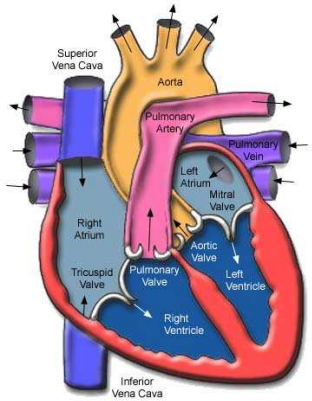
Fisiopatologia cardiaca

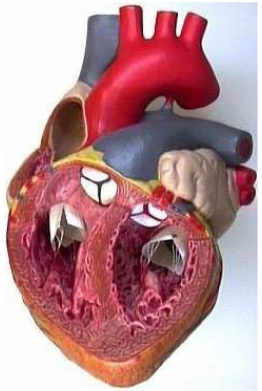
Premessa

La pompa cardiaca



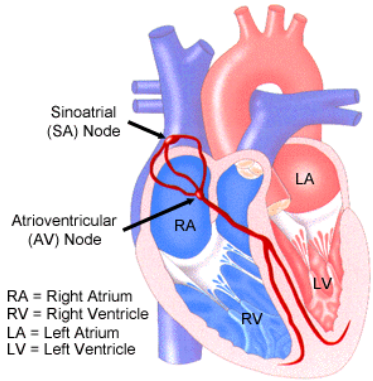
- 100,000 beats/day
- 7,500 L blood/day
- Output 5 L /min at rest
- Powers transport of nutrients, O_2, CO_2, waste and hormones, regulates metabolism.

Il cuore come motore meccanico



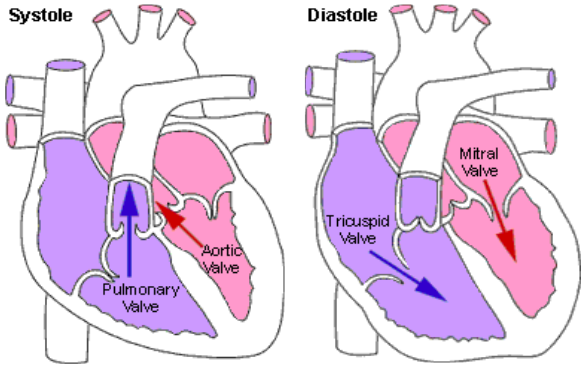
1. miocardio contrattile

Il cuore come motore meccanico



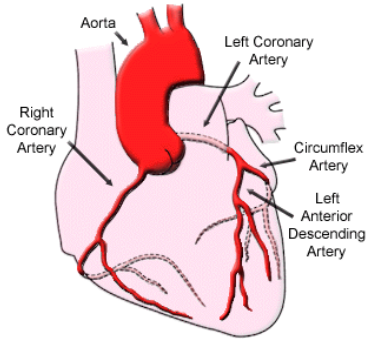
2. Sistema di conduzione

Il cuore come motore meccanico



3. Apparato valvolare

Il cuore come motore meccanico



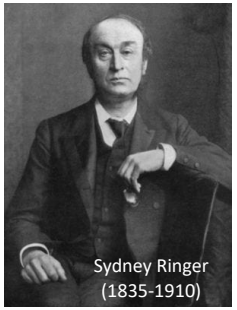
4. Vasi coronarici

Accoppiamento eccitazione-contrazione

Una storia di fortunate intuizioni

Luigi Galvani (1737-1798)

Dissecai una rana, la preparai e la collocai sopra una tavola sulla quale c'era una macchina elettrica … mentre uno dei miei assistenti toccava per caso leggermente con la punta di uno scalpello i nervi crurali di questa rana, a un tratto furono visti contrarsi tutti i muscoli degli arti come se fossero stati presi dalle più veementi convulsioni tossiche ... Ammirato dalle novità della cosa, subito avvertì me che ero completamente assorto e meco stesso d'altre cose ragionavo. Mi accese subito un incredibile desiderio di ripetere l'esperienza e di portare in luce ciò che di occulto c'era ancora nel fenomeno.



Sydney Ringer (1835-1910)

A FURTHER CONTRIBUTION REGARDING THE INFLUENCE OF THE DIFFERENT CONSTITUENTS OF THE BLOOD ON THE CONTRACTION OF THE HEART. By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London.* (Plate I.)

After the publication of a paper in the Journal of Physiology, Vol. III., No. 5, entitled "Concerning the influence exerted by each of the Constituents of the Blood on the Contraction of the Ventricle," I discovered, that the saline solution which I had used had not been prepared with distilled water, but with pipe water supplied by the New River Water Company. As this water contains minute traces of various inorganic substances, I at once tested the action of saline solution made with distilled water and I found that I did not get the effects described in the paper referred to. It is obvious therefore that the effects I had obtained are due to some of the inorganic constituents of the pipe water.

Water supplied by the New River Water Company contains 278·6 parts of solids per million.

They consist of:

Calcium	38·3	per million.
Magnesium	4·5	„
Sodium	23·3	„
Potassium	7·1	„
Combined Carbonic Acid	78·2	„
Sulphuric Acid	55·8	„
Chlorine	15	„
Silicates	7·1	„
Free Carbonic Acid	54·2	„

J. Physiol. (1883) 4, 29-42

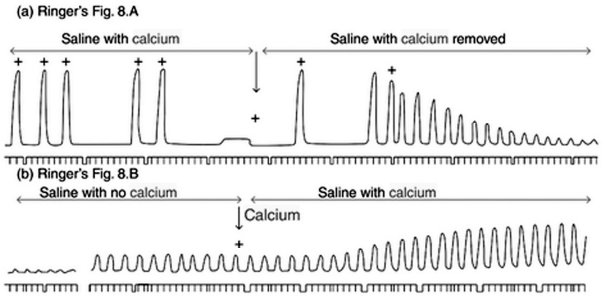
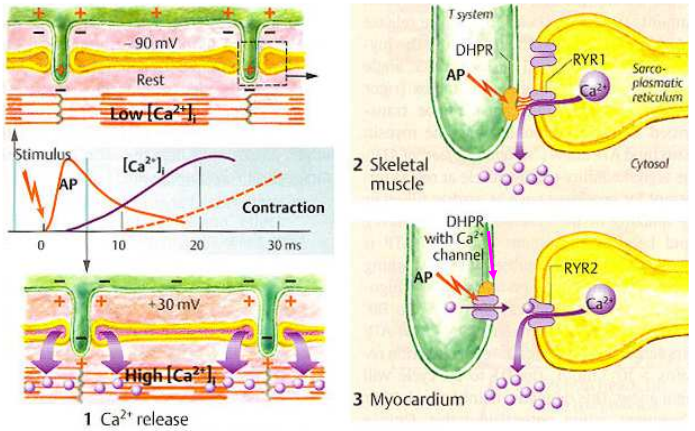
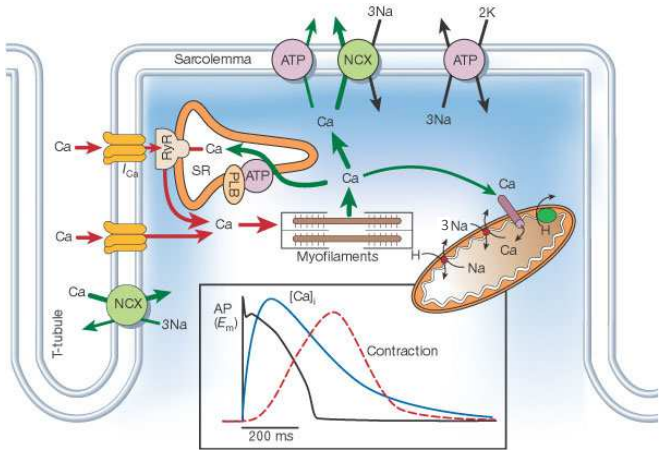


Figure 3.1 Ringer's demonstration that removing calcium stops a heart beat. The preparation was an isolated frog heart with the ventricle connected by a cannula to perfuse the heart with a saline (0.75% NaCl). From Ringer (1883) *J. Physiol.*, **4**, 29–43, Figure VIII.

lo ione Ca^{2+} media l'attivazione del muscolo

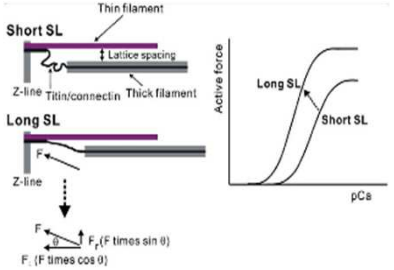


Ruolo del Ca^{2+} nell'attivazione e rilasciamento del muscolo



Aumento della performance sistolica

- L'effetto Frank-Starling è un esempio di inotropismo positivo: Aumento dell'affinità per il calcio del sistema troponinico
- La gittata sistolica aumenta per maggiore utilizzo del volume telesistolico
- Aumenta la FEV perché aumenta la forza contrattile ed il volume telesistolico si riduce



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- L'effetto inotropo delle catecolamine è mediato dalla fosforilazione PKA-dipendente delle proteine del reticolo sarcoplasmatico coinvolte nel ciclo contrazione-rilasciamento

catecolamine ↓

↑ $[cAMP]_i$ → Legame alla subunità regolativa PKA → Fosforilazione →
- RyR
- DHPR ↑ attività
- SERCA

- Fosfolambano (Rimozione effetto inibitorio)

↑ Ca^{2+}-induced Ca^{2+}-release

Aumento della performance sistolica

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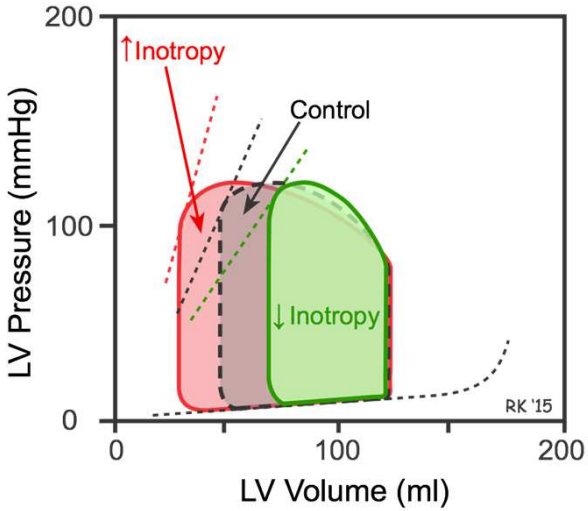
- Aumenta la FEV perché aumenta la forza contrattile ed il volume telesistolico si riduce
- L'effetto inotropo delle catecolamine è mediato dalla fosforilazione PKA-dipendente delle proteine del reticolo sarcoplasmatico coinvolte nel ciclo contrazione-rilasciamento
- Il risultato è un aumento della forza contrattile e della velocità del ciclo contrazione-rilasciamento
- Anche gli inibitori delle fosfodiesterasi hanno effetto inotropo positivo
- In sostanza, le sostanze ad attività inotropa positiva agiscono **aumentando la concentrazione intracellulare del calcio**
- Le catecolamine aumentano la frequenza con un effetto cronotropo positivo (effetto sul nodo SA) e dromotropo positivo (velocità di conduzione)

Ciclo cardiaco

https://www.khanacademy.org/science/health-and-medicine/circulatory-system/pressure-volume-loops/v/end-diastolic-pressure-volume-relationship-edpvr

https://www.khanacademy.org/science/health-and-medicine/circulatory-system/pressure-volume-loops/v/understanding-the-pressure-volume-loop



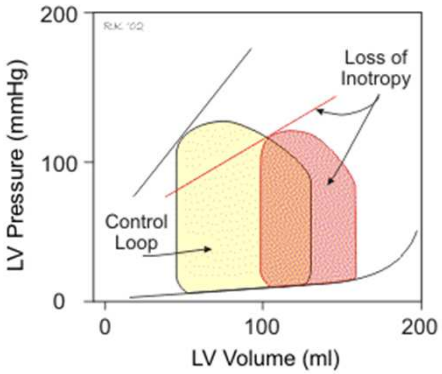
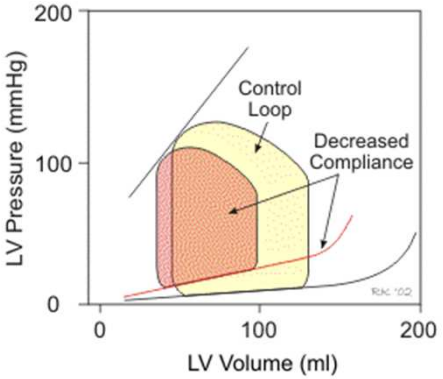
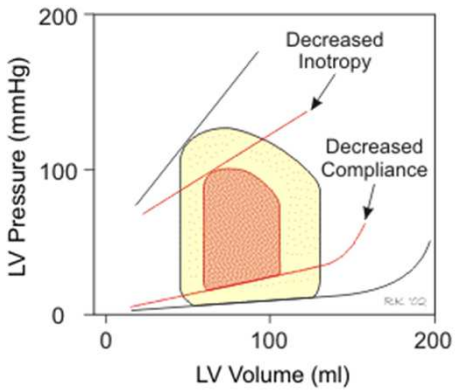


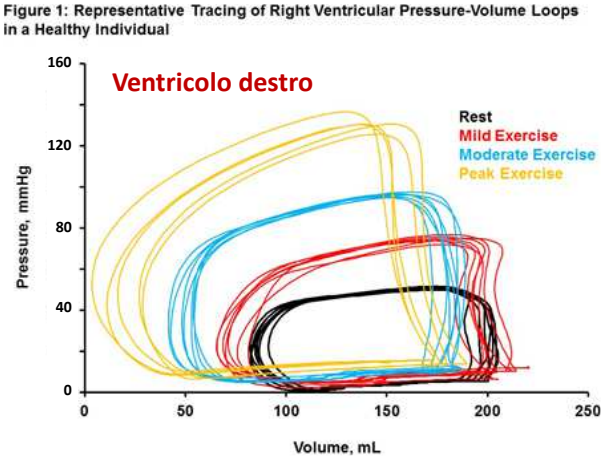
Figure 2. Effects of acute left ventricular failure (loss of inotropy) on left ventricular pressure-volume loop. Heart rate unchanged.



Effects of left ventricular diastolic failure caused by decreased ventricular compliance (e.g., hypertrophy) on left ventricular pressure-volume loop. Heart rate, inotropy and systemic vascular resistance are unchanged.

