



Towards a re-definition of ‘cardiac hypertrophy’ through a rational characterization of left ventricular phenotypes: a position paper of the Working Group ‘Myocardial Function’ of the ESC

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Many primary or secondary diseases of the myocardium are accompanied with complex remodelling of the cardiac tissue that results in increased heart mass, often identified as cardiac ‘hypertrophy’. Although there have been numerous attempts at defining such ‘hypertrophy’, the present paper delineates the reasons as to why current definitions of cardiac hypertrophy remain unsatisfying. Based on a brief review of the underlying pathophysiology and tissue and cellular events driving myocardial remodelling with or without changes in heart dimensions, as well as current techniques to detect such changes, we propose to restrict the use of the currently popular term ‘hypertrophy’ to cardiac myocytes that may or may not accompany the more complex tissue rearrangements leading to changes in shape or size of the ventricles, more broadly referred to as ‘remodelling’. We also discuss the great potential of genetically modified (mouse) models as tools to define the molecular pathways leading to the different forms of left ventricle remodelling. Finally, we present an algorithm for the stepwise assessment of myocardial phenotypes applicable to animal models using well-established imaging techniques and propose a list of parameters most suited for a critical evaluation of such pathophysiological phenomena in mouse models. We believe that this effort is the first step towards a much anticipated unification of the terminology between the experimental and the clinical cardiologists.

Keywords Hypertrophy • Heart failure • Myocardial function

Introduction

The term ‘cardiac hypertrophy’ is used with abandon, but it lacks specificity, precision, and resolution. There have been several attempts to produce satisfactory definitions of ‘hypertrophy’ that are applicable to both clinical and experimental contexts.¹ Although clearly desirable, we are concerned that this may simply not be feasible because of the complexity of the underlying

biological phenomena and differences in our ability to grasp these in the two situations. ‘Hypertrophy’ derives from the Greek *υπερ* (over, beyond) and *τροφη* (food); so even the etymology is not beyond reproach. Cardiac hypertrophy *in vivo* is not a single entity, its development is complex and, as is the case of other multifactorial diseases, such as cancer, it involves multiple aetiologies. There is still a view that any form of cardiac ‘hypertrophy’ is ultimately malign and we would like to challenge this concept.

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Indeed, the terms 'physiological' ('adaptive', 'beneficial', and 'compensated') and 'pathological' ('maladaptive' and 'decompensated') have been applied to so-called 'cardiac hypertrophy' for many years. Thus, the physiological increase in cardiac size following endurance exercise (athlete's heart) or during pregnancy is usually (though not unanimously) differentiated from pathological increase in cardiac size following chronic intrinsic or extrinsic stress. Even within the use of these terms, there are areas of uncertainty and confusion. The Burmese python feeds sporadically (perhaps once a month) but, when it feeds, it ingests a meal equivalent to a significant fraction of its body weight (BW; 25% or more).² The heart mass increases ~40% because of a need to increase metabolic rate and blood supply to peripheral tissues. Is this a physiological enlargement? Yes and no. It is a response to an external stimulus (ingestion of a meal), but is equally a normal part of the python's normal life. Whereas 'physiological' enlargement is largely reversible, pathological enlargement is largely irreversible and is associated with significant increases in morbidity and mortality. There are still areas of debate here. Thus, although wall thickness normalized, a proportion of '5 years deconditioned athletes' who exhibited previous physiological increased left ventricle (LV) size present with ventricular dilatation though with no evidence of dysfunction.³ Some of the dilatation was associated with increased body mass, but it remains possible that prolonged conditioning might be associated with adverse outcomes because of unrecognized (genetic, epigenetic?) influences.

Clearly, there is a need for an operational definition to discriminate between these various situations. 'Hypertrophy' is often indiscriminately designated to ventricular enlargement, with or without ventricular wall thickening (or ventricular wall thickening with or without ventricular enlargement); this is sometimes complemented with calculated left ventricular mass index (LV mass adjusted for body surface area). In the experimental context *ex vivo*, heart weight:body weight (HW/BW) or heart weight:tibial length (HW/TL) is often used. All these terms report changes in the overall volume or mass occupied by the heart in the body. In fact, it is becoming increasingly evident that the mere measurement of these parameters does not allow a complete definition of the possible changes of the heart. Indeed, a variety of changes can be observed in response to a cardiac injury, such as myocardial infarction, pressure or volume overload, some of which do not systematically change the overall volume or mass of the heart or of the ventricle, as depicted in Figure 1. To better define these changes and their functional consequences, at least in an experimental setting, we believe that the different forms of cardiac remodelling need to be clearly delineated using thorough functional, histological, and molecular characterization, as will be detailed below.

