Protective role of olive oil and its major component oleic acid in TNF-α induced remodeling subsequent to myocardial infarction in rats

By

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Dedication

This Thesis is dedicated to

My Love Asma &

My Son Baker

My Family &

in-laws

Contents

List of Abbreviations

ACE: Angiotensin converting enzyme
Ang I: Angiotensin I
Ang II: Angiotensin II
ARB: Angiotensin Receptor Blockers
ATTACH: Anti TNF-α Therapy Against Congestive Heart Failure
cAMP: Cyclic adenosine monophosphate
CAPK: Ceramide activated protein kinase
CD: Cluster of differentiation
CHFN: Canadian Heart Failure Network
CK: Creatine kinase
COPD: Chronic obstructive pulmonary disease
Cu/Zn-SOD: Copper/zinc superoxide dismutase
DCFDA: 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate
ECG: Electrocardiography
Echo: Echocardiography
ELISA: Enzyme-linked immunosorbent assay
FADD: Factors associated with death domain
FAN: Factors associated with neutral sphingomyelinase activation
GSH: Glutathione reduced
GSHPx: Glutathione peroxidase
GSSG: Glutathione oxidized
HR: Heart rate
ICAM-1: Intercellular adhesion molecule 1
IDL: Intermediate density lipoprotein
IFN: Interferon
IL-10: Interleukin-10
LDL: Low density lipoprotein
LPO: Lipid hydroperoxide
LT: Leukotriene
LT-α or TNF-β: Lymphotoxin alpha
LVEF: Left ventricular ejection fraction
LVIDd: Left ventricular internal dimension-diastole
MAPK: Mitogen activated protein kinase
MI: Myocardial infarction
mRNA: Messenger RNA
mTNF-α: Membrane bound TNF-α

NADH: Nicotinamide adenine dinucleotide
NADPH: Nicotinamide adenine dinucleotide phosphate
NIK: NF-κb inducing kinase
NYHA: New York Heart Association
OA: Oleic acid
PBS: Phosphate buffered saline
PLC: Phospholipase C
PLD: Phospholipase D
PMI: Post myocardial infarction
RAAS: Renin–angiotensin–aldosterone system
RANKL: Receptor activator of nuclear factor κB ligand
RAS: Renin angiotensin system
RECOVER: Research into Etanercept: Cytokine antagOnism in VEntriculaR function
RENAISSANCE: Randomised Etanercept North AmerIcan Strategy to Study ANtagonism of Cytokine E
RIP: Receptor interacting protein
ROS: Reactive oxygen species
SOD: Superoxide dismutase
SOLVD: Study of Left Ventricular Dysfunction
sTNF-α: Soluble TNF-α
TACE: TNF-α converting enzyme
TBHQ: Tertiary butylhydroquinone
TCH: Total Cholesterol
TDI: Tissue Doppler imaging
TG: Triglycerides
TGFβ: Transforming growth factor beta
TNF-α: Tumor necrosis factor-α
TNF-R: TNF-α receptors
TRADD: TNF receptor associated death domain
TRAF: TNF-R associated factors
TRAIL: TNF related apoptosis inducing agent
TWEAK: TNF like weak inducers of apoptosis
VCAM-1: Vascular cell adhesion molecule 1
VEGI: Vascular endothelial cell growth inhibitor
vLDL: Very low density lipoprotein

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I. ABSTRACT

Oxidative stress and inflammation are important factors involved in the progression of heart failure. An important cytokine produced during myocardial infarction (MI) is tumor necrosis factor alpha (TNF-α). TNF-α may induce oxidative stress, cell damage, apoptosis and cardiac dysfunction. Considering the anti-inflammatory and anti-oxidant properties of extra-virgin olive oil and its major component (80%) oleic acid (OA), and their benefits to the cardiovascular system, we hypothesized that the negative effects of TNF-α in the pathogenesis of heart failure will be mitigated by olive oil consumption. This hypothesis was tested by examining the effects of a special diet supplemented with 10% olive oil, in coronary artery ligated animal model of MI. Corn oil (10%) supplementation was used as a control for matching caloric intake. Animals in the sham and ligated groups fed regular chow, olive oil, and corn oil were studied at 4 and 16 weeks post myocardial infarction (PMI).

Mortality, diet consumption, weight gain and conduction system abnormalities were comparable among all ligated groups. Echocardiography showed that MI deteriorated cardiac function, and olive oil restored the function. At 16 weeks PMI, only corn oil fed groups showed significant increase in both total cholesterol and HDL. Corn oil was not able to offer protection to the heart, suggesting that the beneficial effects of olive oil are not due to increased caloric intake or increased HDL. MI increased myocardial TNF-α,

oxidative stress, lipid peroxidation, pro-apoptotic protein expression (Bax, cleaved Caspase 3, cleaved PARP, TGFβ, Bnip3), cytochrome C release, MAP kinase activation (p38, JNK) and decreased anti-apoptotic protein Bcl-xL expression at both 4 and 16 weeks PMI, and these changes were modulated by olive oil.

In order to further test the central role of TNF-α PMI, we examined the possible mitigation of TNF-α induced changes by OA in isolated adult rat cardiomyocytes. TNF-α increased oxidative stress, cell damage, cell death, and apoptosis, while OA treatment mitigated these TNF-α induced effects.

We concluded that TNF-α is implicated in the progression of heart failure subsequent to MI and that OA in olive oil may prevent this progression, through its anti-oxidant, anti-inflammatory, anti-hypertensive, and inotropic effects.

II. INTRODUCTION

Cardiovascular diseases account for 30 % of all deaths worldwide. Among different cardiovascular diseases, ischemic heart disease accounts for 80 % of these cases (WHO 2012). Thus a better understanding of the factors contributing to heart failure due to ischemic heart disease is required to develop effective new therapies. Inflammation and oxidative stress are important factors in the progression of heart failure subsequent to myocardial infarction (MI) (Kaur et al. 2006; Bayeva et al. 2013). MI can initiate the inflammatory response, and one of the important cytokines produced after MI is tumor necrosis factor alpha (TNF-α) (Nian et al. 2004). TNF-α plays an important physiologic role in the healing of the heart after injury (Frangogiannis et al. 2002). However, when produced consistently in high levels, TNF-α becomes detrimental and leads to myocardial dysfunction and heart failure subsequent to MI (Kaur et al. 2006; Savic-Radojevic et al. 2013). However, the precise sub-cellular details of TNF-α induced myocardial dysfunction remain to be understood.

Both in humans and animals, many cell types produce TNF-α including macrophages, monocytes and cardiomyocytes (Mizuochi et al. 2007; Chen et al. 2010). Transgenic mice, with the over-expression of myocardial TNF-α develop cardiomyopathy and with time develop congestive heart failure (Kubota et al. 1997). Infusion of TNF-α to treat patients with carcinoma led to cardiac depression and development of cardiomyopathy

(Hegewisch et al. 1990). A rise in TNF-α has also been implicated in other inflammatory diseases including: rheumatoid arthritis, psoriasis, and inflammatory bowel disease (Scheinfeld 2004; Listing et al. 2008). After inflammation, TNF-α can generate reactive oxygen species through cytosolic and mitochondrial pathways (Woo et al. 2000; Ghavami et al. 2009). One of the most important factors involved in the defense mechanisms against oxidative stress is glutathione (Khaper et al. 2010), which is a substrate for the anti-oxidant enzyme glutathione peroxidase that has been shown to inhibit TNF-α induced injury (Oz et al. 2006). Hydrogen peroxide (H_2O_2) has also been shown to increase cardiomyocyte injury, oxidative stress, and apoptosis, all of which are similar to the effects exerted by TNF-α (Dhingra et al. 2007; Al-Shudiefat et al. 2013). This similarity, between H_2O_2 and TNF-α effects on cardiomyocytes, is another piece of supporting evidence that TNF-α may mediate its effects through an increase in the oxidative stress. Study of glutathione and oxidative stress as well as their role in apoptosis in functionally assessed hearts subsequent to MI is lacking.

It is well documented that olive oil and its major component oleic acid (OA) a monounsaturated fatty acid, have anti-inflammatory and antioxidant properties, with a beneficial effect on the cardiovascular system by decreasing both inflammation and oxidative stress (O'Bryne et al. 1998; Lopez-Miranda et al. 2000; Hargrove et al. 2001; Fito et al. 2007; Bermudez et al. 2011; Carrillo et al. 2012; Sales-Campos et al. 2012). Consumption of olive oil in humans has been shown to decrease the serum level of TNF-α significantly (Papageorgiou et al. 2011). A diet enriched with olive oil is reported to decrease the plasma oxidative stress in humans (Bruge et al. 2012). Dietary supplementation of olive oil showed a decrease in the level of the mitochondrial oxidative stress in aging rats

(Huertas et al. 1999). Cardiovascular diseases in Mediterranean people are low potentially due to the consumption of Mediterranean diet enriched with OA present in olive oil (de Lorgeril et al. 2006). A large clinical study including about 74,000 women followed up for 20 years, showed that women consuming a Mediterranean diet containing high amounts of OA, fruits, vegetables, whole grains, fish, and wine, were at lower risk of developing cardiovascular disease and had lower mortality rate compared to other women who were not adherent to Mediterranean diet (Fung et al. 2009). In a more recent clinical trial published in The New England Journal of Medicine on April 2013, involving 7447 persons, with no cardiovascular disease at enrolment, followed for 4.8 years, it has been reported that the consumption of Mediterranean diet enriched with extra-virgin olive oil showed lower incidence of major cardiovascular events (Estruch et al. 2013). It has also been shown that OA decreased TNF-α in human adipose tissue as well as in adipocytes (Murumalla et al. 2012). Furthermore, OA was also able to attenuate TNF-α induced ROS production in endothelial cells (Massaro et al. 2002). The role of olive oil as well as oleic acid in cardiomyocyte injury/ apoptosis and heart failure due to MI has not been described.

Although the inflammatory cytokine TNF-α is produced during heart injury or MI, and its detrimental role is suggested to be mediated through oxidative stress, anti-TNF-α was not cardioprotective (Khaper et al. 2010). In some cases, anti-TNF-α therapy worsened the condition with some side effects including: congestive heart failure, lymphoma, induction of auto-antibodies, demyelinating disease, and a lupus like syndrome (Scheinfeld 2004). Even though the risk of developing these side effects is very low, there is a real need for other alternative approaches to mitigate either the high level of TNF-α or its

effects in such conditions. Olive oil and OA with their anti-inflammatory and anti-oxidant properties may be such an alternative. Although it has been proposed that olive oil consumption reduces cardiovascular diseases in Mediterranean people due to its high content of oleic acid, the molecular mechanisms of this protection on the progression of the heart failure associated with TNF-α subsequent to MI has not been examined.

We proposed and tested the hypothesis "Olive oil and its major component oleic acid will protect against oxidative stress and remodeling of the heart subsequent to myocardial infarction in rats by mitigating TNF-α induced changes in cardiomyocytes". Myocardial infarction in rats was achieved by ligation of the left anterior descending coronary artery. Dietary intervention with regular chow supplemented with 10% olive oil, was started 10 days prior to the surgery. Regular chow, supplemented with 10 % corn oil, was used as a caloric control. Sham and ligated animals fed on regular chow, olive oil, and corn oil were studied at 4 and 16 weeks post myocardial infarction (PMI). Mortality rate, general well being of the animals, weight gain, and diet consumption were recorded. Blood pressure after one week and four weeks of special diet consumption was recorded. Electrocardiography (ECG) of the animals at 4 and 16 weeks PMI was done. For the study of cardiac function, echocardiography [left ventricular ejection fraction (LVEF), left ventricular internal dimension-diastole (LVIDd), and Tissue Doppler Imaging (TDI) parameter for anterior wall], was done at 4 and 16 weeks PMI. Blood samples were taken to analyze plasma TNF-α levels as well as lipid levels (Total cholesterol, HDL, TG). The hearts at 4 and 16 weeks PMI were analyzed for myocardial TNF-α, oxidative stress, level of glutathione, lipid hydroperoxides, apoptotic proteins (Bax, cleaved Caspase 3, cleaved PARP, TGFβ, Bnip3, cytochrome C release), anti-apoptotic protein Bcl-xL, MAP kinases

(p38, JNK, ERK), and fibrosis. To further examine the hypothesis and the central role of TNF-α in the pathogenesis of cardiac dysfunction, isolated adult rat cardiomyocytes were exposed to TNF-α alone or in combination with OA and were analyzed for oxidative stress, cell damage, cell death, and apoptosis.

The results from this study showed that olive oil was able to improve heart function and prevent the progression of heart failure subsequent to MI. This beneficial effect may be due to the ability of OA in the olive oil to attenuate TNF-α induced oxidative stress, cell damage, cell death, and apoptosis. This study raises the real possibility of using a nutritional approach with olive oil to reduce the burden of ischemic heart diseases in the general population.

III. LITERATURE REVIEW

1.0 Heart Failure

Heart failure is a medical condition in which the heart is unable to provide sufficient blood flow to meet the demands of the body. Some of accompanied symptoms are shortness of breath, fatigue and peripheral edema (National Heart, Lung, and Blood Institute 2012; Lee and Moser 2013). About 23 million people worldwide are affected by heart failure (McMurray et al. 1998). In Canada alone, it is estimated that 400,000 people are suffering from this condition (Canadian Heart Failure Network (CHFN) 2013). Although there is a decrease in deaths related to coronary artery disease and stroke, there is an increase in the morbidity and mortality rates related to heart failure (Sharpe and Doughty 1998). Despite the improvement in the treatment for patients with heart failure, the 5 years survival rate is only 50 % (McMurray et al. 2012). Thus, heart failure represents a big burden exists on the health system as well as economy due to the loss of life, loss of productivity and hospitalization costs associated with this disease (McMurray et al. 1998). Global cases of heart failure are increasing due to three factors: aging population with chronic conditions, a shift from pandemic infections to cardiovascular diseases (due to using immunization and antibiotics), and an increase in survival rate due to improvement in treatment (Omran 2001; Lee et al. 2004; Roger et al. 2004).

Heart failure is classified as: diastolic or systolic, depending on the inability of the heart to relax or contract respectively (Bolt et al. 2009); site involved in the heart failure (left versus right heart failure) (Parsons-Smith 1950); or the degree of function impairment (Class I, II, III and IV according to the New York Heart Association classification). Class I: no symptoms with ordinary activity; Class II: symptoms with ordinary activity; Class III: symptoms with less than ordinary activity; Class IV: symptoms at rest (The Criteria Committee of the New York Heart Association 1994). Some of the other types of heart failure include congestive, acute, and ischemic conditions (Parsons-Smith et al. 1950).

1.1 Risk Factors and Mechanisms

There are many risk factors associated with the development of heart failure such as: major clinical risk factors, toxic risk precipitants, minor clinical risk factors, genetic risk predictors, and morphological risk predictors (Coviello 2009). Major clinical risk factors include: myocardial infarction (MI), hypertension, diabetes mellitus, age, male sex, valvular heart diseases, and obesity. Toxic risk precipitants include: alcohol, cocaine, chemotherapy (e.g. anthracycline), thiazolidinediones, and doxazosin. Minor clinical risk factors include: smoking, dyslipidemia, immune activation (e.g. TNF-α, IL-6), chronic kidney disease, anemia, sedentary lifestyle, and psychological stress. Genetic risk predictors include single nucleotide polymorphism (e.g. 1Arg389). Morphological risk predictors include: increased left ventricular internal dimension (LVID), and left ventricular diastolic dysfunction (Coviello et al. 2009).

One of the most important major risk factors for developing heart failure is ischemic heart disease (Teerlink et al. 1991) which happens largely due to atherosclerosis of the coronary arteries (Parsons-Smith et al. 1950). Thirteen clinical trials reported that coronary artery disease was present in 68 % of patients with heart failure in developed countries (Gheorghiade and Bonow 1998). Ischemic heart disease is the leading cause of heart failure in developed countries, while non-ischemic heart diseases, including cardiomyopathy, hypertension and rheumatic heart disease are more common in developing countries (Killip 1985; Mendez and Cowie 2001; Ntusi and Mayosi 2009). Heart failure arises due to remodeling and changes in cardiac structure, function, rhythm or conduction. These changes are caused mainly by ventricular dysfunction in which myocardial infarction (systolic dysfunction) and hypertension (systolic or diastolic dysfunction), or both of them play a major role (McMurray and Pfeffer 2005). Coronary artery disease, may lead to a decrease in the blood supply to the affected region and initiation of ischemia, which with time develop to acute myocardial infarction (Gheorghiade et al. 1998). Cardiac remodeling is related to the progression of heart failure in patients with MI (White et al. 1987). MI leads to the loss of cardiomyocyte function, development of myocardial fibrosis and subsequent left ventricle remodelling. Simultaneously, there are neuro-hormonal adjustments and progressive left ventricular dysfunction in the remaining viable tissue that could develop into heart failure (Gheorghiade et al. 1998).

Hypertension, a major risk factor, increases the risk of developing heart failure two-fold in men and three-fold in women (Levy et al. 1996). Pressure overload causes dilation of the heart chamber and increases in the ratio of the diameter to wall thickness of the chamber after a progress in remodelling of the myocardium (Onodera et al. 1998). In

early compensated pressure overload, there is an increase in the cross sectional area of the cardiomyocytes without changing the cell length (Zierhut et al. 1991). Maladaptive cellular remodelling is characterized by an increase in the cardiomyocyte length in both humans and animals with heart failure associated with hypertension (Gerdes 1995; Gerdes et al. 1996). Another more critical factor that compromises the wall thickness of the ventricles is the lack of transverse growth of cardiomyocytes (Gerdes et al. 1992; Gerdes et al. 1995; Gerdes et al. 1996). Hypertension promotes loss of cardiomyocyte contractility, fibrosis and hypertrophy in heart tissue (Coviello et al. 2009). Treatment with anti-hypertensive drugs including diuretics, beta blockers, and angiotensin converting enzyme inhibitors is important in the prevention of heart failure in patients with hypertension (Turnbull et al. 2003).

Diabetes milletus increases the risk of developing heart failure two-fold to five-fold in human being (Coviello et al. 2009). Diabetes is also associated with increased atherosclerosis (Ginsberg 2000) and/ or hypertension, which also are risk factors for heart failure (Reaven et al. 1996). One in ten adults worldwide has diabetes (WHO 2012). The increase in hemoglobin A1C is highly associated with more hospitalizations and risk to develop heart failure (Iribarren et al. 2001). Diabetes is associated with the development of cardiomyopathy probably due to metabolic changes in heart, and this may lead to the development of heart failure (Kannel et al. 1974). In addition to cardiomyopathy associated with metabolic changes in the heart, diabetes is associated with contractile dysfunction and loss of normal microvasculature (Miki et al. 2012). Other changes related to diabetes include collagen deposition and glycation end products in the heart (Shah et al. 2010).

Moreover, diabetes increases the risk of developing coronary artery disease (van Deursen et al. 2012).

Obesity is also a risk factor for heart failure, it influences blood sugar, cholesterol, lipids, cardiac preload and afterload, thus increasing the chance of developing coronary artery disease (U.S. Department of Health and Human Services 2000; Coviello et al. 2009). Half a billion people or 12 % of the world population are considered obese (WHO 2012). Whether the reduction of weight has beneficial effect after the onset of heart failure or not is still debated (Kenchaiah et al. 2004). Valvular heart disease can increase pressure in ventricles and lead to loss of heart function (Coviello et al. 2009). Repairing stenotic or regurgitant valves can improve the function of the heart and survival (Carabello et al. 1997). Renal dysfunction also affects the development of heart failure. The incidence of heart disease increases in patients with renal disease, and about half of the deaths related to renal disease are due to heart failure (Culleton et al. 1999). Renal disease complications include hypertension, anemia, increase in the inflammatory markers, sodium and water retention, and increase in the levels of homocysteine (Fried et al. 2003). Anemia is present in about 37 % of heart failure patients (Groenveld et al. 2008). Renal dysfunction is a major factor for developing anemia, and it is related to both anemia and heart failure, such a relation called cardiorenal anemia syndrome (Silverberg et al. 2006). Other factors involved in the pathogenesis of anemia in heart failure patients include high central venous pressure, low levels of erythropoietin, iron deficiency, folate deficiency and vitamin B12 deficiency, activation of the renin–angiotensin–aldosterone system (RAAS), and chronic inflammation that reduces bone marrow proliferation (Witte

et al. 2004; Westenbrink et al. 2007; van der Meer et al. 2008; Damman et al. 2009; van der Meer et al. 2009).

In general, chronic obstructive pulmonary disease (COPD) occurs in patients with heart failure. Since both diseases have similarity in symptoms with muscular changes, it is difficult to diagnose if they are present alone or in combination (Gosker et al. 2000). In patients suffering from heart failure, the prevalence of COPD is 10-50 % (Hawkins et al. 2009). A major risk factor for COPD is smoking (Sansores et al. 2013). Smoking has direct toxic effect on cardiomyocytes, and it can induce artery spasm, cholesterol, insulin resistance, and diabetes (Eliasson 2003). Other risk factors for COPD include, inflammation, oxidative stress, malfunction of the sympathetic nervous system, and hypoxia (Iversen et al. 2010). COPD can cause heart failure through chronic inflammation that can play a role in atherosclerosis or by increasing pulmonary blood pressure (Wouters 2005; Sabit et al. 2010).

The prevalence of liver dysfunction in patients with heart failure is 30-60 % (Allen et al. 2009; van Deursen et al. 2010). Reduced cardiac output leads to alteration in the production of liver enzymes (Lau et al. 2002). On the other hand, liver dysfunction can worsen heart failure by increasing fluid overload due to hypoalbuminemia, and low osmolality (van Deursen et al. 2012). Excessive consumption of alcohol can increase the risk of heart failure by 4.5 % through an increase in blood pressure and by the direct toxic effects of alcohol on cardiomyocytes (Walsh et al. 2002). Alcohol is responsible for the increased incidence of heart failure associated morbidity and mortality (Urbano-Marquez et al. 1989). Cognitive impairment due to cerebral dysfunction has a prevalence of 28-58 % in patients suffering from heart failure (Vogels et al. 2007; Pressler 2008; van den

Hurk et al. 2011). Obstructive sleep apnea may cause heart failure by increasing right and left ventricular load, imbalance in myocardial oxygen delivery / consumption ratio, and activation of the sympathetic system (Meguro et al. 2005). Some patients with cardiomyopathy have a high level of coxsakievirus B antibodies, suggesting that viral infections could be another contributor to the development of heart failure (Woodruff 1980). Congenital disorders also could lead to heart failure (Nora et al. 1991).

Some drugs are associated with an increased risk for developing heart failure. One of these drugs implicated in the development and progression of heart failure is doxorubicin, which is an anthracycline used to treat cancer, but has detrimental effects on the heart (Singal et al. 2000). At high doses of anthracycline, about 30 % of patients develop heart failure (Rhoden et al. 1993). Other anti-cancer drugs associated with the development of heart failure include cyclophosphamide, paclitaxel, and trastuzumab (Jekunen et al. 1994; Manthorpe and Svensson 1996; Jassal et al. 2009). Drugs including cocaine and doxazosin are implicated in the damage of cardiac tissue and may result in left ventricular dysfunction (Coviello et al. 2009). Other minor risk factors that can lead to heart failure are coffee consumption, sedentary life style, increased dietary salt, increased resting heart rate, and genetic predisposition (Coviello et al. 2009).

There are several myocardial and humoral adaptive changes in response to stress. One of the myocardial changes is hypertrophy of the heart, which is initially considered as a compensatory mechanism. Activation of the renin angiotensin system (RAS) and inflammatory system are examples of humoral adaptive changes in response to stress (Yamazaki et al. 1999; Paulus 2000; Cunningham et al. 2005). In acute MI, the activation of the neurohormonal system could be treated with ACE inhibitors (Pfeffer et al. 1992).

1.2 Sub-cellular Adjustments in Heart Failure

Many sub-cellular changes occur during the progression of heart failure, and these include a pro-inflammatory response, activation of cytokines, activation of the neurohumoral system, oxidative stress, and apoptosis (Hill and Singal 1996; Murray et al. 2000; Yaoita et al. 2000; Giordano 2005; Kaur et al. 2006). Subsequent to ischemic attack, MI can initiate a pro-inflammatory response via release of endogenous molecules from necrotic cells and the extracellular matrix. In the process, there is also an activation of toll like receptors that recruit inflammatory cells and produce many mediators for myocardial repair, proliferation of fibroblasts, scar formation and healing of the infarct (Liaudet et al. 2013). Prolongation of this inflammatory response after MI also results in adverse remodeling, increased dilatation, sphericity, and can lead to cardiac dysfunction (Frangogiannis 2012). Necrotic cells activate the complement system and generates oxidative stress, that initiates a cytokine cascade by tumor necrosis factor alpha (TNF-α). This cascade activation is important for the proliferation of fibroblasts and scar formation to repair the damaged tissue (Frangogiannis et al. 2002). At this point of acute inflammation and oxidative stress, the anti-inflammatory action of the cytokine interleukin 10 (IL-10) is vital to suppress this action. Otherwise the inflammatory response will become chronic and may lead to heart remodeling and heart failure (Frangogiannis et al. 2002; Kaur et al. 2006). Chronically, these physiologic adaptations in response to injury and stress may turn into pathologic adaptations and may lead to heart failure. Therefore, a part of the therapy to inhibit the progression of heart failure is to use adrenergic blockers, RAS blockers, and anti-inflammatory drugs in patients with heart problems (Frangogiannis et al. 2002; Davila et al. 2005).

Oxidative stress refers to a potential harmful condition due to a relative increase in reactive oxygen species (ROS) in relation to the available antioxidants. Sources for the free radicals include: mitochondrial electron transport chain, xanthine-xanthine oxidase system, nicotinamide adenine dinucleotide / phosphate NADH/NADPH and other redox reactions (Khaper et al. 2010). Free radicals like superoxide anion and hydroxyl radical attack tissues and lead to DNA damage, lipid peroxidation, protein nitration, and activation of matrix metalloproteinases, all of which contribute to cardiac remodeling (Giordano 2005; Matsuzawa et al. 2008). Despite the defense mechanisms exerted by anti-oxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, or non-enzymatic anti-oxidant like glutathione and vitamins, if free radicals exceed the capacity of this system, oxidative stress will occur (Giordano 2005; Seddon et al. 2007). Oxidative stress produced after MI is involved in cardiac remodeling as well as hypertrophy, and can lead to heart failure (Seddon et al. 2007)

Loss of cardiomyocytes leads to a decline in ventricular function and can lead to heart failure (Beltrami et al. 1995; Eichhorn and Bristow 1996). Some studies reported that this loss could be due to programmed cell death (apoptosis), independent of inflammation (Narula et al. 1996). Apoptosis may occur due to prolonged growth stimulation of cardiomyocytes (Katz 1994). In the failing heart, this growth stimulation is compensatory to meet the demand of the body, and it could be mediated by upregulation of adrenergic pathway, renin-angiotensin, and cytokines (Francis et al. 1990; Levine et al. 1990; Sadoshima et al. 1993). These stimulants may increase protein synthesis (Aceto et al. 1990), induce heart hypertrophy (Baker et al. 1990), and upregulate apoptotic proteins (Raynolds et al. 1995). We have reported that TNF-α also up-regulated apoptotic proteins

(Bax, cleaved PARP, cleaved Caspase 3, Bnip3, TGFβ) (Dhingra et al. 2007; Al-Shudiefat et al. 2013). Many studies have shown that mitochondria play important roles in apoptosis by releasing cytochrome C (Liu,X. et al. 1996; Sharma et al. 2007).

Although in the discussion thus far I have identified many gaps in our knowledge, I plan to focus on the analysis of the inflammatory cytokine TNF-α in functionally assessed MI hearts in relation to other sub-cellular changes.

2.0 Tumor Necrosis Factor Alpha (TNF-α)

2.1 Background

Cytokines are small molecular proteins (15–30 kDa), secreted by many cell types in response to different stimuli that act as endocrine, paracrine, juxtacrine, and autocrine signals, at picomolar or even femtomolar effective concentrations (Kaur et al. 2009). Cytokines are responsible for a variety of signalling outcomes including regulation of cell growth, cell differentiation, and cell repair. They are divided into categories, either inflammatory cytokines like TNF-α or anti-inflammatory cytokines like interleukin 10 (IL-10) (Kaur et al. 2006). Although there are over 100 different cytokines, only a few have generated interest in research, probably due to their importance in physiologic and pathologic conditions. For example, some cytokines studied for their role in pathogenesis and / or treatment of heart failure are: TNF-α, IL-1, IL-2, IL-6, IL-10, transforming growth factor beta (TGFβ) and cardiotrophin 1 (Pulkki 1997; Kaur et al. 2006; Dhingra et al. 2007; Yao et al. 2007; Al-Shudiefat et al. 2013).

In the 18th century, Deidier noticed that patients with syphilis were cured from tumors more often than non-infected and patients infected with syphilis had a lower frequency of cancer than the average population. This information drew a connection between microbial infection and cancer (Deidier A (1725).). Recently, this concept was supported by inverse correlation between severe infections and melanoma (Kolmel et al. 1999). Serum taken from mice challenged with a bacterial endotoxin was able to regress tumors in other mice. It was concluded that a certain factor produced by endotoxin was responsible for this cure (O'Malley et al. 1962). In 1968, the anti-tumor response of the immune system was shown by two studies, which revealed a cytotoxic factor produced by lymphocytes named lymphotoxin (Kolb and Granger 1968; Ruddle and Waksman 1968). Another study reported another cytotoxic factor produced by macrophages, and showed that the anti-tumor effect was not due to a direct effect of endotoxin, but instead was due to the production of a factor responsible for necrosis of the tumor called Tumor Necrosis Factor Alpha (TNF-α), which was also called cachexin or cachectin (Carswell et al. 1975; Beutler et al. 1985). Isolated and purified TNF-α protein showed similar characteristics and sequence to lymphotoxin alpha (LT-α or TNF-β) another member of TNF family (Aggarwal et al. 1985). TNF-α is considered a part of the innate immune response and it has a role in trauma, injury, infection, and ischemia/reperfusion. TNF-α upregulates NFκB and genes important for controlling infection and injury (Knuefermann et al. 2002; Frangogiannis 2008). Activation of TNF-α may exacerbate atherosclerosis, myocardial ischemia / reperfusion injury, and heart failure (Frantz et al. 2005), as well as rheumatoid arthritis, acute hepatitis, and neurological disorders (Agostini et al. 1995; Georgopoulos

et al. 1996; Akassoglou et al. 1997). Thus, innate immunity including TNF-α has the potential to heal or destroy the injured tissue.

2.2 TNF-α Protein

TNF-α belongs to a super family with more than 20 cytokines including TNF-α, TNF-beta (LT-α), LT-β, TNF like weak inducers of apoptosis (TWEAK), cluster of differentiation proteins 27, 30, 40 (CD 27, CD 30, CD 40), TNF related apoptosis inducing agent (TRAIL), receptor activator of NFκB ligand (RANKL), vascular endothelial cell growth inhibitor (VEGI) and others (Wajant et al. 2001). The human TNF-α gene was cloned in 1985 and was mapped to chromosome 6p21.3, and comprises 3 kilobases (Nedwin et al. 1985; Old 1985). The protein consists of 212 amino acids with a size of 23 kilodaltons (23 kDa), and is a type II trans-membrane protein with a stable homo-trimer structure (Black et al. 1997; Kriegler et al. 1988; Tang et al. 1996; Black et al. 1997). It exists in membrane bound form (mTNF-α) as well as soluble form (sTNF-α) (Kaur et al. 2006). TNF-α protein is highly conserved in all mammalian species suggesting the important functional role of this cytokine (Fiers 1991). It is synthesized as a trans-membrane precursor, which is either embedded in the membrane or released by proteolytic cleavage via metalloprotease TNF-α converting enzyme (TACE) (Black et al. 1997; Hooper et al. 1997).

2.3 TNF-α Receptors and Signalling

TNF-α has two receptors, tumor necrosis factor alpha receptor type 1 (TNFR1), which is expressed in all tissues including heart, is tumor necrosis factor alpha type 2

(TNFR2), which is expressed in immune cells as well as cardiomyocytes (Kadokami et al. 2000; Blacket al. 2002). TNFR1 is 55 kDa and TNFR2 is 75 kDa. TNFR2 has higher affinity in binding mTNF-α, and TNFR1 has 20 times higher affinity for sTNF-α than TNFR2, therefore, most of the effects exerted by TNF-α are mediated by this receptor (Grell et al. 1995).

Both receptors of TNF-α have an extracellular domain called pre-ligand binding assembly domain (PLAD) important for trimerization during activation by ligand (Chan et al. 2000). TNF-α receptors have sequences that can bind intracellular adaptor proteins that link the TNF-α stimulation with the activation of many signaling cascade, these TNF-R associated factors (TRAFs) and adaptors, transfer the signal of TNF-α in the target cells (Wajant et al. 2001).

TNFR1 contains a death domain important for inducing a death signal in the target cell (Tartaglia et al. 1993). TNF receptor associated death domain (TRADD) is receptor activated and is one of the TNFR related proteins identified early (Hsu et al. 1995). TRADD recruits FADD and RIP and then initiates the apoptotic pathway and cell injury by activating Caspase 2 and Caspase 3 apoptotic proteins (Enari et al. 1996; Duan and Dixit 1997). TNF-α, through activation of factors associated with neutral sphingomyelinase activation (FAN) is an adaptor protein that activates ceramide activated protein kinase (CAPK). The latter activates Raf kinase, which then activates MAP kinase proteins p38 and JNK (Vanden Berghe et al. 1998).

TNF-α activates certain lipases called phospholipase C (PLC) and phospholipase D (PLD), which are important for the breakdown of sphingolipids and ceramide (Kaur et al. 2009). Another phospholipase activated by TNFR1 is phospholipase A2 (PLA 2), which

is responsible for the release of arachidonic acid that could be converted to leukotriene and prostaglandins that induce eicosanoid sensing receptors. These receptors are responsible for the generation of reactive oxygen species (ROS) (Chang et al. 1992). Induction of MAP kinase and PKC by TNFR1 also can activate PLA 2, which is important for TNF-α mediated cell death in the target cells (Chang et al. 1992; Hoeck et al. 1993).

TNFR2 can only be activated by mTNF-α and not sTNF-α, therefore its role is not as clear (Grell et al. 1995). TNFR2 does not contain a death domain, but still recruits TNFR associated factor 2 (TRAF2) which interacts with NFκB inducing kinase (NIK) that phosphorylates inhibitor of κB (IκB), leading to activation of NFκB (Kaur et al. 2009). NFκB activation has a positive feedback on the synthesis of TNF-α as well as other cytokines, while RIP and FADD can directly bind TNFR2 via TRAF2 and promote apoptosis (Kaur et al. 2009). TNFR2 can induce apoptosis with what is called ligand passing, because the low affinity of TNFR2 to sTNF-α make it dissociate easily and become more available to TNFR1, which then initiate apoptosis (Tartaglia et al. 1993).

2.4 TNF-α in the Cardiac System

It was thought earlier that TNF-α is produced only by macrophages (Matthews 1978), but now it is known that cardiomyocytes also can produce this cytokine (Kaur et al. 2006; Chen et al. 2010). In hearts as well as in isolated cardiomyocytes, NFκB activation is able to increase mRNA and protein level of TNF-α in response to angiotensin II, TNF-α, ischemia/ reperfusion, burn injury and lipopolysaccharide (Li et al. 2000; Chanani et al. 2002; Maass et al. 2002; Wright et al. 2002; Hikoso et al. 2004; Yeh et al. 2009; Dai et al. 2010). TNF-α is capable of exerting a variety of cellular responses including an increase

in oxidative stress, apoptosis, inflammation, fibrosis and contractile dysfunction (Frangogiannis et al. 2002; Kaur et al. 2006; Dhingra et al. 2009; Kleinbongard et al. 2011; Al-Shudiefat et al. 2013).

Recruitment of monocytes and macrophages together with cardiomyocytes in the MI region can produce TNF-α (Mizuochi et al. 2007; Chen et al. 2010). Release of TNF-α after MI initiates a cascade of cytokine activation. This activation is important in fibroblast proliferation to repair and clear the injured tissue by forming scar tissue. If this acute inflammation is not eventually inhibited by an anti-inflammatory molecules, like IL-10, it may lead to cardiac remodeling, which may lead to heart failure (Frangogiannis et al. 2002). Patients with dilated or hypertophic cardiomyopathy and myocarditis have shown increased levels of TNF-α and other inflammatory cytokines (Matsumori et al. 1994; Shioi et al. 1997).

TNF-α can produce ROS through cytosolic as well as mitochondrial pathways (Woo et al. 2000; Ghavami et al. 2009). The generation of ROS in the cytosolic pathway is through TNF-α activation of phospholipase A 2 (PLA 2), which is important in TNF-α mediated cell death in the target cells (Chang et al. 1992; Woo et al. 2000) . Inhibition of PLA2 prevented the generation of ROS by TNF-α, suggesting the important role of PLA2 in generating TNF-α mediated oxidative stress (Woo et al. 2000). The second way of TNF-α induced ROS is through mitochondria, by decreasing the membrane potential of mitochondria, and increasing mitochondrial membrane permeabilization, which leads to an increase in the production of ROS. This process is associated with death of cells, while preventing ROS formation with antioxidants is able to inhibit cell death, suggesting the importance of ROS in TNF-α mediated cell death (Ghavami et al. 2009; Rincheval et al.

2012). Our lab showed in many studies that TNF-α induced ROS is an important factor in mediating the deleterious effects of TNF-α including apoptosis, cell damage, and cell death (Kaur et al. 2006; Dhingra et al. 2007; Dhingra et al. 2009; Al-Shudiefat et al. 2013).

There are several hypotheses regarding the source of TNF-α in heart failure. One of them is the activation of immune system release of TNF-α in the myocardium after injury. Another one is that decreased cardiac output leads to reduced perfusion and removal of endotoxins, which in turn increases the production of TNF-α (Rauchhaus et al. 2000). Studies on cancer patients using recombinant TNF-α therapy revealed that TNF-α has a half life of 14 to 18 minutes (Blick et al. 1987). This may suggest that elevated TNF-α in heart failure is related to its local synthesis. The elevated myocardial level of TNF-α may reach the plasma, leading to activation of the immune system, however there was no correlation between intra-cardiac TNF-α in explanted hearts and plasma TNF-α (Kapadia et al. 1995; Kapadia et al. 1998). Furthermore, isolated cardiomyocytes and fibroblasts in response to stress, like ischemia or endotoxins, are able to produce TNF-α in the culture medium (Giroir et al. 1992; Yue et al. 1998). Although neither the protein nor the mRNA of TNF-α are expressed at a constant rate in the unstressed heart, both of them are rapidly synthesized in response to stimuli in animals and humans (Giroir et al. 1992; Torre-Amione et al. 1996; Kapadia et al. 1997; Torre-Amione 2005). In the clinical trial Study of Left Ventricular Dysfunction (SOLVD), it was observed that mortality increased with an increase in the plasma TNF-α levels in heart failure patients (Torre-Amione 2005). With increase expression of TNF-α and its receptors, there was an increase in the mortality in congestive heart failure (CHF) patients (Rauchhaus et al. 2000). After using left

ventricular assist device in patients with end stage heart failure, there was a decrease in TNF-α and improved cardiac function (Torre-Amione et al. 1999).

In an experimental rat model of MI, gene expression of TNF-α rose excessively and was correlated with compromised left ventricular end diastolic dimensions and fibrosis (Irwin et al. 1999). At high but not low left ventricular diastolic pressures in isolated feline hearts, there was an increase in TNF-α level (Kapadia et al. 1997). The change in mRNA as well as protein of TNF-α in this model was apparent at 30 min, suggesting that even increased wall stress can stimulate cardiac TNF-α production rapidly (Kapadia et al. 1995). In rabbits, a brief banding of aorta was able to increase the production of TNF-α and led to deterioration of cardiac function (Stamm et al. 2001).

Administration of TNF-α acutely led to cardiac dysfunction in dogs, rats and pigs (Eichenholz et al. 1992; Heard et al. 1992; Pagani et al. 1992). In dogs, TNF-α infusion caused left ventricular dysfunction, cardiomyopathy, LV remodeling, pulmonary edema, and apoptosis (Heard et al. 1992; Pagani et al. 1992). Administration of TNF-α in the peritoneal cavity of rats chronically appeared to promote left ventricular remodelling (Bozkurt et al. 1998).

The over-expression of TNF-α in transgenic mice induced heart failure, with symptoms including peripheral edema, cardiac dilatation, weight loss, and decreased cardiac contractility (Kubota et al. 1997). In the same model of transgenic mice, over-expression of TNF-α led to cardiomegaly and severe lethal myocarditis (Kubota et al. 1997). However, TNF-α knockout mice showed less cardiac rupture compared to the wild type, with reduced number of apoptotic cardiomyocytes (Sun et al. 2004).

TNF-α is capable of activating matrix metalloproteinases like MMP-1 that are responsible for cardiac remodeling at the site of infarction as well as degradation of extracellular matrix (Rawdanowicz et al. 1994; Cleutjens et al. 1995). There was a correlation between expression of TNF-α and type I and III collagen, one week PMI (Yue et al. 1998). Administration of TNF-α in animal studies decreased fibrillar collagen staining with disrupted myocardial extracellular matrix, suggesting an effect of TNF-α in degradation of matrix (Bozkurt et al. 1998; Li et al. 2000). The degradation of matrix was progressive and associated with left ventricular dilatation. In mice with over-expression of TNF-α, there was an increase in soluble collagen, and use of TNF-α antagonist reduced soluble collagen, and left ventricular remodeling (Li et al. 2000).

2.5 Anti-TNF Therapy and its Failure

Although the suppression of TNF-α was suggested to improve cardiac function in patients with heart disease, an anti-TNF-α therapy approach failed in clinical trials for cardiovascular diseases (Louis et al. 2001; Scheinfeld 2004). TNF-α blockers are either monoclonal anti bodies (MAbs) like Infliximab (Remicade), Adalimumab (Humira), or TNF-α receptor analogues like Etanercept (Enbrel) (Scheinfeld 2004). The FDA has approved the use of these drugs in some auto-immune diseases, such as rheumatoid arthritis, psoriasis, Crohn's disease, and ankylosing spondylitis, but not for heart disease (Listing et al. 2008). There is a risk of developing side effects including: lymphoma, congestive heart failure, demyelinating disease, a lupus like syndrome, induction of autoantibodies and other systemic side effects (Scheinfeld 2004). Research into Etanercept: Cytokine antagOnism in VEntriculaR function (RECOVER) and Randomised Etanercept

North AmerIcan Strategy to Study ANtagonism of Cytokine E (RENAISSANCE) were two major clinical trials of a TNF-α blocker. The first was in Europe and the second was in USA; both were dealing with TNF-α blocker Etanercept. They were stopped because of the lack of evidence of any benefit over the placebo group. Another clinical trial named Anti TNF-α Therapy Against Congestive Heart Failure (ATTACH) using Infliximab, showed a significant increase in hospitalization and death (Chung et al. 2003). This adverse effect may be due to powerful elimination of TNF-α using the MAb Infliximab, compared to the weak elimination of TNF-α by receptor analogue Etanercept. Some explanations for the lack of positive results of clinical trials on Etanercept are: insufficient dose, inclusion of small numbers of patients, and duration of the treatment (Anker and Coats 2002; Mann et al. 2004; Aukrust et al. 2006). Another explanation was that these drugs increased the bioavailability of TNF-α, which may have increased its toxicity (Aderka et al. 1992; Mann et al. 2004).

