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Linoleic Acid: The Code of Life?

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Abstract

Intralipid® 20% is made up of 20% Soybean Oil, 1.2% Egg Yolk Phospholipids, 2.25% Glycerine, and Water for Injection. The major component fatty acids are linoleic acid (44-62%), oleic acid (19-30%), palmitic acid (7-14%), a-linolenic acid (4-11%) and stearic acid (1.4-5.5%). It means that the various effects of Intralipid are based 63% to 92% on linoleic acid and oleic acid.

Are these 2 fatty acids the Code of Life?

Linoleic acid has effects on the mitochondria. Linoleic acid has effects on Cancer. Linoleic acid has effects on Aging.

Keywords Linoleic acid, Oleic acid, Intralipid, Mitochondria, Cancer, Aging.

The Basis of Intralipid

Intralipid® 20% (A 20% I.V. Fat Emulsion) Pharmacy Bulk Package is a sterile, non-pyrogenic fat emulsion intended as a source of calories and essential fatty acids for use in a pharmacy ad- mixture program. It is made up of 20% Soybean Oil, 1.2% Egg Yolk Phospholipids, 2.25% Glycerine, and Water for Injection. In addition, sodium hydroxide has been added to adjust the pH so that the final product pH is 8. pH range is 6 to 8.9.

The soybean oil is a refined natural product consisting of a mixture of neutral triglycerides of predominantly unsaturated fatty acids. The major component fatty acids are linoleic acid (44-62%), oleic acid (19-30%), palmitic acid (7-14%), a-linolenic acid (411%) and stearic acid (1.4-5.5%).

It means that the various effects of Intralipid are based 63% to 92% on linoleic acid and oleic acid. Are these 2 fatty acids the Code of Life?

Linoleic acid belongs to one of the two families of essential fatty acids, which means that the human body cannot synthesize it from other food components.

Linoleic Acid ($C_{18}H_{32}O_2$)

A carboxylic acid, is a polyunsaturated omega-6 fatty acid, an 18-carbon chain with two double bonds in cisconfiguration. A shorthand notation like “18:2 (n-6)” or “18:2 cis-9, 12” may be used in literature. It typically occurs in nature as a triglyceride ester; free fatty acids are typically low in foods.

Linoleic acid belongs to one of the two families of essential fatty acids, which means that the human body cannot synthesize it from other food components. The word “linoleic” derived from the Greek word linon (flax). Oleic means “of, relating to, or derived from oil of olive” or

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"of or relating to oleic acid" because saturating the omega-6 double bond produces oleic acid.

Oleic acid ($C_{18}H_{34}O_2$)

Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is an odourless, colourless oil, though commercial samples may be yellowish. In chemical terms, oleic acid is classified as a monounsaturated omega-9 fatty acid, abbreviated with a lipid number of 18:1 cis-9. It has the formula $CH_3(CH_2)7CH=CH(CH_2)7COOH$. The term "oleic" means related to, or derived from, olive oil which is mostly composed of oleic acid.

Linoleic Acid and Cancer

Conjugated Linoleic Acid (CLA) is fatty acid found endogenously in food sources that prevents new tumor development and reduces the growth of existing tumors in laboratory animals. CLA exerts its anti-carcinogenic effect by reducing VEGF and bFGF serum levels and by blocking flk-1 receptors, thereby inhibiting vascular growth critical to tumor growth and survival. Although the ability of CLA to inhibit angiogenesis in the peripheral nervous system is well characterized, it remains unknown whether CLA also affects vascular morphology in the central nervous system. Therefore, in the present study, exercising and sedentary animals received either standard rat chow or a specially formulated diet consisting of 0.5% CLA for 24 days. The brains were then examined to determine the extent of vascular growth in the cerebellum, a region known to exhibit robust exercise-induced angiogenesis. Our results indicate that CLA administration significantly reduces angiogenesis in the cerebellum. This study is the first to demonstrate the anti-angiogenic effect of CLA in the brain, and suggests that CLA be explored as a therapeutic treatment for cancer and tumors in the brain [1].

Some polyunsaturated fatty acids (PUFAs), if not all, have been shown to have tumoricidal action, but their exact mechanism(s) of action is not clear. In the present study, we observed that n-6 PUFA linoleic acid (LA) inhibited tumor cell growth at high concentrations (above 300 μM); while low concentrations (100200 μM) promoted proliferation [2]. Analysis of cell mitochondrial membrane potential, reactive oxygen species (ROS) formation, malondialdehyde (MDA) accumulation and superoxide dismutase (SOD) activity suggested that anti-cancer action of LA is due to enhanced ROS generation and decreased cell antioxidant capacity that resulted in mitochondrial damage. Of the three cell lines tested, semi-differentiated colorectal cancer cells RKO were most sensitive to the cytotoxic action of LA, followed by undifferentiated colorectal cancer cell line (LOVO) while the normal human umbilical vein endothelial cells (HUVEC) were the most resistant (the degree of sensitivity to LA is as follows: RKO>LOVO>HUVEC). LA induced cell death was primed by mitochondrial apoptotic pathway. Preincubation of cancer cells with 100 μM LA for 24 hr enhanced sensitivity of differentiated and semi-differentiated cells to the subsequent exposure to LA. The relative resistance of LOVO cells to the cytotoxic action of LA is due to a reduction in the activation of caspase-3. Thus, LA induced cancer cell apoptosis by enhancing cellular oxidant status and inducing mitochondrial dysfunction [2].

Colorectal cancer is common in developed countries. Polyunsaturated fatty acids (PUFAs) have been reported to possess tumoricidal action, but the exact mechanism of their action is not clear.

In the present study, we studied the effect of various n-6 and n-3 fatty acids on the survival of the colon cancer cells LoVo and RKO and evaluated the possible involvement of a mitochondrial pathway in their ability to induce apoptosis [3].

It was observed that n-3 α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid (ALA, EPA and DHA respectively) and n-6 linoleic acid, gamma linolenic acid and arachidonic acid (LA, GLA and AA respectively) induced apoptosis of the colon cancer cells LoVo and RKO at concentrations above 120 μM ($p< 0.01$ compared to control). The semi-differentiated colon cancer cell line RKO was more sensitive to the cytotoxic action of PUFAs compared to the undifferentiated colon cancer cell line LoVo. PUFA treated cells showed an increased number of lipid droplets in their cytoplasm. PUFA-induced apoptosis of LoVo and RKO cells is mediated through a mitochondria-mediated pathway as evidenced by loss of mitochondrial membrane potential, generation of ROS, accumulation of intracellular Ca(2+), activation of caspase-9 and caspase-3, decreased ATP level and increase in the Bax/Bcl2 expression ratio.

PUFAs induced apoptosis of colon cancer cells through a mitochondrial dependent pathway [3]. Activity and expression of fatty acid synthase (FAS), a critical enzyme in the de novo biosynthesis of fatty acids in mammals, is exquisitely sensitive to nutritional regulation of lipogenesis in liver or adipose tissue. Surprisingly, a number of studies have demonstrated hyperactivity and overexpression of FAS (oncogenic antigen-519) in a biologically aggressive subset of human breast carcinomas, suggesting that FAS-dependent neoplastic lipogenesis is unresponsive to nutritional regulation. We have assessed the role of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) on the enzymatic activity and protein expression of tumor-associated FAS in SK-Br3 human breast cancer cells, an experimental paradigm of FAS over expressing tumor cells in which FAS enzyme constitutes up to 28%, by weight, of the cytosolic proteins [4]. Of the omega-3 PUFAs tested, alpha-linolenic acid (ALA) dramatically reduced FAS activity in a dose-dependent manner (up to 61%). omega-3 PUFA docosahexaenoic acid (DHA) demonstrated less marked but still significant inhibitory effects on FAS activity (up to 37%), whereas eicosapentaenoic acid (EPA) was not effective. Of the omega-6 fatty acids tested, gamma-linolenic acid (GLA) was the most effective dose-dependent inhibitor of FAS activity, with a greater than 75% FAS activity reduction. Remarkably, omega-6 PUFAs linoleic acid (LA) and arachidonic acid (ARA), suppressors of both hepatic and adipocytic FAS-dependent lipogenesis, had no significant inhibitory effects on the activity of tumor associated FAS in SK-Br3 breast cancer cells. Western blotting studies showed that down-regulation of FAS protein expression tightly correlated with

previously observed inhibition of FAS activity, suggesting that ALA-, DHA-, and GLA-induced changes in FAS activity resulted from effects at the protein level. We investigated whether the FAS inhibitory effect of GLA and omega-3 PUFAs correlated with a cytotoxic effect related to a per-oxidative mechanism [4]. Measurement of cell viability by MTT assay indicated a significant cellular toxicity after ALA and GLA exposures. Furthermore, we observed a significant correlation between the ability of PUFAs to repress FAS and cause cell toxicity. In the presence of anti-oxidants (vitamin E), ALA and GLA dramatically lost their ability to inhibit FAS activity.

