Chapter-1

Review of Literature

Acute Myocardial Infarction

Description and types

AMI occurs when blood supply to the heart muscle is diminished due to thrombus formation and as a result, coronary occlusion creates the metabolic demand of heart muscles due to the less supply of nutrients, minerals and O_2 and cell death is then the obvious effect of the phenomena because cells are unable to perform the normal metabolic activity due to scarcity of fuels. From the morphological and anatomical view, AMI is of two types transmural infarction and non-transmural infarction. In case of transmural infarction; it is widened from endocardium to epicardium and in case of nontransmural, it is stayed in the area of endocardium only and some cases up to the myocardium.

AMI can be classified based on presence/absence of ST-segment elevation on the ECG report; it can further be diagnosed into six types – type-1: infarction caused by atherothrombosis in coronary arteries; type-2: infarction is occurred due to demand-supply mismatch; type-3: sudden death may be happened by infarction; type-4: infarction related to PCI (percutaneous coronary intervention); type-5: infarction related to stent thrombosis; type-6: infarction related to CABG (coronary artery bypass grafting).

We have actually analyzed AMI in our experiment accordance to ECG reports, though it is very difficult to differentiate the categories by ECG. From various previous reports, we differentiated them in ST-elevated myocardial infarction (STEMI) and another is non-STEMI. And Q-wave analysis was also included in the experiment. We included both STEMI and non-STEMI who had the Q waves. And for the confirmation, biomarker (troponin) test have been done and as such, the cause of ischemic discomfort comes out from the serial ECG and biomarker tests.

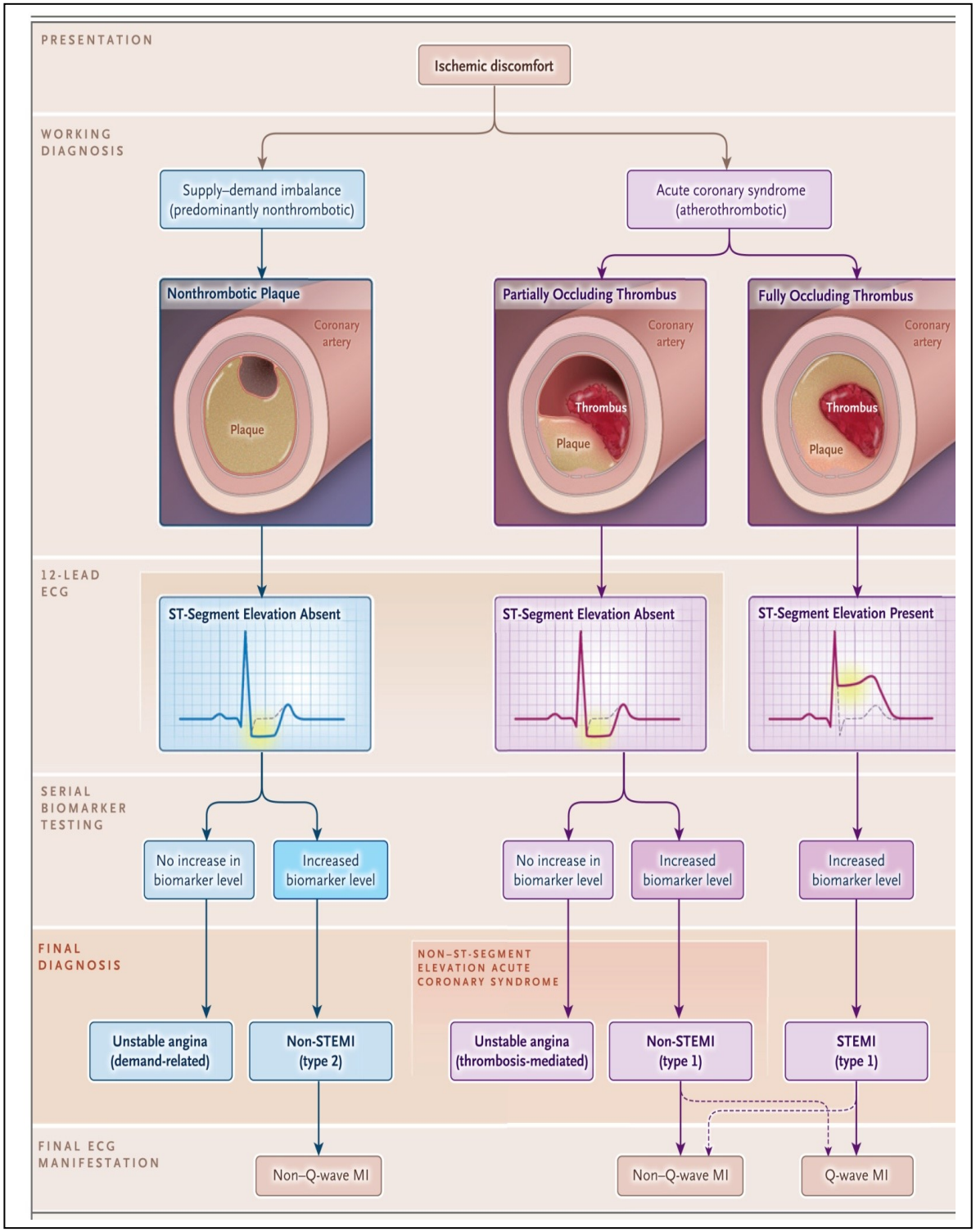


Figure-1.1: Spectrum of Pathologic and Clinical ST-Segment Elevation Acute Myocardial Infarction STEMI and non-STEMI Acute Coronary Syndromes. (Diagram Credit: Morrow ed. Myocardial infarction: a companion to Braunwald's Heart Disease. St. Louis: 2016: 2. & Anderson et al., 2017).

This diagram very clearly demonstrated that when a patient is admitted to the hospital with ischemic discomfort, it might be confirmed from the serial experimentation that the ischemic discomfort is due to the supply-demand miss-match i.e. nonthrombotic plaque or it might be due to the acute coronary syndrome i.e. atherothrombotic plaque and followed by ECG and biomarker test have been performed for the final diagnosis.

Pathophysiology and Risk factors of AMI

There are two types of risk factors for the development of AMI a) Modifiable risk factors b) Nonmodifiable risk factors which are depicted in the below diagram with flow chart. These factors slowly can activate massive platelet aggregation followed by thrombosis and enlargement of thrombus and slowly myocardial cell death occurred.

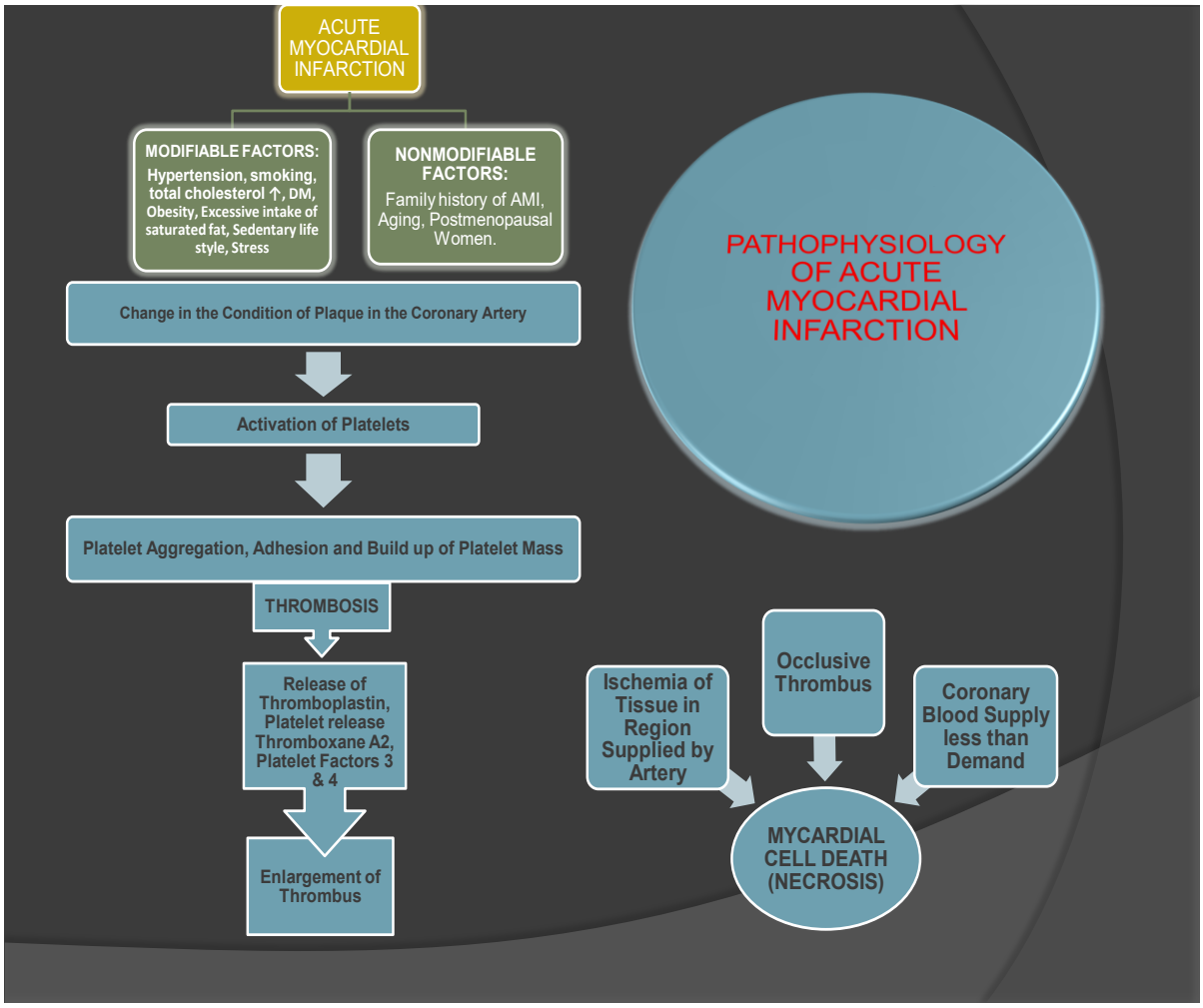


Fig-1.2: Figure demonstrates the formation of myocardial cell death by its risk factors

The above flow-chart diagram explained the mechanism of the development of myocardial cell death triggered by various risk factors that slowly culminate thrombus and its enlargement by induction of severe platelet aggregation and release of platelet factors and thromboxane-A2.

Pathophysiological mechanism

In the occluded artery, the inception of the blood flow (reperfusion) by pharmacological or mechanical induction may protect part of the arterial area of hypoperfused myocardial injury.

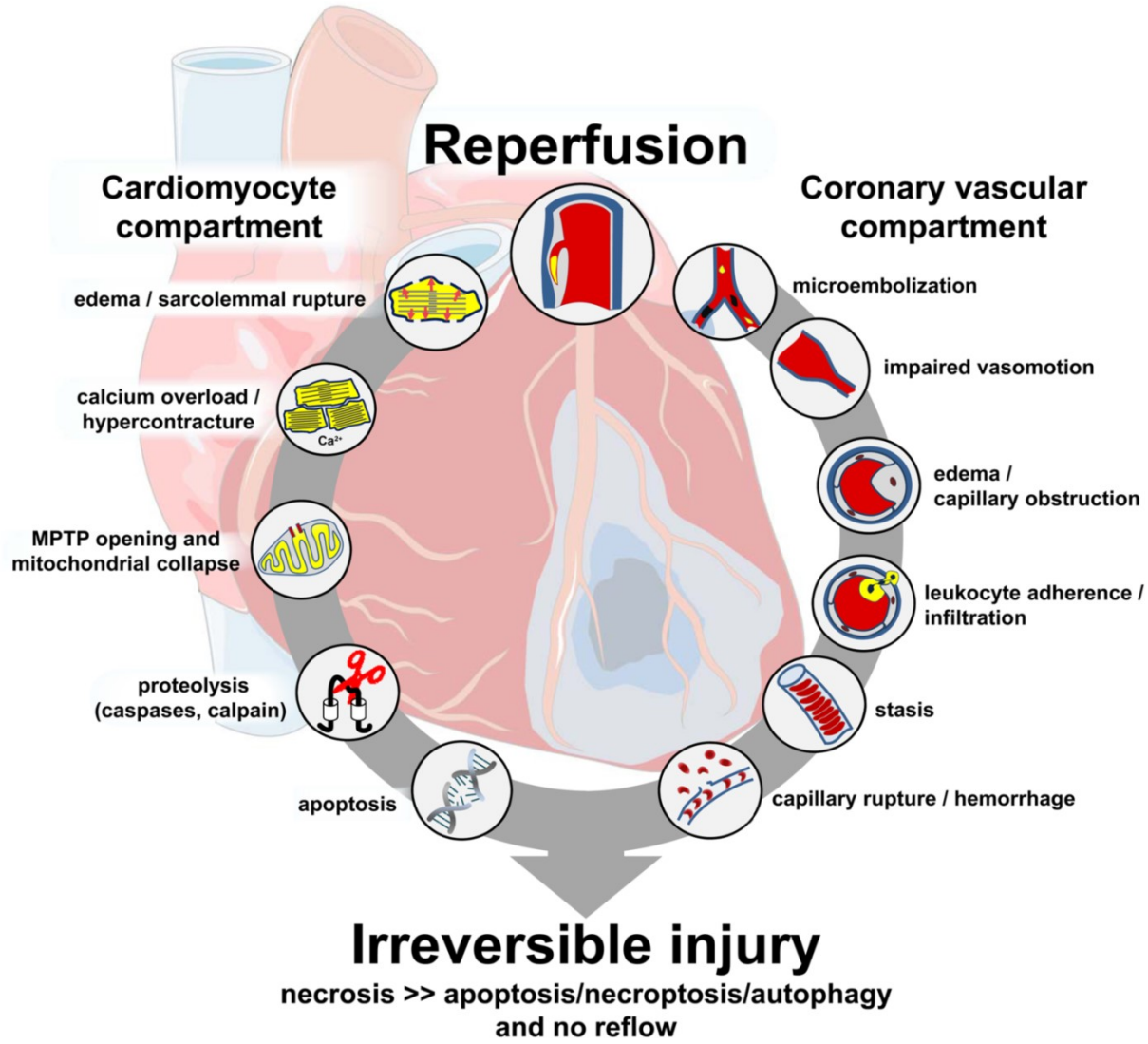


Fig-1.3: Diagram demonstrates the events of cardiomyocyte compartment and coronary vascular compartment which produce irreversible injury/myocardial death (Diagram Credit: Heusch Gerd et al. 2017)

However, reperfusion is not the instrumental at the early stage because during reperfusion there is the possibility of recruitment of inflammatory molecules at the injury site. But the inflammatory mechanism is advantageous a few days later and is also beneficial. Reperfusion induced injury area might be the marked site to improve the post-infarction condition of the left ventricle. The inflammatory cells, neutrophils are recruited first in the area at risk (AAR) after reperfusion, while the macrophages fade from there. Although neutrophils are effective to form a scar to inhibit the adverse transformation/remodelling, these cells may in turn expedite and commemorate myocardial injury (Carbone F *et al*. Thromb Haemost 2013). Generation of reactive oxygen species (ROS) and the release of cytokines trigger a positive feedback loop that increases the recruitment of neutrophils and extends their lifespan. However, after 5–7 days of MI, neutrophil infiltrate resolves and granulocytes undergo apoptosis. Inflammation of neutrophils at the perfect time is a crucial step for optimal healing of the infraction and various terminated signals have developed for the anti-inflammatory cascade following tissue injury (Frangogiannis NG. *et al.* 2012). Along with dying neutrophils and T cells, subpopulations of monocytes are thought to the essential regulators of inflammation in cytokines and growth factor-rich state which regulate angiogenesis and metabolism of the matrix. There are many factors like total ischemic time, hemodynamic status at the time of ischemia, blood flow through the collateral way which can also regulate myocardial salvage pathway. A severe microvascular dysfunction may occur where reflow is impaired in the coronary artery. So, reperfusion is ineffective in that ischaemic myocardial tissue in spite of an orthodox process of reopening of the occluded area of the corresponding epicardial artery (Durante

A. *et al.* 2015). After PCI (percutaneous coronary intervention), no-reflow is the cause of mortality in reperfused ST-elevated acute myocardial infarction (STEMI) at least in part. Ischemia induced endothelial swelling might be the cause of obstruction of microcirculation. Ischaemic endothelium creates a prothrombotic environment by inducing obstructive micro-aggregates of platelets and neutrophils. A diminished level of endothelial nitric oxide in ischaemia and embolism due to thrombus debris may further magnify the obstruction.

Excessive leukocyte extravasation results in interstitial haemorrhages and oedema. Also swelling of myocardial cells and myofibrillar hyper-contraction of myofibril may contribute to compression vessels. However, it is thought that cardiomyocyte destruction is the reason for microvascular dysfunction of coronary artery or it was found that both events are consequently occurring in ischaemia/reperfusion (I/R) injury which is not properly understood. Recently, repetition of leucine-rich domain receptors (NLRs), the damage-conjugated molecular reaction, toll-like receptors (TLRs), found as a prime hot-region of the IR (Vilahur G, *et al*. 2014). After activation, the signaling cascade of TLRs (My88- and TRIF- pathways) triggers nuclear NL sites of NF-kB and as such cytokines (pro-inflammatory) and co-stimulatory molecules are expressed. So, it can be proposed that TLR4 boost immune response through the stimulation of neutrophil recruitment into injured myocardium (Montecucco F *et al*. 2015). Even after primary angioplasty or stenting in TIMI (grade-3), faulty blood flow may produce injury again. This case can be distinguished through a no ST-segment deviation, or it may be partial, and the reason behind it may be the microvascular and tissue injury remodeling and it is related to dreadful cardiac regeneration and surge the rate of mortality (de Lemos JA *et al*. 2000; Nicolau JC *et al.* 2003).

STROKES

A stroke occurs in the brain arteries when there is a blockage due to the clot and blood supply is hampered to the brain cells is called Ischemic Stroke or there is the rupture of the arteries in the brain as such seepage of blood from the artery is called Hemorrhagic Stroke.

