pathway plays an important role in the antioxidative and anti-apoptotic effects of GLP-1 receptor activation in the heart is not known. In this study, we investigated the mechanistic effects of exendin-4 (GLP-1 receptor agonist) and its signaling on the prevention of oxidative stress and apoptosis.

**Methods:** Intracellular reactive oxygen species (ROS) level was measured using 2,7-dichlorofluorescin diacetate (DCF-DA). The mRNA expression of antioxidative genes and anti-apoptotic genes were assessed by RT-qPCR. Apoptotic cells were detected by TUNEL assay.

**Results:** Exendin-4 significantly inhibited H2O2-induced ROS production. The antioxidative activity of exendin-4 was inhibited by a GLP-1 receptor antagonist and an adenyl cyclase inhibitor. The decrease in ROS production by exendin-4 was not affected by PKI, a PKA inhibitor, whereas the depletion of Epac was abolished by pretreatment with either PKA inhibitor or Epac siRNA. Moreover, exendin-4 induced mRNA expression of antioxidative genes, including catalase and MnSOD. GLP-1 receptor activation by exendin-4 also suppressed H2O2-induced apoptosis. Treatment with exendin-4 increased mRNA expression levels of anti-apoptotic genes, including Bcl-2 and Bcl-xl. The antioxidative effect of exendin-4 was abolished by pretreatment with either PKA inhibitor or Epac siRNA.

**Conclusion:** Stimulation of GLP-1 receptor provides the protective effect of oxidative stress via Epac-dependent pathway, and elicits the inhibition of apoptosis through both PKA- and Epac-dependent manners in rat neonatal cardiomyocytes.

### P5040 | BENCH

**Atrial endothelin-1 signalling in hypertensive rats**

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**Purpose:** Hypertension induces cardiac remodeling at the structural and functional level. Endothelin-1 receptor (ET-1) may contribute to cardiac remodeling. It is not known, however, whether atrial ET-1 signaling is altered in hypertension. Here we tested the hypothesis that ET-1 signaling is augmented in the atria of spontaneously hypertensive rats (SHR) and that this may contribute to hypertension-induced atrial arrhythmias.

**Methods:** SHR and control Wistar-Kyoto (WKY) rats were studied at 6-8 months of age. Hearts were removed for morphometric analysis. Atria were snap frozen and analyzed for mRNA and protein expression by qPCR and Western blotting. Isolated atrial myocytes were field stimulated in the presence of 50 nM ET-1, and Ca transients (CaTs) were characterized at the global or subcellular level by epifluorescence (Fura-2/AM) and line scan confocal microscopy (Fluo-4/AM). To mimic atrial tachycardia, atrial tissue slices were stimulated at 5 Hz for up to 20 h as described for Western blotting.

**Results:** At 6-8 months of age, SHR exhibited compensated LV hypertrophy, but left atrial hypertrophy, despite increased atrial mRNA levels of BNP (2-fold) and β-MHC (3.6-fold), while β-MHC was only weakly induced. There were no differences in global CaTs between atrial myocytes from WKY and SHR. Exposure of atrial myocytes to 50 nM ET-1 caused a 35% increase in systolic Ca in SHR (n=14), but only a 14% increase in WKY (n=21; P=0.005). Moreover, in SHR atrial myocytes, ET-1 induced a larger fractional SR Ca release (P<0.05 vs WKY). The ET-1-induced increase in CaTs occurred in the subsarcolemmal as well as in the central cytoplasm. Notably, also atrial ET-1 mRNA and protein expression were higher in SHR and WKY atrial myocytes. Further analysis at the molecular level indicated that the atrial mRNA expression of ET-1 was increased (2-fold) in SHR, ETA receptor expression was unaltered at the mRNA level but upregulated (4-fold) at the protein level in the left atria. Gu and PLCγ (isoforms 1 and 4) mRNA expression were also upregulated in SHR. Simulated atrial tachycardia in atrial tissue slices caused a 2-fold upregulation of ETA receptor expression in both SHR and WKY. Atrial ET-1 further increased ETA receptor expression. At the functional level, atrial myocytes from SHR respond to ET-1 with increases in systolic Ca and the development of arrhythmogenic Ca waves. Thus, atrial ET-1 signaling is enhanced already at early stages of hypertension and may contribute to the initiation of atrial tachycardia in hypertension.

### P5041 | BENCH

**Lymphomonomuclear infiltration and elevated fibrosis extent in the right and left ventricular myocardium is linked to histology of Bachman bundle and associated with history of atrial fibrillation**

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**Background:** Chronic inflammation in the atrial myocardium plays an important role in the development of atrial fibrillation in Atrial Fibrillation (AF). However, it is not clear whether atrial inflammation is associated with fibrosis or remodeling in atrial myocardium. Our aim was to assess the extent of fibrosis and inflammatory reaction in human ventricular myocardium and explore its association with AF.

