ABSTRAKTER FRA NCS' FORSKNINGSSYMPOSIUM – VINTERMØTET 2010

Cancer treatment, survival and premature cardiovascular disease

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Purpose: Mantle field radiotherapy causes premature atherosclerosis in Hodgkin's lymphoma survivors (HLS). We examined if premature atherosclerosis within the radiation field was predicted by traditional risk factors independent of radiation and associated with peripheral endothelial dysfunction and markers of inflammation.

Methods: Forty-three HLS aged median 50 (range 38 – 63) years and treated with mantle field radiotherapy were included. Cardiovascular risk factors were registered in a first follow-up (FU-1) median 10 (range 5 – 13) years after treatment. A second follow-up (FU-2) was undertaken median 23 (18 – 27) years after treatment. At FU-2, in-field atherosclerosis was assessed by computed tomography with the calculation of coronary artery calcium volume score (CACS) and pre-cranial artery atherosclerosis score (PAS). PAS was calculated by scoring the degree of atherosclerotic luminal narrowing in all irradiated pre-cerebral arteries. Peripheral endothelial dysfunction was assessed by strain-gauge plethysmography. Markers of inflammation were CRP and von Willebrand factor.

Results: All registered atherosclerotic lesions were inside the radiation fields. Multiple linear regression analyses showed that cholesterol was a predictor of both CACS (ß 339 (95%CI 244 – 434), p < 0.001) and PAS (ß 3.38 (95%CI 2.01 – 4.74, p < 0.001). An increase in CACS by 100 units was associated with PAS (ß 0.75 (95%CI 0.47 – 1.03), p < 0.001). Reduced peripheral endothelial function was also associated with PAS (ß – 0.20 (95%CI -0.37 - -0.02), p = 0.001).

Conclusion: Premature atherosclerotic lesions develop in all irradiated arteries and were predicted by elevated cholesterol and associated with impairment of endothelial function. Statin treatment may be important to prevent premature atherosclerosis in HLS.

Seasonal variations in the incidence of acute myocardial infarction – myth or reality? The Tromsø Study.

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Background: A seasonal pattern with higher winter morbidity and mortality from acute myocardial infarction (MI) has been reported since the 1920s. The quantity of difference between peak and nadir season has been associated with latitude, with less seasonal variation in colder climates than in warmer climates, but published results are inconsistent. Definitions of seasons have varied and only a few studies are based on adjudicated MI cases in population cohorts. We investigated the seasonal variation in first-ever non-fatal and fatal MI in the population of Tromsø in Northern Norway, a region with a harsh climate and extreme seasonal variation in daylight exposure.

Materials and methods: A total of 37,392 participants from the population-based Tromsø Study

enrolled between 1974 and 2001 were followed from date of study entry throughout 2004. Each incident case of MI was validated with review of full medical records and death certificates. Event ascertainment followed a detailed protocol. MI incidence rates for months and seasons, both as an astronomical model and a temperature model, were analysed for seasonal patterns with Poisson regression. Variation in monthly MI incidence was also analysed with the cosinor procedure. All analyses were stratified in subgroups of sex, age and smoking status.
Results: A total of 1,893 first-ever MI were registered, of which 592 were fatal. No evident seasonal variation in non-fatal or fatal MI incidence or case-fatality was found.
Conclusion: We found no evident seasonal pattern in incident fatal and non-fatal MI. A non-uniform definition of outcome and season may explain some of the inconsistency in results from previous studies. Populations living in the subarctic areas may be more adapted to face winter month exposures through behavioural protection.

Endothelial function in a healthy Norwegian population. The HUNT study.