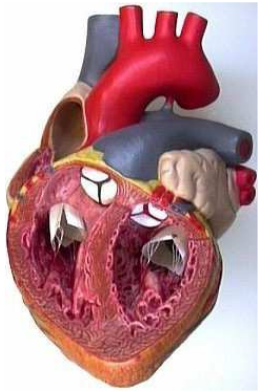


Effects of a combination of systolic dysfunction (decreased inotropy) and diastolic dysfunction (decreased compliance) on left ventricular pressure-volume loop. Heart rate and systemic vascular resistance are unchanged.



Inquadramento: Cause di disfunzione

Il cuore come motore meccanico



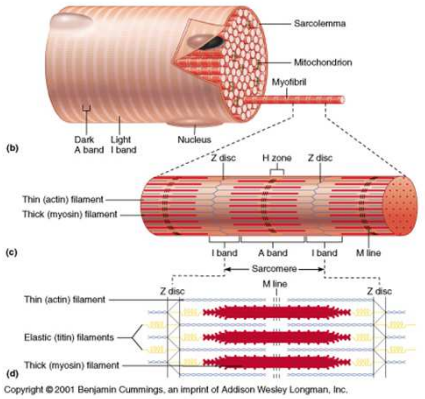
- apparato contrattile del cardiomiocita
  - citoscheletro
- segnali intracellulari che regolano l'attivazione della contrazione

alterazioni congenite (primitive)

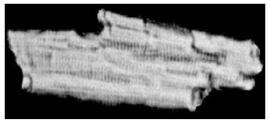
alterazioni acquisite (secondarie)

1. miocardio contrattile

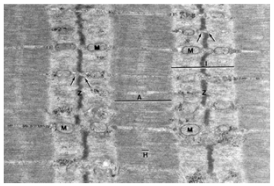
apparato contrattile del cuore: fibre muscolari e sarcomeri



singolo cardiomiocita isolato

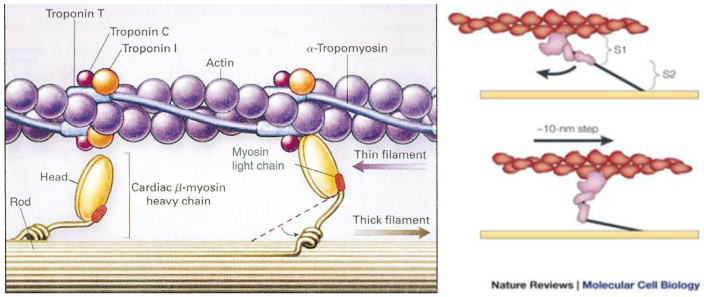


microfotografia al m. elettronico



la lunghezza dei filamenti sottili e dei filamenti spessi rimane costante durante l'accorciamento del sarcomero

Lo scivolamento dei miofilamenti è il motore molecolare della contrazione cardiaca

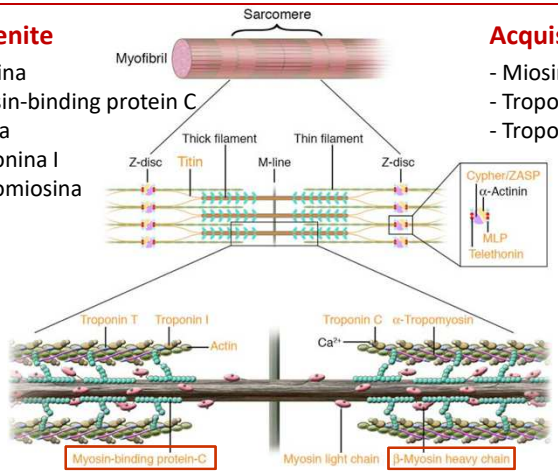


<u>miosina</u>: forma i filamenti spessi, ogni filamento contiene circa 300 molecole di miosina. Le teste globulari hanno attività ATPasica ed interagiscono con l'actina. L'interazione induce il cambiamento conformazionale della testa della miosina, che fa compiere un 'passo' di 10 nm ai miofilamenti.
<u>actina</u>: forma lo scheletro dei filamenti sottili, non ha attività enzimatica.
<u>complesso troponina/tropomiosina</u>: in assenza di Ca^{2+}, inibisce il legame di actina e miosina.

alterazioni dell'**apparato contrattile** sono causa di cardiomiopatie (congenite o acquisite)

Congenite
- Miosina
- Myosin-binding protein C
- Actina
- Troponina I
- Tropomiosina

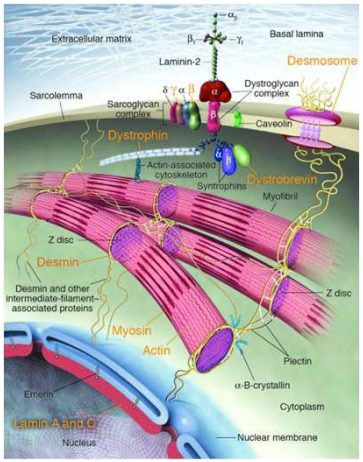
Acquisite
- Miosina
- Troponina I
- Troponina C



alterazioni congenite delle componenti del citoscheletro sono causa di cardiomiopatie primitive

Congenite
- distrofina
- distroglicano
- lamina A/C
- desmina

cardiomiopatie primitive (distrofie cardiache)

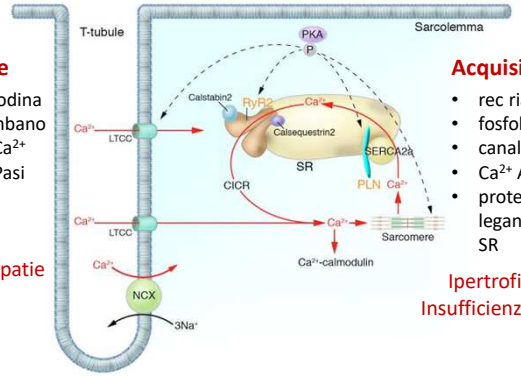


Alterazioni delle proteine che regolano l'omeostasi del calcio sono causa di cardiomiopatie (congenite o acquisite)

Congenite

- rec rianodina
- fosfolambano
- canale Ca^{2+}
- Ca^{2+} ATPasi

Cause di cardiomiopatie primitive



Acquisite

- rec rianodina
- fosfolambano
- canale Ca^{2+}
- Ca^{2+} ATPasi
- proteine che legano Ca^{2+} nel SR

Ipertrofia cardiaca
Insufficienza cardiaca

Journal of Neuromuscular Diseases 3 (2016) 293–308
DOI 10.3233/JND-160158
IOS Press

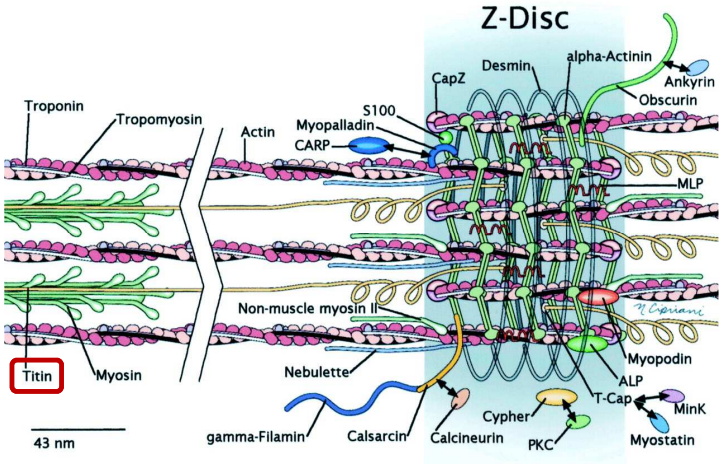
Review

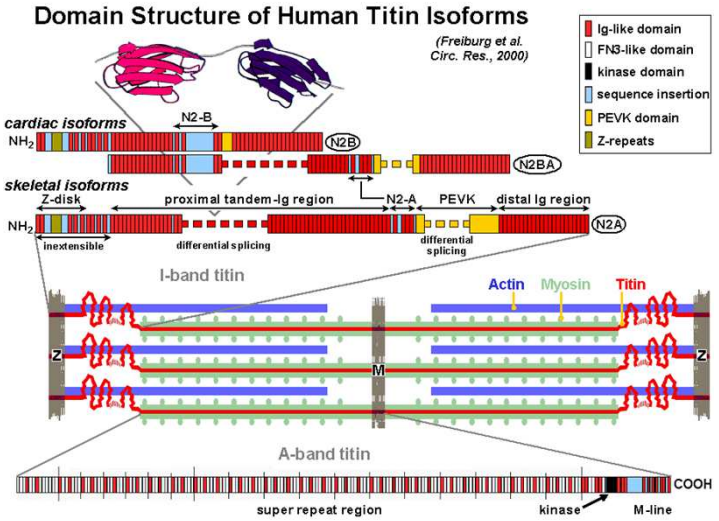
Increasing Role of Titin Mutations in Neuromuscular Disorders

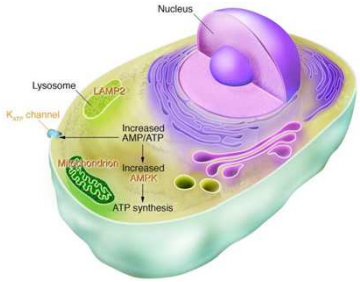
Marco Savarese[a], Jaakko Sarparanta[a,b], Anna Vihola[a], Bjarne Udd[a,c,d] and Peter Hackman[a,*]

- Late-onset autosomal dominant tibial muscular dystrophy (TMD) (MIM #600334)
- Young or early adult onset recessive distal titinopathy
- Limb-girdle muscular dystrophy type 2J (LGMD2J; MIM #608807)
- Congenital centronuclear myopathy (CNM; MIM #255200)
- **Early-onset myopathy with fatal cardiomyopathy, EOMFC (MIM #611705)**
- **Multi-minicore disease with heart disease (MmDHD)** including clinical variations
- Childhood-juvenile onset Emery-Dreifuss-like phenotype without cardiomyopathy
- **Hereditary myopathy with early respiratory failure (HMERF; MIM #603689)**
- Adult onset recessive proximal muscular dystrophy

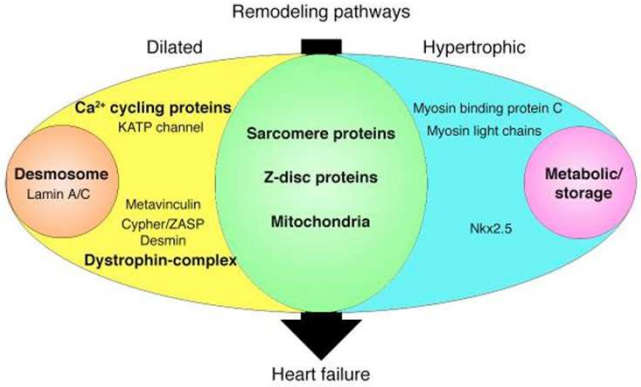
Stretch → Sarcomere length: complex protein machinery





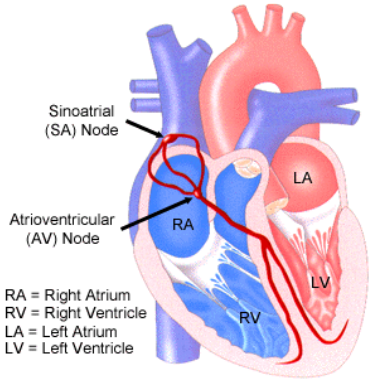


Human gene mutations affecting cardiac energetics and metabolism.
Energy substrate utilization is directed by critical metabolic sensors in myocytes, including AMP-activated protein kinase (AMPK), which, in response to increased AMP/ATP levels, phosphorylates target proteins and thereby regulates glycogen and fatty acid metabolism, critical energy sources for the heart. Glycogen metabolism involves a large number of proteins including α-galactosidase A (mutated in Fabry disease) and LAMP2 (mutated in Danon disease)



Human gene mutations can cause cardiac **hypertrophy (blue)**, **dilation (yellow)**, or **both (green)**. In addition to these two patterns of remodeling, particular gene defects produce hypertrophic remodeling with glycogen accumulation (pink) or dilated remodeling with fibrofatty degeneration of the myocardium (orange). Sarcomere proteins denote β-myosin heavy chain, cardiac troponin T, cardiac troponin I, α-tropomyosin, cardiac actin, and titin.

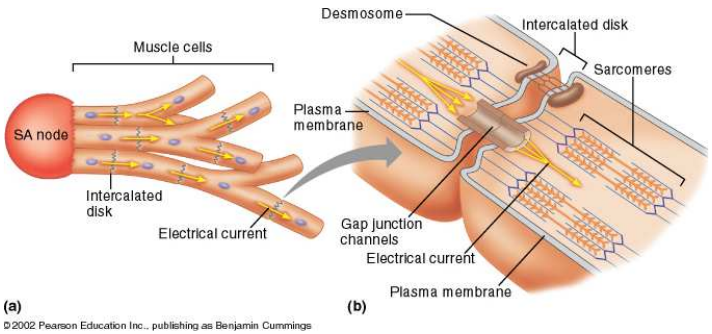
Il cuore come motore meccanico



- Intrinsic pacemaker
- Electrical impulses cause heart to beat (contract).
- Originates in the sinoatrial (SA) node, located at the top of the right atrium.
- SA node electrical impulse causes the atria to contract.
- The signal then passes through the atrioventricular (AV) node.
- The AV node delays the signal allowing for ventricular filling.
- Next the signals travels along the septal muscle fibers of the ventricles and into the walls, causing chamber contraction.
- The heart rate changes depending on physical demands, stress, or hormonal factors.

