Myocardial Substrate Changes in Advanced Ischaemic and Advanced Dilated Human Heart Failure

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There has been renewed interest in cardiac metabolism at least partly driven by the dramatic improvements in heart failure outcomes in recent sodium-glucose cotransporter 2 (SGLT2) inhibitor trials (1). It has been proposed that these agents provide "thrifty" substrates that the failing heart can leverage for more efficient energy supply; however, this process is poorly understood. Most prior reports have used circulating metabolites or lipids to infer conclusions regarding human cardiac substrate use (2). However, it is unclear if these perturbations, remote from the heart, adequately reflect actual myocardial substrate metabolism. Therefore, we investigated changes across multiple metabolic pathways in human left ventricular myocardial tissue from 26 end-stage HF hearts: ischaemic cardiomyopathy (ICM) (n=14) and non-ischaemic dilated cardiomyopathy (DCM) (n=12), compared to matched healthy donor hearts (n=17).

Two targeted platforms were used to measure 210 known metabolites on our platforms at Sydney Mass Spectrometry: hydrophilic interaction chromatography (HILIC) – tandem mass spectrometry with positive electrospray ionisation, and HILIC chromatography / amide stationary phase – tandem mass spectrometry and negative electrospray ionisation (3). These methods incorporate a broad range of metabolites including amino acids, acylcarnitines, ketone bodies, energetics, and central carbon metabolism. These methods are highly reproducible, with an average coefficient of variation of 3.85% across all metabolites (4). Nevertheless, two independent mass spectrometry runs were performed for each method, which were combined and analysed. We focussed on the 40 metabolites reported in the Table that were present in all tissue samples in both independent LC-MS runs, and that passed internal quality control assessment.

Therefore, we did not adjust for multiple comparison. Data were analyzed by Wilcoxon rank-sum test using SPSS (SPSS 20. Chicago, IL, USA). Metabolites and proteins with $P<0.05$ were considered statistically significant.

Mean age in each group were as follows (mean±SD): age ICM 59±8, DCM 47±12, donor 45±15 years. Left ventricular HF samples (ICM and DCM) were procured from hearts of NYHA class III-IV patients with left ventricular ejection fraction (LVEF) <35% at the time of heart transplantation. All patients regardless of the aetiology of heart failure received guideline-directed medical therapy pre-transplant, including angiotensin converting enzyme inhibitor or angiotensin II receptor blocker, beta blocker and spironolactone. No patients were on mechanical assist devices at time of transplantation.

Age-matched donor hearts were procured but not used for heart transplantation (primarily for logistical reasons); they did not have any known cardiovascular risk factors and all hearts underwent formal pathology examination to ensure they were histologically normal – donor hearts did not show histological pathology. Both donor and recipient hearts were procured after preceding cardioplegia. All hearts (donor and heart failure) were immediately processed and stored in liquid nitrogen according to published protocols (5) (Sydney Heart Bank HREC # 2016/923). Fibrotic tissue was avoided when processing samples for metabolomics. Subsequent analysis showed the intra-group variability in metabolite levels to be low, suggesting that myocardial tissue composition was not a significant issue influencing metabolite results. Samples were crushed in Liquid Nitrogen to powder, and exactly 50 mg tissue used for each sample.

We found significant changes in ICM and DCM myocardium in many pathways. Uridine and its metabolites 2-deoxycytidine, cytidine, and cytosine were significantly increased in *both* ICM and DCM. Uridine is reported as being pathological in heart failure: *in vivo* administration of uridine triphosphate induced myocardial fibrosis, and *in vitro* uridine diphosphate initiated fibrosis upon mechanical stretch via its $P2Y_6$ receptor (6).

Branched-chain amino acids (BCAAs) including valine, leucine, and isoleucine were significantly elevated in both ICM and DCM. Mechanical stress upregulates catabolism of BCAAs, causing cardiac dysfunction either through toxic intermediates -- branched-chain ±-keto-acids -- or through direct activation of the protein kinase mTOR (7).

Methionine, homocysteine, and derivatives were also significantly changed. The homocysteine pathway has been widely implicated in cardiovascular disease, particularly in the promotion of atherosclerosis via endothelial dysfunction, LDL oxidation, and inflammation. However, this is the first report, to our knowledge, that demonstrates decreased homocysteine in HF hearts.

There was increased lipid metabolism in HF myocardium. Short-chain acylcarnitines isovalerylcarnitine, butyrylcarnitine, and 2-methylbutyrylcarnitine were significantly decreased in ICM and DCM compared to donor hearts. These data are consistent with previous reports of increased utilisation of short-chain acylcarnitines in advanced HF and a low myocardial:plasma ratio (2). Ketone bodies were also significantly changed – acetoacetate was significantly decreased in DCM compared to donor, whereas ² -hydroxybutryrate was significantly increased

in DCM compared to donor hearts. However, there was no consistent change in ketone bodies across both types of HF, which is at odds with reports suggesting the failing heart is dependent on ketone bodies as its major substrate. Interestingly, recent work examining mechanisms underpinning benefits of SGLT2 inhibitor empagliflozin in the hearts of db/db mice reported increased glucose and fatty acid oxidation, but no change in ketone body oxidation (8).

Deoxyribose-5-phosphate (DRP), a pentose phosphate pathway (PPP) intermediate, was significantly elevated in both ICM and DCM, aligned with reported elevation of PPP intermediates post myocardial infarction (MI). Consistent with previous reports of reduced metabolic flexibility and changes in glycolysis in advanced HF, we found glycolytic intermediates glucose-6-phosphate and fructose-6-phosphate to be significantly reduced in ICM myocardium compared to donor hearts. These and other metabolomic changes are summarised in the Figure.

Herein for the first time, we provide a cross-sectional report on human myocardial substrate perturbations common to heart failure of different aetiologies, as compared to matched, healthy donor hearts. The findings in the current study were limited to those with end-stage, as opposed to earlier-stage, heart failure. Indeed, a spectrum of disease severity and associated metabolite changes are likely to exist, and should be more rigorously explored in animal models in relation to time-course and severity of heart failure development. Our work significantly informs recent work using circulating metabolites and previous murine models of HF.

Furthermore, this work implicates many of the metabolic pathways found to be altered by SGLT2 inhibition.

REFERENCES:

1. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE, Investigators E-RO. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med*. 2015 Nov 26;**373**(22):2117-2128.
2. Bedi KC, Jr., Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, Wang LL, Javaheri A, Blair IA, Margulies KB, Rame JE. Evidence for Intramyocardial Disruption of Lipid Metabolism and Increased Myocardial Ketone Utilization in Advanced Human Heart Failure. *Circulation*. 2016 Feb 23;**133**(8):706-716.
3. Warren CR, O'Sullivan JF, Friesen M, Becker CE, Zhang X, Liu P, Wakabayashi Y, Morningstar JE, Shi X, Choi J, Xia F, Peters DT, Florido MHC, Tsankov AM, Duberow E, Comisar L, Shay J, Jiang X, Meissner A, Musunuru K, Kathiresan S, Daheron L, Zhu J, Gerszten RE, Deo RC, Vasan RS, O'Donnell CJ, Cowan CA. Induced Pluripotent Stem Cell Differentiation Enables Functional Validation of GWAS Variants in Metabolic Disease. *Cell Stem Cell*. 2017 Apr 6;**20**(4):547-557 e547.
4. Kimberly WT, O'Sullivan JF, Nath AK, Keyes M, Shi X, Larson MG, Yang Q, Long MT, Vasan R, Peterson RT, Wang TJ, Corey KE, Gerszten RE. Metabolite profiling identifies anandamide as a biomarker of nonalcoholic steatohepatitis. *JCI Insight*. 2017 May 4;**2**(9).
5. Mamidi R, Li J, Gresham KS, Verma S, Doh CY, Li A, Lal S, Dos Remedios CG, Stelzer JE. Dose-Dependent Effects of the Myosin Activator Omecamtiv Mecarbil on Cross-Bridge Behavior and Force Generation in Failing Human Myocardium. *Circ Heart Fail*. 2017 Oct;**10**(10).
6. Nishida M, Sato Y, Uemura A, Narita Y, Tozaki-Saitoh H, Nakaya M, Ide T, Suzuki K, Inoue K, Nagao T, Kurose H. P2Y6 receptor-Galpha12/13 signalling in cardiomyocytes triggers pressure overload-induced cardiac fibrosis. *The EMBO journal*. 2008 Dec 3;**27**(23):3104-3115.
7. Huang Y, Zhou M, Sun H, Wang Y. Branched-chain amino acid metabolism in heart disease: an epiphenomenon or a real culprit? *Cardiovasc Res*. 2011 May 1;**90**(2):220-223.
8. Verma S, Rawat S, Ho KL, Wagg CS, Zhang L, Teoh H, Dyck JE, Uddin GM, Oudit GY, Mayoux E, Lehrke M, Marx N, Lopaschuk GD. Empagliflozin Increases Cardiac Energy Production in Diabetes: Novel Translational Insights Into the Heart Failure Benefits of SGLT2 Inhibitors. *JACC Basic Transl Sci*. 2018 Oct;**3**(5):575-587.