**Methods:** Medical records from consecutive autopsies were checked for AF history. Heart specimens from consecutive autopsies were checked for AF history. Heart specimens from 30 patients who died from cardiovascular causes (64±12 y, 17 men) were collected in two predefined groups: with AF history (n=20) and without (n=10). Tissue samples were taken from the Bachmann Bundle (BB), Left (LV) and Right (RV) ventricle free walls and stained with Masson’s trichrome for analysis of fibrosis extent. Immunohistochemistry was performed using CD3- and CD45-antibodies and quantified as number of antigen-positive cells per 1 mm².

**Results:** There were no differences between the groups in regard to age or comorbidities as expressed as CHA2DS2-VASc scores (Table). Fibrosis extent, CD3+ and CD45+ immune cell counts were elevated in AF patients regardless of tissue sampling location (Table). Fibrosis extent correlated with both CD3+ (r=0.73, p<0.001 for RV; r=0.40, p=0.028 for LV) and CD45+ (r=0.75, p=0.001 for RV; r=0.49, p=0.006 for LV) cell counts. Fibrosis at BB correlated with RV (r=0.57, p=0.001) but not with LV (r=0.27, p=0.14). Neither fibrosis extent nor inflammation cell count showed any correlation with either age or comorbidities.

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**Table 1**

<table>
<thead>
<tr>
<th>Age</th>
<th>CHA2DS2-VASc score</th>
<th>Left ventricle</th>
<th>Right ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% CD3+</td>
<td>% CD45+</td>
</tr>
<tr>
<td>Any AF</td>
<td>n=20</td>
<td>86±10</td>
<td>83±10</td>
</tr>
</tbody>
</table>

p<0.001 for all comparisons of fibrosis extent, CD3+ and CD45+ cell counts between the groups.
Conclusion: Histological signs of chronic inflammation affecting ventricular myocardium are strongly associated with AF and demonstrate significant correlation with fibrosis extent that cannot be explained by cardiovascular comorbidities otherwise. Since histologic markers of fibrosis and inflammation are not confined to atrial myocardium, AF could be the cause or consequence of diffuse myocardial insult on the entire heart that requires further studies.

P5042 | BENCH
Can moderate training reverse hypertrophic cardiomyopathy in a mouse model of vascular dysfunctional eNOS?
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Purpose: Heart failure is one of the most prevalent causes of death in the western world. Caveolin1 knockout mice (cav1/-) develop a severe hypertrophic cardiomyopathy with functional impairment especially in cardiac output and ejection fraction. Behavior observation during the breeding of this mouse line revealed, that the animals showed a markedly reduced activity. Physiological inactivity can also contribute to heart diseases. The aim of the current study was to test, if moderate physical activity (swim training) can improve heart function and reverse cardiac morphological alterations in this particular model. The efficiency of training was unclear, because caveolin-1 deficiency is associated with eNOS hyperactivation. Hyperactivation of eNOS can lead to massive radical production. Overexpression of eNOS has been described to be counteracting the beneficial effects of training with respect to improved cardiovascular function.

Methods and results: Echocardiographic measurements of 8 week old animals showed that cav1-/- mice had an increased left ventricular wall thickness resulting in a reduced left ventricular volume. Previous data from our group have demonstrated that this left ventricular hypertrophy leads to a reduced cardiac function (reduced stroke volume and cardiac output) in older mice combined with reduced physical ability. Swimming tests compared to wild-type mice. Six weeks of moderate training (2 weeks 5 min/day, 2 weeks 10 min/day and 2 weeks 15 min/day) resulted in markedly prolonged swim duration (untrained: 11±4 min vs. trained: 30±5 min) in cav1-/- mice. In line with this, the functional parameters following training showed that stroke volume and cardiac output (measured by echocardiography) were comparable to wild type littermates. Furthermore, the left ventricular wall thickness was reduced in the during the training period leading to a significantly higher intraventricular volume. None the less, heart and lung weight of these animals were still significantly elevated.

Conclusions: In conclusion the present study documents that moderate training can inhibit the progression of heart failure in a hypertrophic mouse model with elevated eNOS activity. It remains to be established, if the beneficial effects are mediated by direct cardiac mechanisms, or if alterations in the peripheral circulation e.g. in blood viscosity or peripheral resistance are involved here.

P5043 | BENCH
The role of TIMP3 in the development of myocardial fatty accumulation and lipid handling
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Purpose: To assess the role of Tissue Inhibitor of Metalloproteinases (TIMP3), a potent inhibitor of MMP’s, in the development of dysfunctional metabolic handling of lipids and intramyocardial lipid accumulation. In previous research we observed increased atherosclerosis (p<0.01) in ApoE-/-/TIMP3-/- (ET3KO) mice compared to ApoE-/- (EKO) mice. Aortic root analysis revealed increased lesion area, higher lipid deposition, increased necrotic core and F4/80 positive cell infiltration in ET3KO mice. Cholesterol and Triglycerides were not significantly altered, while fasting hypoglycemia was evident (P<0.0002).