Therefore, we propose to restrict the use of the term 'hypertrophy' only in the context of the cardiac myocyte status (*in vivo* or *ex vivo*), rather than the whole heart. Even here, its use in the *in vivo* situation will be restricted to a myocyte whose size is greater than that which would be the case at that given stage of development [i.e. it excludes the normal developmental growth that occurs in this terminally differentiated (non-dividing) cell]. Moreover, we will use the term 'remodelling' to define the reorganization of the different cardiac tissue components (cardiac

myocytes, stroma, and vessels) and this definition is applied to any change in LV shape or size whether it would be an increase or a decrease.

Accordingly, we will describe and discuss:

- the complex biological context of remodelling of cardiac tissue in human subjects;
- the underlying tissue and cellular events responsible for the functional outcome of the remodelled heart;
- how the experimental approaches on animal models should be optimized to gain essential information on the genetic and molecular events controlling the process;
- an algorithm for the stepwise evaluation of the cardiac phenotype in experimental animal models.

Pathophysiology of myocardial remodelling

Despite differences in aetiology, the presence of an insult induces the heart to develop strategies aimed at maintaining cardiac function. The distribution of blood and the perfusion of all organs are the major determinants of regulation of cardiac work. Therefore, any compensatory mechanism will be proportionate to the needs of the whole organism. Two major forms of compensatory

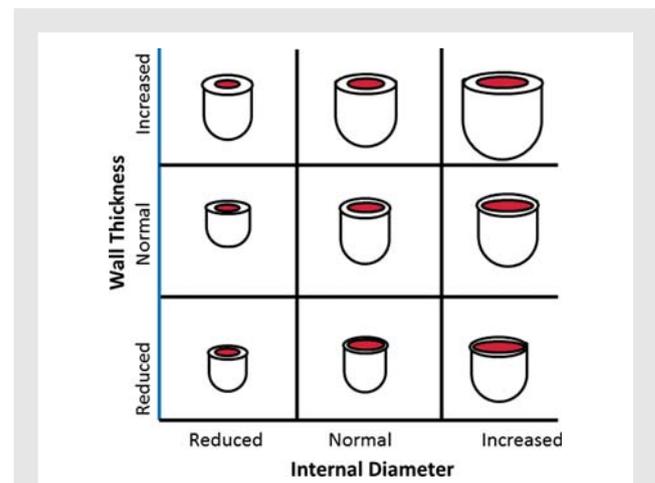


Figure 1 Phenotypes of myocardial remodelling with or without altered left ventricular size. The use of two parameters, i.e. wall thickness and left ventricle internal diameter, allows a more sensitive definition of changes in cardiac dimensions. Indeed, among possibilities, wall thickness remains either normal, is reduced or increases, while internal diameter remains normal, increases, or decreases. Therefore, nine possible combinations of altered cardiac structure may be defined. Changes in wall thickness or diameter can occur in the absence of any change in cardiac mass, but with evident alteration in cardiac structure, and obvious implications in cardiac function. These modifications can all take place at various stages of cardiac disease. The final stage, with decreased wall thickness and increased internal diameter, corresponds to the failing heart, but all other intermediate phenotypes can be accompanied with various degrees of cardiac dysfunction.

