UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE:

CARDIOLOGIA E CIÊNCIAS CARDIOVASCULARES

TESE DE DOUTORADO:

# Diferenças de performance ventrículo esquerdo entre homens e mulheres com e sem disfunção sistólica

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**ÍNDICE**

## LISTA DE ABREVIATURAS

1. VDF: volume diastólico final
2. VSF: volume sistólico final
3. PV: pressão-volume
4. PSF: pressão sistólica final
5. ESF: elastância sistólica final
6. Emax: elastância máxima do ventrículo esquerdo
7. ATP: adenosina trifosfato
8. GLUT 4: transportador de glicose tipo 4

# REVISÃO DA LITERATURA

## 1. INTRODUÇÃO

Está bem estabelecido na literatura que o sexo biológico desempenha importante papel na fisiopatologia cardíaca. Diferenças na incidência e prognóstico das doenças cardiovasculares entre homens e mulheres têm sido extensamente abordadas nos últimos anos. Essas diferenças são mais que esperadas visto que a fisiologia e estrutura cardíaca são sensivelmente diferentes entre os sexos. Apesar de grandes avanços no entendimento da influência do sexo em desfechos cardiovasculares, ainda existem lacunas de conhecimento significativas. Especula-se que, pelo menos em parte, estas lacunas se devam ao fato de que a maioria dos estudos que abordam o assunto são observacionais, sendo estes por definição somente geradores de hipóteses. Além disso, uma análise cuidadosa dos ensaios clínicos randomizados que compararam especificamente homens com mulheres quanto a desfechos cardiovasculares mostra que as mulheres são sub-representadas nestes estudos, de modo que conclusões definitivas ficam prejudicadas (1).

## 2. INFLUÊNCIA DO SEXO NOS DESFECHOS CARDIOVASCULARES:

Quanto à doença arterial coronariana, sabe-se que mulheres pré-menopáusicas apresentam risco significativamente menor de eventos isquêmicos do que homens, no entanto este risco torna-se quase semelhante entre homens e mulheres pós-menopáusicas. Além disso, as mulheres tipicamente apresentam diagnóstico de doença arterial coronariana ou infarto agudo do miocárdio pelo menos 10 anos após os homens (2). Por outro lado, insultos isquêmicos agudos são pobremente tolerados pelas mulheres quando comparados aos homens. A mortalidade das pacientes do sexo feminino, por exemplo, é significativamente maior do que dos pacientes do sexo masculino diante de um evento isquêmico agudo. Nota-se inclusive, que mulheres com menos de 65 anos e diagnóstico de síndrome coronariana aguda tem

mortalidade duas vezes maior que homens de idade semelhante. A morbidade associada ao infarto agudo do miocárdio também é significativamente maior nas mulheres, pois as mesmas tem maior risco de desenvolver instabilidade hemodinâmica, edema pulmonar e choque cardiogênico (3-7).

A diferença de morbi-mortalidade entre os sexos pode ter vários motivos dentre os quais o fato das mulheres apresentarem eventos isquêmicos agudos com idade mais avançada e terem maior prevalência de comorbidades como hipertensão e diabetes. Apesar de claramente se beneficiarem de manejo contemporâneo completo das síndromes coronarianas agudas, estudos demonstram que mulheres muitas vezes podem ter seus sintomas negligenciados, de forma que o diagnóstico e tratamento nem sempre são disponibilizados adequadamente (1). Os ensaios clínicos randomizados no contexto de síndromes coronarianas agudas que empregaram um protocolo de tratamento independente do sexo demonstraram que ainda assim mulheres apresentam maior morbi-mortalidade do que os homens e estas diferenças se mantêm mesmo após ajuste para superfície corporal e fatores de risco (8,9). O fato do sexo feminino ser considerado preditor independente de morbi-mortalidade em pacientes com síndrome coronariana aguda pode indicar que há fatores biológicos associados especificamente ao sexo que contribuem para as diferenças de risco e prognóstico nas doenças cardiovasculares.

No que se refere a epidemiologia da insuficiência cardíaca, Roger et al publicaram um estudo de coorte em que 4357 pacientes foram acompanhados do ano de 1979 até 2000. Este estudo demonstrou que as mulheres apresentaram menor incidência de insuficiência cardíaca e mortalidade do que os homens em mais de 20 anos de seguimento. Homens tendem a apresentar insuficiência cardíaca predominantemente associada à disfunção sistólica do ventrículo esquerdo, visto que a etiologia isquêmica é a mais frequente no sexo masculino. Por outro lado, mulheres tem maior predisposição a desenvolver insuficiência cardíaca com fração de ejeção preservada (10-12). Estudos clínicos demonstraram que hipertensão arterial crônica causa efeitos fisiopatológicos cardíacos diversos entre os sexos, sendo que mulheres hipertensas apresentam prevalência significativamente maior de hipertrofia ventricular (71% versus

56%) e consequentemente de disfunção diastólica (13,14). A análise de uma grande coorte com insuficiência cardíaca do estudo CHARM (15), que incluiu um número significativo de pacientes com função sistólica preservada e disfunção sistólica de diferentes graus de severidade, demonstrou que mesmo após ajuste para heterogeneidades clínicas os homens mantém importante desvantagem quanto à mortalidade nesta doença.

Existem evidências de que o processo de remodelamento cardíaco presente em pacientes com disfunção sistólica do ventrículo esquerdo possa também ser diferente entre os sexos. Luchner et al publicou estudo em que comparou o índice de massa do ventrículo esquerdo com 1888 pacientes. Deste total de pacientes, 23 homens e 25 mulheres apresentavam disfunção sistólica. Os autores identificaram que os homens com disfunção sistólica moderada a grave ( pelo menos 2 desvios-padrão da normalidade) apresentaram índice de massa do ventrículo esquerdo significativamente maior que seus controles saudáveis e do que as mulheres com disfunção sistólica. As mulheres, no entanto, só demonstraram diferenças significativas no remodelamento cardíaco quando foram comparadas pacientes saudáveis com portadoras de disfunção sistólica muito grave ( pelo menos 3 desvios-padrão da normalidade). Os achados deste estudo sugerem que o controle das adaptações hemodinâmicas causadas pela disfunção sistólica pode ser influenciado pelo sexo e que homens desenvolvem hipertrofia ventricular compensatória mais precocemente que as mulheres neste cenário (16).

Outro aspecto importante que pode estar implicado nas diferenças de desfechos clínicos entre homens e mulheres com doenças cardiovasculares é a preservação dos cardiomiócitos em resposta a estímulos patológicos e ao processo de envelhecimento. Estudos *post mortem* mostraram que mulheres apresentam maior preservação do número de miócitos independente da idade ou da presença de insuficiência cardíaca. Outro estudo demonstrou que homens entre 17 e 89 anos perdem uma média de 1g por ano de massa cardíaca, o que é equivalente a 64 milhões de cardiomiócitos/ano e que as mulheres, por sua vez, permanecem com número estável de miócitos com o passar dos anos (17). Grandy et al demonstraram em um estudo experimental em ratos que além da

preservação do número de miócitos, o processo de acoplamento excitação-contração nas fêmeas também sofre menos desgaste com o envelhecimento. Os autores avaliaram medidas de cálcio intracelular e concentração de cálcio no retículo sarcoplasmático, identificando que somente os ratos de sexo masculino considerados idosos ( > 24 meses de idade) apresentaram diminuição significativa das variáveis aferidas quando comparados a ratos do sexo feminino e de menor idade ( <7 meses) (18). O mecanismo de preservação do acoplamento excitação-contração nas mulheres pode ser considerado um fator protetor do sexo feminino. Sabe-se que o dessarranjo do acoplamento excitação-contração é fundamental na fisiopatologia da miocardiopatias e fator causal primordial de disfunção contrátil e arritmias em pacientes com insuficiência cardíaca.

Desse modo, o estudo aprofundado da contratilidade miocárdica e morfologia ventricular podem ajudar no entendimento das diferenças de desfechos clínicos entre os sexos.

## 3. AVALIAÇÃO DA FUNÇÃO SISTÓLICA

A contratilidade miocárdica juntamente com a frequência cardíaca, pré-carga, pós-carga e sinergia são considerados os determinantes da função sistólica do ventrículo esquerdo. Dentre estas variáveis, a contratilidade miocárdica é a que apresenta os maiores desafios para sua aferição correta na prática.

A contratilidade é uma característica intrínseca e independente do miocárdio que pode ser definida como velocidade de encurtamento miocárdico para uma carga específica. Ao longo do século 20, vários índices de contratilidade miocárdica foram utilizados com fins assistenciais e de pesquisa. Neste contexto, deve-se salientar que muitas vezes medidas de função sistólica do ventrículo esquerdo através de índices isovolumétricos ou de ejeção são erroneamente utilizadas como índices de contratilidade. Dentre os índices isovolumétricos deve-se salientar a medida da dP/dt max que representa a máxima velocidade de aumento da pressão intraventricular no instante ou pouco antes da abertura das válvulas semilunares. Diversas correções da medida de dP/dt max vem sendo

utilizadas com relativo sucesso para estimar contratilidade independentemente da pré e pós-carga. Portanto, os índices isovolumétricos (dP/dt) e suas correções apresentam menor interferência dos demais determinantes da função sistólica e podem ser utilizados para estimar contratilidade com alguma margem de erro.

O índice de ejeção mais utilizado é a fração de ejeção do ventrículo esquerdo que necessita aferição do volume diastólico final (VDF) e volume sistólico final (VSF), de forma que pode ser calculada através da fórmula (VDF-VSF)/VDF. A fração de ejeção é uma medida útil e prática, no entanto é diretamente afetada pelos demais determinantes da função sistólica, de modo que não deve ser usada como medida de contratilidade.

Sabe-se que a análise da relação pressão-volume (PV) do ventrículo esquerdo é a maneira mais fidedigna de mensurar contratilidade miocárdica como variável isolada e independente de pré e pós-carga. O vértice superior esquerdo da relação PV representa o ponto de união da pressão sistólica final (PSF) com volume sistólico final (VSF). A inclinação da linha que une os vértices dos ângulos superiores esquerdos das curvas PV representativas de diversas cargas chama-se elastância sistólica final (ESF). Desvio para esquerda da inclinação (ESF) reflete maior contratilidade, enquanto o desvio para direita reflete o oposto. A principal limitação do uso da ESF como parâmetro de contratilidade miocárdica é a justamente a necessidade de construir curvas PV em diversas condições de carga. A metodologia para avaliar ESF é bastante complexa e invasiva, incluindo cateter de condutância para avaliação de volumes ventriculares, cateter de micromanômetro para avaliação de pressões intraventriculares e balão de oclusão da veia cava para causar variações na pré-carga (19-21). Mais recentemente, Shisido et al publicaram um estudo em que avaliação invasiva da pressão intraventricular associada a medidas de volume ventriculares por ecocardiograma em um único batimento foram capazes de estimar ESF com um excelente coeficiente de correlação (r=0.929) quando comparado ao método tradicional citado acima. Desde então, esse método tem ganho adeptos e maior aplicabilidade clínica, visto que a avaliação da contratilidade em um único batimento torna-se mais factível (22).

## 3.1. ECOCARDIOGRAMA E DEFORMAÇÃO MIOCÁRDICA COMO MEDIDA DA FUNÇÃO VENTRICULAR

Determinação da função ventricular é fundamental na avaliação clínica das doenças cardiovasculares. Além de ajudar a estabelecer um diagnóstico, a aferição correta da função ventricular pode dar informações quanto a prognóstico e manejo de diferentes patologias do sistema cardiovascular. Apesar de existirem outros métodos para avaliar função miocárdica, o ecocardiograma continua sendo a modalidade mais utilizada devido a seu caráter não-invasivo, disponibilidade e portabilidade. A fração de ejeção é o parâmetro ecocardiográfico mais difundido para avaliação da função sistólica. Apesar de representar uma boa correlação prognóstica nas miocardiopatias, a fração de ejeção representa uma medida simplista, visto que se correlaciona mais com a performance radial do ventrículo, negligenciando que a deformação ventricular ocorre também nos sentidos longitudinal e circunferencial em cada batimento (23-25). Além disso, a avaliação da função sistólica somente através da fração de ejeção tem outras limitações. Existem potenciais dificuldades na interpretação da motilidade segmentar, pois a mesma é usualmente subjetiva ou semi-quantitiativa através da avaliação do espessamento endocárdico. A disfunção sistólica subclínica é outro problema, visto que pode ocorrer na presença de fração de ejeção preservada, em um momento da história natural da doença em que intervenções terapêuticas seriam potencialmente benéficas (26,27).

Nas últimas duas décadas medidas ecocardiográficas de deformação ventricular chamadas *strain* e *strain rate* surgiram como um método mais completo e acurado de quantificar a função miocárdica. *Strain* é conceituado como a representação da deformação miocárdica ou mudança de extensão (alongamento ou encurtamento) do miocárdio durante a sístole e diástole. *Strain rate* é a medida em que a deformação ocorre por unidade de tempo. Desse modo, *strain* longitudinal e circunferencial que representam encurtamento miocárdico apresentam resultados negativos e *strain* radial que representa alongamento miocárdico tem resultado positivo (28-30). Tais medidas ecocardiográficas foram validadas através de comparação com ressonância nuclear magnética, considerada o padrão ouro para avaliação de deformação miocárdica. Amundsen

et al publicaram um estudo em que medidas de deformação miocárdica por rastreamento de marcadores sonográficos obteve excelente correlação com as medidas obtidas por ressonância magnética ( r=0.87, p<0.001) (31)

É importante ressaltar que embora *strain* e *strain rate* sejam consideradas boas estimativas da função sistólica, não se pode usar estas medidas para fazer avaliação de contratilidade miocárdica, visto que a deformação miocárdica é um fenômeno dependente de pré-carga. Contratilidade, por sua vez, é a propriedade fundamental do miocárdio que descreve sua atividade independente de fatores extrínsecos como a pré e pós-carga (29). O uso das imagens de deformação miocárdica para avaliar contratilidade ventricular e sua modulação continua um desafio. No entanto, sabe-se que o pico sistólico do *strain rate* que ocorre no início da sístole ventricular pode representar o estado contrátil do ventrículo com mais acurácia que medidas da função sistólica como a fração de ejeção. Greenberg et al demonstraram em um estudo experimental em cachorros instrumentados com cateter de condutância para medidas de volume e cateter de micromanômetro para medidas de pressões intraventriculares que existe uma excelente correlação (r=0.94, p<0.01) entre medidas do pico sistólico do *strain rate* e pico de elastância máxima do ventrículo esquerdo (Emax). A partir deste estudo, tem sido sugerido que o pico sistólico do *strain rate* pode ser suficientemente independente da pré-carga para ser considerado um índice de contratilidade miocárdica (32).

As medidas de deformação miocárdica podem ser aferidas através de dois métodos ecocardiográficos: doppler tecidual ou rastreamento de marcadores sonográficos ( speckle tracking). O doppler tecidual é uma técnica que permite avaliar a velocidade tecidual do miocárdio. A primeira descrição do uso das medidas de deformação miocárdica foi realizada utilizando o doppler tecidual. Apesar de ser uma técnica validada por vários estudos, o emprego do doppler tecidual para avaliação de *strain* tem várias desvantagens que o tornou menos confiável. Primeiramente, o doppler tecidual, como todas técnicas baseadas em doppler, é altamente dependente da angulação entre o transdutor e o miocárdio. Se tal ângulo for superior a 20 graus, as medidas de deformação miocárdica podem ficar significativamente subestimadas. Além de apresentar significativa

variabilidade inter-observador (10-15%), a aquisição e processamento dos dados utilizando a técnica do doppler tecidual é mais demorada e necessita de interpretação de especialistas na técnica.

Por outro lado, o rastreamento de marcadores sonográficos é realizado de forma semi-automática através de um programa de computador que utiliza as imagens ultrasonográficas em 2 dimensões para identificar mudança de posição e de velocidade dos marcadores sonográficos quadro a quadro, de forma que a angulação do transdutor não influi nas medidas e a variabilidade intra e inter-observador é consideravelmente menor (5.3%) . Os marcadores sonográficos são simetricamente distribuídos pelo miocárdio e seu tamanho é de aproximadamente 20 a 40 pixels. Sabendo-se a taxa de quadros em que a imagem foi adquirida, pode-se determinar a velocidade da mudança de posição dos marcadores sonográficos e assim calcular os índices de deformação miocárdica. A avaliação de deformação miocárdica através do rastreamento de marcadores sonográficos, no entanto, apresenta uma pequena desvantagem em relação as medidas por doppler tecidual: a necessidade de adquirir imagens com maior qualidade ( 50-70 quadros por segundo) limita sua aplicabilidade clínica de rotina. A aquisição de imagens com mais quadros por segundo que o recomendado resulta em redução de resolução espacial e consequentemente rastreamento menos eficiente pelo programa de computador escolhido para realizar a análise (27-35).