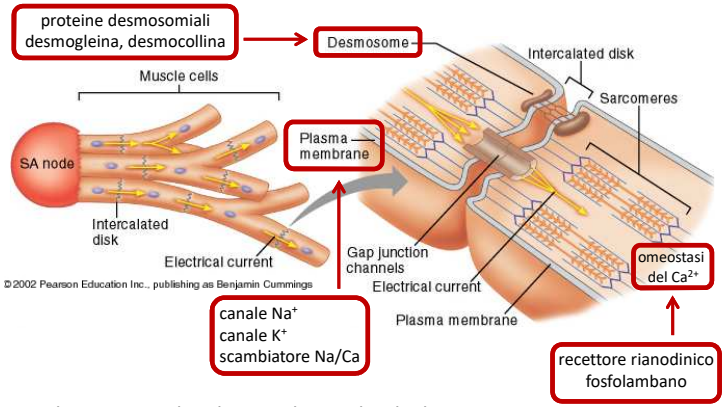
2. Sistema di conduzione

il nodo SA e la conduzione intercellulare dell'impulso



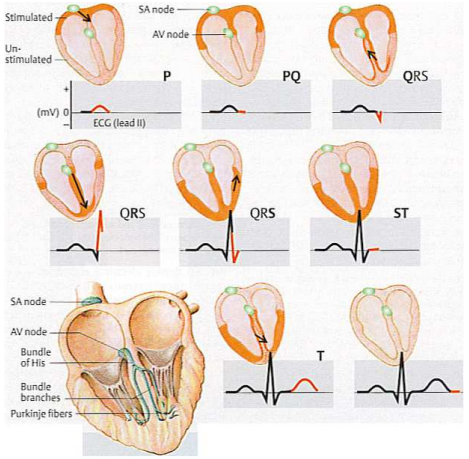
An action potential (AP) is propagated as the result of current transmission through **gap junctions**. Since the cardiac AP is almost as long as cardiac contraction, its **refractory period** prevents propagation of another AP until the muscle relaxes: tetanization is not possible.

Proteine che regolano la conduzione dell'impulso e aritmie cardiache

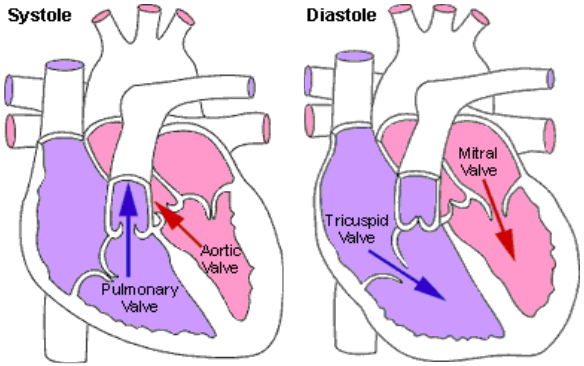


le stesse molecole sono bersaglio di alterazioni secondarie ad ipertrofia o ischemia cardiaca (acquisite)

Excitation Vectors and ECG



Il cuore come motore meccanico



3. Apparato valvolare

L'apparato valvolare cardiaco può andare incontro a disfunzioni che causano principalmente due tipi di alterazione:

1. La valvola non si apre completamente: stenosi

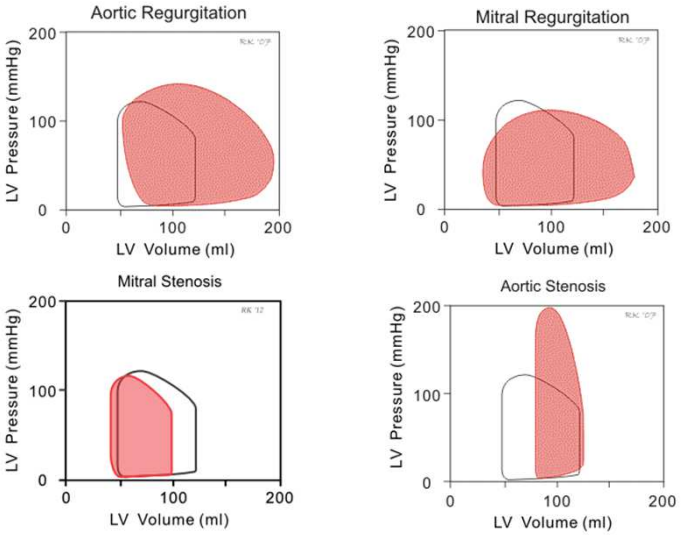
Una valvola che non apre completamente oppone una resistenza maggiore al flusso di sangue che passa attraverso l'ostio valvolare.

2. La valvola non si chiude completamente: insufficienza

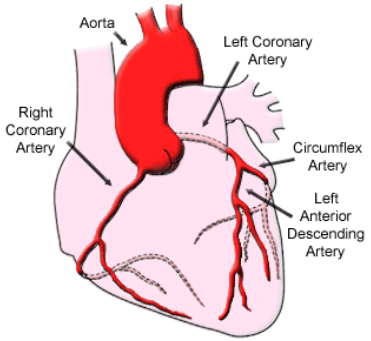
Una valvola che non chiude completamente non contiene il sangue nella camera a valle della stessa.

Le **cause** delle malattie valvolari sono (raro) congenite o acquisite, quasi sempre primitive.

Le **manifestazioni** fisiopatologiche dipendono dalla valvola colpita e dal tipo ed entità del difetto.



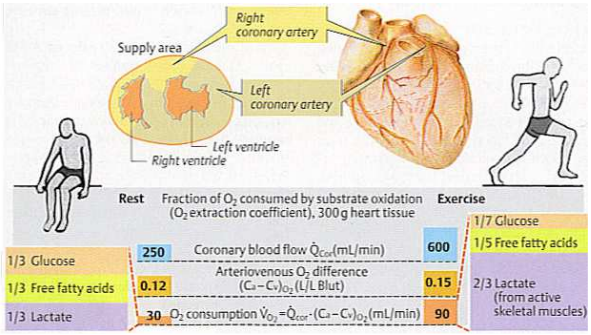
Il cuore come motore meccanico



First vessels branching off the aorta

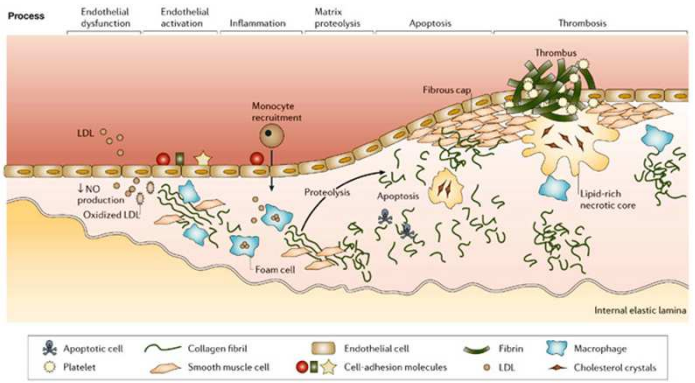
- Left = LCA
- Right = RCA
- LCA = CX + LAD
- LCA perfuses about 2/3 of the heart muscle including the left ventricle
- RCA mainly perfuses smaller right heart

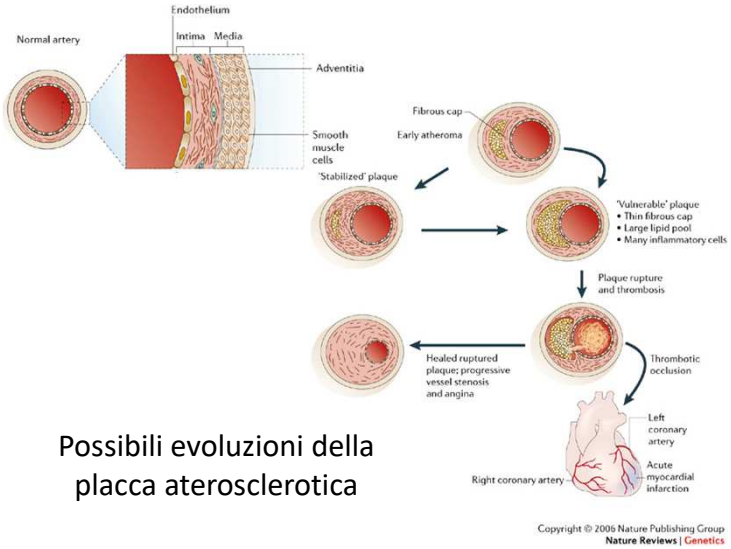
4. Vasi coronarici



Coronary blood flow: LCA = 6/7 x Q_{cor}; phasic; depends on LV pressure.
Myocardial O_2 consumption = is high with 60% oxygen extraction at rest.
Aerobic metabolism → Increase in Q_{cor} only way to adapt to exercise.
Coronary reserve: regulation of vessel resistance → 4-5fold increase of Q_{cor} and O_2

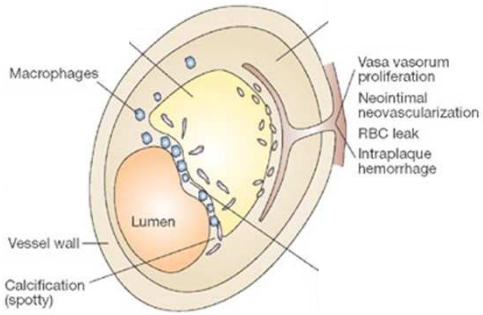
Metabolic: O_2, adenosine, lactate, H^+; **Endothelial:** NO; **Neurohumoral:** NE→ β2AR
Rest: Fueled by FFA, glucose, lactate, → +O_2 → AcCoA → Krebs cycle → ATP
Exercise: Increase of lactate (SM) → inhibit FA uptake → 70% lactate consumption

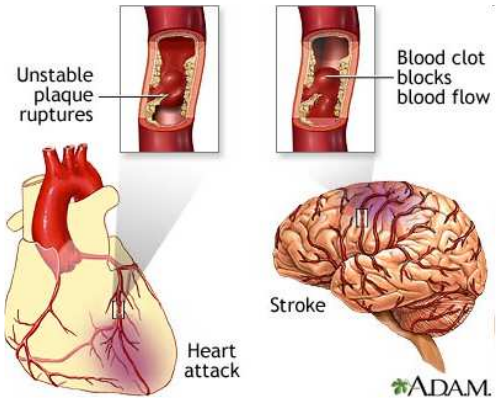


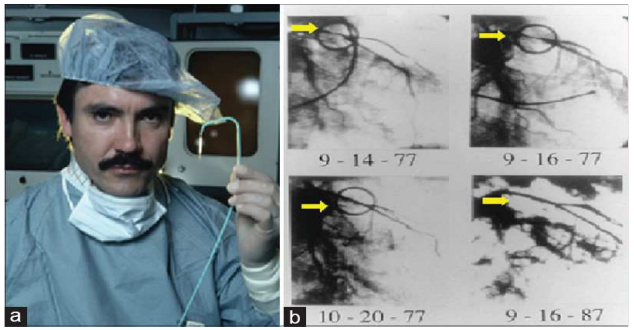


Possibili evoluzioni della placca aterosclerotica

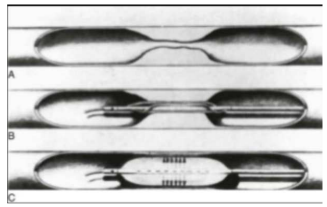
Fattori che determinano l'instabilità della placca

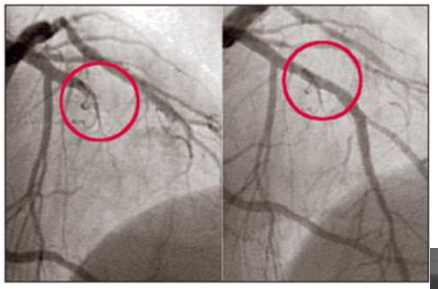




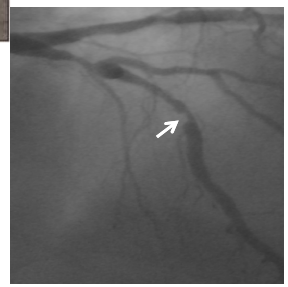


Andreas Roland Grüntzig (Dresda, 25 giugno 1939 – Forsyth, 27 ottobre 1985)





cardiopatia ischemica acuta: occlusione ACUTA della coronaria



cardiopatia ischemica cronica: occlusione CRONICA della coronaria limitante il flusso coronarico massimo

Infarto miocardico acuto: dalla placca all'infarto

Nella maggior parte dei casi dovuto all'occlusione trombotica di una arteria coronaria.

L'evoluzione acuta dipende dalla rottura o fissurazione della placca aterosclerotica, favorita da fattori quali:

- Fumo di sigaretta
- Ipertensione
- Accumulo di lipidi

Le placche coronariche più soggette a rottura sono quelle caratterizzate da un core lipidico soffice e da una placca fibrosa sottile.

Meccanismi di adattamento alla disfunzione cardiaca

In presenza di una disfunzione cardiaca e/o di un carico emodinamico eccessivo il cuore ricorre ad una serie di meccanismi di adattamento:

Adattamento a breve termine (minuti-ore)

- meccanismo di Frank Starling
- attivazione del SNS – rilascio di noradrenalina dai neuroni cardiaci
- attivazione del sistema Renina-Angiotensina-Aldosterone

mantenimento della perfusione di organi vitali

Adattamento a lungo termine (settimane-mesi-anni)

- Rimodellamento miocardico
- **Ipertrofia miocardica**

aumento della massa di cellule contrattili

Ipertrofia e rimodellamento: Meccanismi

Ipertrofia

- L'ipertrofia è un aumento delle dimensioni di un organo o di un tessuto dovuto all'aumento delle dimensioni delle cellule che lo costituiscono
- Il numero delle cellule non varia, quindi il rapporto DNA/peso tissutale diminuisce
- L'ipertrofia può essere fisiologica o patologica
- **Fisiologica**
  - Muscolare in seguito ad allenamento
  - Del muscolo liscio dell'utero in corso di gravidanza
- **Patologica**
  - Il gozzo tiroideo, in caso di anticorpi anti recettore per il TSH (Basedow)
  - L'ipertrofia cardiaca, che può essere causata da un ostacolato efflusso di sangue nell'aorta o da ipertensione sistemica

CAUSE DI IPERTROFIA

- Genetiche
- Aumento della richiesta funzionale
- Ipersecrezione ormonale
- Aumento nutrizionale

Evidente negli organi bilaterali

IPERTROFIA FISIOLOGICA COMPENSATORIA quando uno dei due è eliminato o leso

Ipertrofia cardiaca fisiologica

- Non tutte le forme di ipertrofia cardiaca sono patologiche e nocive, poichè l'esercizio fisico provoca uno stato di crescita fisiologica equilibrata del cuore
- Così come la crescita del cuore che avviene durante lo sviluppo, essa è caratterizzata da un profilo uniforme di crescita della parete ventricolare e del setto, che è accompagnata da un aumento di dimensione delle camere
- Questo fenotipo è associato ad un'ipertrofia dei miociti cardiaci in cui nuovi sarcomeri sono aggiunti **sia in serie che in parallelo**

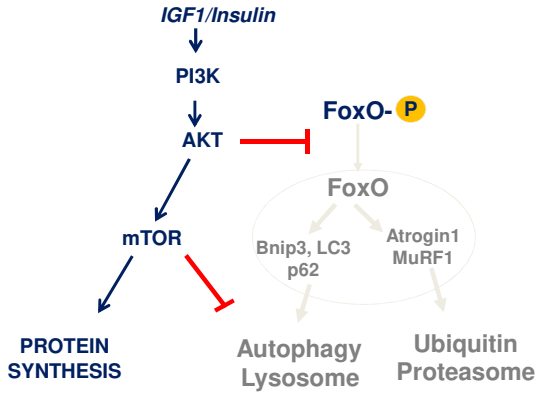
Ipertrofia cardiaca fisiologica

Ipertrofia cardiaca adattativa (atleti): avviene negli atleti in seguito ad allenamenti intensi e prolungati.