Since two large clinical trials including anti-TNF-α therapy failed in the treatment of heart diseases, there is a real need for alternatives to modulate TNF-α function. Olive oil and oleic acid (OA) exert some of their beneficial effects through anti-inflammatory and anti-oxidant properties. They therefore have the potential to mitigate the adverse effect of TNF-α and serve as an alternative. This has been the other focus of the present study.

3.0 Olive Oil and Oleic Acid

3.1 Olive Oil

The olive is the fruit of the olive tree (Olea europaea L). Olive oil is obtained by squeezing the olive fruit under a high pressure using hydraulic press plates (Owen et al. 2000). Olive oil is largely composed of triacylglycerols (fatty acids) and 0.5-1.5 % non glyceridic constituents (Boskou 2000). Italian extra-virgin olive oil contains 79-82.2 % OA (C 18:1), 8.7-11.1 % palmitic acid (C 16:0), 4.2-6.6 % linoleic acid (C 18:2), 1.8-3.4 % stearic acid (C 18:0) and other minor components (Holmann et al. 1977). There are more than 30 bioactive compounds in the minor components of olive oil (Ghanbari et al. 2012). One of these minor components are Phenolic compounds, with concentrations in Italian olive oil ranging from 50-1,000 mg/Kg or less than 1.1 % of olive oil (Montedoro et al. 1992). For the minor components of olive oil, only a few compounds had received attention, and they include: Hydroxytyrosol, tyrosol, oleuropein, ligstroside, and squalene (Owen et al. 2000; Waterman and Lockwood 2007; Bester et al. 2010). Of note, 98% of the phenolic compounds (e.g. hydroxytyrosol, tyrosol, oleuropein and ligstroside) in olive oil are lost in the olive milling waste, and only 2% are bioavailable in the olive oil (Rodis et al. 2002). Oleocanthal, which is the dialdehydic form of ligstroside, is a non selective inhibitor of cyclooxygenase, similar to the mode of action of the non steroidal anti-inflammatory drug (NSAID) ibuprofen (Beauchamp et al. 2005). Olive oil is also used for hair and skin care due to its anti-inflammatory and anti-oxidant contents (Oja olive oil company 2013). It is effective in treating dermatitis in humans (Kiechl-Kohlendorfer et

al. 2008). OA from olive oil is used as an emulsifier or solublizing agent in aerosols (Smolinske, Susan 1992).

Most olive oil, about 99 %, is produced in Mediterranean countries (Ghanbari et al. 2012). Virgin olive oil is obtained by physical means without using chemicals. In contrast, refined olive oil is obtained by using chemical solvents. The quality of olive oil depends on virginity and low acidity (International Olive Council 2013). Virgin olive oil is classified according to its acidity: Extra-virgin (acidity 0.2 %), virgin (acidity 2%), ordinary virgin (acidity 3.3 %), lampante virgin (acidity more than 3.3%- not for human use). Refined olive oil has acidity of 0.3%.

The Seven Countries Study (United States, Finland, the Netherlands, Yugoslavia, Italy, Greece, and Japan) was designed to investigate the relationship between diet and cardiovascular disease. This study revealed that people living in Crete had lower rates of cardiovascular disease and cancer compared to other regions. The authors conclude that this may be related to the low consumption of saturated fats and high consumption of OA present in olive oil (Keys 1970). The first clinical trial that showed the potential beneficial effect of olive oil was The Lyon Diet Heart Study conducted on 605 patients with myocardial infarctions over 27 months (de Lorgeril et al. 1994; de Lorgeril et al. 1999). The results of this study showed a decrease in coronary events (73 %) and total mortality (70 %) in patients on a Mediterranean diet that was enriched in olive oil. Other clinical trials showed beneficial properties of olive oil on the cardiovascular system, including increased HDL, and lowering of cholesterol and LDL levels (Vincent-Baudry et al. 2005; Ahuja et al. 2006; Covas et al. 2006; Estruch et al. 2006). Olive oil also reduces the susceptibility of LDL to oxidation (Ruano et al. 2005; Estruch et al. 2006), improves blood

pressure (Fito et al. 2005; Estruch et al. 2006; Bondia-Pons et al. 2007), enhances endothelial function (Ruano et al. 2005; Fuentes et al. 2008), and reduces susceptibility to thrombosis and platelet aggregation (Pacheco et al. 2006; Ruano et al. 2007; Delgado-Lista et al. 2008).

Protection against cardiovascular diseases in the Mediterranean region is partially due to the consumption of an unsaturated fatty acid diet which contains a high percentage of OA (de Lorgeril et al. 2006). Although the major benefits of olive oil may come from its major compound OA, some other minor compounds have also been reported to offer beneficial effects. Phenolic compounds (oleuropein, hydroxyltyrosol, and tyrosol) showed some cardiovascular benefits including prevention of LDL oxidation, inhibition of platelet aggregation, and scavenging of free radicals (Visioli and Galli 1998).

3.2 Oleic Acid (OA)

The word oleic means related to or derived from olive oil (Wikipedia contributors 2013). Oleic acid is a monounsaturated fatty acid (omega 9 fatty acid) due to the presence of a single double bond on carbon 9 of the 18 carbons presents in its structure (C18:1) (Bermudez et al. 2011; Lim et al. 2013). It is reported to be anti-inflammatory, anti-oxidant, anti-hypertensive, and has a positive inotropic effect in the heart (O'Bryne et al. 1998; Lopez-Miranda et al. 2000; Pi and Walker 2000; Mesa Garcia et al. 2006; Teres et al. 2008). Based on the outcome of twelve clinical trials on the benefit of olive oil in reducing LDL, in 2004, the US Food and Drug Administration (FDA) recommended consumption of two tablespoons (23 g) of olive oil each day to decrease the risk of coronary heart diseases, due to its content of OA (FDA 2004; Ghanbari et al. 2012).

3.2.1 Anti-inflammatory Properties

Inflammation plays a major part in many chronic medical conditions, including cardiovascular disease (Lu et al. 2013). It has been estimated that the number of deaths due to cardiovascular diseases will rise from 17 million in 2008 to 25 million in 2030, and about half of these deaths will be due to coronary artery disease (WHO 2012). The anti-inflammatory property of OA may be due to the inhibition of the release of chemoattractants (Mesa Garcia et al. 2006), as well as a reduction in the TNF-α induced expression of the adhesion molecules vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and E-selectin. Such an inhibitory effect of OA protects the endothelium from inflammation and atherosclerosis by reducing the adhesion of leukocytes (De Caterina et al. 2000).

3.2.2 Anti-oxidant properties

Several epidemiological studies have revealed that diets rich in OA are associated with a decrease in the risk of coronary heart disease (Lopez-Miranda et al. 2010). Oxidized LDL is associated with the progression of atherosclerosis and heart failure, while using anti-oxidants is beneficial for the management of the disease (Patrick and Uzick 2001; Charach et al. 2012). Many studies on the consumption of a high OA diet showed a decrease in the susceptibility of human LDL for oxidation (O'Bryne et al. 1998; Lopez-Miranda et al. 2000; Hargrove et al. 2001). OA has an antioxidant activity greater than the polyunsaturated fatty acid linoleic acid, making olive oil more resistant to oxidation and highly stable, which exerts a greater protection in humans (Owen et al. 2000). Olive oil is resistant to oxidation that is largely due to its high content of OA, which integrates

in the cell membrane and protects it from oxidative stress (Reaven and Witzum 1996). A clinical intervention study has shown that the consumption of diets rich in OA reduced oxidative stress in the plasma of obese subjects (Colette et al. 2003). It has also been suggested that the antioxidant compounds present in extra virgin oil may provide significant protection against cancer (colon, breast, skin) by suppressing oxidative stress (Owen et al. 2000).

3.2.3 Anti-hypertensive properties

Hypertension is a major risk factor for cardiovascular diseases, it is responsible for > 60 % of all strokes and about 50 % of ischemic heart disease (Chrysant et al. 2010). One in three adults worldwide has high blood pressure (WHO 2012). Consumption of olive oil, rich in OA, in hypertensive women decreased blood pressure significantly (Ruiz-Gutierrez et al. 1996). In rats given oleic acid orally, blood pressure decreased significantly (Teres et al. 2008). Olive oil reduces blood pressure by increasing OA content in membranes, which regulates membrane lipid structure and controls G-protein signaling via adenylyl cyclase and phospholipase C (Teres et al. 2008). This mechanism of lowering blood pressure is also supported by others (Yang et al. 2005). Another mechanism of lowering blood pressure by olive oil was suggested to be through the blockade of calcium channels (Gilani et al. 2005). A synthetic derivative of OA, 2-OHOA, is capable of lowering blood pressure via increasing cAMP production in response to Gαs activation (G-protein). This finding supports the previous studies showing that diets rich in OA have beneficial effects on blood pressure in hypertensive patients (Alemany et al. 2004). Elaidic acid, a trans isomer of oleic acid, was not able to reduce blood pressure, suggest-

ing the importance of the cis configuration of the OA double bond in exerting the lowering effect on blood pressure (Teres et al. 2008).

3.2.4 Anti-apoptotic properties

When monounsaturated fatty acids are added to rat cardiomyocytes *in vitro*, the cells remained healthy, whereas addition of saturated fatty acids damaged the cells. Furthermore, the addition of OA counteracted the induction of apoptosis induced by palmitic acid, while elaidic acid delayed apoptosis but did not prevent it (de Vries et al. 1997). In another study, OA was shown to prevent the palmitic acid induced decrease in Bcl2 and the release of cytochrome C, thereby protecting pancreatic cells from apoptosis and death (Maedler et al. 2003). Serum deprivation induced apoptosis in enteroendocrine cell was also inhibited by OA through a reduction in Caspase 3 activity (Katsuma et al. 2005).

3.2.5 Inotropic properties

OA can integrate into cell membrane and affect its physiochemical properties. It has been shown that OA exerts a positive inotropic effect on the heart. It enhanced the contractility of frog cardiomyocytes at a very low concentration. Moreover it diminished the depressive effect of acetylcholine and excessive calcium on cardiomyocytes, probably by normalizing these depressants (Pi and Walker 2000; Loewi 1955). A derivative of OA (2OHOA), has been shown to increase contraction and shortening of isolated cardiomyocytes by increasing $[Ca^{2+}]i$ transient current (Borchert et al. 2008). In another study, OA was able to exert its positive inotropic effect via the activation of protein kinase C (PKC),

which enhanced the contractility of cardiomyocytes, while PKC inhibitors prevented this response (Pi and Walker 2000).

3.2.6 Other properties

In a dietary interventional study, it has been shown that a diet rich in OA was able to reduce the activation of clotting factor VII (Kelly et al. 2001) and decrease LDL levels (Allman-Farinelli et al. 2005). It has been shown that OA was able to prevent TNF-α induced inhibition of insulin release in isolated pancreatic cells as well as in an *in vivo* in rats (Vassiliou et al. 2009). OA protects against inflammation and insulin resistance induced by palmitate in skeletal muscle. This effect occurs by promoting triglyceride formation and upregulating the expression of genes involved in mitochondrial β- oxidation, such as peroxisome proliferator- activated receptor (PPAR) γ –coactivator 1 α and through protein kinase A dependent mechanisms (Coll et al. 2008). A randomized trial of 605 patients with coronary heart diseases consuming either a cardioprotective Mediterranean diet or a diet close to American Heart Association prudent diet reported that the former group has a prolonged survival and was probably protected against cancer (de Lorgeril et al. 1998). In a later study, sequalene, terpenoids and OA in olive oil were considered to be anticancer agents (Owen et al. 2004). OA suppresses the HER2 oncogene which plays an important roles in the development and metastasis in many human tumors through upregulation of PEA3 gene and downregulation of FASN gene (Menendez and Lupu 2006). In 2005, a synthetic OA derivative (Minerval) was used as an anticancer drug, preventing the proliferation of A549 cancer cells through the activation of protein

kinase C (PKC) which increased P53 and decreased cyclins A, B, D3 and cdk2 (Martinez et al. 2005).

The detailed literature review presented here provides a strong rationale for the study of the change as well as the role of TNF-α in heart failure subsequent to myocardial infarction. Given the long list of the beneficial effects of the Mediterranean diet rich in olive oil, a careful study of the potential benefits of olive oil in heart failure is also warranted. This is the main thrust of my thesis. In addition, my study focused on describing the molecular mechanisms by conducting experiments on isolated cardiomyocytes.

IV. HYPOTHESIS

Olive oil and its major component oleic acid will protect against oxidative stress and remodeling of the heart subsequent to myocardial infarction in rats by mitigating TNF-α induced changes in cardiomyocytes.

APPROACH AND SPECIFIC AIMS

Our approach was to analyze first the beneficial effects of olive oil in an *in vivo* myocardial infarction (MI) model of heart failure subsequent to coronary artery ligation in the rat. This was complemented with the study of the effects of corn oil as a control for caloric intake. In the second part of my approach, I examined the beneficial effects of oleic acid (OA) a principal component of olive oil, in isolated adult rat cardiomyocytes challenged with tumor necrosis factor alpha (TNF-α). I chose oleic acid (OA), because most of the beneficial properties of olive oil are due to its major component oleic acid, which comprises about 80 % of extra-virgin olive oil. Some of these properties include lowering of blood pressure (Teres et al. 2008), oxidative stress (Duval et al. 2002), and LDL cholesterol (Allman-Farinelli et al. 2005). The reason for using TNF-α as stimulus is that it is a cytokine known to increase in heart failure and may also be involved in its pathogenesis

subsequent to MI (Torre-Amione et al. 1996; Kubota et al. 1997; Kubota et al. 1997; Bryant et al. 1998; Torre-Amione et al. 1999).

Following are the specific aims of the study:

- Analyze the effects of olive oil and other control diets in coronary ligated rats with respect to:
  - The heart function inclusive of: heart rate, myocardial conduction system abnormalities, blood pressure, left ventricular ejection fraction (LVEF), left ventricular internal dimension-diastole (LVIDd), and Tissue Doppler Imaging (TDI) parameter for the anterior wall.
  - The lipid profile (Total cholesterol, High density lipoprotein (HDL), and Triglycerides (TG)), TNF-α levels in the plasma and myocardial tissue, and oxidative stress (Glutathione, lipid hydroperoxides, and reactive oxygen species (ROS)); and
  - The other cellular and sub-cellular changes in the heart: expression of apoptotic proteins (Bax, Caspase 3, PARP, TGFβ, Bnip3, and cytochrome c), anti-apoptotic protein Bcl-xL, and MAP kinases (p38, JNK, ERK) as well as fibrosis.
- Study the effects of OA in isolated adult rat cardiomyocytes on TNF-α induced oxidative stress (reactive oxygen species production), apoptosis (apoptotic proteins: Bax, cleaved Caspase 3, cleaved PARP, TGFβ, Bnip3 and anti-apoptotic protein Bcl-xL), DNA fragmentation (Hoechst test), cell death (viability of cells), and cell injury (creatine kinase leakage).

V. METHODS

In a comprehensive approach to test our hypothesis, we undertook studies on whole animals (*in vivo* studies) as well as on isolated adult cardiomyocytes (*in vitro* studies).

1.1 *In vivo* studies

1.2 Animal Model

All experiments were done according to protocols approved by the University of Manitoba Animal Care Committee, following the guidelines established by the Canadian Council on Animal Care. Male Sprague-Dawley (SD) rats weighing 100± 10 g were acclimatized for several days. The animals were divided into 3 groups and were started on 3 different diets for 10 days before surgery: rats fed on regular chow, rats fed on regular chow supplemented with (10 % w/w) olive oil, and rats fed on regular chow supplemented with (10 % w/w) corn oil. In each diet group animals were either sham operated or coronary ligated. Post-surgery duration was either 4 weeks or 16 weeks.

Myocardial infarction (MI) was created surgically by ligating the left anterior descending coronary artery as reported earlier with some modifications (Selye et al. 1960; Khaper and Singal 2001). The only change to the procedure was the location of the incision and that the heart was not exteriorized. This procedure was done according to R. O.

Burrel Lab standard operating protocol (SOP MC #7.00). Animals were anaesthetized with 2% isoflurane, and the chest hair on the left side over the heart was shaved and wiped with 3 % hydrogen peroxide and then with 2% iodine for disinfection. A 1-1.5 cm incision was made to the left of, and parallel to the sternum (animal's left side) in the fifth intercostal space, and chest was opened carefully to avoid cutting any blood vessels. The 5^{th} and 6^{th} ribs were separated with a small retractor to visualize the heart. The coronary artery was visualized on the upper left side of the heart with appropriate lighting and gentle pressure with sterile cotton swap wetted with normal sterile saline. The left coronary artery was then tied with a 6-0 monofilament suture. The lungs were re-expanded and the chest was closed by re-approximating the ribs with two intercostal sutures using 3-0 polysorb. The separated muscles were sutured with 3-0 polysorb in a simple continuous pattern. Before closing the skin, the air in the chest was removed using a 20G catheter attached to a 10cc syringe. The skin was then closed with Michel clips. For sham animals, the same procedure was done except, the suture placed around the coronary artery was not tied. The animals were allowed to recover on 100% oxygen upon completion of the surgery. These animals were monitored on a regular basis for their food intake, water intake, body weight, general behaviour and mortality.

1.3 Study Groups

A total of 90 animals were divided into 12 groups, six groups for 4 weeks, and another six groups for the 16 weeks post-surgery duration, as follows:

Table 1: Animal groups for the *in vivo* studies.

Group No.	End point	Operation	Diet	No. of rats
1	4 weeks	Sham	Regular chow	6
2	4 weeks	Ligated	Regular chow	9
3	4 weeks	Sham	Regular chow + 10% olive oil	6
4	4 weeks	Ligated	Regular chow + 10% olive oil	9
5	4 weeks	Sham	Regular chow + 10% corn oil	6
6	4 weeks	Ligated	Regular chow + 10% corn oil	9
7	16 weeks	Sham	Regular chow	6
8	16 weeks	Ligated	Regular chow	9
9	16 weeks	Sham	Regular chow + 10% olive oil	6
10	16 weeks	Ligated	Regular chow + 10% olive oil	9
11	16 weeks	Sham	Regular chow + 10% corn oil	6
12	16 weeks	Ligated	Regular chow + 10% corn oil	9

Rats were started on the special diets 10 days prior to the surgery to establish a stable lipid profiles as well as possible dietary protection. This period of standardization has been used previously for agents like Verapamil, which has an anti-hypertensive property, and epicatechin, which has an anti-oxidant property, to see their protective effects on myocardial infarction. Both of these properties are present in olive oil (Anand et al. 1980; Yamazaki et al. 2010).

1.4 Special Diet Preparation

The regular chow (Prolab RMH 3000 5p00) in a pellet form for regular chow groups, and in powdered form was purchased from Ren's Pet Depot (Guelph, Canada). The diet was reconstituted with either olive oil (10 % wt/wt) or corn oil (10 % wt/wt) for the special diet groups. An antioxidant TBHQ (0.02 %) was added to olive and corn oil stocks to prevent rancidity and preserve freshness of the diet as recommended by the American Institute of Nutrition (American Institute of Nutrition 1980; Fritsche and Johonston 1988; Farhoosh et al. 2012). After adding TBHQ to stock oils (olive and corn) and mixing them very well for 3 hours by magnetic stirrer, the oils (10 % w/w) were mixed with the regular chow powder and 37.5% water was added to the mixture and was mixed with Hobart mixer (Canada) for 3-5 minutes to make a homogenous dough. After that, the diet was bagged (one kilogram per bag) in self sealed plastic bags, and properly labeled as olive oil or corn oil with the date of preparation. After removing the air from the bags, the bags were sealed, and stored at -20 °C as recommended by the American Institute of Nutrition (Reeves et al. 1993). One day before feeding, enough bags were transferred to the cold room at 4 °C to thaw the food for the next day. Food preparation was done every 10 days, to ensure that the diet was always fresh. Animals were fed three times per week on Mondays, Wednesdays, and Fridays. For Mondays and Wednesdays the diet was enough for two days, while for Fridays, the diet was enough for three days until the next Monday. The diet was served in fresh containers (hoppers) each time to prevent mold growth. The remaining diet in the hoppers was weighed for each singly housed rat until the end of the study, to determine the rate of diet consumption for both olive oil and corn oil diet groups.

1.5 Function Assessment

For the study of heart function, two different methods were adopted, CODA non-invasive blood pressure system, and echocardiography (Echo). Electrocardiography (ECG) was also done.

1.5.1 CODA Non-invasive Blood Pressure System

The blood pressure was recorded using CODA non-invasive tail cuff blood pressure system available from Kent Scientific as described previously (Feng and DiPetrillo 2009). This recording was done for all animals at two time points: after one week of the special diet immediately prior to surgery, and after 4 weeks of the special diet (3 weeks post-surgery). Animals were placed on a warm plate to make sure their body temperature was well controlled (35 ^{o}C), as their temperature is critical for the generation of adequate blood flow through vasodilatation of the tail for the blood pressure signal. The blood pressure was recorded in a dark (dim light), quiet room, with doors closed, and rats were gently guided in restrainers to decrease the level of stress and movement. The recording of BP was done through two cuffs: occlusion (O) cuff positioned on the base of the tail, and volume pressure recording (VPR) cuff positioned after occlusion cuff on the rest of the tail to detect changes in the blood flow in the tail which is corresponding to systolic and diastolic blood pressures. VPR cuff and O cuff were inflated to the maximum to push out the blood from the tail and occlude blood, and then the VPR cuff was deflated. When O cuff gradually deflated and systolic blood pressure exceeds the O cuff pressure, the blood flow will increase and detected by VPR cuff. At this point O cuff pressure is the systolic blood pressure. As O cuff deflated more and no increase recorded in the blood

volume of the tail by VPR cuff, at this point, O cuff pressure is the diastolic blood pressure. In order to enhance precision and eliminate false readings, three or more readings were taken for each rat. Rats were trained one to two days prior to the measurements on the system to decrease the level of stress before taking the final readings on the day of the real test.

1.5.2 Echocardiography (Echo)

Transthoracic echocardiography was performed as previously described (Wyatt et al. 1980; Brown et al. 2002; Weytjens et al. 2006; Kaur et al. 2006; Jassal et al. 2009) on all animals at different time points: baseline, 3 days post-surgery, 2 weeks post-surgery, 4 weeks post-surgery, and 16 weeks post-surgery. Cardiac parameters of interest included heart rate (HR), LV cavity dimensions including LV end-diastolic diameter (LVIDd) and LV end-systolic diameter (LVIDs), and LV ejection fraction (LVEF). Additionally, the tissue Doppler imaging parameter endocardial velocity (Vendo) was non-invasively assessed.

Rats were anesthetized with isoflurane (3 %) in a chamber, and then moved to a mask for maintenance (1.5 – 2 % isoflurane). The hair was removed from the chest using clippers, the area was cleaned and a thin layer of acoustic gel is applied. Echocardiography was performed using a 13 MHz probe (Vivid 7, GE Medical Systems, Milwaukee, WI, USA). LVEF was determined by manually tracing the left ventricular end diastolic volume and left ventricular systolic volume. LVIDd was determined using M mode echocardiography through short axis view of the heart. Tissue Doppler imaging was performed in the short axis view of the LV at the level of the papillary muscles. All offline post proc-

essing of images was done using EchoPAC software (Vivid 7, GE Medical Systems, Milwaukee, WI, USA). Once measurements were completed, the animals are allowed to recover on 100 % oxygen via mask.

1.5.3 Electrocardiography (ECG)

Rats were anesthetized with isoflurane (3 %) in a chamber, and then moved to a mask for maintenance (1.5 – 2 % isoflurane). The animals were wiped and wet at the point of insertion with alcohol. Needle electrodes (29 gauge) were inserted subcutaneously in the area of the biceps and inner leg muscles on both sides. Each electrode was labeled as to the location of insertion. Right arm is labeled as RA, left arm is LA, right leg is RL and left leg is LL. The recording was carried out using MP100, a BIOPAC analogue to digital data acquisition system (BIOPAC Systems, Goleta, CA). After the recording, electrodes were removed, cleaned, and disinfected between animals using alcohol. The animals were allowed to recover on 100 % oxygen via mask. ECG was analyzed from lead II as previously mentioned (Singal et al. 1982) to determine myocardial conduction abnormalities compared to sinus rhythm. Final analysis of conduction system abnormalities was done under the guidance of Dr. Davinder Jassal, a cardiologist and clinician scientist in the Institute of Cardiovascular Sciences at Saint Boniface Hospital Research Centre.

1.6 Biochemical Tests

Different biochemical tests were done with the plasma and heart of all animal groups to measure: plasma (lipid profile and TNF-α); and myocardial (TNF-α, oxidative stress, apoptotic and anti-apoptotic proteins, and MAP kinase proteins).

1.6.1 Lipid Profile

For the assessment of lipid profile, blood samples were collected from the jugular vein for all animal groups in heparinised tubes, and mixed very well with the anticoagulant, then centrifuged at 2500 g for 10 minutes to separate plasma from blood. The plasma supernatant was taken carefully without disrupting the pellet, refrigerated immediately and sent to the biochemistry labs at the Saint Boniface Hospital for measuring the lipid profile (Total cholesterol, HDL, TG, and LDL).

1.6.2 Estimation of Myocardial and Plasma TNF-α

Animals were anaesthetized intra-peritoneally by a mixture of 9:1 ketamine (90 mg/kg) to xylazine (10 mg/kg). Animal chests were cleaned and opened to remove the heart carefully. Hearts were washed with PBS to remove blood, atria, fatty tissues and scar tissue was discarded. The remaining heart was gently blotted and using a sharp surgical blade, a thick slice (0.5 cm) from the middle of the heart was cut smoothly and transferred to a container with 10 % formalin buffered solution for histological studies. About 0.1 g of myocardial tissue from the left ventricle was taken for the cytochrome C analysis as well as isolation of mitochondria on the same day. The remaining myocardial tissue from the left ventricle was transferred to Eppendorf tubes quickly and snap frozen in liquid nitrogen, then transferred to the freezer (-70 °C) for biochemical analysis later on.

On the day of analysis, frozen tissue (0.1 g) was washed with PBS and cut into small pieces. The myocardial tissue was homogenized in 1 ml radioimmunoassay (RIPA) buffer, containing protease inhibitor cocktail from Roche, and phosphatase inhibitors

from Sigma, for five seconds, five times, using a PT-3000 Polytron homogenizer (Brinkmann Instruments, Inc), until the mixture was homogenous without apparent pieces. During the homogenization, the tube was on ice in a beaker to minimize any loss of TNF-α protein. Myocardial homogenate was then centrifuged at 10,000 g for 25 minutes at 4 °C. The supernatant containing myocardial TNF-α was then carefully taken into another new Eppendorf tube and properly labeled and assayed for TNF-α using an ELISA kit (R & D Systems, Minneapolis, MN) as previously described (Torre-Amione et al. 1999; Kaur et al. 2006).

For myocardial TNF-α, a 50 µl aliquot (50-80 µg protein) of the sample was added to the assay buffer and was analyzed in duplicate with proper standards and controls, according to the manufacturer's instructions. Spectrophotometric readings of the samples were taken at 450 nm with correction wavelength set at 540 nm. The final concentration of myocardial TNF-α was expressed as picogram/mg (pg/mg) of protein.

For plasma TNF-α, a 50 µl aliquot of twofold dilution of plasma was assayed similarly as myocardial TNF-α. The only difference is that the final concentration for plasma TNF-α was multiplied by two to compensate for the dilution factor. Final concentration of plasma TNF-α was expressed as picogram/ml (pg/ml).

1.6.3 Study of Oxidative Stress

To assess the level of oxidative stress in the hearts of animals, two myocardial parameters were measured: glutathione level, and lipid hydroperoxides.

1.6.3.a Study of Glutathione Level

Myocardial reduced (GSH) and oxidized glutathione (GSSG) were both measured in all animal groups, using a commercial Glutathione Assay kit (Cayman Company, USA). The assay depends on recycling of glutathione by glutathione reductase enzyme (Baker et al. 1990). Myocardial tissue (0.1 g) was homogenized with 1 ml RIPA buffer and centrifuged at 10,000 g for 15 minutes at 4 °C. The supernatant was removed carefully on ice and deproteinized using 10 % metaphosphoric acid and 4 M triethanolamine. 50 µl of each sample along with proper standards were assayed in duplicate according to the manufacturer instructions. The sulfhydryl group of glutathione reacts with DTNB (5, 5'-dithio-*bis*-2-(nitrobenzoic acid), Ellman's reagent) and results in a yellow colored substance 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB is reduced by the enzyme glutathione reductase to recover GSH and make more TNB. The rate of TNB production is proportional to the total GSH concentration in the sample. Spectrophotometric reading of TNB at 405 nm provides a precise evaluation of total glutathione in the sample. Also the assay is capable of measuring only GSSG by dervitizing GSH with 2-vinylpyridine after deproteinization of samples by 10 % metaphosphoric acid and 4 M triethanolamine, and then samples are read at 405 nm. GSH values were calculated as the difference between total glutathione (GSH+GSSG) and GSSG concentrations. Concentration of GSH and GSSG were expressed as µmol/g wet tissue weight. Redox ratio was calculated by dividing GSH over GSSG.

1.6.3.b Study of Lipid Hydroperoxides

Assessment of lipid hydroperoxides in all groups was done using a commercially available kit (LPO-CC assay) from Kamiya Biomedical Company in Seattle, USA. The protocol of this assay was adapted from previously published work (Yagi et al. 1986; Palace et al. 1999; Khaper and Singal 2001). Myocardial tissue (0.1 g) was cut into small pieces after washing with PBS, then homogenized in 1.5 ml chloroform/methanol (2:1, v/v) mixture on ice for five seconds, five times using a PT-3000 Polytron homogenizer (Brinkmann Instruments, Inc), until the mixture was homogeneous without any apparent tissue pieces. Then the mixture was centrifuged at 3000 g for five minutes, and the supernatant was discarded carefully. The chloroform layer containing lipids was evaporated using nitrogen gas for about 20 minutes or until dried. Lipid residue was dissolved in 100 µL isoproponol, and 15 µl of each sample was assayed in duplicate with proper standards according to the kit instructions. The reaction in the assay uses a derivative of methylene blue, which is cleaved by lipid hydroperoxides to yield methylene blue which was measured spectrophotometrically at 675 nm, and a standard curve of cumene hydroperoxide was established to evaluate lipid hydroperoxides in the samples. Concentration of lipid hydroperoxides was expressed as nmol/g myocardial tissue.

1.6.4 Estimation of apoptotic, anti-apoptotic, and MAP Kinase proteins

These proteins were studied by Western blot analysis.

Sample preparation from heart tissue: Frozen myocardial tissue (0.1 g) was washed with PBS and cut into small pieces and then homogenized with 1 ml RIPA buffer (50 mM Tris-HCl pH 7.4, 15 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.5 % sodium deoxy-

cholate, 0.1 % SDS, and 1 % Triton 100X) containing a cocktail of proteases inhibitors from Roche Diagnostics, and phosphatase inhibitors from Sigma. The homogenization was done on ice for five seconds, five times using a PT-3000 Polytron homogenizer (Brinkmann Instruments, Inc), until the mixture was homogeneous without any apparent tissue pieces. The mixture was centrifuged at 10,000 rpm for 20 minutes at 4°C to remove cell debris, and the supernatant was saved for protein estimation and Western blot analysis.

Sample preparation for mitochondrial and cytosolic cytochrome C: Mitochondrial and cytosolic fractions of cytochrome C were prepared from the fresh myocardial tissues using a commercial kit (No 89801, Thermo Scientific). Protease inhibitor cocktail from Roche Diagnostic was added to the working kit reagents. A small amount of myocardial tissue (0.1 g) was cut into small pieces using scissors in a weighing cup on ice and washed two times by PBS, then the tissue was homogenized in a tube on ice containing 800 μL PBS with a Polytron homogenizer three to five times. The homogenate was centrifuged at 1,000 g for 3 minutes at 4°C, and the supernatant was removed carefully and the pellet was suspended in 800 μL BSA/reagent A solution. The pellet was then vortexed at medium speed for 5 seconds, and the tubes were incubated on ice for exactly 2 minutes. Reagent B (10 μL) was added to each tube and vortexed at maximum speed for 5 seconds, the tubes were incubated 5 minutes and vortexed at maximum speed every minute. 800 μL reagent C was added and tubes were inverted several times to mix. Then the tubes were centrifuged at 700 x g for 10 minutes at 4°C. The supernatant was carefully transferred to new tubes, centrifuged at 3,000 x g for 15 minutes at 4°C, and the pellet was discarded. The supernatant was taken and labelled (cytosolic cytochrome C) and

the pellet was washed and labelled (mitochondrial cytochrome C). These two fractions were then processed for protein estimation and Western blot analysis.

Protein estimation: Total proteins were estimated using the Bradford method (Bradford 1976). 10 µl of diluted sample and six standards of bovine serum albumin (0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) were aliquoted in triplicate on flat bottom microtiter plate and 200 µl 1 X protein assay dye (Bio-Rad) was added to these aliquots and mixed very well. After five minutes of incubation, the microtiter plate was read by ELISA reader at 600 nm. A standard curve from absorbance and concentration of standards was established to evaluate the concentration of the protein in the samples.

Western Blot analysis: Protein (35 µg) was taken and mixed with 1 x Laemmli buffer boiled for 5 minutes, cooled on ice and loaded on SDS page electrophoresis gels (Laemmli 1970; Al-Shudiefat et al. 2013). Gel (5 %) was used as stacking gel and gel (12 %) was used for the separation of isolated proteins. Electrophoresis was done using 120 volts for 90 minutes and proteins in the gel were transferred on polyvinylidene difluoride (PVDF) (Roche Diagnostics) at 300 mV for 90 minutes or 30 mV overnight. After transfer, PVDF membrane was quickly checked for the presence of proteins using Ponceau stain and then washed with TBST until the stain was gone. The membrane was blocked for one hour in a blocking solution (either 5% skimmed milk or 1% BSA depending on antibody). Primary antibody diluted (1:1000) (Cell Signalling) in TBS buffer or recommended buffer with recommended concentration was added to the membrane and kept overnight at 4°C with gentle shaking. The membrane was then washed 3 times for 15 minutes each with TBST buffer, and a secondary antibody (goat anti-rabbit IgG horseradish peroxidase conjugate) with recommended dilution (Bio-Rad) was used to detect the

primary antibody, by incubating with the membrane for 1 hour at room temperature, and then washed for 15 minutes three times with TBST. The protein bound to PVDF membrane was detected using Pierce ECL western blotting substrate and bands were visualized using X-ray films (Thermo Scientific). Protein bands were quantified using image analysis software (Quantity One, Bio-Rad Laboratories).

1.7 Histological studies

The hearts from the different study groups were washed with PBS and cleaned and blotted gently with soft tissue. These hearts were then photographed on a scaled paper to examine the scar tissue and the enlargement or hypertrophy of ligated hearts compared to their respective sham hearts. Thick slices of hearts (0.5 cm) preserved in 10 % buffered formalin solutions were checked for flat surfaces by cutting the rough edges with a surgical sharp blade. These slices were sent to the pathology lab in the Faculty of Dentistry at the University of Manitoba for processing and staining with Masson's trichrome stain to explore fibrosis in all ligated animal groups compared to their respective sham groups. Stained cross sections were examined by microscope with a color digital camera, and representative pictures were taken from each animal group at 40 X and 400 X magnification. For the whole cross sections, pictures were taken using a digital camera.

2.0 *In vitro* studies

For isolated cardiomyocytes, normal SD rats (N=90) in the weight range 250-300 g were used. Ventricular cardiomyocytes were isolated from hearts of normal adult male Sprague Dawley rats using a procedure described earlier (Piper et al. 1982; Al-Shudiefat

et al. 2013). Cardiomyocyes were exposed to different treatments and were assessed for oxidative stress, cell damage, viability, and apoptosis.

2.1 Adult Cardiac Myocyte Isolation

Rats (250-300 g) were anaesthetized intra-peritoneally with a mixture of 9:1 ketamine (90 mg/kg) to xylazine (10 mg/kg). Animal chests were cleaned and opened with surgical forceps and scissor. The hearts were removed carefully and transferred quickly to cold Krebs buffer. Rat hearts were mounted on a modified Langendorff perfusion apparatus that allows switching between buffer-collagenase solution and calcium free buffer at 37°C. The heart was washed by calcium free Krebs buffer (110mM NaCl, 2.6mM KCl, 1.2mM KH_2PO_4,1.2mM $MgSO_4$, 25mM $NaHCO_3$, 60mM taurine, 11mM glucose, pH 7.4) for a few minutes to wash out the blood, then the perfusion was switched to buffer-collagenase solution (containing same Krebs buffer, 0.1 % collagenase (Worthington), 0.1 % BSA, 30 μmol $CaCl_2$). The perfusion was for 45-55 minutes. The heart was removed from the apparatus, and incubated in desegregation solution containing 1 % BSA, 19 μmol $CaCl_2$. The atria were removed and discarded and the ventricles were passed through wide mouth pipette tips with different diameters ranging from 1 - 5 mm starting with the larger then passing through the smaller until homogenized cardiomyocytes without any pieces of tissue were obtained. The cell suspension was then passed through a gradual increase in calcium chloride concentration solutions, and each time it was allowed to settle down for 5-7 minutes and the supernatant was discarded each time. The last pellet was re-suspended in M199 medium (Sigma) at 37°C. The cardiomyocytes were plated (1×10^5 myocytes /35 mm dish) on plates pre-coated with 10 μM mouse laminin

purchased from (VWR Canada) at 37°C for 2 hrs. Fresh serum free M199 medium (37°C) was added after washing the dead cells and incubated overnight. M199 medium was supplemented with 10 μg/ml Gentamicin and 0.25 μg/ml amphotericin B both purchased from Invitrogen Canada.

2.2 Cell Treatment

Cardiomyocytes were treated as described previously (Al-Shudiefat et al. 2013). Cardiomyocytes were divided into four groups: A) control; B) OA (50 μM) treated; C) TNF-α (10 ng/ml) treated; and D) TNF-α (10 ng/ml) + OA (50 μM) treated. In some experiments 25 μM H_2O_2 (Sigma) was used as a positive control for oxidative stress. In all groups, cardiomyocytes were incubated for 4 hrs after adding TNF-α or OA except the combination treatment group (TNF-α + OA) where cells were pretreated for 30 minutes with 50 μM OA. These concentrations were based on our pilot studies using TNF-α (0.5, 1, 2, 5, 10, 20, 30, 40, and 50 ng/ml), OA (50, 100, and 200 μM), H_2O_2 (10, 25, 35, 50, 75, and 100 μM), and different exposure times (1, 2, 4, 6, 8, 12, 18, and 24 hrs).

2.3 Assessment of ROS Production

Endogenous reactive oxygen species due to different treatments were assessed as described previously (Swift et al. 2000; Al-Shudiefat et al. 2013). Control and treated cardiomyocytes were washed with warm PBS (37°C) and then incubated with 10 μM solution of the fluorescent dye 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (DCFDA; Invitrogen) dissolved in warm PBS . Cardiomyocytes were incubated for 30 minutes at 37°C and were protected from light. After capturing the fluorescent im-

ages of the cardiomyocytes with an Olympus BX 51 fluorescent microscope (on average 10 fields per plate), the fluorescent intensity was analyzed using the Image Pro Plus software.

2.4 Assessment of Cardiac Cell Viability and Damage

Viability and damage of cardiomyocytes were examined as described previously (Al-Shudiefat et al. 2013). Trypan blue: M199 (1:1) were added to control and treated cells for 5 minutes, the cells were observed for viability with an Olympus microscope equipped with color Infinity X camera (Lumenera Corporation), and were analyzed using Infinity software. Viable cells excluded the dye and remained colorless, whereas the dead cells retained the blue dye.

Damage of cardiomyocytes was assessed indirectly by measuring creatine kinase (CK) release in the medium after treatment. A commercially available kit for measuring the creatine kinase activity through spectrophotometric assay was used according to the manufacturer's specifications (Stanbio Laboratory, Boerne, TX). Briefly, after 4 hours of treatment, 25 μL culture medium from control and treated groups were added to 1 ml prewarmed reconstituted CK reagent and the change in absorbance was recorded at 1 min intervals for 3 minutes at 340 nm.

2.5 Evaluation of Cardiomyocyte Apoptosis

Apoptosis of cardiomyocytes was evaluated using Hoechst stain to explore DNA fragmentation, and Western blot for analyzing different apoptotic proteins (Bax, Caspase 3, PARP, TGFβ, and Bnip3) and anti-apoptotic protein (Bcl-xL).

2.5.1 Assessment of DNA Fragmentation

DNA fragmentation in cardiomyocytes was evaluated according to a method published previously (Dhingra et al. 2009; Al-Shudiefat et al. 2013). For examining DNA fragmentation, one of the hallmarks of apoptosis, control and treated cardiomyocytes were washed with warm PBS and fixed with 4 % paraformaldehyde for 30 minutes. After fixation, paraformaldehyde was discarded and cardiomyocytes were washed with PBS and incubated with Hoechst 33258 (1 μg/ml) for 10 minutes protected from light exposure. After staining of cardiomyocyte nuclei, plates were examined using a fluorescent microscope (Olympus, BX 51).

2.5.2 Assessment of Apoptotic and Anti-Apoptotic Proteins

For the assessment of apoptotic and anti-apoptotic protein expression in cardiomyocytes with different treatments, Western blot analysis was used as published previously (Al-Shudiefat et al. 2013).

Sample preparation: Control and treated cardiomyocytes were washed with warm PBS, scraped gently from the plates placed on ice and homogenized with 200 μL RIPA buffer containing protease (Roche Diagnostics) and phophatase inhibitor cocktail (Santa Cruz). The mixture was sonicated 4 times for about 5 seconds each time on ice. The cell suspension was centrifuged at 10,000 rpm for 10 minutes at 4°C and the pellet was discarded. The supernatant was carefully taken for analysis.

Protein estimation and Western blot analysis: These were done following the methods described earlier for the *in vivo* studies.

3.0 Statistical Analysis

Differences among groups were considered significant if value of $P<0.05$. Standard error was used to express the variations of data compared to its mean. Groups were compared using one way ANOVA for *in vitro* studies and two way ANOVA for *in vivo* studies, and Bonferroni's test was performed to identify differences between groups.

VI. RESULTS

1.1 *In Vivo* Studies

1.1.1 General

Sham control and ligated animals were regularly monitored for their weight and general well being. The mortality rate for the ligated animals was 13 % and most of these deaths were within 24 hrs after surgery. There was a gradual increase in the animal weight ranging from 184.9 ± 10.4 g in the first week to 750 ± 176.5 g in the 18 weeks post myocardial infarction (PMI). There was no significant difference in the body weight of sham regular chow and ligated regular chow, sham olive oil and ligated olive oil, and sham corn oil and ligated corn oil rats at any of the time points in the study (Fig. 1).