Estudos com o uso de medidas ecocardiográficas de deformação miocárdica têm crescido exponencialmente nos últimos anos tanto como objeto de pesquisa como na prática clínica. Medidas de strain e strain rate têm sido utilizadas para melhorar o entendimento e manejo terapêutico de diversas doenças cardiovasculares. Entre elas pode-se citar avaliação de isquemia e viabilidade miocárdica, disfunção diastólica do ventrículo esquerdo, dissincronia ventricular em candidatos a ressincronização miocárdica, valvulopatias e diversas miocardiopatias. Avanços adicionais são esperados com o uso de medidas de deformação miocárdica por rastreamento de marcadores sonográficos em 3 dimensões, especialmente no que se refere a terapia de ressincronização miocárdica. Tais medidas ecocardiográficas em 3D permitem

um melhor mapeamento da ativacão ventricular e posicionamento mais preciso do cabo no ventrículo esquerdo. (28)

## 4. DIFERENÇAS MORFOLÓGICAS E HEMODINÂMICAS DO VENTRÍCULO ESQUERDO ENTRE OS SEXOS

O ventrículo esquerdo é significativamente menor nas mulheres que nos homens, mesmo após ajuste por superfície corporal (36). Estes achados foram confirmados pelo Dallas Heart Study que comparou medidas do ventrículo esquerdo entre homens e mulheres saudáveis através de ressonância nuclear magnética e confirmou que o sexo feminino apresenta volume diastólico final do ventrículo esquerdo significativamente menor. Apesar disso, medidas de índice cardíaco são comparáveis entre os sexos . Tal fenômeno pode ser explicado pelo fato de que as mulheres apresentam função sistólica e frequência cardíaca aumentada em relação aos homens. Os autores do Dallas Heart Study demonstraram que além do volume diastólico final do ventrículo esquerdo ser menor nas mulheres, o volume sistólico final também é proporcionalmente menor, em uma fração de ejeção maior no sexo feminino. A partir destes resultados, os autores deste estudo sugeriram que os valores de referência da fração de ejeção deveriam ser diferenciados entre os sexos (37).

Estudos experimentais com medidas invasivas da função cardíaca em humanos ratificam os achados do Dallas Heart Study. Hayward et al compararam relações pressão-volume do ventrículo esquerdo em 16 homens e 14 mulheres saudáveis. Medidas invasivas de pressão com cateter de micromanômetro e de volume com cateter de condutância foram utilizadas para construir gráficos da relação pressão-volume nos sujeitos do estudo. Os autores demonstraram que a contratilidade miocárdica, medida através da elastância sistólica final do ventrículo esquerdo é significativamente maior nas mulheres, enquanto o volume sistólico indexado pela área de superfície corporal se mantém menor no sexo feminino, de forma que a manutenção de um débito cardíaco semelhante entre os sexos se dá por presença de frequência cardíaca basal maior nas mulheres (38). Mittof et al publicaram outro estudo experimental comparando

função cardíaca entre homens e mulheres com e sem disfunção sistólica. Os sujeitos do estudo foram instrumentados com cateter de termodiluição na artéria pulmonar para realização de cateterismo direito e cateter de micromanômetro no ventrículo esquerdo. Cada paciente do sexo feminino foi pareado com dois pacientes do sexo masculino de idade e comorbidades semelhantes de modo que foram incluídos 73 homens e 39 mulheres com função sistólica normal. Os autores demonstraram que as mulheres apresentaram frequência cardíaca, pressão sistólica e +dP/dt do ventrículo esquerdo significativamente maior. Entretanto, as chamadas pressões de enchimento como a pressão diastólica final do ventrículo esquerdo, pressão capilar e pressão do átrio direito foram significativamente menores nas mulheres (39).

Fu et al encontraram que o estímulo externo através de pressão negativa no membros inferiores causava decrementos progressivamente maiores no volume sistólico associado a um aumento significativamente maior da frequência cardíaca em mulheres do que homens saudáveis. A diminuição mais acentuada da pré-carga em resposta a estímulo de pressão negativa e aumento da frequência cardíaca pode ser responsável pela depressão do mecanismo de Frank-Starling nas mulheres, o que contribui para limitar a reserva contrátil do ventrículo esquerdo no sexo feminino (40). Apesar do estudo de Fu et al ser bastante contundente, o achado de diminuição do volume sistólico com estímulo de pressão negativa no membros inferiores não pode ser aplicado em todos os cenários. Vale lembrar que estes estudos foram realizado em adultos saudáveis, portanto sua generalização em pacientes mais idosos ou com comorbidades pode não ser adequada.

Desse modo, diferenças na frequência cardíaca, função sistólica e pré-carga sugerem que os múltiplos determinantes envolvidos na performance mecânica do ventrículo esquerdo são diretamente influenciados pelo sexo biológico.

## 5. ACOPLAMENTO EXCITAÇÃO-CONTRAÇÃO NO CARDIOMIÓCITO:

### 5.1 EVIDÊNCIAS PRÉ-CLÍNICAS

A sequência de eventos desde a despolarização do cardiomiócito até a liberação de cálcio pelo retículo sarcoplasmático chama-se acoplamento excitação-contração. Evidências científicas sugerem que existem diferenças no acoplamento excitação-contração entre os sexos que se traduzem em diferenças de contratilidade miocárdica. Estudos em preparações de fibras miocárdicas isoladas de animais demonstram que a contratilidade miocárdica pode estar deprimida nas fêmeas. Tais estudos em fibras miocárdicas isoladas evidenciaram que características intrínsecas do sexo feminino atuam em diferentes etapas do acoplamento excitação-contração limitando a entrada de cálcio no cardiomiócito ou a liberação deste íon pelo retículo sarcoplasmático (2). Além disso, Petre el al demonstrou em preparação de trabéculas ventriculares que os gatos machos apresentam reserva contrátil aumentada em resposta a incrementos da frequência da cardíaca quando comparados as fêmeas. Este aumento de reserva contrátil nos machos foi reproduzido em preparações de outros animais e tem sido relacionado à menor entrada de íons cálcio pelos canais tipo L dos cardiomiócitos das fêmeas (41). Especula-se também que a sensibilidade dos miofilamentos ao cálcio possa diferir entre os sexos. Schwertz et al demonstrou que proteínas contráteis em fibras de ratos fêmea exibiram sensibilidade maior ao cálcio do que nos machos. O fato dos miofilamentos serem mais sensíveis ao cálcio no sexo feminino, pode explicar a presença de concentrações menores do íon nos miócitos das fêmeas. Entretanto, os autores não identificaram diferenças de contratilidade entre os sexos (42).

A hipótese de reserva contrátil aumentada em machos evidencia que quando houver aumento significativo da demanda no músculo cardíaco os sujeitos do sexo masculino apresentam maior possibilidade de manter a perfomance cardíaca adequada (43). A menor reserva contrátil nas fêmeas pode estar relacionada a diferenças no metabolismo dos miócitos em relação especificamente a utilização de glicose e consumo de oxigênio. Peterson et al publicaram um estudo em que 25 indivíduos saudáveis ( 13 homens e 12

mulheres) foram submetidos a tomografia por emissão de positrons para investigar diferenças entre os sexos no consumo e extração de oxigênio pelo miocárdio, assim como na extração e metabolismo da glicose e ácidos graxos. Os autores identificaram que o metabolismo cardíaco do sexo feminino apresenta maior consumo de oxigênio e menor consumo de glicose. Pesquisadores acreditam que o estrogênio diminui a utilização de glicose pelo miocárdio através do aumento da atividade da sintase do óxido nítrico, que reduziria a translocação do transportador GLUT-4 para superfície celular, inibindo a captação de glicose pelos cardiomiócitos. Em situações em que a demanda energética do miocárdio fica aumentada como no exercício, insuficiência cardíaca ou isquemia, a menor disponibilidade de glicose no miocárdio feminino é especialmente deletéria, visto que a glicólise é uma fonte anaeróbica de ATP e, portanto, não necessitaria de oxigênio. Concomitantemente, o que ocorre é um aumento do consumo de oxigênio, visto que a fonte principal de energia neste contexto passa a ser oxidação dos ácidos graxos. Portanto, a noção de que o estrogênio apresenta somente efeitos cardioprotetores pode ser equivocada, pois sua ação no metabolismo cardíaco em situações de demanda energética aumentada pode ser deletéria (44).

## 5.2 INFLUÊNCIA DOS HORMÔNIOS SEXUAIS NO ACOPLAMENTO EXCITAÇÃO-CONTRAÇÃO DOS CARDIOMIÓCITOS:

A influência dos hormônios sexuais na função cardíaca é alvo de pesquisas há muitos anos. Sabe-se que fatores sociais e comportamentais podem potencialmente contribuir para as diferenças no metabolismo cardíaco, e em última análise, na função cardíaca. Entretanto, pesquisas identificaram que os efeitos biológicos dos hormônios sexuais podem ser considerados variáveis independentes envolvidas na função cardíaca. O impacto direto dos hormônios sexuais no cardiomiócito é demonstrado pela presença de receptores de estrogênio, progesterona e androgênios nas células cardíacas de várias espécies, inclusive de humanos (45-47). É possível que os hormônios sexuais desempenhem papel importante na mediação do acoplamento excitação-

contração dos cardiomiócitos, contribuindo para diferenças expressivas deste processo entre os sexos.

Chu et al demonstrou em ratos ooforectomizados que a falta de estrogênio aumenta a expressão dos canais tipo L responsáveis pela entrada de cálcio no miócito e inibe a proteína trocadora de sódio-cálcio que está envolvida na retirada de cálcio da célula, o que se traduziu em aumento de contratilidade miocárdica (48). Outros estudos demonstraram também que animais submetidos a ooforectomia apresentam aumento no número de receptores β-adrenérgicos na membrana dos miócitos e liberação aumentada de cálcio pelo retículo sarcoplasmático (49-51). Vale ressaltar que estes efeitos foram reversíveis, de modo que reposição de estrogênio possuiu efeito contrário, inibindo a contratilidade miocárdica .

Entretanto, o papel dos hormônios sexuais não se restringe ao estrogênio. Enquanto o estrogênio limita a entrada de cálcio na célula, a testosterona parece exercer o efeito contrário. Um estudo em ratos machos adultos submetidos a gonadectomia demonstrou diminuição na expressão dos canais de cálcio tipo L e redução nos índices de contratilidade miocárdica. Os efeitos inotrópicos negativos da gonadectomia nos ratos machos também foi revertido com reposição aguda de testosterona nos animais (52,53). Há também alguma evidência de que a progesterona pode afetar o acoplamento excitação-contração. Um estudo demonstrou que o uso de progesterona em altas doses pode aumentar a duração do potencial de ação em coração de coelhos (54) e outro estudo demonstrou que progesterona em baixas doses reduz a duração do potencial de ação em cardiomiócitos isolados de porcos-da-índia (55), de modo que o real papel da progesterona no processo de excitação-contração dos cardiomiócitos ainda é controverso.

## 5.3. DIVERGÊNCIAS ENTRE OS ESTUDOS EXPERIMENTAIS IN VIVO E EX VIVO

O uso de fibras miocárdicas isoladas nos estudos experimentais é uma forma elegante de excluir o efeito das alterações de pré e pós-carga presentes

nos estudos in vivo. Como discutido acima, a maioria dos estudos experimentais em músculo cardíaco isolado de animais demonstrou contratilidade miocárdica diminuída em fêmeas (2). Paradoxalmente, os estudos experimentais realizados em humanos demonstraram contratilidade ventricular aumentada nas pacientes do sexo feminino. Diversas hipóteses tem sido aventadas para explicar o achado paradoxal de contratilidade miocárdica aumentada nas mulheres em estudos clínicos. Estes achados em humanos podem estar relacionados ao fato de que as mulheres apresentam elastância arterial aumentada em relação aos homens, ou seja, o coração das mulheres precisa ejetar sangue através de um sistema vascular com maior rigidez, de forma que para manter o débito cardíaco aceitável o ventrículo esquerdo exerce maior força sistólica ou elastância sistólica final (56,57). A influência do acoplamento arterial-ventricular como explicação das diferenças de contratilidade miocárdica entre os sexos foi testada recentemente em um estudo publicado por Coutinho et al. Neste estudo os autores quantificaram a elastância arterial e ventricular através de diversos marcadores em 189 homens e 272 mulheres com média de idade de 65 ± 10 anos. Tanto a elastância arterial ( 1.30 ± 0.28 vs 1.57 ± 0.36 mmHg/ml) como a elastância sistólica final do ventrículo esquerdo (1.42 ± 0.38 vs 1.73 ± 0.47mmHg/ml) foram significativamente maiores ( $p<0.0001$) nas pacientes do sexo feminino. Os autores concluíram que a rigidez arterial acentuada presente na aorta proximal das mulheres está envolvida na maior incidência de insuficiência cardíaca com fração de ejeção preservada no sexo feminino (58).

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## HIPÓTESE CONCEITUAL

Aumento da frequência cardíaca induzida por estímulo de marcapasso atrial acentua diferenças de performance do ventrículo esquerdo entre homens e mulheres com e sem insuficiência cardíaca

## OBJETIVO GERAL

Avaliar diferenças na performance do ventrículo esquerdo entre homens e mulheres com e sem disfunção sistólica submetidos à aumento da frequência cardíaca através de marcapasso atrial.

OBJETIVOS ESPECÍFICOS:

1. Determinar efeito do aumento da freqüência cardíaca na medida da Elastância Sistólica Final através do método de único batimento ( Shisido) em homens e mulheres com função sistólica preservada do ventrículo esquerdo.

2. Determinar efeito do aumento da frequência cardíaca nas medidas ecocardiográficas de deformação miocárdica em pacientes com e sem disfunção sistólica do ventrículo esquerdo.

# FREQUENCY-DEPENDENT LEFT VENTRICULAR PERFORMANCE IN WOMEN AND MEN

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**Brief title: Left ventricular performance in women and men**

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## ABSTRACT

**Objectives:** We aimed to determine whether sex differences extend to the dynamic response of the left ventricular (LV) chamber to an inotropic and loading intervention.

**Background:** Several observations suggest sex influences LV structure and function in health; moreover, this physiology is also affected in a sex-specific manner by aging.

**Methods** Eight women and 8 men underwent a study to assess the relationship between heart rate (HR) by right atrial pacing and LV $+dP/dt_{max}$ as assessed by micromanometer-tipped catheter and Doppler echocardiography measurements of LV volume. Analysis of approximated LV pressure volume relationships was performed using a time-varying model of elastance. External stroke work was also calculated.

**Results** The relationship between HR and $LV+dP/dt_{max}$ was expressed as $LV+dP/dt_{max} = b + mHR$. The slope (m) of the relationship was steeper in women compared to men ($11.8 \pm 4.0$ versus $6.1 \pm 4.1$ $mmHgs^{-1}$/bpm, $p=0.01$). The greater increase in contractility in women was reproducibly observed after normalizing $LV+dP/dt_{max}$ to LV end-diastolic volume or by measuring end-systolic elastance. LV end-diastolic and stroke volume decreased more in women. Thus despite greater increases in contractility, HR was associated with a lesser rise in cardiac output and a steeper fall in external stroke work in women .

**Conclusions** Compared to men, women exhibit greater inotropic responses to incremental RA pacing, which occurs at the same time as a steeper decline in external stroke work. These differences suggest sexual dimorphism in determinants of LV mechanical performance, which may render women more vulnerable to hemodynamic instability.

Keywords: Sex, left ventricular function, contractility

## INTRODUCTION

The prevalence, presentation, management and outcomes of the broad spectrum of cardiovascular disease appear to be influenced by whether the patient is male or female. The extent to which sexual dimorphism in cardiac and circulatory physiology contributes to clinical sex differences remains incompletely understood. Several observations suggest sex influences left ventricular (LV) structure and function in health; moreover, this physiology is also affected in a sex-specific manner by aging. Understanding these differences may yield insight into sex differences observed among patients with abnormal LV chamber function and resultant HF syndromes.

