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controlled by creatine via activation of MtCK within MI. Knowing that the extremely low apparent Km for free ADP in cancer cells as well as in isolated mitochondria is associated with the lack of tubulin II(β2) isotype, we can assume that tubulin II(β2) can specifically control the voltage dependent anion channel (VDAC) selective permeability resulting in adenine nucleotides micro-compartmentation within the MI. The absence of II(β2) tubulin isotype and MtCK isoforms induce significant alterations of phenotype and regulatory mechanisms of cancer cells energy metabolism. By these mechanisms, all mitochondrial ATP can be captured to maintain activated glycolysis and lactate production, known as Warburg effect.

1618-Pos Board B528
Assessment of Cardiac Function in Chromosome 14 Congenic Strains using Pressure-Volume Measurements
Kirsty Foote, Martin W. McBride, Delyth Graham, Kirsten Douglas, Sarah Kettlewell, Godfrey L. Smith, Anna F. Dominiczak, Christopher M. Loughrey.
Elevated blood pressure (BP) is an important determinate of left ventricular hypertrophy (LVH) however, the intensity of BP load does not always correspond to the degree of LVH. Rat chromosome 14 contains a quantitative trait locus (QTL) associated with left ventricular mass index (LVMI) in stroke-prone spontaneously hypertensive animals (SHRSP). This has been verified through reciprocal congenic strains: (1) WKY.SP_{Gla14a}; in which segments of chromosome 14 from SHRSP rats is introgressed into Wistar Kyoto rats (WKY) resulting in an increased LVMI and (2) SP.WKY_{Gla14a} (the reciprocal) which results in a decreased LVMI. To assess whether changes in LVMI in these congenic strains were related to LV pressure differences, load-independent functional pressure-volume measurements were performed. 17-week old male WKY (n=6), WKY.SP_{Gla14a} (n=5) SHRSP (n=5) and SP.WKY_{Gla14a} (n=8) rats were anaesthetised (1.5-2% isofluorane) and a 1.9F pressure-volume catheter (*SciSense*) inserted into the LV. Pressure-volume measurements were recorded in steady state and during alteration of preload through transient vena cava occlusion. WKY.SP_{Gla14a} rats showed a small but significant increase in end-systolic pressure (ESP) compared to WKY rats (139.9 ± 1.5 *vs.* 120.6 ± 4.7 mmHg, $P<0.05$) however both values were in the normotensive range. End-diastolic pressure (EDP) was significantly increased in WKY.SP_{Gla14a} compared to WKY rats (11.2 ± 1.1 *vs.* 7.5 ± 1.0 mmHg, $P<0.05$ respectively) and end-diastolic stiffness constant (β) measured from the end-diastolic pressure-volume relationship (EDPVR) was also increased (0.034 ± 0.001 *vs.* 0.016 ± 0.004, $P<0.05$). SP.WKY_{Gla14a} and SHRSP were hypertensive to the same degree (ESP: 179.9 ± 8.7 *vs.* 171.2 ± 5.3 mmHg; EDP 9.6 ± 1.1 *vs.* 12.2 ± 1.1 mmHg respectively) however, SP.WKY_{Gla14a} rats have an improved β value. These data demonstrate that the identified QTL on chromosome 14 contributes to a blood pressure independent diastolic dysfunction which corresponds to a change in LVMI.

1619-Pos Board B529
Differentiation of H9c2 Cells Enables to Assess the Insulin-Induced Glucose Uptake: A Novel *ex vivo* Model to Investigate Insulin-Resistant Myocardium
Mamoru Nobuhara, Masao Saotome, Tomoyuki Watanabe, Tsuyoshi Urushida, Hideki Katoh, Hiroshi Satoh, Hideharu Hayashi.
[Purpose] Although the myocardial insulin resistance is a deteriorating factor in failing heart, little is known about the mechanisms of altered cardiac metabolism. We investigated to establish a novel *ex vivo* insulin resistant cardiac model by using a rat cardiac cell line (H9c2 cell).
[Methods] H9c2 cardiac myoblasts were cultured with a differentiation medium (Dulbecco's modified Eagle's medium with 1% of fetal bovine serum) to induce the differentiation to cardiomyocytes. To assess the insulin signal pathway in differentiated H9c2 cells, the expression of insulin receptor substrate-1 (IRS-1) and glucose transporter (GLUT4) was measured. The differentiated H9c2 cardiomyocytes were then cultured with saturated fatty acid (palmitate; 0.2 mM) for 24 hours. To investigate whether differentiated cardiomyocytes were insulin-resistant, the GLUT4 translocation after insulin was assessed by immunocytochemistry, and then the insulin (100 nM)-stimulated glucose uptake was evaluated with 2-deoxy-D-glucose (2DG).
[Results] (1) Expression of IRS-1 and GLUT4 was significantly increased in differentiated cardiomyocytes, compared with that in non-differentiated H9c2 cells. (2) There was neither the insulin-induced GLUT4 translocation nor the 2DG uptake after insulin in non-differentiated H9c2 cells, whereas both the GLUT4 translocation to plasma membrane and the 2DG uptake (1.7 ± 0.1 fold increase from non-stimulation, vs. 1.0 ± 0.1 of control, $p< 0.01$) were activated after insulin (100 nM) in differentiated cardiomyocytes. (3) 2DG uptake after insulin was significantly reduced in palmitate-treated cells (0.5 ± 0.1 fold increase from non-stimulation, vs. 1.7 ± 0.1 of control, $p<0.01$), showing the decrease in glucose uptake.
[Conclusion] We established an *ex vivo* model to investigate metabolic deficiency in cardiac myocytes by using the differentiated H9c2 cardiomyocytes and the treatment with palmitate, which could be a model of insulin-resistant myocardium.

