPA and DHA levels were observed in CRC cells, in contrast with other cancers (Louw *et al.*, 1998a; Louw and Claassen, 2006). The decreased levels of EPA and DHA could be an indication of these FAs trying to potentiate mitochondrial lipid oxidation for apoptotic induction in CRC, as was suggested earlier by Chen and Istfan (2000). Generally, increased requirements of rapidly diving cells can also contribute to n-3 PUFA deficiency in cancer cells. Insufficient dietary consumption of these FAs, typical of the Western diet, may definitely be to the further disadvantage of these CRC patients. Overall, an increased n-6 PUFA: n-3 PUFA ratio is prevalent in the CRC cells of this study, a phenomenon characteristic of all cancers (Simopoulos, 2006).

6.3.9 Lauric acid and myristic acid

Significantly lower LRA and significantly higher MA levels were observed in the CRC cells. Contradictory remarks about these two SFAs are encountered in the literature, since they were suggested to be associated with CRC risk and prevention, according to different studies (Nkondjock *et al.,* 2003). This could account the metabolism-raising properties of coconut oil. In the case of MA it is reported that this FA has high hydrophobicity and becomes incorporated into the PL bilayer of cell membranes where it acts as a lipid anchor in biomembranes (Fatty acids, 2007).

6.4 FATTY ACID THERAPEUTIC OPTIONS:

MAIN OPTIONS: FA therapeutic options to improve CRC management are as follows:

A: In the case where decreased DHA levels in CRC cells occur, it is widely accepted this FA with apoptotic potential (down-regulation of COX-2 and Bcl-2 expression) improves membrane fluidity, which makes it an excellent therapeutic agent in combination with drug therapy. The outcome of clinical trials with concurrent therapy that includes DHA and Celecoxib (COX-2 inhibitor) to induce apoptosis and improve drug therapy after surgery is currently awaited with anticipation.

B: In the case where enhanced PA and OA levels in CRC cells occur, the consequence of up-regulated FAS and Δ^9d activity for maintenance of cell proliferation rates and survival occurred, under circumstances where LA conversion to AA is impeded, the beneficial use of therapeutic GLA to inhibit FAS is supported. GLA and EPA administration is the conventional method to: modulate membrane FA compositions; improve the n-6: n-3 PUFA ratio in the Western diet; and thereby down-regulate FAS activity. In addition, the inclusion of OA (an antioxidant and anti-oncogene regulator) can be considered for evaluation.

C: In the case of enhanced LA levels of CRC cells, a new direction with CLA and EPA is proposed to address immunomodulation of membrane FA compositions for the prevention of carcinogenesis and immunodeficiency.

A rationale for CLA and GLA in combination with EPA is presented in section 6.5.

COMMENTS: Research convincingly proved that FAs may have beneficial use in CRC, base on the literature discussion in CHAPTER 3. Although radiochemotherapy can inhibit cell proliferation and induce apoptosis, FAs as non-toxic chemotherapeutic agents have the advantage that they may eliminate/alleviate undesirable side-effects of these therapeutic approaches. Research also revealed that in the case of concurrent therapy (FAs in combination with drugs), FAs have the potential to enhance the impact of drugs on cancer cells and thereby improve the outcome of therapy. There is sufficient evidence in support of adjuvant FA therapy in combination with standard treatment for improved management of CRC (Reddy, 2005; Kapoor and Huang, 2006; Chapkin *et al.,* 2007a and 2007b; Courtney *et al.,* 2007). Taken together, combination surgery and adjuvant FA therapy with or without radiochemotherapy need to be evaluated.

DOSAGE: Needless to say, the dosage and time duration for optimal efficacy is of the utmost importance. Cheng and co-workers (2003) found that low dosages of n-3 PUFAs

(1mg EPA and 400mg DHA per day) significantly increased rates of apoptosis in the normal colonic mucosa of patients with colorectal adenomas after 2 years of supplementation. Recently, the study of Courtney and co-workers (2007) revealed that 3 months of highly purified EPA in free fatty acid form (2g/day) can reduce crypt cell proliferation and increase apoptosis in normal colonic mucosa in subjects with a history of colorectal adenomas. Findings by Nakamura and co-workers (2005) suggested that oral administration of a supplement rich in n-3 PUFAs for 5 days before surgery may improve not only preoperative nutritional status, but also postoperative inflammatory and immune responses in patients who have cancer. In the case of CLA, proclaimed to be an excellent anti-cancer and immunomodulatory FA agent, a safe dosage of 2,5-6g/day was determined for human consumption (Pariza, 2004). Most recently, a phase II clinical trail on the nutritional and inflammatory status of CRC patients revealed the beneficial use of dietary counseling and EPA oral supplementation to maintain nutritional status during CRC management (Read *et al*., 2007). Proper nutrition remains a problem, since the typical Western diet does not meet with daily recommended requirements. However, a few commercial FA supplementation products are available. Although prevention of cancer risk by FAs may be in doubt, the beneficial use of FAs in therapy is gaining prominence.

6.5 RATIONALE FOR FA THERAPIES

There is compelling evidence available which demonstrates the ability of CLA to modify eicosanoid, cytokine and immunoglobulin production (O'Shea *et al.,* 2004). It is also evident that two CLA products (*c*9,*t*11-18:2 and *t*10,*c*12-18:2) exert distinct effects on T-lymphocyte populations and immunoglobulin subclasses. Of importance is the fact that CLA has the potential to up-regulate reduced IL-2 production and to improve cytotoxic T lymphocyte function, as well as the potential to down-regulate IgE and to improve B lymphocyte maturation (O'Shea, *et al.,* 2004). Immunodeficiency is characteristic of CRC patients and IL-2 is reported to be down-regulated in colorectal polyps (Jacobson-Brown and Neuman, 2003). Pariza reported in 2004 on the efficacy and safety of the use of CLA for inflammatory diseases in humans. CLA is currently widely explored and evidence is being steadily strengthened by results from animal toxicology tests. Research on a pig model with CLA and EPA supplementation revealed that CLA down-regulated the generation of eicosanoids and up-regulated PPARγ expression which delayed the onset of inflammatory bowel disease, whereas EPA accelerated colonic regeneration and

clinical remission by activating PPARδ/β (Bassaganya-Riera *et al* 2002; Bassaganya-Riera and Hontecillas, 2006; Courtney *et al.,* 2007). This implies that the impact of PPARδ/β lies in its ability to induce apoptosis during early triggering of cells destined to become polyp growths and thereby prevent or inhibit polyps and their possible transformation to CRC. It is argued that over-expression of PPARδ/β during the adenoma-carcinoma sequence suppresses apoptosis in CRC cells. From a therapeutic viewpoint, CLA and EPA can activate PPARα and PPARγ for prevention of stress, carcinogenesis and immunodeficiency, based on laboratory studies (Larsson *et al*., 2004; Grau *et al*., 2006; Martinasso *et al*., 2007; Ponferrada *et al*., 2007). CLA has been tested on healthy individuals and the efficiency thereof in diseases such as CRC is on the brink of investigation, but there is still ongoing research to evaluate the use of specific CLA products (different isomer compositions and mixtures). Currently, mixtures of *c9,t*11-18:2 (mostly 50:50) are used for inflammatory conditions (O'Shea *et al*., 2004; Pariza, 2004). The study done by Courtney *et al*. (2007) on healthy subjects with a history of polyps is extremely promising and a paradigm shift to prevent polyp recurrence after surgery in patients with CRC seems to be the next step where CLA and EPA is administered. Despite a promising new direction with CLA therapy, conventional FA therapy with GLA and EPA can not be disregarded. GLA is currently receiving renewed interest and is proclaimed to inhibit inflammation by PGE_1 up-regulation, up-regulate p53 and down-regulate ras and Bcl-2 oncogene expression, and to protect cells against toxic actions of radiation (Das, 2005, 2006a, 2006b, 2007; Das *et al*., 2007). Ras mutations are present in all aberrant crypt foci that progress towards adenomas, whilst the p53 gene is mostly mutated in adenocarcinoma. It seems feasible that GLA and EPA administration to restore membrane FA composition may prevent chronic prolonged inflammation and prevent mutation of the ras oncogene.

COMMENT: From the preceding information it is clear that there is a rationale for different adjuvant FA therapies in the prevention of polyp and CRC recurrence after surgery. Elected candidates are: GLA and EPA to down-regulate FAS activity and ras mutations and thereby prevent / inhibit polyp growths by restoring membrane FA compositions for normal cell functions; and CLA and EPA to prevent / inhibit tumour growth, eliminate viral and bacterial particles or to control / ameliorate viral and bacterial infections and thereby improve immune defences. Different adjuvant FA therapeutic strategies (before, during and after surgery) need to be evaluated in clinical trials for their efficacy.

CHAPTER 7
CONCLUSIONS

7.1 FATTY ACID ROLE-PLAYERS

The *ex vivo* phospholipid FA profile for colorectal adenocarcinoma revealed LA, AA, PA, SA and OA as main role players and there is ample evidence-based literature that confirm their involvement during carcinogenesis and immunune responses. These FAs with a higher or lower prevalence in CRC can be imputed to: excessive dietary intake of LA (maize products); excessive SFAs by exogenous dietary intake (red meat) and *de novo* metabolic production (enhanced FAS activity); and excessive OA by exogenous dietary intake (editable fats and oils) and endogenous production (enhanced Δ^9d activity). Dietary LA is apparently responsible for initiation of cellular over-production, whilst PA maintains cell proliferation rates and cell survival. The link between enhanced OA and CRC is not yet fully unraveled. LA contributes to radical-mediated cell damage and biohydroxygenase products in the gut, whilst AA (beef and pork) contributes to oxidative stress (inflammatory recruited ROS and $NO^{\bullet}$) and radical-mediated cell damage. Iron intake can convincingly be a co-factor in the etiology of CRC, ascribed to high red meat intake and deficient anti-oxidant defense mechanisms (such as metal chelators, among preventative anti-oxidants) which can initiate oxidative stress. It is assumed that high exogenous dietary AA intake and a bevy of factors in command of COX-2 expression (including enhanced SFAs), inevitably manifest in chronic inflammation with depletion of AA. CRC with environmental etiological causes is marked by a shift in lipid metabolism and the altered pathways that are followed are summarized as follows: down-regulation of Δ^6d and Δ^5d pathways during EFAM; down-regulation of the 15-LOX-1 pathway during LA oxidative metabolism; up-regulation of the COX-2 and 15-LOX-2 pathways during AA metabolism; up-regulation of the iNOS pathway during oxidative stress; and up-regulation of the FAS and Δ^9d pathways during the n-7 and n-9 FA metabolism, are encountered. These pathways lead to apoptotic resistance and oxidative stress-related CRC. Apart from the fact that PGE_2 impairs the ability of T lymphocytes to generate anti-tumor Th1 cytokines and tilts the scale towards CRC as a Th2 dominant disease, it is also tempting to speculate that SFAs with a role in oxidative stress not only

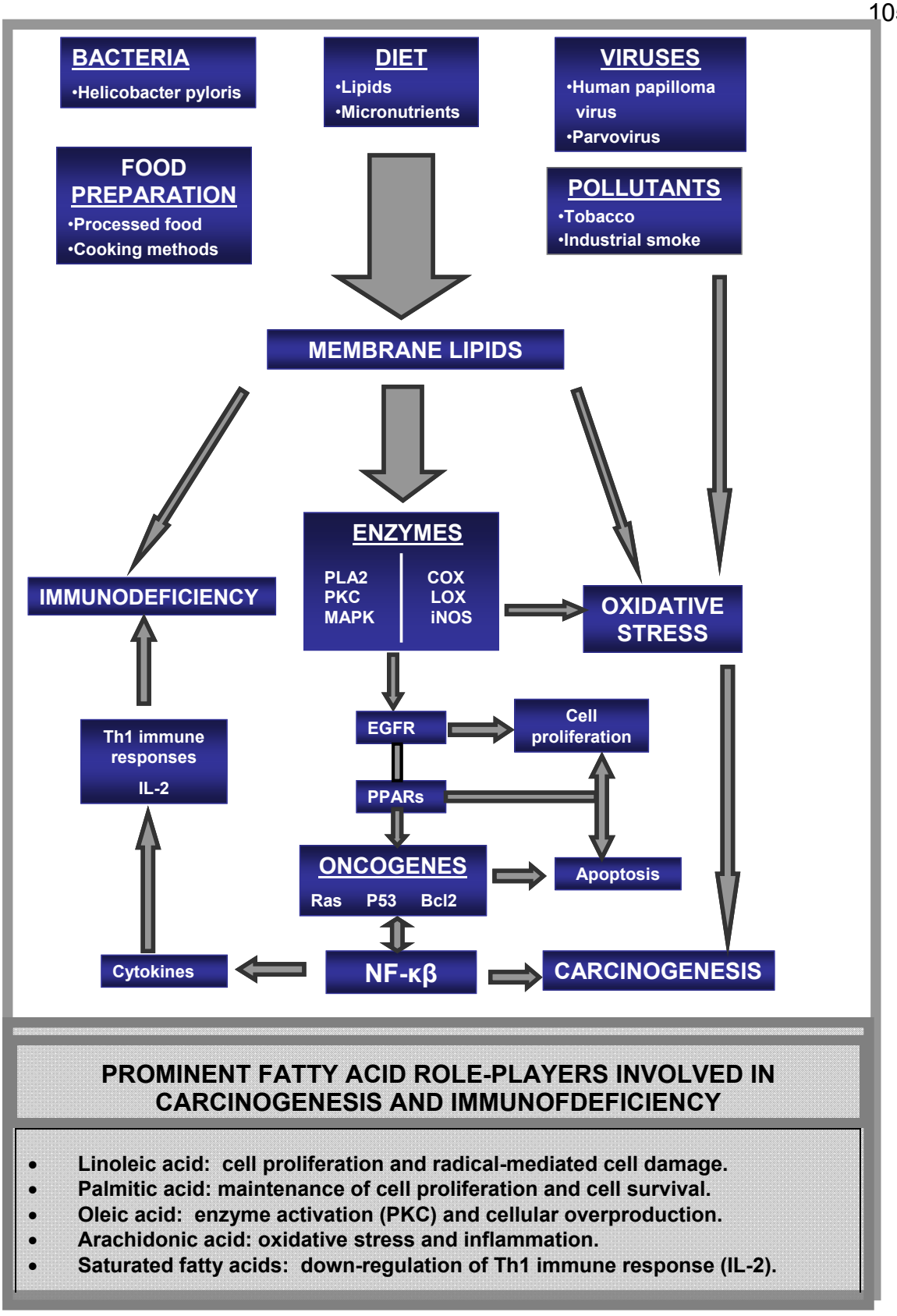

FIGURE 7.1 Summary of factors involved in colorectal cancer and immunodeficiency
Abbreviations: *COX, cyclooxygenase; EGFR, epidermal growth factor receptor; IL-2, interleukin-2; iNOS, inducible nitric oxide; LOX, lipoxygenase MAPK, mitogen-activated protein kinase; NF-κβ, nuclear factor kappa-beta; PKC, protein kinase C; PLA2, phospholipase A_2; PI3K, phosphatidylinositol 3-kinase; PPAR, peroxisome proliferator activated receptor.*

contribute to carcinogenesis, but ultimately also exhaust Th1 cellular-mediated immune responses (IL-2 and T lymphocyte production/function) that manifest in suppression of the immune system. This seems logical if the assumption holds truth that NF-κβ is increased during early carcinogenesis and decreased during advanced CRC cancer. It is clear that FAs contribute significantly to CRC, but it is also clear that no single metabolic pathway in the complex lipid network is solely responsible for this cancer entity and search for knowledge continue. A general overview on factors involved during colorectal carcinogenesis and immunodeficiency, based on integration of lipid findings in this study with applicable research findings in the literature, is given in the above FIGURE 7.1.

7.2 ADJUVANT FA THERAPEUTIC PROPOSALS

GENERAL: It is a personal opinion that "causes" of altered lipid metabolism rather than "consequences" during lipid metabolism need to be addressed. Hence, the emphasis is on modulation of membrane FA compositions with adjuvant FA therapy rather than to target factors during polyp and cancerous growth, despite promising PPAR targeting therapy (Sertzing *et al*., 2007). Considerable work has been done to demonstrate the beneficial use of EPA, DHA, CLA, GLA and OA as therapeutic agents in cancer and research convincingly proved that these FAs have therapeutic use to prevent polyps and CRC. The transition of experimental findings to clinical application in CRC therapy is hampered by a lack of randomized clinical trials with long-term follow-up studies. Nevertheless, available research findings serve as a strong incentive for intervention trials that will test the effect of recommended adjuvant FA therapies in the management of polyps and CRC. The author holds the viewpoint that complete surgical resection combined with chemoradiotherapy of CRC remains the primary mode of therapy and that adjuvant FA therapy in combination with surgery may be the key to improve polyp and CRC management.

STRATEFICATION INTO SUBSETS: It can be argued that stratification of CRC into subsets according to etiological causes may be required for accurate clinical intervention to ensure optimal disease outcome. In the case of environmental causes, patients with polyps and CRC might benefit from different adjuvant FA therapeutic approaches after surgery. In the case of polyps with up-regulated FAS and Δ^9d activities, GLA and EPA is predicted to improve membrane FA compositions and thereby down-regulate the AA cascade, as well as FAS and Δ^9d pathways for normal cell growth. In the case of CRC

where TNM staging (tumor stage/nodular infiltration/ metastasis) and the immunocompetence of the patient need to be considered, CLA is predicted to protect against radical-mediated oxidative damage and to eliminate/ameliorate bacterial and viral particles/infection and thereby prevent polyp recurrence or inhibit polyp growth, as well as improve the immunocompetence of CRC patients.

FATTY ACID THERAPEUTIC STRATEGIES: FA therapy is non-destructive reliable chemotherapy that still does not receive its rightful place in medicine. Different FA therapeutic strategies, inclusive to the existing FA modality with DHA to improve membrane structure and the impact of chemo- or radiochemotherapy, can be proposed. In my opinion, based on the *ex vivo* FA profiles constructed for colorectal adenocarcinoma and all the preceding information provided in this study, I propose: **firstly,** CLA and EPA therapy for patients with CRC to prevent polyp recurrence/ inhibit polyp growth after surgery and to improve the immunocompetence of the patients; and **secondly,** GLA and EPA therapy in the management of patients with a history of polyp growths to prevent polyp recurrence/inhibit polyp growth. The above mentioned proposals need to be properly evaluated.