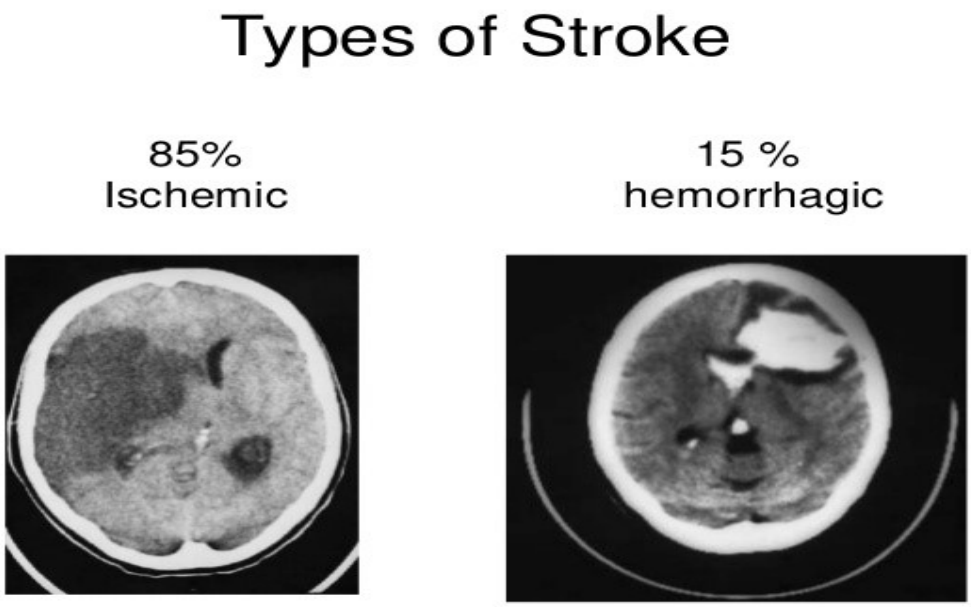


Fig-1.4: Figure represents the ischemic stroke (left) and hemorrhagic stroke (right) (Figure Credit: Management of stroke slide: Dr. Mallum C.B., Neurology Unit, Dept. of Internal Medicine, Juth).

Ischemic stroke: Fatty deposition is one of the prime reasons in ischemic stroke which can cause two kinds of block: **Cerebral thrombosis** is defined as the thrombus i.e. blood clot that develops at the clogged part of the vessel (*American stroke association, a Division of American Heart Association)*. Cerebral embolism is the blood clot or thrombus that forms in generally distant part of the body (large arteries of the neck or upper chest or in the heart). When the fragment of that thrombus or clot tears and flows by the bloodstream and then proceeds through the brain's small blood vessels, it may clog

because of the small radius of the vessels. A second most cause of embolism is atrial fibrillation where an irregular heartbeat happens. In this case where blood clot formation is in the heart, fragmented and flow through the brain (*The American Stroke Association, a division of American Heart Association;https://www.strokeassociation.org/en/about-stroke/types-ostroke/ischemic stroke-clots*). **Silent cerebral infarction** (SCI), or "silent stroke," is a brain injury likely caused by a blood clot interrupting blood flow in the brain. It's a risk factor for future strokes which could lead to progressive brain damage due to these strokes (*American Stroke Association, a division of the American Heart Association; https://www.strok eassociation.org/en/about-stroke/types-of-stroke/ischemic-stroke-clots*).

Hemorrhagic stroke: There are two types of exhausted blood vessels which typically cause hemorrhagic stroke: aneurysms and arteriovenous malformations (AVMs). An **aneurysm** is a dilated part of a blood vessel which is weakened part of a small vessel. If it is not treated properly, pursuing exhaustiveness due to the aneurism and at a time fissure of that portion occur and bleeds into the brain. An **arterio-venous malformation** (AVM) is a clump of blood vessels and their formation is anomalous, so that anyone of those vessels may break, and can be the cause of bleeding into the brain.

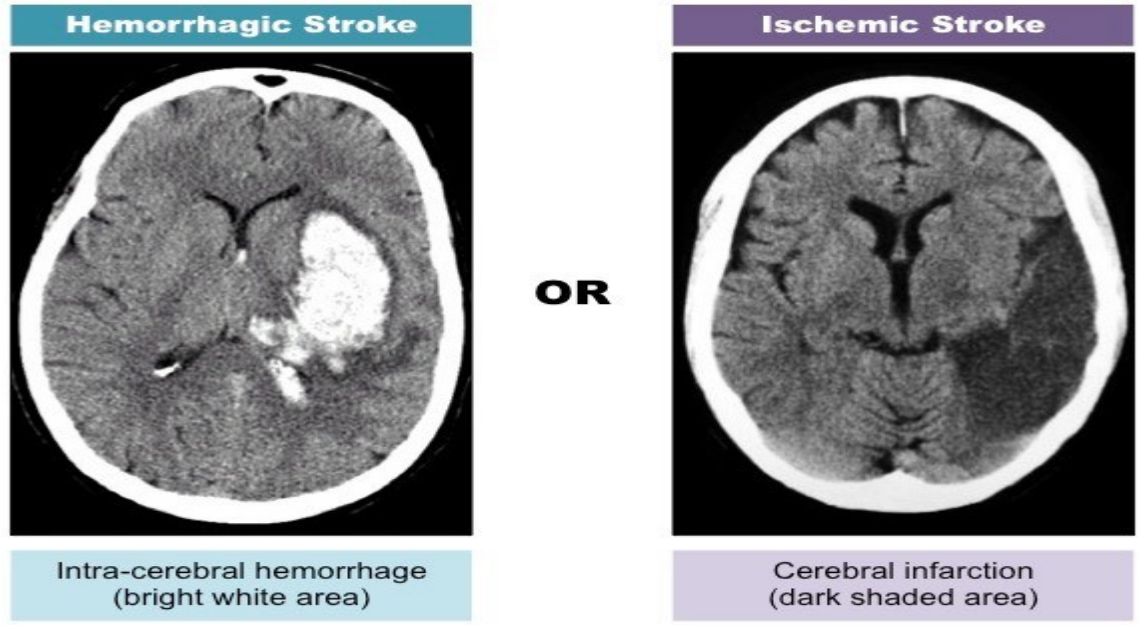


Fig-1.5: Figure demonstrates the difference between hemorrhagic and ischemic stroke, [Credit: Cornell, B. 2016. Referencing [ONLINE] Available at: http://ib.bioninja. com.au. (Accessed 27 January 2019)] *where bright white area indicates the cerebral hemorrhage in hemorrhagic stroke and dark shaded area indicates the brain cell death in ischemic stroke patients due to the blood clot into the arteries, as such blood flow in the arteries of the brain has been stopped.*

PLATELET

The span of life of a platelet is approximately 10 days in our blood circulation. From the previous experiments, it is found that transit time to release platelets from the progenitor cells (megakaryocyte) into the circulation is six day average. In the presence of EDTA and Wright's stain, platelets take color as bluish-gray, shape is oval to round with red-purple granules and its diameter is 1.5-3µm (average diameter). A 14-20 nm fuzzy coat is found around the platelet surface which is composed of membrane glycolipids, glycoproteins, mucopolysaccharides, and absorbed plasma proteins. Platelet has

canalicular system on its membrane which helps in many functions. The presence of sialic acid in protein and lipid may be the major causes of negative charge in platelets and in other cells also (*Williams Hematology, Chapter 112, SUSANS. SMYTH Sidney Whiteheart, Joseph E. Italiano Jr., Paul Bray and BARRY S. COLLER).*

The platelet plasma membrane

Plasma membrane of platelet is a trilaminar which is comprised of glycolipids, glycoproteins and phospholipids forming a bilayer of phospholipids (White *et al*, 1993). The phospholipids are asymmetrically organized in platelet membrane; the negatively charged phospholipid Phosphatidylserine and Phosphatidyl ethanol amine are exclusively present in the inner leaflet of the resting platelets. While platelets are activated aminophospholipids may expose on the surface layer of platelets or on it may be on the microparticle surface and actuate coagulation based on cell-surface reaction (Heemskerk *et al.,* 2002; Solum *et al.,* 1999; Sims *et al.,* 1988; Sims *et al.,* 1989).

Dense granules

Platelets constitute about 3-8 electron dense granules (White *et al*, 1993; McNicol *et al*, 1999). They are dense because they are highly osmophilic observed under transmission electron microscope and they are (McNicol *et al.,* 1999) dense granules maintain lower pH (6.1) and able to concentrate serotonin **(**Brenner *et al.,* 2007) and the concentration of ADP is higher than ATP in these granules.

Shape changes of platelet

Shape change of platelet occurs by different response and there is a hampering of the normal discoid shape of platelet. It is argued, because of the change of shape of platelets,

electrostatic repulsion is reduced among the platelets and also between platelets and cell surface.

Blood flow is the important regulator for platelet aggregation

Platelet aggregation and its adhesion to the neutrophils/platelets/membrane surface of other cells depend on the blood flow condition of the arterial injury site. Stenosed arteries have the shear rates > 40,000 s^{-1} and at that high shear environment, platelets have the capability to form a plug (haemostatic) at the wall of arteries (Bluestein D *et al,* 1997*;* Ruggeri *et al,* 1997). In case of normal blood flow and shear stress, the vascular source of NO acting on platelets is likely derived from biochemical agonist and shear-dependent release of endothelial NO. (Cooke *et al.* 1990).

Receptor-ligand interaction in platelet aggregation

From the advance studies, it has been found that multiple ligands like vonWillebrand factor, fibronectin and fibrinogen lead the main role in platelet-platelet interaction. Platelet –leukocyte interaction.

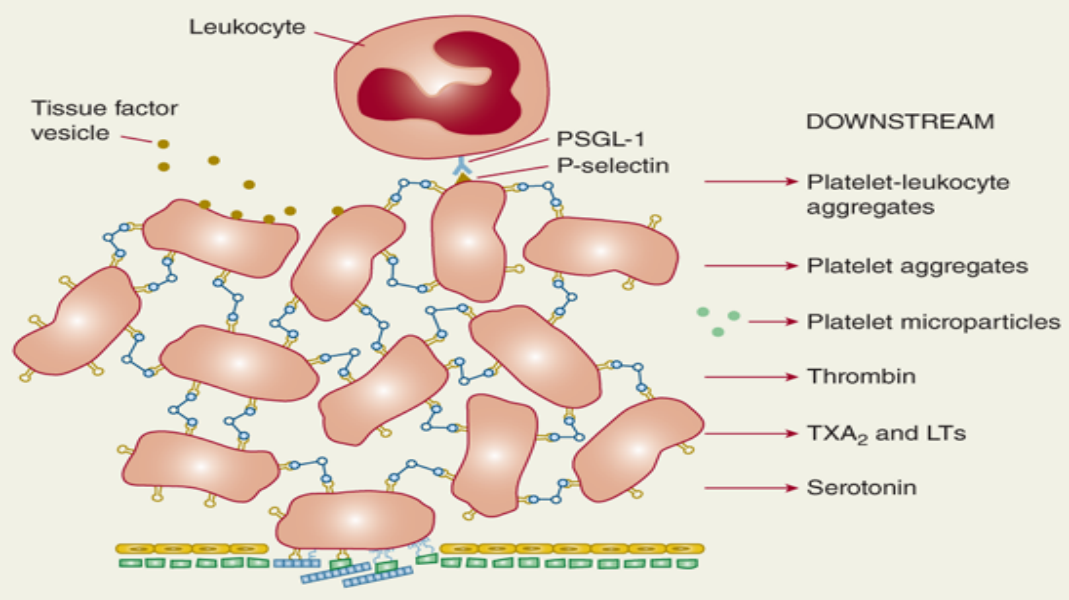


Fig-1.6: Figure demonstrates the platelet-platelet and platelet-leukocyte interaction (Figure credit: Kaushansky et al. Williams Hematology, 9th ed.) *Here this diagram demonstrated the platelet aggregation or platelet-leukocyte interaction in the presence of tissue factors, TXA_2, Leucotrines, and where p-selectin is activated and may interact with PSGL-1.*

ATHEROSCLEROSIS

Virchow first proposed atherosclerosis is an inflammatory and prothrombotic process (Virchow et al, 1863). Most of the lipid of atherosclerotic lesions is because of accumulation of the plasma lipoprotein.

Lipid in atherosclerosis: Lipoprotein transport into the artery wall, this concentration dependent process does not require receptor mediated endocytosis (Steinberg D *et al.* 1989; Young *et al.* 1994). It was reported that the mutation in a single LDL receptor gene is the cause of an increase level of LDL and creates all the components of atherosclerotic reaction (Brown *et al.* 1986).

Lipoprotein concentration and its retention in the arterial walls played the main role here.

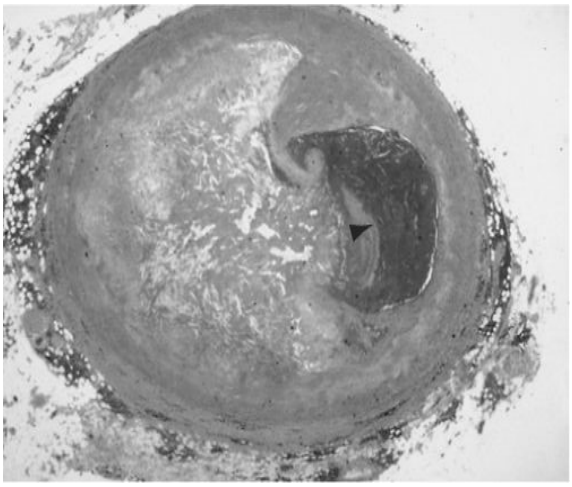


Fig-1.7:Atherosclerosis

Jonas: Mosby's Dictionary of Complementary and Alternative Medicine. (c) 2005, Elsevier.

Causes of Atherosclerosis

The development of atherosclerosis is a complex process, many things and events occur in atherosclerosis but the essential occasion is by all accounts rehashed, unpretentious damage to the supply route's inward coating (endothelium), through different factors and mechanisms. These mechanisms are -

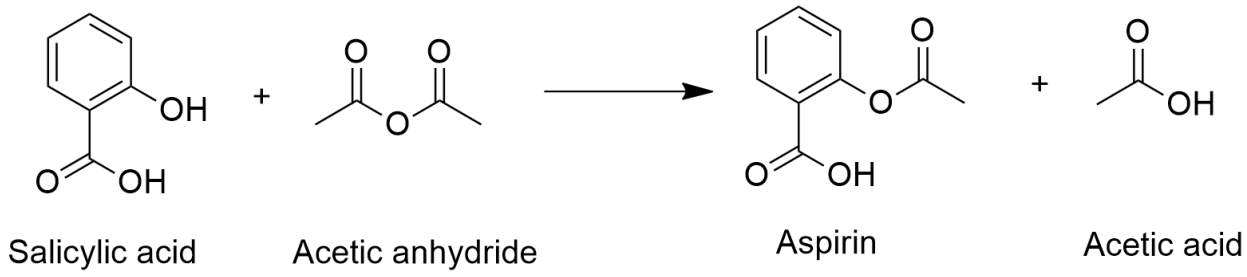
- The persons who have hypertension face physical strains due to the fierce bloodstream.
- The Immune system may be affected by inflammatory stresses (for eg. During cigarette smoke)
- High levels of cholesterol (bad cholesterol LDL) and high blood sugar in diabetes are the abnormalities that influence endothelial abnormality.

Bacterial or viral infections (like *Chlamydia pneumoniae* or cytomegalovirus) may trigger inflammation in the endothelial surface and conduct atherosclerosis.

ASPIRIN (IUPAC name: Acetylsalicylic Acid)

Aspirin (ASA) is a well known and abundantly used medicine all over the world which is used to treat pain, inflammation or fever. Aspirin is also used in specific inflammatory conditions like Kawasaki disease, and pericarditis. Aspirin is also able decrease heart diseases and death due to the diseases. Aspirin is also used in stroke, cancer disease. It is a nonsteroidal anti-inflammatory drug (NSAID) which inhibits platelet aggregation (*"Aspirin". Drugs.com. American Society of Health-System Pharmacists. 6 June 2016. Archived from the original on 25 April 2017. Retrieved 30 August 2016)*. Though aspirin is the corner stone therapy in acute myocardial infarction because of its resistance

effect in that condition, specific low and unique dose of aspirin might be helpful (Bank *et al*. 2014).



So, use of aspirin in AMI is little or not properly known. Although low-dose of aspirin is recommended now-a-days.

Table1.1: Low-dose therapy of aspirin recommendation

Recommended by	Primary Prevention	Secondary Prevention
American Heart Association/American Stroke Association	It is recommended for the high cardiovascular risk persons and the benefits to outweigh the year risk of cardiovascular events greater than 6 – 10%).	Heart attack and stroke survivors are recommended to regularly take low-dose aspirin for the recurrence of the disease i.e. secondary prevention.
American College of Chest Physicians	Recommended to all patients ≥ 50 years of age.	Recommendation of low dose aspirin or clopidogrel for all patients who had prominent with established cardiovascular disease.

Mechanism of aspirin

Aspirin showed its effect through the acetylating of its target and change the activity of the target. Aspirin shows its effect not only through COX pathway but also through the nitric oxide pathway. Through its acylation effect aspirin acts on clot lysis, protein transportation and on oxygen transport.