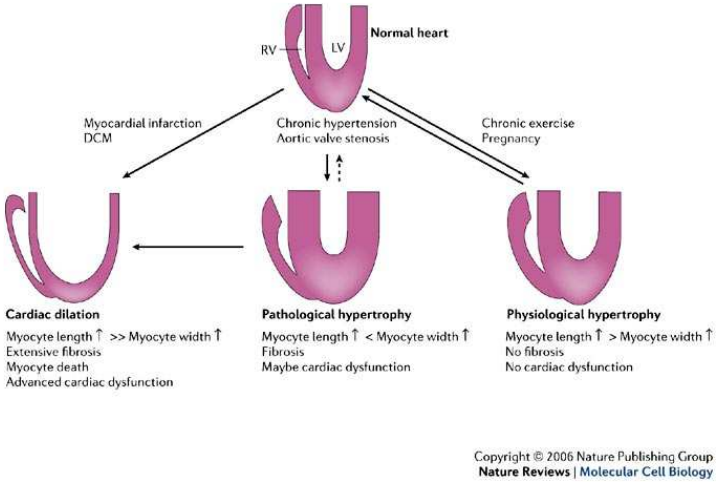
Questi individui hanno:

1. Normale ECG
2. modesto spessore della parete
3. non accompagnato da accumulo di collagene né da riattivazione di programmi genici fetali
4. un'ipertrofia reversibile
5. Dal punto di vista molecolare: no attivazione della via di segnale calcineurina-NFAT ma attivazione della via IGF1-Akt-mTOR

Ipertrofia cardiaca fisiologica



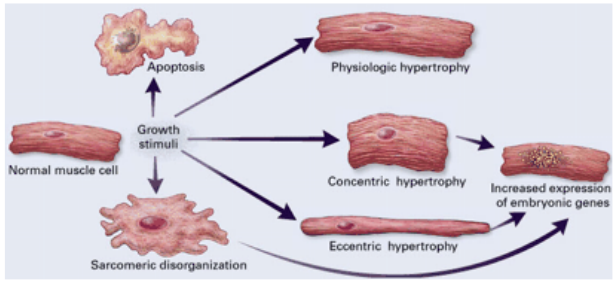
Tipi di ipertrofia cardiaca



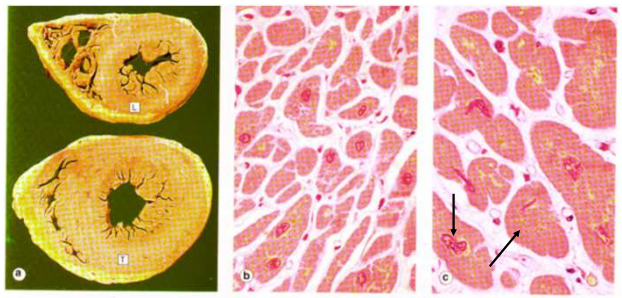
Ipertrofia cardiaca patologica

- L'ipertrofia cardiaca è la risposta del cuore ad una varietà di stimoli intrinseci ed estrinseci che determinano un aumento dello stress biomeccanico
- Il risultato dell'ipertrofia cardiaca patologica è un aumento della massa muscolare e delle dimensioni cellulari, con disorganizzazione dei cardiomiociti e fibrosi
- Non c'è crescita dei vasi creando le premesse per la sofferenza ischemica

Cardiac hypertrophy at the cellular level



Ipertrofia cardiaca



L'ipertrofia cardiaca accompagna lo sviluppo dell'insufficienza cardiaca cronica

- L'insufficienza cardiaca è un difetto nella capacità del cuore di pompare adeguatamente il sangue in risposta alla richiesta sistemica, che ha come conseguenze affaticamento, dispnea e/o edema.
- E' indotto da una serie di stimoli patologici fra cui ipertensione persistente, ischemia associata a patologie coronariche, insufficienza o stenosi valvolare, miocardite, difetti congeniti…..
- La maggior parte di questi stimoli induce prima una fase di ipertrofia cardiaca, nella quale i singoli miociti aumentano di dimensioni compensando la funzione di pompa e diminuendo la tensione della parete ventricolare

Esempio: Patogenesi delle forme genetiche

Espressione della proteina mutante

↓

Incorporazione nei sarcomeri

↓

La proteina mutante determina diversi difetti iniziali come aumentata sensibilità al calcio (troponina C), ridotta attività ATPasica (MyHC) o alterato cross bridge cycling

↓

Bassa contrattilità del miocita

↓

Aumento di stress dei miociti

↓

Upregolazione di risposte trofiche allo stress
(ACE, angiotensina II, IGF-1, TGF-β, calcineurina..)

↓

Ipertrofia, disorganizzazione, sintesi di collagene, altri..

↓

Cardiomiopatia ipertrofica

Ipertrofia cardiaca patologica acquisita

- L'ipertrofia cardiaca patologica produce ipertrofia di tipo **concentrico**, in cui la parete ventricolare e il setto si ispessiscono con una diminuzione netta delle dimensioni delle camere cardiache
- Questo rimodellamento è associato ad un maggiore aumento di spessore del miocita rispetto alla lunghezza
- L'ipertrofia patologica può però produrre anche un fenotipo di crescita eccentrica dilatativa. Tale risposta è il risultato di una crescita patologica del miocita in lunghezza, con aggiunta predominante di sarcomeri in serie

At the cellular level:

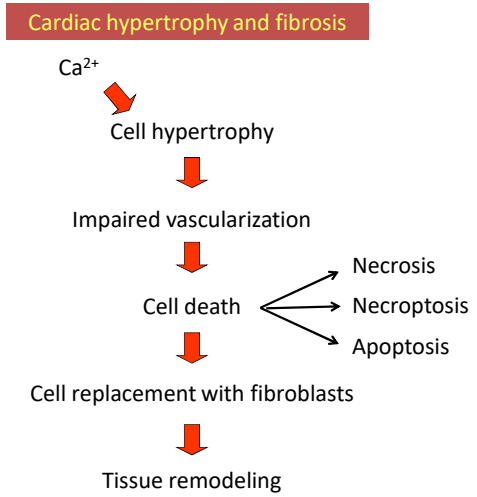
- increase in cell size
- enhanced protein synthesis
- decreased protein degradation
- altered organization of the sarcomere

At the molecular level:

- reinduction of the so-called fetal gene program (fetal MyHC, diminished myofibrillar ATPase activity, slower sliding velocity and impaired contractility, expression of natriuretic factors ANF, BNP)
- activation of specific kinase signaling pathways
- induction of pathways of apoptosis and fibrosis

At the metabolic level:

- Initially increase of lipids usage (requires oxygen)
- In failing hearts the cardiomyocytes use glucose instead of lipids (less dependence on oxygen)

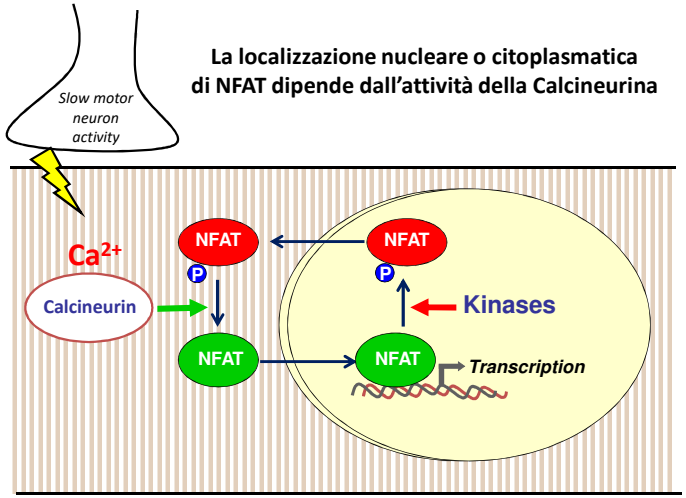


Segnali intracellulari che mediano l'ipertrofia cardiaca (patologica)

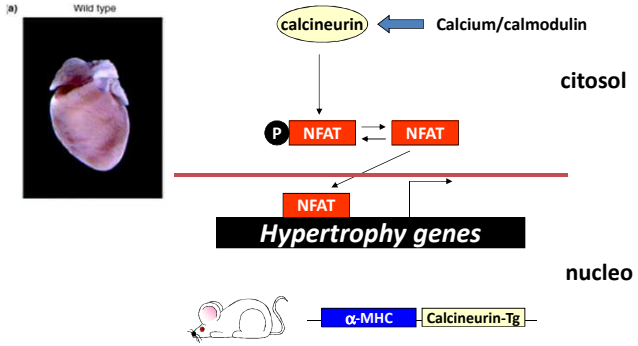
Calcineurina

- ser/thr fosfatasi attivata da calcio-calmodulina
- defosforila NFAT (nuclear factor of activated T cells), una famiglia di fattori trascrizionali
- Attiva la trascrizione dei geni della risposta ipertrofica

La localizzazione nucleare o citoplasmatica di NFAT dipende dall'attività della Calcineurina



Animal models of hypertrophic cardiomyopathy



Molkentin et al., Cell 93: 215 (1998)

Calcineurin-overexpressing mice have heart hypertrophy

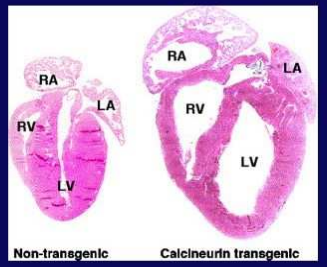
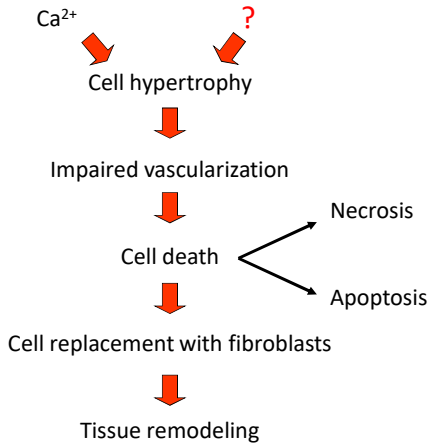
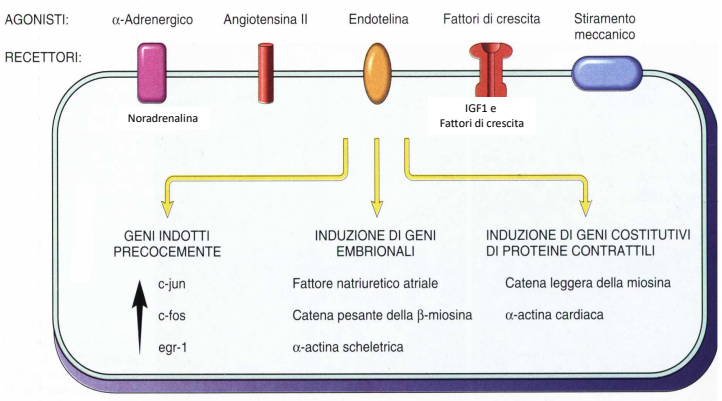


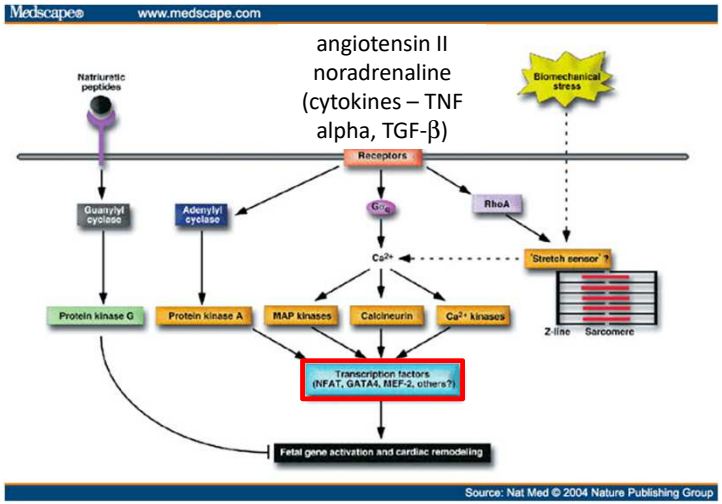
Fig. 3. H&E-stained histological section through a heart at 4 weeks of age from a non-transgenic (left) or activated calcineurin transgenic mouse (right). Abbreviations not defined in text: LV, left ventricle; RV, right ventricle; LA, left atrium; RA, right atrium.

from Wilkins & Molkentin, 2004, BBRC, 322, 1178-1191

Cardiac hypertrophy and fibrosis

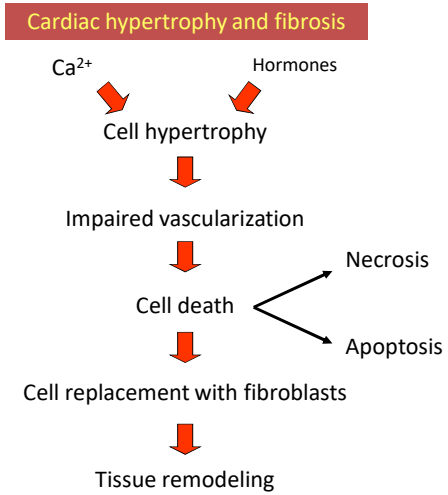






Fattori trascrizionali che inducono ipertrofia cardiaca

- **GATA4.** Fattore trascrizionale (contenente un motivo Zn-finger) che regola lo sviluppo cardiaco. Viene ri-espresso in condizioni di stress e media la trascrizione di geni coinvolti nell'ipertrofia cardiaca
- **MEF2.** Fattore trascrizionale contenente un motivo MAD-box che lega i promotori della maggior parte dei geni muscolari scheletrici e cardiaci
- **NF-kB.** Il signalling attraverso NFkB regolato da una cascata di chinasi e regola l'ipertrofia cardiaca
- **NFAT** (discusso prima)



L'ipertrofia cardiaca a livello molecolare

Gli stimoli che danno inizio al processo di crescita ipertrofica del cardiomiocita possono essere suddivisi in:

1. biomeccanici e stretch-sensitive
2. neuroumorali (ormoni, citochine, chemochine, fattori di crescita…)

Tali ligandi interagiscono con una varietà di G-protein-coupled receptor, recettori ad attività tirosin chinasica o associati a ser/thr kinasi. Si pensa che i vari recettori convergano su di un numero limitato di vie di segnale intracellulari

Segnali

- Canali ionici (calcio) sensibili alla tensione (Mechanosensitive ion channel) che attivano **Calcineurina** e **CaMKII**
- Segnali che attivano la chinasi AKT (a valle dei recettori IGF1/Insulina)
- GSK3β blocca l'ipertrofia
- G-proteins (attivate da adrenalina/noradrenalina, angiotensina II ed endothelin 1) che attivano il G-protein-coupled receptor (GPCR), fosfolipasi C e produzione di IP3 ma possono anche attivare MAPKs ed adrenergic receptors che inducono cAMP e PKA.
- Apoptosi, sia intrinsica che estrinseca, necroptosi.