SUMMARY

INTRODUCTION: The challenge we face today is identification of the mechanisms through which dietary factors perturb fundamental fatty acid (FA) pathways in cancer cells. In principle, insight into a lipid model for the cancer entity under assessment is required. This prompted and investigation into FA role-players that drive cellular over-production and apoptotic pathways under circumstances where environmental factors impede essential fatty acid metabolism (EFAM) that contributes to colorectal cancer (CRC). Ultimately, better understanding of FA metabolic pathways followed under colorectal pathological conditions can contribute to improved therapies by which growth and recurrence of this disease may be obviated. PURPOSE OF STUDY: The primary goal of this study was to construct clinically relevant *ex vivo* FA profiles from a lipid model for colorectal adenocarcinoma, not previously reported in the literature. Aims were to identify prominent FA role-players and to debate their involvement in different signaling pathways. The final purpose of this study was to present a rationale for adjuvant FA therapies to improve the management of CRC. MATERIAL AND METHODS: The study group consisted of CRC biopsies (TNM staging, mostly T2/T3) and normal colorectal mucosal biopsies, (n8 of each group). Biopsy selection was hampered by difficult circumstances, since most patients received treatment prior to surgery. All the lipid analyses were personally conducted at the Biolipid Division of the Department Biochemistry at the University of the Free State (UFS) as follows: lipids were extracted; total lipids (TLs) were fractionated into neutral lipids (NLs) and phospholipids (PLs); phosholipid subclasses, i.e. phosphatidylcholine, (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylinositol (PI), were separated; and fatty acid methyl esters of all the classes and subclasses were analyzed. Lipid analyses were done according to well established and internationally accepted gaschromatography (GLC) and thin layer chromatography (TLC) techniques. Statistical analyses were performed by a biostatistician attached to the UFS, and p-values and 95% confident intervals (95% CI) for median of differences, as well as specific ratios were calculated. MAIN RESULTS AND DISCUSSION: The *ex vivo* phospholipid FA profile for CRC cells revealed linoleic acid (LA), arachidonic acid (AA), palmitic acid (PA) and oleic acid (OA) as main role-players involved in CRC. An extensive literature study revealed that: prolonged inflammatory responses and oxidative events, marked by the up-regulation of cyclooxygenases (COXs) and inducible nitric oxide synthase (iNOS), contribute to colorectal carcinogenesis; and prolonged cytokine

mediated responses, orchestrated by nuclear factor-kappa Beta (NF-κβ) with consequent immunodeficiency, are prevalent in CRC patients. Past research revealed without doubt that high dietary AA intake (meat) and COX-2 metabolism play a pivotal role during colorectal carcinogenesis. CRC is a stress-related cancer and it is plausible that high iron intake and lipids contribute to chronic inflammation and radical-mediated cell damage in this age-related disease. Current research directions in CRC concern LOX metabolism and peroxisome proliferator activator receptors (PPARs) are receiving much attention, especially PPARδ/β that was an enigma and elicited controversial debate in adenomas (polyps) and adenocarcinoma. Taken together at this point in time, the impact of environmental factors on essential fatty acid metabolism (EFAM) that impede LA conversion to AA via delta-6 and -5 desaturase (Δ^6d and Δ^5d) pathways with up-regulation of the fatty acid synthase (FAS) and Δ^9d pathways, and the down-regulation of LA oxidative metabolism via the 15 LOX-1 pathway with up-regulation of the COX-2 and 15-LOX-2 pathways of AA, is characteristic of colorectal carcinogenesis. Seen in context, the lipid findings of this study integrated with evidence-based information in the literature encompasses the following: excessive dietary linoleic acid (LA) intake that can contribute to cell proliferation, radical-mediated cell damage, and the production of conjugated linoleic acid (CLA) in the gut; high dietary AA intake and COX-2 over-expression that play a pivotal role during carcinogenesis and immune responses with consequent exhaustion of the AA source; enhanced FAS and Δ^9d activities that contribute to, respectively, enhanced palmitic acid (PA) and oleic acid (OA), is prevalent in CRC cells. The high PA content in CRC cells apparently activates PPARδ/β and thereby suppresses apoptosis, a crucial factor in the pathobiology of CRC. The link between OA and CRC still needs proper clarification. It is conceivable that the high saturated fatty acid (SFA) content observed in CRC cells, exogenous intake with red meat and endogenous production by enhanced FAS activity, contribute to colorectal carcinogenesis and immunodeficiency in CRC patients, since it can stimulate COX-2 expression (and PGE_2 activity) and down-regulate the Th1 immune pathway that make CRC a Th2 dominant disease. Taken in consideration that lipid rafts rich in SFAs down-regulate the Th1 cytokine subset, particularly interleukin-2 (IL-2) that stimulates lymphocyte production, it is plausible that management of the CRC patient is hampered by immunodeficiency. All the altered cell signaling pathways in CRC debated in this study served as sound foundation for clinical intervention with adjuvant FA therapeutic strategies to improve CRC management. CONCLUSION: The identification of prominent FA role-players in CRC cells that serve as ligands for specific PPAR family members contributed to the assessment of FA driven proliferation and apoptotic pathways characteristic of CRC. It is conceivable that

enhanced PA (and LA) contributes to PPARδ/β over-expression that can suppress PPARγ (and PPARα) activity and then tilts the scale in favor of apoptotic resistance and survival of CRC cells. From an epidemiological viewpoint, excessive iron and SFAs seem to be important co-factors in the multifactorial etiology of CRC. The biohydroxygenation of LA to different CLA products in the gut and to what extent harmful CLA products may pose a danger need to be fully explored, especially since the uptake of beneficial products is apparently limited. Among current therapeutic options in the field of lipids, concurrent therapy with a COX-2 inhibitor (Celecoxib) and docosahexaenoic acid (DHA) to improve membrane fluidity and the impact of drug therapy entered clinical trials and although the outcome is still awaited, the impact of DHA on lymphocyte production may be a concern. Based on a mountain of evidence presented in this study, it is suffice to say that there is a rationale for additional adjuvant FA therapies that can be included in the existing therapeutic regime for CRC management. Firstly, CLA and EPA therapy for prevention of polyp recurrence and improvement of immunocompetence in CRC patients and secondly, GLA and EPA therapy in the management of patients with a history of polyp growths is proposed.

REFERENCES

1. Abbas, A.K., Lichtman, A.H. and Pillai, S. 2007. *Cellular and Molecular Immunology*, 6th edition chapt. 5, pg. 100-107. Ed. W.B. Saunders Elsevier, Philadelphia USA. ISBN 978-0-8089-2358-9.

2. Aggarwal, B.B., Shishodia, S., Sandur, S.K., Pandey, M.K. and Sethi, G. 2006. Inflammation and cancer: How hot is the link? *Biochemical Pharmacology,* **72**:1605-1621.

3. Aggarwal, B.B. 2007. Nuclear factor-kappa B: a transcription factor for all seasons. *Expert Opinion on Therapeutic Targets,***11**(2):109-110.

4. Ahn, J., Park I-S., Lee, K-S., Kim, S-Y., Chum, E.0.J., Kim, J., Kim, D-J., Yoom, S. and Lee-Kim, Y.C. 2001. Fatty acid patterns in gastric mucosa of stomach cancer patients. *Jonsei Medical Journal,* **42**:220-226.

5. Ajuwon, K.M. and Spurlock, M.E. 2005. Palmitate activates the NF-κβ transcription factor and induces IL-6 and TNFα expression in 3T3-L1 adipocytes. *Journal of Nutrition,* **135**(8):1841-1846.

6. Akihisha, T., Tokuda, H., Ogata, M., Ukiya, M., Lizuka, M., Suzuki, T., Metori, K., Shimizu, N. and Nishino, H. 2004. Cancer chemopreventive effects of polyunsaturated fatty acids. *Cancer Letters,* **205**(1):9-13.

7. Al-Daccak, R., Mooney, N. and Charron, D. 2004. MHC class II signaling in antigen-presenting cells. *Current Opinion in Immunology,* **16**:108-113.

8. American Cancer Society, Inc. What are the key statistics for colon and rectum cancer? Date of access: 07/04/2006. Available from RL:http//www.cancer.org/dogroot/home/index.asp?level=0

9. Andersohn, F., Suissa, S. and Garbe, E. 2006. Risks and benefits of celecoxib to prevent colorectal adenomas. *The New England Journal of Medicine,* **355**(22):2371-2373.

10. Andoh, A., Tsujikawa, T. and Fujiyama, Y. 2003. Role of dietary fiber and short-chain fatty acids in the colon. *Current Pharmaceutical Design,* **9**(4):347-358.

11. Andre',T., Tournigand, C., Mineur, L., Fellague-Chebra, R., Flesch, M., Mabro, M., Hebbar, M., Postel Vinay, S., Bidard, F.C., Louvet, C., de Gramont, A. and GERCOR (French Oncology Research Group). 2006. Phase II study of an optimized 5-fluorouracil-oxaliplatin strategy (OPTIMOX2) with celecoxib in metastatic colorectal cancer: a GERCOR study. *Annals of Oncology,* **18**(11):77-81.

12. Angelo, L.S. and Kurzrock, R. 2007. Vascular endothelial growth factor and its relationship to inflammatory mediators. *Clinical Cancer Research,* **13**(10):2825-2830.

13. Araujo de Vizcarrondo, C., Carrillo de Padilla, F. and Martin E. 1998. Fatty acid composition of beef, pork, and poultry fresh cuts, and some of their processed products. *Archivos Latinoamericanos de Nutricion,* **48**(4):354-358.

14. Arber, N. and Levin, B. 2005. Chemoprevention of colorectal cancer: ready for routine use? *Recent Results in Cancer Research,* **166**:213-30.

15. Arber, N., Eagle, C.J., Spicak, J., Racz, I., Dite, P., Hajer, J., Zavoral, M., Lechuga, M.J., Gerletti, P., Tang, J., Rosenstein, R.B., Macdonald, K., Bhadra, P., Fowler, R., Wittes, J., Zauber, A.G., Solomon, S.D., Levin, B. and PreSAP Trial Investigators. 2006. Celecoxib for the prevention of colorectal adenomatous polyps. *The New English Journal of Medicine,* **355**(9**)**:885-895.

16. Astler, V.B. and Coller, F.A. 1954. The prognostic significance of direct extension of carcinoma of the colon and rectum. *Annals of Surgery,* **139**:846-852.

17. Astorg, P. 2005. Dietary fatty acids and colorectal and prostate cancers: epidemiological studies. *Bulletin du Cancer,* **92**(7):670-684.

18. Athar, M. 2002. Oxidative stress and experimental carcinogenesis. *Indian Journal of Experimental Biology,* **40**:656-667.

19. Augenlicht, L.H., Mariadason, J.M., Wilson, A., Arango, D., Yang, W., Heerde, B.G. and Velcich, A. 2002. Short chain fatty acids and colon cancer. *Nutrition,* **132**:3804S-3808S.

20. Babbs, C.F. 1990. Free radicals and the etiology of colon cancer. *Free Radical Biology & Medicine,* **8**:191-200.

21. Backlund, M.G., Mann, J.R., Wang, D. and Dubois, R.N. 2005. Ras up-regulation of cyclooxygenase-2. *Methods in Enzymology,* **407**:401-410.

22. Badawi, A.F., El-Sohemy, A., Stephen, L.L., Ghoshal, A.K. and Archer, M.C. 1998. The effect of dietary n-3 and n-6 polyunsaturated fatty acids on the expression of cyclooxygenase 1 and 2 levels on $p21^{ras}$ in mammary glands. *Carcinogenesis,* **19**:905-910.

23. Baek, S.J. and Eling, T.E. 2006. Changes in gene expression contributes to cancer prevention by COX inhibitors. *Progress in Lipid Research,* **45**:1-16.

24. Baier, P.K., Wolff-Vorbeck, G., Eggstein, S., Baumgartner, U. and Hopt, U.T. 2005. Cytokine expression in colon carcinoma. *Anticancer Research,* **25**:2135-2139.

25. Ballinger, A.B. and Anggiansah, C. 2007. Colorectal cancer. *BMJ,* **335**:715-718.

26. Baniyash, M. 2006. Chronic inflammation, immunosuppression and cancer: New insights and outlook. *Seminars in Cancer Biology,* **16**:80-88.

27. Barber, M.D., Fearon, C. and Ross, J.A. 2005. Eicosapentaenoic acid modulates the immune response but has no effect on mimic of antigen-specific responses. *Nutrition,* **21**:588-503.

28. Bardon, S., Benelli, C., Bernard-Gallon, D., Blottiere, H., Demarquoy, J., Duee, P.H. and Forest, C. 2005. Dietary fatty acids and cancer: potential cellular and molecular mechanisms. *Bulletin du Cancer,* **92**(7):697-707.

29. Bartoli, R., Fernandez-Banares, F., Navarro, F., Castella, E., Mane, J., Alvarez, M., Pastor, C., Cabre, E. and Gassull, M.A. 2000. Effect of olive oil on early and late events of colon carcinogenesis in rats; modulation of arachidonic acid metabolism and local prostaglandin E(2) synthesis**.** *Gut,* **46**(2):191-199.

30. Bartsch, H., Nair, J. and Owen, R. 1999. Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers. *Carcinogenesis,* **20**(12):2209-2218.

31. Bartsch, H. and Nair, J. 2002. Potential role of lipid peroxidation derived DNA damage in human colon carcinogenesis: studies on exocyclic base adducts as stable oxidative stress markers. *Cancer Detection and Prevention,* **26**:308-312

32. Baruch, B.A. 2006. Inflammation-associated immune suppression in cancer: The role played by cytokines, chemokines and additional mediators. *Seminars in Cancer Biology,* **16**:38-52*.*

33. Bassaganya-Riera, J., Hontecillas, R. and Beitz, D.C. 2002. Colonic anti-inflammatory mechanisms of conjugated linoleic acid. *Clinical Nutrition,* **21**:451-459.

34. Bassaganya-Riera, J., Reynolds, K., Martino-Catt, S., Cui, Y., Henninghausen, L., Gonzalec, F., Rohrer, J., Benninghoff, A.U. and Hontecillas, R. 2004. Activation of PPAR gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. *Gastoenterology,* **127**:777-791.

35. Bassaganya-Riera, J. and Hontecillas, R. 2006. CLA and n-3 PUFA differentially modulate clinical activity and colonic PPAR-responsive gene expression in a pig model of experimental IBD. *Clinical Nutrition,* **25**:454-465.

36. Becuwe, P., Bianchi, A., Didelot, C., Barberi-Heyob, M. and Dauqa, M. 2003. Arachidonic acid activates a functional AP-1 and an inactive NFκβ complex in human HepG2 hepatoma cells. *Free Radical Biology & Medicine,* **35**(6):636-647.

37. Beets-Tan, R.G.H., Beets, G.L. and van de Velde, C.J.H. 2005. Staging in colorectal cancer. *European Journal of Cancer Supplements,* **3**(3):361-366.

38. Belury, M.A. 2002. Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action. *Nutritional Journal,* **132**:2995-2998.

39. Beppu, F., Hosokawa, M., Tanak, L., Kohno, H., Tanaka, T. and Miyashita, K. 2006. Potent inhibitory effect of *trans*9,*trans*11 isomer of conjugated linoleic acid on the growth of human colon cancer cells. *Journal of Nutritional Biochemistry,* **17**:830-836.

40. Berger, J. and Moller, D.E. 2002. The mechanisms of action of PPARs. *Annual Reviews in Medicine,* **53**:409-435.

41. Berghella, A.M., Contasta, I., Pellegrini, P., Del Beato, T. and Adorno, D. 2006. Are immunological mechanisms involved in colon cancer and are they possible

markers for biotherapy improvement? *Cancer Biotherapy and Radiopharmaceuticals,* **21**(5**)**:468-487.

42. Bertram, J.S. 2000. The molecular biology of cancer. *Molecular Aspects of Medicine,* **21**(6):167-223.

43. Bhattacharya, A., Banu, J., Rahman, M., Causey, J. and Fernandes, G. 2006. Biological effects of conjugated linoleic acids in health and disease. *Journal of Nutritional Biochemistry,* **17**:789-810.

44. Blanke, C.D., Mattek, N.C., Deloughery, T.G. and Koop, D.R. 2005. A phase I study of 5-fluorouracil, leucovorin, and celecoxib in patients with incurable colorectal cancer. *Prostaglandins and Other Lipid Mediators,* **75**:169-172.

45. Bocher, V., Chinetti, G., Fruchart, J.C. and Staels, B. 2002. Role of the peroxisome proliferator-activated receptors (PPARS) in the regulation of lipids and inflammation control. *Journal de la Societe de Biologie,* **196**(1):47-52.

46. Boone, D.L., Lee, E.G., Libby, S., Gibson, P.J., Chien, M., Chan, F., Madonia, M., Burkett, P.R. and Ma, A. 2002. Recent advances in understanding NF-kappaB regulation. *Inflammatory Bowel Diseases,* **8**:201-212.

47. Borghesi, L. and Milcarek, C. 2007. Innate versus adaptive immunity: a paradigm past its prime? *Cancer Research,* **67**(9):3989-3993.

48. Boudreau, M.D., Sohn, K.H., Thee, S.H., Lee, J.D., Hunt, J.D. and Hwang, D.H. 2001. Suppression of tumor cell growth both in nude mice and in culture by n-3 polyunsaturated fatty acids; mediation through cyclooxygenase-independent pathways. *Cancer Research,* **61**:1386-1391.

49. Bougnoux, P. and Menanteau, J. 2005. Dietary fatty acids and experimental carcinogenesis. *Bulletin du Cancer,* **92**(7**)**:685-696.

50. Bours, V., Bentires-Alj, M., Helin, A-C., Viatour, P., Robe, P., Delhalle, S., Benoit, V. and Merville, M-P. 2000a. Nuclear Factor–κβ, cancer, and apoptosis. *Biochemistry and Pharmacology,* **60**:1085-1090.