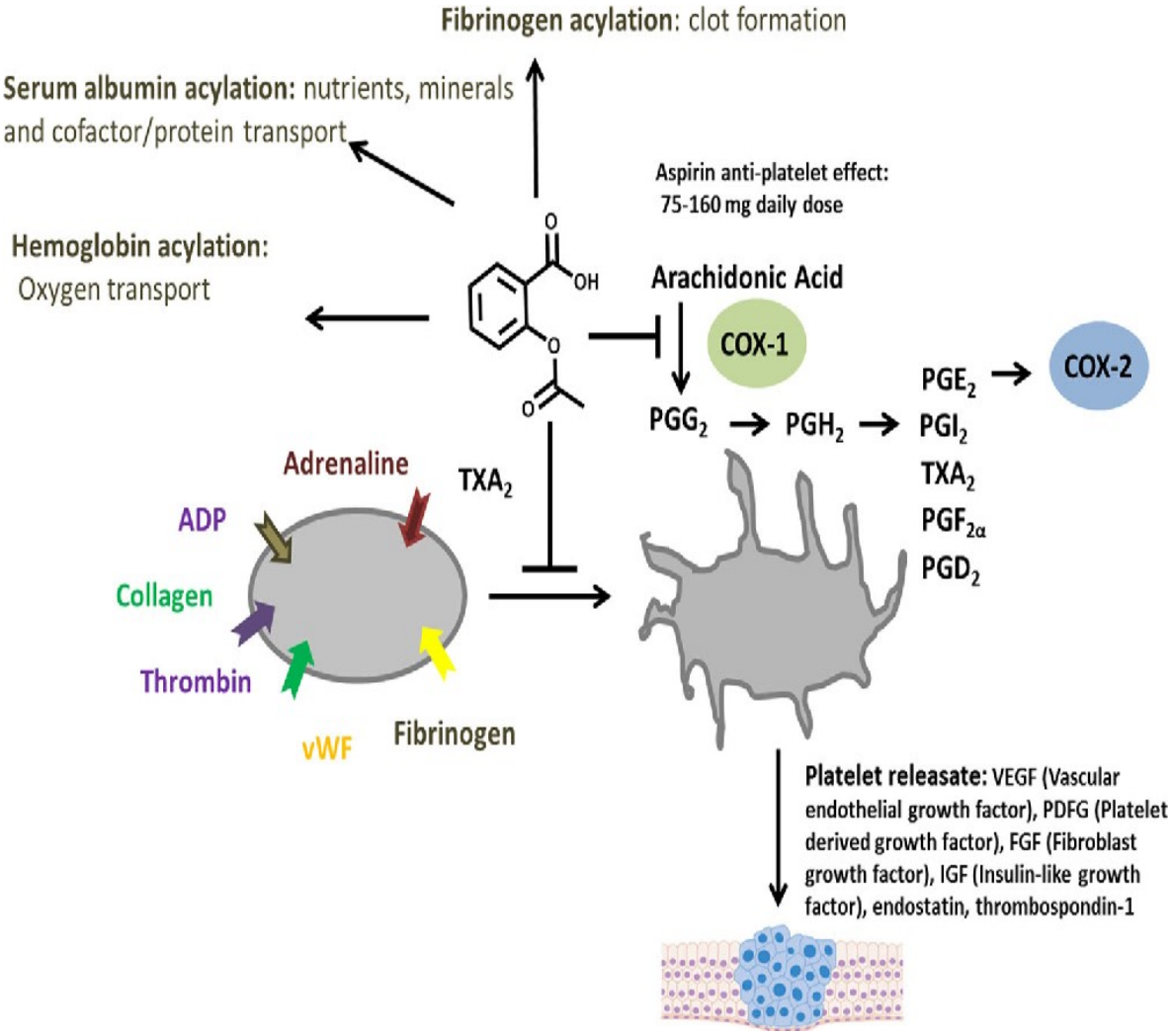


Fig-1.8: Role of aspirin on Cyclooxygenase (Figure Credit: Ornelas et al., 2017).

Platelet activation is initiated by different platelet aggregation agents (ADP, adrenaline, thrombin, collagen) by inducing thromboxane-A2, where as aspirin blocks platelet activation by inhibiting the cyclooxygenase (COX).

NITRIC OXIDE

Chemistry of Nitric Oxide: There are many reports that nitric oxide (NO) is only the toxic compound, but slowly, it has been revealed that NO is one of the keys in physiological regulators, this NO acts as the messenger molecule in many physiological processes. Nitric Oxide i.e. (nitrogen monoxide) is a chemical compound with formula •NO. Nitric oxide is a colorless free radical gas because of its unpaired electron (Lund *et al*. 2011).

Nitric Oxide can be made by the ammonia oxidation at 850°C in the presence of platinum catalyst.

$$4\ NH_3 + 5\ O_2 \rightarrow 4\ \cdot\mathbf{NO} + 6\ H_2O$$

According to Birkeland-Eyde procedure, nitric oxide is formed by the reaction of N2 and O2 at high temperature (>2000°C) and in the presence of lightning.

$$N_2 + O_2 \rightarrow 2\ \cdot\mathbf{NO}$$

Nitric oxide can be produced by the reduction of dilute nitric oxide in the presence of Cu.

1) $8\ HNO_3 + 3\ Cu \rightarrow 3Cu(NO_3)_2 + 4\ H_2O + 2\ \cdot\mathbf{NO}$
2) $2\ NaNO_2 + 2\ FeSO_4 + 3\ H_2SO_4 \rightarrow Fe_2(SO_4)_3 + 2\ NaHSO_4 + 2\ H_2O + 2\ \cdot\mathbf{NO}$

Nitric Oxide Synthase

Nitric oxide (NO) is synthesized by nitric oxide synthase (NOS) which catalyzes the NADPH dependent oxidation of L-arginine in the presence of O2 and produce nitric oxide (NO) and L-citrulline. Three isozymes of NOS are neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS), they are also known as NOS-1, -2, and -3,

respectively. The isozymes possess 50-60% sequence similarity and each consists of two domains:

1) The N-terminal (500 residues) heme domain binds the substrate O_2 and L-arginine and two prosthetic group Fe (III) heme and 5,6,7,8-tetrahydrobiopterin (BH4) which also acts in the hydroxylation of phenylalanine to tyrosine. From the X-ray structure of nNOS, iNOS and eNOS, it was found that they have closely similar heme domain.
2) The C-terminal reductase (600residues) domain supplies the electron for NOS reaction. This domain binds with redox active prosthetic group FMN and FAD and also with NADPH. The C-terminal domain is homologous to cytochrome P450 reductase.

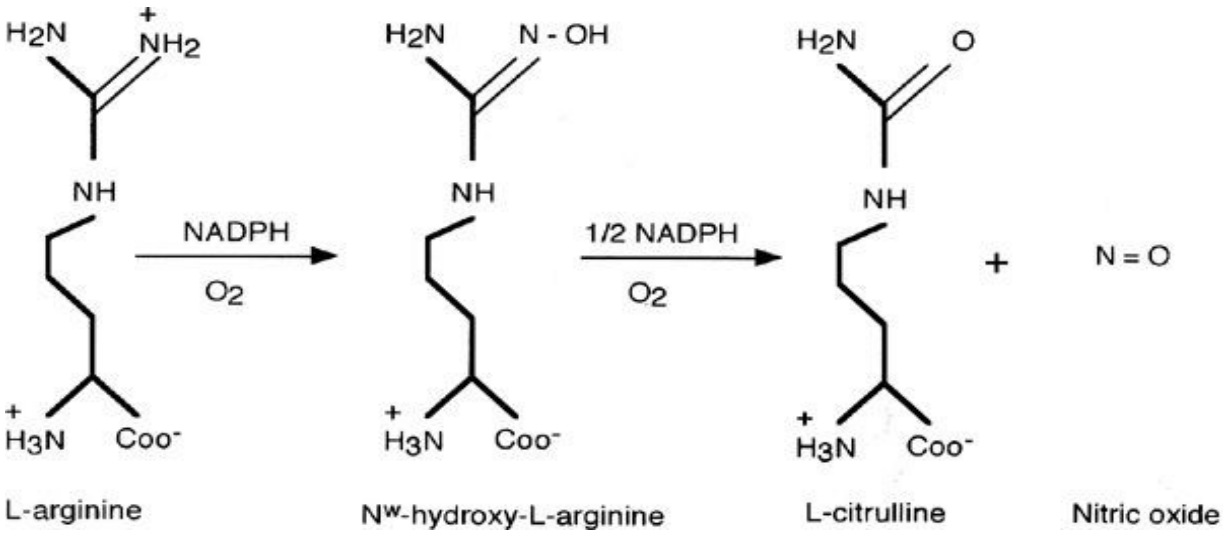


Figure-1.9: Biosynthesis of NO. (Figure credit: Xu et al. 1998). *Here this figure indicates the amino acid reaction of L-arginine in the presence of NADPH and O_2 where L-arginine can produce NO in two steps – in 1st step oxidation of L-arginine to N^w-hydroxy-L-argninie, and then to NO and citrulline. For this reaction the activators Ca^{2+} and calmodulin are essential and tetrahydrobiopterin also needed for the accelrator.*

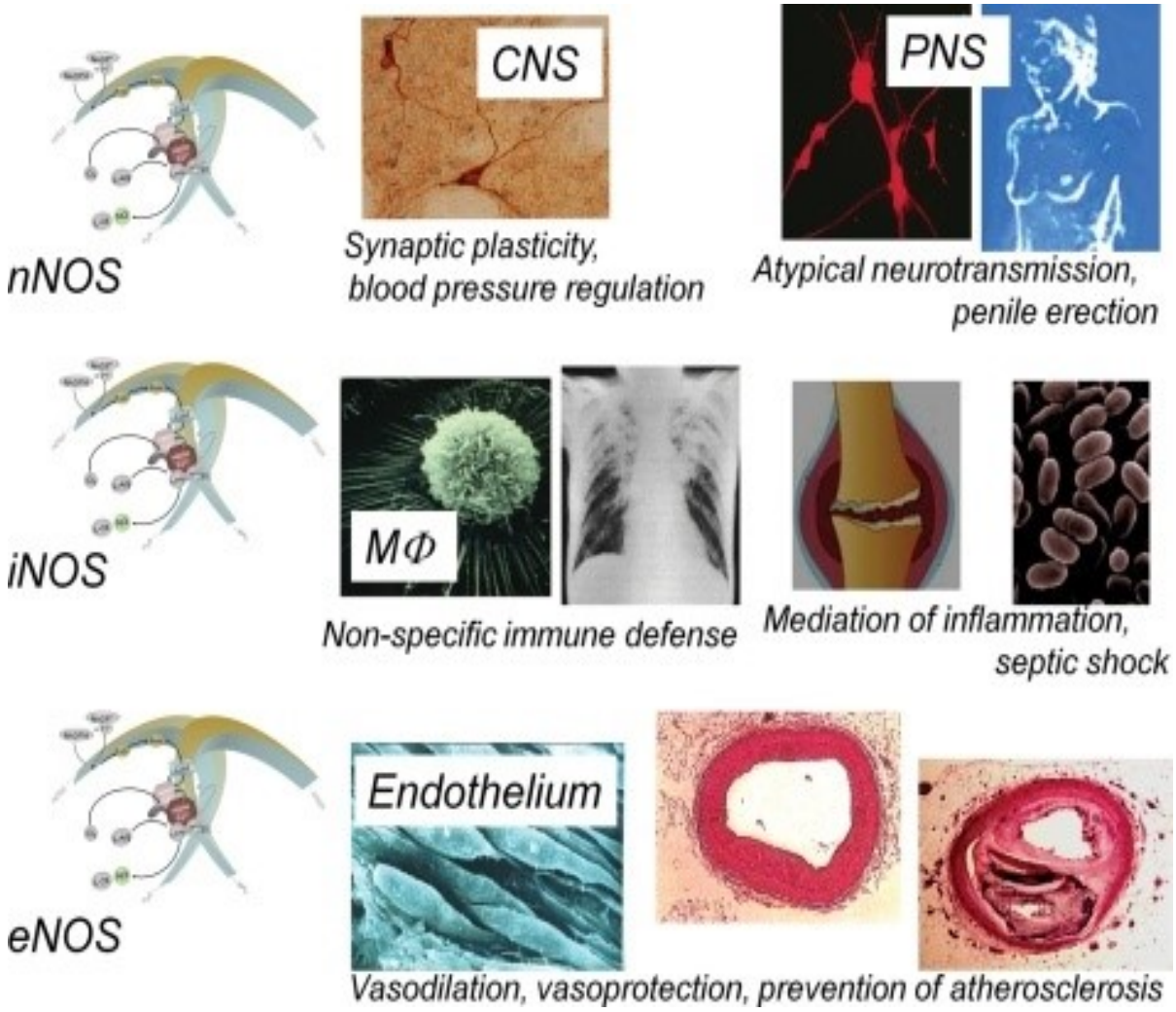


Fig-1.10: Role of different Nitric Oxide Synthase (NOS). (Figure Credit: Förstermann et al. 2012.)

nNOS is manifested specifically in the CNS. This nNOS has an important role in synaptic plasticity (i.e. long-term potentiating and long-term inhibition). The plasticity is related to the memory formation and learning process. The nNOS induced NO acts a major role in controlling blood pressure. In the peripheral nervous system, NO which is derived from nNOS functions as a neurotransmitter in the peripheral nervous system, this NOS induces vasodilation, gut peristalsis and erection (penile). nNOS is essential for the effects of the phosphodiesterase 5 inhibitors sildenafil and tadalafil.

Inducible NOS can be expressed in any cell (almost) by cytokines or other agents. The iNOS induction is essential in macrophage (MΦ) for the control of intracellular *Mycobacterium tuberculosis* or the parasite *Leishmania*. In different inflammatory diseases, iNOS is upregulated. This iNOS is a very important regulator of vasodilation. Endothelial NOS-induced NO is important for physiological vasodilation maintenance. It is the potent inhibitor of platelet aggregation and leukocytes-endothelial interaction by decreasing chemo attractant protein molecules like MCP-1 and other surface molecules which are adhesive types in the vascular wall. This eNOS not only inhibits thrombosis, but also the growth factors derived from platelets are inhibited. So, the eNOS plays a crucial role in anti-atherosclerotic activity and which also inhibits the LDL oxidation. It is shown to protect the later phase of atherogenesis by inhibiting mitogenesis and DNA synthesis. So, from the different reports on eNOS it has been found that eNOS plays an immense role in anti-atherosclerosis (Li and Förstermann, 2013).

Platelet-Derived NO and Inhibition of Platelet Recruitment

At the onset of an acute coronary syndrome, there is the condition of endothelial dysfunction, and then the NO from other different sources may be important for the regulation of platelet responses. It is found that a constitutive NO synthase (cNOS) from both megakaryocyte cells and human platelets (Sase *et al*. 1995; Pigazzi *et al*. 1995) and this isoform is active (Zhou *et al*. 1995).

Nitric Oxide Synthase and Stroke

Previously it was thought that nitric oxide is a free radical gas, which induces toxicity, but with time and recent researches showed that the physiological level of nitric oxide is essential for the various cellular activities and acts as the messenger molecule.

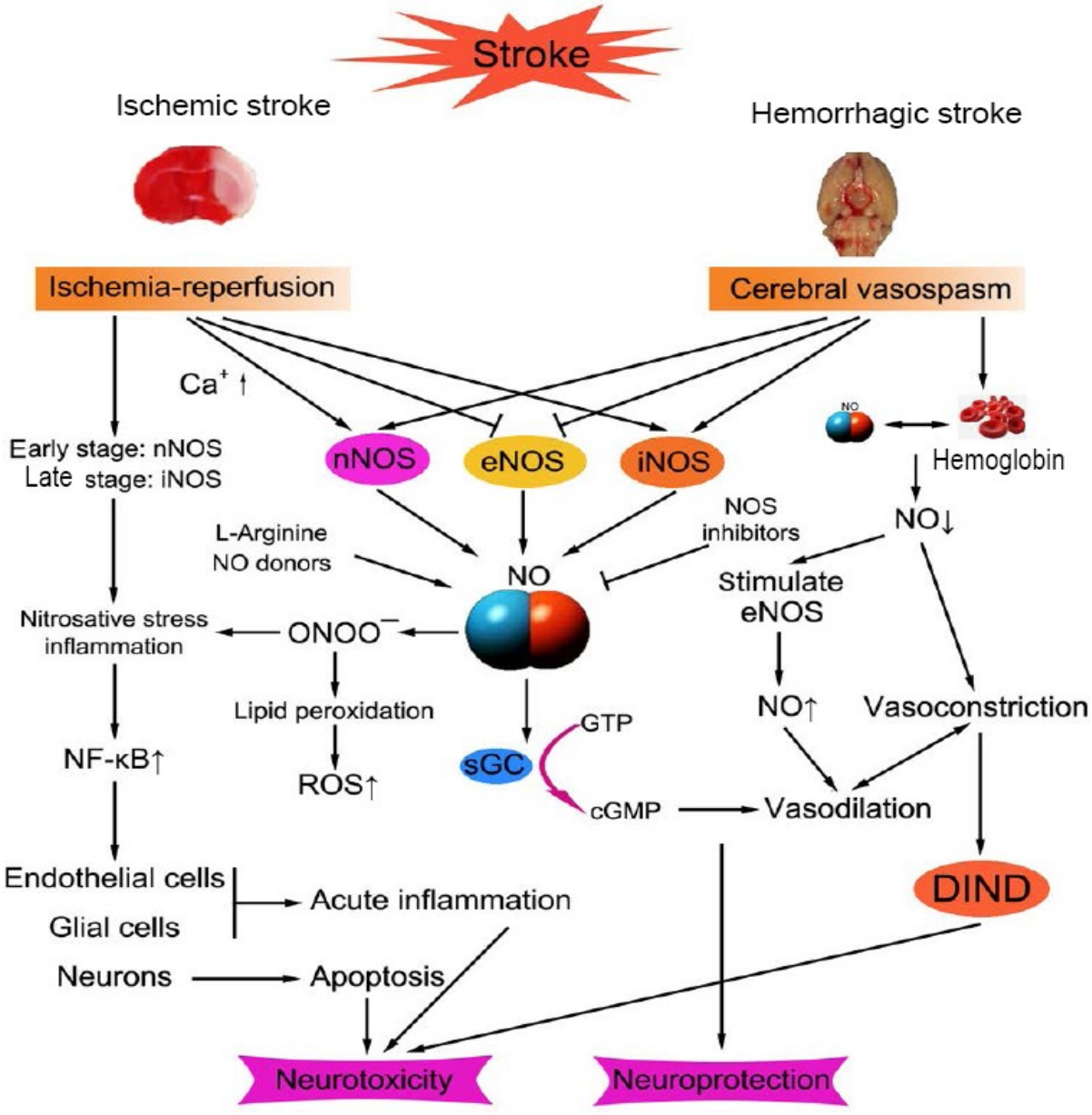


Fig-1.11: Figure demonstrates that how Nitric Oxide imparts a role in Ischemic Stroke and Hemorrhagic Stroke. (Figure Credit: Chen et al. 2017). *From the over induce nitric oxide peroxynitrite anion ($ONOO^-$) is formed which trigger endothelial dysfunction and*

acute inflammation to apoptosis, low level of NO creates vasoconstriction which triggers delayed ischemic neurological deficits (DIND) which ultimately generates neurotoxicity.