Sex differences in determinants of LV performance may be observed at several levels. There is evidence from non-human experiments for sex differences in excitation-contraction coupling[1-3] which may translate to differences in systolic and diastolic function. Our laboratory[4] and others[5-7] have demonstrated that LV systolic function and LV filling pressures differ at rest between adult men and women. Sex differences in LV performance during loading manipulations have also been demonstrated in young healthy subjects.[6] In humans, the LV contractile response to changing stimulation frequency or heart rate (HR), may serve as a quantitative in vivo index of excitation-contraction coupling, known as the force frequency relationship (FFR).[8-11] It is also well understood that changing HR will alter LV loading conditions. We tested the hypothesis that responses in LV performance to increases in HR by right atrial (RA) pacing will differ between men and women. We studied post menopausal women and similarly aged men, an age range relevant to the onset of cardiovascular disease.

## METHODS

### Study Participants

Men and women referred from outpatient clinics for elective cardiac catheterization and the assessment of a chronic chest pain syndrome were recruited. Inclusion criteria included normal LV systolic function (LVEF > 55%) and absence of valvular heart disease demonstrated by standard 2D echocardiography. LV mass was also recorded.[12] All patients were in normal sinus rhythm, and specific exclusions included a QRS duration > 110 ms, evidence of atrio-ventricular block or the presence of a permanent pacemaker. Also excluded were patients with uncontrolled hypertension, acute coronary syndromes or revascularization procedures within the previous 6 months, as were patients taking hormone replacement therapies or supplements including estrogens, estrogen receptor modulators, androgens, testosterones or anabolic steroids. Finally, patients with any history, signs or symptoms of heart failure despite preserved LV systolic function, and patients with an LVEDP > 15mmHg at the time of catheterization were also excluded from this study. All patients gave written informed consent and the study was approved by the Research Ethics Board at the Mount Sinai Hospital. Prior to catheterization, venous blood was sampled for measurements of hemoglobin, creatinine and estimated glomerular filtration rate. Serum samples were obtained to assay sex steroid hormones including estrogen, progesterone and testosterone by high performance liquid chromatography. Patients were minimally sedated, and all medications were withheld on the morning of the procedure.[4]

### Cardiac Catheterization and Instrumentation

Diagnostic cardiac catheterization from the femoral approach and subsequent study procedures were performed at the Cardiac Catheterization Research Laboratory at the Mount Sinai Hospital. To control HR, a 6 Fr bipolar pacing catheter was advanced to the high right atrium from the right femoral vein under fluoroscopic guidance. The pacemaker catheter was

aligned with a programmable stimulator (Prucka GE CardioLab). A 7 Fr micromanometer-tipped catheter (Mikro-tip Catheter, Millar Industries) was then advanced from the femoral artery to the LV. Femoral artery pressure was acquired continuously from the sidearm of the sheath. The LV pressure and the first derivative of LV pressure were continuously displayed and acquired (1000Hz) on line, allowing serial sampling of LV+dP/$dt_{max}$. A rest period was allotted after instrumentation prior to initiating the research pacing protocols.

**Force Frequency Pacing Protocol 1: Measurement of Isovolumic Contractility**

Prior to initiating RA pacing, control measurements were recorded after 3 serial sampling intervals demonstrate LV+dP/$dt_{max}$ values within 5%. RA pacing was then initiated at least 5-14 bpm above the intrinsic rate to a multiple of 10 bpm. Thereafter, HR was increased by increments of 10 bpm every 3 minutes to a maximum of 120 bpm or until Mobitz type 1 or 2:1 AV block occurred. If AV conduction fell below 1:1, the pacing was reduced by increments of 5 bpm until 1:1 conduction returned.

Analysis of the LV pressure waveform was performed off-line using customized software (Labview 8.5, National Instruments) by a research assistant blinded to the characteristics of the patient and purpose of the study. For each heart rate condition, 50 consecutive beats from the start of the second minute of RA pacing were selected. If the preceding RR interval was not within 2% of the planned pacing cycle length, the specific beat and the subsequent 5 beats were discarded. Per beat, LV+dP/$dt_{max}$, peak LV systolic pressure (LVSP), end-diastolic pressure (LVEDP) and the time constant of isovolumic relaxation (Tau) by the logarithmic method were calculated as previously reported.[4] Systolic, diastolic, and mean arterial pressures (SAP/DAP/MAP) were also recorded. Reported values represent the mean of these 50 cardiac cycles. For each patient, the linear relationship between HR and LV+dP/$dt_{max}$ was described, such that LV+dP/$dt_{max}$ = b + mHR, where m represents the slope ($mmHgs^{-1}$/bpm).The intercept b

occurs at a HR of 0 bpm, an irrelevant value in vivo; as such the intercept of LV+dP/$dt_{max}$ at 60 bpm (LV+dp/$dt_{60}$), calculated as [b + m(60)] was also reported.

**Force Frequency Pacing Protocol 2: Measurements of Single Beat Elastance**

LV+dP/$dt_{max}$ is a reproducible and precise measure that is applicable to rapid, repeated acquisition. However, LV+dP/$dt_{max}$ is sensitive to changes in loading conditions and may be affected by potentially more pronounced HR-dependent decreases in LV filling in women. In a second pacing protocol, we attempted to account for loading changes by normalizing LV+dP/dtmax to LV end-diastolic volume (LVVed). We also assessed the FFR using end-systolic elastance (Ees), estimated by analysis of the approximated time-varying elastance suggested by Shishido et al.[13]

The second pacing protocol was initiated after recontrol was established (when LV+dP/$dt_{max}$ sampling was within 10% of the original baseline). Two-dimensional echocardiographic images and Doppler acquisition was performed [GE Vivid 7 Imaging System, M4S Matrix Sector Array Probe (2-5 MHz), GE Healthcare, Canada] by a research echocardiography technician. The chronometers for both the imaging system and the hemodynamic recording system were synchronized to ensure that offline analysis of LV pressure was simultaneous with the timestamped echocardiographic image frames.

Prior to initiating the FFR pacing protocol, the LV outflow tract (LVOT) dimension allowing calculation of its cross-sectional area (CSA) calculated as $(LVOT/2)^2$ x 3.14. Pulsed-wave Doppler aortic flow spectrum was obtained in the apical five chamber view and transducer placement was marked on the chest wall for repeated acquisition. Three RA pacing conditions were acquired at 80, 100 and 120 bpm (CL 750, 600 and 500ms). Within the first 2 minutes of steady state pacing at each HR condition, standard grayscale 2D images were acquired in the apical 4-chamber view at 70-120 fps, with higher frame rates at higher HR, achieved by

optimizing the sector length and width to the region of interest. The images were stored in cineloop format for subsequent off line analysis. Pulsed-wave Doppler flow across the LVOT was then re-established and acquired simultaneously with the LV pressure waveform over 5 consecutive beats between the 2nd and 5$^{th}$ minute of the pacing stage. No pacing stage exceeded 5 minutes.

Analysis of imaging data was performed on an offline EchoPac workstation (GE Healthcare, Canada). From the stored 2D images LV end-diastolic and end-systolic volumes (LVVed and LVVes) were measured using standard echocardiographic techniques. Doppler echocardiographic flow velocity spectra yielded measurement of the time-velocity integral (TVI). SV was calculated as the product of CSA and TVI. Ejection fraction (EF) was calculated as SV/LVVed. Cardiac output (CO) was calculated at SV x HR. Total peripheral resistance (TPR) was calculated as MAP÷CO.

Shishido[13] et al suggested that time-varying elastance of the LV can be estimated as a bilinear relationship reflecting the change from the isovolumic to the ejection phase. The ratio between these two slopes ($\alpha$) can be estimated from the relationship between duration of isovolumic contraction and the ejection period. Per cardiac cycle 3 timed intervals within the LV pressure waveform were determined based on the following definitions: 1) early isovolumic contraction, $t_{ec}$: point at which +dP/dt reached 30% of $+dP/dt_{max}$, 2) transition from isovolumic contraction to ejection, $t_{ej}$: point at which $+dP/dt_{max}$ occurred and 3) end-systole $t_{es}$: point at which –dP/dt exceeded 20% of $-dP/dt_{min}$. The corresponding LV pressure at these timepoints $P_{ec}$, $P_{ej}$, and $P_{es}$ were used as surrogates of elastance at these phases.

Using these 3 timed points, the bilinear, time-varying elastance can be expressed as:

$E_{es} = E_{ej} + (E_{ej} - E_{ec})/PEP \bullet ET \bullet \alpha$, where PEP is the pre-ejection period ($t_{ej}$-$t_{ec}$) and ET is the ejection time ($t_{es}$-$t_{ej}$). Combining this equation with knowledge of the pressure-volume relationship:

$E_{es} = [Pej + (Pej\text{-}Pec/PEP \bullet ET \bullet \alpha - P_{es}]/SV]$

$\alpha$ is strongly correlated to both EF and PEP/(PEP + ET). The empiric estimation can be derived as:

$\alpha = -0.210 + 1.348EF + 0.682\ PEP/(PEP+ET)$.

**Statistics Analysis**

Data are presented as means and standard deviations. Comparisons between men and women were made using unpaired Student ttests for continuous variables or Chi square analysis for categorical variables (Statview). A 2-way analysis of variance for repeated measures was employed to compare hemodynamic and LV function responses to HR stage as one factor and sex as the second factor. As resting heart rate may differ between men and women, changes in hemodynamics and LV function between men and women were analysed in relation to incremental changes in HR above resting. A p value of $< 0.05$ was considered significant.

**RESULTS**

**Patients**

Sixteen patients completed the study, 8 women (60 ± 10 years) and 8 men (59 ± 4 years). Three women and 3 men had a history of hypertension controlled with medications, 1 woman and 2 men had a history of coronary artery disease. All women were peri- or post-menopausal and 7 had serum estradiol levels below the limit of detection of < 50 pmol/L. Other baseline characteristics including hemoglobin, renal function and serum testosterone levels are detailed in Table 1.

**Characteristics of the Left Ventricular Pressure Waveform at Rest**

Resting HR was higher in women than men. Measurements are presented at rest, and at a standardized HR of 80 bpm (Table 2), as 14 of the 16 patients underwent RA pacing at this rate, while 2 women had a resting HR just above 80 bpm. In our study cohort, systemic blood pressure tended to be higher in women, but was within normal limits in both groups. LVSP and LV+dP/$dt_{max}$ tended to be increased in women. Importantly, LVEDP was similar in both groups and not elevated. The isovolumic time constant of relaxation, tau, was also similar at rest in both groups.

**Frequency-dependent Effects on the Left Ventricular Pressure Waveform**

All patients achieved at least 4 RA paced conditions above their resting HR (Table 3). One man and 1 woman did not achieve stable RA pacing at 120 bpm. Increases in HR were associated with decreasing LVEDP, while diastolic and mean arterial pressure increased. There was no significant change systolic arterial pressure. LVSP decreased at the highest increments of RA pacing.

As expected, there was a linear relationship between paced-HR and LV+dP/$dt_{max}$. For all patients, the FFR was described as the linear regression equation LV+dP/$dt_{max}$ = b + mHR. The intercept (b),LV +dP/$dt_{max}$ at theoretical HR 0 bpm, tended to be lower in women compared to men (660 ± 180 versus 920 ± 327 mmHg/s respectively, p=0.07), while predicted LV+dP/$dt_{max}$ at HR 60 bpm was not statistically different between women and men (1367 ± 143 versus 1286 ± 293 mmHg/s, p=0.49) and close to resting values. However, the slope of the FFR as assessed by LV+dP/$dt_{max}$ was significantly steeper in women compared to men (11.8 ± 4.0 versus 6.1 ± 4.1 $mmHgs^{-1}$/bpm, p=0.01). Similarly, two-way analysis of variance demonstrated a significant interaction between sex and increases in LV+dP/$dt_{max}$ with a steeper HR-related rise in contractility in women (Figure 1).

The effect of RA pacing on early diastolic relaxation as assessed by Tau was also different between the women and men in this cohort. Significant frequency-dependent shortening of Tau was observed in men, while no significant change was observed in women. Women demonstrated a tendency toward prolongation of Tau at the highest heart rates.

**Frequency-dependent Effects on Analysis of Left Ventricular Pressure and Doppler-echocardiographic Assessment of the Left Ventricular Chamber**

Prior to the second FFR pacing protocol, recontrol measurements demonstrated LV+dP/$dt_{max}$ had returned to baseline. Data was obtained at resting HR and RA pacing at 80, 100 and 120 bpm (Table 4). Stable pacing could not be established at 120bpm again in 1 man and 1 woman.

Resting echocardiographic measurements demonstrated that LVVed was similar between men and women, while LVVes tended to be smaller, yielding a higher EF in women, as has been observed by others.[7] At resting HR, stroke volume measured by the velocity-time integral and resultant cardiac output tended to be higher at rest in the women. Analysis of the LV pressure waveform and SV yielded the measurement of $P_{ec}$, Pej and $P_{es}$ as described. $P_{es}$ and ET tended to be increased in women compared to men. $E_{es}$ was also slightly higher in women, and similar to LV+dP/$dt_{max}$.

The second RA pacing run confirmed the steeper slope of the FFR in women by additional measurements of contractility (Figure 1). The increase in LV+dP/$dt_{max}$ induced by incremental RA pacing was reproducible and again steeper in women than men. The frequency-dependent increase in LV+dP/$dt_{max}$/Ved was significantly greater in women compared to men. Finally, the frequency dependent increase in Ees was also significantly greater in women.

The increases in Ees were related to decreases in LVVed and SV (Figure 2) with RA pacing. The slope of the decline in LVVed was significantly steeper in women, and a similar

trend was observed with respect to SV. As such, calculated CO increased with increasing HR in men, but in women the decline in SV offset the rise in HR, limiting HR-associated increases in CO. Mean arterial pressure did not change significantly in either women or men. Given the limitation in HR-related increases in CO, TPR did not decrease in women as it did in men.

The mean measurements of $P_{ec}$, $P_{ej}$, $P_{es}$ and LVEDP and the simultaneous acquisition of SV generated 4 points utilized to inscribe approximated LV pressure volume loops at each HR for the men and the women (Figure 3). The change in the configuration of the pressure volume loop with increasing HR was apparent in women, such that the area within the PV loop (or external mechanical stroke work) decreased with increasing HR. Quantitatively, calculated external stroke work declined with increasing HR more steeply in women. This occurred despite the observed greater increases in measurements of inotropy ($LV+dP/dt_{max}$ and $E_{es}$) in women.

**DISCUSSION**

In this model of frequency-stimulated LV function, responses of several determinants of LV chamber performance were significantly different between the men and women studied. The slope of the force frequency relationship was steeper for the women in this cohort compared to the men, as assessed by both $LV+dP/dt_{max}$ as well as the measure of end-systolic elastance. HR-associated increases in contractility were observed at the same time that LVVed (as well as SV) declined more precipitously in women, suggesting a sex-based difference in length-dependant force development. Moreover, the balance of HR-related effects on LV pressure generation and filling were differentiated such that external mechanical stroke work was more negatively affected in women by increases in HR.

The inclusion and exclusion criteria specified ambulatory patients with chronic, usually atypical chest pain syndromes who were clinically stable. This resulted in a population of women and men at an age relevant to the onset of cardiovascular disease, who were well matched for

demographic variables and comorbid illness. Both at rest and at a standardized HR of 80 bpm, LV function and hemodynamic characteristics for the men and women in our study were consistent with previous reports, including a trend to higher HR[4;5;14]smaller LV end-systolic volumes and higher ejection fraction in women.[7] Importantly, we observed a tendency to increased systolic function in women as measured by LVSP, LV+dP/$dt_{max}$ as well as end-systolic elastance. The latter was estimated using a method assuming time-varying elastance adapted from Shishido et al.[13] Measured end-systolic elastances for men and women in this study were quantitatively similar to that previously reported by Hayward et al,[5] who employed conductance catheter methodology to perform pressure-volume analysis. In their study, a preponderance of hypertension in the women studied tempered conclusions regarding intrinsic sex differences. Hypertension was less prevalent in our study although, as a catheterization cohort, comorbid conditions were unavoidable. Single beat methodology adapted for non-invasive measurement of end-systolic elastance has been employed in a large community based, cross-sectional investigation. This study again documented higher end-systolic and arterial elastance in women with and without cardiovascular disease, interpreted as reflective of increased LV and arterial stiffness. The totality of evidence supports that higher systolic function, elastance and/or stiffness is a characteristic of the female LV chamber in middle-aged and older adults.[15]

The current study examined whether sex differences also extend to the dynamic response of the LV chamber to an inotropic and loading intervention. The FFR is considered an intrinsic mechanism by which contractility is augmented in response to increases in HR. Experimentally the FFR has been described in humans[8-11] as an in vivo index of $Ca^{2+}$ handling, given the predominant mechanism is increased $Ca^{2+}$ availability to the myofilaments. Preclinical data have suggested limitations to excitation-coupling in female mammalian animal models. [1-3] In our cohort, the women studied demonstrated a steeper slope of the FFR, reproducibly and using more

than one index of contractility. We also observed a divergence in the pattern of isovolumic relaxation in response to HR. These data may suggest either intrinsic sex differences in $Ca^{2+}$ handling or, more likely, in the modulation of the excitation-contraction coupling.