Interestingly, a combination of ALA and GLA was FAS inhibitory in an additive manner, and this FAS repression was only partially reversible by vitamin E. In examining the molecular mechanisms underlying resistance of breast cancer-associated FAS to normal dietary fatty acid-induced suppression, a dramatic decrease of FAS accumulation was found after exposure of SK-Br3 cells to mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (MAPK ERK1/2) inhibitor U0126, phosphatidylinositol-3'-kinase (PI-3'K) blocker LY294002, and/or anti-HER-2/neu antibody trastuzumab. Interestingly, a long-term exposure to pharmacological inhibitors of FAS activity cerulenin [(2S,3R) 2,3-epoxy-4-oxo7E,10E-dodecadienamide] or C75 also resulted in a significant reduction of FAS accumulation. These data indicate that: a) GLA- and omega-3 PUFA-induced repression of tumor associated FAS may result, at least in part, from a non-specific cytotoxic effect due to per-oxidative mechanisms; b) alternatively, GLA and omega-3 PUFAs have a suppressive effect on FAS expression and activity that can result in the accumulation of toxic fluxes of the FAS substrate malonyl-CoA; c) GLA- and/or omega-3 PUFA-induced repression of tumor associated FAS may represent a novel mechanism of PUFA induced cytotoxicity clinically useful against breast carcinomas carrying overexpression of FAS enzyme; d) fundamental differences in the ability of FAS gene to respond to normal fatty acid's regulatory actions in lipogenic tissues may account for the observed extremely high levels of FAS in breast carcinoma; and e) FAS overexpression in SK-Br3 breast cancer cells is driven by increases in HER-2/neu signaling, acting in major part through a constitutive downstream art through a constitutive downstream activation of the MAPK ERK1/2 and PI-3'K/AKT transduction cascades [4].

The expression and activity of Fatty Acid Synthase (FASN; the sole enzyme capable of the reductive de novo synthesis of long chain fatty acids from acetyl-CoA, malonyl-CoA, and nicotinamide adenine dinucleotide phosphate -NADPH-) is extremely low in nearly all non-malignant adult tissues, whereas it is significantly up-regulated or activated in many cancer types, thus creating the potential for a large therapeutic index. Since the pioneering observation that inhibition of FASN activity by the mycotoxin cerulenin preferentially kills cancer cells and retards the growth of tumors in xenografts models, numerous *in vitro* and *in vivo* studies have confirmed the potential of FASN as a target for antineoplastic intervention. Other FASN inhibitors such as the cerulenin derivative C75, the beta-lactone orlistat, the green tea polyphenol epigallocatechin-3-gallate (EGCG) and other naturally occurring flavonoids (i.e., luteolin, quercetin, and kaempferol), as well as the antibiotic triclosan, have been identified and have been shown to limit cancer cell growth by inducing apoptotic cell death. Though the exact mode of action of these FASN inhibitors is under discussion, it has been revealed that depletion of end-product fatty acids, toxic intracellular accumulation of supra-physiological concentrations of the FASN substrate malonyl-CoA and/or limited membrane synthesis and/or functioning by altered production of phospholipids partitioning into detergent-resistant membrane microdomains (lipid raft-aggregates), can explain, at least in part, the cytostatic, cytotoxic as well as the apoptotic effects occurring upon pharmacological inhibition of FASN activity in cancer cells.

Moreover, several cancer-associated molecular features including nonfunctioning p53, overexpression of the Her-2/neu (erbB-2) oncogene, and hyperactivation of the PI-3'K downstream effector protein kinase B (AKT), appear to determine an exacerbated sensitivity to FASN inhibition-induced cancer cell death. Although few of these inhibitors are expected to be "exclusively" selective for FASN, the potential of FASN as a target for antineoplastic intervention has eventually been confirmed by RNA interference (RNAi)-knockdown of FASN. Certainly, future studies should definitely elucidate the ultimate biochemical link between FASN inhibition and cancer cell death. Although the combination of FASN structural complexity and until recently the lack of X-ray crystallography data of mammalian FASN created a significant challenge in the exploitation of FASN as a valuable target for drug development, it is hoped that the improvement in the selectivity and potency of forthcoming novel FASN-targeted small molecule inhibitors by taking advantage, for instance, of the recent 4.5 A resolution X-ray crystallographic map of mammalian FASN, will direct the foundation of a new family of chemotherapeutic agents in cancer history [5].

Obesity with excessive levels of circulating free fatty acids (FFAs) is tightly linked to the incidence of type 2 diabetes. Insulin resistance of peripheral tissues and pancreatic β-cell dysfunction are two major pathological changes in diabetes and both are facilitated by excessive levels of FFAs and/or glucose. To gain insight into the mitochondrial mediated mechanisms by which long-term exposure of INS-1 cells to excess FFAs causes β-cell dysfunction, the effects of the unsaturated FFA linoleic acid (C 18:2, n-6) on rat insulinoma INS-1 β cells was investigated. INS-1 cells were incubated with 0, 50, 250 or 500 μM linoleic acid/0.5% (w/v) BSA for 48 h under culture conditions of normal (11.1 mM) or high (25 mM) glucose in serum-free RPMI-1640 medium. Cell viability, apoptosis, glucose-stimulated insulin secretion, Bcl-2, and Bax gene expression levels, mitochondrial membrane potential and cytochrome c release were examined. Linoleic acid 500 μM significantly suppressed cell viability and induced apoptosis when administered in 11.1 and 25 mM glucose culture medium. Compared with control, linoleic acid 500 μM significantly increased Bax expression in 25 mM glucose culture medium but not in 11.1 mM glucose culture medium. Linoleic acid also dose-dependently reduced

mitochondrial membrane potential (ΔΨm) and significantly promoted cytochrome c release from mitochondria in both 11.1 mM glucose and 25 mM glucose culture medium, further reducing glucose stimulated insulin secretion, which is dependent on normal mitochondrial function. With the increase in glucose levels in culture medium, INS-1 β-cell insulin secretion function was deteriorated further. The results of this study indicate that chronic exposure to linoleic acid-induced β-cell dysfunction and apoptosis, which involved a mitochondrial-mediated signal pathway, and increased glucose levels enhanced linoleic acid induced β-cell dysfunction [6].

Conjugated linoleic acid, a functional lipid, produced from Lactobacillus plantarum (LPCLA), has been demonstrated to possess apoptotic activity. The antiproliferative and apoptotic potential of LPCLA was here evaluated *in vitro* using the MDAMB231 human breast cancer cell line as a model system. Proliferation of MDA MB231 cells was inhibited with increasing concentrations of LPCLA with altered morphological features like cell detachment, rounding of cells and oligo nucleosomal fragmentation of DNA. Flow cytometry confirmed the apoptotic potential of LPCLA by ANNEXIN V/PI double staining. Furthermore, outcome results indicated that the apoptosis was mediated by downregulation of the NFkB pathway which in turn acted through proteasome degradation of IkBα, inhibition of p65 nuclear translocation, release of cytochromeC from mitochondria and finally overexpression of Bax protein. Thus, conjugated linoleic acid, a natural product derived from probiotics, could therefore be a possible potential chemotherapeutic agent due to its apoptotic activity against estrogen receptor negative breast cancer cells [7].

HAMLET (human α-lactalbumin made lethal to tumour cells) is a complex of α-lactalbumin (aLA) and oleic acid (OA) which kills transformed cells, while leaving fully differentiated cells largely unaffected. Other protein-lipid complexes show similar anticancer potential. We call such complexes liprotides [8]. The cellular impact of liprotides, while intensely investigated, remains unresolved. To address this, we report on the cell killing mechanisms of liprotides prepared by incubating aLA with OA for 1 h at 20 or 80°C (lip20 and lip80, respectively). The liprotides showed similar cytotoxicity against MCF7 cells, though lip80 acts more slowly, possibly due to intermolecular disulphide bonds formed during preparation. Liprotides are known to increase the fluidity of a membrane and transfer OA to vesicles, prompting us to focus on the effect of liprotides on the cell membrane. Extracellular Ca2+ influx is important for activation of the plasma membrane repair system, and we found that removal of Ca2+ from the medium enhanced the liprotides' killing effect. Liprotide cytotoxicity was also increased by knockdown of Annexin A6 (ANXA6), a protein involved in plasma membrane repair. We conclude that MCF7 cells counteract liprotide-induced membrane permeabilization by activating their plasma membrane repair system, which is triggered by extracellular Ca2+ and involves ANXA6 [8].

Glucose consumption in many types of cancer cells, in particular hepatocellular carcinoma (HCC), was followed completely by over-expression of type II hexokinase (HKII). This evidence has been used in modern pharmacotherapy to discover therapeutic target against glycolysis in cancer cells. Bromo pyruvate (BrPA) exhibits antagonist property against HKII and can be used to inhibit glycolysis. However, the clinical application of BrPA is mostly combined with inhibition effect for healthy cells particularly erythrocytes. Our strategy is to encapsulate BrPA in a selected vehicle, without any leakage of BrPA out of vehicle in blood stream. This structure has been constructed from chitosan embedded into oleic acid layer and then coated by dual combination of folic acid (FA) and bovine serum albumin (BSA). With FA as specific ligand for cancer folate receptor and BSA that can be an easy binding for hepatocytes, they can raise the potential selection of carrier system [9].