51. Bours, V., Bonizzi, G., Bentires-Alj, M., Bureau, F., Piette, J., Lekeux, P., Merville, M-P. 2000b. NF-κβ activation in response to toxical and therapeutical agents: role in inflammation and cancer treatment. *Toxicology,* **153**:27-38.

52. Boyle, P. and Leon, M.E. 2002. Epidemiology of colorectal cancer. *British Medical Bulletin,* **64**:1-25.

53. Braga, M., Gianotti, L., Vignali, A. and Carlo, V.D. 2002. Preoperative oral arginine and n-3 fatty acid supplementation improves the immunometabolic host response and outcome after colorectal resection for cancer. *Surgery,* **132**(5): 805-814.

54. Brash, A.R. 2001. Arachidonic acid as a bioactive molecule. *The Journal of Clinical Investigation,* **107**:1339-1345.

55. Brookes, M.J., Hughes, S., Turner, F.E., Reynolds, G., Sharma, N., Ismail, T., Berx, G., McKie, A.T. Hotchin, N., Anderson, G.J., Iqbal, T. and Tselepis, C. 2006. Modulation of iron transport proteins in human colorectal carcinogenesis, *Gut,* **55**(10):1449-1460.

56. Brown, D.A. and London, E. 1998. Functions of lipid rafts in biological membranes*. Annual Review of Cell and Developmental Biology,* **14**:111-136.

57. Buchanan, F.G. and DuBois, R.N. 2006. Connecting COX-2 and Wnt in cancer. uu*Cancer Cell,* **9**(1):6-8.

58. Bull, A.W., Steffensen, K.R., Leers, J. and Rafter, J.J. 2003. Activation of PPAR gamma in colon tumor cell lines by oxidized metabolites of linoleic acid, endogenous ligands for PPAR gamma. *Carcinogenesis,* **11**:1717-1722.

59. Butte, W. 1983. Rapid method for the determination of fatty acid profiles from fats and oils using trimethylsulphonium hydroxide for transesterification. *Journal of Chromatography,* **261**:142-145.

60. Cahill, D.P., Kinzler, K.W., Vogelstein, B. and Lengauer, C. 1999. Genetic instability and darwinian selection in tumours. *Trends in Cellular Biology,* **9**:M57-M60.

61. Calder, P.C. and Grimble, R.F. 2002. Polyunsaturated fatty acids, inflammation and immunity. *European Journal of Clinical Nutrition,* **87**:S31-S48.

62. Calder, P.C., Yaqoob, P., Thies, F. Wallace, F.A. and Miles, E.A. 2002. Fatty acids and lymphocyte functions. *British Journal of Nutrition,* **87**:(1**)**:S31-S48.

63. Calder, P.C. 2006. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *American Journal of Clinical Nutrition,* **83**:1505S-1519S.

64. Calviello, G., Palozza, P., Maggiano, N., Picioni, E., Franceschelli, P., Frattucci, A., Di Nicuolo, F. and Bartoli, G.M. 1999. Cell proliferation, differentiation and apoptosis are modified by n-3 polyunsaturated fatty acids in normal colonic mucosa. *Lipids in Health and Disease*, **34**(6):599-604.

65. Carayannopoulos, N. and Yokoyama, M. 2004. Recognition of infected cells by natural killer cells. *Current Opinion in Immunology,* **16**:26-33.

66. Carrier, F., Georgel, P.T., Pourquier, P., Blake, M. Kontny, H.U., Antinore, M.J., Gariboldi, M., Myers T.G., Weinstein, J.N., Pommier, Y. and Fornace, A.J. 1999. Gadd45, a p53 responsive stress protein, modifies DNA accessibility on damaged chromatin. *Molecular and Cellular Biology,* **19***:*1673-1685.

67. Castellino, F. and Germain, R.N. 2006. Cooperation between CD4+ and CD8+ T cells: When, where and how. *Annual Review of Immunology,* **24**:519-540.

68. Castellone, M.D., Teramoto, H., Williams, B.O. Druey, K.M. and Gutkind, J.S. 2005. Prostaglandin E_2 promotes colon cancer cell growth through a G_S -Axin-β-catenin signaling axis. *Science,* **310**(5753):1504-1510.

69. Chan, T. A., Giovannucci, E.L., Scherhammer, E.S., Colditz, G.A. and Hunter, D.J. 2004. A prospective study of aspirin use and the risk for colorectal adenoma. *Annals of Internal Medicine,* **140**(3):157-166.

70. Chan, T.A. 2006. Prostaglandins and the colon cancer connection. *Trends in Molecular Medicine,* **12**(6):240-244.

71. Chang, T.H. and Szabo, E. 2000. Induction of differentiation and apoptosis by ligands of peroxisome proliferators-activated receptor gamma in non-small cell lung cancer. *Cancer Research,* **60**(4):1129-1138.

72. Chao, A., Thun, M.J., Connell, C.J., Mc Cullough, M.L., Jacobs, E.J., Flanders, W.D., Rodriquez, C., Sinha, R. and Calle, E.E. 2005. Meat consumption and risk of colorectal cancer. *JAMA,* **293**(2**)**: 233-234.

73. Chapkin, R.S., Jiang, Y.H., Davidson, L.A. and Lupton, J.R. 1997. Modulation of intra cellular second messengers by dietary fat during colonic tumour development. *Advanced and Experimental Medical Biology,* **422**:85-96.

74. Chapkin, R.S., Davidson, L.A., Ly, L., Weeks, B.R., Lupton, J.R. and McMurray, D.N. 2007a. Immunomodulatory effects of (n-3) fatty acids: putative link to inflammation and colon cancer. *The Journal of Nutrition,* **137**(1):2005-2045.

75. Chapkin, R.S., McMurray, D.N. and Lupton, J.R. 2007b. Colon cancer, fatty acids and anti-inflammatory compounds. 2007. *Current Opinion in Gastroenterology*, **23**(1):48-54.

76. Chau, I and Cunninghan, D. 2006. Adjuvant therapy in colon cancer-what, when and how? *Annals of Oncolocy,* **17:**1347-1359.

77. Chen, Z., and Istfan, N. 2000. Docosahexaenoic acid is a potent inducer of apoptosis in HT-29 colon cancer cells. *Prostaglandins Leukotrienes and Essential Fatty Acids,* **63**:301-308.

78. Chen, G.G., Lee, J.F.Y., Wang, S.H., Chan, U.P.F., Ip, P.C. and Lau, W.A. 2002. Apoptosis induced by activation of peroxisome-proliferator activated receptor-gamma is associated with Bcl-2 and NF-κβ in human colon cancer. *Life Sciences,* **70**:2631-2646.

79. Cheng, J., Kumiko, O., Kiyonori, K., Yoshifumi, Y., Kamiya, T., Seno, K., Okuyama, H., Wang, J., Luo, C., Fujii, T. Ichikawa, H., Shirai, T. and Tokudome, S. 2003. Increased intake of n-3 polyunsaturated fatty acids elevates the level of apoptosis in the normal sigmoid colon of patients polypectomized for adenomas/tumors. *Cancer Letters,* **193**:17-24.

80. Chinen, J. and Shearer, W.T. 2004. Advances in Asthma, Allergy and Immunology Series 2004: Basic and clinical immunology. *Journal of Allergy and Clinical Immunology,* **114**(2**)**:398-405.

81. Chiu, L.C.M., Oui, V.E.C. and Wan, J.M.F. 2001. Eicosapentaenoic acid modulates cyclin expression and arrests cell cycle progression in human leukemic K-562 cells. *International Journal of Oncology,* **19**:845-849.

82. Chlebowski, R.T., Wactawski-Wende, J., Ritenbaugh, C., Hubbell, F.A. Ascensao, J., Rodabough, R.J. and Rosenberg, C.A. 2004. Estrogen plus progestin and colorectal cancer in postmenopausal women. *New English Journal of Medicine,* **350**(10):991-1004.

83. Cho, H.J., Kim, W.K., Kim, E.J., Jung, K.C., Park, S., Leed, H.S., Tyner, A.L. and Pa, J.H. 2003. Conjugated linoleic acid inhibits cell proliferation and Erb3 signaling in HT-29 human colon cell line. *American Journal of Physiology-Gastrointestinal and Liver Physiology,* **284**(6):G996-1005.

84. Chun, K-S. and Surh, Y-J. 2004. Signal transduction pathways regulating cyclooxygenase-2 expression: potential molecular targets for chemoprevention. *Biochemical Pharmacology,* **68**:1089-1100.

85. Clausen, M.R., Bonnen, H. and Mortensen, P.B. 1991. Colonic fermentation of dietary fibre to short chain fatty acids in patients with adenomatous polyps and colonic cancer. *Gut,* **32**:923-928.

86. Clevers, H. 2006. Colon cancer – understanding how NSAID work. *The New England Journal of Medicine,* **354**(7):761-763.

87. Collet, E.D., Davidson, L.A., Fan, Y.Y., Lupton, J.R. and Chapkin, R.S. 2001. N-6 and n-3 polyunsaturated fatty acids differentially modulate oncogenic Ras activation in colonocytes*.* *American Journal of Physiology and Cell Physiology,* **280**:C1066-1075.

88. Colomer, R. and Menéndez, J.A. 2006. Mediterranean diet, olive oil and cancer. *Clinical and Translational Oncology,* **8**(1):15-21.

89. Colomer, R., Moreno-Nogueira, J.M., Garcia-Luna, P.P., Garcia-Peris, P., Garcia-de-Lorenzo, A., Zarazaga, A., Quecedo, L., Del Llano, J., Usan., L. and Casimiro, C. 2007. n-3 Fatty acids, cancer and cachexia: a systematic review of the literature. *British Journal of Nutrition,* **97**(5):823-831.

90. Colquhoun, A. and Schumacher, R.I. 2001. Gamma-linolenic acid and eicosapentaenoic acid induce modifications in mitochondrial metabolism reactive oxygen species generation, lipid peroxidation and apoptosis in Walker 256 rat carcinosarcoma cells. *Biochimica et Biophysica Acta,* **1533**(3):207-219.

91. Comalada, M., Bailon, E., de Haro, O., Lara-Villoslada, F., Xaus, J., Zarzuelo, A. and Galvez, J. 2006. The effects of short-chain fatty acids on colon epithelial proliferation and survival depend on the cellular phenotype. *Journal of Cancer Research and Clinical Oncology,* **132**:487-497.

92. Compton, C.C. and Greene, F.J. 2004. The staging of colorectal cancer: 2004 and beyond. *CA: A Cancer Journal for Clinicians,* **54**(6):295-308.

93. Contasta, I., Berghella, A., Pellegrini, P. and Adorno, D. 2003. Passage from normal mucosa to adenoma and colon cancer: alteration of normal sCD30 mechanisms regulating Th1/Th2 cell functions. *Cancer Biotheraphy and Radiopharmaceuticals,* **18**(4):549-557.

94. Cotran, Ramzi S., Kumar V., Collins T. 2004. *Robins Pathologic Basis of Disease*. 6th edition pg. 831-837. Ed. W.B. Saunders Company, USA. ISBN: 0808921 64-9.

95. Courtney, E.D., Matthews, S., Finlayson, C., Di Pierro, D., Belluzzi, A., Roda, E., Kang, J.Y. and Leicester, R.J. 2007. Eicosapentaenoic acid (EPA) reduces crypt cell proliferation and increases apoptosis in normal colonic mucosa in subjects with a history of colorectal adenomas. *International Journal of Colorectal Disease,* **22**:765-776.

96. Coussens, L.M. and Werb, W. 2002. Inflammation and cancer. *Nature,* **420**:860-867.

97. Craig-Schmidt, M.C. 2006. World-wide consumption of trans-fatty acids. *Atherosclerosis Supplements,* **7**(2):1-4.

98. Crawford, M., Galli, C., Visioli, F., Renaud, S., Simopoulos. A.P. and Spector, A.A. 2000. Role of plant-derived omega-3 fatty acids in human nutrition. *Annals of Nutrition and Metabolism,* **44***:* 263-265.

99. Cunard, R., Ricote, M., DiCampli, D., Archer, D.C., Kahn, D.A., Glass, C.K. and Kelly, C.J. 2002. Regulation of cytokine expression by ligands of peroxisome proliferator activated receptors. *Journal of Immunology,* **168**: 2795-2802.

100. Cury-Boaventura, M.F., Gorjao, R., de Lima, T.M., Newsholme, P. and Curi, R. 2006. Comparative toxicity of oleic and linoleic acid on human lymphocytes. *Life Sciences,* **78**(13):1448-1456.

101. Dalerba, P., Macalli, C., Casati, C., Castelli, C. and Parmiani, G. 2003. Immunology and immunotherapy of colorectal cancer. *Critical Reviews in Oncology/Hematology,* **46:**33-57.

102. Dalton, T.P., Shertzer, H.G., Puga, A. 1999. Regulation of gene expression by reactive oxygen. *Annual Reviews of Pharmacology and Toxicology,* **39**:67-101.

103. Daly, K., Cuff, M.A. Fung, F. and Shirazi-Beechey, S.P. 2005. The importance of colonic butyrate transport to the regulation of genes associated with colonic tissue homeostasis. *Biochemical Society Transactions*, **33**(4**)**:733-735.

104. Damin, D.C., Caetano, M.B., Rosito, M.A., Schwartsmann, G., Damin, A.S., Frazzon, A.P., Ruppenthal, R.D. and Alexandre, C.O. 2007. Evidence for an association of human papillomavirus infection and colorectal cancer. *European Journal of Surgical Oncology,* **33**:569-574.

105. Das, U.N. 2005. COX-2 inhibitors and metabolism of essential fatty acids. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research,* **11**(7):RA223-237.

106. Das, U.N. 2006a. Tumoricidal and anti-angiogenic actions of gamma-linolenic acid and its derivatives. C*urrent Pharmaceutical Biotechnology,* **7**(6):457-466.

107. Das, U.N. 2006b. Essential Fatty acids – a review. C*urrent Pharmaceutical Biotechnology,* 7(6):467-482.

108. Das, D, Arber, N. and Jankowski, A. 2007. Chemoprevention of colorectal cancer. *Digestion: International Journal of Gastroenterology,* **76**(1):51-67.

109. Das, U.N. 2007. Gamma-linolenic acid therapy of human glioma-a review of in vitro, in vivo, and clinical studies. *Medical Science Monitor,* **13**(7):RA119-131.

110. Davydov, V.V. and Bozhkov, A.I. 2003. Metabolism of endogenous aldehydes: participation in oxidative stress-induced lesions and its age aspects. *Biomed Khim,* **49**(4):374-87.

111. De Antueno, R.J., Knickle, L.C., Smith, H., Elliot, H., Elliot, M.L., Nwaka, A.S. and Winther, M.D. 2001. Activity of human Δ5 and Δ6 desaturases on multiple n-3 and n-6 polyunsaturated fatty acids. *FABS Letters,* **509**:77-80.

112. Dempke, W., Grothey, A. and Schmoll, H-J. 2001. Cyclooxygenase-2: a novel target for cancer chemotherapy? *Journal of Cancer Research and Clinical Oncology,* **127**(7):411-417.

113. De Pablo, M.A. and de Cienfuegos, A. 2000. Modulatory effects of dietary lipids on immune system functions. *Immunology and Cell Biology,* **78**:31-39.

114. De Pablo, M.A., Puertollano, M.A. and Alvarez de Cienfuegos G. 2000. Immune cell functions, lipids and host natural resistance. *FEMS Immunology and Medical Microbiology,* **29**:323-328.

115. De Pablo, M.A., Puertollano, M.A. and Alvarez de Cienfuegos G. 2002. Biological and clinical significance of lipids as modulators of immune system functions. *Clinical and Diagnostic Laboratory Immunology,* **9**(5):945-950.

116. Devillard, E., McIntosh, F.M., Duncan, S.H. and Wallace, R.J. 2007. Metabolism of linoleic acid by human gut bacteria: different routes for biosynthesis of conjugated linoleic acid. *Journal of Bacteriology,* **189**:2566-2570.

117. Diefenbach, A. and Raulet, D.H. 2002. The innate immune response to tumors and its role in the induction of T-cell immunity. *Immunological Reviews,* **188**:9-21.

118. Diggle, C.P. 2002. In vitro studies on the relationship between polyunsaturated fatty acids and cancer: tumour or tissue specific effects? *Progress in Lipid Research,* **41**:240-253.

119. Ding, W, Hennig, R. and Adria T.E. 2003. Lipoxygenase and cyclooxygenase metabolism: new insights in treatment and chemoprevention of pancreatic cancer. *Molecular Cancer,* **2**:10-21.

120. Dolara, P., Caderni, G., Salvadori, M., Morozzi, G., Fabiani, R., Cresci, A., Orpianes, C., Trallori, G., Russo, A. and Palli, D. 2002. Fecal levels of short-chain fatty acids and bile acids as determinants of colonic mucosal cell proliferation in humans. *Nutrition and Cancer,* **42**(2):186-190.

121. Dommels, Y., Alink, G., Linssen, J. and van Ommen, B. 2002a. Effects of n-6 and n-3 polyunsaturated fatty acids on gap junctional intercellular communication during spontaneous differentiation of the human colon adenocarcinoma cell line Caco-2. *Nutrition and Cancer,* **42**:125-30.

122. Dommels, E.M., Alink, G.M., van Bladeren, P.J. and van Ommen, B. 2002b. Dietary n-6 and n-3 polyunsaturated fatty acids and colorectal carcinogenesis: results from cultured colon cells, animal models and human studies. *Enviromental Toxicology and Pharmacology,* **12**(4**)**:233-244.

123. Dommels, Y.E.E., Haring, M.M., Keestra, N.G., Alink, G.M., van Bladeren, P.J. and van Ommen, B. 2003. The role of cyclooxygenase in n-6 and n-3 polyunsaturated fatty acid mediated effects on cell proliferation, PGE_2 synthesis and cytotoxicity in human colorectal carcinoma cell lines. *Carcinogenesis,* **24**:(3):385-392.