response are activated: mechanical and neurohumoral, and the goal of these is to bring about a complete restoration of myocardial function. An example of a short-term mechanical adaptation of the heart is the increase in cardiac volume at the end of the diastole, an attempt to increase cardiac output through the Frank Starling mechanism.⁴ This cardiac adaptation leads to changes in gene expression and cellular composition of three major tissue components of the heart namely cardiac myocytes, fibroblasts of the stroma, and blood vessel cells.^{5,6} Cardiac myocytes undergo hypertrophy that results from at least two separate, but possibly interdependent, stimuli: the stretch imposed on the chamber wall⁷ (and therefore on the individual myocytes), and neurohumoral stimulation by agents such as catecholamines and vasoactive peptides (e.g. angiotensin II or the endothelins).^{8,9} These can be said considered as 'extrinsic' factors. The increased mass of individual myocytes requires a reinforcement of the fibrous scaffold of the heart (the extracellular matrix, ECM), which is sustained by deposition of collagen and other ECM proteins.¹⁰ Current studies challenge the view of the changes in the ECM being only a secondary event in cardiac remodelling. In this context, the fibroblasts (which are responsible for ECM deposition) may be important regulators of the cardiac myocyte hypertrophic response.^{11,12} Endothelial cells, and associated vessel wall components, are additional key elements undergoing important growth during cardiac response to stress stimuli to assure adequate oxygen and nutrient supply in conditions of increased workload. Thus, the cytoarchitectural pattern of myocardial remodelling can greatly influence the functional outcome of the heart response to stress and unbalanced contribution of any of the three major cellular components can promote an inadequate remodelling, leading to heart failure. The natural history of the challenged heart has to be considered as a 'continuum'¹³ in which both loss of cardiac myocytes and cardiac myocyte hypertrophy may co-exist.

It should be pointed out that even though myocyte hypertrophy has traditionally been considered as 'compensatory' of increased load imposed to the myocardium, several clinical studies showed that minimal 'hypertrophy' (i.e. increased LV mass) in patients with severe aortic stenosis predicted a favourable outcome. Increase in LV mass was shown to be dispensable in several genetic mouse models—these animals remained haemodynamically compensated, even after chronic pressure overload.¹⁴ Indeed, ~10% of patients with severe aortic stenosis do not have increased external LV dimensions, while 4% do not present LV mass increase,¹⁵ challenging the paradigm that increased LV thickness is needed to maintain wall stress and contractility. This is consonant with results from experimental studies in animal models of pressure overload that showed that increased LV thickness and LV mass increase are not required to maintain LV function¹⁶ and that deficient increase in LV thickness can, in fact, prevent rather than promote systolic dysfunction.¹⁴ In a prospective cohort of patients with isolated aortic stenosis, increased LV mass alone predicted systolic dysfunction and heart failure, regardless of the severity of the obstruction. Even in patients with critical aortic stenosis, one-fifth did not have LV mass increase and had better preserved ejection fraction and less heart failure when compared with those with increased LV mass.¹⁷ Further reinforcing the negative impact of LV mass increase, in a single-centre

observational study involving more than 3000 patients who underwent aortic valve replacement with a single type of bioprosthesis, severe preoperative LV mass increase ($\geq 185 \text{ g/m}^2$), which preceded symptoms in 17% of patients, decreased long-term survival.¹⁸

In this context, it is relevant to mention physiological conditions where temporary increased LV size ultimately regresses. One example is pregnancy that represents a combination of a mechanical stimulus (volume overload) and hormonal changes¹⁹ inducing a mild and reversible 'eccentric' (volume overload) enlargement associated with chamber expansion and a proportional wall thickening.²⁰ Similarly, endurance exercise-induced increase in LV size largely regresses after cessation of exercise.³ Another example may be aortic regurgitation after correction of valve insufficiency, if performed early enough. In the clinical context, the presence of other co-morbidities (e.g. diabetes), as well as differences in body size, sex, age, exercise regimes, diet, etc. can affect evolution of the disease despite an indistinguishable initial cardiac insult.²¹ It is interesting to note that the modern strategy for the treatment of heart failure is based essentially on the antagonism of the detrimental effects of above-mentioned compensatory mechanisms: beta-blockers and renin angiotensin aldosterone system inhibitors interfere with the neurohormonal mechanisms. Diuretics are used to correct the increases in blood volume, in peripheral resistance, blood pressure (afterload), and to reduce venous return to the heart (preload). When applied with caution in patients with moderate-to-severe heart failure, digitalis provides benefit²² and is recommended by guidelines.²³ Notably, other positive inotropic drugs may provide beneficial effects for a limited period and are useful for the treatment of acute myocardial dysfunction, but they result in detrimental effects in the longer term.²⁴

The issues discussed above raise the need to better define the term hypertrophy avoiding its use to indicate a whole organ remodelling rather than a cellular event regarding exclusively cardiac myocytes.