Erlotinib (ETB) is a well-established therapeutic for non-small cell lung cancer (NSCLC). To overcome drug resistance and severe toxicities in the clinical application, redox-responsive and pH-sensitive nanoparticle drug delivery systems were designed for the encapsulation of ETB.

Poly(acrylic acid)-cystamine-oleic acid (PAA-ss-OA) was synthesized. PAA-ss-OA-modified ETB-loaded lipid nanoparticles (PAA-ETB-NPs) were prepared using the emulsification and solvent evaporation method. The tumor inhibition efficacy of PAA-ETB-NPs was compared with that of ETB-loaded lipid nanoparticles (ETB-NPs) and free ETB anticancer drugs in tumor-bearing mice.

PAA-ETB-NPs had a size of 170 nm, with a zeta potential of -32 mV. The encapsulation efficiency and drug loading capacity of PAA-ETB-NPs were over 85% and 2.6%, respectively. *In vitro* cytotoxicity of ETB-NPs were higher than that of ETB solution. The cytotoxicity of PAA-ETB-NPs was the highest. The *in vivo* tumor growth inhibition by PAA-ETB-NP treatment was significantly higher than that by ETB-NPs and ETB solution. No obvious weight loss was observed in any of the treatment groups, indicating that all the treatments were well tolerated.

PAA-ETB-NPs could enhance the stability and anti-cancer ability of ETB to treat lung cancer and are a promising drug delivery system for lung cancer treatment [10].

Triple negative breast cancer (TNBC) comprises ~20% of all breast cancers and is the most aggressive mammary cancer subtype. Devoid of the estrogen and progesterone receptors, along with the receptor tyrosine kinase ERB2 (HER2) that define most mammary cancers, there are no targeted therapies for patients with TNBC. This, combined with a high metastatic rate and a lower 5-year survival rate than for other breast cancer phenotypes, means there is significant unmet need for new therapeutic strategies. Herein, the anti-neoplastic effects of the electrophilic fatty acid nitro alkene derivative, 10-nitro-octadec-9enoic acid (nitro-oleic acid, NO2-OA), were investigated in multiple preclinical models of TNBC. NO2-OA reduced TNBC cell growth and viability *in vitro*, attenuated tumor necrosis factor α (TNFα)-induced TNBC cell migration and invasion and inhibited the tumor growth of MDA-MB-231 TNBC cell xenografts in the mammary fat pads of female nude mice. The upregulation of these aggressive tumor cell growth,

migration and invasion phenotypes is mediated in part by the constitutive activation of pro-inflammatory nuclear factor kappa B (NF-κB) signaling in TNBC. NO_2-OA inhibited TNFα-induced NF-κB transcriptional activity in human TNBC cells and suppressed downstream NFκB target gene expression, including the metastasis-related proteins intercellular adhesion molecule-1 and urokinase-type plasminogen activator. The mechanisms accounting for NF-κB signaling inhibition by NO2-OA in TNBC cells were multifaceted, as NO_2-OA a) inhibited the inhibitor of NF-κB subunit kinase β phosphorylation and downstream inhibitor of NF-κB degradation, b) alkylated the NF-κB RelA protein to prevent DNA binding and c) promoted RelA polyubiquitination and proteasomal degradation. Comparisons with non-tumorigenic human breast epithelial MCF-10A and MCF7 cells revealed that NO_2-OA more selectively inhibited TNBC function. This was attributed to more facile mechanisms for maintaining redox homeostasis in normal breast epithelium, including a more favorable thiol/disulfide balance, greater extents of multi-drug resistance protein-1 (1) expression and greater MRP1-mediated efflux of NO_2-OA-glutathione conjugates. These observations reveal that electrophilic fatty acid nitro alkenes react with more alkylation sensitive targets in TNBC cells to inhibit growth and viability [11].

Gastric cancer and breast cancer have a clear tendency toward metastasis and invasion to the microenvironment predominantly composed of adipocytes. Oleic acid is an abundant monounsaturated fatty acid that releases from

adipocytes and impinges on different energy metabolism responses. The effect and underlying mechanisms of oleic acid on highly metastatic cancer cells are not completely understood. We reported that AMP-activated protein kinase (AMPK) was obviously activated in highly aggressive carcinoma cell lines treated by oleic acid, including gastric carcinoma HGC-27 and breast carcinoma MDA-MB-231 cell lines [12]. AMPK enhanced the rates of fatty acid oxidation and ATP production and thus significantly promoted cancer growth and migration under serum deprivation. Inactivation of AMPK attenuated these activities of oleic acid. Oleic acid inhibited cancer cell growth and survival in low metastatic carcinoma cells, such as gastric carcinoma SGC7901 and breast carcinoma MCF-7 cell lines. Pharmacological activation of AMPK rescued the cell viability by maintained ATP levels by increasing fatty acid β-oxidation. These results indicate that highly metastatic carcinoma cells could consume oleic acid to maintain malignancy in an AMPK-dependent manner. Our findings demonstrate the important contribution of fatty acid oxidation to cancer cell function [12].

Both oleic acid (OA) and alpha-linolenic acid (ALA) have been proposed to down-regulate cell proliferation of prostate, breast, and bladder cancer cells. However, direct evidence that OA and/or ALA suppresses to the development of esophageal cancer has not been studied. Also, no previous studies have evaluated how OA and/or ALA regulates malignant potential (cell proliferation, migration, colony formation and adhesion) and intracellular signaling pathways, and whether their effects might be synergistic and/or additive in esophageal cancer cells has not yet been elucidated.

We conducted *in vitro* studies and evaluated whether OA and ALA alone or in combination may regulate malignant potential in OE19 and OE33 esophageal cancer cell lines [13]. Both OA and ALA significantly down-regulated cell proliferation, adhesion and/or migration. OA and/or ALA did not change the number of colonies but decrease colony sizes when compared to control. Also, we observed that OA and/or ALA positively cross-regulates the expression levels of AMPK/S6 axis. Moreover, OA and ALA up-regulated tumor suppressor genes (p53, p21, and p27) and these effects are abolished by AMPK siRNA administration. Importantly, we observed that these effects are additively regulated by OA and ALA in combination when compared to control in OE19 and OE33 esophageal cancer cell lines.

Our novel mechanistic studies provide evidence for an important role for OA and ALA in esophageal cancer, and suggest that OA and/or ALA might Our novel mechanistic studies provide evidence for an important role for OA and ALA in esophageal cancer, and suggest that OA and/or ALA might be useful agents in the management or chemoprevention of esophageal cancer [13].

A proper balance between saturated and unsaturated fatty acids (FAs) is required for maintaining cell homeostasis. The increased demand of FAs to assemble the plasma membranes of continuously dividing cancer cells might unbalance this ratio and critically affect tumour outgrowth. We unveiled the role of the stearoyl-CoA desaturase SCD5 in converting saturated FAs into mono-unsaturated FAs during melanoma progression [14]. SCD5 is down-regulated in advanced melanoma and its restored expression significantly reduced melanoma malignancy, both *in vitro* and *in vivo*, through a mechanism governing the secretion of extracellular matrix proteins, such as secreted protein acidic and rich in cysteine (SPARC) and collagen IV and of their proteases, such as cathepsin B. Enforced expression of SCD5 or supplementation of its enzymatic product, oleic acid, reduced the intracellular pH (pHe>pHi) and, in turn, vesicular trafficking across plasma membranes as well as melanoma dissemination. This intracellular acidification appears also to depend on SCD5 induced reduction of the C2 subunit of the vacuolar H(+) ATPase, a proton pump whose inhibition changes the secretion profile of cancer cells. Our data support a role for SCD5 and its enzymatic product, oleic acid, in protection against malignancy, offering an explanation for the beneficial Mediterranean diet. Furthermore, SCD5 appears to functionally connect tumor cells and the surrounding stroma toward modification of the tumor microenvironment, with consequences on tumor spread and resistance to treatment [14].

The excess of saturated free fatty acids, such as palmitic acid, that induces lipotoxicity in hepatocytes, has been implicated in the development of non-alcoholic fatty liver disease also associated with insulin resistance. By contrast, oleic acid, a monounsaturated fatty acid, attenuates the effects of palmitic acid. We evaluated whether palmitic acid is directly associated with both insulin resistance

and lipoapoptosis in mouse and human hepatocytes and the impact of oleic acid in the molecular mechanisms that mediate both processes [15]. In human and mouse hepatocytes palmitic acid at a lipotoxic concentration triggered early activation of endoplasmic reticulum (ER) stress-related kinases, induced the apoptotic transcription factor CHOP, activated caspase 3 and increased the percentage of apoptotic cells. These effects concurred with decreased IR/IRS1/Akt insulin pathway. Oleic acid suppressed the toxic effects of palmitic acid on ER stress activation, lipoapoptosis and insulin resistance. Besides, oleic acid suppressed palmitic acid-induced activation of S6K1. This protection was mimicked by pharmacological or genetic inhibition of S6K1 in hepatocytes. In conclusion, this is the first study highlighting the activation of S6K1 by palmitic acid as a common and novel mechanism by which its inhibition by oleic acid prevents ER stress, lipoapoptosis and insulin resistance in hepatocytes [15].