Animals after 16W PMI appeared more stressed as indicated by the presence of porphyrin stain and ruffled hair, and by week 17 of diet consumption, some animals started to lose weight. The consumption of special diet (olive and corn oils) for all groups (sham and ligated) increased with time from the first week until the fifth week and became steady thereafter. The special diet consumption ranged from 23.03 ± 1.56 g/day (wet weight) or 14.43 ± 0.98 g/day (dry weight) in the first week to 59.76 ± 7.67 g/day (wet weight) or 37.35 ± 4.79 g/day (dry weight) in the 5^{th} week and afterwards.

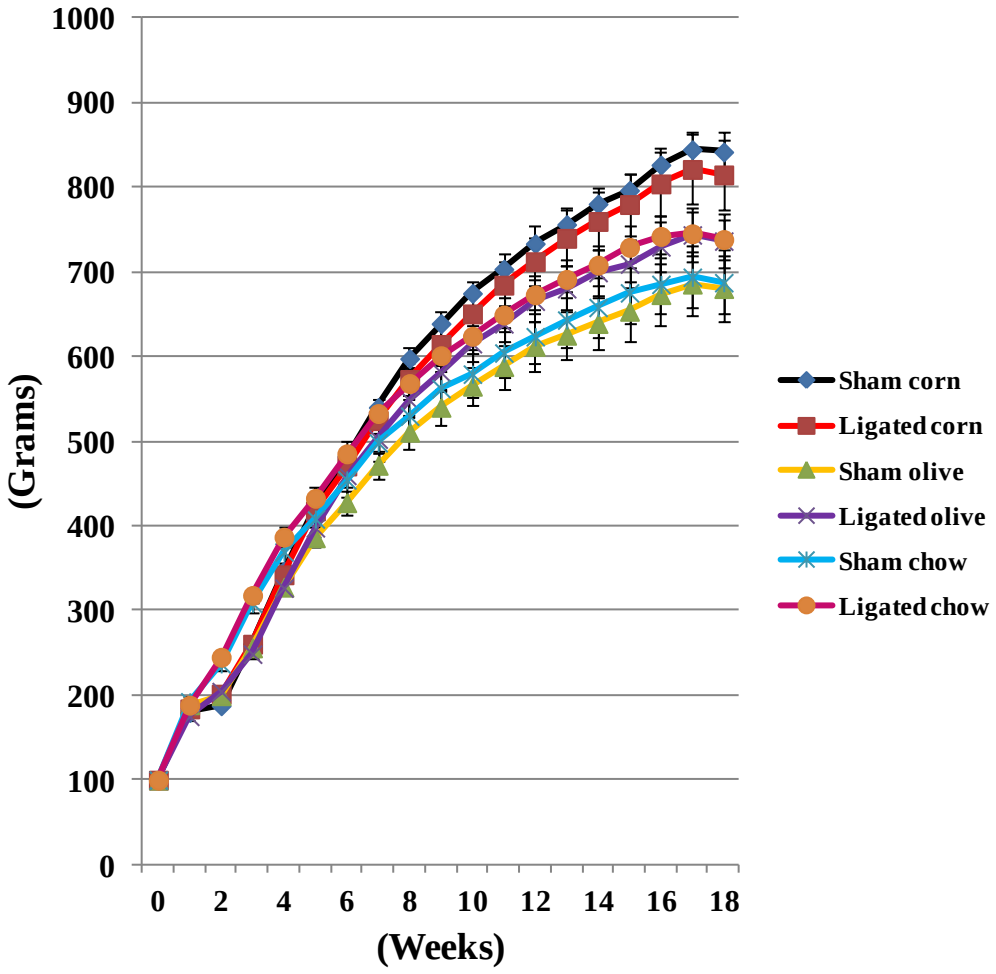


Fig 1: Body weight of sham and ligated animals from 1 week to 18 weeks of diet consumption. Data are presented as mean ± SEM from 6-9 animals for each group. Two way ANOVA analysis was done.

The diet consumption rate for the regular chow group in the SD rats was close to what we have reported earlier for feed consumption (Weinberg and Singal 1987). There was no significant difference in the special diet consumption between any of the sham and their respective ligated groups (Fig 2).

1.2 Function Assessment

1.2.1 Blood pressure

In order to study the effects of diet alone on the blood pressure, the animals were studied for blood pressure changes after one week on the special diets without any coronary artery ligation. Blood pressure in all groups were also recorded after 4 weeks of special diet consumption and 3 weeks post myocardial infarction (PMI). Only groups fed the olive oil rich diet showed a significant decrease in both systolic (15.9 %) and diastolic blood pressure (19.6 %) after four weeks of special diet, regardless of the coronary artery ligation, compared to other groups (Fig 3).

1.2.2 Echocardiographic studies

At 3 days post surgery all the ligated groups showed a significant reduction (ranging from 12.1 % to 17.5 %) in the LVEF as compared to their respective sham control (Table 2 + Fig 4). In the regular chow with the prolongation of the post surgery duration, the ligated group showed a further decline of 9.3 %.

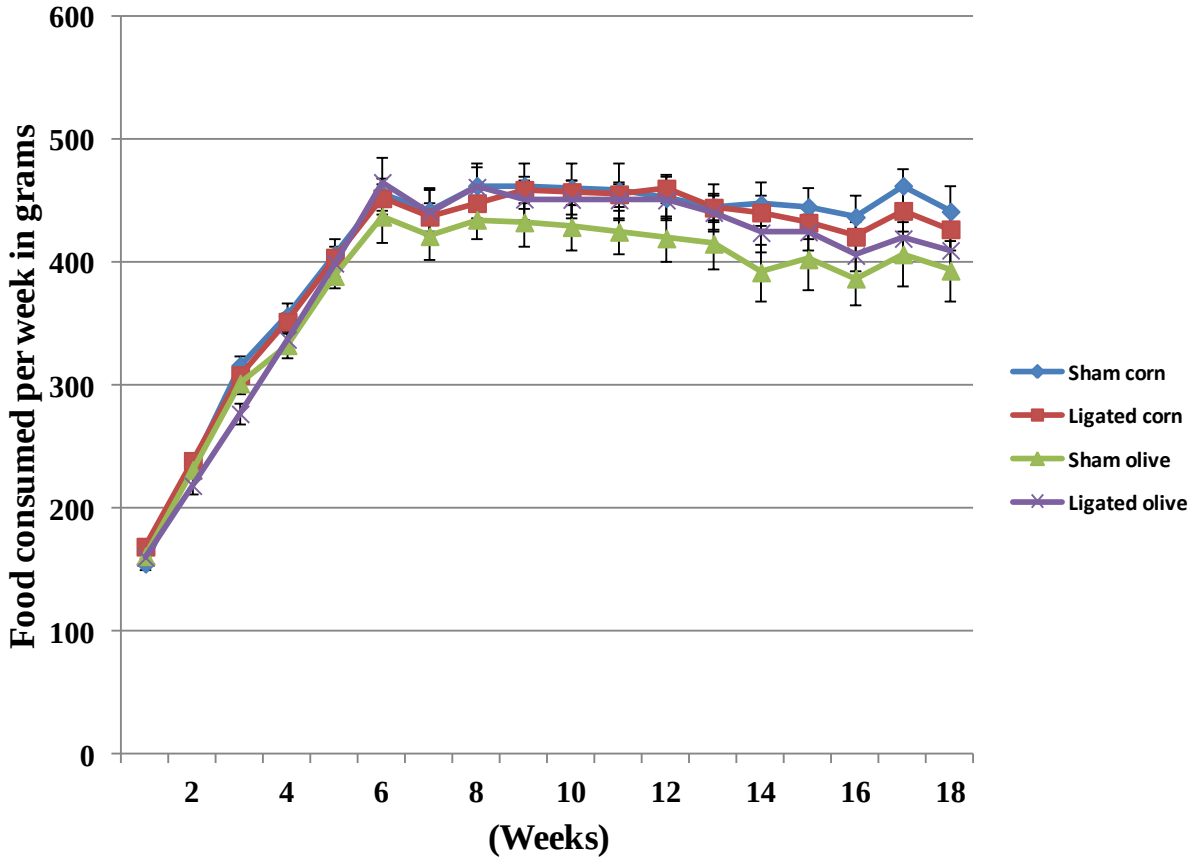


Fig 2: Special diet consumption (olive or corn oil) in all groups (sham and ligated) from 1 to 18 weeks. Data are presented as mean ± SEM from 6-9 animals for each group. Two way ANOVA analysis was done.

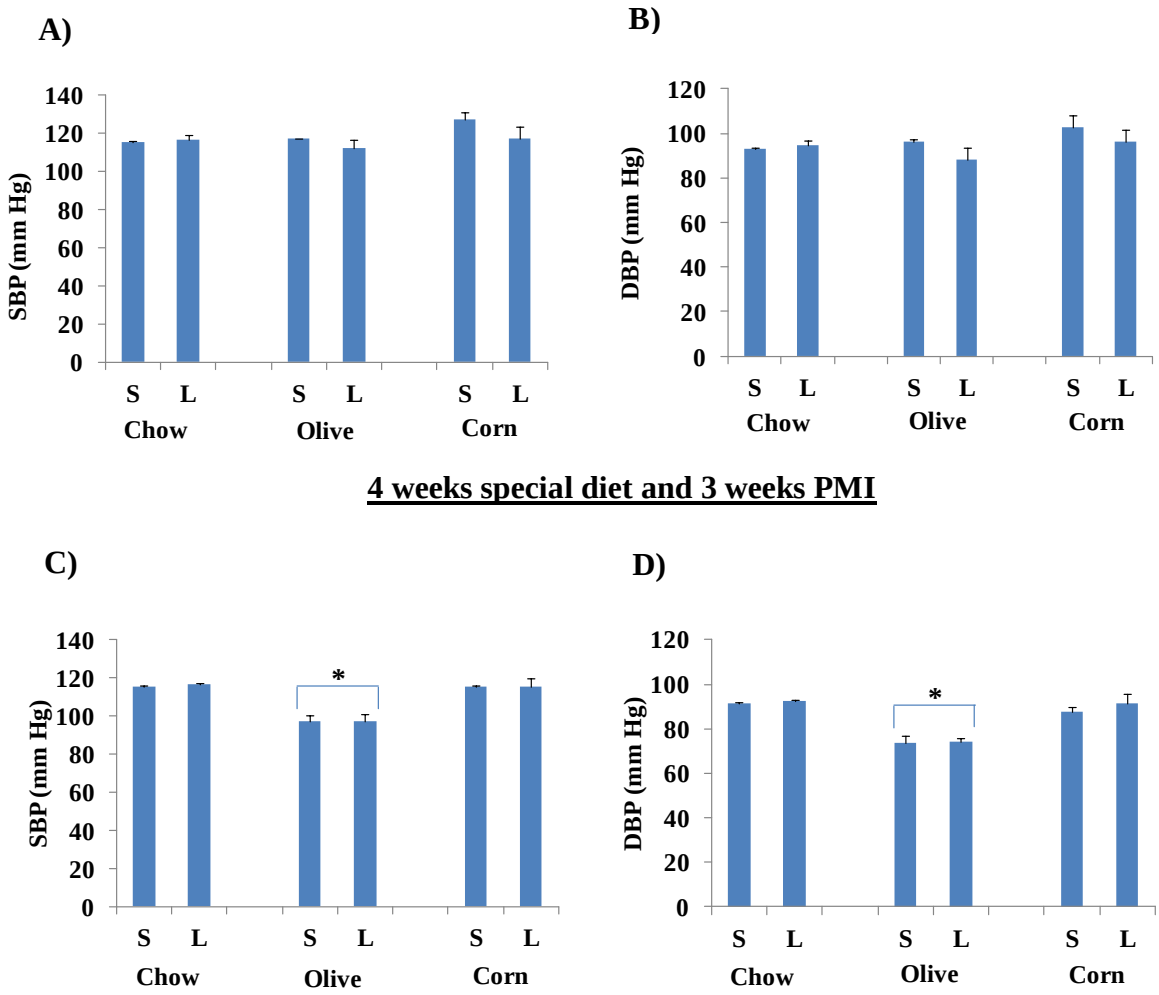


Fig 3: Effect of olive oil and other control diets on blood pressure without any surgery (A and B) and 3 weeks PMI (C and D). Sham (S) and ligated (L) animals were put on special diets for 10 days before surgery until the endpoint. Systolic (A) and diastolic (B) blood pressure were recorded one week after special diet and two days prior to surgery; Systolic (C) and diastolic (D) blood pressure 3 weeks PMI, where the animals were on special diets for about 4 weeks. Data are presented as mean ± SEM from 6-9 animals for each group. Two way ANOVA analysis was done. *) Significantly different from other groups (P< 0.05).

Table 2: Echocardiographic analysis of heart function in rats at different post-surgery time points

Echoparameter	Group	baseline	3 days Post-surgery	2 wks post-surgery	4 wks post surgery	16 wks post- surgery
LVEF (%)	S. Chow	78.33±0.33	78.40±0.68	78.00±0.3	78.63±1.15	74.00±1.78
	L. Chow	77.40±0.76	68.00±4.0 *	64.00±1.69 *	62.67±1.8 *	61.66±0.19 *
	S. Olive	76.50±0.84	78.17±1.62	78.33±0.31	78.67±0.48	72.33±1.47
	L. Olive	77.90±0.60	65.11±2.6 * #	66.11±1.86 *#	67.00±1.17 * #	70.67±2.17 $
	S. Corn	74.83±1.42	74.00±0.68	74.17±1.47	76.5±1.28	71.33± 0.85
	L. Corn	78.67±0.76	64.88±0.96 *#	63.71±2.92 *#	65.57±2.54 *#	63.00± 2.33 *#
LVIDd (mm)	S. Chow	8.20±0.04	8.21±0.04	8.25±.04	8.22±0.04	8.67±0.01
	L. Chow	8.10±0.03	8.89±0.19	8.93±0.2	8.98±0.19	9.99±0.11 *
	S. Olive	8.14±0.02	8.20±0.02	8.22±0.02	8.28±0.04	9.55±0.33
	L. Olive	8.19±0.04	8.95±0.23	9.02±0.25 *	9.02±0.28 #	9.44±0.28
	S. Corn	8.23±0.05	8.24±0.04	8.30±0.04	8.32±0.04	9.30±0.06
	L. Corn	8.18±0.02	9.07±0.17 *#	9.28±0.14 *#	9.38±0.14 *#	9.48±0.29
TDI Ant.(cm/s)	S. Chow	1.72±0.01	1.70±0.03	1.70±0.02	1.73±0.02	1.82±0.11
	L. Chow	1.74±0.02	1.42±0.2	1.19±0.08 *	1.24±0.07 *	0.92±0.06 *
	S. Olive	1.84±0.04	1.86±0.04	1.79±0.07	1.71±0.11	1.25±0.32
	L. Olive	1.78±0.03	1.53±0.1	1.50±0.11	1.53±0.12	1.17±0.17
	S. Corn	1.79±0.02	1.80±0.02	1.76±0.04	1.74±0.06	1.35±0.19
	L. Corn	1.80±0.02	1.32±0.12 *	1.19±0.09 *#	1.21±1.38 *#	0.53±0.06 *#
HR (beats/min)	S. Chow	341.67±8.8	348.33±12.6 $	361.67±7	371.67±7.85	329.33±15.53
	L. Chow	345.20±4.93	407.33±9.1 *	387.67±10	418.33±8.98	367.00±6.69
	S. Olive	368.50±6.3	403.67±6.9 #	393.50±6.7	412.83±10.08	338.00±10.44
	L. Olive	357.90±7.5	405.33±6.2	381.56±11.9	381.44±28.39	310.33± 6.85 $
	S. Corn	362.50±13.6	375.83±7.16	404.17±18.9	390.17±13.23	342.33±7.19
	L. Corn	365.11±4.9	390.13±12.5	374.71±8.6	393.14±8.71	327.33±7.12 $

Data are presented as mean ± SEM from 6-9 animals for each group. Two way ANOVA analysis was done. *) Significant from their corresponding sham, #) significant from sham regular chow, $) significant from ligated regular chow. In all (P< 0.05). S, Sham; L, Ligated; LVEF, Left Ventricular Ejection Fraction; LVIDd, Left Ventricular Internal Dimension-diastole, TDI Ant., Tissue Doppler Imaging parameter of the anterior wall; HR, Heart Rate.

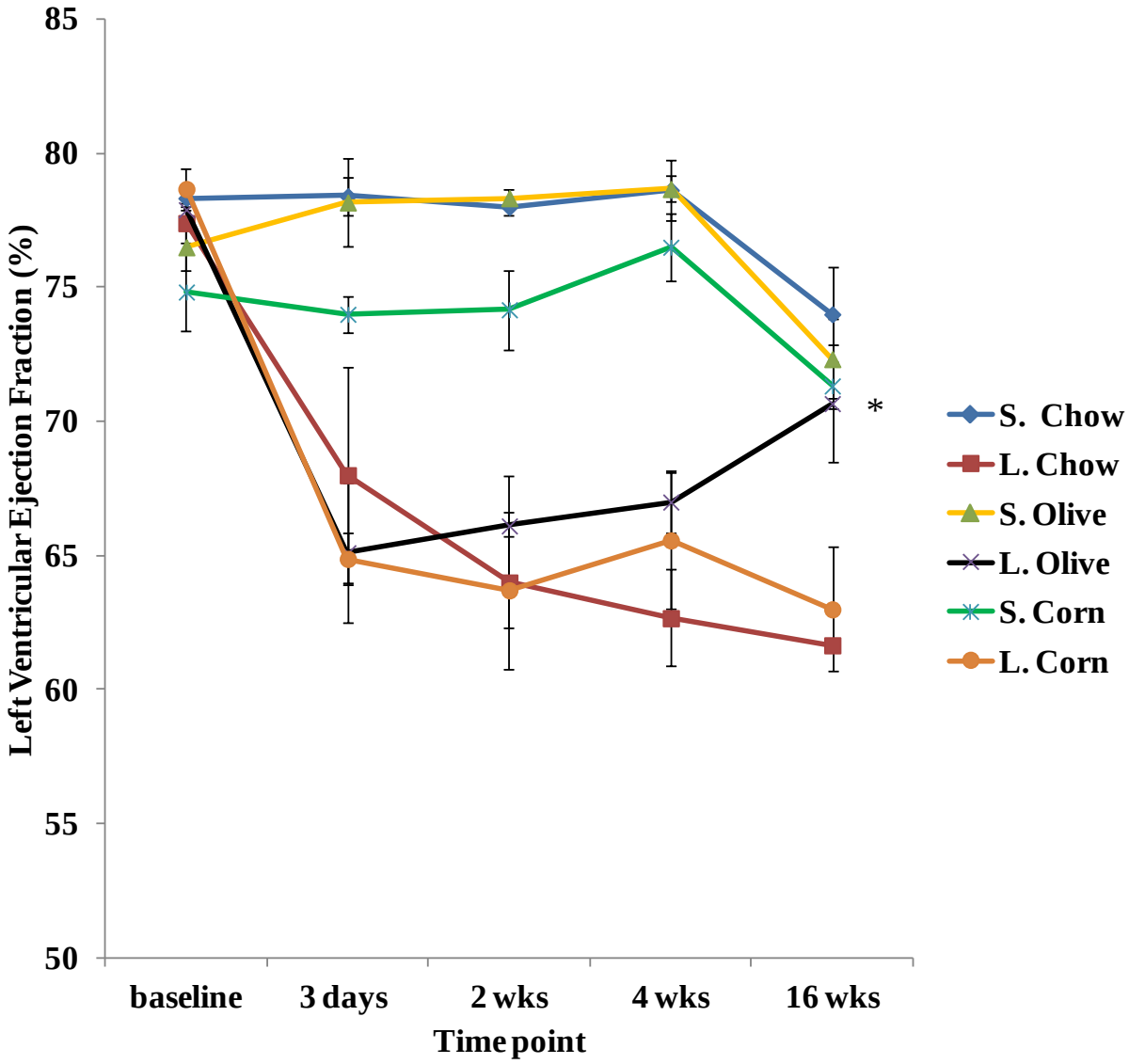


Fig 4: Left ventricular ejection fraction (LVEF) of sham (S) and ligated (L) animals for all groups at different time points of coronary artery ligation. Notice the improvement and restoration of LVEF in the ligated olive oil group (black line) compared to other ligated groups. Data are presented as mean ± SEM of 6-9 animals for each group. Two way ANOVA analysis was done. *) Significant (P< 0.05) from the other ligated groups.

After 3 days post surgery, the ligated olive oil group showed a steady improvement in LVEF and at 16 weeks, there was no significant difference between sham olive oil (LVEF 72.3 ± 1.5) and ligated olive oil (LVEF 70.7 ± 2.2) (Table 2 + Fig 4).

Furthermore at 16 weeks post-surgery, left ventricular internal dimension-diastole (LVIDd) was also significantly better in the ligated olive oil group (Table 2 and Fig 5 F) as compared to the ligated regular chow group (Table 2 and Fig 5 B). Although there was a significant drop (17.5 %) in LVEF in the ligated corn oil group on day 3 after surgery, this condition did not deteriorate further with the post surgery duration. Thus, ligated olive oil group LVEF (70.7 ± 2.2 %) was significantly better than the ligated corn oil group LVEF (63.0 ± 2.3 %).

The LVIDd at 16 weeks in the ligated regular chow group was 9.99 ± 0.11 mm and was significantly higher than its sham control (Table 2). The ligated olive oil group, however, was very much comparable to its own sham olive oil group (Table 2). Although in the ligated corn oil group, the LVIDd was higher up to 4 weeks PMI, this difference was no longer apparent at 16 weeks PMI, perhaps due to an increase in LVIDd with aging in the sham corn oil group. Tissue Doppler imaging (TDI) parameter of the anterior wall showed a significant decline in the ventricular shortening velocity in the ligated groups. At 16 weeks PMI, the ligated olive oil group was not different from its sham olive oil group, whereas ligated regular chow and ligated corn oil showed a significant difference in TDI parameter of the anterior wall as compared to their respective sham groups (Table 2). There was some increase in the heart rate (HR) (11.4 %) in the ligated regular chow group at 16 weeks PMI as compared to its sham control.

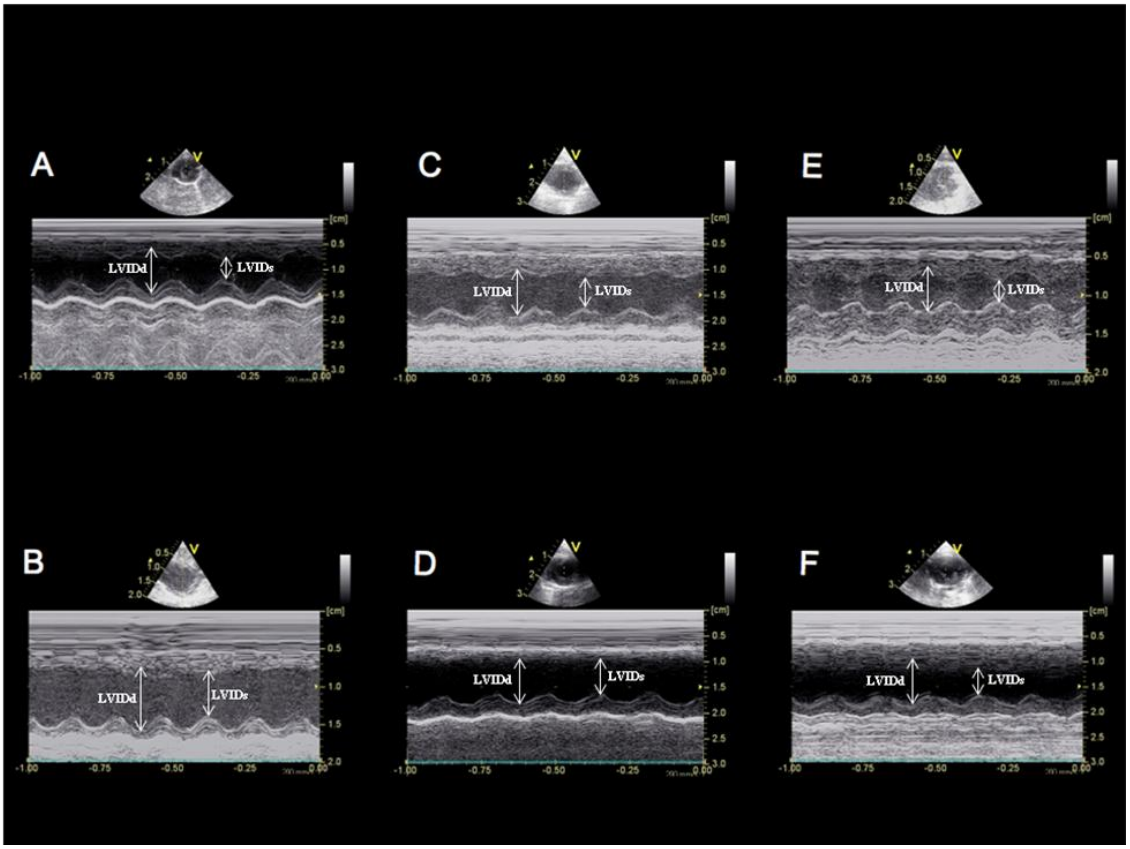


Fig 5: Representative echocardiography images showing left ventricular internal dimension at diastole (LVIDd) and systole (LVIDs) in all groups at 16 weeks PMI. A) Sham regular chow, B) Ligated regular chow; C) Sham corn oil, D) Ligated corn oil, E) Sham olive oil and F) Ligated olive oil. LVIDd and LVIDs are pointed out by white arrows. Olive oil ligated group (F) showed significant improvement in the LVIDd as compared to the ligated regular chow group (B) (see table 2).

Such an increase was not present in the ligated olive oil group (Table 2). In fact at 16 weeks PMI, the hearts in ligated olive oil group beat slower than their sham group. The HR of 310 beats/min in the ligated olive oil group was significantly less ($P<0.05$) than the HR of 367 beats/min in the ligated regular chow group (Table 2).

1.2.3 Electrocardiography Studies

Electrocardiography (ECG) was done at 4 weeks and 16 weeks PMI. The ECG analysis of hearts of the animals showed a significant incidence of conduction system abnormalities in all ligated groups (Fig 6) but not in the sham animals at both 4 and 16 weeks PMI (Table 3). The incidence of conduction abnormalities was 56% in both ligated regular chow and ligated corn oil at 4 and 16 weeks PMI, while it was 43 % for ligated olive oil at 4 weeks PMI and increased to 57 % at 16 weeks PMI. There was no significant difference between all the ligated groups at 4 and 16 weeks PMI (Table 3).

Some of the conduction system abnormalities found in the ligated groups are shown in Fig 6 and these include right bundle branch block (Fig 6 B+C), ST elevation (Fig 6 D), and biatrial enlargement (Fig 6 E) as compared to the sinus rhythm (Fig 6 A) in the sham controls.

1.3 Biochemical analysis

Animals in all groups were analyzed for: lipid profile, myocardial and plasma TNF-α, myocardial oxidative stress, myocardial apoptotic and anti-apoptotic proteins, and myocardial MAP kinases.

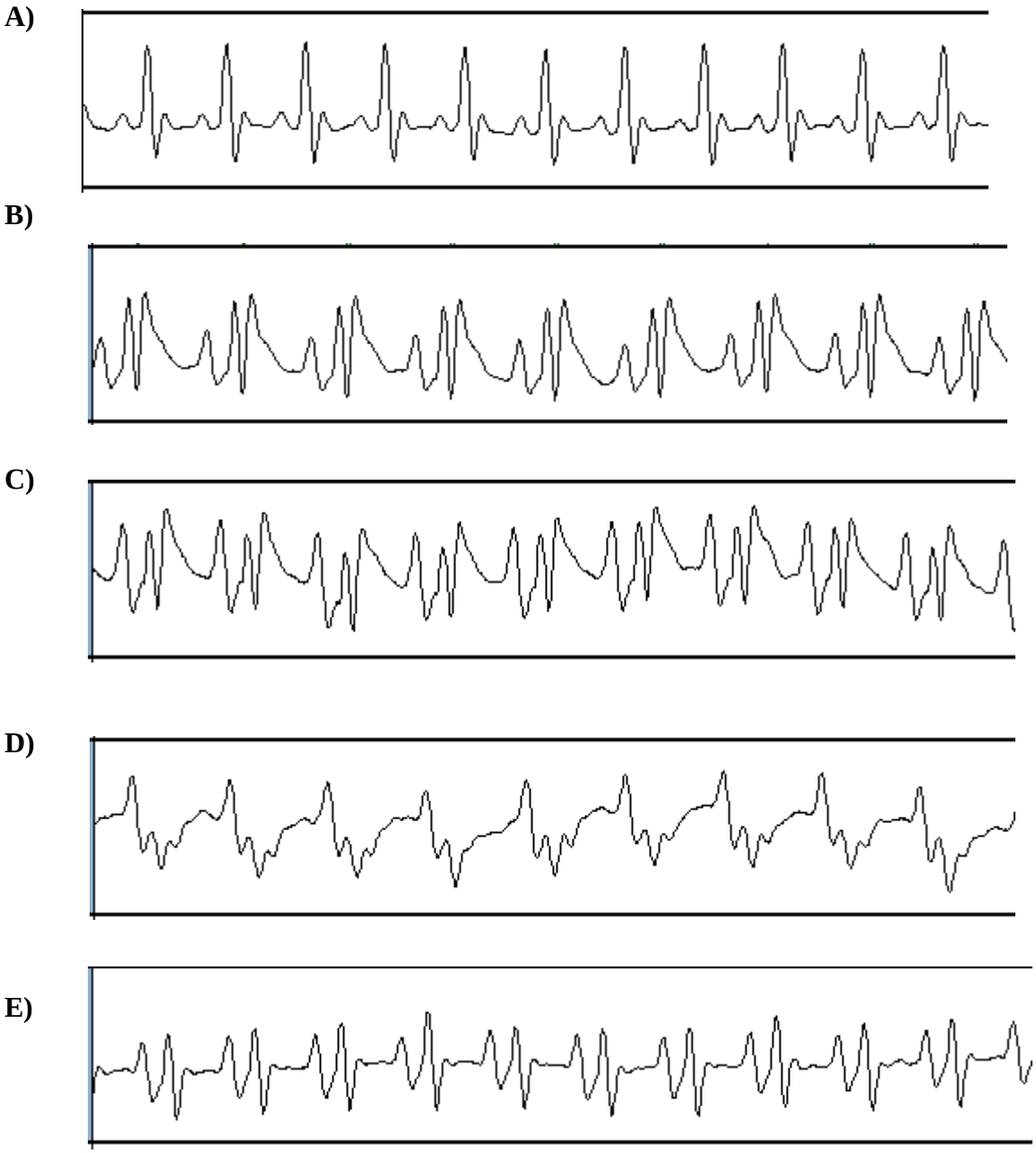


Fig 6: ECG traces showing different types of conduction abnormalities at 4 weeks PMI. A- Sham with sinus rhythm; (B-F) ligated rats: B- Right bundle branch block C- Another right bundle branch block D- ST elevation (sign of ischemia) E- Biatrial enlargement (part of p wave is positive and the other is negative).

Table 3: Incidence of conduction system abnormalities in all groups at 4 and 16 weeks PMI

Group	CS abnormality % at 4 Wks	CS abnormality % at 16 Wks	No rats
Sham Reg. Chow	**0**	**0**	**6**
Ligated Reg. Chow	**56***	**56***	**9**
Sham Olive Oil	**0**	**0**	**6**
Ligated Olive Oil	**43***	**57***	**7**
Sham Corn Oil	**0**	**0**	**6**
Ligated Corn Oil	**56***	**56***	**9**

Data presented as percentage of 6-9 animals for each group. Two way ANOVA analysis was done. Reg. means regular, *) Significant (P< 0.05) from its sham group.

1.3.1 Lipid Profile

For the study of different lipids, blood plasma was collected from all groups and analysed for total cholesterol (T.Ch), high density lipoprotein (HDL), triglycerides (TG) and low density lipoprotein (LDL).

At 4 weeks PMI, total cholesterol, HDL, and TG were comparable in all groups except for a significant increase of HDL in ligated olive oil and ligated corn oil compared to ligated regular chow group (Fig 7 B).

In the 16 weeks PMI groups, only sham and ligated corn oil showed significant increase ($P < 0.05$) in Total and HDL cholesterol levels (Fig 8 A and B).

Interestingly, low density lipoprotein (LDL) levels were almost negligable (data not shown). It is important to note that in rats, the major carrier of cholesterol is HDL, carrying up to 60 % of the total cholesterol, and the remaining 40 % is collectively

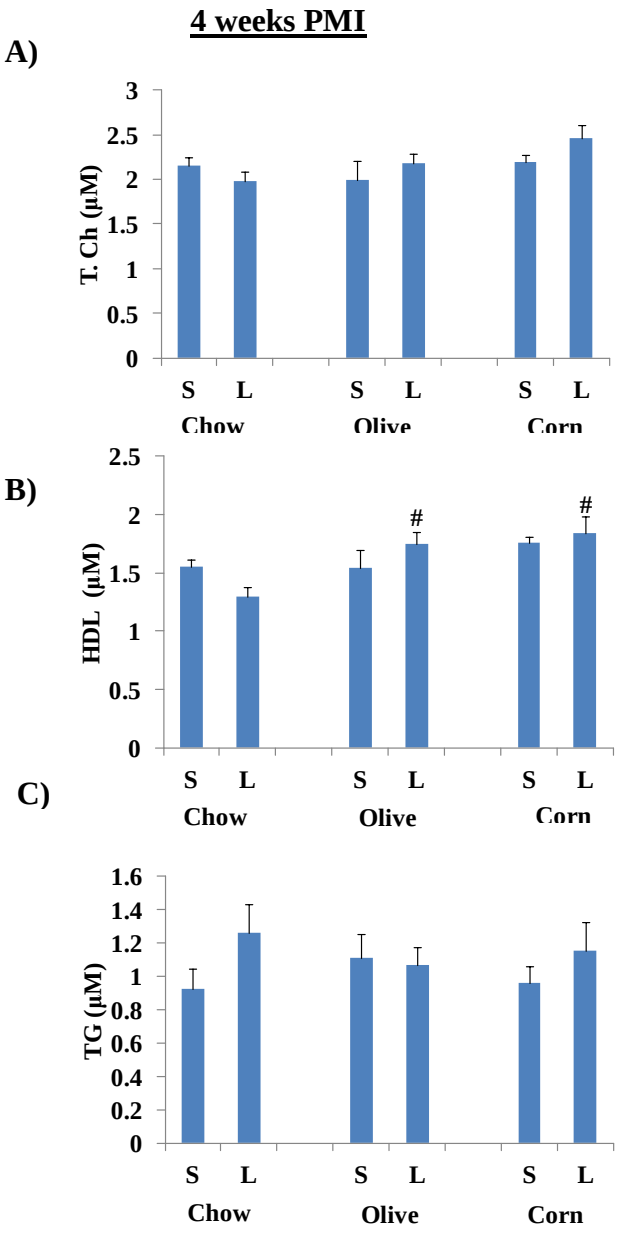


Fig 7: Plasma lipid profile of sham (S) and ligated (L) animals for all groups, 4 weeks PMI. A) Total cholesterol (T. Ch); B) HDL cholesterol; and C) Triglycerides (TG). Data are presented as mean ± SEM of 6-9 animals for each group. Two way ANOVA analysis was done. #) Significant (P< 0.05) from the ligated regular chow.

16 weeks PMI

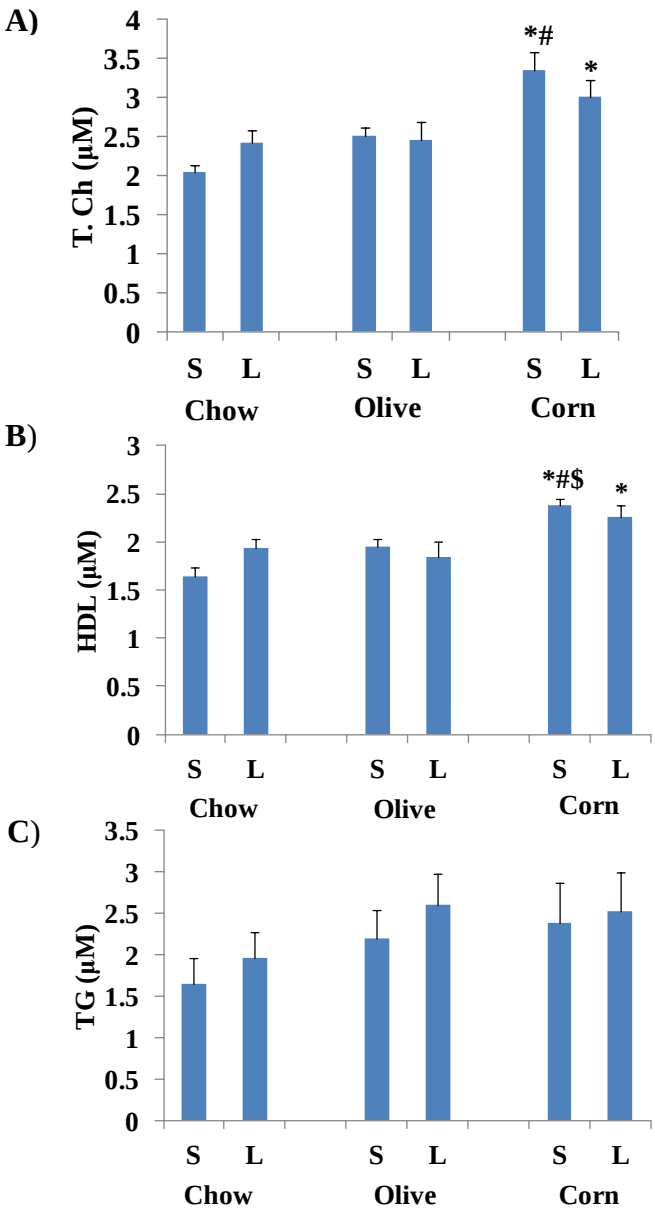


Fig 8: Plasma lipid profile of sham (S) and ligated (L) animals for all groups 16 weeks PMI. A) Total cholesterol (T. Ch); B) HDL cholesterol; and C) Triglycerides (TG). Data are presented as mean ± SEM of 6-9 animals for each group. Two way ANOVA analysis was done. *) Significant from the sham regular chow, #) Significant from ligated regular chow, and $) significant from ligated olive oil. In all groups (P< 0.05).

carried by intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL) and low density lipoptrotein (LDL) (Sonoyama et al. 1994).

1.3.2 Myocardial and Plasma TNF-α

TNF-α protein levels were determined in myocardial tissues as well as in plasma in all groups at both 4 and 16 weeks PMI. Myocardial and plasma TNF-α of sham groups at 4 and 16 weeks PMI were within the range of 2.98 ± 0.08 pg/mg to 4.23 ± 0.15 pg/mg (Fig 9).

At 4 weeks PMI, there was a significant increase (176 %) in the myocardial TNF-α level in the ligated regular chow group (Fig 9 A) from 4.2 ± 0.06 pg/mg in the sham control to 11.58 ± 0.47 pg/mg in the ligated group. There was no change in TNF-α level in the ligated olive oil compared to its sham or sham regular chow, but ligated olive oil group showed significantly lower levels of TNF-α compared to ligated regular chow and ligated corn oil groups. There was a significant increase (251 %) in the TNF-α level from 3.46 ± 0.27 pg/mg in sham to 12.16 ± 0.55 pg/mg in ligated corn oil (Fig 9 A).

At 16 weeks PMI, myocardial TNF-α protein levels were comparable and within the normal range for all groups (Fig 9 C).

At 4 weeks PMI, the plasma level of TNF-α protein was significantly lower in ligated olive oil group, compared to ligated regular chow (Fig 9 B). At 16 weeks PMI, the plasma level of TNF-α protein was comparable with no significant change in any of the groups (Fig 9 D).

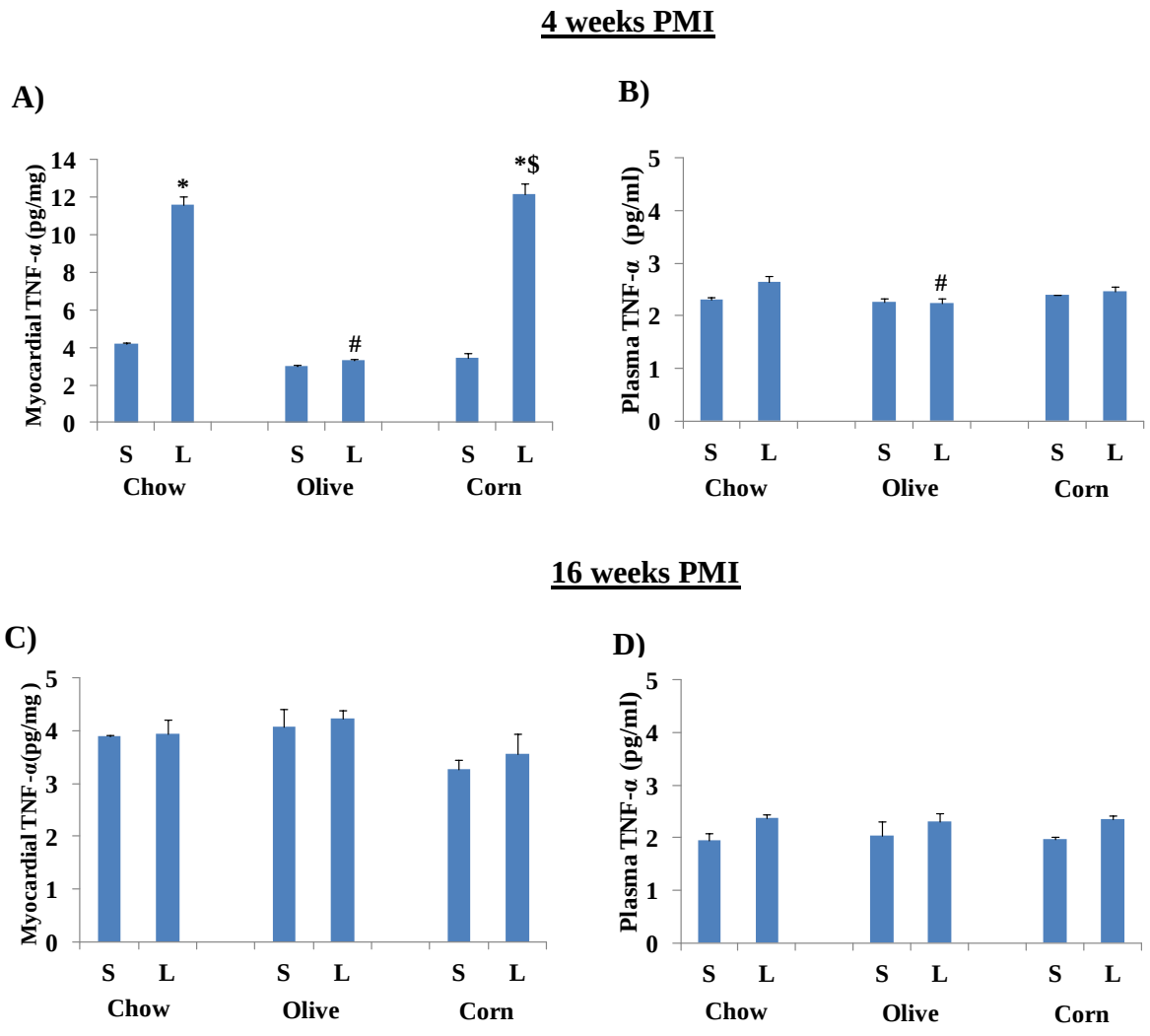


Fig 9: Myocardial and plasma TNF-α protein levels in sham (S) and ligated (L) animals after 4 and 16 weeks PMI. (A) Myocardial and (B) plasma TNF-α protein levels at 4 weeks PMI; (C) Myocardial and (D) plasma TNF-α protein levels at 16 weeks PMI. Data are presented as mean ± SEM of 6-9 animals for each group. Two way ANOVA analysis was done. *) Significant from its own sham, #) Significant from ligated regular chow, $) Significant from ligated olive oil. In all groups P< 0.05. The scale in Fig 9 A is different from the other 3 figures.

