IVO VIEIRA DE SOUSA NETO

Impacto do treinamento de força paterno intergeracional sobre as adaptações moleculares no ventrículo esquerdo, tendão e tecido adiposo da prole exposta à dieta hiperlipídica

Brasília – DF, 2021.

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Área de concentração: Mecanismos Básicos e Tecnologias em Saúde.
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Orientador: Prof. Dra. Rita de Cássia Marqueti Durigan

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BANCA EXAMINADORA

_____

Profª. Dra. Rita de Cássia Marqueti Durigan
Universidade de Brasília

_____

Profª. Dra. Graziella Anselmo Joanitti
Universidade de Brasília

_____

Profª. Dra. Cláudia Regina Cavaglieri
Universidade Estadual de Campinas

_____

Prof. Dr. Adelino Sanchez Ramos da Silva
Universidade de São Paulo

_____

Prof. Dr. Alex Leite Pereira (suplente)
Universidade de Brasília

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“O conhecimento é uma luz que não adianta esconder, pois alguém tomado de curiosidade descobrirá o que foi encoberto.”

Mário Pereira Gomes

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RELAÇÃO DE SIGLAS E ABREVIATURAS

Revisão de literatura

DNA - Ácido Desoxirribonucleico

GLUT4 - Transportador de glicose 4

IRS1 - Receptor de insulina 1

MEC – Matrix extracelular

MicroRNA – Pequenos ácidos ribonucleicos não-codificantes

MMP-2 – Metaloproteinase 2

MMP-9 - Metaloproteinase 2

PI3K - Fosfoinositídeo 3-quinase

RNA - Ácido ribonucleico

RNAm - RNA mensageiro

TF – Treinamento de Força

VE – Ventrículo esquerdo

Manuscrito 1

BDNF - Brain-derived neurotrophic factor

DNA - Deoxyribonucleic acid

DEXA - Dual-energy X-ray absorptiometry

F1 – First offspring

FASN - Fatty acid synthase

HF – High fat

HIIT - High-intensity interval training

IGF-1 -Pro-insulin like growth factor I

mRNA - Messenger RNA

RNA - Ácido ribonucleico

RT – Resistance training

SLC38a2 - Solute carrier family 38 member 2

TNF-α - Tumor necrosis factor-alpha

TRFS - RNA fragments

TRKB - Tyrosine kinase B receptor

VO_2 max - Maximal oxygen uptake

Manuscrito 2

ANOVA - Analysis of variance

AUC - Area under the curve

HF - High fat

LC-MS/MS - High performance liquid chromatography tandem mass spectrometry

LV - Left ventricle

RT – Resistance training

SFO-C- Offspring from sedentary fathers, exposed to control diet

SFO-HF - Offspring from sedentary fathers exposed to a high-fat diet

STRING - Protein-Protein Interaction Networks Functional Enrichment Analysis

TFO-C - Offspring from trained fathers, exposed to control diet

TFO-HF - Offspring from trained fathers exposed to a high-fat diet

Manuscrito 3

ANOVA - Analysis of variance

DNA - Deoxyribonucleic acid

ECM - Extracellular matrix

HF - High fat

LC-MS/MS - High performance liquid chromatography tandem mass spectrometry

MIF - Macrophage migration inhibitory factor

RT – Resistance training

RNA - Ribonucleic acid

SFO-C- Offspring from sedentary fathers, exposed to control diet

SFO-HF - Offspring from sedentary fathers exposed to a high-fat diet

STRING - Protein-Protein Interaction Networks Functional Enrichment Analysis

TFO-C - Offspring from trained fathers, exposed to control diet

TFO-HF - Offspring from trained fathers exposed to a high-fat diet

THBS1 - Cartilage oligomeric matrix protein and thrombospondin

Manuscrito 4

ANOVA - Analysis of variance

CMH - 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine

CAT - Catalase

CEBPA - cCAAT/enhancer binding protein alpha

COL1A1- Type I collagen alpha 1 chain

COL3A1- Type III collagen alpha 1 chain

CTGF- Connective tissue growth factor

DCF – RFU - 2’7’-dichlorodihydrofluorescein diacetate

DNA - Deoxyribonucleic acid

ECM - Extracellular matrix

FABP4- Fatty acid-binding protein 4

HFD - High fat diet

IGF-1A -Pro-insulin like growth factor IA

IL1β - Interleukin 1β

IL-6 - Interleukin 6

MCP1 - Monocyte chemoattractant protein-1

MMP-2 - Matrix Metalloproteinase-2

MMP-9 - Matrix Metalloproteinase-9

NRF-2 Nuclear factor E2-related factor 2

NFκB -Nuclear factor kappa B

NO -Oxide nitric

PPARA -Peroxisome proliferator-activated receptor alpha

RNA - Ribonucleic acid

RPLP0 - Ribosomal protein lateral stalk subunit P0 (Rplp0)