Cellular and tissue events leading to myocardial remodelling

It is clear from the above that ventricular remodelling involves changes in cardiac wall thickness and chamber volume, and the associated histological characteristics of the myocardium. A gross distinction can be drawn between the muscular component of the heart associated with pump function and cardiac circulation. In pathological cases of remodelling, there is a fall in coronary reserve that may reflect changes in the balance between these two components. At a higher resolution, changes in wall thickness and functional properties result from the relative contribution of at least three major components, namely (i) the cardiac myocytes, (ii) the non-cardiac myocytes (fibroblasts, endothelial cells, inflammatory cells, smooth muscle cells, stem cells, etc.), and (iii) the ECM.

Cardiac myocytes

Cardiac myocytes are large cells and are the major constituents of the myocardium in terms of cellular mass (70–80%) but not in

terms of cell number (only 20–30%). They alone are responsible for the external work performed by the heart. Adult cardiac myocytes are terminally differentiated, non-proliferating cells and there is little evidence that they can undergo complete cycles of cell division even in response to stress conditions, although the viewpoint has been challenged.^{25,26} In some cases cardiac myocytes can undergo nuclear division without cytokinesis to produce polynucleated cells. Equally, there is little evidence that cardiac myocytes can be replaced by differentiation of pluripotent cells. A recent paper²⁷ based on radiocarbon enrichment of the atmosphere during nuclear weapon testing and its subsequent reduction in frequency suggests that cardiac myocyte division/replacement in young adult human beings is maximally 1% per year and this proportion declines with age. Given the technical difficulties involved in the study, such as the separation of myocyte and non-myocyte nuclei (non-myocytes can divide or be replaced), and the assumptions that are necessary (such as that 'DNA damage and repair are very limited in differentiated cells'), it seems very unlikely that myocyte division/replacement is significant in adults, at least in the uninjured heart. This is in spite of recent reports that cardiac myocytes can derive from resident stem cells or from stem cell recruitment following their proliferation and differentiation.²⁷

Although it cannot divide, the cardiac myocyte is capable of genuine hypertrophy (i.e. enlargement over that which would be expected of that cell at a given stage of development, in the absence of a complete cycle of cell division). Indeed, cardiac myocyte hypertrophy could be viewed as an attempt of a terminally differentiated cell to divide under the influence of non-developmentally regulated external stimuli. In order to enable the heart to perform increased work, the number of contractile elements (the sarcomeres) in the cardiac myocytes and possibly the efficiency of contraction have to be increased. In some species, both variables are increased whereas in others sarcomere accumulation alone is the major factor. In this context it is probably important to point out that in a mouse model of transaortic constriction, cardiomyocyte hypertrophy *per se* did not predict the decrease in cell shortening in response to β -adrenergic stimulation, unless it was associated with the isoform switch from α to β myosin heavy chain.²⁸

The adult mammalian cardiac myocyte is an asymmetric cell approximating to a cylinder in which the myofibrils (composed of repeating sarcomeric units) are aligned along the long axis of the cell. Pressure overload causes the addition of sarcomeres principally in parallel with the long axis so that cell cross-sectional area increases disproportionately relative to the length, and the chamber wall thickens in the absence of any significant change in chamber volume. Volume overload causes the addition of sarcomeres principally in series with the long axis causing a disproportionate increase in cell length compared with cross-sectional area.²⁹ The chamber enlarges and the internal and external circumferences increase with some wall thickening and increase in myocyte cross-sectional area in proportion to chamber expansion. Depending on the duration and severity of the stresses imposed, cardiac myocytes develop different degrees of hypertrophy.

