Activation of Cannabinoid Receptors Prevents Endothelin-1-induced Cardiac Myocyte Hypertrophy and Mitochondrial Dysfunction

By

Yan Lu

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements of the degree of

Doctorate of Philosophy

College of Pharmacy

Faculty of Health Sciences

University of Manitoba

Winnipeg

Abstract

Objectives: Endocannabinoids are bioactive amides, esters, and ethers of long-chain polyunsaturated fatty acids that activate two cannabinoid receptors, CB1 and CB2. Evidence suggests that activation of the endocannabinoid pathway offers cardioprotection. Cardiac hypertrophy is a convergence point of risk factors for heart failure, and it is associated with aberrant mitochondrial function. We determined a role for endocannabinoids in attenuating endothelin-1 (ET1)-induced hypertrophy and mitochondrial dysfunction, as well as the signaling pathways involved.

Design and Methods: Cardiac myocyte hypertrophy was provoked by ET1 in isolated neonatal rat ventricular myocytes. Effects of four cannabinoid receptor agonists (anandamide, R-methanandamide, JWH-133 and CB-13) on hypertrophic markers (myocyte enlargement and hypertrophic gene expression) were assessed in the presence or absence of selective antagonists of CB1 or CB2 receptors. Mitochondrial function was evaluated by assessing changes in membrane permeability transition (calcein-AM), membrane potential (JC-1 dye), and mitochondrial bioenergetics related to fatty acid and glucose oxidation (seahorse XF24 analyzer). Molecular pathway components were identified by western blot and real-time PCR.

Results: Anandamide and its metabolically-inactive analogue, R-methanandamide, prevented ET1-induced increases in hypertrophic markers, and application of selective CB receptor antagonists revealed a distinct role of each receptor therein. Also, JWH-133, a selective CB2 agonist, and CB-13, a dual agonist of CB1/CB2 receptors with limited brain penetration, were investigated as strategies to theoretically avoid central CB1

receptor-mediated psychoactive effects. CB-13 attenuated all indicators of hypertrophy, whereas JWH-133 did not. The anti-hypertrophic actions of CB-13 were mediated by AMPK-eNOS crosstalk.

ET1 induced mitochondrial membrane depolarization in the presence of either palmitate or glucose as primary energy substrate, decreased mitochondrial bioenergetics and proteins related to fatty acid oxidation (i.e. PGC-1α, a driver of mitochondrial biogenesis, and CPT-1β, facilitator of fatty acid uptake), but did not affect glucose-related bioenergetics. CB-13 corrected all of these parameters, at least in part, via AMPK signaling.

Conclusions: Activation of cannabinoid receptors by CB-13, a peripherally-restricted agonist of CB1/CB2 receptors, offers protective effects on cardiac myocyte hypertrophy and its related mitochondrial disorders. Therefore, a cannabinoid-based treatment for cardiac disease represents a potential therapeutic strategy that warrants further study.

Acknowledgement

I can't believe I'm approaching the finish line, and how much I have gained throughout the Ph.D. program. First, I would like to express my deepest gratitude to my supervisor, Dr. Hope Anderson, who provided me this opportunity to step into the science world, guided and supported me through the journey. She helped and encouraged me during the difficult times of research, spent tons of times in helping me with my writing and presentation skills, gave me trust and freedom in planning and arranging my work. Dr. Anderson not only influenced me as a great scientist, her wisdom in life also made her my role model. It is impossible to finish this thesis without her, and I could not ask for a better supervisor.

Second, I would like to thank my committee members Dr. Yuewen Gong, Dr. Karmin O, and Dr. Grant Pierce. Their valuable advice and feedback made my project better than I expected. Also, I want to thank Dr. Paul Fernyhough and Dr. Subir Chowdhury for their continual help in the mitochondria aspect of my thesis. Their expertise in this area helped me saved a lot of time and resource.

Third, I would like to give my thanks to my colleagues and graduate peers: June, Kim, Basma, Haining, Bolanle, Caroline, Crystal, and Danielle. I'm lucky to have these wonderful people around me to discuss and share ideas. Special thanks go to June, who introduced me to Dr. Anderson, taught me so many techniques when I started, and helped me out with my project whenever I need. She is not just a colleague, but also my teacher and friend. In addition, thanks to R.O. Burrell lab and other support staff, who provided great service and ensured the progression of my project.

Last but not least, I would like to thank my families and friends. My parents and sister have always been supportive and understanding for my decisions, and I know they

always will. A big thank you goes to my aunt Yin, uncle Feng, and cousins (Lucy, Anna, and Rhea) in Winnipeg. They are like my parents and sisters in Canada and they made my life here much easier and happier. Also, thanks to my friends, without them I wouldn't be able to come so far. Pengqi, my dearest roommate, we shared every moment in our life, ups and downs, during the past 5 years, and I know we are lifelong friends. Jiaqi, Yuhua, Zirui, and all of the friends in the College of Pharmacy have brought me a lot of special memories, they made my Ph.D program everything but boring.

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List of Abbreviations

Abbreviation	Full text
2-AG	2-arachidonoylglycerol
ABHD	α/β hydrolase domain
AC	adenylyl cyclase
ACCF	American College of Cardiology Foundation
ACE	angiotensin converting enzyme
AHA	American Heart Association
ALVDD	asymptomatic left ventricular diastolic dysfunction
ALVSD	asymptomatic left ventricular systolic dysfunction
AMPK	AMP-activated protein kinase
ANP	atrial natriuretic peptide
ANT	adenine nucleotide translocator
AP-1	activator protein-1
APAF-1	apoptotic protease activating factor 1
ARB	angiotensin II receptor blocker
α-MHC	α-myosin heavy chain
β-MHC	β-myosin heavy chain
Bcl-2	B-cell lymphoma 2
BNP	brain natriuretic peptide
BSA	bovine serum albumin
calcein-AM	non-fluorescent acetomethoxy derivate of calcein
cAMP	cyclic AMP
CCS	cosmic calf serum

CHD	coronary heart disease
CNS	central nervous system
COX-2	cyclooxygenase-2
CPT-1β	carnitine palmitoyltransferase I-beta
CVD	cardiovascular diseases
DAG	diacylglycerol
DHA	docosahexaenoic acid
DMEM	Dulbecco's Modified Eagle's Medium
ECG	electrocardiogram
ECM	extracellular matrix
eEF2	eukaryotic elongation factor-2
eIF4E	eukaryotic initiation factor 4E
eNOS	endothelial nitric oxide synthase
EPA	eicosapentaenoic acid
ERK	extracellular signal-regulated kinase
ET1	endothelin-1
ETC	electron transport chain
FAAH	fatty acid amide hydrolase
$FADH_2$	flavin adenine dinucleotide (reduced form)
FCCP	carbonyl cyanide-*4*-(trifluoromethoxy)phenylhydrazone
FLAT	FAAH-like anandamide transporter
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GEF	guanine nucleotide exchange factors
GPCR	G protein-coupled receptor

GSK-3β	glycogen synthase kinase-3 beta
HAND 1/2	heart and neural crest derivatives expressed protein 1/2
HDAC	histone deacetylase
HDL	high density lipoprotein
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
IFN-γ	interferon-γ
IL	interleukin
iNOS	inducible nitric oxide synthase
JNK	c-Jun NH_2-terminal kinase
LDL	low density lipoprotein
LVEF	left ventricular ejection fraction
LVH	left ventricular hypertrophy
MAGL	monoacylglycerol lipase
MAPK	mitogen-activated protein kinase
MEF2	myocyte enhancer factor-2
MMP	matrix metalloproteinase
MnSOD	manganese-dependent superoxide dismutase
mPT	mitochondrial permeability transition
mPTP	mitochondrial permeability transition pore
NAAA	N-acylethanolamine-hydrolyzing acid amidase
NADH	nicotinamide adenine dinucleotide (reduced form)
NAPE	N-acylphosphatidylethanolamine
NFAT	nuclear factor of activated T cells

NF-κB	nuclear factor κ-light-chain-enhancer of activated B cells
nNOS	neuronal nitric oxide synthase
NYHA	New York Heart Association
OCR	oxygen consumption rate
p-AMPK	phosphorylated AMP-activated protein kinase
PBS	phosphate-buffered saline
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PKA	protein kinase A
PKB	protein kinase B
PKC	protein kinase C
PLCβ	phospholipase C-β
PPAR	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acid
RAAS	renin-angiotensin-aldosterone system
RhoA	Ras homolog gene family member A
RIPA	radioimmunoprecipitation assay buffer
ROCK	Rho-associated protein kinase
ROS	reactive oxygen species
shRNA	short hairpin RNA
SRF	serum response factor
SOD	superoxide dismutase
SIRT1	sirtuin 1
TAC	transverse aortic constriction
TCA cycle	tricarboxylic acid cycle

TGF-β	transforming growth factor-β
THC	tetrahydrocannabinol
TNF-α	tumor necrosis factor-α
TRPV1	transient receptor potential vanilloid type 1
UCP	uncoupling protein
$\Delta\psi_m$	membrane potential

List of Pharmacological Agents

Agents	Receptor Target	Pharmacodynamics and Working Concentrations
AM251	CB1-selective antagonist	Ki: CB1=7.5 nM; CB2=2290 nM (Lan, Liu, et al., 1999). Working concentration: 0.1 μM
AM281	CB1-selective antagonist	Ki: CB1=12 nM; CB2=4200 nM (Lan, Gatley, et al., 1999). Working concentration: 0.1 μM
AM630	CB2-selective antagonist	Ki: CB1=5200 nM; CB2=31.2 nM (R. A. Ross et al., 1999). Working concentration: 0.1 μM
anandamide	CB1 and CB2 receptor agonist	Ki: CB1=89 nM; CB2=371 nM (Pertwee, 1999). Working concentrations: 0.001-1 μM
CB-13	peripherally-restricted (non-CNS) CB1/CB2 receptor agonist	Ki: CB1=6.1 nM; CB2=27.9 nM (Dziadulewicz et al., 2007). Working concentrations: 0.001-1 μM
compound C	AMPK inhibitor	Ki: 109 nM (G. Zhou et al., 2001). Working concentration: 1 μM

ET1	$ET_{A/B}$ receptor agonist	Hypertrophic stimulus (Sugden & Clerk, 2005). Working concentration: 0.1 μM (Alibin, Kopilas, & Anderson, 2008; Y. Huang et al., 2011).
JWH-133	CB2-selective agonist	Ki: CB1=677 nM; CB2=3.4 nM (Huffman et al., 1999). Working concentrations: 0.001-1 μM
L-NIO	eNOS inhibitor	IC_{50} is 500 nM at eNOS; 10- and 5-fold less potent at nNOS and iNOS, respectively (Moore et al., 1994). Working concentration: 1 μM
R-methanandamide	CB1 and CB2 receptor agonist	Ki: CB1=20 nM; CB2=815 nM (Abadji et al., 1994; Khanolkar et al., 1996). Working concentration: 1 μM

Chapter I: Introduction

Cardiovascular diseases (CVDs) are diseases or injuries occurring within the heart or blood vessels. Global statistics from 2008 indicate that 17 million deaths were due to CVDs, and this accounted for 39% deaths. Thus, CVDs are the leading cause of death worldwide (World Health Organization, 2014). In Canada, CVDs are the second leading cause of death, accounting for 29% deaths in 2008. 80% of these were due to heart diseases (Heart and Stroke Foundation of Canada, 2014a). Although the mortality due to CVDs has declined over the last few decades due to improved diagnosis and treatment, CVDs remain the leading cause of death and hospitalization. 1.3 million Canadians were suffering from heart diseases in 2009, and the hospitalizations due to various heart diseases approached 0.3 million in 2011 (Heart and Stroke Foundation of Canada, 2014b). Approximately $21 billion were spent on CVDs in Canada both directly (hospitalizations, physician services, medication and equipment) and indirectly (loss of productivity) in 2010. In fact, CVDs represent the second greatest economic burden of disease in Canada (Public Health Agency of Canada, 2012).

Ninety percent of Canadians have at least one risk factor for heart disease (for example, smoking, excessive alcohol intake, physical inactivity, obesity, hypertension, hypercholesterolemia, or diabetes (Public Health Agency of Canada, 2009)). Without proper intervention, these risk factors contribute to the development of various heart diseases that may eventually lead to heart failure. Heart failure is defined as impaired heart function that is manifested by a reduced stroke volume that cannot meet the oxygen and nutrient demands of the body. Declining mortality from cardiac events such as acute myocardial infarction and cardiac arrest has increased the prevalence of heart failure (Bui,

Horwich, & Fonarow, 2011). Statistics revealed that more than 500,000 Canadians are living with heart failure and 50,000 new patients are diagnosed each year (H. Ross et al., 2006). In fact, the incidence of heart failure per year was predicted to double by 2025 (Johansen, Strauss, Arnold, Moe, & Liu, 2003). Heart failure develops gradually, but once diagnosed, the average survival time for men is 1.7 years and for women is 3.2 years (K. K. Ho, Anderson, Kannel, Grossman, & Levy, 1993). Overall, approximately 50% of patients die within 5 years of diagnosis (Heart and Stroke Foundation of Canada, 2014b). Therefore, prevention of heart failure has emerged as a research priority.

Heart failure is usually initiated and exacerbated by risk factors such as coronary heart diseases (CHDs), hypertension, and cardiac hypertrophy. In particular, pathological cardiac hypertrophy involves morphological and metabolic changes, and is considered an independent contributor to cardiac dysfunction and mortality from CVD (Benjamin & Levy, 1999; D. W. Brown, Giles, & Croft, 2000). Also, regression of hypertrophy was associated with reduced risks of cardiovascular events and mortality (Wachtell et al., 2007). Therefore, attenuation of cardiac hypertrophy is regarded as a promising strategy to suppress the development of heart failure.

The goals of current heart failure therapies are to relieve symptoms and delay progression by targeting the following objectives: 1) reduce hemodynamic overload (for example, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are prescribed as first-line therapy for hypertension; diuretics, by inhibiting sodium reabsorption and promoting water excretion, are used to relieve symptoms such as pulmonary congestion and peripheral edema; nitrates and hydralazine dilate blood vessels, thereby reducing hemodynamic overload and lowering oxygen demand); and 2) reduce cardiac workload by suppressing heart rate and contractility vis-

à-vis depressing the sympathetic nervous system (β-adrenergic blockers) and Ca^{2+} concentrations (Ca^{2+} channel blockers). Angina is also relieved because of reduced oxygen demand. The costs of managing heart failure patients in Canada are at least $2.3 billion (Bentkover et al., 2003). The increased incidence of heart failure and associated costs suggest that we need to identify new targets and develop novel therapeutic strategies. Since hypertrophy is a leading predictor of heart failure and sudden death, this study investigated novel mechanisms by which we might attenuate cardiac hypertrophy.

Normal functioning of the heart requires an abundant and dynamic energy supply. Thus, mitochondria, as the primary organelle responsible for ATP production, play an important role in maintaining cardiac performance. In healthy cardiac myocytes, mitochondria use predominantly fatty acids as substrate. However, in the diseased heart, a shift to glucose utilization as substrate occurs (Akhmedov, Rybin, & Marin-Garcia, 2015). This is accompanied by a decrease in oxidative phosphorylation, which leads to ATP generation that is inadequate to meet cardiac energy demand (Akhmedov et al., 2015; Allard, Schonekess, Henning, English, & Lopaschuk, 1994). In addition, increased oxidative stress and apoptosis due to mitochondrial disorders are involved in CVDs such as ischemia/reperfusion injury, atherosclerosis, and diabetic cardiomyopathy (Goncharov, Avdonin, Nadeev, Zharkikh, & Jenkins, 2015; Pham, Loiselle, Power, & Hickey, 2014; Teshima et al., 2014). These findings suggest a tight association between mitochondrial function and cardiac health. Thus, this work is based partly on the hypothesis that the correction of mitochondrial function is an important therapeutic target.

Cannabis sativa has a long history of use as medicine to relieve symptoms such as pain, fever, anxiety, and diarrhea in the context of numerous diseases (Grant, Atkinson, Gouaux, & Wilsey, 2012). To improve safety and efficacy, compounds from cannabis

were purified or synthesized and named under an umbrella group as cannabinoids. Currently, several cannabinoids may be prescribed in Canada to mitigate nausea from chemotherapy, relieve pain from cancer, prevent spasticity due to multiple sclerosis and improve appetite in patients with AIDS (Kalant, Porath-Waller, Canadian Centre on Substance Abuse., & Canadian Electronic Library (Firm), 2012). More recently, an increasing number of studies investigated the endogenous cannabinoid system and discovered other possible uses, including cardioprotection (Pacher, Batkai, & Kunos, 2006). The therapeutic potential of cannabinoids is therefore extended; however, evidence is limited and mechanisms remain unclear. In addition, the use of cannabinoids clinically has been hindered due to pronounced psychoactive side effects, such as dizziness, euphoria and addiction (Grant et al., 2012). Clearly, the role of cannabinoids and the endocannabinoid system in cardiac function and diseases remains poorly understood. Furthermore, any attempts to develop cannabinoid-based cardiovascular therapies would require mitigation of adverse psychoactive effects.

This study determined whether activation of the endocannabinoid system prevents cardiac hypertrophy and mitochondrial abnormalities within cardiac myocytes. Significant efforts were focused on identifying synthetic cannabinoid receptor agonists that would theoretically avoid unwanted psychoactive effects.

Chapter II: Literature Review

1. Heart failure

1.1. Definition

Heart failure is a condition characterized by impaired cardiac output, which leads to inadequate blood supply to meet the oxygen and nutrients demands of the body. It may result from any structural or functional abnormality such as valvular disease, myocardial infarction and hypertrophic cardiomyopathy (Yancy et al., 2013).

1.2. Classifications

Based on the left ventricular ejection fraction (i.e. ratio of left ventricular stroke volume to end-diastolic volume; LVEF), heart failure is classified into two categories: i) heart failure with reduced LVEF (HFrEF), and ii) heart failure with preserved LVEF (HFpEF). Normal LVEF varies, but the average value resides between 55-70%. There is no clear cutoff value for reduced LVEF, but it is widely accepted that heart failure with LVEFs of >50%, 40%-50%, and <40% are HFpEF, heart failure with mild systolic dysfunction, and HFrEF respectively (Ahmed et al., 2006; Massie et al., 2008; Steinberg et al., 2012; Yusuf et al., 2003). The American College of Cardiology Foundation/American Heart Association (ACCF/AHA) guideline for the management of heart failure also defined the clinical diagnosis of HFrEF as a LVEF $\leq$ 40% (Yancy et al., 2013).

HFrEF was historically also referred to as systolic heart failure because it is generally associated with systolic dysfunction. Hallmarks of HFrEF include depressed contractility and eccentric hypertrophy (i.e. reduced relative wall thickness, dilated left

ventricular chamber, and increased left ventricular mass) (Devereux et al., 2000). HFrEF accounts for approximately 50% of patients with heart failure. These patients are more likely to have a history of previous myocardial infarction (Devereux et al., 2000; Solomon et al., 2015). Furthermore, lower LVEF was correlated with higher incidence of cardiovascular and all-cause deaths (Solomon et al., 2015).

HFpEF is characterized by a normal or near normal LVEF (>50%). Instead, it is associated with diastolic dysfunction; impaired relaxation of the ventricular wall during diastole leads to a reduced end-diastolic volume, which impacts filling and eventually reduces cardiac output. HFpEF is often accompanied by concentric hypertrophy, with increased relative wall thickness, unchanged chamber volume, and augmented left ventricular mass. The prevalence of HFpEF is approximately half of all heart failure patients, with increased preference in elderly (>65 years old) and female patients (Andersen & Borlaug, 2014; Devereux et al., 2000; Masoudi et al., 2003; Owan et al., 2006). There is some speculation that the postmenopausal phase contributes to the higher risk of developing HFpEF, although the mechanism is unknown (Scantlebury & Borlaug, 2011). In addition, HFpEF patients are more likely to be hypertensive, diabetic and overweight (Devereux et al., 2000; Solomon et al., 2015). Clinical studies observed lower risk of mortality in patients with HFpEF than HFrEF (Meta-analysis Global Group in Chronic Heart, 2012). However, in the elderly population (>65 years old), the total number of HFpEF-related deaths may be greater due to the increasing prevalence compared to HFrEF (Gottdiener et al., 2002).

Heart failure can be asymptomatic or symptomatic. Symptoms include dyspnea, orthopnea, paroxysmal nocturnal dyspnea, fatigue, exercise intolerance and edema. The New York Heart Association (NYHA) classified heart failure into four categories by the

severity of symptoms, whereas the ACCF/AHA classified heart failure into four stages based on the development of structural and functional abnormalities (Yancy et al., 2013). A comparison of ACCF/AHA stages and NYHA classifications is shown in Table 1.

Table 1. Comparison of ACCF/AHA stages and NYHA classifications.

	ACCF/AHA stages of heart failure		NYHA functional classifications
A	At high risk for heart failure but without structural heart disease or symptoms of heart failure	n/a	
B	Structural heart disease but without signs or symptoms of heart failure	I	No limitation of physical activity. Ordinary physical activity does not cause symptoms of heart failure.
C	Structural heart disease with prior or current symptoms of heart failure	I	No limitation of physical activity. Ordinary physical activity does not cause symptoms of heart failure.
		II	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in symptoms of heart failure.
		III	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes symptoms of heart failure.
		IV	Unable to carry on any physical activity without symptoms of heart failure, or symptoms of heart failure at rest.
D	Refractory heart failure requiring specialized interventions	IV	Unable to carry on any physical activity without symptoms of heart failure, or symptoms of heart failure at rest.

Table 1. Adapted from "2013 ACCF/AHA Guideline for the Management of Heart Failure A Report of the American College of Cardiology Foundation/American Heart

Association Task Force on Practice Guidelines," published in the *Journal of the American College of Cardiology*, with permission from Elsevier.

It bears mentioning that the NYHA classes and ACCF/AHA stages do not replace each other, but rather offer complementary descriptions of heart failure progression. Also, the severity of symptoms described in the NYHA system does not necessarily correlate with the stage of heart failure progression defined by ACCF/AHA system. For example, a patient with severe heart failure symptoms (NYHA class III or IV) may exhibit mild symptoms due to treatment, although the progression of heart failure is not reversed (Goldberg & Jessup, 2006). Patients in ACCF/AHA stage B exhibit no previous or current symptoms, but they are still at high risk of developing symptoms and experiencing significant morbidity and mortality. A 12-year follow-up study on participants from the Framingham Heart Study demonstrated only a 7.1-year survival for patients with asymptomatic left ventricular systolic dysfunction (ALVSD) after diagnosis (T. J. Wang et al., 2003). In particular, Echouffo-Tcheugui et al. reported a higher risk of developing overt heart failure in patients with ALVSD than patients with asymptomatic left ventricular diastolic dysfunction (ALVDD) (Echouffo-Tcheugui, Erqou, Butler, Yancy, & Fonarow, 2015). In fact, a population-based study in England found that approximately half (47%) of patients with HFrEF were asymptomatic (Davies et al., 2001). Diagnosis of asymptomatic heart failure is challenging, as it can be easily missed unless patients experienced a prior cardiovascular event such as acute myocardial infarction. Therefore, guidelines on heart failure from the ACCF/AHA and the Heart Failure Society of America (HFSA) recommend a family history recording over three generations, physical examination, and regular clinical screening for populations at high risk (e.g. first-degree relatives of patients with familial dilated cardiomyopathy)

(Hershberger et al., 2009; Yancy et al., 2013). Clinical screening strategies include biomarker assays, electrocardiogram (ECG), and echocardiography (Yancy et al., 2013).

Heart failure is a syndrome that results from a complex of risk conditions; thus the diagnosis requires careful history and physical examinations, a series of diagnostic tests such as ECG, biomarker measurements (e.g. brain natriuretic peptide [BNP] or atrial natriuretic peptide [ANP]), cardiac imaging (e.g. echocardiography and magnetic resonance) and invasive evaluation (e.g. left-heart and right-heart catheterization and endomyocardial biopsy) (Yancy et al., 2013). HFrEF is relatively easier to diagnose; symptoms and biomarkers of heart failure with a reduced LVEF is sufficient to confirm the diagnosis. In contrast, diagnosis of HFpEF has been challenging because of the confusion caused by other conditions that contribute to heart failure symptoms, such as lung diseases, anemia and obesity (Caruana, Petrie, Davie, & McMurray, 2000). Thus current diagnostic guidelines recommend determination of diastolic function by Doppler echocardiography or cardiac catheterization, in addition to heart failure symptoms and a relatively normal LVEF (Paulus et al., 2007; Wachter & Edelmann, 2014).

Extensive research has been conducted on the prevention and treatment of HFrEF, as it was long believed to be the sole cause of heart failure. Current therapies such as ACE inhibitors, ARBs and β-blockers improved patient quality of life and mortality (Flather et al., 2000; Granger et al., 2003; Packer et al., 1996; The SOLVD Investigators, 1992). However, there is currently little evidence of benefits for the more recently recognized HFpEF (Massie et al., 2008; Shah, Desai, & Givertz, 2010; Yusuf et al., 2003), although reduced hospitalization was observed with ARB treatment (candesartan) (Yusuf et al., 2003).

1.3. Risk factors

Heart failure is a progressive developing syndrome that may be initiated by any disorders that relate to cardiac function. Thus, identifying risk factors is a key process in order to prevent or delay the progression of heart failure. The Framingham Heart Study, a longitudinal cohort study that started in 1948 and is ongoing, introduced the concept of identifying risk factors as an aspect of the etiology of CVDs and heart failure. In addition, it established a Framingham risk score system to estimate the risk of an individual developing CVDs. Based on the Framingham heart study, several significant risk factors have been identified: CHDs, hypertension, diabetes, valvular diseases, atrial fibrillation, left ventricular hypertrophy (LVH), smoking, obesity and age (J. E. Ho et al., 2013; D. S. Lee et al., 2009). More recently, many studies have reported a distinction between the risk factors for HFrEF and HFpEF. For example, Lee et al. found CHD significantly increases the risk of HFrEF whereas valvular diseases, hypertension and atrial fibrillation are more highly associated with HFpEF (D. S. Lee et al., 2009).

Among all the risk factors, LVH was associated with 15- and 13-fold increases in heart failure incidence in men and women, respectively (K. K. Ho, Pinsky, Kannel, & Levy, 1993; Mahmood & Wang, 2013). The Framingham Heart Study also observed higher incidences of CVDs and death associated with increased left ventricular mass and relative wall thickness measured by echocardiography and magnetic resonance (Levy, Garrison, Savage, Kannel, & Castelli, 1990; Tsao et al., 2015). Accordingly, the MAVI study (Verdecchia et al., 2001) demonstrated the strong prognostic role of LVH within the primary hypertensive population. Hypertensive patients with greater echocardiographic measurements of LVH have a higher risk of cardiovascular events, regardless of age, cigarette smoking, diabetes, and serum creatinine. Also, the Losartan

Intervention For Endpoint reduction in hypertension study (LIFE) found that regression of LVH (as determined by ECG measurements) is associated with reduced risk of cardiovascular events, cardiovascular and all-cause deaths, irrespective of blood pressure and all other risk factors (Wachtell et al., 2007). In conclusion, cardiac hypertrophy strongly predicts acute cardiovascular events and heart failure even when adjusted for hypertension or prior myocardial infarction (K. K. Ho, Pinsky, et al., 1993; Mahmood & Wang, 2013).

2. Cardiac remodeling

Cardiac remodeling refers to changes in myocardium and extracellular matrix (ECM), which in turn lead to alterations of the size, shape, structure, and ultimately, function of the heart. Cardiac hypertrophy, a term that describes the increase in myocardial mass, is an important component of cardiac remodeling. Given that LVH is a strong predictor of heart failure and cardiovascular death, cardiac remodeling and cardiac hypertrophy are introduced here.

2.1. Categories

Cardiac remodeling is classified into physiological and pathological remodeling based on its etiology. A comparison of characteristics of physiological and pathological cardiac remodeling is found in Table 2.

Table 2. Characteristics of physiological and pathological cardiac remodeling.

	Pathological cardiac remodeling	Physiological cardiac remodeling
Stimuli	Pressure overload (e.g. hypertension, aortic coarction) or volume overload (e.g. valvular disease) in a disease setting	Regular physical activity, chronic exercise training, and pregnancy: Volume load (e.g. pregnancy, running, walking, and swimming); Pressure load (e.g. strength training: weight lifting).
Cardiac morphology	Increased myocyte volume, Formation of new sarcomeres, Interstitial fibrosis, Myocyte necrosis and apoptosis	Increased myocyte volume, Formation of new sarcomeres
Fetal gene expression	Usually up-regulated	Relatively normal
Cardiac function	Depressed over time	Normal or enhanced (the athlete's heart) Depressed for a short time (pregnancy)
Completely reversible	Not usually	Usually
Association with heart failure and mortality	Yes	No

Adapted from "Differences Between Pathological and Physiological Cardiac Hypertrophy: Novel Therapeutic Strategies to Treat Heart Failure" (McMullen & Jennings, 2007), published in *Clinical and Experimental Pharmacology and Physiology*, with permission from John Wiley and Sons.

2.1.1. Physiological cardiac remodeling

Physiological cardiac remodeling, as exemplified by the athlete's heart, occurs as a consequence of a range of adaptive responses to increased cardiac workload (Wasfy & Weiner, 2015). In particular, endurance exercise, such as swimming and running, requires greater blood supply. This is provided by enhanced cardiac output, which is achieved by adaptive modifications including an enlarged left ventricular chamber and increased left ventricular mass (Wasfy & Weiner, 2015). In contrast, strength-based exercise, such as weightlifting, involves intense muscle contraction. This leads to elevated peripheral vascular resistance and hence, increases afterload. The adaptive alterations here include increased left ventricular wall thickness and left ventricular mass (Wasfy & Weiner, 2015). Physiological remodeling that occurs in the athlete's heart is usually mild and is considered a beneficial adaptation. Also, regression of this type of remodeling was observed after detraining (Maron, Pelliccia, Spataro, & Granata, 1993; Pelliccia et al., 2002).

Another example is pregnancy-induced cardiac remodeling. Similar to the athlete's heart, in order to adapt to increased demand during the pregnancy, the heart, and in particular, the left ventricle, undergoes hypertrophy (dilated left ventricular chamber and increased left ventricular mass). However, unlike the maintained or improved cardiac function in the athlete's heart, pregnancy-induced hypertrophy is accompanied by short-term systolic and diastolic dysfunction (Schannwell et al., 2002). Apart from the LVH, sex hormones such as estrogen and testosterone are dramatically enhanced during pregnancy, which are speculated to participate in the development of LVH, although the specific roles remain unclear (J. Li et al., 2012). Pregnancy-induced LVH is considered a physiological phenotype because in most cases, LVH regresses, and systolic and diastolic

function are restored following delivery (Schannwell et al., 2002). However, healthy women may develop peripartum cardiomyopathy during late pregnancy and up to six months postpartum, which could be fatal. The mechanism is unclear, but factors including age >30 years, smoking, hypertension during pregnancy, and African American ethnicity increase the risk of peripartum cardiomyopathy (Sliwa et al., 2010).

2.1.2. Pathological cardiac remodeling

Pathological cardiac remodeling occurs in response to hemodynamic stress or myocardial injury (Parker, Patterson, & Johnson, 2005). It is often associated with other risk conditions, such as ischemia and hypertension, and ultimately results in a decline in cardiac output. Hereafter, cardiac remodeling refers to pathological cardiac remodeling. Cardiac remodeling usually starts as an adaptive response to compensate for decreased cardiac output. Compensatory changes include augmented left ventricular wall thickness or dilated chamber. However, without proper management, sustained remodeling eventually contributes to decreased cardiac function and heart failure, and is known as maladaptive cardiac remodeling (Vasan, Wilson, Colucci, & Yeon, 2010).

2.2. Characteristics of cardiac remodeling

Loss of myocytes, interstitial fibrosis, and cardiac hypertrophy are the three main features involved in cardiac remodeling.

2.2.1. Loss of myocytes

Loss of myocytes by necrosis and apoptosis is a critical feature of pathological remodeling that is distinct from physiological remodeling. In the healthy heart, the growing heart muscle is accompanied by a proliferation of coronary vasculature (Oka,

Akazawa, Naito, & Komuro, 2014), so that the greater oxygen and nutrient demand can be met. However, a reduction of coronary capillary density was observed in the remodeled heart (Anversa, Capasso, Ricci, Sonnenblick, & Olivetti, 1989). This may limit the blood supply and render the myocardium more prone to ischemia. In addition, atherosclerosis-induced ischemia and myocardium infarction are highly associated with the progression of cardiac remodeling. Atherosclerosis and ischemic insults involve oxidative stress due to increased ROS generation and reduced activity of SODs (Sawyer et al., 2002). Increased oxidative stress is a key stimulus that triggers a series of apoptotic cascades; commonly recognized proapoptotic mediators include p38 MAPK, JNKs, p53, and bax (see section 3.2.4) (Nadal-Ginard, Kajstura, Leri, & Anversa, 2003). Consequently, the increased risk of ischemia leads to further cell death. Unfortunately, cardiac myocytes generally stop dividing after birth (although recent evidence implied a very slow rate of proliferation, the rate is too slow to repair the injured area) (Brooks, Poolman, & Li, 1998; Mollova et al., 2013). Eventually, the loss of myocytes may diminish wall thickness, weaken contractile force, and lead to systolic dysfunction.

2.2.2. Myocardial fibrosis

Cardiac fibroblasts are the major cells that constitute the connective tissues in the heart and account for more than 50% of the cell population (Camelliti, Borg, & Kohl, 2005). Fibroblasts play important roles in maintaining myocardial and vascular structure and synchronizing electrical conduction.

First, fibroblasts synthesize and degrade ECM proteins (such as collagen and fibronectin), which serve as a mechanical scaffold for the myocardium. In normal conditions, the synthesis and degradation of ECM are well-regulated by fibroblasts.

However, fibrosis occurs due to over-production of ECM and/or suppression of ECM degrading proteins (i.e. matrix metalloproteinases [MMP]). This in turn impairs myocardial contraction and relaxation, and gives rise to systolic and diastolic dysfunction (Souders, Bowers, & Baudino, 2009). Acute myocardial infarction is a common stimulus of fibrotic scar formation. In addition, many clinical studies showed increased collagen synthesis or fibrosis in patients with hypertrophic cardiomyopathy, either asymptomatic or symptomatic, but mainly in hypertrophic regions, and the severity of fibrosis is negatively correlated with regional contractility (Choudhury et al., 2002; C. Y. Ho et al., 2010).

Second, fibroblasts synchronize electrical signals by interacting with myocytes via gap junction connexins (Goshima, 1970). This electrical coupling with myocytes facilitates the transduction of contractile force (Kohl, Kamkin, Kiseleva, & Noble, 1994). However, overproduction of ECM in fibrosis disrupts gap junction structure, and further interferes with propagation of the action potential. This eventually results in isolation of the fibrotic region from the paced electrical signal and causes arrhythmias (de Jong, van Veen, van Rijen, & de Bakker, 2011; Spach & Boineau, 1997). Studies revealed that cardiac remodeling determined by echocardiographic, ECG and cardiac magnetic resonance measurements is positively correlated with the incidence of atrial fibrillation (Chrispin et al., 2014; Okin et al., 2006), and regression of remodeling in response to anti-hypertensive pharmacotherapy lowered the onset of atrial fibrillation (Okin et al., 2006).

In conclusion, fibrosis, caused by over-production of ECM, leads to stiffening of cardiac tissue, impairs contraction and relaxation, and contributes to arrhythmias.

2.2.3. Cardiac hypertrophy

Cardiac hypertrophy refers to an increase in myocardial mass, and may or may not be accompanied by dilation of heart chambers. Among the four chambers of the heart, hypertrophy of the left ventricle is most common. As LVH significantly increases the risk of heart failure (Levy et al., 1990), it is an important research target.

2.2.3.1. Categories of LVH

Based on the geometric nature of changes, LVH can be classified as concentric LVH or eccentric LVH. Concentric cardiac hypertrophy is usually due to pressure overload, for example, hypertension. In order to accommodate the elevated afterload, sarcomeres are added in parallel and the myocytes grow laterally. This leads to an increase in ventricular mass and relative wall thickness. These characteristics of concentric hypertrophy are very common in HFpEF. In fact, along with myocardial fibrosis, concentric hypertrophy impairs ventricular diastolic function (Andersen & Borlaug, 2014).

In contrast, eccentric hypertrophy is usually due to volume overload, for example, valvular regurgitation. In order to adapt to increased blood filling, sarcomeres are added longitudinally and myocytes are lengthened. This leads to ventricular dilation. According to the Frank-Starling law (Moss & Fitzsimons, 2002), within a certain range, stroke volume positively correlates with the end diastolic volume such that increased sarcomere length contributes to greater contraction force. Therefore, initially, eccentric LVH increases or maintains the cardiac output as an adaptive response. However, when the sarcomeres are overstretched, the stroke volume stops increasing despite the chamber dilation. At this time, ventricular systolic function is impaired, which is a key feature of

HFrEF. In addition, reduced systolic function causes fluid retention in the pulmonary and systemic circulation, thereby leading to edema. In the pulmonary circulation, edema impairs pulmonary diffusion capacity, and this results in dyspnea (Ganau et al., 1992).

The prevalence of concentric and eccentric LVH is similar in patients with primary or essential hypertension, whereas concentric LVH is more common than eccentric LVH in patients with secondary hypertension due to renal disease (Radulescu, Stoicescu, Buzdugan, & Donca, 2013).

2.2.3.2. Myocyte hypertrophy

Cardiac myocytes are the major functional cells in the heart, accounting for approximately 75% of the heart volume (Vliegen, van der Laarse, Cornelisse, & Eulderink, 1991). Myocyte enlargement plays a crucial role in the development of cardiac hypertrophy. Stimuli such as hemodynamic stress and neurohormonal factors induce alterations of the surviving myocytes to maintain stroke volume; these include reactivation of the fetal gene program, acceleration of protein synthesis and increase in myocyte size.

2.2.3.2.1. Fetal gene activation

The fetal gene program regulates a group of proteins that are primarily expressed in the fetal stage, and are replaced by other isoforms after birth. For example, in rodent models, the predominant isoform of the sarcomeric protein, myosin, is switched from β-myosin heavy chain (β-MHC) in the fetal heart to α-myosin heavy chain (α-MHC) in the adult heart. In addition, there is a shift from skeletal α-actin to cardiac α-actin in human hearts from the fetal to adult phase (Cox & Marsh, 2014). However, when the heart undergoes hypertrophy, the fetal gene program is reactivated (Taegtmeyer, Sen, & Vela,

2010). Those reactivated fetal genes include the following (Cox & Marsh, 2014; Dirkx, da Costa Martins, & De Windt, 2013; Fuller, Gillespie-Brown, & Sugden, 1998; Taegtmeyer et al., 2010): i) early response genes for transcriptional factors, such as c-fos, c-jun, c-myc, heart and neural crest derivatives expressed protein 1/2 (HAND 1/2), myocyte enhancer factor 2 (MEF 2), GATA4, NK2 homeobox protein NKX2-5/CSX and serum response factor (SRF), etc., ii) genes for contractile proteins such as β-MHC for rodents, skeletal α-actin and N2BA isoform of titin, 3) genes for ANP and BNP, and 4) genes for proteins involved in fetal energy metabolism, which assist the utilization of glucose over fatty acids as energy substrates, such as malonyl-CoA (Stanley, Recchia, & Lopaschuk, 2005).

It is worth mentioning that ANP and BNP are widely used clinically to diagnose, and as a prognostic marker for heart failure. As implied from their names, ANP is primarily secreted from atrial myocytes. BNP was first discovered in the brain, but is primarily released from the ventricles. Both are secreted from myocytes in response to high mechanical stress and exert diuretic and vasodilatory actions. Studies showed that ANP reduced synthesis of collagen, the inflammatory molecule, tenascin-C, and endothelin-1 (ET1) from cardiac fibroblasts, and attenuated myocyte enlargement stimulated by angiotensin II (Fujita et al., 2013). Inhibition of ANP degradation in heart failure patients reduced aldosterone levels and tended to lessen the hemodynamic load (Elsner, Muntze, Kromer, & Riegger, 1992). Compared to ANP, BNP is more widely used clinically to diagnose heart failure and determine prognosis, because the difference in the plasma concentration of BNP between normal subjects and heart failure patients is greater than ANP (de Lemos, McGuire, & Drazner, 2003). Two forms of BNP are assessed in the clinical measurements; BNP >100 pg/mL and N-terminal pro-BNP >900

pg/mL for patients >50 years old are generally considered as heart failure compared to the normal concentration of 10 pg/mL (Januzzi et al., 2006). In addition, an elevated BNP level independently predicts hospital readmission and cardiac death in heart failure patients (de Lemos et al., 2003).

2.2.3.2.2. Protein synthesis

The second feature of myocyte hypertrophy is enhanced protein synthesis rate. As mentioned above, the fetal gene program is reactivated, and it is followed by protein synthesis. Increased protein synthesis rate is mediated at the transcriptional and translational levels (Hannan, Jenkins, Jenkins, & Brandenburger, 2003). Although there is a switch between different isoforms of specific proteins, for instance, α-MHC is replaced by β-MHC in myocyte hypertrophy of rodent heart, net protein synthesis is also increased (Hannan et al., 2003). For example, Ojamaa et al. found that pressure overload increased β-MHC mRNA by 319% and decreased α-MHC mRNA by only 54%, with a net increase in total MHC mRNA content (Ojamaa, Petrie, Balkman, Hong, & Klein, 1994). Translation is reportedly the key step in up-regulation of protein synthesis rate; both translation capacity (i.e. the amount of ribosomal apparatus) and translation efficiency (i.e. the protein synthesis rate per amount of ribosomes) are improved (Hannan et al., 2003). This is supported by observations that ribosomal synthesis and activity of translation initiate factor eukaryotic initiation factor 4E (eIF4E) (Makhlouf & McDermott, 1998; Wada, Ivester, Carabello, Cooper, & McDermott, 1996) and eukaryotic elongation factor-2 (eEF2) (Browne & Proud, 2002) are increased by hypertrophic stimuli. Therefore, the protein synthesis rate and total protein amount are elevated. Increased rate of transcription contributes to augmented expression of specific genes (i.e. fetal gene program) (Ojamaa

et al., 1994). The availability of mRNA is sufficient in both normal and fast-growing hearts, so increased transcriptional rate may not exert significant effects on protein synthesis rate directly (Morgan et al., 1987). However, early response genes reactivated by hypertrophic stimuli, for example, c-myc, up-regulates the expression of ribosomal apparatus, and thus promotes protein translation (Xiao et al., 2001).

2.2.3.2.3. Myocyte size

Lastly, enhanced protein synthesis contributes to enlarged myocyte volume. Individual myocytes becomes wider or longer due to the different pattern of sarcomere reassembly, leading to concentric hypertrophy or eccentric hypertrophy, respectively. The hypertrophic stimuli, ET1 and angiotensin II, increased the surface area of neonatal and adult isolated cardiac myocytes by approximately 50% and 45% respectively (M. V. Correa et al., 2014; Lu, Akinwumi, Shao, & Anderson, 2014).

Therefore, fetal gene reactivation, enhanced protein synthesis and enlarged myocyte size are considered three typical markers of myocyte hypertrophy. However, unlike physiological hypertrophy, coronary capillaries do not proliferate in proportion. This leads to further tissue infarction and therefore, cell death. Thus, interplay between cell death and myocyte hypertrophy contributes to the decompensatory progression of cardiac hypertrophy.

2.2.3.3. Hypertrophic signaling

Various stimuli cause hypertrophy through common intracellular molecular cascades including, for example, the mitogen-activated protein kinases (MAPKs) pathways. However, the scope of all molecular mechanisms involved in hypertrophy is

complex and not completely understood. Several well-established pathways are introduced here.

G protein-dependent signaling is the most common pro-hypertrophic pathway. Receptors of angiotensin II, ET1, and norepinephrine are all G protein-coupled receptors (GPCR). Binding with ligands induces conformational changes of GPCR that lead to the activation of GPCR-coupled G protein. G proteins are composed of three subunits, Gα, Gβ and Gγ, and once activated, Gα dissociates from Gβ and Gγ. The Gα monomer then initiates a series of specific effector molecules depending on the subtype: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, or $G\alpha_{12/13}$ (Tilley, 2011).

$G\alpha_s$ activates adenylyl cyclase (AC), which catalyzes the production of cAMP. This leads to the activation of cAMP-dependent protein kinase A (PKA) (Zou et al., 1999). cAMP/PKA signaling then phosphorylates extracellular signal-regulated kinases (ERK), which in turn induce myocyte growth by enhancing DNA transcription and RNA translation. The nuclear factor of activated T cells (NFAT) family of transcriptional factors plays a key role in this process by up-regulating transcriptional activity (Dorn & Force, 2005).

In contrast, $G\alpha_i$ activation desensitizes AC. However, instead of preventing cardiac hypertrophy, overexpression of $G\alpha_i$ contributes to impaired myocyte contractility (Bohm et al., 1992; Bohm et al., 1993). Furthermore, $G\alpha_i$ stimulates Ras-dependent signaling by cooperating with the Gβγ subunits (Tilley, 2011; Zou et al., 1999). Ras is a family of small GTPases that is activated while binding with GTP, but inactivated when coupled with GDP. The activity of Ras proteins is facilitated by guanine nucleotide exchange factor (GEF), which exchanges the Ras-liganded GDP to GTP. Activated Ras further stimulates a second effector, Raf-1, with subsequent ERK activation, and

phosphoinositide 3-kinase (PI3K) with downstream protein kinase B (PKB) signaling (Sugden, 2003). Both routes eventually contribute to enhanced DNA transcription and protein expression that promote myocyte hypertrophy.

$G\alpha_{q/11}$ activation initiates phospholipase C-β (PLCβ), primarily PLCβ-1b, which catalyzes the generation of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (Filtz, Grubb, McLeod-Dryden, Luo, & Woodcock, 2009). IP3 activates its receptors on the sarcoplasmic reticulum and triggers the release of Ca^{2+}, which binds to calmodulins in the cytosol. The integrated Ca^{2+}/calmodulin complex in turn activates protein kinase CAMKII and the protein phosphatase, calcineurin. CAMKII, mainly the δ isoform, phosphorylates and then depresses the nuclear import of class II histone deacetylase 4 (HDAC4), which is a MEF2 inhibitor. Therefore, CAMKII promotes MEF2 activity and MEF-2-dependent transcription of hypertrophic genes (Anderson, Brown, & Bers, 2011; Backs, Song, Bezprozvannaya, Chang, & Olson, 2006). Calcineurin activates the NFAT family of transcriptional factors, which enter the nucleus and promote the transcription of hypertrophy-related proteins. DAG activates protein kinase C (PKC), a family that comprises many isoforms. Among those expressed in the heart (α, β1, β2, δ, ε, and λ/ζ), PKCε is best-known to be involved in hypertrophy (Dorn & Force, 2005). PKCs are capable of activating several downstream cascades, among which ERK and ERK-stimulated transcriptional factors are major targets.

$G\alpha_{12/13}$-mediated signaling starts with activation of a group of proteins termed as Rho guanine nucleotide exchange factor (RhoGEF), which in turn activates the RhoA family of GTPase, followed by the activation of downstream cascades c-Jun NH_2-terminal kinases (JNK) and p38 MAPKs (Nishida et al., 2005). Although the mechanisms are not clearly understood, many studies reported that JNK and p38 activate numerous

substrates in the cytosol and nucleus, and exert pro-hypertrophic effects (Rose, Force, & Wang, 2010).

Although different subtypes of the G protein are involved, there are interactions between the protein kinase cascades, and most of them converge at the MAPKs, which are considered the central regulator of cardiac myocyte hypertrophy.

2.3. Hypertrophy Stimuli

Myocardial injury, hemodynamic overload and neurohormonal activation are three main causes of cardiac hypertrophy.

2.3.1. Myocardial injury

Myocardial injury such as myocardial infarction causes cell death directly, and also leads to fibrous scar formation and myocyte hypertrophy, which further exacerbate apoptosis (Konstam, Kramer, Patel, Maron, & Udelson, 2011). Cardiac hypertrophy begins immediately after myocardial infarction. Furthermore, ventricular dilation was observed three hours following the onset of myocardial infarction (Korup et al., 1997), and can deteriorate for as long as 6 to 12 months (Giannuzzi et al., 2001; Rumberger, Behrenbeck, Breen, Reed, & Gersh, 1993). Anterior infarction causes more pronounced ventricular wall dilation and tends to deteriorate for a longer period compared to inferior infarction (Korup et al., 1997; Rumberger et al., 1993). Post-infarction remodeling extends beyond the infarcted zone and into the non-infarcted zone. Early remodeling includes apoptosis, scar formation and myocardial dilation, which collectively result in impaired contractility. The non-infarcted myocardium stretches in an attempt to distribute the extra workload from the infarcted zone, thus yielding overall ventricular hypertrophy (Sutton & Sharpe, 2000).

2.3.2. Hemodynamic overload

Hemodynamic overload includes pressure overload and volume overload. Pressure overload is an increase in afterload, which refers to the pressure that the heart must overcome in order to eject blood into the aorta. Examples include hypertension or aortic stenosis. Volume overload is due to elevated preload, which refers to the pressure generated during passive filling. Conditions that may cause left ventricular volume overload include aortic and mitral regurgitation. Pressure overload and volume overload contribute to concentric hypertrophy and eccentric hypertrophy, respectively (Lorell & Carabello, 2000).

2.3.3. Neurohormonal activation

Neurohormonal activation contributes to cardiac hypertrophy both directly and indirectly by provoking hemodynamic stress and myocardial injury (Sutton & Sharpe, 2000).

2.3.3.1. Angiotensin II

Angiotensin II is an important component of the renin-angiotensin-aldosterone system (RAAS). Production of angiotensin II includes two steps: i) generation of angiotensin I from angiotensinogen catalyzed by renin released from kidney, and ii) conversion to angiotensin II from angiotensin I catalyzed by ACE. ACE is abundantly expressed in endothelial cells from various organs and tissues, and was also detected in vascular smooth muscle cells, fibroblasts, and cardiac myocytes (Fleming, 2006). Moreover, higher levels of ACE have been detected adjacent to the infarct area (Hokimoto et al., 1996). Thus, angiotensin II is synthesized both systemically and locally in the myocardium in response to mechanical stretch (Sadoshima, Xu, Slayter, & Izumo,

1993). Locally, two GPCRs of angiotensin II, that is, AT1 and AT2 receptors, are present in the heart (Griendling, Lassegue, & Alexander, 1996). Angiotensin II induces cardiac myocyte hypertrophy and promotes fibroblast proliferation primarily by activating AT1 receptors (Sadoshima & Izumo, 1993). Systemically, angiotensin II constricts blood vessels and increases sodium and water reabsorption, thereby elevating blood pressure. In addition, angiotensin II stimulates the secretion of aldosterone, a hormone that further promotes sodium reabsorption and water retention. Moreover, aldosterone induces cardiac hypertrophy directly (Okoshi et al., 2004). Accordingly, ACE inhibitors and ARBs are recommended as first-line antihypertensive treatments. In summary, angiotensin II contributes to the progression of cardiac remodeling by directly stimulating myocardial fibrosis and myocyte hypertrophy, as well as by increasing hemodynamic load.

2.3.3.2. ET1

ET1 is a 21-amino acid vasoconstrictor peptide that is primarily secreted by endothelial cells, but is also synthesized by cardiac myocytes and fibroblasts (Chao et al., 2005; Suzuki, Kumazaki, & Mitsui, 1993). Two GPCRs of ET1, ET_A and ET_B, are expressed in cardiac myocytes, in which ET_A receptors are predominant and constitute approximately 82% in adult rat left ventricles (Ito, 1997). $ET_{C,}$ a third receptor with a low affinity for ET1 was also isolated. However, its function remains to be defined (Karne, Jayawickreme, & Lerner, 1993). Increased ET1 levels have been observed after mechanical stretch (Yamazaki et al., 1996) and in a variety of heart diseases such as myocardial infarction (Miyauchi et al., 1989; Stewart, Kubac, Costello, & Cernacek, 1991), dilated cardiomyopathy (Hiroe et al., 1991), and heart failure (Lerman, Kubo,

Tschumperlin, & Burnett, 1992; McMurray, Ray, Abdullah, Dargie, & Morton, 1992; C. M. Wei et al., 1994). *In vitro* and *in vivo* studies confirmed the effects of ET1 on cardiac hypertrophy, manifested by increased cell surface area, protein synthesis rate, transcription of contractile protein genes (Ito et al., 1993; Ito et al., 1991; Suzuki, Hoshi, & Mitsui, 1990), and cardiac fibrosis (Ceylan-Isik et al., 2013; Fujisaki et al., 1995). ET1 also acts as an intermediate signal. Activation of MAPKs and PKC by mechanical stress or angiotensin II is suppressed by an ET_A antagonist (Yamazaki et al., 1993), and ET1 mediates, at least partially, angiotensin II-induced cardiac fibrosis and hypertrophy (Adiarto et al., 2012; Fujisaki et al., 1995). There were also observations that ET_A and/or ET_B receptor antagonists prevent left ventricular dysfunction and hypertrophy (Mishima et al., 2000), as well as improve survival of rats with chronic heart failure (Mulder et al., 1997; Sakai et al., 1996).

2.3.3.3. Norepinephrine

Norepinephrine is the neurotransmitter released from sympathetic neurons that activate adrenergic receptors. Adrenergic receptors are GPCRs, and they are present in the heart as several isoforms. β-adrenergic receptors account for approximately 90% of adrenergic receptors in the heart, and of the three isoforms (β_1, β_2 and β_3), β_1 is the predominant subtype. The remaining 10% are α-adrenergic receptors (α_1 and α_2) (Cotecchia, Del Vescovo, Colella, Caso, & Diviani, 2015). By activating β-adrenergic receptors, norepinephrine exerts sympathetic effects on the cardiovascular system (i.e. promotes contractility, heart rate, electrical conduction, vessel constriction and renin release), and therefore results in high blood pressure and arrhythmia. By activating α-adrenergic receptors, norepinephrine directly promotes cardiac hypertrophy (Simpson,

1983). *In vitro* and *in vivo* studies observed myocyte hypertrophic markers (i.e. enhanced protein synthesis, reactivated fetal gene program and enlarged myocyte size) and increased ventricular mass induced by norepinephrine (Cotecchia et al., 2015; Marino, Cassidy, Marino, Carson, & Houser, 1991; Thandapilly et al., 2011). Norepinephrine may also induce cardiac hypertrophy indirectly. For example, an increase in ET1 mRNA was detected in ventricular myocardium infused with norepinephrine (Kaddoura, Firth, Boheler, Sugden, & Poole-Wilson, 1996).

In closing, cardiac hypertrophy is a complex process that involves the interplay of hemodynamic overload, myocardial injury and neurohormonal factors.

2.4. Cardiac hypertrophy - interventions

As cardiac hypertrophy is usually initiated by myocardial injury or hemodynamic stress (i.e. CHD, hypertension, and heart attack), the current therapies attempt to reduce or reverse these risk factors.

Lifestyle modification is always an important adjunct to pharmacological treatment and surgical intervention. Recommended lifestyle changes include no smoking, balanced diet with high fiber, low sodium and low saturated fat diets, regular exercise, and limited or moderate alcohol consumption (Luz, Nishiyama, & Chagas, 2011).

In addition to life style modifications, blood pressure control medications, especially ACE inhibitors and ARBs, are considered standard therapies for cardiac hypertrophy. Studies showed that ACE inhibitors reduced left ventricular mass index as determined by echocardiography in a time-dependent manner, 17.5% reduction after 7.5 months and 38.6% after 38.3 months. This marked reversal was accompanied by improved diastolic function (Franz, Tonnesmann, & Muller, 1998). The PAMELA

(Pressioni Arteriose Monitorate E Loro Associazioni) study (Mancia et al., 2002) showed that compared to hypertensive patients with inadequate control of blood pressure, effectively treated patients showed a lower prevalence and lesser degree of LVH, suggesting a positive correlation between hypertension and LVH. The LIFE study compared the effects of losartan, an AT1 receptor-selective antagonist, and atenolol, a β-blocker, on multiple cardiovascular outcomes including blood pressure and ECG-measured LVH parameters. Despite similar reductions in blood pressure, losartan produced a greater degree of LVH reversal and lesser morbidity and mortality (Dahlof et al., 1997).

It should be noted, however, that data from the PAMELA study (Mancia et al., 2002) showed a greater prevalence of LVH in hypertensive patients whose blood pressure had already reached the target compared to the normotensive group. This study unveiled the limitation of standard hypertensive therapies on hypertrophic treatment. Therefore, there remains interest in identifying the underlying mechanisms of cardiac hypertrophy from different perspectives to develop new therapeutic approaches. Potential approaches include: 1) manipulating the balance between MMP and its inhibitors to prevent fibrosis (Fingleton, 2007; Mishra, Givvimani, Chavali, & Tyagi, 2013), 2) inhibiting oxidative stress to protect against myocyte apoptosis and fibroblast proliferation (Sawyer et al., 2002; Siwik, Pagano, & Colucci, 2001), and 3) preserving energy metabolism to maintain cardiac performance (Siddiqi, Singh, Beadle, Dawson, & Frenneaux, 2013).

3. Mitochondria

As the key organelle responsible for energy production, mitochondria play a central role in cardiac function. In fact, many CVDs, including hypertrophy and heart failure, are

associated with mitochondrial abnormalities. Thus, mitochondrial dysfunction is emerging as a potential therapeutic target. Mitochondrial physiology and its role in heart diseases are discussed below.

3.1. Mitochondrial physiology

3.1.1. Structure

Mitochondria are organelles that are responsible for aerobic metabolism and the majority of ATP production. In humans, mitochondria exist in all cells except mature red blood cells, and mitochondrial content varies in different cell types. In fact, mitochondria are dynamic organelles that constantly undergo fusion and fission to adapt to various energy situations (Westrate, Drocco, Martin, Hlavacek, & MacKeigan, 2014).

Mitochondria have two membranes - the outer mitochondrial membrane and the inner mitochondrial membrane that divide the mitochondria into the intermembrane space and the matrix. These two membranes are composed of phospholipid bilayers, but are distinct in morphological and physiological properties. The outer membrane has a protein-to-lipid ratio of 1:1, similar to the cell membrane, and contains porins that allow molecules of up to 5 kDa to diffuse freely. Larger proteins are imported into the mitochondria via the outer membrane translocation machinery; this process includes receptor binding and translocation through protein-conducting channels (Herrmann & Neupert, 2000). In contrast, the inner membrane has a higher protein-to-lipid ratio (4:1). Its surface area is extended due to the formation of cristae, and it is impermeable to virtually all molecules. Ions, small molecules and proteins are imported into the matrix through the inner membrane translocase complex machinery. In addition, there exist enzymes within the inner membrane to facilitate the transport of specific molecules. For

example, carnitine palmitoyltransferase I (CPT-1) facilitates transport of fatty acyl-CoA. The inner membrane also carries respiratory chain complexes that are responsible for oxidative phosphorylation. Thus, the inner membrane is a central site for mitochondrial integrity and function.

Enclosed within the mitochondrial outer and inner membranes is the matrix, which serves as the site of metabolism, and therefore contains abundant substrates, enzymes and metabolites. The two major metabolic pathways that occur in the matrix are β-oxidation and the tricarboxylic acid (TCA) cycle. These generate a small amount of ATP and substrates for oxidative phosphorylation, which occurs in the inner membrane (Krauss, 2001).

3.1.2. Mitochondrial functions

Mitochondria are well known for their role in energy metabolism. Other processes such as apoptosis and oxidative stress are regulated by mitochondria as well.

3.1.2.1. Energy metabolism

Fatty acid oxidation and glucose utilization will be introduced here because they serve as major energy sources for cardiac myocytes. Glucose and fatty acids undergo glycolysis and β-oxidation, respectively, before these pathways merge at the TCA cycle and are followed by oxidative phosphorylation.

3.1.2.1.1. Fatty acid oxidation

Fatty acids are oxidized in three stages: β-oxidation, the TCA cycle and oxidative phosphorylation. Briefly, following cellular uptake into the cell, fatty acids are converted to acyl-CoA by fatty acyl-CoA synthetases in the cytosol. However, the mitochondrial

inner membrane is not permeable to acyl-CoA; thus, before entering the matrix for further metabolism, the CoA is replaced by carnitine to form acyl-carnitine. This step is catalyzed by CPT-1, which is located in the outer membrane. Acyl-carnitine is then transported across the inner membrane into the mitochondrial matrix by carnitine:acylcarnitine translocase. Once inside the matrix, acyl-carnitines are converted back to acyl-CoA by CPT-2, which is located on the interior side of the inner membrane, and the free carnitines are transported back to the intermembrane space via carnitine:acylcarnitine translocase (Kerner & Hoppel, 2000).

After entering the matrix, acyl-CoA undergoes β-oxidation, a process that converts acyl-CoA into acetyl-CoA. Enzymes involved in this stage of oxidation are acyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and thiolase enzyme.

The acetyl-CoA then enters the TCA cycle, which is a series of reactions that use acetyl-CoA to release a small amount of ATP and produce NADH and FADH2. From one molecule of acetyl-CoA, 1 ATP, 3 NADH and 2 FADH2 are produced. NADH and FADH2 are proton donors that are ultimately consumed during oxidative phosphorylation (see 3.1.2.1.3).

3.1.2.1.2. Glucose oxidation

The aerobic metabolism of glucose consists of glycolysis, the TCA cycle and oxidative phosphorylation. Glycolysis is the process in which glucose is broken down to two molecules of pyruvate, with a net generation of 2 ATP and 2 NADH. It occurs in the cytosol and does not require oxygen. Pyruvate is then transported into the mitochondrial matrix via members of the mitochondrial pyruvate carrier (MPC1 and MPC2), where it is

converted to acetyl-CoA by pyruvate dehydrogenase (Gray, Tompkins, & Taylor, 2014). Acetyl-CoA then enters the TCA cycle as described above.

3.1.2.1.3. Oxidative phosphorylation

As depicted in Figure 1, NADH and FADH2, yielded from the TCA cycle, serve as electron donors in the electron transport chain (ETC) and initiate oxidative phosphorylation. Briefly, the ETC is composed of several protein complexes (complex I, II, III, and IV) embedded in the mitochondrial inner membrane. ETC couples with ATP synthase to carry out oxidative phosphorylation. Electrons donated from NADH are passed to complex I (NADH dehydrogenase), and then are shuttled to complex III (cytochrome b-c1) by coenzyme Q (ubiquinone). In contrast, complex II accepts electrons from FADH2 and again, electrons are passed to complex III via coenzyme Q. Electrons are then transferred to complex IV (cytochrome oxidase) by cytochrome C. Eventually, electrons are received by oxygen to form water. During electron transport, energy is released to pump protons out to the intermembrane space from complex I, III, and IV and to build a proton gradient across the mitochondrial inner membrane. The energy trapped in this potential is released when the protons flow back down its gradient into the matrix through ATP synthase, whereby ATP is generated.

Each glucose molecule generates approximately 31 ATP with consumption of 6 oxygen molecules. In contrast, for fatty acids such as a single palmitic acid molecule, approximately 105 ATP are generated with consumption of 23 oxygen molecules (Fillmore, Mori, & Lopaschuk, 2014). Therefore, glucose oxidation is commonly regarded as more oxygen efficient than fatty acid oxidation.