Review Article Journal of Molecular and Cellular Cardiology 51 (2011) 468–473

CaMKII in myocardial hypertrophy and heart failure

Mark E. Anderson [a,*], Joan Heller Brown [b], Donald M. Bers [c]

Many signals have risen and fallen in the tide of investigation into mechanisms of myocardial hypertrophy and heart failure (HF). In our opinion, the multifunctional Ca and calmodulin-dependent protein kinase II (CaMKII) has emerged as a molecule to watch, in part because a solid body of accumulated data essentially satisfy Koch's postulates, showing that the CaMKII pathway is a core mechanism for promoting myocardial hypertrophy and heart failure. Multiple groups have now confirmed the following: (1) that CaMKII activity is increased in hypertrophied and failing myocardium from animal models and patients; (2) CaMKII overexpression causes myocardial hypertrophy and HF and (3) CaMKII inhibition (by drugs, inhibitory peptides and gene deletion) improves myocardial hypertrophy and HF. Patients with myocardial disease die in equal proportion from HF and arrhythmias, and a major therapeutic obstacle is that drugs designed to enhance myocardial contraction promote arrhythmias. In contrast, inhibiting the CaMKII pathway appears to reduce arrhythmias and improve myocardial responses to pathological stimuli. This brief paper will introduce the molecular physiology of CaMKII and discuss the impact of CaMKII on ion channels, Ca handling proteins and transcription in myocardium. This article is part of a special issue entitled "Key Signaling Molecules in Hypertrophy and Heart Failure".

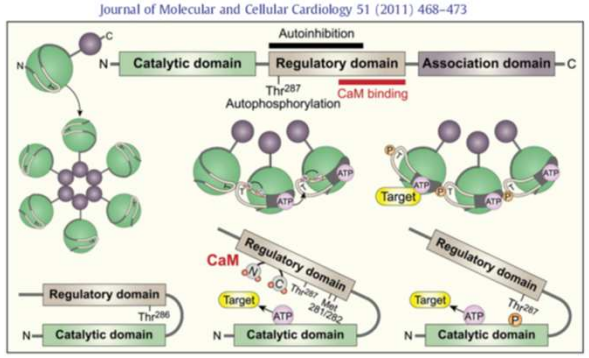
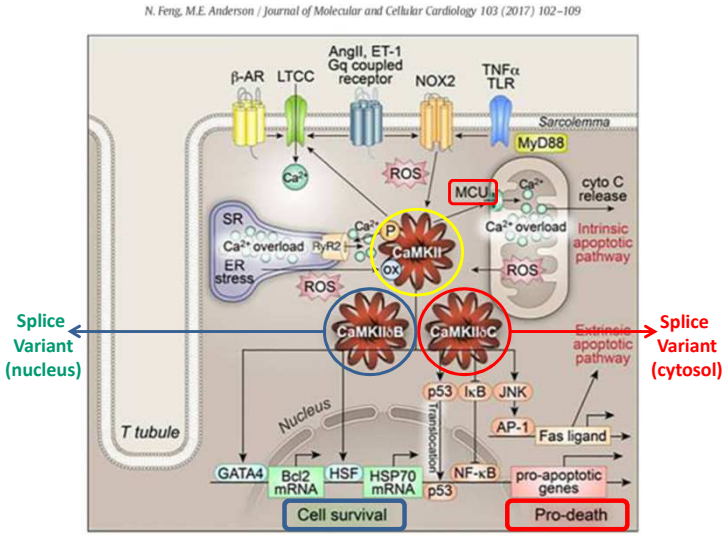


Fig. 1. CaMKII structural domains and regulation. Under resting conditions the catalytic domain is constrained by the regulatory domain (left middle and bottom panels). After intracellular Ca^{2+} rises and complexes with calmodulin (CaM) the Ca^{2+}/CaM binds to the C terminal portion of the CaMKII regulatory domain (mid portion of the top, middle and bottom panels) to prevent autoinhibition of the regulatory domain on the catalytic domain, activating CaMKII. With sustained Ca^{2+}/CaM or increased oxidation, **CaMKII transitions into a Ca^{2+}/CaM-autonomous active enzyme after autophosphorylation (at Thr 287) or oxidation (at Met281/282) of amino acids in the regulatory domain.**



CaMKII determines mitochondrial stress responses in heart

8 NOVEMBER 2012 | VOL 491 | NATURE | 269

Mei-ling A. Joiner[1], Olha M. Koval[1], Jingdong Li[1]†, B. Julie He[1,2], Chantal Allamargot[3], Zhan Gao[1], Elizabeth D. Luczak[1], Duane D. Hall[1], Brian D. Fink[4], Biyi Chen[1], Jinying Yang[1], Steven A. Moore[2,5], Thomas D. Scholz[6], Stefan Strack[7], Peter J. Mohler[1]†, William I. Sivitz[1,4], Long-Sheng Song[1] & Mark E. Anderson[1,2]

Myocardial cell death is initiated by excessive mitochondrial Ca^{2+} entry causing Ca^{2+} overload, mitochondrial permeability transition pore (mPTP) opening and dissipation of the mitochondrial inner membrane potential ($\Delta\Psi m$)[1,2]. However, the signalling pathways that control mitochondrial Ca^{2+} entry through the inner membrane mitochondrial Ca^{2+} uniporter (MCU)[3–5] are not known. The multifunctional Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is activated in ischaemia reperfusion, myocardial infarction and neurohumoral injury, common causes of myocardial death and heart failure; these findings suggest that CaMKII could couple disease stress to mitochondrial injury. Here we show that CaMKII promotes mPTP opening and myocardial death by increasing MCU current (I_{MCU}). Mitochondrial-targeted CaMKII inhibitory protein or cyclosporin A, an mPTP antagonist with clinical efficacy in ischaemia reperfusion injury[6], equivalently prevent mPTP opening, $\Delta\Psi m$ deterioration and diminish mitochondrial disruption and programmed cell death in response to ischaemia reperfusion injury. Mice with myocardial and mitochondrial-targeted CaMKII inhibition have reduced I_{MCU} and are resistant to ischaemia reperfusion injury, myocardial infarction and neurohumoral injury, suggesting that pathological actions of CaMKII are substantially mediated by increasing I_{MCU}. Our findings identify CaMKII activity as a central mechanism for mitochondrial Ca^{2+} entry in myocardial cell death, and indicate that mitochondrial-targeted CaMKII inhibition could prevent or reduce myocardial death and heart failure in response to common experimental forms of pathophysiological stress.

NATURE MEDICINE VOLUME 22 | NUMBER 2 | FEBRUARY 2016 175

CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress–induced myocardial necroptosis

Ting Zhang[1,2,5], Yan Zhang[1,2,5], Mingyao Cui[1,2], Li Jin[1,2], Yimei Wang[1,2], Fengxiang Lv[1,2], Yuli Liu[1,2], Wen Zheng[1,2], Haibao Shang[1,2], Jun Zhang[1,2], Mao Zhang[1,2], Hongkun Wu[1,2], Jiaojiao Guo[1,2], Xiuqin Zhang[1,2], Xinli Hu[1,2], Chun-Mei Cao[1,2] & Rui-Ping Xiao[1–4]

Regulated necrosis (necroptosis) and apoptosis are crucially involved in severe cardiac pathological conditions, including myocardial infarction, ischemia-reperfusion injury and heart failure. Whereas apoptotic signaling is well defined, the mechanisms that underlie cardiomyocyte necroptosis remain elusive. Here we show that receptor-interacting protein 3 (RIP3) triggers myocardial necroptosis, in addition to apoptosis and inflammation, through activation of Ca^{2+}-calmodulin–dependent protein kinase (CaMKII) rather than through the well-established RIP3 partners RIP1 and MLKL. In mice, RIP3 deficiency or CaMKII inhibition ameliorates myocardial necroptosis and heart failure induced by ischemia-reperfusion or by doxorubicin treatment. RIP3-induced activation of CaMKII, via phosphorylation or oxidation or both, triggers opening of the mitochondrial permeability transition pore and myocardial necroptosis. These findings identify CaMKII as a new RIP3 substrate and delineate a RIP3-CaMKII-mPTP myocardial necroptosis pathway, a promising target for the treatment of ischemia- and oxidative stress–induced myocardial damage and heart failure.

Journal of Molecular and Cellular Cardiology 103 (2017) 102–109

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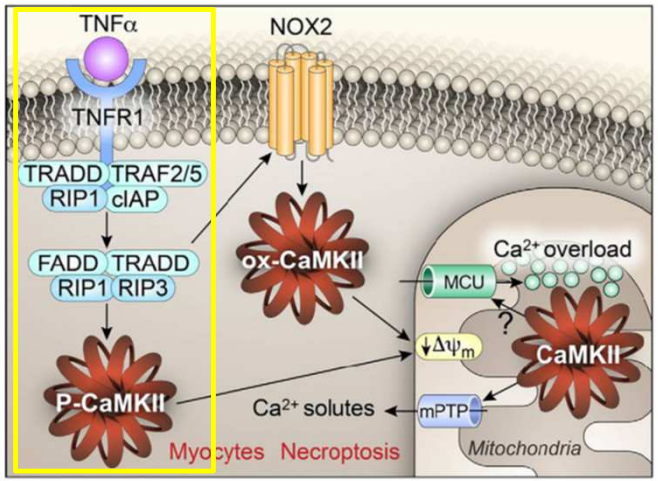
journal homepage: www.elsevier.com/locate/yjmcc

CaMKII is a nodal signal for multiple programmed cell death pathways in heart

Ning Feng[a,*], Mark E. Anderson[a,b,**]

Sustained Ca^{2+}/calmodulin-dependent kinase II (CaMKII) activation plays a central role in the pathogenesis of a variety of cardiac diseases. Emerging evidence suggests CaMKII evoked programmed cell death, including apoptosis and necroptosis, is one of the key underlying mechanisms for the detrimental effect of sustained CaMKII activation. CaMKII integrates β-adrenergic, Gq coupled receptor, reactive oxygen species (ROS), hyperglycemia, and pro-death cytokine signaling to elicit myocardial apoptosis by intrinsic and extrinsic pathways. New evidence demonstrates CaMKII is also a key mediator of receptor interacting serine/threonine kinase 3 (RIP3)-induced myocardial necroptosis. The role of CaMKII in cell death is dependent upon subcellular localization and varies across isoforms and splice variants. While CaMKII is now an extensively validated nodal signal for promoting cardiac myocyte death, the upstream and downstream pathways and targets remain incompletely understood, demanding further investigation.



Molecular mechanisms of CaMKII-mediated myocardial necroptosis. The engagement of tumor necrosis factor alpha (TNFα) with its receptor leads to the formation of necroptosome, mainly consisting of receptor-interacting serine/threonine-protein kinase 1 and 3 (RIP1 and RIP3). **RIP3 activation induces CaMKII activation through direct phosphorylation of CaMKII at threonine 287** (P-CaMKII, CaMKIId numbering) or **oxidation of CaMKII via NOX2 stimulation (ox-CaMKII) at methionines 281 and 282**. Both P-CaMKII and ox-CaMKII contribute to myocardial necroptosis by triggering mitochondrial permeability transition pore (mPTP) opening, which leads to the collapse of the inner mitochondrial membrane potential ($\Delta\psi_m$). The mechanism of CaMKII biased mPTP opening is unclear, but CaMKII-induced mitochondrial Ca^{2+} uniporter (MCU) hyperactivity and Ca^{2+} overload may contribute.

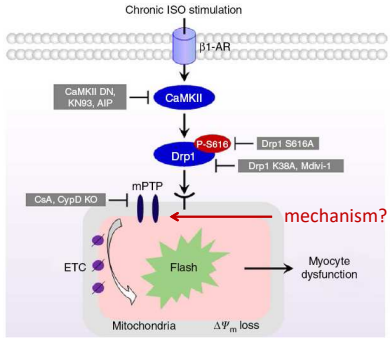


Figure 9 | Schematic model of CaMKII mediating mPTP through $Drp1^{S616}$ phosphorylation during chronic β-AR stimulation. Sustained ISO treatment activates CaMKII pathway, a downstream kinase of β1-AR, and subsequently increases the phosphorylation of Drp1 at S616 ($Drp1^{S616}$), which activates Drp1. After translocating to the outer membrane of mitochondria, the phosphorylated Drp1 triggers fission and mPTP openings, which are recorded by mitochondrial flash events. Finally, chronic activation of this pathway leads to mitochondrial and myocyte dysfunction. Abolishing CaMKII activity (CaMKII DN, KN93 or AIP), inhibiting Drp1 activity (Drp1 K38A or Mdivi-1), preventing $Drp1^{S616}$ phosphorylation (Drp1 S616A), or blocking mPTP openings (CsA or CypD KO) efficiently prevented myocyte damage and cardiac hypertrophy during chronic β1-AR stimulation.

