decreased in AB cardiomyocytes with increasing pacing frequency, revealing a negative shortening-frequency relationship that was attenuated by both drug treatments. Length/width ratio was decreased in AB and AB-TAD animals with concomitant decreases in left ventricular (LV) end diastolic and end systolic volumes. Normal cardiomyocyte size and LV volumes were preserved in the AB-SAX group. Interestingly, all AB groups exhibited similar gross hypertrophic remodeling (heart weight:body weight ratio) despite differences in cardiomyocyte morphology. In conclusion, saxagliptin appears superior for preserving normal cardiomyocyte morphology and overall function versus tadalafil, independent of changes in cGMP-PKG activity.

1463-Pos Board B414
Direct Cardiotoxic Action of Quercetin, a Plant Flavonoid, through Mechanism Independent of its Anti-Oxidative Action
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Quercetin (3, 3′, 4′, 5′, 7-pentahydroxyflavone, QCT) is a major flavonoid of plants, known to exhibit anti-oxidative, anti-inflammatory, and anti-cancer effects. QCT has been demonstrated to have a cardioprotective effect through its antioxidant activity. In the present study, we found that QCT markedly enhanced the contractility of a single cardiomyocyte isolated from mouse hearts in a dynamic fashion even under conditions with no apparent oxidative stress. Simultaneous measurement of Ca2+ transient in a Fura-2 loaded single cardiomyocyte revealed that QCT markedly increased the cytosolic Ca2+ levels both at diastolic and systolic phases under regular electrical stimulation. Echocardiography revealed that intravenous administration of QCT also increased the left ventricular systolic function of the heart evaluated by ejection fraction in mice with reduced cardiac function due to a mutation causing genetic dilated cardiomyopathy (delK210 mutation in cardiac troponin T). QCT did not change the maximum force-generating capability and Ca2+-sensitivity of force generation in skinned (membrane-permeabilized) cardiac muscle fibers prepared from mouse hearts, indicating that QCT has no direct effects on the contractile machinery in cardiomyocytes. These findings indicate that QCT has a direct cardiotoxic effect through enhancing the Ca2+ transient in cardiomyocytes independently of its anti-oxidative action. Studies on the molecular mechanisms underlying these phenomena are in progress.

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DCM Mutation ACTCE361G Causes Uncoupling of Myofibril Sensitivity from TnI Phosphorylation that can be Reversed by Epigallocatechin-3-Gallatate
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We examined the relationships between troponin I phosphorylation and Ca2+-sensitivity of force generation in skinned muscle fibers prepared from mouse hearts, indicating that QCT has no direct effects on the contractile machinery in cardiomyocytes. We found that when the TnI phosphorylation level was reduced from 1.02 to 0.3 the Ca2+-sensitivity of force was increased (EC50/P50=1.8), relaxation parameter kREL was reduced and tREL was increased. ACTCE361G mouse myofibrils were uncoupled: Ca2+-sensitivity and relaxation parameters did not depend on troponin I phosphorylation level (EC50/P50=0.88). Nevertheless, modulation of Ca2+-sensitivity by sarcromere length or due to EMD57033 was retained. The Ca2+-desensitiser Epigallocatechin-3-gallate (EGCG) decreased Ca2+-sensitivity in phosphorylated and unphosphorylated NTG myofibrils equally (EC50 P50= 0.35 ± 0.06 and 0.31 ± 0.07 respectively) but did not change the relaxation parameters tREL and kREL. The rate of force development (kACT), measured at high Ca2+, was unchanged in myofibrils with phosphorylated TnI and 22% decreased in unphosphorylated myofibrils indicating that EGCG affects cross-bridge activation kinetics.