ROS- Reactive oxygen species

RT – Resistance training

SFO-C- Offspring from sedentary fathers, exposed to control diet

SFO-HF - Offspring from sedentary fathers exposed to a high-fat diet

SRBP1 - Sterol regulatory element-binding protein 1

SOD - Superoxide dismutase

TA – Adipose tissue

TGFB1 - Transforming growth factor, beta 1

TBARS - Thiobarbituric acid reactive substances

TFO-C - Offspring from trained fathers, exposed to control diet

TFO-HF - Offspring from trained fathers exposed to a high-fat diet

TIMP-2 - Tissue inhibitor of metalloproteinases 2

RESUMO

A obesidade pode ser programada antes da vida uterina de um indivíduo por meio de herança intergeracional. Estudos recentes revelaram que o treinamento de força (TF) é eficaz e seguro na prevenção desta doença. Já estão bem definidos os impactos negativos da obesidade sobre os resultados perinatais, porém ainda são escassas as informações sobre o papel do exercício paterno sobre as adaptações teciduais da primeira geração. Assim, investigamos os efeitos de 8 semanas de TF realizado apenas pelo pai antes da fecundação sobre as adaptações moleculares no ventrículo esquerdo, tendão e tecido adiposo na prole exposta à dieta padrão e hiperlipídica. Foram usados incialmente quatorze ratos (Wistar) com quatro meses de vida divididos aleatoriamente em dois grupos: TF durante 8 semanas (n=7) e grupo controle (n=7). Os pais realizaram um protocolo de TF durante oito semanas com três sessões semanais com carga atada às suas caudas. Após o TF os animais acasalaram com fêmeas da mesma linhagem que permaneceram sedentárias durante todo o período experimental. Após o nascimento, os animais (machos) foram distribuídos em quatro grupos: prole de pais sedentários exposta à dieta controle (PPS-DC; n= 7) (66 g carboidratos; 22 g de proteínas; 4 g de lipídeos); prole de pais treinados exposta à dieta controle (PPT-DC; n= 7); prole de pais sedentários exposta à dieta hiperlipídica (PPS-DH; n= 7) (25 g de carboidrato; 20 g de proteínas; 50 g de gordura) (Prag soluções®) e refrigerante (Coca-Cola®) (carboidratos: 105,7 g/L; sódio: 51,4 g/L); e prole de pais treinados exposta à dieta hiperlipídica (PPT-DH; n= 7). O TF paterno modifica o perfil proteômico do ventrículo esquerdo independente da dieta da prole. A análise proteômica demonstrou que o TF é um fator crítico capaz de reprogramar proteínas associadas à contração muscular, processos metabólicos, atividade antioxidante, transporte e regulação da transcrição. Similarmente, o TF paterno modula os níveis de abundância de proteínas no tendão da prole, sendo que essa modulação foi mais evidente quando a prole foi submetida à dieta hiperlípica. As proteínas moduladas foram associadas à proteção tecidual, como organização da matriz extracelular, transporte e mediadores inflamatórios. Em relação ao tecido adiposo, o TF paterno atenuou os efeitos deletérios da dieta hiperlipídica na prole, incluindo a diminuição do tamanho do adipócito, fibrose, produção de espécies reativas de oxigênio, citocinas pró-inflamatórias, agentes pró-oxidantes, metaloproteinases e genes associados a adipogênese e inflamação. Estes achados foram acompanhados por aumentos de enzimas antioxidantes e diminuição de marcadores metabólicos (insulina e leptina) na circulação sanguínea. O conjunto dos efeitos protetores

do exercício paterno sobre os diversos sistemas fisiológicos podem potencialmente melhorar a saúde da primeira geração. Por fim, a presente pesquisa fornece informações valiosas sobre os mecanismos moleculares envolvidos na herança intergeracional por meio da linhagem paterna.

Palavras – chave: obesidade, herança epigenética, exercício paterno, gerações, saúde metabólica.

ABSTRACT

Obesity can be programmed before an individual's uterine life through intergenerational inheritance. Recent studies revealed that resistance training (RT) is effective in preventing this disease. The obesity negative impacts on perinatal outcomes are well defined; however evidence of the paternal exercise role on tissue adaptations in the first generation is still scarce. Thus, we investigated the effects of 8 weeks of paternal resistance training preconception on molecular adaptations in the left ventricle (LV), tendon, and adipose tissue in offspring exposed to the standard and high-fat diet. Wistar rats (male) were randomly divided into two groups: sedentary fathers and trained fathers (8 weeks, three times per week, with 8–12 dynamic movements per climb in a stair climbing apparatus). The offspring were obtained by mating with sedentary females. Upon weaning, male offspring were divided into four groups (5 animals per group): offspring from sedentary fathers were exposed either to control diet (SFO-C), or to a high-fat diet (SFO-HF); offspring from trained fathers were exposed to control diet (TFO-C) or to a high-fat diet (TFO-HF). Paternal RT modulates LV proteome independent of offspring diet. Proteomic analysis demonstrated that paternal exercise is a critical factor capable of reprogramming offspring LV proteins associated with muscle contraction, cellular and metabolic processes, antioxidant activity, transport, translation, nucleosome assembly, and transcription regulation. Similarly, paternal RT modulates pathways in the tendon of the offspring, being such modulation more evident when the offspring is subjected to HF diet. Most modulated proteins are associated with biological pathways related to tendon protection and damage recovery, such as ECM organization, transport and inflammatory mediators. Regarding adipose tissue, paternal RT compensated the detrimental effects of offspring HFD by downregulation of genes, ROS production, pro-oxidants agents, enzymes, and ECM structural compounds, linked to adipogenesis, chronic inflammation, oxidative stress, and metabolic dysfunction. These findings were accompanied by the substantial improved antioxidants enzymes and decreased metabolic markers in the blood circulation. The protective effects of paternal exercise on the various physiological systems can potentially improve the health first generation. Finally, this research provides valuable information about the molecular mechanisms involved in intergenerational inheritance through the paternal lineage

Keywords: obesity, epigenetic inheritance, paternal exercise, generations, metabolic health.