Figure 1. Oxidative phosphorylation.

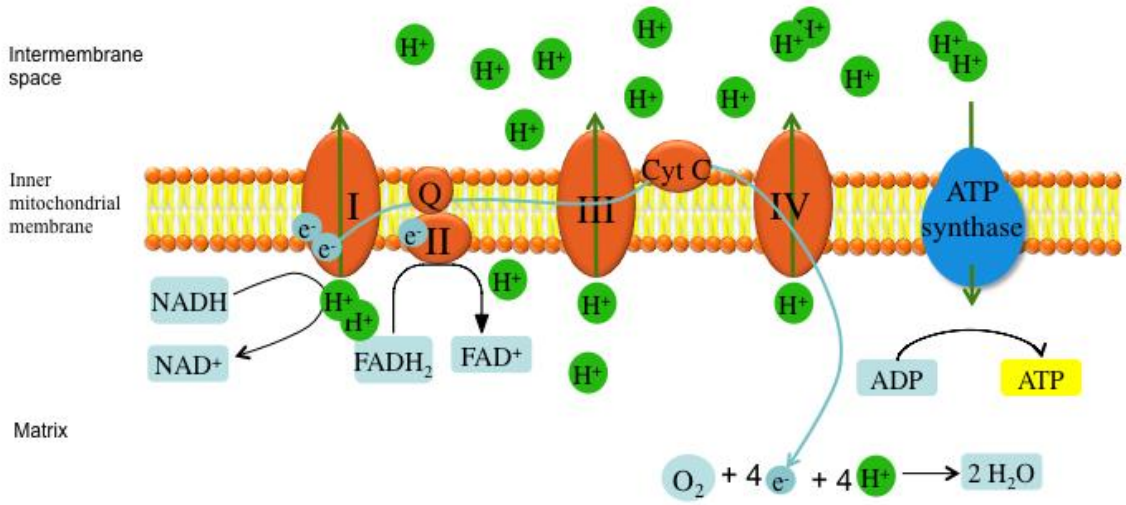


Figure 1. Oxidative phosphorylation, which involves coupling between the electron transport chain and ATP synthase, generates ATP and H_2O.

3.1.2.2. Proton leak

The efficiency of oxidative phosphorylation is defined as the degree of coupling between substrate oxidation (i.e. electron transport) and ATP synthesis. Full efficiency refers to the theoretical scenario in which energy retained in the proton gradient is used entirely to synthesize ATP. However, even under normal conditions, protons leak down its gradient from the intermembrane space to the matrix through uncoupling protein (UCP), specifically, UCP1, thereby generating heat. This is called *proton leak*. Three isoforms of UCP were discovered: UCP1, 2 and 3. UCP1 exists predominantly in high concentrations in adipose tissues, mediating thermogenesis (Nicholls, Bernson, & Heaton, 1978). One hypothesis suggests that, instead of transporting protons directly, UCP1 actually transports fatty acid anions, which then induce proton leak (Garlid, Orosz, Modriansky, Vassanelli, & Jezek, 1996). UCP1 has also emerged as a potential target for

body weight management, as UCP1 deficiency induced obesity in mice (Feldmann, Golozoubova, Cannon, & Nedergaard, 2009). Basal proton leak is essential and significant, playing physiological roles in terms of body temperature maintenance during cold exposure. It was estimated that approximately 26% and 50% of resting respiration is due to basal proton leak in hepatocytes and muscle cells, respectively (Brand et al., 1999). Although the mechanism of basal proton leak is not fully understood, it is widely believed that the magnitude of basal proton leak correlates with the fatty acyl composition and adenine nucleotide translocase (ANT) content of the inner membrane (Brand et al., 2005).

In addition to basal proton leak, inducible proton leak can be activated or inhibited, and is regulated by UCP2 and 3, although the mechanisms are still unclear. UCP2 is expressed widely, whereas UCP3 is predominantly expressed in skeletal muscle and heart tissue at low concentrations, and neither transports protons unless activated (Schrauwen & Hesselink, 2002). In fact, there is abundant evidence to suggest that increased reactive oxygen species (ROS) levels activate UCP2 and UCP3 and induce mild proton leak, which then lowers ROS production as negative feedback (Brand et al., 2004; Mailloux & Harper, 2011). Thus, UCP2/3 activation may serve as a protective approach against oxidative damage and its related conditions, such as aging, inflammation and ischemia reperfusion injury.

In closing, although it impairs the efficiency of oxidative phosphorylation, proton leak plays a significant role with respect to maintaining basal physiological conditions such as body temperature. Also, induction of proton leak mediated by UCP2/3 is a physiological process, to which beneficial effects have been linked.

3.2. Mitochondrial pathology in heart diseases

The heart contracts ceaselessly, and thus requires large amounts of ATP. Mitochondria are the major sites of ATP production, and the ETC embedded within the mitochondrial inner membrane is responsible for oxidative phosphorylation, thereby yielding approximately 95% of the total ATP (Ashrafian, Frenneaux, & Opie, 2007). Accordingly, cardiac myocytes possess the greatest mitochondrial content (30% of myocyte volume) in order to meet the high and dynamic energy demand (Kolwicz, Purohit, & Tian, 2013). A variety of substrates may be used by cardiac myocytes as fuel, including fatty acids, carbohydrates, and ketone bodies (Wallace, 1959). However, it is well-established that under normal conditions, cardiac myocytes use predominantly fatty acids, and this accounts for 50% to 70% of total ATP production (Lopaschuk, Ussher, Folmes, Jaswal, & Stanley, 2010; Opie, 1968).

Given its important function, it is not surprising that mitochondrial dysfunction has been linked to numerous cardiac pathological conditions or diseases, including ischemia reperfusion injury (Stanley, 2004), cardiomyopathy (Taylor, Bhandari, & Seymour, 2015), and heart failure (Doenst, Nguyen, & Abel, 2013). The most commonly discussed mitochondrial abnormalities associated with heart disease are summarized below.

3.2.1. Disrupted energy metabolism

Energy metabolism in the diseased heart is characterized by a shift of energy substrate from fatty acids to glucose. Healthy hearts rely mainly on fatty acid oxidation for ATP production. However, in the hypertrophied heart, the rate of fatty acid oxidation is reduced by 30-40% (Allard et al., 1994; el Alaoui-Talibi, Landormy, Loireau, & Moravec, 1992; Schonekess, Allard, & Lopaschuk, 1995). Meanwhile, reduced fatty acid

oxidation may be accompanied by increased utilization of glucose (Dodd et al., 2012), which involves glycolysis and glucose oxidation. The former is the conversion of glucose to pyruvate in the cytosol, whereas the latter, which involves pyruvate oxidation, occurs in mitochondria.

The shift from fatty acid to glucose as energy substrate is considered a compensatory mechanism, as glucose is a more efficient substrate than fatty acids in terms of ATP production per oxygen molecule (see section 3.1.2.1.3). Moreover, Korvald et al. detected a 48% increase in myocardial oxygen consumption in pigs following lipid infusion compared to glucose infusion (Korvald, Elvenes, & Myrmel, 2000). Mjos also observed a 26% increase in oxygen consumption in intact dog hearts following intravenous infusion of a fatty emulsion, though cardiac output remained unchanged (Mjos, 1971).

Nevertheless, the switch to glucose utilization in the diseased heart, while compensatory, is ultimately inadequate to meet cardiac energy demand. Accumulating evidence suggests that total ATP is decreased in hypertrophied and failing hearts (Akhmedov et al., 2015; Allard et al., 1994). First, complete glucose oxidation generates approximately 20% less ATP per carbon atom than fatty acids (Lou et al., 2013). Second, the increase in glucose utilization in diseased heart is mostly due to accelerated glycolysis, which is not accompanied by a concomitant increase in glucose-dependent oxidative phosphorylation (aka glucose oxidation). In fact, some studies found an unaltered rate while others found decreased rates of glucose oxidation in the hypertrophied heart. For example, Wambolt et al. reported a correlation between ischemia-induced cardiac hypertrophy and enhanced glycolysis, and the latter was normalized with the reversal of hypertrophy. However, glucose oxidation rates remained the same among the groups

(Wambolt, Henning, English, Bondy, & Allard, 1997). Allard et al. (Allard et al., 1994) reported increased glycolysis in the hypertrophied rat heart at both normal and high workloads; however, glucose oxidation was not altered by hypertrophy, although its contribution to ATP production was significantly elevated in response to high workload. Moreover, higher rates of glycolysis and lower rates of glucose oxidation were observed in post-ischemic and pressure overload-induced hypertrophied hearts (Allard et al., 1997; Wambolt, Lopaschuk, Brownsey, & Allard, 2000). In both cases, there exists an uncoupling between glycolysis (markedly increased) and glucose oxidation (unaltered or reduced), which is a critical phenotype of the hypertrophied heart. Mechanisms underlying this uncoupling are not yet fully understood, but increased activity of glycolytic enzymes and reduced pyruvate oxidative enzymes in diseased hearts may play a role. Indeed, expression or activity of glycolytic enzymes such as hexokinase, phosphofructokinase, and lactate dehydrogenase was enhanced in hypertensive and hypertrophic heart (Smith, Kramer, Reis, Bishop, & Ingwall, 1990; Taegtmeyer & Overturf, 1988). Second, activity of the pyruvate dehydrogenase complex, which consists of enzymes that convert pyruvate into acetyl-CoA to initiate the TCA cycle, as well as activity of citrate synthase, which catalyzes acetyl-CoA into citrate, were reduced in ischemic (Patel & Olson, 1984) and hypertrophied heart (Seymour & Chatham, 1997; Smith et al., 1990).

Given this uncoupling between glycolysis and glucose oxidation, and the potential high efficiency of glucose as a substrate (i.e. ATP production per O_2 molecule), improving glucose oxidation has been proposed as a strategy to ameliorate energy deficiency in hypertrophied cardiac myocytes. For example, palmitate/glucose-perfused rat hearts exhibit slower recovery of cardiac performance from ischemia compared to

hearts perfused with glucose alone. Improving glucose oxidation using dichloroacetate, which stimulates pyruvate dehydrogenase, in the palmitate/glucose-perfused hearts attenuated the delay in recovery (Q. Liu, Docherty, Rendell, Clanachan, & Lopaschuk, 2002).

A competitive relationship between fatty acids and glucose as energy substrate was also proposed by Philip Randle, commonly referred to as the Randle cycle (Randle, Garland, Hales, & Newsholme, 1963). Here, enhanced fatty acid oxidation inhibits glucose oxidation, and vice versa. Underlying mechanisms have been reported; for example, acetyl-CoA and NADH originating from fatty acids inhibit the activity of pyruvate dehydrogenase, whereas acetyl-CoA and NADH generated by pyruvate dehydrogenase suppress enzymes involved in β-oxidation (Eaton, Middleton, & Bartlett, 1998; Olowe & Schulz, 1980). Indeed, reduced glucose oxidation and its uncoupling from glycolysis were observed in palmitate-perfused rat hearts, and CVT-4325, an inhibitor of fatty acid oxidation, restored them (Q. Liu et al., 2002; L. Wu, Belardinelli, & Fraser, 2008).

Based on the Randle cycle, the concept of suppressing fatty acid oxidation to stimulate glucose oxidation to achieve cardioprotection was proposed (Lionetti et al., 2005). However, this concept has been challenged by opposite findings. Inhibition of fatty acid oxidation using etomoxir, an inhibitor of mitochondrial fatty acid uptake, failed to rescue cardiac dysfunction induced by pressure overload *in vivo* (Schwarzer et al., 2009). CPT-1β knockout mice also exhibited exacerbated cardiac hypertrophy and contractile dysfunction, and this was associated with reduced fatty acid oxidation (L. He et al., 2012). This agrees with human data in which decreasing fatty acid uptake using acipimox, an inhibitor of lipolysis, reduced stroke volume, cardiac output, and cardiac

efficiency in patients with dilated cardiomyopathy (Tuunanen et al., 2006). In contrast, restoring fatty acid oxidation via deletion of acetyl CoA carboxylase 2, an inhibitor of fatty acid oxidation, enhanced and prevented the development of cardiac hypertrophy (Kolwicz et al., 2012).

In conclusion, uncoupling between glycolysis and glucose oxidation, and reduced fatty acid oxidation, contribute to overall depletion of energy metabolism, which is a main feature of cardiac hypertrophy and heart failure. It is still unclear whether the shift from fatty acids to glucose as substrate is beneficial or detrimental, but the therapeutic strategy of disrupting fatty acid oxidation to improve glucose oxidation is likely to be unviable.

3.2.2. Increased oxidative stress

ROS are byproducts of oxidative phosphorylation, which occur due to incomplete reduction of oxygen by electrons that escape the ETC complexes, mainly complex I and III (Orrenius, Gogvadze, & Zhivotovsky, 2007). Also, succinate, a complex II substrate, stimulates complex I-generated ROS by inducing a reverse electron flow (Y. Liu, Fiskum, & Schubert, 2002). ROS species include the hydroxyl radical ($OH^{\cdot}$), superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and others (Kornfeld et al., 2015), and they are highly reactive via unpaired electrons. Under normal conditions, ROS are balanced by a defense system consisting of superoxide dismutase (SOD) and non-enzymatic antioxidants. However, this balance is disturbed by overproduction of ROS or lack of antioxidant activity. The resulting oxidative stress is detrimental to mitochondria and the intact cell, and contributes to a wide range of pathological conditions such as atherosclerosis,

neurodegeneration, ischemia/reperfusion injuries, cancer, etc.(Goncharov et al., 2015; Ramalingam & Kim, 2012; Tong, Chuang, Wu, & Zuo, 2015).

ROS generation by the ETC is subject to a number of regulatory mechanisms. First, membrane potential ($\Delta\psi_m$) of the inner mitochondrial membrane influences oxidative status. Slight reduction of the inner mitochondrial $\Delta\psi_m$ causes a significant decrease in ROS production (Miwa & Brand, 2003). In contrast, Korshunov et al. reported a threshold (≈140 mV) in rat heart mitochondria, above which small increases in the inner $\Delta\psi_m$ cause significant elevations in ROS generation. This may be due to a reversal in proton and electron flow at complex I caused by extremely high $\Delta\psi_m$ (Korshunov, Skulachev, & Starkov, 1997). In addition, UCP2 and 3 also modulate ROS generation. Once activated by superoxide, UCP2 and 3 dissipate $\Delta\psi_m$, induce proton leak, and in turn prevent ROS production (Echtay et al., 2002; Mailloux & Harper, 2011). Therefore, the mild proton leak regulated by UCPs may be a protective mechanism at the cost of slight loss of ATP (Jastroch, Divakaruni, Mookerjee, Treberg, & Brand, 2010). Finally, there are 3 forms of SOD in humans. SOD1, 2 and 3 are present in the cytosol, mitochondria, and extracellular space respectively. SOD2 is also referred to as MnSOD because of the manganese molecule located in the reactive centre. SOD2 catalyzes the conversion of superoxide to H_2O_2, which is more stable than superoxide, and H_2O_2 is converted to H_2O and O_2 by catalase or glutathione peroxidase (Droge, 2002; Sinha, Das, Pal, & Sil, 2013).

ROS interact with many components of the mitochondria and cause oxidative damage. They are able to degrade mitochondrial DNA, which along with nuclear DNA, encode proteins involved in the ETC; hence, this gives rise to dysfunctional oxidative phosphorylation (Mikhed, Daiber, & Steven, 2015). NADH dehydrogenase, NADH oxidase and ATP synthase are also dramatically inactivated by hydroxyl radicals and

superoxide, though they are more resistant to H_2O_2 (Y. Zhang, Marcillat, Giulivi, Ernster, & Davies, 1990). Furthermore, oxidative stress induces the opening of mitochondrial permeability transition pores (mPTP), releases cytochrome c, and then initiates the apoptosis program (Cadenas & Davies, 2000). Excessive ROS release was observed in a myriad of heart diseases, including atherosclerosis (Hulsmans, Van Dooren, & Holvoet, 2012), ischemia/reperfusion injury (Braunersreuther & Jaquet, 2012), hypertrophy (Amin et al., 2001) and heart failure (Ide et al., 1999).

3.2.3. Increased mitochondrial permeability transition (mPT)

Increased mPT results from the formation of mPTPs. These are non-specific protein channels located on the inner membrane that allow the free transport of molecules of less than 1500 Da across the inner membrane. Unlike the UCPs, which are expressed under normal conditions to maintain heat and regulate ROS production, mPTPs form only in response to pathological stimuli, and then lead to severe proton leak and impaired energy metabolism (Crompton, 1999).

The composition of mPTP is yet to be fully determined, but ANT and cyclophilin D, a mitochondrial matrix peptidyl-prolyl cis-trans isomerase, are likely involved (Brustovetsky, Tropschug, Heimpel, Heidkamper, & Klingenberg, 2002). In support of this, it is known that: i) cyclosporin A suppresses Ca^{2+} overload-induced mPT by inhibiting the interaction between cyclophilin D and ANT (Halestrap & Davidson, 1990); and ii) cyclophilin D null mice are less susceptible to mitochondrial mPT and swelling, as well as cardiac myocyte death, as stimulated by Ca^{2+} overload and oxidative stress (Baines et al., 2005). However, isolated liver mitochondria from ANT-deficient and cyclophilin D-deficient mice still underwent mPT and cell death, but required a higher

concentration of Ca^{2+}. This indicates that ANT and cyclophilin D are not essential components, but contribute to the sensitivity of mPTP to Ca^{2+} (Basso et al., 2005; Kokoszka et al., 2004). Also, the involvement of UCP in mPTP formation was ruled out based on the observation that Ca^{2+}-induced mPT is similar in control and UCP1-deficient mice (Crichton, Parker, Vidal-Puig, & Brand, 2010).

Formation of mPTP compromises the structure of the inner membrane, the most functional compartment of mitochondria, and leads to a universal collapse of mitochondrial function: dissipated mitochondrial inner $\Delta\psi_m$, impaired ATP synthesis, release of apoptotic factors (cycochrome C), and elevated ROS production, etc. These eventually contribute to cell death (Lemasters, Theruvath, Zhong, & Nieminen, 2009).

It is widely accepted that Ca^{2+} overload (Haworth & Hunter, 1979) and oxidative stress (Javadov, Karmazyn, & Escobales, 2009) stimulate mPT. In addition, disrupted inner $\Delta\psi_m$ is thought to be another strong stimulus of mPTP formation (Bernardi, 1992), although a contrasting view is that disrupted $\Delta\psi_m$ is the consequence of mPTP formation. Nonetheless, oxidative stress, mPTP opening, mitochondrial membrane depolarization, and interplay therein contribute to mitochondrial disorders and cell apoptosis, and have been observed in cardiac myocytes in response to hypertrophic stimuli such as ET1 and phenylephrine (Javadov, Rajapurohitam, et al., 2009; Peng & Jou, 2010; Shaheen, Cheema, Shahbaz, Bhattacharya, & Weber, 2011). Conversely, limiting mPT was found to be protective against cardiac diseases such as diabetic cardiomyopathy (Y. Wang et al., 2009) and ischemia/reperfusion injury (Baines et al., 2005).

3.2.4. Increased apoptosis

Mitochondria are heavily involved in the regulation of apoptosis. In fact, it is thought that increased permeability is the convergence point for different apoptotic pathways. Generally, the enhanced inner and outer membrane permeability causes swelling and rupture of the outer membrane, releases apoptotic proteins such as cytochrome c to the cytosol, and initiates a series of cell death cascades (Kinnally, Peixoto, Ryu, & Dejean, 2011).

In response to oxidative stress, p53 tumor suppressor protein contributes to apoptosis by increasing mitochondrial outer membrane permeability (Dashzeveg & Yoshida, 2015). Signaling involved in this process includes interaction with proapoptotic B-cell/lymphoma-2 (Bcl-2) family members, Bax and Bak (Chipuk et al., 2004; Leu, Dumont, Hafey, Murphy, & George, 2004). Once outer membrane permeability is elevated, cytochrome c is released to the cytosol. Cytochrome c is a protein located at the outer edge of the mitochondrial inner membrane, which transfers electrons from complex III to complex IV. It is loosely attached to the ETC due to its electrostatic interaction with an anionic phospholipid, cardiolipin. Under pathological conditions such as oxidative stress, a tight hydrophobic interaction forms between cytochrome c and cardiolipin. This dissociates cytochrome c from the ETC and converts it into a peroxidase (Birk, Chao, Bracken, Warren, & Szeto, 2014). Then, cardiolipin is peroxidized by cytochrome c and facilitates the release of cytochrome c by interacting with the Bcl-2 family members (i.e. Bax, Bak, and Bid) (Kagan et al., 2005). Upon cytochrome c release, downstream pathways are stimulated. These include binding with apoptotic protease activating factor-1 (APAF-1), formation of the apoptosome, interaction with initiator caspase-9, and activation of effector caspases-3 and -7. Finally, degradation of proteins, such as lamins,

cytoskeletal proteins, and focal adhesion complex occurs (Sinha et al., 2013), thereby destroying the structure of nuclear and cell membranes, and causing cell detachment and apoptosis (Spierings et al., 2005).

With respective to inner membrane permeability transition (discussed above), although ANT and cyclophilin D are not essential components of mPTP, they contribute to the sensitivity of the pores to stimuli such as Ca^{2+} overload. Thus, molecules that modify ANT and cyclophilin D are also likely involved in apoptosis. Indeed, the proapoptic Bcl-2 family plays a critical role in inducing mPTP opening. As a consequence, respiratory chain components such as oxidation of NADH and glutathione are affected (Sinha et al., 2013), and this further stimulates ROS production and contributes to apoptosis in a vicious cycle.

As introduced above, attenuation of maladaptive hypertrophy is regarded as a primary research target to suppress the development of heart failure (Frey, Katus, Olson, & Hill, 2004). Meanwhile, mitochondrial function is tightly associated with the progression of hypertrophy and heart failure (Rosca, Tandler, & Hoppel, 2013). Therefore, modulating mitochondrial integrity might be a promising approach towards achieving cardioprotection.

4. Endocannabinoid system.

The endocannabinoid system is a lipid signaling system that is involved in a wide range of physiological and pathological processes such as energy metabolism and inflammatory reactions.

4.1. Components

Three major components constitute the endocannabinoid system: endocannabinoids, cannabinoid receptors, and endocannabinoid degradation (Battista, Fezza, Finazzi-Agro, & Maccarrone, 2006).

4.1.1. Endocannabinoids

Endocannabinoids are endogenously-produced bioactive lipids that activate cannabinoid receptors. N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG) are the best-studied naturally-occurring endocannabinoids, which were initially identified in brain and intestine respectively (Devane et al., 1992; Mechoulam et al., 1995).

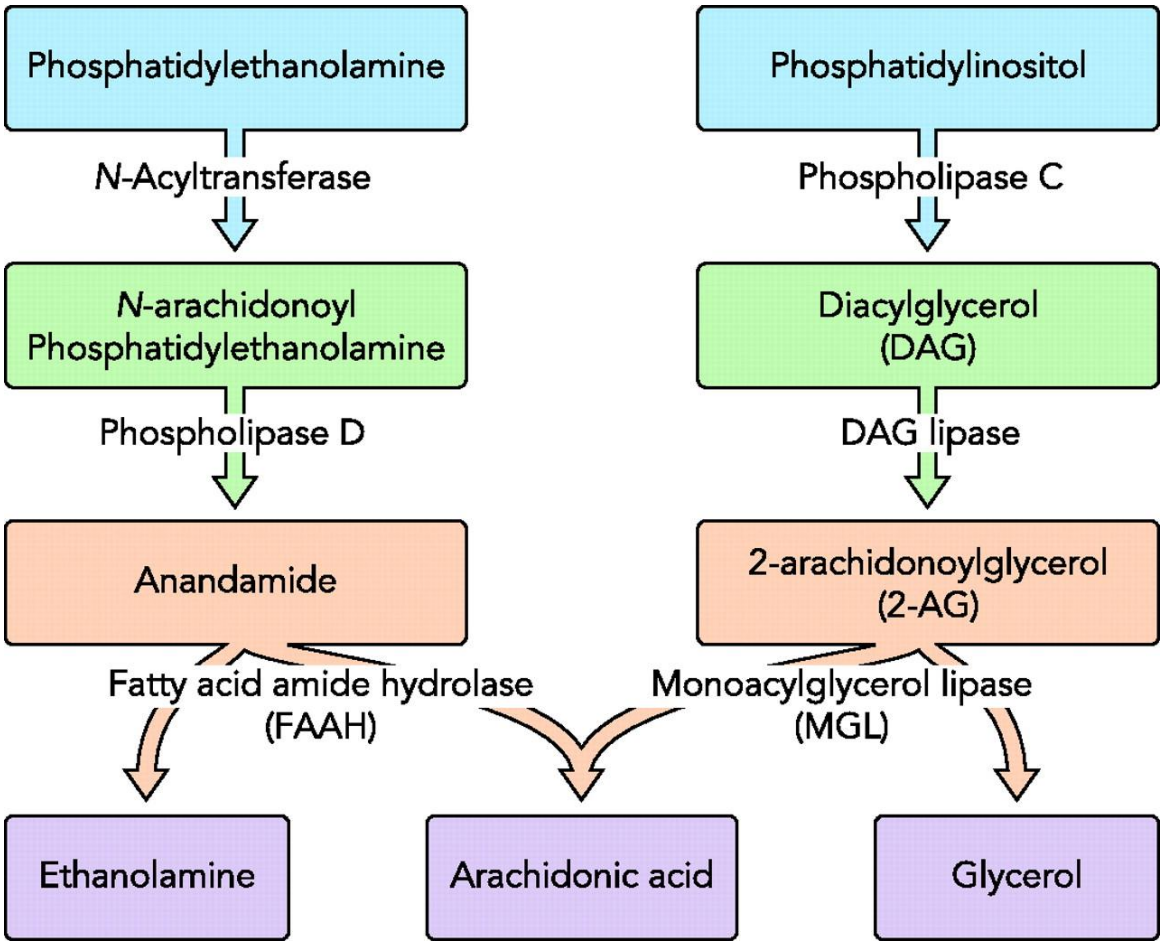
Anandamide was first discovered in porcine brain by Devane et al. in 1992 (Devane et al., 1992), and was then detected in other tissues from a variety of species: brain of bovine and rat, spleen of rat and human, skin of rat, and testis of rat, as well as human heart (≈10 pmol/g) (Felder et al., 1996). Endogenous anandamide synthesis is a two-step process (Figure 2): i) N-arachidonoyl phosphatidylethanolamine (NAPE) formation by transferring arachidonic acid from one phospholipid (phosphatidylcholine) to another (phosphatidylethanolamine) (Sugiura et al., 1996), and ii) anandamide generation from the cleavage of NAPE mainly by NAPE phospholipase D (Basavarajappa, 2007). In fact, NAPE distribution in various regions of rat brain corresponds with that of anandamide, although NAPE concentrations are much higher (Bisogno et al., 1999).

2-AG was isolated from canine intestine by Mechoulam et al. in 1995 (Mechoulam et al., 1995). Later, Kondo et al. detected 2-AG in rat brain (3.36 nmol/g), liver (1.15 nmol/g), spleen (1.17 nmol/g), kidney (0.98 nmol/g) and lung (0.78 nmol/g). Note that the

brain contains considerably higher concentrations (Kondo et al., 1998). Stella et al. also determined the concentration of 2-AG in rat brain (4 nmol/g), and this is approximately 170 times greater than anandamide (23 pmol/g) (Stella, Schweitzer, & Piomelli, 1997). In fact, the amount of 2-AG is higher than anandamide in most tissues (Mechoulam, Fride, & Di Marzo, 1998). There are also two steps in 2-AG synthesis (Figure 2): i) DAG is generated by phospholipid C-regulated hydrolysis of membrane phospholipids, followed by ii) DAG lipase-catalyzed conversion to 2-AG (Basavarajappa, 2007).

It is believed that anandamide and 2-AG are synthesized on demand upon stimulation (for example, by depolarization of postsynaptic neurons and resultant intracellular Ca^{2+} accumulation (Stella et al., 1997)), and released immediately due to their lipophilicity (Battista, Di Tommaso, Bari, & Maccarrone, 2012; Hashimotodani et al., 2013).

Figure 2. Synthesis and degradation of 2-AG and anandamide.



Adapted from "The Role of Endocannabinoid Signaling in Motor Control" published in *Physiology*.

4.1.2. Cannabinoid receptors

4.1.2.1. CB1/CB2 receptors

Two GPCRs for endocannabinoids, CB1 and CB2, have been extensively studied to date.

CB1 receptors are highly expressed in brain (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990), except in the respiratory centers of the brain stem (Howlett et al., 2002).

They are particularly dense in cerebral cortex, hippocampus, basal ganglia and cerebellum. CB1 receptors are also expressed in peripheral sensory nerves and the autonomic nervous system (Quarta et al., 2010). In addition, CB1 receptors are present at measurable levels in several peripheral tissues, including the spleen, lung, thymus, heart (Bonz et al., 2003; Howlett et al., 2002) and vasculature (Gebremedhin, Lange, Campbell, Hillard, & Harder, 1999; J. Liu et al., 2000).

In contrast, CB2 receptors are abundantly expressed in hematopoietic cells (Valk & Delwel, 1998) and in the immune system (Munro, Thomas, & Abu-Shaar, 1993), including spleen, tonsils, bone marrow, and leukocytes. Bouchard et al. reported that expression levels of CB2 receptors are comparable to that of CB1 receptors in rat hearts (Bouchard, Lepicier, & Lamontagne, 2003). It was originally believed that CB2 receptors are absent in the brain (Munro et al., 1993). However, investigators eventually detected CB2 receptor mRNA and protein expression in rat and mouse central nervous systems (CNS), including neurons in various regions of the brain (Skaper et al., 1996), although at a much lower level compared to CB1 receptors (Gong et al., 2006; Van Sickle et al., 2005). More recently, CB2 receptors were detected in cerebrovascular endothelial cells (Golech et al., 2004), microglia (Beltramo et al., 2006) and neurons, with postsynaptic localization (Brusco, Tagliaferro, Saez, & Onaivi, 2008; Callen et al., 2012; Kim & Li, 2015).

Existing evidence suggests that both CB1 and CB2 receptors are coupled to $G\alpha_{i/o}$ protein. Thus, activation of CB1 and CB2 receptors inhibits AC/cAMP/PKA/ERK signaling (Demuth & Molleman, 2006; Jung et al., 1997). In addition, CB1, but not CB2 receptors, also activate $G\alpha_s$ proteins, and stimulate cAMP production (McAllister & Glass, 2002). This dual inhibition and activation effects of CB1 receptor on AC/cAMP was

demonstrated to be ligand-specific (Bonhaus, Chang, Kwan, & Martin, 1998). Furthermore, CB1 receptors reportedly modulate Ca^{2+} channels. Activation of CB1 receptors on presynaptic neurons inhibited Ca^{2+} influx and hence suppressed neurotransmitter release (Shen & Thayer, 1998). It also inhibited Ca^{2+} current through L-type Ca^{2+} channels in arterial smooth muscle cells leading to vasodilation (Gebremedhin et al., 1999).

Anandamide exhibits marked selectivity for CB1 over CB2 receptors (Felder et al., 1995; Khanolkar et al., 1996), whereas 2-AG is less selective (Ben-Shabat et al., 1998; Mechoulam et al., 1995). To investigate the specific role of each receptor, analogues of endocannabinoids have been synthesized (Pertwee, 2006) including, for example, the CB1-selective agonist arachidonyl-2-chloroethylamide (ACEA) (Hillard et al., 1999), and the CB2-selective agonist JWH-133 (Marriott & Huffman, 2008). Synthetic agonists and antagonists are listed in Table 3 and 4, respectively.

Table 3. Cannabinoid receptor agonists.

Cannabinoid agonists	Description	Ki for CB1 (nM)	Ki for CB2 (nM)	References
2-AG	CB1 and CB2 agonist	472	1400	(Stella et al., 1997)
ACEA	CB1-selective agonist	1.4	3100	(Hillard et al., 1999)
AM1241	CB2-selective agonist	280	3.4	(Ibrahim et al., 2003)
Anandamide	CB1 and CB2 agonist	89	371	(Pertwee, 1999)
CB13	Peripherally-restricted CB1 and CB2 agonist	6.1	27.9	(Dziadulewicz et al., 2007)
CP55940	CB1 and CB2 agonist	3.72	2.55	(Felder et al., 1995)
HU-210	CB1 and CB2 agonist	0.061	0.52	(Felder et al., 1995)
JWH015	CB1 and CB2 agonist	383	13.8	(Showalter, Compton, Martin, & Abood, 1996)
JWH133	CB2-selective agonist	677	3.4	(Huffman et al., 1999)
R-methanandamide	CB1 and CB2 agonist	20	815	(Abadji et al., 1994; Khanolkar et al., 1996),
THC	CB1 and CB2 agonist	53.3	75.3	(Felder et al., 1995)
WIN55, 212-2	CB1 and CB2 agonist	3.3	62.3	(Felder et al., 1995)

Table 4. Cannabinoid receptor antagonists.

Cannabinoid antagonists	Description	Ki for CB1 (nM)	Ki for CB2 (nM)	References
AM251	CB1-selective antagonist	7.5	2290	(Lan, Liu, et al., 1999)
AM281	CB1-selective antagonist	12	4200	(Lan, Gatley, et al., 1999)
AM630	CB2-selective antagonist	5200	31.2	(R. A. Ross et al., 1999)
SR141716	CB1-selective antagonist	2	>1000	(Rinaldi-Carmona et al., 1995)
SR144528	CB2-selective antagonist	400	0.6	(Rinaldi-Carmona et al., 1998)
AM6545	Peripherally-restricted CB1-selective antagonist	1.7	523	(Cluny et al., 2010)

4.1.2.2. Other putative cannabinoid receptors

There were observations that some of the effects of anandamide cannot be explained by CB1/CB2 activation, suggesting that there might exist other cannabinoid receptors (A. J. Brown & Robin Hiley, 2009).

G protein-coupled receptor 55 (GPR55) has gained a lot of attention as a potential receptor for cannabinoid ligands that mediated effects independently of CB1 and CB2 receptors. In fact, it was recommended as a potential candidate as a third CB receptor (i.e. CB3) (A. J. Brown & Robin Hiley, 2009). GPR55 was detected in human brain, and peripheral tissues including spleen, adrenal gland and intestine (Yang, Zhou, & Lehmann, 2015). Although considered a potential cannabinoid receptor, GPR55 has a different

ligand profile from classical CB1/CB2 receptors. Ryberg et al. reported that anandamide, 2-AG, Δ^9-tetrahydrocannabinol (THC), HU210 (CB1 agonist), and AM251 (CB1 antagonist) act as agonists of GPR55. Moreover, cannabidiol, which has a restricted affinity for CB1 and CB2 receptors, acted as an antagonist, whereas WIN55, 212-2 (CB1/CB2 agonist), AM281 (CB1 antagonist) exerted neither agonistic nor antagonistic effects (Ryberg et al., 2007). In addition, GPR55 elicits signaling cascades distinct from those of CB1/CB2 receptors. GPR55 activation stimulates the $G\alpha_{12/13}$ pathway (Yang et al., 2015), and downstream effectors include RhoA/Rho-associated protein kinase (ROCK) and then JNK and p38 MAPKs (Nishida et al., 2005), as well as PLC-induced Ca^{2+} release and subsequent transcriptional modification via NFAT (Henstridge et al., 2009).

Transient receptor potential vanilloid type 1 (TRPV1) receptors are non-selective cation channels that also mediate some endocannabinoid effects. Found in central and peripheral neurons (R. A. Ross, 2003; Van Der Stelt & Di Marzo, 2004; Zygmunt et al., 1999), as well as non-neuronal cells (Fernandes, Fernandes, & Keeble, 2012), they are activated by naturally-occurring vanilloids, acid and heat, and signal a painful and burning sensation. For example, myocardial ischemia causes acidification, which then activates TRPV1 and leads to angina pain (W. Huang, Rubinstein, Prieto, Thang, & Wang, 2009). Anandamide and ACEA also activate TRPV1 (R. A. Ross, 2003). Toth et al. summarized features of the interaction between TRPV1 and anandamide: the efficacy of anandamide on TRPV1 activation depends on tissue, species, TRPV1 expression level and phosphorylation status; the concentration of anandamide required to activate TRPV1 is higher (≈10 times) than that to activate CB1; metabolites of anandamide may activate TRPV1; activation of TRPV1 stimulates anandamide synthesis; and CB1-dependent

cascades activated by anandamide (e.g. PKA or MAPKs) stimulate TRPV1 activation (Toth, Blumberg, & Boczan, 2009). The complex interaction between endocannabinoids and TRPV1 renders mechanisms of endocannabinoid-TRPV1 crosstalk difficult to elucidate.

In summary, CB1 and CB2 receptors are broadly distributed and are involved in a wide range of physiological processes. In addition, cannabinoid compounds also activate GPR55 and TRPV1 receptors, making them putative cannabinoid receptors. The profile of cannabinoid receptors requires further investigation.

4.1.3. Endocannabinoid transport and degradation

Endocannabinoid activity is rapidly terminated by cellular uptake and intracellular degradation.

The transport mechanism of anandamide and 2-AG is not completely understood, although hypotheses of passive diffusion and protein transporter facilitated diffusion have been proposed (Basavarajappa, 2007). Beltramo et al. identified an anandamide membrane transporter in rat neurons and astrocytes. Moreover, AM404, a competitive inhibitor of anandamide transport, prolonged and enhanced anandamide-stimulated CB1 activity (Beltramo et al., 1997). More recently, Fu et al. discovered an anandamide-selective transport protein in rat brain and liver. It is an analogue of fatty acid amide hydrolase (FAAH), though it lacks hydrolytic activity, and is therefore named FAAH-like anandamide transporter (FLAT). A competitive FLAT inhibitor, ARN272, also generated CB1-mediated analgesic and anti-inflammation effects by suppressing the cellular uptake of anandamide (Fu et al., 2012).

Two major endocannabinoid-metabolizing enzymes are known: FAAH for anandamide and 2-AG (Deutsch & Chin, 1993; Maccarrone et al., 1998), and monoacylglycerol lipase (MAGL) for 2-AG (Dinh, Carpenter, et al., 2002; Dinh, Freund, & Piomelli, 2002; Saario, Savinainen, Laitinen, Jarvinen, & Niemi, 2004). These enzymes hydrolyze anandamide to arachidonic acid and ethanolamine, and 2-AG to arachidonic acid and glycerol, which are recycled to form phospholipids that might integrate into the cell membrane (Figure 2) (Basavarajappa, 2007).

FAAH is widely expressed in many tissues, such as brain, liver, lung, spleen, testis, and kidney. Its expression was not detected in skeletal muscle and heart (Cravatt & Lichtman, 2002), yet myocardial anandamide levels were elevated in FAAH knockout mice; this provides indirect evidence for the presence of FAAH in the heart (Pacher, Batkai, & Kunos, 2004), perhaps at levels below detection limits. In mouse brain and liver, anandamide hydrolysis rate dropped by 100 and 50 fold, respectively, in FAAH knockout mice (Cravatt et al., 2001).

MAGL mRNA was also detected in a number of rat tissues, including adipose tissue, kidney, brain, heart, lung, liver, skeletal muscle and spleen (Karlsson, Contreras, Hellman, Tornqvist, & Holm, 1997). Blankman et al. reported that MAGL contributes to 85% of 2-AG hydrolysis in mouse brain, whereas FAAH only accounts for 1% (Blankman, Simon, & Cravatt, 2007).

Other enzymes in addition to FAAH and MAGL reportedly to degrade anandamide and 2-AG, but their activity is less clear compare to that of MAGL and FAAH (Basavarajappa, 2007; Pertwee, 2014). These include FAAH-2, N-acylethanolamine-hydrolyzing acid amidase (NAAA), α/β hydrolase domain (ABHD), cyclooxygenase-2 (COX-2), and cytochrome p450. FAAH-2 was identified in primate models, including

humans, but not in rodents. However, its hydrolytic activity for anandamide is approximately 38 times lower than FAAH (B. Q. Wei, Mikkelsen, McKinney, Lander, & Cravatt, 2006). NAAA was also identified in various human, rat, and mouse tissues (eg. lung, spleen and small intestine). Using N-palmitoylethanolamine, an anandamide analogue, as reference, rat FAAH catalytic activity shows a preference towards anandamide (V_{max}=5700 nmol/min/mg; K_m=30 μM) over N-palmitoylethanolamine (V_{max}=1800 nmol/min/mg; K_m=70 μM) (Katayama, Ueda, Katoh, & Yamamoto, 1999), whereas rat NAAA hydrolase activity for anandamide was only 8% of that for N-palmitoylethanolamine (V_{max}=1847 nmol/min/mg; K_m=35 μM) (Ueda, Yamanaka, & Yamamoto, 2001), suggesting that NAAA exerts weak hydrolase activity for anandamide compared to FAAH. Furthermore, NAAA exhibits no hydrolase activity on 2-AG (Ueda et al., 2001). Thus, it is reasonable to conclude that the role of NAAA as a hydrolase of endocannabinoids is insignificant.

Proteins that contain the ABHD (i.e. ABHD 6 and ABHD 12) are serine hydrolases that can hydrolyze 2-AG. In fact, ABHD 6 and ABHD 12 account for 4% and 9% of total 2-AG hydrolysis in mouse brain, respectively (Blankman et al., 2007). COX-2 is well known for its ability to convert arachidonic acid to the pro-inflammatory lipid, prostaglandin. COX-2 also oxygenates anandamide and 2-AG to produce ethanolamide and glycerol ester derivatives of prostaglandin respectively (Rouzer & Marnett, 2011). Likewise, various families (3A4, 4F2, and 2D6 etc.) of cytochrome p450 oxidize anandamide into different isoforms of epoxyeicosatrienoic acids ethanolamides (EET-EAs) and hydroxy-eicosatetraenoic acids ethanolamides (HETE-EAs), which exhibit diverse effects in inflammation and vascular tone modulation (Rouzer & Marnett, 2011). Cytochrome p450 was only recently implicated in 2-AG oxidation in 2014, when

McDougle et al. detected 2-AG metabolite (2-EET-glycerols) production by CYP2J2, a predominant cytochrome p450 in the heart. CYP2J2 also hydrolyzes 2-AG to glycerol and arachidonic acids in a NADPH-dependent manner (McDougle, Kambalyal, Meling, & Das, 2014). Reports of COX-2- and cytochrome p450-dependent metabolism of endocannabinoids have only recently emerged and represent novel research areas that warrant further investigation.

The aforementioned enzymes responsible for degrading endocannabinoids play an important role in terminating endocannabinoid signaling. Indeed, manipulating the levels of FAAH and MAGL by overexpression, knockdown or using their inhibitors has been an area of intense study. For example, Hohmann et al. reported that inhibition of MAGL and FAAH increased 2-AG and anandamide levels in rat brain and enhanced anti-hyperalgesic effects (Hohmann et al., 2005). Ho et al. found that FAAH and MAGL inhibitors enhanced the vasodilatory actions of anandamide and 2-AG in rat isolated small mesenteric arteries (W. S. Ho & Randall, 2007). Carnevali et al. demonstrated an anti-depressant effect of FAAH inhibition, which was associated with increases in central and peripheral anandamide levels (Carnevali et al., 2015).

Collectively, endocannabinoid signaling includes endocannabinoid biosynthesis, receptor activation, membrane transport and degradation.

4.2. (Patho-)physiological functions of the endocannabinoid systems

Due to its wide distribution throughout the body, the endocannabinoid system has been implicated in multiple physiological or pathological processes. Whether acting at CB receptor or non-CB receptor sites, cannabinoid-related compounds exert inhibitory effects on obesity, inflammation, pain, and chemotherapy-induced nausea or vomiting,

and may alleviate the symptoms of neurodenegerative diseases and multiple sclerosis. Nabilone, dronabinol, and sativex are cannabinoid-based drugs that have been approved to treat pain, appetite loss, spasticity and chemotherapy-induced nausea (Grant et al., 2012). However, it should be noted that cannabinoids are linked to unwanted side effects, particularly psychoactive in nature. Therefore, the endocannabinoid system is a convergence of benefits and risks that requires careful and comprehensive study.

4.2.1. Appetite and energy expenditure

Central CB1 receptors play an important role in appetite regulation. Kirkham et al. found that injection of 2-AG to limbic forebrain, a brain area that controls eating motivation, stimulated appetite, and this was inhibited by SR141716, an CB1 antagonist (Kirkham, Williams, Fezza, & Di Marzo, 2002). Appetite stimulation by CB1 receptors was confirmed by Cota et al., who observed reductions in food intake and body weight in CB1-deficient mice (Cota et al., 2003). In contrast, FAAH-deficient mice exhibited enhanced appetite and this was accompanied by elevated anandamide levels in hypothalamus, liver, and small intestine (Tourino, Oveisi, Lockney, Piomelli, & Maldonado, 2010). The stimulatory effect on appetite corresponds with the finding that anandamide and 2-AG levels in limbic forebrain and hypothalamus were highest during fasting, but dropped during eating (Kirkham et al., 2002). A clinical trial on dronabinol, a cannabinoid-based drug, showed improved appetite in AIDS patients, and dronabinol was subsequently approved to treat AIDS-associated anorexia (Beal et al., 1995).

In addition to appetite regulation, CB1 receptors also modulate energy expenditure. Verty et al. reported that CB1 blockade with rimonabant (SR141716) in rats enhanced thermogenesis in brown adipose tissue, and this was associated with up-regulation of

UCP1, a protein that stimulates heat production. These changes were partially attenuated by denervation, implying the role of central CB1 receptors in restricting energy expenditure (Verty, Allen, & Oldfield, 2009). A clinical trial that involved obese patients with hypertension or dyslipidemia showed that rimonabant (SR141716), a CB1 antagonist, at 20 mg/day reduced body weight and waist circumference, and also improved several cardiovascular metabolic parameters (i.e. increased high-density lipoprotein and decreased triglycerides and insulin resistance) (Van Gaal et al., 2005). These findings were in agreement with similar clinical trials on rimonabant (Pi-Sunyer et al., 2006; Scheen et al., 2006). In 2006, rimonabant was released into the European market as an anti-obesity drug, although it was quickly withdrawn due to reports of adverse effects such as nausea, depression and anxiety (Di Marzo & Despres, 2009).

To eliminate the psychiatric effects mediated by central CB1 receptors, attempts were made to evaluate the contribution of peripheral CB1 receptors to obesity. Cluny et al. observed a transient reduction in food intake and sustained body weight loss in response to a peripherally-restricted CB1 receptor antagonist, AM6545, in rats and mice (Cluny et al., 2010). Reduced hepatic triglycerides, increased expression of fatty acid oxidation genes, and improved insulin sensitivity were also confirmed with AM6545 (Tam et al., 2010). Hsiao et al. compared the effects of SR141716 and BPR0912, a peripherally-resticted CB1 receptor antagonist, on obesity parameters in diet-induced obese mice. Similar reductions in body weight, serum insulin, triglycerides, and hepatic triglycerides were observed with both compounds, but compared to SR141716, BPR0912 raised fatty acid oxidation-related gene expression and thermogenesis more significantly (Hsiao et al., 2015). In summary, activation of CB1 receptors contributes to increased appetite, suppressed energy expenditure, and when deranged, obesity. Therefore, antagonism of

CB1 receptors, particularly in the periphery, remains a promising approach to enhance adipocyte lipolysis, reduce food intake and decrease body weight (Arrabal et al., 2015; Mastinu, Pira, Pani, Pinna, & Lazzari, 2012).

The role of CB2 receptors in food intake and energy metabolism is not as extensively studied as that of CB1, but appears to oppose CB1 effects. The observation that the food intake inhibitory effect of AM6545, a CB1 antagonist, was abolished in CB1/CB2 knockout mice, but not in CB1 knockout mice indicated that CB2 receptors might be involved (Cluny et al., 2010). Onaivi et al. reported increased appetite in C57Bl/6 mice treated with the CB2 receptor antagonist AM630 after 12 h food-deprivation, but not in other strains (i.e. Balb/c and DBA/2) (Onaivi et al., 2008). Similarly, the CB2 receptor agonist JWH015 induced a transient reduction in food intake in C57Bl/6 mice, which was restored by AM630. In addition, JWH-015-induced body weight loss, reduced white adipose tissue weight and adipocyte cell size, as well as increased triglyceride lipase expression were also reported (Verty, Stefanidis, McAinch, Hryciw, & Oldfield, 2015).

4.2.2. Inflammation

Since CB2 receptors are abundantly expressed in the immune system (Munro et al., 1993), the involvement of CB2 receptors in inflammation is well-documented. Indeed, CB2 receptors play an important role in preventing gastrointestinal inflammation. Storr et al. reported that the CB2 receptor agonists, JWH133 and AM1241, attenuated colitis in mice, and pretreatment with a CB2 receptor antagonist or CB2 knockout abrogated this effect (M. Storr et al., 2009). Similar findings were observed in human colonic mucosa, where tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β)-induced inflammation,

as evidenced by luminal epithelial and crypt damage and increased lymphocyte density, was attenuated by CB2 activation (Harvey, Nicotra, Vu, & Smid, 2013). Overexpression of CB2 receptors in intestine was detected in models of inflammatory bowel disease, suggesting the important role of CB2 receptors as a compensatory anti-inflammatory response (M. Storr et al., 2009; Wright et al., 2005).

Anti-inflammatory actions of CB2 receptors were also observed in other tissue or cells. For example, anandamide and 2-AG alleviated inflammation in cultured human retinal explant, which was reflected by increased viability of retinal neurons and Muller glia, as well as reduced Muller glia proliferation. This anti-inflammatory effect was achieved by inhibiting proinflammatory cytokines (e.g. IL-6, interferon-γ (IFN-γ), and TNF-α) while elevating anti-inflammatory molecules (e.g. IL-10 and transforming growth factor-β (TGF-β)) (Krishnan & Chatterjee, 2012). CB2 receptor activation also relieved rheumatoid arthritis symptoms via multiple pathways, including inhibition of fibroblast proliferation, suppression of proinflammatory cytokine release in fibroblast-like synoviocytes, T cells and macrophages, as well as prevention of bone erosion via stimulating osteoblasts and reducing osteoclasts (Gui, Tong, Qu, Mao, & Dai, 2015).

Incidentally, the anti-inflammatory properties of cannabinoids are also an important aspect of treating neurodegenerative diseases, and cardiovascular risk factors, where inflammation is a key factor. This is discussed below.

4.2.3. Emesis

Emesis can be triggered centrally or peripherally. Stimuli such as food toxins and chemotherapeutic agents evoke vomiting primarily by inducing serotonin (5-HT) release from the epithelium of the gastrointestinal tract. By activating 5-HT receptors in afferent

nerves, signals are communicated to the emesis center in the medulla, followed by a series of motor responses. This emesis center can also be directly activated by central stimuli, for instance, aversive memories (Becker, 2010). Cannabinoids have traditionally been used to treat nausea and vomiting. Nabilone and dronabinol, synthetic analogues of THC, are approved to treat chemotherapy-induced vomiting (Sharkey, Darmani, & Parker, 2014). A clinical trial compared dronabinol, ondansetron (5-HT antagonist), and their combination on chemotherapy-induced nausea and emesis, and found similar anti-emetic effects, However, dronabinol alone showed a better effect on reducing the severity of nausea (Meiri et al., 2007). Sativex, a 1:1 combination of THC and cannabidiol, is not yet approved to treat chemotherapy-induced vomiting. However, a clinical study observed that in combination with standard anti-emetic therapy, sativex improved chemotherapy-induced nausea and vomiting compared to standard therapy alone (Duran et al., 2010). The role of the endocannabinoid system in anti-emetic effects was investigated in animal models. Hu et al. showed that CB1 receptor activation inhibited enterotoxin-induced 5-HT release from the intestine of musk shrew, suggesting a peripheral action of CB1 in the anti-emetic effect of cannabinoids (Hu et al., 2007). The role of CB1 receptors was confirmed by other studies (Darmani, 2001; O'Brien et al., 2013), but evidence on CB2 receptors is lacking.

4.2.4. Pain

Pain is regulated by the endocannabinoid system at both central and peripheral sites. Following electrical stimulation, Walker et al. detected release of anandamide in periaqueductal gray, the primary brain region for pain modulation, which coincided with the central CB1-mediated analgesic effect (Walker, Huang, Strangman, Tsou, & Sanudo-

Pena, 1999). Clapper et al. reported that URB937, a peripheral FAAH inhibitor, generated a peripheral accumulation of anandamide, and suppressed both neuropathic and inflammatory pain-related behavior responses as well as neuron activation in the spinal cord; a CB1 receptor antagonist reversed these effects. The ability of URB937 to modulate pain signals despite its lack of CNS penetration implies that activation of peripheral CB1 receptors exhibits an analgesic effect by blocking the transduction of pain signals into the CNS (Clapper et al., 2010). The CB2 receptor was originally found to suppress pain sensation by attenuating the release of pro-inflammatory molecules, which increase the sensitivity of primary afferent neurons (Malan et al., 2003). Beltramo et al. later observed a direct inhibition on pain neurotransmitter production by CB2 receptors as well, in parallel to its analgesic effects in a rat model of neuropathic pain and a mouse model of central sensitization (Beltramo et al., 2006). Finally, Romero et al. confirmed the analgesic actions of CB1 and CB2 receptors, in a manner that requires activation of peripheral adrenergic receptors by norepinephrine (Romero, Resende, Guzzo, & Duarte, 2013).

4.2.5. Endocannabinoid signaling in the CNS

The endocannabinoid system has been extensively studied in the brain. Anandamide is found in brains of animal models at concentrations of 173 pmol/g, 101 pmol/g and 30 pmol/g in porcine, bovine and rat brain, respectively (Bisogno et al., 1999; Schmid et al., 1995). Felder et al. measured anandamide concentrations in specific regions of human brain, and identified a range of approximately 35 pmol/g in cerebellum to 107 pmol/g in hippocampus (Felder et al., 1996). A brief overview on the important role of endocannabinoid signaling in a few CNS disorders is discussed below.

4.2.5.1. Multiple sclerosis

Multiple sclerosis is an autoimmune disorder characterized by axon demyelination of neurons within CNS. Cannabis has traditionally been used to relieve symptoms, and current thinking attributes the beneficial effects to immunosuppressive and neuroprotective properties of cannabinoid receptors. However, whether cannabinoid ligands delay multiple sclerosis progression remains unknown, though preclinical studies are supportive. For example, microglial cells, which are the primary immune cells in the CNS, can be activated to secrete pro-inflammatory factors, including IL-12 and IL-23, thereby contributing to the progression of multiple sclerosis. Anandamide inhibited secretion of IL-12 and IL-23 in microglial cells in a partial CB2-dependent ERK1/2 and JNK pathway (F. Correa et al., 2009). An increase in anandamide concentration was detected in inflammatory brain tissue from multiple sclerosis patients (Eljaschewitsch et al., 2006), and in experimental autoimmune encephalomyelitis mice, a model of multiple sclerosis, WIN55, 212-2 (CB1/CB2 agonist) attenuated the up-regulation of inflammatory cytokines (COX-2, iNOS and TNF-α) and microglial-induced cell aggregation in spinal cord and brainstem. These effects of WIN55, 212-2 were reversed by a CB1 receptor antagonist (de Lago, Moreno-Martet, Cabranes, Ramos, & Fernandez-Ruiz, 2012). Increasing 2-AG levels in mice spinal cord using a MAGL inhibitor also slowed down the progression of multiple sclerosis, and was associated with decreased leukocyte infiltration and microglial activity (Hernandez-Torres et al., 2014). However, the Cannabinoid Use in Progressive Inflammatory brain Disease (CUPID) trial failed to show benefits of dronabinol on multiple sclerosis progression, perhaps due to slow progression rate which confounded statistical detection of group differences (Zajicek et al., 2013).

4.2.5.2. Neurodegenerative diseases

Parkinson's disease, Huntington's disease, and Alzheimer's disease are three common neurodegenerative diseases characterized by progressive degeneration and/or death of neurons. No therapy has been discovered to cure these diseases yet. However, manipulation of the endocannabinoid system has shown promising effects to alleviate symptoms.

4.2.5.2.1. Parkinson's disease

Price et al. showed that administration of WIN55, 212-2 improved survival of dopamine-producing neurons in a mouse model of Parkinson's disease (i.e. 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced), in parallel with improved motor performance. This was mediated by CB2 receptors, whose expression was increased in the disease model (Price et al., 2009). Similar findings were observed in a lipopolysaccharide-induced mouse model of Parkinson's disease (Garcia et al., 2011). Unlike the beneficial effects of CB2 receptor activation, CB1 contributed to disease progression. Rimonabant, a CB1 antagonist, improved motor coordination, but did not alter neurodegeneration (Gonzalez et al., 2006; Kelsey, Harris, & Cassin, 2009). In addition, neuroprotection provided by some cannabinoid agents such as THC, cannabidiol, and AM404, is due to antioxidant actions (Garcia-Arencibia et al., 2007). For example, cannabidiol, a naturally-occurring cannabinoid with limited affinity for CB1 and CB2 receptors, attenuated dopamine reduction and increased SOD expression (Garcia-Arencibia et al., 2007). In addition, two anandamide uptake inhibitors, AM404 and UCM707, which would increase anandamide levels, were compared. AM404, which possesses antioxidant properties, rescued dopamine levels in a rat model of Parkinson's

disease, whereas UCM707, which lacks antioxidant properties, did not (Garcia-Arencibia et al., 2007). In summary, agonism of CB2 receptors, antagonism of CB1 receptors, and cannabinoids that exert antioxidant property might improve symptoms of Parkinson's disease.

4.2.5.2.2. Huntington's disease

Huntington's disease is a genetic neurodegenerative disease that is characterized by impaired muscle coordination and cognitive ability, as well as behavior changes, such as anxiety, depression, apathy and aggression. It is caused by a mutation of the Huntington gene, which leads to neuron degeneration mainly in the striatum. No treatment is known to slow disease progression. Cannabinoid compounds may exert symptom-relieving effects. The malonate-induced rat model of Huntington's disease is associated with increased proinflammatory molecules, edema, and microglial activity. A combination of THC and cannabidiol reversed all of these in a CB1- and CB2-dependent manner (Valdeolivas, Satta, Pertwee, Fernandez-Ruiz, & Sagredo, 2012). The role of CB2 receptors was confirmed in CB2 knockout mice, which responded more severely to malonate (Sagredo et al., 2009). CB1 receptor activity declines dramatically in basal ganglia and striatum during the progression of Huntington's disease, and this decline was proposed as a contributor to disease progression. However, markedly reduced CB1 levels render the CB1 receptor a poor therapeutic target (Lastres-Becker et al., 2001), although cannabinoid (anandamide, methanandamide, and ACEA) treatments increase CB1 mRNA in mouse striatal progenitor cell lines that model features of Huntington's disease (Laprairie, Kelly, & Denovan-Wright, 2013). A double-blind, placebo-controlled, cross-over clinical trial involving 44 patients with Huntington's disease showed that while

nabilone failed to improve motor score, the chorea score was improved significantly (Curtis, Mitchell, Patel, Ives, & Rickards, 2009).

4.2.5.2.3. Alzheimer's disease

Alzheimer's disease is characterized by the excessive deposition of β-amyloid peptide and activation of microglial cells in senile plaques, which lead to neuron degeneration mainly in the hippocampus and prefrontal cortex. Symptoms include cognitive impairment, memory loss, mood swings, behavior changes and so on. Both CB1 and CB2 receptors were detected in senile plaques (Ramirez, Blazquez, Gomez del Pulgar, Guzman, & de Ceballos, 2005). CB2 receptor agonism attenuated β-amyloid-induced microglia activation and microglia-induced neurotoxicity in rats, and it preserved cognitive ability (Ramirez et al., 2005). However, studies on CB1 receptors in the progression of Alzheimer's disease are controversial. Some found CB1 is detrimental to memory and learning ability, fostering interest in CB1 antagonism as a treatment approach. For example, Mazzola et al. reported that the CB1 antagonist, SR141716, reversed β-amyloid peptide-induced memory deficit in mice (Mazzola, Micale, & Drago, 2003). In contrast, others demonstrated beneficial effects of CB1 receptors. First, CB1 levels are markedly reduced in brains of various animal models with Alzheimer's disease (Aso et al., 2012; Ramirez et al., 2005). Also, in patients, CB1 activity is increased in the earlier stage of Alzheimer's disease, followed by a reduction in advanced stages of the disease. This implies an initial compensatory response mediated by CB1, which was impaired as neurodegeneration developed (Manuel, Gonzalez de San Roman, Giralt, Ferrer, & Rodriguez-Puertas, 2014). Second, Aso et al. showed that the CB1 agonist ACEA, at a non-amnesic dose, prevented cognitive retardation in a mouse model of

Alzheimer's disease, particularly in the early stage. Mechanisms include inhibition of glycogen synthase kinase 3β (GSK-3β), microglial activation, and subsequent release of pro-inflammatory factors (Aso et al., 2012). There is little evidence derived from clinical trials to support the use of cannabinoid-based compounds to treat Alzheimer's disease. However, dronabinol improved adverse psychiatric effects such as agitation, insomnia, and appetite loss in a few small clinical trials (Ahmed, van der Marck, van den Elsen, & Olde Rikkert, 2015).

4.2.5.3. Mood

It is well known that marijuana use elicits a feeling described as "high." In fact, this is a complex of psychoactive effects due mainly to THC and cannabidiol, the majior cannabinoids in marijuana (Fitzgerald, Bronstein, & Newquist, 2013). Thus, it was speculated that endocannabinoid system exerts anti-depressant and anxiolytic effects. The role of CB1 receptors is well-established. The anti-depressant properties of low dose WIN55, 212-2 in rat were blocked by a CB1 receptor antagonist (Bambico, Katz, Debonnel, & Gobbi, 2007). Injection of anandamide and a CB1 selective agonist, ACEA, into midbrain dorsolateral periaqueductal gray, a region that regulates anxiety responses, also elicited anxiolytic effects in rats, whereas a CB1 antagonist abolished these effects (Moreira, Aguiar, & Guimaraes, 2007). However, in these two studies, high doses of cannabinoids failed to elicit the same effects. In fact, evidence suggests that the effects of cannabinoids on anxiety are bidirectional; anxiolytic at low doses whereas anxiogenic at high doses (Rubino et al., 2008; Viveros, Marco, & File, 2005). In addition, Rubino et al. found that the anxiety-regulation profile of cannabinoids varies in different brain regions; for example, low and high doses of THC injected into the prefrontal cortex and ventral

hippocampus elicit anxiolytic and anxiogenic effects, respectively; however, low doses of THC in basolateral amygdala generate anxiogenic effects whereas high doses of THC were ineffective (Rubino et al., 2008).

Despite the complex activity profile of cannabinoids on anxiety and depression, it is consensus thinking that disruption of CB1 signaling leads to depressive- and anxiogenic-like responses (Moreira, Grieb, & Lutz, 2009). Also, activation of CB1 receptors contributes to the removal of aversive memories (Marsicano et al., 2002). Therefore, CB1 inhibition causes retention of aversive memories and may exacerbate depressive feelings. Patients treated with rimonabant, a CB1 antagonist, as an anti-obesity drug, exhibited depression and anxiety symptoms, and even increased risk of suicide, leading to withdrawal from the market (Christensen, Kristensen, Bartels, Bliddal, & Astrup, 2007).

In summary, both suppression and hyperactivity of the endocannabinoid system may elicit adverse effects, such as anxiety and depression. These effects are mediated by CB1 receptors in CNS, which should be considered during drug development.