Higher ventricular elastance or stiffness in women would predict limitations to LV filling with increased HR, which was confirmed in the present study. Both LV end-diastolic volume and stroke volume decreased more steeply as shortening of diastolic filling time appeared to compromise preload to greater degree in women. Our findings were consistent with the elegant work of Fu et al concerning orthostatic intolerance in younger women,[6] that demonstrated sex differences in the control of cardiac filling extend to young adults. Utilizing lower body negative pressure to lower LV preload, these investigators found that incremental decreases in SV were greater in healthy women compared to men. These investigators also observed a steeper rate of increase in HR, thought to be a compensatory reflex response to decreased cardiac filling and SV with unloading of arterial baroreceptors. We have demonstrated that the reciprocal relationship between HR with RA pacing and SV is also steeper in older women.

The coupling of higher ventricular to vascular elastance would predict increased sensitivity to preload changes and increased lability of blood pressure.[16] In younger adults, Fu et al[6] demonstrated that unloading contributed to hypotension observed in women subjected to orthostatic stress. However, significant changes in blood pressure were not observed in either men or women throughout the RA pacing protocol in the current study. Maintenance of stable blood pressure despite greater HR-mediated decline in SV indicated that the TPR response to RA pacing was also different between women and men. We speculate that baroreflex activation was greater in women related to the decline in both stroke volume and cardiac filling. A sympathetic mechanism may also contribute to our observation that the slope of the FFR was increased in women. It has been demonstrated in humans that the FFR can be modulated by the autonomic

nervous system such that the slope is augmented by sympathetic stimulation.[17] This mechanism merits further exploration as we have recently demonstrated evidence that cardiac-specific sympathetic nervous system activation is increased in women compared to men.[18]

Our findings have clinical implications. Although changes in HR alone are a limited mechanism by which to increase cardiac output,[10] our study shows this limitation is greater in women compared to men. In women, increases in HR were associated with a greater fall in external mechanical stroke work at the same time that augmented responses in contractility, and possibly myocardial oxygen demand, were observed. The counter-directional responses between work performed and contractility raises the possibility that myocardial efficiency may be more compromised by increases in HR in women. Sex differences in LV mechanical performance may thus explain increased vulnerability to hemodynamic instability and myocardial ischemia observed in women suffering acute myocardial infarction.[19;20] In this study, we attempted to avoid selection of patients with overt or a high likelihood of significant diastolic LV impairment, as in this patient population, compromised systolic and diastolic reserve in response to RA pacing is well established.[10;21] Our findings suggest this phenotype extends to women with normal filling pressures and may provide insight into the female predilection for this condition. Age related impairment of diastolic function and increased arterial stiffness affect proportionally more women[15] and would further disadvantage mechanical performance of the LV chamber. Our data suggests that the spectrum of women with the potential to demonstrate hemodynamic limitations is broad. Finally, it is important to note that hemodynamic phenotype we describe is likely overrepresented in, but not specific to, women.

A limitation inherent to studies of this nature is relatively small sample size dictated by the methodology. Our findings provide complementary mechanistic insights that may account for observations made by larger population-based analyses. It should be stated that caution is

required not to overinterpret our findings as reflective of intrinsic biologic sex differences. We did not study healthy volunteers, although the men and women who participated were representative of a typical cardiovascular outpatient clinic. There are limitations to the assessment of ventricular volume by 2D echocardiography particularly given the supine positioning of catheterization patients. We pursued pressure-volume analyses by a method which may be limited by several assumptions that are made regarding time-varying elastance within the cardiac cycle. Although a viable alternative was to utilize a conductance catheter,[10;22] the acquisition of LV waveform and measurement of stroke volume determined by Doppler-echocardiography are well suited to our multiple repeated measures design. Further, this method can be easily adapted for non-invasive application.[15]

In conclusion, we provide evidence that gender affects determinants of LV mechanical performance in an age group relevant to the onset of cardiovascular disease. Cardiovascular medicine often requires optimization of cardiac output by manipulation of heart rate, preload and/or contractile state and the potential response of sex-specific hemodynamic phenotypes should be considered. Our study provides support for ongoing initiatives to enroll sufficient numbers of men and women in cardiovascular clinical trials, particularly if sex-specific pathophysiology effects are suspected.

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## FIGURE LEGEND

**Figure 1. The force frequency relationship in women and men**

Left ventricular peak positive dP/dt ($LV+dP/dt_{max}$), LV $+dP/dt_{max}$/LV end diastolic volume (LVVed) and End systolic elastance (Ees) increase with incremental increases in heart rate by right atrial pacing. The slope of the force frequency relationship is steeper in women.

**Figure 2. Heart rate related changes in left ventricular volumes**

Changes in left ventricular end-systolic (LVVes), end-diastolic (LVVed) and stroke volume (SV) at resting heart rate and then right atrial pacing at 80, 100 and 120 bpm. The decrease in left ventricular end-diastolic volume is steeper in women yielding a trend to a steeper decline in stroke volume.

**Figure 3. Effect of heart rate of left ventricular pressure and volume**

Analysis of the left ventricular pressure waveform and stroke volume using assumptions of time-varying elastance yields 4 points on an approximated pressure volume loop. Left ventricular end-diastolic pressure (LVEDP), pressure during isovolumic systole or early contraction (Pec), pressure during ejection phase (Pej) and pressure at end-systole (Pes). The area inscribed within this approximated loop is an estimation of external mechanical stroke work (upper left panel). The decline in external work with increased HR is greater in women (upper right panel). The lower panels display the changes in external mechanical stroke work (EW) graphically. Approximated pressure volume loops are generated from the mean values for LVEDP, Pec, Pej, Pes and stroke volume for men and women at resting heart rate and right atrial pacing at 80, 100 and 120 bpm. See Table 4 for mean values and standard deviations of these measurements.

**Table 1. Baseline Characteristics**

| | Women | Men | P value |
|---|---|---|---|
| Age (y) | 60 ± 10 | 59 ± 4 | 0.66 |
| Height (cm) | 165 ± 7 | 175 ± 5 | 0.01 |
| Weight (kg) | 84 ± 22 | 77 ± 8 | 0.38 |
| Body surface area ($m^2$) | 1.9 ± 0.2 | 1.9 ± 0.1 | 0.91 |
| Hemoglobin (g/L) | 129 ± 14 | 149 ± 15 | 0.01 |
| Creatinine (mmol/L) | 70 ± 16 | 93 ± 24 | 0.04 |
| Est glomerular filtration rate (ml/min) | 80 ± 21 | 83 ± 21 | 0.75 |
| Testosterone (pmol/L) | 1.2 ± 0.6 | 10.6 ± 2.8 | <0.001 |
| Left ventricular mass ($g/m^2$) | 91 ± 37 | 98 ± 37 | 0.73 |

**Table 2. Resting Hemodynamic Measurements**

| | Resting Heart Rate | | | RA pacing 80 bpm | | |
|---|---|---|---|---|---|---|
| | **Women** | **Men** | **P value** | **Women** | **Men** | **P value** |
| Heart Rate (bpm) | 69 ± 12 | 65 ± 9 | 0.50 | 80 | 80 | |
| SAP (mmHg) | 129 ± 24 | 118 ± 17 | 0.29 | 130 ± 30 | 125 ± 15 | 0.67 |
| DAP (mmHg) | 61 ± 12 | 62 ± 7 | 0.75 | 65 ± 14 | 69 ± 7 | 0.49 |
| MAP (mmHg) | 88 ± 16 | 84 ± 10 | 0.54 | 92 ± 20 | 91 ± 10 | 0.89 |
| LVSP (mmHg) | 119 ± 20 | 107 ± 18 | 0.22 | 120 ± 24 | 112 ± 18 | 0.45 |
| LVEDP (mmHg) | 11 ± 6 | 9 ± 4 | 0.58 | 8 ± 6 | 8 ± 3 | 0.73 |
| LV+dP/$dt_{max}$ (mmHg/s) | 1465 ± 216 | 1301 ± 339 | 0.27 | 1581 ± 151 | 1437 ± 312 | 0.26 |
| Tau (ms) | 39 ± 8 | 43 ± 8 | 0.30 | 37 ± 8 | 39 ± 5 | 0.51 |

SAP-Systolic Arterial Pressure, DAP-Diastolic Arterial Pressure, MAP-Mean Arterial Pressure, LVSP-Left ventricular systolic pressure, LVEDP-Left ventricular end-diastolic pressure, LV+dP/$dt_{max}$-LV peak positive dP/dt

**Table 3. Frequency-dependent Effects on the Left Ventricular Pressure Waveform**

| RA Paced HR (bpm) | | Control + 10 | Control + 20 | Control + 30 | Control + 40 | Control + 50 |
|---|---|---|---|---|---|---|
| SAP (mmHg) | | | | | | |
| | Women | 130 ± 36 | 131 ± 31 | 131 ± 35 | 128 ± 38 | 125 ± 38 |
| | Men | 124 ± 17 | 124 ± 17 | 124 ± 15 | 124 ± 13 | 120 ± 18 |
| DAP (mmHg)* | | | | | | |
| | Women | 61 ± 15 | 64 ± 14 | 67 ± 16 | 68 ± 18 | 70 ± 19 |
| | Men | 66 ± 7 | 69 ± 7 | 72 ± 8 | 74 ± 7 | 74 ± 10 |
| MAP (mmHg)* | | | | | | |
| | Women | 90 ± 23 | 92 ± 21 | 94 ± 24 | 94 ± 26 | 94 ± 27 |
| | Men | 89 ± 10 | 91 ± 11 | 93 ± 11 | 94 ± 9 | 93 ± 14 |
| LVEDP (mmHg)* | | | | | | |
| | Women | 10 ± 6 | 8 ± 5 | 7 ± 5 | 6 ± 4 | 6 ± 4 |
| | Men | 9 ± 4 | 7 ± 4 | 5 ± 4 | 5 ± 3 | 5 ± 3 |
| LV $+dP/dt_{max}$ (mmHg/s)*† | | | | | | |
| | Women | 1508 ± 204 | 1664 ± 213 | 1756 ± 257 | 1894 ± 277 | 1851 ± 287 |
| | Men | 1377 ± 343 | 1445 ± 350 | 1511 ± 381 | 1571 ± 382 | 1609 ± 481 |
| LVSP (mmHg)* | | | | | | |
| | Women | 121 ± 26 | 121 ± 24 | 121 ± 27 | 118 ± 25 | 115 ± 25 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Men | 112 ± 18 | 113 ± 19 | 112 ± 17 | 111 ± 14 | 108 ± 20 |
| Tau (ms) *† | | | | | | |
| | Women | 38 ± 5 | 38 ± 7 | 38 ± 8 | 39 ± 9 | 46 ± 13 |
| | Men | 42 ± 8 | 40 ± 7 | 37 ± 7 | 36 ± 7 | 38 ± 10 |

*$p<0.001$ effect of heart rate,† $p<0.01$ interaction of sex and heart rate, 2W RM ANOVA
For abbreviations, see Table 2

**Table 4. Frequency-dependent Effects on Left Ventricular Pressure and Doppler-echocardiographic Measurements**

| RA Paced HR (bpm) | Control | 80 | 100 | 120 |
|---|---|---|---|---|
| LV +$dP/dt_{max}$ ($mmHgs^{-1}$)*† | | | | |
| Women | 1404 ± 207 | 1529 ± 172 | 1769 ± 203 | 1902 ± 268 |
| Men | 1327 ± 234 | 1460 ± 239 | 1529 ± 302 | 1602 ± 343 |
| LVVed (ml)* † | | | | |
| Women | 97 ± 17 | 90 ± 15 | 79 ± 16 | 62 ± 12 |
| Men | 97 ± 15 | 89 ± 17 | 84 ± 18 | 79 ± 6 |
| LVVes (ml)* | | | | |
| Women | 22 ± 9 | 22 ± 8 | 19 ± 9 | 16 ± 9 |
| Men | 30 ± 8 | 28 ± 9 | 29 ± 12 | 28 ± 19 |
| LV +$dP/dt_{max}$/LVVed* † | | | | |
| Women | 14 ± 2 | 17 ± 2 | 22 ± 3 | 29 ± 3 |
| Men | 14 ± 2 | 16 ± 2 | 17 ± 3 | 21 ± 4 |
| Stroke Volume (ml)* | | | | |
| Women | 75 ± 11 | 68 ± 11 | 59 ± 11 | 46 ± 9 |
| Men | 66 ± 13 | 60 ± 14 | 55 ± 14 | 51 ± 14 |
| Ejection Fraction (%) | | | | |
| Women | 78 ± 6 | 76 ± 7 | 76 ± 10 | 75 ± 11 |
| Men | 68 ± 8 | 68 ± 8 | 66 ± 10 | 65 ± 14 |
| Cardiac output ($Lmin^{-1}$) | | | | |
| Women | 5.3 ± 1.3 | 5.5 ± 1.2 | 5.9 ± 1.1 | 5.5 ± 1.0 |
| Men | 4.1 ± 0.8 | 4.8 ± 1.1 | 5.5 ± 1.4 | 6.2 ± 1.7 |
| Total peripheral resistance($dynescm^{-5}$) † | | | | |
| Women | 1537 ± 435 | 1546 ± 429 | 1456 ± 367 | 1352 ± 305 |
| Men | 1838 ± 329 | 1621 ± 331 | 1455 ± 286 | 1377 ± 388 |
| $P_{ej}$ (mmHg) | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Women | 52 ± 9 | 53 ± 10 | 55 ± 10 | 51 ± 9 |
| | Men | 53 ± 8 | 52 ± 8 | 54 ± 9 | 52 ± 13 |
| $P_{es}$ (mmHg)* | | | | | |
| | Women | 118 ± 22 | 123 ± 23 | 118 ± 21 | 103 ± 29 |
| | Men | 111 ± 19 | 108 ± 18 | 107 ± 19 | 104 ± 15 |
| Pre ejection period (ms) | | | | | |
| | Women | 34 ± 3 | 36 ± 3 | 34 ± 4 | 31 ± 6 |
| | Men | 44 ± 11 | 43 ± 10 | 45 ± 12 | 40 ± 12 |
| Ejection time (ms)* | | | | | |
| | Women | 307 ± 35 | 282 ± 24 | 249 ± 13 | 210 ± 14 |
| | Men | 297 ± 23 | 264 ± 17 | 237 ± 13 | 211 ± 11 |
| $E_{es}$ ($mmHgml^{-1}$)*† | | | | | |
| | Women | 2.90 ± 1.02 | 2.97 ± 0.97 | 3.67 ± 1.17 | 3.94 ± 0.54 |
| | Men | 2.24 ± 0.61 | 2.52 ± 0.63 | 2.55 ± 0.76 | 2.56 ± 1.16 |
| External Mechanical Stroke Work (mmHgml) | | | | | |
| | Women | 5918 ± 1538 | 5355 ± 1079 | 4461 ± 922 | 3346 ± 717 |
| | Men | 5833 ± 962 | 5292 ± 872 | 4876 ± 828 | 3995 ± 1074 |

*$p<0.001$ effect of heart rate,† $p<0.05$ interaction of sex and heart rate, 2W RM ANOVA
For abbreviations, see Table 2