Figure Legend

A. Significantly changed metabolites. FC: Fold change.

B. Pathway visualisation.

HILIC Metabolites

Glycine
Alanine
Serine
Proline
Valine
Threonine
Cysteine
Isoleucine_Leucine
Isoleucine
Leucine
Aspartate
Lysine
Glutamine
Glutamate
Methionine
Histidine
Glucose
Phenylalanine
Arginine
Tyrosine
Methionine sulfone (MetSul)
Methylsulfone (MES)
Tryptophan
Asparagine
Valine-d8
Phenylalanine-d8
1-methylhistamine
2'-deoxyadenosine
2'-deoxycytidine
3-hydroxykynurenine (3-HK)
5-hydroxyindoleacetic acid (5-HIAA)
Adenosine
Asymmetric dimethylarginine (ADMA)
±-keto-´-(NG,NG-dimethylguanidino)valeric acid (DMGV)
Anserine
beta-Alanine
Betaine
Butyrlcarnitine
Cyclic adenosine monophosphate (cAMP)
Carnosine
Choline
Citrulline
Cotinine
Creatine
Cystamine
Cysteamine
Cytidine
Cytosine

Gamma aminobutyric acid (GABA.1)
GABA.2
Glycerol
Histamine
Kynurenic acid
Ornithine
Phosphocholine
Phosphoethanolamine
Serotonin
Spermine
Taurine
Thiamine
Thyroxine
trans-hydroxyproline (trans-HYP)
Triidothyronine
Trimethylamine N-oxide (TMAO)
Thymidine
Uridine
3-deaazadenosine
3-indolepropionic acid (3-IPA)
² -aminoisobutyric acid (BAIBA)
Acetylcarnitine
Acetylcholine
Carnitine
L-Homoserine
N-acetylglutamine
NG-monomethyl-L-arginine (L-NMMA)
Purine
Pyridoxine
Riboflavin
Anandamide
2-Arachidonyl glycerol
Arachidonic acid
Aminoadipic acid (AAD)
Colchicine
Homocysteine
Acetoacetic Acid
Glyoxylic acid
5-Aminolevulinic Acid
a-ketoisocaproic acid.1
a-ketoisocaproic acid.2
Isovalerylcarnitine.1
Isovalerylcarnitine.2
a-keto-b-methylvaleric acid.1
a-keto-b-methylvaleric acid.2
2-methylbutyrlcarnitine.1
2-methylbutyrlcarnitine.2
2-methylbutyrlcarnitine.3
±-ketoisovaleric acid (KIV)
Propylcarnitine

Amide Metabolites

2-hydroxy-2-methylbutyrate (2H2MB)
2-hydroxyglutarate (2-HG)
2-isopropylmalate (2-IPM)
2-ketohexanoate (2-KH)
2-methylacetoacetate (2-MAA)
2-oxobutanoate (2-OB)
3-methyl-2-oxobutyrate (3M2OB)
2-oxodipate (2-OD)
3-hydroxy-3-methylglutaryl coenzyme A
3-hydroxybutyrate (3-HB)
3-hydroxykynurenine (3-HK)
3-phosphoglycerate (3-PG)
4-hydroxyphenylacetate (4-HPA)
5-hydroxyindoleacetate (5-HIAA)
Acetylphosphate (ACP)
Aconitate
ADP.1
ADP.2
Allantoin
2-aminoadipate (AAD)
Ascorbate
ATP.1
ATP.2
Cholesteryl sulfate (CholSO4)
Cyclic adenosine monophosphate (cAMP)
Cholate
Citraconate
Deoxycholate (DCA)
Deoxyribose-phosphate (DRP)
Fructose 1,6-bisphosphate (Fruc-1,6-bP)
Fructose 6-phosphate
oxidized glutathione (GSSG)
reduced glutathione (GSSH)
Glyceraldehyde
Guanidoacetate (GAA)
Homocysteate (HCA)
Homogentisate
Hydroxyisocaproate (HICA)
Isocitrate
Kynurenate
Kynurenine
Malate
Fumarate
Malonyl-coenzyme A
Mevaloate (MEV)
N-Acetyl-L-Alanine
Nicotinate

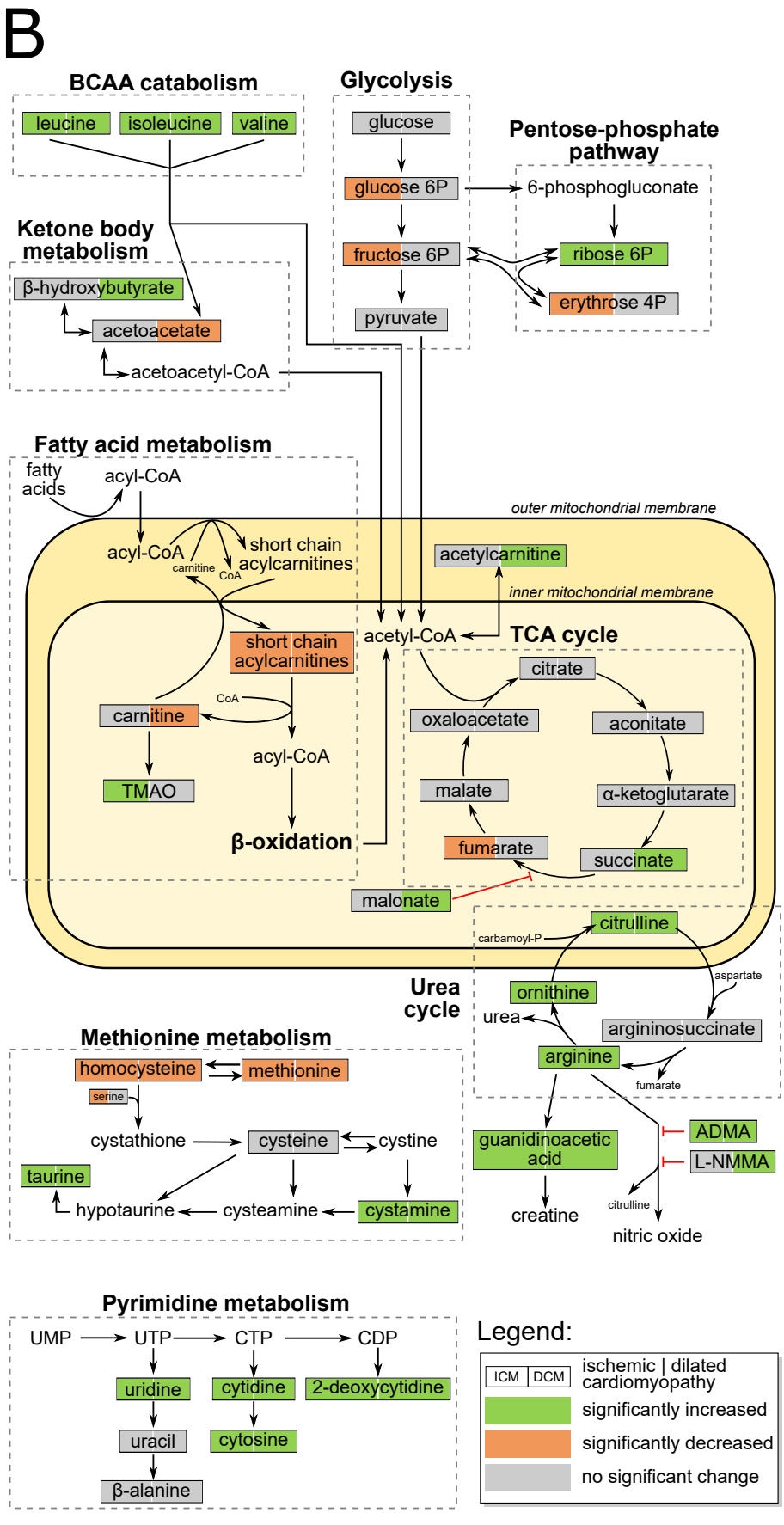
p-aminobenzoate (p-AB)
Phenyllactic
Phenylpyruvate
Phosphoenolpyruvate (PEP)
Pyroglutamate
Pyrophosphate
Pyruvate
Saccharopine
S-adenosyl-L-homocysteine
Taurine
Taurodeoxycholate (TDCA)
Thiamine pyrophosphate (TPP)
Thymidine
Uracil
Uridine
Xanthosine
Xanthurenate (XAN)
Acetyl-coenzyme A
Adenosine monophosphate (AMP.1)
Adenosine monophosphate (AMP.2)
Anthranilate (AA)
Arginosuccinate
Biotin
Erythrose 4-phosphate (E4P.1)
E4P.2
Glyceraldehyde 3-phosphate (G3P)
Glutarylcarnitine (C5-DC)
Inosine
Malonate
N-carbomyl-aspartate
Niacinamide
Oxaloacetate
Phytonadione
Prephenate
Quercetin
Riboflavin 5'-monophosphate (R5'MP.1)
R5'MP.2
Tropisetron
UDP-N-acetyl-glucosamine
Ureidopropionate
Uridine 5'-diphosphate (U5'dP)
Phenylalanine-d8
Thymine-d4
Glucose 6-phosphate
Succinate
Lactate
Camphorsulfonate (CSA)
NADH
Glucose
Nicotinamide Adenine Dinucleotide (NAD)