Telomerase is a ribonucleoprotein complex that elongates telomeric DNA and appears to play an important part in the cellular immortalization of cancers. In the screening of potent inhibitors of human telomerase, several inhibitors have been discovered from natural and chemical sources. Some compounds potently inhibit the activity of human telomerase. Rubromycins and fatty acids such as β-rubromycin and oleic acid, respectively, were found to be inhibitors of human telomerase. The IC(50) values of β-rubromycin and oleic acid were 8.60 and 8.78 μM, respectively. A kinetic study revealed that these compounds competitively inhibited the activity of telomerase with respect to the substrate of the primer and dNTP. The energy-minimized three-dimensional structure of β-rubromycin and oleic acid was calculated and designed. The V-shaped curve and molecule length of 18.7-20.3 Å in these compound structures were suggested to be important for telomerase inhibition. The three-dimensional structure of the active site of telomerase (i.e., the binding site of the primer and dNTP substrate) might have a “pocket” that could “join” these compounds. These results appear to suggest a potential structure for the development of more potent inhibitors of human telomerase [16].

Oleic Acid (OA) has been shown to have anticancer properties mediated by interaction with proteins such as α-lactalbumin and lactoferrins. Therefore, we synthesized complexes of OA and Gc protein-derived macrophage activating factor (GcMAF) that inhibits per se cancer cell proliferation and metastatic potential [17]. We hypothesised that OA-GcMAF complexes could exploit the anticancer properties of both OA and GcMAF in a synergistic manner. We postulated that the stimulating effects of GcMAF on macrophages might lead to release of nitric oxide (NO).

Patients with advanced cancer were treated at the Immuno Biotech Treatment Centre with OA-GcMAF-based integrative immunotherapy in combination with a low-carbohydrate, highprotein diet, fermented milk products containing naturallyproduced GcMAF, Vitamin D3, omega-3 fatty acids and lowdose acetylsalicylic acid.

Measuring the tumor by ultrasonographic techniques, we observed a decrease of tumour volume of about 25%. These observations demonstrate that OA, GcMAF and NO can be properly combined and specifically delivered to advanced cancer patients with significant effects on immune system stimulation and tumor volume reduction avoiding harmful side-effects [17].

The beneficial effects of oleic acid in cancer processes can no longer be doubted, but little is known about the mechanisms of action behind this phenomenon. The aim of the present review is to clarify whether oleic acid has an effect on important mechanisms related to the carcinogenic processes [18]. We searched electronic databases and bibliographies of selected articles were inspected for further reference. We focused our research on two cellular transformations characterizing cancer development: proliferation and cell death or apoptosis.

Numerous studies have reported an inhibition in cell proliferation induced by oleic acid in different tumor cell lines. Herein, oleic acid could suppress the over-expression of HER2 (erbB-2), a well-characterized oncogene which plays a key role in the etiology, invasive progression and metastasis in several human cancers. In addition, oleic acid could play a role in intracellular calcium signaling pathways linked to the proliferation event. Regarding cell death, oleic acid has been shown to induce apoptosis in carcinoma cells. The mechanisms behind the apoptotic event induced by oleic acid could be related to an increase in intracellular ROS production or caspase 3 activity. Several unsaturated fatty acids have been reported to induce apoptosis through a release of calcium from intracellular stores. However, evidence regarding such a role in oleic acid is lacking.

Oleic acid plays a role in the activation of different intracellular pathways involved in carcinoma cell development. Such a role could be the root of its antitumoral effects reported in clinical studies [18].

Linoleic Acid Effects on the Mitochondria

Cardiolipin is a signature phospholipid of major functional significance in mitochondria. In heart mitochondria the fatty acid composition of cardiolipin is commonly viewed as highly regulated due to its high levels of linoleic acid (18:2n-6) and the dominant presence of a 4×18:2 molecular species. However, analysis of data from a comprehensive compilation of studies reporting changes in fatty acid composition of cardiolipin in heart and liver mitochondria in response to dietary fat shows that, in heart the accrual of 18:2 into cardiolipin conforms strongly to its dietary availability at up to 20% of total dietary fatty acid and thereafter is regulated. In liver, no dietary conformer trend is apparent for 18:2 with regulated lower levels across the dietary range for 18:2. When 18:2 and docosahexaenoic acid (22:6n-3) are present in the same diet, 22:6 is incorporated into cardiolipin of heart and liver at the expense of 18:2 when 22:6 is up to ~20% and 10% of total dietary fatty acid respectively. Changes in fatty acid composition in response to dietary fat are also compared for the two other main mitochondrial phospholipids, phosphatidylcholine and phosphatidylethanolamine, and the potential consequences of replacement of 18:2 with 22:6 in cardiolipin are discussed [19].

The oxidation and nitration of unsaturated fatty acids by oxides of nitrogen yield electrophilic derivatives that can modulate protein function via post-translational protein modifications. The biological mechanisms accounting for fatty acid nitration and the specific structural characteristics of products remain to be defined. Herein, conjugated linoleic acid (CLA) is identified as the primary endogenous substrate for fatty acid nitration *in vitro* and *in vivo*, yielding up to 10 greater extent of nitration products as compared with bis-allylic linoleic acid [5]. Multiple enzymatic and cellular mechanisms account for CLA nitration, including reactions catalyzed by mitochondria, activated macrophages, and gastric acidification. Nitroalkene derivatives of CLA and their metabolites are detected in the plasma of healthy humans and are increased in tissues undergoing episodes of ischemia reperfusion. Dietary CLA and nitrite supplementation in rodents elevates NO(2)-CLA levels in plasma, urine, and tissues, which in turn induces heme oxygenase-1 (HO-1) expression in the colonic epithelium. These results affirm that metabolic and inflammatory reactions yield electrophilic products that can modulate adaptive cell signaling mechanisms [20].

Airway epithelial injury is the hallmark of various respiratory diseases, but its mechanisms remain poorly understood. While 13-S-hydroxyoctadecadienoic acid (13-S-HODE) is produced in high concentration during mitochondrial degradation in reticulocytes little is known about its role in asthma pathogenesis. We show that extracellular 13-S-HODE induces mitochondrial dysfunction and airway epithelial apoptosis. This is associated with features of severe airway obstruction, lung remodeling, increase in epithelial stress related pro-inflammatory cytokines and drastic airway neutrophilia in mouse. Further, 13-S-HODE induced features are attenuated by inhibiting Transient Receptor Potential Cation Channel, Vanilloid-type 1 (TRPV1) both in mouse model and human bronchial epithelial cells. These findings are relevant to human asthma, as 13-S-HODE levels are increased in human asthmatic airways. Blocking of 13-S-HODE activity or disruption of TRPV1 activity attenuated airway injury and asthma mimicking features in murine allergic airway inflammation. These findings indicate that 13-S-HODE induces mitochondrial dysfunction and airway epithelial injury [21].

Cardiolipin (CL) is an inner-mitochondrial membrane phospholipid that is important for optimal mitochondrial function. Specifically, CL and CL linoleic (18:2ω6) content are known to be positively associated with cytochrome c oxidase (COX) activity. However, this association has not been examined in skeletal muscle. In this study, rats were fed high fat diets with a naturally occurring gradient in linoleic acid (coconut oil [CO], 5.8%; flaxseed oil [FO], 13.2%; safflower oil [SO], 75.1%) in an attempt to alter both mitochondrial CL fatty acyl composition and COX activity in rat mixed hind-limb muscle. In general, mitochondrial membrane lipid composition was fairly resistant to dietary treatments as only modest changes in fatty acyl composition were detected in CL and other major mitochondrial phospholipids such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE). As a result of this resistance, CL 18:2ω6 content was not different between the dietary groups. Consistent with the lack of changes in CL 18:2ω6 content, mitochondrial COX activity was also not different between the dietary groups. However, correlational analysis using data obtained from rats across the dietary groups showed a significant relationship ($p=0.009$, $R(2)=0.21$). Specifically, the results suggest that CL 18:2ω6 content may positively influence mitochondrial COX activity thereby making this lipid molecule a potential factor related to mitochondrial health and function in skeletal muscle [22]. P450 epoxidation of linoleic acid has been associated with many pathological conditions that often lead to acute renal failure. However, there is only suggestive evidence that linoleic acid mono epoxides and/or linoleic diols directly induce mitochondrial dysfunction. Using isolated rabbit renal cortical mitochondria (RCM), we found that linoleic acid (50 microM) and the linoleic acid monoepoxide, cis-12,13-epoxy-9octadecenoic acid (12,13-EOA, 50 microM) increased state 4 and oligomycin-insensitive respiration and reduced state 3 and oligomycin-sensitive respiration. Concomitant with these effects, linoleic acid and 12,13-EOA decreased mitochondrial membrane potential (DeltaPsi). In contrast, the hydrolyzed product of 12,13-EOA, 12,13dihydroxyoctadecenoic acid (12,13-DHOA, 50 microM), had no effect on state 3, state 4, oligomycin-sensitive, and oligomycin insensitive respiration, and DeltaPsi. Neither linoleic acid nor its metabolites altered uncoupled respiration, which suggests that these compounds have no effect on electron transport chain in RCM. Nucleotides such as ATP (0.5 mM) and GDP (0.5 mM) partially prevented the decrease in DeltaPsi but did not attenuate the increase in oligomycin-insensitive respiration after exposure to linoleic acid (50 microM) and 12,13-EOA (50 microM). These results demonstrate that linoleic acid metabolism to the 12,13-DHOA is a detoxification pathway that prevents mitochondrial dysfunction in RCM. The increase in state 4 respiration concomitant with decreases in state 3 respiration and DeltaPsi suggest that, in addition to uncoupling effects, linoleic acid and 12,13-EOA may have other effects, such as alterations of mitochondrial membranes. The inability of ATP and GDP to fully attenuate the uncoupling effects of linoleic acid and 12,13-EOA suggests that these effects are mediated through a nucleotide-independent mechanism [23]. Cardiac ischaemia-reperfusion (IR) injury remains a significant clinical problem with limited treatment options available. We previously showed that cardio protection against IR injury by nitro-fatty acids, such as nitro-linoleate (LNO2), involves covalent modification of mitochondrial adenine nucleotide translocase 1 (ANT1). Thus, it was hypothesized that conjugation of LNO2 to the mito-chondriotropic triphenylphosphonium (TPP(+)) moiety would enhance its protective properties. TPP(+) -LNO2 was synthesized from aminopropyl-TPP(+) and LNO2 , and characterized by direct infusion MS/MS. Its effects were assayed in primary cultures of cardio myocytes from adult C57BL/6 mice and in mitochondria from these cells, exposed to stimulated IR (SIR) conditions (oxygen and metabolite deprivation for 1h followed by normal conditions for 1h) by measuring viability by LDH release and exclusion of Trypan blue. Nitro-alkylated mitochondrial proteins were also measured by Western