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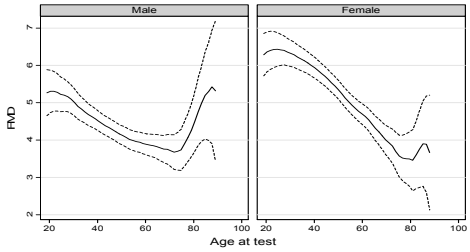
Purpose: Endothelial dysfunction is a central risk factor for atherosclerosis and may be present long before clinical manifestation. Normal values for endothelial function have not been established. The aim of our study was to find normal values for FMD in age and gender groups in a population without clinical atherosclerosis.
Methods: As part of the Health Survey in Nord-Trøndelag County 2007/2008, we tested endothelial function as flow mediated dilation (FMD) in 4737 healthy adults and 164 healthy teenagers. All had a brief interview with a physician. FMD was tested in the left brachial artery with the cuff placed on forearm. A baseline rest image was performed. Arterial occlusion was created by cuff inflation to 250 mmHg for 5 minutes. The diameter measured 60 sec after cuff deflation was registered as post diameter. Difference between post diameter and baseline diameter gives maximal dilation (FMD) of the artery.
Results: Mean FMD (%) in men was 4.29 (95 % CI: 4.13 – 4.45) and in women 5.33 (95 % CI: 5.15 – 5.50). There were significant differences in FMD across gender ($p < 0.001$) and age groups ($p < 0.001$). Mean baseline diameter was 4.69 mm (95 %CI: 4.67 – 4.71) in men, and 3.63 mm (95%CI: 3.61 – 3.64) in women. Analysis using absolute change in diameter showed the same pattern with significant difference among age groups ($p<0.001$ in women and men), and a strong trend with gender ($p=0.1$); adjusting FMD for baseline diameter and post test flow, did not change the results. 16 % of women and 17 % of men had endothelial dysfunction i.e FMD$\leq$ 0 %. The prevalence of endothelial dysfunction increased with age, and this trend was highly significant (Chi-squared test for linear trend of proportions, $p < 0.001$, for both genders).



Conclusion: Women have significantly higher FMD than men; values are decreasing for both genders with increasing age, genders crossing at age around 65 years. Men older than 80 years have higher FMD than men between 50 – 79 years; this suggests a selection of survival in favour of preserved FMD. The lower values in men age 50 – 79 indicate a proportion in this group with subclinical atherosclerosis. This pattern is the same as seen in the incidence of coronary heart disease in both genders, with the 10 – 15 years of delay in women compared with men.

Use of contrast echocardiography in NSTEMI to identify patients with high risk angiographic coronary artery disease

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Background:
Thrombolysis In Myocardial Infarction (TIMI) score is used for risk prediction in non-ST-elevation myocardial infarction (NSTEMI). We evaluated contrast echocardiography in relation to TIMI score and severity of coronary artery disease (CAD).
Methods:
Echocardiography was performed prior to coronary angiography in 110 patients (age 67 years, 31% women) with NSTEMI. Segmental myocardial perfusion was visually scored using a 17 segment left ventricular model. CAD was assessed by quantitative coronary angiography (QCA).
Results:
Troponin T was elevated in all patients (mean 0.68 ± 1.2 mmol/l). In the total study population, TIMI score was 3.1 ± 1.5, 29% had ST- depression, 35% prior CAD, 45% hypertension, 19% diabetes, 40% family history of CAD, 50% hypercholesterolemia, and 28% were current smokers. By QCA 14% had normal angiography, while 1-, 2-,and 3- vessel disease (VD) was present in 36%, 27% and 23%, respectively. Prognostically severe CAD (left main stem, proximal LAD, acute occlusions and 3-VD) was found in 50%. Number of hypoperfused segments by echocardiography increased with extent of CAD from 3.8±2.2 in patients without QCA stenosis to 5.8±2.4, 7.9±3.5 and 10.5±2.7 in patients with 1-, 2- and 3-VD, respectively (all $p<0.05$). Also TIMI score increased with increasing extent of CAD (2.3±0.9, 3.3±1.4, 3.5±1.6 and 3.7±1.4 in 0-,1-,2- and 3-vessel disease respectively, $p<0.05$). In univariate analysis, no significant correlation between TIMI score and number of hypoperfused segments were found. In multiple logistic regression the risk of prognostically severe CAD increased by 39% for each additional hypoperfused segment by echocardiography, independent of TIMI score (Table).
Conclusion:
Contrast echocardiography in acute NSTEMI can predict extent and severity of CAD independent of TIMI score.