Reduced nicotinamide adenine dinucleotide phosphate (NADPH)
Nicotinamide adenine dinucleotide phosphate (NADP.1)
NADP.2
Citrate-D4
Citrate
3-hydroxyanthranilate (3-HAA)

A

Pathway	Metabolite	ICM vs. Control FC	ICM vs. Control P Value	DCM vs. Control FC	DCM vs. Control P Value
Pyrimidines	Uridine	2.76	1.00E-06	2.72	7.30E-07
	2-deoxycytidine	4.2	1.20E-05	3.4	1.62E-04
	Cytosine	3.21	1.20E-05	2.79	2.60E-04
	Cytidine	4.87	1.90E-05	4.24	2.60E-04
	N-Carbamoyl aspartate	3.99	1.00E-03	2.75	7.00E-03
Methionine Metabolism	Homocysteine	0.77	8.00E-03	0.86	2.40E-02
	Taurine	1.73	5.00E-03	1.97	1.40E-05
	Methionine	0.66	1.00E-03	0.69	7.00E-03
	Cystamine	1.94	1.30E-02	2.66	2.04E-04
BCAA	Valine	1.65	3.00E-03	1.63	6.00E-03
	Leucine	1.74	3.00E-03	1.82	4.00E-03
	Isoleucine	1.48	5.00E-02	1.75	7.00E-03
Nitric Oxide Metabolism	Arginine	1.47	2.00E-03	1.46	2.70E-02
	ADMA	1.44	2.00E-03	1.49	6.00E-03
	Citrulline	2.14	2.60E-02	2.1	1.20E-02
	L-NMMA	1.35	1.00E-01	1.46	4.80E-02
Urea Cycle	Ornithine	2.23	1.56E-04	1.51	6.00E-03
	Guanidinoacetic acid	1.38	2.60E-02	1.51	1.00E-03
Free Carnitines	Carnitine	0.88	2.10E-01	0.75	8.00E-03
Short Chain Acylcarnitines	Acetylcarnitine	1.17	8.40E-02	1.21	4.30E-02
	Isovalerylcarnitine	0.64	3.20E-02	0.47	4.00E-03
	2-methylbutyrylcarnitine	0.45	6.00E-03	0.37	2.00E-03
	Butyrylcarnitine	0.83	1.00E-03	0.91	6.00E-03
	Glutarylcarnitine	0.61	2.20E-02	0.68	7.89E-02
Ketone Bodies	Acetoacetate	0.85	5.60E-02	0.78	1.80E-02
	3-hydroxybutyrate	1.06	4.70E-01	1.57	2.20E-02
TCA	Succinate	1.27	1.90E-01	1.32	3.40E-02
	Fumarate	0.75	4.70E-02	0.82	1.70E-01
	Malonate	1.05	5.50E-01	1.44	2.70E-02
Glycolysis (intermed and related)	Glucose-6-Phosphate	0.43	3.50E-02	0.61	2.40E-01
	Fructose-6-Phosphate	0.41	3.50E-02	0.57	1.80E-01
	Serine	0.6	9.00E-03	1.03	1.00E+00
Pentose Phosphate Pathway	Deoxyribose-5-phosphate	1.88	1.00E-03	1.71	4.00E-03
	Erythrose 4-phosphate	0.47	4.60E-02	0.51	1.30E-01
Energetic	5-Aminolevulinic	0.78	4.95E-04	0.74	8.00E-06
Second Messenger	cAMP	0.63	8.00E-03	0.72	1.80E-01
Tryptophan Metabolites	Serotonin	2.21	3.00E-03	1.71	5.90E-02
	Tryptophan	1.63	8.00E-03	1.83	2.00E-03
Hormone	Thyroxine	1.53	2.00E-03	1.38	2.10E-01
Amine oxide (microbiome)	TMAO	2.56	5.00E-03	2.06	3.50E-01

B



1. Trahair T, Yeoh T, Cartmill T, Keogh A, Spratt P, Chang V, dos Remedios CG, Gunning P. (1993) Myosin light chain gene expression associated with diseased states of the human heart. *Journal of Molecular and Cellular Cardiology* **26**: 577-585.
2. Yao M, Keogh A, Spratt P, dos Remedios CG, Kiessling PC. (1996) Elevated DNase I levels in human idiopathic dilated cardiomyopathy: An indicator of apoptosis? *Journal of Molecular and Cellular Cardiology* **28**: 95-101.
3. dos Remedios CG, Berry D, Carter LK, Coumans JV, Heinke ME, Kiessling PC, Seeto RK, Trahair T, Yao M. (1996) Different electrophoretic techniques produce conflicting data in the analysis of myocardial samples from dilated cardiomyopathy patients. *Electrophoresis* **17**: 235-238.
4. Berry D, Yao M, Barden JA, Balcar VJ, Hansen MA, Bennett MR, Keogh A, dos Remedios CG. (1998) Alterations in the expression of P2X1 receptors in failing and non-diseased human atria. *Electrophoresis* **19**: 856-859.
5. Berry D, Barden JA, Balcar VJ, Keogh A, dos Remedios CG. (1999) Increase in expression of P2X1 receptors in the atria of patients suffering from dilated cardiomyopathy. *Electrophoresis* **20**: 2059-2064.
6. Berry D, Balcar VJ, Barden JA. Keogh A, dos Remedios CG. (2000) Changes in $P2X_1$ receptors in human left ventricles and their relationship to the acto-ATPase α-sarcoglycan (adhalin). *Electrophoresis* **17**: 3857-3862.
7. Berry DA, Keogh A, dos Remedios CG. (2001) Nuclear membrane proteins in failing human dilated cardiomyopathy. *Proteomics* **1**: 1507-1512.
8. Court NW, dos Remedios CG, Cordell J, Bogoyevitch MA. (2002) Cardiac expression and subcellular localization of the p38 mitogen-activated protein kinase member, Stress-activated protein kinase-3 (SAPK3). *Journal of Molecular and Cellular Cardiology* **34**: 413-426.
9. dos Remedios CG, Chhabra D, Kekic M, Dedova IV, Tsubakihara M, Berry DA, Nosworthy NJ. (2003) Actin and actin binding proteins: Regulation of cytoskeletal microfilaments. *Physiological Reviews* **83**: 433-473.
10. dos Remedios CG, Liew CC, Allen PD, Winslow RL, Van Eyk JE, Dunn MJ. (2003) Genomics, proteomics and bioinformatics of human heart failure. *Journal of Muscle Research and Cell Motility* **24**: 251-260.
11. Ng DCH, Court NW, dos Remedios CG, Bogoyevitch MA. (2003) Activation of signal transducer and activator of transcription (STAT) pathways in failing human hearts. *Cardiovascular Research* **57**: 333-346.
12. Jiang L, Huang, Y, Hunyor S, dos Remedios CG. (2003) Cardiomyocyte apoptosis is associated with increased wall stress in chronic failing left ventricle. *European Heart Journal* **24**: 742-751.
13. Tsubakihara M. Williams NK, Keogh A, dos Remedios CG. (2004) Comparison of gene expression between left atria and left ventricles from non-diseased humans. *Proteomics* **4:** 261-270.
14. Lal S, Lui R, Nguyen L, Macdonald P, Denyer G, dos Remedios CG. (2004) Increases in leukocyte CD antigen expression during cardiopulmonary bypass in patients undergoing heart transplantation. *Proteomics* **4**: 1918-1926.
15. Jiang L, Bardini M, dos Remedios CG, Burnstock G. (2005) P2X1 receptors are closely associated with connexin 43 in human ventricular myocardium. *International Journal of Cardiology* **98**: 291-297.
16. Narolska NA, Piroddi N, Belus A, Boontje NM, Scellini B, Deppemann S, Zaremba R, Musters RJ, dos Remedios CG, Jaquet K, Foster DB, Murphy AM, van Eyk JE, Tesi C, Poggesi C, van der Velden J, Stienen GJM. (2006) Impaired diastolic function after