blots, using antibodies to TPP(+). TPP(+) -LNO2 protected cardio myocytes from SIR injury more potently than the parent compound LNO2. In addition, TPP(+) LNO2 modified mitochondrial proteins, including ANT1, in a manner sensitive to the mitochondrial uncoupler carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP) and the ANT1 inhibitor carboxyatractyloside. Similar protein nitro-alkylation was obtained in cells and in isolated mitochondria, indicating the cell membrane was not a significant barrier to TPP(+) -LNO2 . Together, these results emphasize the importance of ANT1 as a target for the protective effects of LNO2, and suggest that TPP(+) -conjugated electrophilic lipid compounds may yield novel tools for the investigation of cardioprotection [24].

The therapeutic use of polyunsaturated fatty acids (PUFA) in preserving insulin sensitivity has gained interest in recent decades; however, the roles of linoleic acid (LA) and α linolenic acid (ALA) remain poorly understood. We investigated the efficacy of diets enriched with either LA or ALA on attenuating the development of insulin resistance (IR) in obesity. Following a 12-wk intervention, LA and ALA both prevented the shift toward an IR phenotype and maintained muscle-specific insulin sensitivity otherwise lost in obese control animals. The beneficial effects of ALA were independent of changes in skeletal muscle mitochondrial content and oxidative capacity, as obese control and ALA treated rats showed similar increases in these parameters. However, ALA increased the propensity for mitochondrial H_2O_2 emission and catalase content within whole muscle and reduced markers of oxidative stress (4-HNE and protein carbonylation). In contrast, LA prevented changes in markers of mitochondrial content, respiratory function, H_2O_2 emission, and oxidative stress in obese animals, thereby resembling levels seen in lean animals. Together, the data suggest that LA and ALA are efficacious in preventing IR but have divergent impacts on skeletal muscle mitochondrial content and function. Moreover, we propose that LA has value in preserving insulin sensitivity in the development of obesity, thereby challenging the classical view that n-6 PUFAs are detrimental [25]. Obesity with excessive levels of circulating free fatty acids (FFAs) is tightly linked to the incidence of type 2 diabetes. Insulin resistance of peripheral tissues and pancreatic β-cell dysfunction are two major pathological changes in diabetes and both are facilitated by excessive levels of FFAs and/or glucose. To gain insight into the mitochondrial mediated mechanisms by which long-term exposure of INS-1 cells to excess FFAs causes β-cell dysfunction, the effects of the unsaturated FFA linoleic acid (C 18:2, n-6) on rat insulinoma INS-1 β cells was investigated. INS-1 cells were incubated with 0, 50, 250 or 500 μM linoleic acid/0.5% (w/v) BSA for 48 h under culture conditions of normal (11.1 mM) or high (25 mM) glucose in serum-free RPMI-1640 medium. Cell viability, apoptosis, glucose-stimulated insulin secretion, Bcl-2, and Bax gene expression levels, mitochondrial membrane potential and cytochrome c release were examined. Linoleic acid 500 μM significantly suppressed cell viability and induced apoptosis when administered in 11.1 and 25 mM glucose culture medium. Compared with control, linoleic acid 500 μM significantly increased Bax expression in 25 mM glucose culture medium but not in 11.1 mM glucose culture medium. Linoleic acid also dose-dependently reduced mitochondrial membrane potential ($\Delta\Psi m$) and significantly promoted cytochrome c release from mitochondria in both 11.1 mM glucose and 25 mM glucose culture medium, further reducing glucose stimulated insulin secretion, which is dependent on normal mitochondrial function. With the increase in glucose levels in culture medium, INS-1 β-cell insulin secretion function was deteriorated further. The results of this study indicate that chronic exposure to linoleic acid-induced β-cell dysfunction and apoptosis, which involved a mitochondrial-mediated signal pathway, and increased glucose levels enhanced linoleic acid induced β-cell dysfunction [26].

An uncoupling protein was recently discovered in plant mitochondria and demonstrated to function similarly to the uncoupling protein of brown adipose tissue. In this work, green tomato fruit mitochondria were purified on a self-generating Percoll gradient in the presence of 0.5% bovine serum albumin to deplete mitochondria of endogenous free fatty acids. The uncoupling protein activity was induced by the addition of linoleic acid during the resting state, and in the progressively uncoupled state, as well as during phosphorylating respiration in the presence of benzohydroxamic acid, an inhibitor of the alternative oxidase and with succinate (+ rotenone) as oxidizable substrate. Linoleic acid strongly stimulated the resting respiration in fatty acid-depleted mitochondria but had no effect on phosphorylating respiration, suggesting no activity of the uncoupling protein in this respiratory state. Progressive uncoupling of state 4 respiration decreased the stimulation by linoleic acid. The similar respiratory rates in phosphorylating and fully uncoupled respiration in the presence and absence of linoleic acid suggested that a rate-limiting step on the dehydrogenase side of the respiratory chain was responsible for the insensitivity of phosphorylating respiration to linoleic acid. Indeed, the ADP/O ratio determined by ADP/O pulse method was decreased by linoleic acid, indicating that uncoupling protein was active during phosphorylating respiration and was able to divert energy from oxidative phosphorylation. Moreover, the respiration rates appeared to be determined by membrane potential independently of the presence of linoleic acid, indicating that linoleic acid-induced stimulation of respiration is due to a pure protonophoric activity without any direct effect on the electron transport chain [27].

The beneficial effects exerted by low amounts of conjugated linoleic acids (CLA) suggest that CLA are maximally conserved and raise the question about their mitochondrial oxidizability. Cis-9,trans-11-C(18:2) (CLA1) and trans-10,cis-12C(18:2) (CLA2) were compared to cis-9,cis-12-C(18:2) (linoleic acid; LA) and cis-9-C(16:1) (palmitoleic acid; PA), as substrates for total fatty acid (FA) oxidation and for the enzymatic steps required for the entry of FA into rat liver mitochondria. Oxygen consumption rate was lowest when CLA1 was used as a substrate with that on CLA2 being intermediate between it and the respiration on LA and PA. The order of the radio labelled FA oxidation rate was PA>>LA>CLA2>CLA1. Transesterification to