1.3.3 Oxidative stress studies

For the study of oxidative stress, we analyzed myocardial reduced and oxidized glutathione and lipid hydroperoxides.

1.3.3.a Glutathione levels

Reduced (GSH) and oxidize glutathione GSSG were measured for all sham and ligated groups at 4 and 16 weeks PMI and these redox ratios were also calculated.

At 4 weeks PMI, GSH decreased significantly (24.92 %) from 85.4 ± 1.34 μmol/g in the sham regular chow to 64.1 ± 4.55 μmol/g in the ligated regular chow (Fig 10 A). GSSG increased significantly (35 %) from 7 ± 0.4 μmol/g in sham regular chow to 9.45 ± 0.30 μmol/g in ligated regular chow (Fig 10 B). The drop in GSH was insignificant (10.32 %) at 4 weeks PMI from 92.3 ± 19.3 μmol/g in sham olive oil to 82.8 ± 14.9 μmol/g in ligated olive oil (Fig 10 A). There was a significant increase (7.4 %) in GSSG from 7.16 ± 0.33 μmol/g in sham olive oil to 7.69 ± 0.34 μmol/g in the ligated olive oil (Fig 10 B). GSH decreased significantly (20.4 %) at 4 weeks PMI from 84.16 ± 5.08 μmol/g in sham corn oil to 66.95 ± 3.37 μmol/g in the ligated corn oil (Fig 10 A). GSSG increased significantly (29.3 %) from 7.35 ± 0.15 μmol/g in sham corn oil to 9.5 ± 0.23 μmol/g in the ligated corn oil (Fig 10 B).

At 16 weeks PMI, GSH decreased significantly (46.3 %) from 90 ± 1.4 μmol/g in the sham regular chow to 48.3 ± 7.36 μmol/g in the ligated regular chow (Fig 10 C), while GSSG increased significantly (63.35 %) from 7.75 ± 0.21 μmol/g in sham regular chow to 12.7 ± 0.3 μmol/g in ligated regular chow (Fig 10 D).

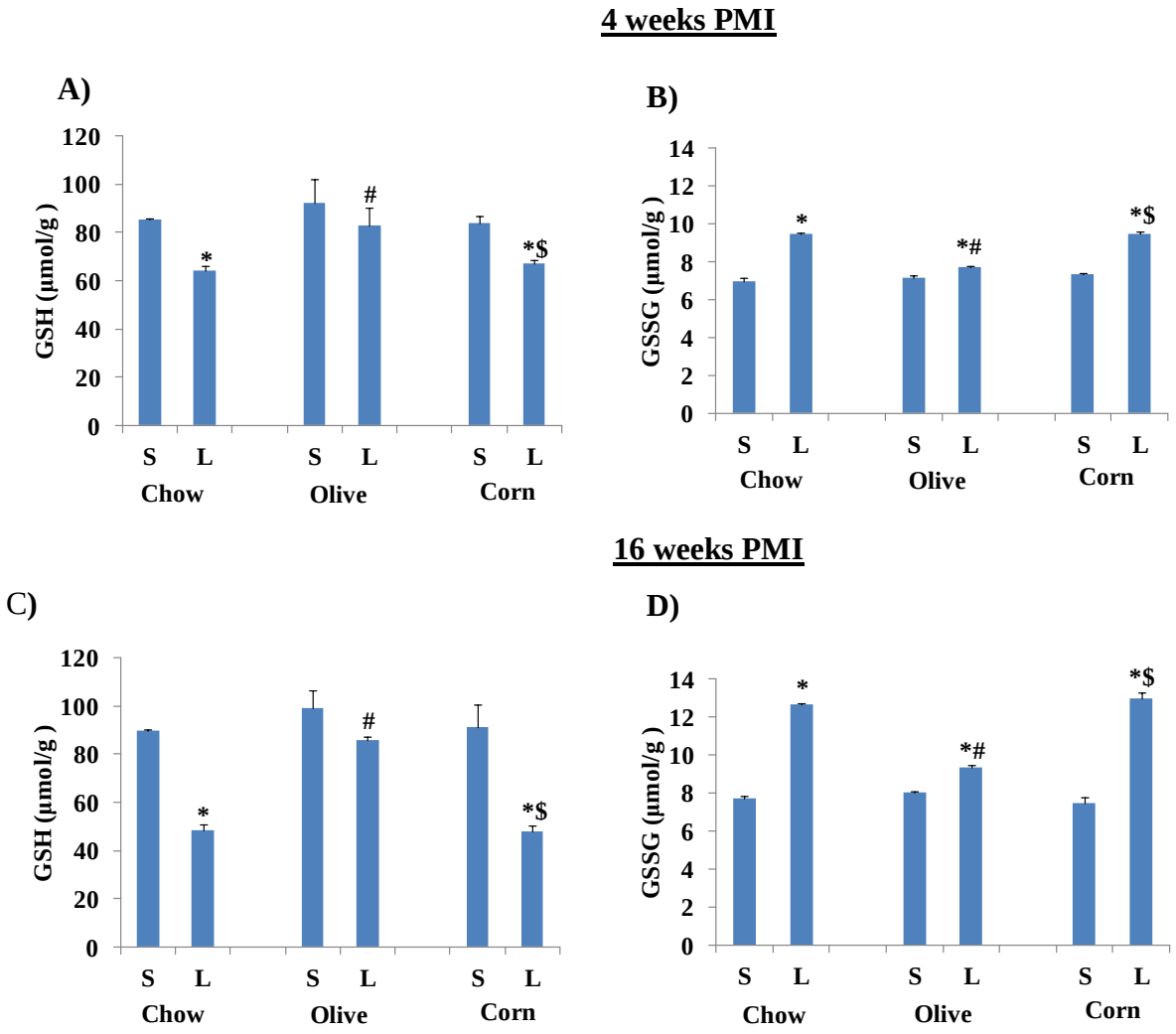


Fig 10: Reduced (GSH) and oxidized glutathione (GSSG) in Sham (S) and ligated (L) animals after 4 and 16 weeks PMI expressed as µmol/g wet tissue weight. A) GSH and ; B) GSSG both after 4 weeks PMI. C) GSH; and D) GSSG both after 16 weeks PMI. Data are presented as mean ± SEM from 6-9 animals for each group. Two way ANOVA analysis was done. *) Significant from its sham, #) Significant from ligated regular chow, $) Significant from ligated olive oil. In all groups (P< 0.05).

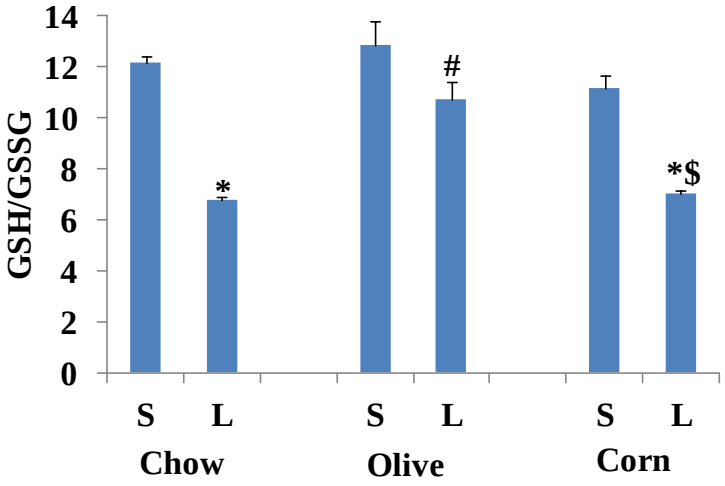
In the olive oil group, GSH reduced (13.2 %) from 99.1 ± 18.1 μmol/g in sham olive oil to 86 ± 3.6 μmol/g in ligated group (Fig 10 C), but the change was not statistically significant.

In the olive oil group, there was a significant increase (16 %) in GSSG from 8 ± 0.15 μmol/g in the sham olive oil to 9.35 ± 0.45 μmol/g in the ligated olive oil group (Fig 10 D). In the corn oil group, GSH decreased significantly (47.8 %) at 16 weeks from 91.46 ± 22.7 μmol/g in sham corn oil to 47.7 ± 7.4 μmol/g in the ligated corn oil (Fig 10 C), while GSSG increased significantly (73.33 %) from 7.5± 0.63 μmol/g in sham corn oil to 13 ± 0.94 μmol/g in the ligated corn oil (Fig 10 D).

Redox ratio (GSH/GSSG) among all sham animals at 4 and 16 weeks PMI was comparable without significant difference (Fig 11). Redox ratio reduced significantly in all ligated animals at 4 and 16 weeks PMI except ligated olive oil at 4 weeks PMI.

At 4 weeks PMI, redox ratio decreased (44.4%) in ligated regular chow, by 16.4 % in ligated olive oil, and by 36.9 % in ligated corn oil (Fig 11 A). At 16 weeks PMI, the ratio decreased significantly (67.2 %) in ligated regular chow, by 25.2 % in ligated olive oil, and 70 % in the ligated corn oil group (Fig 11 B). Overall, olive oil was better in maintaining redox ratio in ligated animals compared to both regular chow and corn oil (Fig 11 A and B).

A) **4 weeks PMI**



B) **16 weeks PMI**

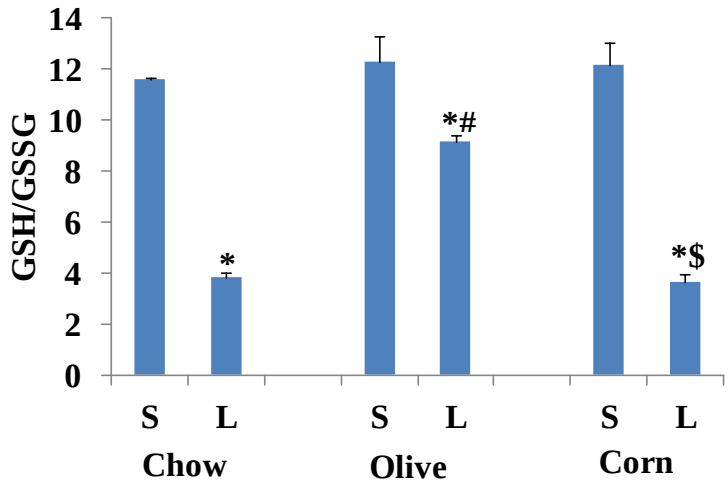


Fig 11: Redox ratio (GSH/GSSG) for sham (S) and ligated (L) animals after 4 and 16 weeks PMI. A) GSH/GSSG after 4 weeks PMI; and B) GSH/GSSG after 16 weeks PMI. Data are presented as mean ± SEM from 6-9 animals for each group . Two way ANOVA analysis was done. *) Significant from its own sham, #) significant from ligated regular chow, and $) significant from ligated olive oil. In all groups P< 0.05.

1.3.3.b Lipid Hydroperoxides

Lipid hydroperoxides were assessed in all groups to evaluate the myocardial oxidative stress in sham and ligated animals at 4 and 16 weeks PMI. Lipid hydroperoxide levels were comparable without any significant difference among all sham animals at 4 and 16 weeks (Fig 12 A and B). However, lipid hydroperoxides levels were significantly increased in all ligated groups at 4 and 16 weeks PMI except in the ligated olive oil group (Fig 12 A and B).

At 4 weeks PMI, lipid hydroperoxides levels increased 137.43 % significantly from 5.44 ± 0.94 nmol/g myocardial tissue in sham regular chow to 12.93 ± 0.96 nmol/g in its ligated group. However in the olive oil group, lipid hydroperoxides were increased 14.68 % from 6.06 ± 0.43 nmol/g in the sham to 6.95 ± 0.69 nmol/g in the ligated olive oil group and the change was not significant. There was a significant increase (97.10 %) in lipid hydroperoxides from 5.88 ± 0.76 nmol/g in sham corn oil to 11.59 ± 0.71 nmol/g in its ligated group (Fig 12 A).

At 16 weeks PMI, lipid hydroperoxides levels increased (272 %) significantly from 5.35 ± 0.45 nmol/g in sham regular chow to 19.89 ± 0.35 nmol/g in ligated regular chow. Lipid hydroperoxides in the olive oil group increased (51.86 %) from 4.55 ± 0.60 nmol/g in sham group to 6.91 ± 0.91 nmol/g in the ligated group but the change was not significant. In the corn oil group, lipid hydroperoxides increased (187 %) significantly from 6.87 ± 0.93 nmol/g in sham to 19.74 ± 0.74 nmol/g in the ligated corn oil (Fig 12 B). In the ligated olive oil group lipid hyrdoperoxides were significantly less than the ligated regular chow and ligated corn oil after 4 and 16 weeks PMI (Fig 12 A and B).

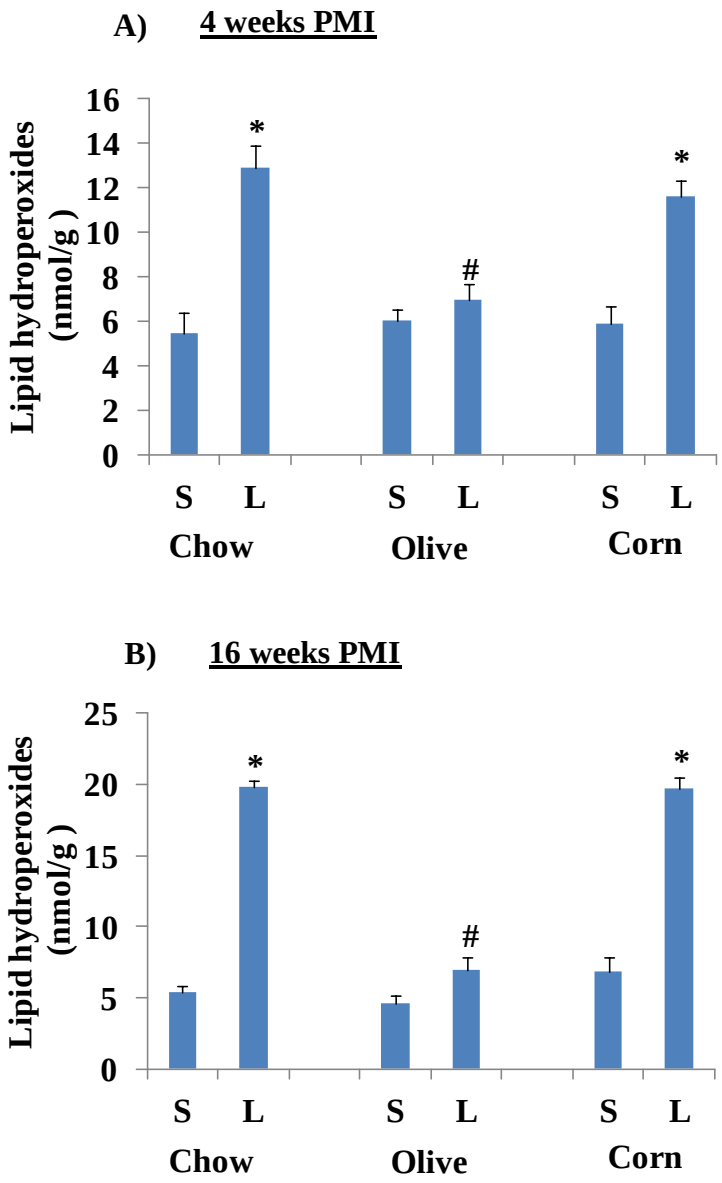


Fig 12: Lipid hydroperoxide levels in sham (S) and ligated (L) animals after 4 (A) and 16 (B) weeks PMI. Data are presented as mean ± SEM from 6-9 animals for each group . Two way ANOVA analysis was done. *) Significant from its own sham, #) significant from ligated regular chow and from ligated corn oil. In all groups, P< 0.05.

1.3.4 Apoptotic and Anti-Apoptotic Proteins

In order to assess apoptosis level in heart, we anlayzed the expression of anti-apoptotic protein Bcl-xL and apototic proteins (Bax, Caspase 3, PARP, TGFβ, Bnip3, and cytochrome C) in the sham and ligated animals using Western blot analysis at 4 and 16 weeks PMI.

1.3.4.a Bax/Bcl-xL Ratio

Bax/Bcl-xL ratio was comparable among all the sham animal groups at 4 and 16 weeks PMI (Fig 13). There was a significant increase in all ligated groups after 4 and 16 weeks PMI, except in ligated olive oil group at 16 weeks PMI (Fig 13)

At 4 weeks PMI, the Bax/Bcl-xL ratio increased significantly in all the ligated groups as compared to their respective sham groups (Fig 13 A). The increase was: 68 % in the ligated regular chow; 37 % in the ligated olive oil group; and 76 % in the ligated corn oil (Fig 13 A). The ligated corn oil group was not significantly different from the ligated regular chow. However, the ligated olive oil group had a significantly lower ratio than the other two ligated groups (Fig 13 A).

At 16 weeks PMI, Bax/Bcl-xL ratio increased (60 %) significantly in the ligated regular chow, and 74 % in the ligated corn oil (Fig 13 B). In the ligated olive oil group, the ratio was not different from its sham group, and it was significantly lower than the ligated regular chow and the ligated corn oil group (Fig 13 B).

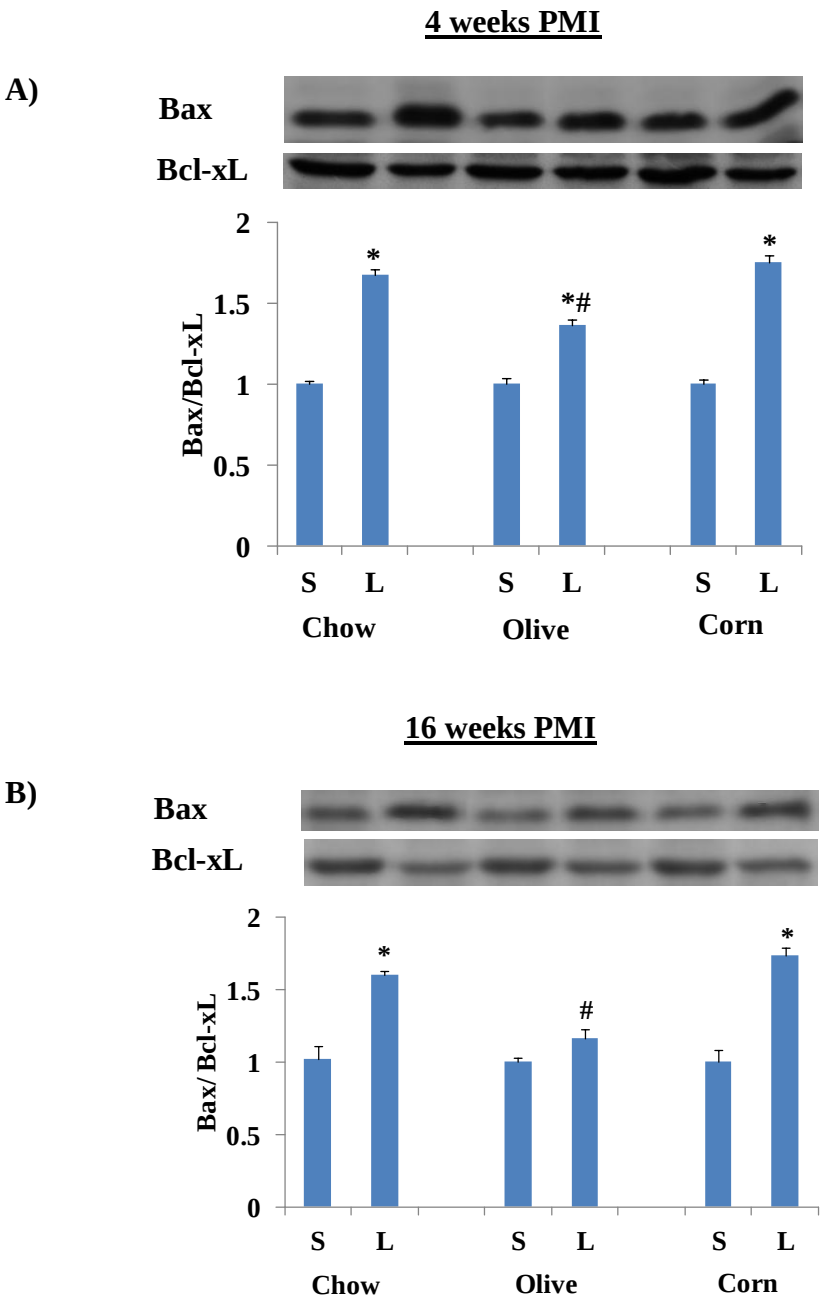


Fig 13: Bax/Bcl-xL expression in sham (S) and ligated (L) animals after 4 (A) and 16 weeks (B) PMI. Data are presented as mean ± SEM of 6-9 animals for each group . Two way ANOVA analysis was done. *) Significant from its own sham, and #) significant from ligated regular chow as well as ligated corn oil. In all groups P< 0.05.

1.3.4.b Cleaved Caspase 3 Expression

There was no significant difference in cleaved Caspase 3 levels among all sham groups at 4 and 16 weeks (Fig 14). There was a significant increase in all ligated groups at 4 and 16 weeks PMI except ligated olive oil at 16 weeks PMI which was comparable to its sham group (Fig 14).

At 4 weeks PMI, cleaved Caspase 3 expression increased significantly by 266 % in the ligated regular chow, 101 % in the ligated olive oil, and by 233 % in the ligated corn oil as compared to their respective sham controls (Fig 14 A). The ligated olive oil group showed significantly lower Caspase 3 cleavage as compared to the ligated regular chow and ligated corn oil (Fig 14 A).

At 16 weeks PMI, the expression of cleaved Caspase 3 increased significantly by 49 % in the ligated regular chow, and by 109 % in the ligated corn oil compared to their respective sham control (Fig 14 B). However, in the ligated olive oil, Caspase 3 cleavage was not different from its sham and was significantly lower than ligated regular chow and ligated corn oil (Fig 14 B). Moreover, the ligated corn oil showed significantly higher Caspase 3 cleavage than the ligated regular chow (Fig 14 B).

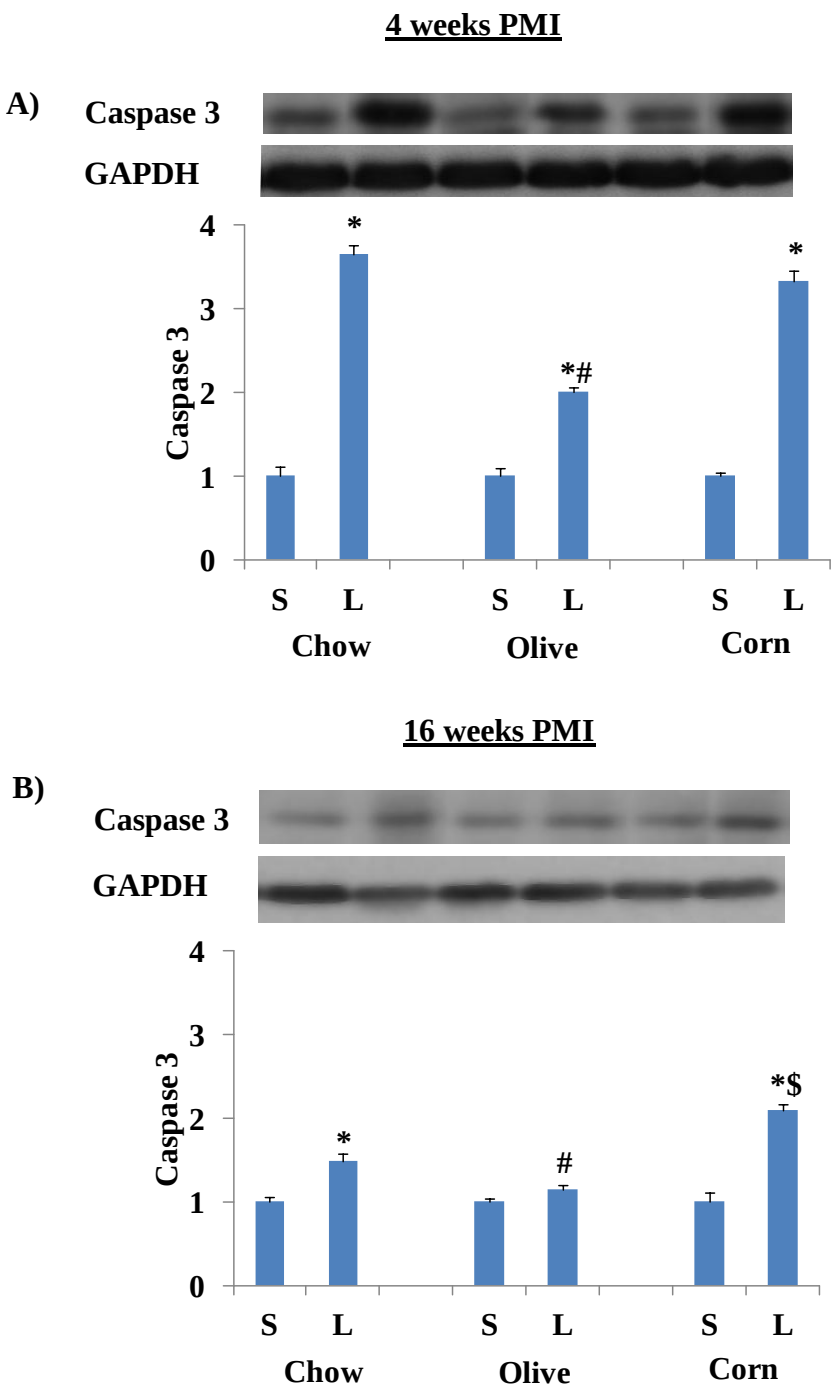


Fig 14: Cleaved Caspase 3 expression in sham (S) and ligated (L) animals after 4 (A) and 16 weeks (B) PMI. Data are presented as mean ± SEM from 6-9 animals for each group. Two way ANOVA analysis was done. *) Significant from its own sham, #) significant from ligated regular chow and ligated corn oil, and $) significant from ligated regular chow. In all groups P< 0.05.

1.3.4.c Cleaved PARP Expression

There was no difference in the expression of cleaved PARP among all sham animals at 4 and 16 weeks PMI (Fig 15). There was a significant increase in cleaved PARP in all ligated animals at 4 and 16 weeks PMI except ligated olive oil which was comparable to its sham control (Fig 15).

At 4 weeks PMI, the expression of cleaved PARP increased significantly by 41 % in the ligated regular chow, and 105 % in the ligated corn oil compared to their respective sham control (Fig 15 A). In the ligated olive oil, the cleavage of PARP was comparable to its sham control (Fig 15 A). The ligated corn oil showed significantly higher cleavage of PARP than the ligated regular chow and the ligated olive oil (Fig 15 A).

At 16 weeks PMI, cleaved PARP expression increased significantly by 31 % in the ligated regular chow, and 91 % in the ligated corn oil compared to their respective sham control (Fig 15 B). In the ligated olive oil, cleaved PARP was comparable to its sham control (Fig 15 B). The ligated corn oil showed significantly higher cleavage of PARP than both, the ligated regular chow and the ligated olive oil groups (Fig 15 B).

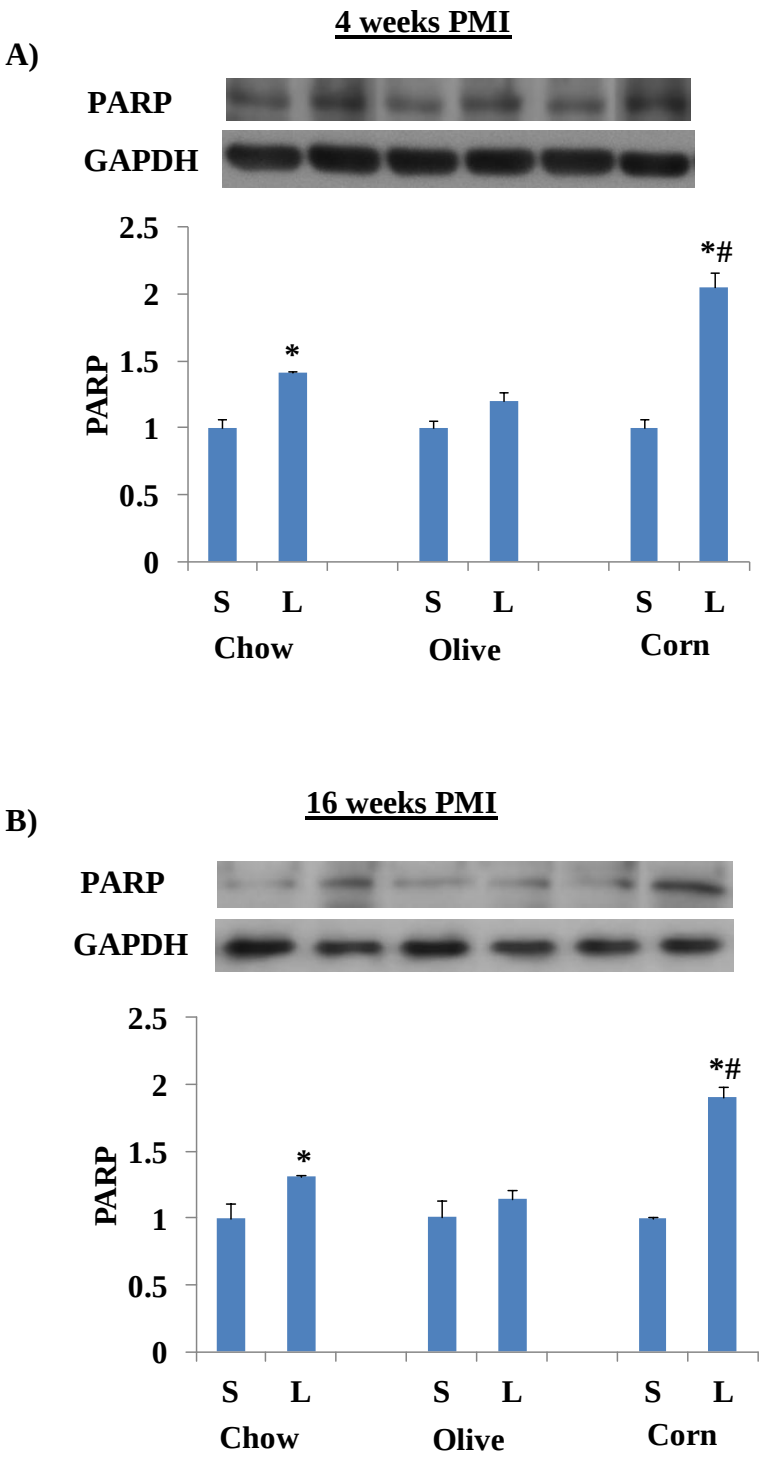


Fig 15: Cleaved PARP expression in sham and ligated animals after 4 (A) and 16 weeks (B) PMI. Data are presented as mean ± SEM from 6-9 animals for each group . Two way ANOVA analysis was done. *) Significant from its own sham, and #) significant from ligated regular chow and ligated olive oil. In all groups P< 0.05.

1.3.4.d TGFß Expression

There was no difference in the expression of TGFβ among all sham animals (Fig 16). The expression of TGFβ increased in all the ligated animals at 4 and 16 weeks PMI except for the ligated olive oil animals (Fig 16).

At 4 weeks PMI, expression of TGFβ increased significantly by 50 % in the ligated regular chow, and 73 % in the ligated corn oil compared to their respective sham control (Fig 16 A). In the ligated olive oil, the TGFβ expression was comparable to its sham control and was significantly lower than the ligated regular chow and ligated corn oil groups (Fig 16 A).

At 16 weeks PMI, the expression of TGFβ increased significantly by 28 % in the ligated regular chow, and 48 % in the ligated corn oil group (Fig 16 B). In the ligated olive oil animals, the TGFβ expression was comparable to its sham control and was significantly lower than ligated regular chow and ligated corn oil groups (Fig 16 B).

1.3.4.e Bnip3 Expression

There were no significant differences in the Bnip3 expression among all sham animals at 4 and 16 weeks PMI (Fig 17). There was a significant increase in Bnip3 expression in all the ligated animals at 4 and 16 weeks PMI except the ligated olive oil (Fig 17).

At 4 weeks PMI, expression of Bnip3 increased significantly by 23 % in the ligated regular chow, and 67 % in the ligated corn oil compared to their respective sham control (Fig 17 A).

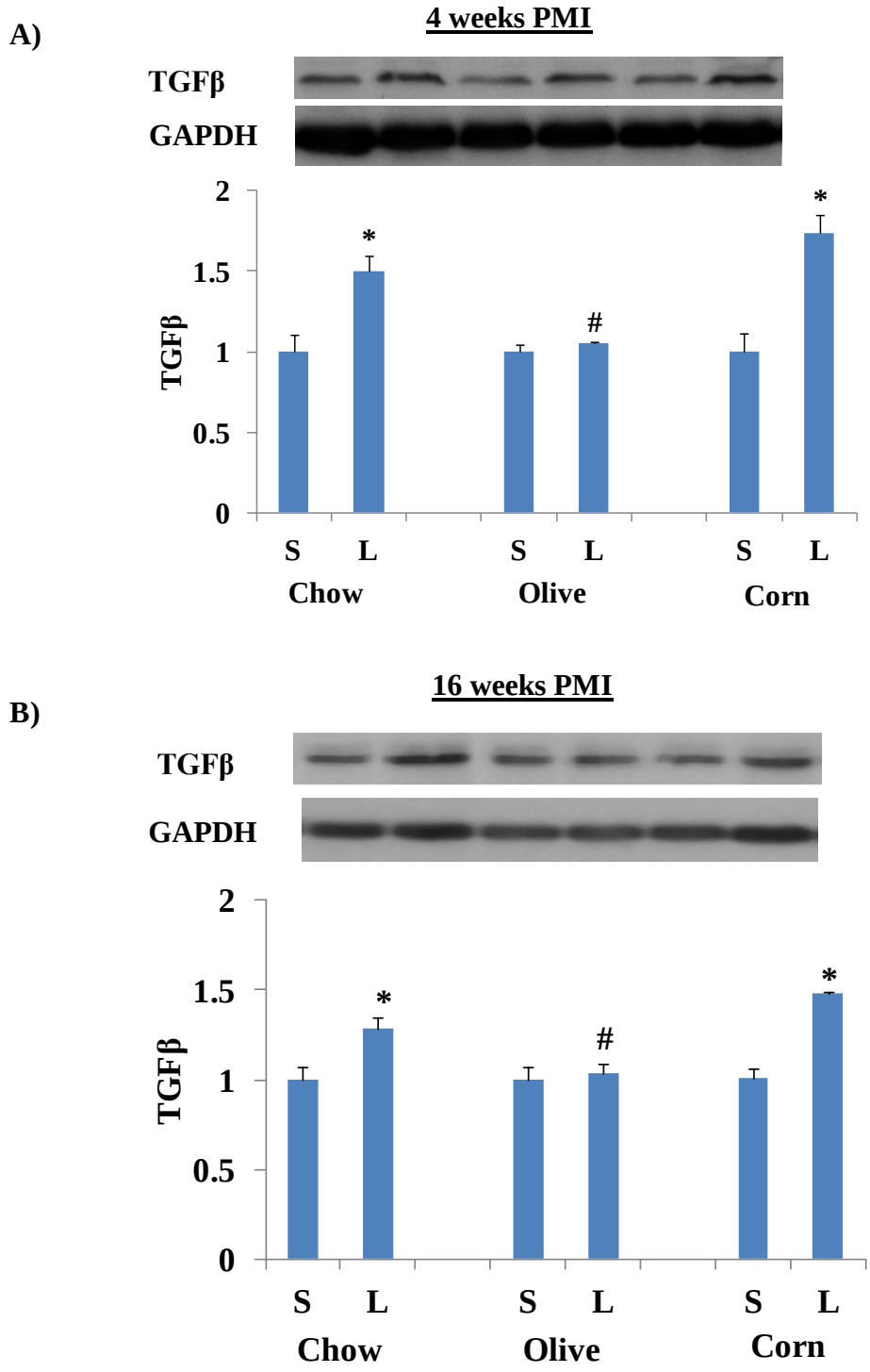


Fig 16: TGFβ expression in sham (S) and ligated (L) animals after 4 (A) and 16 weeks (B) PMI. Data are presented as mean ± SEM from 6-9 animals for each group. Two way ANOVA analysis was done. *) Significant from its own sham, and #) significant from ligated regular chow and ligated corn oil. In all groups, P< 0.05.

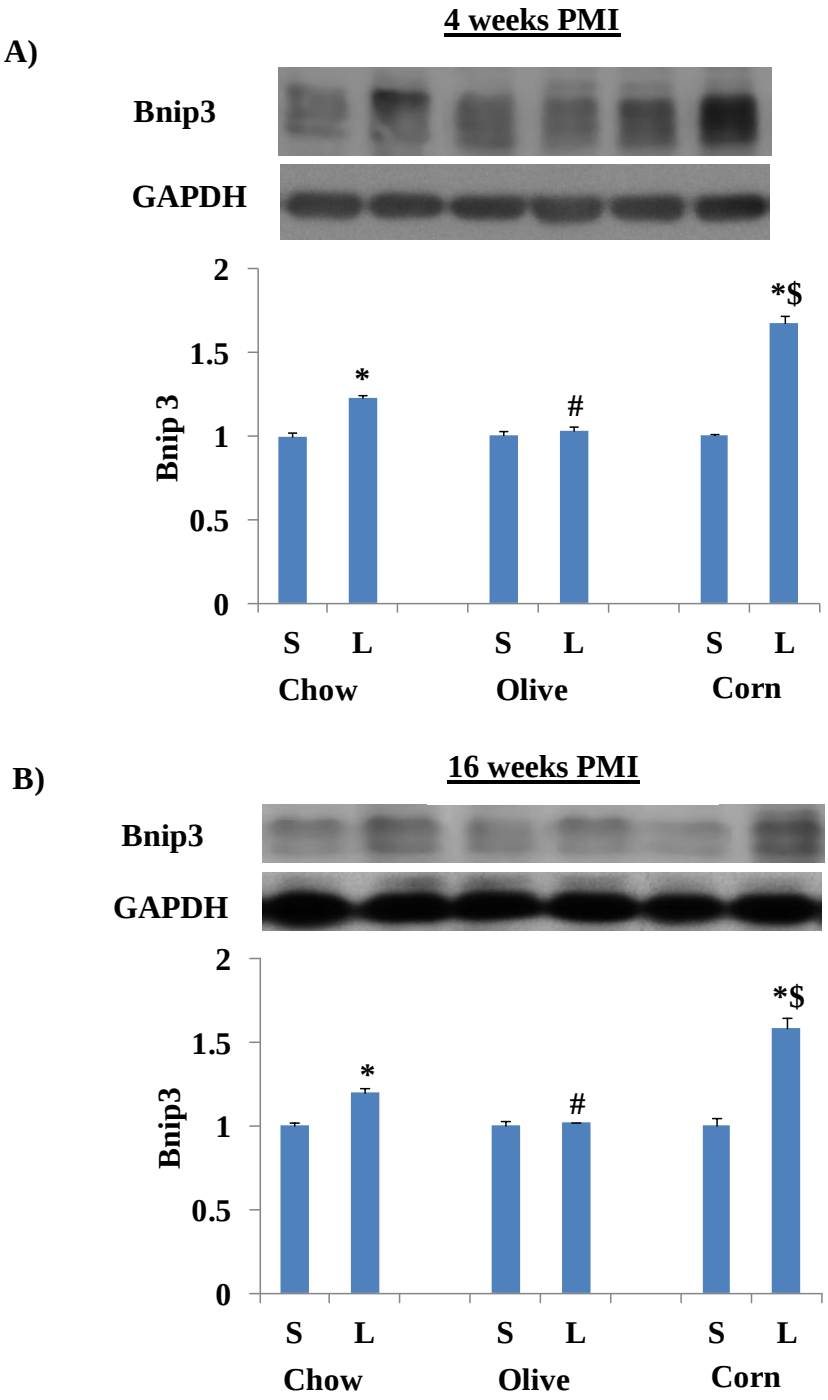


Fig 17: Bnip3 expression in sham (S) and ligated (L) animals after 4 (A) and 16 weeks (B) PMI. Data are presented as mean ± SEM from 6-9 animals for each group. Two way ANOVA analysis was done. *) Significant from its own sham, #) significant from ligated regular chow and from ligated corn oil, and $) significant from ligated regular chow. In all groups (P< 0.05).

In the ligated olive oil, the Bnip3 expression was comparable to its sham control, and was significantly lower than the ligated regular chow as well as the ligated corn oil (Fig 17 A). The ligated corn oil showed higher Bnip3 expression than the ligated regular chow (Fig 17 A).

At 16 weeks PMI, the expression of Bnip3 increased significantly by 20 % in the ligated regular chow, and 59 % in the ligated corn oil compared to their respective sham control (Fig 17 B). In the ligated olive oil, the Bnip3 expression was comparable to its sham control, and was significantly lower than the ligated regular chow as well as the ligated corn oil (Fig 17 B). The ligated corn oil showed higher Bnip3 expression than the ligated regular chow (Fig 17 B).

1.3.4.f Cytochrome C Release

Mitochondrial cytochrome C content was comparable among all sham groups without any significant differences at 4 and 16 weeks PMI (Fig 18). Mitochondrial cytochrome C content decreased significantly in all the ligated groups at 4 and 16 weeks PMI except the ligated olive oil where it was comparable to its sham control (Fig 18).

Cyclophilin D, a protein present only in mitochondria, was used as an internal control in the mitochondrial fraction for Western blot, which has a little GAPDH in normal conditions, while GAPDH was used in cytosolic fraction as internal control for Western blot, where normally no cyclophilin D is present (Tarze et al. 2007; Tristan et al. 2011; Menazza et al. 2012).