exchange of endogenous troponin I with C-terminal truncated troponin I in human cardiac muscle. *Circulation Research* **99**: 1012-1020.
17. dos Remedios, CG. (2007) The regulation of muscle contraction: As in life, it keeps getting more complex. *Biophysical Journal* **93**: 4097-4098.
18. Brown A, Lattimore J-D, McGrady M, Sullivan D, Dyer W, Braet F, dos Remedios CG. (2007) CD antibody arrays identify significant differences in leukocytes between stable and unstable angina. *Proteomics Clinical Applications* **1**: 90-98.
19. Zaremba R, Merkus D, Hamdani N, Lamers JMJ, Paulus WJ, dos Remedios C, Duncker DJ, Stienen GJM, van der Velden J. (2007) Quantitative analysis of myofilament protein phosphorylation in small cardiac biopsies. *Proteomics Clinical Applications* **1**: 1285-1290.
20. Hamdani N, Kooji Vj, van Dijk S, Merkus D, Paulus WJ, dos Remedios CG, Duncker DJ, Stienen GJM, van der Velden J. (2008) Sarcomeric dysfunction in heart failure. *Cardiovascular Research* **77**: 649–658.
21. Fermin DR, Barac A, Lee S, Polster SP, Hannenhalli S, Grindle S, Dyke DB, Pagani F, Miller LW, Tan S, dos Remedios CG, Cappola TP, Margulies KB, Hall JL. (2008) Sex and age dimorphism of myocardial expression in nonischemic human heart failure. *Circulation: Cardiovascular Genetics* **1**: 117-125.
22. Lal S, Brown A, Nguyen L, Braet F, dos Remedios CG. (2008) Using antibody microarrays to detect microparticles from acute coronary syndrome patients based on CD antigen expression. *Molecular and Cellular Proteomics* **8**: 799-804.
23. Ho JWK, Koundinya R, TS Caetano TS, dos Remedios CG, Charleston MA. (2008) Inferring differential leukocyte activity from antibody microarrays using a latent variable model. *Genome Informatics* **21**:126-137.
24. Kruger M, Kotter S, Grutzner A, Lang P, Andresen C, Redfield MM, Butt E, dos Remedios CG, Linke WA. (2009) Protein kinase G modulates human myocardial passive stiffness by phosphorylation of the titin springs. *Circulation Research* **104**: 87-94.
25. Estigoy CB, Ponten F, Odeberg J, Herbert B, Guilhaus M, Charleston M, Ho JWK, Cameron D, dos Remedios CG. (2009) Intercalated discs: Multiple proteins perform multiple functions in non-failing and failing human hearts. *Biophysical Reviews* **1**: 43-49.
26. van Dijk SJ, Dooijes D, dos Remedios CG, Lamers JMJ, ten Cate FJ, Stienen GJM, van der Velden J. (2009) Myosin binding protein C mutations and hypertrophic cardiomyopathy: Haploinsufficiency, deranged phosphorylation and cardiomyocyte dysfunction. *Circulation* **119**:1473-1483.
27. Hamdani N, de Waard M, Messer AE, Boontji NM, Kooij V, van Dijk S, Versteilen A, Lamberts R, Merkus D, dos Remedios CG, Dunker D, Borbely A, Papp Z, Paulus W, Stienen GLM, Marston SB, van der Velden J. (2009) Myofilament dysfunction in cardiac disease from mice to men. *Journal of Muscle Research and Cell Motility* **29**:189-201.
28. Messer AE, Gallon CE, McKenna J, dos Remedios CG, Marston SB. (2009) The use of phosphate-affinity SDS-PAGE to measure the cardiac troponin I phosphorylation distribution in human heart muscle. *Proteomics Clinical Applications* **3**: 1371-1382.
29. van Dijk SJ, Holewijn RA, Tebeest A, dos Remedios C, Stienen GJ, van der Velden J. (2009) A piece of the human heart: variance of protein phosphorylation in left ventricular samples from end-stage primary cardiomyopathy patients. *Journal of Muscle Research and Cell Motility* **30**: 299-302.
30. Kooij V, Saes M, Jaquet K, Zaremba R, Foster DB, Murphy AM, dos Remedios C, van der Velden J, Stienen GJM. (2010) Effect of troponin I Ser23/24 phosphorylation on Ca^{2+}-sensitivity in human myocardium depends on the phosphorylation background. *Journal of Molecular and Cellular Cardiology* **48**: 954-963.
31. Kooji V, Boontji N, Zaremba R, Jacquet K, dos Remedios C, Stienen GJM, van der Velden J. (2010) Protein kinase C a and e phosphorylation of troponin and myosin

binding protein C reduce Ca^{2+}-sensitivity in human myocardium. *Research in Cardiology* **105**: 289-300.
32. Kong SW, Hu Y, Ho J, Ikeda S, Polster S, John R, Hall JL, Bisping E, Pieske B, dos Remedios CG, Pu WT. (2010) Heart failure associated changes in RNA splicing of sarcomere genes. *Circulation: Cardiovascular Genetics* **3**: 138-146.
33. Lu Z, Xu W, Hu W, Lee S, Traverse JH, Zhu G, Fassett J, Tao Y, Zhang P, Hall JL, dos Remedios CG, Garry DJ, Chen Y. (2010) Oxidative stress regulates left ventricular PDE5 expression in the failing heart. *Circulation* **121**: 1474-1483.
34. Hoskins AC, Jacques A, Bardswell SC, McKenna W, Tsang V, dos Remedios CG, Ehler E, Adams K, Jalilzadeh S, Avkiran M, Watkins H, Redwood C, Marston SB, Kentish JC. (2010) Normal passive viscoelasticity but abnormal myofibrillar force generation in human hypertrophic cardiomyopathy. *Journal of Molecular and Cellular Cardiology* **49**: 737-745.
35. Robinson AA, Dunn MJ, McCormack A, dos Remedios CG, Rose ML. (2010) Protective effect of phosphorylated Hsp27 in coronary arteries through actin stabilization. *Journal of Molecular and Cellular Cardiology* **49:**370–379.
36. Lal S, dos Remedios CG. (2010) Is there a connection between ablation of atrial fibrillation and reduced apoptosis? *Cardiology* **117**: 161-162.
37. Iskratsch T, Lange S, Kho AL, dos Remedios CG, Ehler E. (2010) Formin follows function: A muscle specific isoform of FHOD3 is regulated by CK2 phosphorylation and promotes myofibril maintenance. *Journal of Cell Biology* **191**: 1159-1172.
38. Hamdani N, Borbely A, Veenstra SPGR, Kooji V, Vrydag R, Zaremba R, dos Remedios C, Niessen HWM, Michel MC, Paulus WJ, Stienen GJM, van der Velden J. (2010) More severe cellular phenotype in human idiopathic dilated cardiomyopathy compared to ischemic heart disease. *Journal of Muscle Research & Cell Motility* **31**: 289-301.
39. Maeder M, Khammy O, dos Remedios C, Kaye D. (2011) Myocardial and systemic iron depletion in heart failure implications for anemia accompanying heart failure. *Journal of the American College of Cardiology.* **58**: 474-480.
40. Changsirivathanathamrong D, Wang Y, Rajbhandari D, Maghzal GJ, Mak WM, Woolfe C, Anzaca FF, Duflou J, Gebski V, dos Remedios CG, Celermajer DS, Stocker R. (2011) Tryptophan metabolism to kynurenine is a potential novel contributor to hypotension in human sepsis. *Critical Care Medicine* **39**: 2679-2683.
41. Bilek M, Bax D, Kondyurin A, Yin Y, Nosworthy N, Fisher K, Waterhouse A, Weiss A, dos Remedios C, McKenzie D. (2011) Free radical functionalization of surfaces to prevent adverse responses to biomedical devices. *Proceedings of the National Academy of Science USA* **108**: 14405-14410.
42. Polden J, McManus CA, dos Remedios C, Dunn MJ. (2011) A 2-D gel reference map of the basic human heart proteome. *Proteomics* **11**: 3582-3586.
43. Wijnker PJM, Bolnik P, Gergs U, Muller F, Neumann J, dos Remedios C, Schmitz W, Sindermann JR, Stienen GJM, van der Velden J, Kirchhefer U. (2011) Protein phosphatase 2A affects myofilament contractility in non-failing but not in failing myocardium. *Journal of Muscle Research Cell Motility* **32**: 221-223.
44. van Dijk SJ, Paalberends RE, Najafi A, Michels M, Sadayappan S, Carrier L, Boontje NM, Kuster D, van Slegtenhorst M, Dooijes D, dos Remedios CG, ten Cate FJ, Stienen GJM, van der Velden J. (2012) Contractile dysfunction irrespective of the mutant protein in human hypertrophic cardiomyopathy with normal systolic function. *Circulation Heart Failure* **5**: 36-46.
45. Gomez-Arroyo J, Mizuno S, Szczepanek K, Van Tassell BW, Natarajan R, dos Remedios C, Drake JI, Farkas L, Kraskauskas D, Wijesinghe DS, Chalfant CE, Bigbee JW, Abbate A, Lesnefsky EJ, Bogaard HJ, Voelkel NF. (2012) Metabolic gene remodeling and mitochondrial dysfunction in failing right ventricular hypertrophy due to pulmonary arterial