Table

Variable	Odds ratio	95% CI	P value
Number of hypo-perfused segments	1.39	1.20-1.61	<0.01
TIMI risk score	1.25	0.83-1.89	0.29

The homeostatic chemokine CXCL13 and its receptor CXCR5 are important for maintaining cardiac structure by regulating proteoglycans crucial for correct collagen matrix

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Rationale: The chemokine CXCL13 and its receptor CXCR5 are crucial for lymphocyte trafficking. Inflammatory mechanisms have been suggested to play a role in the development of heart failure (HF).
Objective: The aim of the present study was to identify if CXCL13 and CXCR5 has a role in HF and remodelling of the heart.
Methods and Resluts: We found increased plasma levels of CXCL13 and CXCR5 both in HF patients ($p<0.05$) and in an experimental mouse model of HF ($p<0.01$). We exposed CXCR5 deficient ($CXCR5^{-/-}$) and wild type (WT) mice to aortic banding (AB) for 3 weeks. We found higher mortality in the AB $CXCR5^{-/-}$ group ($p<0.05$). Echocardiography demonstrated increased left ventricular (LV) inner diameter in AB $CXCR5^{-/-}$ as compared to AB WT mice ($p<0.01$). Microarray analysis (Affymetrix) revealed altered expression of genes encoding proteins found in extracellular matrix (ECM). Protein levels of fibromodulin, decorin and lumican, all members of the small leucine-rich (SLRP) proteoglycans, were reduced in the AB $CXCR5^{-/-}$ mice. We also found that CXCL13 had a direct effect on myocardial fibroblasts by enhancing the expression of the SLRPs. SLRPs bind to different types of collagens, thereby regulating fibril assembly, organization and degradation, potentially influencing the quality of ECM. Electron microscopic analysis revealed that the extracellular matrix of AB $CXCR5^{-/-}$ mice was loosely packed, with huge areas without fibrous components or proteoglycans.
Conclusions: Our data demonstrates that CXCL13 and CXCR5 are regulated in experimental and human HF. Lack of CXCR5 leads to LV dilatation following aortic banding, possibly via altered levels of SLRPs. We suggest that CXCL13 and CXCR5

are important for maintaining cardiac structure by regulating proteoglycans crucial for correct collagen matrix.

Inhibiting phosphorylation of SMAD2 preserves cardiac function during pressure overload by attenuating left ventricular SERCA2 loss

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Background
Aortic stenosis (AS) leads to left ventricular pressure overload, which induces myocardial remodeling and may reduce cardiac function. A role has been suggested for TGF-ß/SMAD signaling, however it remains unclear whether this pathway affects cardiac function. We have studied the effects of pharmacological inhibition of the SMAD2/3 pathway in a mouse model of left ventricular pressure overload.
Methods
C57BL/6 mice were subjected to banding of the ascending aorta or sham procedure and pharmacological inhibition of SMAD2/3 signaling using a novel drug (SM16) or placebo. Cardiac function was evaluated by echocardiography. We analyzed gene expression by QPCR and protein levels by Western blotting. Myocardial collagen was quantified using hydroxyproline measurements.
Results
Aortic banding increased phosphorylation of SMAD2 by 3-fold, while phosphorylation of SMAD3 was unaltered. SM16 treatment attenuated the phosphorylation of SMAD2 and improved cardiac function. Echocardiography demonstrated a 38 % increase in fractional shortening ($p < 0.05$) and a 28 % reduction in mitral deceleration slope ($p < 0.05$), indicating improved systolic and diastolic function by SM16. Aortic banding resulted in a 21 % increase in lung weight, suggesting pulmonary congestion, a finding which was totally abolished by SM16 treatment ($p < 0.05$). SM16 prevented left ventricular Serca2 loss and studies on isolated cardiomyocytes demonstrated enhanced Ca^{2+} transient decay. Mechanistically, this suggests beneficial effects of SMAD2 inhibition by improved Ca^{2+} handling.
Conclusions
In pressure overload, cardiac function is preserved by pharmacological inhibition of SMAD2 signaling, possibly mediated by effects on cardiomyocyte calcium handling.