In some circumstances, excessive cardiac myocyte hypertrophy eventually leads to heart failure with or without dilatation. Mutations in components of the sarcomeres, Z-discs, or

cytoskeleton are not uncommon causes of myocardial remodelling with increased LV size and heart failure, although the severity of symptoms associated with a particular mutation can vary between individuals. These conditions include the (familial) 'hypertrophic' cardiomyopathies (FHC or HCM) with or without obstruction of the outflow tract, and certain dilated cardiomyopathies (DCM).¹ The cellular organization of the heart is abnormal in these conditions with HCM displaying myofibrillar disarray and DCM showing loss of myocytes and increased proportions of ECM. Although HCM and DCM can (easily) be differentiated in the clinical setting, under experimental conditions this differentiation is not trivial because the presence of dilation does not necessarily exclude the presence of myocyte hypertrophy. Moreover, a fraction of HCM patients (10–20%) develop a DCM phenotype after several years.³⁰ In addition, gene dosage might affect the phenotype as well. Thus, in humans, homozygosity for a mutation that would normally produce HCM when heterozygous is instead associated with DCM.³¹ Furthermore, in mice, heterozygosity in two genes, each of which individually produces HCM, does not in fact cause HCM but rather results in a DCM-like phenotype.^{32,33} As such, genetically modified animal models can provide information useful to understand the relative contribution of cardiac myocyte hypertrophy (either in diameter or in length) and disproportionate tissue remodelling in the pathological phenotype.

In contrast to increased cardiac size, the processes of reduction in heart size and of cardiac myocyte size (atrophy) have been less intensively investigated. Although mainly beneficial roles have been attributed to reductions in the size of failing hearts (e.g. left ventricular assist device support in acute myocarditis), new studies have shown that cardiac muscle atrophy and reduction in cardiac size in systemic inflammation, the presence of metastatic tumours, or immobilization can be as detrimental to cardiac functionality as pathological increased cardiac size.³⁴

Non-myocytes

As indicated above, non-myocytes outnumber cardiac myocytes in the heart but they are small cells and therefore constitute only a minor proportion of the heart mass. Most of these cells are able to divide and they do so during remodelling. Remodelling thus involves an element of non-myocyte hyperplasia. In addition, there may be an invasion of inflammatory cells. Non-myocytes such as fibroblasts are not passive 'bystanders'. They are involved in ECM synthesis and deposition and as such may modify the progression of LV enlargement following different stimuli and may be an important source of cardiac insulin like growth factor 1 (IGF-1) production.¹¹ IGF-1 is probably a major regulator of 'adaptive' or 'physiological' hypertrophy in cardiac myocytes and promotes survival of these cells in response to cytotoxic interventions. However, in contrast to IGF-1, transforming growth factor β 1 (TGF- β 1) may be an important mediator of 'maladaptive remodelling', particularly during the development of HCM due to sarcomeric mutations in animal models as well as in human individuals. TGF- β 1 acts in an autocrine and/or paracrine fashion, thus highlighting cardio myocyte—non-myocyte communication, and might represent a possible target for future therapies.^{35,36}

Angiogenesis

Angiogenesis is another important aspect of LV remodelling. Left ventricle enlargement is associated with increased angiogenesis and inhibition of angiogenesis is associated with a decrease in LV size and the development of heart failure. However, the coronary reserve is reduced in the enlarged LV situation and one interpretation is that although angiogenesis is increased, it is still inadequate, or the function of coronary vessels is altered. The cardiac myocytes may be one determinant of the extent of angiogenesis through expression of the vascular endothelial growth factor, an important and powerful promoter of angiogenesis.³⁷

Inflammation

Left ventricle remodelling is not only the effect of biophysical forces and/or the effects of neurohumoral and angiogenic factors on different cell systems but is also the result of the effects of a multitude of cytokines and different immune cell populations on the myocardium.³⁸ Consequently, myocardial remodelling may also be associated with a significant inflammatory response. However, our understanding of LV remodelling in the context of myocardial inflammation remains rudimentary.³⁹

What can we learn from animal models of remodelling?