1. REVISÃO DE LITERATURA

1.1 Obesidade

Os índices de obesidade vêm crescendo de forma alarmante na maioria dos países, chegando a mais de 1 bilhão de pessoas em todo mundo (WHO, 2013). Segundo a Organização Mundial de Saúde (OMS), a obesidade é uma doença crônica epidêmica, que atinge crianças, adolescentes e adultos de países com diferentes níveis de desenvolvimento, inclusive superando a desnutrição e doenças infecciosas. Esta epidemia global requer atenção especial dos serviços de saúde, pois até 2025, estima-se que aproximadamente 2,3 bilhões indivíduos apresentarão sobrepeso e mais de 700 milhões de obesos (WHO, 2013). De acordo com essas projeções, caso os indivíduos continuem a engordar, cerca de um quinto da população mundial estará acima do peso saudável em menos de 10 anos (WHO, 2013).

A obesidade e os fatores de risco cardiovasculares relacionados são geralmente atribuídos ao alto consumo de alimentos ricos em gorduras e carboidratos refinados que contém grandes quantidades de calorias e sal, combinado com a falta de atividade física (VANDEVIJVERE, 2015). A obesidade tem sido associada a vários efeitos deletérios, incluindo incapacidade funcional, redução da expectativa de vida e aumento da mortalidade (VANDEVIJVERE, 2015).

No Brasil, um levantamento recente da Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico (VIGITEL - BRASIL, 2018) demonstrou que houve aumento de 67,8% nos índices de obesidade nos últimos treze anos, saindo de 11,8% em 2006 para 19,8% em 2018. De forma preocupante, mais da metade da população brasileira, tem excesso de peso (55,7%). O aumento da prevalência foi maior entre as faixas etárias de 18 a 24 anos, com 55,7%. Quando verificado o sexo, os homens apresentam crescimento de 21,7% e as mulheres de 40% (VIGITEL - BRASIL, 2018). Outro dado mostra que, em 2019, uma em cada quatro pessoas de 18 anos ou mais anos de idade no Brasil estava obesa, o equivalente a 41 milhões de pessoas. Já o excesso de peso atingiu 60,3% da população de 18 anos ou mais de idade, o que corresponde a 96 milhões de pessoas, sendo 62,6% das mulheres e 57,5% dos homens (IBGE, 2019).

Em crianças, os dados mais atuais e de abrangência nacional são as notificações do Sistema de Vigilância Alimentar e Nutricional, de 2019 que revelaram que 16,33% das crianças brasileiras entre cinco e dez anos estão com sobrepeso; 9,38% com obesidade; e

5,22% com obesidade grave. Em relação aos adolescentes, 18% apresentam sobrepeso; 9,53% são obesos; e 3,98% têm obesidade grave. Nesse aspecto, uma questão preocupante é que existe a estimativa de que metade das crianças obesas torna-se adultos obesos (YUSUF et al., 2020; VANHALA et al., 1998) com grandes chances de sofrerem as consequências deste agravo, como diabetes tipo II, doenças cardiovasculares, doença aterosclerótica, hipertensão arterial, transtornos ortopédicos e articulares, doenças de pele, maior risco cirúrgico, dentre outras complicações (YUSUF et al., 2020).

Em consequência do aumento preocupante das taxas de sobrepeso em crianças e bebês (TYBOR et al., 2019), há uma necessidade urgente de entender suas causas, a fim de desenvolver estratégias e tratamentos preventivos. Fatores de risco que predispõem a criança ou adolescente à obesidade devem ser identificados e esclarecidos. Nesta perspectiva, estudos prévios evidenciaram que a obesidade e os fatores de risco relacionados a ela podem ser programados antes ou durante a vida uterina de um indivíduo (DENHAM, 2018; BAYOL et al., 2005; OBEN et al., 2010).

1.2 Programação da obesidade através da linhagem paterna

Estudos de associação em todo o genoma mostraram que, embora a obesidade e a síndrome metabólica sejam parcialmente herdáveis, suas ocorrências não podem ser totalmente explicadas pelo modelo mendeliano clássico (KUSUYAMA et al., 2020). As variantes alélicas podem ser responsáveis por apenas uma pequena fração dessa herdabilidade. Desse modo, as mudanças substanciais na expressão gênica e consequentemente das proteínas podem ser transmitidas através de gerações, sem mudanças na sequência primária do DNA (DENHAM, 2018; TIBANA et al. 2017). A programação de doenças degenerativas crônicas pode estar associada a mecanismos intergeracionais e trangeracionais epigenéticos, como o remodelamento da cromatina, metilação e acetilação das histonas e DNA, bem como RNAs não codificadores (ex: MicroRNAs e RNA de transporte e interferência) que resultam em modificações fenotípicas da reprogramação individual do genoma (TIBANA et al. 2017) (figura 1). A transmissão intergeracional ou transgeracional de informação é sugerida ser outra forma de herança, complementar à herança genética canônica.