124. Dong, C. and Flavell, R.A. 2001. Th1 and Th2 cells. *Current Opinion en Hematology,* **8**(1):47-51.

125. Dröge, W. 2002. Free radicals in the physiological control of cell function. *Physiological Reviews,* **82**(1):47-95.

126. Drozina, G., Kohoutek, J., Jabrane-Ferrat, N. and Peterlin, B.M. 2005. Expression of MHC II genes. *Current topics in Microbiology and Immunology,* **290**:147-170

127. Du Bois, R.N., Gupta, R., Brockman, J., Reddy, B.S., Krakow, S.L. and Laza, M.A. 1998. The nuclear eicosanoid receptor, PPARgamma, is aberrantly expressed in colonic cancers. *Carcinogenesis,* **19**(1):49-53.

128. Dukes, C.E. 1932. The classification of cancer of the rectum. *The Journal of Pathology and Bacteriology,* **35**:323-332.

129. Duplus, E. and Forest, C. 2002. Is there a single mechanism for fatty acid regulation of gene transcription? *Biochemistry and Pharmacology,* **64**:893-901.

130. Du Toit, J-L. 2006. The modulation of various signal transduction pathways in colorectal carcinoma cells by docosahexaenoic acid. M.Sc. dissertation, University of Stellenbosch, South Africa.

131. Eberhart, C.E., Coffey, R.J. Radhika, A., Giardiello, F.M., Ferrenbach, S. and DuBois, R.N. 1994. Up-regulation of cycloocxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology,* **107:**1183-1188.

132. Engelbrecht, A-M., Gebhardt, S. and Louw, L. 2006. Ex vivo study of MAPK profiles correlated with parameters of apoptosis during cervical carcinogenesis. *Cancer Letters,* **235**(1**)**:93-99.

133. Epand, R.F., Martinou, J.C., Montessuit, S. and Epand, R.M. 2004. Fatty acids enhance membrane permeabilization by pro-apoptotic Bax. *Biochemistry Journal,* **15(**377):509-516.

134. Escrich, E., Solanas, M., Moral, R., Costa, I. and Grau, L. 2006. Are the olive oil and other dietary lipids related to cancer? Experimental evidence. *Clinical and Translational Oncology,* **8**(12):868-883.

135. Esrich, E., Morai, R., Grau, L., Costa, I. and Solanas, M. 2007. Molecular mechanisms of the effects of olive oil and other dietary lipids on cancer. *Molecular Nutrition and Food Research,* **51**:1279-1292.

136. Evans, C., Dalgleish, A.G. and Kumar, D. 2006. Immune suppression and colorectal cancer. *Alimentary Pharmacology and Therapeutics,* **24**:1163-1177.

137. Evertsson, S. and Sun, X.F. 2002. Protein expression of NF-kappa B in human colorectal adenocarcinoma. *International Journal of Molecular Medicine,* **10**(5**)**:547-50.

138. Ewaschuk, J.B., Walker, J.W., Diaz, H. and Madsen, L. 2006. Bioproduction of conjugated linoleic acid by probiotic bacteria occurs in vitro and in vivo in mice. *Journal of Nutrition,* **136**: 1483-1487.

139. Eynard, A.R. 2003. Potential of essential fatty acids as natural therapeutic products for human tumors. *Nutrition,* **19**(4):386-388.

140. Eynard, A.R. and Lopez, C.B. 2003. Conjugated linoleic acid (CLA) versus saturated fats/cholesterol: their proportion in fatty and lean meats may affect the risk of developing colon cancer. *Lipids in Health and Disease,* **2**:6.

141. Fan, Y.Y. 2003. Dietary n-3 polyunsaturated fatty acids remodel mouse T-cell lipid rafts. *Journal of Nutrition,* **133**:1913-1920.

142. Farreras, N., Artigas, V., Cardona, D., Rius, X., Trias, M. and Gonzalez, J.A. 2005. Effect of early postoperative enteral immunonutrition on wound healing in patients undergoing surgery for gastric cancer. *Clinical Nutrition,* **24**(1):55-65.

143. Fatty acids (Last updated 20 August 2007). Wikipedia, The Free Encyclopedia Retrieved 23 August 2007 from http://en.wikipedia.org/wiki/Fatty_acids.

144. Fatty acids (Last updated 20 August 2007). Wikipedia, The Free Encyclopedia Retrieved 31 October 2007 from http://en.wikipedia.org/wiki/Lauric_acid.

145. Fearon, E.R. and Vogelstein, B. 1990. A genetic model for colorectal tumorigenesis. *Cell,* **61**(5):759-767.

146. Feilchenfeldt, J., Bründler, M-A., Soravia, C., Tötsch, M. and Meier, C.A. 2004. Peroxisome proliferator-activatored receptors (PPARs) and associated transcription factors in colon cancer: reduced expression of PPARγ-coactivator 1 (PGC-1). *Cancer Letters,* **203**:25-33.

147. Feller, S.E., Gawrisch, K. and MacKerell, A.D. 2002. Polyunsaturated fatty acids in lipid bilayers: intrinsic and environmental contributions to their unique physical properties. *Journal of the American Chemical Society,* **124**(2):318-326.

148. Fernandes, G., Chandra, S.B., Luan, X. and Troyer, D.A. 1996. Modulation of antioxidant enzymes and programmed cell death by n-3 fatty acids. *Lipids in Health and Disease,* **31**:591-596.

149. Field, C.J. and Schley, P.D. 2004. Evidence for potential mechanisms for the effect of conjugated linoleic acid on tumor metabolism and immune function: lessons from n-3 fatty acids. *American Journal of Clinical Nutrition,* **79**:1190S-1198S.

150. Folch, J., Lee, M. and Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry,* **226**:497-509.

151. Fong, L.Y.Y., Zhang, L., Jiang, Y. and Farber, J.L. 2005. Dietary zinc modulation of COX-2 expression and lingual and esophageal carcinogenesis in rats. *National. Cancer Institute,* **97**:40-50.

152. Funk, C.D. 2001. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science,* **294**(5548)**:**1871-1875.

153. Furstenberger, G., Krieg, P., Muller-Decker, K. and Habenicht, A.J. 2006. What are cyclooxygenases and lipoxygenases doing in the driver's seat of carcinogenesis? *International Journal of Cancer,* **119**(10):2247-2254.

154. Garcia-Lora, A., Algarra, I. and Garride, F. 2003. MHC class I antigens, immune surveillance, and tumor immune escape. *Journal of Cell Physiology,* **195**(3**)**:346-355

155. Gate, L., Paul, J., Ba, G., Tew, K.D. and Tapiero, H. 1999. Oxidative stress induced in pathologies: the role of antioxidants. *Biomedicine and Pharmacotherapy,* **53**:169-180.

156. Gatenby, R.A. and Vincent, T.L. 2003. An evolutionary model of carcinogenesis. *Cancer Research,* **63**(19):6212-6220.

157. Geleijnse, J.M., Brouwer, E.J. and Feskens, E.J. 2006. Risks and benefits of omega-3 fats: health benefits of omega-3 fats are in doubt. *British Medical Journal,* **332**:915-916.

158. Gelman, L., Fruchart, J.C. and Auwerx, J. 1999. An update on the mechanisms of action of the peroxisome proliferator-activated receptors (PPARs) and their roles in inflammation and cancer. *Cellular and Molecular Life Sciences,* **55**(6-7): 932-943.

159. Giles, R.H., van Es, J.H. and Clever, H. 2003. Caught up in a WNT storm: Wnt signaling in cancer. *Biochimica et Biophysica Acta,–Reviews on Cancer.* **1653**(1):1-24.

160. Giovannucci, E. 1995. Insulin and colon cancer. *Cancer Causes Control,* **6**:164-179.

161. Gokmen-Polar, Y., Murray, N.R., Velasco, M.A., Getalica, S. and Fields, A.P. 2001. Elevated protein kinase CβII is an early promotive event in colon carcinogenesis. *Cancer Research,* **61**:1375-1381.

162. Gomez, C.R., Boehmer, E.D. and Kovacs, E.J. 2005. The aging innate immune system. *Current Opinion in Immunology,* **17**:547-462.

163. Goossens, L., Pommery, N. and Henichart, J.P. 2007. COX-2/5-LOX dual acting anti-inflammatory drugs in cancer chemotherapy. *Current Topics in Medical Chemistry,* **7**(3):283-296.

164. Grau, R., Punzon, C., Fresno, M. and Iniguez, M.A. 2006. Peroxisome-proliferator-activated receptor alpha agonists inhibit cyclooxygenase 2 and vascular endothelial growh factor transcriptional activation in human colorectal carcinoma cells via inhibition of activator protein-1. *The Biochemical Journal,* **395**(1):81-88.

165. Greenhough, A., Patsos, H.A., Williams, A.C. and Paraskeva, C. 2007. The cannabinoid delta(9)-tetrahydrocannabinol inhibits RAS-MAPK and PI3-AKT survival signaling and induces BAD-mediated apoptosis in colorectal cancer cells. *International Journal of Cancer,* **121**(10):2172-2180.

166. Grimaldi, P.A. 2001. Fatty acid regulation of gene expression. *Current Opinion in Clinical Nutrition and Metabolic Care,* **4**:433-437.

167. Grimm, H., Mayer, K., Mayser, P. and Eigenbrodt, E. 2002. Regulatory potential of n-3 fatty acids in immunological and inflammatory processes. *British Nutritional Journal,* **87**:S59-67.

168. Gunter, M.J. and Leitzmann, M.F. 2005. Obesity and colorectal cancer: epidemiology, mechanisms and candidate genes. *Journal of Nutritional Biochemistry,* **17**(3):145-156.

169. Gurr, M. 1996. Fats. In: J.S. Garrow and W.P.T. James, Editors, *Human Nutrition and Dietetics,* Churchill Linigstone, London 1996, 77-102.

170. Guy, A.C. 2005. Lipids as bioeffectors in the immune system. *Life Sciences,* **77**: 1699-1710.

171. Haddad, J.J. 2004. On the antioxidant mechanisms of Bcl-2: a retrospective of NF-κβ signaling and oxidative stress. *Biochemistry and Biophysics Research Communication,* **322**:355-363.

172. Hall, J. and Angele, S. 1999. Radiation, DNA damage and cancer. *Molecular Medicine Today,* **5**:157-164.

173. Han, J.C., Eun, J.K., Soon, S.L., Mi, K.K., Sung, M-K., Kim, J-S. and Park, J.H.Y. 2006. Trans-10,cis-12, not cis9,trans-11, conjugated linoleic acid inhibits G1-S progression in HT-29 human colon cancer cells. *Journal of Nutrition,* **136**: 893-898.

174. Hansen-Petrik, M., McEntee, M.F., Johnson, B.T., Obukowicz, M.G. and Whelan, J. 2000. Highly unsaturated (n-2) fatty acids, but not α-linolenic conjugated linoleic or γ-linolenic acids, reduce tumorigenesis in APC min/+ mice. *Nutritional Journal,* **130**:2434-2443.

175. Hardman, W.E. 2002. Omega-3 fatty acids to augment cancer therapy. *Nutritional Journal,* **132**:3508S-3512S.

176. Hardy, S., Langelier, Y. and Prentki, M. 2000. Oleate activates phosphatidylinositol 3-kinase and promotes proliferation and reduces apoptosis of MDA-MB-231 breast cancer cells, whereas palmitate has opposite effects. *Cancer Research,* **60**(22**)**:6353-6358.

177. Heerdt, B.G., Houston, M.A., Anthony, G.M. and Augenlicht, L.H. 1998. Mitochondrial membrane potential in the coordination op p53-independent proliferation and apoptosis pathways in human colonic carcinoma cells. *Cancer Research,* **58**:2869-2875.

178. Hensley, K., Robinson, K.A., Gabbita, S.P., Salsman, S. and Floyd, R.A. 2000. Reactive oxygen species, cell signaling, and cell injury. *Free Radical Biology and Medicine,* **15**(28): 1456-1462.

179. Hersh, E.V., Lally, E.T. and Moore, P.A. 2005. Update on cyclooxygenase inhibitors: has a third COX isoform entered the fray? *Current Medical Research and Opinion,* **21**(8):1217-1226.

180. Heslin, M.J., Hawkins, A., Boedefeld, W., Arnoletti, J.P., Frolov, A., Soong, R., Urist, M.M. and Bland, K.I. 2005. Tumor-associated down-regulation of 15-lipoxygenase-1 is reversed by celecoxib in colorectal cancer. *Annals of Surgery,* **241**(6):941-946.

181. Heyland, D. and Dhaliwal, R. 2005. Immunonutrition in the critically ill: from old approaches to new paradigms. *Intensive Care Medicine,* **31**(4):501-503.

182. Hinnebusch, B.F., Meng, S., Wu., J.T., Archer, S.Y. and Hodin, R.A. 2002. The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation. *Nutritional Journal,* **132**(5):1012-1017.

183. Hogg, N. and Kalyanaraman, B. 1999. Nitric oxide and lipid peroxidation. *Biochimica et Biophysica Acta,* **1411**:378-384.

184. Hollingshead, H.E., Borland, M.G., Billimn, A.N., Willson, T.M., Gonzalez, F.J. and Peter, J.M. 2007. Ligand activation of peroxisome proliferator-activated receptor-{beta}/{delta} (PPAR{beta}/{delta}) and induction of cyclooxygenase-2 (COX-2) attenuate colon carcinogenesis through independent signaling mechanisms. *Carcinogenesis,* Published online 2007 Sep 24.

185. Horejsi, V. 2003. The roles of membrane microdomains (rafts) in T cell activation. *Immunology Reviews,* **191**:148-164.

186. Horrobin, D.F. 1993. Nutritional and medical importance of gamma-linolenic acid, *Progress in Lipid Research,* **31**:163-194.

187. Horrobin, D.F. 1994. Medical uses of essential fatty acids, *Veterinary Dermatology,* **4**:161-166.

188. Hsu, T-C., Matthew, R., Young, J.C. and Colburn, N.H. 2000. Activator protein 1 (AP-1)- and nuclear factor κβ (NF-κβ) dependent transcriptional events in carcinogenesis. *Free Radical Biology and Medicine,* **28**:1338-1348.

189. Hulbert, A.J., Turner, N., Storlien, L.H. and Else, P.L. 2005. Dietary fats and membrane function: implications for metabolism and disease. *Biological Reviews of the Cambridge Philosophical Society,* **80**(1):155-169.

190. Hume, D. and Hertzog, P. 2006. Innate immunity. *Current Opinion in Immunology,* **18**:1-2.

191. Hussain, S.P., Hofseth, L.J. and Harris, C.C. 2003. Radical causes of cancer. *Nature Reviews Cancer,* **3**:276-285.

192. Hwang, D. and Rhee, S.S. 1999. Receptor-mediated signaling pathways: potential targets of modulation by dietary fatty acids. *American Journal of Clinical Nutrition,* **70**:545-556.

193. Irons, R. and Fritsche, K.L. 2005. Omega-3 polyunsaturated fatty acids impair in vivo interferon-gamma responsiveness via diminished receptor signaling. *The Journal of Infectious Diseases,* **191**(3**)**:481-486.

194. Jacobson-Brown, P. and Neuman M.G. 2003. Colon polyps and cytokines: emerging immunological mechanisms. *Romanian Journal of Gastroenterology,* **12**:207-214.

195. Jho, D.H., Shawn, L., Cole, S.M., Lee, E.M. and Espat, J.N. 2004. Role of omega-3 fatty acid supplementation in inflammation and malignancy. *Integrative Cancer Therapies,* **3**(2):98-111

196. Jaiswal, M. LaRusso, N., Burgart, L. and Gores, G. 2000. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Research,* **60**:184-190

197. Jiang, Y., Lupton, J., Chang, W., Jolly, C., Aukema, H. and Chapkin, R. 1996. Dietary fat and fiber differentially alter intracellular second messengers during tumor development in rat colon. *Carcinogenesis,* **17**:1227-1233.

198. Jiang, W.G., Bryce, R.P. and Horrobin, D.F. 1998. Essential fatty acids: molecular and cellular basis of their anti-cancer action and clinical implications. *Critical Reviews in Oncology/Hematology,* **27**:179-209.

199. Johanson, G.L. and Lapadat, R. 2002. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science,* **298**:1911-1912.

200. Johnson I. 2002. Anticarcinogenic effects of diet-related apoptosis in the colorectal mucosa. *Food Chemistry and Toxicology,* **40**:1171-1178.

201. Jones, R., Adel-Alvarez, L.A., Alvarez, O.R., Broaddus, R. and Das, S. 2003. Arachidonic acid and colorectal carcinogenesis. *Molecular and Cellular Biochemistry,* **253**(1-2):141-149.

202. Jones, P.M., Butt, Y., Messmer, B., Boriak, R. and Bennett, M.J. 2006. Medium-chain fatty acids undergo elongation before β-oxidation in fibroblasts. *Biochemical and Biophysical Research Communications,* **346**(1):193-197.

203. Jones, M., Helliwell, P., Pritchard, C., Tharakan, J. and Mathew, J. 2007. Helicobacter pylori in colorectal neoplamsa: is there an aetiological relationship? *World Journal of Surgical Oncology,* **5**:51-56.

204. Jove, M., Planavila, A., Sanchz, R.M., Merlos, M., Laguna, J.C. and Vazquez-Carrera, M. 2006. Palmitate induces tumor necrosis factor-alpha expression in C2C12 skeletal muscle cells by a mechanism involing protein kinase C and nuclear factor-kappaβ activation. *Endocrinology,* **147**(1):552-561.

205. Jump, D.B. and Clarke, S.D. 1999. Regulation of gene expression by dietary fat. *Annual Review of Nutrition,* **19**:90-96.

206. Jump, D.B. 2002. The biochemistry of n-3 polyunsaturated fatty acids. *Journal of Biological Chemistry, **277**:* 8755-8758.

207. Kahmann, L., Uciechowski, P., Warmuth, S., Malavolta, M., Mocchegiani, E. and Rink, L. 2006. Effect of improved zinc status on T helper cell activation and Th1/Th2 ratio in healthy elderly individuals. *Biogerontology,* **7**(5-6):429-435.