Several animal models of both adaptive and maladaptive LV remodelling (with or without changes in LV size or shape) are available for experimental investigation. These include voluntary or involuntary exercise training or pregnancy as well as surgical interventions to induce haemodynamic overload (pressure overload by constriction of the transverse aorta, volume overload by aortocaval shunt and other forms of interference, i.e. infarction by coronary artery ligation and failure induced by rapid ventricular pacing). Notably, animal models of distinctive types of haemodynamic overload (preload vs. afterload) have unveiled remodelling with completely different underlying biology.⁴⁰ Additional models of heart failure have been developed such as inheritable tendency towards chronic hypertension in various animal strains ('cor hypertensivum') or 'diabetic cardiomyopathy', both of which are clinically highly relevant.^{41,42} These two models may be useful because they mimic common pathological conditions observed in human beings. Even though large animals like pigs and dogs represent important models with pathophysiological features closer to humans, mice are widely used because gene-targeting technologies have allowed mutation of any given gene *in vivo*.⁴³ Mice strains carrying mutations, at least partly mimicking those responsible for different forms of human HCM and DCM, are currently available.^{44,45} The natural history of any pathology and the evolution of phenotypes can be monitored and this is a unique advantage of the animal models. For obvious reasons, such analysis is less practicable in the clinical situation, although the broad use of non-invasive echocardiographic techniques allows such prospective monitoring. In addition, genetic manipulation in mice allows exploration of the functional roles of genes encoding structural, signaling, or regulatory proteins, and definition of their impact on heart pathophysiology. Often, overexpression or deletion of a single gene induces changes in cardiac size and function in mice without further intervention. In other cases, the genetic intervention

Table 1 Proposed parameters to ensure accurate evaluation of experimental cardiac phenotypes

- (1) Select appropriate genetic strain/substrain
- (2) Separate analysis for gender
- (3) Select defined age
- (4) Cardiac mass (HW/BW or left ventricular weight/BW) as measured by actual HW; to avoid confounding effects on BW, it is better normalized to TL
- (5) Morphological (RVWT) and functional (fractional shortening/ejection fraction) echo-cardio parameters. Transaortic constriction models need measurement of the pressure gradient
- (6) Morpho-functional parameters need to be measured for a period of time appropriate to define the evolution in long run of the initial response (1–16 weeks depending on intervention type, mouse strain)
- (7) Blood pressure and heart rate need to be evaluated because these parameters may reflect altered myocardial function
- (8) Pressure, volume, and LV response to stress to define systolic and diastolic function
- (9) Histology: cardiac myocyte cross-sectional area; fibrosis, inflammation, and capillary density
- (10) Cardiac myocyte hypertrophy markers (real-time polymerase chain reaction or protein level; or more comprehensive transcriptomic analysis of gene expression (arrays), including e.g. beta-myosin heavy chain and beta-adrenergic receptors

per se does not result in a specific phenotype and only time or a superimposed cardiac stress unveils maladaptive remodelling with loss of contractile function and heart failure or it may unveil adaptive and/or protective effects. Sometimes overexpression or deletion of a gene is embryonically lethal necessitating temporal control of expression to observe a phenotype and occasionally there is a gender bias in the development of a phenotype. Although there are advantages in transgenic animal studies, any model should be critically analysed because of possible artefacts associated with the genetic manipulation.⁴⁶ Considerations should include the effects of graded overexpression on phenotype, the problems associated with introduction of supposedly inert foreign proteins for technical reasons and variation in phenotype and response dependent on strain (both in terms of the genetic change introduced or the effects of any superimposed interventions). However, genetically modified animals represent the best technology currently available allowing characterization of the *in vivo* role of different novel genes in heart pathophysiology.^{47,48} The power of this tool, however, can be fully exploited only if a number of rules are adopted in the planning of the experimental setting and of the phenotyping, as underlined in *Table 1* and *Figure 2*, as detailed below.

Non-invasive evaluation of left ventricle remodelling: algorithm for the stepwise evaluation of cardiac phenotype

From the discussion above, the need clearly emerges for non-invasive techniques to accurately measure the morphologic and