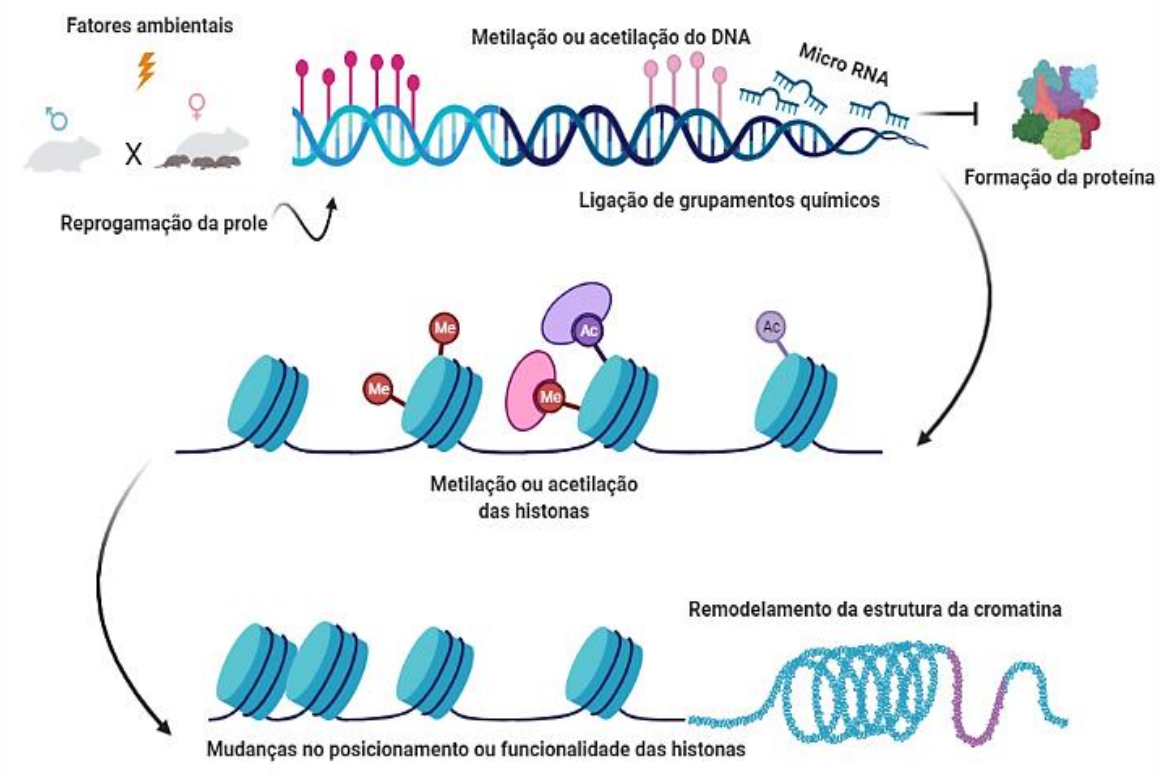

FIGURA 1. Representação ilustrativa dos principais mecanismos da herança intergeracional epigenética que regulam a expressão dos genes e proteínas sem alterar a sequência do DNA. Adaptado de TIBANA et al. 2017

Estudos em animais e humanos sugerem que perturbações ambientais, como dieta, sedentarismo, estresse, drogas, toxinas, álcool e tabagismo antes do nascimento, podem contribuir para o desenvolvimento de consequências metabólicas permanentes nos filhos e resultar em efeitos negativos sobre o fenótipo (KUSUYAMA et al., 2020). A associação entre distúrbios metabólicos em filhos de mães obesas ganhou destaque nos últimos anos (KUSUYAMA et al., 2020), porém pesquisas recentes indicam que os pais também podem desempenhar um papel significativo sobre o desenvolvimento da prole (HUR et al., 2017). A exposição dos pais a dietas ricas em gorduras saturadas pode afetar os resultados de saúde ao longo da vida da prole, independentemente dos genótipos herdados (DENHAM, 2018). Tais efeitos ocorrem em resposta a uma ampla gama de eventos ambientais estressores, incluindo principalmente defeitos ou modificações no metabolismo primário.

As modificações no esperma fornecem uma potencial base molecular para explicar a contribuição do estilo de vida do pai sobre a fisiologia da prole (HUR et al.,

2017). Modificações no DNA, proteínas da cromatina, bem como o RNA derivado nos espermatozoides (em particular o RNA não-codificante) constituem os principais mecanismos desta transmissão (HUR et al., 2017). Foi demostrado que a maturação espermática pode ser um estágio criticamente sensível durante o qual a programação paterna é estabelecida. A espermatogênese é um processo contínuo e as experiências de vida do pai podem reprogramar a qualidade do sêmen e o conteúdo epigenético do esperma e consequentemente levam informações que modificam a fertilização e o fenótipo adulto da prole (HUR et al., 2017). Portanto, o pai desempenha o papel de fornecer a herança epigenética específica do espermatozoide para o ovócito, afetando a trajetória de desenvolvimento embrionário e a saúde da prole adulta, o que veio consolidar o possível envolvimento do pai na transmissão de suscetibilidade ao desenvolvimento de doenças nas gerações futuras (HUR et al., 2017) (Figura 2).