On the other-hand eNOS is inhibited during ischemic and hemorrhagic stroke as such cGMP is inhibited due to the inhibition of the sGC. So, NO leads a major role in the maintenance of neuroprotection and neurotoxicity.

NITROGLYCERIN IN CARDIOVASCULAR DISEASES

Nitroglycerin has been used in the cardiovascular disorder, since long time before. It has been used as an antianginal compound in coronary vasospasm, angina pectoris, and in congestive heart failure. It has very short-life time and it can neutralize the chest pain very well.

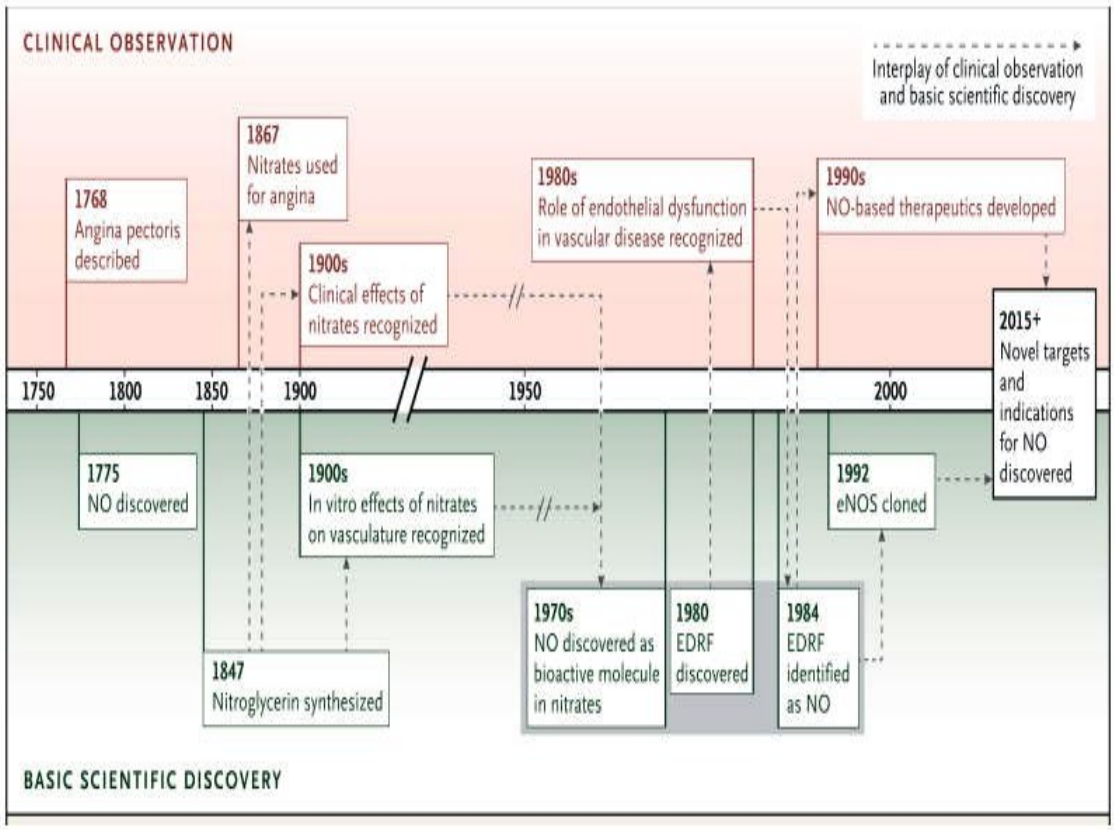


Fig-1.12: Here the timeline demonstrates interaction between basic scientific discoveries and clinical observations involving nitric oxide (NO), nitroglycerin and endothelial signaling roots. Picture Credit: Steinhorn et al, 2015, N Engl J Med.

Effects of Nitric oxide

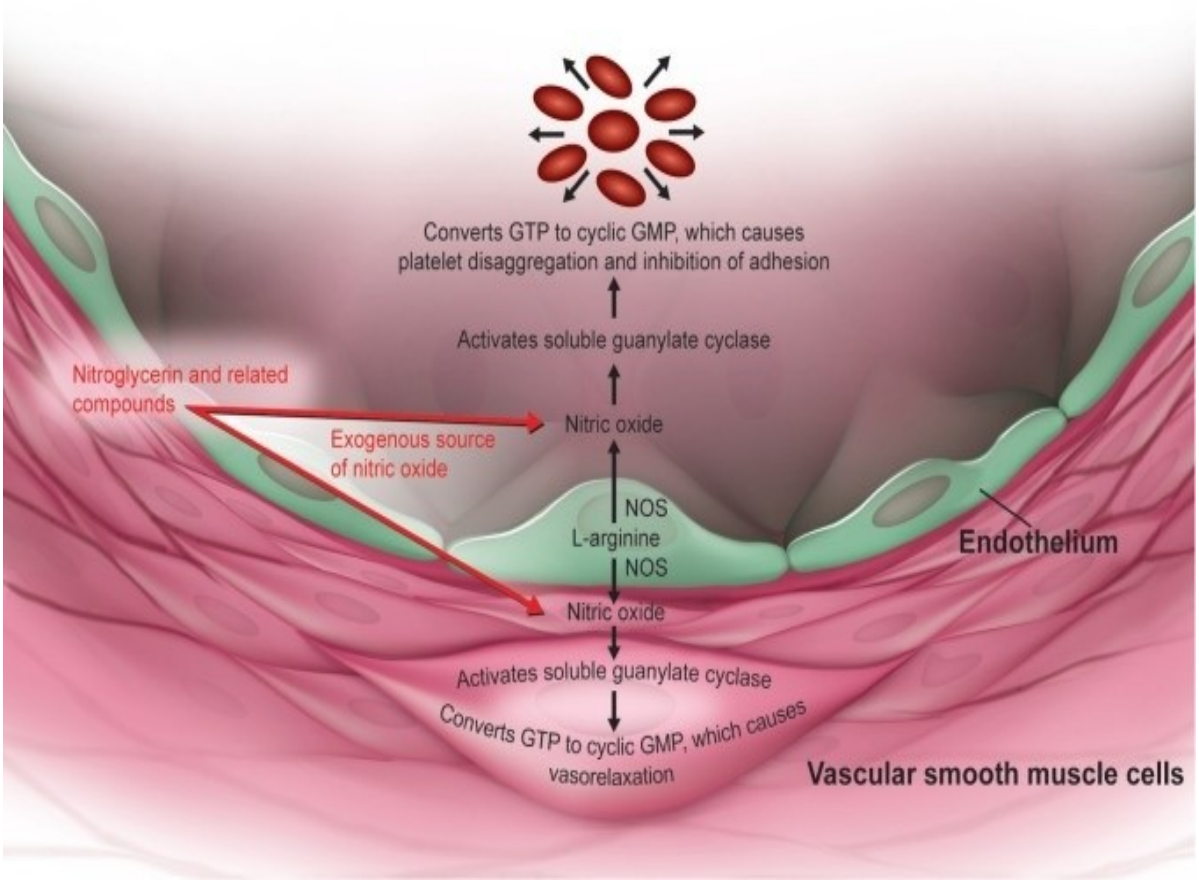


Fig-1.13: NO & cGMP reaction on endothelial cells induced by Nitro compounds (Picture Credit: Boden et al. 2015,). Nitroglycerin is one of the oldest used short-acting nitro compounds in antiangina. This nitroglycerin induces nitric oxide and activates cGMP which triggers vasorelaxation.

DERMCIDIN ISOFORM-2

Dermcidin is an anti-microbial protein found in the sweat gland of human as a part of the innate immune defense system (Schittek *et al*. 2001). This proteolytically processed 47 kDa protein is expressed in sweat gland and showed anti-microbial activity against different types of pathogenic microorganism (Schittek *et al*. 2001). Dermcidin is a proteolysis inducing factor; its C-terminal end is expressed in sweat as anti-microbial and anti-fungal activity and its N-terminal peptide is expressed in severe oxidative stress and associated with cachexia in cancer (Bancovik *et al*. 2015). After production of dermcidin protein, it undergoes extensive proteolytic modification. It was found that differential proteolysis is responsible for different DCD peptides (Flad *et al*. 2002).

Dermcidin structure activity

Dermcidin or proteolysis inducing factor (PIF) is located in chromosome 12, locus 12q13.1. This 11kDa protein is processed into different length of peptide (47amino acid) fragment derived from the C terminal region; function as antibiotic (Schittek *et al*. 2001) 30 amino acids and 20 amino acids fragment derived from N-terminus region as survival evasion peptide (Cunningham *et al*. 1998, 2002).

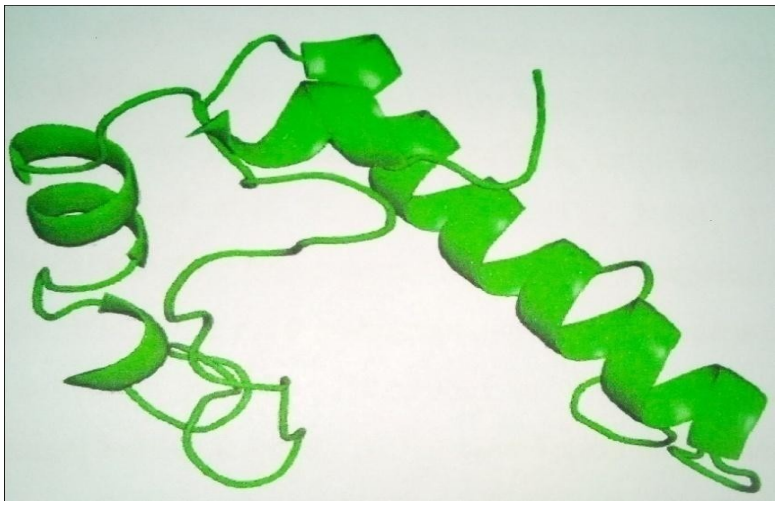


***Fig-1.14**: 3dimensional structure of dermcidin*

Dermcidin in acute myocardial infarction

Dermcidin, a stress induced protein in the circulation of AIHD patients might play the pivotal role in the disease. Though its molecular mechanistic function in the formation of a thrombus is not completely revealed, biochemistry of the protein was observed in our laboratory. The protein was separated in SDS-PAGE at 11kDa position. This dermcidin can inhibit NO and insulin production (Ghosh *et al* 2011; Bank *et al* 2014).

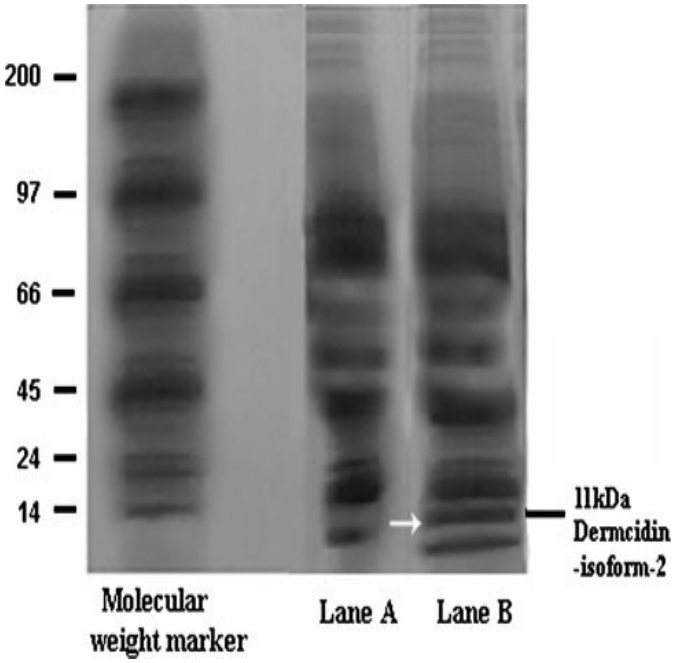


***Fig-1.15: 11kDa dermcidin on SDS-PAGE; Figure Credit: Ghosh et al . 2011** AMI plasma was run on SDS-PAGE (Lane-B) and while (Lane-A) indicated normal plasma and molecualr wt. marker proteins as indicated were also electrophoresed. The white arrow (→) denoted the 11 kDa protein band in the AMI plasma.*

Dermcidin and nitric oxide

It was found that acetyl salicylic acid has the ability to nullify the effect of dermcidin (Ghosh *et al.,* 2010*;* Bank *et al.,* 2014) and it has been also claimed in our laboratory that aspirin was able to inhibit the effect dermcidin through the nitric oxide (NO) production (Karmahapatra *et al.,* 2007; Ghosh *et al.,* 2010; Bank *et al.,* 2014) and it has been found that in presence of NAME (N^G- nitro-L-arginine methyl ester), NO production by aspirin

was inhibited in that case and addition of NAME decreased the stimulation of insulin synthesis in islets in presence of dermcidin with the simultaneous inhibition of aspirin induced nitric oxide synthesis (Ghosh *et al.,* 2010).

Dermcidin in Hypertension

From our laboratory, the role of (r)-Cortexin was found to play a key role in hypertension. It was reported that the protein is involved in blood pressure and the protein possessed the anti-hypertensive property (Chakraborty S *et al*. 2009). This (r)-cortexin, which was isolated from goat kidney cortex cells, acts through nitric oxide signaling pathway. And we also found that aspirin can induce the (r)-cortexin protein and the supply of this protein in endothelial cells can trigger nitric oxide. In another aspect, dermcidin protein was identified from AMI plasma. This protein was found to inhibit (r)-cortexin and nitric oxide.

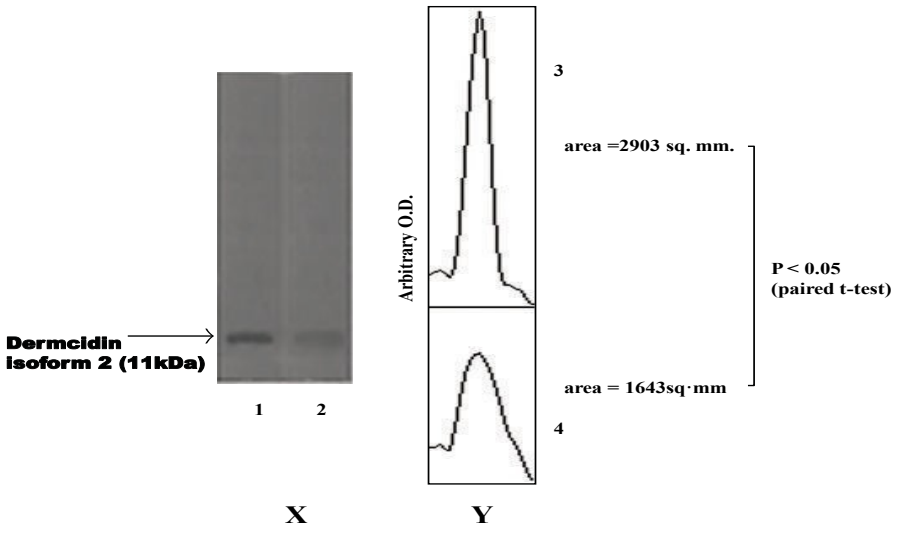


***Fig-1.16: Figure demonstrates the Immunoblot analysis of the dermcidin in the plasma of hypertensive subjects after and before the ingestion of aspirin (Figure adapted from**: **Ghosh, Bank et al., Cardiol Res Pract. 2014).** And the immunoblot was performed in the presence of dermcidin antibody as indicated in panel X. The band intensities of the blot were determined by using the Image J software as in panel Y. Panel (X): 1 denotes the band of dermcidin before the Aspirin ingestion, 2 represents band of dermcidin after the oral ingestion of aspirin. Panel (Y): 3 indicates profile plot of the dermcidin band intensity in the hypertensive patients before the ingestion of aspirin, whereas 4 represents the corresponding band intensity of the dermcidin protein after oral ingestion of aspirin in the same patients. The figure is a typical representative of at least 10 different experiments using 10 different samples from 10 different volunteers (male = 5, female = 5).*

Role of dermcidin in ADP induced thrombosis in animals

Dermcidin, a stress protein induces thrombosis formation

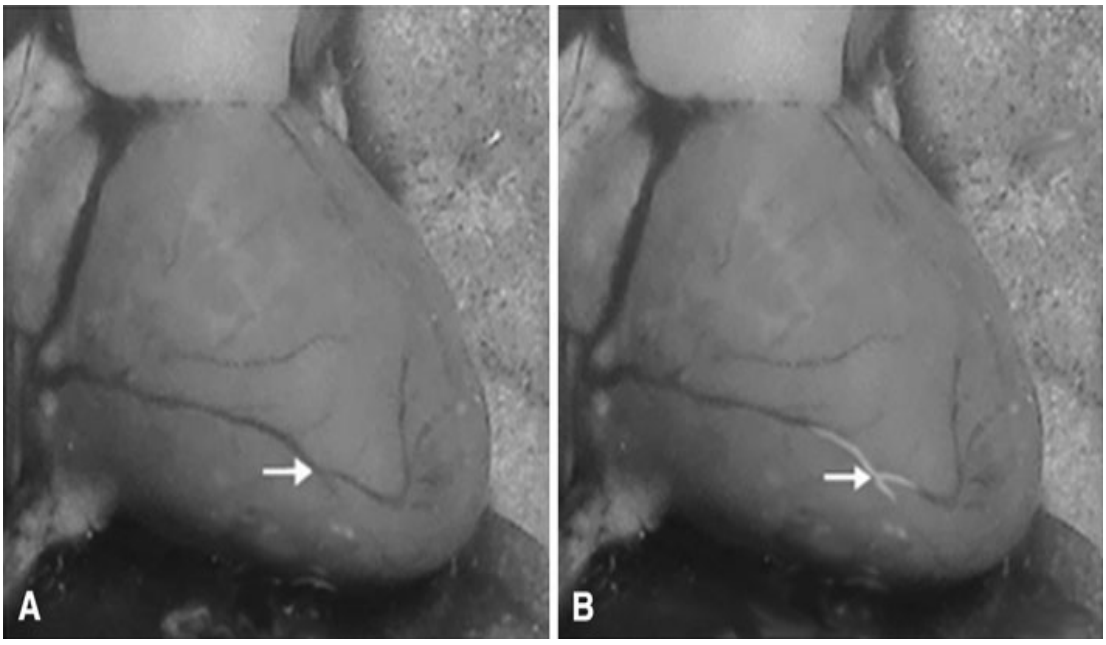


Fig-1.17: This figure demonstrated the consequence of dermcidin injection with ADP in the circulation of mice in the development of coronary thrombosis. Figure Credit:

Ghosh et al. 2011. *The injection of either 0.25 ± 0.03 nmol ADP/g body weight or 3 ± 0.05 nmol dermcidin/g body weight of the mice (n = 10) alone did not result in the formation of thrombus (panel A, white arrow showing the clear coronary artery). The injection of 3 nmol dermcidin/g body weight followed by the injection of 0.25 nmol ADP/g body weight resulted in the formation of thrombus in the coronary artery in the test animal (panel B, the arrow showing the thrombi in the coronary artery as white streaks).*

Insulin

Insulin is a small peptide, it is composed of 51 amino acids and its molecular weight is 5808 Da. It is a very important hormone which is secreted from the beta cells of pancreatic islet which plays the major role in the metabolism of carbohydrate, fat and protein. This is the only peptide which regulates glucose in the circulation. Insulin helps in maintaining glucose homeostasis through GLUT-4 translocation.