4.2.6. Endocannabinoid system and the cardiovascular system

Components of the endocannabinoid system are elevated in various aspects of CVDs, including atherosclerosis, myocardial infarction and cardiac hypertrophy (Duerr et al., 2013; Lin et al., 2015). The following sections discuss the potential roles of endocannabinoids and their receptors in the regulation of cardiovascular health.

4.2.6.1. Hemodynamic parameters

Marijuana use leads to blood pressure changes, and the influence of endocannabinoids on hemodynamics has been extensively studied. However, the results are complex. THC induced biphasic changes in blood pressure and heart rate in

anesthetized rats, which were characterized by an immediate and transient blood pressure increase followed by a marked drop and prolonged hypotension and bradycardia (Lake, Compton, Varga, Martin, & Kunos, 1997). Intravenous injection of anandamide caused a three-phase hemodynamic change in anesthetized rats, including i) phase 1 – a transient reduction in blood pressure, heart rate and cardiac contractility, ii) phase 2 - an elevation of diastolic blood pressure and blood flow in mesenteric and renal vascular beds, followed by iii) phase 3 - a more prolonged and significant decrease in blood pressure and contractility, and a slight reduction in heart rate (Malinowska, Kwolek, & Gothert, 2001; Pacher et al., 2004). Other synthetic compounds, such as HU210, WIN55, 212-2, and CP-55940, also induced prolonged hypotension and bradycardia, although without the initial phases that were observed with THC and anandamide (Lake, Compton, et al., 1997).

Possible mechanisms include CB1 or CB2 receptor activation, TRPV1 activation and metabolite-induced pathways. A similar three-phase action was observed with methanandamide, a stable analogue of anandamide, indicating the involvement of cannabinoid receptors. In addition, the CB1 receptor antagonist SR171416 blocked the phase 3 response, but not the transient pressor effect of THC nor the first two phases of anandamide. This suggests that CB1 is responsible for the prolonged hypotension and bradycardia (Lake, Compton, et al., 1997; Malinowska et al., 2001), perhaps by suppressing the sympathetic nervous system (Niederhoffer, Hansen, Fernandez-Ruiz, & Szabo, 2001). In contrast, a TRPV1 selective antagonist diminished the phase 1 responses induced by anandamide and methanandamide, suggesting that TRPV1 mediates the initial transient drop of blood pressure and heart rate (Malinowska et al., 2001). Phase 2 may also be induced by TRPV1 receptors, as evidenced by the observation that capsaicin, a potent TRPV1 agonist, also generated the phase 2 increase in blood pressure in

anesthetized rats, and this increase was absent in TRPV1 knockout mice compared with wild-type mice (Pacher et al., 2004).

It bears mentioning that the influences of cannabinoids on hemodynamic parameters are different in conscious animals. Unlike the three-phase changes described in anesthetized rats, anandamide elicited the first two phases (i.e. transient depressor and pressor responses) in conscious rats, but not the prolonged hypotension and bradycardia (Lake, Martin, Kunos, & Varga, 1997). This might be explained by the anesthetic agent, urethane, which attenuated the sympathetic suppression of CB1 (Kurz et al., 2009), or the relative high resting sympathetic tone in anesthetic animals, which makes the hypotensive action of cannabinoids more evident (Carruba, Bondiolotti, Picotti, Catteruccia, & Da Prada, 1987).

In humans, marijuana use and cannabinoid agents (sativex and nabione) were associated with an acute acceleration of heart rate that usually peaks at 10 to 30 min after smoking (Karschner et al., 2011; Lile, Kelly, & Hays, 2011). This is regarded as an important biomarker of cannabinoid use (Zuurman, Ippel, Moin, & van Gerven, 2009). The CB1 antagonist rimonabant ameliorated the tachycardia caused by cannabis use (Huestis et al., 2007). Marijuana use also caused hypotension and dizziness in standing position (Mathew, Wilson, & Davis, 2003), which was attenuated by rimonabant (Gorelick et al., 2006). An *in vitro* study demonstrated that CB1 receptor activation by anandamide dilates human vessels by stimulating endothelial nitric oxide release (Bilfinger et al., 1998).

4.2.6.2. Atherosclerosis

Manipulation of cannabinoid receptors (CB2 receptor activation and CB1 receptor inhibition) might also limit atherosclerotic progression, as suggested by animal studies. The anti-atherosclerotic effects of CB2 receptors might be due, at least in part, to its anti-inflammatory actions. Steffens et al. detected CB2 expression in atherosclerotic plaques within human coronary arteries and mouse aorta, but not in regions free of atherosclerotic lesions. They also reported that THC, at a non-psychiatric dose, ameliorated the progression of atherosclerosis, reduced macrophage content and migration within atherosclerotic plaques, and suppressed T cell activation in apoprotein E-deficient mice, a common model of atherosclerosis. A CB2 receptor antagonist blocked all of these effects, indicating the protective role of CB2 receptors (Steffens et al., 2005). Similar effects were reported with WIN55, 212-2, which reduced atherosclerotic size, macrophage infiltration, adhesion molecule expression (i.e. vascular cellular adhesion molecule-1, intracellular adhesion molecule-1, and P-selectin), and expression of pro-inflammatory mediators (i.e. TNF-α, IL-6, and monocyte chemotactic protein 1) in a CB2-dependent manner (Zhao, Liu, et al., 2010; Zhao, Yuan, et al., 2010). Also, it attenuated oxidized low-density lipoprotein (oxLDL)-induced activation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), which in turn up-regulates the pro-inflammatory factors (Zhao, Liu, et al., 2010). In addition, CB2 receptor activation reversed TNF-α-induced proliferation of human coronary artery smooth muscle cells and the underlying MAPK pathway (Rajesh, Mukhopadhyay, Hasko, Huffman, et al., 2008).

In contrast, evidence suggests that CB1 signaling contributes to the atherosclerotic process. First, CB1 antagonism reduced cholesterol deposition in macrophages. Jiang et al. reported suppression of PPARγ by the CB1 antagonist AM251; PPARγ up-regulates

fatty acid translocase/CD36 receptor, which mediates macrophage cholesterol influx, and down-regulates ATP-binding cassette protein A1, which mediates cholesterol efflux (Jiang, Pu, Han, Hu, & He, 2009). Sugamura et al. also showed that rimonabant reduced atherosclerotic lesions, and this was associated with an increase in serum adiponectin, a protein involved in fatty acid degradation, and HDL cholesterol (Sugamura et al., 2010). Second, CB1 antagonism inhibits proliferation and migration of vascular smooth muscle cells. Reduced proliferation and migration of rimonabant-treated human coronary artery smooth muscle cells was observed in parallel to decreased ERK1/2 activation (Rajesh, Mukhopadhyay, Hasko, & Pacher, 2008). Note, however, that clinical trials (STRADIVARIUS and AUDITOR) failed to demonstrate the ability of rimonabant to delay atherosclerotic progression (Nissen et al., 2008; O'Leary et al., 2011), but the STRADIVARIUS trial showed a favorable effect of rimonabant on HDL elevation and triglyceride decrease.

4.2.6.3. Ischemia/reperfusion injury

It is generally accepted that activation of cannabinoid receptors protects the heart against ischemia-reperfusion injury, primarily via CB2 receptor activation. Lagneux and Lamontagne first reported that the cardioprotective effects of lipopolysaccharide following ischemia/reperfusion, namely infarct size reduction and improved myocardial contractility, were blocked by a CB2 receptor antagonist (Lagneux & Lamontagne, 2001). Similar effects were reported subsequently for various endocannabinoids (anandamide, 2-AG and palmitoylethanolamide) (Lepicier, Bouchard, Lagneux, & Lamontagne, 2003; Q. Li, Shi, & Li, 2013) and synthetic agonists (JWH-015 and ACEA) (Lepicier et al., 2003). A CB2 receptor antagonist completely abolished the reduction of infarct size by all of

these compounds, whereas a CB1 receptor antagonist only partially blocked 2-AG-elicited effects (Lepicier et al., 2003). Possible mechanisms include CB2-dependent activation of Pl3K/Akt, p38/ERK1/2, and PKC, as well as inhibition of TNF-α and ROS (Lepicier et al., 2003; Q. Li, Shi, et al., 2013; P. F. Wang et al., 2012).

4.2.6.4. Cardiac hypertrophy and heart failure

As discussed above, the endocannabinoid system is involved in modulation of blood pressure, heart rate and coronary artery conditions, which are important factors that influence cardiac performance. Thus, it was speculated that the endocannabinoid system might regulate cardiac function. However, any evidence of endocannabinoid system effects on cardiac hypertrophy, the convergent point of risk factors to heart failure, is limited and unclear.

Regarding CB1 receptors, Liao et al. queried a potential protective role of CB1 receptors using CB1-deficient mice, and exposing them to acute heart failure model by transverse aortic constriction (TAC). CB1-deficient TAC mice exhibited higher mortality, more severe lung edema, and greater epinephrine and norepinephrine levels compared to wild-type TAC mice and CB1-deficient sham groups. They further demonstrated more advanced LV hypertrophy and contractile impairment, associated with augmented MAPKs (p38 and ERK1/2) activation. In wild-type TAC mice, CB1 agonism ameliorated lung edema, reduced plasma epinephrine and norepinephrine levels, and activated AMP-activated protein (AMPK) (Liao et al., 2012). CB1 receptor activation also suppressed MAPKs in cultured neonatal rat cardiac myocytes treated by isoproterenol (Liao et al., 2013). Results generated by Wagner et al. agreed with the protective role of CB1 in a rat model of post-infarction cardiac remodeling (Wagner et al., 2003). However,

contradictory results suggest that CB1 antagonism improves cardiac performance. Mukhopadhyay et al. generated heart failure in mice using doxorubicin, an anti-cancer drug with severe cardiotoxicity. Cardiac performance-related parameters, including ejection fraction, cardiac output, contractility and apoptosis, deteriorated in response to doxorubicin, whereas CB1 antagonists rimonabant and AM281 were protective (Mukhopadhyay et al., 2007). More recently, Lin et al. showed that the LVH and fibrosis found in mouse model of uremic cardiomyopathy was attenuated by a CB1 antagonist. Also, in an *in vitro* model (indoxyl sulfate treated H9c2 cells), expression of fibrotic markers (collagen I, TGF-β and α-smooth muscle actin), was attenuated by a CB1 receptor antagonist or siRNA knockdown of CB1, vis-à-vis inhibition of Akt (Lin et al., 2015).

There is little evidence regarding CB2 receptors in cardiac hypertrophy, although Weis et al. observed significant elevation of CB2 receptor expression in LV myocardium and endocannabinoids in blood circulation from patients with chronic heart failure, whereas CB1 receptor expression was down-regulated. These results suggest activation of the endocannabinoid system during chronic heart failure, and in particular, of CB2 receptors (Weis et al., 2010). Increased CB2 receptor expression also occurs in patients with aortic stenosis and severe hypertrophic markers (Duerr et al., 2013). However, it is not clear whether this is a compensatory defense mechanism or a detrimental factor.

4.2.7. Endocannabinoid system and mitochondrial function

The endocannabinoid system is involved in various energy regulation processes, and it has been implicated in the regulation of appetite, body weight and diabetes (Horvath, Mukhopadhyay, Hasko, & Pacher, 2012; C. Li, Jones, & Persaud, 2011). For

example, CB1 antagonism reduced hepatic triglycerides, increased expression of genes involved in fatty acid oxidation, and improved insulin sensitivity (Tam et al., 2010). Mitochondria are therefore proposed as reasonable targets of the endocannabinoid system. Recently, studies on the endocannabinoid system and mitochondrial function have emerged. For example, Zaccagnino et al. observed reduced ATP synthesis without mitochondrial $\Delta\psi_m$ loss in isolated liver mitochondria treated by anandamide (Zaccagnino, Corcelli, Baronio, & Lorusso, 2011). Athanasiou et al. reported decreased mitochondrial $\Delta\psi_m$ and oxygen consumption in response to three cannabinoids: anandamide, THC, and the synthetic analog HU 210. Also, activities of ETC complexes I-III and cell viability were reduced, but only at concentrations higher than 10 μM, indicating concentration-dependent effects on mitochondrial function and integrity (Athanasiou et al., 2007). A few studies found cannabinoid receptor-independent effects of endocannabinoids on mitochondrial-dependent apoptosis by modulating the membrane fluidity, but again, at concentrations higher than 10 μM (Catanzaro, Rapino, Oddi, & Maccarrone, 2009; Siegmund et al., 2007). Nevertheless, there is evidence to suggest that CB receptors regulate mitochondrial function, as discussed below.

4.2.7.1. Mitochondrial effects of CB1 receptors

CB1 receptors have been identified on mitochondrial membranes of mouse neuron cells, and account for approximately 15% of the total cellular amount (Benard et al., 2012). Fisar et al. reported that activation of CB1 receptors significantly reduced the activity of ETC complex I and II, but not complex IV in isolated mitochondria from pig brain (Fisar, Singh, & Hroudova, 2014). CB1 receptor activation also reduced mitochondrial oxygen consumption and biogenesis parameters, such as mitochondrial

mass and mitochondrial DNA amount, in mouse muscle and liver, as well as human white adipose tissue (Tedesco et al., 2010). In contrast, a CB1 antagonist increased fatty acid oxidation, reduced obesity in high-fat-diet mice (Jbilo et al., 2005), and prevented high-fat-induced cardiometabolic abnormalities in diabetic rats (Vijayakumar et al., 2012). This deregulation of mitochondrial function by CB1 receptors might be attributed to depressed p-AMPK and eNOS (Tedesco et al., 2010). In summary, existing evidence suggests that CB1 receptors negatively mediate mitochondrial biogenesis and fatty acid oxidation.

4.2.7.2. Mitochondrial effects of CB2 receptors

In contrast, studies suggest protective effects of CB2 receptor activation on mitochondrial performance. CB2 receptor activation slowed down neuron degeneration by preventing mitochondrial apoptotic pathways (Latini et al., 2014). In a rat model of myocardial ischemia/reperfusion, CB2 receptor activation by JWH133 inhibited mPT, mitochondrial membrane depolarization, cytochrome c release, and apoptosis, which were abolished by ERK1/2 inhibitor. These effects were used to explain the cardioprotective actions of CB2 receptors against ischemia/reperfusion injury (Q. Li et al., 2014; Q. Li, Wang, Zhang, Zhou, & Zhang, 2013). Contrary to CB1, CB2 receptor activation exhibits anti-obesity effects (Agudo et al., 2010; Verty et al., 2015); a possible mechanism is the stimulation of palmitate oxidation and related proteins, which is mediated by cAMP/PKA/sirtuin 1 (SIRT1)/PGC-1α signaling cascades (Zheng, Sun, & Wang, 2013).

4.3. Regulation of endocannabinoid system level

Endocannabinoids and cannabinoid receptor levels can be altered by various stimuli, such as stress, inflammation, high-fat diet, obesity, diabetes, and dietary fatty acid composition.

4.3.1. Stress

Stress, depression, and anxiety are known to alter endocannabinoid levels. Memory retrieval in rats that underwent stressful training elicited an increase in 2-AG and a corresponding decrease in the activity of the 2-AG-degrading enzyme MAGL (Morena et al., 2015). A rat model of early life stress created by maternal deprivation also increased levels of endocannabinoid system components (CB1, CB2, TRPV1, GPR55, as well as endocannabinoid synthase and hydrolase) in frontal cortex and hippocampus of adolescent male and female rats respectively (Marco et al., 2014). In addition, serum levels of endocannabinoids were evaluated in female patients with depression and anxiety. This study reported an increase in anandamide and 2-AG in patients with mild depression; however, 2-AG levels were markedly reduced in patients with advanced depression, and tended to decline with prolonged progression. Unlike 2-AG levels that associated with depression, anandamide was found to be negatively correlated with degree of anxiety (M. N. Hill, Miller, Ho, Gorzalka, & Hillard, 2008).

4.3.2. Inflammation

Inflammatory conditions are often associated with elevated CB2 receptor expression. Multiple sclerosis patients have higher CB2 expression in B cells, and higher anandamide levels in B cells, natural killer cells, and T cells (Sanchez Lopez, Roman-Vega, Ramil Tojeiro, Giuffrida, & Garcia-Merino, 2015). CB2 expression was also up-

regulated in mouse model and human model of colitis (M. A. Storr et al., 2009; Wright et al., 2005). Finally, anandamide, 2-AG, CB1 and CB2 receptors were detected in synovial membranes from patients with rheumatoid arthritis, but not healthy volunteers (Gui et al., 2015).

4.3.3. High-fat diet, obesity, and diabetes

High-fat diet, obesity, and diabetes are well-known conditions that involve altered levels of endocannabinoid system components. A marked increase in hepatic anandamide levels was detected in mice fed high-fat diets (60 en%) for three weeks, although the extent of increase declined after 14 weeks. This elevation of anandamide was associated with a reduction in FAAH activity (Osei-Hyiaman et al., 2005). In contrast, no changes of anandamide and 2-AG were observed in rats fed high-saturated fat diets (palm oil-rich diets, 38 en%) for one week (Artmann et al., 2008). Nevertheless, obese patients exhibit higher endocannabinoid levels in visceral fat and serum (Matias et al., 2006). Engeli et al. reported that compared to lean female subjects, obese females exhibited 35% and 52% increases in circulating anandamide and 2-AG respectively, and a reduction of FAAH expression in adipose tissue (Engeli et al., 2005). Cote et al. observed a positive correlation between plasma 2-AG level and body mass index, intra-abdominal adiposity and fasting insulin level in males. However, a negative correlation was found between anandamide and intra-abdominal adiposity (Cote et al., 2007). Interestingly, Annuzzi et al. assessed the levels of endocannabinoids in subcutaneous adipose tissue, and found increased anandamide and decreased 2-AG in patients with both obesity and type 2 diabetes, but not in non-diabetic obese patients (Annuzzi et al., 2010). Similarly, a post-hoc analysis of postmenopausal women reported a higher plasma level of 2-AG in

insulin-resistant obese women compared to insulin-sensitive obese women (Abdulnour et al., 2014). Increased FAAH and MAGL were detected in various adipose tissue (subcutaneous abdominal, visceral, and epididymal) in obese rats with or without diabetes. However, no changes in FAAH and MAGL were found in obese humans (Cable, Tan, Alexander, & O'Sullivan, 2014). The interaction between obesity and endocannabinoid levels remains unclear and existing evidence appears to be controversial. However, it seems like endocannabinoid levels are not simply influenced by high-saturated fat diet, but also by mediators such as leptin and insulin (Matias et al., 2006). Indeed, intravenous injection of leptin significantly reduced anandamide and 2-AG levels in hypothalamus of normal rats and obese mice (Di Marzo et al., 2001). Insulin treatment decreased anandamide and 2-AG levels in healthy adipocytes, but not in insulin-resistant adipocytes (D'Eon et al., 2008).

4.3.4. Dietary consumption of polyunsaturated fatty acid (PUFA)

Anandamide and 2-AG are derived from arachidonic acid, an omega-6 PUFA. Thus, endocannabinoid levels can be modified by diets that affect the arachidonic acid content in tissue phospholipids. Indeed, increasing dietary linoleic acid (omega-6) (from 1 en% to 8 en%) elevated anandamide and 2-AG levels in mouse liver and resulted in weight gain, although the total dietary fat remained unchanged (Alvheim et al., 2012). Also, the effects of omega-3 PUFA on endocannabinoid levels were evaluated in mice brain. Watanabe et al. found that an omega-3 PUFA-deficient diet significantly increased 2-AG content in brain. However, short-term consumption of a docosahexaenoic acid (DHA)-rich diet reduced the arachidonic acid content in phospholipids and brain 2-AG levels (Watanabe, Doshi, & Hamazaki, 2003). These results were similar to the findings of Wood et al.,

which revealed that fish oil supplementation for 2 weeks is sufficient to affect fatty acid composition by enhancing DHA and eicosapentaenoic acid (EPA; omega-3 PUFA) levels, while down-regulating arachidonic acid and anandamide content in mouse brain and plasma (Wood et al., 2010). In addition, a recent human study investigated the effects of an omega-3-rich diet on endocannabinoid levels in obese men. In this study, krill powder, which contains 61.8% krill oil (omega-3-rich oil), was provided to mildly obese men for 24 weeks. After 24 weeks, plasma levels of anandamide and its analogues plmitoylethanolamide and oleoylethanolamide were significantly reduced, as were triglycerides, but no weight loss was observed (Berge et al., 2013). Although more studies are needed, the existing evidence implies that dietary fatty acid composition influences endocannabinoid levels by manipulating omega-3/omega-6 balance, which in turn regulates the level of arachidonic acid. Therefore, modulation of dietary fatty acid composition might be a promising approach to achieve endocannabinoid-mediated health benefits.

Chapter III: Rationale and Hypothesis

1. Rationale

1.1. Determine and characterize the effects of cannabinoid receptor agonists on myocyte hypertrophy.

There is growing interest in the medical benefits of cannabinoid receptor activation. Although there is much research on the cardiovascular role of the endocannabinoid system, few have investigated endocannabinoid effects on cardiac hypertrophy. Therefore, the first objective of this study was to investigate the role of endocannabinoids in ventricular myocyte hypertrophy.

We began by testing the hypothesis that endocannabinoids and synthetic analogs prevent the development of cardiac myocyte hypertrophy via cannabinoid receptor activation.

Anandamide is one of the most-studied endogenously secreted cannabinoids. It was identified as a natural ligand for cannabinoid receptors by Devane et al. in 1992 (Devane et al., 1992). Anandamide is a ligand for both CB1 and CB2 receptors, with a higher selectivity for CB1 (Ki are 89 nM for CB1 and 371 nM for CB2) (Pertwee, 1999). It is detectable in the brain and various peripheral tissues, including the heart (Felder et al., 1996). It has been shown that anandamide elicits vaso-relaxation in rat hepatic artery (Randall & Kendall, 1998), pulmonary arteries (Baranowska-Kuczko et al., 2014), and coronary arteries (White, Ho, Bottrill, Ford, & Hiley, 2001), although the mechanism is still unclear. Also, there are reports that suggest anandamide protects against cardiac ischemic insult (Q. Li, Shi, et al., 2013). There also exists evidence that endocannabinoids may influence the development of hypertrophy in cardiac and non-cardiac cells. For

example, Duerr et al. observed increases in concentration of anandamide and expression of CB2 receptors in patients with aortic stenosis and cardiac hypertrophy (Duerr et al., 2013). Jenkin et al found that anandamide induced hypertrophy in human proximal tubular cells by activating CB1 receptors, whereas CB2 receptors exhibited opposite action (Jenkin, McAinch, Grinfeld, & Hryciw, 2010).

ET1 (0.1 μM) treated neonatal rat ventricular myocytes were used as our hypertrophic model. ET1 is a vasoconstrictor peptide that is primarily synthesized by endothelial cells, and is also produced from cardiac myocytes (Suzuki et al., 1993). In addition to its vaso-constrictive effect on vascular smooth muscle cells, it also acts as a growth factor in cardiac myocytes (Ito et al., 1991; Suzuki et al., 1990). Actually, ET1-treated neonatal rat ventricular myocytes is a well-established model for myocyte hypertrophy (Ito et al., 1991). Myocyte size and fetal gene expression (BNP) are measured as markers of cardiac myocyte hypertrophy. BNP is usually only expressed in late embryonic and early neonatal life, but is reactivated during hypertrophic growth (Kohno et al., 1995). Thus, in the first series of experiments, we assessed the effects of anandamide on ET1-induced cardiac myocyte hypertrophy.

It bears mentioning that endocannabinoid actions may be direct (via cannabinoid receptors) or indirect (via endocannabinoid metabolites). For example, Wenzel et al. demonstrated that the constrictive effect of anandamide on pulmonary arteries relies on FAAH-dependent metabolites (Wenzel et al., 2013). Arachidonic acid is a critical product of anandamide degradation. Studies on arachidonic acid and its metabolites demonstrated conflicting effects on CVDs. Arachidonic acid exhibits vasodilatory and anti-inflammatory properties via its metabolites epoxyeicosatrienoic acids (EETs), and hence protect the heart from ischemic injury and cardiac hypertrophy (Althurwi, Elshenawy, &

El-Kadi, 2014). In contrast, another group of arachidonic acid metabolites, 20-hydroxyeicosatetraenoic acids (20-HETE), promote vasoconstriction and inflammation, thus rendering the heart more susceptible to myocyte hypertrophy and fibrosis (El-Sherbeni & El-Kadi, 2014). Therefore, to determine the contribution of metabolites to anandamide effects, we conducted a series of experiments in which we used a non-hydrolysable analog of anandamide, R-methanandamide. R-methanandamide is a synthetic cannabinoid receptor agonist; the addition of a methyl group to the first carbon of anandamide renders resistance to hydrolysis by FAAH (Howlett et al., 2002).

We were also cognizant that CB1 and CB2 receptors may regulate distinct aspects of the hypertrophic process, and thus we sought to further distinguish between the role of CB1 and CB2 receptors using pharmacological approaches. To do this, we used commercially available CB1- and CB2-selective antagonists. AM251 is selective for CB1 (Ki: CB1=7.5 nM; CB2=2290 nM) (Lan, Liu, et al., 1999) and AM630 for CB2 (Ki: CB1=5200 nM; CB2=31.2 nM) (R. A. Ross et al., 1999).

Finally, we investigated approaches that might render endocannabinoid-based treatment of CVD as clinically viable. Historically, the application of cannabinoid-based therapies has been limited due to psychoactive adverse effects mediated by central CB1 receptors, such as memory impairment, disorientation and possibly addiction. (Hosking & Zajicek, 2008; Kunos, Osei-Hyiaman, Batkai, Sharkey, & Makriyannis, 2009). Alternate strategies like using CB2-selective agonists and/or peripherally-restricted CB1 agonists have been proposed (Gertsch et al., 2008; Hosking & Zajicek, 2008; Kunos et al., 2009; Palazuelos et al., 2006).

We postulated that both CB1 and CB2 receptors are required to suppress hypertrophy. However, activation of CB2 receptors reportedly mediates a variety of

cardioprotective effects, such as reduced risk of ischemia (Duerr et al., 2014; Q. Li, Wang, et al., 2013), deceleration of atherosclerosis by reducing inflammation and macrophage infiltration (Carbone, Mach, Vuilleumier, & Montecucco, 2014), and induction of cardiac myocyte differentiation (Y. Wang et al., 2014). Thus, we proceeded with experiments to assess the ability of JWH-133, a CB2 selective agonist, to protect against cardiac myocyte hypertrophy. JWH-133 was discovered by, and named after John W. Huffman, and exhibits ≈ 200-fold selectivity for CB2 (Ki: CB1=677 nM vs. CB2=3.4 nM) (Huffman, 2005).

The second approach is to selectively target peripheral CB1 and CB2 receptors. We utilized CB-13, a peripherally-restricted dual CB1/CB2 agonist (Ki: CB1=6.1 nM vs. CB2=27.9 nM) (Dziadulewicz et al., 2007). Dziadulewicz et al. reported that CB-13 exhibits anti-hyperalgesic effects through CB1 receptors with very limited penetration into the CNS (Dziadulewicz et al., 2007). In fact, by performing a catalepsy test, negligible CNS effects were detected at a concentration that was 170-fold greater than the dose required to reverse hyperalgesia (0.2 mg/kg) (Dziadulewicz et al., 2007). A catalepsy test records the time of a rodent to correct an externally imposed posture, and it is a commonly used behavioral test to evaluate cannabinoids-induced effects (Fox et al., 2001; Little, Compton, Johnson, Melvin, & Martin, 1988). Furthermore, slow penetration and poor accumulation of CB-13 in the CNS was demonstrated (Dziadulewicz et al., 2007).

1.2. Determine the signaling mediators of endocannabinoid actions.

1.2.1. AMPK

AMPK is a heterotrimeric protein consisting of α, β, and γ subunits. The catalytic domain, which is activated by phosphorylation at threonine-172, is situated on the α subunit (Hawley et al., 1996). Two AMPKα isoforms exist in the heart, α_1 and α_2, and α_2 appears to predominate over α_1 (Sakamoto et al., 2006; Stapleton et al., 1996). It is reported that α_2 accounts for 70-80% of total AMPKα activity in rat cardiac tissue (Cheung, Salt, Davies, Hardie, & Carling, 2000), and it is expressed dramatically more than the α_1 subunit in human heart (Quentin et al., 2011).

AMPK is well known for its ability to maintain cellular energy homeostasis; it senses the energy status of the cell through the ratio of AMP to ATP (Salt et al., 1998) and, in response to energy deprivation, coordinates metabolic reactions to conserve ATP. Specifically in the heart, AMPK is an important regulator of cardiomyocyte energy homeostasis by mechanisms which may include i) increasing fatty acid uptake and oxidation, ii) accelerating glucose uptake, iii) stimulating glycolysis, and iv) inhibition of energy-consuming pathways such as protein synthesis (Dolinsky & Dyck, 2006).

There is evidence to suggest that activated AMPK is anti-hypertrophic. *In vitro*, AMPK activation blocked cardiomyocyte enlargement, protein synthesis, and hypertrophic gene expression in response to phenylephrine (Chan, Soltys, Young, Proud, & Dyck, 2004; B. L. Chen et al., 2010) and angiotensin II (Stuck, Lenski, Bohm, & Laufs, 2008), and also inhibited pro-hypertrophic mediators such as NFAT, NF-κB, and MAPK (H. L. Li et al., 2007). *In vivo*, AICAR, a chemical activator of AMPK, attenuated pressure-overload hypertrophy in rats (H. L. Li et al., 2007), and pressure-overload

hypertrophy was exaggerated in AMPKα_2 gene knockout mice (P. Zhang et al., 2008). Also, the ability of resveratrol (Chan et al., 2008) and calorie restriction (Dolinsky et al., 2010) to impede hypertrophy has been attributed to AMPK signaling.

Notably, there is evidence to suggest that endocannabinoids activate AMPK, albeit derived mostly from the brain. Cannabinoid receptor ligands such as 2-AG (Kola et al., 2005), THC (Kola et al., 2005), and HU210 (Lim et al., 2013) increase AMPK phosphorylation and enzymatic activity in rat hippocampus via CB1 and CB2 receptors (Dagon, Avraham, Ilan, Mechoulam, & Berry, 2007; Lim et al., 2013). There is less evidence from the heart, although WIN55, 212-22 (synthetic CB1 agonist) (Liao et al., 2012) and THC (Kola et al., 2005) were reported to activate AMPK in mice and rats respectively.

1.2.2. Nitric oxide

There is likewise evidence of crosstalk between endocannabinoids and nitric oxide in the nervous system (Ortega-Gutierrez, Molina-Holgado, & Guaza, 2005), immune cells (Vannacci et al., 2004), adipocytes (Gasperi et al., 2007), and vasculature (Harris, McCulloch, Kendall, & Randall, 2002). Nitric oxide contributes to the ability of cannabinoid receptors to reduce myocardial infarct size (Lepicier, Bibeau-Poirier, Lagneux, Servant, & Lamontagne, 2006; Lepicier et al., 2003). Neuronal NOS (nNOS), eNOS and inducible NOS (iNOS) are expressed in myocytes (Balligand & Cannon, 1997), and low levels of nitric oxide derived from eNOS protect the heart from hypertrophy (Barouch et al., 2003; Barouch et al., 2002; Khan et al., 2003; Wollert & Drexler, 2002; Ziolo & Bers, 2003). There are several sites on eNOS that might be phosphorylated to modulate its activity. The best-studied sites are serine-1177 and

threonine-495. Activation of eNOS requires phosphorylation of serine-1177 whereas nitric oxide synthesis is inhibited by phosphorylation of threonine-495 (Mount, Kemp, & Power, 2007). In contrast to eNOS, iNOS activation in the cytosol contributes to deleterious cardiac effects, whether due to high nitric oxide levels or superoxide/peroxynitrite formation (Arstall, Sawyer, Fukazawa, & Kelly, 1999; Feng, Lu, Jones, Shen, & Arnold, 2001; Mungrue et al., 2002; Sam et al., 2001).

1.2.3. AMPK/eNOS crosstalk

As activation of AMPK promotes phosphorylation and activation of eNOS at Ser1177 (Z. P. Chen et al., 1999; Morrow et al., 2003), and AMPK-eNOS signaling has been implicated in the anti-growth effects of resveratrol (Thandapilly et al., 2011) and metformin (C. X. Zhang et al., 2011), we tested the hypothesis that cannabinoid receptor signaling stimulates an AMPK-eNOS signaling axis that contributes to the attenuation of hypertrophy. To achieve this objective, the following experiments were performed:

a) Determine if activation of CB receptors leads to stimulation of AMPK.

CB-13 (1 μM) was used to treat neonatal cardiac myocytes for multiple time points (0-24 h). Western blotting was performed to detect native and phosphorylated AMPKα, and β-actin as internal control.

b) Determine if AMPK mediates the anti-hypertrophic effects of CB receptors.

Cardiac myocytes were pretreated with a chemical inhibitor of AMPK, compound C (1 μM), prior to the addition of CB-13 and ET1. Hypertrophic indicators were assessed as described above.

We extended our findings using lentiviral-based shRNA knockdown to implicate AMPKα, and we predicted that inhibition or knockdown of AMPK would preserve the

hypertrophic response to ET1, which would indicate that the anti-hypertrophic actions of CB receptors rely on AMPK signaling.

c) Determine if eNOS contributes to CB-AMPK signaling.

To assess the effect of CB-AMPK signaling on eNOS activity, cardiac myocytes were treated with CB-13 (1 μM) for multiple time points (0-24 h) with and without AMPK knockdown. Western blotting was performed to detect eNOS phosphorylation at the activation site, Ser-1177 (Z. P. Chen et al., 1999). The ability of CB-13 to block hypertrophy was determined in the presence of an eNOS-selective inhibitor. A chemical inhibitor of eNOS, L-NIO (Rees, Palmer, Schulz, Hodson, & Moncada, 1990), was used to implicate eNOS as having a role in the anti-hypertrophic actions. Overall, we anticipated that eNOS is downstream of CB-AMPK signaling and plays a critical role protecting the heart against hypertrophy induced by ET1.

1.3. Elucidate the alterations of mitochondrial function in ET1-treated cardiac myocytes

As the energy factory of the cell, mitochondrial dysfunction is considered a critical feature of cardiac myocyte hypertrophy; in fact, aberrant energy production by impaired mitochondria might be involved in the development of hypertrophy (L. Y. Zhou et al., 2012). Thus, we assessed myocyte mitochondrial function in ET1-treated cardiac myocytes.

Mitochondrial membrane depolarization is an important aspect of mitochondrial dysfunction. In fact, inner $\Delta\psi_m$ is considered an indicator of mitochondrial health because it provides the driving force for ATP synthesis; thus, the loss of inner $\Delta\psi_m$ impairs ATP production. Membrane depolarization is tightly associated with mPT, although it is controversial whether membrane depolarization or mPT is the initiator. Stimuli such as

oxidative stress and calcium overload cause mPTP to open, thereby increasing permeability of the mitochondrial inner membrane (Javadov, Karmazyn, et al., 2009). This is always accompanied by mitochondrial inner membrane depolarization, which in turn diminishes ATP production. mPTP opening and membrane depolarization have been observed in cardiac myocytes in response to hypertrophic stimuli such as angiotensin II (M. Li, Ma, Han, & Li, 2014), phenylephrine (Javadov, Rajapurohitam, et al., 2009), and ET1 (Javadov, Rajapurohitam, et al., 2009).

Mitochondrial dysfunction also presents as abnormal energy metabolism. Impaired ATP production and a shift of energy substrate from fatty acids to glucose are generally identified in hypertrophied heart (Lehman & Kelly, 2002; Leong, Brownsey, Kulpa, & Allard, 2003). Proteins that have been implicated in the regulation of mitochondrial bioenergetics were investigated in this study. First, peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) is a key regulator of mitochondrial function, as it drives mitochondrial biogenesis (Lehman et al., 2000), and improves fatty acid oxidation (Ventura-Clapier, Garnier, & Veksler, 2008; L. Y. Zhou et al., 2012). However, studies have shown that hypertrophic stimuli such as pressure overload (Sack, Disch, Rockman, & Kelly, 1997) and phenylephrine (Garnier et al., 2009) down-regulate PGC-1α, and the reduction in PGC-1α expression promotes heart failure (Arany et al., 2006; Sano et al., 2004). In contrast, AMPK activators increase PGC-1α (B. Huang et al., 2014; W. J. Lee et al., 2006). Second, we examined CPT-1. CPT-1 facilitates the transport of long chain fatty acids into the mitochondria by catalyzing the formation of fatty acyl-carnitine, and is therefore an initial step in the fatty acid oxidation pathway (Kerner & Hoppel, 2000).

In this study, we assessed mitochondrial disorder in ET1-treated cardiac myocytes by examining the following aspects: i) mitochondrial inner membrane integrity, and ii) energy metabolism and its related proteins.

1.4. Determine the effects of cannabinoid receptor activation on mitochondrial dysfunction.

Activation of cannabinoid receptors regulates energy metabolism. For example, CB1 inhibition increases thermogenesis (Cardinal et al., 2015) and gene expression of fatty acid oxidation-related enzymes and lipase (Hsiao et al., 2015; Tam et al., 2010). In contrast, similar effects were observed in CB2 receptor activation (Zheng et al., 2013) (details were reviewed in chapter II section 4.2.1 and 4.2.7). Accordingly, we speculate that activation of CB1/CB2 receptors regulate mitochondrial function. Our preceding objective was to examine the effects of CB-13 on ET1-induced cardiac myocyte hypertrophy, which is usually characterized by mitochondrial dysfunction (L. Y. Zhou et al., 2012). Here, the effects of CB-13 on ET1-induced mitochondrial abnormalities were investigated.

1.5. Determine the role of AMPK signaling in mitochondrial regulation by cannabinoid receptors

This objective is based on our finding that dual activation of CB1/CB2 receptors using CB-13 prevents the development of cardiac myocyte hypertrophy via AMPK signaling (Lu et al., 2014). AMPK senses energy status of the cell and might be involved in mitochondrial function regulation (Hardie, 2007). Indeed, Toyama et al. discovered that AMPK is required in the mitochondrial fission process in response to energy stress (Toyama et al., 2016). Shin et al. reported that AMPK activation protects hepatocytes

against antimycin-induced mPT and apoptosis (Shin & Kim, 2009). Ido et al. found that hyperglycemia-induced mitochondrial membrane depolarization, reduction of fatty acid oxidation and ATP content, and apoptosis in human umbilical endothelial cells were attenuated by AMPK activation (Ido, Carling, & Ruderman, 2002). Therefore, the role of AMPK in mitochondrial protective actions of CB-13 was investigated.

2. Hypotheses

2.1. Endocannabinoids and synthetic analogs prevent the development of cardiac myocyte hypertrophy via cannabinoid receptor activation.

2.2. AMPK/eNOS signaling mediates the anti-hypertrophic actions of ligand-activated cannabinoid receptors.

2.3. ET1 induces early mitochondrial abnormalities in terms of membrane integrity and energy metabolism.

2.4. Cannabinoid receptor activation rescues mitochondrial function in ET1-treated myocytes.

2.5. Protective effects of cannabinoid receptor activation on mitochondria involve AMPK, PGC-1α, and CPT-1β.

Chapter IV: Objectives

1. Determine and characterize the effects of cannabinoid receptor agonists on myocyte hypertrophy.

    1.1. Determine if endocannabinoids prevent cardiac myocyte hypertrophy via cannabinoid receptors.

    1.2. Determine if selective agonism of CB2 receptors prevents cardiac myocyte hypertrophy.

    1.3. Determine if dual agonism of CB1/B2 receptors using a peripherally-restricted agonist prevents cardiac myocyte hypertrophy.

2. Determine the signaling mediators of endocannabinoid actions.

    2.1. Determine the role of AMPK/eNOS crosstalk in the anti-hypertrophic actions of ligand-activated CB receptors.

3. Elucidate the effects of ET1 treatment on mitochondrial function in cardiac myocytes.

    3.1. Determine if ET1 induces mitochondrial membrane depolarization in isolated ventricular myocytes.

    3.2. Determine if ET1 depresses fatty acid oxidation-dependent mitochondrial bioenergetics.

    3.3. Determine if ET1 depresses glucose oxidation-dependent mitochondrial bioenergetics.

    3.4. Determine if ET1 suppresses the expression of PGC-1α and CPT-1β.

4. Determine the effects of cannabinoid receptor activation on mitochondrial dysfunction.

5. Determine the role of AMPK signaling in mitochondrial regulation by cannabinoid receptors.

Chapter V: Materials and Methods

1. Animals

This study was conducted according to recommendations from the Animal Care Committee of the University of Manitoba and the Canadian Council of Animal Care. Neonatal rat pups were produced from a larger in house Sprague-Dawley rat breeding colony. The pups were born in an aspen bedding-enriched polycarbonate rat cage suspended in a racking system that forms the cage lid. Pups were subsequently housed alone with the mother. Animals were maintained at 22-25°C, 55-60% humidity, and a 12 h light-dark cycle, and allowed free access to water and food (PMI RMH-3000 feed).

2. Materials

Anandamide, R-methanandamide, JWH-133, ET1, compound C, sarcomeric α-actinin antibody, β-actin antibody, sodium pyruvate solution (100 mM), L-carnitine hydrochloride, oligomycin, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP), rotenone, antimycin A and etomoxir were from Sigma–Aldrich (Missouri, USA). AM251, AM281, AM630, and L-NIO were from Tocris Cookson (Minnesota, USA). CB-13 and the JC-1 mitochondrial membrane potential assay kit were from Cayman Chemical (Michigan, USA). Lipofectin, calcein AM (Molecular Probes), propidium iodide (PI) and CPT-1β primers were from Life Technologies (California, USA). Alexa Fluor® 488-conjugated anti-mouse IgG1 antibody was from Abcam (California, USA). The -1595 human brain natriuretic peptide (BNP)-luciferase reporter construct was kindly provided by Dr. David Gardner (University of California, San Francisco, CA). Antibodies against phosphorylated AMPK (p-AMPK), AMPK, and phosphorylated eNOS (p-eNOS) were from Cell Signaling (Whitby, Canada). PGC-1α antibody was from EMD Millipore

(California, USA). XF24 FluxPak was from Seahorse Bioscience (Massachusetts, USA). The conjugated palmitate/BSA substrates were kindly provided by Dr. Paul Fernyhough (University of Manitoba, Winnipeg).

3. Methods

3.1. Treatments

As applicable, myocytes were subjected to transfection or lentiviral infection. Then, unless otherwise indicated, myocytes were rendered quiescent by serum deprivation (0.5% CCS) or starvation (no serum) for 24 h and pre-treated for 1 h or 2 h with vehicle or anandamide (0.001-1 μM), R-methanandamide (1 μM), JWH-133 (0.001-1 μM) and CB-13 (0.001-1 μM) in the presence or absence of cannabinoid receptor antagonists (AM251 and AM281 for CB1, AM630 for CB2; 0.1 μM; 1 h), or chemical inhibitors of AMPK (compound C; 1 μM; 1 h) or eNOS (L-NIO; 1 μM; 1 h). Following the 1 h or 2 h pretreatment, ligands remained in the culture media for the remainder of the experiment. Hypertrophy and mitochondrial dysfunction were stimulated by addition of ET1 (0.1 μM; 24-48 h or 4 h, respectively) (Alibin et al., 2008; Sun et al., 2014). The concentrations of CB antagonists used in these experiments are predicated on reports that sub- or low micromolar concentrations of AM251 and AM630 ablate previously described cardiac or vascular effects of endocannabinoids (Lam, Luk, & Ng, 2007; Romano & Lograno, 2006; Underdown, Hiley, & Ford, 2005). The treatment times of CB-13 and ET1 in mitochondrial function experiments were based on preliminary data suggesting improved or impaired mitochondrial function (data not shown).

3.2. Isolation and cell culture of neonatal rat ventricular myocytes

Ventricular cardiomyocytes were isolated from 1-day-old neonatal Sprague-Dawley rats by digestion of minced ventricles with several cycles of 0.1% trypsin and 0.002% DNase in calcium bicarbonate-free Hanks with HEPES buffer (CBFHH) and mechanical disruption (Alibin et al., 2008). Supernatant was collected in BSA and centrifuged to acquire the cell pellets. To distinguish non-myocytes, cells were collected in Dulbecco's modified Eagle medium (DMEM) containing 10% cosmic calf serum (CCS) (Hyclone) on tissue culture plates for 1 hour (i.e. pre-plating). During pre-plating, non-myocytes (i.e. fibroblasts, endothelial cells, and vascular smooth muscle cells) adhered to the plate, whereas the majority of myocytes remained in suspension. Myocytes were then collected and cultured on gelatin-coated plates in DMEM containing 10% CCS for 18 - 24 h prior to experimentation.

3.3. Isolation and cell culture of adult rat ventricular myocytes

Adult male Sprague Dawley rats (200-250 g) were anesthetized with 3% isoflurane and injected with heparin into the saphenous vein (1000 U/mL at 1 mL/Kg body weight). The heart was immediately removed and placed into a perfusion chamber and cannulated through the aorta. The heart was washed of blood with calcium-free buffer (mM: NaCl 90, KCl 10, KH2PO4 1.2, MgSO4. 7H2O 5.0, NaHCO3 15, taurine 30, glucose 20, pH 7.4) for 5 minutes. The heart was then perfused for 20 minutes (at 37°C) with calcium free buffer containing 179 U/mL collagenase II. After perfusion, ventricles were removed, minced, and incubated for five minutes at 37°C with re-circulated collagenase buffer for further digestion. Isolated cardiomyocytes were then plated on plates precoated with laminin (10 µg/mL) and maintained for 2 hours at 37°C and 5% CO2 in a medium

consisting of medium 199 containing 5% fetal bovine serum, 5% horse serum, and 1% penicillin/streptomycin. After two hours, the medium was replaced with medium 199 supplemented with 5 mM taurine, 2 mM L-carnitine, 1 mM creatine, 2 μM insulin, and 100 IU/mL penicillin/streptomycin.

3.4. Myocyte size measurement

Myocytes were cultured in 12-well plates (1×10^6 cells/well) and serum-starved for 24 h. Following treatments, cells were fixed with 4% paraformaldehyde for 30 min followed by permeabilization with phosphate-buffered saline (PBS) containing 0.1% Triton X-100 for 5 min. Cells were blocked with PBS containing 2% non-fat dry milk for 1 h and incubated with mouse anti-rat sarcomeric α-actinin antibody in blocking solution at 4 °C for overnight. Cells were washed three times with PBS and incubated with Alexa Fluor® 488-conjugated anti-mouse IgG1 antibody at room temperature for 1 h. After three PBS washes, myocytes were viewed using fluorescence microscopy. Cell surface areas of individual cells were quantified using Image J planimetry software from two-dimensional images.

3.5. Transfection and luciferase assay

Myocytes were cultured in 12-well plates (1×10^6 cells/well) then co-transfected with Renilla luciferase and -1595 human hBNP-luciferase with Lipofectin according to the manufacturer's protocol. Myocytes were maintained in DMEM with 10% CCS for 24 h, and then serum-deprived for 24 h. After treatments, luciferase activity was measured from lysates using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activity was normalized to Renilla luciferase activity.

3.6. RNA extraction and real-time PCR

Myocytes were cultured in 12-well plates (1×10^6 cells/well) and serum deprived (0.5% CCS) for 24 h. Following treatments, total RNA was extracted from myocytes using the RNeasy mini kit (QIAGEN, Hilden, Germany). Real-time PCR was performed using the iScript™ One-Step RT-PCR SYBR® Green kit (Bio-Rad, Ontario, Canada) in the presence of BNP primers (forward: CAGCTCTCAAAGGACCAAGG; reverse: CGATCCGGTCTATCTTCTGC), and CPT-1β primers (forward: 5'-CTTCTCAGTATGGTTCATCTTCTC-3'; reverse: 5'-CGAACATCCACCCATGATAG-3'). GAPDH was employed as the internal control. (forward: CTCATGACCACAGTCCATGC; reverse: TTCAGCTCTGGGATGACCT).

3.7. Lentiviral preparation and infection

Lentiviral vectors expressing shRNA against AMPK α1 and α2 were obtained from the University of Manitoba OpenBiosystems library (AMPK α1: TRCN0000000860, TRCN0000024003; AMPK α2: V2LMM_73754, V2LMM_71195). Scrambled sequences served as non-silencing controls. Lentivirus vector plasmids were co-transfected with psPAX2 (packaging) and pMD2.G (enveloping) vectors using FuGENE6 Reagent (Roche; Indianapolis, Indiana). High-titer lentiviral stock was produced in HEK-293T cells 48 h after transfection. Myocytes were infected for 24 h by application of the lentivirus to the culture medium, and then cultured for a further 72 h (to activate knockdown) prior to treatments and further experimentation. Knockdown was confirmed by western blotting.

3.8. Western blotting

Myocytes were cultured in 6-well plates (2×10^6 cells/plate). Following treatments, cell lysates were prepared in radioimmune precipitation assay (RIPA) buffer and clarified by centrifugation. Antibodies against p-AMPK (1:1000), AMPK (1:1000), p-eNOS (1:1000), eNOS (1:1000), PGC-1α (1:1000) and SOD2 (1:50000) were used for detection by conventional western blotting. Membranes were stripped and reprobed with β-actin antibody to account for loading variations among lanes.

3.9. Measurement of cardiomyocyte viability

Cardiomyocyte viability was assessed by double staining with calcein-AM and PI. Calcein AM is a membrane-permeant dye that is converted to a green-fluorescent calcein by intracellular esterases in viable cells. PI is a membrane-impermeant intercalating agent. While excluded from viable cells with intact membranes, PI enters dead cells and fluoresces upon binding to nucleic acids. After treatments and removal of media, 400 µL of a mixture of 3 uM calcein AM and 2.5 µM PI in warm PBS was added to each well (24-well plate, 350,000 cells/well). The following were used as controls: background - no cells + calcein AM/PI; live controls - vehicle-treated cells; dead cell controls - cells treated with 0.2% Triton X-100 (15 min). Following dark incubation 37°C, 30 min), fluorescence was measured using a plate reader using excitation/emission wavelengths 485nm/535nm for calcein and 530 nm/620 nm for PI.

3.10. Measurement of ventricular myocyte contractile function

Contractile properties of adult rat cardiac myocytes were assessed using a video-based edge-detection system (Ionoptix HyperSwitch Myocyte System). Cardiac myocytes were cultured on coverslips (0.3×10^6 cells/coverslip) and rendered quiescent. Following

treatments, coverslips were placed on a chamber mounted on the stage of an inverted microscope and perfused with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl2, 1 MgCl2, 10 glucose, 10 HEPES, at pH7.4 and maintained at 37°C. Cells were stimulated to contract using the IonOptix Myopacer at a frequency of 0.5 Hz. Cardiomyocytes were displayed on a monitor display using an IonOptix Myocam camera. SoftEdge software (IonOptix) was used to compare changes in cell length during shortening (contraction) and relengthening (relaxation). Indices used to evaluate cell contractility included maximal velocity of shortening (+dL/dt) and maximal velocity of relengthening (-dL/dt). These are representations of systolic contraction and diastolic relaxation, respectively. Contractility was also measured as peak shortening.

3.11. Measurement of mPT

Myocytes were cultured in 48-well plates (0.25×10^6 cells/well), and pre-treated with CB-13 (1 μM; 1 h) or vehicle in Krebs-Henseleit buffer supplemented with palmitate/BSA substrates (200 μM), L-carnitine hydrochloride (0.4 mM) and glucose (2.5 mM). Myocytes were then coloaded with calcein-AM (2 μM), $CoCl_2$ (2 mM) and MitoTracker-Red (0.1 μM) for 15 min, followed by 5 min wash with PBS. Images were acquired using an Olympus brightfield fluorescence microscope. The excitation/emission wavelengths for calcein and MitoTracker-Red are 494/517 nm and 579/599 nm respectively. Images were acquired pre- and post-treatment (i.e. 0, 1, 2, 5, 15 and 30 min) with ET1 (0.1 μM) or vehicle. Cells were incubated with ionomycin (2 μM) for 5 min at the end of the experiment; this causes mPT and therefore served as positive control.

3.12. Measurement of changes in $\Delta\psi_m$

The lipophilic fluorescent probe JC-1 (Cayman Chemical Company) was used to investigate $\Delta\psi_m$ in cardiac myocytes, as per the manufacturer's protocol. Myocytes were loaded with JC-1 for 60 min at 37ºC in specific working buffers: 1) Krebs-Henseleit buffer containing palmitate/BSA substrates (200 μM), L-carnitine hydrochloride (0.4 mM) and glucose (2.5 mM) was used to measure fatty acid-dependent $\Delta\psi_m$, or 2) DMEM with glucose (10 mM) and pyruvate (1 mM) was used to measure glucose-dependent $\Delta\psi_m$. Myocytes were then washed with PBS for 5 min, and images were acquired using an Olympus brightfield fluorescence microscope. Samples were excited at 485 nm for monomer fluorescence and at 560 nm for JC-1 aggregate fluorescence. Emission fluorescence images were recorded at 535 nm for JC-1 monomer and 595 nm for JC-1 aggregates. Fluorescence intensity was also quantified using the SpectraMax Gemini XS fluorescence microplate reader. The ratio of aggregate to monomer fluorescence was measured as an indicator of changes in in $\Delta\psi_m$. Also, as JC-1 may respond to plasma membrane depolarization, the mitochondrial uncoupler p-trifluoro-methoxy carbonyl cyanide phenyl hydrazine (FCCP; 1 μM) was added at the end of each experiment to achieve maximal dissipation of $\Delta\psi_m$, and therefore served as positive control.

3.13. Measurement of mitochondrial respiration

A Seahorse XF24 Bioanalyzer (Seahorse Biosciences, North Billerica, MA) was used to measure bioenergetic function in isolated neonatal rat cardiac myocytes. The XF24 creates a transient 7 μL chamber in specialized microplates that allows for oxygen consumption rate (OCR) to be monitored in real time (Roy Chowdhury et al., 2012). Fatty acid- and glucose-dependent OCR, driven by palmitate/BSA conjugate or

pyruvate/glucose respectively, were measured under basal conditions. Briefly, myocytes were seeded at 100,000 cells/well and exposed to assay media 1 h prior to the assay. Krebs-Henseleit buffer containing L-carnitine hydrochloride (0.4 mM), glucose (2.5 mM) and palmitate/BSA conjugate (200 μM) was used to measure fatty acid-dependent respiration, whereas DMEM (pH 7.4) supplemented with pyruvate (1 mM) and glucose (10 mM) was used to assess glucose-dependent respiration. Following the measurement of *basal OCR*, oligomycin (1 μM), FCCP (palmitate assays – 10 μM; glucose assays – 2 μM), and rotenone + antimycin A (1 μM each) were sequentially injected, permitting determination of multiple parameters of mitochondrial bioenergetics. First, addition of oligomycin, an inhibitor of ATP synthase, decreases basal OCR due to any oxygen consumption linked to ATP synthesis; thus, the magnitude of decrease reflects *ATP-linked OCR*, and the remaining portion of basal OCR represents OCR linked to *proton leak*. *Mitochondrial coupling efficiency*, or the efficiency with which mitochondria convert oxygen into ATP, is therefore determined as the ratio of ATP-linked OCR/basal OCR. Second, FCCP uncouples the ETC and allows protons to flow back into the mitochondrial matrix to reduce oxygen; thus, OCR in the presence of FCCP reflects the *maximal respiratory capacity*, and the difference between the maximal OCR and basal OCR reflects the *spare respiratory capacity*. Finally, rotenone + antimycin A abolish electron flow through complexes I to III, such that no oxygen is consumed at cytochrome c oxidase. The remaining oxygen rate consumption after this intervention is therefore due primarily to non-mitochondrial respiration (Brand & Nicholls, 2011).

Oxidation of exogenous palmitate was validated using a CPT1 inhibitor, etomoxir (40 μM). Also, BSA in the presence or absence of etomoxir served as negative control.

3.14. Statistics

All data are presented as means ± SEM. Statistical analyses were performed throughout using GraphPad Prism version 5. One-way ANOVA followed by a Newman–Keuls Multiple Comparison test was used to detect between-group differences. Differences were considered significant at $p<0.05$.

Chapter VI: Results

1. Anandamide suppresses ET1-induced cardiac myocyte hypertrophy.

At the cardiomyocyte level, hypertrophy is characterized by increased cell size and reactivation of the fetal gene program (Chien, Knowlton, Zhu, & Chien, 1991). As re-induction of fetal genes such as BNP is one of the most consistent markers of hypertrophy, expression of the BNP gene and BNP promoter-reporter constructs are used as experimental indicators of hypertrophy (LaPointe, 2005). To begin, we determined the effects of increasing concentrations of anandamide on ET1-induced myocyte enlargement. These first experiments suggested that 1 μM might be anti-hypertrophic while not affecting untreated cells (Figure 3). Therefore, this concentration was selected for further study. ET1 treatment (0.1 μM; 24-48 h) elicited cardiomyocyte hypertrophy, as evidenced by significant enlargement of myocytes (124±8%, $p<0.05$ *vs.* control) (Figure 4A) and activation of the BNP promoter (340±118%, $p<0.05$ *vs.* control) (Figure 4B). These ET1-induced hypertrophic indicators were attenuated by anandamide (myocyte size: 106±6%, $p<0.05$ *vs.* ET1; BNP: 119±44%, $p<0.05$ *vs.* ET1) (Figure 4).

Figure 3. Effect of increasing concentrations of anandamide on ET1-induced myocyte enlargement.

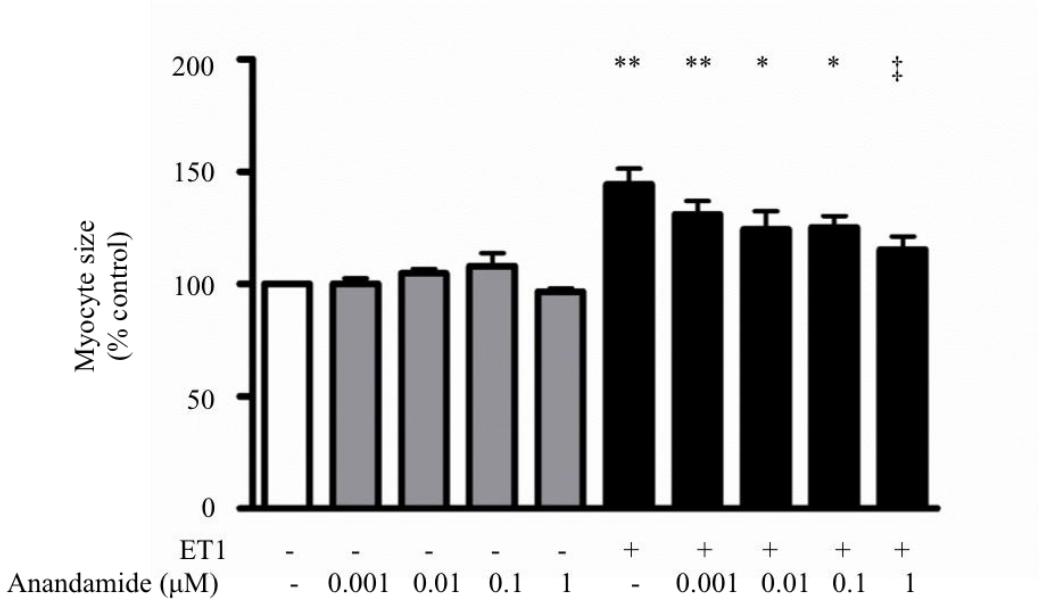


Figure 3. Serum-deprived myocytes were pre-treated with vehicle or increasing concentrations of anandamide (0.001-1 μM; 1 h), followed by addition of ET1 (0.1 μM; 48 h). ET1-induced myocyte enlargement was attenuated by anandamide at a concentration of 1 μM, while this concentration did not affect untreated cells. n=5. *p<0.05 and **p<0.01 *vs.* control (open bar); ‡p<0.01 *vs.* ET1.

Figure 4. Anandamide inhibits ET1-induced myocyte hypertrophy.

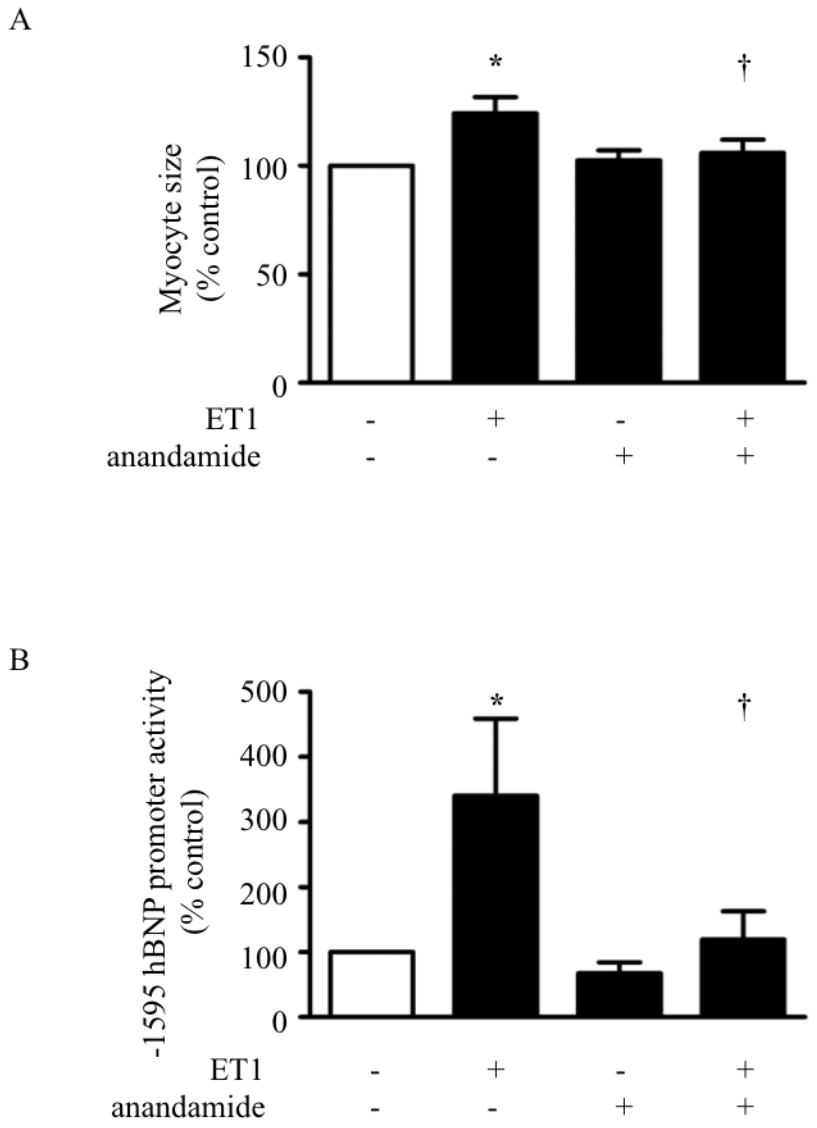


Figure 4. Serum-deprived myocytes were pre-treated with vehicle or anandamide (1 μM; 1 h), followed by addition of ET1 (0.1 μM; 24-48 h). *A*, Myocyte size (surface area) and *B*, fetal gene activation (-1595 human BNP promoter activity) were attenuated by anandamide. n=4-5. *$p<0.05$ *vs.* control (open bars); †$p<0.05$ *vs.* ET1.