Methods: We investigated the role of TIMP3 through the use of Metabolomics, RNA and protein expression as well as histological analyses, between EKO and ET3KO mice. LAD ligation was performed to investigate its role during myocardial ischaemia.

Results: Metabolomic analysis of the serum showed dysfunctional metabolic handling of fatty acids (FA) as evidenced by reductions in long-chain monounsaturated fatty acids (MUFA) such as 18:1n9 and 19:0heptadecenoate, medium-chain acyl-carnitines (e.g., hexanoylcarnitine), medium-chain acyl-glycine (i.e., valerylglutamine) and the medium-chain fatty acid marker of beta-oxidation, 3-hydroxyoctanoyl. The long-chain acyl-carnitine stearoylcarnitine was elevated. A depletion of the antioxidant, ergothioneine, and a liver-modified form of a soy-derived phytoestrogen, equol sulfate, indicated a loss of antioxidant and inflammatory regulatory pathways in ET3KO. Markers of insulin resistance such as 2-hydroxybutyrate and 2-aminobutyrate were also increased. The reduction of SCD-1, as suggested by the metabolomics data, was confirmed at RNA level in the heart of ET3KO mice.

Histological analysis of the ET3KO hearts showed increased intramyocardial lipid deposition as well as areas of extensive fibrosis. Hearts showed reduced levels of PPARα-alphs suggesting an inability to handle lipids (P<0.05 for both). LAD ligation to investigate defective FA oxidation and its impact in the setting of acute ischaemia showed increased peri-operative mortality was in the ET3KO group (13 out of 19 v 2 out of 13 p<0.005) with no apparent bleeding or other cause being identified suggesting an inability of these hearts to withstand ischaemia and its acute change in metabolic demand.

Conclusion: Our data suggests that TIMP3 loss on the background of EKO leads to an inability of the heart to handle lipids leading to the accumulation of intramyocardial lipids and finally resulting in fibrosis and an inability to handle the acute metabolic switch during ischaemia.

P5044 | BENCH
Immunotherapy with an agonistic anti-CD40 antibody limits adipose tissue inflammation and protects from pre-established metabolic disease in mice
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Background: Immune and inflammatory cell recruitment critically drives adipose tissue inflammation during the metabolic syndrome. Co-stimulatory pathways such as the CD40L-CD40 pathway have recently been identified as a potent modulator of immune cell function in adipose tissue and polarization. We recently showed that CD40-deficiency induces an impaired anti-inflammatory and metabolic phenotype in hypertensive mice (in KO). Here, we tested the novel therapeutic concept of immune modulation with a stimulating agonistic anti-CD40 antibody in mice.

Methods and results: We recently reported that CD40-/- mice presented with increased inflammation and dysmetabolism during the metabolic syndrome. To test whether this phenotype depends on immune cell expressed CD40, we generated genetically chimeric mice (Rag1/-/CD40) lacking CD40 on T- and B-cells by reconstituting lethally irradiated WT mice with a mixture of bone marrow from T- and B-lacking Rag1-/- and CD40-/- mice. Interestingly, Rag1/-/CD40 mice fed a high fat diet for 12 weeks presented with increased immune cell infiltration in adipose tissue, elevated weight gain and aggravated insulin resistance compared with mice reconstituted with WT bone marrow, indicating that CD40 on T- or B-cells accounts for its hyper-inflammmatory phenotype in DIO. To test if post-mitotic activation of CD40 signaling is effective to modulate pre-existing metabolic disease, mice were fed a high fat diet (HFD) for 6 weeks and randomized to treatment with the activating CD40 antibody FGK45 (n=12 per group) or a control for 6 weeks. Activation of CD40 signaling with FGK45 abolished further weight gain during diet consumption, lowered glucose and improved insulin resistance, suggestive of an improved metabolic phenotype. Pro-inflammatory cell accumulation in adipose tissue was substantially reduced. Mechanistically, expression of the pro-inflammatory TH1 cytokine INF-γ in Thelper cells decreased after CD40 activation. To further prove a clinical association, 183 patients with a high prevalence of the metabolic syn- drome were screened for plasma levels of soluble CD40. Levels of were elevated in obese patients with a high BMI and high waist circumference, indicating clinical relevance of our findings.

Conclusion: We present the novel finding that immunotherapy with an activating CD40 antibody improves adipose tissue inflammation and its metabolic complications in pre-established disease in mice. In line with this concept, levels of soluble CD40 associated with obesity in humans. Positive modulation of the co-stimulatory CD40 pathway might therefore constitute a promising novel therapeutic concept in the fight against cardiometabolic disease.