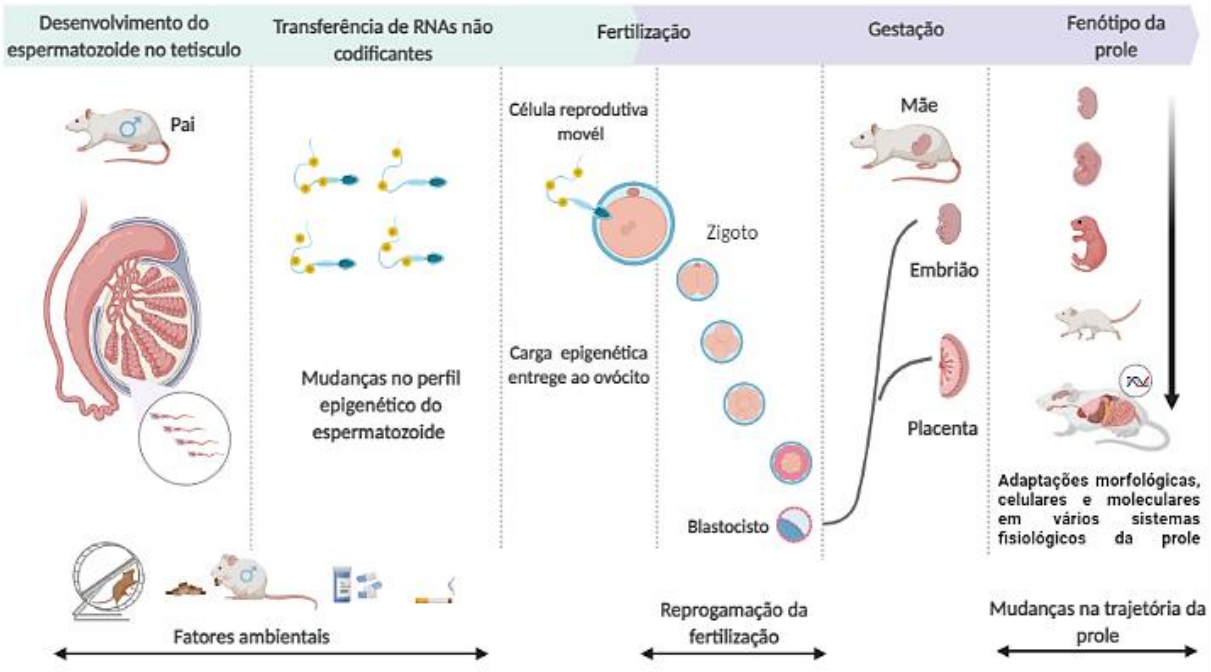

FIGURA 2. Representação do envolvimento de pequenos RNA não codificantes nos espermatozoides. Conteúdo de RNA do espermatozoide pode ser alterado pelas condições ambientais e consequentemente modificar o fenótipo da prole.

Modificações epigenéticas que atuam na capacidade de plasticidade celular preparam o indivíduo para o ambiente extra-uterino e podem potencializar uma vantagem de sobrevivência ao regular os genes diferenciais que codificam proteínas envolvidas no metabolismo energético e na adipogênese (STANFORD et al., 2018). No entanto, diante de uma condição metabólica deletéria, como a obesidade e doenças relacionadas, essas

modificações podem ser exacerbadas ou silenciadas, modificando o fenótipo das gerações futuras (CHAMORRO-GARCIA et al., 2017; LUCAS et al., 217). A obesidade paterna inicia distúrbios metabólicos em até duas gerações futuras, alterando o perfil transcricional do conteúdo de miRNA dos testículos (STANFORD et al., 2018). O conteúdo diferencial de miRNAs canônicos nos espermatozoides induziu uma desregulação na espermatogênese, e modificou o desenvolvimento embrionário e disfunção metabólica na prole (CHAMPROUX et al., 2018; FULLSTON et al., 2013). Além disso, a dieta rica em gordura paterna afetou o status metabólico da prole através de alterações epigenéticas nos genes da adiponectina e da leptina (MASUYAMA et al., 2016). Curiosamente, foi demonstrado que 13 miRNAs de espermatozoides foram modulados pela dieta paterna com alto teor de gordura e transferidos para o embrião na fertilização, alterando a trajetória de crescimento da prole (CHAMPROUX et al., 2018).

Nos últimos anos, novas evidências demonstraram que os efeitos intergeracionais associadas à exposição da obesidade nos pais modifica a plasticidade tecidual na prole. Por exemplo, Ng et al., (2010) demonstraram que a obesidade paterna induziu disfunção das células betas pancreáticas nos filhotes. Além disso, foi associada com maior massa corporal, adiposidade e sensibilidade à insulina nos filhotes quando comparado aos filhotes de pais submetidos à dieta normolipídica**.** Está bem estabelecido que a obesidade paterna é um fator de risco para o aumento da adiposidade dos filhos, predispondo à obesidade (NG et al., 2010). As alterações deletérias do metabolismo estão associadas à intolerância à glicose pela disfunção das ilhotas pancreáticas, com consequências evidentes na primeira geração (NG et al., 2010). Além disso, os pais expostos a dietas ricas em gordura podem aumentar o peso gonadal com efeitos nocivos da função metabólica do esperma e fertilidade na prole, possivelmente pela modificação do microRNA do esperma do pai (MC PHERSON et a., 2014).