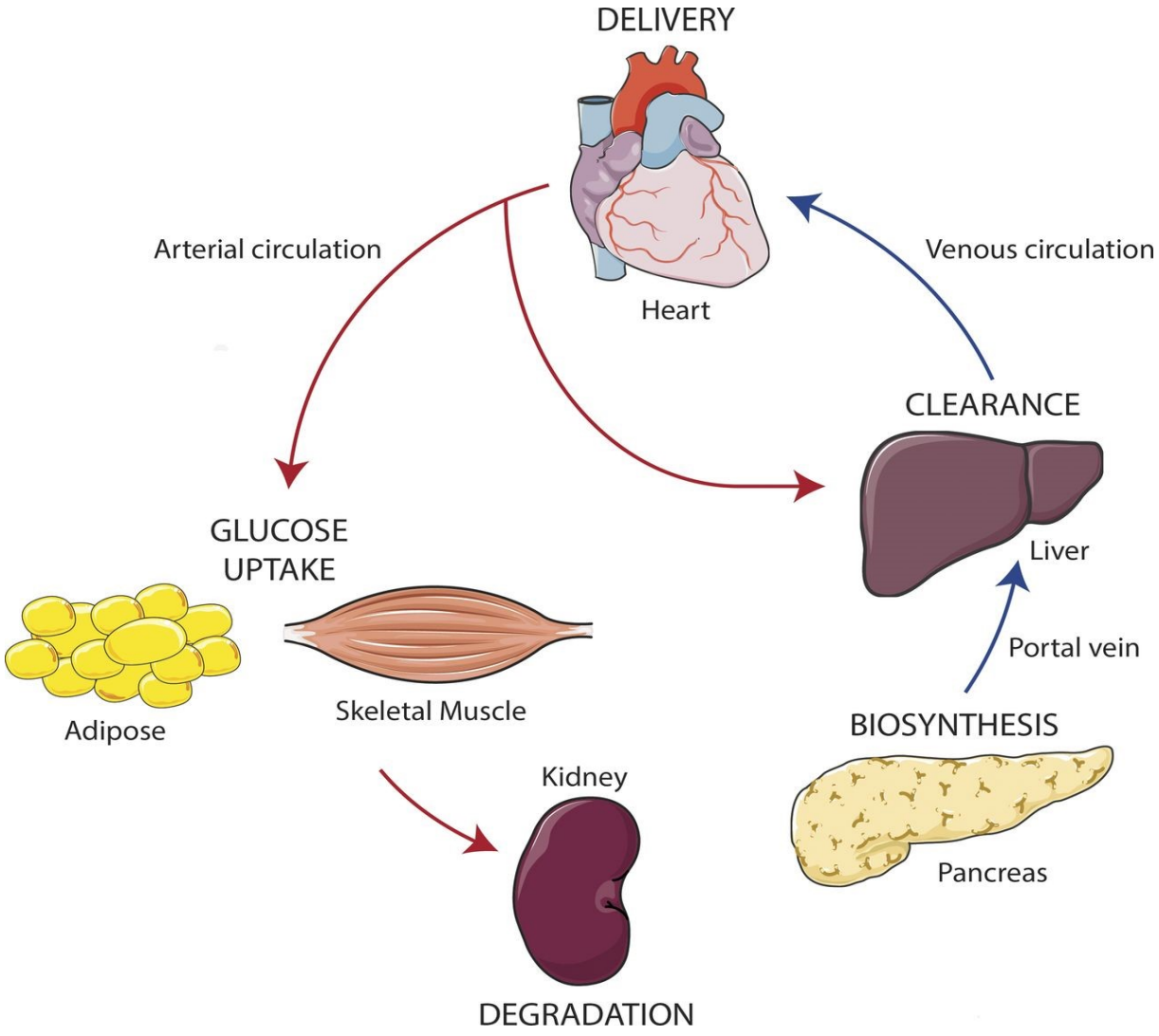


Fig-1.18: Journey of insulin in the body (Figure Credit: Tokarz et al. 2018). *Insulin is synthesized from the pancreatic β-cells, and the synthesized insulin is exported to the liver through the portal vein. During the portal vein transportation, almost half of the insulin is cleared in the liver by the hepatocytes and the rest of the insulin exported from the liver to the heart through the hepatic vein. Then the insulin is disseminated in the circulation through the artery. Insulin in the arterial walls helps in vasodilation. Arterially delivered insulin exerts its metabolic actions in the liver and is further cleared (2^{nd} time pass). Insulin when comes to the fat cells and muscle cells through the circulation, it triggers*

glucose uptake by the cells through GLUT4 translocation. And ultimately the rest of the insulin is destroyed by the kidney. This figure was developed by using Servier Medical Art (available at https://smart.servier.com/)

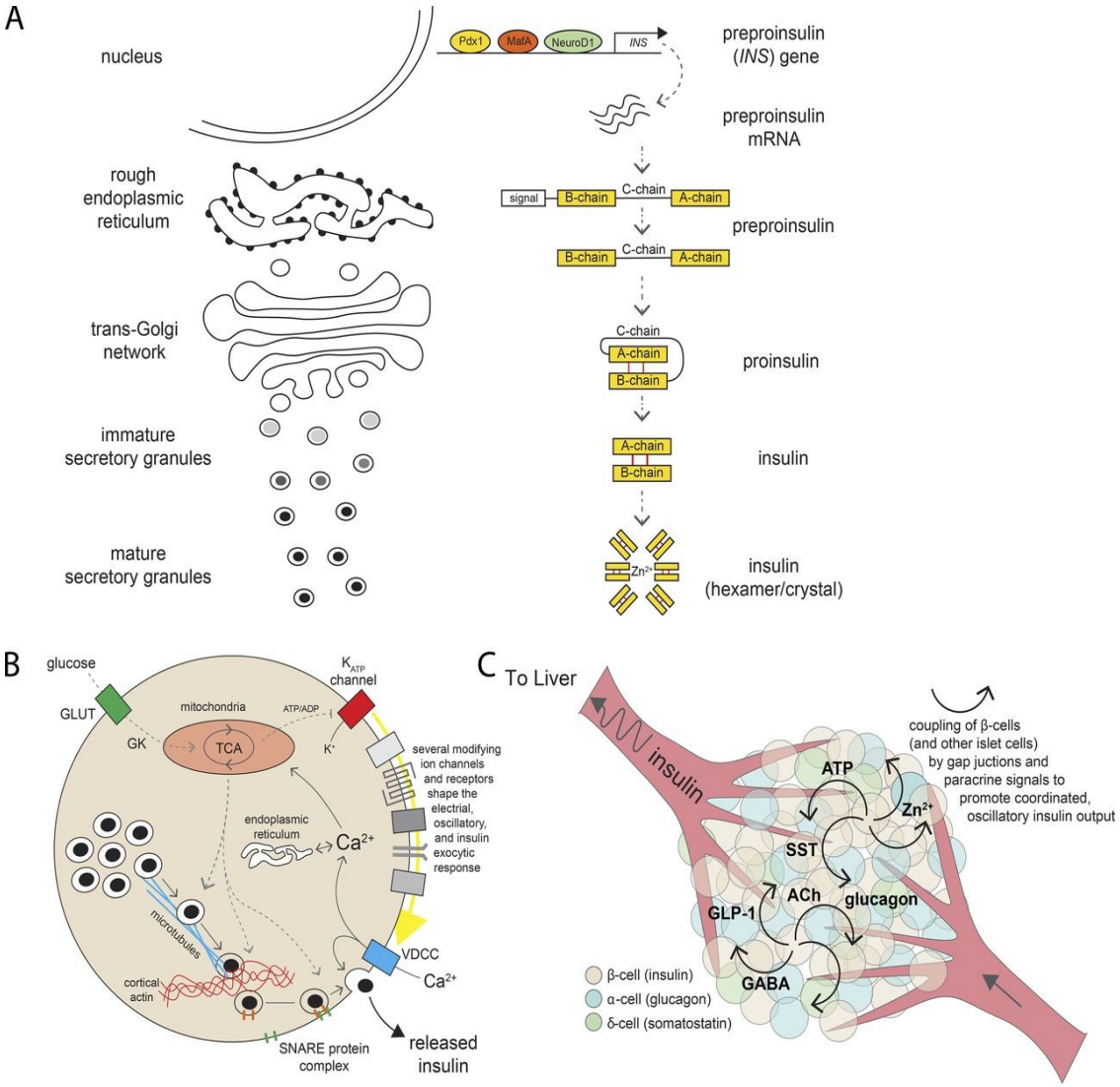


Fig-1.19: Insulin biosynthesis and secretion (Figure Credit: Tokarz et al. 2018). *(A) Maturation of insulin through the granule secretory pathway. At the start of the pathway, preproinsulin (INS) gene is transcripted to preproinsulin mRNA which is translated to preproinsulin peptide and the transition is through the RER and TGN,*

through this transition maturation of the prepropeptide is happened and finally stored as insulin/Zn^{2+} crystals in the hexameric form within mature secretory granules. (B) Secretion of insulin induced by metabolic signaling and glucose sensing. Metabolic signaling and the exocytosis process of insulin releasing from the granules (secretary) of β-cells are tuned by a cascade of electrical and metabolic signals and as a result, glucose molecules enter into the cells by GLUTs translocation, GK mediated phosphorylation, and then enter into the TCA cycle. When ATP dependent K^+ (K_{ATP}) channels close, it triggers the entry of Ca^{2+} through VDCC by which SNARE complex induced exocytosis is happenes. So, the total response is modulated by numerous channels, receptors and intracellular storage of Ca^{2+}, different metabolic signals. (C) Insulin secretion in a pulsatile fashion. β-cells of islet communicate with α-cell, which produce glucagon and with somatostatin, which produce from δ-cells to coordinate their activity. It is supposed that many other messengers like Zn^{2+}, ATP, γ-aminobutyric acid (GABA), GLP-1, acetylcholine (Ach) have been involved therein.

Insulin signaling

Insulin signaling cascade is an important incident in the cell biology. It bind on the cell surface receptors then goes through step by step signaling cascade and through various proteins insulin helps in the translocation of GLUT-4 in adipocytes and muscle cells.

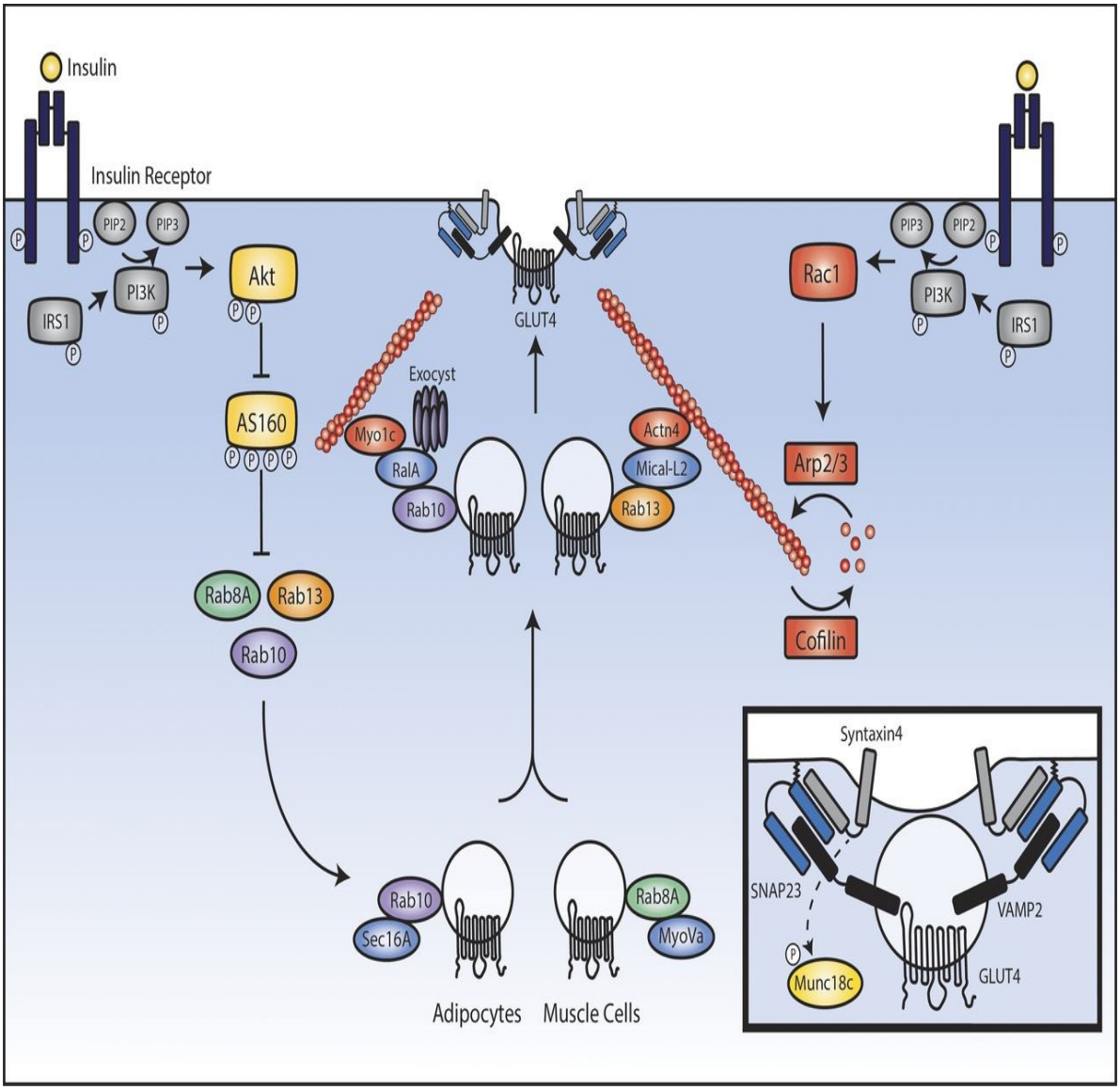


Fig-1.20: Insulin signaling cascade through the GLUT4 to the plasma membrane*. (**Figure Credit: Tokarz et al. 2018).** When insulin attaches to the receptor on the muscle cell surface or to the fat cells, this creates insulin-signaling cascade to Akt and PI3K. In the muscle cells, Akt and phosphorylation of AS160 activates Rab8A and Rab13 (in muscle*

cells) and Rab10 (in adipocytes). In the perinuclear region, Rab8A involves with its effector, MyoVa, and Rab10 with its effector, Sec16A, to promote outward vesicle traffic. Rab13 is involved with Actinin-4 and MICAL-L2 near to the plasma membrane, whereas Rab10 engages with RalA, Myo1c, and Exocyst components. Simultaneously, downstream of PI3K, insulin leads to activation of Rac1 that promotes a dynamic cycle of cortical actin remodeling. Close to the plasma membrane, all the functions together can tie GLUT4 vesicles to the actin cytoskeleton. Inset: Here in inset anchored GLUT4 vesicle is ready to merge with the plasma membrane. Immobilized GLUT4 vesicles fuse with the membrane through formation of a SNARE complex between vesicular VAMP2 and syntaxin4 and SNAP23 on the plasma membrane.

GLUT-4

Energy is required for the body and glucose is the primary source for the fueling. All cells need glucose for the activity of the cell, though the brain cells are the main consumer of glucose. It is distributed in most cells by the membrane protein, which facilitates the diffusive transportation of glucose from the circulation into the cell. Insulin plays the lead role in glucose homeostasis of a cell by triggering one of these transporter proteins, GLUT-4 which helps in the uptake of glucose from the blood into the skeletal muscle.