208. Kamlage, B.L., Hartmann, B.G., Gruhl, B. and Blaut, M. 1999. Intestinal microorganisms do not supply associated gnotobiotic rats with conjugated linoleic acid. *Journal of Nutrition*, **129**(12):2212-2217.

209. Kapoor, R. and Huang, Y.S. 2006. Gamma-linolenic acid: an anti-inflammatory omega-6 fatty acid. *Current Pharmaceutical Biotechnology,* **7**(6):531-534.

210. Katagiri, Y.U., Kiyokawa, N. and Fujimoto, J. 2001. A role for lipid rafts in immune cell signaling. *Microbiology and Immunology,* **45**(1**)**:1-8.

211. Kato, T., Kolenic, N. and Pardini, R.S. 2007. Docosahxaenoic acid (DHA), a primary tumor suppressive omega-3 fatty acid, inhibits growth of colorectal cancer independent of p53 mutational status. *Nutrition and Cancer,* **58**(2):178-187.

212. Katsumata, K., Sumi, T., Tomioka, H., Aoki, T. and Koyanagi, Y. 2003. Induction of apoptosis by p53, bax, bcl-2 and p21 expression in colorectal cancer. *International Journal of Clinical Oncology/Japan,* **8**: 352-356.

213. Kawai,N., Tsujii, M. and Tsuji, S. 2002. Cyclooxygenase and colon cancer. *Prostaglandins and other Lipid Mediators,* **68-69**: 187-196.

214. Kazanietz, M.G. 2005. Targeting protein kinase C and “non-kinase” phorbol ester receptors: emerging concepts and therapeutic implications. *Biochimica et Biophysica Acta,* **1754**(1-2):296-304.

215. Keenan, C. and Kelleher, D. 1998. Protein Kinase C and the Cytoskeleton. *Cellular Signaling,* **10**(4):225-232.

216. Kelavkar, U., Lin, Y., Landsittel, D., Chandran, U. and Dhir, R. 2006. The yin and yang of 15-lipoxygenase-1 and delta-desaturases: Dietary omega-6 linoleic acid metabolic pathway in prostate. *Journal of Carcinogenesis,* **5**:9-13.

217. Kelley, D.S., Taylor, P.C. and Nelson, G.J. 1999. Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. *Lipids in Health and Disease,* **34**:317

218. Kelley, D.S. 2001. Modulation of human immune and inflammatory responses by dietary fatty acids. *Nutrition,* **17**:669-673.

219. Kemp, M.Q., Jeffy, B.D. and Romagnolo, D.F. 2003. Conjugated linoleic acid inhibits cell proliferation through a p53-dependent mechanism: effects on the expression of G1-restriction points in breast and colon cancer cells. *Journal of Nutrition,* **133**:3670-3677.

220. Kew, S., Mesa, M.D., Tricon, S., Buckley, R., Minihane, A.M. and Yaqoob, P. 2004. Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell composition and function in healthy humans. *American Journal of Clinical Nutrition*, **79**:674-681.

221. Kidd, P. 2003. Th1/Th2 balance: The hypothesis, its limitations, and implications for health and disease. *Alternative Medicine Review,* **8**(3);223-246.

222. Kim, E., Kim, W., Kang, Y., Ha, Y., Bach, L., and Park, J. 2000. Inhibition of Caco-2 cell proliferation by (n-3) fatty acids: possible mediation by increased secretion of insulin-like growth factor binding protein-6. *Nutrition Research,* **20**:1409-1421.

223. Kimura, Y., Kono, S., Toyomura, K., Nagano, J., Mizoue, T., Moore, M.A., Mibu, R., Tanaka, M., Kakeji, Y., Maehara, Y., Okamura, T., Ikejiri, K., Futami, K., Yasunami, Y., Maekawa, T., Takenaka, K., Ichimiya, H. and Imaizumi, N. 2007. Meat, fish and fat intake in relation to subsite-specific risk of colorectal cancer: The Fukuoka Colorectal Cancer Study. *Cancer Science,* **98**(4):590-597.

224. King, I.B., Kristal A.R., Schaffer, S., Thornquist, M. and Goodman, G.E. 2005. Serum trans-fatty acids are associated with risk of prostate cancer in β-carotene and retinol efficacy trial. *Cancer Epidemiology, Biomarkers & Prevention,***14**(4):988-992.

225. Kitamura, S., Miyazaki, Y., Shinomura, Y., Konde, S., Kanayama, S. and Matsuzawa, Y. 1999. Peroxisome proliferator-activated receptor gamma induces growth arrest and differentiation markers of human colon cancer cells. *Japanese Journal of Cancer Research,* **90**:75-80.

226. Klaunig, J.E. and Kamendulis, L.M. 2004. The role of oxidative stress in carcinogenesis. *Annual Reviews of Pharmacology and Toxicology,* **44**:239-267.

227. Klaus, W.J., Heys, S.D. and Rotondo, S. 2004. Conjugated linoleic acids: are they beneficial or detrimental to health? *Progress in Lipid Research,* **43**:553-587.

228. Koch, J.L.F. and Ratledge, C. 1993. Changes in lipid composition and arachidonic acid turnover during the life cycle of the yeast Dipodascopsi uninucleata. *Journal of General Microbiology,* **139**:459-464.

229. Kojima, M., Morisaki, T., Uchiyama, A., Doi, F., Mibu, R., Katano, M. and Tanaka, M. 2001. Association of enhanced cyclooxygenase-2 expresssion with possible

local immunosuppression in human colorectal carcinomas. *Annals of Surgical Oncology,* **15**:787-800.

230. Kondo, S., Toyokundi, S., Iwasa, Y., Tanaka, T., Onodera, H. and Imamura, M. 1999. Persistent oxidative stress in human colorectal carcinoma, but not in adenoma. *Free Radical Biology & Medicine,* **271**:401-410.

231. Kountourakis, P., Pvlakis, K., Psyrri, A., Rontogianni, D., Xiros, N., Patsouris, E., Pectasides, D. and Economopoulos, T. 2006. Clinicopathologic significance of EGFR and Her-2/neu in colorectal adenocarcinomas. *Cancer Journal,* **12**:229-236.

232. Koyama, Y. and Kotake, K. 1997. Overview of colorectal cancer in Japan: report from the Registry of the Japanese Society for Cancer of the colon and rectum. *Diseases of the Colon and Rectum,* **40:**S2-S9.

233. Kris-Etherton, P.M., Taylor, D.S., Yu-Poth, S., Huth, P., Moriarty, K., Fishell, V., Hargrove, R.L., Zhao, G. and Etherton, D. 2000. Polyunsaturated fatty acids in the food chain in the United States. *American Journal of Clinical Nutrition,* **71**:179S-88S.

234. Kuhajda, F.P. 2000. Fatty-Acid Synthase and Human Cancer: New perspectives on its role in tumor biology. *Nutrition,* **16**:202-208.

235. Kumar, V. and McNerney, M.E. 2005. A new self: MHC-class-I independant natural-killer-cell self-tolerance. *Immunology,* **5**:563-374.

236. Kummerow, F.A., Zhou, Q., Mahfouz, M.M., Smiricky, M.R., Grieshop, C.M. and Schaeffer, D.J. 2004. Trans fatty acids in hydrogenated fat inhibited the synthesis of the polyunsaturated fatty acids in the phospholipids of arterial cells. *Life Science,* **16**;74(22):2707-2723.

237. Kuriki, K., Wakai, K., Hirose, K., Matsuo, K., Ito, H., Suzuki, T., Saito, T., Kanemitsu, Y., Hirai, T., Kaot, T., Tatematsu, M. and Tajima, K. 2006. Risk of colorectal cancer is linked to erythrocyte compositions of fatty acids as biomarkers for dietary intakes of fish, fat, and fatty acids. *Cancer Epidemiology, Biomarkers and Prevention,* **15**(10):1791-1798.

238. Lagares-Garcia, J., Moore, R. Collier, B. Heggere, M., Diaz, F. and Qian, F. 2001. Nitric oxide synthase as a marker in colorectal carcinoma. *American Journal of Surgery,* **67**:709-713.

239. Laggergren, J., Ye, W. and Ekbom, A. 2001. Intestinal cancer after cholecystectomy: is bile involved in carcinogenesis? *Gastroenterology,* **121**(3**)**:542-547.

240. Lakin, N.D. and Jackson, S.P. 1999. Regulation of p53 in response to DNA damage. *Oncogen,* **18**:7944-7655.

241. Lala, P.K. and Chakraborty, C. 2001. Role of nitric oxide in carcinogenesis and tumor progression. *Lancet Oncology,* **2**:149-156.

242. Lane, J., Mansel, R.E., and Jiang, W.G. 2003. Expression of human delta-6-desaturase is associated with aggressiveness of human breast cancer. *International Journal of Molecular Medicine,* **12**(2):253-257.

243. Lapillonne, A., Clarke, S.D. and Heird, W.C. 2004. Polyunsaturated fatty acids and gene expression. *Current Opinion in Clinical Nutrition and Metabolic Care,* **7**(2):151-156.

244. Larsson, S.C., Kumlin, M., Ingelman–Sundberg, M. and Wolk, A. 2004. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *American Journal of Clinical Nutrition,* **79**:935-945.

245. Larsson, S.C., Bergkvist, L. and Wolk, A. 2005a. High-fat dairy food and conjugated linoleic acid intakes in relation to colorectal cancer incidence in the Swedish Mammography Cohort. *American Journal of Clinical Nutrition,* **82**:894-900.

246. Larsson, S.C., Rafter, J., Holmber, L. Bergkvist, L. and Wolk, A. 2005b. Red meat consumption and risk of cancers of the proximal colon, distal colon and rectum: the Swedish Mammography Cohort. *International Journal of Cancer,* **113**(5):829-834.

247. Larsson, S.C., Giovannucci, E. and Wolk, A. 2006. Long-term aspirin use and colorectal cancer risk: a cohort study in Sweden. *British Journal of Cancer,* **6**;95(9**):**1277-1279.

248. Larsson, S.C. and Wolk, A. 2006. Meat consumption and risk of colorectal cancer a meta-analysis of prospective studies. *International Journal of Cancer,* **119**(11):2657-2664.

249. Lee, J.Y., Sohn, K.H., Rhee, S.H and Hwang, D. 2001. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through toll-like receptor 4. *Journal of Biological Chemistry,* **31A**:1077-1080.

250. Lee, J.Y. and Hwang, D.H. 2002. Docosahexanoic acid suppresses the activity of peroxisome proliferator-activator receptors in a colon tumor cell line. *Biochemistry and Biophysics Research Communication,* **298**:667-674.

251. Lee, D.H., Anderson, K.E., Harnack, L.J., Folsom, A.R. and Jacobs, D.R. Jr. 2004. Heme iron, zinc, alcohol consumption, and colon cancer: Iowa Women's Health Study. *Journal of the National Cancer Institute,* **97**(3):232-233.

252. Lee, K.W., Lee, H.J., Cho, H.Y. and Kim, Y.J. 2005. Role of conjugated linoleic acid in the prevention of cancer*. Critical Reviews in Food Science and Nutrition,* **42**(2):135-144.

253. Leppa, S., Saffrich, R., Ansorge, W. and Bohmann, D. 1998. Differential regulation of cJun by ERK and JNK during PC12 cell differentiation. *European Molecular Biology Organization Journal,* **17**:4404-4413.

254. Li, Q., Tan, L., Wang, C., Li, N., Li, Y., Xu, G. and Li, J. 2006. Polyunsaturated eicosapentaenoic acid changes lipid composition in lipid rafts. *European Nutritional Journal,* **45**:144-151.

255. Li, Y., Wang, J., Zhu, G., Zhang, X., Zhai, H., Zhang, W., Wang, W. and Huang, G. 2007. Detection of parvovirus B19 nucleic acids and expression of viral VP1/VP2 antigen in human colon carcinoma. *American Journal of Gastroenterology,* **102**:1489-1498.

256. Lim, S.C., Lee, T.B., Choi, C.H., Ryu, S.Y., Kim, K.J. and Min, Y.D. 2007. Expression of cyclooxygenase-2 and its relationship to p53 accumulation in colorectal cancers. *Yonsei Medical Journal,* **48**(3):495-501.

257. Litman, B.J., Niu, S.L., Polozova, A. and Mitchell, D.C. 2001. The role of docosahexaenoic acid containing phopsholipids in modulating G protein-coupled signaling pathways: visual transduction. *Journal of Molecular Neuroscience,* **16**(2-3):237-242.

258. Liu, Q., Chan, S.T. and Mahendran, R. 2003. Nitric oxide induces cyclooxygenase expression and inhibits cell growth in colon cancer cell lines. *Carcinogenesis.* **24**(4):637-642.

259. Llor, X., Pons, E., Roca, A., Alvarez, M., Mane, J., Fernandez-Banares, F. and Gassull, M.A. 2003. The effects of fish oil, olive oil, oleic acid and linoleic acid on colorectal neoplastic processes. *Clinical Nutrition,* **22**(1):71-79.

260. Louw, L., Engelbrecht, A-M. and Cloete, F. 1998a. Comparison of the fatty acid composition in intraepithelial and infiltrating lesions of the cervix: I, Total fatty acid profiles*. Prostaglandins, Leukotrienes and Esssential Fatty Acids*, **59**(4):253-257.

261. Louw, L., Engelbrech, A-M. and Cloete, F. 1998b. Comparison of the fatty acid composition in intraepithelial and infiltrating lesions of the cervix: II, Free fatty acid profiles. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* **59**(4):253-257

262. Louw, L., Engelbrecht, A-M. and Cloete, F. 1998c. Comparison of the fatty acid compositions in intraepithelial and infiltrating lesions of the cervix: III, Saturated and unsaturated fatty acid profiles. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **59**(4): 259-264

263. Louw L. and Claassen, A. 2006. Clinically relevant lipid model for laryngeal cancer. In: Dietary fats and chronic diseases. Eds. Huang, Y-S., Yanagita, T. and, Knapp, H.R. *AOCS Press,Champaign, Illinois.*

264. Lupton, J.R. 2004. Microbial degradation products influence colon cancer risk: the Butyrate controversy. *Journal of Nutrition,* **134**:479-482.

265. Lynch, J.P. and Hoops, T.C. 2002 The genetic pathogenesis of colorectal cancer. *Hematology/oncology clinics of North America,* **16**(4):775-810.

266. Maedler, K., Oberholzer, J., Bucher, P., Spinas, G.A. and Donath, M.Y. 2003. Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic beta-cell turnover and function. *Diabetes,* **52**(3):726-733.

267. Mamalakis, G., Kafatos, A. Kalogeropoulos, N., Andrikopoulos, N., Daskalopulos, G. and Kranidis, A. 2002. Prostate cancer vs hyperplasia: relationships with

prostatic and adipose tissue fatty acid composition. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* **66**(5-6**)**:467-477.

268. Marks, F., Müller-Decker, K. and Fürstenberger G. 2000. A casual relationship between unscheduled eicosanoid signaling and tumor development: cancer chemoprevention by inhibitors of arachidonic acid metabolism. *Toxicology,* **153**:11-26.

269. Marnett, L.J. 2002. Oxy-radicals, lipid peroxidation and DNA damage. *Toxicology,* **181-182**:219-222.

270. Marques-Vidal, P., Ravasco, P. and Ermelind, C.M. 2006. Foodstuffs and colorectal cancer risk: a review. *Clinical Nutrition,* **25**(1)14-36.

271. Martenson, J.A., Willett, C.G., Sargent, D.J., Mailliard, J.A., Donohue, L.L., Gunderson, L., Thomas C.R., Fisher, B., Benson, A.B., Myerson, R. and Goldberg, R.M. 2004. Phase III study of adjuvant chemotherapy and radiation therapy compared with chemotherapy alone in the surgical adjuvant treatment of colon cancer: results of intergroup protocol 0130. *Journal of Clinical Oncology,* **22**:3277-3283.

272. Martinasso, G., Oraldi, G., Trombetta, A., Maggiora, M. and Bertetto, M. 2007. Involvement of PPARs in cell proliferation and apoptosis in human colon cancer specimens and in normal and cancer cell lines. *PPAR Research,* http//www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmedid=17389773. Published online 2007. March 13, doi: 10.1155/2007/93416.

273. Martinez, S.R., Shawn, E.Y., Hoedema, R.E., Foshag, L.J. and Bilchik, A.J. 2006. Colorectal cancer screening and surveillance: Current standards and future trends. *Annals of Surgical Oncology,* **13**:768-775.

274. Mathew, A., Peters, U., Chatterjee, N., Kulldorff, M. and Sinha, R. 2004. Fat, fiber, fruits, vegetables, and risk of colorectal adenomas. *International Journal of Cancer,* **10**:108(2):287-292.

275. Matsuda, A., Furukawa, K., Takasaki, H., Suzuki, H., Kan, H., Tsuruta, H., Shinji, S. and Tajiri, T. 2006. Preoperative oral immune-enhancing nutritional supplementation corrects Th1/Th2 imbalance in patients undergoing elective surgery for colorectal cancer. *Diseases of the Colon and Rectum,* **49**(4):507-516.

276. Matsuzaki, K., Seki, T. and Okazaki, K. 2006. TGF-beta during human colorectal carcinogenesis: the shift from epithelial to mesenchymal signaling. *Inflammopharmacology,* **14**(5-6):198-203.

277. May, M.J. and Ghosh, S. 1998. Signal transduction through NFκβ. *Immunology Today,* **80**(19)(2):80-88.

278. McCall, M.R. and Frei, B. 1999. Can antioxidant vitamins materially reduce oxidative damage in humans? *Free Radical Biology and Medicine,* **26**(7-8):1034-1053.

279. McEntee, M.F. and Whelan, J. 2002. Dietary polyunsaturated fatty acids and colorectal neoplasia. *Biomedical Pharmacotherapy,* **56**:380-387.

280. McMillan, L., Buthcer, S.K., Pongracz, J. and Lord, J.M. 2003. Opposing effects of butyrate and bile acids on apoptosis of human colon adenoma cells: differential activation of PKC and MAP kinases. *British Journal of Cancer,* **10**;88(5):748-753.