hypertension. *Circulation: Heart Failure* **6**: 136-144.
46. Liang LH, Khammy O, Byrne M, Amirahmadi F, Foster A, Li G, Zhang L, dos Remedios C, Chen C, Kaye DM. (2012) Irregular rhythm adversely influences calcium handling in ventricular myocardium: Implications for the interaction between heart failure and atrial fibrillation. *Circulation Heart Failure* **5**:786-793.
47. Li A, Ponten F, dos Remedios CG. (2012) The interactome of LIM domain proteins: The contributions of LIM domain proteins to heart failure and heart development. *Proteomics* **12**: 203-225.
48. Wijnker P, Foster D, Allison D, Tsao A, Frazier A, dos Remedios C, Murphy A, Stienen G, van der Velden J. (2013) Impact of site-specific phosphorylation of the protein kinase A sites Ser23 and Ser24 of cardiac troponin I in human cardiomyocytes. *American Journal of Physiology Heart and Circulatory* **304**: H260-H268.
49. Zhang P, Kirk JA, Ji W, dos Remedios CG, Kass DA, Van Eyk JE, Murphy AM. (2012) Multiple reaction monitoring to identify site-specific troponin I phosphorylated residues in the failing human heart. *Circulation* **126**: 828-1837.
50. Kuster D, Sequeira V, Najafi A, Boontje N, Wijnker P, Paalberends E, Marston S, dos Remedios C, Carrier L, Demmers J, Redwood C, Sadayappan S, van Der Velden J. (2012) GSK3β phosphorylates newly identified site in the Pro-Ala rich region of cardiac myosin binding protein C and alters cross-bridge cycling kinetics in humans. *Circulation Research* **112**: 633-639.
51. Sequeira V, Wijnker PJM, Nijenkamp LAM, Najafi A, Rosalie Witjas-Paalberends R, Regan JA, Boontje N, ten Cate F, Carrier L, Sadayappan S, van Slegtenhorst M, Zaremba R, Foster DB, Murphy A, Poggesi C, dos Remedios C, Stienen GJM, Michels M, van der Velden J. (2013) Perturbed length-dependent activation in human hypertrophic cardiomyopathy with sarcomere mutations in myosin and thin filament proteins. *Circulation Research* **112**: 1491-1505.
52. Bayliss CR, Jacques AM, Leung MC, Ward DG, Redwood CS, Gallon CE, Copeland O, McKenna WJ, dos Remedios C, Marston SB, Messer AE. (2013) Myofibrillar Ca2+-sensitivity is uncoupled from troponin I phosphorylation in hypertrophic obstructive cardiomyopathy due to abnormal troponin T. *Cardiovascular Research* **97**: 500-508.
53. Hamdani N, Krysiak J, Kreusser M, Neef S, dos Remedios C, Maier L, Krüger M, Backs J, Linke W. (2013) Crucial role for Ca2+/calmodulin dependent protein kinase-II in regulating diastolic stress of normal and failing hearts via titin phosphorylation. *Circulation Research* **112**: 664-674.
54. Mollova M, Bersell K, Walsh S, Savla J, Das LT, Park SY, Silberstein LE, dos Remedios CG, Graham D, Colan S, Kühn B. (2013) Cardiomyocyte proliferation contributes to heart growth in young humans. *Proceedings of the National Academy of Science USA*. **110**: 1446-1451.
55. Memo M, Leung MC, Ward DG, dos Remedios C, Morimoto S, Zhang L, Ravenscroft G, McNamara E, Nowak KJ, Marston SB, Messer AE. (2013) Familial dilated cardiomyopathy mutations uncouple troponin I phosphorylation from changes in myofibrillar Ca2+-sensitivity. *Cardiovascular Research* **99**: 65-73.
56. Witjas-Paalberends P, Tencate FJ, Michels M, Niessen JWM, Poggesi C, Piroddi N, Ferrara C, Scellini B, Hazebroek M, Heymans S, Tesi C, Stienen GJM, Vanslegtenhorst M, van Dijk S, Olivera S, dos Remedios CG, van der (2013) J. (2013) Cellular dysfunction in hypertrophic cardiomyopathy. *Cardiovascular Res*earch **99**: 432-441.
57. Li A, Estigoy C, Raftery M, Cameron D, Odeberg J, Ponten F, Lal S, dos Remedios CG (2013) Heart research advances using database search engines, Human Protein Atlas and the Sydney Heart Bank. *Heart Lung Circulation* **22**: 819-826.