A programação do metabolismo paterno pode ser observada em várias espécies nos filos de animais (KUSUYAMA et al., 2020), sugerindo que esse fenômeno pode ter um significado evolutivo único. A herança intergeracional relacionada à linhagem paterna tem implicações significativas na etiologia da obesidade e pode explicar distúrbios relacionados com a obesidade na prole (CHAMPROUX et al., 2018; FULLSTON et al., 2013). Tanto a dieta paterna quanto o exercício foram identificados como fatores que afetam os aspectos morfológicos, fisiológicos e epigenéticos do sêmen e, consequentemente, a saúde metabólica da prole. Portanto, o entendimento dos aspectos

específicos da morfologia do esperma é necessário para esclarecer o real papel da célula reprodutiva móvel (KUSUYAMA et al., 2020).

1.3 Exercício físico paterno e respostas intergeracionais relacionadas à obesidade.

A proteção das futuras gerações não nascidas deve ser uma consideração primordial, pois pode contribuir para uma série de implicações para a saúde das gerações futuras. A possibilidade de estilos de vida saudáveis como o exercício físico está diretamente relacionada à redução dos riscos de desenvolvimento de diversas doenças crônicas não transmissíveis, de tal modo que pode ser um importante fator ambiental sobre a modulação da prole (STANFORD et al., 2018).

As possíveis modificações intergeracionais aumentam os programas transcricionais de proteção e diminuem o risco de disfunção metabólica e doenças cardiovasculares. Por exemplo McPherson et al. (2015) analisaram os efeitos da obesidade e do treinamento aeróbio (8 semanas / natação /3 vezes por semana com duração de 30 minutos) paterno na saúde cardiovascular das proles e o possível papel dos microRNAs na resposta desses fenótipos. Os resultados encontrados pelos pesquisadores demonstraram que tanto a dieta controle como a prática de exercício físico foram capazes de normalizar a expressão de microRNAs que possuem papel sobre a obesidade, o que potencialmente pode diminuir a probabilidade das proles de desenvolver doenças metabólicas. Além disso, o exercício paterno promoveu efeitos positivos na composição corporal, colesterol, leptina plasmática e concentração de proteína C-reativa em descendentes fêmeas expostas à dieta hiperlipídica. Essas alterações foram associadas a uma abundância normalizada de microRNAs de espermatozoides ligados ao X que têm como alvo genes que regulam o ciclo celular, apoptose e a homeostase celular.

Não obstante, Murashov et al. (2016) investigaram os efeitos de doze semanas de exercício voluntário em camundogos machos sobre a predisposição de fatores de risco em sua prole induzida a obesidade. Os autores demostaram que o exercício paternal alterou o peso corporal, adiposidade e resistência a insulina da prole. Ademais, alterou os padrões de metilação e expressão de vários genes metabólicos, incluindo *Ogt, Oga, Pdk4, H19, Glut4* e *Ptpn1* no músculo esquelético. Similarmente, Krout et al. (2018) relataram que o exercício pré-conceitual paterno (2 semanas de corrida voluntária) diminuía o risco de diabetes tipo 2 na prole após a dieta hiperlipídica paterna. Os autores sugerem que esse efeito protetor ocorreu possivelmente devido ao aumento na expressão das vias de

sinalização da insulina (GLUT4, IRS1 e PI3K) no músculo esquelético (KROUT et al. 2018).

Recentemente, um estudo conduzido por Stanford et al. (2018) demonstrou que o exercício voluntário paterno em camundongos reverte os efeitos deletérios associado ao consumo da dieta hiperlipídica paterna na prole, reduzindo os efeitos nocivos sobre a tolerância à glicose, ganho de gordura corporal e captação de glicose em diferentes músculos esqueléticos. Essas mudanças no fenótipo da prole são acompanhadas por mudanças na fisiologia do esperma; como por exemplo, a alimentação rica em gordura resulta em diminuição da motilidade espermática, porém o treinamento paterno normaliza tal efeito (STANFORD et al., 2018). O sequenciamento realizado nos espermatozoides revelou efeitos pronunciados do treinamento físico em várias classes de pequenos RNAs, pois várias alterações na carga útil do RNA espermático observadas em animais que consomem dieta rica em gordura foram suprimidas pelo treinamento paterno (STANFORD et al., 2018). Assim, o treinamento voluntário paterno resulta em melhorias pronunciadas na saúde metabólica da prole, através da modulação acentuada nos níveis de múltiplos pequenos RNAs no esperma, os quais apresentam um efeito potencial sobre o fenótipo da primeira geração.

Embora estejam bem definidos na literatura os impactos negativos da obesidade sobre os resultados obstétricos e perinatais (DENHAM, 2018), os efeitos do estilo de vida paterno nas adaptações moleculares do ventrículo esquerdo, tendão e tecido adiposo da prole expostas a diferentes dietas continuam desconhecidos, bem como são escassos os mecanismos inerentes à modulação paterna. Considerando que o consumo de dieta rica em gordura promove efeitos deletérios nas propriedades morfológicas e bioquímicas destes tecidos, é necessário entender como o treinamento de força (TF) antes da gestação poderá promover efeitos protetores nestes diferentes tecidos. Entender distintos tecidos da prole em resposta ao exercício paterno permitirá a compreensão de uma imagem mais completa da sinalização celular, além de um entendimento mais aprofundado sobre os mecanismos moleculares envolvidos na herança intergeracional relacionado a linhagem paterna.