Secretoneurin: a novel protective agent increased in the myocardium and circulation in heart failure

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Background: Secretoneurin (SN) is a peptide from the granin protein family. As other granin proteins, chromogranin (Cg) A and B, are regulated during heart failure (HF) development, we hypothesized that SN would be increased in HF.
Methods: SN production, levels, and localization were examined in a post-myocardial infarction HF mouse model. Plasma SN levels in 58 patients with chronic, stable HF were compared to levels in 20 age- and gender-matched healthy control subjects. Effect of SN on apoptosis and ischemia/reperfusion injury was also investigated.
Results: Pro-SN mRNA levels were 11.5 fold upregulated in the left ventricle (LV) of HF animals compared to sham-operated animals ($p<0.001$). SN protein levels were also increased in the non-infarcted (35%) and infarcted region (85%) of the LV in HF animals, while there was no increase in other

tissues investigated. Myocardial SN production was confined to the cardiomyocytes. Patients with HF of mainly moderate severity had increased circulating SN levels compared to control subjects (0.17 ± 0.01 vs. 0.12 ± 0.01 nmol/L, p<0.001), and SN levels were superior to CgA, an established cardiac biomarker, for diagnosing HF (ROC-AUC 0.84 vs. 0.57, p=0.001). Adding SN to the perfusate in a global ischemia model of the isolated rat heart reduced ischemia/reperfusion injury by 30% (p<0.05). SN also increased Erk1/2 and Stat3 phosphorylation in cardiomyocytes after short-term stimulation, and protected against hydrogen peroxide-induced cardiomyocyte apoptosis *in vitro*.
Conclusion: SN is regulated in the myocardium and circulation in HF and seems to have protective properties during myocardial ischemia and stress.

Gap junction uncoupling protects the heart against ischemia by mitoK$_{ATP}$ and/or mitoK$_{Ca}$ channel activation

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Connexin 43 (Cx43) is localised in the cell membrane as gap junction, but also in organelles like mitochondria and is proposed important in ischemic preconditioning (IPC). The aim of this study was 1) to investigate if heptanol, a gap junction uncoupler, reduces infarct size when given as a pre-treatment and 2) if potential protection is triggered by gap junction uncoupling or by mitochondrial mechanisms.
In Langendorff perfused rat hearts, heptanol 2mM was given as pre-treatment (10min x 3, with 5min x 3 reperfusion) before regional ischemia. This resulted in reduced infarct size compared to controls (29.7±3.4% vs.12.6±2.1% P< 0.001). Mitochondrial potassium channel blockers (5HD blocking mitoK$_{ATP}$ and PAX blocking mitoK$_{Ca}$) abolished the cardio protective effect of heptanol (5HD 36.7±2.9% and PAX 40.2±2.8%). Measurements of mitochondrial oxygen consumption (oxygraph) showed that heptanol significantly reduced respiratory control ratio in both subsarcolemmal (SSM) and interfibrillar mitochondria (IFM) in a dose dependent manner (0.5-5.0 mM). The P/O-ratio was significant reduces both in SSM and IFM by 2mM heptanol. Western blot analysis showed that pre-treatment with 2 mM heptanol significantly increased phosphorylation of AKT and GSK-3ß compared to controls, and the difference in phosphorylation was maintained at the end of the ischemic period.
In conclusion, the results show that pre-treatment with heptanol protects the heart against ischemia. This protection is most likely mediated via mitochondrial mechanisms and not via gap junction uncoupling.