58. Rain S, Handoko ML, Westerhof N, Stienen GJM, Paulus WJ, Ottenheijm CAC, Marcus JT, Dorfmuller P, Guignabert C, Humbert M, McDonald P, dos Remedios CG, Postmus PE, Vonk-Noordegraaf A, Van der Velden J, De Man FS. (2013) Right ventricular diastolic impairment in patients with pulmonary arterial hypertension. *Circulation* **128**: 2016-2025.
59. Cordell HJ, Bentham J, Topf A, Zelenika D, Heath S, Mamasoula C, Cosgrove C, Blue G, Granados-Riveron J, Setchfield K, Thornborough C, Breckpot J, Soemedi R, Martin R, Rahman TJ, Hall D, van Engelen K, Moorman AF, Zwinderman AH, Barnett P, Koopmann TT, Adriaens ME, Varro A, George AL Jr, dos Remedios C, Bishopric NH, Bezzina CR, O'Sullivan J, Gewillig M, Bu'lock FA, Winlaw D, Bhattacharya S, Devriendt K, Brook JD, Mulder BJ, Mital S, Postma AV, Lathrop GM, Farrall M, Goodship JA, Keavney BD. (2013) Genome-wide association study of multiple congenital heart disease phenotypes identifies a susceptibility locus for atrial septal defect at chromosome 4p16. *Nature Genetics* **45**: 822-824.
60. Koetter S, Gout L, Van Frieling-Selawsky M, Müller AE, Helling S, Marcus K, dos Remedios C., Linke W, Krüger M. (2013) Differential changes in titin domain phosphorylation increase myofilament stiffness in failing human hearts. *Cardiovascular Research* **99**: 648-656.
61. Kooij V, Zhang P, Piersma SR, Sequeira V, Boontje NM, Wijnker PJM, Jiménez KE, dos Remedios C, Murphy AM, Van Eyk JE, van der Velden J, Stienen GJM. (2013) PKCα-specific phosphorylation of the troponin complex in human myocardium: A functional and proteomics analysis. *PLoS ONE* **8**: e74847 (doi: 10.1371/journal.pone.0074847).
62. Marsman RFJ, Bezzina C, Freiberg F, Verkerk A, Adriaens M, Podliesna S, Chen C, Purfürst B, Spallek B, Koopmann T. Baczko I, dos Remedios CG, George A Jr, Bishopric N, Lodder EM, de Bakker J, Fischer R, Coronel R, Wilde A, Gotthardt M, Remme CA. (2013) Coxsackie and adenovirus receptor (CAR) is a modifier of cardiac conduction and arrhythmia vulnerability in the setting of myocardial ischemia. *Journal of Molecular and Cellular Cardiology* **63**: 549-559.
63. Kuster DW, Mulders J, Ten Cate FJ, Michels M, dos Remedios CG, da Costa Martins PA, van der Velden J, Oudejans CB. (2013) MicroRNA transcriptome profiling in cardiac tissue of hypertrophic cardiomyopathy patients with MYBPC3 mutations. *Journal of Molecular and Cellular Cardiology* **65**: 59-66.
64. van Dijk SJ, Boontje NM, Heymans MW, ten Cat FJ, Michels M, dos Remedios C, Dooijes D, van Slegtenhorst MA, van der Velden J, Stienen GJM. (2013) Preserved cross-bridge kinetics in human hypertrophic cardiomyopathy patients with *MYBPC3* mutations. *Pflügers Archiv - European Journal of Physiology* **466**: 1619-1633.
65. Wijnker PJ, Sequeira V, Witjas-Paalberends ER, Foster DB, dos Remedios CG, Murphy AM, Stienen GJ, van der Velden J (2014) Phosphorylation of protein kinase C sites Ser42/44 decreases Ca2+-sensitivity and blunts enhanced length-dependent activation in response to protein kinase A in human cardiomyocytes. *Archives of Biochemistry and Biophysics* **554**: 11-21.
66. Saba A, Donzelli R, Colligiani D, Raffaelli A, Nannipien M, Kusmic C, dos Remedios C, Simonides WS, Iervasi G, Zucchi R. (2014) Quantification of thyroxine and 3,5,3'-triiodothyronine in human and animal heart by a novel liquid chromatography - tandem mass spectrometry method. *Hormone and Metabolic Research* **46**: 628-634. Doi: 10.1055/s-0034-1368717
67. Manders E, Bogaard H-J, Handoko ML, van de Veerdonk M, Keogh A, Westerhof N, Stienen GJM, dos Remedios CG, Humbert M, Dorfmüller P, Fadel E, Guignabert C, van der Velden J, Vonk-Noordegraaf A, de Man FS, Ottenheijm CAC. (2014) Contractile

dysfunction of left ventricular cardiomyocytes in patients with pulmonary arterial hypertension. *Journal of the American College of Cardiology* **64**: 28-37.

68. Wijnker PJ, Sequeira V, Witjas-Paalberends ER, Foster DB, dos Remedios CG, Murphy AM, Stienen GJ, can der Velden J. (2014) Phosphorylation of protein kinase C sites Ser42/44 decreases Ca2+-sensitivity and blunts enhanced length-dependent activation in response to protein kinase A in human cardiomyocytes. *Archives of Biochemistry and Biophysics* **554**: 11-21.
69. Rain S, Denielli, Bos D, Handoko ML, Westerhof N, Ger Stienen G, Ottenheijm C, Goebel, Dorfmüller P, Guignabert C, Humbert M, Bogaard HJ, dos Remedios C, Saripalli C, Hidalgo CG, Henk L. Granzier, Vonk-Noordegraaf A, van der Velden J, de Man FS. (2014) Protein changes contributing to right ventricular cardiomyocyte diastolic dysfunction in pulmonary arterial hypertension. *Journal of the American Heart Association* **3**: e000716. (doi: 10.1161/JAHA.113.000716).
70. Witjas-Paalberends ER, Güçlü A, Germans T, Knaapen P, Harms HJ, Vermeer AMC, Christiaans I, Wilde AAM, dos Remedios C, Lammertsma AA. van Rossum AC, Stienen GJM, van Slegtenhorst M, Schinkel AF, Michels M, Ho CY, Poggesi C, van der Velden J. (2014) Gene-specific increase in energetic cost of contraction in hypertrophic cardiomyopathy caused by thick filament mutations. *Cardiovascular Research* **103**: 248-257.
71. Walweel K, J. Li J, Molenaar P, Imtiaz1 MS, Quail A, dos Remedios CG, Beard NA, Dulhunty AF, van Helden1 DF, Laver DR. (2014) Regulation of human RyR2 by Ca2+ and Mg2+ in the cytoplasm and in the lumen of the sarcoplasmic reticulum. *Journal of General Physiology* **144**: 263-271.
72. Chahine MN, Mioulane M, Sikkel MB, O'Gara P, dos Remedios CG, Pierce GN, Lyon AR, Foldes G, Harding SE. (2014) Nuclear pore rearrangements and nuclear trafficking in cardiomyocytes from rat and human failing hearts. *Cardiovascular Research* **105**: 31-43. doi: 10.1093/cvr/cvu218.
73. Koopmann T, Adriaens M, Moerland P, Marsman R, Westerveld M, Lal S, Zhang T, Simmons C, Baczko I, dos Remedios C, Bishopric N, Varro A, George A, Lodder E, Bezzina C. (2014) Genome-wide identification of expression quantitative trait loci (eQTLs) in human heart. *PLoS ONE* **9**: e97380. doi: 10.1371/journal.pone.0097380. eCollection.
74. Dwyer J, Pluess M, Iskratsch T, dos Remedios CG, Ehler E. (2015) The formin FHOD1 in cardiomyocytes. *Anatomical Record* **297**: 1560-1570.
75. McNamara JW, Li A, dos Remedios CG, Cooke R. (2015) The role of super-relaxed myosin in skeletal and cardiac muscle. *Biophysical Reviews* **7**: 5-14.
76. Kagemoto T, Li, A, dos Remedios CG, Ishiwata S. (2015) Spontaneous oscillatory contraction (SPOC) in cardiomyocytes. *Biophysical Reviews* **7**: 15-24.
77. Pleuss M, Daeubler G, dos Remedios CG, Ehler E. (2015) Adaptations of cytoarchitecture in human dilated cardiomyopathy. *Biophysical Reviews* **7**: 25-32.
78. Bergmann O, Zdunek S, Felker A, Salehpour M, Alkass K, Bernard S, Szewczykowska M, dos Remedios C, Malm T, Nyengaard R, Possnert G, Jovinge S, Klinikapediatrii K, Sjos S, Jashari R, Druid H, Andrae M, Frisen J. (2015) Dynamics of cell generation and turnover in the human heart. *Cell* **161**: 1566-1575. doi: 10.1016/j.cell.2015.05.026.
79. Ge Z, Lal S, Le TYL, dos Remedios CG, Chong JJH (2015) Cardiac stem cells: translation to human studies. *Biophysical Reviews* **7**: 127-139. DOI 10.1007/s12551-014-0148-0.
80. Polizzotti BD, Ganapathy B, Walsh S, Choudhury S, Ammanamanchi N, Bennett DG, dos Remedios CG, Haubner BJ, Penninger JM, Kühn B. (2015) Stimulation of cardiomyocyte regeneration in neonatal mice and in human myocardium with neuregulin reveals a

therapeutic window. *Science Translational Medicine* **7**: doi: 10.1126/scitranslmed.aaa5171.