acylcarnitines of the octadecadienoic acids were similar, while uptake across inner membranes of CLA1 and, to a lesser extent, of CLA2 was greater than that of LA or PA. Prior oxidation of CLA1 or CLA2 made re-isolated mitochondria much less capable of oxidising PA or LA under carnitine-dependent conditions, but without altering the carnitine-independent oxidation of octanoic acid. Therefore, the CLA studied appeared to be both poorly oxidizable and capable of interfering with the oxidation of usual FA at a step close to the beginning of the beta-oxidative cycle [28]. Using specific stains and confocal microscope imaging, the patterns of mitochondrial distribution, mitochondrial inner membrane potential and reactive oxygen species (ROS) levels during bovine oocyte maturation were determined in the presence or absence of physiological concentrations of linoleic acid (LA; 100μM) or α-linolenic acid (ALA; 50μM). Mitochondrial distribution in control oocytes at 0h was mainly peripheral and changed to a diffused pattern after 1h of culture; this was maintained up to 24h. Mitochondrial clusters were observed during the early hours of maturation (1-4h); the majority of these were arranged in perinuclear fashion. LA supplementation resulted in: (1) delayed redistribution of the mitochondria from a peripheral to a diffuse pattern and a decreased percentages of oocytes showing perinuclear mitochondrial clusters, (2) decreased mitochondrial inner membrane potential at 1 and 24h compared with the control and (3) higher ROS levels, associated with a lower nuclear maturation rate. In contrast, ALA supplementation had no effect on mitochondrial distribution and activity and decreased ROS levels compared with the control; this was associated with an increased nuclear maturation rate. In conclusion, LA induced alterations in mitochondrial distribution and activity as well as increasing ROS levels, which mediate, at least in part, the inhibitory effect on oocyte maturation [29]. Cardiolipin (CL) is a tetra-acyl phospholipid that provides structural and functional support to several proteins in the inner mitochondrial membrane. The majority of CL in the healthy mammalian heart contains four linoleic acid acyl chains (L(4)CL). A selective loss of L(4) CL is associated with mitochondrial dysfunction and heart failure in humans and animal models. We examined whether supplementing the diet with linoleic acid would preserve cardiac L(4)CL and attenuate mitochondrial dysfunction and contractile failure in rats with hypertensive heart failure. Male spontaneously hypertensive heart failure rats (21 months of age) were administered diets supplemented with high linoleate safflower oil (HLSO) or lard (10% w/w; 28% kilocalorie fat) or without supplemental fat (control) for 4 weeks. HLSO preserved L(4)CL and total CL to 90% of non-failing levels (vs. 61-75% in control and lard groups), and attenuated 17-22% decreases in state 3 mitochondrial respiration observed in the control and lard groups ($P<0.05$). Left ventricular fractional shortening was significantly higher in HLSO vs. control (33 ± 2 vs. 29 ± 2%, $P < 0.05$), while plasma insulin levels were lower (5.4 ± 1.1 vs. 9.1 ± 2.3 ng/mL; $P<0.05$), with no significant effect of lard supplementation. HLSO also increased serum concentrations of several eicosanoid species compared with control and lard diets, but had no effect on plasma glucose or blood pressure. Moderate consumption of HLSO preserves CL and mitochondrial function in the failing heart and may be a useful adjuvant therapy for this condition [30]. In the study reported here the effect of conjugated linoleic acid (CLA) and vitamin A on the polyunsaturated fatty acid composition, chemiluminescence and per-oxidizability index of microsomes and mitochondria isolated from rat liver was analyzed. The effect of CLA on the polyunsaturated fatty acid composition of native microsomes was evidenced by an statistically significant $p<0.007$ decrease of linoleic acid C18:2 n6, whereas in mitochondria it was observed a decrease $p<0.0001$ of arachidonic acid C20:4 n6 when compared with vitamin A and control groups. Docosahexaenoic acid C22:6 n3 in mitochondria was reduced $p < 0.04$ in CLA and vitamin A groups when compared with control. After incubation of microsomes or mitochondria in an ascorbate (0.4 mM)-Fe++ (2.15 microM) system (120 min at 37 degrees C) it was observed that the total cpm/mg protein originated from light emission: chemiluminescence was lower in liver microsomes or mitochondria obtained from CLA group (received orally: 12.5 mg/daily during 10 days) than in the vitamin A group (received intraperitoneal injection: daily 0.195 g/ kg during 10 days). CLA reduced significantly maximal induced chemiluminescence in microsomes relative to vitamin A and control groups, whereas in mitochondria the effect was observed relative to control group. The polyunsaturated fatty acid composition of liver microsomes or mitochondria changed by CLA and vitamin A treatment. The polyunsaturated fatty acids mainly affected when microsomes native and per-oxidized from control group were compared were linoleic, linolenic and arachidonic acids, while in vitamin A group linoleic and arachidonic acid were mainly per-oxidized, whereas in CLA group only arachidonic acid was altered. In mitochondria obtained from the three groups arachidonic acid and docosahexaenoic acid showed a significant decrease when native and per-oxidized groups were compared. As a consequence the per-oxidizability index, a parameter based on the maximal rate of oxidation of fatty acids, show significant changes in the CLA group compare vitamin A and control groups. The simultaneous analysis of per-oxidizability index, chemiluminescence and fatty acid composition demonstrated that CLA is more effective than vitamin A protecting microsomes or mitochondria from per-oxidative damage [31].

A novel mitochondria-targeted antioxidant (TPP-OH) was synthesized by attaching the natural hydrophilic antioxidant caffeic acid to an aliphatic lipophilic carbon chain containing a triphenylphosphonium (TPP) cation. This compound has similar antioxidant activity to caffeic acid as demonstrated by measurement of DPPH/ABTS radical quenching and redox potentials, but is significantly more hydrophobic than its precursor as indicated by the relative partition coefficients. The antioxidant activity of both compounds was intrinsic related to the ortho-catechol system, as the methoxylation of the phenolic functions, namely in TPP-OCH and dimethoxycinnamic acid, gave compounds with negligible antioxidant action [3]. The incorporation of the lipophilic TPP cation to form TTP-OH and TPP-OCH allowed the cinnamic derivatives to accumulate within mitochondria in a process driven by the membrane potential [3]. However,

only TPP-OH was an effective antioxidant: TPP-OH protected cells against H_2O_2 and linoleic acid hydroperoxide-induced oxidative stress. As mitochondrial oxidative damage is associated with a number of clinical disorders, TPP-OH may be a useful lead that could be added to the family of mitochondria-targeted antioxidants that can decrease mitochondrial oxidative damage [32].

Linoleic acid (LA) improves insulin resistance and prevents diabetes. To investigate whether linoleic acid could protect against streptozotocin (STZ)-induced cell death, rat RIN-m5F cells were exposed to STZ. SL and SO groups consisted of cells treated with STZ and then LA or oleic acid (OA) respectively. STZ treatment decreased the mitochondrial membrane potential in the STZ, SO, and SL groups. Cells of the SL group had more intact mitochondria. Increased mRNA expression of mitochondrial DNA (mtDNA) and nuclear DNA (nDNA), as well as of the mitochondrial biogenesis regulators peroxisome proliferator activated receptor gamma coactivator-1alpha (PGC-1alpha), and mitochondrial transcription factor A (Tfam), were found in the LA group. The insulin content was significantly decreased in all three groups. These results suggest that the effects of LA on cell viability after STZ damage occur through maintenance of mitochondrial structure and increased mitochondrial biogenesis [33]. Despite many studies elucidating the mechanisms of necrotic cell death, the role of fatty acids released during necrosis remains to be determined. The goals of this study were to determine whether linoleic acid could protect rabbit renal proximal tubules (RPT) from necrotic cell death associated with mitochondrial dysfunction and oxidative injury and to determine the mechanisms involved. Exposure to antimycin A (10 microM) for 1 h or hypoxia (perfusion with 95% N_2/5% CO_2) for 1 or 2 h induced approximately 70% cellular lysis, as measured by lactate dehyrogenase release, versus 10% in controls. Preincubation with linoleic acid (100 microM) fully protected RPT from cellular lysis. RPT were also protected from lysis if linoleic acid was added 15 min after the addition of antimycin A. Measurements of free intracellular Ca(2+) concentrations showed that linoleic acid did not prevent the rise in intracellular Ca(2+) associated with a 30-min exposure to antimycin A. However, the influx of extracellular Cl(-) following a 30-min exposure to antimycin A was ameliorated in the presence of linoleic acid [34]. Linoleic acid did not prevent cellular lysis after exposure to hypoxia/reoxygenation (1h/1h) or t-butyl hydroperoxide (500 microM, 3h). These data suggest that linoleic acid protects RPT during the late phase of cell death associated with inhibition of the electron transport chain but not oxidative injury. Several other fatty acids also protected RPT from lysis, and structure-activity relationship studies suggest that a free carboxyl terminus and at least one double bond are required for this action [34].

Linoleic Acid Effects on the Mitochondrial Aging Process

Aging results in a redistribution of polyunsaturated fatty acids (PUFAs) in myocardial phospholipids. In particular, a selective loss of linoleic acid (18:2n6) with reciprocal increases of long-chain PUFAs (eg, arachidonic and docosahexaenoic acids) in the mitochondrial phospholipid cardio lipin correlates with cardiac mitochondrial dysfunction and contractile impairment in aging and related pathologies. In this study, we demonstrate a reversal of this aged-related PUFA redistribution pattern in cardiac mitochondria from aged (25 months) C57Bl/6 mice by inhibition of delta-6 desaturase, the rate limiting enzyme in long-chain PUFA biosynthesis. Interestingly, delta-6 desaturase inhibition had no effect on age-related mitochondrial respiratory dysfunction, H2O2 release, or lipid peroxidation but markedly attenuated cardiac dilatation, hypertrophy, and contractile dysfunction in aged mice. Taken together, the studies indicate that PUFA metabolism strongly influences phospholipid remodeling and cardiac function but dissociates these processes from mitochondrial respiratory dysfunction and oxidant production in the aged mouse heart [35].