Improved diastolic myocardial function in elderly after aerobic interval training

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Background: Myocardial diastolic function decreases with age. We hypothesised that aerobic interval training (AIT) improves diastolic function and that lifelong endurance training ameliorate age-associated reduction of the myocardial function.
Methods: 16 sedentary elderly (72 ±1 years, 13 men) performed AIT (4x4minutes) at ~90% of maximal heart rate 3 times/week for 12 weeks. Results were compared with 11 male master athletes (MA), 74±2 years) and 9 young controls who also performed AIT (24 ± 2 years, 5 men). Echocardiography, including tissue Doppler imaging (TDI), was recorded at rest and during submaximal bicycle exercise stress echocardiography (70% of maximal heart rate).
Results: Diastolic function measured by traditional Doppler and TDI, increased significantly after AIT, E/A with 44% and early diastolic tissue velocity (E') with 11% (Table). There was no effect on systolic tissue velocity (S') at rest, but a 26% increase during exercise stress test (p<0.001).
Conclusion: AIT improved left ventricular diastolic function in an elderly population almost to the same level as master athletes. However, exercise training

Table. Effect of aerobic interval training; elderly compared to young and master athletes

	ELDERLY (n=16)			YOUNG (n=9)			MASTER ATHLETES (n=11)		
	Before	After	P-value within group	Before	After	P-value within group	Value	P-value A	P-value B
VO_{2max} (ml/kg/min)	32.5±5.5	37±6.1	<0.01	51.5±5.5	57.0±6.0	<0.01	49.5±5.6	<0.01	<0.01
SBP mmHg	144±15	126±10	<0.01	122±8	121±8	0.78	123±16	<0.01	0.54
DBP mmHg	80±9	73±5	<0.05	71±5	70±7.	0.78	71±9	0.02	0.9
Heart rate, beats/min	69±8	59±7	<0.01	57±6	56±9	0.85	53±4	<0.01	0.04
EDV, ml	92±17	106±28	<0.01	120±29	129±39	0.60	142±21	<0.01	<0.01
SV, ml	74.5±14	81.5±19	0.03	76±21	92±17	0.04	102.0±26	<0.01	0.02
EF, %	60.7±7.0	68.7±6.4	<0.01	58.0±5.8	64.3±4.7	0.10	63.7±4.8	0.20	0.03
E/A	0.89±0.3	1.28±0.7	0.02	2.4±0.66	2.7±0.84	0.56	1.33±0.70	0.06	0.90
E', cm/s	7.3±1.8	8.1±1.9	0.02	15.8±2.2	16.2±2.2	0.73	9.0±2.1	0.04	0.32
A' cm/s	11.1±1.6	9.7±1.5	<0.01	6.7±1.3	6.4±1.4	0.76	10.3±28.0	0.43	0.54
S' submax exercise cm/s	8.0±1.5	10.1±2.1	<0.01	8.9±0.8	9.7±0.8	0.06	11.9±1.2	<0.01	0.01

VO_{2max} -maximal oxygen uptake. SBP-systolic blood pressure. DBP-diastolic blood pressure EDV- end diastolic volume, SV-stroke volume, EF-ejection fraction, E/A-early/late diastolic mitral filling, S'-systolic tissue velocity, E'-early diastolic tissue velocity, A'-late diastolic tissue velocity, p-value A and B indicates comparison between elderly and MA before and after the training period

only partially improves diastolic function as age related diastolic changes are found both in sedentary and well trained old subjects. It seems like exercise delay the aging process and to a certain level rejuvenate the myocardial function

Exercise-induced cardiometabolic effects - the role of intensity

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Although exercise has beneficial effects in patients with cardiovascular disease, the effect of exercise with respect to myocardial substrate metabolism, oxygen consumption (MVO_2) and cardiac efficiency is not known. New studies have indicated more beneficial cardiovascular effects of high- as compared to low- and moderate-intensity levels. The role of intensity on exercise-induced cardiometabolic effects, however, is not known. Thus, the aim of this study was to compare the cardiometabolic effects of high-intensity interval training (HIT) and moderate-intensity continuous training (MIT).
Male C57BL/6J mice were subjected to high-intensity (85-95% of VO_{2max}) interval treadmill running or moderate-intensity (50-70% of VO_{2max}) continuous running for 10 wks (5 days/wk), as described by Kemi et al. (*J Applied Physiol,* 2002). Both groups covered the same running distance and age-matched sedate mice served as controls. Myocardial substrate utilization (radioisotope technique), cardiac efficiency (regression analysis of cardiac work -MVO_2 relationships) and the oxygen cost for basal metabolism and E-C coupling were measured in isolated perfused hearts.
HIT increased max running speed by 60%, which was accompanied with an 18% increase in aerobic capacity (VO_{2max}). The corresponding numbers for MIT were 30% and 10%. Both modes of exercise were associated with a 10% increase in heart weight to body weight ratio, but altered cardiac substrate utilization was observed only in the HIT group, as revealed by a 36% increase in glucose oxidation and a concomitant reduction in fatty acid oxidation. In addition, only HIT significantly increased cardiac efficiency, due to a decrease in the oxygen cost for basal metabolism.
In conclusion, high, but not moderate intensity training was associated with a shift in myocardial substrate utilization in favour of glucose oxidation, combined with increased cardiac efficiency. Improvement of cardiac metabolism and efficiency may in part explain the superiority of high-intensity exercise training with regard to cardioprotection in patients with cardiovascular disease, an issue that should be followed up with further investigations.