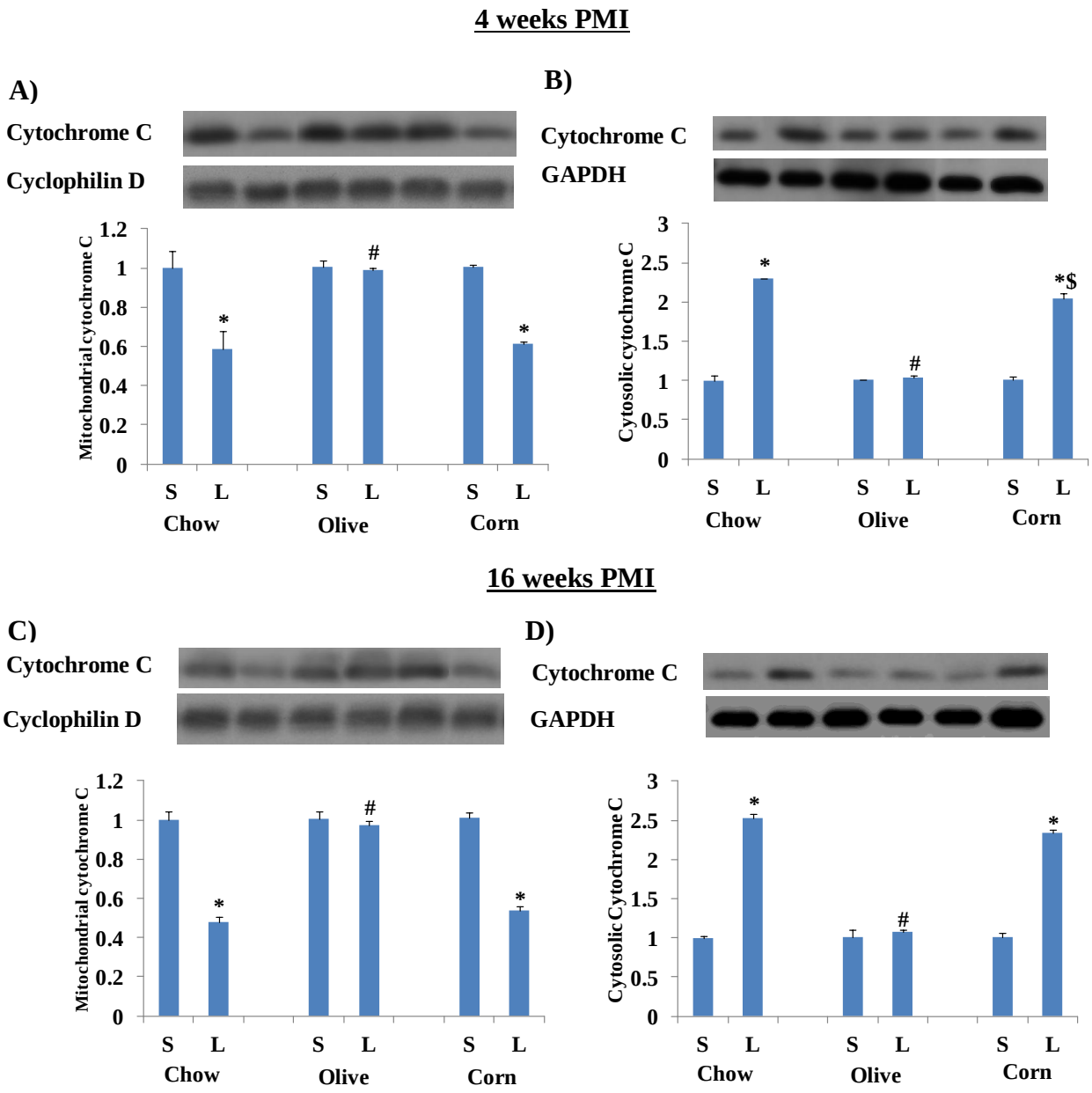


Fig 18: Mitochondrial and cytosolic cytochrome C content in sham (S) and ligated (L) animals after 4 (A+B) and 16 weeks (C+D) PMI. Data are presented as mean ± SEM from 6-9 animals for each group . Two way ANOVA analysis was done. *) Significant from its own sham, #) significant from ligated regular chow and ligated corn oil, and $) significant from ligated regular chow. In all groups (P< 0.05).

At 4 weeks PMI, mitochondrial cytochrome C content decreased significantly by 41 % in the ligated regular chow, and by 39 % in the ligated corn oil compared to their respective sham control (Fig 18 A). In the ligated olive oil, mitochondrial cytochrome C content was comparable to its sham control, and significantly higher than ligated regular chow and ligated corn oil (Fig 18 A).

At 16 weeks PMI, mitochondrial cytochrome C content decreased significantly by 52 % in ligated regular chow, and 46 % in the ligated corn oil compared to their respective sham control (Fig 18 C). In the ligated olive oil, mitochondrial cytochrome C content was comparable to its sham control, and significantly higher than ligated regular chow and ligated corn oil (Fig 18 C).

Cytosolic cytochrome C content was not significantly different among sham animals at 4 and 16 weeks PMI (Fig 18). There was a significant increase in cytosolic cytochrome C content in all ligated animals at 4 and 16 weeks PMI except ligated olive oil (Fig 18).

At 4 weeks PMI, cytosolic cytochrome C content increased significantly by 129 % in the ligated regular chow, and 103 % in the ligated corn oil compared to their respective sham control (Fig 18 B). In the ligated olive oil group, cytosolic cytochrome C content was comparable to its sham control, and significantly lower than ligated regular chow and ligated corn oil (Fig 18 B). In the ligated corn oil group, cytosolic cytochrome C content was significantly lower than the ligated regular chow (Fig 18 B).

At 16 weeks PMI, cytosolic cytochrome C content increased significantly by 152 % in the ligated regular chow, and 134 % in the ligated corn oil compared to their respective sham control (Fig 18 D). In the ligated olive oil animals, cytosolic cytochrome C content

was comparable to its sham control, and significantly lower than ligated regular chow and ligated corn oil (Fig 18 D).

1.3.5 Mitogen Activated Protein Kinases (MAP kinases)

For the study of MAP kinase activation, we analyzed the ratio of myocardial phosphorylated p38 to total p38 (P-p38/T-p38), phosphorylated JNK to total JNK (P-JNK/ T-JNK), and phosphorylated ERK to total ERK (P-ERK/T-ERK).

1.3.5.a Ratio of Phosphorylated p38 to Total p38

There was no significant difference in the ratio of P-p38/T-p38 among all sham animals at 4 and 16 weeks PMI (Fig 19). There was a significant increase in the ratio of P-p38/T-p38 in all ligated animals at 4 and 16 weeks PMI except for the ligated olive oil group at 16 weeks PMI (Fig 19).

At 4 weeks PMI, the ratio of P-p38/T-p38 increased significantly by 81 % in the ligated regular chow, 55 % in the ligated olive oil, and 158 % in the ligated corn oil group compared to their respective sham control (Fig 19 A). The ligated corn oil showed a significantly higher ratio of P-p38/T-p38 than the ligated regular chow and the ligated olive oil (Fig 19 A).

At 16 weeks PMI, the ratio of P-p38/T-p38 increased significantly by 53 % in the ligated regular chow, and 109 % in the ligated corn oil compared to their respective sham control (Fig 19 B). The ligated olive oil showed a comparable ratio compared to its sham control, and a significantly lower ratio than ligated regular chow and ligated corn oil (Fig 19 B).

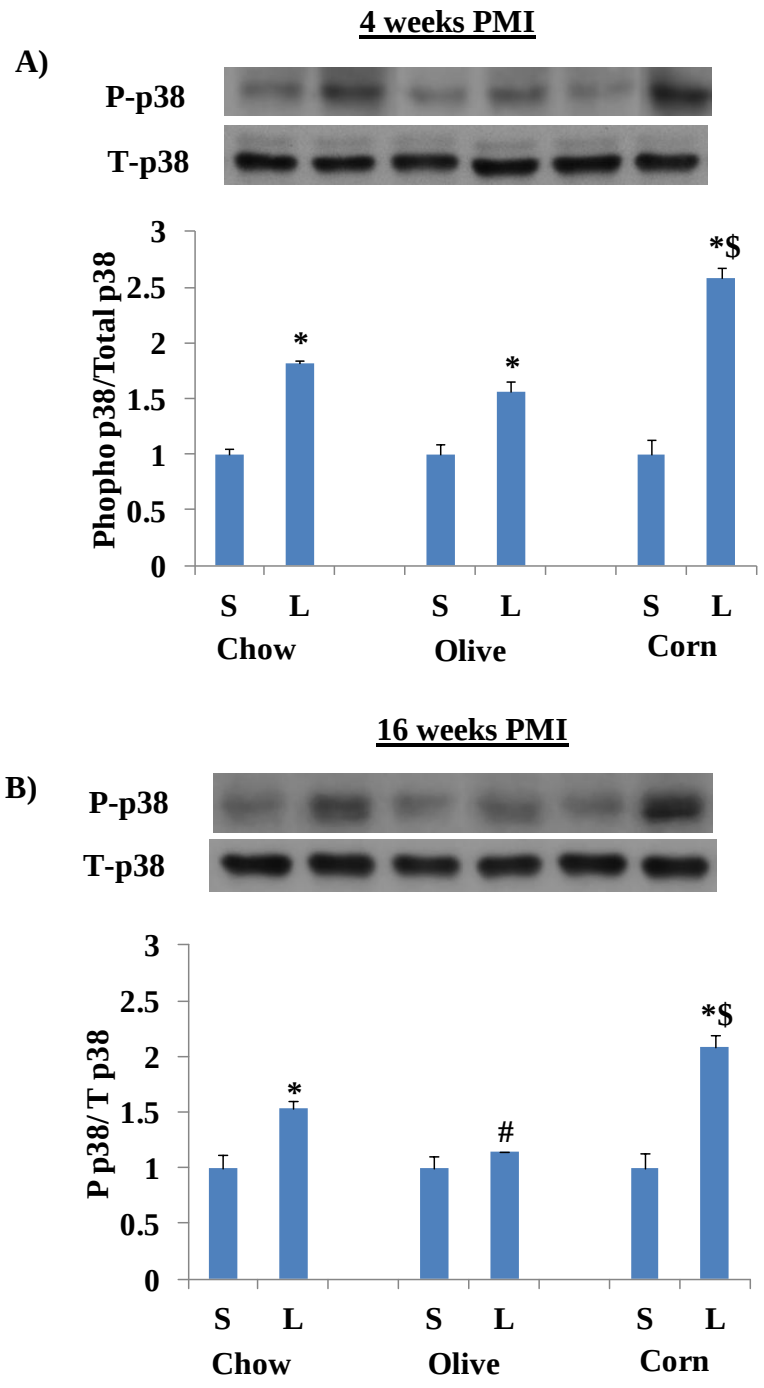


Fig 19: Phosphorylated p38 to total p38 (Pp38/Tp38) activation in sham (S) and ligated (L) animals after 4 (A) and 16 weeks (B) PMI. Data are presented as mean ± SEM from 6-9 animals for each group . Two way ANOVA analysis was done. *) Significant from its own sham, #) significant from ligated regular chow and ligated corn oil, and $) significant from ligated regular chow and ligated olive oil. In all groups P< 0.05.

The ligated corn oil animals, showed significantly higher ratio than the ligated regular chow and the ligated olive oil animals (Fig 19 B).

1.3.5.b Ratio of Phosphorylated JNK to Total JNK

The ratio of P-JNK/T-JNK was not significantly different among all sham animals (Fig 20). There was a significant increase in the ratio of P-JNK/T-JNK in all ligated animals at 4 and 16 weeks PMI except for the ligated olive oil at 16 weeks PMI (Fig 20).

At 4 weeks PMI, the ratio of P-JNK/T-JNK increased significantly by 260 % in the ligated regular chow, 87 % in the ligated olive oil, and 259 % in the ligated corn oil group compared to their respective sham control (Fig 20 A). Even though the ligated olive oil showed a higher ratio than its sham control, this was significantly lower than both, the ligated regular chow and the ligated corn oil (Fig 20 A).

At 16 weeks PMI, the ratio of P-JNK/T-JNK increased significantly by 80 % in the ligated regular chow, and 215 % in the ligated corn oil group compared to their respective sham control (Fig 20 B). In the ligated olive oil, the ratio was comparable to its sham control (Fig 20 B). The ligated corn oil showed significantly higher ratio than the ligated regular chow and the ligated olive oil (Fig 20 B).

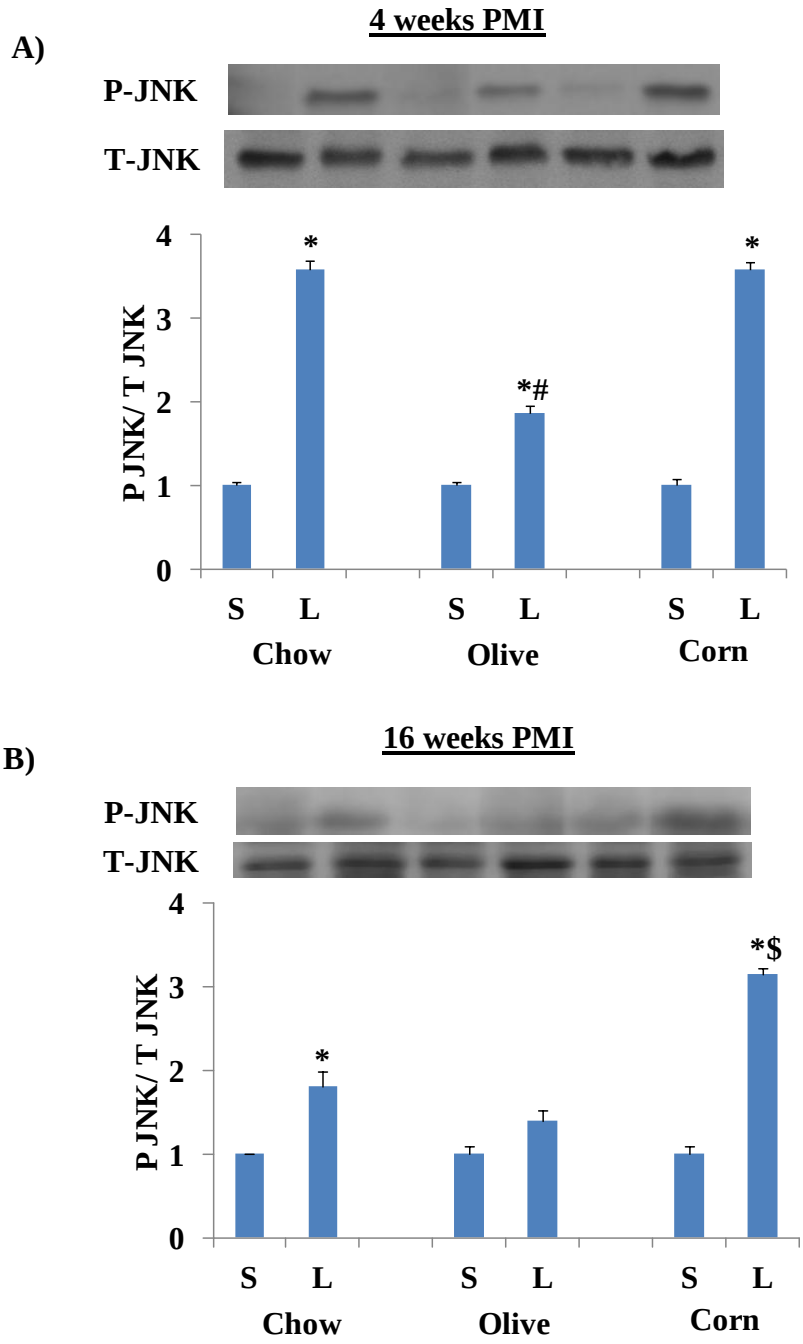


Fig 20: Phosphorylated JNK to total JNK (P-JNK/T-JNK) activation in sham (S) and ligated (L) animals after 4 (A) and 16 weeks (B) PMI. Data are presented as mean ± SEM from 6-9 animals for each group. Two way ANOVA analysis was done. *) Significant from its own sham, #) significant from ligated regular chow and ligated corn oil, and $) significant from ligated regular chow and ligated olive oil. In all groups P< 0.05.

1.3.5.c Ratio of Phosphorylated ERK to Total ERK

The ratio of P-ERK/T-ERK was not significantly changed among all sham animals at 4 and 16 weeks PMI (Fig 21). There was a significant increase in the ratio P-ERK/T-ERK in all ligated animals at 4 and 16 weeks PMI except the ligated regular chow at 16 weeks PMI (Fig 21).

At 4 weeks PMI, the ratio of P-ERK/T-ERK increased significantly by 26 % in the ligated regular chow, 106 % in the ligated olive oil, and 73 % in the ligated corn oil compared to their respective sham control (Fig 21 A). This ratio was significantly higher in the ligated olive oil compared to the ligated regular chow and the ligated corn oil (Fig 21 A). This ratio was also significantly higher in the ligated corn oil compared to the ligated regular chow (Fig 21 A).

At 16 weeks PMI, the ratio of P-ERK/T-ERK increased significantly by 43 % in the ligated olive oil, and by 27 % in the ligated corn oil compared to their respective sham control (Fig 21 B). This ratio was comparable in the ligated regular chow to its sham control (Fig 21 B). This ratio was significantly higher in the ligated olive oil compared to the ligated regular chow and the ligated corn oil (Fig 21 B).

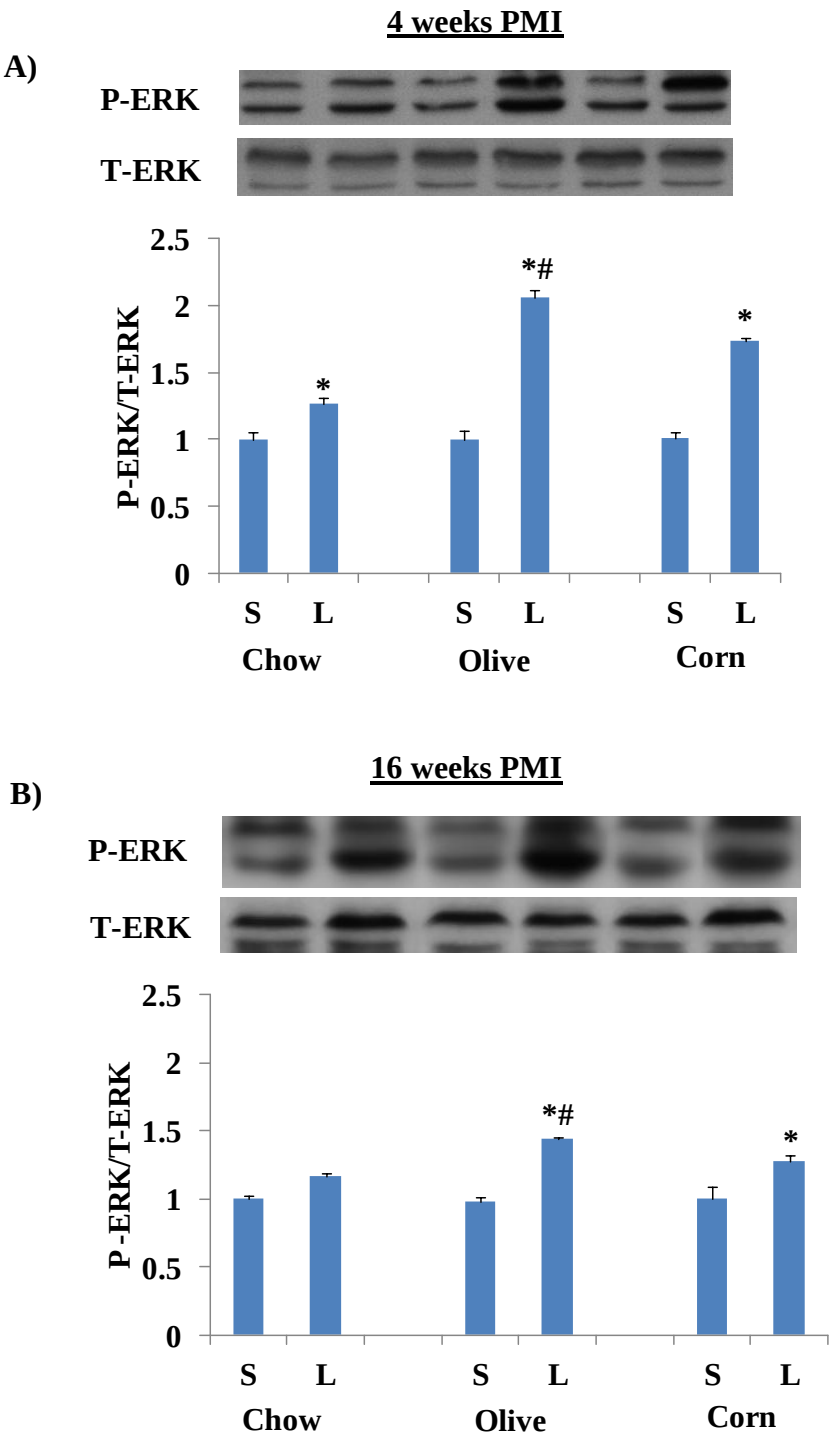


Fig 21: Phosphorylated ERK to total ERK (P-ERK/T-ERK) activation in sham (S) and ligated (L) animals after 4 (A) and 16 weeks (B) PMI. Data are presented as mean ± SEM from 6-9 animals for each group . Two way ANOVA analysis was done. *) Significant from its own sham, and #) significant from ligated regular chow and ligated corn oil. In all groups P< 0.05.

1.4 Histological analysis

Whole hearts from the sham and ligated groups were photographed prior to processing of these hearts for biochemical analysis at 4 and 16 weeks PMI to document the size of the hearts (Fig 22 and Fig 23). Sections from the heart of all sham and ligated groups were taken, and stained with Masson's Trichrome to compare groups in terms of fibrosis as well as gross changes (Fig 24). Scar tissue is shown in these pictures in white bluish color (fibrotic under microscope).

Ligation resulted in the formation of scar as well as hypertrophy of the left ventricular wall and septum. Whole heart hypertrophy was apparent at 4 and 16 weeks PMI in the ligated regular chow as well as in the ligated corn oil group (Fig 22 B and F; Fig 23 B and F). In comparison, the ligated olive oil group showed a trend towards lesser hypertrophy. In low magnification cross section view, the scar was principally fibrotic and stained blue, whereas the left ventricle wall as well as septum were hypertrophied (Fig 24 B and F). In the ligated regular chow and corn oil, there was a trend of more fibrosis and relatively less myocardial tissue (Fig 25 B and F) as compared to ligated olive oil group (Fig 25 D). At a higher magnification, these differences between the ligated olive oil group (Fig 26 D) and ligated regular chow (Fig 26 B) and ligated corn oil (Fig 26 F) were confirmed.

At 16 weeks PMI, the scar formation as well as resulting collagen deposition were more pronounced visually in both the ligated regular chow (Fig 27 B and Fig 28 B) and ligated corn oil group (Fig 27 F and Fig 28 F) at both low and high magnifications. In the olive oil group, there was a trend of less deposition of the collagen (Fig 27 D and Fig 28 D).

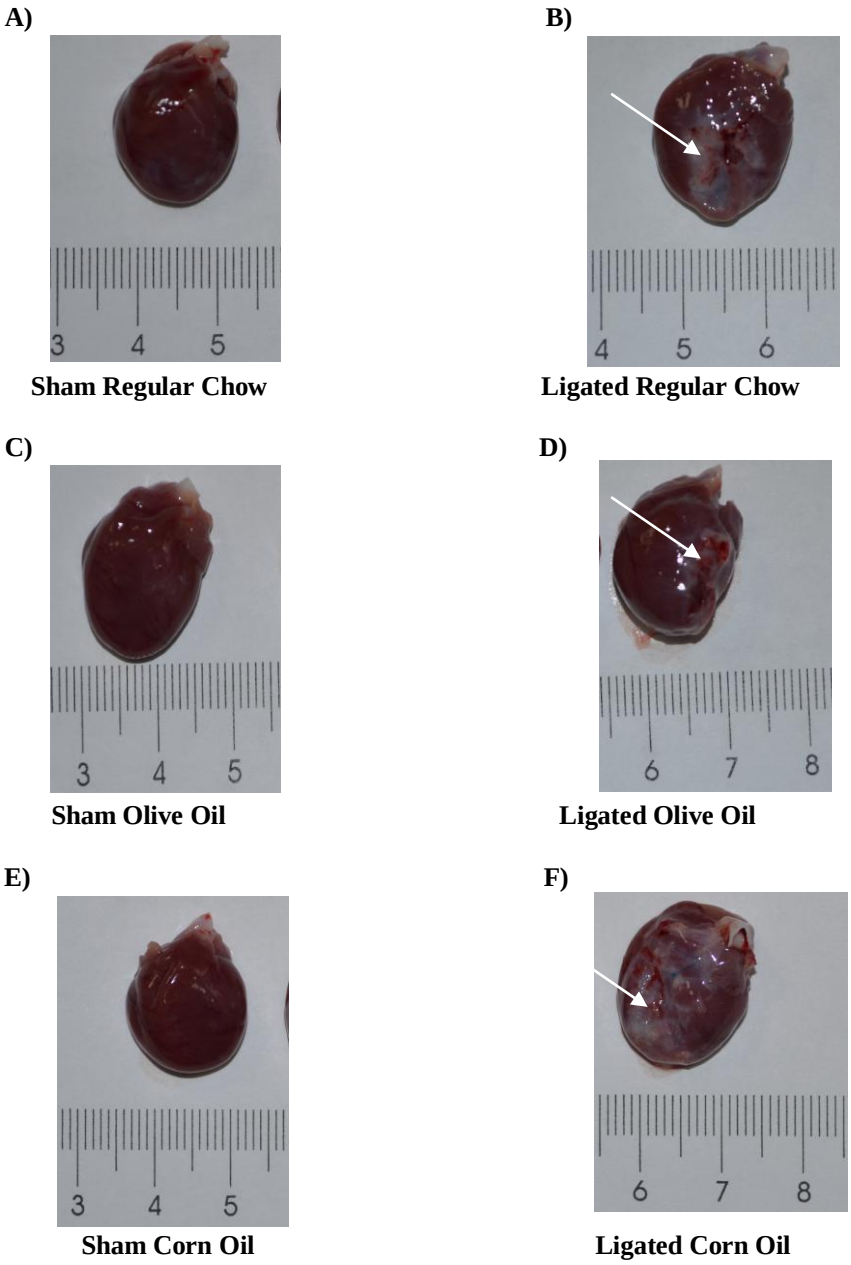
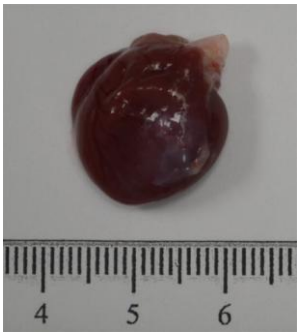


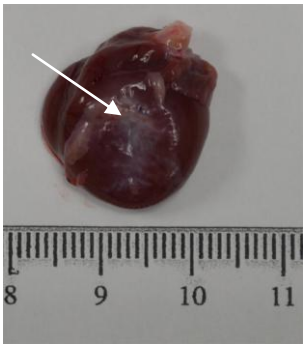
Fig 22: Representative photos of the whole heart from each group at 4 weeks PMI. Photos are in the same scale shown in each figure. Some hypertrophy is apparent in the ligated regular chow (B) as well as in the ligated corn oil groups. White bluish scar tissue (fibrotic under microscope) is pointed by white arrow (for confirmation of fibrosis see Fig 24-28).

A)



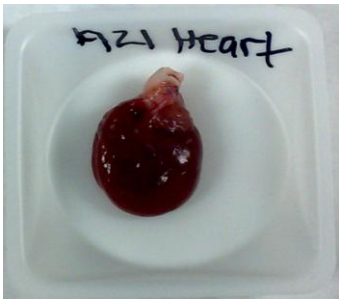
Sham Regular Chow

B)



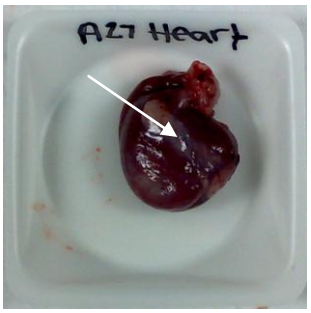
Ligated Regular Chow

C)



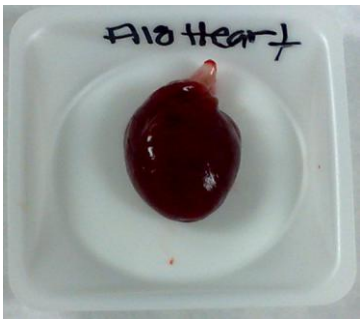
Sham Olive Oil

D)



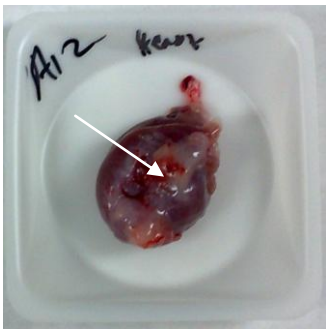
Ligated Olive Oil

E)



Sham Corn Oil

F)

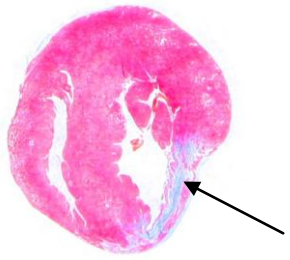


Ligated Corn Oil

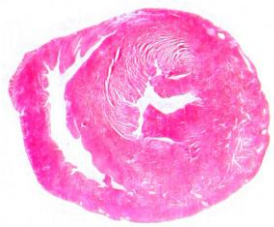
Fig 23: Representative photos of the whole hearts from each group at 16 weeks PMI. Notice the hypertrophy in the ligated regular chow group. White bluish scar tissue (fibrotic under microscope) is pointed by white arrow (for confirmation of fibrosis see Fig 24-28).

A) Sham 4 weeks PMI

B) Ligated 4 weeks PMI



C) Sham 16 weeks PMI



D) Ligated 16 weeks PMI

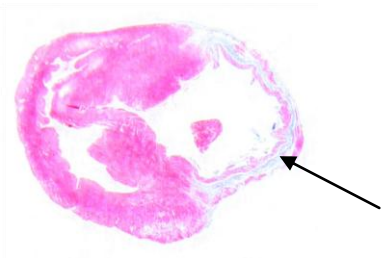
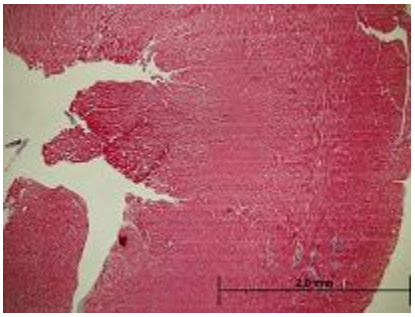


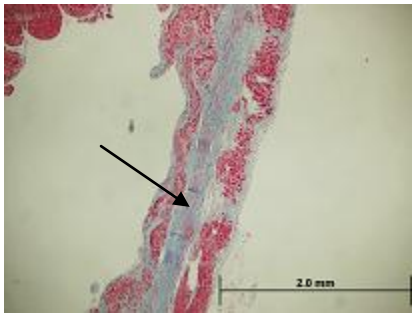
Fig 24: Representative pictures of the left ventricle wall of heart cross sections at 4 and 16 weeks PMI for animals on standard regular chow. The sections were stained with Masson's Trichrome: A) Sham 4 weeks, B) Ligated 4 weeks, C) Sham 16 weeks, and D) Ligated 16 weeks PMI. Scar is seen in ligated (B and D) groups. Fibrosis and left ventricular wall damage and thinning are pointed by black arrow.

A)



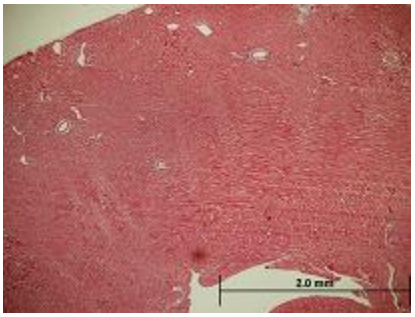
Sham Regular Chow

B)



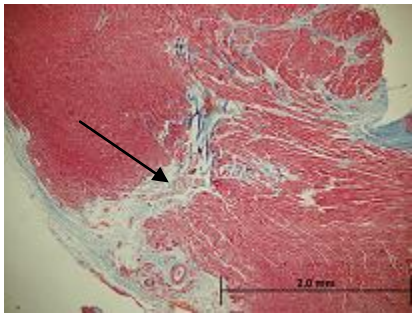
Ligated Regular Chow

C)



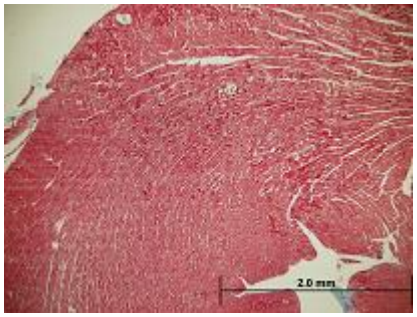
Sham Olive Oil

D)



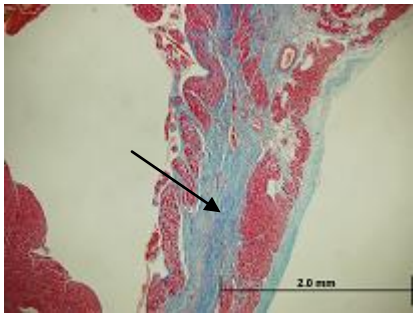
Ligated Olive Oil

E)



Sham Corn Oil

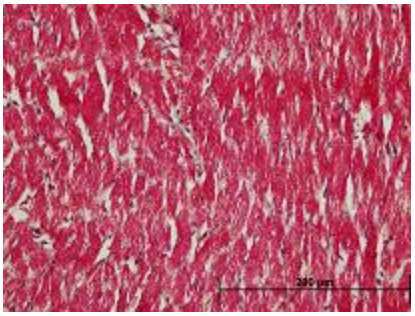
F)



Ligated Corn Oil

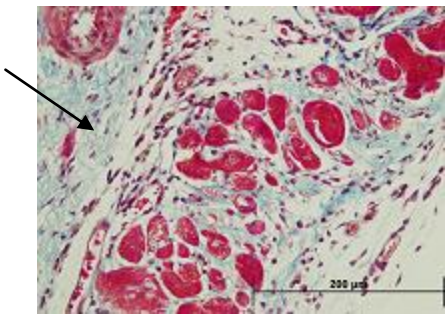
Fig 25: Representative micrographs of the left ventricle wall of heart cross sections at 4 weeks PMI. The sections were stained with Masson's Trichrome: A) Sham Regular Chow, B) Ligated Regular Chow, C) Sham Olive Oil, D) Ligated Olive Oil, E) Sham Corn Oil, and F) Ligated Corn Oil. Fibrosis is shown here in blue color. Fibrosis and left ventricular wall damage and thinning is pointed by black arrow. Magnification bar shown in black is 2 mm (40X).

A)



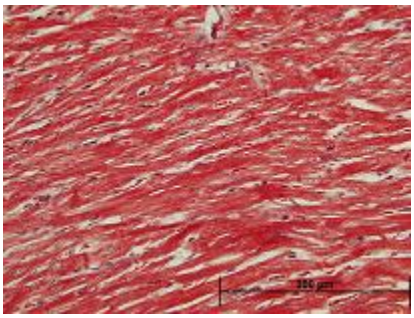
Sham Regular Chow

B)



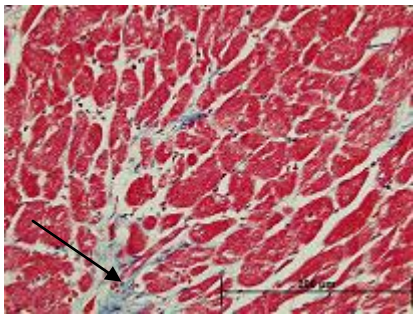
Ligated Regular Chow

C)



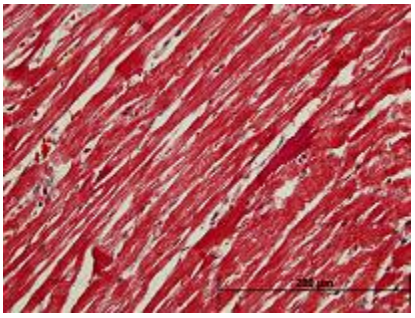
Sham Olive Oil

D)



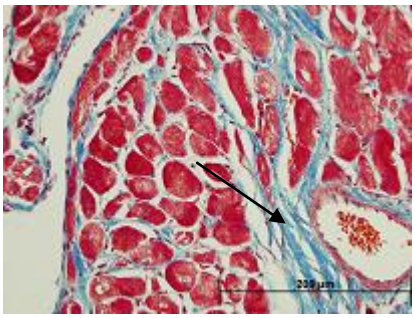
Ligated Olive Oil

E)



Sham Corn Oil

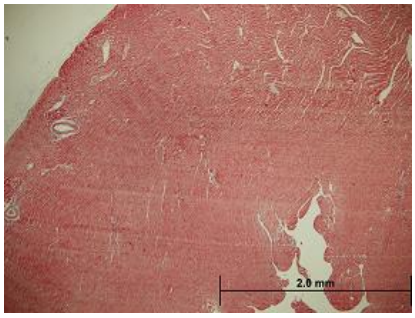
F)



Ligated Corn Oil

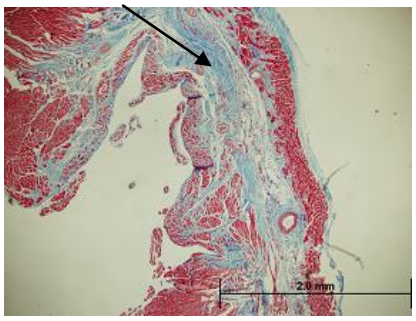
Fig 26: Representative micrographs of the left ventricle wall of heart cross sections at 4 weeks PMI. The sections were stained with Masson's Trichrome: A) Sham Regular Chow, B) Ligated Regular Chow, C) Sham Olive Oil, D) Ligated Olive Oil, E) Sham Corn Oil, and F) Ligated Corn Oil. Fibrosis is shown here in blue color. Fibrosis is pointed by black arrow. Magnification bar shown in black is 200 µm (400X).

A)



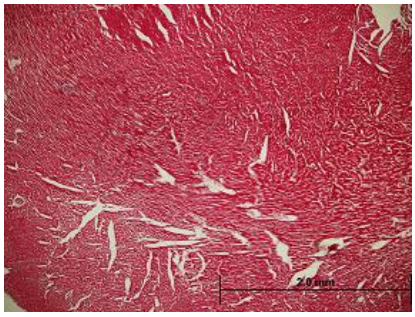
Sham Regular Chow

B)



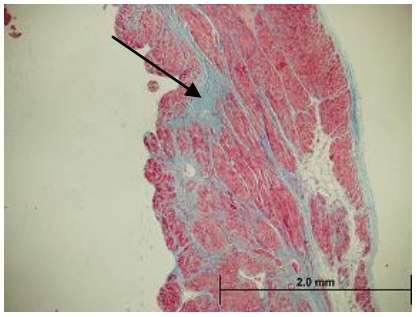
Ligated Regular Chow

C)



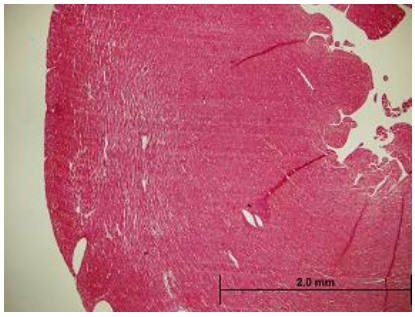
Sham Olive Oil

D)



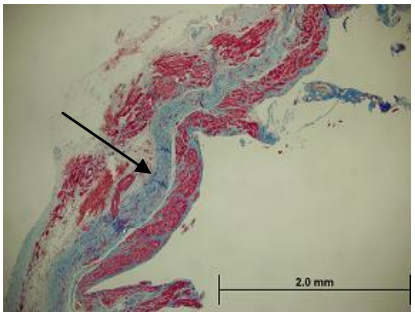
Ligated Olive Oil

E)



Sham Corn Oil

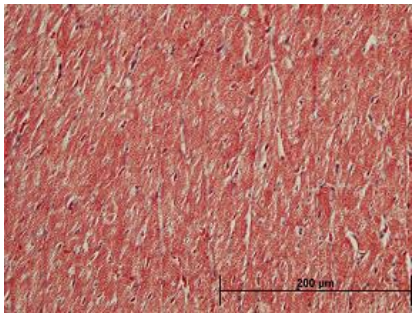
F)



Ligated Corn Oil

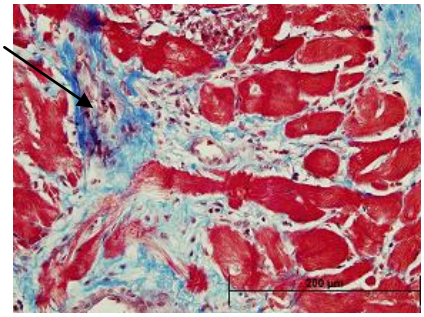
Figure 27: Representative micrographs of the left ventricle wall of heart cross sections at 16 weeks PMI. The sections were stained with Masson's Trichrome: A) Sham Regular Chow, B) Ligated Regular Chow, C) Sham Olive Oil, D) Ligated Olive Oil, E) Sham Corn Oil, and F) Ligated Corn Oil. Fibrosis is shown here in blue color and pointed by black arrow. Magnification bar shown in black is 2 mm (40X).

A)



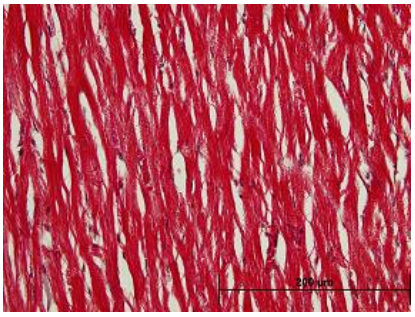
Sham Regular Chow

B)



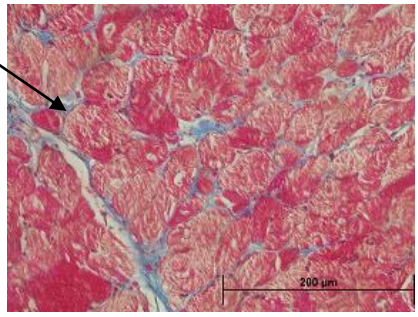
Ligated Regular Chow

C)



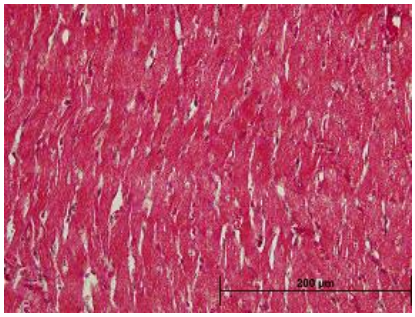
Sham Olive Oil

D)



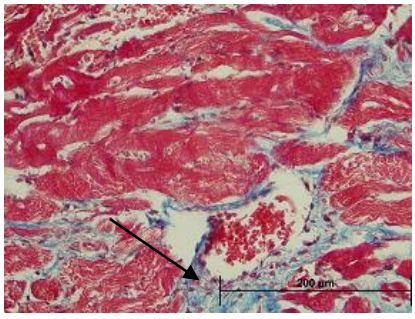
Ligated Olive Oil

E)



Sham Corn Oil

F)



Ligated Corn Oil

Figure 28: Representative micrographs of the left ventricle wall of heart cross sections at 16 weeks PMI. The sections were stained with Masson's Trichrome: A) Sham Regular Chow, B) Ligated Regular Chow, C) Sham Olive Oil, D) Ligated Olive Oil, E) Sham Corn Oil, and F) Ligated Corn Oil. Fibrosis is shown here in blue color and pointed by black arrow. Magnification bar shown in black is 200 µm (400X).