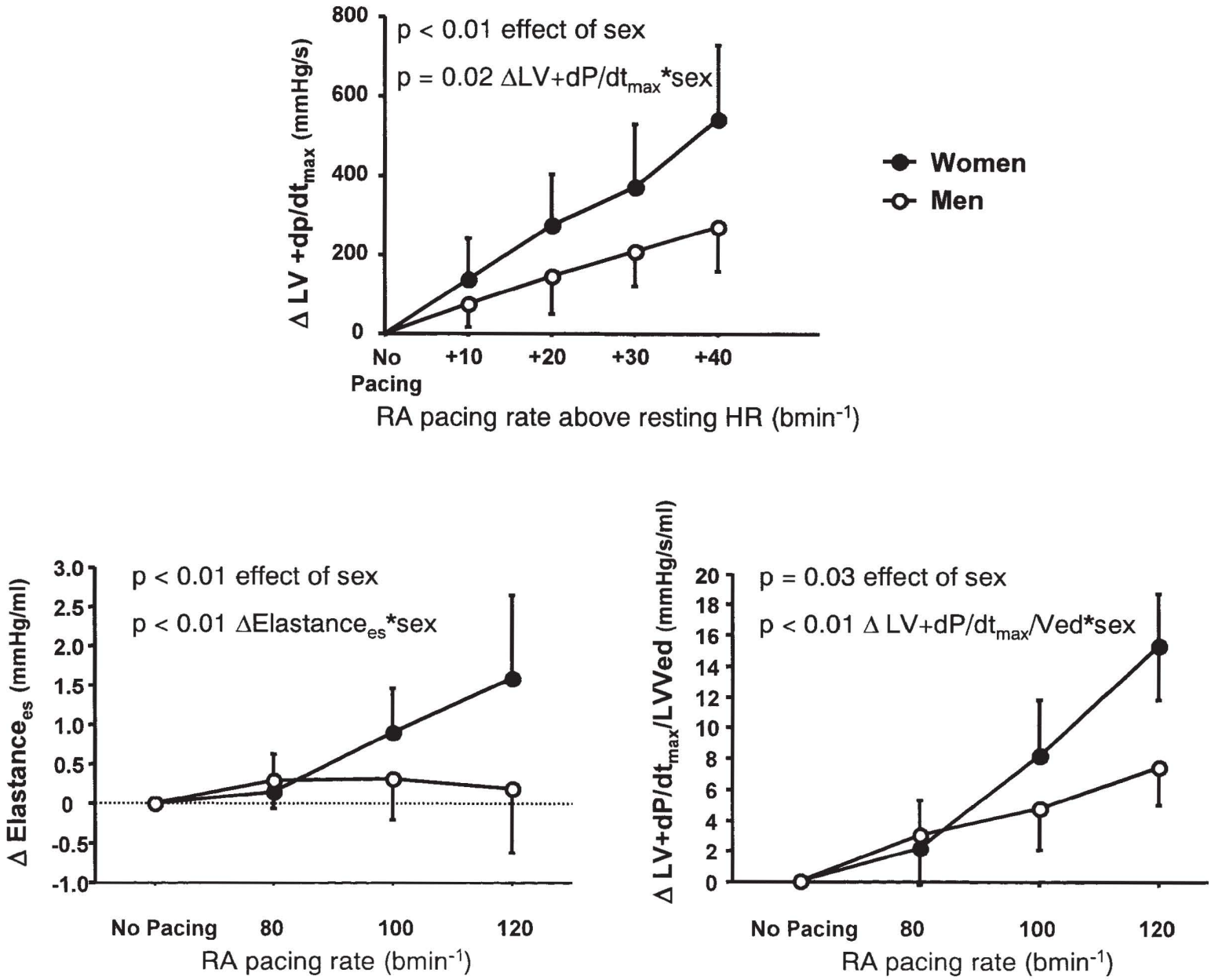
800
600
400
200
0
Δ LV +dp/dtmax (mmHg/s)
p < 0.01 effect of sex
p = 0.02 ΔLV+dP/dtmax*sex
Women
Men
No Pacing
+10
+20
+30
+40
RA pacing rate above resting HR (bmin-1)
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
-1.0
Δ Elastancees (mmHg/ml)
p < 0.01 effect of sex
p < 0.01 ΔElastancees*sex
No Pacing
80
100
120
RA pacing rate (bmin-1)
20
18
16
14
12
10
8
6
4
2
0
Δ LV+dP/dtmax/LVVed (mmHg/s/ml)
p = 0.03 effect of sex
p < 0.01 Δ LV+dP/dtmax/Ved*sex
No Pacing
80
100
120
RA pacing rate (bmin-1)

Figure 1

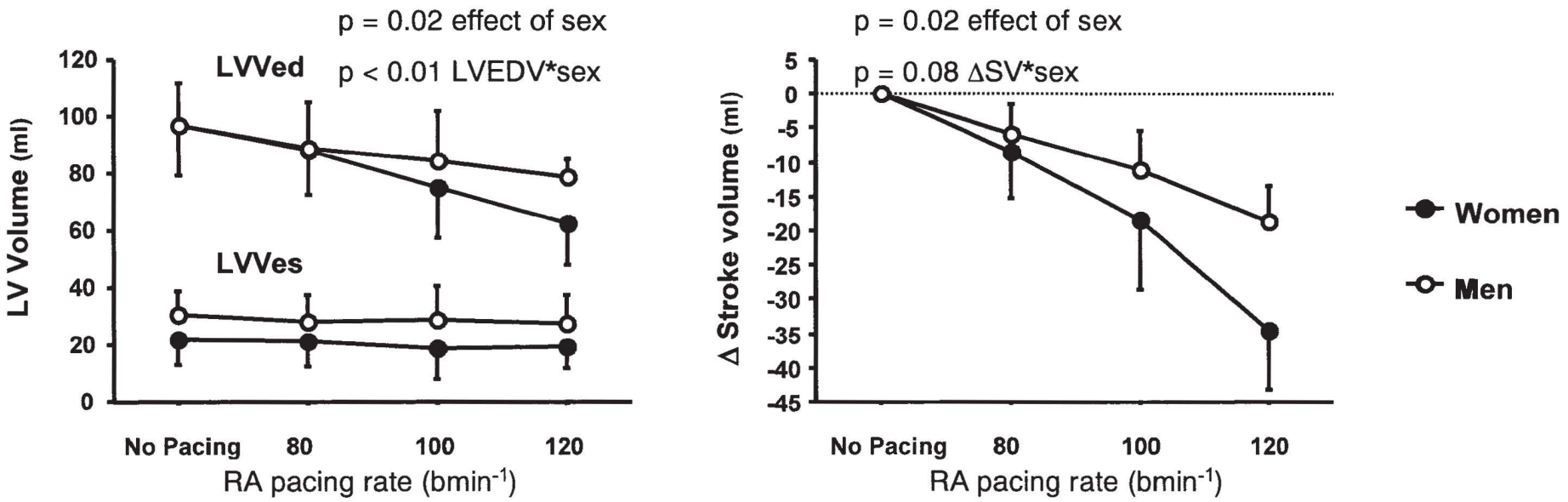
p = 0.02 effect of sex
p < 0.01 LVEDV*sex
LVVed
LVVes
LV Volume (ml)
120
100
80
60
40
20
0
No Pacing
80
100
120
RA pacing rate (bmin-1)
p = 0.02 effect of sex
p = 0.08 ΔSV*sex
Δ Stroke volume (ml)
5
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
No Pacing
80
100
120
RA pacing rate (bmin-1)
Women
Men

Figure 2

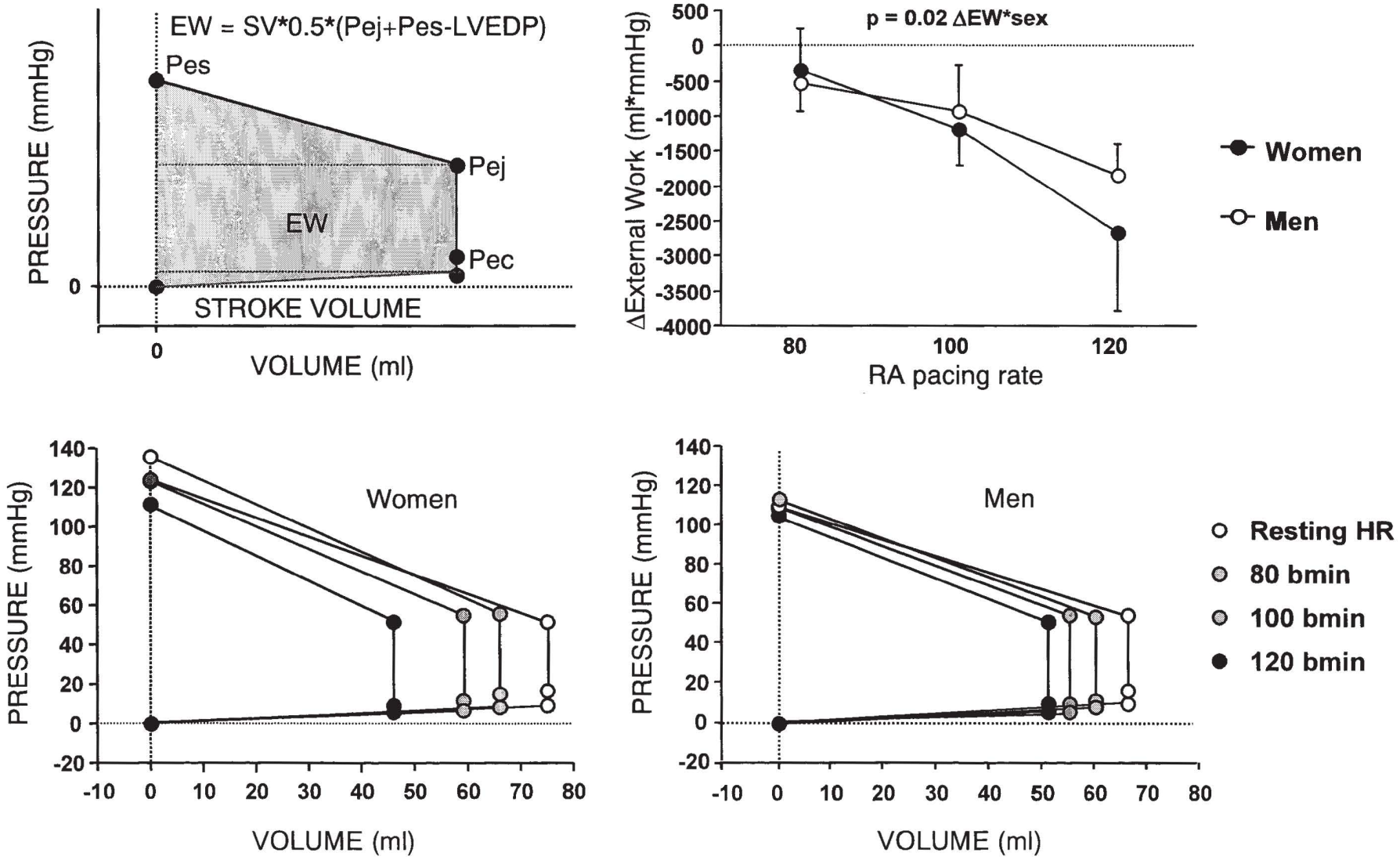
EW = SV*0.5*(Pej+Pes-LVEDP)
Pes
Pej
EW
Pec
STROKE VOLUME
PRESSURE (mmHg)
0
0
VOLUME (ml)
p = 0.02 ΔEW*sex
ΔExternal Work (ml*mmHg)
500
0
-500
-1000
-1500
-2000
-2500
-3000
-3500
-4000
80
100
120
RA pacing rate
Women
Men
Women
PRESSURE (mmHg)
140
120
100
80
60
40
20
0
-20
-10
0
10
20
30
40
50
60
70
80
VOLUME (ml)
Men
PRESSURE (mmHg)
VOLUME (ml)
Resting HR
80 bmin
100 bmin
120 bmin

Figure 3

# TWO DIMENSIONAL ECHOCARDIOGRAPHIC DEFORMATION IMAGING AND STIMULATED LEFT VENTRICULAR FUNCTION IN HUMANS WITH AND WITHOUT HEART FAILURE: Insights from the Force Frequency Relationship

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**ABSTRACT**

We examined indices of systolic LV performance derived from two dimensional speckle tracking analysis of longitudinal strain and high fidelity recordings of LV pressure acquired simultaneously in patients undergoing cardiac catheterization. Measurements were performed at rest and in response to increasing heart rate (HR) by right atrial pacing, the force frequency relationship.

Twenty-five patients were recruited for this study, 13 with normal LV function and 12 with systolic heart failure (HF) (LV ejection fraction 31 ± 13%). At rest, HF patients had impaired isovolumic contractility [peak positive LV dP/dt (LV+dP/dtmax) 1345 ± 286 mmHg/s normal LV function versus 970 ± 178 mmHg/s HF, $p<0.001$)] and lower absolute values of peak longitudinal strain (17.5 ± 3.9% normal LV function versus 9.1 ± 5.3% HF $p<0.001$) and strain rate (1.2 ± 0.3%/s normal LV function versus 0.9 ± 02%/s HF, $p<0.01$). In response to incremental RA pacing, LV+dP/dtmax increased significantly in both groups (Normal LV group, no pacing, 80 and 100 bpm; 1335 ± 296, 1437 ± 264 and 1564 ± 320 mmHg/s $p<0.0001$ and HF group, no pacing and 100 bpm; 960 ± 207 and 1048 ± 223 mmHg/s $p<0.01$). In contrast, peak longitudinal strain decreased significantly with incremental RA pacing (Normal LV group, no pacing, 80 and 100bpm 18.0 ± 3.5, 13.2 ± 6.3 and 10.8 ± 6.0% $p<0.001$ and HF group 9.9 ± 3.7 and 6.9 ± 3.8% $p<0.01$) while strain rate did not change significantly. The decrease in longitudinal strain was related to the HR-related decrease in LV end-diastolic dimensions. Longitudinal strain and strain rate as assessed by two dimensional speckle tracking did not reflect changes in contractility within an in vivo model of stimulated LV function.

## INTRODUCTION

The evolution of deformation imaging by two-dimensional echocardiography has expanded the methodology for non-invasive assessment of left ventricular (LV) chamber function.[1] Strain and strain rate can be measured by tissue Doppler or speckle-tracking to evaluate systolic function of the LV chamber both globally as well as regionally. Assessment of regional myocardial deformation may provide increased sensitivity to discriminate abnormal LV chamber performance. Moreover to evaluate LV function with stress, strain and strain rate have been examined as potentially valuable endpoints as they provide reproducible, repeatable, regional quantitative information.[2]

At rest and in response to stimuli such as exercise or adrenergic agonists, analysis of ventricular wall motion is employed to represent global and regional contractile responses. Contractility is an important determinant of systolic LV performance describing the inotropic state of the heart.[3] Accurate measurement of ventricular contractility in vivo remains an important achievement and reflects myocardial contractility, the fundamental property of heart muscle that reflects the intensity of cross bridge activity and fibre shortening. Accepted methods for assessment of global LV contractility require cardiac catheterization and high fidelity recordings of LV pressure with or without the measurement of LV volume.[4;5]

Non-invasively, systolic LV function is often assessed after opening of the aortic valve during ejection, which is subject to modulation by both preload and afterload.[3;6] As such, indices such as ejection fraction or systolic wall stress are referred to as load dependent. Although myocardial and ventricular deformation also occurs in part during ejection, it has been hypothesized that strain and strain rate may be less load-dependent, sufficient to index ventricular contractility non-invasively.[7-9] We examined indices of systolic LV performance

including deformation imaging and isovolumic contractility, measured simultaneously, in patients undergoing cardiac catheterization. We examined these indices in a range of patients with and without heart failure (HF) due to LV systolic dysfunction, and assessed the acute effect of increasing heart rate (HR), a well-understood modest inotropic stimulus, the force frequency relationship.

## METHODS

### Patient Selection

This prospective study had the overall purpose of comparing LV function and force frequency relationships in women and men with normal LV function as well as in a population with systolic HF. As such, we attempted to balance the number of men and women participating in each group. The sex-based analysis is presented elsewhere. Patients referred for assessment of a chronic, mostly atypical, chest pain syndromes were recruited into the Normal LV function group if normal LV systolic function was documented by two dimensional echocardiography. Symptoms of a HF syndrome, i.e. HF with preserved ejection fraction, were specific exclusion criteria for patients in this group. Patients in the HF group were recruited from a specialized HF clinic and underwent catheterization as part of the clinical evaluation of HF. Inclusion into this group included stable NYHA class 2 or 3 symptoms and an LV ejection fraction (LVEF) < 35% documented either by an echocardiogram or radionuclide ventriculogram. Measurement of LVEF occurred within 3 months of the catheterization and was confirmed on the day of the study. Patients with HF on the basis of valvular heart disease or acute ischemia were specifically excluded. In both the NLV and HF groups, patients with acute coronary syndromes, myocardial infarction, or coronary revascularization within 6 months, or patients with severe triple vessel CAD were specifically excluded. Women of childbearing potential were also excluded.