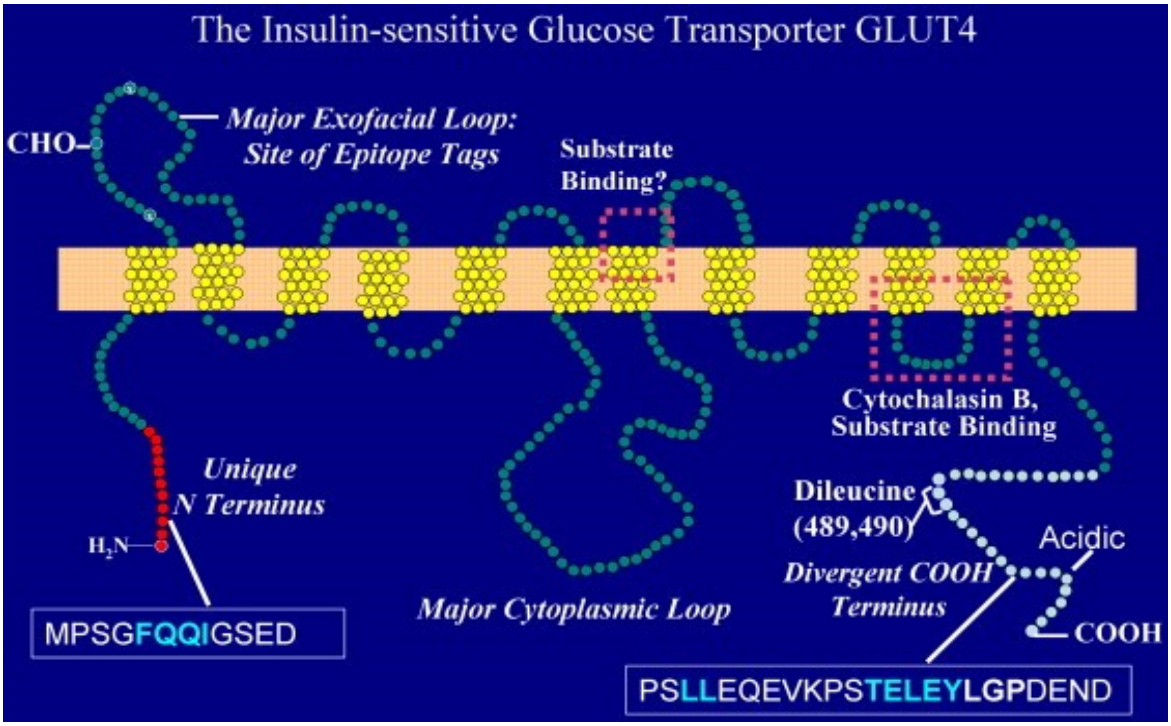


Fig-1.21: Figure demonstrates that Structural Features of the Insulin-Regulated GLUT4 Glucose Transporter Protein. [Figure Credit: Huang et al., 2007, The GLUT4 glucose transporter]. *The unique sensitivity of GLUT4 to insulin-mediated translocation appears to derive from sequences shown in the N-terminal (required phenylalanine) and COOH-terminal (required dileucine and acidic residues) regions. These sequences are likely involved in rapid internalization and sorting of GLUT4 in intracellular membranes termed GLUT4 storage vesicles (GSV).*

GLUT-4 protein has unique N terminal sequence with a phenylalanine residue (Al-Hasani *et al*., 2002) and with dileucine and acidic motif at the C terminus helps it exocytosis and endocytosis trafficking system. The C-terminal dileucine and acidic motifs are also found in insulin regulated amino peptidase (IRAP) protein, which is the major protein of GLUT-4 containing vesicles (Keller *et al*., 1995) and from the various biochemical studies, it was found that GLUT-4 and IRAP share the very similar trafficking route in adiposities (Ross *et al*., 1996; Garza and Birnbaum, 2000). So, GLUT-4 contributes for the major role both in insulin signaling and membrane trafficking system

GLUT-4 distribution

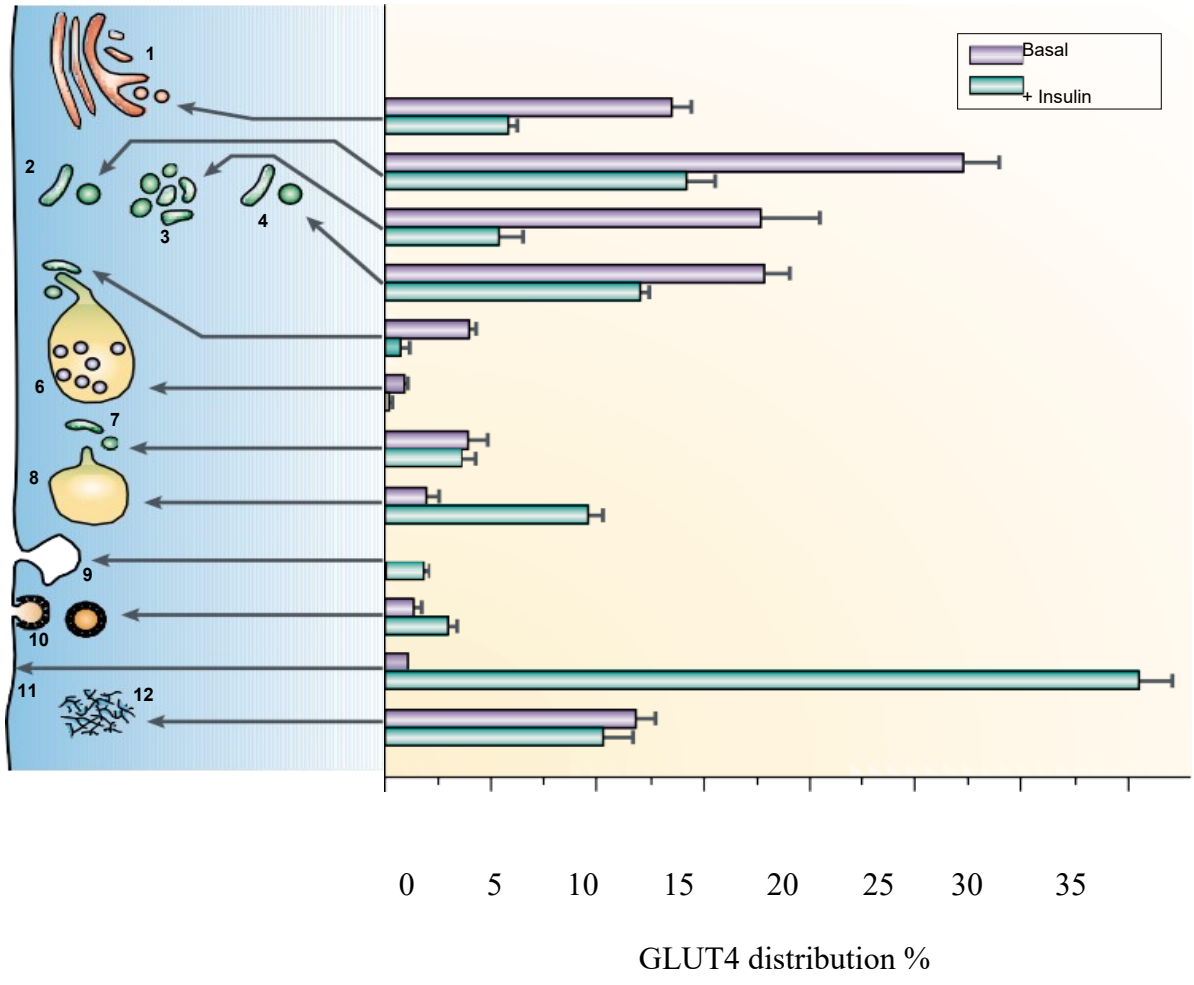


***Fig-1.22**: **This figure demonstrated: Distribution of GLUT4 in the organelles of cells in insulin stimulated or non-stimulated brown adipose tissue (Figure Credit: Bryant et al. 2002).** Brown adipose tissue were labeled and cryosectioned with Protein A and GLUT4 antibody which are gold-conjugated. Those Gold particles were enumareted and assigned to the following organelles: (1) trans-Golgi network (TGN); (2) tubulo–vesicular (T–V) elements which are placed beneath the plasma membrane; (3) clump of tubulo–vesicular elements; (4) dispersed tubulo-vesicular (T-V) elements throughout the cytoplasm; (5) attachment of T–V elements to late endosomal vacuoles (6); (7) T–V elements attached or closely situated to early endosomal vacuoles (8); (9) non-coated invaginations of the plasma membrane; (10) vesicles and coated pits; (11) plasma membrane; (12) cytoplasm. Here, the right-side graph shows the relative distribution of GLUT4 throughout the above described organelles. From REF. 16 ©1991 The Rockefeller University Press.*

GLUT-4 translocation

GLUT-4 translocation maintains glucose homoeostasis in the cell but the GLUT-4 translocation is the incident which is occurred by two cycle phenomena according to the following model. Cycle are among the endosome – cell surface and endosome – TGN.

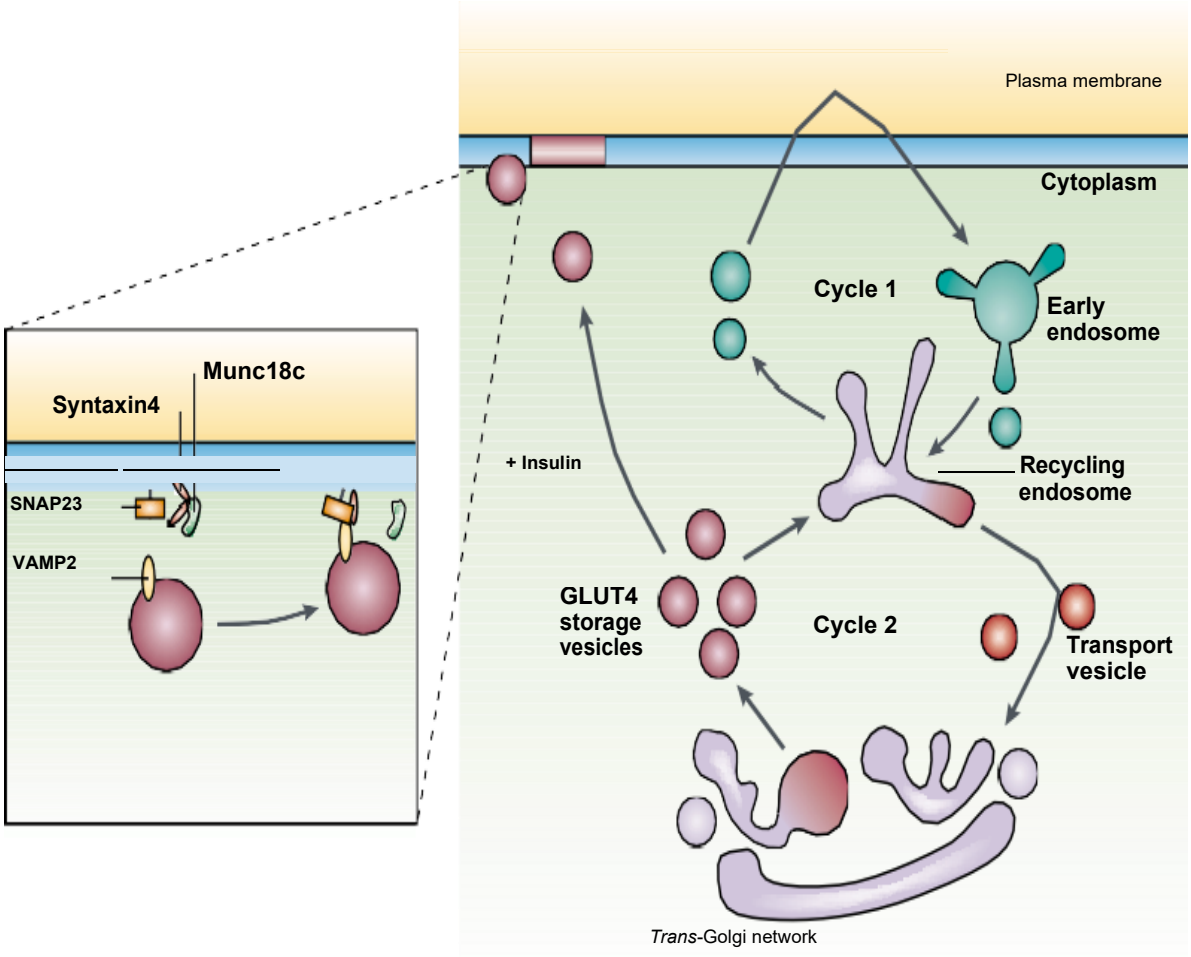


Fig1.-23: A model that depicts the transport of GLUT4 in insulin-responsive cells. (Figure Credit: Bryant et al., 2002). *The above model which is depicted in Nature Reviews: Molecular Cell Biology by Bryant NJ, demonstrates two recycling pathways in the cells and GLUT4: cycle 1 represents the recycling between the endosomes and cell surface; and cycle 2 represents between the endosomes and trans-Golgi network (TGN). Transportation of GLUT4 is regulated at many places in these cycles. On entry into the endosomal system, GLUT4 is selectively retained at the expense of other recycling*

transport, such as the transferrin receptor that constitutively moves through cycle 1. This retention mechanism might predispose GLUT4 for sorting into transport vesicles that bud slowly from the endosome and that are targeted to the TGN. GLUT4 is sorted into a secretory pathway in the TGN. This sorting step probably involves a specialized population of secretory vesicles that excludes other secretory cargo, and that does not fuse constitutively with the plasma membrane. Vesicles that emerge from this sorting step, which we have previously referred to as GLUT4 storage vesicles or GSVs, might constitute most of the GLUT4 that is excluded from the endosomal system. In the absence of insulin, GSVs might slowly fuse with endosomes, thereby accounting for the presence of a significant but small pool of GLUT4 in endosomes, even in the absence of insulin. Insulin would then shift GLUT4 from this TGN–endosome cycle to a pathway that takes GLUT4 directly to the cell surface. The inset shows the SNARE proteins that are thought to regulate docking and fusion of GSVs with the cell surface. The t-SNAREs Syntaxin 4 and SNAP23 in the plasma membrane of fat and muscle cells form a ternary complex with the v-SNARE VAMP2, which is present on GSVs. Munc18c has been identified as the SM (Sec1-like/Munc18 family) protein that controls the formation of this ternary complex.

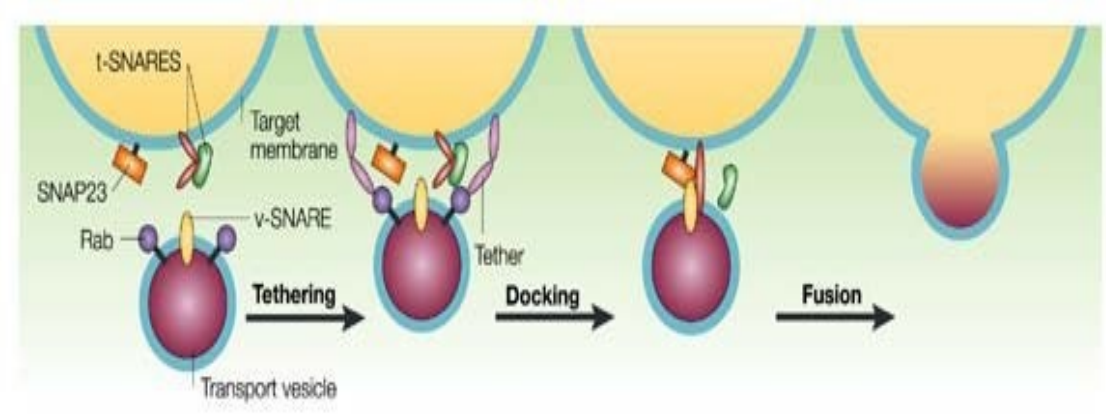


Fig-1.2: SNARE helps in the regulation of docking and fusion of GSV to the membrane (Figure Credit: Bryant et al., 2002).

The membrane transport incidents are controlled by SNAREs and SNARE-associated proteins. v-SNAREs are found in transport vesicles, they can bind to t-SNAREs (membrane proteins that are found on the relevant target membrane) in a definite specific way. So, it is very crucial to form a stable complex between the two sets of SNARE proteins (perfect) which actually assists the target membrane and transport vesicles in closeness and helps to their fusion. Although there is not a clear proof on membrane docking and fusion, these molecules and their associated proteins clearly have an important role in membrane fusion. Membrane fusion can be broken down into the three distinct stages, as outlined in the figure. Vesicle tethering: Rab family proteins and small GTPase is the cause for the vesicles (which transport) to its perfect target membrane.

Vesicle docking: After a transport vesicle is tethered to its target membrane, the formation of a stable ternary SNARE complex docks the transport vesicle onto the target membrane. The Sec1-like/Munc18 (SM) family adds a further level of regulation to membrane fusion at this stage. SM proteins seem to have both a positive and negative role in SNARE complex assembly. These proteins bind tightly to t-SNARE molecules and prevent ternary-complex formation. However, their binding also seems to be required to activate the t-SNARE for entry into the ternary complex. The formation of a stable SNARE complex completes the docking stage of vesicular transport.

If we look at the family of SNARE, Rab and SM proteins and cells throughout evolution, we can see that they are all highly conserved. A situation is emerging in many cellular systems, in each member they have different transport vesicles and a target (or acceptor)

membranes. And the coordination and fusion with the target membrane is maintained by a highly regulated process.

PROTEIN DISULFIDE ISOMERASE (PDI)

Protein folding is an essential step for the survival of a cell and this function is assisted by proper protein folding and chaperones that inhibit aggregation. Disulfide bond formation is one of the key steps for the proper functioning of many proteins.