2.0 *In Vitro* Studies

Based on our *in vivo* whole animal studies, it was established that heart hypertrophy and heart failure subsequent to myocardial infarction were associated with an increase in TNF-α, oxidative stress, apoptosis and associated subcellular changes. It was also observed that diet with olive oil supplementation mitigated these sub-cellular changes coupled with an improvement in the function of the ligated hearts. Therefore, we decided to test whether oleic acid (OA) (which comprises approximately 80 % of extra-virgin olive oil) could block the deleterious effects of TNF-α by challenging adult rat isolated cardiomyocytes in an *in vitro* system with TNF-α in the presence or absence of OA.

Myocardial cells were isolated from Sprague-Dawley (SD) rats, and cultured according to standard protocols in our lab (Al-Shudiefat et al. 2013). Cells were divided into four groups as follows: A) control; B) TNF-α (10 ng/ml) treated; C) OA (50 μM) treated and D) TNF-α (10 ng/ml) + OA (50 μM) treated. In all groups, the incubation with the agent was for 4 hrs except for group D where the cells were pretreated for 30 minutes with OA, followed by 4 hrs of incubation with TNF-α. In order to examine the role of oxidative stress, cells were also treated with 25 μM H_2O_2 for 4 hrs as a positive control. Cells in all groups were assessed for oxidative stress, cell viability, cell damage, and apoptosis.

2.1 Oxidative stress Assessment

The level of oxidative stress was assessed by measuring the level of reactive oxygen species (ROS) produced after exposure of isolated cardiomyocytes to TNF-α (Fig. 29). ROS production in TNF-α treated cardiomyocytes increased (163%) significantly compared to control. OA treated cardiomyocytes showed comparable ROS production with control. In contrast, treatment of cardiomyocytes with OA before addition of TNF-α, prevented the TNF-α induced increase in ROS generation. Hydrogen peroxide (H_2O_2) [25 μM] was able to increase ROS significantly, which was inhibited by OA (data not shown) and this concentration of H_2O_2, was used as a positive control for oxidative stress.

2.2 Assessment of Cell Viability and Damage

TNF-α treated cardiomyocytes showed a significant decrease (43%) in cell viability as compared to control (Fig. 30 a1+a2). Cardiomyocytes treated with OA alone showed comparable cell viability with control, whereas OA treatment blocked TNF-α induced cell death (Fig. 30 a1+a2). TNF-α treatment caused a significant increase (148%) in creatine kinase release in the culture medium due to damaging of cardiomyocyte membranes compared to control group (Fig. 30 b). OA-treated cardiomyocytes showed comparable creatine Kinase release compared to control. However, OA was able to mitigate the TNF-α –induced damage in cardiomyocytes significantly (Fig. 30 b).

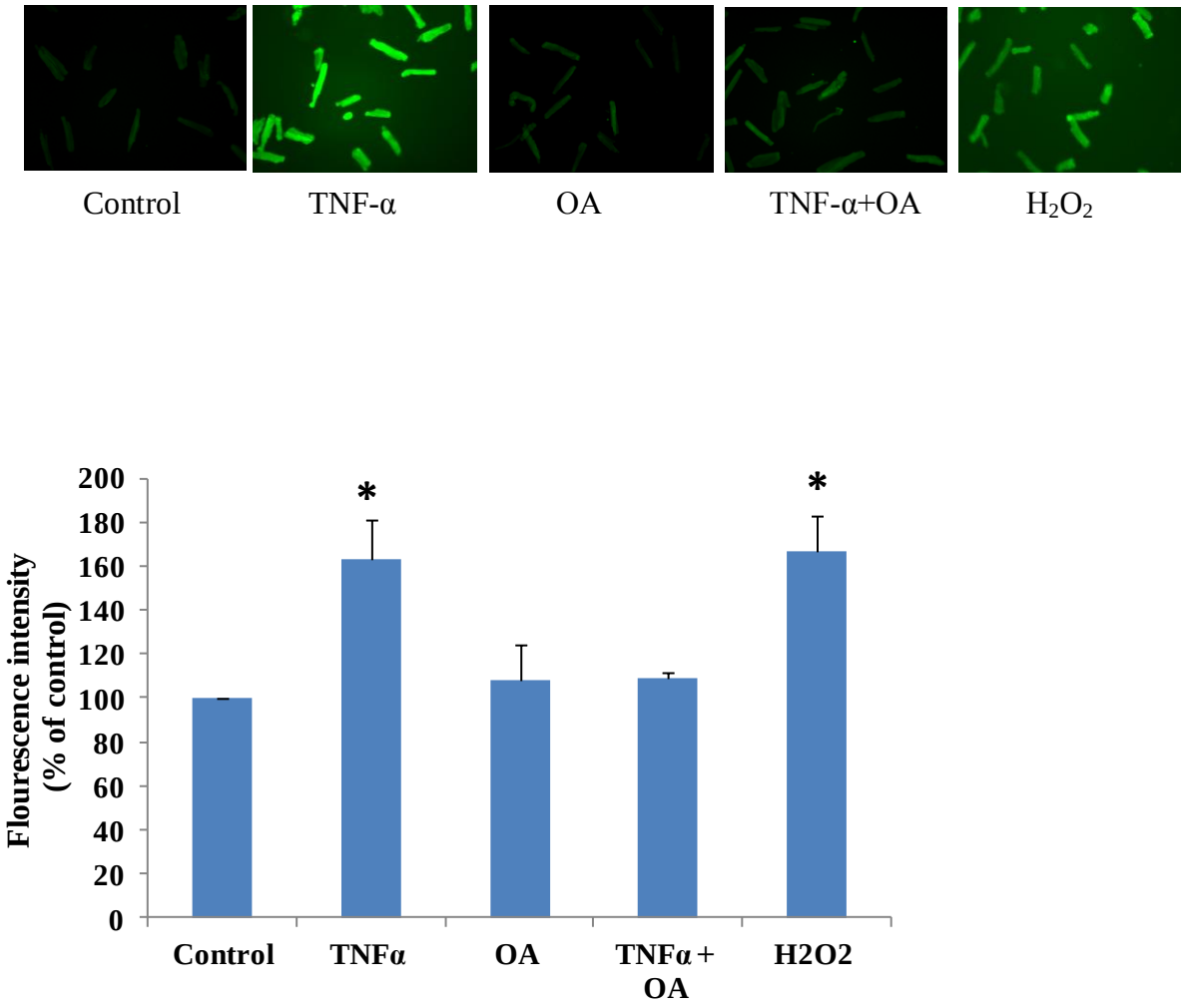
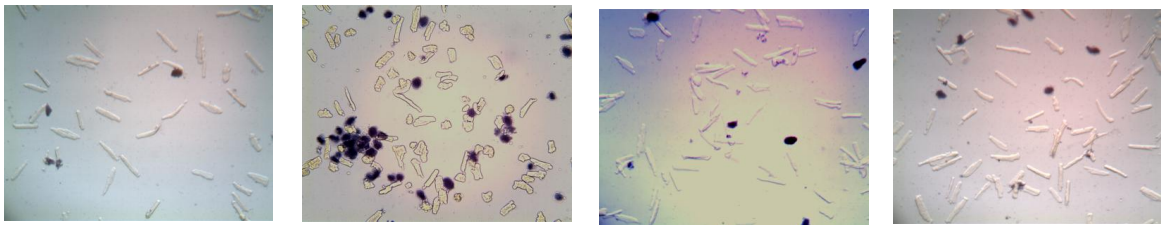


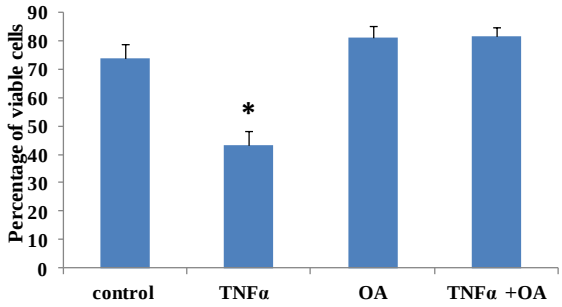
Fig 29: Effects of the exposure to TNF-α (10 ng/ml), OA (50 µM), and TNF-α + OA for 4 hrs on ROS generation in adult rat cardiac myocytes using DCFDA dye: Upper panel fluorescence microscope images; lower panel is a fluorescent intensity analysis. H_2O_2 (25 µM) was used as a positive control. N=5, * P<0.05, significantly different from control.

a1



Control TNF-α OA OA+TNF-α

a2



b

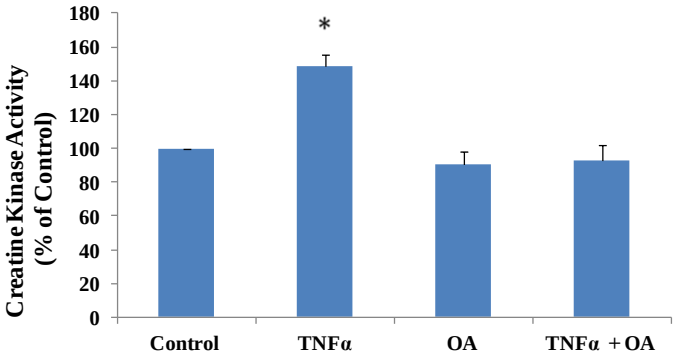


Fig 30: Effects of the exposure to TNF-α (10 ng/ml), OA (50 µM), and TNF-α +OA for 4 hrs on: a) Cell viability as shown by trypan blue exclusion test; and b) Cell damage assessed by creatine kinase release from adult rat cardiac myocytes. N=5, * P<0.05, significantly different from control.

2.3 Apoptosis

Assessment of apoptosis was done through two approaches, the first was to measure level of nuclear fragmentation, and the second was to measure the expression of pro-apoptotic proteins.

2.3.1 Nuclear Fragmentation

TNF-α treated cardiomyocytes showed a significantly increased number of apoptotic cells (22.4%) as compared to the control group (7.5%). OA treated cardiomyocytes showed comparable proportion apoptotic cells compared to the control group, whereas OA pre-treatment was able to significantly inhibit the TNF-α induced apoptosis (Fig 31 a+b). H_2O_2 (25 μM) was used as a positive control for apoptosis; it was able to induce increase of 25.6 % in apoptotic cells.

2.3.2 Expression of pro-apoptotic proteins

TNF-α treated cardiomyocytes showed a significant increase in the expression of pro-apoptotic proteins compared to control (Fig. 32). TNF-α increased significantly: the Bax/Bcl-xL ratio (1.53), cleaved Caspase 3 (140%), cleaved PARP (148 %), Bnip3 (152 %) and TGFβ (150 %). OA treatment alone did not alter the expression of pro-apoptotic proteins, but was able to prevent TNF-α induced expression of pro-apoptotic proteins.

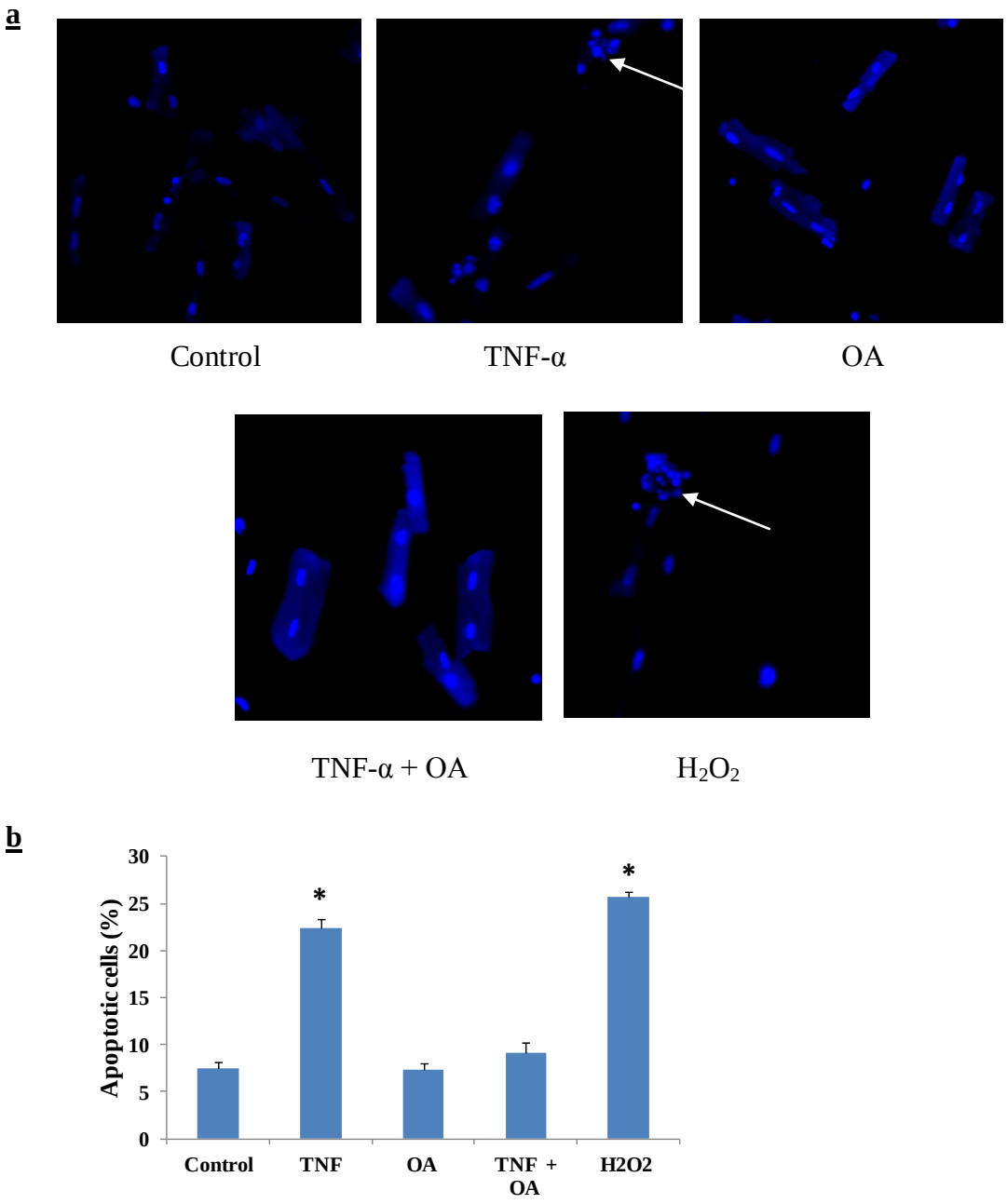


Fig 31: Apoptosis in isolated cardiac myocytes assessed by Hoechst 33258 staining after cells had been treated with TNF-α (10 ng/ml), OA (50 µM), and TNF-α +OA for 4 hrs. H_2O_2 (25 µM) was used as a positive control. Nuclear fragmentation in panel a is marked by white arrows. Panel b represents quantitative analysis of apoptotic cells. N=5, * P<0.05, significantly different from control.

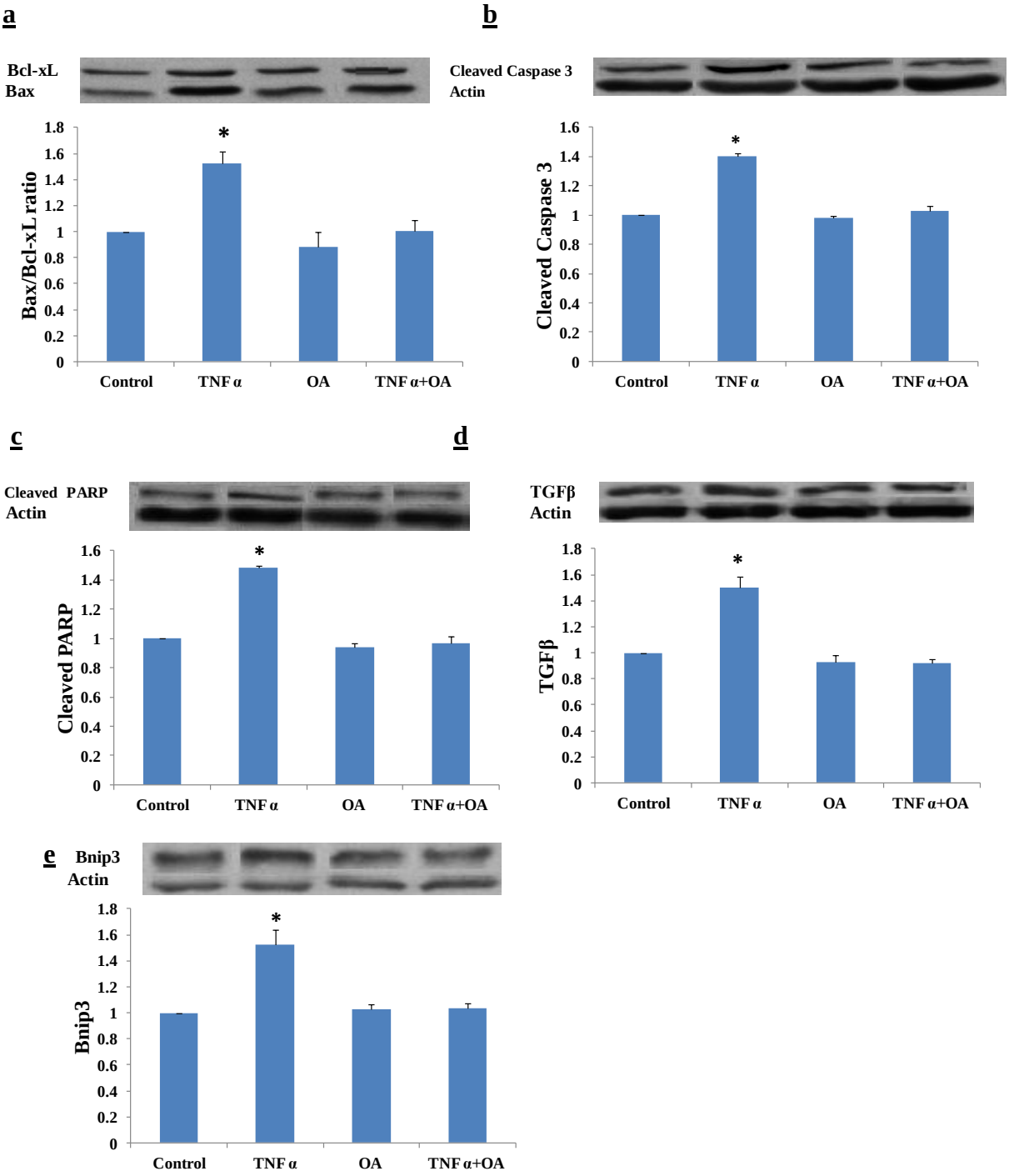


Fig 32: Western blot analysis of the effects of treatment with TNF-α (10 ng/ml), OA (50 µM), and TNF-α +OA for 4 hrs in adult rat cardiac myocytes on: a) Pro-apoptotic Bax and anti-apoptotic Bcl-xL protein expression; b) Cleaved Caspase 3; c) Cleaved PARP; d) TGF β expression; and e) Bnip3 expression. All expressions of proteins were assessed using arbitrary units. N=5, * P<0.05, significantly different from control.

VII. DISCUSSION

It is now known that most chronic medical conditions such as diabetes, atherosclerosis, obesity and cardiovascular diseases, including myocardial infarction, may involve dysregulation of the inflammatory processes (Kaur et al. 2006; Verma et al. 2012; Ahmad et al. 2012; Baldzizhar et al. 2012). Since olive oil is known to have anti-inflammatory properties (Lucas et al. 2011), the goal of my study was to investigate the beneficial effects of olive oil in the pathogenesis of heart failure subsequent to myocardial infarction (MI). The hypothesis of this study was that olive oil and its major component oleic acid (OA) will protect against oxidative stress and remodeling of the heart subsequent to myocardial infarction in rats by mitigating the deleterious effects of TNF-α on cardiomyocytes. The approach was to first examine the effects of olive oil in the whole animal subsequent to coronary artery ligation. To test the hypothesis, we examined the effects of OA a major component of olive oil, in isolated cardiomyocytes challenged with TNF-α. The latter is one of the most important inflammatory cytokines released by cardiomyocytes due to injury or subsequent to MI and it is implicated in the pathogenesis of heart failure (Torre-Amione et al. 1996; Kubota et al. 1997; Kubota et al. 1997; Bryant et al. 1998; Torre-Amione et al. 1999).

The *in vivo* studies in rats clearly showed the beneficial effects of olive oil in mitigating the heart failure subsequent to coronary artery ligation. The special diet supplemented

with olive oil was protective against MI induced myocardial dysfunction and myocardial increases in TNF-α, oxidative stress, apoptosis, and fibrosis. The data also showed that olive oil had a beneficial effect on blood pressure and improved heart function. *In vitro* studies showed that OA had a beneficial effect on cardiomyocytes challenged with TNF-α. OA reduced TNF-α induced oxidative stress, cell damage, cell death, and apoptosis.

We did not use the synthetic antioxidant TBHQ for the regular chow control, because this antioxidant is used at very low concentrations (0.02 %) and only for oils to prevent its rancidity and to maintain freshness of the prepared diet (American Institute of Nutrition 1980). Also, TBHQ was used in the corn oil, which was used as caloric intake control for olive oil, which did not show any benefit for the heart from TBHQ compared to regular chow. Furthermore, the use of TBHQ at concentration of 200 ppm (0.02 %) has been shown not to increase the oxidative stability of virgin olive oil and no synergistic effect of TBHQ as antioxidant was observed with the antioxidant property of virgin olive oil (Farhoosh et al. 2012).

The mortality rate for ligated animals was about 13 %, while most of the deaths were within 24 hours of surgery as a result of trauma, and the olive oil enriched diet had no effect on this early mortality. Animals in all groups gained weight and there was no significant difference between the sham and ligated animals for the entire duration of the study. Although there was no difference in the weight gain, the sham corn oil group showed the highest average weight (842 g) and this gain of weight was even higher than sham olive oil (681 g). However, the difference in the weight was not significant due to a large standard deviation. The lack of significant difference in the weights between sham and ligated animals has been reported previously (Khaper and Singal 2001). It is impor-

tant to note that the consumption of diet (olive oil or corn oil enriched) was comparable among all groups. This was also comparable to the regular chow consumption as reported earlier (Weinberg and Singal 1987). Furthermore, in studies done on mice investigating the beneficial effects of the olive oil (10 % in diet) on obesity, the food consumption and body weight were comparable to the mice fed control chow (Cintra et al. 2012) which is in accordance with our data.

TNF-α can induce cardiomyocyte injury, apoptosis, collagen deposition, fibrosis, and myocardial remodelling (Murtaza et al. 2008; Jobe et al. 2009; Kassiri et al. 2009). Cardiomyocytes are able to produce TNF-α under stress conditions such as ischemia, which may lead to cardiomyocyte dysfunction and cell death (Squadrito et al. 1993; Chen et al. 2010). In hypertensive rats, consumption of olive oil was able to decrease BP, interstitial fibrosis in heart tissues, and cardiomyocyte size and this was probably by the anti-inflammatory property of olive oil (Medeiros et al. 2005). In my *in vivo* study, coronary artery ligation increased both TNF-α and fibrosis, while olive oil was protective against cell damage and fibrosis, which could be result from a reduction in the up-regulation of TNF-α in response to infarction as well as TNF-α induced damage and fibrosis. In our *in vitro* study, OA was able to prevent TNF-α induced cell damage and death. In a previous study, OA was shown to prevent carbon tetrachloride induced liver fibrosis (Tanaka et al. 2009). OA was also able to suppress expression of adhesion molecules VCAM-1 and ICAM-1 induced by TNF-α (Sanadgol et al. 2010). Moreover, OA was able to suppress the inhibitory effect of TNF-α on insulin production in pancreatic cells and preserved its viability of these cells against TNF-α induced cell death (Vassiliou et al. 2009). Nitrated oleic acid, which occurs due to stress, was able to prevent inflammatory and atherogenic

response stimulated by TNF-α in endothelial cells (Hwang et al. 2009). It has been documented that TGFβ has a role in the formation of fibrosis in heart tissue (Rosin et al. 2012), which is consistent with our study, in which olive oil was able to reduce TGFβ, which may reduced the fibrosis formation in the heart tissues.

At concentrations lower than 200 μM, OA has been shown to protect against stearic acid-induced cardiomyocyte cell death and lipotoxicity in cardiomyocytes isolated from chick embryo hearts (Rabkin et al. 2009). OA was able to protect isolated cardiomyocytes from ischemic injury induced cell death via protein kinase C epsilon activation (Mackay et al. 2001). In another study, OA was able to restore cardiomyocytes and mitochondrial function due to ischemia, at a faster rate and more effectively than Elaidic acid, a trans isomer of OA (Tissier et al. 2005; Sanadgol et al. 2010). These studies underscore the importance of the cis configuration of the double bond in OA for the protection of cardiomyocytes. Animals fed on oleate-enriched diet showed a decrease in their systemic and adipose tissue inflammatory cytokines (TNF-α and IL- 1β) (Tardif et al. 2011).

We examined total cholesterol, HDL, and TG in all groups at 4 and 16 weeks PMI. The only change seen at 4 weeks was an increase in HDL in the ligated olive oil and the ligated corn oil groups. In contrast at 16 weeks PMI, only the corn oil groups, regardless of coronary artery ligation, showed highly significant increases in total cholesterol as well as HDL at 16 weeks PMI. The lack of increase in HDL in the 16 weeks PMI olive oil group as compared to the corn oil group may suggest that the beneficial effects of olive oil seen in this study may be not due to changes in HDL. It is known that in rats, LDL levels are extremely low and most of the total cholesterol is carried in HDL, and this may explain the resistance of rats to develop atherosclerosis under certain experimen-

tal conditions (Bhattacharya et al. 1986; Sonoyama et al. 1994). It is important to note that consumption of corn oil, which contains linoleic acid (LA), can promote endocannabinoid hyperactivity and thereby stimulate obesity (Alvheim et al. 2012) which has a positive correlation with total cholesterol (Rizk and Yousef 2012).

Although one week of olive oil-enriched diet consumption prior to surgery had no effect on blood pressure, there was a significant decrease in both systolic (SBP) and diastolic blood pressures (DBP) after 3 weeks PMI and 4 weeks of diet consumption as compared to regular chow and corn oil groups with or without coronary ligation. The drop in SBP (15.9%) and DBP (19.6%) was significant as compared to the regular chow and corn oil. This result is in accordance with the previously published work where virgin olive oil given orally to rats decreased SBP significantly (Teres et al. 2008). In the same study, OA also showed similar effects on blood pressure, suggesting that this beneficial effect of olive oil may have been due to OA. The specificity of the effect due to OA, was further studied using elaidic acid, a trans isomer of OA, which was not able to decrease SBP or DBP (Teres et al. 2008). A diet rich in OA from olive oil was also shown to have beneficial effects on blood pressure in hypertensive patients (Alemany et al. 2004). Other studies also showed this hypotensive property of OA in both humans and animals (Ruiz-Gutierrez et al. 1996; Zhang et al. 2010).

The mechanism of this hypotensive property of OA is suggested to be due to its effect on membrane lipids and G- proteins that control adenylyl cyclase and phospholipase C activity (Yang et al. 2005; Teres et al. 2008). Another mechanism of lowering of blood pressure by olive oil was suggested to be through the blockade of calcium channels (Gilani et al. 2005). A synthetic derivative of OA- 2-hydroxyoleic acid (2-OHOA), is capa-

ble of lowering blood pressure via increasing cAMP production in response to Gαs activation (Alemany et al. 2004). Furthermore, reducing blood pressure even by 5 mm Hg was able to reduce the risk of stroke, the risk of ischemic heart disease and the development of myocardial infarction and heart failure (Law et al. 2003). All these studies confirm that the hypotensive property of virgin olive oil is most likely due to OA.

Myocardial infarction is associated with conduction system abnormalities including arrhythmias (Abramson 1964; Lisowska et al. 2011). Our results demonstrated that all ligated groups including ligated olive oil had comparable conduction system abnormalities. Both olive oil and corn oil treatments were not able to reduce these conduction system abnormalities (McLennan 1993; Isensee and Jacob1994; Lau et al. 2011). This may mean that the beneficial effects of olive oil seen in this study may not involve any improvement in conduction system abnormalities.

In the ligated regular chow and ligated corn oil groups, the left ventricular ejection fraction (LVEF) at 3 days, 2 weeks, 4 weeks and 16 weeks PMI showed a progressive decline. Olive oil significantly improved the function of the ligated hearts, particularly at 16 weeks PMI. Tissue Doppler Imaging (TDI) parameter of the anterior wall was significantly decreased in the ligated regular chow as well as the ligated corn oil group, but the ligated olive oil group showed a sustained TDI parameter of the anterior wall. Left ventricular internal dimension-diastole (LVIDd) deteriorated in the ligated regular chow as well as the ligated corn oil, while ligated olive oil showed significant improvement in this parameter. It is important to note that contractile function was only improved in the ligated olive oil group compared to other ligated groups.

One possible explanation for the depressed heart function is the elaboration of TNF-α after MI which may lead to impairment of heart contractility (Hegewisch et al. 1990), and in our study olive oil was able to decrease TNF-α expression post-MI. One of the most important cytokines released after MI is TNF-α, which may play a critical role in cardiac remodeling and heart failure (Nian et al. 2004). It was demonstrated previously that TNF-α decreased left ventricular ejection fraction in humans (Suffredini et al. 1989), dogs (Natanson et al. 1989) and in rats (Li et al. 2010). Depression and dilation of the heart due to TNF-α could be due to the production of nitric oxide (Finkel et al. 1992) or through a decrease in intracellular calcium and the Ca^{2+} transients (Kapadia et al. 1995; Duncan et al. 2007). The infusion of TNF-α in carcinoma patients caused a depression of the heart function leading to cardiomyopathy (Hegewisch et al. 1990). In another study, olive oil consumption was shown to decrease TNF-α receptors in patients with a high risk for cardiovascular diseases (Urpi-Sarda et al. 2012) which could be another explanation for the protective role of olive oil. Intraperitoneal injection of TNF-α in mice depressed contractile force of the diaphragm via the activation of TNF- α receptor 1 (TNFR1) and an increase in oxidative stress at the myofibril level (Hardin et al. 2008). A pre-treatment with Trolox, a potent antioxidant, was able to restore contractile function in this muscle. In this study, olive oil was able to decrease oxidative stress by decreasing lipid peroxidation and maintaining glutathione level, thus restoring contractile function. Furthermore, heart function improvement could be due to the positive inotropic effect of OA in olive oil, which is suggested to normalize the depressants of heart contractility (Pi and Walker 2000; Loewi 1955).

Some of the beneficial effects of olive oil with respect to improved cardiac function may also come from a reduction in TNF-α induced oxidative stress. TNF-α, which increased after MI, has been shown to generate reactive oxygen species as demonstrated in my study as well as by others (Kaur et al. 2006; Li et al. 2007; Dhingra et al. 2009; Liu et al. 2011; Al-Shudiefat et al. 2013). Such an increase in TNF-α induced ROS production may occur via altering the mitochondrial membrane potential by the impairment of membrane permeability transition pore (MPTP) adenosine nucleotide translocator as well as the voltage dependent anion channel (Mariappan et al. 2007). TNF-α induced oxidative stress in cardiomyocytes is mediated via increasing the production of mitochondrial and cytosolic ROS (Woo et al. 2000; Dhingra et al. 2007; Ghavami et al. 2009). Olive oil consumption in humans is reported to decrease oxidative stress markers in their plasma (Covas et al. 2006). Olive oil may prevent oxidative stress in the hearts of mice through upregulation of nuclear transcription related factor 2 (Nrf2)-dependent gene expression (Bayram et al. 2012), which induces anti-inflammatory and anti-oxidative outcomes (Sporn and Liby 2012). In contrast, corn oil consumption increased carbon tetrachloride-induced lipid peroxidation in rats (Fang and Lin 2008).

Our studies on isolated cardiomyocytes also showed that OA decreased TNF-α induced production of ROS, which was associated with reduced cell injury and apoptosis. It is well documented that TNF-α induced cardiomyocyte damage and death are mediated in part through an increase in oxidative stress (Kaur et al. 2006; Li et al. 2007; Dhingra et al. 2009; Liu et al. 2011; Al-Shudiefat et al. 2013). Treatment of umbilical vein endothelial cells with OA was able to blunt the TNF-α induced ROS production via scavenging of free radicals through OA (Massaro et al. 2002). It has been reported that mitochondrial

oxidative stress in umbilical vein endothelial cells was reduced by OA via the epidermal growth factor receptor mediated activation of glutathione peroxidase and the subsequent improved degradation of reactive oxygen species (Duval et al. 2002). It has been shown that pre-incubating hepatic HepG2 cells with OA reduced TNF-α cytotoxicity and increased superoxide dismutase activity (Damelin et al. 2007). TNF-α may also produce reactive oxygen species through the upregulation of NADPH oxidase subunits 2 and 4 (Nox2 and Nox4) (Moe et al. 2011). Ginseng, known to have anti-oxidant properties, was able to reduce TNF-α induced oxidative stress and cardiomyocyte death in rats via activating nuclear erythroid derived factor 2 (Nrf2)- an important endogenous transcription factor for restoring antioxidant reserve (Li et al. 2010). In total, this published work as well as my studies *in vivo* and cardiomyocytes suggest that olive oil and thus OA may act as antioxidants.

Another explanation for enhanced heart function in this study could be the ability of olive oil to decrease both systolic and diastolic blood pressures, thus reducing preload as well as afterload, which might improve cardiac pumping. In this regard, it is documented that metoprolol, a beta-blocker and anti-hypertensive drug, was able to preserve contractility of the heart in coronary microembolized rats, and this effect was also attributed to the reduced expression of TNF-α (Lu et al. 2011). It is reported that using a nonpeptide angiotensin-(1-7) analog AVE 0991, another antihypertensive drug, also led to protection against cardiac contractility dysfunction in rats after MI, by decreasing the expression of both TNF-α and TGFβ (Zeng et al. 2012). A similar mechanism may also be involved in our study as there was a significant reduction in both TNF-α and TGFβ in the ligated olive oil group. A long term clinical trial conducted over one year showed that olive oil

reduced TNF alpha receptors, IL-6 and intercellular adhesion molecule 1 (ICAM 1) (Urpi-Sarda et al. 2012). A randomized clinical pilot trial showed that extra-virgin olive oil reduced inflammation better than piroxicam gel, a drug used to treat the inflammatory disease osteoarthritis (Bohlooli et al. 2012). Olive oil also reduces hypothalamic inflammation and insulin resistance (Cintra et al. 2012).

Corn oil has a high percentage (59%) of polyunsaturated linoleic acid (LA) with a double bond on carbon number six (omega 6 fatty acid). In Jurkat cells, LA induced apoptosis and upregulated pro-inflammatory molecules like interleukin 8 (IL-8) and chemokine (C-C motif) ligand 11 (CCL11) (Ion et al. 2011). The ratio of omega 6 fatty acid (e.g LA in corn oil) to omega 3 (e.g alpha linoleic acid in fish oil or flax) is very important for the beneficial effect on the cardiovascular system. If the ratio is low, it is protective, and if the ratio is high it causes allergy, atheroma, thrombus, and inflammatory problems (Simopoulos 2002). Consuming less LA and more OA and alpha-LA is protective against cardiovascular events and deaths (de Lorgeril et al. 1994). Consumption of LA can promote platelet aggregation (Renaud 1990), which may lead to myocardial infarction (Elwood et al. 1990). Dietary LA induced inflammation in mice (Alvheim et al. 2012). Omega 6 fatty acids like LA increased apoptosis and its mediators in lymphocytes of mice spleen (Avula et al. 1999). Thus it is not surprising that corn oil was unable to offer any significant benefit to the ligated hearts in the present study.

Many studies have reported that both olive oil and OA reduce expression of several apoptotic proteins and increase anti-apoptotic Bcl 2 expression (de Vries et al. 1997; Dyntar et al. 2001; Harvey et al. 2010; Cintra et al. 2012). These reports are in agreement with my findings that both olive oil and OA reduce apoptotic protein expression. The

ability of olive oil to decrease the expression of these proteins may be due to the anti-inflammatory properties of OA (Borniquel et al. 2010; Lucas et al. 2011; Eidi et al. 2012). Caspase 3 and PARP cleavage, induced by stearic acid in human aortic endothelial cells (Harvey et al. 2010) as well as palmitic acid-induced cardiomyocytes apoptosis in rats were both reduced by OA (de Vries et al. 1997; Dyntar et al. 2001). In our study, we used H_2O_2 as a positive control to generate reactive oxygen species, which also resulted in an increase in both oxidative stress and apoptosis. These findings suggest that TNF-α induced detrimental effects may be mediated through an increase in oxidative stress. In our study, olive oil reduced the inflammatory cytokine TNF-α as well as TNF-α induced expression of pro-apoptotic proteins (Bax, cleaved Caspase 3, cleaved PARP, TGFβ, Bnip3, cytochrome C release from mitochondria). In contrast olive oil enhanced the expression of the anti-apoptotic protein Bcl-xl. A similar effect was seen with OA in isolated cardiomyocytes, which mitigated the TNF-α induced changes in pro-apoptotic proteins (Bax, cleaved Caspase 3, cleaved PARP), whereas Bcl-xL was promoted by OA.

Both JNK and p38 are reported to be upregulated by injury, while phosphorylation of ERK is considered to play a role in cell growth and cell repair (Chye et al. 2012; Esteras et al. 2012). In our study, olive oil increased the phosphorylation of ERK and decreased the phosphorylation of JNK and p38 in its respective ligated group, compared to the ligated regular chow and ligated corn oil groups. This deactivation of JNK and p38 may play a protective role against the promotion of apoptosis (Chye et al. 2012). Furthermore, it has also been reported that the inhibition of p38 MAP kinase could suppress the expression of TNF-α and preserve LVEF in coronary microembolization induced myocardial contractile dysfunction in rats (Li et al. 2011).

Olive oil and / or OA has also been reported to offer protection in a variety of other conditions. Extra-virgin olive oil decreased Ras expression and upregulated the Raf/ERK cascade in a rat model of breast cancer (Solanas et al. 2010). Oleate can decrease JNK induced oxidative stress, apoptosis and Caspase 3 cleavage in skeletal muscle cells (Yuzefovych et al. 2010). Olive oil and OA also have been reported to increase the activity of many antioxidants in the body including catalase and superoxide dismutase (Faine et al. 2006).

Although the focus of my study was OA, which is the major component (about 80%) of extra-virgin olive oil, there are some reports showing beneficial effects of some of the other minor components present in olive oil. For example, Oleuropein, a polyphenolic component of olive oil decreased the expression of inflammatory cytokines TNF-α and interleukin-1β (IL-1β) in mice (Impellizzeri et al. 2012). Oleuropein decreased the histological damage of spinal cord injury in mice, and reduced oxidative stress and inflammatory cytokines (Bello et al. 2006; Cumaoglu et al. 2011; Impellizzeri et al. 2012). Administration (i.p.) of oleuropein aglycon in mice decreased the expression of inducible nitric oxide synthase, Bax, Caspase 3, PARP, and apoptosis (Impellizzeri et al. 2012). Hyrdoxytyrosol derived from olive oil protected against dietary acrylamide-induced activity of Caspase 3, JNK activation and H_2O_2 induced oxidative stress (Rodriguez-Ramiro et al. 2011; Zrelli et al. 2011). Thus, in addition to OA, these other components may also hold therapeutic potential.

VIII. CONCLUSIONS

Myocardial infarction subsequent to coronary artery ligation resulted in depressed cardiac function. There was significantly increased myocardial TNF-α post MI. This change was associated with an increase in oxidative stress, lipid hydroperoxides, apoptosis, cell damage and fibrosis. There was also a decrease in the glutathione levels and anti-apoptotic protein Bcl-xL. All these changes led to cardiomyocyte remodeling and deteriorated cardiac function, and this process was prevented by olive oil. Based on the data presented in this study, the suggested sequence of events is outlined in Figure 33. Consumption of olive oil lowered blood pressure, decreased TNF-α, and mitigated other TNF-α induced sub-cellular changes in the ligated rats. Corn oil, used as a control to compensate for caloric intake, failed to offer protection, suggesting that olive oil protection against heart failure was not due to an increase in caloric intake.

It is suggested that olive oil protected against TNF-α induced remodelling through its anti-inflammatory and anti-oxidant properties, either directly by decreasing the production and expression of TNF-α, or indirectly by mitigating TNF-α induced oxidative stress, lipid peroxidation, apoptosis, cell damage, fibrosis, and cardiac depression. Furthermore, olive oil improved cardiac function and restored it completely in the ligated animals. This recovery by pathway can be explained other than olive oil mediated protection against TNF-α and its deleterious effects such as: lowering blood pressure, enhancement of heart

contractility by its positive inotropic effects, both of which are present in OA, or by preserving cardiomyocytes from loss through decreasing TGFβ expression, which plays an important role in the formation of fibrosis.

Since TNF-α plays a central role in the above listed changes and in the progression of heart failure subsequent to MI, and since olive oil offered protection, we examined the protective role of OA, a major component in olive in isolated adult rat cardiomyocytes challenged with TNF-α. Results showed that OA was able to mitigate TNF-α induced oxidative stress, cell death, apoptosis, and cell damage. Thus this provides clear supporting evidence at the sub-cellular level that the protective role of olive oil against the progression of heart failure may be due to its active ingredient OA. It is suggested that these effects of olive oil are due to anti-inflammatory, antioxidant, anti-hypertensive, and inotropic properties, all of which are present in OA.