This protocol was approved by the University of Toronto Ethics Review Committee for experimentation involving human subjects. All patients gave written informed consent.

### Cardiac Catheterization Procedures

Diagnostic cardiac catheterization from the femoral approach and subsequent study procedures were performed at the Cardiac Catheterization Research Laboratory at the Mount

Sinai Hospital. Prior to catheterization, venous blood was sampled for measurements of hemoglobin, creatinine and estimated glomerular filtration rate. Patients were minimally sedated, fasting and all medications were withheld on the morning of the procedure. These protocols are well-established within our laboratory.[10] To control HR, a 6 Fr bipolar pacing catheter was advanced to the high right atrium from the right femoral vein under fluoroscopic guidance. The pacemaker catheter was aligned with a programmable stimulator (Prucka GE CardioLab). A 7 Fr micromanometer-tipped catheter was then positioned in the LV from the femoral artery. Femoral artery pressure was recorded from the sidearm of the sheath. The LV pressure and the first derivative of LV pressure are continuously displayed and acquired (1000Hz) on line, allowing serial sampling of LV+dP/$dt_{max}$. Control measurements prior to intervention are recorded only after 3 serial sampling intervals demonstrate LV+dP/$dt_{max}$ values within 5%.

**Echocardiographic image acquisition**

Two-dimensional echocardiographic images and Doppler acquisition was performed using a GE Vivid 7 Imaging System, M4S Matrix Sector Array Probe (2-5 MHz) by a research echocardiography technician who did not have knowledge of the clinical history or hemodynamics. Patients were imaged in the supine position. The chronometers for both the imaging system and the hemodynamic recording system were synchronized for offline analysis of simultaneous hemodynamic recordings and timestamped echocardiographic image frames. Analysis of imaging data was performed on an offline EchoPac workstation (GE Healthcare, Canada).

**Pacing Protocol**

After instrumentation and determination of the threshold for the pacing catheter, patients were instructed to maintain quiet tidal respiration and a 10 minute rest period was allotted. Prior

to pacing, for the measurement of SV, echocardiographic imaging is employed to determine the LV outflow tract (LVOT) dimension and cross-sectional area (CSA) calculated at $(LVOT/2)^2$ x 3.14. The pulsed-wave Doppler aortic flow spectrum was then obtained in the apical five chamber view and transducer placement was marked on the chest wall for repeated acquisition. If possible, 4 HR conditions were then studied, resting HR, then RA pacing at 80, 100, and 120 bpm (CL 750, 600 and 500ms). If resting HR was >75 bpm, pacing at 80bpm was omitted. If Mobitz type 1 or 2:1 AV block occurred at any pacing condition, the pacing rate was reduced by increments of 5 bpm until 1:1 conduction was reestablished for 30s; RA pacing at the prespecified rate was then reattempted.

Within the first minute of each HR condition, stability of RA pacing capture was established. The continuous hemodynamic recording was annotated at the beginning of the second minute of each HR condition for the acquisition of femoral arterial pressure and the LV pressure waveform inscribed by the micromanometer-tippled catheter. Within the $2^{nd}$ to $5^{th}$ minutes of each HR condition, standard grayscale 2D images were acquired in the apical 4-chamber view at 60-90 fps. The images were stored in cineloop format from 3-5 beats, for subsequent speckle tracking analysis, as well as end-systolic and end-diastolic LV volumes using standard echo techniques. Pulsed-wave Doppler aortic flow spectrum was also re-established, and further simultaneous acquisition of the TVI and LV pressure over 3-5 consecutive beats was performed. No pacing condition exceeded 5 minutes.

**Data Analysis**

For each HR condition, the annotated interval for analysis was interrogated off-line by the research assistant in our laboratory, who was blinded to the characteristics of the patient and purpose of the study. Per beat of the LV pressure recording, $LV+dP/dt_{max}$, peak LV systolic

pressure (LVSP), end-diastolic pressure (LVEDP) and the time constant of isovolumic relaxation (tau, t) by the logarithmic method and pressure half time method were calculated as previously reported in our laboratory. Contractility was also expressed as LV+dP/dtmax normalized to Ved, a less load-sensitive index of inotropic state. Arterial pressures (systolic/diastolic/mean, SAP/DAP/MAP) were analysed from the femoral arterial recording. For each beat, the preceding RR interval was recorded. If the preceding RR interval was not within 2% of the planned pacing cycle length, the specific beat and the subsequent 5 beats were discarded.

LV longitudinal strain as assessed by speckle tracking was analysed from the stored apical 4-chamber images. For each heart rate, the best quality image was selected and the endocardium was traced. The region of interest was accepted for analysis after adequacy of the tracing for speckle-tracking was determined by an automated tracking quality system. The ventricular chamber was divided into 6 segments (apical/mid/basal septum and apical/mid/basal lateral wall) and 6 segmental strain curves were analysed. Peak segmental and global longitudinal systolic strain measurements, reported as absolute values, were determined from these curves. Stored images and Doppler measurements were also used to calculate SV, end-diastolic and end-systolic volumes. Ejection fraction was calculated using modified Simpson's method. The time-stamp from echocardiographic frames identified the cardiac cycle from which to obtain simultaneous LV and arterial pressure. Hemodynamic measurements were not significantly different when derived from the single cardiac cycle, or taken as a mean of 10 cycles either preceding or following the beat analysed for echocardiographic variables.

Per beat, 3 timed intervals within the LV pressure waveform are determined based on the following definitions: 1) early isovolumic contraction, $t_{ec}$: point at which +dP/dt exceeds 30% of $+dP/dt_{max}$, 2) transition from isovolumic contraction to ejection, $t_{ej}$: point at which $+dP/dt_{max}$ occurs

and 3) end-systole $t_{es}$: point at which –dP/dt exceeds 20% of $-dP/dt_{min}$. ET is the ejection time and is calculated as $t_{es}$-$t_{ej}$. The corresponding LV pressures at these timepoints $P_{ec}$, $P_{ej}$, and $P_{es}$ can then also be determined. Simultaneous acquisition the Doppler-derived stroke volume allows the generation of approximated pressure volume loops.[11] External stroke work (ml*mmHg) can then be calculated from the area of the loop as SV*0.5*(Pej+Pes-LVEDP).

**Statistical Analysis**

All data are presented as mean ± SEM. Analysis was performed with a statistical software package (Statview). Between group comparisons of baseline characteristics were made with a Student t-test for continuous variables and Chi square analysis for categorical variables. Responses to changes in HR within groups were analysed using a repeated measures ANOVA. Where applicable, post hoc pairwise comparisons were then performed with Bonaferroni-Dunn tests. Relationships between indices of deformation and other echocardiographic and hemodynamic variables were examined using linear regression analysis. Variables related to changes in deformation by HR were similarly examined. Variables demonstrating significant correlations with indices of deformation were selected to enter into stepwise regression. A p value of less than 0.05 was required for statistical significance.

**RESULTS**

**Study Groups**

Twenty-five patients were recruited for this study, 13 in the normal LV function group (7 men, 6 women) and 12 in the HF group (7 men, 5 women). The baseline characteristics of the study patients, comorbid conditions and medical therapy are described in Table 1. Patients in both groups were similar with respect to age, body size, hemoglobin and measures of renal function. The majority of patients in the HF group were treated with angiotensin receptor

blocking agents, adrenergic receptor antagonists and diuretics more frequently then the Normal LV Function group. Hemodynamic and echocardiographic measurements at rest are presented in Table 2. As expected, patients with HF demonstrated impairment of LV isovolumic contractility as measured by LV+dP/$dt_{max}$ and significantly higher filling pressures. LV chamber enlargement and impaired LVEF was also evident in the HF group. Global peak longitudinal systolic strain and peak strain rate were significantly depressed in the HF group.

**Right Atrial Pacing and LV Contractility: The Force Frequency Relationship**

Stable pacing and adequate image acquisition was obtained from 12 NLV function patients at control, RA pacing at 80bpm and 100bpm, and 9 HF patients at control and RA pacing at 100bpm. The RA pacing condition at 80bpm was omitted in the HF group, as resting heart rate in all except one patient ranged from 72bpm to 87 bpm. Patients in both groups exhibited typical inotropic and hemodynamic responses to increases in HR, presented in Table 3. In patients with normal LV function, the Treppe or positive staircase effect was clearly demonstrated with a significant increase in LV+dP/dtmax as RA pacing rate increased from control to 80 and 100bpm. The increase in LV+dP/dtmax was observed despite a significant fall in LVEDP as well as LV end-diastolic and stroke volume. Although stroke volume decreased, the effect on CO was offset by the increase in HR, such that CO increased. Per beat, stroke work decreases significantly, mostly attributable to the fall in stroke volume. Ejection time falls significantly in response to increasing HR.

In HF patients, the typical response to increases in HR was again observed. A significant increase in LV+dP/dtmax was observed as RA pacing rate increased from 80 to 100bpm, although the incremental increase was apparently attenuated when contrasted to patients with normal LV function. HF patients demonstrated a trend to decreases in LVEDP, although in

contrast to the normal LV function patients, there was little change in LV end-diastolic volume and a modest non-significant fall in stroke volume. Similar to the normal LV function group, ejection time decreased significantly. Stroke work also tended to decrease with increases in HR, although again, the net effect of HR was to yield an increase in CO.

**Right Atrial Pacing and Global and Regional Longitudinal Strain and Strain Rate**

For each patient who underwent RA pacing, regional analysis of six segments of the LV chamber were performed at 3 HR conditions in patients with normal LV function (12) and 2 HR conditions in HF patients (9) and thus a total of 336 segments were available for analysis. In total, 2.4% were unsatisfactory for analysis, 5.4% of basal septum, 1.8% of the mid septum, apical septum, apical lateral and mid lateral segments, and 3.6% of basal lateral segments.

The effect of HR on longitudinal strain and strain rate are presented in Table 3. With increases in HR due to RA pacing, the quantity of peak systolic strain moved in the opposite direction of LV+dP/dtmax and decreased significantly in both patients with normal LV function and HF patients. Figure 1 contrasts the effect of incremental RA pacing to increase LV+dP/dtmax with the decline in stroke work and longitudinal strain in both the HF and Normal LV Function groups. In both groups, there were not significant changes in peak strain rate with increasing RA pacing rate despite the increase in LV+dP/dtmax. Ejection fraction tended to decrease in both groups with increases in RA pacing rate, but the changes did not reach statistical significance. The decrease in longitudinal strain was apparent when analyzed as a global measurement, but also as regional segments (Figure 2). In patients with normal LV function, the decrease in strain with increases in HR reached statistical significance in all segments analysed. In HF patients, the decrease in strain was also apparent in all segments analysed, and reached

statistical significance in all segments except apical septal, apical lateral and basal lateral segments.

**Measurements of Deformation and Indices of LV function**

Several measurements of LV function were related to longitudinal strain and strain rate over the range of HRs tested in the two study groups (Table 4). LV+dP/dtmax and ejection time were significantly correlated with longitudinal strain, while LVEDP, stroke volume, end-diastolic and end systolic volume were inversely related to longitudinal strain. Stepwise regression demonstrated that ejection time, stroke volume and end-systolic volume maintained significant independent relationships to peak systolic strain. LV +dP/dtmax was significantly related to longitudinal strain rate, while LVEDP, end-diastolic volume and end systolic volume were inversely related to longitudinal strain rate. Stepwise regression demonstrated that end-systolic volume remained independently related to longitudinal strain rate.

**Determinants of the Change in Longitudinal Strain with Changes in Heart Rate**

Although longitudinal strain was correlated with LV+dP/dtmax, it was apparent this quantity did not index the acute inotropic effect of increased HR or force frequency relationship. We attempted to identify the factors related to the systematic HR-related decrease in peak longitudinal strain observed. As others have demonstrated, increased HR was associated with a decrease in Ved without significant changes in Ves, particularly in the Normal LV Function group. Analysis of speckle tracking yields the assessment of Lagrangian stain, or the change in length expressed as a percentage of the initial length between two locations within the myocardium. The Lagrangian strain relationship would predict that a decrease in initial length without change in the end-systolic length will result in a decrease in the quantity of strain. The decline in strain values should be related to a term defined by the change in end-diastolic

dimension (ΔVed) expressed as a proportion of the new end-diastolic dimension (Ved –ΔVed) (see Figure 4). Patients with NLV function underwent assessment at 2 pacing increments and HF patients at 1 pacing increment. Analysis of changes in strain and calculation of the term ΔVed / (Ved –ΔVed) was possible for 32 pacing increments in our patient population. The relationships between the change in other variables related to strain and strain rate were also examined (Table 5). As predicted, a linear relationship was present between the HR related decline in strain and the calculated term reflecting the change in end-diastolic LV dimension (Figure 4). The change in ejection time with HR was the only other variable significantly related to the decrease in longitudinal strain (Figure 4).

**DISCUSSION**

The measurement of strain and strain rate have generated interest as novel and improved non-invasive indices of LV function with clinical relevance.[1;2] The current study demonstrates that, while correlating with other measures of LV function, in humans with and without HF, deformation imaging as an index to assess contractile responses to stimulated LV function may be confounded.

The force frequency relationship is an intrinsic property of normal cardiac muscle by which peak tension is enhanced by increasing stimulation frequency, the Treppe or positive staircase effect.[12] The mechanism is related primarily to increased $Ca^{2+}$ availability to the myofilaments, and experimentally the FFR has been described in humans as an in vivo index of $Ca^{2+}$ handling.[13-15] In humans with normal LV function, RA pacing rates up to 140-150 bpm increases LV+dP/dtmax by approximately 30%.[15-17] As reproduced in our study, this occurs with little change in LV systolic pressure and despite a decrease in the LVEDP and stroke volume, which would tend to oppose increases in LV+dP/dtmax. Thus LV+dP/dtmax still indexed

frequency-dependant increases in contractility even though the measure is load sensitive as predicted by the Frank Starling relationship. Frequency-dependent increases in contractility was also demonstrated with the less load-dependent measure of LV+dP/dtmax/Ved and the end-systolic pressure volume relationship.[16]

Increases in HR are a modest stimulus to contractility, and the overall effect on LV systolic performance is also modified by shortened diastolic filling period and a fall in preload.[15-17] This was most apparent in the normal LV function group in whom end-diastolic volume decreased with increases in HR. As others have shown, end-systolic volumes in this population did not change significantly with increased HR and as such, stroke volume and stroke work decrease despite increased contractility. Therefore, as a stimulus to LV systolic function, HR is associated with a divergent response between pre-load dependent indices of ejection (stroke volume and stroke work) and both load dependent and independent indices of contractility (LV+dP/dtmax, LV+dP/dtmax/Ved and end-systolic elastance). In this study, it was apparent that longitudinal strain analyzed by two dimensional speckle tracking as a measure of LV systolic performance did not gauge the acute inotropic effect of increased HR. Both globally and regionally, the quantity of strain appeared to decrease systematically in response to increased HR, and was predicted by the change in ejection time as well as a term defined by the decrease in end-diastolic dimension with HR. In our cohort, the changes in contractility were not reflected in longitudinal strain rate either, as this quantity did not change with HR.

The potential modulation of strain by load has been acknowledged.[18] Our findings with respect to longitudinal strain are very consistent with preclinical studies.[19-21] Weideman et al[19] performed tissue-doppler strain and strain rate imaging at the same time as acquiring micromanometer-tipped catheter measurements in anesthetized pigs. HR manipulation was

performed with RA pacing and effects of both dobutamine and esmolol infusion were also examined. As in our study, stroke volume decreased significantly with HR, driven mostly by a fall in end-diastolic dimensions without change in end-systolic dimensions. The fall in stroke volume was closely related to the decrease in radial ventricular strain. The authors concluded that strain rate may be a good index of regional contractile function based on its ability to index the positive and negative inotropic effects of dobutamine and esmolol respectively. Rosner et al[20] reproduced the findings of the Weideman study in another pig study, showing a significant decline in longitudinal strain values with no change in strain rate in response to incremental RA pacing. These investigators also showed that while the effect on strain within subjects was likely related to decreases in LV filling at higher heart rates, differences in left ventricular size between subjects also influenced the measurement of strain and strain rate. Despite similar contractile function, increasing LV dimensions were related to smaller values for strain and strain rate.