Alterations in left ventricular systolic and diastolic function during normal pregnancy

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Background
During pregnancy, the cardiovascular system adapts to the metabolic needs of mother and fetus in order to sustain adequate tissue perfusion. The effects on left ventricular (LV) function have not been well documented in larger patient groups. Thus, the aim of this study was, by use of serial echocardiography with tissue Doppler to evaluate LV systolic and diastolic function during normal pregnancy.

Methods
65 women (33.0 ± 1.2 years), with normal pregnancies at first trimester, were studied with transthoracic echocardiography (GE Vingmed Vivid 7) including grey scale imaging, Doppler and tissue Doppler, at gestational weeks 14-16, 22-24, 36 and 6 months postpartum.

Results
Cardiac output (CO) increased significantly during pregnancy, also when controlled for changes in body surface area ($p < 0.001$). LV end-diastolic volume (EDV) increased and LV ejection fraction (EF) decreased significantly. Whereas left atrial (LA) size increased, E/e' was unchanged during pregnancy indicating unaltered LV filling pressures. e', a marker of diastolic function was slightly, but significantly reduced. Mitral deceleration time was not significantly changed ($p = 0.11$). All women were asymptomatic.

Conclusion
During normal pregnancies in healthy asymptomatic women, LV EF was reduced without concordant increase in filling pressures. These findings can be explained by volume shifts during pregnancy, and may be used as a comparative standard in evaluation of pregnant woman with heart disease.

	14-16 weeks	22-24 weeks	36 weeks	6 months post-partum	ANOVA p
CO (L/min)	5.7 ± 1.1	6.0 ± 1.0	6.0 ± 0.9	4.8 ± 1.0# * §	< 0.001
LV EDV (mL)	92 ± 22	97 ± 23	101 ± 26	78 ± 19# * §	< 0.001
LV EF biplane (%)	61 ± 6	59 ± 9	54 ± 8 # *	60 ± 7 §	< 0.001
Mitral E (m/s)	0.80 ± 0.15	0.78 ± 0.13	0.66 ± 0.15# *	0.67 ± 0.12# *	< 0.001
Mitral E/A	1.66 ± 0.37	1.61 ± 0.33	1.41 ± 0.34#	1.53 ± 0.33	0.001
LA Area (cm^2)	16.0 ± 2.5	16.7 ± 2.3	17.5 ± 2.1#	14.6 ± 2.3 # * §	< 0.001
E/e'	6.7 ± 2.2	7.0 ± 2.4	6.6 ± 3.0	6.6 ± 1.8	0.780
e' (m/s)	0.14 ± 0.03	0.13 ± 0.03	0.12 ± 0.03 #	0.13 ± 0.02 #	< 0.001

*Mean ± SD. $p < 0.05$ vs #14-16 w,*22-24 w, §36 w. E = peak early transmitral flow velocity and e' = mean peak lateral and septal mitral annulus velocity.*

Inotropic effects of prostanoids are attenuated in failing human and rat ventricular myocardium

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Aims: Prostaglandin-modulatory approaches in heart failure (HF) patients have not shown beneficial effects in clinical studies. Both treatment with prostaglandins and inhibition of prostaglandin synthesis (non-selective COX-inhibitors and COX-2 inhibitors) have resulted in increased mortality in heart failure patients. Currently, it remains unknown if prostaglandin-mediated effects upon contractility are detrimental or favourable in the failing heart. Therefore, the objectives of this study were to determine if prostanoids could elicit direct inotropic effects in human ventricle, and if so, determine if they are modified in failing ventricle.