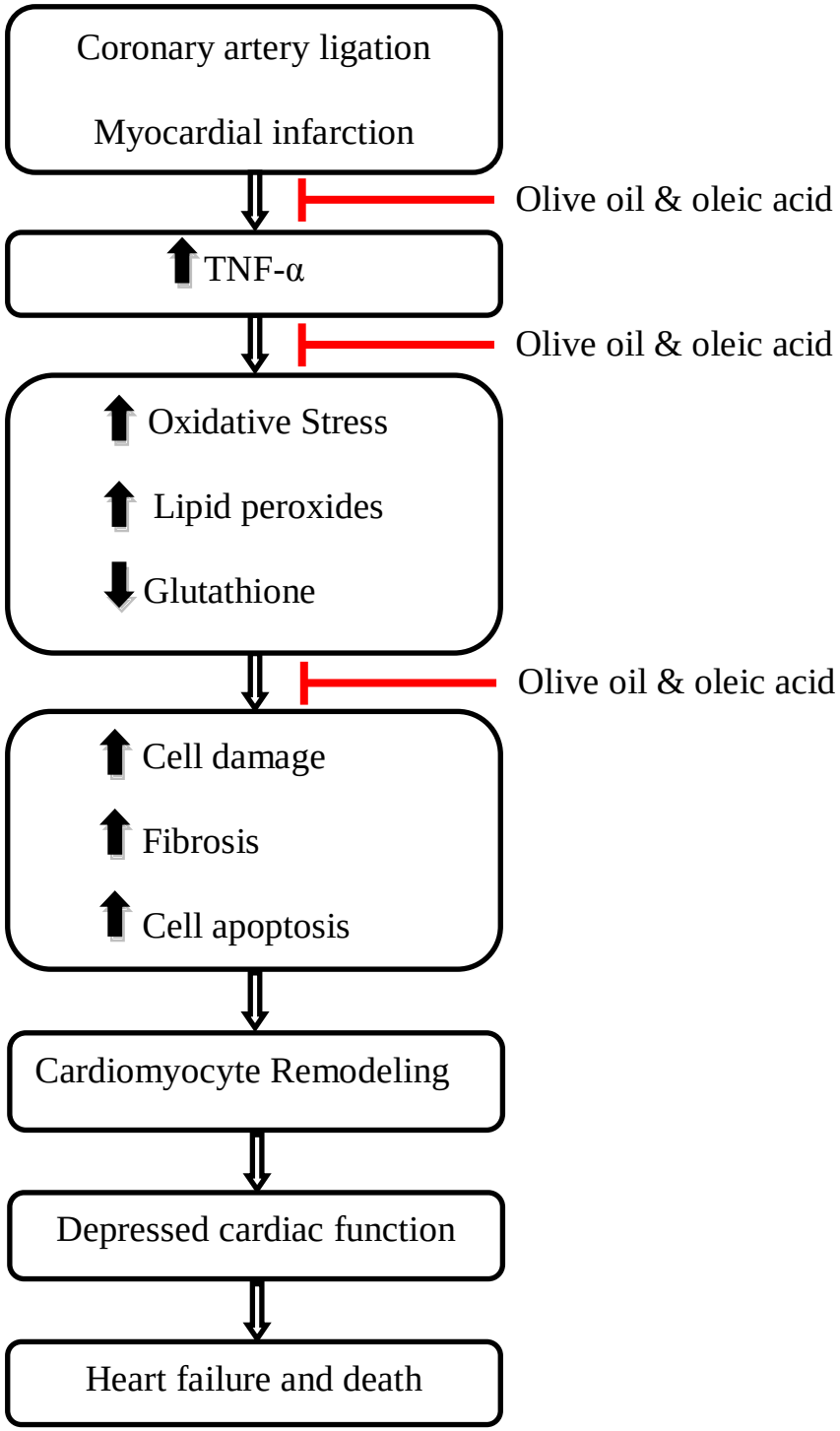


Fig 33: Schematic representation of possible role of tumor necrosis factor alpha (TNF-α) in the pathogenesis of cardiomyocyte dysfunction and heart failure and its mitigation by olive oil and oleic acid.

IX. FUTURE DIRECTIONS

Although my study has provided very clear evidence that oleic acid in olive oil (80% of extra-virgin olive oil) had beneficial effects in mitigating the progression of heart failure subsequent to MI, the beneficial roles of other active ingredients present in olive oil besides OA needs to be explored. In this context, the minor components that compose 20 % of olive oil need to be examined. Further studies to elucidate the molecular mechanism of oleic acid protection against TNF-α induced oxidative stress are also warranted. Although our study showed that TNF-α increased oxidative stress, apoptosis, cell damage and fibrosis, the role of other cytokines such as IL-10, IL-1β, IFN-β needs to be studied. There is a real need to study the applied values of the findings reported in this thesis by clinical trials using olive oil enriched diet. Even though clinical trials are very expensive, potentially costing up to millions of dollars, the applied value of the data generated in the thesis cannot be stated without such population studies.

X. REFERENCES

1. Report of the American Institute of Nurtition ad hoc committee on standards for nutritional studies. J Nutr. 1977 Jul;107(7):1340-8.

2. Abramson H. Vectorcardiographic abnormalities simulating myocardial infarction in the normal heart. Can Med Assoc J. 1964 Apr 11;90:903-9.

3. Aceto JF, Baker KM. Sar1]angiotensin II receptor-mediated stimulation of protein synthesis in chick heart cells. Am J Physiol. 1990 Mar;258(3 Pt 2):H806-13.

4. Aderka D, Engelmann H, Maor Y, Brakebusch C, Wallach D. Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J Exp Med. 1992 Feb 1;175(2):323-9.

5. Aggarwal BB, Kohr WJ, Hass PE, Moffat B, Spencer SA, Henzel WJ, et al. Human tumor necrosis factor. production, purification, and characterization. J Biol Chem. 1985 Feb 25;260(4):2345-54.

6. Agostini C, Zambello R, Trentin L, Cerutti A, Enthammer C, Facco M, et al. Expression of TNF receptors by T cells and membrane TNF-alpha by alveolar macrophages suggests a role for TNF-alpha in the regulation of the local immune responses in the lung of HIV-1-infected patients. J Immunol. 1995 Mar 15;154(6):2928-38.

7. Ahmad R, Al-Mass A, Atizado V, Al-Hubail A, Al-Ghimlas F, Al-Arouj M, et al. Elevated expression of the toll like receptors 2 and 4 in obese individuals: Its significance for obesity-induced inflammation. J Inflamm (Lond). 2012 Nov 28;9(1):48.

8. Ahuja KD, Pittaway JK, Ball MJ. Effects of olive oil and tomato lycopene combination on serum lycopene, lipid profile, and lipid oxidation. Nutrition. 2006 Mar;22(3):259-65.

9. Akassoglou K, Probert L, Kontogeorgos G, Kollias G. Astrocyte-specific but not neuron-specific transmembrane TNF triggers inflammation and degeneration in the central nervous system of transgenic mice. J Immunol. 1997 Jan 1;158(1):438-45.

10. Alemany R, Teres S, Baamonde C, Benet M, Vogler O, Escriba PV. 2-hydroxyoleic acid: A new hypotensive molecule. Hypertension. 2004 Feb;43(2):249-54.

11. Allen LA, Felker GM, Pocock S, McMurray JJ, Pfeffer MA, Swedberg K, et al. Liver function abnormalities and outcome in patients with chronic heart failure: Data from the candesartan in heart failure: Assessment of reduction in mortality and morbidity (CHARM) program. Eur J Heart Fail. 2009 Feb;11(2):170-7.

12. Allman-Farinelli MA, Gomes K, Favaloro EJ, Petocz P. A diet rich in high-oleic-acid sunflower oil favorably alters low-density lipoprotein cholesterol, triglycerides, and factor VII coagulant activity. J Am Diet Assoc. 2005 Jul;105(7):1071-9.

13. Al-Shudiefat AA, Sharma AK, Bagchi AK, Dhingra S, Singal PK. Oleic acid mitigates TNF-alpha-induced oxidative stress in rat cardiomyocytes. Mol Cell Biochem. 2013 Jan;372(1-2):75-82.

14. Alvheim AR, Torstensen BE, Lin YH, Lillefosse HH, Lock EJ, Madsen L, et al. Dietary linoleic acid elevates endogenous 2-arachidonoylglycerol and anandamide in atlantic salmon (salmo salar L.) and mice, and induces weight gain and inflammation in mice. Br J Nutr. 2012 Aug 10:1-10.

15. American Institute of Nutrition. Second report of the ad hoc committee on standards for nutritional studies. J Nutr. 1980;110(8):1726.

16. Anand IS, Sharma PL, Chakravarti RN, Wahi PL. Experimental myocardial infarction in rhesus monkeys. verapamil pretreatment in the reduction of infarct size. Adv Myocardiol. 1980;2:425-33.

17. Anker SD, Coats AJ. How to RECOVER from RENAISSANCE? the significance of the results of RECOVER, RENAISSANCE, RENEWAL and ATTACH. Int J Cardiol. 2002 Dec;86(2-3):123-30.

18. Aukrust P, Yndestad A, Ueland T, Damas JK, Froland SS, Gullestad L. The role of intravenous immunoglobulin in the treatment of chronic heart failure. Int J Cardiol. 2006 Sep 10;112(1):40-5.

19. Avula CP, Zaman AK, Lawrence R, Fernandes G. Induction of apoptosis and apoptotic mediators in Balb/C splenic lymphocytes by dietary n-3 and n-6 fatty acids. Lipids. 1999 Sep;34(9):921-7.

20. Baker KM, Chernin MI, Wixson SK, Aceto JF. Renin-angiotensin system involvement in pressure-overload cardiac hypertrophy in rats. Am J Physiol. 1990 Aug;259(2 Pt 2):H324-32.

21. Baker MA, Cerniglia GJ, Zaman A. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. Anal Biochem. 1990 Nov 1;190(2):360-5.

22. Baldzizhar R, Fedorchuk C, Jha M, Rathinam C, Henegariu O, Czyzyk J. Anti-serpin antibody-mediated regulation of proteases in autoimmune diabetes. J Biol Chem. 2012 Nov 29.

23. Bayeva M, Gheorghiade M, Ardehali H. Mitochondria as a therapeutic target in heart failure. J Am Coll Cardiol. 2013 Feb 12;61(6):599-610.

24. Bayram B, Ozcelik B, Grimm S, Roeder T, Schrader C, Ernst IM, et al. A diet rich in olive oil phenolics reduces oxidative stress in the heart of SAMP8 mice by induction of Nrf2-dependent gene expression. Rejuvenation Res. 2012 Feb;15(1):71-81.

25. Beauchamp GK, Keast RS, Morel D, Lin J, Pika J, Han Q, et al. Phytochemistry: Ibuprofen-like activity in extra-virgin olive oil. Nature. 2005 Sep 1;437(7055):45-6.

26. Bello RI, Gomez-Diaz C, Buron MI, Navas P, Villalba JM. Differential regulation of hepatic apoptotic pathways by dietary olive and sunflower oils in the aging rat. Exp Gerontol. 2006 Nov;41(11):1174-84.

27. Beltrami CA, Finato N, Rocco M, Feruglio GA, Puricelli C, Cigola E, et al. The cellular basis of dilated cardiomyopathy in humans. J Mol Cell Cardiol. 1995 Jan;27(1):291-305.

28. Bermudez B, Lopez S, Ortega A, Varela LM, Pacheco YM, Abia R, et al. Oleic acid in olive oil: From a metabolic framework toward a clinical perspective. Curr Pharm Des. 2011;17(8):831-43.

29. Bester D, Esterhuyse AJ, Truter EJ, van Rooyen J. Cardiovascular effects of edible oils: A comparison between four popular edible oils. Nutr Res Rev. 2010 Dec;23(2):334-48.

30. Beutler B, Greenwald D, Hulmes JD, Chang M, Pan YC, Mathison J, et al. Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. Nature. 1985 Aug 8-14;316(6028):552-4.

31. Bhattacharya S, Balasubramaniam S, Simons LA. Regulation of low-density-lipoprotein metabolism in the rat. Biochem J. 1986 Mar 1;234(2):493-6.

32. Black RA. Tumor necrosis factor-alpha converting enzyme. Int J Biochem Cell Biol. 2002 Jan;34(1):1-5.

33. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, et al. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature. 1997 Feb 20;385(6618):729-33.

34. Blick M, Sherwin SA, Rosenblum M, Gutterman J. Phase I study of recombinant tumor necrosis factor in cancer patients. Cancer Res. 1987 Jun 1;47(11):2986-9.

35. Bohlooli S, Jastan M, Nakhostin-Roohi B, Mohammadi S, Baghaei Z. A pilot double-blinded, randomized, clinical trial of topical virgin olive oil versus piroxicam gel in osteoarthritis of the knee. J Clin Rheumatol. 2012 Mar;18(2):99-101.

36. Bolt CL, Baur L, Stoffers J, Lenderink T, Winkens R. Novel strategies for the detection of systolic and diastolic heart failure. Curr Cardiol Rev. 2009 May;5(2):112-8.

37. Bondia-Pons I, Schroder H, Covas MI, Castellote AI, Kaikkonen J, Poulsen HE, et al. Moderate consumption of olive oil by healthy european men reduces systolic blood pressure in non-mediterranean participants. J Nutr. 2007 Jan;137(1):84-7.

38. Borchert GH, Giggey M, Kolar F, Wong TM, Backx PH, Escriba PV. 2-hydroxyoleic acid affects cardiomyocyte [Ca2+]i transient and contractility in a region-dependent manner. Am J Physiol Heart Circ Physiol. 2008 Apr;294(4):H1948-55.

39. Borniquel S, Jansson EA, Cole MP, Freeman BA, Lundberg JO. Nitrated oleic acid up-regulates PPARgamma and attenuates experimental inflammatory bowel disease. Free Radic Biol Med. 2010 Feb 15;48(4):499-505.

40. Boskou D. Olive oil. World Rev Nutr Diet. 2000;87:56-77.

41. Bozkurt B, Kribbs SB, Clubb FJ,Jr, Michael LH, Didenko VV, Hornsby PJ, et al. Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats. Circulation. 1998 Apr 14;97(14):1382-91.

42. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976 May 7;72:248-54.

43. Brasil D, Temsah RM, Kumar K, Kumamoto H, Takeda N, Dhalla NS. Blockade of 5-HT(2A) receptors by sarpogrelate protects the heart against myocardial infarction in rats. J Cardiovasc Pharmacol Ther. 2002 Jan;7(1):53-9.

44. Brown L, Fenning A, Chan V, Loch D, Wilson K, Anderson B, et al. Echocardiographic assessment of cardiac structure and function in rats. Heart Lung Circ. 2002;11(3):167-73.

45. Bruge F, Bacchetti T, Principi F, Scarpa ES, Littarru GP, Tiano L. Olive oil supplemented with coenzyme Q(10): Effect on plasma and lipoprotein oxidative status. Biofactors. 2012 May-Jun;38(3):249-56.

46. Bryant D, Becker L, Richardson J, Shelton J, Franco F, Peshock R, et al. Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor-alpha. Circulation. 1998 Apr 14;97(14):1375-81.

47. Burton GW, Foster DO, Perly B, Slater TF, Smith IC, Ingold KU. Biological antioxidants. Philos Trans R Soc Lond B Biol Sci. 1985 Dec 17;311(1152):565-78.

48. **What is heart failure?** [Internet].; 2013. Available from: http://www.chfn.ca/what-is-heart-failure.

49. Carabello BA, Crawford FA,Jr. Valvular heart disease. N Engl J Med. 1997 Jul 3;337(1):32-41.

50. Carrillo C, Cavia Mdel M, Alonso-Torre S. Role of oleic acid in immune system; mechanism of action; a review. Nutr Hosp. 2012 Jul-Aug;27(4):978-90.

51. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A. 1975 Sep;72(9):3666-70.

52. Chan FK, Chun HJ, Zheng L, Siegel RM, Bui KL, Lenardo MJ. A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. Science. 2000 Jun 30;288(5475):2351-4.

53. Chanani NK, Cowan DB, Takeuchi K, Poutias DN, Garcia LM, del Nido PJ, et al. Differential effects of amrinone and milrinone upon myocardial inflammatory signaling. Circulation. 2002 Sep 24;106(12 Suppl 1):I284-9.

54. Chang DJ, Ringold GM, Heller RA. Cell killing and induction of manganous superoxide dismutase by tumor necrosis factor-alpha is mediated by lipoxygenase metabolites of arachidonic acid. Biochem Biophys Res Commun. 1992 Oct 30;188(2):538-46.

55. Charach G, Rabinovich A, Argov O, Weintraub M, Charach L, Ayzenberg O, et al. Anti-oxidized low-density lipoprotein antibodies in chronic heart failure. World J Cardiol. 2012 Nov 26;4(11):302-8.

56. Chen Y, Pat B, Zheng J, Cain L, Powell P, Shi K, et al. Tumor necrosis factor-alpha produced in cardiomyocytes mediates a predominant myocardial inflammatory response to stretch in early volume overload. J Mol Cell Cardiol. 2010 Jul;49(1):70-8.

57. Chrysant SG, Chrysant GS. Effectiveness of lowering blood pressure to prevent stroke versus to prevent coronary events. Am J Cardiol. 2010 Sep 15;106(6):825-9.

58. Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT, Anti-TNF Therapy Against Congestive Heart Failure Investigators. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: Results of the anti-TNF therapy against congestive heart failure (ATTACH) trial. Circulation. 2003 Jul 1;107(25):3133-40.

59. Chye SM, Tiong YL, Yip WK, Koh RY, Len YW, Seow HF, et al. Apoptosis induced by para-phenylenediamine involves formation of ROS and activation of p38 and JNK in chang liver cells. Environ Toxicol. 2012 Nov 22.

60. Cintra DE, Ropelle ER, Moraes JC, Pauli JR, Morari J, Souza CT, et al. Unsaturated fatty acids revert diet-induced hypothalamic inflammation in obesity. PLoS One. 2012;7(1):e30571.

61. Cleutjens JP, Kandala JC, Guarda E, Guntaka RV, Weber KT. Regulation of collagen degradation in the rat myocardium after infarction. J Mol Cell Cardiol. 1995 Jun;27(6):1281-92.

62. Colette C, Percheron C, Pares-Herbute N, Michel F, Pham TC, Brillant L, et al. Ex-changing carbohydrates for monounsaturated fats in energy-restricted diets: Effects on metabolic profile and other cardiovascular risk factors. Int J Obes Relat Metab Disord. 2003 Jun;27(6):648-56.

63. Coll T, Eyre E, Rodriguez-Calvo R, Palomer X, Sanchez RM, Merlos M, et al. Oleate reverses palmitate-induced insulin resistance and inflammation in skeletal muscle cells. J Biol Chem. 2008 Apr 25;283(17):11107-16.

64. Covas MI, Nyyssonen K, Poulsen HE, Kaikkonen J, Zunft HJ, Kiesewetter H, et al. The effect of polyphenols in olive oil on heart disease risk factors: A randomized trial. Ann Intern Med. 2006 Sep 5;145(5):333-41.

65. Coviello J. Heart failure: An update. Home Healthc Nurse. 2009 Jun;27(6):354,61; quiz 362-3.

66. Culleton BF, Larson MG, Wilson PW, Evans JC, Parfrey PS, Levy D. Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. Kidney Int. 1999 Dec;56(6):2214-9.

67. Cumaoglu A, Ari N, Kartal M, Karasu C. Polyphenolic extracts from olea europea L. protect against cytokine-induced beta-cell damage through maintenance of redox homeo-stasis. Rejuvenation Res. 2011 Jun;14(3):325-34.

68. Cunningham KS, Gotlieb AI. The role of shear stress in the pathogenesis of athero-sclerosis. Lab Invest. 2005 Jan;85(1):9-23.

69. Dai W, Chen H, Jiang J, Kong W, Wang Y. Silencing MR-1 attenuates inflammatory damage in mice heart induced by AngII. Biochem Biophys Res Commun. 2010 Jan 15;391(3):1573-8.

70. Damelin LH, Coward S, Kirwan M, Collins P, Selden C, Hodgson HJ. Fat-loaded HepG2 spheroids exhibit enhanced protection from pro-oxidant and cytokine induced damage. J Cell Biochem. 2007 Jun 1;101(3):723-34.

71. Damman K, van Deursen VM, Navis G, Voors AA, van Veldhuisen DJ, Hillege HL. Increased central venous pressure is associated with impaired renal function and mortality in a broad spectrum of patients with cardiovascular disease. J Am Coll Cardiol. 2009 Feb 17;53(7):582-8.

72. Davila DF, Nunez TJ, Odreman R, de Davila CA. Mechanisms of neurohormonal activation in chronic congestive heart failure: Pathophysiology and therapeutic implications. Int J Cardiol. 2005 Jun 8;101(3):343-6.

73. De Biase L, Pignatelli P, Lenti L, Tocci G, Piccioni F, Riondino S, et al. Enhanced TNF alpha and oxidative stress in patients with heart failure: Effect of TNF alpha on platelet O2- production. Thromb Haemost. 2003 Aug;90(2):317-25.

74. De Caterina R, Liao JK, Libby P. Fatty acid modulation of endothelial activation. Am J Clin Nutr. 2000 Jan;71(1 Suppl):213S-23S.

75. de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet. 1994 Jun 11;343(8911):1454-9.

76. de Lorgeril M, Salen P. The mediterranean-style diet for the prevention of cardiovascular diseases. Public Health Nutr. 2006 Feb;9(1A):118-23.

77. de Lorgeril M, Salen P, Martin JL, Monjaud I, Boucher P, Mamelle N. Mediterranean dietary pattern in a randomized trial: Prolonged survival and possible reduced cancer rate. Arch Intern Med. 1998 Jun 8;158(11):1181-7.

78. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: Final report of the lyon diet heart study. Circulation. 1999 Feb 16;99(6):779-85.

79. de Vries JE, Vork MM, Roemen TH, de Jong YF, Cleutjens JP, van der Vusse GJ, et al. Saturated but not mono-unsaturated fatty acids induce apoptotic cell death in neonatal rat ventricular myocytes. J Lipid Res. 1997 Jul;38(7):1384-94.

80. Deidier A (1725).
Dissertation medecinal et chirurgical sur les tumeurs, paris. [dissertation].

81. Delgado-Lista J, Lopez-Miranda J, Cortes B, Perez-Martinez P, Lozano A, Gomez-Luna R, et al. Chronic dietary fat intake modifies the postprandial response of hemostatic markers to a single fatty test meal. Am J Clin Nutr. 2008 Feb;87(2):317-22.

82. Dhingra S, Sharma AK, Arora RC, Slezak J, Singal PK. IL-10 attenuates TNF-alpha-induced NF kappaB pathway activation and cardiomyocyte apoptosis. Cardiovasc Res. 2009 Apr 1;82(1):59-66.

83. Dhingra S, Sharma AK, Singla DK, Singal PK. p38 and ERK1/2 MAPKs mediate the interplay of TNF-alpha and IL-10 in regulating oxidative stress and cardiac myocyte apoptosis. Am J Physiol Heart Circ Physiol. 2007 Dec;293(6):H3524-31.

84. Duan H, Dixit VM. RAIDD is a new 'death' adaptor molecule. Nature. 1997 Jan 2;385(6611):86-9.

85. Duncan DJ, Hopkins PM, Harrison SM. Negative inotropic effects of tumour necrosis factor-alpha and interleukin-1beta are ameliorated by alfentanil in rat ventricular myocytes. Br J Pharmacol. 2007 Mar;150(6):720-6.

86. Duval C, Auge N, Frisach MF, Casteilla L, Salvayre R, Negre-Salvayre A. Mitochondrial oxidative stress is modulated by oleic acid via an epidermal growth factor receptor-dependent activation of glutathione peroxidase. Biochem J. 2002 Nov 1;367(Pt 3):889-94.

87. Dyntar D, Eppenberger-Eberhardt M, Maedler K, Pruschy M, Eppenberger HM, Spinas GA, et al. Glucose and palmitic acid induce degeneration of myofibrils and modulate apoptosis in rat adult cardiomyocytes. Diabetes. 2001 Sep;50(9):2105-13.

88. Ebaid GM, Seiva FR, Rocha KK, Souza GA, Novelli EL. Effects of olive oil and its minor phenolic constituents on obesity-induced cardiac metabolic changes. Nutr J. 2010 Oct 19;9:46.

89. Eichenholz PW, Eichacker PQ, Hoffman WD, Banks SM, Parrillo JE, Danner RL, et al. Tumor necrosis factor challenges in canines: Patterns of cardiovascular dysfunction. Am J Physiol. 1992 Sep;263(3 Pt 2):H668-75.

90. Eichhorn EJ, Bristow MR. Medical therapy can improve the biological properties of the chronically failing heart. A new era in the treatment of heart failure. Circulation. 1996 Nov 1;94(9):2285-96.

91. Eidi A, Moghadam-kia S, Moghadam JZ, Eidi M, Rezazadeh S. Antinociceptive and anti-inflammatory effects of olive oil (olea europeae L.) in mice. Pharm Biol. 2012 Mar;50(3):332-7.

92. Eliasson B. Cigarette smoking and diabetes. Prog Cardiovasc Dis. 2003 Mar-Apr;45(5):405-13.

93. Elwood PC, Beswick AD, Sharp DS, Yarnell JW, Rogers S, Renaud S. Whole blood impedance platelet aggregometry and ischemic heart disease. the caerphilly collaborative heart disease study. Arteriosclerosis. 1990 Nov-Dec;10(6):1032-6.

94. Enari M, Talanian RV, Wong WW, Nagata S. Sequential activation of ICE-like and CPP32-like proteases during fas-mediated apoptosis. Nature. 1996 Apr 25;380(6576):723-6.

95. Esteras N, Alquezar C, Bermejo-Pareja F, Bialopiotrowicz E, Wojda U, Martin-Requero A. Downregulation of extracellular signal-regulated kinase 1/2 activity by calmodulin KII modulates p21(Cip1) levels and survival of immortalized lymphocytes from alzheimer's disease patients. Neurobiol Aging. 2012 Nov 12.

96. Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ruiz-Gutierrez V, Covas MI, et al. Effects of a mediterranean-style diet on cardiovascular risk factors: A randomized trial. Ann Intern Med. 2006 Jul 4;145(1):1-11.

97. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, et al. Primary prevention of cardiovascular disease with a mediterranean diet. N Engl J Med. 2013 Apr 4;368(14):1279-90.

98. Evangelista LS, Miller PS. Overweight and obesity in the context of heart failure: Implications for practice and future research. J Cardiovasc Nurs. 2006 Jan-Feb;21(1):27-33.

99. Faine LA, Rodrigues HG, Galhardi CM, Ebaid GM, Diniz YS, Padovani CR, et al. Effects of olive oil and its minor constituents on serum lipids, oxidative stress, and energy metabolism in cardiac muscle. Can J Physiol Pharmacol. 2006 Feb;84(2):239-45.

100. Fang HL, Lin WC. Lipid peroxidation products do not activate hepatic stellate cells. Toxicology. 2008 Nov 20;253(1-3):36-45.

101. Farhoosh R, Haddad Khodaparast MH, Sharif A, Zamani-Ghalehshahi A, Hoseini-Yazdi SZ. Oxidative stability of virgin olive oil as affected by the bene unsaponifiable matters and tertiary-butylhydroquinone. J Food Sci. 2012 Jun;77(6):C697-702.

102. FDA. Monounsaturated fatty acids from olive oil and coronary heart disease. in health claim petition docket no. 2003Q-0559; FDA: Rome, italy, 2004. . 2004.

103. Feng M, DiPetrillo K. Non-invasive blood pressure measurement in mice. Methods Mol Biol. 2009;573:45-55.

104. Fernandez-Jarne E, Martinez-Losa E, Prado-Santamaria M, Brugarolas-Brufau C, Serrano-Martinez M, Martinez-Gonzalez MA. Risk of first non-fatal myocardial infarction negatively associated with olive oil consumption: A case-control study in spain. Int J Epidemiol. 2002 Apr;31(2):474-80.

105. Fiers W. Tumor necrosis factor. characterization at the molecular, cellular and in vivo level. FEBS Lett. 1991 Jul 22;285(2):199-212.

106. Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. Science. 1992 Jul 17;257(5068):387-9.

107. Fito M, Cladellas M, de la Torre R, Marti J, Alcantara M, Pujadas-Bastardes M, et al. Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: A randomized, crossover, controlled, clinical trial. Atherosclerosis. 2005 Jul;181(1):149-58.

108. Fito M, de la Torre R, Covas MI. Olive oil and oxidative stress. Mol Nutr Food Res. 2007 Oct;51(10):1215-24.

109. Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang CS, et al. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the studies of left ventricular dysfunction (SOLVD). Circulation. 1990 Nov;82(5):1724-9.

110. Frangogiannis NG. Regulation of the inflammatory response in cardiac repair. Circ Res. 2012 Jan 6;110(1):159-73.

111. Frangogiannis NG. The immune system and cardiac repair. Pharmacol Res. 2008 Aug;58(2):88-111.

112. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res. 2002 Jan;53(1):31-47.

113. Frankel EN. Nutritional and biological properties of extra virgin olive oil. J Agric Food Chem. 2011 Feb 9;59(3):785-92.

114. Frantz S, Bauersachs J, Kelly RA. Innate immunity and the heart. Curr Pharm Des. 2005;11(10):1279-90.

115. Fried LF, Shlipak MG, Crump C, Bleyer AJ, Gottdiener JS, Kronmal RA, et al. Renal insufficiency as a predictor of cardiovascular outcomes and mortality in elderly individuals. J Am Coll Cardiol. 2003 Apr 16;41(8):1364-72.

116. Fritsche KL, Johnston PV. Rapid autoxidation of fish oil in diets without added antioxidants. J Nutr. 1988 Apr;118(4):425-6.

117. Fuentes F, Lopez-Miranda J, Perez-Martinez P, Jimenez Y, Marin C, Gomez P, et al. Chronic effects of a high-fat diet enriched with virgin olive oil and a low-fat diet enriched with alpha-linolenic acid on postprandial endothelial function in healthy men. Br J Nutr. 2008 Jul;100(1):159-65.

118. Georgopoulos S, Plows D, Kollias G. Transmembrane TNF is sufficient to induce localized tissue toxicity and chronic inflammatory arthritis in transgenic mice. J Inflamm. 1996;46(2):86-97.

119. Gerdes AM., editor. Chronic ischemic heart disease. in: Weber KT, ed. wound healing in cardiovascular disease. Futura Publishing Co.; 1995.

120. Gerdes AM, Capasso JM. Structural remodeling and mechanical dysfunction of cardiac myocytes in heart failure. J Mol Cell Cardiol. 1995 Mar;27(3):849-56.

121. Gerdes AM, Kellerman SE, Moore JA, Muffly KE, Clark LC, Reaves PY, et al. Structural remodeling of cardiac myocytes in patients with ischemic cardiomyopathy. Circulation. 1992 Aug;86(2):426-30.

122. Gerdes AM, Onodera T, Wang X, McCune SA. Myocyte remodeling during the progression to failure in rats with hypertension. Hypertension. 1996 Oct;28(4):609-14.

123. Ghanbari R, Anwar F, Alkharfy KM, Gilani AH, Saari N. Valuable nutrients and functional bioactives in different parts of olive (olea europaea L.)-A review. Int J Mol Sci. 2012;13(3):3291-340.

124. Ghavami S, Eshraghi M, Kadkhoda K, Mutawe MM, Maddika S, Bay GH, et al. Role of BNIP3 in TNF-induced cell death--TNF upregulates BNIP3 expression. Biochim Biophys Acta. 2009 Mar;1793(3):546-60.

125. Gheorghiade M, Bonow RO. Chronic heart failure in the united states: A manifestation of coronary artery disease. Circulation. 1998 Jan 27;97(3):282-9.

126. Gilani AH, Khan AU, Shah AJ, Connor J, Jabeen Q. Blood pressure lowering effect of olive is mediated through calcium channel blockade. Int J Food Sci Nutr. 2005 Dec;56(8):613-20.

127. Ginsberg HN. Insulin resistance and cardiovascular disease. J Clin Invest. 2000 Aug;106(4):453-8.

128. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. J Clin Invest. 2005 Mar;115(3):500-8.

129. Giroir BP, Johnson JH, Brown T, Allen GL, Beutler B. The tissue distribution of tumor necrosis factor biosynthesis during endotoxemia. J Clin Invest. 1992 Sep;90(3):693-8.

130. Gosker HR, Wouters EF, van der Vusse GJ, Schols AM. Skeletal muscle dysfunction in chronic obstructive pulmonary disease and chronic heart failure: Underlying mechanisms and therapy perspectives. Am J Clin Nutr. 2000 May;71(5):1033-47.

131. Grell M, Douni E, Wajant H, Lohden M, Clauss M, Maxeiner B, et al. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. Cell. 1995 Dec 1;83(5):793-802.

132. Groenveld HF, Januzzi JL, Damman K, van Wijngaarden J, Hillege HL, van Veldhuisen DJ, et al. Anemia and mortality in heart failure patients a systematic review and meta-analysis. J Am Coll Cardiol. 2008 Sep 2;52(10):818-27.

133. Grundy SM. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N Engl J Med. 1986 Mar 20;314(12):745-8.

134. Hardin BJ, Campbell KS, Smith JD, Arbogast S, Smith J, Moylan JS, et al. TNF-alpha acts via TNFR1 and muscle-derived oxidants to depress myofibrillar force in murine skeletal muscle. J Appl Physiol. 2008 Mar;104(3):694-9.

135. Hargrove RL, Etherton TD, Pearson TA, Harrison EH, Kris-Etherton PM. Low fat and high monounsaturated fat diets decrease human low density lipoprotein oxidative susceptibility in vitro. J Nutr. 2001 Jun;131(6):1758-63.

136. Harvey KA, Walker CL, Xu Z, Whitley P, Pavlina TM, Hise M, et al. Oleic acid inhibits stearic acid-induced inhibition of cell growth and pro-inflammatory responses in human aortic endothelial cells. J Lipid Res. 2010 Dec;51(12):3470-80.

137. Hausman DB, Lu J, Ryan DH, Flatt WP, Harris RB. Compensatory growth of adipose tissue after partial lipectomy: Involvement of serum factors. Exp Biol Med (Maywood). 2004 Jun;229(6):512-20.

138. Hawkins NM, Petrie MC, Jhund PS, Chalmers GW, Dunn FG, McMurray JJ. Heart failure and chronic obstructive pulmonary disease: Diagnostic pitfalls and epidemiology. Eur J Heart Fail. 2009 Feb;11(2):130-9.

139. Heard SO, Perkins MW, Fink MP. Tumor necrosis factor-alpha causes myocardial depression in guinea pigs. Crit Care Med. 1992 Apr;20(4):523-7.

140. Hegewisch S, Weh HJ, Hossfeld DK. TNF-induced cardiomyopathy. Lancet. 1990 Feb 3;335(8684):294-5.

141. Hikoso S, Yamaguchi O, Higuchi Y, Hirotani S, Takeda T, Kashiwase K, et al. Pressure overload induces cardiac dysfunction and dilation in signal transducer and activator of transcription 6-deficient mice. Circulation. 2004 Oct 26;110(17):2631-7.

142. Hill MF, Singal PK. Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infarction in rats. Am J Pathol. 1996 Jan;148(1):291-300.

143. Hoeck WG, Ramesha CS, Chang DJ, Fan N, Heller RA. Cytoplasmic phospholipase A2 activity and gene expression are stimulated by tumor necrosis factor: Dexamethasone blocks the induced synthesis. Proc Natl Acad Sci U S A. 1993 May 15;90(10):4475-9.

144. Holmann R, editor. Fedeli, E. lipids of olives; in :Progress in the chemistry of fats and other lipids. pp 57-74. Oxford: Oxford, Academic Press; 1977.

145. Hooper NM, Karran EH, Turner AJ. Membrane protein secretases. Biochem J. 1997 Jan 15;321 (Pt 2)(Pt 2):265-79.

146. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. Cell. 1995 May 19;81(4):495-504.

147. Huertas JR, Martinez-Velasco E, Ibanez S, Lopez-Frias M, Ochoa JJ, Quiles J, et al. Virgin olive oil and coenzyme Q10 protect heart mitochondria from peroxidative damage during aging. Biofactors. 1999;9(2-4):337-43.

148. Hwang J, Lee KE, Lim JY, Park SI. Nitrated fatty acids prevent TNFalpha-stimulated inflammatory and atherogenic responses in endothelial cells. Biochem Biophys Res Commun. 2009 Oct 2;387(4):633-40.

149. Impellizzeri D, Esposito E, Mazzon E, Paterniti I, Di Paola R, Bramanti P, et al. The effects of a polyphenol present in olive oil, oleuropein aglycone, in an experimental model of spinal cord injury in mice. Biochem Pharmacol. 2012 May 15;83(10):1413-26.

150. **Designations and definitions of olive oils** [Internet].; 2013. Available from: http://www.internationaloliveoil.org/estaticos/view/83-designations-and-definitions-of-olive-oils.

151. Ion G, Fazio K, Akinsete JA, Hardman WE. Effects of canola and corn oil mimetic on jurkat cells. Lipids Health Dis. 2011 Jun 1;10:90.

152. Iribarren C, Karter AJ, Go AS, Ferrara A, Liu JY, Sidney S, et al. Glycemic control and heart failure among adult patients with diabetes. Circulation. 2001 Jun 5;103(22):2668-73.

153. Irwin MW, Mak S, Mann DL, Qu R, Penninger JM, Yan A, et al. Tissue expression and immunolocalization of tumor necrosis factor-alpha in postinfarction dysfunctional myocardium. Circulation. 1999 Mar 23;99(11):1492-8.

154. Isensee H, Jacob R. Differential effects of various oil diets on the risk of cardiac arrhythmias in rats. J Cardiovasc Risk. 1994 Dec;1(4):353-9.

155. Iversen KK, Kjaergaard J, Akkan D, Kober L, Torp-Pedersen C, Hassager C, et al. The prognostic importance of lung function in patients admitted with heart failure. Eur J Heart Fail. 2010 Jul;12(7):685-91.

156. Jassal DS, Han SY, Hans C, Sharma A, Fang T, Ahmadie R, et al. Utility of tissue doppler and strain rate imaging in the early detection of trastuzumab and anthracycline mediated cardiomyopathy. J Am Soc Echocardiogr. 2009 Apr;22(4):418-24.

157. Jassal DS, Othman RA, Ahmadie R, Fang T, Zieroth S, Fischer G, et al. The role of tissue doppler imaging in the noninvasive detection of chronic rejection after heterotopic cardiac transplantation in rats. Echocardiography. 2009 Jan;26(1):37-43.

158. Jekunen A, Heikkila P, Maiche A, Pyrhonen S. Paclitaxel-induced myocardial damage detected by electron microscopy. Lancet. 1994 Mar 19;343(8899):727-8.

159. Jobe LJ, Melendez GC, Levick SP, Du Y, Brower GL, Janicki JS. TNF-alpha inhibition attenuates adverse myocardial remodeling in a rat model of volume overload. Am J Physiol Heart Circ Physiol. 2009 Oct;297(4):H1462-8.

160. Kadokami T, McTiernan CF, Kubota T, Frye CS, Feldman AM. Sex-related survival differences in murine cardiomyopathy are associated with differences in TNF-receptor expression. J Clin Invest. 2000 Aug;106(4):589-97.

161. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: The framingham study. Am J Cardiol. 1974 Jul;34(1):29-34.

162. Kapadia S, Dibbs Z, Kurrelmeyer K, Kalra D, Seta Y, Wang F, et al. The role of cytokines in the failing human heart. Cardiol Clin. 1998 Nov;16(4):645,56, viii.

163. Kapadia S, Lee J, Torre-Amione G, Birdsall HH, Ma TS, Mann DL. Tumor necrosis factor-alpha gene and protein expression in adult feline myocardium after endotoxin administration. J Clin Invest. 1995 Aug;96(2):1042-52.

164. Kapadia S, Torre-Amione G, Yokoyama T, Mann DL. Soluble TNF binding proteins modulate the negative inotropic properties of TNF-alpha in vitro. Am J Physiol. 1995 Feb;268(2 Pt 2):H517-25.

165. Kapadia SR, Oral H, Lee J, Nakano M, Taffet GE, Mann DL. Hemodynamic regulation of tumor necrosis factor-alpha gene and protein expression in adult feline myocardium. Circ Res. 1997 Aug;81(2):187-95.

166. Kassiri Z, Defamie V, Hariri M, Oudit GY, Anthwal S, Dawood F, et al. Simultaneous transforming growth factor beta-tumor necrosis factor activation and cross-talk cause aberrant remodeling response and myocardial fibrosis in Timp3-deficient heart. J Biol Chem. 2009 Oct 23;284(43):29893-904.

167. Katsuma S, Hatae N, Yano T, Ruike Y, Kimura M, Hirasawa A, et al. Free fatty acids inhibit serum deprivation-induced apoptosis through GPR120 in a murine enteroendocrine cell line STC-1. J Biol Chem. 2005 May 20;280(20):19507-15.

168. Katz AM. The cardiomyopathy of overload: An unnatural growth response in the hypertrophied heart. Ann Intern Med. 1994 Sep 1;121(5):363-71.

169. Kaur K, Dhingra S, Slezak J, Sharma AK, Bajaj A, Singal PK. Biology of TNFalpha and IL-10, and their imbalance in heart failure. Heart Fail Rev. 2009 Jun;14(2):113-23.

170. Kaur K, Sharma AK, Dhingra S, Singal PK. Interplay of TNF-alpha and IL-10 in regulating oxidative stress in isolated adult cardiac myocytes. J Mol Cell Cardiol. 2006 Dec;41(6):1023-30.

171. Kaur K, Sharma AK, Singal PK. Significance of changes in TNF-alpha and IL-10 levels in the progression of heart failure subsequent to myocardial infarction. Am J Physiol Heart Circ Physiol. 2006 Jul;291(1):H106-13.

172. Kelly CM, Smith RD, Williams CM. Dietary monounsaturated fatty acids and haemostasis. Proc Nutr Soc. 2001 May;60(2):161-70.

173. Kenchaiah S, Gaziano JM, Vasan RS. Impact of obesity on the risk of heart failure and survival after the onset of heart failure. Med Clin North Am. 2004 Sep;88(5):1273-94.

174. Keys A. Coronary heart disease in seven countries. (suppl to vol.41) 1-211. Circulation. 1970;Supp to vol. 41:1-211.

175. Khaper N, Bryan S, Dhingra S, Singal R, Bajaj A, Pathak CM, et al. Targeting the vicious inflammation-oxidative stress cycle for the management of heart failure. Antioxid Redox Signal. 2010 Oct 1;13(7):1033-49.

176. Khaper N, Singal PK. Modulation of oxidative stress by a selective inhibition of angiotensin II type 1 receptors in MI rats. J Am Coll Cardiol. 2001 Apr;37(5):1461-6.

177. Kiechl-Kohlendorfer U, Berger C, Inzinger R. The effect of daily treatment with an olive oil/lanolin emollient on skin integrity in preterm infants: A randomized controlled trial. Pediatr Dermatol. 2008 Mar-Apr;25(2):174-8.

178. Killip T. Epidemiology of congestive heart failure. Am J Cardiol. 1985 Jul 10;56(2):2A-6A.

179. Kleinbongard P, Schulz R, Heusch G. TNFalpha in myocardial ischemia/reperfusion, remodeling and heart failure. Heart Fail Rev. 2011 Jan;16(1):49-69.

180. Knuefermann P, Nemoto S, Baumgarten G, Misra A, Sivasubramanian N, Carabello BA, et al. Cardiac inflammation and innate immunity in septic shock: Is there a role for toll-like receptors? Chest. 2002 Apr;121(4):1329-36.

181. Kolb WP, Granger GA. Lymphocyte in vitro cytotoxicity: Characterization of human lymphotoxin. Proc Natl Acad Sci U S A. 1968 Dec;61(4):1250-5.

182. Kolmel KF, Pfahlberg A, Mastrangelo G, Niin M, Botev IN, Seebacher C, et al. Infections and melanoma risk: Results of a multicentre EORTC case-control study. european organization for research and treatment of cancer. Melanoma Res. 1999 Oct;9(5):511-9.

183. Kriegler M, Perez C, DeFay K, Albert I, Lu SD. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: Ramifications for the complex physiology of TNF. Cell. 1988 Apr 8;53(1):45-53.

184. Kubota T, McTiernan CF, Frye CS, Demetris AJ, Feldman AM. Cardiac-specific overexpression of tumor necrosis factor-alpha causes lethal myocarditis in transgenic mice. J Card Fail. 1997 Jun;3(2):117-24.

185. Kubota T, McTiernan CF, Frye CS, Slawson SE, Lemster BH, Koretsky AP, et al. Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor-alpha. Circ Res. 1997 Oct;81(4):627-35.