Outro fator relevante que deve ser considerado é que estudos prévios realizaram somente treinamentos físicos com predominância ou componente aeróbio (exercício voluntário na roda ou esteira) e não manipularam estritamente as variáveis prescritoras do treinamento (duração, intensidade, intervalo de descanso, volume e frequência do

treinamento). Assim, elucidar outras modalidades de exercício paterno e os possíveis mecanismos adaptativos do exercício monitorado/programado que controla as variáveis prescritoras é necessário ampliar a compreensão da dose resposta sobre variáveis metabólicas e vias moleculares responsáveis pelas adaptações teciduais na prole.

Em relação ao TF, estudos realizados pelo nosso grupo de pesquisa revelaram que este tipo de exercício apresenta segurança e efetividade em mulheres com síndrome metabólica e obesidade (PEREIRA et al., 2012; TIBANA et al., 2012). Pereira et al. (2012), monstraram que o TF realizado em mulheres com síndrome metabólica não aumentou as citocinas inflamatórias. Tibana et al. (2012), encontraram reduções na pressão arterial de 24h em mulheres com sobrepeso e obesidade. Além disso, outros estudos demonstraram associações entre a força muscular e a pressão arterial (TIBANA et al., 2013), síndrome metabólica e obesidade (TIBANA et al., 2012) em mulheres de meia-idade sedentárias.

Estudo com modelo animal demonstrou que o TF realizado em ratas ovariectomizadas foi efetivo para controlar os efeitos negativos da ovariectomia sobre depósitos de gorduras, perfil lipídico e conteúdo tecidual de lipídios (LEITE, 2010). Ademais, descobrimos recentemente que animais que realizavam o TF de alto volume (oito subidas na escada) demonstravam uma menor atividade da metaloproteinase do tipo 2 (MMP-2) no tecido adiposo visceral, quando comparado ao grupo sedentário (SOUSA NETO et al., 2017). Essa descoberta pode ser importante, para elucidar os mecanismos induzidos pelo TF sobre as modulações na diferenciação de adipócitos, angiogênese e melhora dos processos inflamatórios locais.

Não obstante, nosso grupo de pesquisa demonstrou que o TF é um agente potencial na prevenção de danos sobre a matriz extracelular (MEC) e que atenua os efeitos prejudiciais do envelhecimento sobre os tendões, como rupturas e tendinopatias (MARQUETI et al., 2017). Tais efeitos benéficos foram associados a mudanças no perfil proteômico do tendão calcâneo, incluindo proteínas envolvidas nas vias de remodelamento da MEC, transporte celular e metabolismo primário (BARIN et al., 2017). Adicionalmente, o TF pode induzir melhorias na massa do tecido cardíaco, volume sistólico, densidade da cavidade e espessura da parede, o que pode ser uma estratégia terapêutica eficaz para combater a função cardíaca adversa e o remodelamento patológico causado por doenças cardiovasculares associadas ao sobrepeso e obesidade (WEINER et a., 2015).

Desse modo, os possíveis benefícios do TF sobre o ventrículo esquerdo, tendão e tecido adiposo justificam sua aplicabilidade na melhora da saúde e na prevenção de doenças crônicas degenerativas, assim é relevante esclareceremos a importância desse tipo de treinamento nas gerações futuras. As investigações no campo da epigenética em resposta ao exercício elucidam novas possibilidades sobre a hereditariedade, ampliando as formas de abordagem em relação a diagnóstico e tratamento de doenças crônicas não transmissíveis. Além disso, auxiliam a compreensão aprofundada sobre a saúde e comportamento da prole.

A metilação do DNA e da histona e os microRNAs são os mecanismos intergeracionais mais estudados em reposta ao exercício paterno (STANFORD et al., 2018). No entanto, outros processos moleculares adjacentes podem ser relevantes. Por exemplo, as proteínas são efetoras de mecanismos celulares de diferentes organelas e são moléculas críticas na manutenção da homeostase celular (MALIPATIL et al., 2019). A análise proteômica e ferramentas de bioinformática em diferentes tecidos da prole elucidarão uma visão integrada das vias moleculares moduladas por dieta e exercício paterno. Considerando que diferentes dietas e os efeitos intergeracionais podem regular a transcriptômica da prole (STANFORD et al., 2018), consequentemente o perfil proteômico poderá ser modulado. Uma abordagem de larga escala como a proteômica, permitiria a identificação e quantificação de muitas proteínas simultaneamente envolvidas nas diferentes organelas, processos biológicos e funções moleculares (PETRIZ et al., 2012). Tal análise pode esclarecer profundamente as redes moleculares por trás das adaptações fisiológicas promovidas pelo efeito paterno sobre a prole e permite a geração de novos alvos terapêuticos e aplicações clínicas.