Methods: Contractile force was measured in ventricular strips from non-failing or failing human and rat hearts. The ratio of phosphorylated to non-phosphorylated myosin light chain 2 and myosin phosphatase targeting subunit-2 protein were measured by Western blotting in rat myocardial strips, and the levels of prostenoid F receptor (FPR) mRNA and protein were measured by real time RT-PCR and receptor binding assays.

Results: Iloprost and PGE_1 evoked a positive inotropic effect, through a cAMP-independent mechanism in non-failing human hearts, which was absent in failing human heart. Similarly, the FPR-mediated inotropic effect was reduced (~50%), but the fluprostenol-induced MLC-2 phosphorylation

levels were unchanged in failing compared to non-failing rat heart. FPR-density was significantly reduced (~40%) and mRNA trended higher in failing compared to non-failing rat heart.
Conclusions: Prostanoids may provide inotropic support in non-failing human heart, likely by enhancing myofilament calcium sensitivity, representing a less energy-demanding mechanism than beta-adrenergic activation of cAMP signalling. Possibly, the significant reduction of prostanoid-mediated inotropic support observed in both human and rat failing heart may be detrimental.

Depressed contractile function, SERCA-activity and reduced T-tubule density in myocytes isolated from the free left ventricular wall from patients with post-infarction heart failure

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Purpose
Most cellular and molecular data for depressed cardiac function in man comes from well-established animal models and biopsies from transplant hearts (end-stage heart failure). These studies suggest impaired SERCA-2 activity and reduced T-tubule density as central mechanisms behind a failing heart. The aim of the present study was to determine SERCA-function in tissue samples, and contractile function, calcium handling and T-tubule density in cardiomyocytes isolated from free left ventricular wall in patients with- and without post-infarction heart failure (post-MI HF-patients) undergoing coronary artery bypass graft (CABG).
Methods
Ten (63±6 years) patients without myocardial infarction and an EF>50 and 10 (69±5 years) post-MI HF-patients with EF<30 (NYHA 2-4) scheduled for CAGB were included. Small muscle biopsies were taken during the surgery. One small sample was processed for measurements of SERCA-activity whereas one was used for enzymatic cell isolation and one saved for molecular analysis. Tissue and cardiomyocytes were studied using fluorescence and confocal imaging.
Results
Cardiomyocyte shortening was similar between groups at 0.5Hz (i.e. 30 beats/min), but in contrast to cardiomyocytes from heart with normal EF, we found a negative shortening-frequency relation in cardiomyocytes from failing hearts. At 2 Hz stimulation, cardiomyocyte shortening was clearly depressed ($p<0.001$) in post-MI HF. Diastolic calcium was increased ($p<0.01$), calcium amplitude ~45% lower, and time to peak contraction and time to relaxation were slower at all stimulation frequencies ($p<0.01$) in cardiomyocytes from post-MI HF-patients. Synchrony of calcium release is closely linked to the density and organization of T-tubules in the cardiomyocyte. We found that the T-tubule density was reduced by ~35% in myocytes from post-MI HF patients, which may contribute to the slower time to calcium release and lower amplitude. The rate of calcium removal via SERCA was 35% slower in tissue from post-MI HF-patients ($p<0.01$), which may explain the prolonged time to calcium removal and time to relenghtening in cardiomyocytes from failing hearts.
Conclusion
This study demonstrate that patients selected for CAGB with post-MI HF have impaired cardiomyocyte function and depressed calcium handling. Reduced T-tubule density contributes to reduced calcium release and hence reduced cardiomyocyte shortening. Impaired SERCA function influence upon calcium removal and thereby relaxation during diastole. The findings substantiate that impaired SERCA-2 function and reduced T-tubule density is central mechanisms behind heart failure.