2. R-methanandamide suppresses ET1-dependent induction of hypertrophic markers.

We also considered the possibility that the anti-hypertrophic effects of anandamide might be related to anandamide metabolism through the arachidonic acid cascade. Thus, to determine if metabolites contribute to anandamide-mediated anti-hypertrophic actions, we conducted experiments in which we replaced anandamide with R-methanandamide, which is a non-hydrolysable, metabolically stable analog of anandamide (Abadji et al., 1994). R-methanandamide also suppressed ET1-dependent myocyte enlargement (107±4%, $p<0.01$ *vs.* ET1 (131±6%, $p<0.01$ *vs.* control)) (Figure 5A) and BNP promoter activity (212±20%, $p<0.01$ *vs.* ET1 (388±61%, $p<0.01$ *vs.* control)) (Figure 5B). These results demonstrate that the capacity of anandamide (and R-methanandamide) to suppress cardiac myocyte hypertrophy is not mediated by metabolites, but instead involves ligand activation of CB receptors.

Figure 5. Anti-hypertrophic actions of endocannabinoids are not mediated by metabolites generated through the arachidonic acid cascade

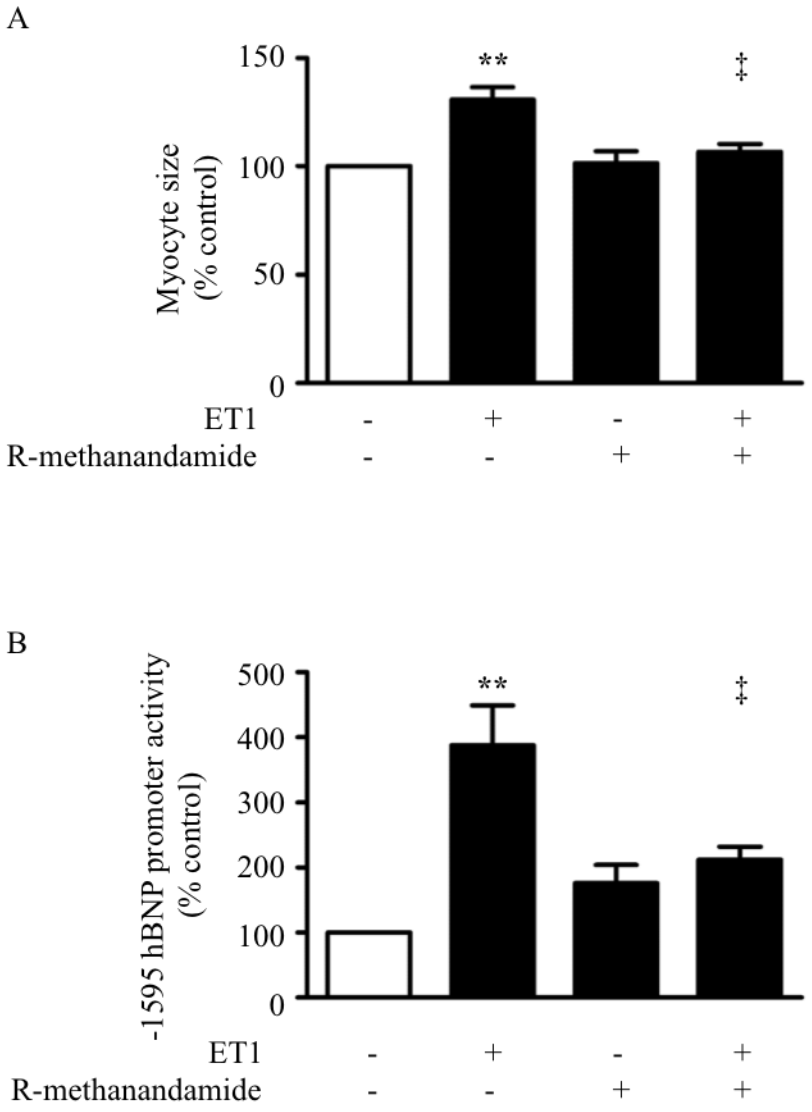


Figure 5. Serum-deprived myocytes were pre-treated with vehicle or metabolically-stable R-methanandamide (1 μM; 1 h), followed by addition of ET1 (0.1 μM; 24-48 h). *A*, Myocyte size (surface area), and *B*, fetal gene activation (-1595 human BNP promoter activity) were attenuated by R-methanandamide. n=4-5. **p<0.01 *vs.* control (open bars); ‡p<0.01 *vs.* ET1.

3. CB1 and CB2 receptors mediate distinct aspects of the anti-hypertrophic actions of R-methanandamide.

To determine whether CB1 and CB2 receptors regulate distinct aspects of the hypertrophic profile, we used selective pharmacological antagonists of CB1 and CB2 receptors. AM251 is a selective CB1 receptor antagonist (Pacher et al., 2006). Ki values for AM251 are CB1=7.49 nM *vs.* CB2=2290 nM (Lan, Gatley, et al., 1999). In contrast, AM630 is selective for CB2 receptors; Ki values are CB1=5152 nM vs. CB2=31.2 nM (Pacher et al., 2006; R. A. Ross et al., 1999). The ability of R-methanandamide to suppress myocyte enlargement was abolished by AM630 (124±6%, $p<0.01$ *vs.* control), but was not affected by AM251 (Figure 6A). In contrast, the ability of R-methanandamide to suppress ET1-dependent BNP promoter activation was abolished by AM251 (217±40%, $p<0.05$ *vs.* control) but not by AM630 (Figure 6B). Given reports that AM251 might also act as a GPR55 agonist, we verified the involvement of CB1 receptors using AM281 (Ki: CB1=12 nM *vs.* CB2=4200 nM) (Lan, Gatley, et al., 1999), as AM281 exerts neither agonistic nor antagonistic effects on GPR55 (Ryberg et al., 2007). In fact, the effects of AM281 agree with those of AM251. As with AM251, AM281 failed to affect the ability of R-methanandamide to suppress myocyte enlargement (Figure 7A). In contrast, AM281 abolished the inhibitory effects of R-methanandamide on ET1-induced BNP mRNA expression (365±56%, $p<0.05$ *vs.* control) (Figure 7B). These results show dissociation of the trophic effect from the gene-expression effect of ET1 in cardiomyocytes, and that agonism of both CB receptor subtypes is essential to attenuate both hypertrophic events (i.e. CB1 inhibits BNP gene activation, whereas CB2 inhibits myocyte enlargement).

Figure 6. Distinct CB receptor subtypes mediate the inhibitory effects of R-methanandamide on ET1-induced myocyte enlargement and fetal gene activation.

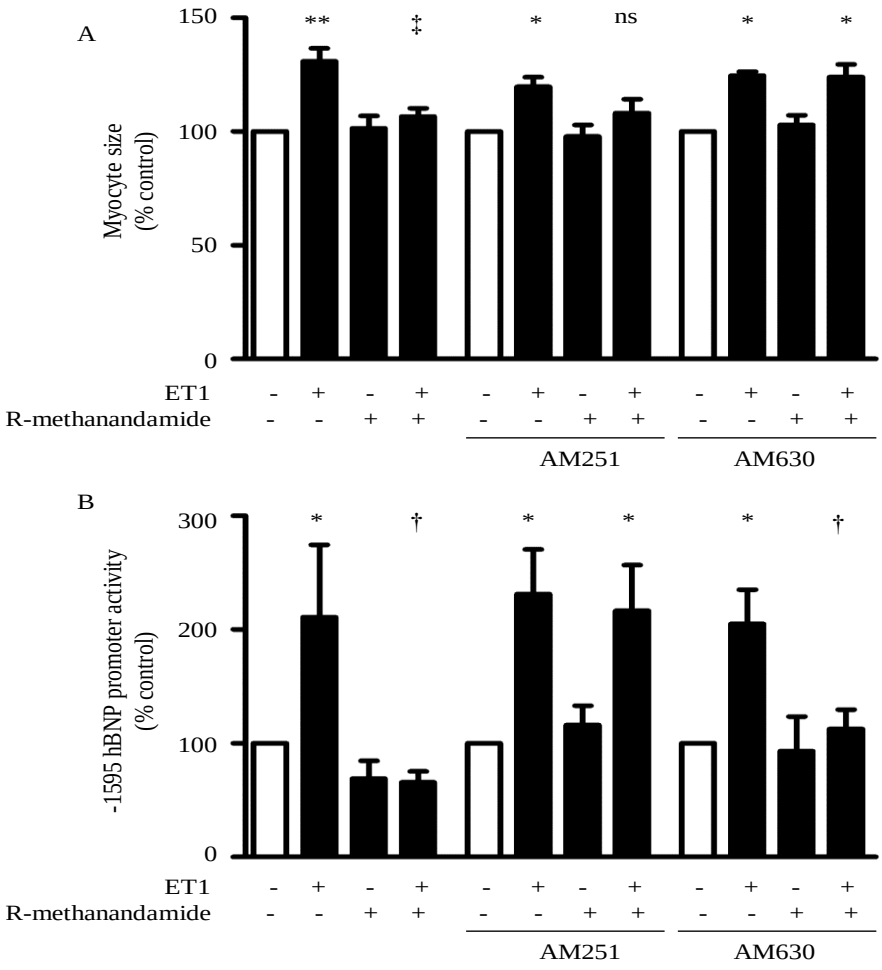


Figure 6. *A,* The ability of R-methanandamide (1 μM; 1 h) to suppress ET1-dependent (0.1 μM; 24-48 h) myocyte enlargement was unaffected by antagonism of CB1 receptors (AM251; 0.1 μM; 1 h), but was blocked by antagonism of CB2 receptors (AM630; 0.1 μM; 1 h). *B,* In contrast, R-methanandamide-dependent suppression of ET1-induced BNP promoter activation was blocked by AM251, but was unaffected by AM630. n=4-6. ns=not significant *vs.* control (open bars); *p<0.05 and **p<0.01 *vs.* control (open bars); †p<0.05 and ‡p<0.01 *vs.* ET1.

Figure 7. AM281, a CB1-selective antagonist, suppresses hypertrophic gene expression, but not myocyte enlargement.

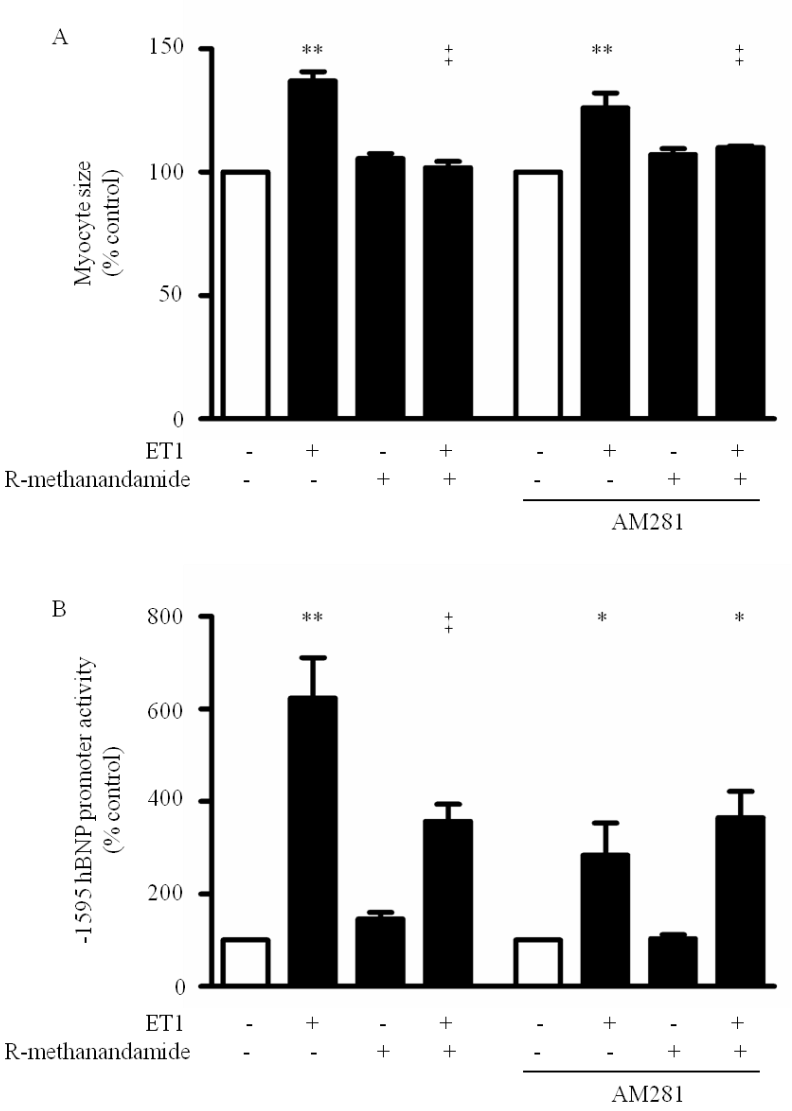


Figure 7. *A,* The ability of R-methanandamide (1 μM; 1 h) to suppress ET1-dependent (0.1 μM; 24-48 h) myocyte enlargement was unaffected by antagonism of CB1 receptors (AM281; 0.1 μM; 1 h). *B,* In contrast, R-methanandamide-dependent suppression of ET1-induced BNP promoter activation was blocked by AM281. n=3. *p<0.05 and **p<0.01 *vs.* control (open bars); ‡p<0.01 *vs.* ET1. These data suggest that the ability of AM251 to inhibit hypertrophy involved CB1 antagonism rather than GPR55 agonism.

4. Selective agonism of CB2 receptors attenuated ET1-induced myocyte enlargement, but not BNP promoter activation.

Clinical use of cannabinoid-based therapies has been impeded due to a series of psychoactive adverse effects (e.g. memory impairment, dizziness, and drug addiction) mediated by central CB1 receptors (Hosking & Zajicek, 2008; Kunos et al., 2009). One previously proposed approach is to selectively target CB2 receptors (Gertsch et al., 2008; Palazuelos et al., 2006), and cardioprotective effects of CB2 ligands have been reported (Lagneux & Lamontagne, 2001; Lepicier et al., 2003). Therefore, we assessed the effects of selective activation of CB2 receptors using JWH-133, a CB2 receptor-selective agonist. (Based on the role of CB1 and CB2 receptors in the anti-hypertrophic actions of R-methanandamide, we anticipated that JWH-133 would fail to completely abolish hypertrophy).

We determined the effects of increasing concentrations of JWH-133 on ET1-induced myocyte enlargement. As with anandamide and R-methanandamide, a JWH-133 concentration of 1 μM significantly attenuated hypertrophic growth (100±3%, $p<0.01$ *vs.* ET1 (127±3%, $p<0.01$ *vs.* control)) (Figure 8 and Figure 10A). This concentration was used in further experiments. To verify the involvement of CB2 and not CB1 receptors in JWH-133 effects, selective antagonists of CB1 and CB2 receptors were applied. The ability of JWH-133 to attenuate cell enlargement was not affected by AM251 (i.e. CB1 receptor antagonist), but was attenuated by AM630 (i.e. CB2 receptor antagonist) (117±2%, $p<0.01$ *vs.* control) (Figure 9).

However, JWH-133 did not attenuate ET1-induced increase in BNP mRNA expression (Figure 10B). These data suggest that ligand activation of CB2 receptors alone is not sufficient to completely prevent cardiac hypertrophy.

Figure 8. Effect of increasing concentrations of JWH-133, a CB2-selective agonist, on ET1-induced myocyte enlargement.

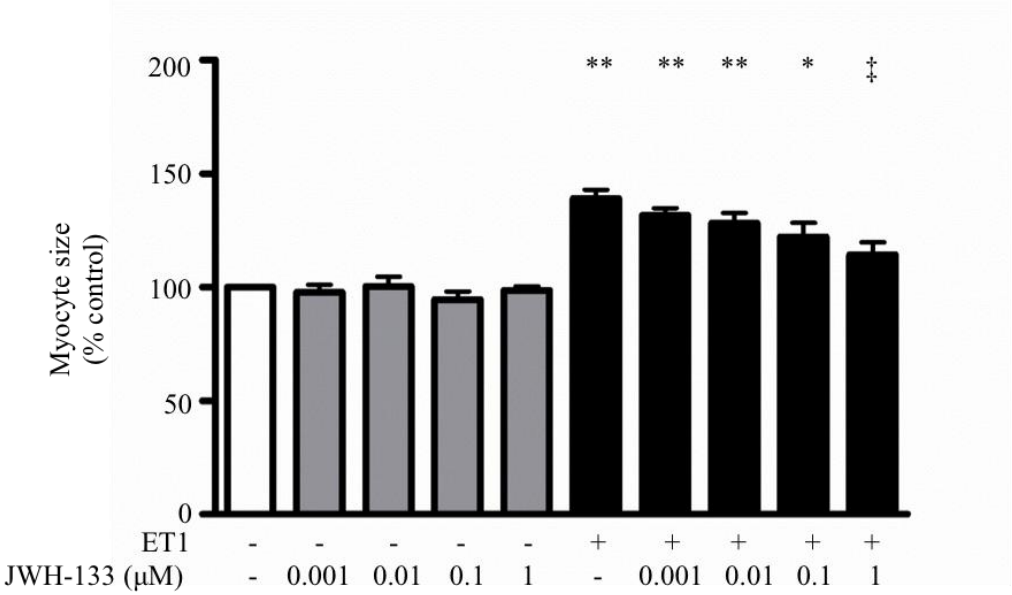


Figure 8. Serum-deprived myocytes were pre-treated with vehicle or increasing concentrations of JWH-133 (0.001-1 μM; 1 h), followed by addition of ET1 (0.1 μM; 48 h). Myocyte size was attenuated by JWH-133 at a concentration of 1 μM. n=5. *$p<0.05$ and **$p<0.01$ *vs.* control (open bars); ‡$p<0.01$ *vs.* ET1.

Figure 9. The inhibitory effects of JWH-133 on ET1-induced myocyte enlargement are mediated by the CB2 receptor.

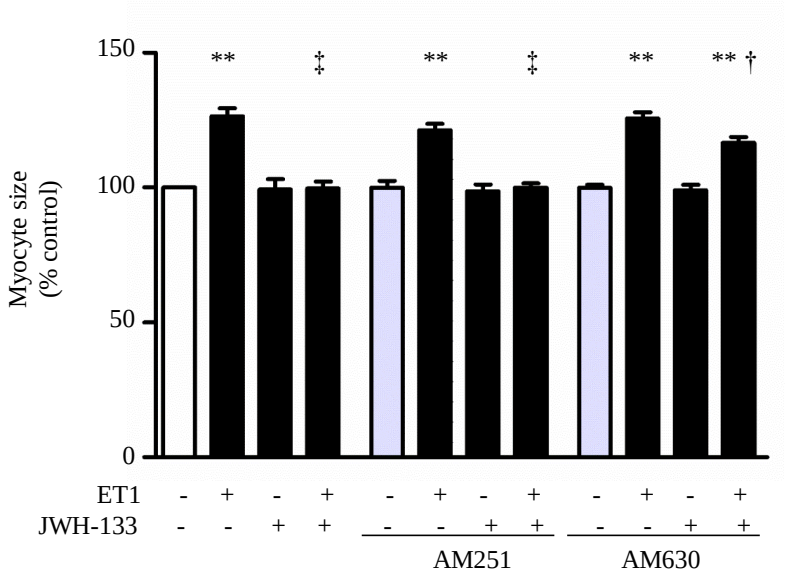


Figure 9. The ability of JWH-133 (1 μM; 1 h) to suppress ET1-dependent (0.1 μM; 48 h) myocyte enlargement was unaffected by antagonism of CB1 receptors (AM251; 0.1 μM; 1 h), but was blocked by antagonism of CB2 receptors (AM630; 0.1 μM; 1 h). n=3-5. **p<0.01 *vs.* control (open bars); †p<0.05 and ‡p<0.01 *vs.* ET1.

Figure 10. JWH-133 only partially suppresses ET1-induced hypertrophic processes.

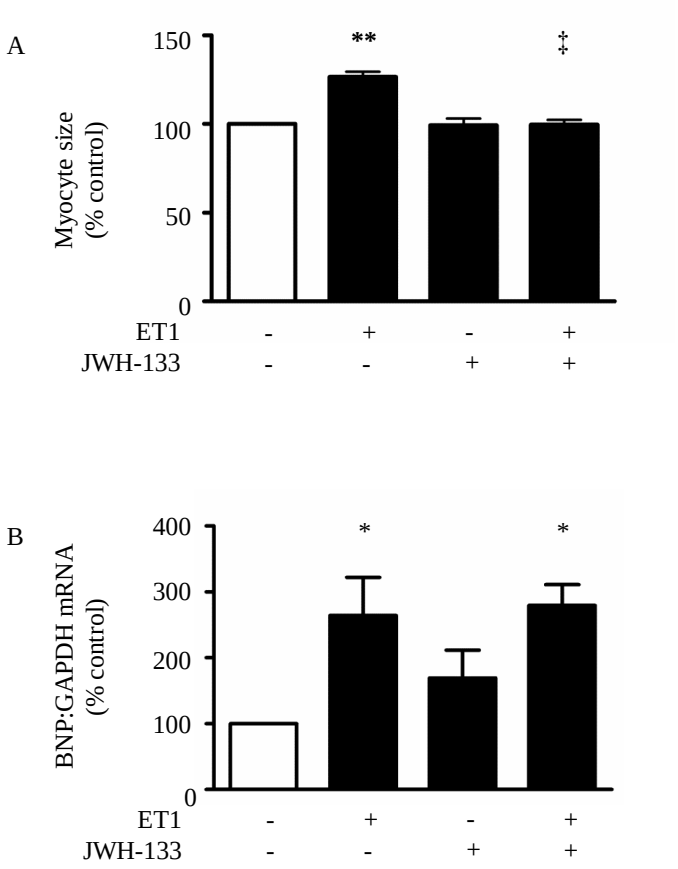


Figure 10. Serum-deprived myocytes were pre-treated with vehicle or JWH-133 (1 μM;1 h), followed by addition of ET-1 (0.1 μM; 24-48 h). JWH-133 attenuated *A*, myocyte size (surface area), but not *B*, fetal gene activation (BNP mRNA expression), n=3-9. *p<0.05 and **p<0.01 *vs*. control (open bars); ‡p<0.01 *vs*. ET1.

5. CB-13 attenuates all of the hypertrophy markers stimulated by ET1.

As we expected, selectively agonism of CB2 receptors failed to completely prevent cardiac myocyte hypertrophy. We then focused our efforts on an alternative strategy: selective targeting of peripheral (i.e. non-central) CB1 and CB2 receptors. To perform these experiments, we selected CB-13, a peripherally-restricted dual agonist of CB1 and CB2 receptors.

First, we determined the effects of increasing concentrations of CB-13 on ET1-induced myocyte enlargement. As with anandamide, R-methanandamide, and JWH-133, a CB-13 concentration of 1 μM significantly attenuated hypertrophic growth (105±3%, $p<0.01$ *vs.* ET1 (122±4%, $p<0.01$ *vs.* control)) (Figure 11 and Figure 12A). This concentration was used in further experiments.

In addition, CB-13 prevented the ET1-dependent induction of BNP mRNA expression (210±31%, $p<0.05$ *vs.* ET1 (426±89%, $p<0.01$ *vs.* control)) (Figure 12B). These results suggest that dual agonism of CB1 and CB2 receptors is able to prevent the hypertrophic process in cardiac myocytes.

Furthermore, we found that micromolar CB-13 had no adverse effects on myocyte viability (Figure 13). Moreover, although ET1 treatment prolonged shortening and relengthening velocities, CB-13 had no adverse effects on contractile function either in untreated or ET1-treated cardiac myocytes (Table 5).

Figure 11. CB-13 suppresses ET1-induced myocyte enlargement at 1 μM.

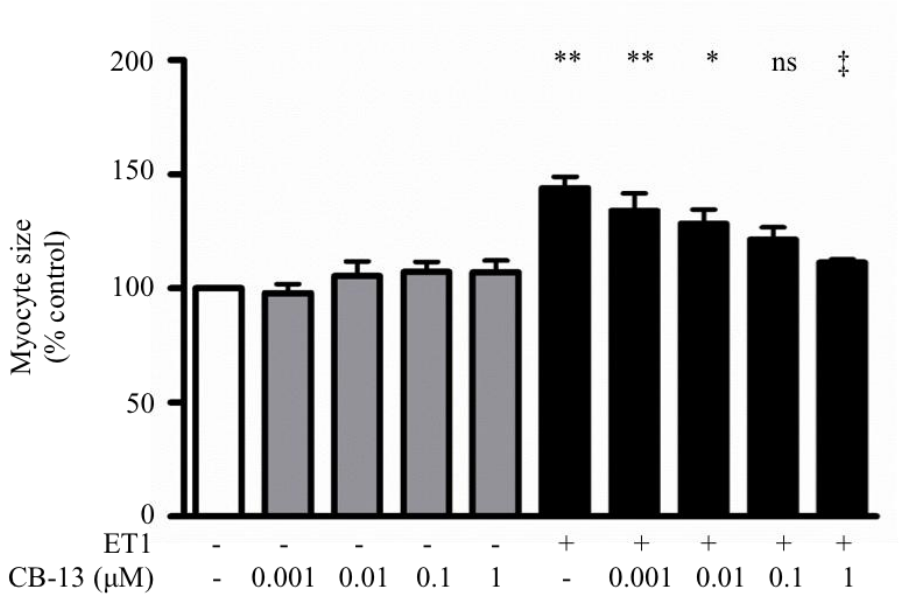


Figure 11. Serum-deprived myocytes were pre-treated with vehicle or increasing concentrations of CB-13 (0.001-1 μM; 1 h), followed by addition of ET1 (0.1 μM; 48 h). Myocyte size was attenuated by CB-13, in part at a concentration of 0.1 μM, and completely at 1 μM. n=4. ns=not significant *vs.* ET1. *p<0.05 and **p<0.01 *vs.* control (open bars); ‡p<0.01 *vs.* ET1.

Figure 12. Dual agonism of CB1/CB2 receptors by CB-13 suppresses ET1-induced myocyte hypertrophy.

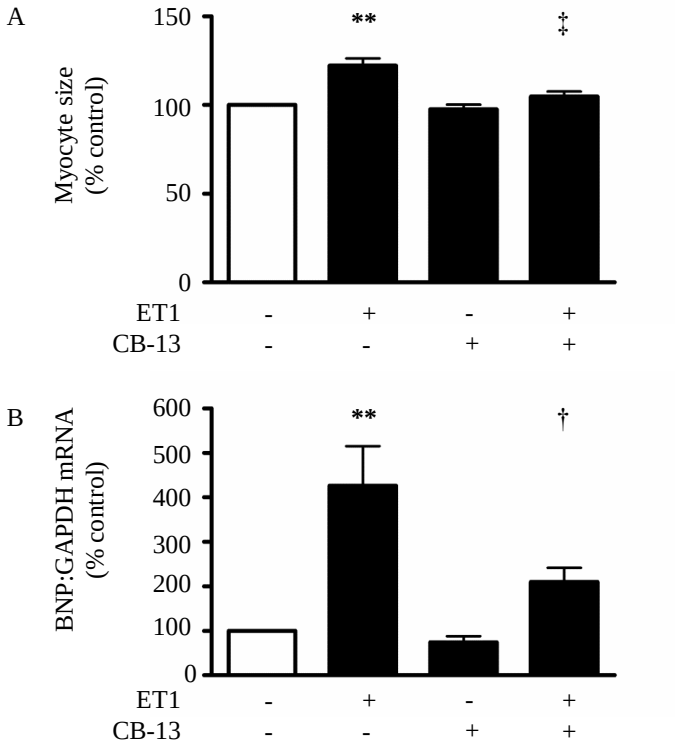


Figure 12. Serum-deprived myocytes were pre-treated with vehicle or CB-13 (1 μM; 1 h), followed by addition of ET1 (0.1 μM; 24-48 h). *A*, Myocyte size (surface area), and *B*, fetal gene activation (BNP mRNA expression) were attenuated by CB-13. n=5-7. **p<0.01 *vs.* control (open bars); †p<0.05 and ‡p<0.01 *vs.* ET1.

Figure 13. CB-13 does not adversely affect cardiac myocyte viability.

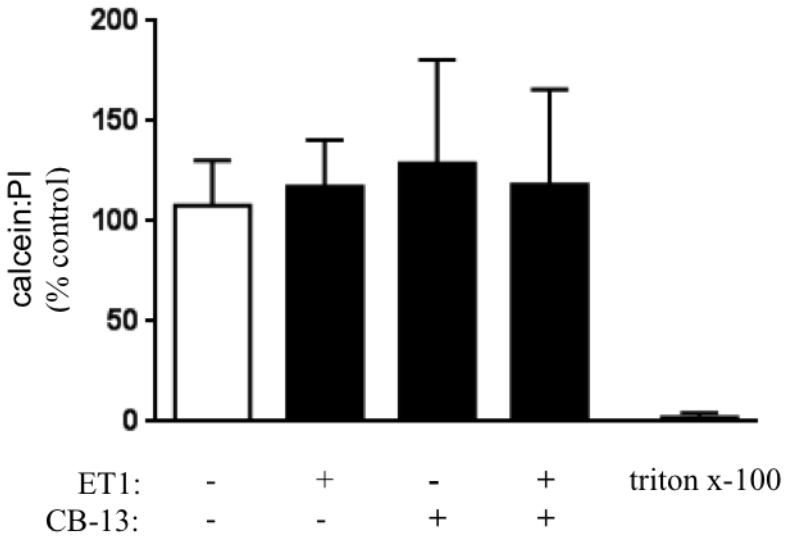


Figure 13. Neither CB-13 (1 μM) nor ET1 (0.1 μM) affected myocyte viability, as measured by calcein/propidium iodide (PI) fluorescence staining. n=7. This experiment was designed by me and performed by Ms. Bolanle Akinwumi (Ph.D. student as of 2016).

Table 5. CB-13 does not adversely affect cardiac myocyte contractile function.

Parameter	Control	ET1	CB-13	CB-13+ET1
Maximal velocity of shortening (+dL/dt; μm/s)	133 ± 10	86 ± 6*	147 ± 14	79 ± 6*
Maximal velocity of relengthening (-dL/dt; μm/s)	-92 ± 9	-51 ± 5*	-97 ± 15	-54 ± 9*
Peak shortening (% control)	100 ± 0	88 ± 10	116 ± 19	93 ± 10

Table 5. ET1 (0.1 μM) significantly prolonged shortening (contraction) and relengthening (relaxation) velocities of adult rat cardiac myocytes. However, CB-13 (1 μM) had no adverse effects on contractile function either in untreated or ET1-treated cardiac myocytes. *$p<0.01$ *vs.* control. This experiment was designed by me and conducted by Ms. Bolanle Akinwumi (Ph.D. student as of 2016).

6. AMPK-eNOS signaling contributes to CB-13 effects.

As discussed above, we identified the AMPK-eNOS signaling axis as a candidate mediator of CB-13 effects. CB-13 significantly increased phosphorylation of AMPKα at Thr172 (CB-13 4h: 354±58% and CB-13 8h: 321±64%, $p<0.01$ *vs.* control) (Figure 14A), which is an indicator of AMPK activation status (Beauloye, Bertrand, Horman, & Hue, 2011; Witters, Kemp, & Means, 2006). Consistent with reports that activated AMPK promotes phosphorylation and activation of eNOS at Ser1177 (Z. P. Chen et al., 1999; Morrow et al., 2003), CB-13 also activated eNOS (CB-13 4h: 237±51%, $p<0.01$ *vs.* control) (Figures 14B). We next performed knockdown of AMPK$\alpha_{1/2}$ to ascertain its role in eNOS phosphorylation by CB-13. Infection of cardiomyocytes with lentiviral constructs expressing shRNA against AMPKα_1/α_2 produced significant, simultaneous reductions of AMPKα_1, AMPKα_2 and total AMPKα to 17±6, 28±14% and 20±4%, respectively (Figure 15). The ability of CB-13 to induce eNOS phosphorylation (CB-13 (in the presence of empty vector): 143±8%, $p<0.05$ *vs.* control) was abolished by AMPK knockdown (CB-13 (in the presence of AMPK$\alpha_{1/2}$ shRNA): 83±23%, $p>0.05$ *vs.* control (83±10%)) (Figure 16). These findings suggest that CB-13 stimulates AMPK-eNOS signaling.

Moreover, the ability of CB-13 to attenuate cardiomyocyte hypertrophy was abolished by disruption of AMPK signaling using a chemical inhibitor (compound C/dorsomorphin: Ki = 109 nM; 1 μM) (myocyte size: 123±5%, $p<0.01$ *vs.* control, BNP mRNA: 232±44%, $p<0.01$ *vs.* control) or by shRNA knockdown of AMPK$\alpha_{1/2}$ (myocyte size: 124±4%, $p<0.01$ *vs.* control; BNP mRNA: 148±17%, $p<0.05$ *vs.* control) when normalized to their respective controls, (Figure 17 and 18). The selective eNOS inhibitor N5-(1-iminoethyl)-L-ornithine (L-NIO; IC50 = 500 nM; 10x and 5x less potent at nNOS

and iNOS, respectively; 1 μM) (Moore et al., 1994) also ablated the anti-hypertrophic effects of CB-13 (myocyte size: 124±6%, $p<0.01$ *vs.* control; BNP mRNA: 318±76%, $p<0.05$ *vs.* control) (Figure 19). It bears mentioning that shRNA knockdown of AMPK$\alpha_{1/2}$ and L-NIO increased baseline cardiomyocyte size to 161±20% ($n=3$, $p<0.05$) and 159±17% ($n=5$, $p<0.01$) *vs*. control, respectively, suggesting basal anti-growth activities of AMPK and eNOS in cardiomyocytes. Collectively, these findings indicate that CB-13 attenuates cardiomyocyte hypertrophy via AMPK-eNOS signaling.

Figure 14. CB-13 stimulates AMPK and eNOS phosphorylation.

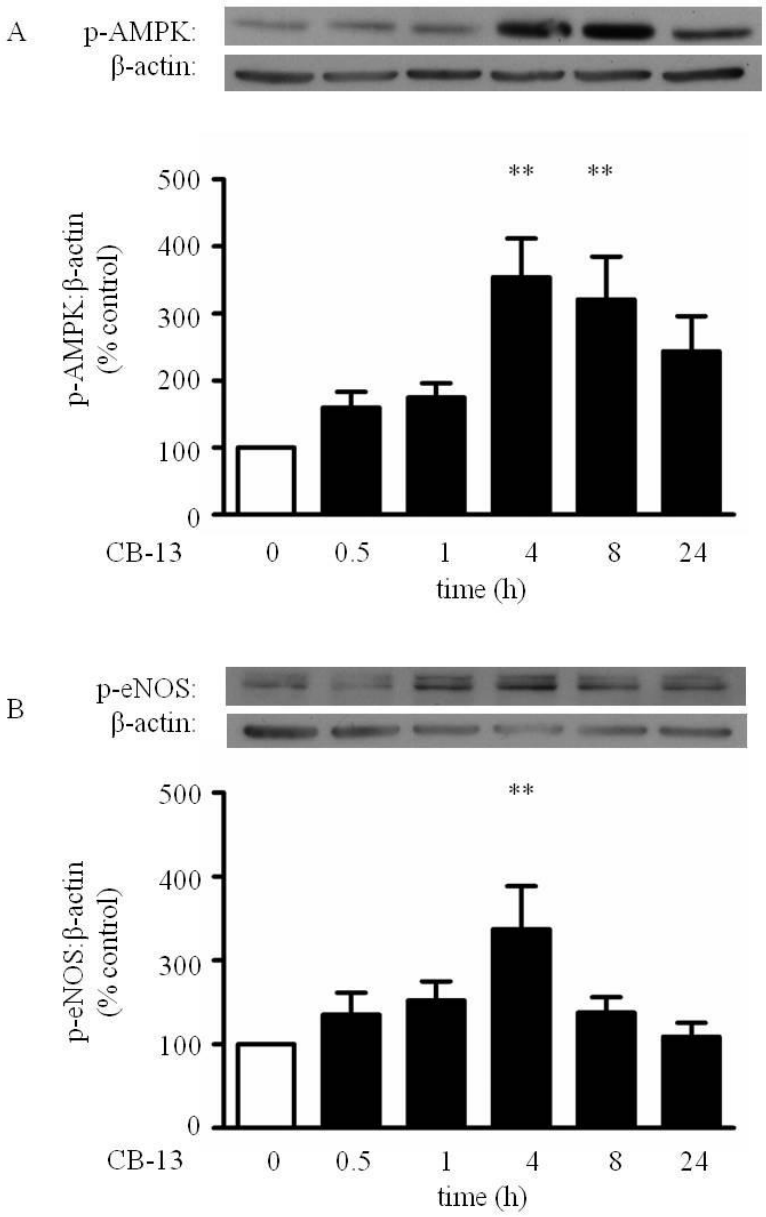


Figure 14. Serum-deprived myocytes were treated with vehicle or CB-13 (1 μM) for 0-24 h. *A,* AMPKα phosphorylation (Thr172), and *B,* eNOS phosphorylation (Ser1177), measured by conventional western blotting, were stimulated by CB-13 at 4 h (p-AMPK and p-eNOS) and 8 h (p-AMPK). Results are presented as percent of normalized protein *vs.* vehicle-treated controls. n=10. **p<0.01 *vs.* vehicle-treated control (open bars).

Figure 15. shRNA silencing of AMPKα isoforms.

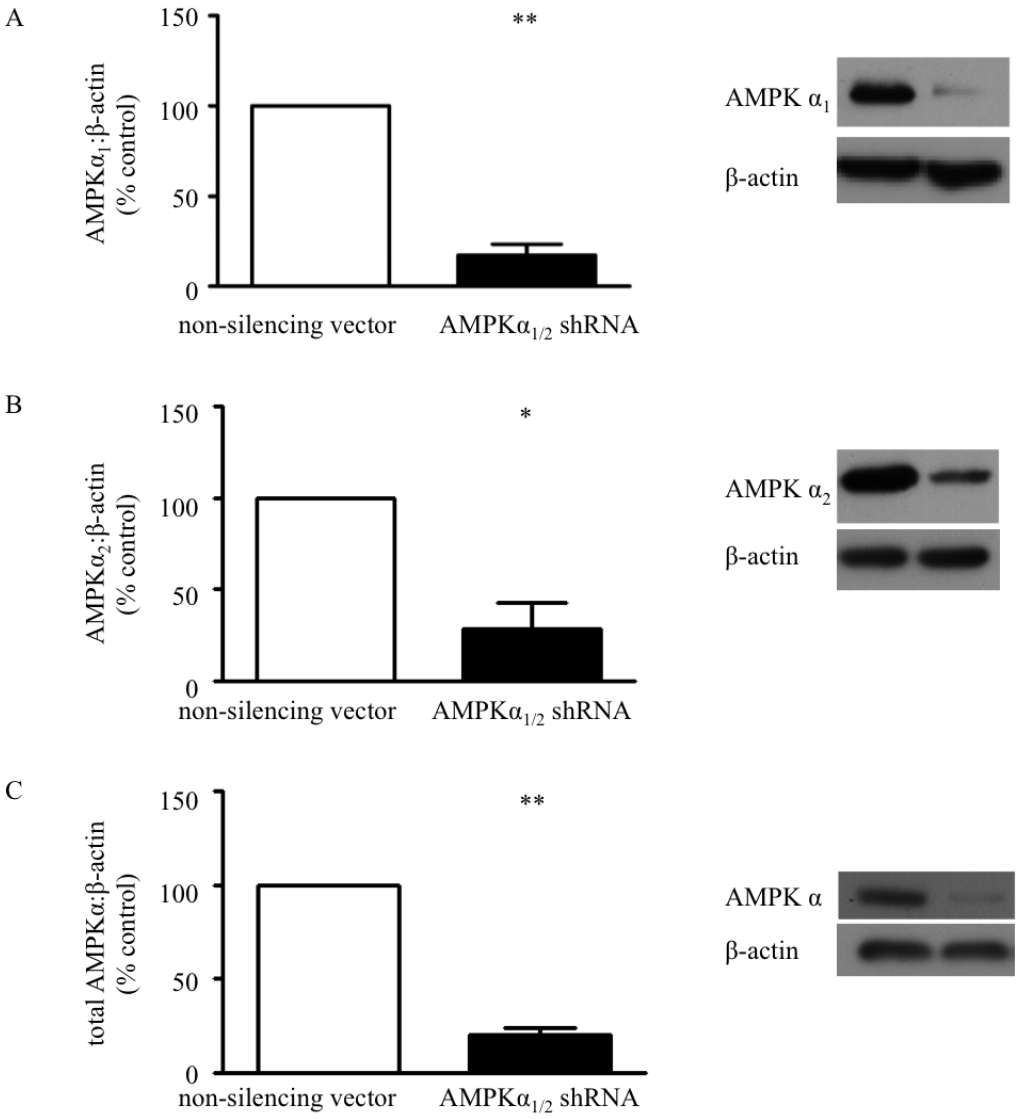


Figure 15. Serum-starved myocytes were infected with lentivirus carrying shRNA against AMPK α_1 and α_2 or non-silencing empty vector. Five days post-infection, infection efficiencies were 89±3% and 88±1% for non-silencing vector and AMPKα_1/α_2 shRNA, respectively. *A,* AMPK α_1, *B,* AMPK α_2, and *C,* AMPK α_1/α_2, measured by conventional western blotting, achieved significant knockdown in response to relevant shRNA. Results are presented as percent of normalized protein *vs.* empty vector-treated controls (open bars). n=3. *p<0.05 and **p<0.01 *vs.* non-silencing vector (open bars).

Figure 16. AMPK knockdown abolishes CB-13-dependent eNOS phosphorylation.

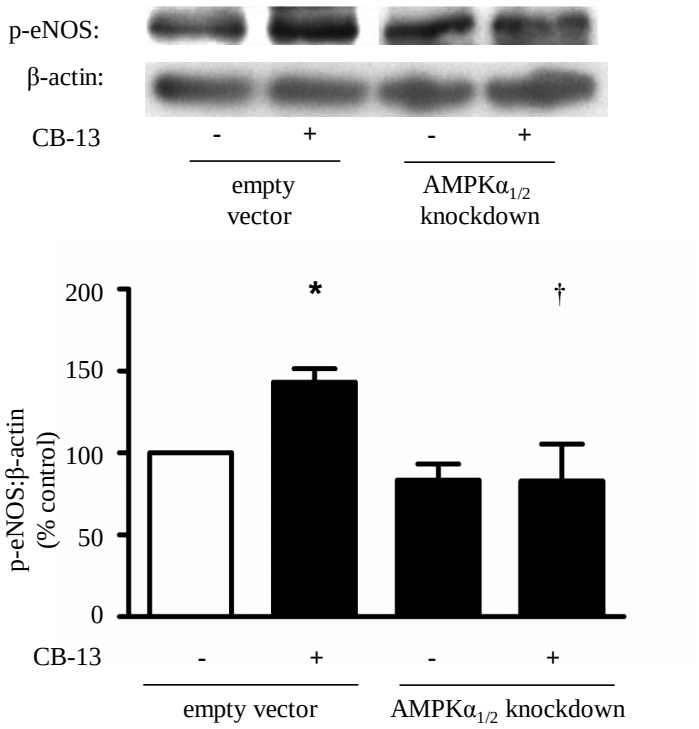


Figure 16. Simultaneous knockdown of AMPKα_1 and AMPKα_2 abrogated the ability of CB-13 to phosphorylate eNOS at Ser1177, suggesting that eNOS activation lies downstream of AMPKα. n=3.*p<0.05 and *vs.* control (open bars); †p<0.05 *vs.* CB-13.

Figure 17. Chemical inhibition of AMPK using compound C blocked the anti-hypertrophic actions of CB-13.

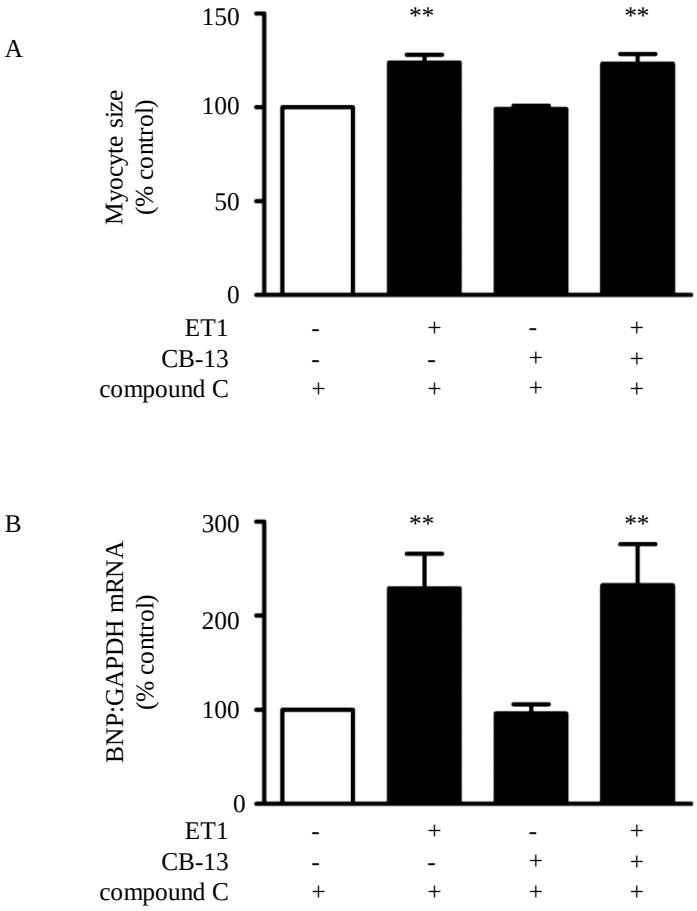


Figure 17. Serum-deprived myocytes were pre-treated with vehicle or CB-13 (1 μM; 1 h) in the presence and absence of compound C (AMPK inhibitor; 1 μM; 1 h), followed by addition of ET1 (0.1 μM; 24-48 h). The ability of CB-13 to attenuate ET1-induced increases in *A*, myocyte size (surface area), and *B*, fetal gene activation (BNP mRNA expression) was abolished by compound C. n=6-7. **p<0.01 *vs.* control (open bars).

Figure 18. shRNA knockdown of AMPK$\alpha_{1/2}$ blocked the anti-hypertrophic actions of CB-13.

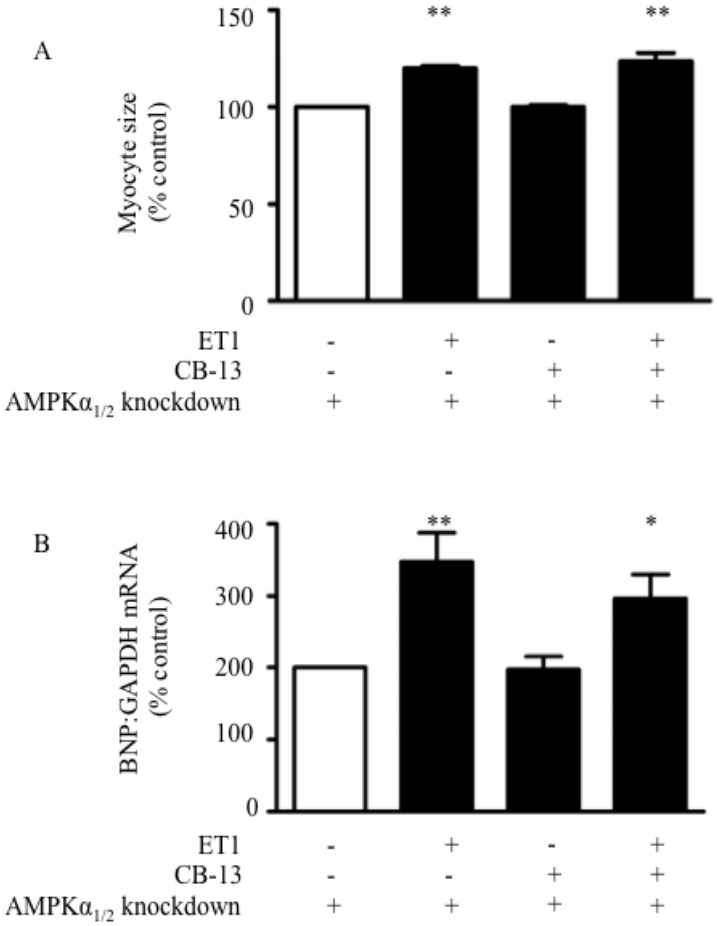


Figure 18. Serum-deprived myocytes were pre-treated with vehicle or CB-13 (1 μM; 1 h) in the presence and absence of lentivirus containing shRNA against AMPK α_1 and α_2, followed by addition of ET1 (0.1 μM; 24-48 h). The ability of CB-13 to attenuate ET1-induced increases in *A*, myocyte size (surface area), and *B*, fetal gene activation (BNP mRNA expression) was abolished by AMPK$\alpha_{1/2}$ knockdown. n=3-10. *p<0.05 and **p<0.01 *vs.* control (open bars).

Figure 19. Inhibition of eNOS using L-NIO blocked the anti-hypertrophic actions of CB-13.

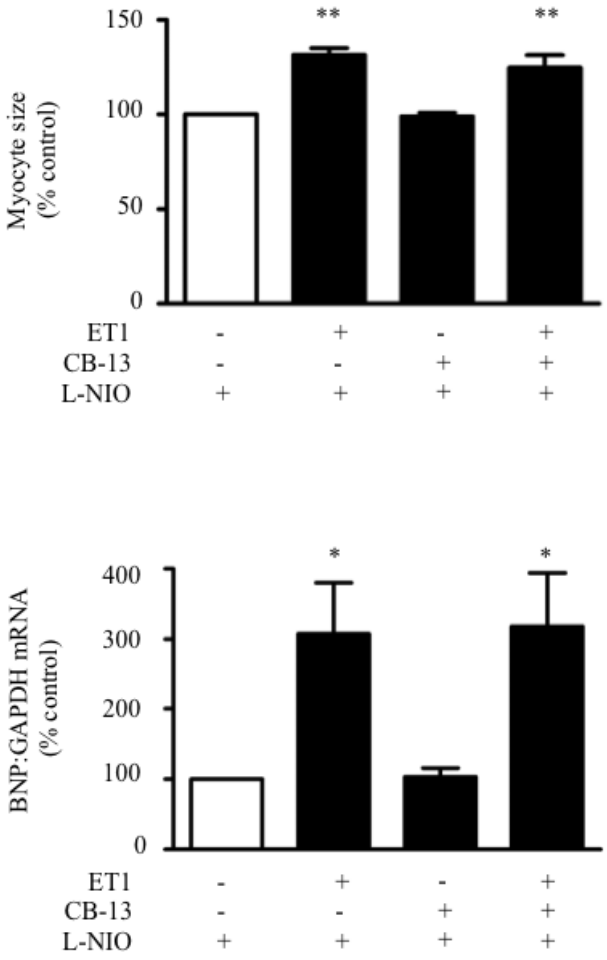


Figure 19. Serum-deprived myocytes were pre-treated with vehicle or CB-13 (1 μM; 1 h) in the presence and absence of L-NIO (eNOS inhibitor; 1 μM; 1 h), followed by addition of ET1 (0.1 μM; 24-48 h). The ability of CB-13 to attenuate ET1-induced increases in *A*, myocyte size (surface area), and *B*, fetal gene activation (BNP mRNA expression) was abolished by L-NIO. n=4-9. *p<0.05 and **p<0.01 *vs.* control (open bars).

7. With palmitate as the primary substrate, ET1-induced mPT is prevented by CB-13.

Calcein-AM and $CoCl_2$ dual staining is a well-established method used to assess the extent of mPT in intact cells (Petronilli et al., 1999). Calcein-AM is a membrane-permeant dye that, once inside the cell, is then converted to a fluorescent calcein (green) by intracellular esterases. Fluorescent calcein is then trapped in cellular compartments and unable to cross cellular membranes, including mitochondrial membranes. $CoCl_2$, a quencher of calcein fluorescence, selectively quenches calcein fluorescence in cytosol, thereby enabling detection of the fluorescent calcein signals accumulated in the mitochondria (manifested as bright fluorescence particles). However, in response to pathological stimuli, mPTPs open, thereby allowing fluorescent calcein to leak from mitochondria into the cytosol; this hence decreases the fluorescent contrast between mitochondria and cytosol, leading to a smeared appearance within cells. Mitotracker-Red (0.1 μM) was also co-loaded to verify the location of mitochondria (pictures not shown). The fluorescence signal of calcein and Mitotracker-Red overlapped, suggesting the bright fluorescent particles were within mitochondria.

Whether ET1 promotes mPT in the presence or absence of CB-13 was studied. First, we detected whether ET1 induces mPT in the absence of CB-13. Myocytes were pretreated with DMSO (vehicle of CB-13), and co-loaded with calcein AM and $CoCl_2$. Mitochondria stained with calcein fluorescence were presented as glowing particles (Figure 20A-1 and 20B-1); Addition of ET1 (0.1 μM) for 15 min dramatically disrupted the fluorescence density and dissolved those particles (Figure 20B-2), suggesting an increase in mPT. This mPT stimulation did not occur in myocytes treated with H_2O (ET1 vehicle) (Figure 20A-2). Second, we examined whether CB-13 pretreatment (1 μM; 1 h) prevented ET1-induced mPT. In the presence of CB-13, mitochondria stained with

calcein fluorescence were presented as glowing fluorescent particles (Figure 20C-1 and 20D-1). However, these particles were not affected by addition of ET1 or H_2O (Figure 20D-2 and 20C-2), indicating preserved mPT in the presence of CB-13. At the end of the experiment, myocytes from all of the groups were treated with ionomycin. Ionomycin causes Ca^{2+} overload and thereby induces mPT, and thus served as positive control. Addition of ionomycin (2 μM; 5 min) disrupted the fluorescence particles in all groups (Figure 20A-3, 20B-3, 20C-3, and 20D-3). These results suggest protective effects of CB receptor activation on ET1-induced mPT.

Figure 20. CB-13 ameliorates ET1-induced mPT.

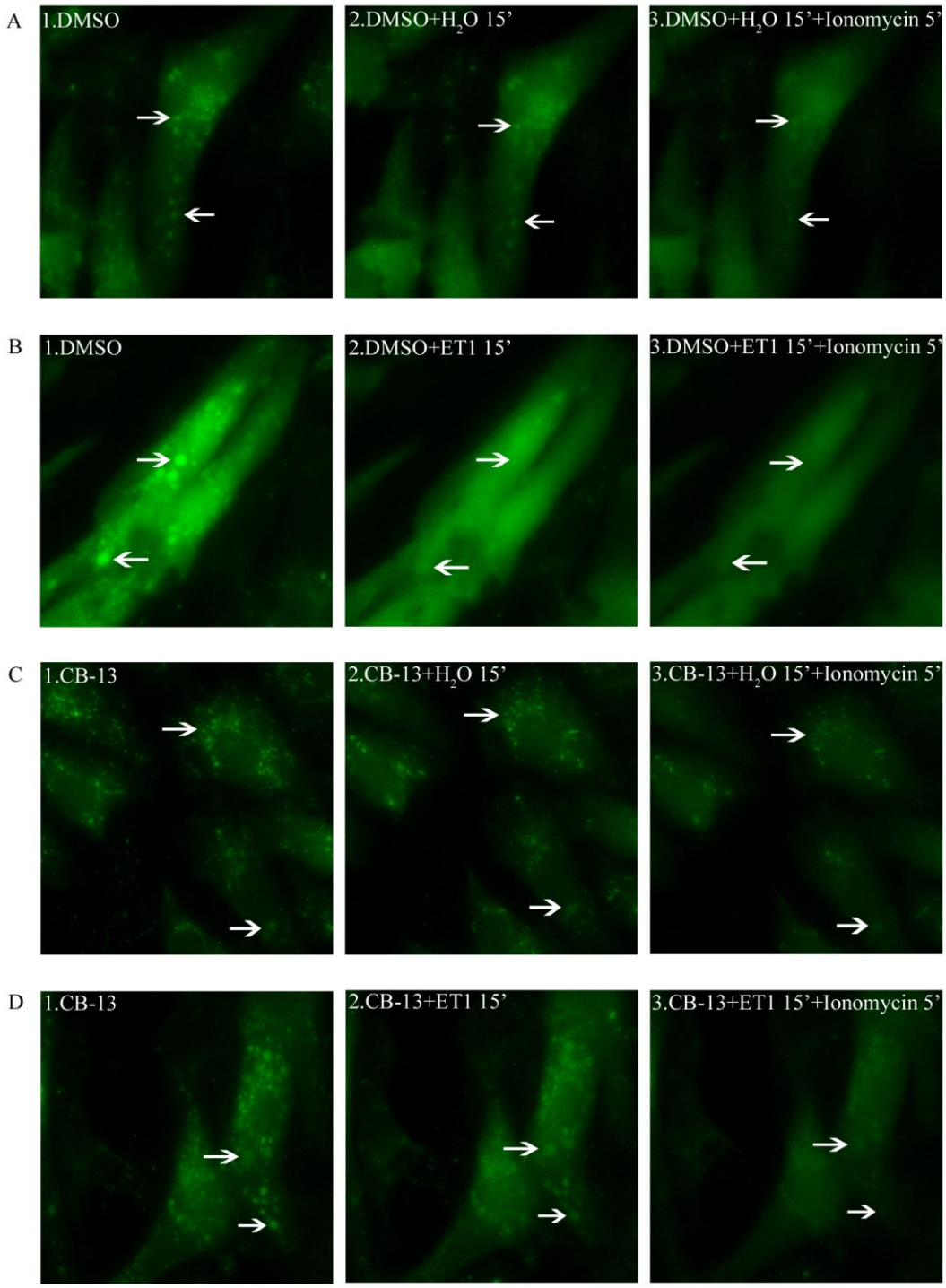


Figure 20. Myocytes were pre-treated with vehicle (DMSO) or CB-13 (1 μM; 1 h) in Krebs-Henseleit buffer containing palmitate/BSA (200 μM), L-carnitine hydrochloride (0.4 mM) and glucose (2.5 mM). Myocytes were then co-loaded with Calcein-AM (2 μM; 15 min), $CoCl_2$ (2 mM; 15 min) and Mitotracker-Red (0.1 μM; 15min). After washing with PBS three times for 5 min, images were acquired pre- and post-treatement with ET1 (0.1 μM) or its vehicle H_2O. Ionomycin (2 μM) was added as a positive control at the end of each experiment. $CoCl_2$ quenches the fluorescence in cytosol but not mitochondria, thus allowing detection of brighter mitochondrial particles. *B-2,* addition of ET1 for 15 min significantly dissipated the mitochondrial particles compared to *A-2,* H_2O-treatment, suggesting calcein fluorescence leaked from the mitochondria. This is interpreted as increased mPT. In the presence of CB-13, neither *C-2,* H_2O nor *D-2,* ET1 affected mitochondrial fluorescence, suggesting preserved mPT. *A/B/C/D-3,* as expected, ionomycin induced mPT in all groups.

8. With palmitate as the primary substrate, CB-13 prevents ET1-induced mitochondrial membrane depolarization in an AMPK-independent manner.

The ratio of aggregate to monomer fluorescence of JC-1 was measured as an indicator of changes in $\Delta\psi_m$. In healthy mitochondria with relatively high $\Delta\psi_m$, JC-1 concentrates as J-aggregates and shows intense red fluorescence. In contrast, in unhealthy mitochondria with reduced $\Delta\psi_m$, JC-1 presents mainly as monomeric form due to decreased concentration and emits green fluorescence. As shown in figure 21, the ratio of red J-aggregates/green monomer declined in ET1-treated cells (80±3%, $p<0.05$ *vs.* control), reflecting membrane depolarization. This depolarization was attenuated in the presence of CB-13 pretreatment (106±10%, $p<0.05$ *vs.* ET1). However, the ability of CB-13 to prevent mitochondrial depolarization was not affected by AMPK chemical inhibitor, compound C (Figure 22). These data indicate that $\Delta\psi_m$ is dissipated in ET1-treated cardiac myocytes where fatty acids are the primary substrates; CB receptor activation preserves $\Delta\psi_m$ in an AMPK-independent manner.

Figure 21. CB-13 prevents ET1-induced mitochondrial membrane depolarization in the presence of palmitate as substrate.

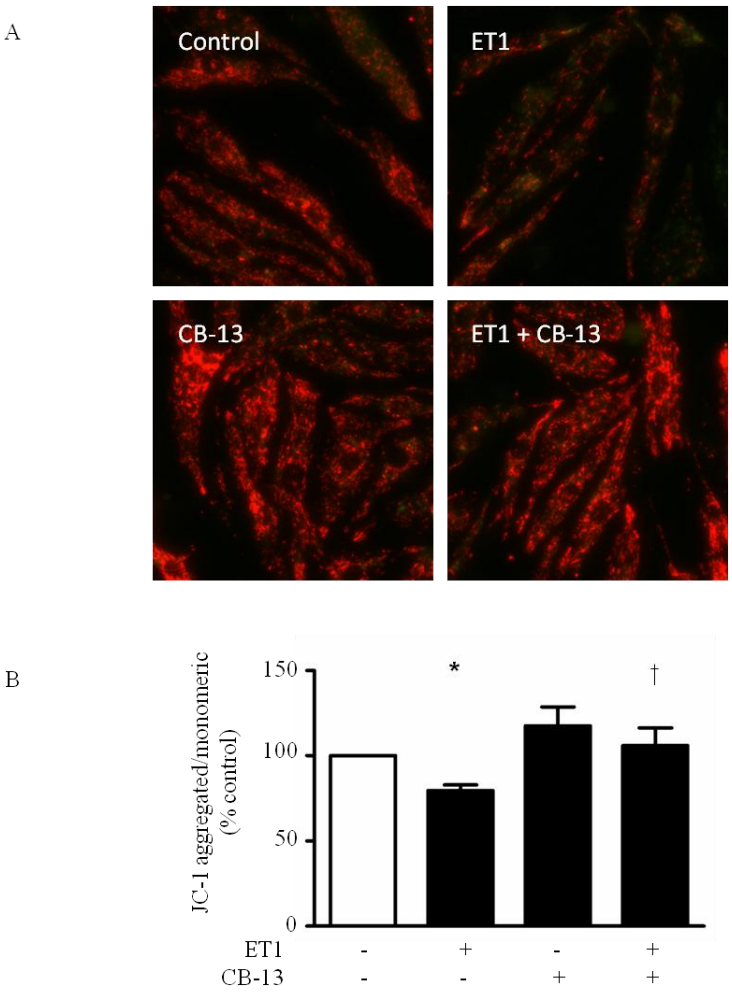
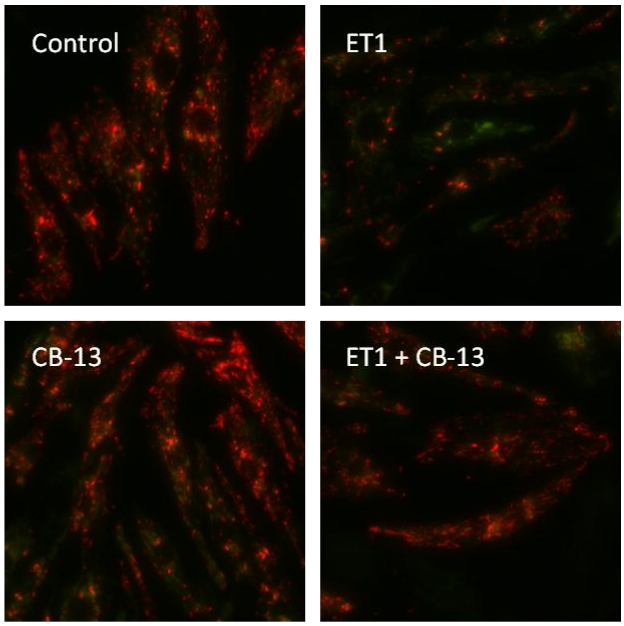


Figure 21. Serum-deprived myocytes were pre-treated with CB-13 (1 μM, 2 h), followed by addition of ET1 (0.1 μM; 4 h) in media containing palmitate/BSA (200 μM) as substrate. The ability of ET1 to induce mitochondrial membrane depolarization, indicated by decreased ratio of JC-1 aggregated red signal to monomeric green signal, was attenuated by pre-treatment with CB-13 (1 μM; 2 h). Results are presented as *A*, representative fluorescent images and *B*, percent of normalized red/green fluorescence ratio *vs.* control (open bar). n=7. *p<0.05 and *vs.* control (open bars); †p<0.05 *vs.* ET1.