EGCG reduced Ca2+-sensitivity and kACT in both phosphorylated and unphosphorylated ACTCE361G myofibrils. The change in EC50 was more in phosphorylated myofibrils, consequently EGCG reversed the lost difference in EC50 values between phosphorylated and unphosphorylated myofibrils and also the difference in relaxation parameters tREL and kREL. The observation that EGCG does not affect either the EC50 P/EC50 unP or tREL in NTG myofibrils but changes them in ACTG E361G suggests that EGCG can restore modulation of cardiac contractile function by TnI phosphorylation to DCM mutant myofibrils independently of its Ca2+-desensitising function.

1465-Pos Board B416
Obscurin Mutations Cause Haploinsufficiency and are Common in Patients with Familial Dilated Cardiomyopathy (FDCM)
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Whole exome sequence data from 28 FDCM patient cardiac muscle samples was screened for potentially disease-causing mutations in 58 genes previously implicated in HCM or DCM. We identified OBS CN gene mutations in 5 samples; one sample had two OBS CN mutations, one also had a DSP mutation and another also had a SCN5A mutation. Also identified were 6 truncating mutations in TTN, 3 mutations in MYH7, 2 in DSP and one each in TNNC1, TNM3, MYOM1, VCL, GLA, PLB, PKP2 and LAMA4.

The mean level of obscurin mRNA was significantly greater and more variable in donor samples than the FDCM samples (1.69 ± 0.53, n=58 compared with 0.57 ± 0.10,n=68, p=0.0025). The mRNA content of FDCM samples was not significantly different with and without OBS CN mutations. The obscurin protein band was estimated to be <1% of the abundance of titin; it was identified and quantified with antibodies. The apparent mass was 960 ± 60 kDa. The OBS CN mutation samples had levels of expression, significantly different from FDCM samples without obscurin mutations; both at diastole and systole under regular electrical stimulation. Immunofluorescence microscopy using obscurin, myomesin and α-actinin-specific antibodies showed that obscurin was located at the level of the M-line and preferentially labelled the sides of the myofibrils. There was no apparent differences between wild-type and mutant samples. Mutations in the obscurin gene should be considered as a significant cause of FDCM, alone or in concert with another mutation. Disease-related OBS CN mutations cause demonstrably abnormal expression in myofibrils that could account for the development of a DCM phenotype.

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Differential Involvement of Various Sources of Reactive Oxygen Species in Thyroxin-Induced Hemodynamic Changes and Contractile Dysfunction of the Heart and Diaphragm Muscles
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Thyroid hormones are key regulators of basal metabolic state and oxidative metabolism. Hyperthyroidism has been reported to cause significant alterations in hemodynamics, and cardiac and diaphragm muscle function, all of which have been linked to increased oxidative stress. Previously, we have shown that thyroxin (T4) treatment in mice resulted in hypertension, increased cardiac reactive oxygen species (ROS), cardiac hypertrophy, and cardiac contractile dysfunction. Here, we sought to investigate the functional impact of T4 on diaphragm muscle function as well as to identify the role and the source(s) of ROS in these distinct phenotypes of our model. Wild-type and T4 mice with and without 2-week treatments with allopurinol (xanthine oxidase inhibitor), apocynin (NADPH-oxidase inhibitor), L-NIO (nitric oxide synthase inhibitor), or MitoTEMPO (mitochondria-targeted antioxidant) were studied. Blood pressure and echocardiography were non-invasively evaluated, followed by ex-vivo assessments of isolated heart and diaphragm muscle functions. Treatment with L-NIO attenuated the T4-induced hypertension in mice. Apocynin improved the left ventricular (LV) dysfunction without preventing the cardiac hypertrophy in these mice. Both allopurinol and MitoTEMPO reduced the T4-induced fatigue of the diaphragm muscles. In conclusion, we show here for the first time that T4 exerts differential effects on various sources of ROS to induce distinct cardiovascular and skeletal muscle phenotypes. Additionally, we find that T4-induced LV dysfunction is independent of cardiac hypertrophy, while xanthine-oxidase is a key player in this process. Furthermore, we prove the significance of both xanthine-oxidase and mitochondrial ROS pathways in T4-induced fatigue of diaphragm muscles.