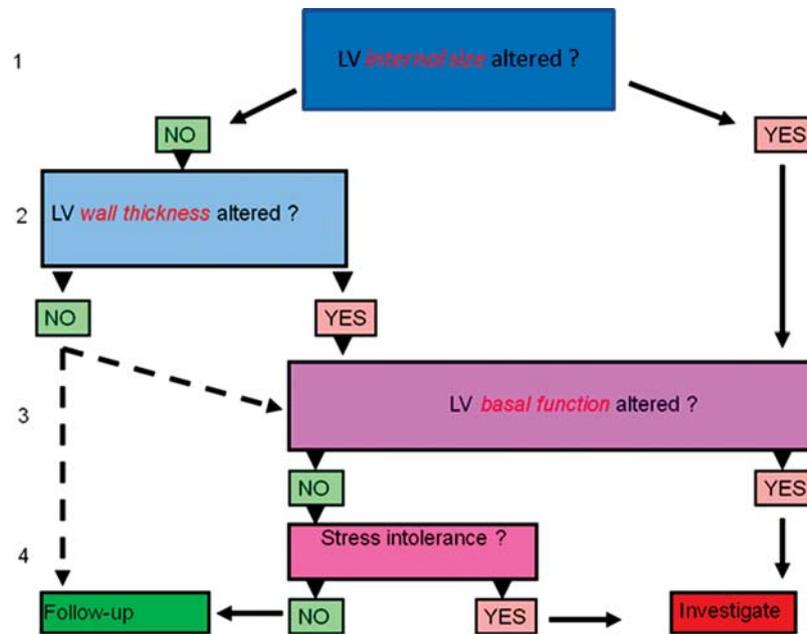


Figure 2 Algorithm for the detection of abnormal ventricular phenotypes. The proposed algorithm proceeds through four steps (steps 1–4). Steps 1 and 2 apply morphometric measurements (by echocardiography and/or magnetic resonance imaging). Steps 3 and 4 apply techniques for functional assessment of ventricular performance (by echocardiography and/or magnetic resonance imaging for ejection fraction /fractional shortening as well as regional wall motion at baseline in step 3; and more thorough assessment in step 4, including response to stress). Stepwise characterization results in a decision either to further investigate ('Investigate') because of probable disease, or mere follow-up ('Follow-up'). Note that accuracy in step 2 may be limited in case of segmental left ventricle infarction or changes in left ventricle thickness limited to one segment of the left ventricle, where complete assessment of left ventricle morphology is needed.

histologic components of remodelling that can be applied in animal models to serially monitor its evolution during the course of disease, as is done in the clinic.

In the large majority of cases, imaging technologies such as echocardiography allows the accurate measurement of LV thickness in all LV segments, LV cavity size, LV systolic function, and LV relaxation by Doppler techniques; occasionally, this is complemented by cardiac magnetic resonance imaging (MRI), if needed. These parameters can be used in a formula to derive an estimate of LV mass.^{49,50} This parameter can be refined by the calculation of the relative wall thickness, by dividing the sum of septal and wall thickness by the internal diameter, and has been widely applied for patients' stratification, e.g. in hypertension.⁵¹ In addition, both echocardiography and MRI can provide qualitative information on myocardial composition, i.e. the extent of fibrosis and other pathological alterations, although the performance of MRI is generally considered superior in this aspect. However, neither can estimate the extent of cardiac myocyte hypertrophy, which can only be determined histologically. Finally, both echocardiography and MRI are used to evaluate LV function, and recent developments with echocardiography using speckle tracking may provide a non-invasive assessment of contractility that may become more accessible; Doppler measurements of mitral flow provides an additional means to study LV relaxation and compliance, which may become affected in the course of LV remodelling and contribute to the development of heart failure with normal ejection fraction (the

reader is referred to excellent reviews and consensus papers for further elaboration on this topic).^{52,53}

Using these (mostly echocardiographic) tools, which are now available with the high-frequency ultrasound machines for small animals, we propose an algorithm (Figure 2) for the stepwise assessment of LV phenotypes that takes into account (i) LV size and (ii) LV wall thickness that allows the discrimination between the different phenotypes as considered in Figure 1. As morphometric criteria alone cannot predict the impact on LV performance (which bears on the progression of disease), subsequent evaluation of LV function adds additional information to detect alterations requiring additional evaluation. A first-step non-invasive evaluation could be the tolerance to stress, i.e. exercise test or catecholamines injection. A second step, invasive evaluation may include haemodynamic measurements, with full assessment of contractile function. Such functional assessment will more clearly locate the phenotype on the continuum of the natural history of the challenged heart. For instance, remodelling with an increased LV size and thickness with preserved cardiac function would define an initial, compensated condition, while an increased LV size and thinning with depressed cardiac function would depict the end stage of the spectrum of the cardiac phenotype. More complete phenotyping can be done with subsequent *ex vivo* analysis. For the latter, we propose the assessment of the parameters as indicated in Table 1 when describing an experimental set-up.