Despite their high metabolic rates, birds have a much higher maximum longevity (MLSP) than mammals of similar body size, and thus represent ideal models for identifying longevity characteristics not linked to low metabolic rates. This study shows that the fatty acid double bond content of both canary (MLSP=24 years) and parakeet (MLSP=21 years) hearts is intrinsically lower than in mouse (MLSP=3.5 years) heart. This is caused by a redistribution between types of unsaturated fatty acids, mainly due to a lower content of the most highly unsaturated docosahexaenoic acid (22:6n-3) in the two birds in relation to the mammal. The lower double bond content leads to a lower sensitivity to lipid peroxidation, and to a lower level of *in vivo* lipid peroxidation in the heart of parakeets and canaries than in that of mice. Similar results have been previously found comparing liver mitochondria of rats and pigeons and tissues of different mammalian species. All these results taken together suggest that a low degree of fatty acid unsaturation is a general characteristic of longevous homeothermic vertebrate animals, both when they have low metabolic rates (mammals of large body size) or high metabolic rates (the studied birds); this constitutive trait protects their tissues and organelles against free radical mediated lipid peroxidation, and can contribute to their slow aging rate [36].

The cholesterol, phospholipid, and fatty acid compositions in synaptic and non-synaptic mitochondria from rat brains and the effect of aging were studied. Both cholesterol and phospholipid contents were found to be significantly different in synaptic compared to non-synaptic mitochondria. In both types of brain mitochondria, aging decreases the cholesterol content by 27% and the phospholipid content by approximately 12%. The difference between these decreases observed in the organelles causes decreases in the cholesterol/phospholipid molar ratios for synaptic and non-synaptic mitochondria of 17 and 19%, respectively. Also, the phospholipid composition is significantly different in synaptic compared to non-synaptic mitochondria. Among phospholipids, only the cardio lipin fraction showed a significant decrease (26%) in non-synaptic mitochondria from the brains of aged rats. Instead, the fatty acid composition was not significantly different

in synaptic compared to non-synaptic mitochondria. The 21% aging decrease in linoleic acid (18:2), observed only in non-synaptic mitochondria, may be related to a decrease in cardio lipin, which contains a large amount of this fatty acid [37].

Birds have a much higher maximum longevity (MLSP) than mammals of similar metabolic rate. Recent data showed that pigeon mitochondria produce oxygen radicals at a rate much slower than rat mitochondria, in spite of showing similar levels of oxygen consumption. Since oxidative damage from and to mitochondria seems important in relation to aging and longevity, and mitochondrial membranes are situated at the place where oxygen radicals are generated, we studied protein and lipid peroxidation and fatty acid composition of the three main membrane phospholipids of liver mitochondria from rats (MLSP=4 years) and pigeons (MLSP=35 years). It was found that pigeon mitochondria show lower levels of fatty acid unsaturation than rat mitochondria in the three lipid fractions, mainly due to a substitution of highly unsaturated fatty acids (20:4 and 22:6) by linoleic acid (18:2), and that these mitochondria are more resistant to lipid peroxidation. Previous research has also obtained exactly the same major difference in fatty acid composition in human mitochondria when compared to those of rat. Thus, present information suggests that the liver mitochondrial membranes of especially long-lived species show both a low level of free radical production and a low degree of fatty acid unsaturation as important constitutive protective traits to slow down aging [38]. The aim of this investigation was to study the connection between body size, fatty acid composition and sensitivity to lipid peroxidation of heart mitochondria and microsomes isolated from different size bird species: manon (Lonchura striata), quail (Coturnix coturnix var japonica), pigeon (Columba livia), duck (Cairina moschata) and goose (Anser anser), representing a 372-fold range of body mass. Fatty acids of total lipids were determined using gas chromatography and lipid peroxidation was evaluated with a chemiluminescence assay. The fatty acids present in heart organelles of the different bird species analyzed showed a small number of significant allometric trends. In mitochondria, from the individual fatty acid data, palmitoleic acid (C16:1 n7) increased allometrically (r=0.878), while stearic acid (C18:0) was negatively related to body mass (r=-0.903). Interestingly, none of the calculated fatty acid variables, the average fatty acid saturated, monounsaturated, polyunsaturated (PUFA) and the unsaturation index (UI) was established to show significant body size-related variations. In heart microsomes, the content of C18:0 was significantly smaller (r=-0.970) in the birds of greater size. A significant allometric increase in linoleic acid (C18:2 n6) (r=0.986), polyunsaturated (r=0.990) and UI (r=0.904) was observed in the larger birds. The total n6 fatty acids of heart mitochondria did not show significant differences when it was correlated to body mass of the birds. Moreover, positive allometric relationships were shown for microsomes. The total n3 fatty acids of heart mitochondria and microsomes indicated no significant correlations to body mass of birds. The C16:1 n7, C18:0 in mitochondria and C18:0, C18:2 n6, PUFA, UI and PUFA n6 in microsomes showed significant differences when they were correlated to maximum life span (MLSP) of birds. As light emission=chemiluminescence originated from heart organelles was not statistically significant, a lack of correlation between the sensitivity to lipid peroxidation and body size or maximum life span was obtained. These results indicate that the high resistance of bird hearts to the attack by free radicals is body size-independent and would be related to the preservation of cardiac function [39]. In this study, we examined the effect of CLA isomers in preventing age-associated muscle loss and the mechanisms underlying this effect, using 12-months-old C57BL/6 mice fed 10% corn oil (CO) or a diet supplemented with 0.5% c9t11-CLA, t10c12-CLA, or c9t11-CLA+t10c12-CLA (CLA-mix) for 6 months. Both t10c12-CLA and CLA-mix groups showed significantly higher muscle mass, as compared to CO and c9t11-CLA groups, measured by dual-energy X-ray absorptiometry and muscle wet weight. Enhanced mitochondrial ATP production, with higher membrane potential, and elevated muscle antioxidant enzymes (catalase and glutathione peroxidase) production, accompanied by slight increase in H(2)O(2) production was noted in t10c12CLA and CLA-mix groups, as compared to that of CO and c9t11CLA groups. Oxidative stress, as measured by serum malondialdehyde and inflammation, as measured by LPStreated splenocyte IL-6 and TNF-alpha, were significantly less in CLA isomers groups. Thus, CLA may be a novel dietary supplement that will prevent sarcopenia by maintaining redox balance during aging [40]. In this communication, we show that the plant uncoupling mitochondrial protein (PUMP) present in potato tuber mitochondria is induced by aging at 28 degrees C and that this induction is strongly stimulated when the potato tubers are stored at low temperature (4 degrees C). PUMP activity was detected by the degree of linoleic acid (LA)-induced ATP-sensitive mitochondrial uncoupling measured as a function of the decrease in membrane potential (delta psi). The PUMP content was evaluated by immunoblot analysis using polyclonal antibodies raised against potato PUMP that specifically detected a 32 kDa band. In agreement with the effect of LA on delta psi, the content of the 32 kDa band increased during storage and was stimulated by low temperature. These results support the proposed role of PUMP in plant thermogenesis and possibly in fruit ripening and senescence [41].

Free radical damage is considered a determinant factor in the rate of aging. Unsaturated fatty acids are the tissue macromolecules that are most sensitive to oxidative damage. Therefore, the presence of low proportions of fatty acid unsaturation is expected in the tissues of long-lived animals. Accordingly, the fatty acid compositions of the major liver mitochondrial phospholipid classes from eight mammals, ranging in maximum life span potential (MLSP) from 3.5 to 46 yr, show that the total number of double bonds is inversely correlated with MLSP in both phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) ($r=0.757$, $P<0.03$, and $r=0.862$, $P< 0.006$, respectively), but not in cardio lipin ($P=0.323$). This is due not to a low content of unsaturated fatty acids in long-lived animals, but mainly to a redistribution between kinds of fatty acids on PtdCho and