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CaMKII induces permeability transition through Drp1 phosphorylation during chronic β-AR stimulation

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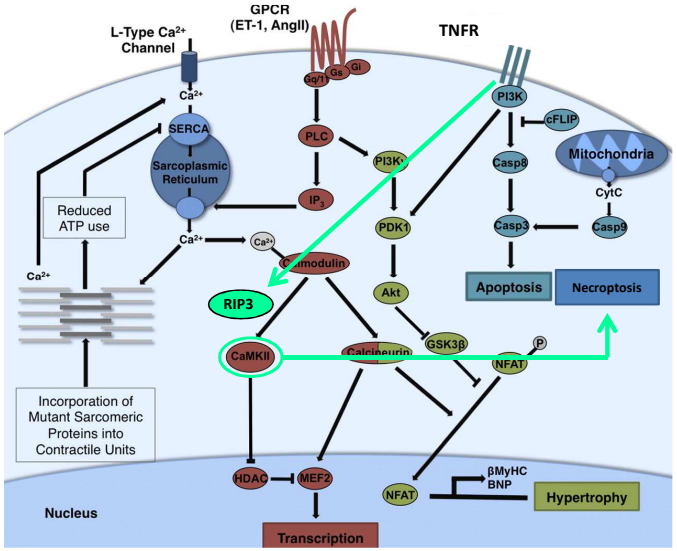

Il ruolo dei fibroblasti

Premessa: A **bromodomain** is an approx. 110 amino acid protein domain that recognizes **acetylated lysine residues**, such as those on the N-terminal tails of histones. The name "bromodomain" is derived from the relationship of this domain with the Drosophila gene *Brahma* and is unrelated to the chemical element bromine. A well-known example of a bromodomain family is the **BET** (Bromodomain and extraterminal domain) **family**. Members of this family include BRD2, BRD3, BRD4 and BRDT.

Article

A transcriptional switch governs fibroblast activation in heart disease

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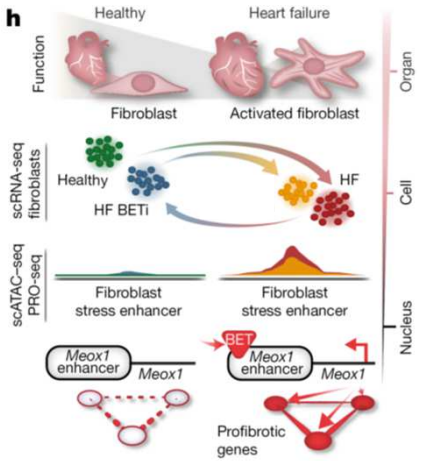
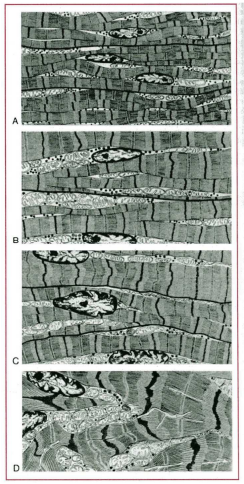
Michael Alexanian[1], Pawel F. Przytycki[1], Rudi Micheletti[2], Arun Padmanabhan[1,3], Lin Ye[1], Joshua G. Travers[4], Barbara Gonzalez-Teran[1], Ana Catarina Silva[1], Qiming Duan[1], Sanjeev S. Ranade[1], Franco Felix[1], Ricardo Linares-Saldana[5], Li Li[5], Clara Youngna Lee[1], Nandhini Sadagopan[1,3], Angelo Pelonero[1], Yu Huang[1], Gaia Andreoletti[6], Rajan Jain[5], Timothy A. McKinsey[4], Michael G. Rosenfeld[6], Casey A. Gifford[7], Katherine S. Pollard[6,7,8,9], Saptarsi M. Haldar[1,3,10] ✉ & Deepak Srivastava[1,10,11] ✉

- *Meox1* is a homeodomain-containing transcription factor necessary for TGFβ-induced fibroblast activation.
- It was highly upregulated via BET in myofibroblasts after transverse aortic constriction (TAC).
- Resident cardiac fibroblasts demonstrated robust toggling between the quiescent and activated state in a manner directly correlating with BET inhibitor (BETi) exposure and cardiac function.
- We identify MEOX1 as a central regulator of fibroblast activation associated with cardiac dysfunction and demonstrate its upregulation after activation of human lung, liver and kidney fibroblasts.
- The plasticity and specificity of BET-dependent regulation of MEOX1 in tissue fibroblasts provide previously unknown *trans*- and *cis*-targets for treating fibrotic disease.



ipertrofia del miocardio: aspetti istopatologici



aumento volume cellulare
aumento miofibrille
aspetto normale conservato

aumento volume mitocondri
aumento irregolare miofibrille
eterogeneità cellulare

disorganizzazione tissutale
nuclei ingranditi
disarray delle miofibrille

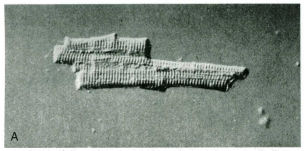
disorganizzazione bande Z
disorganizzazione tubuli T
disarray totale delle miofibrille

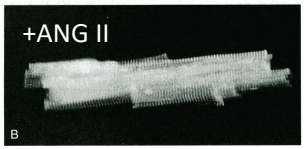
progressione insufficienza

Effetto della prolungata stimolazione con angiotensina

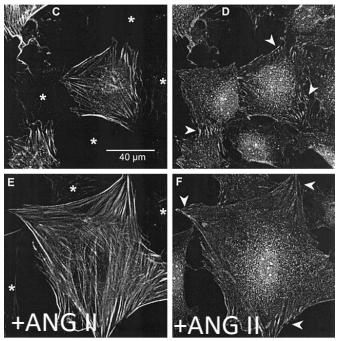
control sham

control +AngII





fibroblasti cardiaci



VIEWPOINTS J Am Heart Assoc. 2022;11:e024656. DOI: 10.1161/JAHA.121.024656

Cardiac Myosin Inhibitors as a Novel Treatment Option for Obstructive Hypertrophic Cardiomyopathy: Addressing the Core of the Matter

Ahmad Masri, MD, MS; Iacopo Olivotto, MD

Over the past 2 decades, the need for more effective and less invasive therapies combined with advances in our understanding of HCM pathophysiology have set the stage for developing new agents targeting the molecular basis of the disease. HCM-associated mutations affecting sarcomere protein genes have been shown to cause myocardial hyper-contractility, due to excessive availability of myosin heads ready to form cross-bridges with actin, with a reduced proportion remaining in the energy-sparing super-relaxed state not available for engagement. This is thought to represent the core pathophysiological abnormality ultimately generating the HCM phenotypes, from compensatory hypertrophy to diastolic impairment, from LVOTO to arrhythmias, and from energy depletion to fibrosis. Inhibiting the myosin ATPase via selective cardiac myosin inhibitors (CMI) counters this state of things by reducing the number of myosin heads available for engagement with resultant return to a normal or quasi-normal contractile state, relief of LVOT obstruction, decrease in wall stress, and improvement in lusitropy.[19]

Currently, there are 2 main CMIs currently in various stages of development, mavacamten and aficamten. In a murine model harboring heterozygous pathogenic mutations in the cardiac myosin heavy chain, chronic administration of mavacamten suppressed the development of ventricular hypertrophy, cardiomyocyte disarray and myocardial fibrosis, and attenuated hypertrophic and profibrotic gene expression.[20] These potent and protean effects support a disease-modifying potential for CMI. The Table summarizes clinical trials of CMIs and the current available therapies for oHCM and the Figure summarizes the landscape of CMIs with current and potential future applications.

Mavacamten is an allosteric, selective, and reversible **inhibitor of cardiac myosin ATPase**. It is a first-in-class molecule that reduces the formation of actin-myosin cross-bridges, thus reducing the probability of systolic and diastolic cross-bridge formation. Excessive myosin actin cross-bridge arrangement and dysregulation of the relaxed state are the pathophysiologic and mechanistic hallmarks of HCM. Mavacamten promotes an energy-sparing and super-relaxed state that translates as a reduction in LVOT obstruction and improvement of cardiac filling pressures.

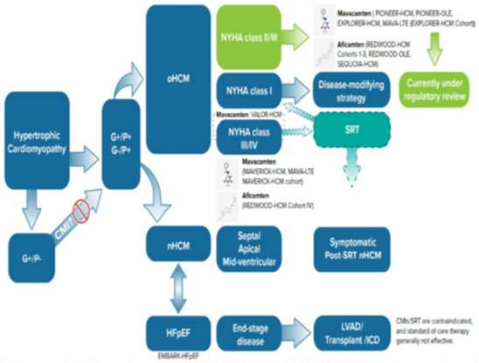


Figure. The landscape of current and future applications of cardiac myosin inhibitors (CMI) for the whole spectrum of hypertrophic cardiomyopathy.
Currently, the most mature application for CMI is symptomatic oHCM with NYHA class II/III, where mavacamten is under regulatory review (green boxes). Other possible future applications of CMIs include disease modifying strategies in asymptomatic individuals (de-novo and post-SRT), in oHCM who are referred for SRT, and in nHCM with septal, apical, mid-ventricular, and post-SRT phenotypes. Patients with heart failure with preserved ejection fraction also represent a future target for CMIs given their mechanism of action. Finally, in the minority of patients who present or progress to end-stage disease (defined as a left ventricular ejection fraction ≤50%), CMIs and SRT are contraindicated and/or not beneficial, and standard of care therapies are not typically effective. In these scenarios, advanced heart failure therapies are required. CMs indicates cardiac myosin inhibitors; G–, genotype negative; G+, genotype positive; HFpEF, heart failure with preserved ejection fraction; ICD, internal cardioverter defibrillator; LVAD, left ventricular assist device; nHCM, non-obstructive hypertrophic cardiomyopathy; NYHA, New Yok Heart Association; oHCM, obstructive hypertrophic cardiomyopathy; P–, phenotype negative; P+, phenotype positive; and SRT, septal reduction therapies.

New York Heart Association (NYHA) Classification

Class I - No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc.

Class II - Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.

Class III - Marked limitation in activity due to symptoms, even during less-than-ordinary activity, *e.g.* walking short distances. Comfortable only at rest.

Class IV - Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients.

Response to Masri and Olivotto

Barry J. Maron, MD; Martin S. Maron, MD; Mark V. Sherrid, MD; Ethan J. Rowin, MD

We welcome therapeutic advances that benefit HCM patients. Nevertheless, we do not agree with some arguments presented regarding myosin inhibitors.

The authors have not completely described current management of HCM, the arena mavacamten would build upon. Surgical myectomy and alcohol ablation are highly effective at reversing heart failure in most obstructive patients with low risk in expert centers. Performed as a one-time procedure, myectomy eliminates need for long-term medical therapy, inevitably associated with high cost typical of novel drug treatments. Indeed, the maturation of myectomy/ablation has been partly responsible for reduced HCM mortality (to only 0.5%/year).

Highly favorable Real world outcome data in thousands of HCM patients over decades does not align with Authors' assertion that somehow the present "status quo" is unacceptable, fails to "empower patients" or personalize care. Claims that myosin inhibitor drugs address the "core (molecular) mechanism of disease" is an attractive hypothesis but without substantiating evidence. Mavacamten actions beyond its negative inotropic gradient reduction are speculative at present.

The EXPLORER-HCM data clearly document that mavacamten can reduce LV outflow gradients. However, there are two other areas that did not receive proper attention. First, most patients (two-thirds) did not meet the short-term pre-defined primary end-point of subjectively improved symptoms and/or modest increases in peak VO_2, suggesting that many of these patients with limited clinical benefit may be (or soon become) potential candidates for myectomy/ablation. Second, risk for systolic dysfunction is understated, odd considering the importance FDA has already placed on this safety issue. If unrecognized in practice, reduced EF <50% could be associated with heart failure symptoms, underscoring importance of long-term vigilance with frequent echocardiography.

Myosin-inhibitors will have a role in management of symptomatic obstructive HCM. However, based on our extensive experience with HCM, prudent perspectives regarding mavacamten (rather than unbridled enthusiasm) would be in the best interests of patients.

Fisiopatologia generale

Insufficienza cardiaca

La condizione in cui la gittata cardiaca si riduce, in assoluto o relativamente alle richieste periferiche, nonostante un normale riempimento venoso atriale

Escluse dalla definizione

Le condizioni di ridotto ritorno venoso

↓

Shock distributivo

Cause di insufficienza cardiaca

	♀	♂
• Ipertensione	37	30
• Coronaropatia	8	19
• Coronaropatia + ipertensione	40	40
• Altre	15	11

Classificazione in base a

1. Tempo: acuta e cronica

2. Sede: sinistra, destra e globale

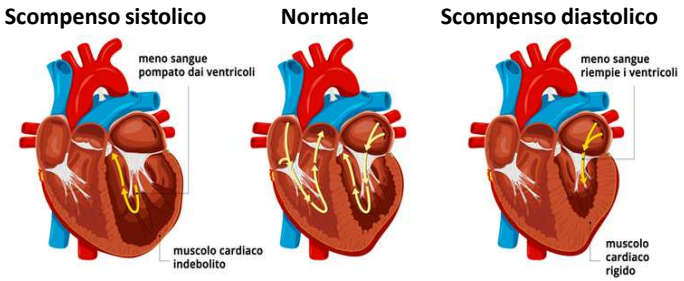
3. Fase del ciclo cardiaco compromessa:

- diastolica (↓ distensione in diastole)
- sistolica (↓ contrazione in sistole)

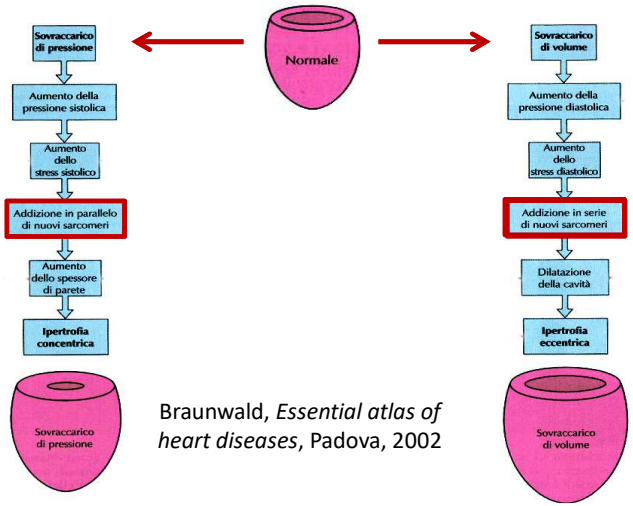
4. Modificazioni della gittata:

- assoluta (bassa gittata <5 l/min)
- relativa (alta gittata >5 l/min)

Correlazione anatomo-clinica della disfunzione cardiaca



Dilatazione con cardiomegalia		Ipertrofia concentrica
++++	Malattia coronarica	+
++	Ipertensione	++++
+++	Diabete	+