With this algorithm, we propose a stepwise approach where functional assessment will further discriminate abnormal phenotypes despite normal morphometry (and conversely, normal function despite abnormal morphometry). Admittedly, observation of a 'normal function' at the whole organ level may not necessarily predict normal contractility at the myocyte level. For example, remodelling with increased LV wall thickness (with or without increased LV size) may be accompanied with normal or even supranormal parameters of LV function despite impairment of contractile properties of individual cardiac myocytes. Nevertheless, following the algorithm, such situation would be detected as 'remodelling with increased LV thickness (with or without increased LV size)' and lead to further assessment of baseline LV function (which may be normal or supranormal, as pointed out), but, possibly to stress intolerance (due to either increased stiffness or altered relaxation) and lead to further investigation (Figure 2). A clinical illustration of such situation would be aortic stenosis, as discussed previously, where a proportion of patients will develop LV remodelling with no initial change in LV internal size (step 1), but increased LV thickness (step 2); despite unaltered LV basal function (step 3), an exercise test (step 4) may uncover abnormal functional adaptation and lead to further investigations (Figure 2). As another example, a post-ischaemic, dilated LV (step 1) with altered basal function (step 3) would directly undergo further investigation. Conversely, a subject with normal parameters in steps 1 to 4 would be dismissed.

Limitations

Admittedly, the proposed algorithm would have some limitations when applied to LV with large infarcts and/or aneurysm development, where a combination of asymmetric dilatation and segmental wall thinning may confound the interpretation. This would also be the case for asymmetric LV remodelling with or without dilatation. There, global assessment of all LV segments and LV function by echocardiography should nevertheless guide the diagnosis. Most other situations, however, would be discriminated and take into account the various scenarios leading to remodelling with 'increased LV size' or 'changes in LV wall thickness with normal LV size'.

Conclusion

In summary, hypertrophy of cardiac myocytes and hyperplasia of non-cardiac myocyte components occur simultaneously in the heart in response to a variety of intrinsic and extrinsic factors. As a consequence, we prefer using the general term 'myocardial remodelling' (which may or may not result in changes in LV shape or size) to describe the morphological changes associated with these pathophysiological conditions. This will circumvent the confusion when the term 'hypertrophy', which specifically refers to the cardiac myocyte component, is applied to the complex situation in the whole heart.

Based on the use of these preferred definitions and to better translate the results of animal studies to the human pathology, we propose an algorithm (Figure 2) for the stepwise assessment of LV phenotypes by the use of echocardiography (and/or other

imaging techniques) as first-line tool and suggest the evaluation of a number of parameters for accurate and critical definition of cardiac phenotype in experimental animal models.

The lack of a common terminology between the clinical world and the experimental set-up represents an important obstacle for the mutual understanding of physicians and researchers. We believe that our proposal, in line with another recent review emphasizing the better predictive value of LV volume and mass in the clinical assessment of heart failure⁵⁴ represents an important step towards the integration of the semantic that will improve the exchange of information between clinical and experimental cardiologists.

Finally, additional terminology is proposed to define a number of situations in which the heart is undergoing important morpho-functional changes:

Developmental or maturational growth of the heart: To be used in growing or immature animals or even human beings in which growth occurs primarily by growth of individual cardiac myocytes in the absence of division, but also by expansion of the non-cardiomyocytic elements of the heart often by cell division.

Perinatal growth of the heart: A subsidiary stage of developmental growth during which myocytes retain some capacity to divide. The age at which human cardiac myocytes withdraw from the cell cycle is unknown.

Hypertrophy: A term that should be used primarily in the context of single cells (here in the context of the cardiac myocyte rather than the whole heart).

Atrophy: The reverse of hypertrophy (of cardiac myocytes)

Remodelling: Defines the reorganization of the different cardiac tissue components (cardiac myocytes, stroma, and vessels) in response to cardiac stress induced by, e.g. myocardial infarction, pressure or volume overload, mutations of sarcomeric genes, etc. and this definition is applied to any change in LV shape or size be it an increase or a decrease.

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