Actin & Actin-binding Proteins I

1620-Pos Board B530
Observation of Dynamical Conformational Changes of Skeletal Muscle Actin Filament
Atsuko H. Iwane, Masatoshi Morimatsu, Toshio Yanagida.
Actin is a ubiquitous and essential protein that has a primary role in a number of important cellular phenomenon including muscle contraction, cell motility, and cell division. It has two forms, monomeric G-actin and filamentous F-actin, which are determined by the cellular environment. Understanding the dynamic conformations between the two is important for understanding actin's cellular functions, yet only recent advancements have been made. Oda and Maeda used X-ray fiber diffraction to describe the multiple conformations of F-actin. To investigate the dynamics of these F-actin states, we here describe the preparation of fully active α-actin obtained from a baculovirus expression system suitable for single molecule FRET (smFRET) measurements. We developed α-actin recombinants in which two domains of the tetramers have specific sites for fluorescent probes. Actin filaments showed different dynamic conformational changes between these domains in the presence and absence of myosin S1. Specially, we observed several sequential transition states on a second or sub-second order. This technique, which combines specific labeling and smFRET, offers to significantly expand the information acquired in actin studies.

1621-Pos Board B531
Modeling the Mechanical Property of Single Actin Filament
Jin Seob Kim, Osman N. Yogurtcu, Sean X. Sun.
Actin filaments play many important roles in the cellular processes including motility, morphogenesis, and mechanosensing of the environment. One of the keys to better understanding of how actin filaments perform those roles lies in understanding the mechanical properties of actin filament, such as persistence length. There have been intensive studies on the mechanical properties of actin filament and its network. The measurements so far show the diversity of persistence length, ranging from several to a few tens of microns, also dependent upon the chemical states of actin molecules. Another interesting issue is the description of actin filament breaking. In order to understand these, we built up a simple model where each actin monomer is treated as a spherical particle connected by a set of springs. These spring stiffness parameters are determined from the known information on the chemical bonds in the actin filament and stretching deformation of the actin filament as an elastic rod. Our results show the length dependency of the persistence length, especially in a shorter length range which is relevant to the physiological conditions. They also show that the diversity of persistence length measurements is closely related to the breaking of the bonds in the actin filament, as well as the chemical states of actin monomers in the filament. Finally the mechanism of actin filament breaking is discussed.

1622-Pos Board B532
Probing F-actin Stability and Mechanics using Structure-Based Computational Modeling
Philip Bransford, Roger Kamm, Mark Bathe.
Filamentous actin (F-actin) is a core component of the cytoskeleton that is active in both homeostatic and dynamic cellular processes and interacts with a broad range of actin-binding proteins that regulate its stability, higher-order structure, and mechanical properties. Recent structural studies utilizing cryo-electron microscopy and 3D particle reconstruction have provided novel structural models of F-actin in isolation as well as with various bound actin-binding proteins, offering new opportunities to understand the molecular basis of F-actin stability and mechanics. Here we apply a recently introduced computational framework based on the finite element method to model the bending, twisting, and stretching deformation of 150 nm F-actin in experimentally observed states at near atomic resolution. We use the model to address two questions: (1) are bare F-actin modes of deformation conserved among the experimentally observed models and (2) do actin-binding proteins change F-actin's flexibility along one or more low modes of deformation? We find that the lowest mode shapes of the molecule are conserved across distinct F-actin models, as well as the bacterial homolog ParM, and that the actin-bundling protein fimbrin decreases significantly the torsional stiffness of F-actin while the cross-linking protein α-actinin does not. Because F-actin is itself highly conserved, these actin-binding proteins may provide a means to tune its stability and mechanical properties for specific cellular processes.