Braunwald, *Essential atlas of heart diseases*, Padova, 2002

Insufficienza cardiaca a bassa gittata

Cardiomiopatie e malattie cardiache

Primitive: Ipertrofiche e Dilatative

Secondarie:

Ischemiche	Aterosclerosi Amiloidosi Leucemia
Metaboliche	Morbo di Pompe Avitaminosi B1 (beri-beri) Avitaminosi B2
Infettive	Miocardite virale Difterite Ascesso miocardico (Sepsi)
Immunopat.	Febbre reumatica Artrite reumatoide
Doxorubicina	

Compromissione prevalentemente **sistolica**

↓

↓ forza contrattile (scarso compenso)

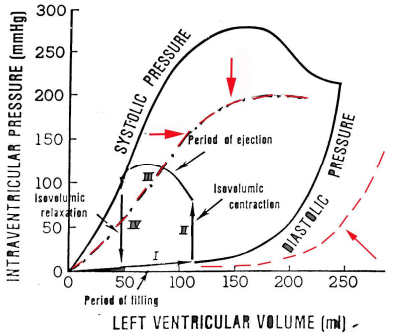
↓

↓ gittata sistolica (FEV < 50%)

↓

Insufficienza

Modificazione del diagramma volume-pressione nell'insufficienza sistolica



Insufficienza cardiaca a bassa gittata

Sovraccarico pressorio

Ipertensione arteriosa
Ipertensione polmonare
Coartazione aortica

Stenosi valvolari (congenite o acquisite)

Malformazioni congenite

Compromissione prevalentemente **diastolica**

↓

Sovraccarico pressorio cronico

↓

Elevata capacità di compenso tramite ipertrofia

↓

gittata sistolica mantenuta a lungo (FEV > 50%)

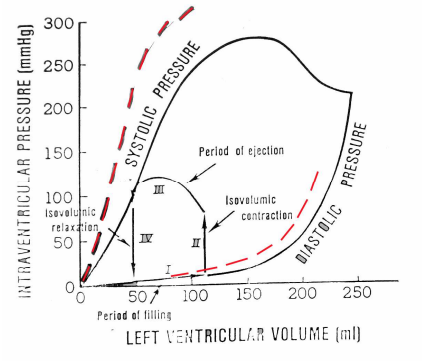
↓

Nel tempo si sviluppa ischemia

↓

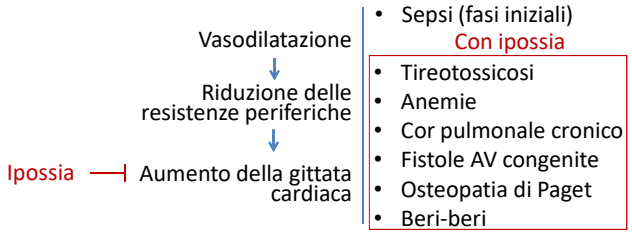
Insufficienza

Modificazione del diagramma volume-pressione nell'insufficienza diastolica

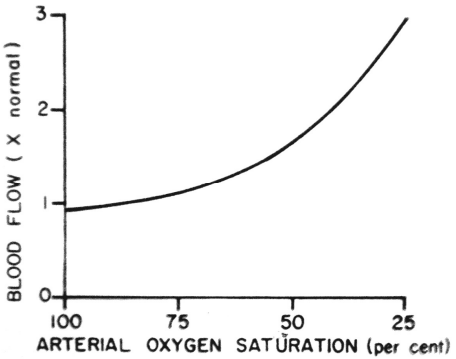


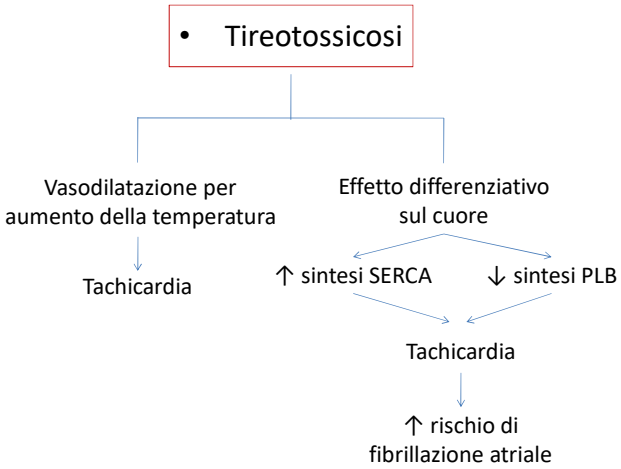
Insufficienza cardiaca ad alta gittata

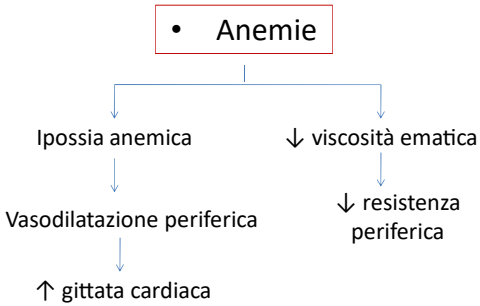
Gittata aumentata in valore assoluto, tachicardia, aumento della pressione differenziale



Effetto della saturazione in ossigeno sul flusso periferico







- Cor pulmonale cronico

Ipertrofia ventricolare destra da ipertensione polmonare

Patologia polmonare

Ipossia ipossica → Vasodilatazione periferica → ↑ gittata cardiaca

Ipertrofia ventricolare → Favorisce l'ischemia

- Fistole arterovenose congenite
- Osteopatia di Paget

shunt artero-venoso

Ipossia periferica → Vasodilatazione periferica → ↑ gittata cardiaca

↑ ritorno venoso → ↑ gittata cardiaca

- Beri-beri

- Da carenza di vitamina B1 (tiamina)
- Dieta monotona in paesi asiatici
- Alcolisti in Occidente
- Correggibile con somministrazione di vit. B1
- *Dry beri beri* (neuropatia periferica)
- *Wet beri beri* (malattia cardiovascolare)
  - Forma acuta fulminante
  - Forma cronica

Wet beri-beri

Ipossia istotossica da blocco della decarbossilazione ossidativa del piruvato

Acidosi lattica + ↑ sintesi adenosina → Vasodilatazione periferica (ipotensione)

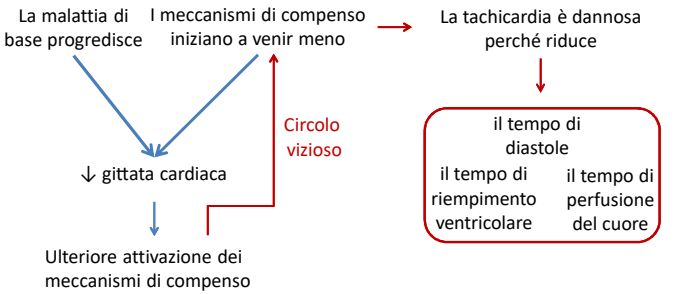
↑ gittata cardiaca tachicardia

Rapido sviluppo di insufficienza cardiaca ad alta gittata (forma fulminante Shoshin beriberi)

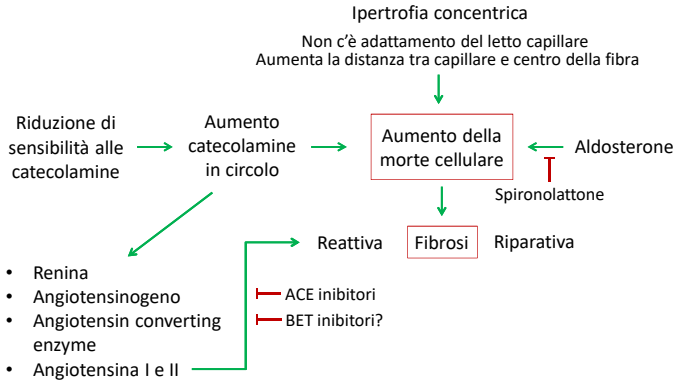
Meccanismi di compenso

- Effetto Starling
- Stimolazione simpatica
- Ipertrofia

Passaggio allo scompenso



Passaggio allo scompenso



Effetti dell'insufficienza cardiaca

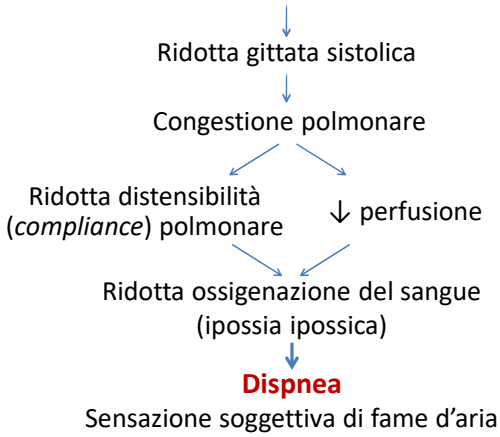
Sinistra

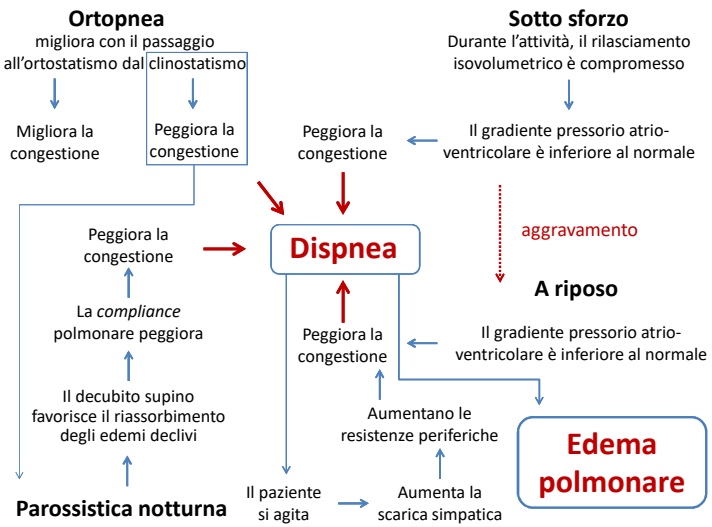
- Effetti retrogradi sul polmone
- Effetti anterogradi sul rene e su altri organi

Destra

- Effetti retrogradi sul fegato e sul circolo venoso sistemico
- Effetti anterogradi sul polmone

Effetti retrogradi da insufficienza sinistra



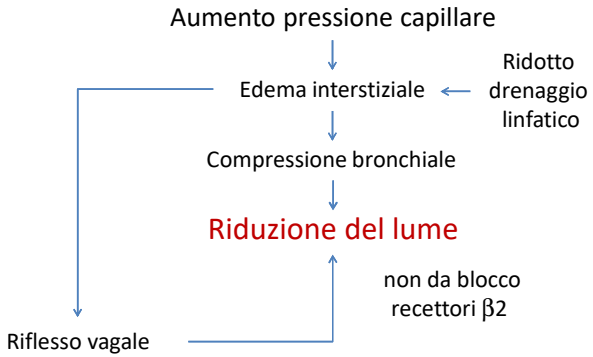


Classificazione NYHA

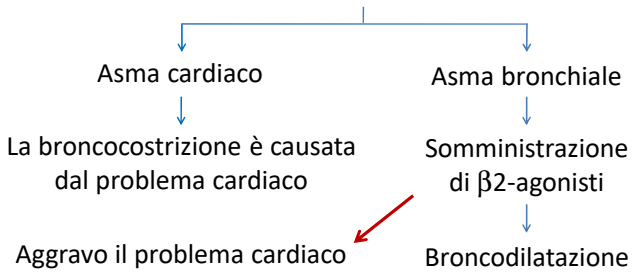
Classe I	Nessuna dispnea durante le comuni attività fisiche
Classe II	Nessuna dispnea a riposo, dispnea moderata durante le comuni attività fisiche
Classe III	Marcata dispnea moderata durante le comuni attività fisiche e per attività inferiori alle comuni
Classe IV	Dispnea a riposo

Asma cardiaco

In un paziente cardiaco, nel contesto di una crisi notturna, si sviluppa dispnea espiratoria da broncospasmo



Attenzione!



La terapia è quella dell'edema polmonare acuto

Indurimento bruno del polmone

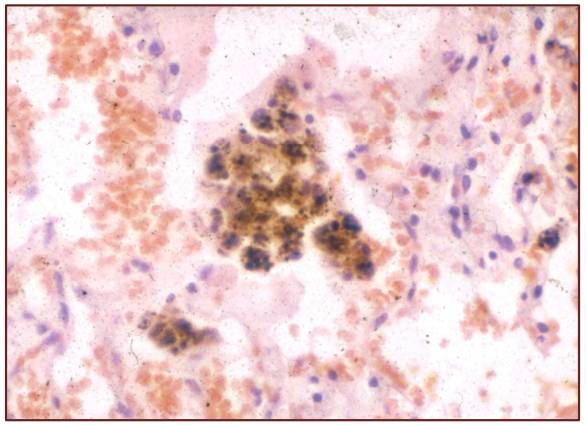
Quadro anatomo-patologico polmonare conseguente alla congestione di lunga durata

Indurimento → Fibrosi

Bruno → Accumulo di «cellule cardiache» → Macrofagi che hanno fagocitato i globuli rossi extravasati in seguito alla congestione, e che si sono caricati di pigmento ferrico

L'indurimento bruno causa ipertensione polmonare → *Cor pulmonale cronico*

«cellule cardiache»



Effetti anterogradi da insufficienza sinistra

Ridotta gittata sistolica

Ridotta perfusione renale

Attivazione del RAAS

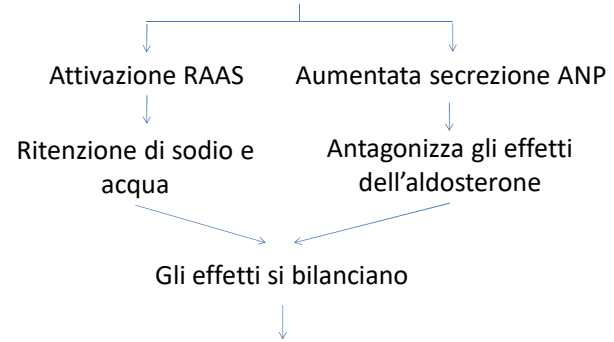
Ritenzione di sodio e acqua

Edema generalizzato anasarcatico

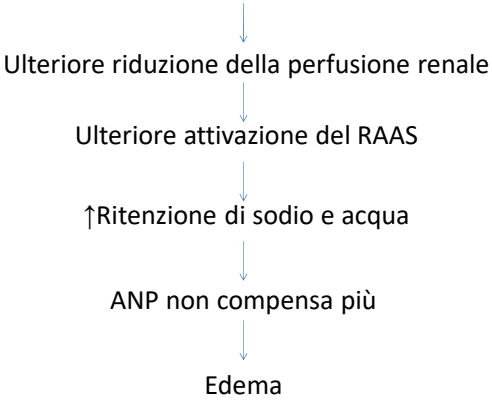
L'edema cardiaco generalizzato si manifesta prevalentemente agli arti inferiori