186. Kusters S, Tiegs G, Alexopoulou L, Pasparakis M, Douni E, Kunstle G, et al. In vivo evidence for a functional role of both tumor necrosis factor (TNF) receptors and transmembrane TNF in experimental hepatitis. Eur J Immunol. 1997 Nov;27(11):2870-5.

187. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970 Aug 15;227(5259):680-5.

188. Lau DH, Psaltis PJ, Carbone A, Kelly DJ, Mackenzie L, Worthington M, et al. Atrial protective effects of n-3 polyunsaturated fatty acids: A long-term study in ovine chronic heart failure. Heart Rhythm. 2011 Apr;8(4):575-82.

189. Lau GT, Tan HC, Kritharides L. Type of liver dysfunction in heart failure and its relation to the severity of tricuspid regurgitation. Am J Cardiol. 2002 Dec 15;90(12):1405-9.

190. Law M, Wald N, Morris J. Lowering blood pressure to prevent myocardial infarction and stroke: A new preventive strategy. Health Technol Assess. 2003;7(31):1-94.

191. Lee DS, Mamdani MM, Austin PC, Gong Y, Liu PP, Rouleau JL, et al. Trends in heart failure outcomes and pharmacotherapy: 1992 to 2000. Am J Med. 2004 May 1;116(9):581-9.

192. Lee KS, Moser DK. Heart failure symptom measures: Critical review. Eur J Cardiovasc Nurs. 2013 Jan 8.

193. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med. 1990 Jul 26;323(4):236-41.

194. Levy D, Larson MG, Vasan RS, Kannel WB, Ho KK. The progression from hypertension to congestive heart failure. JAMA. 1996 May 22-29;275(20):1557-62.

195. Li C, Ha T, Liu L, Browder W, Kao RL. Adenosine prevents activation of transcription factor NF-kappa B and enhances activator protein-1 binding activity in ischemic rat heart. Surgery. 2000 Feb;127(2):161-9.

196. Li J, Ichikawa T, Jin Y, Hofseth LJ, Nagarkatti P, Nagarkatti M, et al. An essential role of Nrf2 in american ginseng-mediated anti-oxidative actions in cardiomyocytes. J Ethnopharmacol. 2010 Jul 20;130(2):222-30.

197. Li L, Qu N, Li DH, Wen WM, Huang WQ. Coronary microembolization induced myocardial contractile dysfunction and tumor necrosis factor-alpha mRNA expression partly inhibited by SB203580 through a p38 mitogen-activated protein kinase pathway. Chin Med J (Engl). 2011 Jan;124(1):100-5.

198. Li L, Zhao X, Lu Y, Huang W, Wen W. Altered expression of pro- and anti-inflammatory cytokines is associated with reduced cardiac function in rats following coronary microembolization. Mol Cell Biochem. 2010 Sep;342(1-2):183-90.

199. Li S, Tao L, Jiao X, Liu H, Cao Y, Lopez B, et al. TNFalpha-initiated oxidative/nitrative stress mediates cardiomyocyte apoptosis in traumatic animals. Apoptosis. 2007 Oct;12(10):1795-802.

200. Li YY, Feng YQ, Kadokami T, McTiernan CF, Draviam R, Watkins SC, et al. Myocardial extracellular matrix remodeling in transgenic mice overexpressing tumor necrosis factor alpha can be modulated by anti-tumor necrosis factor alpha therapy. Proc Natl Acad Sci U S A. 2000 Nov 7;97(23):12746-51.

201. Liaudet L, Rosenblatt-Velin N. Role of innate immunity in cardiac inflammation after myocardial infarction. Front Biosci (Schol Ed). 2013 Jan 1;5:86-104.

202. Lim JH, Gerhart-Hines Z, Dominy JE, Lee Y, Kim S, Tabata M, et al. Oleic acid stimulates complete oxidation of fatty acids through PKA-dependent activation of SIRT1/PGC1alpha complex. J Biol Chem. 2013 Jan 17.

203. Lisowska A, Tycinska A, Knapp M, Lisowski P, Musial WJ. The incidence and prognostic significance of cardiac arrhythmias and conduction abnormalities in patients with acute coronary syndromes and renal dysfunction. Kardiol Pol. 2011;69(12):1242-7.

204. Listing J, Strangfeld A, Kekow J, Schneider M, Kapelle A, Wassenberg S, et al. Does tumor necrosis factor alpha inhibition promote or prevent heart failure in patients with rheumatoid arthritis? Arthritis Rheum. 2008 Mar;58(3):667-77.

205. Liu S, Yin T, Wei X, Yi W, Qu Y, Liu Y, et al. Downregulation of adiponectin induced by tumor necrosis factor alpha is involved in the aggravation of posttraumatic myocardial ischemia/reperfusion injury. Crit Care Med. 2011 Aug;39(8):1935-43.

206. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: Requirement for dATP and cytochrome c. Cell. 1996 Jul 12;86(1):147-57.

207. Loewi O. On the mechanism of the positive inotropic action of fluoride, oleate, and calcium on the frog's heart. J Pharmacol Exp Ther. 1955 May;114(1):90-9.

208. Lopez-Miranda J, Gomez P, Castro P, Marin C, Paz E, Bravo MD, et al. Mediterranean diet improves low density lipoprotein susceptibility to oxidative modifications. Med Clin (Barc). 2000 Sep 30;115(10):361-5.

209. Lopez-Miranda J, Perez-Jimenez F, Ros E, De Caterina R, Badimon L, Covas MI, et al. Olive oil and health: Summary of the II international conference on olive oil and health consensus report, jaen and cordoba (spain) 2008. Nutr Metab Cardiovasc Dis. 2010 May;20(4):284-94.

210. Louis A, Cleland JG, Crabbe S, Ford S, Thackray S, Houghton T, et al. Clinical trials update: CAPRICORN, COPERNICUS, MIRACLE, STAF, RITZ-2, RECOVER and RENAISSANCE and cachexia and cholesterol in heart failure. highlights of the scientific sessions of the american college of cardiology, 2001. Eur J Heart Fail. 2001 Jun;3(3):381-7.

211. Lu XT, Zhao YX, Zhang Y, Jiang F. Psychological stress, vascular inflammation and atherogenesis: Potential roles of circulating cytokines. J Cardiovasc Pharmacol. 2013 Jan 11.

212. Lu Y, Li L, Zhao X, Huang W, Wen W. Beta blocker metoprolol protects against contractile dysfunction in rats after coronary microembolization by regulating expression of myocardial inflammatory cytokines. Life Sci. 2011 Jun 6;88(23-24):1009-15.

213. Lucas L, Russell A, Keast R. Molecular mechanisms of inflammation. anti-inflammatory benefits of virgin olive oil and the phenolic compound oleocanthal. Curr Pharm Des. 2011;17(8):754-68.

214. Maass DL, Hybki DP, White J, Horton JW. The time course of cardiac NF-kappaB activation and TNF-alpha secretion by cardiac myocytes after burn injury: Contribution to burn-related cardiac contractile dysfunction. Shock. 2002 Apr;17(4):293-9.

215. Mackay K, Mochly-Rosen D. Arachidonic acid protects neonatal rat cardiac myocytes from ischaemic injury through epsilon protein kinase C. Cardiovasc Res. 2001 Apr;50(1):65-74.

216. Maedler K, Oberholzer J, Bucher P, Spinas GA, Donath MY. Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic beta-cell turnover and function. Diabetes. 2003 Mar;52(3):726-33.

217. Mann DL, McMurray JJ, Packer M, Swedberg K, Borer JS, Colucci WS, et al. Targeted anticytokine therapy in patients with chronic heart failure: Results of the randomized etanercept worldwide evaluation (RENEWAL). Circulation. 2004 Apr 6;109(13):1594-602.

218. Manthorpe R, Svensson O. Cardiomyopathy following intravenous cyclophosphamide therapy in a patient with wegener's granulomatosis. Clin Exp Rheumatol. 1996 Nov-Dec;14(6):702-3.

219. Mariappan N, Soorappan RN, Haque M, Sriramula S, Francis J. TNF-alpha-induced mitochondrial oxidative stress and cardiac dysfunction: Restoration by superoxide dismutase mimetic tempol. Am J Physiol Heart Circ Physiol. 2007 Nov;293(5):H2726-37.

220. Martinez J, Vogler O, Casas J, Barcelo F, Alemany R, Prades J, et al. Membrane structure modulation, protein kinase C alpha activation, and anticancer activity of minerval. Mol Pharmacol. 2005 Feb;67(2):531-40.

221. Martinez-Gonzalez MA, Fernandez-Jarne E, Serrano-Martinez M, Marti A, Martinez JA, Martin-Moreno JM. Mediterranean diet and reduction in the risk of a first acute myocardial infarction: An operational healthy dietary score. Eur J Nutr. 2002 Aug;41(4):153-60.

222. Massaro M, De Caterina R. Vasculoprotective effects of oleic acid: Epidemiological background and direct vascular antiatherogenic properties. Nutr Metab Cardiovasc Dis. 2002 Feb;12(1):42-51.

223. Matsumori A, Yamada T, Suzuki H, Matoba Y, Sasayama S. Increased circulating cytokines in patients with myocarditis and cardiomyopathy. Br Heart J. 1994 Dec;72(6):561-6.

224. Matsuzawa A, Ichijo H. Redox control of cell fate by MAP kinase: Physiological roles of ASK1-MAP kinase pathway in stress signaling. Biochim Biophys Acta. 2008 Nov;1780(11):1325-36.

225. Matthews N. Tumour-necrosis factor from the rabbit. II. production by monocytes. Br J Cancer. 1978 Aug;38(2):310-5.

226. McLennan PL. Relative effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on cardiac arrhythmias in rats. Am J Clin Nutr. 1993 Feb;57(2):207-12.

227. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Bohm M, Dickstein K, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The task force for the diagnosis and treatment of acute and chronic heart failure 2012 of the european society of cardiology. developed in collaboration with the heart failure association (HFA) of the ESC. Eur J Heart Fail. 2012 Aug;14(8):803-69.

228. McMurray JJ, Petrie MC, Murdoch DR, Davie AP. Clinical epidemiology of heart failure: Public and private health burden. Eur Heart J. 1998 Dec;19 Suppl P:P9-16.

229. McMurray JJ, Pfeffer MA. Heart failure. Lancet. 2005 May 28-Jun 3;365(9474):1877-89.

230. Medeiros FJ, Mothe CG, Aguila MB, Mandarim-de-Lacerda CA. Long-term intake of edible oils benefits blood pressure and myocardial structure in spontaneously hypertensive rat (SHR) and streptozotocin diabetic SHR. Prostaglandins Other Lipid Mediat. 2005 Dec;78(1-4):231-48.

231. Meguro K, Adachi H, Oshima S, Taniguchi K, Nagai R. Exercise tolerance, exercise hyperpnea and central chemosensitivity to carbon dioxide in sleep apnea syndrome in heart failure patients. Circ J. 2005 Jun;69(6):695-9.

232. Menazza S, Wong R, Nguyen T, Wang G, Gucek M, Murphy E. CypD(-/-) hearts have altered levels of proteins involved in krebs cycle, branch chain amino acid degradation and pyruvate metabolism. J Mol Cell Cardiol. 2012 Dec 19.

233. Mendez GF, Cowie MR. The epidemiological features of heart failure in developing countries: A review of the literature. Int J Cardiol. 2001 Sep-Oct;80(2-3):213-9.

234. Menendez JA, Lupu R. 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). Curr Pharm Biotechnol. 2006 Dec;7(6):495-502.

235. Mesa Garcia MD, Aguilera Garcia CM, Gil Hernandez A. Importance of lipids in the nutritional treatment of inflammatory diseases. Nutr Hosp. 2006 May;21 Suppl 2:28,41, 30-43.

236. Miki T, Yuda S, Kouzu H, Miura T. Diabetic cardiomyopathy: Pathophysiology and clinical features. Heart Fail Rev. 2012 Mar 28.

237. Mizuochi Y, Okajima K, Harada N, Molor-Erdene P, Uchiba M, Komura H, et al. Carvedilol, a nonselective beta-blocker, suppresses the production of tumor necrosis factor and tissue factor by inhibiting early growth response factor-1 expression in human monocytes in vitro. Transl Res. 2007 Apr;149(4):223-30.

238. Moe KT, Yin NO, Naylynn TM, Khairunnisa K, Wutyi MA, Gu Y, et al. Nox2 and Nox4 mediate tumour necrosis factor-alpha-induced ventricular remodelling in mice. J Cell Mol Med. 2011 Dec;15(12):2601-13.

239. Montedoro G, Servili M, Baldioli M, Miniati E. Simple and hydrolyzable phenolic compounds in virgin olive oil. 2. initial characterization of the hydrolyzable fraction. J Agric Food Chem. 1992 09/01; 2013/02;40(9):1577-80.

240. Murray DR, Prabhu SD, Chandrasekar B. Chronic beta-adrenergic stimulation induces myocardial proinflammatory cytokine expression. Circulation. 2000 May 23;101(20):2338-41.

241. Murtaza I, Wang HX, Feng X, Alenina N, Bader M, Prabhakar BS, et al. Down-regulation of catalase and oxidative modification of protein kinase CK2 lead to the failure of apoptosis repressor with caspase recruitment domain to inhibit cardiomyocyte hypertrophy. J Biol Chem. 2008 Mar 7;283(10):5996-6004.

242. Murumalla RK, Gunasekaran MK, Padhan JK, Bencharif K, Gence L, Festy F, et al. Fatty acids do not pay the toll: Effect of SFA and PUFA on human adipose tissue and mature adipocytes inflammation. Lipids Health Dis. 2012 Dec 21;11:175,511X-11-175.

243. Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, et al. Apoptosis in myocytes in end-stage heart failure. N Engl J Med. 1996 Oct 17;335(16):1182-9.

244. Natanson C, Eichenholz PW, Danner RL, Eichacker PQ, Hoffman WD, Kuo GC, et al. Endotoxin and tumor necrosis factor challenges in dogs simulate the cardiovascular profile of human septic shock. J Exp Med. 1989 Mar 1;169(3):823-32.

245. What is heart failure? [Internet].; 2012. Available from: http://www.nhlbi.nih.gov/health/health-topics/topics/hf/signs.html.

246. Nedwin GE, Naylor SL, Sakaguchi AY, Smith D, Jarrett-Nedwin J, Pennica D, et al. Human lymphotoxin and tumor necrosis factor genes: Structure, homology and chromosomal localization. Nucleic Acids Res. 1985 Sep 11;13(17):6361-73.

247. Nian M, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial infarction remodeling. Circ Res. 2004 Jun 25;94(12):1543-53.

248. Nora J, Berg K, Nora A. *Cardiovascular diseases*: *Genetics, epidemiology* and *prevention*. New York: Oxford University Press; 1991.

249. Novoyatleva T, Schymura Y, Janssen W, Strobl F, Swiercz JM, Patra C, et al. Deletion of Fn14 receptor protects from right heart fibrosis and dysfunction. Basic Res Cardiol. 2013 Mar;108(2):325,012-0325-x. Epub 2013 Jan 17.

250. Ntusi NB, Mayosi BM. Epidemiology of heart failure in sub-saharan africa. Expert Rev Cardiovasc Ther. 2009 Feb;7(2):169-80.

251. O'Bryne DJ, O'Keefe SF, Shireman RB. Low-fat, monounsaturate-rich diets reduce susceptibility of low density lipoproteins to peroxidation ex vivo. Lipids. 1998 Feb;33(2):149-57.

252. **Olive oil for hair** [Internet].; 2013. Available from: http://www.ojaioliveoil.com/olive-oil-for-hair-s/1840.htm.

253. Old LJ. Tumor necrosis factor (TNF). Science. 1985 Nov 8;230(4726):630-2.

254. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, bax, that accelerates programmed cell death. Cell. 1993 Aug 27;74(4):609-19.

255. O'Malley W, Achinstein B, Shear M. Action of bacterial polysaccharide on tumors. II. damage of sarcoma 37 by serum of mice treated with serratia marcescens polysaccharide, and induced tolerance. J Natl Cancer Inst. 1962;29:1169-75.

256. O'Malley WE, Achinstein B, Shear MJ. Journal of the national cancer institute, vol. 29, 1962: Action of bacterial polysaccharide on tumors. II. damage of sarcoma 37 by serum of mice treated with serratia marcescens polysaccharide, and induced tolerance. Nutr Rev. 1988 Nov;46(11):389-91.

257. Omran AR. The epidemiologic transition. A theory of the epidemiology of population change. 1971. Bull World Health Organ. 2001;79(2):161-70.

258. Onodera T, Tamura T, Said S, McCune SA, Gerdes AM. Maladaptive remodeling of cardiac myocyte shape begins long before failure in hypertension. Hypertension. 1998 Oct;32(4):753-7.

259. Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B, Bartsch H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. Eur J Cancer. 2000 Jun;36(10):1235-47.

260. Owen RW, Giacosa A, Hull WE, Haubner R, Wurtele G, Spiegelhalder B, et al. Olive-oil consumption and health: The possible role of antioxidants. Lancet Oncol. 2000 Oct;1:107-12.

261. Owen RW, Haubner R, Wurtele G, Hull E, Spiegelhalder B, Bartsch H. Olives and olive oil in cancer prevention. Eur J Cancer Prev. 2004 Aug;13(4):319-26.

262. Oz HS, Im HJ, Chen TS, de Villiers WJ, McClain CJ. Glutathione-enhancing agents protect against steatohepatitis in a dietary model. J Biochem Mol Toxicol. 2006;20(1):39-47.

263. Pacheco YM, Lopez S, Bermudez B, Abia R, Muriana FJ. Extra-virgin vs. refined olive oil on postprandial hemostatic markers in healthy subjects. J Thromb Haemost. 2006 Jun;4(6):1421-2.

264. Pagani FD, Baker LS, Hsi C, Knox M, Fink MP, Visner MS. Left ventricular systolic and diastolic dysfunction after infusion of tumor necrosis factor-alpha in conscious dogs. J Clin Invest. 1992 Aug;90(2):389-98.

265. Palace V, Kumar D, Hill MF, Khaper N, Singal PK. Regional differences in non-enzymatic antioxidants in the heart under control and oxidative stress conditions. J Mol Cell Cardiol. 1999 Jan;31(1):193-202.

266. Papageorgiou N, Tousoulis D, Psaltopoulou T, Giolis A, Antoniades C, Tsiamis E, et al. Divergent anti-inflammatory effects of different oil acute consumption on healthy individuals. Eur J Clin Nutr. 2011 Apr;65(4):514-9.

267. Parsons-Smith BT. Cardiac failure. Lancet. 1950 May 13;1(6611):889-94.

268. Patrick L, Uzick M. Cardiovascular disease: C-reactive protein and the inflammatory disease paradigm: HMG-CoA reductase inhibitors, alpha-tocopherol, red yeast rice, and olive oil polyphenols. A review of the literature. Altern Med Rev. 2001 Jun;6(3):248-71.

269. Paulus WJ. Cytokines and heart failure. Heart Fail Monit. 2000;1(2):50-6.

270. Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ,Jr, Cuddy TE, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. results of the survival and ventricular enlargement trial. the SAVE investigators. N Engl J Med. 1992 Sep 3;327(10):669-77.

271. Pi Y, Walker JW. Diacylglycerol and fatty acids synergistically increase cardiomyocyte contraction via activation of PKC. Am J Physiol Heart Circ Physiol. 2000 Jul;279(1):H26-34.

272. Piper HM, Probst I, Schwartz P, Hutter FJ, Spieckermann PG. Culturing of calcium stable adult cardiac myocytes. J Mol Cell Cardiol. 1982 Jul;14(7):397-412.

273. Pressler SJ. Cognitive functioning and chronic heart failure: A review of the literature (2002-july 2007). J Cardiovasc Nurs. 2008 May-Jun;23(3):239-49.

274. Pulkki KJ. Cytokines and cardiomyocyte death. Ann Med. 1997 Aug;29(4):339-43.

275. Rabkin SW, Lodhia P. Stearic acid-induced cardiac lipotoxicity is independent of cellular lipid and is mitigated by the fatty acids oleic and capric acid but not by the PPAR agonist troglitazone. Exp Physiol. 2009 Aug;94(8):877-87.

276. Rauchhaus M, Coats AJ, Anker SD. The endotoxin-lipoprotein hypothesis. Lancet. 2000 Sep 9;356(9233):930-3.

277. Rauchhaus M, Doehner W, Francis DP, Davos C, Kemp M, Liebenthal C, et al. Plasma cytokine parameters and mortality in patients with chronic heart failure. Circulation. 2000 Dec 19;102(25):3060-7.

278. Rawdanowicz TJ, Hampton AL, Nagase H, Woolley DE, Salamonsen LA. Matrix metalloproteinase production by cultured human endometrial stromal cells: Identification of interstitial collagenase, gelatinase-A, gelatinase-B, and stromelysin-1 and their differential regulation by interleukin-1 alpha and tumor necrosis factor-alpha. J Clin Endocrinol Metab. 1994 Aug;79(2):530-6.

279. Raynolds,M., Peacock, S. J., Roden, R. L. FASEB abtract. 1995;9:1281.

280. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities--the role of insulin resistance and the sympathoadrenal system. N Engl J Med. 1996 Feb 8;334(6):374-81.

281. Reaven P, Parthasarathy S, Grasse BJ, Miller E, Steinberg D, Witztum JL. Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects. J Clin Invest. 1993 Feb;91(2):668-76.

282. Reaven PD, Witztum JL. Oxidized low density lipoproteins in atherogenesis: Role of dietary modification. Annu Rev Nutr. 1996;16:51-71.

283. Reeves PG, Nielsen FH, Fahey GC,Jr. AIN-93 purified diets for laboratory rodents: Final report of the american institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 1993 Nov;123(11):1939-51.

284. Renaud S. Linoleic acid, platelet aggregation and myocardial infarction. Atherosclerosis. 1990 Jan;80(3):255-6.

285. Rhoden W, Hasleton P, Brooks N. Anthracyclines and the heart. Br Heart J. 1993 Dec;70(6):499-502.

286. Rincheval V, Bergeaud M, Mathieu L, Leroy J, Guillaume A, Mignotte B, et al. Differential effects of bcl-2 and caspases on mitochondrial permeabilization during endogenous or exogenous reactive oxygen species-induced cell death: A comparative study of H(2)O(2), paraquat, t-BHP, etoposide and TNF-alpha-induced cell death. Cell Biol Toxicol. 2012 Aug;28(4):239-53.

287. Rizk NM, Yousef M. Association of lipid profile and waist circumference as cardiovascular risk factors for overweight and obesity among school children in qatar. Diabetes Metab Syndr Obes. 2012;5:425-32.

288. Rodis PS, Karathanos VT, Mantzavinou A. Partitioning of olive oil antioxidants between oil and water phases. J Agric Food Chem. 2002 Jan 30;50(3):596-601.

289. Rodriguez-Ramiro I, Martin MA, Ramos S, Bravo L, Goya L. Olive oil hydroxytyrosol reduces toxicity evoked by acrylamide in human caco-2 cells by preventing oxidative stress. Toxicology. 2011 Oct 9;288(1-3):43-8.

290. Roger VL, Weston SA, Redfield MM, Hellermann-Homan JP, Killian J, Yawn BP, et al. Trends in heart failure incidence and survival in a community-based population. JAMA. 2004 Jul 21;292(3):344-50.

291. Rosin NL, Falkenham A, Sopel MJ, Lee TD, Legare JF. Regulation and role of connective tissue growth factor in AngII-induced myocardial fibrosis. Am J Pathol. 2012 Dec 31.

292. Ruano J, Lopez-Miranda J, de la Torre R, Delgado-Lista J, Fernandez J, Caballero J, et al. Intake of phenol-rich virgin olive oil improves the postprandial prothrombotic profile in hypercholesterolemic patients. Am J Clin Nutr. 2007 Aug;86(2):341-6.

293. Ruano J, Lopez-Miranda J, Fuentes F, Moreno JA, Bellido C, Perez-Martinez P, et al. Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. J Am Coll Cardiol. 2005 Nov 15;46(10):1864-8.

294. Ruddle NH, Waksman BH. Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. 3. analysis of mechanism. J Exp Med. 1968 Dec 1;128(6):1267-79.

295. Ruiz-Gutierrez V, Muriana FJ, Guerrero A, Cert AM, Villar J. Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. J Hypertens. 1996 Dec;14(12):1483-90.

296. Sabit R, Bolton CE, Fraser AG, Edwards JM, Edwards PH, Ionescu AA, et al. Subclinical left and right ventricular dysfunction in patients with COPD. Respir Med. 2010 Aug;104(8):1171-8.

297. Sadoshima J, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. Cell. 1993 Dec 3;75(5):977-84.

298. Sales-Campos H, de Souza PR, Peghini BC, da Silva JS, Cardoso CR. An overview of the modulatory effects of oleic acid in health and disease. Mini Rev Med Chem. 2012 Dec 31.

299. Sanadgol N, Mostafaie A, Bahrami G, Mansouri K, Ghanbari F, Bidmeshkipour A. Elaidic acid sustains LPS and TNF-alpha induced ICAM-1 and VCAM-I expression on human bone marrow endothelial cells (HBMEC). Clin Biochem. 2010 Aug;43(12):968-72.

300. Sansores RH, Ramirez-Venegas A, Hernandez-Zenteno R, Mayar-Maya ME, Perez-Bautista OG, Velazquez Uncal M. Prevalence and diagnosis of chronic obstructive pulmonary disease among smokers at risk. A comparative study of case-finding vs. screening strategies. Respir Med. 2013 Jan 8.

301. Savic-Radojevic A, Radovanovic S, Pekmezovic T, Pljesa-Ercegovac M, Simic D, Djukic T, et al. The role of serum VCAM-1 and TNF-alpha as predictors of mortality and morbidity in patients with chronic heart failure. J Clin Lab Anal. 2013 Jan 24.

302. Schwartz B, Birk Y, Raz A, Madar Z. Nutritional-pharmacological combinations--a novel approach to reducing colon cancer incidence. Eur J Nutr. 2004 Aug;43(4):221-9.

303. Seddon M, Looi YH, Shah AM. Oxidative stress and redox signalling in cardiac hypertrophy and heart failure. Heart. 2007 Aug;93(8):903-7.

304. Selye H, Bajusz E, Grasso S, Mendell P. Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology. 1960 Oct;11:398-407.

305. Shah AM, Uno H, Kober L, Velazquez EJ, Maggioni AP, MacDonald MR, et al. The inter-relationship of diabetes and left ventricular systolic function on outcome after high-risk myocardial infarction. Eur J Heart Fail. 2010 Nov;12(11):1229-37.

306. Sharma AK, Dhingra S, Khaper N, Singal PK. Activation of apoptotic processes during transition from hypertrophy to heart failure in guinea pigs. Am J Physiol Heart Circ Physiol. 2007 Sep;293(3):H1384-90.

307. Sharpe N, Doughty R. Epidemiology of heart failure and ventricular dysfunction. Lancet. 1998 Aug;352 Suppl 1:SI3-7.

308. Shioi T, Matsumori A, Kihara Y, Inoko M, Ono K, Iwanaga Y, et al. Increased expression of interleukin-1 beta and monocyte chemotactic and activating factor/monocyte chemoattractant protein-1 in the hypertrophied and failing heart with pressure overload. Circ Res. 1997 Nov;81(5):664-71.

309. Silverberg DS, Wexler D, Iaina A, Steinbruch S, Wollman Y, Schwartz D. Anemia, chronic renal disease and congestive heart failure--the cardio renal anemia syndrome: The need for cooperation between cardiologists and nephrologists. Int Urol Nephrol. 2006;38(2):295-310.

310. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2002 Oct;56(8):365-79.

311. Singal PK, Dhillon KS, Beamish RE, Kapur N, Dhalla NS. Myocardial cell damage and cardiovascular changes due to i.v. infusion of adrenochrome in rats. Br J Exp Pathol. 1982 Apr;63(2):167-76.

312. Singal PK, Khaper N, Farahmand F, Bello-Klein A. Oxidative stress in congestive heart failure. Curr Cardiol Rep. 2000 May;2(3):206-11.

313. Smolinske SC. *Handbook of food, drug, and cosmetic excipients*. ; 1992.

314. Solanas M, Grau L, Moral R, Vela E, Escrich R, Escrich E. Dietary olive oil and corn oil differentially affect experimental breast cancer through distinct modulation of the p21Ras signaling and the proliferation-apoptosis balance. Carcinogenesis. 2010 May;31(5):871-9.

315. Sonoyama K, Nishikawa H, Kiriyama S, Niki R. Cholestyramine and a fat-free diet lower apolipoprotein A-IV mRNA in jejunum and cholestyramine lowers apolipoprotein A-I mRNA in ileum of rats. J Nutr. 1994 May;124(5):621-7.

316. Sporn MB, Liby KT. NRF2 and cancer: The good, the bad and the importance of context. Nat Rev Cancer. 2012 Jul 19;12(8):564-71.

317. Squadrito F, Altavilla D, Zingarelli B, Ioculano M, Calapai G, Campo GM, et al. Tumor necrosis factor involvement in myocardial ischaemia-reperfusion injury. Eur J Pharmacol. 1993 Jun 24;237(2-3):223-30.

318. Stamm C, Friehs I, Cowan DB, Moran AM, Cao-Danh H, Duebener LF, et al. Inhibition of tumor necrosis factor-alpha improves postischemic recovery of hypertrophied hearts. Circulation. 2001 Sep 18;104(12 Suppl 1):I350-5.

319. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, et al. The cardiovascular response of normal humans to the administration of endotoxin. N Engl J Med. 1989 Aug 3;321(5):280-7.

320. Sun M, Dawood F, Wen WH, Chen M, Dixon I, Kirshenbaum LA, et al. Excessive tumor necrosis factor activation after infarction contributes to susceptibility of myocardial rupture and left ventricular dysfunction. Circulation. 2004 Nov 16;110(20):3221-8.

321. Swift LM, Sarvazyan N. Localization of dichlorofluorescin in cardiac myocytes: Implications for assessment of oxidative stress. Am J Physiol Heart Circ Physiol. 2000 Mar;278(3):H982-90.

322. Tanaka N, Kono H, Ishii K, Hosomura N, Fujii H. Dietary olive oil prevents carbon tetrachloride-induced hepatic fibrosis in mice. J Gastroenterol. 2009;44(9):983-90.

323. Tang P, Hung M-, Klostergaard J. Human pro-tumor necrosis factor is a homotrimer. Biochemistry. 1996 Jun 25;35(25):8216-25.

324. Tardif N, Salles J, Landrier JF, Mothe-Satney I, Guillet C, Boue-Vaysse C, et al. Oleate-enriched diet improves insulin sensitivity and restores muscle protein synthesis in old rats. Clin Nutr. 2011 Dec;30(6):799-806.

325. Tartaglia LA, Ayres TM, Wong GH, Goeddel DV. A novel domain within the 55 kd TNF receptor signals cell death. Cell. 1993 Sep 10;74(5):845-53.

326. Tartaglia LA, Pennica D, Goeddel DV. Ligand passing: The 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. J Biol Chem. 1993 Sep 5;268(25):18542-8.

327. Tarze A, Deniaud A, Le Bras M, Maillier E, Molle D, Larochette N, et al. GAPDH, a novel regulator of the pro-apoptotic mitochondrial membrane permeabilization. Oncogene. 2007 Apr 19;26(18):2606-20.

328. Teerlink JR, Goldhaber SZ, Pfeffer MA. An overview of contemporary etiologies of congestive heart failure. Am Heart J. 1991 Jun;121(6 Pt 1):1852-3.

329. Teres S, Barcelo-Coblijn G, Benet M, Alvarez R, Bressani R, Halver JE, et al. Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. Proc Natl Acad Sci U S A. 2008 Sep 16;105(37):13811-6.

330. The Criteria Committee of the New York Heart Association. Nomenclature and criteria for diagnosis of diseases of the heart and great vessels. 9th ed. Boston, Mass: Little, Brown & Co; 1994.

331. Tissier C, Vandroux D, Devillard L, Brochot A, Moreau D, Rochette L, et al. Substrate dependence of the postischemic cardiomyocyte recovery: Dissociation between functional, metabolic and injury markers. Mol Cell Biochem. 2005 May;273(1-2):43-55.

332. Torre-Amione G. Immune activation in chronic heart failure. Am J Cardiol. 2005 Jun 6;95(11A):3C,8C; discussion 38C-40C.

333. Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: A report from the studies of left ventricular dysfunction (SOLVD). J Am Coll Cardiol. 1996 Apr;27(5):1201-6.

334. Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, et al. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. Circulation. 1996 Feb 15;93(4):704-11.

335. Torre-Amione G, Stetson SJ, Youker KA, Durand JB, Radovancevic B, Delgado RM, et al. Decreased expression of tumor necrosis factor-alpha in failing human myocardium after mechanical circulatory support : A potential mechanism for cardiac recovery. Circulation. 1999 Sep 14;100(11):1189-93.

336. Tristan C, Shahani N, Sedlak TW, Sawa A. The diverse functions of GAPDH: Views from different subcellular compartments. Cell Signal. 2011 Feb;23(2):317-23.

337. Turnbull F, Blood Pressure Lowering Treatment Trialists' Collaboration. Effects of different blood-pressure-lowering regimens on major cardiovascular events: Results of

prospectively-designed overviews of randomised trials. Lancet. 2003 Nov 8;362(9395):1527-35.

338. U.S. Department of Health and Human Services. Healthy people 2010: Understanding and improving health.
Washington, DC: Government Printing Office; 2000.

339. Urbano-Marquez A, Estruch R, Navarro-Lopez F, Grau JM, Mont L, Rubin E. The effects of alcoholism on skeletal and cardiac muscle. N Engl J Med. 1989 Feb 16;320(7):409-15.

340. Urpi-Sarda M, Casas R, Chiva-Blanch G, Romero-Mamani ES, Valderas-Martinez P, Arranz S, et al. Virgin olive oil and nuts as key foods of the mediterranean diet effects on inflammatory biomakers related to atherosclerosis. Pharmacol Res. 2012 Jun;65(6):577-83.

341. van den Hurk K, Reijmer YD, van den Berg E, Alssema M, Nijpels G, Kostense PJ, et al. Heart failure and cognitive function in the general population: The hoorn study. Eur J Heart Fail. 2011 Dec;13(12):1362-9.

342. van der Meer P, Lok DJ, Januzzi JL, de la Porte PW, Lipsic E, van Wijngaarden J, et al. Adequacy of endogenous erythropoietin levels and mortality in anaemic heart failure patients. Eur Heart J. 2008 Jun;29(12):1510-5.

343. van der Meer P, van Veldhuisen DJ. Anaemia and renal dysfunction in chronic heart failure. Heart. 2009 Nov;95(21):1808-12.

344. van Deursen VM, Damman K, Hillege HL, van Beek AP, van Veldhuisen DJ, Voors AA. Abnormal liver function in relation to hemodynamic profile in heart failure patients. J Card Fail. 2010 Jan;16(1):84-90.

345. van Deursen VM, Damman K, van der Meer P, Wijkstra PJ, Luijckx GJ, van Beek A, et al. Co-morbidities in heart failure. Heart Fail Rev. 2012 Dec 25.

346. Vanden Berghe W, Plaisance S, Boone E, De Bosscher K, Schmitz ML, Fiers W, et al. p38 and extracellular signal-regulated kinase mitogen-activated protein kinase pathways are required for nuclear factor-kappaB p65 transactivation mediated by tumor necrosis factor. J Biol Chem. 1998 Feb 6;273(6):3285-90.

347. Vassiliou EK, Gonzalez A, Garcia C, Tadros JH, Chakraborty G, Toney JH. Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNF-alpha both in vitro and in vivo systems. Lipids Health Dis. 2009 Jun 26;8:25,511X-8-25.

348. Verma S, Gupta M, Ridker PM. Therapeutic targeting of inflammation in atherosclerosis: We are getting closer. Can J Cardiol. 2012 Nov;28(6):619-22.

349. Vincent-Baudry S, Defoort C, Gerber M, Bernard MC, Verger P, Helal O, et al. The medi-RIVAGE study: Reduction of cardiovascular disease risk factors after a 3-mo intervention with a mediterranean-type diet or a low-fat diet. Am J Clin Nutr. 2005 Nov;82(5):964-71.

350. Visioli F, Galli C. The effect of minor constituents of olive oil on cardiovascular disease: New findings. Nutr Rev. 1998 May;56(5 Pt 1):142-7.

351. Vogels RL, Scheltens P, Schroeder-Tanka JM, Weinstein HC. Cognitive impairment in heart failure: A systematic review of the literature. Eur J Heart Fail. 2007 May;9(5):440-9.

352. Wajant H, Henkler F, Scheurich P. The TNF-receptor-associated factor family: Scaffold molecules for cytokine receptors, kinases and their regulators. Cell Signal. 2001 Jun;13(6):389-400.

353. Walsh CR, Larson MG, Evans JC, Djousse L, Ellison RC, Vasan RS, et al. Alcohol consumption and risk for congestive heart failure in the framingham heart study. Ann Intern Med. 2002 Feb 5;136(3):181-91.

354. Waterman E, Lockwood B. Active components and clinical applications of olive oil. Altern Med Rev. 2007 Dec;12(4):331-42.

355. Weinberg LE, Singal PK. Refractory heart failure and age-related differences in adriamycin-induced myocardial changes in rats. Can J Physiol Pharmacol. 1987 Sep;65(9):1957-65.

356. Westenbrink BD, Visser FW, Voors AA, Smilde TD, Lipsic E, Navis G, et al. Anaemia in chronic heart failure is not only related to impaired renal perfusion and blunted erythropoietin production, but to fluid retention as well. Eur Heart J. 2007 Jan;28(2):166-71.

357. Weytjens C, Cosyns B, D'hooge J, Gallez C, Droogmans S, Lahoute T, et al. Doppler myocardial imaging in adult male rats: Reference values and reproducibility of velocity and deformation parameters. Eur J Echocardiogr. 2006 Dec;7(6):411-7.

358. White HD, Norris RM, Brown MA, Brandt PW, Whitlock RM, Wild CJ. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. Circulation. 1987 Jul;76(1):44-51.

359. **New data highlight increases in hypertension, diabetes incidence** [Internet].; 2012. Available from: http://www.who.int/mediacentre/news/releases/2012/world_health_statistics_20120516/en/.

360. World_Health_statistics brochure 2012. [Internet].; 2012. Available from: http://www.who.int/gho/publications/world_health_statistics/EN_WHS2012_Brochure.pdf.

361. **Cardiovascular diseases (CVDs)** [Internet].; 2012 [updated 2012; cited 12/2/2013]. Available from: http://www.who.int/mediacentre/factsheets/fs317/en/.

362. Oleic acid [Internet].; 2013. Available from: http://en.wikipedia.org/wiki/Oleic_acid#cite_note-Ullmann-2.

363. Witte KK, Desilva R, Chattopadhyay S, Ghosh J, Cleland JG, Clark AL. Are hematinic deficiencies the cause of anemia in chronic heart failure? Am Heart J. 2004 May;147(5):924-30.

364. Woo CH, Eom YW, Yoo MH, You HJ, Han HJ, Song WK, et al. Tumor necrosis factor-alpha generates reactive oxygen species via a cytosolic phospholipase A2-linked cascade. J Biol Chem. 2000 Oct 13;275(41):32357-62.

365. Woodruff JF. Viral myocarditis. A review. Am J Pathol. 1980 Nov;101(2):425-84.

366. Wouters EF. Local and systemic inflammation in chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2005;2(1):26-33.

367. Wright G, Singh IS, Hasday JD, Farrance IK, Hall G, Cross AS, et al. Endotoxin stress-response in cardiomyocytes: NF-kappaB activation and tumor necrosis factor-alpha expression. Am J Physiol Heart Circ Physiol. 2002 Mar;282(3):H872-9.

368. Wyatt HL, Meerbaum S, Heng MK, Gueret P, Corday E. Cross-sectional echocardiography. III. analysis of mathematic models for quantifying volume of symmetric and asymmetric left ventricles. Am Heart J. 1980 Dec;100(6 Pt 1):821-8.

369. Yagi K, Kiuchi K, Saito Y, Miike A, Kayahara N, Tatano T, et al. Use of a new methylene blue derivative for determination of lipid peroxides in foods. Biochem Int. 1986 Feb;12(2):367-71.

370. Yamazaki KG, Taub PR, Barraza-Hidalgo M, Rivas MM, Zambon AC, Ceballos G, et al. Effects of (-)-epicatechin on myocardial infarct size and left ventricular remodeling after permanent coronary occlusion. J Am Coll Cardiol. 2010 Jun 22;55(25):2869-76.

371. Yamazaki T, Yazaki Y. Role of tissue angiotensin II in myocardial remodelling induced by mechanical stress. J Hum Hypertens. 1999 Jan;13 Suppl 1:S43,7; discussion S49-50.

372. Yang Q, Alemany R, Casas J, Kitajka K, Lanier SM, Escriba PV. Influence of the membrane lipid structure on signal processing via G protein-coupled receptors. Mol Pharmacol. 2005 Jul;68(1):210-7.

373. Yao YY, Yin H, Shen B, Chao L, Chao J. Tissue kallikrein and kinin infusion rescues failing myocardium after myocardial infarction. J Card Fail. 2007 Sep;13(7):588-96.

374. Yaoita H, Ogawa K, Maehara K, Maruyama Y. Apoptosis in relevant clinical situations: Contribution of apoptosis in myocardial infarction. Cardiovasc Res. 2000 Feb;45(3):630-41.

375. Yeh CH, Chen TP, Wang YC, Lin YM, Lin PJ. HO-1 activation can attenuate cardiomyocytic apoptosis via inhibition of NF-kappaB and AP-1 translocation following cardiac global ischemia and reperfusion. J Surg Res. 2009 Jul;155(1):147-56.

376. Yue P, Massie BM, Simpson PC, Long CS. Cytokine expression increases in nonmyocytes from rats with postinfarction heart failure. Am J Physiol. 1998 Jul;275(1 Pt 2):H250-8.

377. Yuzefovych L, Wilson G, Rachek L. Different effects of oleate vs. palmitate on mitochondrial function, apoptosis, and insulin signaling in L6 skeletal muscle cells: Role of oxidative stress. Am J Physiol Endocrinol Metab. 2010 Dec;299(6):E1096-105.

378. Zeng WT, Chen WY, Leng XY, Tang LL, Sun XT, Li CL, et al. Impairment of cardiac function and remodeling induced by myocardial infarction in rats are attenuated by the nonpeptide angiotensin-(1-7) analog AVE 0991. Cardiovasc Ther. 2012 Jun;30(3):152-61.

379. Zhang J, Villacorta L, Chang L, Fan Z, Hamblin M, Zhu T, et al. Nitro-oleic acid inhibits angiotensin II-induced hypertension. Circ Res. 2010 Aug 20;107(4):540-8.

380. Zierhut W, Zimmer HG, Gerdes AM. Effect of angiotensin converting enzyme inhibition on pressure-induced left ventricular hypertrophy in rats. Circ Res. 1991 Sep;69(3):609-17.

381. Zrelli H, Matsuoka M, Kitazaki S, Zarrouk M, Miyazaki H. Hydroxytyrosol reduces intracellular reactive oxygen species levels in vascular endothelial cells by upregulating catalase expression through the AMPK-FOXO3a pathway. Eur J Pharmacol. 2011 Jun 25;660(2-3):275-82.