Figure 22. AMPK inhibitor, compound C, does not influence the inhibitory effect of CB-13 on ET1-induced mitochondrial membrane depolarization in the presence of palmitate as substrate.

A



B

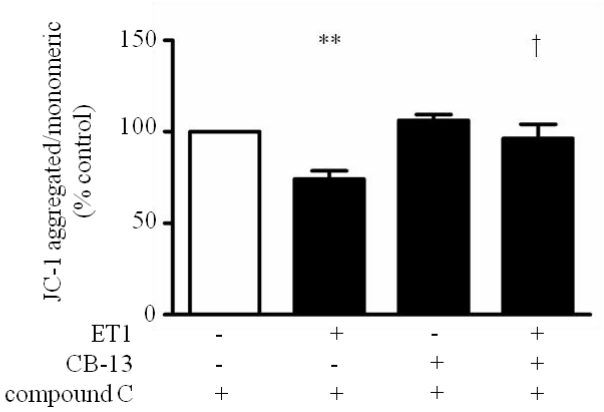


Figure 22. The ability of CB-13 (1 μM; 2 h) to attenuate ET1-induced (0.1 μM; 4 h) mitochondrial membrane depolarization was preserved in the presence of compound C (AMPK inhibitor, 1 μM; 1 h). Results are presented as *A,* representative fluorescent image and *B,* percent of normalized red/green fluorescence ratio *vs.* control (open bar). n=8. **p<0.01 and *vs.* control (open bars); †p<0.05 *vs.* ET1.

9. Optimization of working conditions for mitochondrial bioenergetics assay.

Mitochondrial bioenergetics of cardiac myocytes were assessed in the presence of glucose/pyruvate and palmitate as major substrates. It was important to first determine the optimal conditions for mitochondrial bioenergetics assays. Therefore, the glucose/pyruvate model was used to achieve the maximal responses, and to optimize cell seeding density and oligomycin concentration. Furthermore, as the maximal effective concentration of FCCP may vary depending on the different energy substrate provided (Abe et al., 2010), optimal concentrations of FCCP were ascertained for both glucose and palmitate.

9.1. Optimization of myocyte seeding density for mitochondrial bioenergetics assays.

It is critical to determine the optical cell density for mitochondrial bioenergetics assays. The optimal density of cells should present as a monolayer and generate a maximal OCR. In this study, primary neonatal rat cardiac myocytes were seeded into 24-well seahorse culture plate with test densities: 100,000 cells/well, 200,000 cells/well, 300,000 cells/well and 400,000 cells/well. As shown in figure 23, the OCRs declined with increases in cell density in this range (100,000 to 400,000 cells/well). In addition, Hill et al. detected a linear increase in OCR in neonatal rat ventricular myocytes within a range of 25,000 to 75,000 cells/well (B. G. Hill, Dranka, Zou, Chatham, & Darley-Usmar, 2009). Taken both results into consideration, the seeding density of 75,000-100,000 cells/well reaches a peak of OCR, whereas underseeding or overseeding impacts the accuracy of OCR measurements. Here, 100,000 cells/well was chosen for future experiments.

Figure 23. Optimization of myocyte seeding density for mitochondrial bioenergetics assays (glucose-based working buffer).

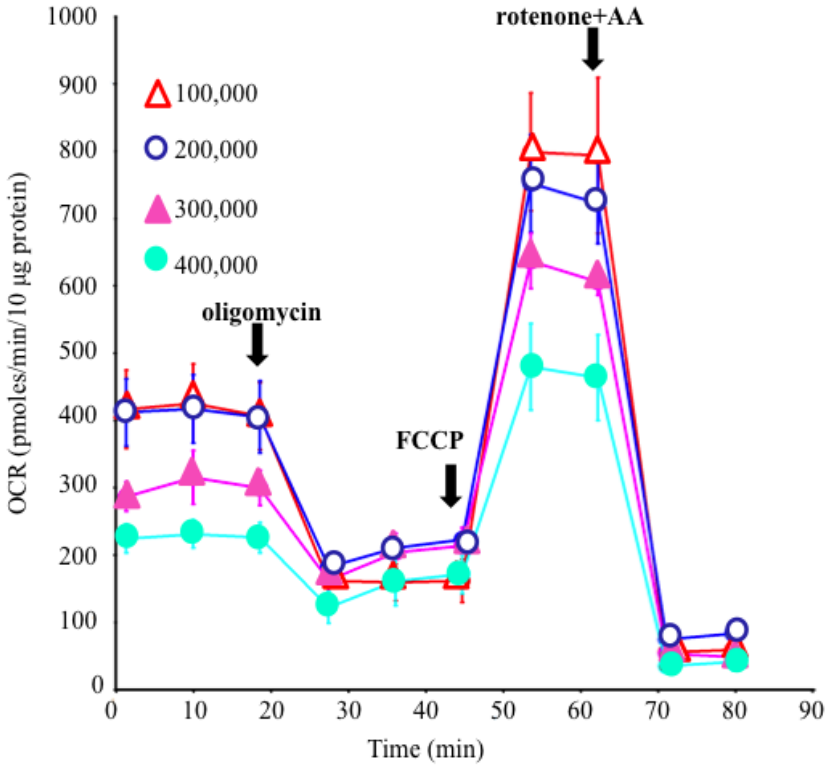


Figure 23. OCR were measured using the Seahorse Bioscience XF24 analyzer in cultured neonatal rat cardiac myocytes at cell densities ranging from 100,000 to 400,000 myocytes per well. The results are presented as the OCR per 10 µg protein. A cell density of 100,000 myocytes per well resulted in the maximal protein-normalized OCR. n=5 replicate cultures.

9.2. Optimization of oligomycin concentration in the presence of glucose/pyruvate.

Oligomycin inhibits ATP synthesis; therefore, injection of oligomycin causes an OCR drop that reflects ATP-linked OCR, and the remaining portion of basal OCR represents OCR linked to proton leak. Increasing concentrations of oligomycin were titrated with glucose/pyruvate as provided substrates. OCR plots were presented as percent values normalized to basal levels (basal OCR was set as a baseline of 100%) (Figure 24). Addition of increasing concentrations of oligomycin (0.5 μg/mL, 1 μg/mL, 5μg/mL and 10 μg/mL) reduced OCRs to similar degrees. A working oligomycin concentration of 1 μg/mL was selected for the subsequent experiments.

Figure 24. Optimization of oligomycin concentration for mitochondrial bioenergetics assays (glucose-based working buffer).

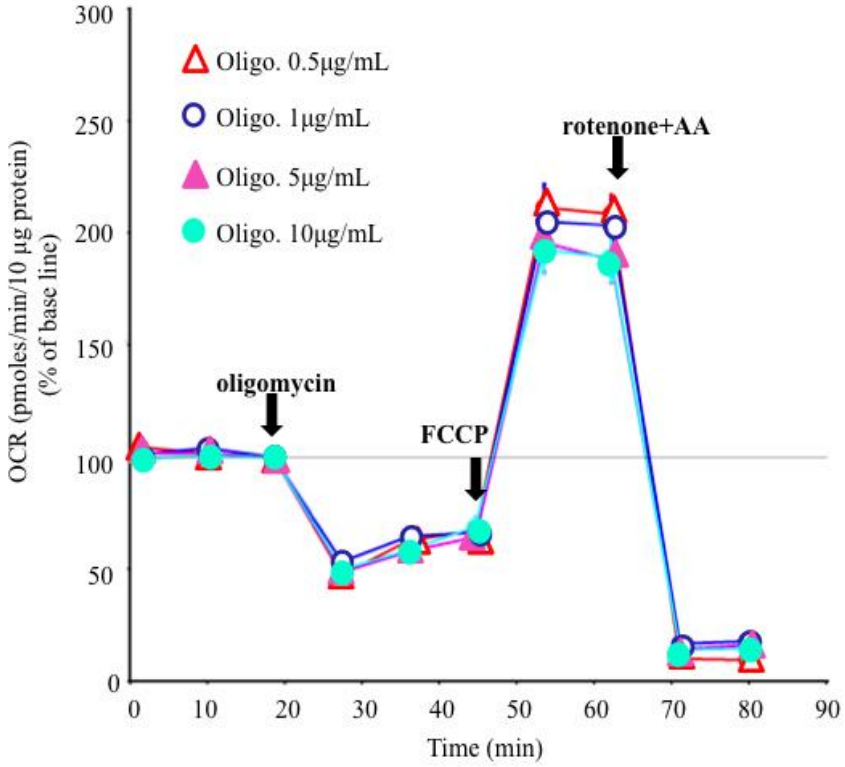


Figure 24. OCRs of cultured myocytes at basal levels were measured using the Seahorse Bioscience XF24 Analyzer in media containing 10 mM glucose and 1 mM pyruvate, followed by the sequential addition of increasing concentrations of oligomycin (0.5-10 µg/mL), then FCCP (2 µM) and R/AA (rotenone, 1 µM and antimycin A, 1 µM). Results are presented as the percent values of basal OCR. There were no concentration-dependent differences in oligomycin-induced OCR reductions between groups. Oligomycin at 1 µg/mL was chosen for subsequent experiments. n=5 replicate cultures.

9.3. Optimization of FCCP concentrations in the presence of glucose/pyruvate or palmitate as substrate.

FCCP uncouples the ETC and allows protons to flow back into the mitochondrial matrix to reduce oxygen, thereby reflecting a maximal respiratory capacity. Effective concentrations of FCCP vary in different cell types and with energy substrates (Abe et al., 2010; Choi, Gerencser, & Nicholls, 2009; B. G. Hill et al., 2009; J. Liu et al., 2009). Hence, it is important to optimize its concentration in cardiac myocytes in response to glucose or palmitate.

Increasing concentrations of FCCP were tested in the presence of either palmitate/BSA or glucose/pyruvate. As shown in figure 25, during glucose oxidation, FCCP concentrations ranging from 1 μM to 10 μM did not generate significant differences, except perhaps only a minor increase at 2 μM. In contrast, the effect of FCCP on fatty acid oxidation was concentration-dependent, and 10 μM achieved the maximal response (Figure 26). Therefore, 2 μM and 10 μM of FCCP were selected for glucose- and fatty acid-dependent OCR respectively.

Figure 25. Optimization of FCCP concentration for mitochondrial bioenergetics assays (glucose-based working buffer).

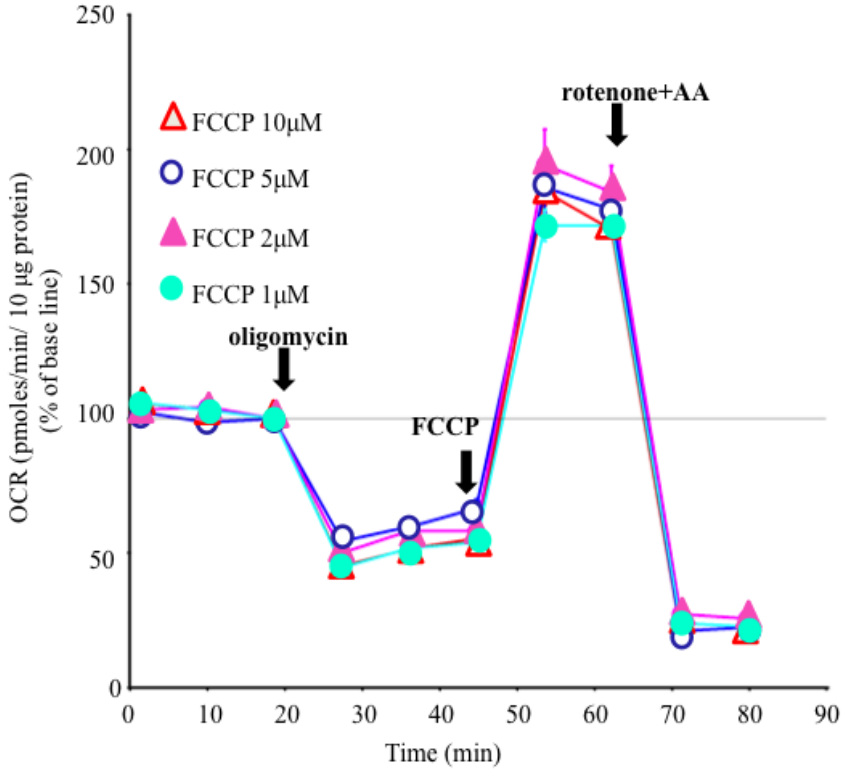


Figure 25. OCRs of cultured myocytes at basal levels were measured using the Seahorse Bioscience XF24 Analyzer in media containing 10 mM glucose and 1 mM pyruvate, followed by the sequential addition of oligomycin (1 µg/mL), FCCP (1-10 µM) and R/AA (rotenone, 1 µM and antimycin A, 1 µM). Results are presented as the percent of basal OCR. Differences between groups are subtle; FCCP of 2 µM gives an apparent maximal rate of OCR. n=5 replicate cultures.

Figure 26. Optimization of FCCP concentration for mitochondrial bioenergetics assays (palmitate-based working buffer).

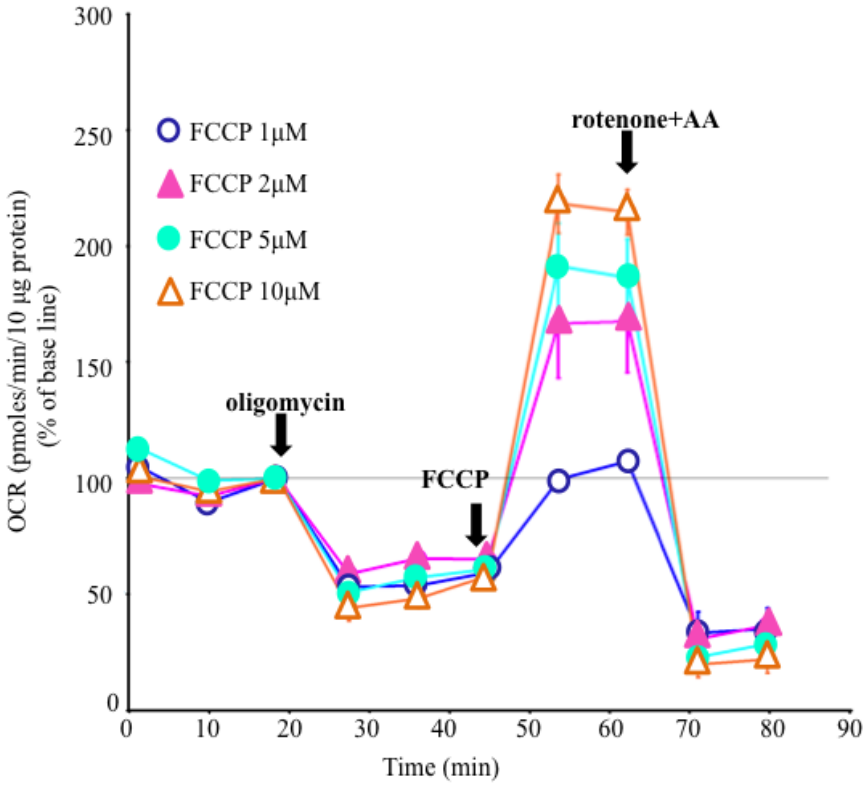


Figure 26. OCRs of cultured myocytes at basal levels were measured using the Seahorse Bioscience XF24 Analyzer with palmitate/BSA (200 µM) as substrates, followed by the sequential addition of oligomycin (1 µg/mL), FCCP (1-10 µM) and R/AA (rotenone, 1 µM and antimycin A, 1 µM). Results are presented as the percent of basal OCR. FCCP at 10 µM gives a maximal response of increased OCR. n=5 replicate cultures.

10. CB-13 rescues ET1-induced aberrations of fatty acid-related mitochondrial bioenergetics.

OCR was measured using the Seahorse Bioscience XF24 analyzer to determine the capacity of mitochondrial respiration. Cultured neonatal rat cardiac myocytes were exposed to palmitate/BSA conjugates to facilitate the study of fatty acid-dependent respiration. To verify that responses were due to utilization of exogenous palmitate by mitochondria, subgroups were pretreated with etomoxir (40 μM) 15 min prior to the assay to inhibit CPT-1. As CPT-1 facilitates fatty acid transport across the mitochondrial membrane, etomoxir would inhibit oxidation of exogenous fatty acids. BSA, the carrier of palmitate, was injected to the cells in the presence or absence of etomoxir, and served as negative control. As shown in Figure 27, the addition of etomoxir dramatically inhibited palmitate-related OCR, and BSA only generated negligible OCR. These data confirm that here, oxygen consumption was due to oxidation of exogenous palmitate.

As shown in Figure 28 (representative plot) and Figure 29 (quantitative data), ET1 reduced a number of parameters pertaining to fatty acid-associated mitochondrial bioenergetics, including (*vs.* control) basal OCR (82±5%, $p<0.05$), coupling efficiency (86±6%, $p<0.05$), maximal (78±4%, $p<0.01$) and spare (72±5%, $p<0.01$) respiratory capacity, as well as respiratory control ratio (81±5%, $p<0.01$). Basal OCR consists of both ATP-linked and proton leak-linked OCR; Figure 29B and C suggest that ET1-induced reduction of basal OCR was attributed mainly to a decrease in ATP-linked OCR (74±7%, $p<0.05$ *vs.* control). CB-13 pretreatment partially attenuated the depression of basal OCR (95±3%, not significant (ns) from control or ET1) and coupling efficiency (97±2%, ns from control or ET1), and significantly restored maximal (97±5%, $p<0.05$ *vs.* ET1), spare respiratory capacity (97±4%, $p<0.01$ *vs.* ET1) and respiratory control ratio

(94±2%, $p<0.05$ vs. ET1). Proton leak-related OCR was not affected by either ET1 or CB-13. These results show that mitochondria within ET1-treated cardiac myocytes exhibit reduced efficiency and impaired potential capacity in terms of fatty acid utilization to generate ATP. Activation of cannabinoid receptors using CB-13 improves fatty acid utilization in ET1-treated cardiac myocytes.

Figure 27. Determination of fatty acid-associated OCR in cultured neonatal rat cardiac myocytes.

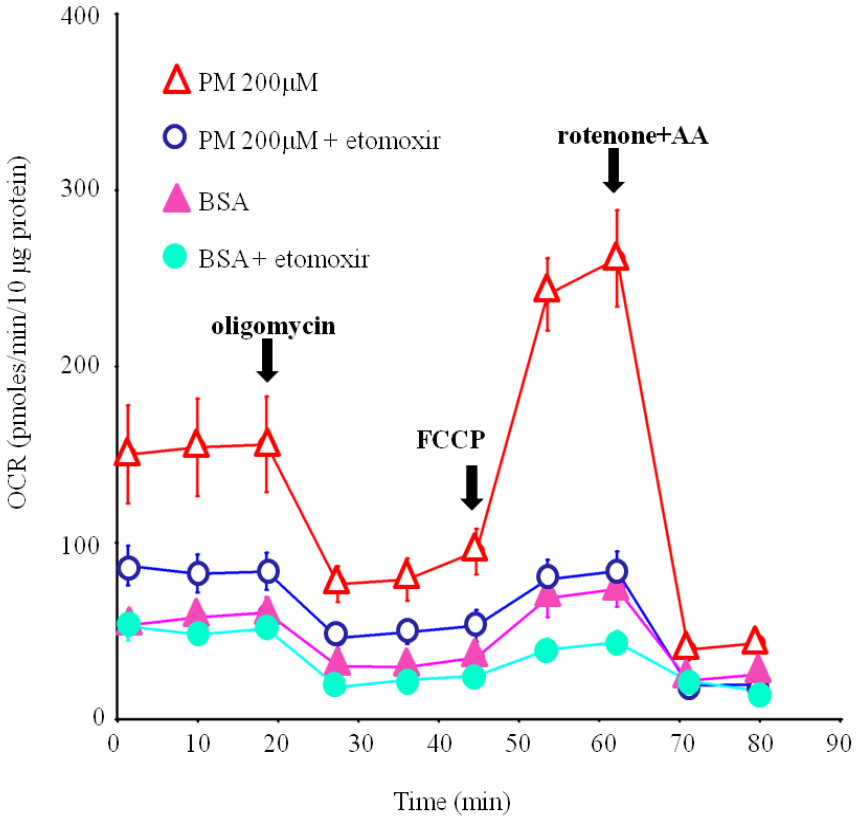


Figure 27. OCRs at basal levels were measured in the Seahorse Bioscience XF24 Analyzer in cultured myocytes (100,000 cells per well) with palmitate/BSA complex (PM, 200 µM) or BSA (vehicle carrier) as the substrate, in the absence or presence of etomoxir (inhibits CPT-1 and therefore mitochondrial fatty acid uptake), followed by the sequential addition of oligomycin (1 µg/mL), FCCP (2 µM) and R/AA (rotenone, 1 µM and antimycin A, 1 µM). The difference between PM (Δ) and PM + etomoxir (○) sugguests that exogenous fatty acids were utilized as substrate. n=5 replicate cultures.

Figure 28. CB-13 attenuates ET1-induced depression of fatty acid-related respiration (representative plot).

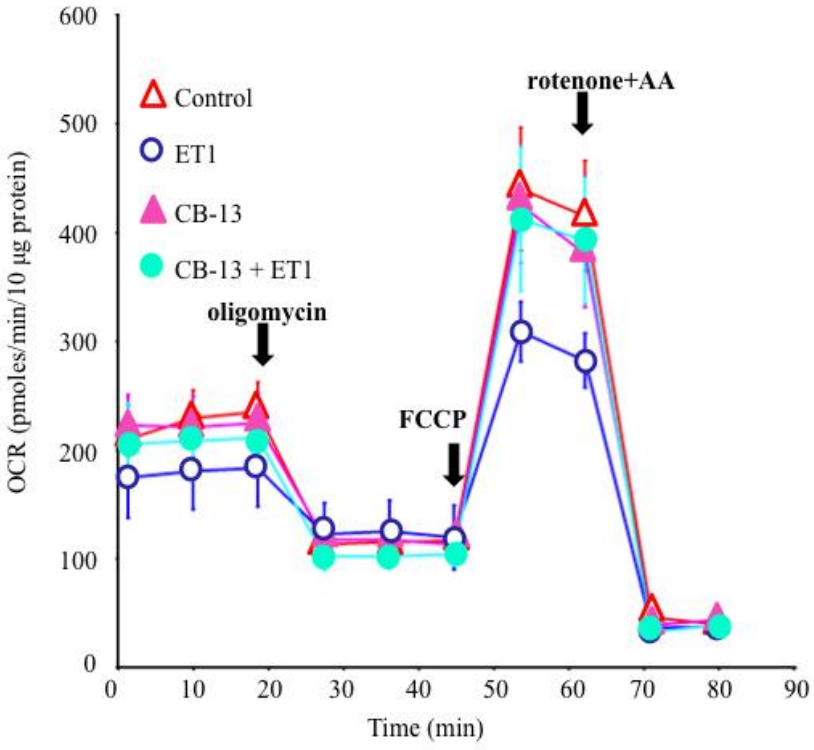


Figure 28. Serum-deprived myocytes were pre-treated with CB-13 (1 μM; 2 h) followed by addition of ET1 (0.1 μM; 4 h). OCRs were assessed in cultured neonatal rat cardiac myocytes with palmitate/BSA conjugates (200 μM) as substrate. Basal OCR were measured followed by addition of oligomycin (1 μg/mL), FCCP (10 μM) and R/AA (rotenone, 1 μM and antimycin A, 1 μM).

Figure 29. CB-13 attenuates ET1-induced depression of fatty acid-related respiration (quantitative data).

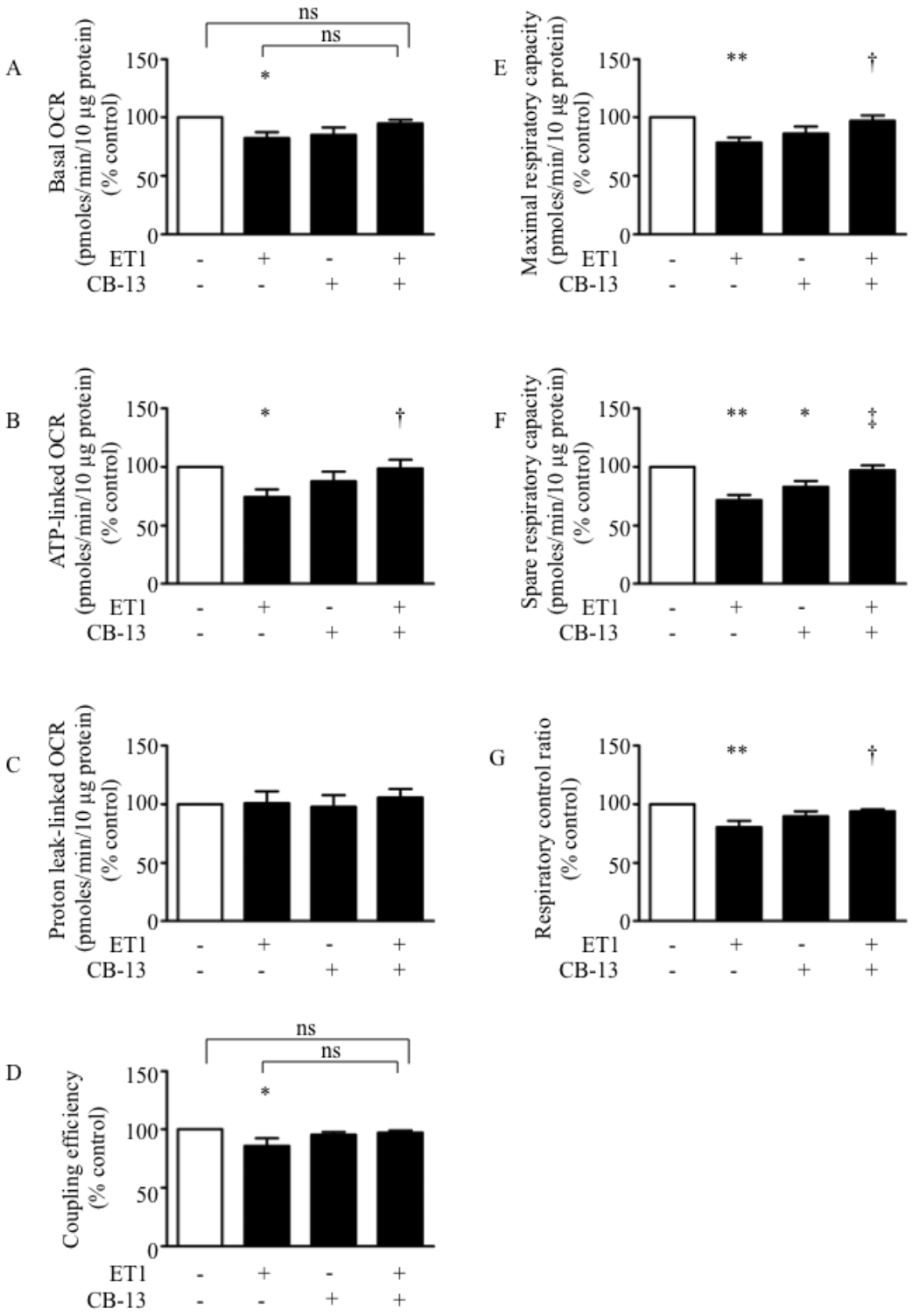


Figure 29. Mitochondrial respiratory parameters including *A,* basal OCR, *B,* ATP-linked OCR, *D,* coupling efficiency, *E,* maximal and *F,* spare respiratory capacity, as well as *G,* respiratory control ratio were reduced by ET1 (0.1 μM; 4 h). Pretreatment with CB-13 (1 μM; 2 h) attenuated ET1-induced reductions in these parameters. *C,* Proton leak-linked OCR was not affected by ET1 nor CB-13. n=7, *p<0.05, **p<0.01*vs.* control (open bars); ns=not significant; †p<0.05 and ‡p<0.01 *vs.* ET1.

11. AMPK contributes to CB-13-dependent correction of fatty acid oxidation-related mitochondrial bioenergetics in ET1-treated myocytes.

As discussed in chapter III, section 1.2.1, AMPK is well known for its crucial role in energy homeostatic regulation; one such aspect is to maintain or promote ATP production by improving fatty acid oxidation. Thus, we examined the role of AMPK in preservation of fatty acid oxidation by CB-13. Indeed, the ability of CB-13 to correct fatty acid-dependent mitochondrial bioenergetics in ET1-treated myocytes was abolished by disruption of AMPK signaling using its chemical inhibitor, compound C (1 μM; 1 h). Specifically, parameters (*vs.* control) include basal OCR (66±6%, $p<0.01$), ATP-linked OCR (64±9%, $p<0.01$), maximal (67±4%, $p<0.01$) and spare (65±6%, $p<0.01$) respiratory capacity were not rescued by CB-13 in the presence of compound C. Interestingly, fatty acid-related respiration was also impaired in the CB-13 + compound C group, namely (*vs.* control) basal OCR (81±3%, $p<0.05$), ATP-linked OCR (77±4%, $p<0.05$), coupling efficiency (92±2%, $p<0.05$), maximal (78±4%, $p<0.01$) and spare (71±7%, $p<0.01$) respiratory capacity, as well as respiratory control ratio (88±2%, $p<0.01$) (Figure 30 and 31).

Figure 30. The ability of CB-13 to attenuate ET1-induced depression of fatty acid-related respiration requires AMPK activity (representative plot).

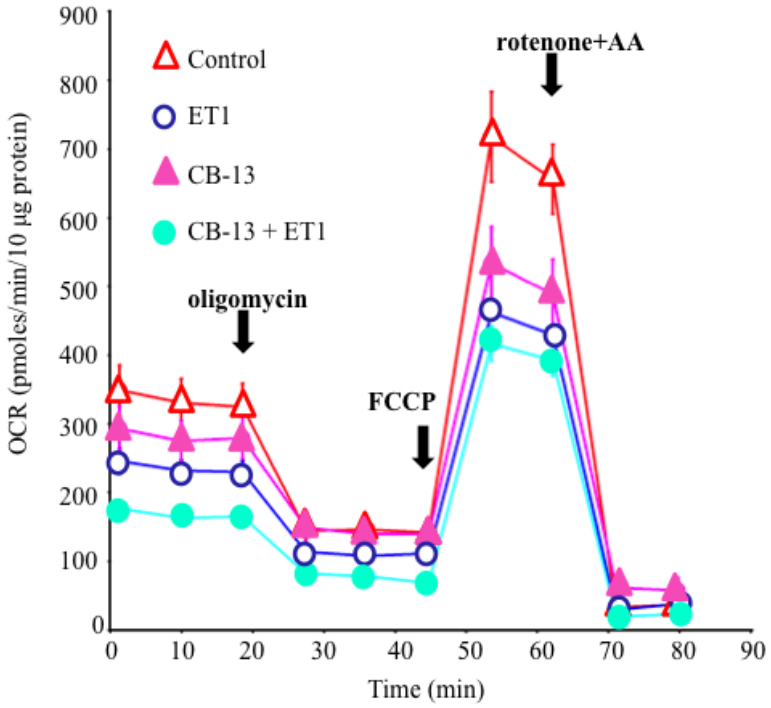


Figure 30. Serum-deprived myocytes were pre-treated with CB-13 (1 µM; 2 h) followed by addition of ET1 (0.1 µM; 4 h) in the presence of compound C (AMPK inhibitor; 1 µM; 1 h) OCR were assessed in cultured neonatal rat cardiac myocytes with palmitate/BSA conjugates (200 µM) as substrates. Basal OCRs were measured followed by addition of oligomycin (1 µg/mL), FCCP (10 µM) and R/AA (rotenone, 1 µM and antimycin A, 1 µM).

Figure 31. The ability of CB-13 to attenuate ET1-induced depression of fatty acid-related respiration requires AMPK activity (quantitative data).

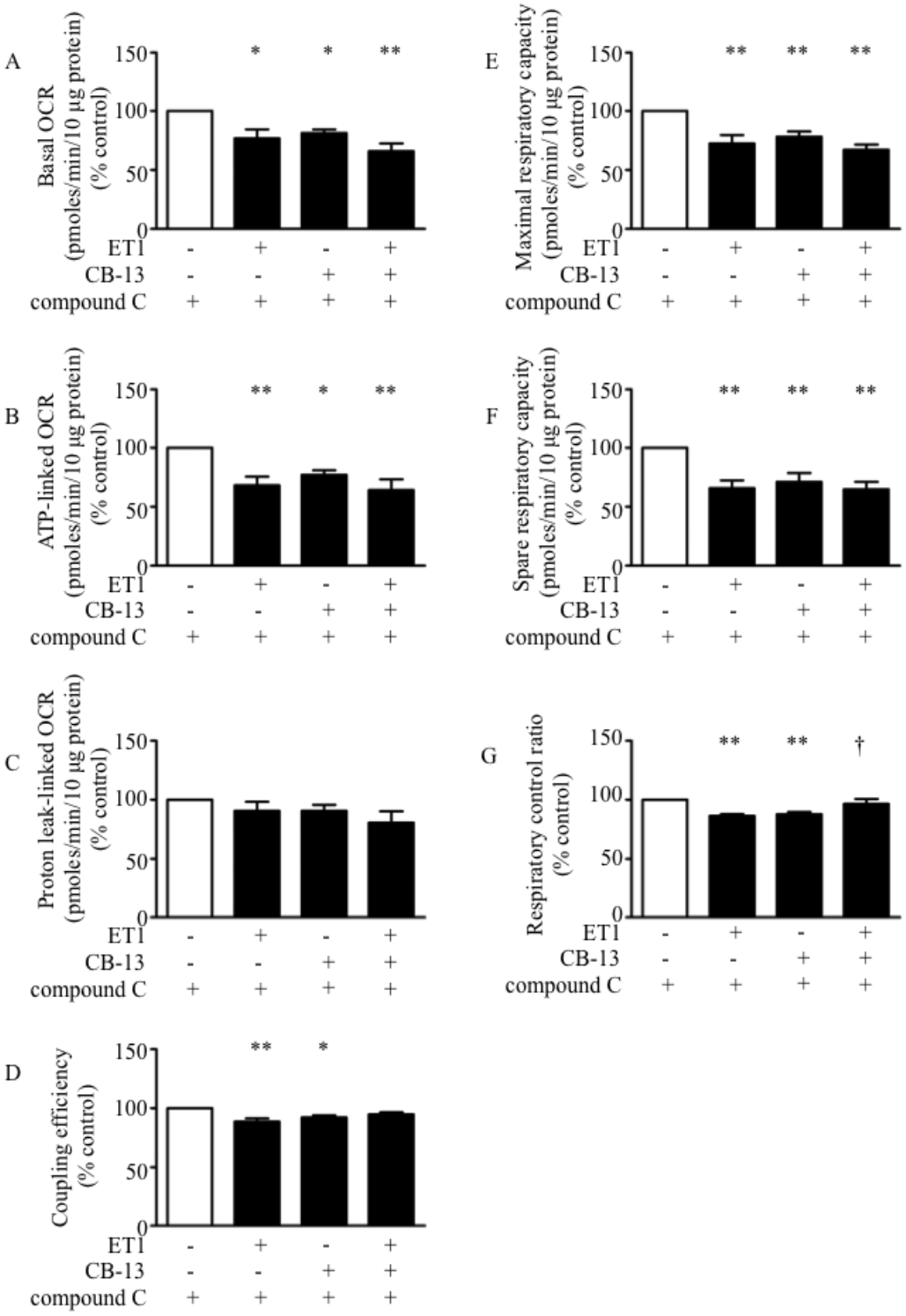


Figure 31. The ability of CB-13 to attenuate ET1-induced reductions in *A,* basal OCR, *B,* ATP-linked OCR, *D,* coupling efficiency, *E,* maximal and *F,* spare respiratory capacity were blocked by compound C (AMPK inhibitor; 1 μM). *C,* Proton leak-linked OCR and *G,* respiratory control ratio were not affected. n=4, *p<0.05, **p<0.01 *vs.* control (open bars); ns=not significant; †p <0.05 *vs.* ET1.

12. With glucose as the primary substrate, CB-13 prevents ET1-induced mitochondrial membrane depolarization in an AMPK-dependent manner.

As with palmitate, when glucose was provided as the primary substrate, ET1 reduced the ratio of red J-aggregates/green monomer (80±5%, $p<0.05$ *vs.* control), reflecting membrane depolarization. This depolarization was attenuated by CB-13 pre-treatment, as evidenced by a normalized J-aggregates/green monomer ratio (98±4%, $p<0.05$ *vs.* ET1) (Figure 32). The ability of CB-13 to prevent mitochondrial depolarization was abolished by compound C (AMPK inhibitor) (81±9%, $p<0.05$ *vs.* control) (Figure 33).

Figure 32. CB-13 prevents ET1-induced mitochondrial membrane depolarization in the presence of glucose/pyruvate as substrates.

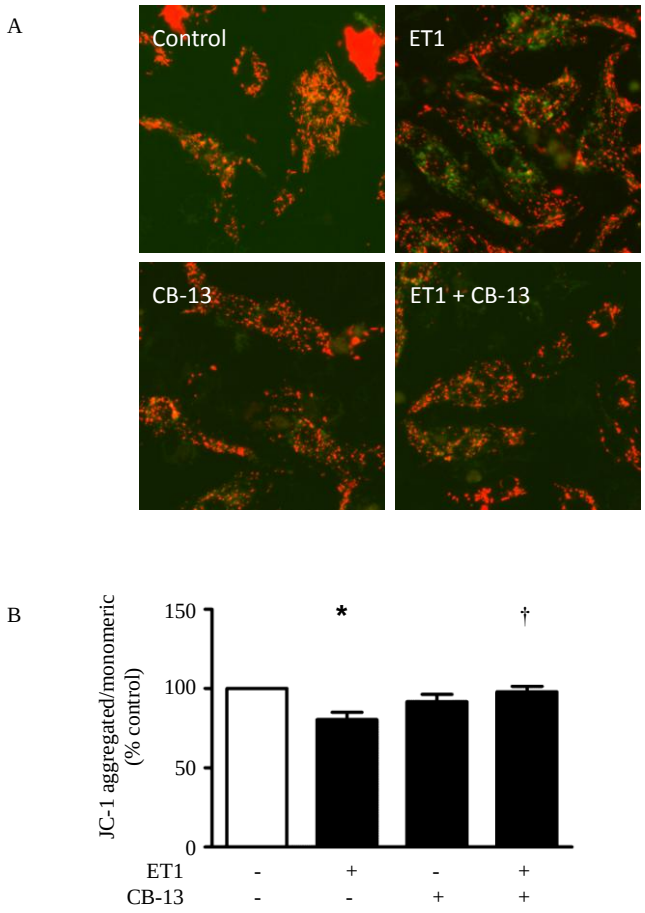


Figure 32. Serum-deprived myocytes were pre-treated with CB-13 (1 μM, 2 h), followed by addition of ET1 (0.1 μM; 4 h) in media that contained glucose (10 mM) and pyruvate (1 mM) as substrates. The ability of ET1 to induce mitochondrial membrane depolarization, indicated by decreased ratio of JC-1 aggregated red signal to monomeric green signal, was attenuated by pre-treatment with CB-13. Results are presented as *A,* representative fluorescent images and *B,* percent of normalized red/green fluorescence ratio *vs.* control (open bar). n=3. *p<0.05 and *vs.* control (open bars); †p<0.05 *vs.* ET1.

Figure 33. Inhibition of AMPK using compound C abolishes the inhibitory effect of CB-13 on ET1-induced mitochondrial membrane depolarization in the presence of glucose/pyruvate as substrates.

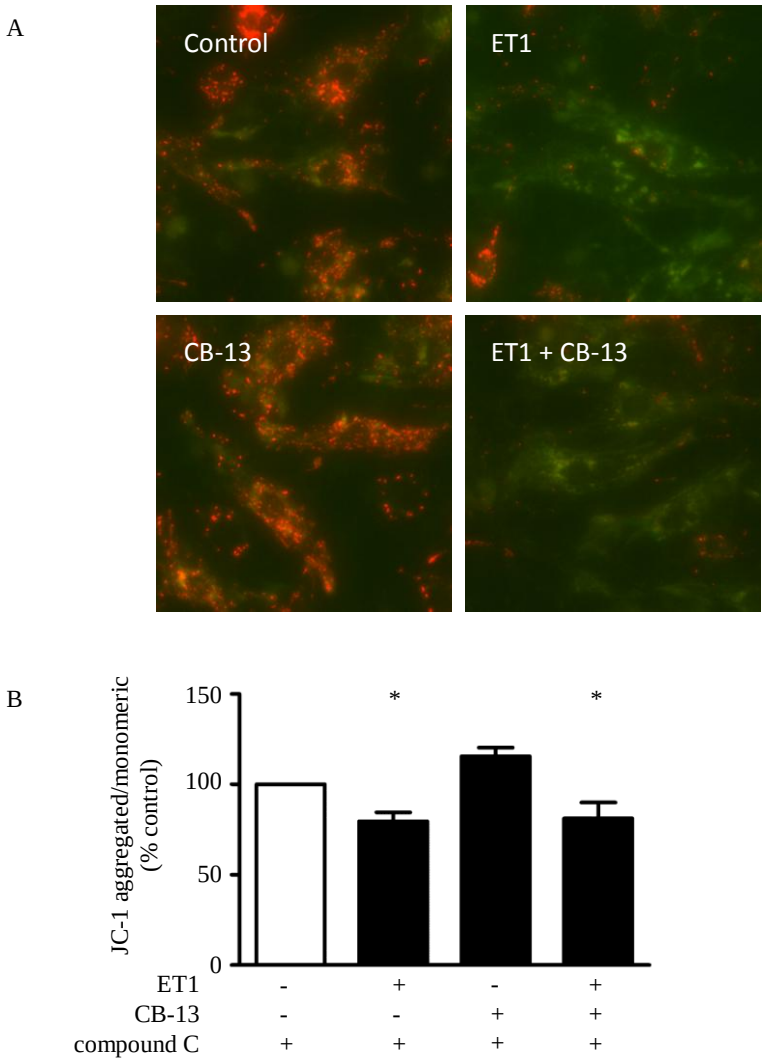


Figure 33. The ability of CB-13 (1 μM; 2 h) to attenuate ET1-induced (0.1 μM; 4 h) mitochondrial membrane depolarization was ablated in the presence of compound C (AMPK inhibitor, 1 μM; 1 h). Results are presented as *A*, representative fluorescent images and *B*, percent of normalized red/green fluorescence ratio *vs.* control (open bar). n=7. *p<0.05 and *vs.* control (open bars).

13. Neither ET1 nor CB-13 affects glucose-related mitochondrial bioenergetics.

Cultured neonatal rat cardiac myocytes were exposed to glucose (10 mM) and pyruvate (1 mM) to facilitate the study of glucose-related respiration. Basal OCR, coupling efficiency, maximal and spare respiratory capacity, and respiratory control ratio were determined following treatment with ET1 for 0-24 h. No significant changes in bioenergetics were observed in response to ET1 (data not shown). Likewise, CB-13 pre-treatment did not alter glucose oxidation-related respiration (Figure 34 and 35).

Figure 34. Neither ET1 nor CB-13 affects glucose-related mitochondrial respiration (representative plot).

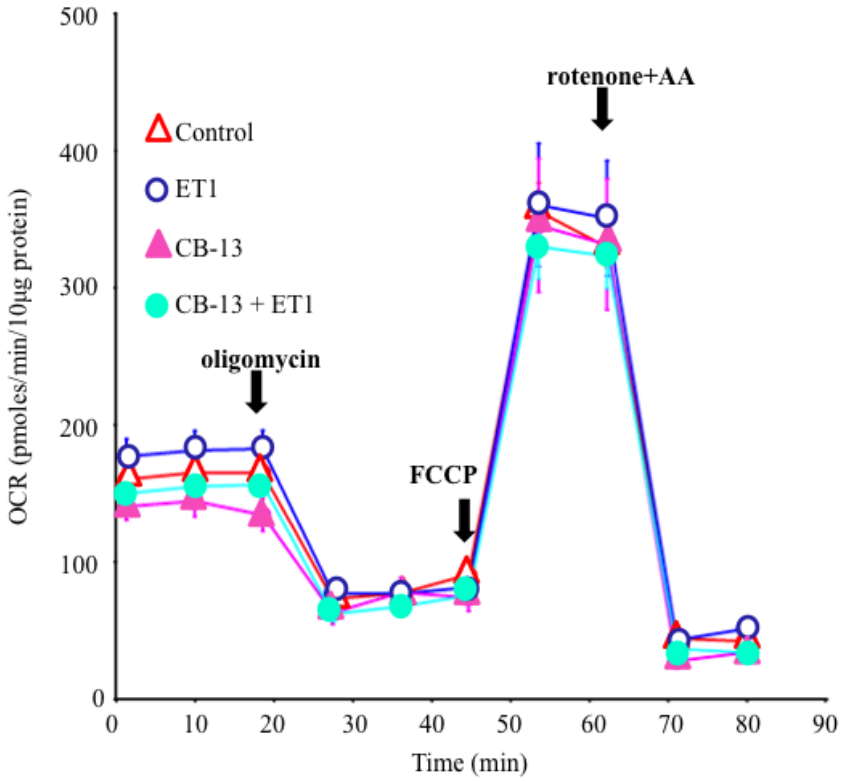


Figure 34. Serum-deprived myocytes were pre-treated with CB-13 (1 µM; 2 h) followed by addition of ET1 (0.1 µM; 4 h). OCRs were assessed in cultured neonatal rat cardiac myocytes with glucose (10 mM) and pyruvate (1 mM) as primary substrates. Basal OCR was measured followed by addition of oligomycin (1 µg/mL), FCCP (10 µM) and R/AA (rotenone, 1 µM and antimycin A, 1 µM).

Figure 35. Neither ET1 nor CB-13 affects glucose-related mitochondrial respiration (quantitative data).

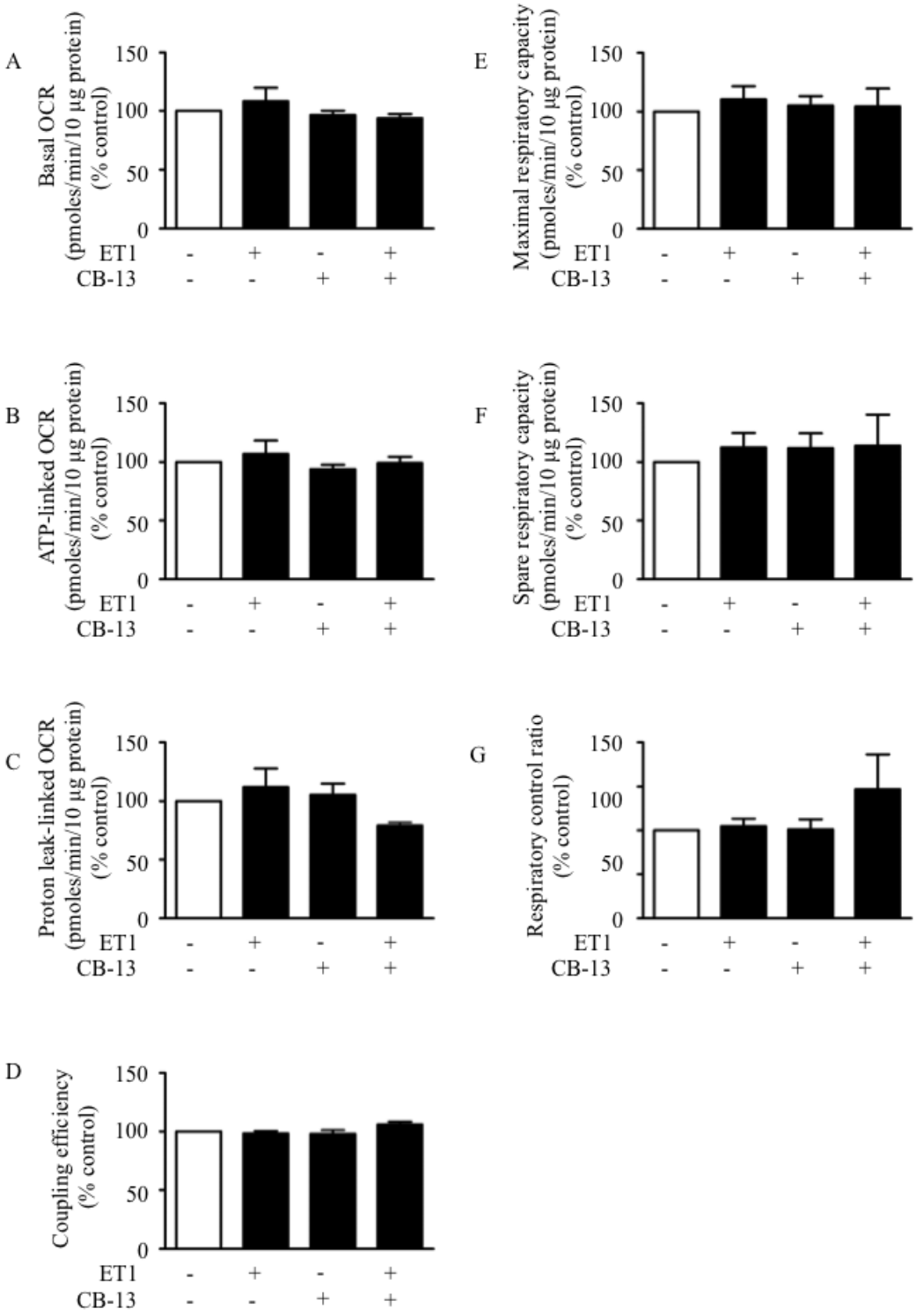


Figure 35. Mitochondrial respiratory parameters including *A*, basal OCR, *B*, ATP-linked OCR, *C.* proton leak-linked OCR, *D*, coupling efficiency, *E*, maximal and *F*, spare respiratory capacity, as well as *G*, respiratory control ratio were unaffected by ET1 or CB-13. n=7.

14. CB-13 attenuates ET1-reduced PGC-1α expression through AMPK.

We next turned our attention to candidate mediators of CB-13 effects on mitochondria. We began by examining PGC-1α, the central regulator of mitochondrial metabolism, by conventional western blotting. As shown in Figure 36A, ET1 reduced PGC-1α expression by 41±7% ($p<0.01$ *vs.* control), and 2 h CB-13 pretreatment ablated the reduction (96±2%, $p<0.05$ *vs.* ET1). In addition, CB-13 alone increased PGC-1α to 140±27% ($p<0.01$ *vs.* control). Simultaneous knockdown of AMPKα_1 and AMPKα_2 abrogated the ability of CB-13 to increase PGC-1α (Figure 36B), and in fact, in the absence of AMPK$\alpha_{1/2}$, CB-13 actually reduced PGC-1α expression compared to control, whether in the presence (32±7%, $p<0.01$ *vs.* control) or absence (59±10%, $p<0.01$ *vs.* control) of ET1 (Figure 36B and C).

Figure 36. ET1-induced down-regulation of PGC-1α is rescued by CB-13 in an AMPK-dependent manner.

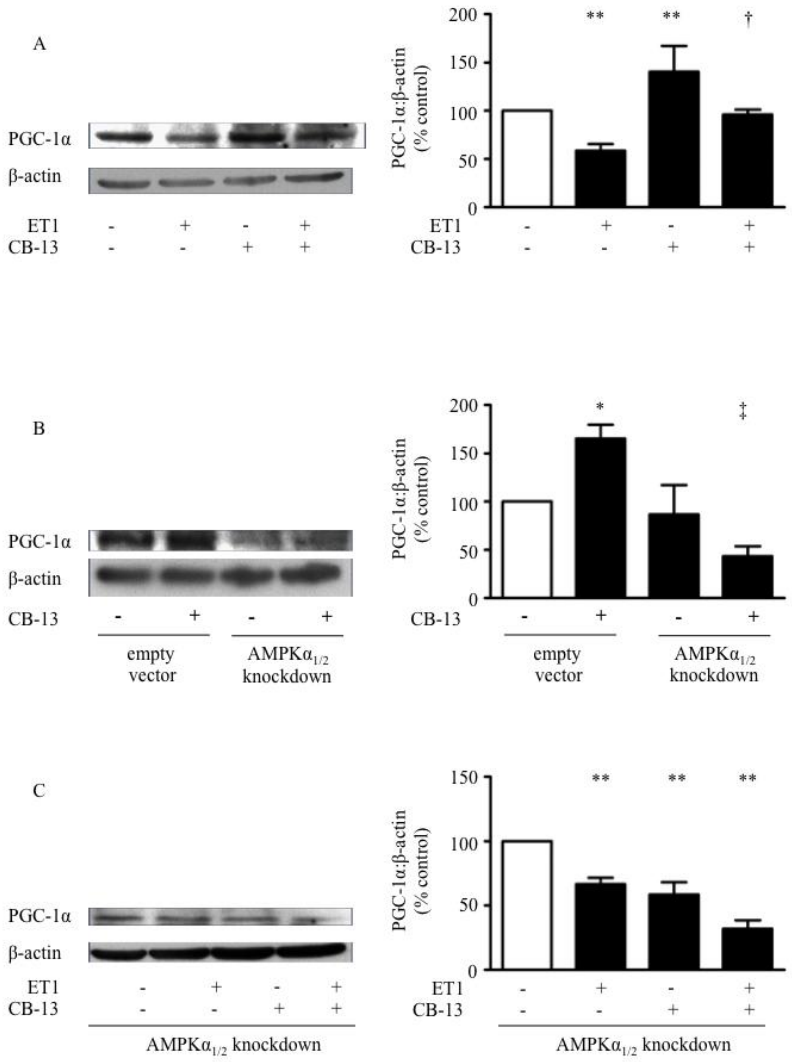


Figure 36. *A*, PGC-1α was reduced by ET1 (0.1 μM; 4 h), and this was prevented by CB-13 (1 μM; 2 h) pretreatment. *B*, The up-regulation of PGC-1α by CB-13 (1 μM; 4 h) was abolished by AMPKα_1/α_2 knockdown. *C*, The ability of CB-13 to restore PGC-1α reduced by ET1 was blocked by AMPKα_1/α_2 knockdown. n=3-12. *p<0.05 and **p<0.01 *vs.* control (open bars); †p<0.05 *vs.* ET1, and ‡p<0.01 *vs.* CB-13 with empty vector.

15. ET1-induced down-regulation of CPT-1β is attenuated by CB-13.

The second candidate mediator of CB-13 effects on mitochondria that we considered was CPT-1. CPT-1 is a rate-limiting enzyme that facilitates the transport of fatty acids into the mitochondria by catalyzing the formation of acyl-carnitines. This therefore facilitates the use of fatty acids by mitochondria as substrate for energy generation. Thus, CPT-1 is a potentially critical mediator that might be targeted by CB-13. CPT-1β, the predominant isoform of CPT-1 in the heart (N. F. Brown, Weis, Husti, Foster, & McGarry, 1995), was assessed by real-time PCR. ET1 reduced CPT-1β to 82±4% ($p<0.05$ vs. control), and this was attenuated by CB-13 (91±6%, ns from control or ET1) (Figure 37A). However, simultaneous knockdown of AMPKα_1 and AMPKα_2 abrogated the ability of CB-13 to rescue, at least in part, CPT-1β (73±8%, $p<0.05$ *vs.* control) (Figure 37B). This finding suggests that the ability of ET1 to reduce fatty acid transport into the mitochondria is partially prevented by CB-13 in an AMPK-dependent manner.

Figure 37. CB-13 blunts ET1-induced reduction of CPT-1β via AMPK.

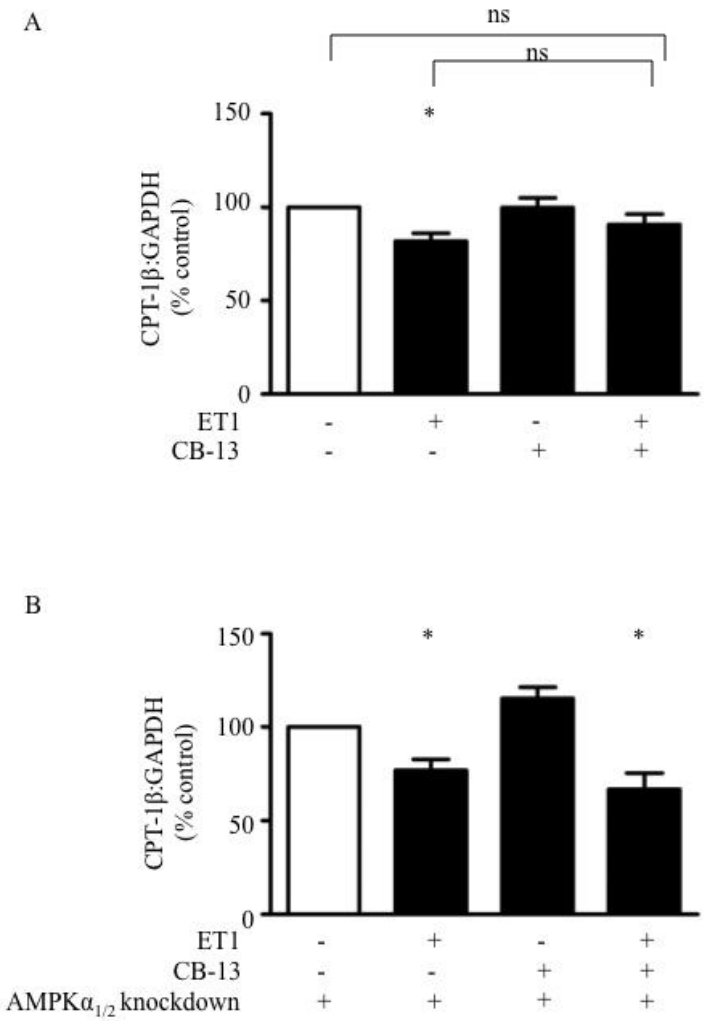


Figure 37. *A*, The ability of ET1 (0.1 μM; 4 h) to decrease CPT-1β RNA expression was partially attenuated by CB-13 (1 μM; 2 h), whereas *B*, AMPKα_1/α_2 knockdown blocked CB-13 effects. n=5-8, *p<0.05 *vs.* control (open bars); ns=not significant *vs.* control (open bars).

Chapter VII: Discussion

1. Effect of cannabinoid receptor activation on cardiac myocyte hypertrophy.

To our knowledge, the present study shows for the first time that ligand activation of cannabinoid receptors attenuates hypertrophy of isolated cardiomyocytes via AMPK-eNOS signaling. Anandamide, a naturally-occurring endocannabinoid (Devane et al., 1992), and three synthetic CB receptor ligands (R-methanandamide, JWH-133, and CB-13) prevented ET1-induced cardiomyocyte enlargement. As cardiac hypertrophy is a major risk factor for heart failure, we further investigated here the signaling mechanisms that underlie the anti-hypertrophic effects of CB receptors.

There is significant interest in manipulation of the endocannabinoid system as a therapeutic approach to treat disorders such as metabolic syndrome, inflammatory and neuropathic pain, and multiple sclerosis (Hosking & Zajicek, 2008; Kunos et al., 2009; Palazuelos et al., 2006). Unfortunately, therapeutic use of cannabinoids is impeded by psychotropic side effects including dysphoria, memory impairment, reduced concentration, disorientation, motor incoordination, and possibly addiction (Hosking & Zajicek, 2008; Kunos et al., 2009). This undesirable psychoactivity is mediated by CNS CB1 receptors (Hosking & Zajicek, 2008; Howlett et al., 2002; Kunos et al., 2009; Piomelli, 2003), so alternate strategies like using CB2-selective agonists and/or peripherally-restricted CB1/CB2 dual agonists have been proposed (Gertsch et al., 2008; Hosking & Zajicek, 2008; Kunos et al., 2009; Palazuelos et al., 2006)

We therefore investigated the role of CB1 and CB2 receptors and found that distinct CB receptor subtypes mediate the anti-hypertrophic actions of R-methanandamide. In fact, selective antagonism of CB receptor subtypes dissociated the

inhibitory effects on ET1-induced myocyte growth from effects on hypertrophic gene expression. In particular, R-methanandamide prevents myocyte enlargement through CB2 receptor and was sensitive to AM630 (Figure 6A). In contrast, inhibition of fetal gene activation trafficked through CB1 receptors and was sensitive to AM251 (Figure 6B). We also considered the possibility that the ability of AM251 to block R-methanandamide effects might be due to agonism of GPR55 rather than antagonism of CB1 (Kapur et al., 2009). However, it is likely that AM251 was functioning here solely as a CB1 receptor antagonist. First, the concentration of AM251 that blocked R-methanandamide effects on BNP gene activation was 0.1 μM in accordance with its K_i at CB1 receptors (7.5 nM) (Lan, Liu, et al., 1999). In contrast, Kapur et al. (Kapur et al., 2009) demonstrated GPR55-mediaed effects of AM251 at 30 μM, with an EC50 of about 10 μM, whereas at 0.1 μM, and even a logarithmic increment higher at 1 μM, AM251 failed to invoke GPR55 signaling. This information strongly suggests that the inhibitory effects of AM251 are attributable to antagonism of CB1 receptors. In support of this notion, we verified that AM281, a CB1 receptor antagonist lacking effects on GPR55, also abolished the ability of R-methanandamide to suppress BNP gene activation (Figure 7).

There exist literature precedents in which morphological changes are uncoupled from the hypertrophic gene program. For example, Thorburn et al. (Thorburn, Frost, & Thorburn, 1994) reported that in isolated myocytes, ERK signaling mediates phenylephrine-dependent ANP promoter activation but not organization of contractile proteins such as actin. Furthermore, activator protein 1 (AP-1), and in particular c-Fos, is a key mediator of hypertrophic gene expression but not myocyte growth (Jeong, Kinugawa, Vinson, & Long, 2005). *In vivo*, activated GSK-3β can dissociate the expression of hypertrophic genes from cardiac growth overexpression; transgenic mice

that overexpress calcineurin exhibit cardiac growth and BNP expression, whereas coexpression of activated GSK-3β inhibited cardiac growth but not BNP expression (Antos et al., 2002). Interestingly, activation of CB1 receptors suppressed phosphorylation of MAPKs (i.e. ERK and p38) in neonatal rat cardiac myocytes (Liao et al., 2013). Moreover, there is evidence that endocannabinoids might interfere with c-Fos signaling (Greco et al., 2010) through CB1 receptors (Soderstrom & Tian, 2008). We therefore speculate that activated CB1 receptors may attenuate BNP expression by suppressing ERK/AP-1 signaling, though this remains to be determined.