Edema colonnare

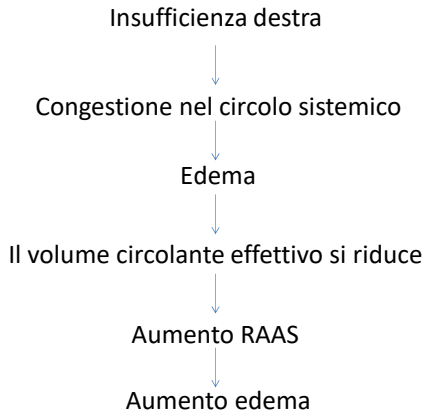
La patogenesi dell'edema cardiaco è complessa



La comparsa dell'edema riflette il peggioramento della situazione



Nella patogenesi dell'edema cardiaco c'è anche una componente retrograda



Effetti dell'insufficienza cardiaca

Sinistra	Destra
• Effetti retrogradi sul polmone • Effetti anterogradi sul rene e su altri organi	• Effetti retrogradi sul fegato e sul circolo venoso sistemico • Effetti anterogradi sul polmone

Effetti retrogradi da insufficienza destra

↓

Congestione sul circolo cavale inferiore

↓

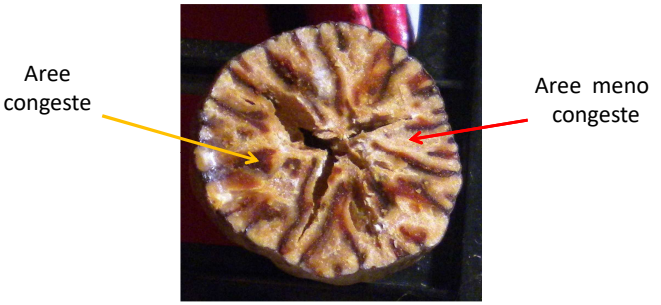
Congestione epatica

↓

Epatomegalia

Fegato a noce moscata

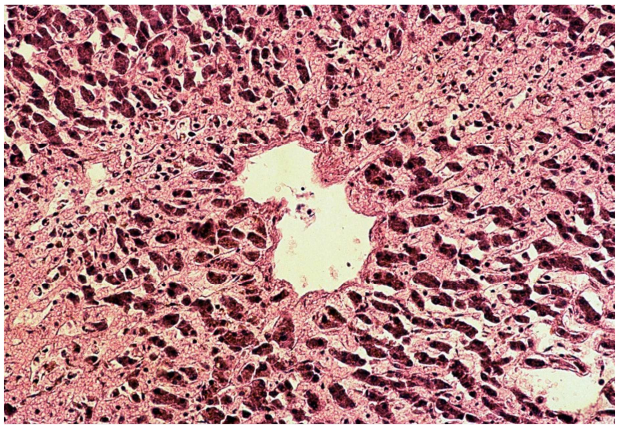
- Il quadro anatomo-patologico macroscopico del fegato da stasi cronica
- La superficie di taglio è caratterizzata dall'alternanza di aree scure congeste e aree chiare non congeste

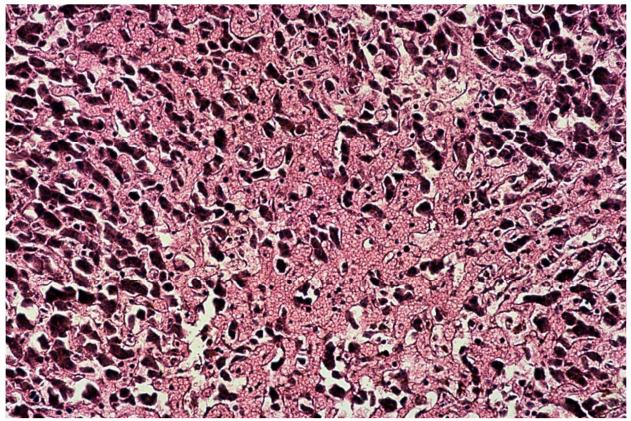


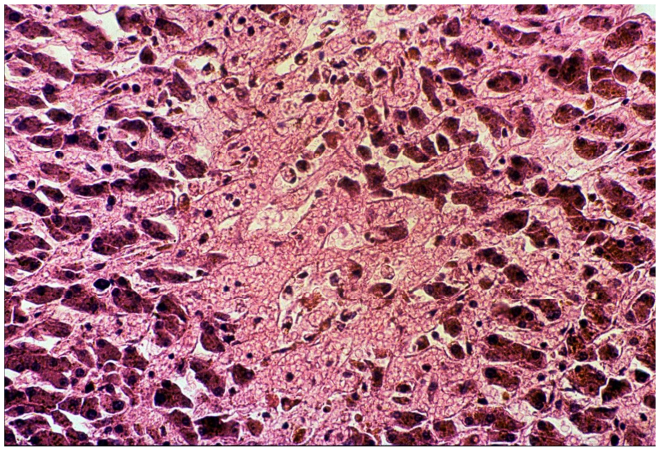
Microscopicamente la congestione è peri-vena centrolobulare.

Gli epatociti attorno alla vena centrolobulare sono atrofici.

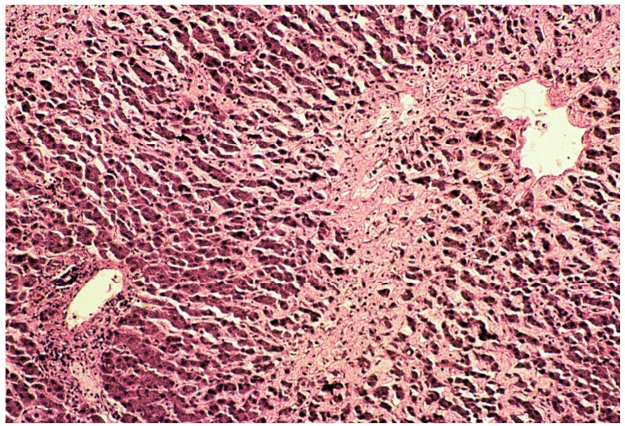
I cordoni di epatociti si assottigliano fino a scomparire

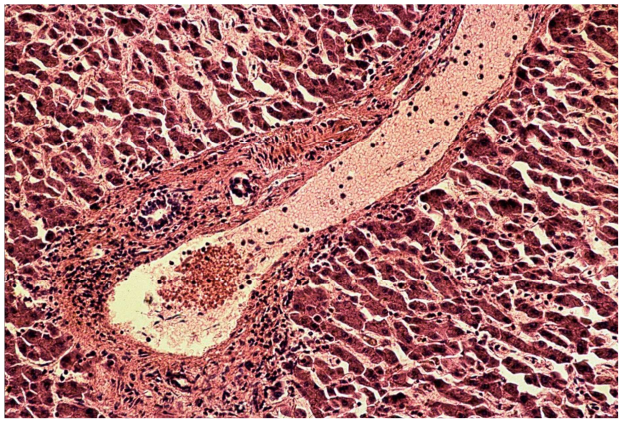






Le aree adiacenti agli spazi portali
sono meglio conservate





Cirrosi cardiaca

Termine improprio per indicare l'eventuale sviluppo di fibrosi attorno alla vena centrolobulare

La differente localizzazione del danno dipende dalla vascolarizzazione epatica

- 20% sangue arterioso
- 80% sangue venoso portale
  - Decorre dagli spazi portali alla vena centrolobulare
    - Epatociti peri-spazi portali più ossigenati
    - Epatociti peri-vena centrolobulare meno ossigenati

Effetti retrogradi da insufficienza destra

- Congestione sul circolo cavale
  - Superiore
    - Turgore delle giugulari
    - Idrotorace
  - Inferiore
    - Stasi agli arti inferiori
      - Edema

Cachessia cardiaca

La situazione di generale deperimento organico con atrofia muscolare associata all'insufficienza cardiaca, in particolare destra.

Patogenesi della cachessia

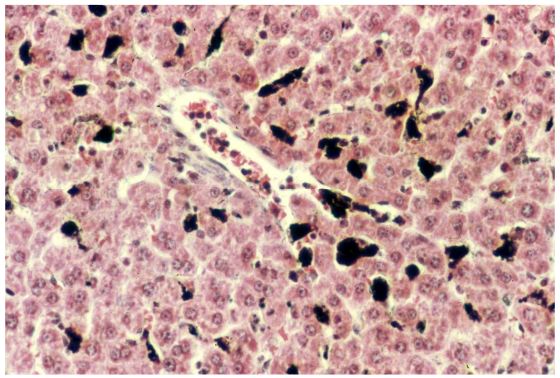
TFNα

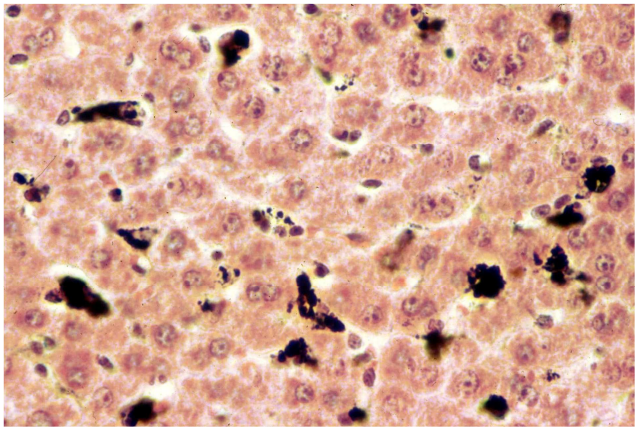
↑ livelli ematici

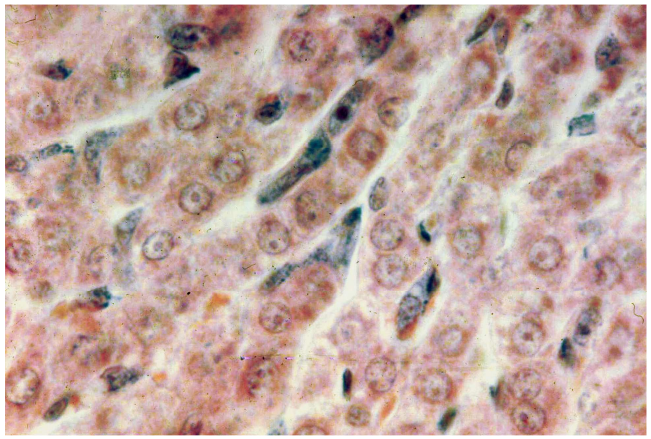
Indicatore prognostico negativo

Ruolo delle cellule di Kupffer?

Cellule di Kupffer







Heart Failure

Philip A Poole-Wilson, *Imperial College School of Medicine, London, UK*
Rakesh Sharma, *Imperial College School of Medicine, London, UK*
Aidan Bolger, *Imperial College School of Medicine, London, UK*

Heart failure is a medical condition in which reduced function of the heart causes symptoms. The commonest symptoms are shortness of breath and tiredness or fatigue.

A pathophysiological state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirements of the metabolising tissues

Eugene Braunwald, 1980

A clinical syndrome caused by an abnormality of the heart and recognised by a characteristic pattern of haemodynamic, renal, neural and hormonal responses

Philip A. Poole-Wilson, 1985

...syndrome... which arises when the heart is chronically unable to maintain an appropriate blood pressure without support

Peter Harris, 1987

Symptoms of heart failure, objective evidence of cardiac dysfunction and response to treatment directed towards heart failure.

Task Force of the European Society of Cardiology, 1995

Figure 1 Heart failure – some definitions.

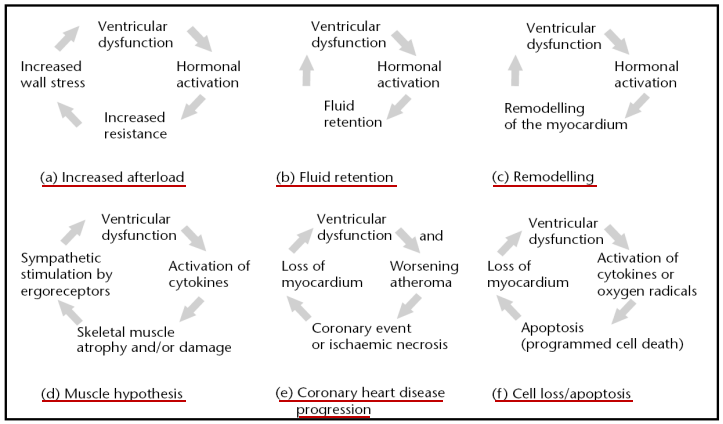
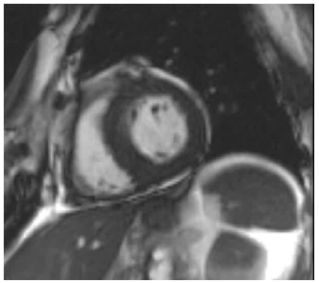
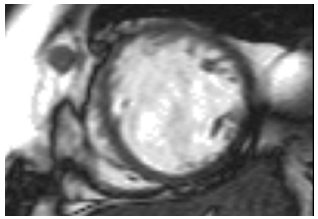


Figure 6 Spirals of heart failure.

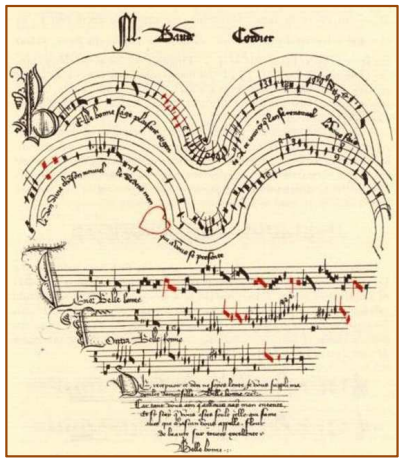
cuore normale



insufficienza cardiaca



danno iniziale → attivazione cronica compenso



Baude Cordier's chanson 'Belle, bonne, sage,' from The Chantilly Manuscript, ca. 1350-1400