Several animal studies have demonstrated that strain rate may serve as a good index of contractility.[19-22] It has been acknowledged that strain rate remains sensitive to loading conditions, as would be expected for an index of deformation without consideration of myocardial stress. Nevertheless, studies performed in muscle strips as well as in vivo have shown strain rate closely tracked changes in contractility as assessed by high fidelity catheter measures of either isovolumic contractility or the end-systolic pressure-volume relationship in response to the positive and negative inotropic effect of dobutamine and esmolol respectively. [19-22] The effects of changes in HR on strain rate were studied by both Weideman[19] and Rosner.[20] In these pig models, at the RA pacing increments studied, changes in heart rate were not associated with significant changes in strain rate, leading the authors to conclude that strain rate is a HR-independent index of contractility. Unlike humans, in these animals no frequency-dependent

increases in LV+dP/$dt_{max}$ or other measures of contractility were observed either. As discussed, in humans, the net inotropic effect of HR is the sum of the positive effects of increased intracellular $Ca^{2+}$ that is only partially offset by the negative effect of decreased LV filling. Within human subjects in our study, no significant changes in strain rate were observed despite demonstrating a force frequency relationship with the expected increase in LV+dP/dtmax. Thus, the measurement of longitudinal strain rate did not appear sufficiently sensitive to detect the modest HR-related increase in +LVdP/dt observed in humans.

The current study is among a smaller number of human investigations examining the effects of specific acute loading manipulations on strain and strain rate. Andersen et al[23] examined the effect of sublingual nitroglycerin and the Trendlenburg position on indices of longitudinal deformation measured by tissue Doppler in healthy adults. The investigators suggested that strain was not a load-sensitive measure of LV systolic function, as it did not change significantly in response to the interventions. However, comparative indices of ventricular performance were not obtained in this study. Burns et al[24] also examined strain and strain rate in response to glyceryl trinitrate and saline loading in an invasive study in which high fidelity LV pressure measurements were also obtained. In contrast to the previous study, these investigators showed that circumferential strain and strain rate were affected by the loading interventions. LV+dP/dt, strain, and strain rate were augmented by the nitrate intervention, although unexpectedly, saline loading appeared to depress LV+dP/dtmax. Strain rate in particular appeared to diminish in a linear fashion with increases in end-diastolic pressure in this study, contrary to what would be predicted based on the Frank-Starling relationship. The authors suggested this observation was likely related to the use of nitrates to decrease preload, an intervention which would also decrease afterload. Finally, Boettler et al[25] demonstrated that

decreases in HR and ejection time in children during growth similarly impact the measurement of myocardial strain and values increased with increasing age. Strain rate, however, did not change. The augmentation of strain values during growth is observed despite the increase in LV dimension which may tend to depress strain values as noted.

Our findings have implications for the interpretation of deformation imaging in diagnostic procedures dependent on LV function stimulated by interventions associated with an increase in HR. Dobutamine stress echocardiography and particularly exercise echocardiography merit consideration in this regard. Strain and strain rate have been examined as valuable endpoints as they may provide reproducible, repeatable, regional quantitative information.[2] Both of these interventions exert chronotropic effects, but additionally decrease end-systolic volume and increase stroke work and stroke volume by substantial increases in inotropic state, as well as afterload reduction and ventricular filling via enhanced diastolic relaxation.[26-28] Thus, unlike heart rate, both exercise and dobutamine yield measurable increases in myocardial strain and strain rate.[19-22;29] However, our findings suggest that strain and strain rate may be impacted in the opposite direction based on the relative changes in end-diastolic and end-systolic dimensions. The resultant quantity would thus reflect the variable balance between inotropic and chronotropic effects and may misrepresent the contractile response of the myocardium to stimulation. This does not negate the utility of strain imaging for the assessment of stimulated LV chamber function, particularly for identification of impaired regional stroke work responses provoked by stress. In our study, strain reflected the HR-related change in stroke work more accurately than the measurement of LVEF, which did not change significantly with RA pacing.

We had the opportunity to examine resting and stimulated LV function in HF patients. Including patients with and without HF demonstrated that strain and strain rate serve as useful

descriptors of LV function, clearly discriminating normal from abnormal myocardium.[1] Between the two groups studied, strain and strain rate were directly related to other measures of ventricular function, including ejection phase indices such as LVEF or stroke work, as well as the measurement of isovolumic contractility, LV+dP/dtmax. However, the current study and work of others suggest that interpretation of deformation imaging as an index of myocardial function may be complex in HF. Values for strain and strain rate may be negatively impacted simply by higher resting HRs in this patient population.[25] Similarly, the demonstration that LV dimension[20] is inversely related to values for strain and strain rate, independent of contractility, may also be confounding. We observed a systematic HR-related decline in longitudinal strain values in this population, similar to the Normal LV function group. However, the mechanisms by which the quantity of strain decreased with RA pacing was less evident in the HF population. Interpretation was more limited in the HF group which was smaller, and underwent only one pacing increment. The decrease in strain was not adequately explained by changes in LV volumes, which did not change significantly in response to changes in HR. Similar to the Normal LV Function group, the modest increase in LV+dP/dt was also not reflected in the measurement of longitudinal strain rate. Thus, although deformation imaging may be of potential interest in the assessment of contractile reserve in the HF population, interpretation may be confounded by factors that are currently poorly understood.

Some limitations of this study merit discussion. Acquisition of echocardiographic images was limited to the supine position during the simultaneous catheterization procedure. Our findings appeared linked to HR-related changes in end-diastolic and end-systolic volumes, for which there are limitations when assessed by 2D echocardiography. However, our observations of the effect of RA pacing on LV volumes and hemodynamics replicate the findings of several

investigations using different methodologies [15-17] We employed high fidelity recordings of LV pressure which are highly reproducible and are sensitive to small changes in LV inotropic state.[30;31] However, LV+dP/dtmax is itself load-dependent and affected by decreases in LV end-diastolic volume.[30] Although a combined high fidelity and conductance catheter could have been employed for analysis of the end-systolic pressure-volume relationship, LV+dP/dtmax adequately describes the force frequency relationship despite load sensitivity. In other words, in characterizing the force frequency relationship, unlike longitudinal strain, LV+dP/dtmax is not confounded by load-dependence. As shown by animal studies,[19-22] strain rate be more representative of ventricular contractility than the measurement of strain. In our study however, strain rate did not change significantly with HR. Strain rate may be less sensitive to changes in contractility that LV+dP/dt and it is likely that we lacked power to demonstrate HR-related differences in strain rate.

In conclusion, in patients with and without HF, we demonstrated that longitudinal strain and strain rate as assessed by two dimensional speckle tracking did not reflect changes in contractility within an in vivo model of stimulated LV function, the force frequency relationship. The loading changes induced by incremental RA-pacing appear to confound the measurement of strain as an index of contractility in this setting of moderate stimulation of LV function. The measurement of strain rate did not appear sensitive enough to detect modest changes in contractility. These findings bear consideration in the use of strain imaging as an index of dynamic LV systolic performance, particularly in the setting of interventions that have significant chronotropic or other loading effects. This study reinforces that strain and strain rate should not be presented as indices of contractility, but considered a parameter of LV performance in an integrated load dependent system.

**TABLE 1: Baseline Characteristics**

| | **Normal LV Function (n=13)** | **Heart Failure (n=12)** | **p-value** |
|---|---|---|---|
| Age (y) | 59 ± 8 | 56 ± 11 | 0.54 |
| Height (cm) | 172 ± 8 | 171 ± 8 | 0.67 |
| Weight (kg) | 79 ± 14 | 79 ± 21 | 0.99 |
| BSA ($m^2$) | 1.91 ± 0.17 | 1.91 ± 0.27 | 0.92 |
| Hemoglobin (g/L) | 139 ± 17 | 143 ± 13 | 0.59 |
| Cholesterol (mmol/L) | 5.3 ± 1.5 | 4.9 ± 1.0 | 0.57 |
| eGFR | 81 ± 22 | 79 ± 25 | 0.81 |
| Creatinine (μmol/L) | 84 ± 25 | 88 ± 22 | 0.65 |
| Hypertension (n) | 6 | 4 | 0.51 |
| Diabetes mellitus | 2 | 4 | 0.29 |
| Hypercholesterolemia | 8 | 1 | 0.005 |
| Coronary disease | 1 | 3 | 0.49 |
| Adrenergic Antagonists | 4 | 10 | 0.008 |
| Angiotensin system antagonists | 5 | 10 | 0.008 |
| Diuretics | 2 | 8 | 0.009 |
| Digoxin | 0 | 3 | 0.05 |

**TABLE 2. Hemodynamic and Echocardiographic Measurements**

| | Normal LV Function | Heart Failure | p value |
|---|---|---|---|
| Heart rate (bpm) | 66 ± 11 | 76 ± 10 | 0.03 |
| Mean arterial pressure (mmHg) | 94 ± 17 | 95 ± 17 | 0.88 |
| LV end-diastolic pressure (mmHg) | 9 ± 4 | 16 ± 8 | 0.02 |
| LV +dP/dtmax (mmHgs) | 1348 ± 286 | 970 ± 178 | <0.001 |
| End systolic volume (ml) | 26 ± 9 | 120 ± 58 | <0.0001 |
| End diastolic volume (ml) | 96 ± 16 | 168 ± 69 | <0.001 |
| Stroke volume (ml) | 70 ± 13 | 49 ± 25 | 0.02 |
| Ejection fraction (%) | 73 ± 8 | 31 ± 13 | <0.0001 |
| Cardiac Output (L/min) | 4.6 ± 1.2 | 3.5 ± 1.9 | 0.12 |
| Stroke Work (ml*mmHg) | 5678 ± 1414 | 4066 ± 2435 | 0.06 |
| Peak systolic strain (%) | 17.5 ± 3.9 | 9.1 ± 5.3 | <0.001 |
| Peak systolic strain rate (%/s) | 1.2 ± 0.3 | 0.9 ± 0.2 | <0.01 |

**TABLE 3. Effect of Increasing Heart Rate by Right Atrial Pacing**

| | Normal LV function (n=12) | | | | Heart Failure (n=9) | | |
|---|---|---|---|---|---|---|---|
| **Pacing condition** | **Control** | **80** | **100** | **p value** | **Control** | **100** | **p value** |
| MAP (mmHg) | 94 ± 17 | 96 ± 15 | 99 ± 16* | 0.01 | 96 ± 18 | 99 ± 20 | 0.05 |
| LVSP (mmHg) | 123 ± 24 | 122 ± 22 | 120 ± 22 | 0.22 | 127 ± 18 | 124 ± 19* | 0.01 |
| LVEDP (mmHg) | 9 ± 4 | 7 ± 4* | 5 ± 5*† | <0.0001 | 16 ± 9 | 13 ± 10 | 0.09 |
| LV+dP/$dt_{max}$ | 1335 ± 296 | 1437 ± 264* | 1564 ± 320*† | <0.0001 | 960 ± 207 | 1048 ± 223* | <0.01 |
| Ves (ml) | 28 ± 8 | 26 ± 8 | 26 ± 10 | 0.55 | 123 ± 60 | 131 ± 62 | 0.26 |
| Ved (ml) | 98 ± 15 | 89 ± 16* | 83 ± 16* | <0.0001 | 167 ± 68 | 166 ± 62 | 0.86 |
| SV (ml) | 70 ± 13 | 62 ± 14* | 57 ± 13*† | <0.0001 | 52 ± 27 | 45 ± 20 | 0.13 |
| LV+dP/$dt_{max}$/Ved | 14 ± 3 | 17 ± 2* | 19 ± 4*† | <0.0001 | 7 ± 3 | 7 ± 3 | 0.12 |
| Ejection Time (ms) | 298 ± 32 | 269 ± 22* | 243 ± 16*† | <0.0001 | 237 ± 32 | 205 ± 19* | <0.01 |
| EF (%) | 72 ± 7 | 70 ± 8 | 69 ± 10 | 0.20 | 31 ± 11 | 28 ± 11 | 0.11 |
| CO (L/min) | 4.6 ± 1.3 | 5.1 ± 1.2* | 5.7 ± 1.3* | <0.001 | 3.8 ± 2.0 | 4.5 ± 2.0* | 0.04 |
| SW (mmHg*ml) | 5614 ± 1457 | 4973 ± 1419* | 4580 ± 1290*† | <0.0001 | 4181 ± 2554 | 3640 ± 1814 | 0.14 |
| Peak SS (%) | 18.0 ± 3.5 | 13.2 ± 6.3* | 10.8 ± 6.0* | <0.001 | 9.9 ± 3.7 | 6.9 ± 3.8* | <0.01 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Peak SSR (%/s) | 1.1 ± 0.3 | 1.2 ± 0.2 | 1.1 ± 0.9 | 0.97 | 0.8 ± 0.2 | 0.9 ± 0.3 | 0.26 |

**Table 4. Measurements of Deformation and Descriptors of LV Function**

| Variable | Longitudinal Strain | | Longitudinal Strain Rate | |
|---|---|---|---|---|
| | $r^2$ | p-value | $r^2$ | p-value |
| dP/dt max | 0.194 | 0.0006 | 0.254 | 0.0002 |
| LVEDP | 0.145 | 0.0053 | 0.251 | 0.0002 |
| Ejection time | 0.451 | <0.0001 | 0.051 | 0.12 |
| Stroke Volume | 0.164 | 0.0035 | 0.016 | 0.39 |
| Ved | 0.190 | 0.0015 | 0.239 | 0.0004 |
| Ves | 0.356 | <0.0001 | 0.304 | <0.0001 |
| Longitudinal Strain | 1.00 | 0.000 | 0.255 | 0.0002 |
| Longitudinal Strain rate | 0.255 | 0.0002 | 1.00 | 0.000 |

**Table 5. Determinants of the Change in Longitudinal Strain with Increased HR**

| **Δ Variable with Increased HR** | **$r^2$** | **p** |
|---|---|---|
| ΔEjection time | 0.255 | 0.004 |
| ΔLV $+dP/dt_{max}$ | 0.113 | 0.07 |
| ΔLongitudinal strain rate | 0.083 | 0.12 |
| ΔLV-end diastolic pressure | 0.092 | 0.10 |
| ΔStroke Volume | 0.057 | 0.20 |
| ΔVed | 0.172 | 0.02 |
| ΔVed / (Ved –ΔVed) | 0.25 | 0.005 |
| ΔVes | 0.075 | 0.14 |

## FIGURE LEGEND

**Figure 1.** Individual patient and mean responses to incremental RA pacing in patients with Normal LV function and patients with HF. Within group comparisons performed with one way RMANOVA. Post hoc pairwise comparisons performed with Bonaferroni-Dunn testing. *$p<0.05$ versus No pacing, †$p<0.05$ versus RA pacing at 80 bpm.

**Figure 2.** Regional absolute strain values in response to incremental RA pacing. In patients with normal LV function, the decrease in strain with increases in HR reached statistical significance in all segments analysed. In HF patients, the decrease in strain was also apparent in all segments analysed, and reached statistical significance in all segments except apical septal, apical lateral and basal lateral segments. Analysis was performed with one way RMANOVA.

**Figure 3.** The relationship between LV+dP/dtmax and both strain and strain rate in patients with and without heart failure at varying heart rates.

**Figure 4.** The Lagrangian strain relationship would predict that a decrease in initial length without change in the end-systolic length (as occurs with increasing HR) will result in a decrease in the quantity of strain. The decline in strain values should be related to a term defined by the change in end-diastolic dimension ($\Delta$Ved) expressed as a proportion of the new end-diastolic dimension (Ved $-\Delta$Ved). There was a linear relationship between this term $\Delta$Ved / (Ved $-\Delta$Ved) and the change in strain values with increasing heart rate. The change in strain values was also related to the HR-related decrease in ejection time.