Selective agonism of CB2 receptors with JWH-133 suppressed myocyte enlargement but failed to prevent fetal gene activation (Figure 9). In contrast, we found that the anti-hypertrophic actions of a dual CB1/CB2 agonist, CB-13, extended beyond those of JWH-133, such that both myocyte enlargement and BNP gene expression were inhibited (Figure 12). These findings are consistent with the notion that, rather than a CB2-selective agonist, a viable strategy which might dissociate the peripheral cardioprotective effects from the unwanted central effects is to use a CB1/CB2 receptor dual agonist that does not cross the blood-brain barrier.

Other cardioprotective attributes have been reported and pertain predominantly to activation of CB2 receptors. These include protection from ischemic insult (Di Filippo, Rossi, Rossi, & D'Amico, 2004; Lagneux & Lamontagne, 2001; Lepicier et al., 2003; Underdown et al., 2005), anti-arrhythmic effects (Krylatov et al., 2001), and prevention of endothelial dysfunction of coronary arteries (Bouchard et al., 2003; Wagner et al., 2003). However, while CB2 signaling may be cardioprotective, there is also evidence that activation of CB1 receptors is associated with dysfunction of vascular endothelium (Tiyerili et al., 2010), exerts pro-atherosclerotic actions (Dol-Gleizes et al., 2009; Molica

et al., 2013; Sugamura et al., 2009), and promotes oxidative stress, inflammation and/or cell death *in vitro* (Rajesh et al., 2010) and in pathological conditions such as diabetic cardiomyopathy (Rajesh et al., 2012), and doxorubicin-induced cardiomyopathy (Mukhopadhyay et al., 2007; Mukhopadhyay et al., 2010). Our data are consistent with the notion that sole activation of CB1 receptors is cardio-deleterious; in the context of cardiomyocyte hypertrophy, myocyte enlargement was suppressed by CB2 receptors, whereas only fetal gene activation was prevented by CB1 receptors. Thus, in the presence of sole activation of CB1 receptors and absence of CB2 signaling, myocyte hypertrophy would persist and potentially give rise to adverse endpoints such as ischemia (vis-à-vis reduced capillary density), myocyte misalignment, and cardiac stiffening. Activation of CB2 receptors is therefore a necessary component of cannabinoid-based anti-hypertrophic therapy which we speculate may ameliorate adverse effects of unopposed activation of CB1 receptors alone.

CB-13 stimulated phosphorylation of AMPKα (Thr172) and eNOS (Ser1177) at known activation sites (Figures 14A and B) (Z. P. Chen et al., 1999; Morrow et al., 2003) (Beauloye et al., 2011; Witters et al., 2006), and we determined that eNOS phosphorylation is downstream of AMPK since it was inhibited by AMPKα knockdown (Figures 16). Inhibition of AMPK or eNOS signaling abolished the anti-hypertrophic effects of CB-13 (Figure 17-19). Taken together, our findings suggest that signaling through AMPK-eNOS crosstalk might play a role in the anti-hypertrophic effects of CB receptors. This is consistent with reports by others, in which AMPK-eNOS crosstalk underlies the anti-growth effects of non-cannabinoid interventions such as metformin (C. X. Zhang et al., 2011), calorie restriction (Dolinsky et al., 2010), and resveratrol (Thandapilly et al., 2011). Interestingly, phosphorylative activation of AMPK and eNOS

peaked by 4 h, and returned to baseline by 24 h. This suggests that AMPK-eNOS crosstalk might occur at a pivotal, early point with downstream effects that will collectively prevent the hypertrophic response. Attenuation of pro-hypertrophic RhoA/RhoA kinase (ROCK) signaling might be one such downstream event. These members of the Rho family GTPases are well-established mediators of cardiac hypertrophy (J. H. Brown, Del Re, & Sussman, 2006; Loirand, Guerin, & Pacaud, 2006; Zeidan, Gan, Thomas, & Karmazyn, 2014). In fact, Hunter et al. reported that nitric oxide inhibits ET1-induced cardiac myocyte hypertrophy by blocking the RhoA/ROCK cascade (Hunter et al., 2009). Moreover, CB receptor activation inhibits RhoA signaling (Kurihara et al., 2006; Nithipatikom et al., 2012; Rajesh et al., 2007). We therefore speculate that CB-13, by transiently activating AMPK/eNOS crosstalk, elicits NO-dependent blockade of RhoA/ROCK although the effects of CB-13 on RhoA/ROCK signaling remain to be determined.

We tested the anti-hypertrophic potential of a wide range of concentrations of anandamide (Figure 3), JWH-133 (Figure 8), and CB-13 (Figure 11). Clearly, low micromolar concentrations are required; CB-13 partially attenuated or completely abolished ET1-induced myocyte enlargement at 0.1 μM and 1 μM, respectively. While seemingly high, these concentrations are achievable in blood as CB-13 exhibits good oral bioavailability. Dziadulewicz et al. reported that following oral administration of CB-13 (3 mg/kg), a C_{max} of 1.13 μM was observed at 1 h post-dose, whereas a maximal brain concentration of only 0.24 μmol/kg was reached 4 h post-dose (Dziadulewicz et al., 2007). Moreover, CB-13 exerted biological effects (anti-hyperalgesia) in a rat model of neuropathic pain, but produced no CNS effects at this dose (Dziadulewicz et al., 2007). Importantly, our results suggest that this concentration does not adversely affect cardiac

myocyte viability (Figure 13). Also, although ET1 reduces conduction velocity in isolated myocytes (Reisner et al., 2009), and this is associated with increased expression and density of L-type Ca^{2+} channels (Yu et al., 2013), CB-13 did not worsen contractile function (Table 5). Therefore, our experimental concentration of CB-13 (1 μM) should be physiological attainable and relevant.

To summarize, these results suggest that: 1) endocannabinoids and their synthetic analogues may suppress cardiac myocyte hypertrophy by activating cannabinoid receptors; 2) CB1 and CB2 receptors contribute to the anti-hypertrophic action from distinct angles: CB1 suppresses fetal gene reactivation (possibly by ERK-AP-1 inhibition), whereas CB2 inhibits myocyte enlargement. Thus, dual activation of CB1 and CB2 is required to elicit complete prevention of cardiac myocyte hypertrophy; 3) Indeed, CB-13, a peripherally-restricted dual agonist of CB1/CB2 might be a potential candidate, due to its ability to attenuate all aspects of cardiac myocyte hypertrophy, while avoiding CNS-mediated adverse effects theoretically; and 4) AMPK-eNOS crosstalk and potential downstream cascades (possibly RhoA/ROCK inhibition) contribute to the anti-hypertrophic action of CB-13.

2. Effect of cannabinoid receptor activation on ET1-induced mitochondrial dysfunction.

We previously showed that ET1 induces hypertrophy in neonatal rat cardiac myocytes, and the activation of cannabinoid receptors CB1/CB2 attenuated it via the AMPK/eNOS pathway. Numerous studies reported that pressure overload and various hypertrophic stimuli caused mitochondrial dysfunction and energy substrate shift (Abel & Doenst, 2011; Q. He, Harris, Ren, & Han, 2014; Lang et al., 2014; Westenbrink et al., 2015). However, the mitochondrial bioenergetics profile associated with specific energy

substrates has not been clearly demonstrated. Also, the effects of cannabinoid receptor activation on cardiac hypertrophy-related mitochondrial dysfunction have not been studied.

Because it is widely accepted that there is a switch from fatty acids to glucose as the primary energy source during the progression of hypertrophy, this study investigated the effects of ET1, as a hypertrophic stimulus, on mitochondrial respiration in the context of palmitate or glucose/pyruvate as primary substrate. Mitochondrial $\Delta\psi_m$ and proteins involved in energy metabolism were also detected to evaluate mitochondrial function. In addition, this study examined whether CB-13, a peripherally-restricted agonist of CB1/CB2 receptors, prevents ET1-induced mitochondrial disorder.

2.1. Glucose oxidation- or fatty acids oxidation-dependent mitochondrial bioenergetics in ET1-induced hypertrophy.

First, mitochondrial bioenergetics during fatty acid oxidation was assessed with palmitate/BSA conjugates provided as substrate. In the ET1-treated group, the basal OCR, which consists of ATP synthesis and proton leak-related OCR, was reduced solely due to the decline in ATP synthesis-linked OCR. Coupling efficiency, which indicates the efficiency of ATP production, was correspondingly decreased. The maximal and spare respiration capacity that reflects the ability of the mitochondria to respond to stress or higher energy demand was also decreased (Figure 29).

Second, mitochondrial bioenergetics in glucose oxidation was assessed with glucose and pyruvate provided as substrates. Interestingly, the ability of mitochondria to oxidize glucose was unaffected by ET1 or CB-13 (Figure 35).

Decreased fatty acid oxidation and unchanged glucose oxidation revealed a net reduced ability of the mitochondria to oxidize substrates in ET1-treated cardiac myocytes. ET1 is able to induce cardiac myocyte hypertrophy, which is characterized by stimulated fetal gene program, accelerated protein synthesis and increased cell size, and this requires more ATP. However, the overall ATP production derived from fatty acids and glucose was impaired by ET1. These results predict disruption of other myocyte functions such as contraction. Indeed, our results (Table 5) showed that ET1 impaired contractile function of cardiac myocytes, as evidenced by reductions in shortening and relengthening velocity. Maximal and spare respiration capacity was also impaired by ET1. This predicts a limited capacity of mitochondria to adjust to conditions that requires extra ATP, such as hemodynamic overload, rendering the cells more susceptible to secondary stress (Roy Chowdhury et al., 2012), and more prone to heart failure. This may explain the contribution of hypertrophy to the development of heart failure from a bioenergetics aspect.

CB-13 partially or completely corrected fatty acid oxidation-related mitochondrial bioenergetics (Figure 29). However, inhibition of AMPK using compound C dissipated the protective effects of CB-13 (Figure 31). Thus, AMPK signaling contributes to the ability of CB-13 to improve fatty acid-dependent bioenergetics.

2.2. Mitochondrial membrane integrity in ET1-treated cardiac myocytes in the presence of palmitate vs. glucose.

Mitochondrial $\Delta\psi_m$ is regarded as a key marker of mitochondrial performance. It is the electrochemical gradient across the inner membrane, and is established when protons are pumped out to the intermembrane space by the ETC. Two parallel ways to dissipate

the $\Delta\psi_m$ are proton leak and ATP synthesis. Thus, the $\Delta\psi_m$ is determined by the rate of proton extrusion and reentry (Figure 38). This notion led us to a more comprehensive understanding of the profile of mitochondrial oxidative phosphorylation.

$$\Delta\psi_m = \text{Rate}_{\text{proton extrusion}} - \text{Rate}_{\text{proton reentry}}$$

Figure 38. Determination of mitochondrial $\Delta\psi_m$.

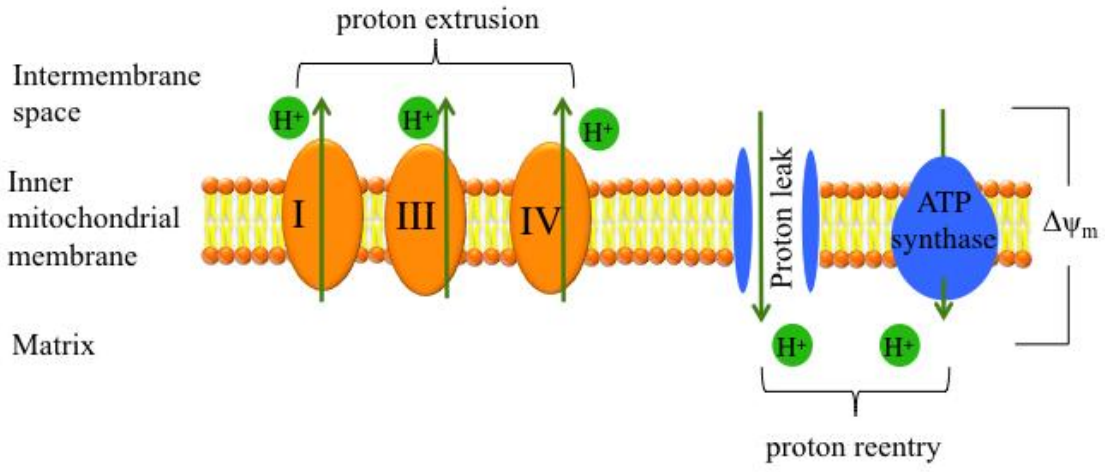


Figure 38. Mitochondrial $\Delta\psi_m$ is established when protons are pumped to the intermembrane space by ETC complexes I, III, and IV. Meanwhile, $\Delta\psi_m$ is dissipated by proton reentry through proton leak and ATP synthesis. Therefore, mitochondrial $\Delta\psi_m$ is determined by proton extrusion and reentry.

ET1 dissipated the mitochondrial $\Delta\psi_m$ whether cardiac myocytes were using fatty acids or glucose as energy substrate (Figure 21 and 32). However, when palmitate was provided as energy substrate, ET1 reduced net proton reentry (as evidenced by less ATP production and accompanied by unaffected proton leak) (Figure 29). Nevertheless, mitochondrial $\Delta\psi_m$ was significantly decreased, indicating that the decline in active proton extrusion exceeded the decline in proton reentry. In contrast, when glucose/pyruvate served as the substrate, ET1 did not influence proton reentry (as suggested by unaltered ATP production and proton leak) (Figure 35). However, $\Delta\psi_m$ was

still dissipated, suggesting again a reduction in active proton extrusion. These data indicate that ET1-induced mitochondrial depolarization was attributable to reductions in active proton extrusion from the mitochondrial ETC, regardless of the substrate utilized.

As expected, AMPK inhibition by compound C ablated the ability of CB-13 to restore mitochondrial $\Delta\psi_m$ when glucose served as substrates (Figure 33), but not with palmitate (Figure 22). Actually, when AMPK was inhibited, CB-13 reduced proton reentry (i.e. decreased ATP synthesis) (Figure 31), but the $\Delta\psi_m$ was unaltered, suggesting a parallel reduction in proton extrusion. Possible explanations include AMPK inhibition causing blunted activity of complex I-IV, and/or reduced NADH/$FADH_2$ concentrations due to decreased fatty acid entry vis-a-vis decreased CPT-1β levels. Further determination of complex I-IV activity can be performed to confirm this hypothesis, but evidence from other studies lend support. For example, Afolayan et al. observed decreased AMPK activity and expression of ETC complexes in pulmonary artery endothelial cells from newborn lambs with persistent pulmonary hypertension (Afolayan et al., 2015). Preston et al. also observed decreased gene expression of complex I and ATP synthase in ventricles from aged rats (Preston et al., 2008), and it is well known that aging is associated with decreased AMPK activity (Salminen & Kaarniranta, 2012; Y. Zhang et al., 2014). Enzyme activities of complex I and II, as well as AMPK signaling, were also reduced in spontaneously hypertensive rats with hypertrophy (Tang, Mi, Liu, Gao, & Long, 2014). Overall, this study showed that AMPK signaling contributes to the maintenance of both proton extrusion rate and ATP production (proton reentry process) during fatty acid oxidation, although its inhibition may not significantly influence mitochondrial $\Delta\psi_m$. Thus, it is incorrect to simply conclude that preserved mitochondrial $\Delta\psi_m$ reflects normal ETC function.

We then queried which downstream cascades might underlie the protective effects of CB-13/AMPK signaling on mitochondria. We first assessed PGC-1α, a master regulator of mitochondrial function, the expression of which is increased by AMPK activation via sirtuin-1 (B. Huang et al., 2014; W. J. Lee et al., 2006). A variety of roles of PGC-1α in multiple tissues have been reported, including increasing mitochondrial biogenesis, improving thermogenesis, and enhancing fatty acid oxidation and glucose uptake (Z. Wu & Boss, 2007). For example, PGC-1α knockout mice developed obesity, exhibited lower levels of mitochondria density, ETC protein expression, and oxidative phosphorylation in skeletal muscle, as well as impaired exercise tolerance that was associated with reduced cardiac output. Also, PGC-1α knockout mice failed to maintain body temperature when exposed to cold environment (Leone et al., 2005). Our results showed that PGC-1α was reduced by ET1 and restored by CB-13 (Figure 36A). However, knockdown of AMPK$\alpha_{1/2}$ abrogated the ability of CB-13 to increase PGC-1α (Figure 36B and C). Given that CB-13 increases AMPK activity, AMPK/PGC-1α could be a potential downstream pathway of CB receptor activation that contributes to correction of ET1-induced mitochondrial dysfunction. CPT-1β is another key enzyme involved in fatty acid oxidation. It regulates the rate-limiting step of mitochondrial fatty acid uptake by introducing a carnitine group to none-permeable fatty acid acyl-CoA. Evidence demonstrated up-regulation of CPT-1 mediated by AMPK activation (L. Li, Wu, Wang, Liu, & Zhao, 2007). Our results showed that CPT-1β was significantly depressed by ET1, and this was prevented, at least in part, by CB-13 (Figure 37A). The attenuation of CPT-1β suppression by CB-13 was disrupted by shRNA knockdown of AMPK$\alpha_{1/2}$ (Figure 37B). These data support a predominant role of AMPK in protecting mitochondrial function by up-regulating PGC-1α and CPT-1β.

2.3. Distinct roles of CB1/CB2 receptors on mitochondrial function.

Interestingly, AMPK$\alpha_{1/2}$ knockdown did not just block CB-13-induced PGC-1α up-regulation, but also caused a CB-13-dependent decrease of PGC-1α in the presence and absence of ET1 (Figure 36C). This is consistent with the finding that CB-13 on its own reduced fatty acid oxidation-related mitochondrial respiration in the presence of an AMPK inhibitor (Figure 31). This suggests that without AMPK signaling, CB-13 might be stimulating other signaling cascades to reduce PGC-1α, thus depressing fatty acid oxidation-related mitochondrial bioenergetics. CB-13 is a dual agonist of CB1 and CB2 receptors, and our previous data showed that JWH-133, a CB2-selective agonist stimulates AMPK activity (data not shown). In addition, Zheng et al. reported that a CB2 agonist activated PGC-1α (Zheng et al., 2013). Therefore, we speculate that CB1-induced signaling emerges to exert opposing effects when CB2/AMPK signaling is inhibited. Indeed, Tedesco et al. observed decreased AMPK activity and eNOS expression, as well as depressed mitochondrial biogenesis in liver, muscle and white adipose tissues in mice treated with a CB1-selective agonist (Tedesco et al., 2010). Perwitz et al. also showed that blockage of CB1 receptors enhanced mitochondrial respiration and increased AMPK activity and PGC-1α expression in adipocytes (Perwitz et al., 2010). Other studies also reported the opposite effects of CB1 and CB2 receptors, where CB1 is detrimental and CB2 is beneficial (Fisar et al., 2014; Q. Li et al., 2014; Q. Li, Wang, et al., 2013). Therefore, an explanation for our finding that CB-13 impairs fatty acid oxidation when AMPK signaling is inhibited might be that CB1 (deleterious) and CB2 (salutary) act in opposition, at least in cardiac myocyte mitochondria, and that CB2 receptor-stimulated AMPK pathways dominate over CB1 receptor signaling in cardiac myocytes to achieve regulation of mitochondrial function by CB-13.

2.4. Is it protective to sustain fatty acid oxidation in the hypertrophied heart?

It has been long known that decreased fatty acid oxidation and increased glycolysis are features of cardiac hypertrophy and heart failure, as glycolysis is more efficient regarding oxygen requirement (Bishop & Altschuld, 1970; Fillmore et al., 2014). However, uncoupling of glycolysis and glucose oxidation was observed in diseased heart in many studies (Allard et al., 1997; Allard et al., 1994; Leong et al., 2003; Wambolt et al., 1997). Briefly, unlike the significant increase in glycolysis, the rate of pyruvate oxidation that occurs in mitochondria either does not change or accelerates to a lesser extent than glycolysis. Accordingly, we observed decreased fatty acid oxidation and unaltered glucose oxidation as early responses to a hypertrophic stimulus, which is consistent with these literature precedents.

Although the substrate shift from fatty acid to glucose is well-recognized, whether this shift is beneficial is still under debate. The preference for glucose may be favorable to the failing heart based on the fact that glucose is more efficiently coupled to ATP production/O_2 than fatty acids (B. G. Hill & Schulze, 2014). However, others insist that the shift to glucose oxidation cannot satisfy energy demand because glucose metabolism generates approximately 20% less ATP per carbon atom than fatty acids (Lou et al., 2013). A clinical study on dilated cardiomyopathy patients observed the substrate switch from fatty acids to glucose, and it was associated with deceased mechanical performance both at rest and in response to acute stress (Neglia et al., 2007). Also, in dilated cardiomyopathy patients, limited availability of fatty acids leads to decreased cardiac performance and efficiency (Tuunanen et al., 2006). In addition, Lou et al. used infarction-induced remodeling of rat heart followed by ischemia/reperfusion with or without protective pretreatment (i.e. conditioning with sevoflurane), and found that the

protected heart stimulated fatty acid oxidation, but not glucose oxidation after the ischemic injury. Furthermore, the increased fatty acid oxidation improved cardiac function and recovery (Lou et al., 2013). In the present study, we observed decreased fatty acid oxidation in ET1-treated (4 h) myocytes (Figure 29), and we previously showed that ET1 induced a hypertrophic myocyte phenotype and slowed myocyte contraction after 24 h (Figure 12 and Table 4). Thus we support the notion that instead of being a compensatory mechanism, the shift away from fatty acid to glucose is detrimental and contributes to the development of cardiac dysfunction. In addition, various concentrations of FCCP were injected into myocytes to identify the clearest uncoupling response. FCCP is an oxidative phosphorylation uncoupler that induces proton leak across the inner membrane instead of generating ATP. FCCP achieved maximal uncoupling at 2 μM and 10 μM in the presence of glucose and palmitate/BSA conjugates respectively (Figure 25 and Figure 26). This may imply that with supplementation of fatty acids, the mitochondrial respiratory chain is more resistant to FCCP-induced uncoupling, whereas glucose renders the mitochondria more sensitive to FCCP. Fatty acid substrates may therefore be protective at the inner mitochondrial membrane.

To summarize, this series of experiments found that: 1) short exposure to ET1, a hypertrophic stimulus, reduced the ability of mitochondria to perform fatty acid oxidation, but did not alter glucose oxidation in cardiac myocytes; 2) ET1 disrupted the mitochondrial $\Delta\psi_m$; 3) CB-13, a peripherally-restricted dual agonist of CB1/CB2 receptors, rescued fatty acid oxidation and mitochondrial $\Delta\psi_m$, which were depressed by ET1; 4) The protective actions of CB-13 on mitochondria involve AMPK and downstream signaling mediators, PGC-1α and CPT-1β. Based on these findings and pertinent literature, we further propose the following: 1) AMPK contributes to CB-13-

protected proton extrusion and ATP synthesis in fatty acid oxidation, but does not directly regulate $\Delta\psi_m$; 2) The use of only $\Delta\psi_m$ to evaluate mitochondrial performance is not recommended, as preserved $\Delta\psi_m$ does not guarantee proper mitochondrial function; 3) Compared to CB1 receptor signaling (detrimental), CB2 receptor signaling (protective) predonminates in terms of mitochondrial regulation by CB-13; 4) Decreased fatty acid oxidation is detrimental to energy production, rather than compensatory to improve ATP/O_2 efficiency.

To our knowledge, this study is the first to demonstrate the effects of cannabinoid receptor activation on mitochondrial function in the context of cardiac myocytes stimulated to undergo hypertrophy. CB-13, a peripherally-restricted dual agonist of CB1/CB2 receptors, corrected mitochondrial function parameters that were impaired by ET1, including mitochondrial $\Delta\psi_m$ and energy metabolism. AMPK, a key energy sensor, is a coordinator of various mitochondrial protective actions and serves as a link to the anti-hypertrophic effects of CB-13. This study also reveals the limitation of using a single parameter to evaluate mitochondrial function (such as $\Delta\psi_m$), and suggests that mitochondrial bioenergetics must also be considered.

Chapter VIII: Conclusion

There is significant interest in manipulation of the endocannabinoid system as a therapeutic approach to treat disorders such as metabolic syndrome, inflammatory and neuropathic pain, and multiple sclerosis (Hosking & Zajicek, 2008; Palazuelos et al., 2006). Unfortunately, therapeutic use of cannabinoids is impeded by psychotropic side effects including dysphoria, memory impairment, reduced concentration, disorientation, motor incoordination, and possibly addiction. This undesirable psychoactivity is mediated by central CB1 receptors (Hosking & Zajicek, 2008; Howlett et al., 2002; Kunos et al., 2009; Piomelli, 2003), so alternate strategies like using CB2-selective agonists and/or peripherally-restricted CB1/CB2 dual agonists have been proposed (Gertsch et al., 2008; Hosking & Zajicek, 2008; Kunos et al., 2009; Palazuelos et al., 2006). Activation of the endocannabinoid system remains a viable strategy to prevent cardiac hypertrophy, a major risk factor for heart failure. Overall, our findings suggest that to achieve cannabinoid-based cardioprotection devoid of undesirable central CB1-mediated side effects, the best approach warranting further study would be a CB1/CB2 receptor dual agonist with negligible brain penetration.

The heart requires abundant ATP to maintain its optimal performance. Mitochondria mediate a variety of processes including ATP generation and apoptosis signaling. In fact, mitochondrial disorders were implicated in multiple cardiac pathological conditions, including the maladaptive progression of hypertrophy. Here, activation of cannabinoid receptors was found to prevent the development of cardiac myocyte hypertrophy, and protective effects on mitochondrial function were subsequently identified.

Activation of peripheral CB1 and CB2 receptors by CB-13 restored mitochondrial $\Delta\psi_m$ irrespective of the substrates provided and prevented the depression of fatty acid oxidation-related mitochondrial bioenergetics. AMPK played a central role in these beneficial actions, at least partially by up-regulating PGC-1α and CPT-1β, which are key regulators of fatty acid oxidation. Given that fatty acids are the primary energy source in the heart and are replaced by glucose under pathological conditions, the ability of CB-13 to restore fatty acid oxidation and subsequent ATP generation significantly amplifies and strengthens its cardioprotective potential. Thus, it is possible that activation of peripheral CB1/CB2 receptors will be a new therapeutic approach to mitigate the maladaptive effects of cardiac hypertrophy at both the cellular and subcellular level.

Chapter IX. Future Directions

This present study provided evidence for the protective effects of CB receptor activation on cardiac hypertrophy and its related mitochondrial dysfunction *in vitro* using neonatal rat cardiac myocytes. Strengths and limitations of this study are listed below.

Strengths: i) we detected a comprehensive profile of mitochondrial bioenergetics within different energy metabolic phenotypes (i.e. glucose and palmitates), and ii) instead of measuring bioenergetics in isolated mitochondria, we conducted experiments in intact myocytes, which theoretically avoided disruption of cellular environments and preserved better physiological relevance.

Limitations: i) we did not perform *in vivo* experiments, and ii) we did not specify the distinct signaling cascades mediated by CB1 and CB2 receptors, respectively.

Therefore, future studies could be performed as follows:

1. Characterize the effects of CB receptor activation on hypertrophy and mitochondrial function *in vivo*.

Cardiac hypertrophy primarily develops in adulthood. Thus, future studies might verify the anti-hypertrophic action of CB signaling *in vivo* within an adult model, such as the spontaneously hypertensive heart failure rat or TAC-induced mouse model of pressure overload. Blood pressure, cardiac dimensions, and left ventricular performance could be monitored in intact animals. Also, OCRs could be measured in isolated mitochondria from hearts. In addition, limited penetration of CB-13 to the brain could be verified, and compared to CB-13 levels in plasma and heart.

2. Determine molecular signaling involved in protective actions of CB-13.

As discussed on page 164-165, ERK1/2-AP-1 and RhoA/ROCK warrant further study as potential targets of CB1 and/or CB2 receptor activation by CB-13. Activity or

expression of mitochondrial ETC complexes I, III, and IV, as well as ROS production and MnSOD expression should be assessed to further investigate mitochondrial function.

Chapter X. Reference

Abadji, V., Lin, S., Taha, G., Griffin, G., Stevenson, L. A., Pertwee, R. G., & Makriyannis, A. (1994). (R)-methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. *J Med Chem, 37*(12), 1889-1893. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8021930

Abdulnour, J., Yasari, S., Rabasa-Lhoret, R., Faraj, M., Petrosino, S., Piscitelli, F., . . . Di Marzo, V. (2014). Circulating endocannabinoids in insulin sensitive vs. insulin resistant obese postmenopausal women. A MONET group study. *Obesity (Silver Spring), 22*(1), 211-216. doi:10.1002/oby.20498

Abe, Y., Sakairi, T., Kajiyama, H., Shrivastav, S., Beeson, C., & Kopp, J. B. (2010). Bioenergetic characterization of mouse podocytes. *Am J Physiol Cell Physiol, 299*(2), C464-476. doi:10.1152/ajpcell.00563.2009

Abel, E. D., & Doenst, T. (2011). Mitochondrial adaptations to physiological vs. pathological cardiac hypertrophy. *Cardiovasc Res, 90*(2), 234-242. doi:10.1093/cvr/cvr015

Adiarto, S., Heiden, S., Vignon-Zellweger, N., Nakayama, K., Yagi, K., Yanagisawa, M., & Emoto, N. (2012). ET-1 from endothelial cells is required for complete angiotensin II-induced cardiac fibrosis and hypertrophy. *Life Sci, 91*(13-14), 651-657. doi:10.1016/j.lfs.2012.02.006

Afolayan, A. J., Eis, A., Alexander, M., Michalkiewicz, T., Teng, R. J., Lakshminrusimha, S., & Konduri, G. G. (2015). Decreased Endothelial NOS Expression and Function Contributes to Impaired Mitochondrial Biogenesis and Oxidative Stress in Fetal Lambs with PPHN. *Am J Physiol Lung Cell Mol Physiol*, ajplung 00392 02014. doi:10.1152/ajplung.00392.2014

Agudo, J., Martin, M., Roca, C., Molas, M., Bura, A. S., Zimmer, A., . . . Maldonado, R. (2010). Deficiency of CB2 cannabinoid receptor in mice improves insulin sensitivity but increases food intake and obesity with age. *Diabetologia, 53*(12), 2629-2640. doi:10.1007/s00125-010-1894-6

Ahmed, A., Rich, M. W., Fleg, J. L., Zile, M. R., Young, J. B., Kitzman, D. W., . . . Gheorghiade, M. (2006). Effects of digoxin on morbidity and mortality in diastolic heart failure: the ancillary digitalis investigation group trial. *Circulation, 114*(5), 397-403. doi:10.1161/CIRCULATIONAHA.106.628347

Ahmed, A., van der Marck, M. A., van den Elsen, G., & Olde Rikkert, M. (2015). Cannabinoids in late-onset Alzheimer's disease. *Clin Pharmacol Ther, 97*(6), 597-606. doi:10.1002/cpt.117

Akhmedov, A. T., Rybin, V., & Marin-Garcia, J. (2015). Mitochondrial oxidative metabolism and uncoupling proteins in the failing heart. *Heart Fail Rev, 20*(2), 227-249. doi:10.1007/s10741-014-9457-4

Alibin, C. P., Kopilas, M. A., & Anderson, H. D. (2008). Suppression of cardiac myocyte hypertrophy by conjugated linoleic acid: role of peroxisome proliferator-activated receptors alpha and gamma. *J Biol Chem, 283*(16), 10707-10715. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18283099

Allard, M. F., Henning, S. L., Wambolt, R. B., Granleese, S. R., English, D. R., & Lopaschuk, G. D. (1997). Glycogen metabolism in the aerobic hypertrophied rat

heart. *Circulation, 96*(2), 676-682. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9244242

Allard, M. F., Schonekess, B. O., Henning, S. L., English, D. R., & Lopaschuk, G. D. (1994). Contribution of oxidative metabolism and glycolysis to ATP production in hypertrophied hearts. *Am J Physiol, 267*(2 Pt 2), H742-750. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8067430

Althurwi, H. N., Elshenawy, O. H., & El-Kadi, A. O. (2014). Fenofibrate modulates cytochrome P450 and arachidonic acid metabolism in the heart and protects against isoproterenol-induced cardiac hypertrophy. *J Cardiovasc Pharmacol, 63*(2), 167-177. doi:10.1097/FJC.0000000000000036

Alvheim, A. R., Malde, M. K., Osei-Hyiaman, D., Lin, Y. H., Pawlosky, R. J., Madsen, L., . . . Hibbeln, J. R. (2012). Dietary linoleic acid elevates endogenous 2-AG and anandamide and induces obesity. *Obesity (Silver Spring), 20*(10), 1984-1994. doi:10.1038/oby.2012.38

Amin, J. K., Xiao, L., Pimental, D. R., Pagano, P. J., Singh, K., Sawyer, D. B., & Colucci, W. S. (2001). Reactive oxygen species mediate alpha-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes. *J Mol Cell Cardiol, 33*(1), 131-139. doi:10.1006/jmcc.2000.1285

Andersen, M. J., & Borlaug, B. A. (2014). Heart failure with preserved ejection fraction: current understandings and challenges. *Curr Cardiol Rep, 16*(7), 501. doi:10.1007/s11886-014-0501-8

Anderson, M. E., Brown, J. H., & Bers, D. M. (2011). CaMKII in myocardial hypertrophy and heart failure. *J Mol Cell Cardiol, 51*(4), 468-473. doi:10.1016/j.yjmcc.2011.01.012

Annuzzi, G., Piscitelli, F., Di Marino, L., Patti, L., Giacco, R., Costabile, G., . . . Di Marzo, V. (2010). Differential alterations of the concentrations of endocannabinoids and related lipids in the subcutaneous adipose tissue of obese diabetic patients. *Lipids Health Dis, 9*, 43. doi:10.1186/1476-511X-9-43

Antos, C. L., McKinsey, T. A., Frey, N., Kutschke, W., McAnally, J., Shelton, J. M., . . . Olson, E. N. (2002). Activated glycogen synthase-3 beta suppresses cardiac hypertrophy in vivo. *Proc Natl Acad Sci U S A, 99*(2), 907-912. doi:10.1073/pnas.231619298

Anversa, P., Capasso, J. M., Ricci, R., Sonnenblick, E. H., & Olivetti, G. (1989). Morphometric analysis of coronary capillaries during physiologic myocardial growth and induced cardiac hypertrophy: a review. *Int J Microcirc Clin Exp, 8*(4), 353-363. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2532627

Arany, Z., Novikov, M., Chin, S., Ma, Y., Rosenzweig, A., & Spiegelman, B. M. (2006). Transverse aortic constriction leads to accelerated heart failure in mice lacking PPAR-gamma coactivator 1alpha. *Proc Natl Acad Sci U S A, 103*(26), 10086-10091. doi:10.1073/pnas.0603615103

Arrabal, S., Lucena, M. A., Canduela, M. J., Ramos-Uriarte, A., Rivera, P., Serrano, A., . . . Suarez, J. (2015). Pharmacological Blockade of Cannabinoid CB1 Receptors in Diet-Induced Obesity Regulates Mitochondrial Dihydrolipoamide Dehydrogenase in Muscle. *PLoS One, 10*(12), e0145244. doi:10.1371/journal.pone.0145244

Arstall, M. A., Sawyer, D. B., Fukazawa, R., & Kelly, R. A. (1999). Cytokine-mediated apoptosis in cardiac myocytes: the role of inducible nitric oxide synthase induction and peroxynitrite generation. *Circ Res, 85*(9), 829-840. Retrieved from

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10532951

Artmann, A., Petersen, G., Hellgren, L. I., Boberg, J., Skonberg, C., Nellemann, C., . . . Hansen, H. S. (2008). Influence of dietary fatty acids on endocannabinoid and N-acylethanolamine levels in rat brain, liver and small intestine. *Biochim Biophys Acta, 1781*(4), 200-212. doi:10.1016/j.bbalip.2008.01.006

Ashrafian, H., Frenneaux, M. P., & Opie, L. H. (2007). Metabolic mechanisms in heart failure. *Circulation, 116*(4), 434-448. doi:10.1161/CIRCULATIONAHA.107.702795

Aso, E., Palomer, E., Juves, S., Maldonado, R., Munoz, F. J., & Ferrer, I. (2012). CB1 agonist ACEA protects neurons and reduces the cognitive impairment of AbetaPP/PS1 mice. *J Alzheimers Dis, 30*(2), 439-459. doi:10.3233/JAD-2012-111862

Athanasiou, A., Clarke, A. B., Turner, A. E., Kumaran, N. M., Vakilpour, S., Smith, P. A., . . . Bates, T. E. (2007). Cannabinoid receptor agonists are mitochondrial inhibitors: a unified hypothesis of how cannabinoids modulate mitochondrial function and induce cell death. *Biochem Biophys Res Commun, 364*(1), 131-137. doi:10.1016/j.bbrc.2007.09.107

Backs, J., Song, K., Bezprozvannaya, S., Chang, S., & Olson, E. N. (2006). CaM kinase II selectively signals to histone deacetylase 4 during cardiomyocyte hypertrophy. *J Clin Invest, 116*(7), 1853-1864. doi:10.1172/JCI27438

Baines, C. P., Kaiser, R. A., Purcell, N. H., Blair, N. S., Osinska, H., Hambleton, M. A., . . . Molkentin, J. D. (2005). Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature, 434*(7033), 658-662. doi:10.1038/nature03434

Balligand, J. L., & Cannon, P. J. (1997). Nitric oxide synthases and cardiac muscle. Autocrine and paracrine influences. *Arterioscler Thromb Vasc Biol, 17*(10), 1846-1858. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9351345

Bambico, F. R., Katz, N., Debonnel, G., & Gobbi, G. (2007). Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. *J Neurosci, 27*(43), 11700-11711. doi:10.1523/JNEUROSCI.1636-07.2007

Baranowska-Kuczko, M., Kozlowska, H., Kozlowski, M., Schlicker, E., Kloza, M., Surazynski, A., . . . Malinowska, B. (2014). Mechanisms of endothelium-dependent relaxation evoked by anandamide in isolated human pulmonary arteries. *Naunyn Schmiedebergs Arch Pharmacol, 387*(5), 477-486. doi:10.1007/s00210-014-0961-9

Barouch, L. A., Cappola, T. P., Harrison, R. W., Crone, J. K., Rodriguez, E. R., Burnett, A. L., & Hare, J. M. (2003). Combined loss of neuronal and endothelial nitric oxide synthase causes premature mortality and age-related hypertrophic cardiac remodeling in mice. *J Mol Cell Cardiol, 35*(6), 637-644. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12788381

Barouch, L. A., Harrison, R. W., Skaf, M. W., Rosas, G. O., Cappola, T. P., Kobeissi, Z. A., . . . Hare, J. M. (2002). Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. *Nature, 416*(6878), 337-339. Retrieved from

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11907582

Basavarajappa, B. S. (2007). Critical enzymes involved in endocannabinoid metabolism. *Protein Pept Lett, 14*(3), 237-246. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17346227

Basso, E., Fante, L., Fowlkes, J., Petronilli, V., Forte, M. A., & Bernardi, P. (2005). Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. *J Biol Chem, 280*(19), 18558-18561. doi:10.1074/jbc.C500089200

Battista, N., Di Tommaso, M., Bari, M., & Maccarrone, M. (2012). The endocannabinoid system: an overview. *Front Behav Neurosci, 6*, 9. doi:10.3389/fnbeh.2012.00009

Battista, N., Fezza, F., Finazzi-Agro, A., & Maccarrone, M. (2006). The endocannabinoid system in neurodegeneration. *Ital J Biochem, 55*(3-4), 283-289. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17274532

Beal, J. E., Olson, R., Laubenstein, L., Morales, J. O., Bellman, P., Yangco, B., . . . Shepard, K. V. (1995). Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. *J Pain Symptom Manage, 10*(2), 89-97. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7730690

Beauloye, C., Bertrand, L., Horman, S., & Hue, L. (2011). AMPK activation, a preventive therapeutic target in the transition from cardiac injury to heart failure. *Cardiovasc Res, 90*(2), 224-233. doi:10.1093/cvr/cvr034

Becker, D. E. (2010). Nausea, vomiting, and hiccups: a review of mechanisms and treatment. *Anesth Prog, 57*(4), 150-156; quiz 157. doi:10.2344/0003-3006-57.4.150

Beltramo, M., Bernardini, N., Bertorelli, R., Campanella, M., Nicolussi, E., Fredduzzi, S., & Reggiani, A. (2006). CB2 receptor-mediated antihyperalgesia: possible direct involvement of neural mechanisms. *Eur J Neurosci, 23*(6), 1530-1538.

Beltramo, M., Stella, N., Calignano, A., Lin, S. Y., Makriyannis, A., & Piomelli, D. (1997). Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science, 277*(5329), 1094-1097. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9262477

Ben-Shabat, S., Fride, E., Sheskin, T., Tamiri, T., Rhee, M. H., Vogel, Z., . . . Mechoulam, R. (1998). An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol, 353*(1), 23-31. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9721036

Benard, G., Massa, F., Puente, N., Lourenco, J., Bellocchio, L., Soria-Gomez, E., . . . Marsicano, G. (2012). Mitochondrial CB(1) receptors regulate neuronal energy metabolism. *Nat Neurosci, 15*(4), 558-564. doi:10.1038/nn.3053

Benjamin, E. J., & Levy, D. (1999). Why is left ventricular hypertrophy so predictive of morbidity and mortality? *Am J Med Sci, 317*(3), 168-175. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10100690

Bentkover, J. D., Stewart, E. J., Ignaszewski, A., Lepage, S., Liu, P., & Cooper, J. (2003). New technologies and potential cost savings related to morbidity and mortality reduction in Class III/IV heart failure patients in Canada. *Int J Cardiol, 88*(1), 33-41. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12659982

Berge, K., Piscitelli, F., Hoem, N., Silvestri, C., Meyer, I., Banni, S., & Di Marzo, V. (2013). Chronic treatment with krill powder reduces plasma triglyceride and anandamide levels in mildly obese men. *Lipids Health Dis, 12*, 78. doi:10.1186/1476-511X-12-78

Bernardi, P. (1992). Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient. Evidence that the pore can be opened by membrane depolarization. *J Biol Chem, 267*(13), 8834-8839. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1374381

Bilfinger, T. V., Salzet, M., Fimiani, C., Deutsch, D. G., Tramu, G., & Stefano, G. B. (1998). Pharmacological evidence for anandamide amidase in human cardiac and vascular tissues. *Int J Cardiol, 64 Suppl 1*, S15-22. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9687088

Birk, A. V., Chao, W. M., Bracken, C., Warren, J. D., & Szeto, H. H. (2014). Targeting mitochondrial cardiolipin and the cytochrome c/cardiolipin complex to promote electron transport and optimize mitochondrial ATP synthesis. *Br J Pharmacol, 171*(8), 2017-2028. doi:10.1111/bph.12468

Bishop, S. P., & Altschuld, R. A. (1970). Increased glycolytic metabolism in cardiac hypertrophy and congestive failure. *Am J Physiol, 218*(1), 153-159. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4243400

Bisogno, T., Berrendero, F., Ambrosino, G., Cebeira, M., Ramos, J. A., Fernandez-Ruiz, J. J., & Di Marzo, V. (1999). Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. *Biochem Biophys Res Commun, 256*(2), 377-380. doi:10.1006/bbrc.1999.0254

Blankman, J. L., Simon, G. M., & Cravatt, B. F. (2007). A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol, 14*(12), 1347-1356. doi:10.1016/j.chembiol.2007.11.006

Bohm, M., Gierschik, P., Knorr, A., Larisch, K., Weismann, K., & Erdmann, E. (1992). Desensitization of adenylate cyclase and increase of Gi alpha in cardiac hypertrophy due to acquired hypertension. *Hypertension, 20*(1), 103-112. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1319958

Bohm, M., Gierschik, P., Knorr, A., Schmidt, U., Weismann, K., & Erdmann, E. (1993). Cardiac adenylyl cyclase, beta-adrenergic receptors, and G proteins in salt-sensitive hypertension. *Hypertension, 22*(5), 715-727. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8225531

Bonhaus, D. W., Chang, L. K., Kwan, J., & Martin, G. R. (1998). Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. *J Pharmacol Exp Ther, 287*(3), 884-888. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9864268

Bonz, A., Laser, M., Kullmer, S., Kniesch, S., Babin-Ebell, J., Popp, V., . . . Wagner, J. A. (2003). Cannabinoids acting on CB1 receptors decrease contractile performance in human atrial muscle. *J Cardiovasc Pharmacol, 41*(4), 657-664. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12658069

Bouchard, J. F., Lepicier, P., & Lamontagne, D. (2003). Contribution of endocannabinoids in the endothelial protection afforded by ischemic preconditioning in the isolated rat heart. *Life Sci, 72*(16), 1859-1870. Retrieved

from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12586223

Brand, M. D., Brindle, K. M., Buckingham, J. A., Harper, J. A., Rolfe, D. F., & Stuart, J. A. (1999). The significance and mechanism of mitochondrial proton conductance. *Int J Obes Relat Metab Disord, 23 Suppl 6*, S4-11. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10454114

Brand, M. D., Buckingham, J. A., Esteves, T. C., Green, K., Lambert, A. J., Miwa, S., . . . Echtay, K. S. (2004). Mitochondrial superoxide and aging: uncoupling-protein activity and superoxide production. *Biochem Soc Symp*(71), 203-213. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15777023

Brand, M. D., & Nicholls, D. G. (2011). Assessing mitochondrial dysfunction in cells. *Biochem J, 435*(2), 297-312. doi:10.1042/BJ20110162

Brand, M. D., Pakay, J. L., Ocloo, A., Kokoszka, J., Wallace, D. C., Brookes, P. S., & Cornwall, E. J. (2005). The basal proton conductance of mitochondria depends on adenine nucleotide translocase content. *Biochem J, 392*(Pt 2), 353-362. doi:10.1042/BJ20050890

Braunersreuther, V., & Jaquet, V. (2012). Reactive oxygen species in myocardial reperfusion injury: from physiopathology to therapeutic approaches. *Curr Pharm Biotechnol, 13*(1), 97-114. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21470157

Brooks, G., Poolman, R. A., & Li, J. M. (1998). Arresting developments in the cardiac myocyte cell cycle: role of cyclin-dependent kinase inhibitors. *Cardiovasc Res, 39*(2), 301-311. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9798515

Brown, A. J., & Robin Hiley, C. (2009). Is GPR55 an anandamide receptor? *Vitam Horm, 81*, 111-137. doi:10.1016/S0083-6729(09)81005-4

Brown, D. W., Giles, W. H., & Croft, J. B. (2000). Left ventricular hypertrophy as a predictor of coronary heart disease mortality and the effect of hypertension. *Am Heart J, 140*(6), 848-856. doi:10.1067/mhj.2000.111112

Brown, J. H., Del Re, D. P., & Sussman, M. A. (2006). The Rac and Rho hall of fame: a decade of hypertrophic signaling hits. *Circ Res, 98*(6), 730-742. doi:10.1161/01.RES.0000216039.75913.9e

Brown, N. F., Weis, B. C., Husti, J. E., Foster, D. W., & McGarry, J. D. (1995). Mitochondrial carnitine palmitoyltransferase I isoform switching in the developing rat heart. *J Biol Chem, 270*(15), 8952-8957. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7721804

Browne, G. J., & Proud, C. G. (2002). Regulation of peptide-chain elongation in mammalian cells. *Eur J Biochem, 269*(22), 5360-5368. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12423334

Brusco, A., Tagliaferro, P., Saez, T., & Onaivi, E. S. (2008). Postsynaptic localization of CB2 cannabinoid receptors in the rat hippocampus. *Synapse, 62*(12), 944-949. doi:10.1002/syn.20569

Brustovetsky, N., Tropschug, M., Heimpel, S., Heidkamper, D., & Klingenberg, M. (2002). A large Ca2+-dependent channel formed by recombinant ADP/ATP carrier from Neurospora crassa resembles the mitochondrial permeability transition pore. *Biochemistry, 41*(39), 11804-11811. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12269823

Bui, A. L., Horwich, T. B., & Fonarow, G. C. (2011). Epidemiology and risk profile of heart failure. *Nat Rev Cardiol, 8*(1), 30-41. doi:10.1038/nrcardio.2010.165

Cable, J. C., Tan, G. D., Alexander, S. P., & O'Sullivan, S. E. (2014). The effects of obesity, diabetes and metabolic syndrome on the hydrolytic enzymes of the endocannabinoid system in animal and human adipocytes. *Lipids Health Dis, 13*, 43. doi:10.1186/1476-511X-13-43

Cadenas, E., & Davies, K. J. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med, 29*(3-4), 222-230. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11035250

Callen, L., Moreno, E., Barroso-Chinea, P., Moreno-Delgado, D., Cortes, A., Mallol, J., . . . McCormick, P. J. (2012). Cannabinoid receptors CB1 and CB2 form functional heteromers in brain. *J Biol Chem, 287*(25), 20851-20865. doi:10.1074/jbc.M111.335273

Camelliti, P., Borg, T. K., & Kohl, P. (2005). Structural and functional characterisation of cardiac fibroblasts. *Cardiovasc Res, 65*(1), 40-51. doi:10.1016/j.cardiores.2004.08.020

Carbone, F., Mach, F., Vuilleumier, N., & Montecucco, F. (2014). Cannabinoid receptor type 2 activation in atherosclerosis and acute cardiovascular diseases. *Curr Med Chem, 21*(35), 4046-4058. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25245379

Cardinal, P., Bellocchio, L., Guzman-Quevedo, O., Andre, C., Clark, S., Elie, M., . . . Cota, D. (2015). Cannabinoid type 1 (CB1) receptors on Sim1-expressing neurons regulate energy expenditure in male mice. *Endocrinology, 156*(2), 411-418. doi:10.1210/en.2014-1437

Carnevali, L., Vacondio, F., Rossi, S., Callegari, S., Macchi, E., Spadoni, G., . . . Sgoifo, A. (2015). Antidepressant-like activity and cardioprotective effects of fatty acid amide hydrolase inhibitor URB694 in socially stressed Wistar Kyoto rats. *Eur Neuropsychopharmacol, 25*(11), 2157-2169. doi:10.1016/j.euroneuro.2015.07.015

Carruba, M. O., Bondiolotti, G., Picotti, G. B., Catteruccia, N., & Da Prada, M. (1987). Effects of diethyl ether, halothane, ketamine and urethane on sympathetic activity in the rat. *Eur J Pharmacol, 134*(1), 15-24. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3556398

Caruana, L., Petrie, M. C., Davie, A. P., & McMurray, J. J. (2000). Do patients with suspected heart failure and preserved left ventricular systolic function suffer from "diastolic heart failure" or from misdiagnosis? A prospective descriptive study. *BMJ, 321*(7255), 215-218. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10903655

Catanzaro, G., Rapino, C., Oddi, S., & Maccarrone, M. (2009). Anandamide increases swelling and reduces calcium sensitivity of mitochondria. *Biochem Biophys Res Commun, 388*(2), 439-442. doi:10.1016/j.bbrc.2009.08.037

Ceylan-Isik, A. F., Dong, M., Zhang, Y., Dong, F., Turdi, S., Nair, S., . . . Ren, J. (2013). Cardiomyocyte-specific deletion of endothelin receptor A rescues aging-associated cardiac hypertrophy and contractile dysfunction: role of autophagy. *Basic Res Cardiol, 108*(2), 335. doi:10.1007/s00395-013-0335-3

Chan, A. Y., Dolinsky, V. W., Soltys, C. L., Viollet, B., Baksh, S., Light, P. E., & Dyck, J. R. (2008). Resveratrol inhibits cardiac hypertrophy via AMP-activated protein kinase and Akt. *J Biol Chem*. Retrieved from

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18562309

Chan, A. Y., Soltys, C. L., Young, M. E., Proud, C. G., & Dyck, J. R. (2004). Activation of AMP-activated protein kinase inhibits protein synthesis associated with hypertrophy in the cardiac myocyte. *J Biol Chem, 279*(31), 32771-32779. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15159410

Chao, H. H., Chen, J. J., Chen, C. H., Lin, H., Cheng, C. F., Lian, W. S., . . . Cheng, T. H. (2005). Inhibition of angiotensin II induced endothelin-1 gene expression by 17-beta-oestradiol in rat cardiac fibroblasts. *Heart, 91*(5), 664-669. doi:10.1136/hrt.2003.031898

Chen, B. L., Ma, Y. D., Meng, R. S., Xiong, Z. J., Wang, H. N., Zeng, J. Y., . . . Dong, Y. G. (2010). Activation of AMPK inhibits cardiomyocyte hypertrophy by modulating of the FOXO1/MuRF1 signaling pathway in vitro. *Acta Pharmacol Sin, 31*(7), 798-804. doi:10.1038/aps.2010.73

Chen, Z. P., Mitchelhill, K. I., Michell, B. J., Stapleton, D., Rodriguez-Crespo, I., Witters, L. A., . . . Kemp, B. E. (1999). AMP-activated protein kinase phosphorylation of endothelial NO synthase. *FEBS Lett, 443*(3), 285-289. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10025949

Cheung, P. C., Salt, I. P., Davies, S. P., Hardie, D. G., & Carling, D. (2000). Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. *Biochem J, 346 Pt 3*, 659-669. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10698692

Chien, K. R., Knowlton, K. U., Zhu, H., & Chien, S. (1991). Regulation of cardiac gene expression during myocardial growth and hypertrophy: molecular studies of an adaptive physiologic response. *FASEB J, 5*(15), 3037-3046. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1835945

Chipuk, J. E., Kuwana, T., Bouchier-Hayes, L., Droin, N. M., Newmeyer, D. D., Schuler, M., & Green, D. R. (2004). Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science, 303*(5660), 1010-1014. doi:10.1126/science.1092734

Choi, S. W., Gerencser, A. A., & Nicholls, D. G. (2009). Bioenergetic analysis of isolated cerebrocortical nerve terminals on a microgram scale: spare respiratory capacity and stochastic mitochondrial failure. *J Neurochem, 109*(4), 1179-1191. doi:10.1111/j.1471-4159.2009.06055.x

Choudhury, L., Mahrholdt, H., Wagner, A., Choi, K. M., Elliott, M. D., Klocke, F. J., . . . Kim, R. J. (2002). Myocardial scarring in asymptomatic or mildly symptomatic patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol, 40*(12), 2156-2164. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12505229

Chrispin, J., Jain, A., Soliman, E. Z., Guallar, E., Alonso, A., Heckbert, S. R., . . . Nazarian, S. (2014). Association of electrocardiographic and imaging surrogates of left ventricular hypertrophy with incident atrial fibrillation: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol, 63*(19), 2007-2013. doi:10.1016/j.jacc.2014.01.066

Christensen, R., Kristensen, P. K., Bartels, E. M., Bliddal, H., & Astrup, A. (2007). Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of

randomised trials. *Lancet, 370*(9600), 1706-1713. doi:10.1016/S0140-6736(07)61721-8

Clapper, J. R., Moreno-Sanz, G., Russo, R., Guijarro, A., Vacondio, F., Duranti, A., . . . Piomelli, D. (2010). Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism. *Nat Neurosci, 13*(10), 1265-1270. doi:10.1038/nn.2632

Cluny, N. L., Vemuri, V. K., Chambers, A. P., Limebeer, C. L., Bedard, H., Wood, J. T., . . . Sharkey, K. A. (2010). A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause malaise, in rodents. *Br J Pharmacol, 161*(3), 629-642. doi:10.1111/j.1476-5381.2010.00908.x

Correa, F., Docagne, F., Mestre, L., Clemente, D., Hernangomez, M., Loria, F., & Guaza, C. (2009). A role for CB2 receptors in anandamide signalling pathways involved in the regulation of IL-12 and IL-23 in microglial cells. *Biochem Pharmacol, 77*(1), 86-100. doi:10.1016/j.bcp.2008.09.014

Correa, M. V., Nolly, M. B., Caldiz, C. I., de Cingolani, G. E., Cingolani, H. E., & Ennis, I. L. (2014). Endogenous endothelin 1 mediates angiotensin II-induced hypertrophy in electrically paced cardiac myocytes through EGFR transactivation, reactive oxygen species and NHE-1. *Pflugers Arch, 466*(9), 1819-1830. doi:10.1007/s00424-013-1413-y

Cota, D., Marsicano, G., Tschop, M., Grubler, Y., Flachskamm, C., Schubert, M., . . . Pagotto, U. (2003). The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest, 112*(3), 423-431. doi:10.1172/JCI17725

Cote, M., Matias, I., Lemieux, I., Petrosino, S., Almeras, N., Despres, J. P., & Di Marzo, V. (2007). Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *Int J Obes (Lond), 31*(4), 692-699. doi:10.1038/sj.ijo.0803539

Cotecchia, S., Del Vescovo, C. D., Colella, M., Caso, S., & Diviani, D. (2015). The alpha1-adrenergic receptors in cardiac hypertrophy: signaling mechanisms and functional implications. *Cell Signal, 27*(10), 1984-1993. doi:10.1016/j.cellsig.2015.06.009

Cox, E. J., & Marsh, S. A. (2014). A systematic review of fetal genes as biomarkers of cardiac hypertrophy in rodent models of diabetes. *PLoS One, 9*(3), e92903. doi:10.1371/journal.pone.0092903

Cravatt, B. F., Demarest, K., Patricelli, M. P., Bracey, M. H., Giang, D. K., Martin, B. R., & Lichtman, A. H. (2001). Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci U S A, 98*(16), 9371-9376. doi:10.1073/pnas.161191698

Cravatt, B. F., & Lichtman, A. H. (2002). The enzymatic inactivation of the fatty acid amide class of signaling lipids. *Chem Phys Lipids, 121*(1-2), 135-148. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12505696

Crichton, P. G., Parker, N., Vidal-Puig, A. J., & Brand, M. D. (2010). Not all mitochondrial carrier proteins support permeability transition pore formation: no involvement of uncoupling protein 1. *Biosci Rep, 30*(3), 187-192. doi:10.1042/BSR20090063

Crompton, M. (1999). The mitochondrial permeability transition pore and its role in cell death. *Biochem J, 341 (Pt 2)*, 233-249. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10393078

Curtis, A., Mitchell, I., Patel, S., Ives, N., & Rickards, H. (2009). A pilot study using nabilone for symptomatic treatment in Huntington's disease. *Mov Disord, 24*(15), 2254-2259. doi:10.1002/mds.22809

D'Eon, T. M., Pierce, K. A., Roix, J. J., Tyler, A., Chen, H., & Teixeira, S. R. (2008). The role of adipocyte insulin resistance in the pathogenesis of obesity-related elevations in endocannabinoids. *Diabetes, 57*(5), 1262-1268. doi:10.2337/db07-1186

Dagon, Y., Avraham, Y., Ilan, Y., Mechoulam, R., & Berry, E. M. (2007). Cannabinoids ameliorate cerebral dysfunction following liver failure via AMP-activated protein kinase. *FASEB J, 21*(10), 2431-2441. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17431095

Dahlof, B., Devereux, R., de Faire, U., Fyhrquist, F., Hedner, T., Ibsen, H., . . . Wedel, H. (1997). The Losartan Intervention For Endpoint reduction (LIFE) in Hypertension study: rationale, design, and methods. The LIFE Study Group. *Am J Hypertens, 10*(7 Pt 1), 705-713. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9234823

Darmani, N. A. (2001). Delta-9-tetrahydrocannabinol differentially suppresses cisplatin-induced emesis and indices of motor function via cannabinoid CB(1) receptors in the least shrew. *Pharmacol Biochem Behav, 69*(1-2), 239-249. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11420092

Dashzeveg, N., & Yoshida, K. (2015). Cell death decision by p53 via control of the mitochondrial membrane. *Cancer Lett, 367*(2), 108-112. doi:10.1016/j.canlet.2015.07.019

Davies, M., Hobbs, F., Davis, R., Kenkre, J., Roalfe, A. K., Hare, R., . . . Lancashire, R. J. (2001). Prevalence of left-ventricular systolic dysfunction and heart failure in the Echocardiographic Heart of England Screening study: a population based study. *Lancet, 358*(9280), 439-444. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11513906

de Jong, S., van Veen, T. A., van Rijen, H. V., & de Bakker, J. M. (2011). Fibrosis and cardiac arrhythmias. *J Cardiovasc Pharmacol, 57*(6), 630-638. doi:10.1097/FJC.0b013e318207a35f

de Lago, E., Moreno-Martet, M., Cabranes, A., Ramos, J. A., & Fernandez-Ruiz, J. (2012). Cannabinoids ameliorate disease progression in a model of multiple sclerosis in mice, acting preferentially through CB1 receptor-mediated anti-inflammatory effects. *Neuropharmacology, 62*(7), 2299-2308. doi:10.1016/j.neuropharm.2012.01.030

de Lemos, J. A., McGuire, D. K., & Drazner, M. H. (2003). B-type natriuretic peptide in cardiovascular disease. *Lancet, 362*(9380), 316-322. doi:10.1016/S0140-6736(03)13976-1

Demuth, D. G., & Molleman, A. (2006). Cannabinoid signalling. *Life Sci, 78*(6), 549-563. doi:10.1016/j.lfs.2005.05.055

Deutsch, D. G., & Chin, S. A. (1993). Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem Pharmacol, 46*(5), 791-796.

Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8373432

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., . . . Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science, 258*(5090), 1946-1949. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1470919

Devereux, R. B., Roman, M. J., Liu, J. E., Welty, T. K., Lee, E. T., Rodeheffer, R., . . . Howard, B. V. (2000). Congestive heart failure despite normal left ventricular systolic function in a population-based sample: the Strong Heart Study. *Am J Cardiol, 86*(10), 1090-1096. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11074205

Di Filippo, C., Rossi, F., Rossi, S., & D'Amico, M. (2004). Cannabinoid CB2 receptor activation reduces mouse myocardial ischemia-reperfusion injury: involvement of cytokine/chemokines and PMN. *J Leukoc Biol, 75*(3), 453-459. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14657208

Di Marzo, V., & Despres, J. P. (2009). CB1 antagonists for obesity--what lessons have we learned from rimonabant? *Nat Rev Endocrinol, 5*(11), 633-638. doi:10.1038/nrendo.2009.197

Di Marzo, V., Goparaju, S. K., Wang, L., Liu, J., Batkai, S., Jarai, Z., . . . Kunos, G. (2001). Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature, 410*(6830), 822-825. doi:10.1038/35071088

Dinh, T. P., Carpenter, D., Leslie, F. M., Freund, T. F., Katona, I., Sensi, S. L., . . . Piomelli, D. (2002). Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A, 99*(16), 10819-10824. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12136125

Dinh, T. P., Freund, T. F., & Piomelli, D. (2002). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem Phys Lipids, 121*(1-2), 149-158. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12505697

Dirkx, E., da Costa Martins, P. A., & De Windt, L. J. (2013). Regulation of fetal gene expression in heart failure. *Biochim Biophys Acta, 1832*(12), 2414-2424. doi:10.1016/j.bbadis.2013.07.023

Dodd, M. S., Ball, D. R., Schroeder, M. A., Le Page, L. M., Atherton, H. J., Heather, L. C., . . . Tyler, D. J. (2012). In vivo alterations in cardiac metabolism and function in the spontaneously hypertensive rat heart. *Cardiovasc Res, 95*(1), 69-76. doi:10.1093/cvr/cvs164

Doenst, T., Nguyen, T. D., & Abel, E. D. (2013). Cardiac metabolism in heart failure: implications beyond ATP production. *Circ Res, 113*(6), 709-724. doi:10.1161/CIRCRESAHA.113.300376

Dol-Gleizes, F., Paumelle, R., Visentin, V., Mares, A. M., Desitter, P., Hennuyer, N., . . . Bono, F. (2009). Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol, 29*(1), 12-18. doi:10.1161/ATVBAHA.108.168757

Dolinsky, V. W., & Dyck, J. R. (2006). Role of AMP-activated protein kinase in healthy and diseased hearts. *Am J Physiol Heart Circ Physiol, 291*(6), H2557-2569. doi:10.1152/ajpheart.00329.2006

Dolinsky, V. W., Morton, J. S., Oka, T., Robillard-Frayne, I., Bagdan, M., Lopaschuk, G. D., . . . Dyck, J. R. (2010). Calorie restriction prevents hypertension and cardiac hypertrophy in the spontaneously hypertensive rat. *Hypertension, 56*(3), 412-421. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20696994

Dorn, G. W., 2nd, & Force, T. (2005). Protein kinase cascades in the regulation of cardiac hypertrophy. *J Clin Invest, 115*(3), 527-537. doi:10.1172/JCI24178

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiol Rev, 82*(1), 47-95. doi:10.1152/physrev.00018.2001

Duerr, G. D., Heinemann, J. C., Dunkel, S., Zimmer, A., Lutz, B., Lerner, R., . . . Dewald, O. (2013). Myocardial hypertrophy is associated with inflammation and activation of endocannabinoid system in patients with aortic valve stenosis. *Life Sci, 92*(20-21), 976-983. doi:10.1016/j.lfs.2013.03.014

Duerr, G. D., Heinemann, J. C., Suchan, G., Kolobara, E., Wenzel, D., Geisen, C., . . . Dewald, O. (2014). The endocannabinoid-CB2 receptor axis protects the ischemic heart at the early stage of cardiomyopathy. *Basic Res Cardiol, 109*(4), 425. doi:10.1007/s00395-014-0425-x

Duran, M., Perez, E., Abanades, S., Vidal, X., Saura, C., Majem, M., . . . Capella, D. (2010). Preliminary efficacy and safety of an oromucosal standardized cannabis extract in chemotherapy-induced nausea and vomiting. *Br J Clin Pharmacol, 70*(5), 656-663. doi:10.1111/j.1365-2125.2010.03743.x

Dziadulewicz, E. K., Bevan, S. J., Brain, C. T., Coote, P. R., Culshaw, A. J., Davis, A. J., . . . Zadrobilek, J. (2007). Naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone: a potent, orally bioavailable human CB1/CB2 dual agonist with antihyperalgesic properties and restricted central nervous system penetration. *J Med Chem, 50*(16), 3851-3856. doi:10.1021/jm070317a

Eaton, S., Middleton, B., & Bartlett, K. (1998). Control of mitochondrial beta-oxidation: sensitivity of the trifunctional protein to [NAD+]/[NADH] and [acetyl-CoA]/[CoA]. *Biochim Biophys Acta, 1429*(1), 230-238. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9920399

Echouffo-Tcheugui, J. B., Erqou, S., Butler, J., Yancy, C. W., & Fonarow, G. C. (2015). Assessing the Risk of Progression From Asymptomatic Left Ventricular Dysfunction to Overt Heart Failure: A Systematic Overview and Meta-Analysis. *JACC Heart Fail*. doi:10.1016/j.jchf.2015.09.015

Echtay, K. S., Roussel, D., St-Pierre, J., Jekabsons, M. B., Cadenas, S., Stuart, J. A., . . . Brand, M. D. (2002). Superoxide activates mitochondrial uncoupling proteins. *Nature, 415*(6867), 96-99. doi:10.1038/415096a

el Alaoui-Talibi, Z., Landormy, S., Loireau, A., & Moravec, J. (1992). Fatty acid oxidation and mechanical performance of volume-overloaded rat hearts. *Am J Physiol, 262*(4 Pt 2), H1068-1074. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1533101

El-Sherbeni, A. A., & El-Kadi, A. O. (2014). Alterations in cytochrome P450-derived arachidonic acid metabolism during pressure overload-induced cardiac hypertrophy. *Biochem Pharmacol, 87*(3), 456-466. doi:10.1016/j.bcp.2013.11.015

Eljaschewitsch, E., Witting, A., Mawrin, C., Lee, T., Schmidt, P. M., Wolf, S., . . . Ullrich, O. (2006). The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. *Neuron, 49*(1), 67-79. doi:10.1016/j.neuron.2005.11.027

Elsner, D., Muntze, A., Kromer, E. P., & Riegger, G. A. (1992). Effectiveness of endopeptidase inhibition (candoxatril) in congestive heart failure. *Am J Cardiol, 70*(4), 494-498. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1386491

Engeli, S., Bohnke, J., Feldpausch, M., Gorzelniak, K., Janke, J., Batkai, S., . . . Jordan, J. (2005). Activation of the peripheral endocannabinoid system in human obesity. *Diabetes, 54*(10), 2838-2843. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16186383

Felder, C. C., Joyce, K. E., Briley, E. M., Mansouri, J., Mackie, K., Blond, O., . . . Mitchell, R. L. (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol Pharmacol, 48*(3), 443-450. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7565624

Felder, C. C., Nielsen, A., Briley, E. M., Palkovits, M., Priller, J., Axelrod, J., . . . Becker, G. W. (1996). Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. *FEBS Lett, 393*(2-3), 231-235. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8814296

Feldmann, H. M., Golozoubova, V., Cannon, B., & Nedergaard, J. (2009). UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab, 9*(2), 203-209. doi:10.1016/j.cmet.2008.12.014

Feng, Q., Lu, X., Jones, D. L., Shen, J., & Arnold, J. M. (2001). Increased inducible nitric oxide synthase expression contributes to myocardial dysfunction and higher mortality after myocardial infarction in mice. *Circulation, 104*(6), 700-704. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11489778

Fernandes, E. S., Fernandes, M. A., & Keeble, J. E. (2012). The functions of TRPA1 and TRPV1: moving away from sensory nerves. *Br J Pharmacol, 166*(2), 510-521. doi:10.1111/j.1476-5381.2012.01851.x

Fillmore, N., Mori, J., & Lopaschuk, G. D. (2014). Mitochondrial fatty acid oxidation alterations in heart failure, ischaemic heart disease and diabetic cardiomyopathy. *Br J Pharmacol, 171*(8), 2080-2090. doi:10.1111/bph.12475

Filtz, T. M., Grubb, D. R., McLeod-Dryden, T. J., Luo, J., & Woodcock, E. A. (2009). Gq-initiated cardiomyocyte hypertrophy is mediated by phospholipase Cbeta1b. *FASEB J, 23*(10), 3564-3570. doi:10.1096/fj.09-133983

Fingleton, B. (2007). Matrix metalloproteinases as valid clinical targets. *Curr Pharm Des, 13*(3), 333-346. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17313364

Fisar, Z., Singh, N., & Hroudova, J. (2014). Cannabinoid-induced changes in respiration of brain mitochondria. *Toxicol Lett, 231*(1), 62-71. doi:10.1016/j.toxlet.2014.09.002

Fitzgerald, K. T., Bronstein, A. C., & Newquist, K. L. (2013). Marijuana poisoning. *Top Companion Anim Med, 28*(1), 8-12. doi:10.1053/j.tcam.2013.03.004

Flather, M. D., Yusuf, S., Kober, L., Pfeffer, M., Hall, A., Murray, G., . . . Braunwald, E. (2000). Long-term ACE-inhibitor therapy in patients with heart failure or left-ventricular dysfunction: a systematic overview of data from individual patients. ACE-Inhibitor Myocardial Infarction Collaborative Group. *Lancet, 355*(9215), 1575-1581. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10821360

Fleming, I. (2006). Signaling by the angiotensin-converting enzyme. *Circ Res, 98*(7), 887-896. doi:10.1161/01.RES.0000217340.40936.53

Fox, A., Kesingland, A., Gentry, C., McNair, K., Patel, S., Urban, L., & James, I. (2001). The role of central and peripheral Cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. *Pain, 92*(1-2), 91-100. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11323130

Franz, I. W., Tonnesmann, U., & Muller, J. F. (1998). Time course of complete normalization of left ventricular hypertrophy during long-term antihypertensive therapy with angiotensin converting enzyme inhibitors. *Am J Hypertens, 11*(6 Pt 1), 631-639. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9657621

Frey, N., Katus, H. A., Olson, E. N., & Hill, J. A. (2004). Hypertrophy of the heart: a new therapeutic target? *Circulation, 109*(13), 1580-1589. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15066961

Fu, J., Bottegoni, G., Sasso, O., Bertorelli, R., Rocchia, W., Masetti, M., . . . Piomelli, D. (2012). A catalytically silent FAAH-1 variant drives anandamide transport in neurons. *Nat Neurosci, 15*(1), 64-69. doi:10.1038/nn.2986

Fujisaki, H., Ito, H., Hirata, Y., Tanaka, M., Hata, M., Lin, M., . . . Hiroe, M. (1995). Natriuretic peptides inhibit angiotensin II-induced proliferation of rat cardiac fibroblasts by blocking endothelin-1 gene expression. *J Clin Invest, 96*(2), 1059-1065. doi:10.1172/JCI118092

Fujita, S., Shimojo, N., Terasaki, F., Otsuka, K., Hosotani, N., Kohda, Y., . . . Imanaka-Yoshida, K. (2013). Atrial natriuretic peptide exerts protective action against angiotensin II-induced cardiac remodeling by attenuating inflammation via endothelin-1/endothelin receptor A cascade. *Heart Vessels, 28*(5), 646-657. doi:10.1007/s00380-012-0311-0

Fuller, S. J., Gillespie-Brown, J., & Sugden, P. H. (1998). Oncogenic src, raf, and ras stimulate a hypertrophic pattern of gene expression and increase cell size in neonatal rat ventricular myocytes. *J Biol Chem, 273*(29), 18146-18152. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9660773

Ganau, A., Devereux, R. B., Roman, M. J., de Simone, G., Pickering, T. G., Saba, P. S., . . . Laragh, J. H. (1992). Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension. *J Am Coll Cardiol, 19*(7), 1550-1558. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1534335

Garcia, C., Palomo-Garo, C., Garcia-Arencibia, M., Ramos, J., Pertwee, R., & Fernandez-Ruiz, J. (2011). Symptom-relieving and neuroprotective effects of the phytocannabinoid Delta(9)-THCV in animal models of Parkinson's disease. *Br J Pharmacol, 163*(7), 1495-1506. doi:10.1111/j.1476-5381.2011.01278.x

Garcia-Arencibia, M., Gonzalez, S., de Lago, E., Ramos, J. A., Mechoulam, R., & Fernandez-Ruiz, J. (2007). Evaluation of the neuroprotective effect of

cannabinoids in a rat model of Parkinson's disease: importance of antioxidant and cannabinoid receptor-independent properties. *Brain Res, 1134*(1), 162-170. doi:10.1016/j.brainres.2006.11.063

Garlid, K. D., Orosz, D. E., Modriansky, M., Vassanelli, S., & Jezek, P. (1996). On the mechanism of fatty acid-induced proton transport by mitochondrial uncoupling protein. *J Biol Chem, 271*(5), 2615-2620. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8576230

Garnier, A., Zoll, J., Fortin, D., N'Guessan, B., Lefebvre, F., Geny, B., . . . Ventura-Clapier, R. (2009). Control by circulating factors of mitochondrial function and transcription cascade in heart failure: a role for endothelin-1 and angiotensin II. *Circ Heart Fail, 2*(4), 342-350. doi:10.1161/CIRCHEARTFAILURE.108.812099

Gasperi, V., Fezza, F., Pasquariello, N., Bari, M., Oddi, S., Agro, A. F., & Maccarrone, M. (2007). Endocannabinoids in adipocytes during differentiation and their role in glucose uptake. *Cell Mol Life Sci, 64*(2), 219-229. doi:10.1007/s00018-006-6445-4

Gebremedhin, D., Lange, A. R., Campbell, W. B., Hillard, C. J., & Harder, D. R. (1999). Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca2+ channel current. *Am J Physiol, 276*(6 Pt 2), H2085-2093. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10362691

Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J. Z., Xie, X. Q., . . . Zimmer, A. (2008). Beta-caryophyllene is a dietary cannabinoid. *Proc Natl Acad Sci U S A, 105*(26), 9099-9104. doi:10.1073/pnas.0803601105

Giannuzzi, P., Temporelli, P. L., Bosimini, E., Gentile, F., Lucci, D., Maggioni, A. P., . . . Nicolosi, G. L. (2001). Heterogeneity of left ventricular remodeling after acute myocardial infarction: results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-3 Echo Substudy. *Am Heart J, 141*(1), 131-138. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11136498

Goldberg, L. R., & Jessup, M. (2006). Stage B heart failure: management of asymptomatic left ventricular systolic dysfunction. *Circulation, 113*(24), 2851-2860. doi:10.1161/CIRCULATIONAHA.105.600437

Golech, S. A., McCarron, R. M., Chen, Y., Bembry, J., Lenz, F., Mechoulam, R., . . . Spatz, M. (2004). Human brain endothelium: coexpression and function of vanilloid and endocannabinoid receptors. *Brain Res Mol Brain Res, 132*(1), 87-92. doi:10.1016/j.molbrainres.2004.08.025

Goncharov, N. V., Avdonin, P. V., Nadeev, A. D., Zharkikh, I. L., & Jenkins, R. O. (2015). Reactive oxygen species in pathogenesis of atherosclerosis. *Curr Pharm Des, 21*(9), 1134-1146. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25312724

Gong, J. P., Onaivi, E. S., Ishiguro, H., Liu, Q. R., Tagliaferro, P. A., Brusco, A., & Uhl, G. R. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res, 1071*(1), 10-23. doi:10.1016/j.brainres.2005.11.035

Gonzalez, S., Scorticati, C., Garcia-Arencibia, M., de Miguel, R., Ramos, J. A., & Fernandez-Ruiz, J. (2006). Effects of rimonabant, a selective cannabinoid CB1 receptor antagonist, in a rat model of Parkinson's disease. *Brain Res, 1073-1074*, 209-219. doi:10.1016/j.brainres.2005.12.014

Gorelick, D. A., Heishman, S. J., Preston, K. L., Nelson, R. A., Moolchan, E. T., & Huestis, M. A. (2006). The cannabinoid CB1 receptor antagonist rimonabant attenuates the hypotensive effect of smoked marijuana in male smokers. *Am Heart J, 151*(3), 754 e751-754 e755. doi:10.1016/j.ahj.2005.11.006

Goshima, K. (1970). Formation of nexuses and electrotonic transmission between myocardial and FL cells in monolayer culture. *Exp Cell Res, 63*(1), 124-130. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5531475

Gottdiener, J. S., McClelland, R. L., Marshall, R., Shemanski, L., Furberg, C. D., Kitzman, D. W., . . . Manolio, T. A. (2002). Outcome of congestive heart failure in elderly persons: influence of left ventricular systolic function. The Cardiovascular Health Study. *Ann Intern Med, 137*(8), 631-639. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12379062

Granger, C. B., McMurray, J. J., Yusuf, S., Held, P., Michelson, E. L., Olofsson, B., . . . Committees. (2003). Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. *Lancet, 362*(9386), 772-776. doi:10.1016/S0140-6736(03)14284-5

Grant, I., Atkinson, J. H., Gouaux, B., & Wilsey, B. (2012). Medical marijuana: clearing away the smoke. *Open Neurol J, 6*, 18-25. doi:10.2174/1874205X01206010018

Gray, L. R., Tompkins, S. C., & Taylor, E. B. (2014). Regulation of pyruvate metabolism and human disease. *Cell Mol Life Sci, 71*(14), 2577-2604. doi:10.1007/s00018-013-1539-2

Greco, R., Gasperi, V., Sandrini, G., Bagetta, G., Nappi, G., Maccarrone, M., & Tassorelli, C. (2010). Alterations of the endocannabinoid system in an animal model of migraine: evaluation in cerebral areas of rat. *Cephalalgia, 30*(3), 296-302. doi:10.1111/j.1468-2982.2009.01924.x

Griendling, K. K., Lassegue, B., & Alexander, R. W. (1996). Angiotensin receptors and their therapeutic implications. *Annu Rev Pharmacol Toxicol, 36*, 281-306. doi:10.1146/annurev.pa.36.040196.001433

Gui, H., Tong, Q., Qu, W., Mao, C. M., & Dai, S. M. (2015). The endocannabinoid system and its therapeutic implications in rheumatoid arthritis. *Int Immunopharmacol, 26*(1), 86-91. doi:10.1016/j.intimp.2015.03.006

Halestrap, A. P., & Davidson, A. M. (1990). Inhibition of Ca2(+)-induced large-amplitude swelling of liver and heart mitochondria by cyclosporin is probably caused by the inhibitor binding to mitochondrial-matrix peptidyl-prolyl cis-trans isomerase and preventing it interacting with the adenine nucleotide translocase. *Biochem J, 268*(1), 153-160. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2160810

Hannan, R. D., Jenkins, A., Jenkins, A. K., & Brandenburger, Y. (2003). Cardiac hypertrophy: a matter of translation. *Clin Exp Pharmacol Physiol, 30*(8), 517-527. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12890171

Hardie, D. G. (2007). AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol, 8*(10), 774-785. doi:10.1038/nrm2249

Harris, D., McCulloch, A. I., Kendall, D. A., & Randall, M. D. (2002). Characterization of vasorelaxant responses to anandamide in the rat mesenteric arterial bed. *J Physiol, 539*(Pt 3), 893-902. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11897858

Harvey, B. S., Nicotra, L. L., Vu, M., & Smid, S. D. (2013). Cannabinoid CB2 receptor activation attenuates cytokine-evoked mucosal damage in a human colonic explant model without changing epithelial permeability. *Cytokine, 63*(2), 209-217. doi:10.1016/j.cyto.2013.04.032

Hashimotodani, Y., Ohno-Shosaku, T., Tanimura, A., Kita, Y., Sano, Y., Shimizu, T., . . . Kano, M. (2013). Acute inhibition of diacylglycerol lipase blocks endocannabinoid-mediated retrograde signalling: evidence for on-demand biosynthesis of 2-arachidonoylglycerol. *J Physiol, 591*(19), 4765-4776. doi:10.1113/jphysiol.2013.254474

Hawley, S. A., Davison, M., Woods, A., Davies, S. P., Beri, R. K., Carling, D., & Hardie, D. G. (1996). Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. *J Biol Chem, 271*(44), 27879-27887. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8910387

Haworth, R. A., & Hunter, D. R. (1979). The Ca2+-induced membrane transition in mitochondria. II. Nature of the Ca2+ trigger site. *Arch Biochem Biophys, 195*(2), 460-467. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/38751

He, L., Kim, T., Long, Q., Liu, J., Wang, P., Zhou, Y., . . . Yang, Q. (2012). Carnitine palmitoyltransferase-1b deficiency aggravates pressure overload-induced cardiac hypertrophy caused by lipotoxicity. *Circulation, 126*(14), 1705-1716. doi:10.1161/CIRCULATIONAHA.111.075978

He, Q., Harris, N., Ren, J., & Han, X. (2014). Mitochondria-targeted antioxidant prevents cardiac dysfunction induced by tafazzin gene knockdown in cardiac myocytes. *Oxid Med Cell Longev, 2014*, 654198. doi:10.1155/2014/654198

Heart and Stroke Foundation of Canada. (2014a). cardiovascular disease deaths. Retrieved from http://www.heartandstroke.com/site/c.ikIQLcMWJtE/b.3483991/k.34A8/Statistics.htm - heartdisease

Heart and Stroke Foundation of Canada. (2014b). http://www.heartandstroke.com/site/c.ikIQLcMWJtE/b.3483991/k.34A8/Statistics.htm - chf. Retrieved from http://www.heartandstroke.com/site/c.ikIQLcMWJtE/b.3483991/k.34A8/Statistics.htm - chf

Henstridge, C. M., Balenga, N. A., Ford, L. A., Ross, R. A., Waldhoer, M., & Irving, A. J. (2009). The GPR55 ligand L-alpha-lysophosphatidylinositol promotes RhoA-dependent Ca2+ signaling and NFAT activation. *FASEB J, 23*(1), 183-193. doi:10.1096/fj.08-108670

Hernandez-Torres, G., Cipriano, M., Heden, E., Bjorklund, E., Canales, A., Zian, D., . . . Lopez-Rodriguez, M. L. (2014). A reversible and selective inhibitor of monoacylglycerol lipase ameliorates multiple sclerosis. *Angew Chem Int Ed Engl, 53*(50), 13765-13770. doi:10.1002/anie.201407807

Herrmann, J. M., & Neupert, W. (2000). Protein transport into mitochondria. *Curr Opin Microbiol, 3*(2), 210-214. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10744987

Hershberger, R. E., Lindenfeld, J., Mestroni, L., Seidman, C. E., Taylor, M. R., Towbin, J. A., & Heart Failure Society of, A. (2009). Genetic evaluation of

cardiomyopathy--a Heart Failure Society of America practice guideline. *J Card Fail, 15*(2), 83-97. doi:10.1016/j.cardfail.2009.01.006

Hill, B. G., Dranka, B. P., Zou, L., Chatham, J. C., & Darley-Usmar, V. M. (2009). Importance of the bioenergetic reserve capacity in response to cardiomyocyte stress induced by 4-hydroxynonenal. *Biochem J, 424*(1), 99-107. doi:10.1042/BJ20090934

Hill, B. G., & Schulze, P. C. (2014). Insights into metabolic remodeling of the hypertrophic and failing myocardium. *Circ Heart Fail, 7*(6), 874-876. doi:10.1161/CIRCHEARTFAILURE.114.001803

Hill, M. N., Miller, G. E., Ho, W. S., Gorzalka, B. B., & Hillard, C. J. (2008). Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. *Pharmacopsychiatry, 41*(2), 48-53. doi:10.1055/s-2007-993211

Hillard, C. J., Manna, S., Greenberg, M. J., DiCamelli, R., Ross, R. A., Stevenson, L. A., . . . Campbell, W. B. (1999). Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). *J Pharmacol Exp Ther, 289*(3), 1427-1433. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10336536

Hiroe, M., Hirata, Y., Fujita, N., Umezawa, S., Ito, H., Tsujino, M., . . . Marumo, F. (1991). Plasma endothelin-1 levels in idiopathic dilated cardiomyopathy. *Am J Cardiol, 68*(10), 1114-1115. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1927934

Ho, C. Y., Lopez, B., Coelho-Filho, O. R., Lakdawala, N. K., Cirino, A. L., Jarolim, P., . . . Seidman, C. E. (2010). Myocardial fibrosis as an early manifestation of hypertrophic cardiomyopathy. *N Engl J Med, 363*(6), 552-563. doi:10.1056/NEJMoa1002659

Ho, J. E., Lyass, A., Lee, D. S., Vasan, R. S., Kannel, W. B., Larson, M. G., & Levy, D. (2013). Predictors of new-onset heart failure: differences in preserved versus reduced ejection fraction. *Circ Heart Fail, 6*(2), 279-286. doi:10.1161/CIRCHEARTFAILURE.112.972828

Ho, K. K., Anderson, K. M., Kannel, W. B., Grossman, W., & Levy, D. (1993). Survival after the onset of congestive heart failure in Framingham Heart Study subjects. *Circulation, 88*(1), 107-115. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8319323

Ho, K. K., Pinsky, J. L., Kannel, W. B., & Levy, D. (1993). The epidemiology of heart failure: the Framingham Study. *J Am Coll Cardiol, 22*(4 Suppl A), 6A-13A. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8376698

Ho, W. S., & Randall, M. D. (2007). Endothelium-dependent metabolism by endocannabinoid hydrolases and cyclooxygenases limits vasorelaxation to anandamide and 2-arachidonoylglycerol. *Br J Pharmacol, 150*(5), 641-651. doi:10.1038/sj.bjp.0707141

Hohmann, A. G., Suplita, R. L., Bolton, N. M., Neely, M. H., Fegley, D., Mangieri, R., . . . Piomelli, D. (2005). An endocannabinoid mechanism for stress-induced analgesia. *Nature, 435*(7045), 1108-1112. doi:10.1038/nature03658

Hokimoto, S., Yasue, H., Fujimoto, K., Yamamoto, H., Nakao, K., Kaikita, K., . . . Miyamoto, E. (1996). Expression of angiotensin-converting enzyme in remaining

viable myocytes of human ventricles after myocardial infarction. *Circulation, 94*(7), 1513-1518. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8840838

Horvath, B., Mukhopadhyay, P., Hasko, G., & Pacher, P. (2012). The endocannabinoid system and plant-derived cannabinoids in diabetes and diabetic complications. *Am J Pathol, 180*(2), 432-442. doi:10.1016/j.ajpath.2011.11.003

Hosking, R. D., & Zajicek, J. P. (2008). Therapeutic potential of cannabis in pain medicine. *Br J Anaesth, 101*(1), 59-68. doi:10.1093/bja/aen119

Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., Devane, W. A., . . . Pertwee, R. G. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev, 54*(2), 161-202. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12037135

Hsiao, W. C., Shia, K. S., Wang, Y. T., Yeh, Y. N., Chang, C. P., Lin, Y., . . . Hung, M. S. (2015). A novel peripheral cannabinoid receptor 1 antagonist, BPR0912, reduces weight independently of food intake and modulates thermogenesis. *Diabetes Obes Metab, 17*(5), 495-504. doi:10.1111/dom.12447

Hu, D. L., Zhu, G., Mori, F., Omoe, K., Okada, M., Wakabayashi, K., . . . Nakane, A. (2007). Staphylococcal enterotoxin induces emesis through increasing serotonin release in intestine and it is downregulated by cannabinoid receptor 1. *Cell Microbiol, 9*(9), 2267-2277. doi:10.1111/j.1462-5822.2007.00957.x

Huang, B., Cheng, X., Wang, D., Peng, M., Xue, Z., Da, Y., . . . Zhang, R. (2014). Adiponectin promotes pancreatic cancer progression by inhibiting apoptosis via the activation of AMPK/Sirt1/PGC-1alpha signaling. *Oncotarget, 5*(13), 4732-4745. doi:10.18632/oncotarget.1963

Huang, W., Rubinstein, J., Prieto, A. R., Thang, L. V., & Wang, D. H. (2009). Transient receptor potential vanilloid gene deletion exacerbates inflammation and atypical cardiac remodeling after myocardial infarction. *Hypertension, 53*(2), 243-250. doi:10.1161/HYPERTENSIONAHA.108.118349

Huang, Y., Zhang, H., Shao, Z., O'Hara, K. A., Kopilas, M. A., Yu, L., . . . Anderson, H. D. (2011). Suppression of endothelin-1-induced cardiac myocyte hypertrophy by PPAR agonists: role of diacylglycerol kinase zeta. *Cardiovasc Res, 90*(2), 267-275. doi:10.1093/cvr/cvq401

Huestis, M. A., Boyd, S. J., Heishman, S. J., Preston, K. L., Bonnet, D., Le Fur, G., & Gorelick, D. A. (2007). Single and multiple doses of rimonabant antagonize acute effects of smoked cannabis in male cannabis users. *Psychopharmacology (Berl), 194*(4), 505-515. doi:10.1007/s00213-007-0861-5

Huffman, J. W. (2005). CB2 receptor ligands. *Mini Rev Med Chem, 5*(7), 641-649. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16026310

Huffman, J. W., Liddle, J., Yu, S., Aung, M. M., Abood, M. E., Wiley, J. L., & Martin, B. R. (1999). 3-(1',1'-Dimethylbutyl)-1-deoxy-delta8-THC and related compounds: synthesis of selective ligands for the CB2 receptor. *Bioorg Med Chem, 7*(12), 2905-2914. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10658595

Hulsmans, M., Van Dooren, E., & Holvoet, P. (2012). Mitochondrial reactive oxygen species and risk of atherosclerosis. *Curr Atheroscler Rep, 14*(3), 264-276. doi:10.1007/s11883-012-0237-0

Hunter, J. C., Zeidan, A., Javadov, S., Kilic, A., Rajapurohitam, V., & Karmazyn, M. (2009). Nitric oxide inhibits endothelin-1-induced neonatal cardiomyocyte hypertrophy via a RhoA-ROCK-dependent pathway. *J Mol Cell Cardiol, 47*(6), 810-818. doi:10.1016/j.yjmcc.2009.09.012

Ibrahim, M. M., Deng, H., Zvonok, A., Cockayne, D. A., Kwan, J., Mata, H. P., . . . Malan, T. P., Jr. (2003). Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci U S A, 100*(18), 10529-10533. doi:10.1073/pnas.1834309100

Ide, T., Tsutsui, H., Kinugawa, S., Utsumi, H., Kang, D., Hattori, N., . . . Takeshita, A. (1999). Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. *Circ Res, 85*(4), 357-363. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10455064

Ido, Y., Carling, D., & Ruderman, N. (2002). Hyperglycemia-induced apoptosis in human umbilical vein endothelial cells: inhibition by the AMP-activated protein kinase activation. *Diabetes, 51*(1), 159-167. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11756336

Ito, H. (1997). Endothelins and cardiac hypertrophy. *Life Sci, 61*(6), 585-593. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9250714

Ito, H., Hirata, Y., Adachi, S., Tanaka, M., Tsujino, M., Koike, A., . . . Hiroe, M. (1993). Endothelin-1 is an autocrine/paracrine factor in the mechanism of angiotensin II-induced hypertrophy in cultured rat cardiomyocytes. *J Clin Invest, 92*(1), 398-403. doi:10.1172/JCI116579

Ito, H., Hirata, Y., Hiroe, M., Tsujino, M., Adachi, S., Takamoto, T., . . . Marumo, F. (1991). Endothelin-1 induces hypertrophy with enhanced expression of muscle-specific genes in cultured neonatal rat cardiomyocytes. *Circ Res, 69*(1), 209-215. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2054934

Januzzi, J. L., van Kimmenade, R., Lainchbury, J., Bayes-Genis, A., Ordonez-Llanos, J., Santalo-Bel, M., . . . Richards, M. (2006). NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study. *Eur Heart J, 27*(3), 330-337. doi:10.1093/eurheartj/ehi631

Jastroch, M., Divakaruni, A. S., Mookerjee, S., Treberg, J. R., & Brand, M. D. (2010). Mitochondrial proton and electron leaks. *Essays Biochem, 47*, 53-67. doi:10.1042/bse0470053

Javadov, S., Karmazyn, M., & Escobales, N. (2009). Mitochondrial permeability transition pore opening as a promising therapeutic target in cardiac diseases. *J Pharmacol Exp Ther, 330*(3), 670-678. doi:10.1124/jpet.109.153213

Javadov, S., Rajapurohitam, V., Kilic, A., Zeidan, A., Choi, A., & Karmazyn, M. (2009). Anti-hypertrophic effect of NHE-1 inhibition involves GSK-3beta-dependent attenuation of mitochondrial dysfunction. *J Mol Cell Cardiol, 46*(6), 998-1007. doi:10.1016/j.yjmcc.2008.12.023

Jbilo, O., Ravinet-Trillou, C., Arnone, M., Buisson, I., Bribes, E., Peleraux, A., . . . Casellas, P. (2005). The CB1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. *FASEB J, 19*(11), 1567-1569. doi:10.1096/fj.04-3177fje

Jenkin, K. A., McAinch, A. J., Grinfeld, E., & Hryciw, D. H. (2010). Role for cannabinoid receptors in human proximal tubular hypertrophy. *Cell Physiol Biochem, 26*(6), 879-886. doi:10.1159/000323997

Jeong, M. Y., Kinugawa, K., Vinson, C., & Long, C. S. (2005). AFos dissociates cardiac myocyte hypertrophy and expression of the pathological gene program. *Circulation, 111*(13), 1645-1651. doi:10.1161/01.CIR.0000160367.99928.87

Jiang, L. S., Pu, J., Han, Z. H., Hu, L. H., & He, B. (2009). Role of activated endocannabinoid system in regulation of cellular cholesterol metabolism in macrophages. *Cardiovasc Res, 81*(4), 805-813. doi:10.1093/cvr/cvn344

Johansen, H., Strauss, B., Arnold, J. M., Moe, G., & Liu, P. (2003). On the rise: The current and projected future burden of congestive heart failure hospitalization in Canada. *Can J Cardiol, 19*(4), 430-435. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12704491

Jung, M., Calassi, R., Rinaldi-Carmona, M., Chardenot, P., Le Fur, G., Soubrie, P., & Oury-Donat, F. (1997). Characterization of CB1 receptors on rat neuronal cell cultures: binding and functional studies using the selective receptor antagonist SR 141716A. *J Neurochem, 68*(1), 402-409. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8978752

Kaddoura, S., Firth, J. D., Boheler, K. R., Sugden, P. H., & Poole-Wilson, P. A. (1996). Endothelin-1 is involved in norepinephrine-induced ventricular hypertrophy in vivo. Acute effects of bosentan, an orally active, mixed endothelin ETA and ETB receptor antagonist. *Circulation, 93*(11), 2068-2079. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8640984

Kagan, V. E., Tyurin, V. A., Jiang, J., Tyurina, Y. Y., Ritov, V. B., Amoscato, A. A., . . . Borisenko, G. G. (2005). Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. *Nat Chem Biol, 1*(4), 223-232. doi:10.1038/nchembio727

Kalant, H., Porath-Waller, A. J., Canadian Centre on Substance Abuse., & Canadian Electronic Library (Firm). (2012). *Clearing the smoke on cannabis medical use of cannabis and cannabinoids Clearing the smoke on cannabis ; 5* (pp. 1 electronic text (10 p.)). Retrieved from http://site.ebrary.com/lib/umanitoba/Doc?id=10585752 Retrieved from http://site.ebrary.com/lib/umanitoba/Doc?id=10585752

Kapur, A., Zhao, P., Sharir, H., Bai, Y., Caron, M. G., Barak, L. S., & Abood, M. E. (2009). Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *J Biol Chem, 284*(43), 29817-29827. doi:10.1074/jbc.M109.050187

Karlsson, M., Contreras, J. A., Hellman, U., Tornqvist, H., & Holm, C. (1997). cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J Biol Chem, 272*(43), 27218-27223. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9341166

Karne, S., Jayawickreme, C. K., & Lerner, M. R. (1993). Cloning and characterization of an endothelin-3 specific receptor (ETC receptor) from Xenopus laevis dermal melanophores. *J Biol Chem, 268*(25), 19126-19133. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8360195

Karschner, E. L., Darwin, W. D., McMahon, R. P., Liu, F., Wright, S., Goodwin, R. S., & Huestis, M. A. (2011). Subjective and physiological effects after controlled

Sativex and oral THC administration. *Clin Pharmacol Ther, 89*(3), 400-407. doi:10.1038/clpt.2010.318

Katayama, K., Ueda, N., Katoh, I., & Yamamoto, S. (1999). Equilibrium in the hydrolysis and synthesis of cannabimimetic anandamide demonstrated by a purified enzyme. *Biochim Biophys Acta, 1440*(2-3), 205-214. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10521704

Kelsey, J. E., Harris, O., & Cassin, J. (2009). The CB(1) antagonist rimonabant is adjunctively therapeutic as well as monotherapeutic in an animal model of Parkinson's disease. *Behav Brain Res, 203*(2), 304-307. doi:10.1016/j.bbr.2009.04.035

Kerner, J., & Hoppel, C. (2000). Fatty acid import into mitochondria. *Biochim Biophys Acta, 1486*(1), 1-17. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10856709

Khan, S. A., Skaf, M. W., Harrison, R. W., Lee, K., Minhas, K. M., Kumar, A., . . . Hare, J. M. (2003). Nitric oxide regulation of myocardial contractility and calcium cycling: independent impact of neuronal and endothelial nitric oxide synthases. *Circ Res, 92*(12), 1322-1329. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12764022

Khanolkar, A. D., Abadji, V., Lin, S., Hill, W. A., Taha, G., Abouzid, K., . . . Makriyannis, A. (1996). Head group analogs of arachidonylethanolamide, the endogenous cannabinoid ligand. *J Med Chem, 39*(22), 4515-4519. doi:10.1021/jm960152y

Kim, J., & Li, Y. (2015). Chronic activation of CB2 cannabinoid receptors in the hippocampus increases excitatory synaptic transmission. *J Physiol, 593*(4), 871-886. doi:10.1113/jphysiol.2014.286633

Kinnally, K. W., Peixoto, P. M., Ryu, S. Y., & Dejean, L. M. (2011). Is mPTP the gatekeeper for necrosis, apoptosis, or both? *Biochim Biophys Acta, 1813*(4), 616-622. doi:10.1016/j.bbamcr.2010.09.013

Kirkham, T. C., Williams, C. M., Fezza, F., & Di Marzo, V. (2002). Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br J Pharmacol, 136*(4), 550-557. doi:10.1038/sj.bjp.0704767

Kohl, P., Kamkin, A. G., Kiseleva, I. S., & Noble, D. (1994). Mechanosensitive fibroblasts in the sino-atrial node region of rat heart: interaction with cardiomyocytes and possible role. *Exp Physiol, 79*(6), 943-956. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7873162

Kohno, M., Horio, T., Yokokawa, K., Yasunari, K., Ikeda, M., Minami, M., . . . Takeda, T. (1995). Brain natriuretic peptide as a marker for hypertensive left ventricular hypertrophy: changes during 1-year antihypertensive therapy with angiotensin-converting enzyme inhibitor. *Am J Med, 98*(3), 257-265. doi:10.1016/S0002-9343(99)80372-6

Kokoszka, J. E., Waymire, K. G., Levy, S. E., Sligh, J. E., Cai, J., Jones, D. P., . . . Wallace, D. C. (2004). The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature, 427*(6973), 461-465. doi:10.1038/nature02229

Kola, B., Hubina, E., Tucci, S. A., Kirkham, T. C., Garcia, E. A., Mitchell, S. E., . . . Korbonits, M. (2005). Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. *J Biol Chem, 280*(26), 25196-25201. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15899896

Kolwicz, S. C., Jr., Olson, D. P., Marney, L. C., Garcia-Menendez, L., Synovec, R. E., & Tian, R. (2012). Cardiac-specific deletion of acetyl CoA carboxylase 2 prevents metabolic remodeling during pressure-overload hypertrophy. *Circ Res, 111*(6), 728-738. doi:10.1161/CIRCRESAHA.112.268128

Kolwicz, S. C., Jr., Purohit, S., & Tian, R. (2013). Cardiac metabolism and its interactions with contraction, growth, and survival of cardiomyocytes. *Circ Res, 113*(5), 603-616. doi:10.1161/CIRCRESAHA.113.302095

Kondo, S., Kondo, H., Nakane, S., Kodaka, T., Tokumura, A., Waku, K., & Sugiura, T. (1998). 2-Arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through CA2+-dependent and -independent mechanisms. *FEBS Lett, 429*(2), 152-156. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9650580

Konstam, M. A., Kramer, D. G., Patel, A. R., Maron, M. S., & Udelson, J. E. Left ventricular remodeling in heart failure current concepts in clinical significance and assessment. *JACC Cardiovasc Imaging, 4*(1), 98-108. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=21232712

Konstam, M. A., Kramer, D. G., Patel, A. R., Maron, M. S., & Udelson, J. E. (2011). Left ventricular remodeling in heart failure: current concepts in clinical significance and assessment. *JACC Cardiovasc Imaging, 4*(1), 98-108. doi:10.1016/j.jcmg.2010.10.008

Kornfeld, O. S., Hwang, S., Disatnik, M. H., Chen, C. H., Qvit, N., & Mochly-Rosen, D. (2015). Mitochondrial reactive oxygen species at the heart of the matter: new therapeutic approaches for cardiovascular diseases. *Circ Res, 116*(11), 1783-1799. doi:10.1161/CIRCRESAHA.116.305432

Korshunov, S. S., Skulachev, V. P., & Starkov, A. A. (1997). High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett, 416*(1), 15-18. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9369223

Korup, E., Dalsgaard, D., Nyvad, O., Jensen, T. M., Toft, E., & Berning, J. (1997). Comparison of degrees of left ventricular dilation within three hours and up to six days after onset of first acute myocardial infarction. *Am J Cardiol, 80*(4), 449-453. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9285656

Korvald, C., Elvenes, O. P., & Myrmel, T. (2000). Myocardial substrate metabolism influences left ventricular energetics in vivo. *Am J Physiol Heart Circ Physiol, 278*(4), H1345-1351. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10749732

Krauss, S. (2001). Mitochondria: Structure and Role in Respiration. *eLS.* doi:10.1038/npg.els.0001380

Krishnan, G., & Chatterjee, N. (2012). Endocannabinoids alleviate proinflammatory conditions by modulating innate immune response in muller glia during inflammation. *Glia, 60*(11), 1629-1645. doi:10.1002/glia.22380

Krylatov, A. V., Ugdyzhekova, D. S., Bernatskaya, N. A., Maslov, L. N., Mekhoulam, R., Pertwee, R. G., & Stephano, G. B. (2001). Activation of type II cannabinoid receptors improves myocardial tolerance to arrhythmogenic effects of coronary occlusion and reperfusion. *Bull Exp Biol Med, 131*(6), 523-525. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11586395

Kunos, G., Osei-Hyiaman, D., Batkai, S., Sharkey, K. A., & Makriyannis, A. (2009). Should peripheral CB(1) cannabinoid receptors be selectively targeted for therapeutic gain? *Trends Pharmacol Sci, 30*(1), 1-7. doi:10.1016/j.tips.2008.10.001

Kurihara, R., Tohyama, Y., Matsusaka, S., Naruse, H., Kinoshita, E., Tsujioka, T., . . . Yamamura, H. (2006). Effects of peripheral cannabinoid receptor ligands on motility and polarization in neutrophil-like HL60 cells and human neutrophils. *J Biol Chem, 281*(18), 12908-12918. doi:10.1074/jbc.M510871200

Kurz, C., Baranowska, U., Lupinski, S., Gothert, M., Malinowska, B., & Schlicker, E. (2009). Urethane, but not pentobarbitone, attenuates presynaptic receptor function in rats: a contribution to the choice of anaesthetic. *Br J Pharmacol, 157*(8), 1474-1482. doi:10.1111/j.1476-5381.2009.00315.x

Lagneux, C., & Lamontagne, D. (2001). Involvement of cannabinoids in the cardioprotection induced by lipopolysaccharide. *Br J Pharmacol, 132*(4), 793-796. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11181418

Lake, K. D., Compton, D. R., Varga, K., Martin, B. R., & Kunos, G. (1997). Cannabinoid-induced hypotension and bradycardia in rats mediated by CB1-like cannabinoid receptors. *J Pharmacol Exp Ther, 281*(3), 1030-1037. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9190833

Lake, K. D., Martin, B. R., Kunos, G., & Varga, K. (1997). Cardiovascular effects of anandamide in anesthetized and conscious normotensive and hypertensive rats. *Hypertension, 29*(5), 1204-1210. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9149688

Lam, F. F., Luk, P. W., & Ng, E. S. (2007). Pharmacological characterization of receptor types mediating the dilator action of anandamide on blood vessels of the rat knee joint. *Life Sci, 80*(16), 1495-1502. doi:10.1016/j.lfs.2007.01.009

Lan, R., Gatley, J., Lu, Q., Fan, P., Fernando, S. R., Volkow, N. D., . . . Makriyannis, A. (1999). Design and synthesis of the CB1 selective cannabinoid antagonist AM281: a potential human SPECT ligand. *AAPS PharmSci, 1*(2), E4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11741201

Lan, R., Liu, Q., Fan, P., Lin, S., Fernando, S. R., McCallion, D., . . . Makriyannis, A. (1999). Structure-activity relationships of pyrazole derivatives as cannabinoid receptor antagonists. *J Med Chem, 42*(4), 769-776. doi:10.1021/jm980363y

Lang, H., Li, Q., Yu, H., Li, P., Lu, Z., Xiong, S., . . . Zhu, Z. (2014). Activation of TRPV1 attenuates high salt-induced cardiac hypertrophy through improvement of mitochondrial function. *Br J Pharmacol*. doi:10.1111/bph.12987

LaPointe, M. C. (2005). Molecular regulation of the brain natriuretic peptide gene. *Peptides, 26*(6), 944-956. doi:10.1016/j.peptides.2004.08.028

Laprairie, R. B., Kelly, M. E., & Denovan-Wright, E. M. (2013). Cannabinoids increase type 1 cannabinoid receptor expression in a cell culture model of striatal neurons: implications for Huntington's disease. *Neuropharmacology, 72*, 47-57. doi:10.1016/j.neuropharm.2013.04.006

Lastres-Becker, I., Fezza, F., Cebeira, M., Bisogno, T., Ramos, J. A., Milone, A., . . . Di Marzo, V. (2001). Changes in endocannabinoid transmission in the basal ganglia in a rat model of Huntington's disease. *Neuroreport, 12*(10), 2125-2129. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11447320

Latini, L., Bisicchia, E., Sasso, V., Chiurchiu, V., Cavallucci, V., Molinari, M., . . . Viscomi, M. T. (2014). Cannabinoid CB2 receptor (CB2R) stimulation delays rubrospinal mitochondrial-dependent degeneration and improves functional recovery after spinal cord hemisection by ERK1/2 inactivation. *Cell Death Dis, 5*, e1404. doi:10.1038/cddis.2014.364

Lee, D. S., Gona, P., Vasan, R. S., Larson, M. G., Benjamin, E. J., Wang, T. J., . . . Levy, D. (2009). Relation of disease pathogenesis and risk factors to heart failure with preserved or reduced ejection fraction: insights from the framingham heart study of the national heart, lung, and blood institute. *Circulation, 119*(24), 3070-3077. doi:10.1161/CIRCULATIONAHA.108.815944

Lee, W. J., Kim, M., Park, H. S., Kim, H. S., Jeon, M. J., Oh, K. S., . . . Park, J. Y. (2006). AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARalpha and PGC-1. *Biochem Biophys Res Commun, 340*(1), 291-295. doi:10.1016/j.bbrc.2005.12.011

Lehman, J. J., Barger, P. M., Kovacs, A., Saffitz, J. E., Medeiros, D. M., & Kelly, D. P. (2000). Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest, 106*(7), 847-856. doi:10.1172/JCI10268

Lehman, J. J., & Kelly, D. P. (2002). Gene regulatory mechanisms governing energy metabolism during cardiac hypertrophic growth. *Heart Fail Rev, 7*(2), 175-185. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11988641

Lemasters, J. J., Theruvath, T. P., Zhong, Z., & Nieminen, A. L. (2009). Mitochondrial calcium and the permeability transition in cell death. *Biochim Biophys Acta, 1787*(11), 1395-1401. doi:10.1016/j.bbabio.2009.06.009

Leone, T. C., Lehman, J. J., Finck, B. N., Schaeffer, P. J., Wende, A. R., Boudina, S., . . . Kelly, D. P. (2005). PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. *PLoS Biol, 3*(4), e101. doi:10.1371/journal.pbio.0030101

Leong, H. S., Brownsey, R. W., Kulpa, J. E., & Allard, M. F. (2003). Glycolysis and pyruvate oxidation in cardiac hypertrophy--why so unbalanced? *Comp Biochem Physiol A Mol Integr Physiol, 135*(4), 499-513. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12890541

Lepicier, P., Bibeau-Poirier, A., Lagneux, C., Servant, M. J., & Lamontagne, D. (2006). Signaling pathways involved in the cardioprotective effects of cannabinoids. *J Pharmacol Sci, 102*(2), 155-166. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17031075

Lepicier, P., Bouchard, J. F., Lagneux, C., & Lamontagne, D. (2003). Endocannabinoids protect the rat isolated heart against ischaemia. *Br J Pharmacol, 139*(4), 805-815. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12813004

Lerman, A., Kubo, S. H., Tschumperlin, L. K., & Burnett, J. C., Jr. (1992). Plasma endothelin concentrations in humans with end-stage heart failure and after heart transplantation. *J Am Coll Cardiol, 20*(4), 849-853. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1527295

Leu, J. I., Dumont, P., Hafey, M., Murphy, M. E., & George, D. L. (2004). Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. *Nat Cell Biol, 6*(5), 443-450. doi:10.1038/ncb1123

Levy, D., Garrison, R. J., Savage, D. D., Kannel, W. B., & Castelli, W. P. (1990). Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med, 322*(22), 1561-1566. Retrieved from http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=m&form=6&dopt=r&uid=2139921

Li, C., Jones, P. M., & Persaud, S. J. (2011). Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas. *Pharmacol Ther, 129*(3), 307-320. doi:10.1016/j.pharmthera.2010.10.006

Li, H. L., Yin, R., Chen, D., Liu, D., Wang, D., Yang, Q., & Dong, Y. G. (2007). Long-term activation of adenosine monophosphate-activated protein kinase attenuates pressure-overload-induced cardiac hypertrophy. *J Cell Biochem, 100*(5), 1086-1099. doi:10.1002/jcb.21197

Li, J., Umar, S., Amjedi, M., Iorga, A., Sharma, S., Nadadur, R. D., . . . Eghbali, M. (2012). New frontiers in heart hypertrophy during pregnancy. *Am J Cardiovasc Dis, 2*(3), 192-207. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22937489

Li, L., Wu, L., Wang, C., Liu, L., & Zhao, Y. (2007). Adiponectin modulates carnitine palmitoyltransferase-1 through AMPK signaling cascade in rat cardiomyocytes. *Regul Pept, 139*(1-3), 72-79. doi:10.1016/j.regpep.2006.10.007

Li, M., Ma, G., Han, L., & Li, J. (2014). Regulating effect of tea polyphenols on endothelin, intracellular calcium concentration, and mitochondrial membrane potential in vascular endothelial cells injured by Angiotensin II. *Ann Vasc Surg, 28*(4), 1016-1022. doi:10.1016/j.avsg.2013.11.003

Li, Q., Guo, H. C., Maslov, L. N., Qiao, X. W., Zhou, J. J., & Zhang, Y. (2014). Mitochondrial permeability transition pore plays a role in the cardioprotection of CB2 receptor against ischemia-reperfusion injury. *Can J Physiol Pharmacol, 92*(3), 205-214. doi:10.1139/cjpp-2013-0293

Li, Q., Shi, M., & Li, B. (2013). Anandamide enhances expression of heat shock protein 72 to protect against ischemia-reperfusion injury in rat heart. *J Physiol Sci, 63*(1), 47-53. doi:10.1007/s12576-012-0228-5

Li, Q., Wang, F., Zhang, Y. M., Zhou, J. J., & Zhang, Y. (2013). Activation of cannabinoid type 2 receptor by JWH133 protects heart against ischemia/reperfusion-induced apoptosis. *Cell Physiol Biochem, 31*(4-5), 693-702. doi:10.1159/000350088

Liao, Y., Bin, J., Asakura, M., Xuan, W., Chen, B., Huang, Q., . . . Kitakaze, M. (2012). Deficiency of type 1 cannabinoid receptors worsens acute heart failure induced by pressure overload in mice. *Eur Heart J, 33*(24), 3124-3133. doi:10.1093/eurheartj/ehr246

Liao, Y., Bin, J., Luo, T., Zhao, H., Ledent, C., Asakura, M., . . . Kitakaze, M. (2013). CB1 cannabinoid receptor deficiency promotes cardiac remodeling induced by pressure overload in mice. *Int J Cardiol, 167*(5), 1936-1944. doi:10.1016/j.ijcard.2012.05.033

Lile, J. A., Kelly, T. H., & Hays, L. R. (2011). Separate and combined effects of the cannabinoid agonists nabilone and Delta(9)-THC in humans discriminating Delta(9)-THC. *Drug Alcohol Depend, 116*(1-3), 86-92. doi:10.1016/j.drugalcdep.2010.11.019

Lim, C. T., Kola, B., Feltrin, D., Perez-Tilve, D., Tschop, M. H., Grossman, A. B., & Korbonits, M. (2013). Ghrelin and cannabinoids require the ghrelin receptor to affect cellular energy metabolism. *Mol Cell Endocrinol, 365*(2), 303-308. doi:10.1016/j.mce.2012.11.007

Lin, C. Y., Hsu, Y. J., Hsu, S. C., Chen, Y., Lee, H. S., Lin, S. H., . . . Shih, C. C. (2015). CB1 cannabinoid receptor antagonist attenuates left ventricular hypertrophy and Akt-mediated cardiac fibrosis in experimental uremia. *J Mol Cell Cardiol, 85*, 249-261. doi:10.1016/j.yjmcc.2015.06.010

Lionetti, V., Linke, A., Chandler, M. P., Young, M. E., Penn, M. S., Gupte, S., . . . Recchia, F. A. (2005). Carnitine palmitoyl transferase-I inhibition prevents ventricular remodeling and delays decompensation in pacing-induced heart failure. *Cardiovasc Res, 66*(3), 454-461. doi:10.1016/j.cardiores.2005.02.004

Little, P. J., Compton, D. R., Johnson, M. R., Melvin, L. S., & Martin, B. R. (1988). Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J Pharmacol Exp Ther, 247*(3), 1046-1051. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2849657

Liu, J., Cao, L., Chen, J., Song, S., Lee, I. H., Quijano, C., . . . Finkel, T. (2009). Bmi1 regulates mitochondrial function and the DNA damage response pathway. *Nature, 459*(7245), 387-392. doi:10.1038/nature08040

Liu, J., Gao, B., Mirshahi, F., Sanyal, A. J., Khanolkar, A. D., Makriyannis, A., & Kunos, G. (2000). Functional CB1 cannabinoid receptors in human vascular endothelial cells. *Biochem J, 346 Pt 3*, 835-840. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10698714

Liu, Q., Docherty, J. C., Rendell, J. C., Clanachan, A. S., & Lopaschuk, G. D. (2002). High levels of fatty acids delay the recovery of intracellular pH and cardiac efficiency in post-ischemic hearts by inhibiting glucose oxidation. *J Am Coll Cardiol, 39*(4), 718-725. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11849874

Liu, Y., Fiskum, G., & Schubert, D. (2002). Generation of reactive oxygen species by the mitochondrial electron transport chain. *J Neurochem, 80*(5), 780-787. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11948241

Loirand, G., Guerin, P., & Pacaud, P. (2006). Rho kinases in cardiovascular physiology and pathophysiology. *Circ Res, 98*(3), 322-334. doi:10.1161/01.RES.0000201960.04223.3c

Lopaschuk, G. D., Ussher, J. R., Folmes, C. D., Jaswal, J. S., & Stanley, W. C. (2010). Myocardial fatty acid metabolism in health and disease. *Physiol Rev, 90*(1), 207-258. doi:10.1152/physrev.00015.2009

Lorell, B. H., & Carabello, B. A. (2000). Left ventricular hypertrophy: pathogenesis, detection, and prognosis. *Circulation, 102*(4), 470-479. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10908222

Lou, P. H., Zhang, L., Lucchinetti, E., Heck, M., Affolter, A., Gandhi, M., . . . Zaugg, M. (2013). Infarct-remodelled hearts with limited oxidative capacity boost fatty acid oxidation after conditioning against ischaemia/reperfusion injury. *Cardiovasc Res, 97*(2), 251-261. doi:10.1093/cvr/cvs323

Lu, Y., Akinwumi, B. C., Shao, Z., & Anderson, H. D. (2014). Ligand activation of cannabinoid receptors attenuates hypertrophy of neonatal rat cardiomyocytes. *J Cardiovasc Pharmacol, 64*(5), 420-430. doi:10.1097/FJC.0000000000000134

Luz, P. L., Nishiyama, M., & Chagas, A. C. (2011). Drugs and lifestyle for the treatment and prevention of coronary artery disease: comparative analysis of the scientific basis. *Braz J Med Biol Res, 44*(10), 973-991. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21876872

Maccarrone, M., van der Stelt, M., Rossi, A., Veldink, G. A., Vliegenthart, J. F., & Agro, A. F. (1998). Anandamide hydrolysis by human cells in culture and brain. *J Biol Chem, 273*(48), 32332-32339. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9822713

Mahmood, S. S., & Wang, T. J. (2013). The epidemiology of congestive heart failure: the Framingham Heart Study perspective. *Glob Heart, 8*(1), 77-82. doi:10.1016/j.gheart.2012.12.006

Mailloux, R. J., & Harper, M. E. (2011). Uncoupling proteins and the control of mitochondrial reactive oxygen species production. *Free Radic Biol Med, 51*(6), 1106-1115. doi:10.1016/j.freeradbiomed.2011.06.022

Makhlouf, A. A., & McDermott, P. J. (1998). Increased expression of eukaryotic initiation factor 4E during growth of neonatal rat cardiocytes in vitro. *Am J Physiol, 274*(6 Pt 2), H2133-2142. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9841540

Malan, T. P., Jr., Ibrahim, M. M., Lai, J., Vanderah, T. W., Makriyannis, A., & Porreca, F. (2003). CB2 cannabinoid receptor agonists: pain relief without psychoactive effects? *Curr Opin Pharmacol, 3*(1), 62-67. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12550743

Malinowska, B., Kwolek, G., & Gothert, M. (2001). Anandamide and methanandamide induce both vanilloid VR1- and cannabinoid CB1 receptor-mediated changes in heart rate and blood pressure in anaesthetized rats. *Naunyn Schmiedebergs Arch Pharmacol, 364*(6), 562-569. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11770012

Mancia, G., Carugo, S., Grassi, G., Lanzarotti, A., Schiavina, R., Cesana, G., . . . Pressioni Arteriose Monitorate, E. L. A. S. (2002). Prevalence of left ventricular hypertrophy in hypertensive patients without and with blood pressure control: data from the PAMELA population. Pressioni Arteriose Monitorate E Loro Associazioni. *Hypertension, 39*(3), 744-749. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11897756

Manuel, I., Gonzalez de San Roman, E., Giralt, M. T., Ferrer, I., & Rodriguez-Puertas, R. (2014). Type-1 cannabinoid receptor activity during Alzheimer's disease progression. *J Alzheimers Dis, 42*(3), 761-766. doi:10.3233/JAD-140492

Marco, E. M., Echeverry-Alzate, V., Lopez-Moreno, J. A., Gine, E., Penasco, S., & Viveros, M. P. (2014). Consequences of early life stress on the expression of endocannabinoid-related genes in the rat brain. *Behav Pharmacol, 25*(5-6), 547-556. doi:10.1097/FBP.0000000000000068

Marino, T. A., Cassidy, M., Marino, D. R., Carson, N. L., & Houser, S. (1991). Norepinephrine-induced cardiac hypertrophy of the cat heart. *Anat Rec, 229*(4), 505-510. doi:10.1002/ar.1092290411

Maron, B. J., Pelliccia, A., Spataro, A., & Granata, M. (1993). Reduction in left ventricular wall thickness after deconditioning in highly trained Olympic athletes. *Br Heart J, 69*(2), 125-128. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8435237

Marriott, K. S., & Huffman, J. W. (2008). Recent advances in the development of selective ligands for the cannabinoid CB(2) receptor. *Curr Top Med Chem, 8*(3), 187-204. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18289088

Marsicano, G., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascio, M. G., . . . Lutz, B. (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature, 418*(6897), 530-534. doi:10.1038/nature00839

Masoudi, F. A., Havranek, E. P., Smith, G., Fish, R. H., Steiner, J. F., Ordin, D. L., & Krumholz, H. M. (2003). Gender, age, and heart failure with preserved left ventricular systolic function. *J Am Coll Cardiol, 41*(2), 217-223. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12535812

Massie, B. M., Carson, P. E., McMurray, J. J., Komajda, M., McKelvie, R., Zile, M. R., . . . Investigators, I. P. (2008). Irbesartan in patients with heart failure and preserved ejection fraction. *N Engl J Med, 359*(23), 2456-2467. doi:10.1056/NEJMoa0805450

Mastinu, A., Pira, M., Pani, L., Pinna, G. A., & Lazzari, P. (2012). NESS038C6, a novel selective CB1 antagonist agent with anti-obesity activity and improved molecular profile. *Behav Brain Res, 234*(2), 192-204. doi:10.1016/j.bbr.2012.06.033

Mathew, R. J., Wilson, W. H., & Davis, R. (2003). Postural syncope after marijuana: a transcranial Doppler study of the hemodynamics. *Pharmacol Biochem Behav, 75*(2), 309-318. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12873621

Matias, I., Gonthier, M. P., Orlando, P., Martiadis, V., De Petrocellis, L., Cervino, C., . . . Di Marzo, V. (2006). Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. *J Clin Endocrinol Metab, 91*(8), 3171-3180. doi:10.1210/jc.2005-2679

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature, 346*(6284), 561-564. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2165569

Mazzola, C., Micale, V., & Drago, F. (2003). Amnesia induced by beta-amyloid fragments is counteracted by cannabinoid CB1 receptor blockade. *Eur J*

Pharmacol, 477(3), 219-225. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14522360

McAllister, S. D., & Glass, M. (2002). CB(1) and CB(2) receptor-mediated signalling: a focus on endocannabinoids. *Prostaglandins Leukot Essent Fatty Acids, 66*(2-3), 161-171. doi:10.1054/plef.2001.0344

McDougle, D. R., Kambalyal, A., Meling, D. D., & Das, A. (2014). Endocannabinoids anandamide and 2-arachidonoylglycerol are substrates for human CYP2J2 epoxygenase. *J Pharmacol Exp Ther, 351*(3), 616-627. doi:10.1124/jpet.114.216598

McMullen, J. R., & Jennings, G. L. (2007). Differences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat heart failure. *Clin Exp Pharmacol Physiol, 34*(4), 255-262. doi:10.1111/j.1440-1681.2007.04585.x

McMurray, J. J., Ray, S. G., Abdullah, I., Dargie, H. J., & Morton, J. J. (1992). Plasma endothelin in chronic heart failure. *Circulation, 85*(4), 1374-1379. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1532540

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., . . . et al. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol, 50*(1), 83-90. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7605349

Mechoulam, R., Fride, E., & Di Marzo, V. (1998). Endocannabinoids. *Eur J Pharmacol, 359*(1), 1-18. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9831287

Meiri, E., Jhangiani, H., Vredenburgh, J. J., Barbato, L. M., Carter, F. J., Yang, H. M., & Baranowski, V. (2007). Efficacy of dronabinol alone and in combination with ondansetron versus ondansetron alone for delayed chemotherapy-induced nausea and vomiting. *Curr Med Res Opin, 23*(3), 533-543. doi:10.1185/030079907X167525

Meta-analysis Global Group in Chronic Heart, F. (2012). The survival of patients with heart failure with preserved or reduced left ventricular ejection fraction: an individual patient data meta-analysis. *Eur Heart J, 33*(14), 1750-1757. doi:10.1093/eurheartj/ehr254

Mikhed, Y., Daiber, A., & Steven, S. (2015). Mitochondrial Oxidative Stress, Mitochondrial DNA Damage and Their Role in Age-Related Vascular Dysfunction. *Int J Mol Sci, 16*(7), 15918-15953. doi:10.3390/ijms160715918

Mishima, T., Tanimura, M., Suzuki, G., Todor, A., Sharov, V. G., Goldstein, S., & Sabbah, H. N. (2000). Effects of long-term therapy with bosentan on the progression of left ventricular dysfunction and remodeling in dogs with heart failure. *J Am Coll Cardiol, 35*(1), 222-229. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10636284

Mishra, P. K., Givvimani, S., Chavali, V., & Tyagi, S. C. (2013). Cardiac matrix: a clue for future therapy. *Biochim Biophys Acta, 1832*(12), 2271-2276. doi:10.1016/j.bbadis.2013.09.004