281. Meade, C.J. and Mertin, J. 1978. Fatty acids and immunity. *Advanced Lipid Research,* **16**:127-165.

282. Mehigan, B.J., Ashman, J.N., Baker, R.P. Macdonald, A., Greenman, J., Monson, J.R. and Cawkwell, L. 2006. Mismatch repair, p53 and chromosomal aberrations in primary colorectal carcinomas. *Acta Oncologica,* **45**(1):61-66.

283. Menendez, J., Roperto, S., Mahmi, E., Atlas, E., Colomer, R. and Lupu, R. 2004a. Overexpression and hyperactivity of breast cancer-associated fatty acid synthase (oncogenic antigen-519) is insensitive to normal arachidonic fatty acid-induced suppression in lipogenic tissues but it is selectively inhibited by tumoricidal alpha-linolenic and gamma-linolenic fatty acids: a novel mechanism by which dietary fat can alter mammary tumorigenesis. *International Journal of Oncology,* **24**:1369-1383.

284. Menendez, J.A., Vellon, L., Mehmi, I., Oza, B.P., Ropero, S., Colomer, R. and Lupu, R. 2004b. Inhibition of fatty acid synthase (FAS) suppresses HER2/neu (erB-2) oncogene overexpression in cancer cells. *Proceedings of the National Academy of Sciences of the USA,* **101**(29):10715-10720.

285. Menendez, J., Colomer, R. and Lupu, R. 2005a. Why does tumor-associated fatty acid syntahse (oncogenic antigen-519) ignore dietary fatty acids? *Medical Hypotheses,* **64**:342-349.

286. Menendez, J.A., Vellon, L. and Lupu, R. 2005b. Oleic acid the main monounsaturated fatty acid of olive oil, suppresses Her-2/neu (erbB-2) expression and synergistically enhances the growth inhibitory affects of trastuzumab (Herceptin) in breast cancer cells with Her-2/*neu* oncogene amplification. *Annals of Oncology,* **16**:359-371.

287. Menendez, J.A. and Lupu, R. 2006. Mediterranean dietary traditions for the molecular treatment of human cancer: anti-oncogenic actions of the main olive oil's monounsaturated fatty acid oleic acid (18:1n-9). *Current Pharmaceutical Biotechnology,* **7**: 495-502.

288. Menendez, J.A., Papadimitropoulou, A., Vellon, L., Colomer, R. and Lupu, R. 2006a. A genomic explanation connecting "Mediterranean diet", olive oil and cancer: Oleic acid, the main monounsaturated Fatty acid of olive oil, induces formation of inhibitory " PEA3 transcription factor-PEA3 DNA binding site " complexes at the Her-2/neu (erbB-2) oncogene promoter in breast, ovarian and stomach cancer cells. *European Journal of Cancer,* **42**:2425-2432.

289. Menendez, J.A., Vazquez-Martin, A., Ropero, S., Colomer, R. and Lupu, R. 2006b. HER2 (erB-2)-targeted effects of the omega-3 polyunsaturated fatty acid, alpha-linolenic acid (ALA; 18:3n-3), in breast cancer cells: the "fat features" of the "Mediterranean diet" as an "anti-HER2 cocktail". *Clinical Translational Oncology,* **8**:812-820.

290. Menzel, T., Schauber, J., Kreth, F., Kudlich, T., Melcher, R., Gostner, A., Scheppach, W. and Luhrs, H. 2002. Butyrate and aspirin in combination have an

enhanced effect on apoptosis in human colorectal cancer cells. *European Journal of Cancer Prevention,* **11**(3):271-281.

291. Michi, P. and Downward, J. 2005. Mechanisms of disease: PI3/AKT signaling in gastrointestinal cancers. *Zeitschrift für Gastroeneterologie,* **43**:1133-1139.

292. Miller, TA., Le Brasseur, N.K., Cote, G.M., Trucillo, M.P. Pimentel, D.R., Ido, Y., Ruderman, N.B. and Sawyer, D.B. 2005. Oleate prevents palmitate-induce cytotoxic stress in cardiac myocytes. *Biochemical and Biophysical Research Communications,* **336**(1):309-315.

293. Mills, S.C., Windsor, A.C. and Knight, S.C. 2005. The potential interactions between polyunsaturated fatty acids and colonic inflammatory processes. *Clinical and Experimental Immunology,* **142:**216-228.

294. Minguet, S., Swamy, M., Alarcon, B., Luescher, I.F and Schamel, W.W.A. 2007. Full activation of the T cell receptor requires both clustering and conformational changes at CD3. *Immunity,* **26**(1):43-54.

295. Mirnikjoo, B., Brown, S.E., Kim, F.H.F.S. Marangell, L.B., Sweatt, J.D. and Weeber, E.J. 2001. Protein kinase inhibition by ω-3 fatty acids. *The Journal of Biological Chemistry,* **276**(14):10888-10896.

296. Mishra, R. and Simonson, M.S. 2005. Saturated free fatty acids and apoptosis in microvascular mesangial cells: palmitate activates pro-apoptotic signaling involving caspase 9 and mitochondrial release of endonuclease G. *Cardiovascular Diabetology,* **4**(1):2

297. Mitchell, J.A., Warner, T.T. 1999. Cyclo-oxygenase-2: Pharmacology, physiology, biochemistry and relevance to NSAID therapy. *British Journal of Pharmacology,* **128**:1121-1132.

298. Moretta, A. 2006. The dialogue between human natural killer cells and dendritic cells. *Current Opinion in Imunology,* **17**:306-311.

299. Mossman, T.R., Herwinski, H., Bond, W., Giedlin, M.A. and Cofman, R.L. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *Journal of Immunology,* **136**(7):2348-2357.

300. Mosmann, T.R. and Sad, S. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunology Today,* **17**(3):138-146.

301. Mozaffarian, D., Pischon, T., Hankinson, S.E., Rifai, N., Joshipura, K., Willett, W.C. and Rimm, E.B. 2004. Dietary intake of trans-fatty acids and systemic inflammation in women. *American Journal of Clinical Nutrition,* **79**(4):606-612.

302. Mozaffarian, D. 2006. Trans-fatty acids: Effects on systemic inflammation and endothelial function. *Atherosclerosis Supplements,* **7**:29-32.

303. Mulcahy, H.E., Hyland, J. and O'Donoghue, D.P. 2003. From dinosaurs to DNA: a history of colorectal cancer. *International Journal of Colorectal Disease,* **18**:210-215.

304. Murphy, M.G. 1999. Dietary fatty acids and membrane protein function. *Journal of Nutritional Biochemistry,* **1**:68–79

305. Murray, N.R., Davidson, R.S., Chapkin, R.S., Gustafson, W.C., Schattenber, D.G. and Fields A.P. 1999. Overexpression of protein kinase C βII induces colonic hyperproliferation and increased sensitivity to colon carcinogenesis. *Journal of Cell Biology,* **145**:699-711.

306. Murray, N.R., Weems, C., Chem, L., Leon, J., Yu, W., Davidson, L.A., Jamieson, L., Chapkin, R.S., Thompson, E.A. and Fields, A.P. 2002. Protein kinase CβII and TGFβRII in n-3 fatty acid mediated inhibition of colon carcinogenesis. *Journal of Cell Biology,* **157**(6):915-920.

307. Murray, R.K., Granner, D.K., Mayes, P.A., Rodwell, V.W. 2003. *Harper's illustrated biochemistry.* Twenty-sixth edition, Lange Medial Books/ McGraw Hill, ISBN 0-07-138901-6 Chapter 14:111-121; Chapter 25:217-220.

308. Murray, N.R., Jamieson, L., Yu, W., Zhang, J., Gökmen-Polar, Y., Sier, D., Anastasiadis, P., Gatalica, Z., Thompson, A., and Fields, A.P. 2004. Protein kinase α, is required for Ras transformation and colon carcinogensis in vivo. *Journal of Cell Biology,* **164**(6):797-802

309. Musashi, M., Ota, S. and Shiroshita, N. 2000. The role of protein kinase C isoforms in cell proliferation and apoptosis. *International Journal of Hematology,* **72**:12-19.

310. Nakamura, K., Kariyazono, H., Komokata, T., Hamada, N., Sakata, R. and Yamada, K. 2005. Influence of preoperative administration of ω-3 fatty acid-enriched supplement on inflammatory and immune responses in patients undergoing major surgery for cancer. *Nutrition,* **21**:639-649.

311. Narayanan, B., Narayana, N., Reddy, B. 2001. Docosahexaenoic acid regulated genes and transcription factors inducing apoptosis in human colon cancer cells. *International Journal of Oncology,* **19**:1255-1262.

312. Narayanan, B., Narayanan, N., Simi, B. and Reddy, B. 2003. Modulation of inducible nitric oxide synthase and related pro-inflammatory genes by the omega-3 fatty acid docosahexaenoic acid in human colorectal cancer cells. *Cancer Research,* **63**:972-9.

313. Nebert, D. 2002. Transcription factors and cancer: an overview. *Toxicology,* **181-182**:131-141.

314. Nelson, R.L. 2001. Iron and colorectal cancer risk: human studies. *Nutrition Reviews,* **59**(5):344-346.

315. Ng, Y., Barhoumi, R., Tjalkens, R.B., Fan, Y.Y., Kolar, S., Wang, N., Lupton, J.R. and Chapkin, R.S. 2005. The role of docosahexaenoic acid in mediating mitochondrial membrane lipid oxidation and apoptosis in colonocytes. *Carcinogenesis,* **26**(11**)**:1914-1921.

316. Nguyen, K.A., Cao, Y., Chen, J.R., Townsend, C.M. Jr and Ko, T.C. 2006. Dietary fiber enhances a tumor suppressor signaling pathway in the gut. *Annals of Surgery,* **243**(5):619-625.

317. Niba, L.L. and Niba, S.H. 2003. Role of non-digestible carbohydrates in colon cancer protection. *Nutrition and Food Science,* **33**(1):28-33.

318. Nigam, S. and Schewe, T. 2000. Phospholipase A2 and lipid peroxidation. *Biochimica et Biophysica Acta,* **1488**:167-181.

319. Niki, E., Yoshida, Y., Saito, Y and Noguchi, N. 2005. Lipid peroxidation: Mechanisms, inhibition, and biological effects. *Biochemical and Biophysical Research Communications,* **338:**668-676.

320. Nilsen, T.I. and Vatten, L.J. 2001. Prospective study of colorectal cancer risk and physical activity, diabetes, blood glucose and BMI: exploring the hyperinsulinaemia hypothesis. *British Journal of Cancer,* **84**(3):417-422.

321. Nixon, J.B., Kamitani, H., Baek, S.J. and Eling, T.E. 2003. Evaluation of eicosanoids and NSAIDs as PPARγ ligands in colorectal carcinoma cells. *Prostaglandins, Leukotrienes and Fatty Acids.* **68**:323-330.

322. Nixon, J.B., Kim, K.S., Lamb, P.W. Bottone, F.G. and Eling, T.E. 2004. 15-Lipoxygenase-1 has anti-tumorigenic effects in colorectal cancer. *Prostaglandins Leukotrienes and Essential Fatty Acids,* **70**(1):7-15.

323. Nkondjock, A., Shatenstein, B., Maisonneuve, P. and Ghadirian, P. 2003. Specific fatty acids and human colorectal cancer: an overview. *Cancer Detection and Prevention,* **27**:55-66.

324. Norat, T., Bingham, S., Ferrari, P. Slimani, N. and Jenab, M. 2005. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. *Journal of the National Cancer Institute,* **97**(12**)**:906-916.

325. Novak, T.E., Babcock, T.A., Jho, D.H., Helton, W.S. and Espat, N.J. 2003. NF-kappa B inhibition by omega- fatty acids modulates LPS-stimulated macrophage TNF-alpha transcription. *American Journal of Physiology,* **284**:L84-89.

326. Ntambi, J.M., Miyazaki, M. and Dobrzyn, A. 2004. Regulation of stearoyl-CoA desaturase expression. *Lipids,* **39**(11**)**:1061-1065.

327. Obata, T., Brown, G.E. and Yaffe, M.B. 2000. MAPK kinase pathways activated by stress: the p38 MAPK pathway. *Critical Care Medicine,* **28**:N67-N77.

328. Oberley, T.D. 2002. Oxidative damage and cancer. *American Journal of Pathology*, **160**:403-408.

329. Ogino, S., Kawasaki, T., Ogawa, A., Kirkner, G.J., Loda, M. and Fuchs, C.S. 2007. Fatty acid synthase overexpression in colorectal cancer is associated with microsatellite instability, independent of CpG island methylator phenotype. *Human Pathology,* **38**: 842-849.

330. O'Hagan, S. and Menzel, A. 2003. A subchronic 90-day oral rat toxicity study and in vitro genotoxicity studies with a conjugated linoleic acid product. *Food and Chemical Toxicology,* **41**:1749-1760.

331. Ohta, T., Takahashi, M. and Ochiai, A. 2005. Increased protein expression of both inducible nitric oxide synthase and cyclooxygenase-2 in human colon cancers. *Cancer Letters,* **239**:246-253.

332. Ohtsu, H., Ho, E., Huang, Y-S., Chuang, L-T. and Bray, T.M. 2005. Conjugated linoleic acid decreases cellular proliferation and inhibits nuclear factor–κβ and activator protein 1activation in PC3 cancerous prostate epithelial cells. *Nutrition Research,* **25**(7**)**:655-662.

333. Okada, F. 2002. Inflammation and free radicals in tumor development and progression. *Redox Report,* **7**:357-368.

334. Okajuma, H. 1998. Is the excess linoleic acid syndrome confined to Japan? *ISSFAL Newsletter,* **5**:3-8.

335. O'Keefe, S.J., Kidd, M., Espitalier-Noel, G. and Owira, P. 2001. Rarity of colon cancer in Africans is associated with low animal product consumption, not fiber. *American Journal of Gastroenterology,* **94**(5):1373-1380.

336. O'Neill, L.A.J. 2006. How Toll-like receptors signal: what we know and what we don't know. *Current Opinion in Immunology,* **18**:3-9.

337. Oppenheim, J.J., Biragyn, A., Kwak, L.W. and Yang, D. 2003. Roles of antimicrobial peptides such as defensins in innate and adaptive immunity. *Annals of the Rheumatic Diseases,* **62**(2**)**:17-21.

338. Ordemann, J., Jacobi, C.A., Braumann, C., Schwenk, W., Volk, H.D. and Müller, J.M. 2002. Immunomodulatory changes in patients with colorectal cancer. *International Journal of Colorectal Disease,* **17**:37-41.

339. Osawa, E., Nakajima, A., Fujisawa, T., Kawamura, Y.I., Toyama-Sorimachi, N., Nakagama, H. and Dohi, T. 2006. Predominant T helper type-2-inflammatory responses promote murine colon cancers. *International Journal of Cancer,* **118**(9):2232-2236.

340. O'Shea, M., Bassagaya-Riera, J. and Mohede, I.C. 2004. Immunomodulatory properties of conjugated linoleic acid. *The American Journal of Clinical Nutrition,* **79**:1199S-1206S.

341. Owen, R.W., Haubner, R., Wurtele, G., Hulle, E., Spiegelhalder, B. and Bartsch, H. 2004. Olives and olive oil in cancer prevention. *European Journal of Cancer Prevention,* **13**(4):319-326.

342. Owuor, E.D. and Kong, A-N.T. 2002. Antioxidants and oxidants regulated signal transduction pathways. *Biochemical Pharmacology,* **64**:765-770.

343. Pahl, H.L. 1999. Activators and target gene of Rel/NF-kappaB trancription factors. *Oncogene,* **18**:6867-6874.

344. Pai, R., Nakamura, T., Moon, W.S., Tarnawski, A.S. 2003. Prostaglandins promote colon cancer cell invasion; signaling cross-talk between two distinct growth factor receptors. *Federation of American Societies for Experimental Biology Journal,* **17**:1640-1647.

345. Pandey, M., Sharma, L.B., Singh, S. and Shukla, V.H. 2003. Erythrocyte membrane fatty acid profile and saturation index in gallbladder carcinogenesis: a case-control study. *World Journal of Surgical Oncoloy,* **1**:5-8

346. Pariza, M.W. 2004. Perspective on the safety and effectiveness of conjugated linoleic acid. *The American Journal of Clinical Nutrition,* **79**(6):1132S-1136S.

347. Park, H.S., Cho, H.Y., Ha, Y.L. and Park, J.H.Y. 2004. Dietary conjugated linoleic acid increases the mRNA ratio of Bax/Bcl-2 in the colonic mucosa of rats. *The Journal of Nutritional Biochemistry,* **15**:229-235.

348. Petrik, M., McEntee, M., Chiu, C. and Whelan, J. 2000. Antagonism of arachidonic acid is linked to the antitumorigenic effect of dietary eicosapentaenoic acid in Apc(Min/+) mice. *The Journal of Nutrition*, **130**:1153-1158.

349. Philpott, M. and Ferguson, L.R. 2004. Immunonutrition and cancer. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* **551**:29-42.

350. Pierce, S.K. 2002. Lipid rafts and B cell activation. *Nature Reviews. Immunology,* **2**:96-105.

351. Playfair, J.J.L. and Chain, B.M. 2005. Immunology at a Glance. 8th edition. Blackwell Publishing Ltd. ISBN: 978-0-8089, Pg 10.

352. Ponferrada, A., Caso, J.R., Alou, L., Colon, A., Sevillano, D., Moro, M.A., Lizasoain, I., Menchen, P., Gomez-Lus, M.L., Lorenzo, P., Cos, E., Leza, J.C. and Menchen, L. 2007. The role of PPARgamma on restoration of colonic homeostasis after experimental stress-induced inflammation and dysfunction. *Gastroenterology,* **132**(5):1791-1803.

353. Ponz de Leon, M. and Roncucci, L. 2000. The cause of colorectal cancer. *Digestive and Liver Disease,* **32**(5):426-439.

354. Ponz de Leon, M. and Di Gregoria, C. 2001. Pathology of colorectal cancer. *Digestive and Liver Disease,* **33**(4**)**:372-388.

355. Pool-Zobel, B., Loo, J., Rowland, I. and Roberfroid, M.B. 2002. "Experimental evidences on the potential of prebiotic fructans to reduce risk of colon cancer". *British Journal of Nutrition,* **87**(2):273-281.