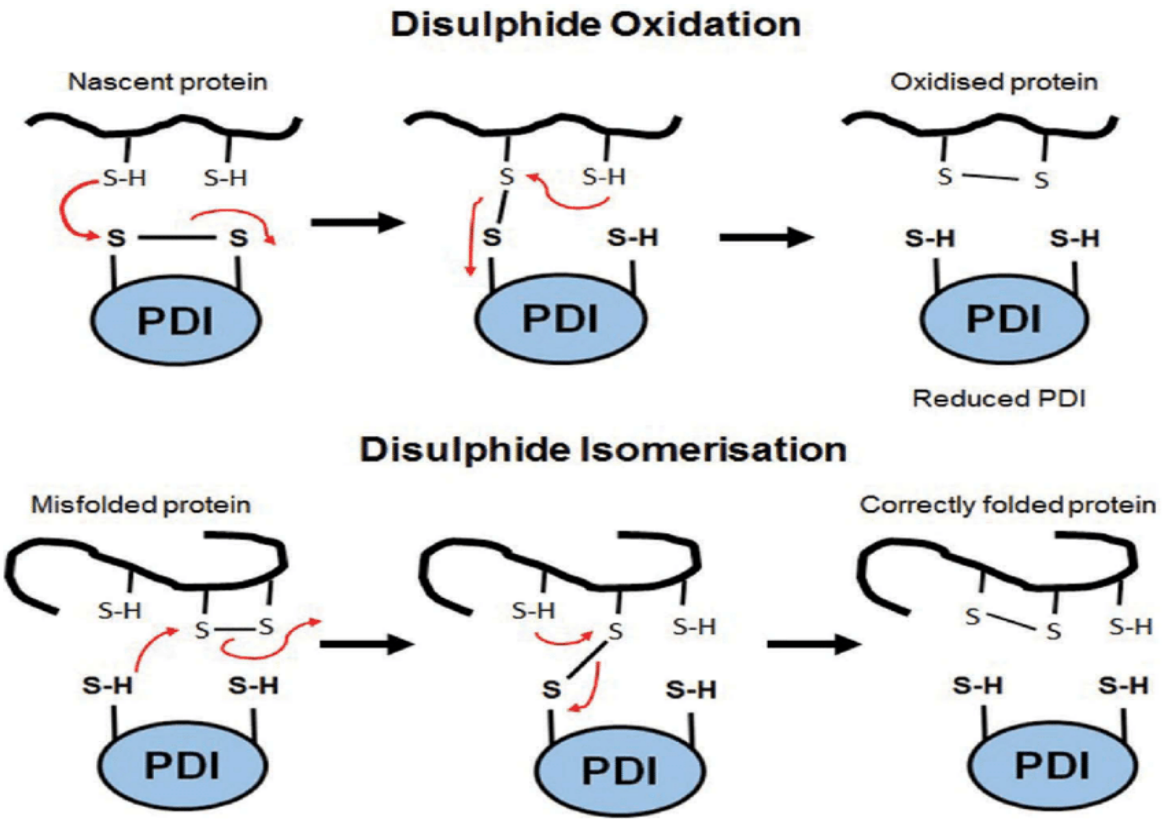


Fig-1.25: PDI has the disulphide interchange activity (Adapted from Benhar et al., 2006 Nitrosative stress in the ER: a new role for S-nitrosylation in neurodegenerative diseases. ACS Chem Biol. 1(6):355-8). *PDI can oxidize (form), reduce (break down), and isomerise (rearrange) the disulphide bonds of misfolded proteins. These events by PDI actually help and maintain the perfect conformation.*

So, protein folding is an important step; misfolding induces protein aggregation and creates neurodegenerative diseases - Alzheimer's disease (Koo *et al*., 1999; Harper *et al*., 1997), emphysema (Cabral *et al*., 2001), Prion protein disease (DeBurman *et al*., 1997), Cystic fibrosis disease (Qu *et al.*, 1997).

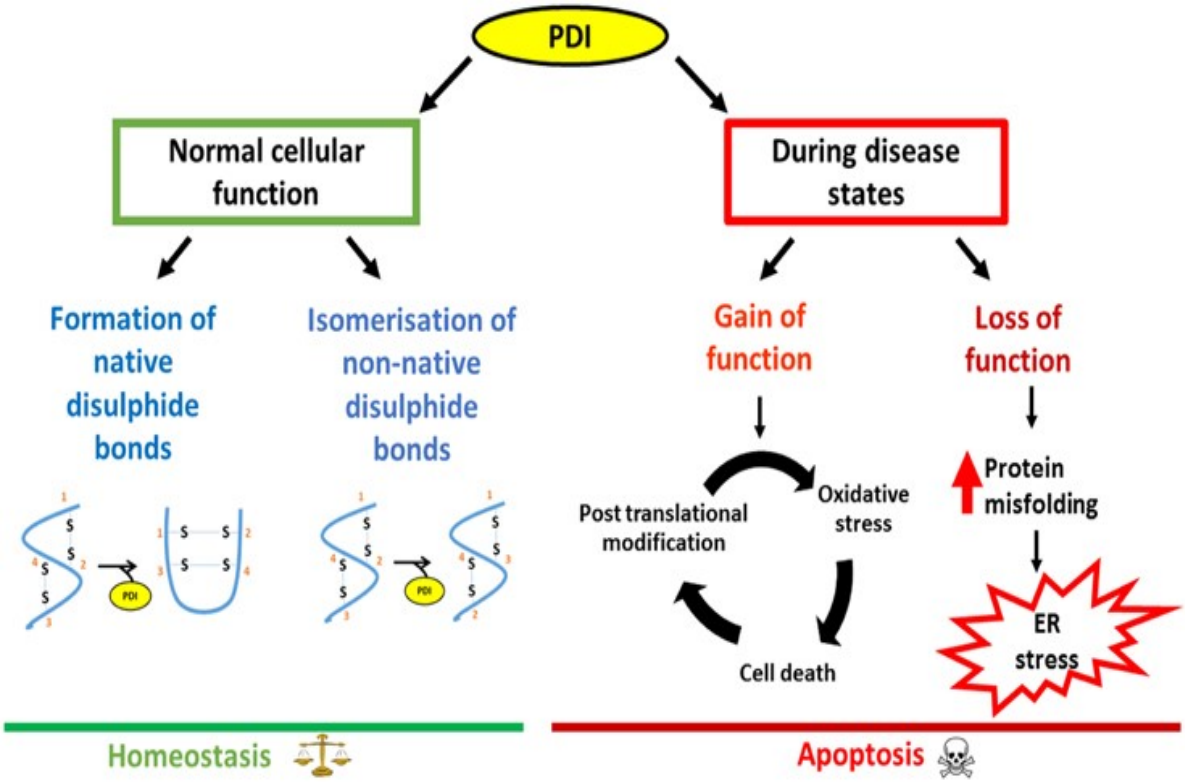


Fig-1.26: Schematic diagram: Twin nature of PDI on neurodegenerative disorders as an example. (Figure credit: Parakh et al., 2015). *Under normal conditions, PDI reduces the load of misfolded proteins either by its chaperone activity or by isomerization of non-native bonds. However, during disease states, loss of the normal protective function of PDI or the gain of additional, toxic functions, leads the cell to apoptosis, thus contributing to pathology.*

TRIAD EFFECT IN ACUTE MYOCARDIAL INFARCTION

Cell death occur due to the atherosclerotic plaque rupture because blood supply is very less than demand and as a consequence, there is a lack of O_2, nutrients, glucose (Uryga *et al*., 2015), cells loss their normal activity.

Different types of oxidative stress and H_2O_2 can cause platelet aggregation, and these are the factors responsible for the AMI formation. Different type of free radicals and H_2O_2 are generated at the time of platelet aggregation, and then catalase acts as a defender (Del Principe *et al*., 1985) and saves the cells from death also by the caspase inhibition via catalase activation (Murtaza *et al*., 2008). We previously claimed that dermcidin is involved in the prognosis of AMI and it's significantly higher in AMI circulation as compared to acute coronary syndrome patients and normal plasma (Bank *et al*., 2014). Insulin and low-dose-aspirin are decisively considered to be inhibitors of DCN-2 (Bank *et al*. 2014). Actually, DCN was able to obstruct the action of insulin and aspirin. Thus, aspirin was unable to inhibit the platelet aggregation in AMI patients.

Both of these organic (aspirin) and physiological (insulin) compounds were able to produce NO through the activation of aspirin-activated NOS (Karmohapatra *et al*., 2007) and insulin-activated NOS (Ray *et al*., 2012), respectively, and eliminated the effect of dermcidin (Bank *et al*., 2014). We have already reported that exact amount of aspirin or insulin was able to produce NO. Precisely regulated physiological level of NO is a very important messenger molecule that plays an instrumental role in different cells and tissues of various functions. One of the essential roles of NO is to inhibit the unnatural cell death in AMI by impairing the Fas expression (Mannick *et al*., 1997).

On the other hand, if the availability of catalase in an AMI patient remains normal, cell death in AMI might be inhibited due to the reduction of free radicals during platelet aggregation. Therefore, by the interactive role of the TRIAD system (catalase, insulin,

and aspirin), the cell death in AMI can be stopped and even the consequence of the recurrence of AMI due to the

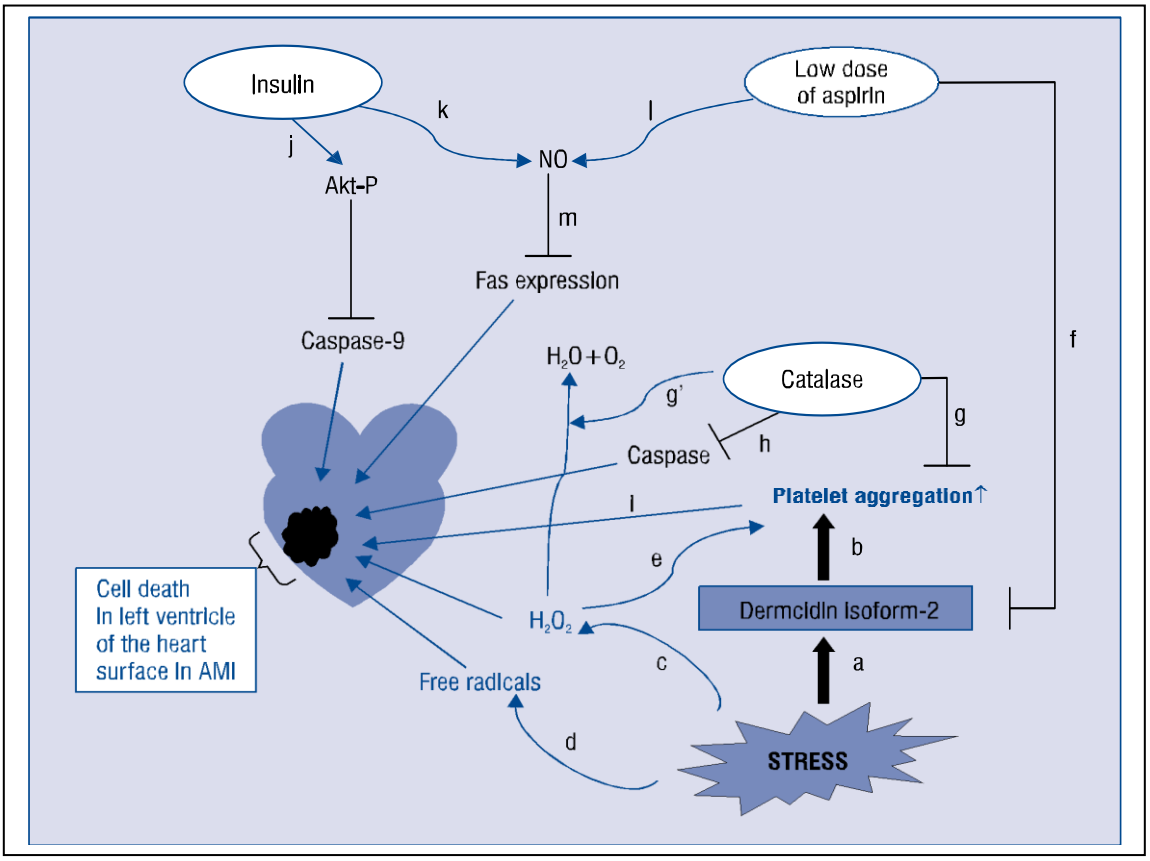


Fig-1.27: TRIAD effect on Dermcidin in AIHD; (Figure credit: Bank et al., 2016). *This diagram is indicated that how TRIAD system of low dose of aspirin, insulin and catalase inhibit cell death mainly through NO signaling pathway.*

negative effect of DCN-2 could be prevented. As a result, it can also be argued that if we were able to inhibit NOS in the heart by gene silencing through double stranded RNA-interference pathway, it would be better to understand the efficacy of the TRIAD system on DCN-2. In this context, our report explains the mechanism of cell death occurrence in AMI patients by the stress-induced protein DCN-2 and provides explanation how cell

death can be prevented, and recurrence of the disease stopped by a unique solution of maintaining catalase, insulin, and low-dose-aspirin in AMI patients.

SYNTHESIS OF DERMCIDIN IN LOW PARTIAL PRESSURE OF OXYGEN

In this context, we have also analyzed the high altitude illness (HAI), which is a cluster of syndromes which develop in the population who are not acclimatized to the reduced partial pressure of O_2 in the atmosphere. While for syndromes such as thrombophlebitis at high altitude, water and electrolyte imbalance and increased capillary permeability lead to the accumulation of fluid at different locations of the body, the reduction of the partial pressure of O_2 in the ambient atmosphere is also known to cause central nervous dysfunction leading to dimmed vision and hemorrhages in nails, kidney, and brain. While these syndromes, although alarming in nature, usually disappear rapidly after decent to the plain, HAI is also known to cause the far more life threatening condition of prothrombotic disease due to the development of prediabetic/hypertensive consequences leading to acute coronary syndromes (ACS) where the descent to sea level from the high altitude condition usually produced little or no effect on the ensuing ACS. As such, persons stationed at high altitude are particularly vulnerable to an attack of ACS. Unfortunately, however, neither the mechanism of the development of ACS due to prothrombotic condition, nor any diagnosis for the occurrence of prothrombotic condition, which could be used as a warning to stave off the ominous event that may precipitate ACS has yet been identified.

The feasibility of developing such diagnosis for HAI induced ACS was attempted by our earlier studies that demonstrated a protein of 11 kDa, identified to be dermcidin isoform 2 (dermicdin) which was produced in the system due to various environmental stresses (Ghosh *et al*., 2011; Ghosh *et al*., 2012). Dermcidin was found to result in the

development of acute type 1 diabetes mellitus on the injection of the protein (0.2 μM final) in mice as a test animal (Ghosh *et al*., 2012). Furthermore, dermcidin at the above concentration was also found to increase both systolic and diastolic pressures within 60 minutes of injection in rabbits (Ghosh *et al*., 2012). This stress induced protein was also found to be a potent activator of platelet cyclooxygenase and was found to potentiate the ADP induced platelet aggregation by 70%. As platelet aggregation, particularly by ADP (Chakraborty *et al*., 2003) on the atherosclerotic plaque rupture site in the coronary artery has been reported to cause ACS inducing acute myocardial infarction, the role of dermcidin as a diabetogenic and hypertensive agent, as well as platelet cyclooxygenase activator could be of pathologic significance on the precipitation of ACS at high altitude.

FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET)

FRET is a physical process which depends on the distance and require a dipole-dipole coupling between the fluorophores where energy is transferred from an excited fluorophore molecule (donor) to other fluorophore (acceptor) and the efficacy will be higher when the molecules are positioned within the Förster radius (the distance at which half the excitation energy of the donor is transferred to the acceptor, typically 3–6 nm). The efficiency of this process is proportional to the inverse of the sixth power of the distance between the two fluorophores (Förster, 1965; Clegg, 1996; Lakowicz, 1999), making it a sensitive technique for investigating a variety of biological phenomena that produce changes in molecular proximity (dos Remedios *et al*., 1987). Technological advances in light microscopy imaging, combined with the availability of genetically encoded fluorescent proteins provide the tools necessary to obtain spatial and temporal

distribution of protein associations inside living cells (Heim and Tsien, 1996; Day, 1998; Elangovan *et al*., 2002, 2003).

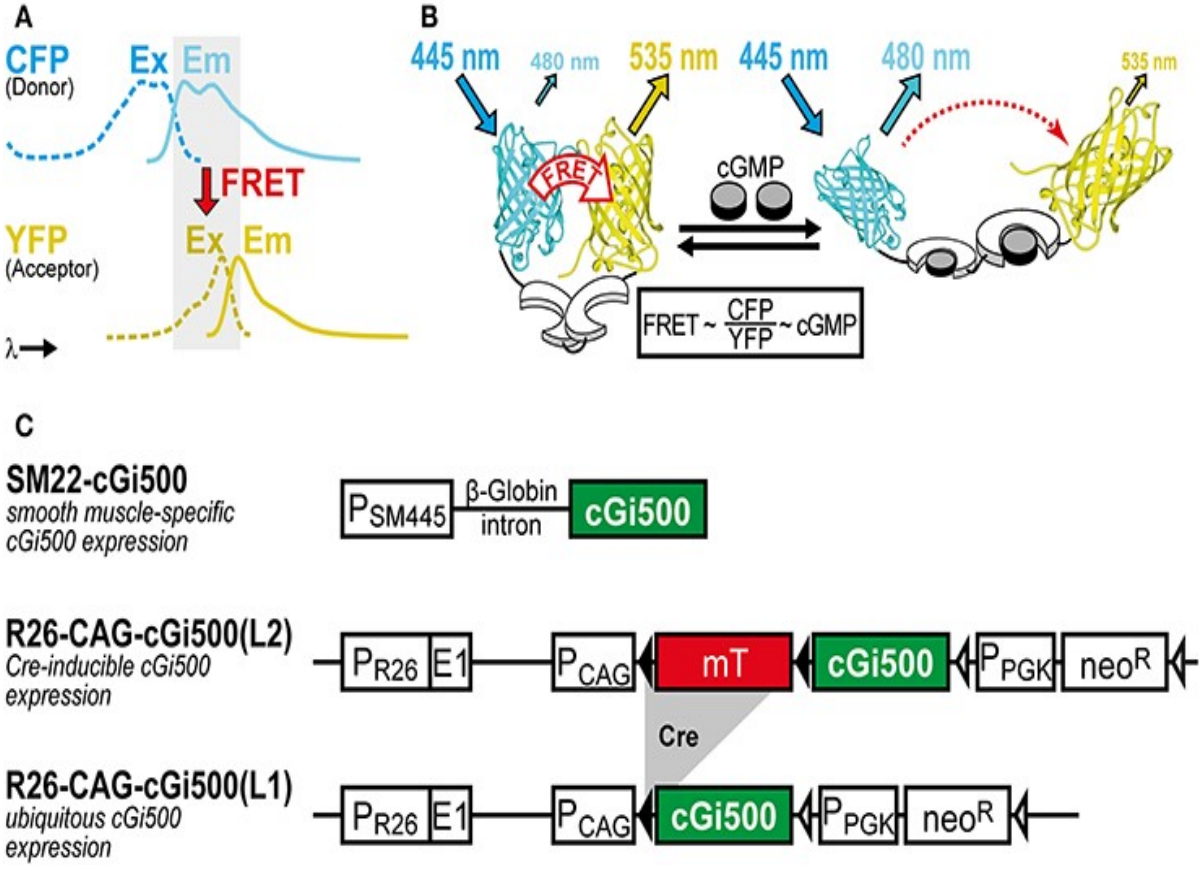


Fig-1.28: Basics of FRET (Figure Credit*: *Thunemann et al., Front Physiol. 2014).