Miwa, S., & Brand, M. D. (2003). Mitochondrial matrix reactive oxygen species production is very sensitive to mild uncoupling. *Biochem Soc Trans, 31*(Pt 6), 1300-1301. doi:10.1042/

Miyauchi, T., Yanagisawa, M., Tomizawa, T., Sugishita, Y., Suzuki, N., Fujino, M., . . . Masaki, T. (1989). Increased plasma concentrations of endothelin-1 and big

endothelin-1 in acute myocardial infarction. *Lancet, 2*(8653), 53-54. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2567834

Mjos, O. D. (1971). Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs. *J Clin Invest, 50*(7), 1386-1389. doi:10.1172/JCI106621

Molica, F., Burger, F., Thomas, A., Staub, C., Tailleux, A., Staels, B., . . . Steffens, S. (2013). Endogenous cannabinoid receptor CB1 activation promotes vascular smooth-muscle cell proliferation and neointima formation. *J Lipid Res, 54*(5), 1360-1368. doi:10.1194/jlr.M035147

Mollova, M., Bersell, K., Walsh, S., Savla, J., Das, L. T., Park, S. Y., . . . Kuhn, B. (2013). Cardiomyocyte proliferation contributes to heart growth in young humans. *Proc Natl Acad Sci U S A, 110*(4), 1446-1451. doi:10.1073/pnas.1214608110

Moore, W. M., Webber, R. K., Jerome, G. M., Tjoeng, F. S., Misko, T. P., & Currie, M. G. (1994). L-N6-(1-iminoethyl)lysine: a selective inhibitor of inducible nitric oxide synthase. *J Med Chem, 37*(23), 3886-3888. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7525961

Moreira, F. A., Aguiar, D. C., & Guimaraes, F. S. (2007). Anxiolytic-like effect of cannabinoids injected into the rat dorsolateral periaqueductal gray. *Neuropharmacology, 52*(3), 958-965. doi:10.1016/j.neuropharm.2006.10.013

Moreira, F. A., Grieb, M., & Lutz, B. (2009). Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. *Best Pract Res Clin Endocrinol Metab, 23*(1), 133-144. doi:10.1016/j.beem.2008.09.003

Morena, M., De Castro, V., Gray, J. M., Palmery, M., Trezza, V., Roozendaal, B., . . . Campolongo, P. (2015). Training-Associated Emotional Arousal Shapes Endocannabinoid Modulation of Spatial Memory Retrieval in Rats. *J Neurosci, 35*(41), 13962-13974. doi:10.1523/JNEUROSCI.1983-15.2015

Morgan, H. E., Gordon, E. E., Kira, Y., Chua, H. L., Russo, L. A., Peterson, C. J., . . . Watson, P. A. (1987). Biochemical mechanisms of cardiac hypertrophy. *Annu Rev Physiol, 49*, 533-543. doi:10.1146/annurev.ph.49.030187.002533

Morrow, V. A., Foufelle, F., Connell, J. M., Petrie, J. R., Gould, G. W., & Salt, I. P. (2003). Direct activation of AMP-activated protein kinase stimulates nitric-oxide synthesis in human aortic endothelial cells. *J Biol Chem, 278*(34), 31629-31639. doi:10.1074/jbc.M212831200

Moss, R. L., & Fitzsimons, D. P. (2002). Frank-Starling relationship: long on importance, short on mechanism. *Circ Res, 90*(1), 11-13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11786511

Mount, P. F., Kemp, B. E., & Power, D. A. (2007). Regulation of endothelial and myocardial NO synthesis by multi-site eNOS phosphorylation. *J Mol Cell Cardiol, 42*(2), 271-279. doi:10.1016/j.yjmcc.2006.05.023

Mukhopadhyay, P., Batkai, S., Rajesh, M., Czifra, N., Harvey-White, J., Hasko, G., . . . Pacher, P. (2007). Pharmacological inhibition of CB1 cannabinoid receptor protects against doxorubicin-induced cardiotoxicity. *J Am Coll Cardiol, 50*(6), 528-536. doi:10.1016/j.jacc.2007.03.057

Mukhopadhyay, P., Rajesh, M., Batkai, S., Patel, V., Kashiwaya, Y., Liaudet, L., . . . Pacher, P. (2010). CB1 cannabinoid receptors promote oxidative stress and cell

death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. *Cardiovasc Res, 85*(4), 773-784. doi:10.1093/cvr/cvp369

Mulder, P., Richard, V., Derumeaux, G., Hogie, M., Henry, J. P., Lallemand, F., . . . Thuillez, C. (1997). Role of endogenous endothelin in chronic heart failure: effect of long-term treatment with an endothelin antagonist on survival, hemodynamics, and cardiac remodeling. *Circulation, 96*(6), 1976-1982. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9323089

Mungrue, I. N., Gros, R., You, X., Pirani, A., Azad, A., Csont, T., . . . Husain, M. (2002). Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death. *J Clin Invest, 109*(6), 735-743. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11901182

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature, 365*(6441), 61-65. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7689702

Nadal-Ginard, B., Kajstura, J., Leri, A., & Anversa, P. (2003). Myocyte death, growth, and regeneration in cardiac hypertrophy and failure. *Circ Res, 92*(2), 139-150. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12574141

Neglia, D., De Caterina, A., Marraccini, P., Natali, A., Ciardetti, M., Vecoli, C., . . . Recchia, F. A. (2007). Impaired myocardial metabolic reserve and substrate selection flexibility during stress in patients with idiopathic dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol, 293*(6), H3270-3278. doi:10.1152/ajpheart.00887.2007

Nicholls, D. G., Bernson, V. S., & Heaton, G. M. (1978). The identification of the component in the inner membrane of brown adipose tissue mitochondria responsible for regulating energy dissipation. *Experientia Suppl, 32*, 89-93. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/348493

Niederhoffer, N., Hansen, H. H., Fernandez-Ruiz, J. J., & Szabo, B. (2001). Effects of cannabinoids on adrenaline release from adrenal medullary cells. *Br J Pharmacol, 134*(6), 1319-1327. doi:10.1038/sj.bjp.0704359

Nishida, M., Tanabe, S., Maruyama, Y., Mangmool, S., Urayama, K., Nagamatsu, Y., . . . Kurose, H. (2005). G alpha 12/13- and reactive oxygen species-dependent activation of c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase by angiotensin receptor stimulation in rat neonatal cardiomyocytes. *J Biol Chem, 280*(18), 18434-18441. doi:10.1074/jbc.M409710200

Nissen, S. E., Nicholls, S. J., Wolski, K., Rodes-Cabau, J., Cannon, C. P., Deanfield, J. E., . . . Investigators, S. (2008). Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. *JAMA, 299*(13), 1547-1560. doi:10.1001/jama.299.13.1547

Nithipatikom, K., Gomez-Granados, A. D., Tang, A. T., Pfeiffer, A. W., Williams, C. L., & Campbell, W. B. (2012). Cannabinoid receptor type 1 (CB1) activation inhibits small GTPase RhoA activity and regulates motility of prostate carcinoma cells. *Endocrinology, 153*(1), 29-41. doi:10.1210/en.2011-1144

O'Brien, L. D., Limebeer, C. L., Rock, E. M., Bottegoni, G., Piomelli, D., & Parker, L. A. (2013). Anandamide transport inhibition by ARN272 attenuates nausea-induced behaviour in rats, and vomiting in shrews (Suncus murinus). *Br J Pharmacol, 170*(5), 1130-1136. doi:10.1111/bph.12360

O'Leary, D. H., Reuwer, A. Q., Nissen, S. E., Despres, J. P., Deanfield, J. E., Brown, M. W., . . . investigators, A. (2011). Effect of rimonabant on carotid intima-media thickness (CIMT) progression in patients with abdominal obesity and metabolic syndrome: the AUDITOR Trial. *Heart, 97*(14), 1143-1150. doi:10.1136/hrt.2011.223446

Ojamaa, K., Petrie, J. F., Balkman, C., Hong, C., & Klein, I. (1994). Posttranscriptional modification of myosin heavy-chain gene expression in the hypertrophied rat myocardium. *Proc Natl Acad Sci U S A, 91*(8), 3468-3472. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8159771

Oka, T., Akazawa, H., Naito, A. T., & Komuro, I. (2014). Angiogenesis and cardiac hypertrophy: maintenance of cardiac function and causative roles in heart failure. *Circ Res, 114*(3), 565-571. doi:10.1161/CIRCRESAHA.114.300507

Okin, P. M., Wachtell, K., Devereux, R. B., Harris, K. E., Jern, S., Kjeldsen, S. E., . . . Dahlof, B. (2006). Regression of electrocardiographic left ventricular hypertrophy and decreased incidence of new-onset atrial fibrillation in patients with hypertension. *JAMA, 296*(10), 1242-1248. doi:10.1001/jama.296.10.1242

Okoshi, M. P., Yan, X., Okoshi, K., Nakayama, M., Schuldt, A. J., O'Connell, T. D., . . . Lorell, B. H. (2004). Aldosterone directly stimulates cardiac myocyte hypertrophy. *J Card Fail, 10*(6), 511-518. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15599842

Olowe, Y., & Schulz, H. (1980). Regulation of thiolases from pig heart. Control of fatty acid oxidation in heart. *Eur J Biochem, 109*(2), 425-429. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6105961

Onaivi, E. S., Carpio, O., Ishiguro, H., Schanz, N., Uhl, G. R., & Benno, R. (2008). Behavioral effects of CB2 cannabinoid receptor activation and its influence on food and alcohol consumption. *Ann N Y Acad Sci, 1139*, 426-433. doi:10.1196/annals.1432.035

Opie, L. H. (1968). Metabolism of the heart in health and disease. I. *Am Heart J, 76*(5), 685-698. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4235250

Orrenius, S., Gogvadze, V., & Zhivotovsky, B. (2007). Mitochondrial oxidative stress: implications for cell death. *Annu Rev Pharmacol Toxicol, 47*, 143-183. doi:10.1146/annurev.pharmtox.47.120505.105122

Ortega-Gutierrez, S., Molina-Holgado, E., & Guaza, C. (2005). Effect of anandamide uptake inhibition in the production of nitric oxide and in the release of cytokines in astrocyte cultures. *Glia, 52*(2), 163-168. doi:10.1002/glia.20229

Osei-Hyiaman, D., DePetrillo, M., Pacher, P., Liu, J., Radaeva, S., Batkai, S., . . . Kunos, G. (2005). Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest, 115*(5), 1298-1305. doi:10.1172/JCI23057

Owan, T. E., Hodge, D. O., Herges, R. M., Jacobsen, S. J., Roger, V. L., & Redfield, M. M. (2006). Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med, 355*(3), 251-259. doi:10.1056/NEJMoa052256

Pacher, P., Batkai, S., & Kunos, G. (2004). Haemodynamic profile and responsiveness to anandamide of TRPV1 receptor knock-out mice. *J Physiol, 558*(Pt 2), 647-657. doi:10.1113/jphysiol.2004.064824

Pacher, P., Batkai, S., & Kunos, G. (2006). The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev, 58*(3), 389-462. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16968947

Packer, M., Bristow, M. R., Cohn, J. N., Colucci, W. S., Fowler, M. B., Gilbert, E. M., & Shusterman, N. H. (1996). The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. U.S. Carvedilol Heart Failure Study Group. *N Engl J Med, 334*(21), 1349-1355. doi:10.1056/NEJM199605233342101

Palazuelos, J., Aguado, T., Egia, A., Mechoulam, R., Guzman, M., & Galve-Roperh, I. (2006). Non-psychoactive CB2 cannabinoid agonists stimulate neural progenitor proliferation. *FASEB J, 20*(13), 2405-2407. doi:10.1096/fj.06-6164fje

Parker, P., Patterson, J., & Johnson, J. (2005). *Pharmacotherapy. A Pathophysiologic Approach: Heart Failure.* (Sixth ed.): The McGraw-Hill Companies, Inc.

Patel, T. B., & Olson, M. S. (1984). Regulation of pyruvate dehydrogenase complex in ischemic rat heart. *Am J Physiol, 246*(6 Pt 2), H858-864. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6742152

Paulus, W. J., Tschope, C., Sanderson, J. E., Rusconi, C., Flachskampf, F. A., Rademakers, F. E., . . . Brutsaert, D. L. (2007). How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. *Eur Heart J, 28*(20), 2539-2550. doi:10.1093/eurheartj/ehm037

Pelliccia, A., Maron, B. J., De Luca, R., Di Paolo, F. M., Spataro, A., & Culasso, F. (2002). Remodeling of left ventricular hypertrophy in elite athletes after long-term deconditioning. *Circulation, 105*(8), 944-949. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11864923

Peng, T. I., & Jou, M. J. (2010). Oxidative stress caused by mitochondrial calcium overload. *Ann N Y Acad Sci, 1201*, 183-188. doi:10.1111/j.1749-6632.2010.05634.x

Pertwee, R. G. (1999). Pharmacology of cannabinoid receptor ligands. *Curr Med Chem, 6*(8), 635-664. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10469884

Pertwee, R. G. (2006). The pharmacology of cannabinoid receptors and their ligands: an overview. *Int J Obes (Lond), 30 Suppl 1*, S13-18. doi:10.1038/sj.ijo.0803272

Pertwee, R. G. (2014). Elevating endocannabinoid levels: pharmacological strategies and potential therapeutic applications. *Proc Nutr Soc, 73*(1), 96-105. doi:10.1017/S0029665113003649

Perwitz, N., Wenzel, J., Wagner, I., Buning, J., Drenckhan, M., Zarse, K., . . . Klein, J. (2010). Cannabinoid type 1 receptor blockade induces transdifferentiation towards a brown fat phenotype in white adipocytes. *Diabetes Obes Metab, 12*(2), 158-166. doi:10.1111/j.1463-1326.2009.01133.x

Petronilli, V., Miotto, G., Canton, M., Brini, M., Colonna, R., Bernardi, P., & Di Lisa, F. (1999). Transient and long-lasting openings of the mitochondrial permeability transition pore can be monitored directly in intact cells by changes in

mitochondrial calcein fluorescence. *Biophys J, 76*(2), 725-734. doi:10.1016/S0006-3495(99)77239-5

Pham, T., Loiselle, D., Power, A., & Hickey, A. J. (2014). Mitochondrial inefficiencies and anoxic ATP hydrolysis capacities in diabetic rat heart. *Am J Physiol Cell Physiol, 307*(6), C499-507. doi:10.1152/ajpcell.00006.2014

Pi-Sunyer, F. X., Aronne, L. J., Heshmati, H. M., Devin, J., Rosenstock, J., & Group, R. I.-N. A. S. (2006). Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *JAMA, 295*(7), 761-775. doi:10.1001/jama.295.7.761

Piomelli, D. (2003). The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci, 4*(11), 873-884. doi:10.1038/nrn1247

Preston, C. C., Oberlin, A. S., Holmuhamedov, E. L., Gupta, A., Sagar, S., Syed, R. H., . . . Jahangir, A. (2008). Aging-induced alterations in gene transcripts and functional activity of mitochondrial oxidative phosphorylation complexes in the heart. *Mech Ageing Dev, 129*(6), 304-312. doi:10.1016/j.mad.2008.02.010

Price, D. A., Martinez, A. A., Seillier, A., Koek, W., Acosta, Y., Fernandez, E., . . . Giuffrida, A. (2009). WIN55,212-2, a cannabinoid receptor agonist, protects against nigrostriatal cell loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Eur J Neurosci, 29*(11), 2177-2186. doi:10.1111/j.1460-9568.2009.06764.x

Public Health Agency of Canada. (2009). Tracking Heart Disease and Stroke in Canada. Retrieved from http://www.phac-aspc.gc.ca/publicat/2009/cvd-avc/pdf/cvd-avs-2009-eng.pdf

Public Health Agency of Canada. (2012). Cardiovascular Disease-Economic Burden of Illness.

Quarta, C., Bellocchio, L., Mancini, G., Mazza, R., Cervino, C., Braulke, L. J., . . . Pagotto, U. (2010). CB(1) signaling in forebrain and sympathetic neurons is a key determinant of endocannabinoid actions on energy balance. *Cell Metab, 11*(4), 273-285. doi:10.1016/j.cmet.2010.02.015

Quentin, T., Kitz, J., Steinmetz, M., Poppe, A., Bar, K., & Kratzner, R. (2011). Different expression of the catalytic alpha subunits of the AMP activated protein kinase--an immunohistochemical study in human tissue. *Histol Histopathol, 26*(5), 589-596. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21432774

Radulescu, D., Stoicescu, L., Buzdugan, E., & Donca, V. (2013). Patterns of left ventricular remodeling among patients with essential and secondary hypertension. *Rev Med Chil, 141*(12), 1520-1527. doi:10.4067/S0034-98872013001200004

Rajesh, M., Batkai, S., Kechrid, M., Mukhopadhyay, P., Lee, W. S., Horvath, B., . . . Pacher, P. (2012). Cannabinoid 1 receptor promotes cardiac dysfunction, oxidative stress, inflammation, and fibrosis in diabetic cardiomyopathy. *Diabetes, 61*(3), 716-727. doi:10.2337/db11-0477

Rajesh, M., Mukhopadhyay, P., Batkai, S., Hasko, G., Liaudet, L., Huffman, J. W., . . . Pacher, P. (2007). CB2-receptor stimulation attenuates TNF-alpha-induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. *Am J Physiol Heart Circ Physiol, 293*(4), H2210-2218. doi:10.1152/ajpheart.00688.2007

Rajesh, M., Mukhopadhyay, P., Hasko, G., Huffman, J. W., Mackie, K., & Pacher, P. (2008). CB2 cannabinoid receptor agonists attenuate TNF-alpha-induced human vascular smooth muscle cell proliferation and migration. *Br J Pharmacol, 153*(2), 347-357. doi:10.1038/sj.bjp.0707569

Rajesh, M., Mukhopadhyay, P., Hasko, G., Liaudet, L., Mackie, K., & Pacher, P. (2010). Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. *Br J Pharmacol, 160*(3), 688-700. doi:10.1111/j.1476-5381.2010.00712.x

Rajesh, M., Mukhopadhyay, P., Hasko, G., & Pacher, P. (2008). Cannabinoid CB1 receptor inhibition decreases vascular smooth muscle migration and proliferation. *Biochem Biophys Res Commun, 377*(4), 1248-1252. doi:10.1016/j.bbrc.2008.10.159

Ramalingam, M., & Kim, S. J. (2012). Reactive oxygen/nitrogen species and their functional correlations in neurodegenerative diseases. *J Neural Transm (Vienna), 119*(8), 891-910. doi:10.1007/s00702-011-0758-7

Ramirez, B. G., Blazquez, C., Gomez del Pulgar, T., Guzman, M., & de Ceballos, M. L. (2005). Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci, 25*(8), 1904-1913. doi:10.1523/JNEUROSCI.4540-04.2005

Randall, M. D., & Kendall, D. A. (1998). Endocannabinoids: a new class of vasoactive substances. *Trends Pharmacol Sci, 19*(2), 55-58. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9550942

Randle, P. J., Garland, P. B., Hales, C. N., & Newsholme, E. A. (1963). The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet, 1*(7285), 785-789. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/13990765

Rees, D. D., Palmer, R. M., Schulz, R., Hodson, H. F., & Moncada, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br J Pharmacol, 101*(3), 746-752. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1706208

Reisner, Y., Meiry, G., Zeevi-Levin, N., Barac, D. Y., Reiter, I., Abassi, Z., . . . Binah, O. (2009). Impulse conduction and gap junctional remodelling by endothelin-1 in cultured neonatal rat ventricular myocytes. *J Cell Mol Med, 13*(3), 562-573. doi:10.1111/j.1582-4934.2008.00361.x

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Alonso, R., Shire, D., Congy, C., . . . Le Fur, G. (1995). Biochemical and pharmacological characterisation of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. *Life Sci, 56*(23-24), 1941-1947. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7776817

Rinaldi-Carmona, M., Barth, F., Millan, J., Derocq, J. M., Casellas, P., Congy, C., . . . Le Fur, G. L. (1998). SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J Pharmacol Exp Ther, 284*(2), 644-650. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9454810

Romano, M. R., & Lograno, M. D. (2006). Cannabinoid agonists induce relaxation in the bovine ophthalmic artery: evidences for CB1 receptors, nitric oxide and potassium channels. *Br J Pharmacol, 147*(8), 917-925. doi:10.1038/sj.bjp.0706687

Romero, T. R. L., Resende, L. C., Guzzo, L. S., & Duarte, I. D. G. (2013). CB1 and CB2 cannabinoid receptor agonists induce peripheral antinociception by activation of the endogenous noradrenergic system. *Anesth Analg., 116*(2), 463-472.

Rosca, M. G., Tandler, B., & Hoppel, C. L. (2013). Mitochondria in cardiac hypertrophy and heart failure. *J Mol Cell Cardiol, 55*, 31-41. doi:10.1016/j.yjmcc.2012.09.002

Rose, B. A., Force, T., & Wang, Y. (2010). Mitogen-activated protein kinase signaling in the heart: angels versus demons in a heart-breaking tale. *Physiol Rev, 90*(4), 1507-1546. doi:10.1152/physrev.00054.2009

Ross, H., Howlett, J., Arnold, J. M., Liu, P., O'Neill, B. J., Brophy, J. M., . . . Canadian Cardiovascular Society Access to Care Working, G. (2006). Treating the right patient at the right time: access to heart failure care. *Can J Cardiol, 22*(9), 749-754. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16835668

Ross, R. A. (2003). Anandamide and vanilloid TRPV1 receptors. *Br J Pharmacol, 140*(5), 790-801. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14517174

Ross, R. A., Brockie, H. C., Stevenson, L. A., Murphy, V. L., Templeton, F., Makriyannis, A., & Pertwee, R. G. (1999). Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656, and AM630. *Br J Pharmacol, 126*(3), 665-672. doi:10.1038/sj.bjp.0702351

Rouzer, C. A., & Marnett, L. J. (2011). Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chem Rev, 111*(10), 5899-5921. doi:10.1021/cr2002799

Roy Chowdhury, S. K., Smith, D. R., Saleh, A., Schapansky, J., Marquez, A., Gomes, S., . . . Fernyhough, P. (2012). Impaired adenosine monophosphate-activated protein kinase signalling in dorsal root ganglia neurons is linked to mitochondrial dysfunction and peripheral neuropathy in diabetes. *Brain, 135*(Pt 6), 1751-1766. doi:10.1093/brain/aws097

Rubino, T., Guidali, C., Vigano, D., Realini, N., Valenti, M., Massi, P., & Parolaro, D. (2008). CB1 receptor stimulation in specific brain areas differently modulate anxiety-related behaviour. *Neuropharmacology, 54*(1), 151-160. doi:10.1016/j.neuropharm.2007.06.024

Rumberger, J. A., Behrenbeck, T., Breen, J. R., Reed, J. E., & Gersh, B. J. (1993). Nonparallel changes in global left ventricular chamber volume and muscle mass during the first year after transmural myocardial infarction in humans. *J Am Coll Cardiol, 21*(3), 673-682. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8436749

Ryberg, E., Larsson, N., Sjogren, S., Hjorth, S., Hermansson, N. O., Leonova, J., . . . Greasley, P. J. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol, 152*(7), 1092-1101. doi:10.1038/sj.bjp.0707460

Saario, S. M., Savinainen, J. R., Laitinen, J. T., Jarvinen, T., & Niemi, R. (2004). Monoglyceride lipase-like enzymatic activity is responsible for hydrolysis of 2-arachidonoylglycerol in rat cerebellar membranes. *Biochem Pharmacol, 67*(7), 1381-1387. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15013854

Sack, M. N., Disch, D. L., Rockman, H. A., & Kelly, D. P. (1997). A role for Sp and nuclear receptor transcription factors in a cardiac hypertrophic growth program. *Proc Natl Acad Sci U S A, 94*(12), 6438-6443. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9177236

Sadoshima, J., & Izumo, S. (1993). Molecular characterization of angiotensin II--induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT1 receptor subtype. *Circ Res, 73*(3), 413-423. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8348686

Sadoshima, J., Xu, Y., Slayter, H. S., & Izumo, S. (1993). Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell, 75*(5), 977-984. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8252633

Sagredo, O., Gonzalez, S., Aroyo, I., Pazos, M. R., Benito, C., Lastres-Becker, I., . . . Fernandez-Ruiz, J. (2009). Cannabinoid CB2 receptor agonists protect the striatum against malonate toxicity: relevance for Huntington's disease. *Glia, 57*(11), 1154-1167. doi:10.1002/glia.20838

Sakai, S., Miyauchi, T., Kobayashi, M., Yamaguchi, I., Goto, K., & Sugishita, Y. (1996). Inhibition of myocardial endothelin pathway improves long-term survival in heart failure. *Nature, 384*(6607), 353-355. doi:10.1038/384353a0

Sakamoto, K., Zarrinpashneh, E., Budas, G. R., Pouleur, A. C., Dutta, A., Prescott, A. R., . . . Bertrand, L. (2006). Deficiency of LKB1 in heart prevents ischemia-mediated activation of AMPKalpha2 but not AMPKalpha1. *Am J Physiol Endocrinol Metab, 290*(5), E780-788. doi:10.1152/ajpendo.00443.2005

Salminen, A., & Kaarniranta, K. (2012). AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. *Ageing Res Rev, 11*(2), 230-241. doi:10.1016/j.arr.2011.12.005

Salt, I., Celler, J. W., Hawley, S. A., Prescott, A., Woods, A., Carling, D., & Hardie, D. G. (1998). AMP-activated protein kinase: greater AMP dependence, and preferential nuclear localization, of complexes containing the alpha2 isoform. *Biochem J, 334 (Pt 1)*, 177-187. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9693118

Sam, F., Sawyer, D. B., Xie, Z., Chang, D. L., Ngoy, S., Brenner, D. A., . . . Colucci, W. S. (2001). Mice lacking inducible nitric oxide synthase have improved left ventricular contractile function and reduced apoptotic cell death late after myocardial infarction. *Circ Res, 89*(4), 351-356. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11509452

Sanchez Lopez, A. J., Roman-Vega, L., Ramil Tojeiro, E., Giuffrida, A., & Garcia-Merino, A. (2015). Regulation of cannabinoid receptor gene expression and endocannabinoid levels in lymphocyte subsets by interferon-beta: a longitudinal study in multiple sclerosis patients. *Clin Exp Immunol, 179*(1), 119-127. doi:10.1111/cei.12443

Sano, M., Wang, S. C., Shirai, M., Scaglia, F., Xie, M., Sakai, S., . . . Schneider, M. D. (2004). Activation of cardiac Cdk9 represses PGC-1 and confers a predisposition to heart failure. *EMBO J, 23*(17), 3559-3569. doi:10.1038/sj.emboj.7600351

Sawyer, D. B., Siwik, D. A., Xiao, L., Pimentel, D. R., Singh, K., & Colucci, W. S. (2002). Role of oxidative stress in myocardial hypertrophy and failure. *J Mol Cell Cardiol, 34*(4), 379-388. doi:10.1006/jmcc.2002.1526

Scantlebury, D. C., & Borlaug, B. A. (2011). Why are women more likely than men to develop heart failure with preserved ejection fraction? *Curr Opin Cardiol, 26*(6), 562-568. doi:10.1097/HCO.0b013e32834b7faf

Schannwell, C. M., Zimmermann, T., Schneppenheim, M., Plehn, G., Marx, R., & Strauer, B. E. (2002). Left ventricular hypertrophy and diastolic dysfunction in healthy pregnant women. *Cardiology, 97*(2), 73-78. doi:57675

Scheen, A. J., Finer, N., Hollander, P., Jensen, M. D., Van Gaal, L. F., & Group, R. I.-D. S. (2006). Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet, 368*(9548), 1660-1672. doi:10.1016/S0140-6736(06)69571-8

Schmid, P. C., Krebsbach, R. J., Perry, S. R., Dettmer, T. M., Maasson, J. L., & Schmid, H. H. (1995). Occurrence and postmortem generation of anandamide and other long-chain N-acylethanolamines in mammalian brain. *FEBS Lett, 375*(1-2), 117-120. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7498458

Schonekess, B. O., Allard, M. F., & Lopaschuk, G. D. (1995). Propionyl L-carnitine improvement of hypertrophied rat heart function is associated with an increase in cardiac efficiency. *Eur J Pharmacol, 286*(2), 155-166. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8605952

Schrauwen, P., & Hesselink, M. (2002). UCP2 and UCP3 in muscle controlling body metabolism. *J Exp Biol, 205*(Pt 15), 2275-2285. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12110661

Schwarzer, M., Faerber, G., Rueckauer, T., Blum, D., Pytel, G., Mohr, F. W., & Doenst, T. (2009). The metabolic modulators, Etomoxir and NVP-LAB121, fail to reverse pressure overload induced heart failure in vivo. *Basic Res Cardiol, 104*(5), 547-557. doi:10.1007/s00395-009-0015-5

Seymour, A. M., & Chatham, J. C. (1997). The effects of hypertrophy and diabetes on cardiac pyruvate dehydrogenase activity. *J Mol Cell Cardiol, 29*(10), 2771-2778. doi:10.1006/jmcc.1997.0512

Shah, R. V., Desai, A. S., & Givertz, M. M. (2010). The effect of renin-angiotensin system inhibitors on mortality and heart failure hospitalization in patients with heart failure and preserved ejection fraction: a systematic review and meta-analysis. *J Card Fail, 16*(3), 260-267. doi:10.1016/j.cardfail.2009.11.007

Shaheen, M., Cheema, Y., Shahbaz, A. U., Bhattacharya, S. K., & Weber, K. T. (2011). Intracellular calcium overloading and oxidative stress in cardiomyocyte necrosis via a mitochondriocentric signal-transducer-effector pathway. *Exp Clin Cardiol, 16*(4), 109-115. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22131852

Sharkey, K. A., Darmani, N. A., & Parker, L. A. (2014). Regulation of nausea and vomiting by cannabinoids and the endocannabinoid system. *Eur J Pharmacol, 722*, 134-146. doi:10.1016/j.ejphar.2013.09.068

Shen, M., & Thayer, S. A. (1998). The cannabinoid agonist Win55,212-2 inhibits calcium channels by receptor-mediated and direct pathways in cultured rat hippocampal neurons. *Brain Res, 783*(1), 77-84. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9479052

Shin, S. M., & Kim, S. G. (2009). Inhibition of arachidonic acid and iron-induced mitochondrial dysfunction and apoptosis by oltipraz and novel 1,2-dithiole-3-thione congeners. *Mol Pharmacol, 75*(1), 242-253. doi:10.1124/mol.108.051128

Showalter, V. M., Compton, D. R., Martin, B. R., & Abood, M. E. (1996). Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *J Pharmacol Exp Ther, 278*(3), 989-999. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8819477

Siddiqi, N., Singh, S., Beadle, R., Dawson, D., & Frenneaux, M. (2013). Cardiac metabolism in hypertrophy and heart failure: implications for therapy. *Heart Fail Rev, 18*(5), 595-606. doi:10.1007/s10741-012-9359-2

Siegmund, S. V., Qian, T., de Minicis, S., Harvey-White, J., Kunos, G., Vinod, K. Y., . . . Schwabe, R. F. (2007). The endocannabinoid 2-arachidonoyl glycerol induces death of hepatic stellate cells via mitochondrial reactive oxygen species. *FASEB J, 21*(11), 2798-2806. doi:10.1096/fj.06-7717com

Simpson, P. (1983). Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an alpha 1 adrenergic response. *J Clin Invest, 72*(2), 732-738. doi:10.1172/JCI111023

Sinha, K., Das, J., Pal, P. B., & Sil, P. C. (2013). Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. *Arch Toxicol, 87*(7), 1157-1180. doi:10.1007/s00204-013-1034-4

Siwik, D. A., Pagano, P. J., & Colucci, W. S. (2001). Oxidative stress regulates collagen synthesis and matrix metalloproteinase activity in cardiac fibroblasts. *Am J Physiol Cell Physiol, 280*(1), C53-60. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11121376

Skaper, S. D., Buriani, A., Dal Toso, R., Petrelli, L., Romanello, S., Facci, L., & Leon, A. (1996). The ALIAmide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. *Proc Natl Acad Sci U S A, 93*(9), 3984-3989. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8633002

Sliwa, K., Hilfiker-Kleiner, D., Petrie, M. C., Mebazaa, A., Pieske, B., Buchmann, E., . . . Heart Failure Association of the European Society of Cardiology Working Group on Peripartum, C. (2010). Current state of knowledge on aetiology, diagnosis, management, and therapy of peripartum cardiomyopathy: a position statement from the Heart Failure Association of the European Society of Cardiology Working Group on peripartum cardiomyopathy. *Eur J Heart Fail, 12*(8), 767-778. doi:10.1093/eurjhf/hfq120

Smith, S. H., Kramer, M. F., Reis, I., Bishop, S. P., & Ingwall, J. S. (1990). Regional changes in creatine kinase and myocyte size in hypertensive and nonhypertensive cardiac hypertrophy. *Circ Res, 67*(6), 1334-1344. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2147129

Soderstrom, K., & Tian, Q. (2008). CB(1) cannabinoid receptor activation dose dependently modulates neuronal activity within caudal but not rostral song control regions of adult zebra finch telencephalon. *Psychopharmacology (Berl), 199*(2), 265-273. doi:10.1007/s00213-008-1190-z

Solomon, S. D., Claggett, B., Lewis, E. F., Desai, A., Anand, I., Sweitzer, N. K., . . . Investigators, T. (2015). Influence of ejection fraction on outcomes and efficacy

of spironolactone in patients with heart failure with preserved ejection fraction. *Eur Heart J*. doi:10.1093/eurheartj/ehv464

Souders, C. A., Bowers, S. L., & Baudino, T. A. (2009). Cardiac fibroblast: the renaissance cell. *Circ Res, 105*(12), 1164-1176. doi:10.1161/CIRCRESAHA.109.209809

Spach, M. S., & Boineau, J. P. (1997). Microfibrosis produces electrical load variations due to loss of side-to-side cell connections: a major mechanism of structural heart disease arrhythmias. *Pacing Clin Electrophysiol, 20*(2 Pt 2), 397-413. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9058844

Spierings, D., McStay, G., Saleh, M., Bender, C., Chipuk, J., Maurer, U., & Green, D. R. (2005). Connected to death: the (unexpurgated) mitochondrial pathway of apoptosis. *Science, 310*(5745), 66-67. doi:10.1126/science.1117105

Stanley, W. C. (2004). Myocardial energy metabolism during ischemia and the mechanisms of metabolic therapies. *J Cardiovasc Pharmacol Ther, 9 Suppl 1*, S31-45. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15378130

Stanley, W. C., Recchia, F. A., & Lopaschuk, G. D. (2005). Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev, 85*(3), 1093-1129. doi:10.1152/physrev.00006.2004

Stapleton, D., Mitchelhill, K. I., Gao, G., Widmer, J., Michell, B. J., Teh, T., . . . Kemp, B. E. (1996). Mammalian AMP-activated protein kinase subfamily. *J Biol Chem, 271*(2), 611-614. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8557660

Steffens, S., Veillard, N. R., Arnaud, C., Pelli, G., Burger, F., Staub, C., . . . Mach, F. (2005). Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature, 434*(7034), 782-786. doi:10.1038/nature03389

Steinberg, B. A., Zhao, X., Heidenreich, P. A., Peterson, E. D., Bhatt, D. L., Cannon, C. P., . . . Investigators. (2012). Trends in patients hospitalized with heart failure and preserved left ventricular ejection fraction: prevalence, therapies, and outcomes. *Circulation, 126*(1), 65-75. doi:10.1161/CIRCULATIONAHA.111.080770

Stella, N., Schweitzer, P., & Piomelli, D. (1997). A second endogenous cannabinoid that modulates long-term potentiation. *Nature, 388*(6644), 773-778. doi:10.1038/42015

Stewart, D. J., Kubac, G., Costello, K. B., & Cernacek, P. (1991). Increased plasma endothelin-1 in the early hours of acute myocardial infarction. *J Am Coll Cardiol, 18*(1), 38-43. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2050938

Storr, M., Keenan, C., Zhang, H., Patel, K., Makriyannis, A., & Sharkey, K. (2009). Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. *Inflamm Bowel Dis, 15*(11), 1678-1685.

Storr, M. A., Keenan, C. M., Zhang, H., Patel, K. D., Makriyannis, A., & Sharkey, K. A. (2009). Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. *Inflamm Bowel Dis, 15*(11), 1678-1685. doi:10.1002/ibd.20960

Stuck, B. J., Lenski, M., Bohm, M., & Laufs, U. (2008). Metabolic switch and hypertrophy of cardiomyocytes following treatment with angiotensin II are prevented by AMP-activated protein kinase. *J Biol Chem, 283*(47), 32562-32569. doi:10.1074/jbc.M801904200

Sugamura, K., Sugiyama, S., Fujiwara, Y., Matsubara, J., Akiyama, E., Maeda, H., . . . Ogawa, H. (2010). Cannabinoid 1 receptor blockade reduces atherosclerosis with

enhances reverse cholesterol transport. *J Atheroscler Thromb, 17*(2), 141-147. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20124735

Sugamura, K., Sugiyama, S., Nozaki, T., Matsuzawa, Y., Izumiya, Y., Miyata, K., . . . Ogawa, H. (2009). Activated endocannabinoid system in coronary artery disease and antiinflammatory effects of cannabinoid 1 receptor blockade on macrophages. *Circulation, 119*(1), 28-36. doi:10.1161/CIRCULATIONAHA.108.811992

Sugden, P. H. (2003). Ras, Akt, and mechanotransduction in the cardiac myocyte. *Circ Res, 93*(12), 1179-1192. doi:10.1161/01.RES.0000106132.04301.F5

Sugden, P. H., & Clerk, A. (2005). Endothelin signalling in the cardiac myocyte and its pathophysiological relevance. *Curr Vasc Pharmacol, 3*(4), 343-351. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16248777

Sugiura, T., Kondo, S., Sukagawa, A., Tonegawa, T., Nakane, S., Yamashita, A., . . . Waku, K. (1996). Transacylase-mediated and phosphodiesterase-mediated synthesis of N-arachidonoylethanolamine, an endogenous cannabinoid-receptor ligand, in rat brain microsomes. Comparison with synthesis from free arachidonic acid and ethanolamine. *Eur J Biochem, 240*(1), 53-62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8797835

Sun, X., Kumar, S., Sharma, S., Aggarwal, S., Lu, Q., Gross, C., . . . Black, S. M. (2014). Endothelin-1 induces a glycolytic switch in pulmonary arterial endothelial cells via the mitochondrial translocation of endothelial nitric oxide synthase. *Am J Respir Cell Mol Biol, 50*(6), 1084-1095. doi:10.1165/rcmb.2013-0187OC

Sutton, M. G., & Sharpe, N. (2000). Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. *Circulation, 101*(25), 2981-2988. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10869273

Suzuki, T., Hoshi, H., & Mitsui, Y. (1990). Endothelin stimulates hypertrophy and contractility of neonatal rat cardiac myocytes in a serum-free medium. *FEBS Lett, 268*(1), 149-151. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2200708

Suzuki, T., Kumazaki, T., & Mitsui, Y. (1993). Endothelin-1 is produced and secreted by neonatal rat cardiac myocytes in vitro. *Biochem Biophys Res Commun, 191*(3), 823-830. doi:10.1006/bbrc.1993.1291

Taegtmeyer, H., & Overturf, M. L. (1988). Effects of moderate hypertension on cardiac function and metabolism in the rabbit. *Hypertension, 11*(5), 416-426. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3366475

Taegtmeyer, H., Sen, S., & Vela, D. (2010). Return to the fetal gene program: a suggested metabolic link to gene expression in the heart. *Ann N Y Acad Sci, 1188*, 191-198. doi:10.1111/j.1749-6632.2009.05100.x

Tam, J., Vemuri, V. K., Liu, J., Batkai, S., Mukhopadhyay, B., Godlewski, G., . . . Kunos, G. (2010). Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *J Clin Invest, 120*(8), 2953-2966. doi:10.1172/JCI42551

Tang, Y., Mi, C., Liu, J., Gao, F., & Long, J. (2014). Compromised mitochondrial remodeling in compensatory hypertrophied myocardium of spontaneously hypertensive rat. *Cardiovasc Pathol, 23*(2), 101-106. doi:10.1016/j.carpath.2013.11.002

Taylor, D., Bhandari, S., & Seymour, A. M. (2015). Mitochondrial dysfunction in uremic cardiomyopathy. *Am J Physiol Renal Physiol*, ajprenal 00442 02014. doi:10.1152/ajprenal.00442.2014

Tedesco, L., Valerio, A., Dossena, M., Cardile, A., Ragni, M., Pagano, C., . . . Nisoli, E. (2010). Cannabinoid receptor stimulation impairs mitochondrial biogenesis in mouse white adipose tissue, muscle, and liver: the role of eNOS, p38 MAPK, and AMPK pathways. *Diabetes, 59*(11), 2826-2836. doi:10.2337/db09-1881

Teshima, Y., Takahashi, N., Nishio, S., Saito, S., Kondo, H., Fukui, A., . . . Saikawa, T. (2014). Production of reactive oxygen species in the diabetic heart. Roles of mitochondria and NADPH oxidase. *Circ J, 78*(2), 300-306. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24334638

Thandapilly, S. J., Louis, X. L., Yang, T., Stringer, D. M., Yu, L., Zhang, S., . . . Netticadan, T. (2011). Resveratrol prevents norepinephrine induced hypertrophy in adult rat cardiomyocytes, by activating NO-AMPK pathway. *Eur J Pharmacol, 668*(1-2), 217-224. doi:10.1016/j.ejphar.2011.06.042

The SOLVD Investigators. (1992). Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N Engl J Med, 327*(10), 685-691. doi:10.1056/NEJM199209033271003

Thorburn, J., Frost, J. A., & Thorburn, A. (1994). Mitogen-activated protein kinases mediate changes in gene expression, but not cytoskeletal organization associated with cardiac muscle cell hypertrophy. *J Cell Biol, 126*(6), 1565-1572. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8089186

Tilley, D. G. (2011). G protein-dependent and G protein-independent signaling pathways and their impact on cardiac function. *Circ Res, 109*(2), 217-230. doi:10.1161/CIRCRESAHA.110.231225

Tiyerili, V., Zimmer, S., Jung, S., Wassmann, K., Naehle, C. P., Lutjohann, D., . . . Wassmann, S. (2010). CB1 receptor inhibition leads to decreased vascular AT1 receptor expression, inhibition of oxidative stress and improved endothelial function. *Basic Res Cardiol, 105*(4), 465-477. doi:10.1007/s00395-010-0090-7

Tong, L., Chuang, C. C., Wu, S., & Zuo, L. (2015). Reactive oxygen species in redox cancer therapy. *Cancer Lett, 367*(1), 18-25. doi:10.1016/j.canlet.2015.07.008

Toth, A., Blumberg, P. M., & Boczan, J. (2009). Anandamide and the vanilloid receptor (TRPV1). *Vitam Horm, 81*, 389-419. doi:10.1016/S0083-6729(09)81015-7

Tourino, C., Oveisi, F., Lockney, J., Piomelli, D., & Maldonado, R. (2010). FAAH deficiency promotes energy storage and enhances the motivation for food. *Int J Obes (Lond), 34*(3), 557-568. doi:10.1038/ijo.2009.262

Toyama, E. Q., Herzig, S., Courchet, J., Lewis, T. L., Jr., Loson, O. C., Hellberg, K., . . . Shaw, R. J. (2016). Metabolism. AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. *Science, 351*(6270), 275-281. doi:10.1126/science.aab4138

Tsao, C. W., Gona, P. N., Salton, C. J., Chuang, M. L., Levy, D., Manning, W. J., & O'Donnell, C. J. (2015). Left Ventricular Structure and Risk of Cardiovascular Events: A Framingham Heart Study Cardiac Magnetic Resonance Study. *J Am Heart Assoc, 4*(9), e002188. doi:10.1161/JAHA.115.002188

Tuunanen, H., Engblom, E., Naum, A., Nagren, K., Hesse, B., Airaksinen, K. E., . . . Knuuti, J. (2006). Free fatty acid depletion acutely decreases cardiac work and

efficiency in cardiomyopathic heart failure. *Circulation, 114*(20), 2130-2137. doi:10.1161/CIRCULATIONAHA.106.645184

Ueda, N., Yamanaka, K., & Yamamoto, S. (2001). Purification and characterization of an acid amidase selective for N-palmitoylethanolamine, a putative endogenous anti-inflammatory substance. *J Biol Chem, 276*(38), 35552-35557. doi:10.1074/jbc.M106261200

Underdown, N. J., Hiley, C. R., & Ford, W. R. (2005). Anandamide reduces infarct size in rat isolated hearts subjected to ischaemia-reperfusion by a novel cannabinoid mechanism. *Br J Pharmacol, 146*(6), 809-816. doi:10.1038/sj.bjp.0706391

Valdeolivas, S., Satta, V., Pertwee, R. G., Fernandez-Ruiz, J., & Sagredo, O. (2012). Sativex-like combination of phytocannabinoids is neuroprotective in malonate-lesioned rats, an inflammatory model of Huntington's disease: role of CB1 and CB2 receptors. *ACS Chem Neurosci, 3*(5), 400-406. doi:10.1021/cn200114w

Valk, P. J., & Delwel, R. (1998). The peripheral cannabinoid receptor, Cb2, in retrovirally-induced leukemic transformation and normal hematopoiesis. *Leuk Lymphoma, 32*(1-2), 29-43. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10036999

Van Der Stelt, M., & Di Marzo, V. (2004). Endovanilloids. Putative endogenous ligands of transient receptor potential vanilloid 1 channels. *Eur J Biochem, 271*(10), 1827-1834. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15128293

Van Gaal, L. F., Rissanen, A. M., Scheen, A. J., Ziegler, O., Rossner, S., & Group, R. I.-E. S. (2005). Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet, 365*(9468), 1389-1397. doi:10.1016/S0140-6736(05)66374-X

Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., . . . Sharkey, K. A. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science, 310*(5746), 329-332. doi:10.1126/science.1115740

Vannacci, A., Giannini, L., Passani, M. B., Di Felice, A., Pierpaoli, S., Zagli, G., . . . Mannaioni, P. F. (2004). The endocannabinoid 2-arachidonylglycerol decreases the immunological activation of Guinea pig mast cells: involvement of nitric oxide and eicosanoids. *J Pharmacol Exp Ther, 311*(1), 256-264. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15187170

Vasan, R. S., Wilson, P. W., Colucci, W. S., & Yeon, S. B. (2010). Epidemiology and causes of heart failure. *UpToDate*.

Ventura-Clapier, R., Garnier, A., & Veksler, V. (2008). Transcriptional control of mitochondrial biogenesis: the central role of PGC-1alpha. *Cardiovasc Res, 79*(2), 208-217. doi:10.1093/cvr/cvn098

Verdecchia, P., Carini, G., Circo, A., Dovellini, E., Giovannini, E., Lombardo, M., . . . Group, M. S. (2001). Left ventricular mass and cardiovascular morbidity in essential hypertension: the MAVI study. *J Am Coll Cardiol, 38*(7), 1829-1835. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11738281

Verty, A. N., Allen, A. M., & Oldfield, B. J. (2009). The effects of rimonabant on brown adipose tissue in rat: implications for energy expenditure. *Obesity (Silver Spring), 17*(2), 254-261. doi:10.1038/oby.2008.509

Verty, A. N., Stefanidis, A., McAinch, A. J., Hryciw, D. H., & Oldfield, B. (2015). Anti-Obesity Effect of the CB2 Receptor Agonist JWH-015 in Diet-Induced Obese Mice. *PLoS One, 10*(11), e0140592. doi:10.1371/journal.pone.0140592

Vijayakumar, R. S., Lin, Y., Shia, K. S., Yeh, Y. N., Hsieh, W. P., Hsiao, W. C., . . . Hung, M. S. (2012). Induction of fatty acid oxidation resists weight gain, ameliorates hepatic steatosis and reduces cardiometabolic risk factors. *Int J Obes (Lond), 36*(7), 999-1006. doi:10.1038/ijo.2011.171

Viveros, M. P., Marco, E. M., & File, S. E. (2005). Endocannabinoid system and stress and anxiety responses. *Pharmacol Biochem Behav, 81*(2), 331-342. doi:10.1016/j.pbb.2005.01.029

Vliegen, H. W., van der Laarse, A., Cornelisse, C. J., & Eulderink, F. (1991). Myocardial changes in pressure overload-induced left ventricular hypertrophy. A study on tissue composition, polyploidization and multinucleation. *Eur Heart J, 12*(4), 488-494. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1829680

Wachtell, K., Okin, P. M., Olsen, M. H., Dahlof, B., Devereux, R. B., Ibsen, H., . . . Thygesen, K. (2007). Regression of electrocardiographic left ventricular hypertrophy during antihypertensive therapy and reduction in sudden cardiac death: the LIFE Study. *Circulation, 116*(7), 700-705. doi:10.1161/CIRCULATIONAHA.106.666594

Wachter, R., & Edelmann, F. (2014). Diagnosis of heart failure with preserved ejection fraction. *Heart Fail Clin, 10*(3), 399-406. doi:10.1016/j.hfc.2014.04.010

Wada, H., Ivester, C. T., Carabello, B. A., Cooper, G. t., & McDermott, P. J. (1996). Translational initiation factor eIF-4E. A link between cardiac load and protein synthesis. *J Biol Chem, 271*(14), 8359-8364. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8626533

Wagner, J. A., Hu, K., Karcher, J., Bauersachs, J., Schafer, A., Laser, M., . . . Ertl, G. (2003). CB(1) cannabinoid receptor antagonism promotes remodeling and cannabinoid treatment prevents endothelial dysfunction and hypotension in rats with myocardial infarction. *Br J Pharmacol, 138*(7), 1251-1258. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12711625

Walker, J. M., Huang, S. M., Strangman, N. M., Tsou, K., & Sanudo-Pena, M. C. (1999). Pain modulation by release of the endogenous cannabinoid anandamide. *Proc Natl Acad Sci U S A, 96*(21), 12198-12203. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10518599

Wallace, H. W. (1959). Cardiac metabolism. *The New England Journal of Medicine, 261*, 1322-1324.

Wambolt, R. B., Henning, S. L., English, D. R., Bondy, G. P., & Allard, M. F. (1997). Regression of cardiac hypertrophy normalizes glucose metabolism and left ventricular function during reperfusion. *J Mol Cell Cardiol, 29*(3), 939-948. doi:10.1006/jmcc.1996.0336

Wambolt, R. B., Lopaschuk, G. D., Brownsey, R. W., & Allard, M. F. (2000). Dichloroacetate improves postischemic function of hypertrophied rat hearts. *J Am*

Coll Cardiol, 36(4), 1378-1385. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11028498

Wang, P. F., Jiang, L. S., Bu, J., Huang, X. J., Song, W., Du, Y. P., & He, B. (2012). Cannabinoid-2 receptor activation protects against infarct and ischemia-reperfusion heart injury. *J Cardiovasc Pharmacol, 59*(4), 301-307. doi:10.1097/FJC.0b013e3182418997

Wang, T. J., Evans, J. C., Benjamin, E. J., Levy, D., LeRoy, E. C., & Vasan, R. S. (2003). Natural history of asymptomatic left ventricular systolic dysfunction in the community. *Circulation, 108*(8), 977-982. doi:10.1161/01.CIR.0000085166.44904.79

Wang, Y., Ebermann, L., Sterner-Kock, A., Wika, S., Schultheiss, H. P., Dorner, A., & Walther, T. (2009). Myocardial overexpression of adenine nucleotide translocase 1 ameliorates diabetic cardiomyopathy in mice. *Exp Physiol, 94*(2), 220-227. doi:10.1113/expphysiol.2008.044800

Wang, Y., Ma, S., Wang, Q., Hu, W., Wang, D., Li, X., . . . Cao, F. (2014). Effects of cannabinoid receptor type 2 on endogenous myocardial regeneration by activating cardiac progenitor cells in mouse infarcted heart. *Sci China Life Sci, 57*(2), 201-208. doi:10.1007/s11427-013-4604-z

Wasfy, M. M., & Weiner, R. B. (2015). Differentiating the athlete's heart from hypertrophic cardiomyopathy. *Curr Opin Cardiol, 30*(5), 500-505. doi:10.1097/HCO.0000000000000203

Watanabe, S., Doshi, M., & Hamazaki, T. (2003). n-3 Polyunsaturated fatty acid (PUFA) deficiency elevates and n-3 PUFA enrichment reduces brain 2-arachidonoylglycerol level in mice. *Prostaglandins Leukot Essent Fatty Acids, 69*(1), 51-59. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12878451

Wei, B. Q., Mikkelsen, T. S., McKinney, M. K., Lander, E. S., & Cravatt, B. F. (2006). A second fatty acid amide hydrolase with variable distribution among placental mammals. *J Biol Chem, 281*(48), 36569-36578. doi:10.1074/jbc.M606646200

Wei, C. M., Lerman, A., Rodeheffer, R. J., McGregor, C. G., Brandt, R. R., Wright, S., . . . Burnett, J. C., Jr. (1994). Endothelin in human congestive heart failure. *Circulation, 89*(4), 1580-1586. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8149524

Weis, F., Beiras-Fernandez, A., Sodian, R., Kaczmarek, I., Reichart, B., Beiras, A., . . . Kreth, S. (2010). Substantially altered expression pattern of cannabinoid receptor 2 and activated endocannabinoid system in patients with severe heart failure. *J Mol Cell Cardiol, 48*(6), 1187-1193. doi:10.1016/j.yjmcc.2009.10.025

Wenzel, D., Matthey, M., Bindila, L., Lerner, R., Lutz, B., Zimmer, A., & Fleischmann, B. K. (2013). Endocannabinoid anandamide mediates hypoxic pulmonary vasoconstriction. *Proc Natl Acad Sci U S A, 110*(46), 18710-18715. doi:10.1073/pnas.1308130110

Westenbrink, B. D., Ling, H., Divakaruni, A. S., Gray, C. B., Zambon, A. C., Dalton, N. D., . . . Heller Brown, J. (2015). Mitochondrial Reprogramming Induced by CaMKIIdelta Mediates Hypertrophy Decompensation. *Circ Res, 116*(5), e28-39. doi:10.1161/CIRCRESAHA.116.304682

Westrate, L. M., Drocco, J. A., Martin, K. R., Hlavacek, W. S., & MacKeigan, J. P. (2014). Mitochondrial morphological features are associated with fission and fusion events. *PLoS One, 9*(4), e95265. doi:10.1371/journal.pone.0095265

White, R., Ho, W. S., Bottrill, F. E., Ford, W. R., & Hiley, C. R. (2001). Mechanisms of anandamide-induced vasorelaxation in rat isolated coronary arteries. *Br J Pharmacol, 134*(4), 921-929. doi:10.1038/sj.bjp.0704333

Witters, L. A., Kemp, B. E., & Means, A. R. (2006). Chutes and Ladders: the search for protein kinases that act on AMPK. *Trends Biochem Sci, 31*(1), 13-16. doi:10.1016/j.tibs.2005.11.009

Wollert, K. C., & Drexler, H. (2002). Regulation of cardiac remodeling by nitric oxide: focus on cardiac myocyte hypertrophy and apoptosis. *Heart Fail Rev, 7*(4), 317-325. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12379817

Wood, J. T., Williams, J. S., Pandarinathan, L., Janero, D. R., Lammi-Keefe, C. J., & Makriyannis, A. (2010). Dietary docosahexaenoic acid supplementation alters select physiological endocannabinoid-system metabolites in brain and plasma. *J Lipid Res, 51*(6), 1416-1423. doi:10.1194/jlr.M002436

World Health Organization. (2014). Deaths from CVD and diabetes Retrieved from http://www.who.int/gho/ncd/mortality_morbidity/cvd_text/en/

Wright, K., Rooney, N., Feeney, M., Tate, J., Robertson, D., Welham, M., & Ward, S. (2005). Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing. *Gastroenterology, 129*(2), 437-453. doi:10.1016/j.gastro.2005.05.026

Wu, L., Belardinelli, L., & Fraser, H. (2008). A novel partial fatty acid oxidation inhibitor decreases myocardial oxygen consumption and improves cardiac efficiency in demand-induced ischemic heart. *J Cardiovasc Pharmacol, 51*(4), 372-379. doi:10.1097/FJC.0b013e318166803b

Wu, Z., & Boss, O. (2007). Targeting PGC-1 alpha to control energy homeostasis. *Expert Opin Ther Targets, 11*(10), 1329-1338. doi:10.1517/14728222.11.10.1329

Xiao, G., Mao, S., Baumgarten, G., Serrano, J., Jordan, M. C., Roos, K. P., . . . MacLellan, W. R. (2001). Inducible activation of c-Myc in adult myocardium in vivo provokes cardiac myocyte hypertrophy and reactivation of DNA synthesis. *Circ Res, 89*(12), 1122-1129. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11739276

Yamazaki, T., Komuro, I., Kudoh, S., Zou, Y., Shiojima, I., Hiroi, Y., . . . Yazaki, Y. (1996). Endothelin-1 is involved in mechanical stress-induced cardiomyocyte hypertrophy. *J Biol Chem, 271*(6), 3221-3228. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8621724

Yamazaki, T., Tobe, K., Hoh, E., Maemura, K., Kaida, T., Komuro, I., . . . Yazaki, Y. (1993). Mechanical loading activates mitogen-activated protein kinase and S6 peptide kinase in cultured rat cardiac myocytes. *J Biol Chem, 268*(16), 12069-12076. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7685031

Yancy, C. W., Jessup, M., Bozkurt, B., Butler, J., Casey, D. E., Jr., Drazner, M. H., . . . American Heart Association Task Force on Practice, G. (2013). 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol, 62*(16), e147-239. doi:10.1016/j.jacc.2013.05.019

Yang, H., Zhou, J., & Lehmann, C. (2015). GPR55 - a putative "type 3" cannabinoid receptor in inflammation. *J Basic Clin Physiol Pharmacol*. doi:10.1515/jbcpp-2015-0080

Yu, L., Li, M., She, T., Shi, C., Meng, W., Wang, B., & Cheng, M. (2013). Endothelin-1 stimulates the expression of L-type Ca2+ channels in neonatal rat cardiomyocytes via the extracellular signal-regulated kinase 1/2 pathway. *J Membr Biol, 246*(4), 343-353. doi:10.1007/s00232-013-9538-7

Yusuf, S., Pfeffer, M. A., Swedberg, K., Granger, C. B., Held, P., McMurray, J. J., . . . Committees. (2003). Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: the CHARM-Preserved Trial. *Lancet, 362*(9386), 777-781. doi:10.1016/S0140-6736(03)14285-7

Zaccagnino, P., Corcelli, A., Baronio, M., & Lorusso, M. (2011). Anandamide inhibits oxidative phosphorylation in isolated liver mitochondria. *FEBS Lett, 585*(2), 429-434. doi:10.1016/j.febslet.2010.12.032

Zajicek, J., Ball, S., Wright, D., Vickery, J., Nunn, A., Miller, D., . . . group, C. i. (2013). Effect of dronabinol on progression in progressive multiple sclerosis (CUPID): a randomised, placebo-controlled trial. *Lancet Neurol, 12*(9), 857-865. doi:10.1016/S1474-4422(13)70159-5

Zeidan, A., Gan, X. T., Thomas, A., & Karmazyn, M. (2014). Prevention of RhoA activation and cofilin-mediated actin polymerization mediates the antihypertrophic effect of adenosine receptor agonists in angiotensin II- and endothelin-1-treated cardiomyocytes. *Mol Cell Biochem, 385*(1-2), 239-248. doi:10.1007/s11010-013-1832-2

Zhang, C. X., Pan, S. N., Meng, R. S., Peng, C. Q., Xiong, Z. J., Chen, B. L., . . . Dong, Y. G. (2011). Metformin attenuates ventricular hypertrophy by activating the AMP-activated protein kinase-endothelial nitric oxide synthase pathway in rats. *Clin Exp Pharmacol Physiol, 38*(1), 55-62. doi:10.1111/j.1440-1681.2010.05461.x

Zhang, P., Hu, X., Xu, X., Fassett, J., Zhu, G., Viollet, B., . . . Chen, Y. (2008). AMP activated protein kinase-alpha2 deficiency exacerbates pressure-overload-induced left ventricular hypertrophy and dysfunction in mice. *Hypertension, 52*(5), 918-924. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18838626

Zhang, Y., Marcillat, O., Giulivi, C., Ernster, L., & Davies, K. J. (1990). The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *J Biol Chem, 265*(27), 16330-16336. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2168888

Zhang, Y., Mi, S. L., Hu, N., Doser, T. A., Sun, A., Ge, J., & Ren, J. (2014). Mitochondrial aldehyde dehydrogenase 2 accentuates aging-induced cardiac remodeling and contractile dysfunction: role of AMPK, Sirt1, and mitochondrial function. *Free Radic Biol Med, 71*, 208-220. doi:10.1016/j.freeradbiomed.2014.03.018

Zhao, Y., Liu, Y., Zhang, W., Xue, J., Wu, Y. Z., Xu, W., . . . Yuan, Z. (2010). WIN55212-2 ameliorates atherosclerosis associated with suppression of pro-inflammatory responses in ApoE-knockout mice. *Eur J Pharmacol, 649*(1-3), 285-292. doi:10.1016/j.ejphar.2010.09.027

Zhao, Y., Yuan, Z., Liu, Y., Xue, J., Tian, Y., Liu, W., . . . Chen, T. (2010). Activation of cannabinoid CB2 receptor ameliorates atherosclerosis associated with suppression of adhesion molecules. *J Cardiovasc Pharmacol, 55*(3), 292-298. doi:10.1097/FJC.0b013e3181d2644d

Zheng, X., Sun, T., & Wang, X. (2013). Activation of type 2 cannabinoid receptors (CB2R) promotes fatty acid oxidation through the SIRT1/PGC-1alpha pathway. *Biochem Biophys Res Commun, 436*(3), 377-381. doi:10.1016/j.bbrc.2013.05.108

Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., . . . Moller, D. E. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest, 108*(8), 1167-1174. doi:10.1172/JCI13505

Zhou, L. Y., Liu, J. P., Wang, K., Gao, J., Ding, S. L., Jiao, J. Q., & Li, P. F. (2012). Mitochondrial function in cardiac hypertrophy. *Int J Cardiol*. doi:10.1016/j.ijcard.2012.09.082

Ziolo, M. T., & Bers, D. M. (2003). The real estate of NOS signaling: location, location, location. *Circ Res, 92*(12), 1279-1281. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12829613

Zou, Y., Komuro, I., Yamazaki, T., Kudoh, S., Uozumi, H., Kadowaki, T., & Yazaki, Y. (1999). Both Gs and Gi proteins are critically involved in isoproterenol-induced cardiomyocyte hypertrophy. *J Biol Chem, 274*(14), 9760-9770. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10092665

Zuurman, L., Ippel, A. E., Moin, E., & van Gerven, J. M. (2009). Biomarkers for the effects of cannabis and THC in healthy volunteers. *Br J Clin Pharmacol, 67*(1), 5-21. doi:10.1111/j.1365-2125.2008.03329.x

Zygmunt, P. M., Petersson, J., Andersson, D. A., Chuang, H., Sorgard, M., Di Marzo, V., . . . Hogestatt, E. D. (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature, 400*(6743), 452-457. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10440374