356. Prasad, A.S. and Kucuk, O. 2002. Zinc in cancer prevention. *Cancer Metastasis Rev.* **21**:291-295

357. Rakheja, D., Kaur, P. and Hoang, M.P. 2005. Increased ratio of saturated to unsaturated C18 fatty acids in colonic adenocarcinoma: Implication for cryotherapy and lipid raft function. *Medical Hypotheses,* **65**:1120 -1123.

358. Rao, C.V., Hirose, Y., Indranie, C. and Reddy, B.S. 2001. Modulation of experimental colon tumorigenesis by types and amounts of dietary fatty acids. *Cancer Research,* **61**(5):1927-1933.

359. Rao, M., Yang, W., Seifalian, A. and Winslet, M. 2004. Role of cyclooxygenase-2 in the angiogenesis of colorectal cancer. *International Journal of Colorectal Disease,* **19**:1-11.

360. Rashid, A., Pizer, E.S., Moga, M., Milgraum, L.Z., Zahurak, M., Pasternack, G.R., Kuhajda, F.P. and Hamilton, S.R. 1997. Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. *The American Journal of Pathology,* **150**;(1):201-208.

361. Read, J.A., Beale, P.J., Volker, D.H., Smith, N., Childs, A. and Clarke, S.J. 2007. Nutrition intervention using an eicosapentaenoic acid (EPA)-containing supplement in patients with advances colorectal cancer. Effects on nutritional and inflammatory status: a phase II trial. *Supportive Care in Cancer,* **15**(3):301-307.

362. Reddy, B.S. 2000. Novel approaches to the prevention of colon cancer by nutritional manipulation and chemoprevention. *Cancer Epidemiology, Biomarkers and Prevention,* **9**:239-247.

363. Reddy, B.S., Patlolla, J.M., Simi, B., Want, S.H. and Rao, C.V. 2005. Prevention of colon cancer by low doses of celecoxib, a cyclooxygenase inhibitor, administered in diet rich in omega-3 polyunsaturated fatty acids. *Cancer Research,* **65**(17**)**:8022-8027.

364. Ries, L.A.G., Wingo, P.A., Miller, D.S., Howe, H.L., Weir, H.K., Rosenberg, H.M., Vernon, S.W., Cronin, K. and Edward, B.K. 2000. The Annual report to the Nation on the Status of cancer, 1973-1997 with a special section on Colorectal Cancer. *Cancer,* **88**:2398-2424.

365. Romagnani, S. 2006. Regulation of the T cell response. *Clinical and Experimental Allergy,* **36** (11**)**:1357-1366.

366. Rose, D.P. and Connolly, J.M. 1999. Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacology and Therapeutics,* **83**:217-244.

367. Roynette, C.E., Calder, P.C., Dupertuis, Y.M. and Pichard, C. 2004. N-3 Polyunsaturated fatty acids and colon cancer prevention. *Clinical Nutrition,* **23**:139-151.

368. Saether, T., Tran, T.N. Rootwelt, H., Grav, H.J. Christophersen, B.O. and Haugen, T.B. 2007. Essential fatty acid deficiency induces fatty acid desaturase expression in rat epididymis, but no in testis. *Reproduction,* **133**(2):467-477.

369. Saez, E., Tontonoz, P., Nelson, M.C., Alvarez, J.G., Ming, U.T., Baird, S.M., Thomazy, V.A. and Evans, R.M. 1998. Activators of the nuclear receptor PPARgamma enhance colon polyp formation. *Nature Medicine,* **4**:1058-1061.

370. Sakuragi, N., Salah-eldin, A., Watari, H., Itoh, T., Inoue, S., Moriuchi, T. and Fujimoto, S. 2002. Bax, Bcl-2 and p53 expression in endometrial cancer. *Gynecologic Oncology,* **86**:288-296.

371. Salas-Salvado, J., Marquez-Sandoval, F. and Bullo, M. 2006. Conjugated linoleic acid intake in humans: a systematic review focusing on its effect on body composition, glucose, and lipid metabolism. *Critical Reviews in Food Science and Nutrition,* **46**(6):479-488.

372. Sampath, H. and Ntambi, J.M. 2004. Polyunsaturated fatty acid regulation of gene expression. *Nutrition Reviews,* **62**(9):333-339.

373. Sampath, H. and Ntambi, J.M. 2005a. Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annual Review of Nutrition,* **25**:317-340.

374. Sampath, H. and Ntambi, J.M. 2005b. The fate and intermediary metabolism of stearic acid. *Lipids,* **40**(12):1187-1191.

375. Sanborn, R. and Blanke, C.D. 2005. Cycloocxygenase-2 inhibition in colorectal cancer: boom or bust? *Seminars in Oncology,* **32**(1):69-75.

376. Sandel, M.H., Speetjens, F.M., Menon, A.G., Albertsson, P.A., Basse, P.H., Hokland, M., Nagelkerke, J.F., Tollenaar, R.A.E.M., van de Velde, C.J.H. and Kuppern, P.J.K. 2005. Natural killer cells infiltrating colorectal cancer and MHC class I expression. *Molecular Immunology,* **42**:541-546.

377. Sano, H., Kawahito, Y., Wilder, R.L., Hashiramoto, A., Mukai, S. and Asai, K. 1995. Expression of cyclooxygenase-1 and –2 in human colorectal cancer. *Cancer Research,* **55**:3785-9378.

378. Sander, C.S., Chang, H., Hamm, F., Elsner, P. and Thiele, J.J. 2004a. Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. *Internal Journal of Dematology,* **43**:326-335.

379. Sander, L.M., Henderson, C.E., Hong, M.Y. Barhoumi, R., Burghardt, R.C., Wang, N., Spinka, C.M., Carroll, R.J., Turner, N.D., Chapkin, R.S. and Lupton JR. 2004b. An increase in reactive oxygen species by dietary fish oil coupled with the attenuation of antioxidant defenses by dietary pectin enhances rat colonocyte apoptosis. *Journal of Nutrition,* **134**:3233-3238.

380. Scaglia, N., Caviglia, J.M. and Igal, R.A. 2005. High stearoyl-CoA desaturase protein and activity levels in simian virus 40 transformed-human lung fibroblasts. *Biochimica et Biophysica Acta,* **1687**(1-3):141-151.

381. Scaglia, N. and Igal, R.A. 2005. Stearoyl-CoA desaturase is involved in the control of proliferation, anchorage-independent growth, and survival in human transformed cells. *The Journal of Biological Chemistry,* **280**(27**)**:25339-25349.

382. Schaffer, J.E. 2002. Fatty acid transport: the roads taken. *American Journal of Physiology: Endocrinology and Metabolism,* **282**: E239-E246.

383. Scheppach, W., Bertratm., H.P. and Richter, F. 1995. Role of short-chain fatty acids in the prevention of colorectal cancer. *European Journal of Cancer,* **31**(7-8):1077-1080.

384. Schmiegel, N.A. 2005. Chemoradiotherapy for colorectal cancer. *Gut,* **54**:1194-1202.

385. Schottelius, A.J. and Dinter, H. 2006. Cytokines, NF-kappa β, microenvironment, intestinal inflammation and cancer. *Cancer Treatment and Research,* **130**:67-87.

386. Schuell, B., Gruenberger, T., Scheithauer, W., Zielinski, C. and Wrba, F. 2006. HER-2/neu protein expression in colorectal cancer. *BMC Cancer,* **6**(1):123.

387. Schwartz, S.A., Hernandez, A. and Mark Evers, B. 1999. The role of NF-kappaβ/Ikappaβ proteins in cancer; implications for novel treatment strategies. *Surgical Oncology,* **8**:143-153.

388. Schwartz, B., Birek, Y., Raz, A. and Madar, Z. 2004. Nutrition-pharmacological combinations. *European Journal of Nutrition,* **43**:221-229.

389. Screpanti, V., Wallin, R.P.A., Grandien, A. and Ljunggren, H-G. 2004. Impact of FASL-induced apoptosis in the elimination of tumor cells by NK cells. *Moleular Immunology,* **42**(4):49549-49570.

390. Sebolt-Leopold, J. S., Dudley, D. T., Herrera, R., Van Becelaere, K., Wiland, A., Gowan, R., C., Tecle, H., Barrett, S. D., Bridges, A., Przybranowski, S., Leopold, W. R. and Saltiel, A. R. 1999. Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo. *Nature Medicine,* **5**:736-737.

391. Seed. B. 1998. PPARgamma and colorectal carcinoma: conflicts in a nuclear family. *Nature Medicine,* **4**(9):1004-5.

392. Sengupta, S., Muir, J.G. and Gibson, P.R. 2006. Does butyrate protect from colorectal cancer? *Journal of Gastroenterology and Hepatology,* **21***:*209-218.

393. Seril, D.N., Liao, J., West, A.B. and Yang, G.Y. 2006. High-iron diet: foe or feat in ulcerative colitis and ulcerative colitis-associated carcinogenesis. *Journal of Clinical Gastroenterology,* **40**(5):397-397.

394. Sertznig, P., Seifert, M., Tilgen, W. and Reichrath, J. 2007. Present concepts and future outlook: Function of peroxisome proliferator-activated receptors (PPARs) for pathogenesis, progression, and therapy of cancer. *Journal of Cellular Physiology,* **212**:1-12.

395. Sesink, A.L.A., Termont, D.S.M.L., Kleibeuker, J.H. and Van der Meer, R. 2000. Red meat and colon cancer: dietary haem, but not fat, has cytotoxic and hyperproliferative effects on rat colonic epithelium. *Carcinogenesis,* **21**:1909-1915.

396. Seung Kim, H.F., Weeber, E.J., Sweatt, J.D., Stoll, A.L. and Marangell L.B. 2001. Inhibitory effects of omega-3 fatty acids on protein kinase C activity in vitro. *Molecular Psychiatry,* **6**:246-248.

397. Shah, A.M. and Channon, K.M. 2004. Free radicals and redox signaling in cardiovascular disease. *Heart,* **90**:486-487.

398. Shah, U.S., Dhir, R., Gollin, S.M., Chandran, U.R., Lewis, D., Acquafondata, M. and Pflug, B.R. 2006. Fatty acid synthase gene overexpression and copy number gain in prostate adenocarcinoma. *Human Pathology,* **37**:401-409.

399. Shao, J., Lee, S.B., Guo, H., Evers,B.M. and Sheng, H. 2003. Prostaglandin E_2 stimulates the growth of colon cancer cells via induction of Amphiregulin. *Cancer Research,* **63**:5218-5223.

400. Shao, J., Evers, B.M. and Sheng, H. 2004. Prostaglandin E2 synergistically enhances receptor tyrosine kinase-dependent signaling system in colon cancer cells. *Biological Chemistry,* **279**:4287-4293.

401. Shaulian, E. and Karin, M. 2002. AP-1 as a regulator of cell life and death. *Nature Cell Biology,* **4**:E131-E136.

402. Sheng, H., Shao, J., Morrow, D.J.D., Beauchamp, R.D. and Du Bois, R.N. 1998. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Research,* **58**:362-366.

403. Shim, Y.J., Choi, K.Y., Lee, W.C., Kim, M.K., Lee, S.Y. and Lee-Kim, Y.C. 2005. Phospholipid fatty acid patterns in the mucosa of human colorectal adenomas and carcinomas. *Nutrition Research,* **25**(3):261-269.

404. Shishodia, S. and Aggarwal, B.B. 2004. Nuclear factor-κβ a friend or a foe in cancer? *Biochemical Pharmacology,* **68**:1071-1080.

405. Shureiqi, I., Jian, W., Zuo, X., Wu, Y. and Stimmel, J.B. 2003. The 15-lipoxygenase-1 product 13-s-hydroxyoctadecadienoic acid down-regulates PPAR-δ to induce apoptosis in colorectal cancer cells. *Proceeding of the National Academy of Sciences of the USA,* **100**(17**)**: 9968-9973.

406. Shureqei, I., Wu, Y., Chen, D., Yang, W.L., Guan, B., Morris, J.S., Yang, P. Newman, R.A., Broaddus, R., Hamilton, S.R., Lynch, P., Levin, B., Fischer, S.M. and Lippman, S.M. 2005. The critical role of 15-lipoxygenase-1 in colorectal epithelial cell terminal differentiation and tumorigenesis. *Cancer Research,* **65**(24):11486-11492.

407. Silverthorn, D.U., Ober, W.C., Garrison, C.W., Silverthorn, A.C. and Johnson, B.R. 2004. Human Physiology an integrated approach. Chapt 6., Pg. 175-179. Pearson Education, Inc., publishing as Benjamin Cummings, San Francisco. ISBN 0-321-22687-9.

408. Simons, K. and Toomre, D. 2000. Lipid rafts and signal transduction. *Nature Reviews. Molecular Cell Biology,* **1**:31-39.

409. Simopoulos, A.P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy,* **56**: 365- 379.

410. Simopoulos, A.P. 2004. The traditional diet of Greece and cancer. *European Journal of Cancer Prevention,* **13**(3):219-230.

411. Simopoulos, A.P. 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomedicine and Pharmacotherapy,* **60**(9):502-507.

412. Sing, H. 2007. Shaping a helper T cell identity. *Nature Immunology,* **8**(2):119-120.

413. Skrzydlewska, E., Kozuszko, B., Sulkowska, M., Bogdan, Z, Kozlowski, M., Snarska, J. Puchalski, Z., Sulkowski, S. and Skrzydlewski, Z. 2003. Anitoxidant potential in esophageal, stomach and colorectal cancers. *Hepatogastroenterology,* **50**:126-131.

414. Skrzydlewska, E., Sulkowski, S, Koda, M., Zalewski, B., Kanczuga-Koda, L. and Sulkowska, M. 2005. Lipid peroxidation and antioxidant status in colorectal cancer. *World Journal of Gastroenterology,* **11**:403-406.

415. Slattery, M.L., Benson, J. Ma, K-N., Schaffer, D. and Potter, J.D. 2001. *Trans*-fatty acids and colon cancer. *Nutrition and Cancer,* **39**(2):170-175.

416. Smith, W.L. Dewitt, D.L. and Garavito, R.M. 2000. Cyclooxygenases: structural, cellular and molecular biology. *Annual Review of Biochemistry,* **69**:145-82.

417. Smith, C., Marks, A.D. and Lieberman, M. 2005. Mark's Basic Medical Biochemistry: A Clinical Approach. Second edition. Ed. Lippincott: Williams & Wilkins. Chapter 33. Pg 596-613. ISBN: 0-7817-2145-8.

418. Soel, S.M., Choi, O.S., Bang, M.H., Yoon Park, J. H. and Kim, W.K. 2007. Influence of conjugated linoleic acid isomers on the metastasis of colon cancer cells in vitro and in vivo. *Journal of Nutritional Biochemistry,* **18**:650-657.

419. Song, H-J., Grant, I., Rotondo, D., Mohede, I., Sattar, N., Heys, S.D. and Wahle, K.W.J. 2005. Effect of CLA supplementation on immune function in young healthy volunteers. *European Journal of Clinical Nutrition,* **59**:508-517.

420. Soumaoro, L.T. Iida, S., Uetake, H., Ishiguro, M., Takagi, Y., Higuchi, T., Yasuno, M., Enomoto, M. and Sugihara, K. 2006. Expression of 5-lipoxygenase in human colorectal cancer. *World Journal of Gastroenterology,* **12**(39):6355-6360.

421. Stack, E. and Dubois, R.N. 2001. Novel approaches to the prevention of colon cancer by nutritional manipulation and chemoprevention. *Best Practice and Research. Clinical Gastroenterology,* **15***:*787-800.

422. Steele, R.J. 2006. Modern challenges in colorectal cancer. *Surgeon,* **4**(5):285-291.

423. Steinke, J.W. and Borish, M.D. 2006. Cytokines and chemokines. *Journal of Allergy and Clinical Immunology,* **117**:S441-445.

424. Stremmel, W., Kleiners, H. and Fitscher, B.A. 1992. Mechanisms of cellular fatty acid uptake. *Biochemical Society Transaction,* **20**:817-817.

425. Stilwell, W. and Wassall, S.R. 2003. Docosahexanoic acid: membrane properties of a unique fatty acid. *Chemistry and Physics of Lipids,* **126** (1):1-27.

426. Stulnig, T.M., Huber, J., Leitinger, N., Imre E-M., Angelisova', P., Nowotny, P. and Waldhäusl, W. 2001. Polyunsaturated eicosapentaenoic acid displaces proteins from membrane rafts by altering raft lipid composition. *Journal of Biological Chemistry,* **276**:37335-37340.

427. Stulnig, T.M. 2003. Immunomodulation by Polyunsaturated Fatty Acids: Mechanisms and Effects. *International Archives of Allergy and Immunology,* **132**:310-321.

428. Subbaramaiah, K. and Dannenberg, A.J. 2003. Cyclooxygenase 2: a molecular target for cancer prevention and treatment. *Trends in Pharmacological Sciences,* **24**(2):96-102.

429. Swamy, M.V., Cooma, I., Patlolla, J.M.R., Simi, B., Bandaru, S. and Chinthalapally, V.R. 2004. Modulation of cyclooxygenase-2 activities by the combined action of celecoxib and decosahexaenoic acid: Novel strategies for colon cancer prevention and treatment. *Molecular Cancer Therapeutics*, **3**:215-221.

430. Swinnen, J.V., Van Veldhoven, P.P., Timmermans, L., De Schrijver, E., Brusselmans, K., Vanderhoydonc, F., Van de Sande, T., Heemers, H., Heyns, W. and Verhoeven, G. 2003. Fatty acid synthase drives the synthesis of phospholipids partitioning into detergent-resistant membrane microdomains. *Biochemical and Biophysical Research Communications,* **302**:898-903.

431. Takayama, T., Ohi, M., Hayashi, T., Miyanishi, K., Nobuoka, A., Nakajima, T., Satoh, T., Takimoto, R., Kato, J., Sakamaki, S. and Niitsu, Y. 2001. Analysis of K-ras, APC, and beta-catenin in aberrant crypt foci in sporadic adenoma, cancer, and familial adenomatous polyposis. *Gastroenterology,* **121**(3):599-611.