81. Sequeira V, Najafi A, McConnell M, Fowler ED, Bollen IAE, Wüst RCI, dos Remedios CG, Helmes M, White E, Stienen GJM, Tardiff JC, Kuster DWD, Van der Velden J. (2015) Synergistic role of ADP and Ca^{2+} in diastolic myocardial stiffness. *Journal of Physiology* **593**:3899-3916. doi: 10.1113/JP270354.
82. Sequeira V, Najafi A, Wijnker PJM, dos Remedios CG, Michels M, Kuster DWK, van der Velden J. (2015) ADP-stimulated contraction: A predictor of thin-filament activation in cardiac disease. *Proceedings of the National Academy*
83. *of Science USA* **112**: E7003-E7012. doi: 10.1073/pnas.1513843112.
84. Mamidi R, Gresham KS, Li A, dos Remedios CG, Stelzer JE. (2015) Molecular effects of the myosin activator omecamtiv mecarbil on contractile properties of skinned myocardium lacking cardiac myosin binding protein-C. *Journal of Molecular and Cellular Cardiology* **85**: 262-272. Doi:10.1016/j.yjmcc.2015.06.011.
85. Wijnker PJM , Li Y, Zhang P, Foster B, dosRemedios C, VanEyk JE, Stienen GJM, Murphy AM, van der Velden J. (2015) A novel phosphorylation site, Serine 199, in the C-terminus of cardiac troponin I regulates calcium sensitivity and susceptibility to calpain-induced proteolysis. *Journal of Molecular and Cellular Cardiology* **82**: 93-103.
86. Marston S, Montgiraud C, Munster AB, Copeland O, Onjee C, dos Remedios C, Messer AE, Ehler, E, Knőll, R. (2015) OBSCN mutations associated with dilated cardiomyopathy and haploinsufficiency. *PLoS One* **10**:e0138568.doi: 10.1371/journal.pone.0138568.
87. Lynch T, Sivaguru M, Velayutham M, Cardounel A, Michels M, Barefield D, Govindan S, dos Remedios C, van der Velden J, Sadayappan S. (2015) Contractile dysfunction in dilated cardiomyopathy caused by MYBPC3 mutation exacerbates cardiac oxidative stress. *Oxidative Medicine and Cellular Longevity* (accepted Aug 11, 2015). doi:10.1155/2015/424751.7.050
88. Lal S, Li A, Allen D, Allen PD, Bannon P, Cartmill T, Cooke R, Farnsworth A, Keogh A, dos Remedios C. (2015) Best practice biobanking of human heart tissue. *Biophysical Reviews* **7**: 399-406. DOI: 10.1007/s12551-015-0182-6.
89. Foster SR, Porrello ER, Stefani M, Smith NJ, Molenaar P, dos Remedios CG, Thomas WG, Ramialison M. (2015) Cardiac gene expression data and in silico analysis provide novel insights into human and mouse taste receptor gene regulation. *Naunyn_Schmiedeberg's Archives of Pharmacology* **388**: 1009-1027. Doi: 10.1007/s0021-015-1118-1.
90. Huang Z-P, Ding Y, Chen J, Wu G, Kataoka M, Hu Y, Yang J-H, Liu J, Drakos SG, Selzman CH, Kyselovic J, Qu L-H, dos Remedios CG, Pu WT, Wang D-Z (2016) Long non-coding RNAs link extracellular matrix gene expression to ischemic cardiomyopathy. *Cardiovascular Research* **112**: 543-554. DOI: 10.1093/cvr/cvw201
91. Lal S, Li A, dos Remedios C. (2016) Limitations in translating animal studies to humans in cardiovascular disease. *Journal of Cardiovascular Translational Research* **9**:165-166. doi: 10.1007/s12265-016-9676-2.
92. Lal S, Nguyen L, Tezone R, Ponten F, Odeberg J, Li A, dos Remedios C. (2016) Tissue microarray (TMA) profiling in human heart failure. *Proteomics* **16**:2319-2326. doi: 10.1002/pmic.201600135.
93. Lange S, Gehmlich K, Lun AS, Blondelle J, Hooper C, Dalton ND, Alvarez EA, Zhang X, Bang ML, Abassi YA, dos Remedios CG, Peterson KL, Chen J, Ehler E. (2016) MLP and CARP are linked to chronic PKCα signalling in dilated cardiomyopathy. *Nature Communications* **7**: 12120. doi: 10.1038/ncomms12120.
94. McNamara J, Li A, Smith NJ, Lal S, Graham RM, Kooiker KB, van Dijk SJ, dos Remedios CG, Harris SP, Cooke R. (2016) Ablation of cardiac myosin binding protein-C disrupts the

super-relaxed state of myosin in murine cardiomyocytes. *Journal of Molecular and Cellular Cardiology* **94**: 65-71. (doi: 10.1016/j.yjmcc.2016.03.009).

95. Messer AE, Bayliss CR, El-Mezgueldi M, Redwood CS, Ward DG, Leung MC, Papadaki M, dos Remedios C, Marston SB. (2016) Mutations in troponin T associated with hypertrophic cardiomyopathy increase Ca2+-sensitivity and suppress the modulation of Ca2+-sensitivity by troponin I phosphorylation. *Archives of Biochemistry and Biophysics* **601**:113-120. (10.1016/j.abb.2016.03.027).
96. Lin Z, Guo H, Cao Y, Zohrabian S, Zhou P, Ma Q, VanDusen N, Guo Y, Zhang J, Stevens SM, Liang F, Quan Q, van Gorp PR, Li A, dos Remedios C, He A, Bezzerides VJ, Pu WT. (2016) Acetylation of VGLL4 regulates Hippo-YAP signaling and postnatal growth. *Developmental Cell* **39**: 466-479. doi: 10.1016/j.devcel.2016.09.005.
97. Crossman DJ, Shen X, Jüllig M, Munro M, Hou Y, Middleditch M, Shrestha D, Li A, Lal , dos Remedios CG, Baddeley D, Ruygrok P, Soeller C. (2017) Increased collagen within the transverse tubules in human heart failure. *Cardiovascular Research* doi: 10.1093/cvr/cvx055.
98. Walweel K, Molenaar P, Imtiaz MS, A Denniss A., dos Remedios C, van Helden DF, Dulhunty AF, Laver DR, Beard N, (2017) Ryanodine receptor modification and regulation by intracellular Ca^{2+} and Mg^{2+} in healthy and failing human hearts. *Journal of Molecular and Cellular Cardiology* **144**: 263-271. Doi: 10.1085/jgp.201311157.
99. Land S, Park-Holohan SJ, Smith NP, dos Remedios CG, Kentish J, Niederer SA. (2017) A model of cardiac contraction based on novel measurements of tension development in human cardiomyocytes. *Journal of Molecular and Cellular Cardiology* **106**: 68-83. doi: 10.1016/j.yjmcc.2017.03.008.
100. Walweel K, Gomez-Hurtado N, Oo YW, Beard NA, dos Remedios C, Johnson CN, Chazin WJ, van Helden DF, Knollmann BC, Laver DR. (2017) Calmodulin mutants linked to catecholaminergic polymorphic ventricular tachycardia fail to inhibit human RyR2 channels during adrenergic stress. *Journal of the American College of Cardiology*. **70**: 115-117. Doi 10.1016/j.jacc.2017.04.055
101. dos Remedios G, Gilmour D. (2017) An historical perspective of the discovery of titin filaments. *Biophysical Reviews* DOI: 10.1007/s12551-017-0269-3.
102. Li A, dos Remedios C. (2017) Special issue on titin and its binding proteins in striated muscle. *Biophysical Reviews* **9**:177-178. DOI 10.1007/s12551-017-0270-x
103. Lin BL, Li A, Mun JY, Previs MJ, Previs SB, Colson BA, Campbell SG, Thomas DD, dos Remedios CG, de Tombe P, Craig R, Warshaw DM, Sadayappan S. (2018) Cardiac and skeletal myosin binding protein-C isoforms differentially regulate thin filament muscle activity in a Ca2+-dependent manner. *eLife* (in press 2.4.2017).
104. dos Remedios CG, Gilmour D. (2017) An historical perspective of the discovery of titin filaments. *Biophysical Reviews* **9**:179-188. DOI: 10.1007/s12551-017-0269-3.
105. McNamara JW, Li A, Lal S, Bos JM, Harris SP, van der Velden J, Ackerman MJ, Cooke R, dos Remedios CG. (2017) *MYBPC3* mutations are associated with a reduced super-relaxed state in patients with hypertrophic cardiomyopathy. *PLoS One* **12**: e0180064. doi: 10.1371/journal.pone.0180064.
106. dos Remedios CG, Peckham M. (2017) 3Rs and Biophysics. *Biophysical Reviews* **9**: 277-278. doi: 10.1007/s12551-017-0291-5.
107. dos Remedios CG, et al. (2017) The Sydney Heart Bank: Improving translational research while eliminating or reducing the use of animal models of human heart disease. *Biophysical Reviews*. **9**: DOI: 10.1007/s12551-017-0305-3.
108. Heinig M, Adriaens ME, Schafer S, van Deutekom H, Lodder EM, Ware JS, Schneider V, Felkin LE, Creemers EE, Meder B, Katus HA, Rühle F, Stoll M, Cambien F,

Villard E, Charron P, Varro A, Bishopric NH, George AL, dos Remedios C, Moral AM, Pesce F, Bauerfeind A, Rüschendorf F, Rintisch C, Petretto E, Barton P, Pinto Y, Cook SA, Bezzina CR, Hubner N. (2017) Natural genetic variation of the cardiac transcriptome in non-diseased donors and patients with dilated cardiomyopathy. *Genome Biology* **18**:170 (doi: 10.1186/s13059-017-11286-z).