PtdEtn, shifting from arachidonic (r=0.911, P<0.002, and r=0.681, P=0.05, respectively), docosahexaenoic (r=0.931 and r= 0.965, P<0.0001, respectively) and palmitic (r=0.944 and r=0.974, P<0.0001, respectively) acids to linoleic acid (r=0.942, P<0.0001, for PtdCho; and r=0.957, P<0.0001, for PtdEtn). For cardio lipin, only arachidonic acid showed a significantly inverse correlation with MLSP (r=0.904, P<0.002). This pattern strongly suggests the presence of a species-specific desaturation pathway and deacylation-reacylation cycle in determining the mitochondrial membrane composition, maintaining a low degree of fatty acid unsaturation in long-lived animals [42]. The aged heart sustains greater injury during ischemia (ISC) and reperfusion (REP) compared to the adult heart. In the Fischer 344 (F344) rat, aging decreases oxidative phosphorylation and complex III activity increasing the production of reactive oxygen species in interfibrillar mitochondria (IFM) located among the myofibrils. In the isolated, perfused 24 month old elderly F344 rat heart 25 min of stop-flow ISC causes additional damage to complex III, further decreasing the rate of oxidative phosphorylation. We did not observe further progressive mitochondrial damage during REP. We next asked if ISC or REP increased oxidative damage within mitochondria of the aged heart. Cardiolipin (CL) is a phospholipid unique to mitochondria consisting predominantly of four linoleic acid residues (C18:2). Following ISC and REP in the aged heart, there is a new CL species containing three oxygen atoms added to one linoleic residue. ISC alone was sufficient to generate this new oxidized molecular species of CL. Based upon oxidative damage to CL, complex III activity, and oxidative phosphorylation, mitochondrial damage thus occurs in the aged heart mainly during ISC, rather than during REP. Mitochondrial damage during ischemia sets the stage for mitochondrial-driven cardio myocyte injury during reperfusion in the aged heart [43]. Cardiolipin was first isolated from beef heart and was shown to contain an unusually high content of linoleic acid ester residues. Cardiolipin is found throughout the eukaryotes including animals, plants and fungi. In mammalian tissue and in yeast, cardiolipin is found exclusively in mitochondria. Mitochondrial synthesis of cardiolipin utilizes phosphatidylglycerol and CDP-diacylglycerol as substrates in a reaction which requires a divalent cation (Mg^{2+}, Mn^{2+} or Co^{2+}). Cardiolipin synthase has been purified to nearhomogeneity from rat liver by solubilization with Zwittergent 314 followed by FPLC anion exchange, gel permeation and chromatofocusing steps. Cardiolipin synthase has a molecular mass of 50 kDa, a pH optimum of 8.0, and requires added phospholipids (phosphatidylethanolamine and cardiolipin) and 4 mM Co^{2+} for optimal activity. Except for the effects of divalent cations and the requirement for phospholipids, little is known about the regulation of cardiolipin synthase. Cardiolipin deficiency in aging mitochondria has been linked to decreased metabolite transport across the inner membrane. Both cardiolipin levels and cardiolipin synthase activity are increased in hyperthyroidism and decreased in hypothyroidism suggesting regulation by thyroid hormone. Mammalian cardiolipin synthase has not been sequenced or cloned and its biological role in mitochondria is not yet fully understood [44]. Phospholipids from liver mitochondrial and microsomal membrane preparations were analyzed to further assess the effects of age and lifelong calorie restriction on membrane lipid composition. Results showed that the major phospholipid classes, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol and cardiolipin did not vary significantly with age or diet. The fatty acid composition of the phospholipids was determined in PC and PE and ages of 6, 12 and 24 months. The data revealed characteristic patterns of age-related changes in ad libitum (AL) fed rats: membrane levels of long-chain polyunsaturated fatty acids, 22:4 and 22:5, increased progressively, while membrane linoleic acid (18:2) decreased steadily with age. Levels of 18:2 fell by approximately 40%, and 22:5 content almost doubled making the peroxidizability index increase with age. In addition, levels of 16:1 and 18:1 decreased significantly with age, indicating a possible change in delta 9-desaturase activity coefficient. Food restriction resulted in a significant increase in levels of essential fatty acids while attenuating levels of 22:4, 22:5, 22:6 and peroxidizability. We concluded that the membrane-stabilizing action of long-term calorie restriction relates to the selective modification of membrane long-chain polyunsaturated fatty acids during aging [45]. The effects of almitrine on ATPase/ATPsynthase previously described in beef heart mitochondria are also observed in liver mitochondria isolated from rats older than 7 weeks. In contrast, in rats younger than 5 weeks, almitrine at the same concentration has no effect on the ATPase/ATPsynthase complex. This age-dependent action of almitrine is well correlated with age-dependent modifications of two fatty acids: linoleic and docosahexaenoic acids. The possibility of a change in H^+/ATP stoichiometry of the ATPase/ATPsynthase induced by almitrine seems related to more general modifications of membrane properties during growth of the rat [46].

Aging enhances cardiac injury during ischemia and reperfusion compared to the adult heart, including in the Fischer 344 rat model of aging (F344). In interfibrillar cardiac mitochondria obtained from the elderly F344 rat, the rate of oxidative phosphorylation and the activity of electron transport complex III is decreased, concomitant with an increase in the production of reactive oxygen species. In the isolated, perfused heart, 25 min of global ischemia results in additional damage to complex III. We proposed that ischemic damage superimposed upon the aging defect augments production of reactive oxygen species leading to greater oxidative damage in the aged heart. Cardiolipin is an oxidatively sensitive phospholipid located in the inner mitochondrial membrane. Oxidative damage to cardiolipin was assessed by characterization of the individual molecular species of cardiolipin via reverse phase HPLC and electrospray mass spectrometry (MS). The predominant molecular species of cardiolipin (95%) contains four linoleic acid residues (C18:2). Ischemia and reperfusion did not alter the content or composition of cardiolipin in the adult heart. Following ischemia and reperfusion in the aged heart, a new molecular species of cardiolipin was present with mass increased by 48 Da, suggesting the addition of three oxygen atoms. MS fragmentation localized the added

mass to the C18:2 residues. Ischemia alone was sufficient to modify cardiolipin in the aged heart whereas cardiolipin in the adult heart remained unaltered. Thus, age-enhanced oxidative damage occurs within mitochondria in the heart during ischemia and reperfusion, especially during ischemia [47]. A comparative study was made on the fate of linoleic, arachidonic, and docosa-7,10,13,16-tetraenoic acids in various subcellular fractions of liver and testis from rats of different ages. It was demonstrated that testicular microsomes can desaturate and elongate linoleic and arachidonic acids in a manner similar to liver microsomes, and that testicular mitochondria can convert docosa-7,10,13,16-tetraenoic acid to arachidonic acid. Testicular or liver microsomes actively desaturate linoleic acid to gamma linolenic acid and eicosa-8,11,14-trienoic acid to arachidonic acid. However, it was impossible to measure *in vitro* any direct conversion of adrenic acid (22:4 [n-6]) to docosapentaenoic acid (22:5 [n-6]) by either liver or testicular microsomes. Docosa-7,10,13,16-tetraenoic acid is incorporated preferentially into the triglyceride fraction of total testis, mitochondria, and microsomes, while linoleic and arachidonic acids are incorporated more into phospholipids. The capacity of testicular microsomes, but not of liver microsomes, to synthesize polyunsaturated fatty acids declines with age. It is proposed that the synthesis of acids of the linoleic family proceeds in two stages, a rapid one in which arachidonic acid is made and a second, slower, one in which C(22) and C(24) acids are synthesized. In addition, there appears to be a cycle between microsomes and mitochondria that acts to conserve essential polyunsaturated C(20) and C(22) fatty acids by means of synthesis and partial degradation, respectively. This cycle would restrict the loss of essential fatty acids and might be of importance for the supply of arachidonic acid in testis under specific requirements and especially in older animals [48]. We fed male Wistar rats lifelong on virgin olive (rich in the monounsaturated oleic acid) or sunflower (rich in the polyunsaturated linoleic acid) oil-based diets. At 6 and 24 months, liver mitochondria were analyzed for a mitochondrial DNA (mtDNA) deletion, reactive oxygen species, antioxidants, and ultrastructural alterations. An aging-related increase in the relative amount of the deletion was observed for both dietary groups, being higher in animals fed sunflower oil. Oxidative stress was lower in virgin olive oil-fed animals. Aging led to higher superoxide dismutase, catalase, and glutathione peroxidase activities and increased alpha-tocopherol and coenzyme Q. Mitochondria from aged animals fed sunflower oil exhibited a lower number of cristae and a higher circularity. Results suggest that the age-related increase of the relative amount of deleted mtDNA depends on fat unsaturation. Moreover, the studied mtDNA deletion was correlated with mitochondrial oxidative stress and ultrastructural alterations [49]. The lipid contents of the microsomal and mitochondrial membrane fractions of liver and kidney were determined in 6 and 24 month old rats. A significant age related increase in the molar ratio of cholesterol/phospholipid was observed in all membrane fractions. A significant age related reduction of phospholipid was noted in the microsomal fractions of liver and kidney. The relative amount of phosphatidylethanolamine was found to decrease in all membrane fractions during aging. Membrane glyceride content, however, remained relatively constant with age. Significant increase in oleic acid was seen in the neutral lipid of both liver and kidney and in the polar lipid of kidney. Significant increase in docosahexaenoic acid and significant decrease in linoleic acid were seen in the polar lipid of the liver membrane fractions. Possible alterations in membrane physiochemical properties and in membrane function due to these age related lipid changes are discussed [50]. The effects of cold adaptation upon the brown adipose tissue have been studied in rats, hamsters, mice, and guinea pigs. Striking effects were found for total tissue as well as at the mitochondrial level, e.g., increases in protein and phospholipid contents, changes in phospholipid fatty acid composition (a decrease in the percentage of palmitic and palmitoleic acids and an increase in stearic and linoleic acids), and a change in the mitochondrial polypeptide composition (a marked increase in a 32000 molecular weight polypeptide, except for hamsters). In situations where animals exhibit a greatly enhanced capacity for non-shivering thermogenesis (cold adaptation for rats, mice, and guinea pigs, birth for guinea pigs, and hibernation ability for hamsters, dormice, and garden dormice), brown fat mitochondria are characterized by the occurrence of large amounts of the 32000 molecular weight polypeptide characteristic of these mitochondria [51].

Conclusion

Linoleic acid has effects on the mitochondria. It has effects on Cancer and has effects on Aging.

Is Linoleic acid the Code of life?

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