432. Takayama, O., Yamamoto, H., Damdinsuren, B., Sugita, Y., Ngan, C.Y., Xu, X., Tsujino, T., Takemasa, I., Ikeda, M., Sekimoto, M., Matsuura, N. and Monden, M. 2006. Expression of PPARdelta in multistage carcinogenesis of the colorectum: implications of malignant cancer morphology. *British Journal of Cancer,* **9**;95(7):889-895.

433. Taketo, M.M. and Sonoshita, M. 2002. Phospolipase A_2 and apoptosis. *Biochimica et Biophysica Acta,* **1585**:72-76.

434. Tan, N.S., Shaw, N.S., Vinckenbosch, N., Liu, P., Yasmin, R., Desvergne, B., Wahli, W. and Noy, N. 2002. Selective cooperation between fatty acid binding proteins and peroxisome proliferator-activated receptors in regulating transcription. *Molecular and Cellular Biology,* **22**(14**)**:5114-5127

435. Tan, T-T. and Coussens, L.M. 2007. Humoral immunity, inflammation and cancer. *Current Opinion in Immunology,* **19**(2):209-216.

436. Tapiero, H., Nguyen, B.G., Couvreur, P. and Tew, K.D. 2002. Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomedicine and Pharmacotherapy,* **56**:215-222.

437. Tedeling, S., Westberg, F., Kjerrulf, M. and Vidal, A. 2007. Anti-inflammatory properties of the short-chain fatty acids acetate and proprionate: a study with relevance to inflammatory bowel disease. *Journal of Gastroenterology,* **13**(20):2826-2832.

438. Thies, F., Nebe-von-Caron, G., Powell, J., Yaqoob, P., Newsholme, E.A. and Calder, P. 2001a. Dietary supplementation with eicosapentaenoic acid, but not with other long chain n-3 of n-6 polyunsaturated fatty acids, decreases natural killer cell activity in healthy subjects aged > 55 years. *American Journal of Clinical Nutrition,* **73**:539-548.

439. Thies, F., Nebe-von-Caron, G., Powell, J., Yaqoob, P., Newsholme, E.A. and Calder. 2001b. Dietary supplementation with gamma-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. *Nutritional Journal,* **131**:1918-1927.

440. Thum, M.J., Henley, S.J. and Patrono, C. 2002. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. *Journal of the National Cancer Institute,* **94**:252-266.

441. Topping, D.L. and Clifton, P.M. 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews,* **81**(3):1031-1064.

442. Tortola, S.; Marcuello, E.; Gonzalez, I.; Reyes, G.; Arribas, R.; Aiza, G.; Sancho, F.J.; Peinado, M.A.; Capella, G. 1999. p53 and K-ras gene mutations correlate with tumor aggressiveness but are not of routine prognostic value in colorectal cancer. *Journal of Clinical Oncology,* **17**(5):1375-1381.

443. Tosi, M.F. 2005. Innate immune responses to infection. *Journal of Allergy and Clinical Immunology,* **116**:241-249.

444. Tricon, S. and Yaqoob, P. 2006. Conjugated linoleic acid and human health: a critical evaluation of the evidence. *Current Opinion in Clinical Nutrition and Metabolic Care,* **9**(2);105-110.

445. Trombetta, E.S. and Mellman, I. 2005. Cell biology of antigen processing in vitro and in vivo. *Annual Review of Immunology,* **23**:975-1028.

446. Tsan, M-F. 2006. Toll-like receptors, inflammation and cancer. *Seminars in Cancer Biology,* **16**:32-37.

447. Tsujii, M., Kawana, S. and DuBois, R.N. 1997. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proceeding of the National Academy of Science of the United States of America,* **94**:33336-33340.

448. Turner, N., Zhang, J., Davidson, L. Lupton, J. and Chapkin. R. 2002. Oncogenic ras alters sensitivity of mouse colonocytes to butyrate and fatty acid mediated growth arrest and apoptosis. *Cancer Letters,* **186**: 29-35.

449. Tuynman, J.B., Peppelenbosch, M.P. and Richel, D.J. 2004. COX-2 inhibition as a tool to treat and prevent colorectal cancer. *Critical Reviews in Oncology/Hematology,* **52**:81-101.

450. Turpaev, K.T. and Litvinov, D. 2004. Redox-dependent regulation of gene expression induced by nitric oxide. *Molecular Biology (Moskou),* **38**:56-68.

451. Underwood J.C.E. General and Systematic Pathology. 4th edition. Harcourt Publishers Ltd. 2004:393-397. ISBN: 0-443-07335-X.

452. Valko, M., Izakovic, M., Mazue, M., Rhodes, C.J. and Telser, J. 2004. Role of oxygen radicals in DNA damage and cancer incidence. *Molecular and Cellular Biochemistry,* **266**(1-2):37-56.

453. Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. and Mazur, M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions,* **160**:1-40.

454. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M. and Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology,* **39**:44-84.

455. Vamecq, J. and Latruffe, N. 1999. Medical significance of peroxisome proliferator-activated receptors. *Lancet,* **354**:141-148.

456. Van den Elsen, P.J., Hollin, T.M., Kuipers, H. and van der Stoep, N. 2004. Transcriptional regulation of antigen presentation. *Current Opinion in Immunology,* **16**:67-75.

457. Van den Elsen, P.J. and Rudensky, A. 2004. Antigen processing and recognition. Recent developments. *Current Opinion in Immunology,* **16**:63-66.

458. Van der Logt, E.M.J., Roelofs, H.M.J., Wobbes, T., Nagengast, F.M. and Peters, W.H.M. 2005. High oxygen radical production in patients with sporadic colorectal cancer. *Free Radical Biology and Medicine,* **39**(2):182-187.

459. Van Es, J.H., Barker, N. and Clevers, H. 2003. You Wnt some, you lose some: oncogenes in the Wnt signaling pathway. *Current Opinion in Genetics & Development,* **13**(1):28-33.

460. Vang, K. and Ziboh, V.A. 2005. 15-lipoxygenase metabolites of γ-linolenic acid/eicosapentaenoic acid suppress growth and arachidonic acid metabolism in human prostatic adenocarcinoma cells: Possible implication of dietary fatty acids. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* **72**:363-372.

461. Verlengia, R., Gorjao, R. ,Kanunfre, C.C., Bordin, S., De Lima, T.M., Martins, E.F. and Curi, R. 2004. Comparitive effects of eicosapentaenoic acid and docosahexaenoic acid on proliferation, cytokine production, and pleiotropic gene expression in Jurkat cells. *Journal of Nutritional Biochemistry,* **15**:657-665.

462. Vermeulen, L., Van den Berghe, W. and Haegeman, G. 2006. Regulation of NF-kappa B transcriptional activity**.** *Cancer Treatment and Research,* **130**:89-102.

463. Vora, P., Thomas, A.L.S., Fukata, M., Tesfay, S.Y., Lukasek, K., Michelsen, K.S., Wada, A.T., Hirayama, T., Arditi, M. and Abreu, M.T. 2004. Beta-defensin-3 expression is regulated by TLR signaling in intestinal epithelial cells. *Journal of Immunology.* **173**:5398-405.

464. Wahle, K.W.J., Heys, S.D. and Rotondo, D. 2004. Conjugated linoleic acids: are they beneficial or detrimental to health? *Progress in Lipid Research,* **43**:553-587.

465. Wansheng, Y., Murray, N.R., Weems, C., Chen, L., Huiping, G., Ethridge, R., Ceci, J.D., Evers, B.M., Thompson E.A. and Fields, A.P. 2003. Role of cyclooxygenase 2 in Protein Kinase CβII-mediated colon carcinogenesis. *Journal of Biological Chemistry,* **278**(13):11167-11174.

466. Watson, A.J.M. 2006. An overview of apoptosis and the prevention of colorectal cancer. *Critical Reviews in Oncology/Haematology,* **57**(2):107-121.

467. Webster, G.A. and Perkins, N.D. 1999. Transcriptional cross talk between Nuclear Factor –κB and p53. *Molecular and Cellular Biology,* **19**:3485-3495.

468. Wei, E.K., Giovannucci, E., Wu, K., Rosner, B., Fuchs, C.S., Wilett, W.C. and Colditz, G.A. 2004. Comparison of risk factors for colon and rectal cancer. *International Journal of Cancer,* **20**:108(3):433-442.

469. Weitz, J., Koch, M., Debus, J., Höhler, T., Galle, P.R. and Bochler, M.W. 2005. Colorectal cancer. *The Lancet,* **365**:153-165.

470. Welters, H.J., Diakogiannaki, E., Mordue, J.M., Tadayyon, M., Smith, S.A. and Morgan, N.G. 2006. Differential protective effects of palmitoleic acid and cAMP on caspase activation and cell viability in pancreatic beta-cells exposed to palmitate. *Apoptosis,* **11**:1231-1238.

471. Willert, K. and Jones, K.A. 2006. Wnt signaling: is the party in the nucleus? *Genes and Development,* **20**:1394-1404.

472. Wollowski, I., Reckhemmer, G. and Pool-Zobel, B.L. 2001. Protective role of probiotics and prebiotics in colon cancer. *American Journal of Clinical Nutrition,* **73**(2):4515-55.

473. Wu, K., Willett, W.C., Fuchs, C.S., Colditz, G.A. and Giovannucci, E.L. 2002. Calcium intake and risk of colon cancer in women and men. *Journal of the National Cancer Institute,* **94**(6):437-446.

474. Wu, R.; Abramson, A.L; Shikowitz, M.J; Dannnenberg, A.J. and Steinberg, B.M. 2005. Epidermal growth factor-induced cyclooxygenase-2 expression is mediated through phoshatidylinositol-3 kinase, in recurrent respiratory papillomas, *Clinical Cancer Research,* **11**:6155-6161

475. Wynder, E.L., Kajitani, T., Ishikawa, S., Dodo, H. and Takano, A. 1969. Environmental factors of cancer of the colon and rectum. II. Japanese epidemiological data. *Cancer,* **23**(5):1210-1220.

476. Yamac, D., Celenkoglu, G., Coskum, U., Akyurek, N, Akcali, Z., Dursun, A. and Koybasioglu, F. 2005. Prognostic importance of COX-2 expression in patients with colorectal cancer. *Pathology, Research and Practice,* **201**(7):497-502.

477. Yaqoob, P. 2003a. Fatty acids as gatekeepers of immune cell regulation. *Trends in Immunology,* **24**:639-645.

478. Yaqoob, P. 2003b. Lipids and the immune response: from molecular mechanism to clinical applications. *Current Opinion in Clinical Nutrition and Metabolic Care,* **6**:133-150.

479. Yaqoob, P. 2004. Fatty acids and the immune system: from basic science to clinical applications. *The Proceedings of the Nutritional Society,* **63**(1):89-104.

480. Yeh, C-S., Wang, J-W., Cheng, T-L., Juan, C-H., Wu, C-H. and Lin, S-R. 2006. Fatty acid metabolism pathways play an important role in carcinogenesis of human colorectal cancers by Microarray-Bioinformatics analysis. *Cancer Letters,* **233**(2):297-308.

481. Yona, D. and Arber, N. 2006. Coxibs and cancer prevention. *Journal of Cardiovascular Pharmacology,* **47**:S76-S81.

482. Yoshinaga, M., Murao, H., Kitamura, Y., Koga, K., Tsuruta, S., Igarashi, H., Nakamura, K. and Tkayanagi, R. 2007. The 15-lipoxygenase-1 expression may enhance the sensitivity to non-steroidal anti-inflammatory drug-induced apoptosis in colorectal cancers from patients who are treated with the compounds. *Journal of Gastroenterology and Hepatology,* Published online 2007 Jun 7.

483. Young, M.R., Yang, H-S. and Colburn N.H. 2003. Promising molecular targets for cancer prevention: AP-1, NF-KB and Pdcd4. *Trends in Molecular Medicine,* **9**:36-41.

484. Yu, L. 2001. Free radical scavenging properties of conjugated linoleic acids. *Journal of Agricultural and Food Chemistry,* **49**:3452-3456.

485. Yu, H.G., Yu, L.L., Yang, Y., Luo, H.S., Yu, J.P., Meier, J.J., Schrader, H., Bast, A., Schmidt, W.E. and Schmitz, F. 2003a. Increased expression of RelA/nuclear factor-kappa B protein correlates with colorectal tumorigenesis. *Oncology,* **65**(1):37-45.

486. Yu, W., Murray, N.R., Weems, C., Chen, L., Guo, H., Ethridge, R., Cecei, D.C., Evers, B.M., Thompson, E.A. and Fields, A.P. 2003b. Role of cyclooxygenase-2 in Protein Kinase C βII-mediated colon carcinogenesis. *The Journal of Biological Chemistry,* **278**(13):11167-11174.

487. Yuri, M., Sasahira, T., Nakai, K., Ishimaru, S., Ohmori, H. and Kuniyasu, H. 2007. Reversal of expression of 15-lipoxygenase-1 to cyclooxygenase-2 is associated with development of colonic cancer. *Histopathology,* **51**(4):520-527.

488. Yuspa, S.H. 2000. Overview of carcinogenesis: past, present and future. *Carcinogenesis,* **21**:341-344.

489. Zbar, A.P. 2004. The immunology of colorectal cancer. *Surgical Oncology,* **13**(2-3):45-53.

490. Zeyda, M., Szekers, A.B., Säemann, M.D., Geyeregger, R., Stockinger, H., Zlabinger, G.J., Waldhäusl, W., and Stulnig T.M 2003. Suppression of T cell signaling by polyunsaturated fatty acids: Selectivity in inhibition of mitogen-activated protein kinase and nuclear factor activation. *Journal of Immunology,* **170**:6033-6039.

491. Zhang, X. and Young, H.A. 2002. PPAR and immune system-what do we know? *International Immunopharmacology,* **2**(8**)**:1029-1044.

492. Zhang, P., Smith, R., Chapkin, R.S. and McMurray, D.N. 2005. Dietary (n-3) polyunsaturated fatty acids modulate murine Th1/Th2 balance toward the Th2 pole by suppression of Th1 development. *The Journal of Nutrition,* **135**(7):1745-1751.

493. Zhang, P., Kim, W., Zhou, L., Wang, N., Ly, L.H., McMurray, D.N. and Chapkin, R.S. 2006. Dietary fish oil inhibits antigen-specific murine Th1 cell development by suppression of clonal expression. *The Journal of Nutrition,* **136**(9**)**:2391-2398.

494. Zuo, X., Wu, Y., Morris, J.S., Stimmel, J.B., Leesnitzer, L.M., Fischer, S.M., Lippman, S.M. and Shureiqi, I. 2006. Oxidative metabolism of linoleic acid modulates PPAR-beta/delta suppression of PPAR-gamma activity. *Oncogene,* **25**: 1225-1241.

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ACF	Aberrant crypt foci
ALA	Alpha-linolenic acid
AP-1	Activator protein -1
APC-gene	Adenomatous polyposis coli-gene
APC	Antigen-presenting cell
ATP	Adenosine triphosphate
BA	Butyric acid
CLA	Conjugated linoleic acid
COX-2	Cyclooxygenase-2
PLA_2	Phospholipase A2
Ca	Calsium
CRC	Colorectal cancer
CSF	Colony-stimulating factor
CTL	Cytotoxic T-lymphocyte
DAG	Diacylglycerol
DGLA	Dihomo-gamma-linolenic acid
DHA	Docosahexaenoic acid
EFA	Essential fatty acid
EFAD	Essential fatty acid deficiency
EFAM	Essential fatty acid metabolism
EGF	Epidermal growth factor
EPA	Eicosapentaenoic acid
ERK	Extracellular regulated kinase
FAP	Familial adenomatous polyposis
FA	Fatty acid
FAS	Fatty acid synthase
FFAs	Free fatty acids
FGF	Fibroblast growth factor
GLA	Gamma-linolenic acid
HBD	Human β-defensin
HNPCC	Hereditary nonpolyposis colorectal cancer
HETE	Hydroxyeicosatetraenoic acid

HEPE	Hydroxyeicosapentaenoic acid
HLA	Human leucocyte antigen
HODE	Hydroxyoctadecadienoic acid
HPETE	Hydroperoxyeicosatetraenoic acid
IFN	Interferon
IGF	Insulin growth factor
IGFBP	Insulin growth factor binding protein
IGF-II	Insulin-like growth factor-II
Ig	Immunoglobulin
IL	Interleukin
iNOS	Inducible nitric oxide synthase
JNK	c-Jun N-terminal kinase
LA	Linoleic acid
LOX	Lipoxygenase
5-LOX	5-lipoxygenase
LPS	Lipopolysaccharids
LRA	Lauric acid
LT	Leukotriene
LX	Lipoxins
MA	Myristic acid
MHC	Major histocompatibility complex
Mg	Magnesium
MAPKS	Mitogen-activated protein kinase
OA	Oleic acid
ODC	Ornithine decarboxylase
NDC	Nuclear dehydrogenating clostridia
NF-kβ	Nuclear factor kappa-Beta
NK	Natural Killer
NO	Nitric oxide
NSAID	Non-steroid anti- inflammatory drug
PA	Palmitic acid
PAMP	Pathogen associated molecular pattern
PBS	Phosphate buffered saline
PC	Phosphatidylcholine
PDGF	Platelet derived growth factor
PE	Phosphatidylethanolamine

PG	Prostaglandin
PGE_2	Prostaglandin E_2
PI	Phosphatidylinositol
PKC	Protein kinase
PL	Phospholipase
PLA	Palmitoleic acid
PS	Phosphatidylserine
PPAR	Peroxisome proliferator activated receptor
PRR	Pattern recognition receptor
PUFA	Polyunsaturated fatty acid
RER	Rough endoplasmic reticulum
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
SA	Stearic acid
SCFA	Short chain fatty acid
Se	Selinium
sIg	Surface membrane
TCR	T cell receptor
Tcf	T-cell factor
TGF-β	Transforming growth factor- Beta
TLR	Toll-like receptor
TMSH	Trimethyl sulphonium hydroxide
TNF	Tumor necrosis factor
TX	Thromboxane
VA	Vaccenic acid
VEGF	Vascular endothelial growth factor
WNT	Wingless-type
Zn	Zinc

LIST OF TABLES

LIST OF FIGURES