109. Bollen IAE, Ehler E, Fleischandel K, Bouwman F, Kempers L, Ricke-Hoch M, Hilfiker-Kleiner D, dos Remedios CG, Krüger M, Vink A, Wouter Asselbergs F, van Spaendonck-Zwarts KY, Pinto YM, Kuster DWD, van der Velden J. (2017) Myofilament remodeling and function is more impaired in peripartum cardiomyopathy compared to dilated cardiomyopathy and ischemic heart disease. *The American Journal of Pathology*. (accepted 25.8.2017).

110. Bos da Silva Goncalves D, Van Der Bruggen C, Kurakula K, Xiao-Qing Sun, Casali K, Adenauer Casali, Rol N, Szulcek R, dos Remedios C, Guignabert C, Tu L, Dorfmüller P, Humbert M, Wijnker PJM, Kuster D, van der Velden J, Goumans JM-J, Jan Bogaard H, Vonk Noordegraaf, de Man AF, Handoko ML. (2017) Contribution of impaired parasympathetic activity to right ventricular dysfunction and pulmonary vascular remodeling in pulmonary arterial hypertension. *Circulation* (accepted 25 Aug 2017).

111. Coats CJ, Heywood WE, Virasami A, Syrris P, dos Remedios C, Treibel TA, Moon JC, McGregor CG, Ashworth M, Sebire N, McKenna WJ, Mills K, Elliott PM. (2017). A proteomic analysis of the myocardium in hypertrophic obstructive cardiomyopathy. *PLoS Medicine* Doi*:* PMEDICINE-D-17-02782.

112. Mamidi Li J, Gresham KS, Verma S, Doh CY, Li A, Lal S, Dos Remedios CG, Stelzer JE. (2017) Dose-dependent effects of the myosin activator omecamtiv mecarbil on cross-bridge behavior and force generation in failing human myocardium. *Circulation: Heart Failure* Doi: 10.1161/CIRCHEARTFAILURE.117.004257/-/DC1.

113. Krysiak J, Andreas Unger A, Beckendorf L, Hamdani N, dos Remedios CG, Margaret M. Redfield[3], Farah Sheikh[4], Gergs U, Boknik P, Linke WA. (2017) Protein phosphatase 5 regulates titin phosphorylation and function at a sarcomere-associated mechanosensor complex in cardiomyocytes. *Nature Communications* **9**: 262. doi: 10.1038/s41467-017-02483-3.

114. Montag J, Syring M, Becker E, Rose J, Weber A-L, Ernstberger P, Mayer A-K, Keyser B, Perrot FAA, dos Remedios C, van der Velden J, Ho CY, Navarro-Lopez F, Brenner B, Kraft T. (2017) Intrinsic MYH7 expression regulation contributes to tissue level allelic imbalance in hypertrophic cardiomyopathy. *Journal of Muscle Research and Cell Motility*. Doi 10.10074-017-9486-4.

115. Vikhorev PG, Smolktunowicz, N, Munster AB, Copeland O, Kostin S, Montgiraud, C, Messer AE, Toliat MR, Li A, dos Remedios CG, Lal S, Blair CA, Campbell KS, Guilin M, Knoll R, Marston SB. (2017) Abnormal contractility in human heart myofibrils from patients with dilated cardiomyopathy due to mutations in TTN and contractile protein genes. *Scientific Reports* **7**: 1-11. doi:10.1038/541598-017-13675-8.

116. Lin, BL, Li A, Mun JY, Previs MJ, Previs SB, Campbell SG, dos Remedios CG, de Tombe P, Craig R, Warshaw DM, Sadayappan S. (2017) Skeletal myosin binding protein-C isoforms regulate thin filament activity in a Ca^{2+}-dependent manner. *Scientific Reports* (accepted 12/2017).

117. Iorga B, Schwanke K, Weber N, Wendland M, Greten S, Birgit Piep, dos Remedios CG, Martin U, Zweigest RT, Kraft T, Bernhard B. (2018) Differences in contractile function of myofibrils within human embryonic stem cell derived cardiomyocytes vs. adult ventricular myofibrils are related to distinct sarcomeric protein isoforms. *Frontiers in Physiology* doi.org/10.3389/fphys.2017.01111

118. dos Remedios CG. (2018) An historical perspective of the discovery of titin filaments – Part 2 *Biophysical Reviews* **10:**1-3 Doi: 10.1007/s12551-017-0393-0.
119. Montag J, Kowalski K, Makul M, Ernstberger P, Radocaj A, Beck, Becker JE, Tripathi S, Keyser B, Mühlfeld C, Wissel K, Pich A, van der Velden J, dos Remedios CG, Perrot A, Francino A, Navarro-Lopez F, Brenner B, Kraft T. Burst-like transcription of mutant and wildtype MYH7-alleles as possible origin of cell-to-cell contractile imbalance in hypertrophic cardiomyopathy. *Frontiers in Physiology* doi: 10.3389/fphys.2018.00359.
120. Martin-Garrido A, Biesiadecki BJ, Salhi HE, Yasin Shaifta Y, dos Remedios C, Ayaz-Guner S, Cai W, Ge Y, Avkiran M, Kentish JC. (2017) Monophosphorylation of cardiac troponin-I at Ser23/24 regulates cardiac myofibrillar Ca^{2+}sensitivity and modulates calpain-induced proteolysis. (accepted 4/2018).
121. Le TYL, Pickett HA, Ho JWK, Yang A, Thavapalachandran S, Igoor S, Yang SF, Farraha M, Voges H, Hudson JE, dos Remedios CG, Bryan T, Kizana E, Chong JJH. (2018) Enhanced cardiac repair by human telomerase reverse transcriptase over-expression in human cardiac mesenchymal stem cells.
122. Li L, Pickett HA, dos Remedios CG, Barbaro PM, Kizana E, Chong JJH. (2018) Platelet-derived growth factor receptor-alpha expressing cardiac progenitor cells can be derived from previously cryopreserved human heart samples. *Stem Cells and Development* **27**:184-198. doi: 10.1089/scd.2017.0082.
123. dos Remedios CG, Li A, Lal S (2018) Non-Sarcomeric causes of heart failure: A Sydney Heart Bank perspective. (in press). *Biophysical Reviews* DOI: 10.1007/s12551-018-0441-4.
124. Kagemoto T, Oyama K, Yamane M, Tsukamoto S, Kobirumaki-Shimozawa F, Li A, dos Remedios C, Fukuda N, Ishiwata S. (2018) Sarcomeric Auto-Oscillations in Single Myofibrils From the Heart of Patients With Dilated Cardiomyopathy. *Circulation Heart Failure* **11**: e004333. doi: 10.1161/CIRCHEARTFAILURE.117.004333.
125. Coleman JLJ, Mouat MA, Wu J, Jancovski N, Bassi JK, Chan AY, Humphreys DT, Mrad N, Yu ZY, Ngo T, Iismaa S, dos Remedios CG, Feneley MP, Allen AM, Graham RM, Smith NJ. (2018) Orphan receptor GPR37L1 contributes to the sexual dimorphism of central cardiovascular control. *Biology of Sex Differences* **9**: 14 doi: 10.1186/s13293-018-0173-y.
126. Gotzmann M, Grabbe S, Schöne D, von Frieling-Salewsky M, dos Remedios CG, Strauch J, Bechtel M, Dietrich JW, Tannapfel A, Mügge A, Linke WA. (2018) Alterations in Titin Properties and Myocardial Fibrosis Correlate With Clinical Phenotypes in Hemodynamic Subgroups of Severe Aortic Stenosis. *Journal of the American College of Cardiology Basic Translational Science* **3(3)**: (accepted February 13, 2018). doi
127. Coats C, Heywood W, Virasami A, Ashrafi N, Syrris P, dos Remedios C, Treibel T, Moon J, Lopes L, McGregor C, Ashworth M, Sebire N, McKenna W, Mills K, Elliott P. (2018) A Proteomic Analysis of the Myocardium in Hypertrophic Obstructive Cardiomyopathy. *Circulation: Genomic and Precision Medicine* (in press).

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