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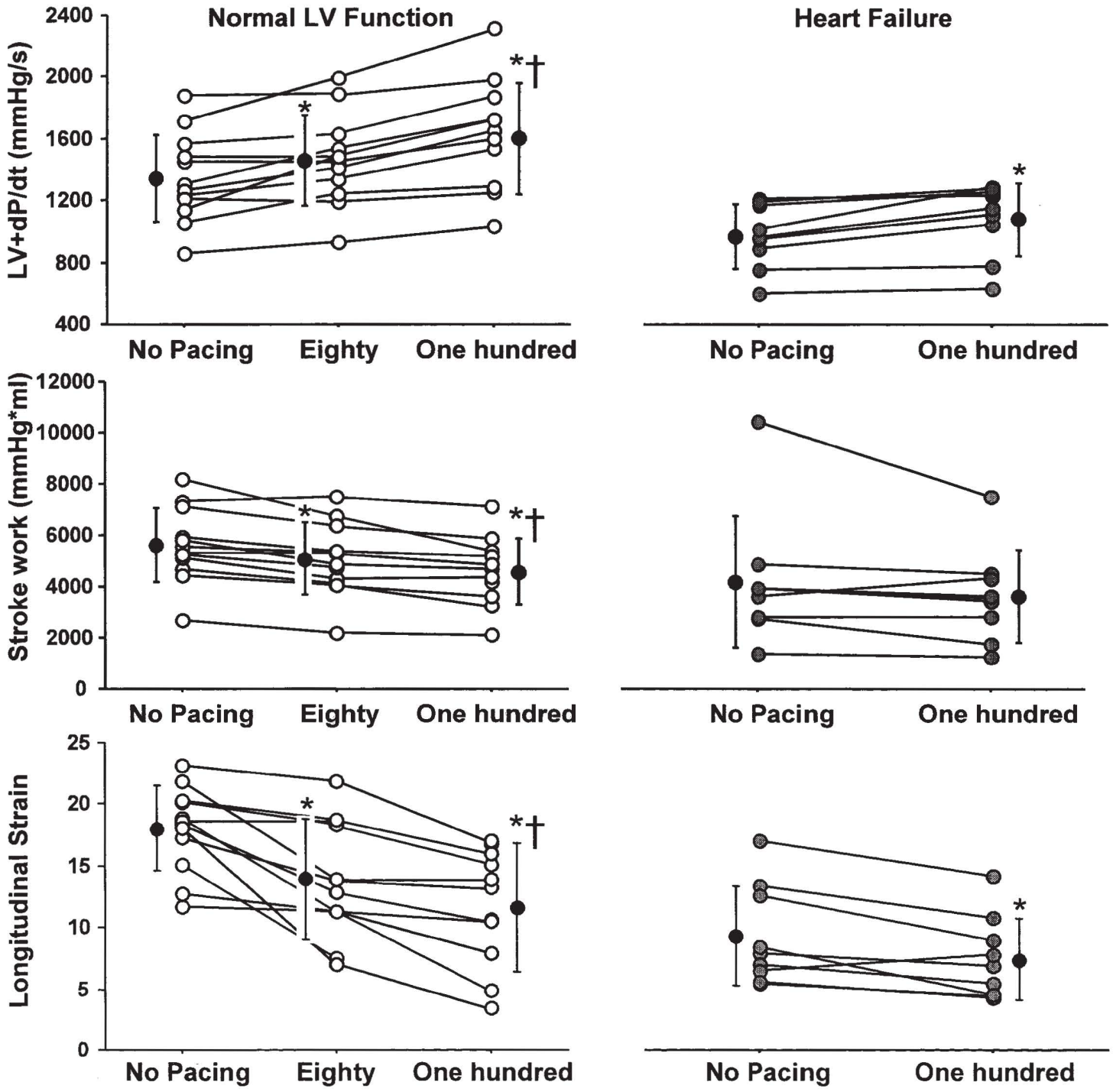
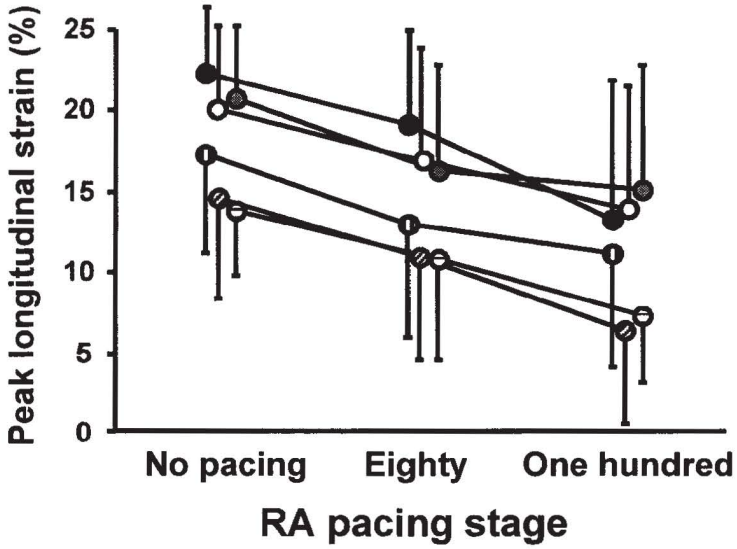
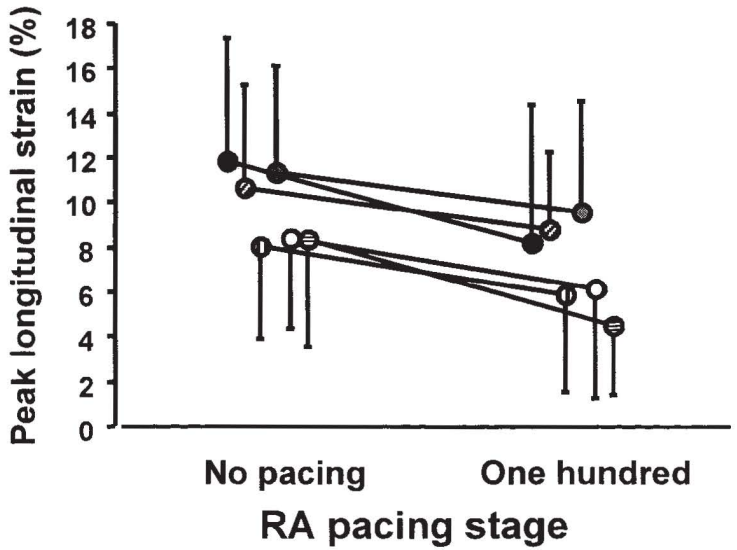

Normal LV Function
Heart Failure
LV+dP/dt (mmHg/s)
2400
2000
1600
1200
800
400
No Pacing
Eighty
One hundred
Stroke work (mmHg*ml)
12000
10000
8000
6000
4000
2000
0
Longitudinal Strain
25
20
15
10
5
0
*
*†

Figure 1.

**Normal LV Function**




**Heart Failure**



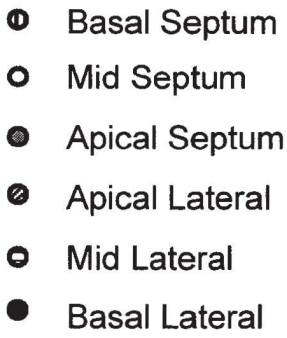




**Figure 2.**

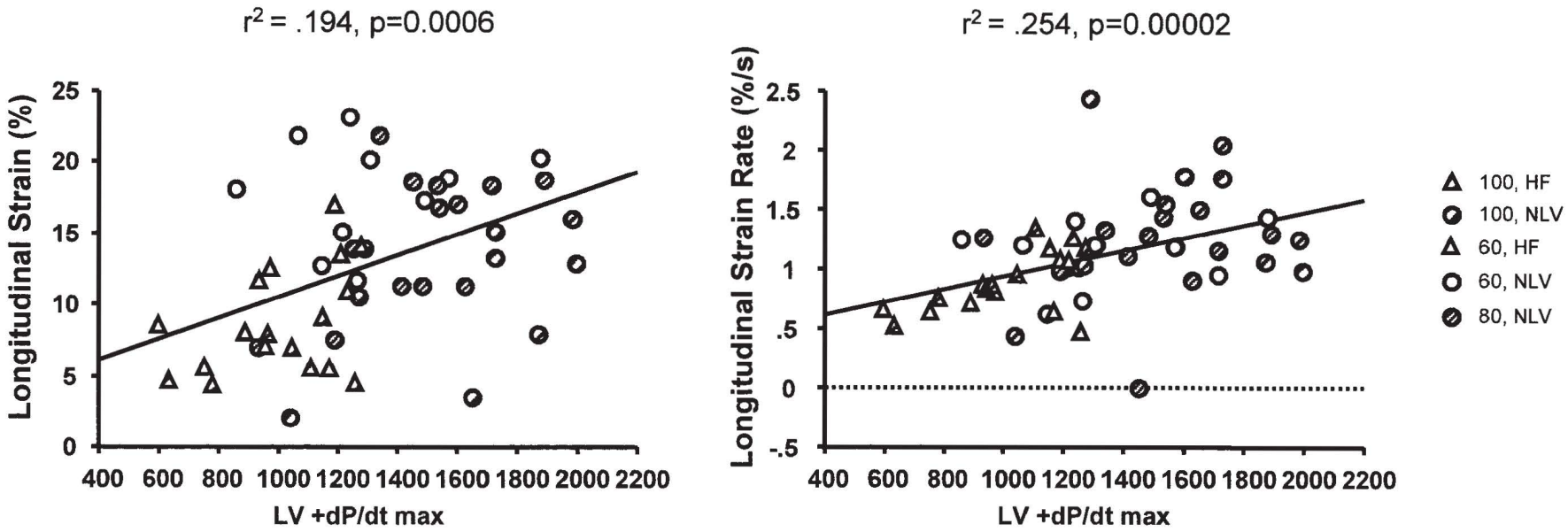
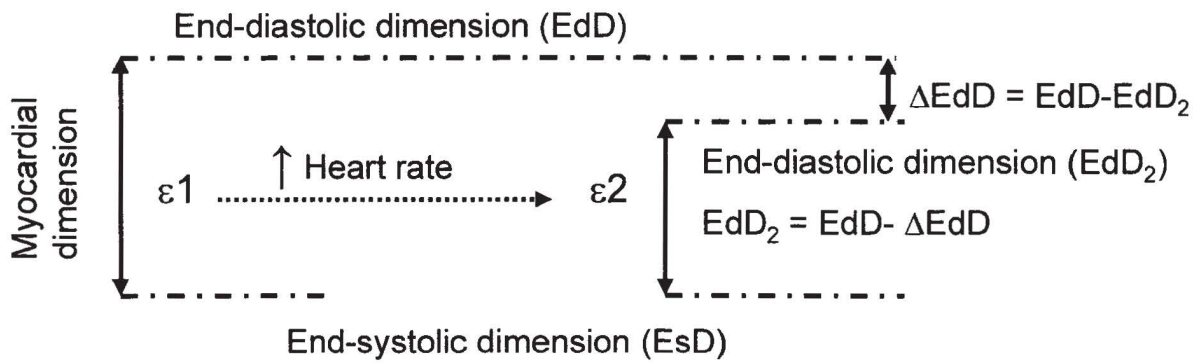

r² = .194, p=0.0006
Longitudinal Strain (%)
25
20
15
10
5
0
400 600 800 1000 1200 1400 1600 1800 2000 2200
LV +dP/dt max
r² = .254, p=0.00002
Longitudinal Strain Rate (%/s)
2.5
2
1.5
1
.5
0
-.5
400 600 800 1000 1200 1400 1600 1800 2000 2200
LV +dP/dt max
100, HF
100, NLV
60, HF
60, NLV
80, NLV

**Figure 3.**




$\varepsilon 1 = (EdD-EsD) / EdD$

$= 1 - (EsD / EdD)$

$\varepsilon 2 = (EdD_2-EsD) / EdD_2$

$= [(EdD-\Delta EdD)-EsD] / (EdD- \Delta EdD)$

$= 1 - [EsD / (EdD-\Delta EdD)]$

$\varepsilon 1-\varepsilon 2 = EsD / (EdD-\Delta EdD) - EsD / EdD$

$\Delta \varepsilon$ α $1 / (EdD-\Delta EdD) - 1 / EdD$

α $\Delta EdD / (EdD -\Delta EdD)$

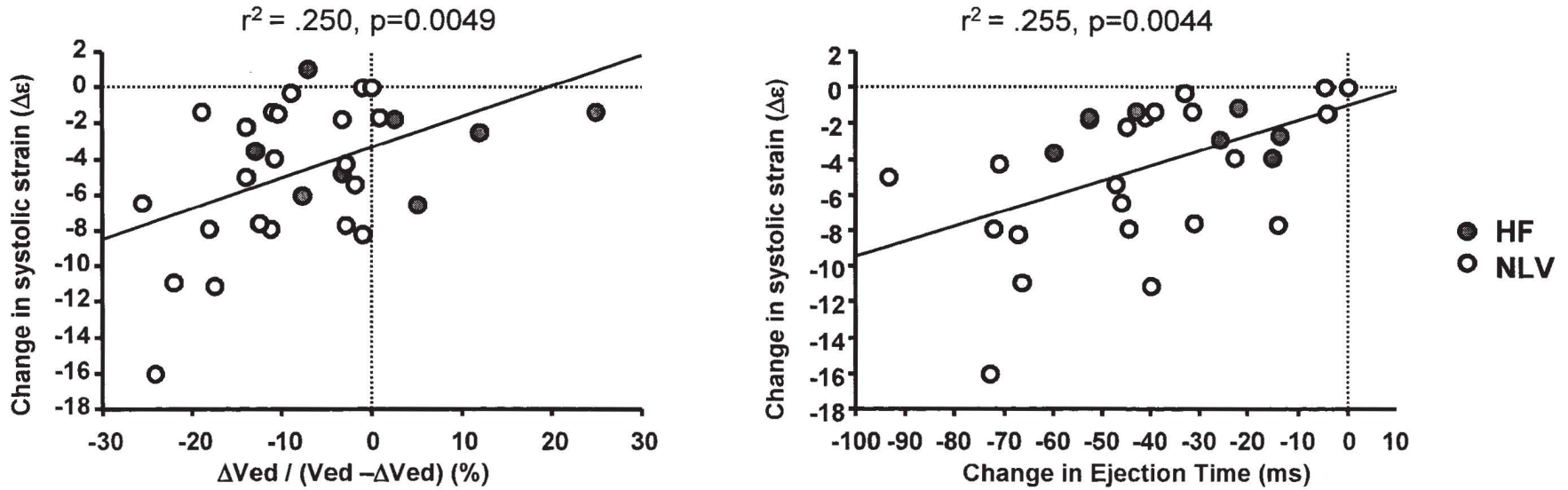



**Figure 4.**

CONCLUSÃO:

Os estudos experimentais incluídos neste manuscrito apresentam resultados em comum, visto que há uma interpolação de metodologia entre ambos. A relação força-frequência é uma parâmetro hemodinâmico que serve como um índice *in vivo* do processo de acoplamento excitação-contração dos cardiomiócitos. Esta relação que consiste no aumento contratilidade miocárdica induzido por aumento da freqüência cardíaca foi identificada com sucesso nos dois estudos.

O artigo número 1 (Frequency-dependent left ventricular performance in women and men) demonstrou em pacientes com função sistólica preservada que a inclinação da relação força-frequência nas mulheres é mais acentuada que nos homens. A contratilidade nas pacientes do sexo feminino se manteve mais elevada que nos homens mesmo quando o índice de contratilidade isovolumétrico +dP/dt max foi normalizado para volume diastólico final ou quando foi aferida através da elastância sistólica final medida através do método de batimento único preconizado por Shisido et al. Ao mesmo tempo, o estudo demonstrou que apesar de aumentar a contratilidade, o estímulo de aumento da frequência cardíaca induzido por marcapasso atrial causou decréscimos mais significativos no volume diastólico final e volume sistólico de ejeção das mulheres, de modo que o aumento do débito cardíaco foi menos pronunciado nas mulheres também.

O artigo número 2 ( Strain, Strain Rate, and the Force Frequency Relantionship in Patients with and withou Heart Failure) demonstrou que os pacientes com disfunção sistólica do ventrículo esquerdo apresentam um relação força-frequência atenuada quando comparados a pacientes com função sistólica preservada. As medidas ecocardiográficas de deformação miocárdica avaliadas através do método de rastreamento de marcadores sonográficos demonstrou que o strain longitudinal diminuiu significativamente em ambos os grupos submetidos à aumento da freqüência cardíaca e que este resultado foi diretamente associado à diminuição do volume diastólico final do ventrículo

esquerdo. A medida de Strain rate, por sua vez, não apresentou alteração significativa associada a aumento da frequência cardíaca.

Conclui-se então que o aumento da frequência cardíaca induzida pelo estímulo de marcapasso atrial acentua significativamente as diferenças de performance mecânica do ventrículo esquerdo entre homens e mulheres com e sem disfunção sistólica do ventrículo esquerdo e que o strain longitudinal é uma medida de deformação miocárdica dependente de pré e pós-carga, visto que acompanha a diminuição do volume sistólico de ejeção causado pelo aumento da frequência cardíaca.