A, B) indicate the fundamental of FRET-based cGi500 sensor and (C) demonstrates the generation of cGi500-expressing mice by using the exogenous gene. (A) Spectral overlap (gray) of YFP excitation (Ex, dashed lines) and CFP emission (Em, solid lines) spectra that is necessary for FRET to occur. (B) The cGMP indicator protein cGi500 consists of the tandem cGMP-binding sites from bovine cGK type I (white) flanked by CFP and YFP. Without cGMP, FRET occurs from excited CFP to YFP, leading to light emission from YFP. Binding of cGMP (gray) causes a conformational change and a decrease in FRET

efficiency, so that light emitted from YFP at 535 nm is reduced and emission from CFP at 480 nm is increased.

(B) is reproduced from Thunemann et al. (2013b). (C) Constructs used to generate transgenic cGi500-expressing mice. Abbreviations: E1, first exon of the endogenous Rosa26 gene; mT, membrane-targeted tandem-dimer tomato red fluorescent protein; neoR, neomycin resistance gene; PCAG, chicken actin/β-globin promoter; PPGK, phosphoglycerate kinase promoter; PSM445, 445-bp promoter fragment of the Transgelin/SM22 gene; PR26, endogenous Rosa26 promoter. Black triangles represent loxP sites, open triangles represent FRET sites

DIABETES

Overview: Diabetes mellitus refers to an assembly of the diseases which affect our body to use blood sugar (glucose). Glucose is the vital for the energy source for the activity of cells and it is also the main fuel for the brain. It is not the matter of diabetes type because diabetes means the excess blood sugar in the circulation and excess sugar (glucose) creates a serious health problem. There is two kinds of diabetes - type 1 diabetes and type 2 diabetes. Diabetes condition may be higher than normal, but it is not high such as classified diabetes, it is the prediabetes and another is gestational diabetes mellitus (GDM) that appears during pregnancy but becomes normal after birth of baby (Adapted from:*https://www.mayoclinic.org/diseases-conditions/diabetes/symptoms-causes/syc-20371444*).

Insulin and Glucose: Insulin hormone plays the main role in diabetes because this protein hormone has an immense role in glucose homoeostasis in the cell.

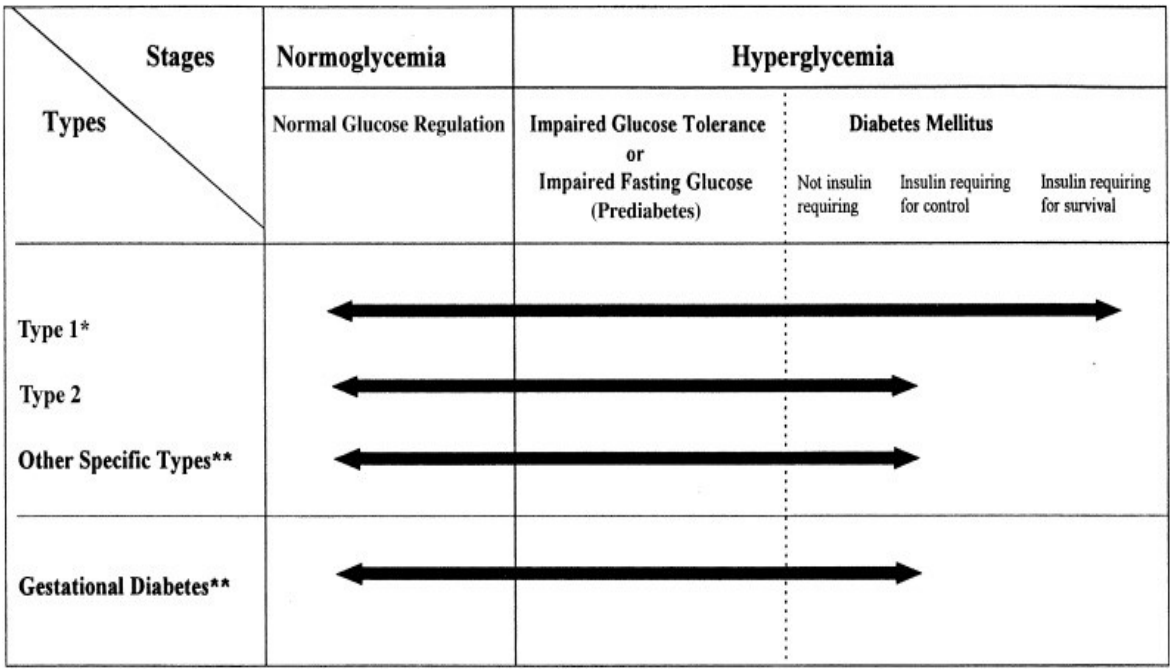


Fig-1.29: Diagnosis and Classification of Diabetes Mellitus

Glycemic disorder: etiologic types and stages. *Here in this case when patients were presented with ketoacidosis, they can shortly return to normoglycemia without any required therapy continuously (i.e., "honeymoon" remission); **in rare incidents, patients of some cases (e.g., Vacor toxicity, type 1 diabetes presenting in pregnancy) insulin may act as a lifesaver where insulin is obviously needed. .

HYPOXIA

Constant oxygen supply is crucial for the viability of cardiac function. The mammalian heart is completely dependent on aerobic condition; it is not possible for the survival of the heart without oxygen.

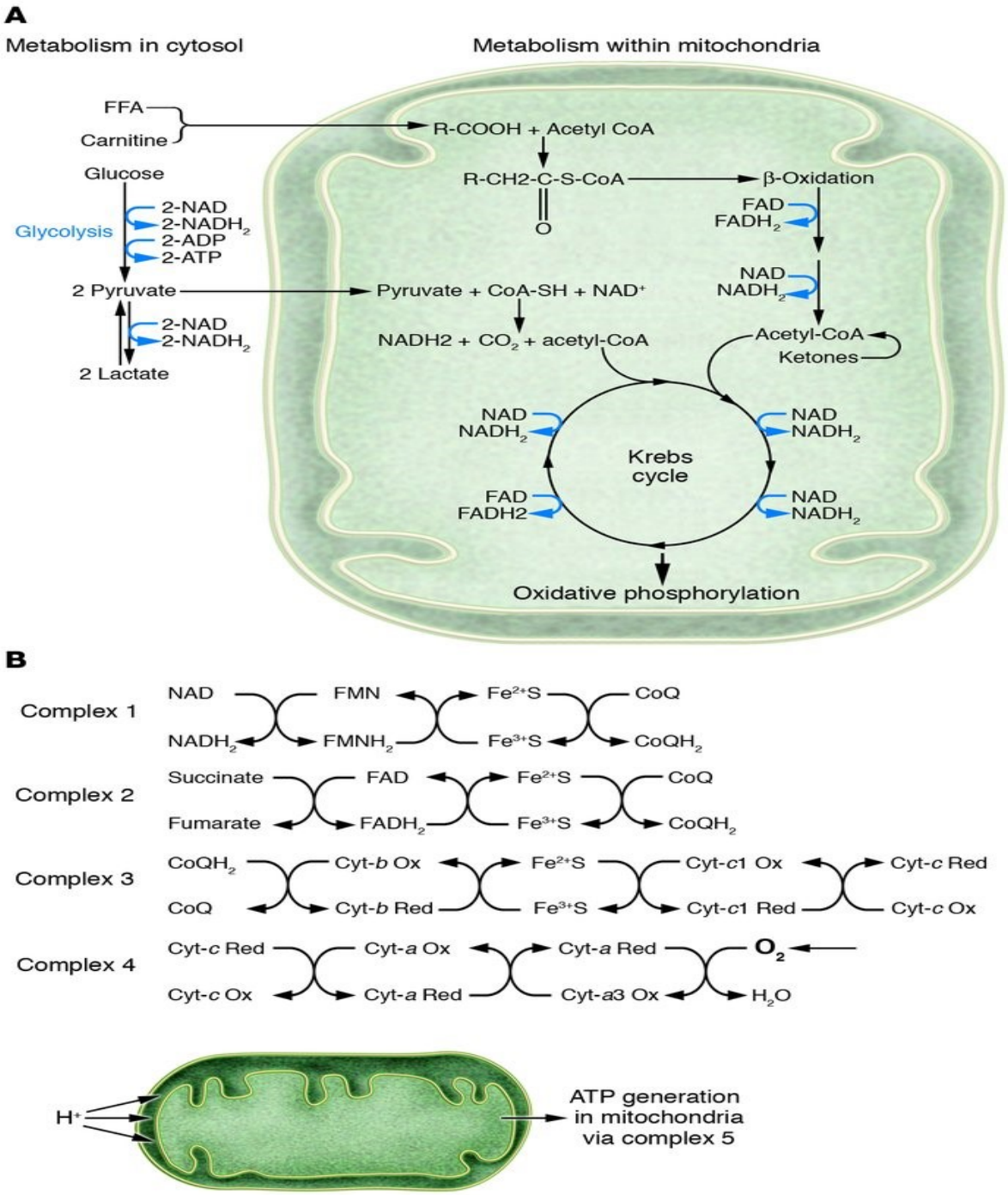


***Fig-1.30: Contribution of oxygen in the metabolism of myocardial cells (Figure Credit**: **Frank J. Giordano. Oxygen, oxidative stress, hypoxia, and heart failure. J Clin Invest. 2005).** (A) depicted the pathways of the utilization of various fuels (glucose, fatty acid, ketones and lactate) by cardiac muscle cells. Oxygen is not needed for the glycolysis process in the cytosol, Ketone metabolism, β-Oxidation of fatty acid and the intermediates derived from glucose metabolism – all produce reduced flavoproteins ($FADH_2$ and $NADH_2$). (B) Schematic diagram of the fundamental oxidative phosphorylation in the mitochondria. In mitochondria each complex from I-IV refer to specific electron transfer steps among the flavoproteins ($NADH_2$, $FMNH_2$, $FADH_2$), coenzyme Q, iron-sulfur and the cytochromes a - c1, and as a result proton is accumulated between the outer and inner mitochondrial membranes. And this proton gradient acts as the energy supplier for the generation of ATP through complex V. So, to maintain and support of this critical process continuous supply of oxygen is required at the end-stage electron acceptor.*

At rest stage heart cells devour almost 8–15 ml O_2/ 100 g tissue/min which significantly higher than the brain's O_2 consumption (about 3 ml O2/min/100 g tissue) and even during strenuous exercise the consumption of O_2 can be increased to more than 70 ml O2/100 g myocardial tissue/min (Braunwald, 2001). Oxygen can change the gene expression pattern in myocardial cell as such in hypoxia due to the decrease oxygen myocardial gene expression pattern would be changed (Huang *et al*. 2004).

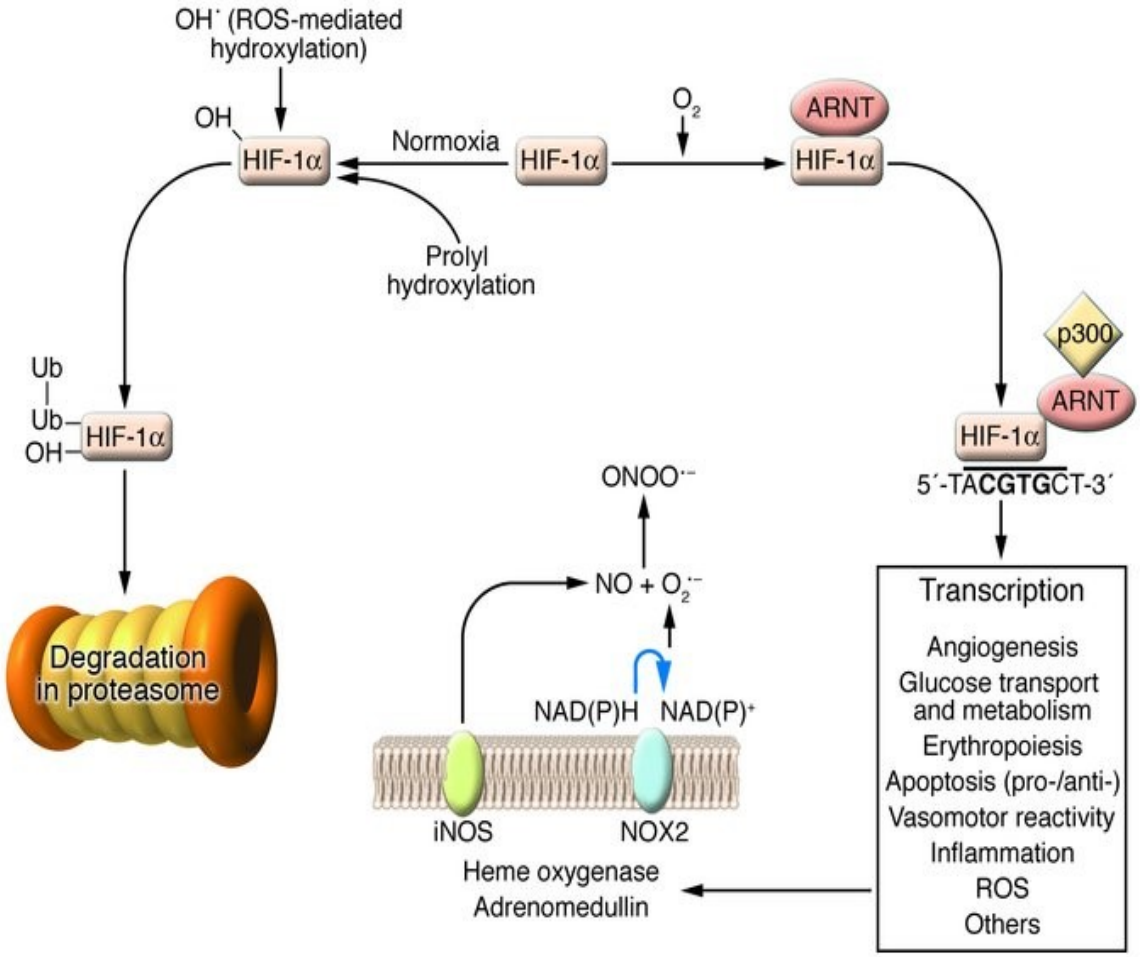


***Fig-1.31: Gene regulation by the HIF-1α. (Figure Credit**: **Giordano et al. 2005).** The HIF-1α protein undergoes rapid prolyl hydroxylation under normoxic conditions by specific cellular prolyl hydroxylases. Direct hydroxylation by ROS is a purported alternative pathway. Hydroxylated HIF interacts with the VHL, a critical member of an E3 ubiquitin ligase complex that polyubiquitylates HIF (Ub, ubiquitin). Polyubiquitylation targets HIF-1α for destruction by the proteosome. Under hypoxia hydroxylation does not occur and HIF-1α is stabilized. Heterodimerization with ARNT forms the active HIF complex that binds to a core hypoxia response element in a wide array of genes involved in a diversity of biological processes germane to cardiovascular function. Transcriptional activation of iNOS expression is shown as an example of how*

HIF-mediated gene expression can affect ROS generation by generating NO that interacts with O_2^{-} to form $ONOO^{-}$. NOX2 is shown as a cellular source of O_2^{-}.

On the basis of this review of literature, it was found that dermcidin might be involved in the genesis of diabetes and predisposing to cardiovascular disease but there is a lacking of particular interrelation. We were intended to formulate our present aims and objectives to reveal the role of the protein in that context in my research work.

AIMS AND OBJECTIVE

- To demonstrate the role of dermcidin in the development of resistant of AMI platelets to aspirin induced attenuation of platelet aggregation and the regulation of NOS on the platelet surface.
- To determine the role of aspirin, insulin and NO on dermcidin and to study the platelet aggregation in platelet rich plasma from AMI patients of diabetic and non-diabetic history.
- To test the role of dermcidin in the genesis of diabetes even in the presence of insulin and dermcidin associated mechanism of the development of diabetes mellitus in the presence of various stressors.

STUDY DESIGN

In this thesis the following studies were carried out -

- The concentration of dermcidin protein was quantified by ELISA in acute myocardial infarction and acute coronary syndrome patients to ascertain the level of the protein in the circulation in the disease state.

- Role of dermcidin protein in platelet aggregation and the abolition of the effect of aspirin (i.e. aspirin resistance) in the presence of protein in AMI were determined. So, the mechanism of resistance of platelets to the inhibitory effect of aspirin and possible solution to overcome was analyzed.

- Acetyl salicylic acid induced production of NO in platelet-rich plasma (PRP) was investigated and analyzed its implication in cardiovascular disease. It was also observed the role of acetyl salicylic acid (aspirin) to inhibit ADP induced platelet aggregation.

- Binding of dermcidin in the presence of aspirin, insulin and NO on the platelet surface were analyzed by determining dissociation constant (K_d) and number of ligand molecules bound/molecule of protein (n).

- The identical experiment was determined in case of atherosclerotic plaque formation in cerebrovascular accident to study the action of dermcidin in the stroke where the physiological phenomena is the same only the site is different.

- We also wanted to reveal the cause of chest pain in AMI and this pain is actually a distinctive feature in ACS or AMI, and our working was on NOS enzyme, so we aimed to investigate the involvement of any NOS in this case.

- As “nitro” compounds are used in chest-pain in cardiac patients, so the role of these compounds and even aspirin, insulin, glucose was determined in the NO production in goat arterial endothelial cells.
- We wanted to testify the role of dermcidin in the glucose uptake by the mice epitrochlearis muscle.
- The Dermcidin gene expression was analyzed under various stressful conditions.
- So, the mechanism of the effect of dermcidin in acute myocardial infarction and diabetes was analyzed and possible solution to overcome that effect was determined.