Slowing of Ca^{2+} release in failing cardiomyocytes

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Contractile power is reduced in heart failure. At the level of single cardiomyocytes, this has widely been reported to result from reduced magnitude of contraction. However, we have observed that contraction is also slowed in failing myocytes, which results in a slowed, less powerful contraction *in vivo*. We investigated the underlying mechanisms in a murine model of congestive heart failure (CHF). Myocardial infarction (MI) was induced by left coronary artery ligation, and at 10 weeks post-MI, mice exhibited marked symptoms of CHF. Thickening of the non-infarcted posterior wall was slowed in CHF hearts, as was contraction in cardiomyocytes isolated from viable regions of the septum. Confocal line-scan

imaging revealed a slowed rate of rise of Ca^{2+} transients (fluo-4 AM, 1 Hz) in CHF cells, which largely resulted from spatially non-uniform Ca^{2+} release. Ca^{2+} sparks were also slower to peak in CHF than SHAM (11.5±0.6 ms vs 9.5±0.6 ms, P<0.05) and longer lasting (FWHM=24.5±0.7 ms vs 21.6±1.0 ms, P<0.05). The mean increase in these measurements resulted from a sub-population of sparks in CHF with very long rise times probably reflecting altered function of local Ca^{2+} release channels (ryanodine receptors). Local Ca^{2+} transients (width=2 μm) measured at the same coordinates as these sparks were also slow to rise, indicating that altered Ca^{2+} spark kinetics contributed to the dyssynchronous Ca^{2+} release pattern in CHF. As well, di-8-ANEPPS staining revealed disorganized T-tubular structure in failing myocytes, which promoted Ca^{2+} release dyssynchrony. Thus, slowed contraction in failing myocytes results from slowed, dyssynchronous Ca^{2+} release due to a combination of altered ryanodine receptor function and T-tubule disorganization.

Defining electromechanical delay and its potential role in electrical LV dyssynchrony.

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Background: Previous studies have suggested that the latest activated segments during left bundle branch block (LBBB) display prolonged electromechanical delay (EMD). In the present study we investigated EMD by two different methods in the septum and lateral LV wall during baseline, LBBB and biventricular pacing (BVP).
Methods: In 5 anesthetized dogs with LV micromanometers we measured intramyocardial electromyograms (IM-EMG) and myocardial segment lengths by sonomicrometry. As reference method for onset of mechanical activation we used onset of regional active force generation (AFG), defined as the time when the myocardial pressure-segment length coordinate deviated upward from its passive-elastic curve. In addition we measured onset of mechanical activation as first onset of shortening (FOS) (Fig.1). EMD was calculated as time from regional electrical activation (onset R in IM-EMG) to onset AFG and FOS during baseline, LBBB and BVP.
Results: Electrical activation delay of the lateral wall relative to septum was 3±5 ms (±SEM) at baseline, 53±4 ms during LBBB, and -5±2 ms during BVP. EMD measured by AFG was essentially constant in both walls during all interventions (13±1ms Fig. 2). EMD assessed by FOS showed an increase in the lateral wall from baseline (17±2 ms) to LBBB (43±8 ms, p<0.05) but was near normalized by BVP (25±24 ms).
Conclusions: Electromechanical delay assessed by onset AFG was constant during all interventions and did therefore not contribute to dyssynchrony. The large changes between interventions in electromechanical delay assessed by first onset of shortening in the lateral wall suggest that this is an inaccurate measure of mechanical activation.

Fig. 1

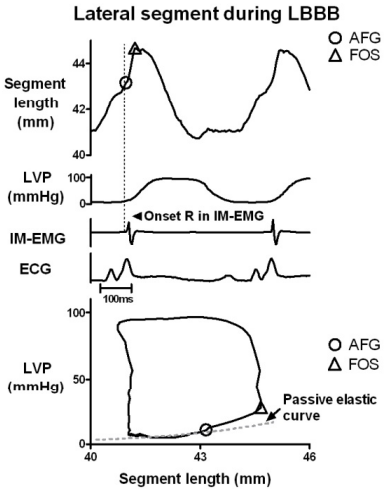

Fig. 2