A exposição à dieta rica em lipídeos ancestral também é capaz de desencadear o início e / ou induzir alterações importantes no tecido adiposo da prole. Por exemplo, Šnajder et al. (2018) investigaram os efeitos de diferentes combinações dietéticas materna pós-natal sobre a morfologia no tecido adiposo da prole. Os autores demostraram um maior número de adipócitos no tecido adiposo perirenal no grupo em que tanto a mãe quanto os filhos foram expostos à dieta hiperlipídica quando comparado ao grupo onde somente a prole foi exposta a dieta hiperlipídica (ŠNAJDER et al. 2018). Tais resultados sugerem que as mudanças dietéticas materna tem um efeito importante na reprogramação do remodelamento do tecido adiposo (ŠNAJDER et al. 2018). Não obstante, o exercício (esteira, 3 vezes por semana) realizado por mães obesas durante a gravidez aumentou a expressão de genes e proteínas relacionados a termogênese (Ucp1, Ppargc1a, and

Prdm16) no tecido adiposo da prole, além de aumentar os níveis de apelina no feto e circulação sanguínea (SON et al., 2020). Ademais, diminui o tamanho dos adipócitos e níveis de glicose plasmáticos, sugerindo um efeito protetor contra o início de doenças crônicas na prole (SON et al., 2020). No entanto, o impacto do estilo de vida do pai sobre as adaptações teciduais da prole permanece pouco compreendido. Além disso, os mecanismos moleculares e celulares inerentes aos efeitos paternos não foram elucidados neste tecido.

Considerando que cada sistema fisiológico tem sua função intrínseca, bem como é formado por uma hierarquia estrutural e agrupamento de células que exercem atividades específicas, desvendar as particularidades moleculares em diferentes tecidos é relevante para distinguir o reais efeitos e limitações do exercício paterno sobre a saúde da prole. Consequentemente, o TF paterno pode modular uma "assinatura molecular" particular em cada tecido e este conhecimento é importante para desvendar as especificidades fenotípicas teciduais na prole.

A vista disso, a presente tese pretende abordar uma nova ótica do papel do TF praticado pelos pais na contribuição não farmacológica da prevenção dos efeitos relacionados ao sobrepeso e obesidade. Nesse sentido, novas tecnologias e ferramentas podem ser desenvolvidas para auxiliar na prescrição e avaliação do programa de treinamento, otimizando os benefícios obtidos com a prática regular de exercícios. A compreensão das vias de sinalização intracelulares que modulam o desenvolvimento de diferentes tecidos da prole decorrente do TF realizado nos pais, será um achado valioso para área da fisiologia molecular do exercício. A identificação de marcadores associados a informação deletéria para a descendência pode ser assim uma estratégia importante para prevenir doenças não genéticas. Por fim, dentro dessa linha de pesquisa há uma expectativa promissora para mapear vias moleculares específicas que podem ser moduladas pelos possíveis efeitos protetores do treinamento físico paterno.

1.4 Relação entre ventrículo esquerdo, obesidade e exercício físico

O coração é definido como um órgão oco, que possui como principal atividade disseminar o sangue para o organismo (PUTZ, 1993). Sendo o principal tecido do aparelho circulatório, o coração é de extrema importância para a manutenção da homeostasia do organismo em virtude que distribui sangue oxigenado (PUTZ, 1993). Em

relação a sua morfologia, o ventrículo esquerdo (VE) forma o ápice do coração e está localizado abaixo do átrio esquerdo (PUTZ, 1993). No óstio atrioventricular esquerdo, encontramos a valva atrioventricular esquerda, constituída apenas por duas lâminas, denominadas cúspides. Ademais, o VE é constituído de trabéculas carnosas e cordas tendíneas, que fixam as cúspides da valva bicúspide aos músculos papilares (PUTZ, 1993). A principal função do VE é bombear o sangue para todo circulação sistêmica, através da artéria aorta. Assim, a avaliação acurada da função do VE torna-se de importância fundamental para o diagnóstico, tratamento e acompanhamento da saúde cardiovascular.

A hipertrofia ventricular esquerda induzida pelo exercício físico é um importante mecanismo compensatório fisiológico em resposta a aumentos crônicos da sobrecarga hemodinâmica durante o stress (DEJGAARD et al., 2018). Este fenótipo está associado a sarcômeros adicionados em série para alongar a célula cardíaca, bem como em paralelo (DEJGAARD et al., 2018). O aumento da área transversal contribui para o aumento do volume sistólico ventricular e do débito cardíaco, o que melhora a capacidade energética do coração (MÜLLER et al., 2013). Estudos prévios demonstraram que o VE de indivíduos treinados apresenta maior eficiência mecânica da musculatura cardíaca, bem como aumento da capilarização e atividade enzimática, as quais resultam de uma complexa interação de mecanismos centrais e periféricos, operados em níveis estruturais, eletrofisiológicos, bioquímicos, metabólicos e neurogênicos (HAYKOWSKY et al., 2011; KOLWICZ et al., 2009. Em contraste, a hipertrofia ventricular esquerda patológica em doenças cardiovasculares e obesidade está associada ao aumento da fibrose e à redução da capacidade aeróbia, aumentando o risco de mortalidade e risco de doenças crônicas (POWELL et al., 2006).

Estudos experimentais com modelo animal são fundamentais na investigação da hipertrofia ventricular e dos mecanismos moleculares inerentes as doenças cardíacas. Determinar as funções das proteínas e suas interações representa um grande desafio na descoberta de biomarcadores terapêuticos em resposta a disfunções cardiovasculares e exercício físico. A proteômica pode ajudar a estudar a complexidade das proteínas e seu respectivo papel na saúde e função biológica (BURNISTON et al., 2011). Proteínas estão diretamente envolvidas em processos celulares, influenciando assim o fenótipo tecidual (BURNISTON et al., 2011). Uma das grandes inovações prometidas pelo conhecimento do proteoma, além do mapeamento das vias metabólicas específicas, é a possibilidade de

identificação de moléculas novas que podem ser utilizadas como alvos específicos ou como marcadores biológicos (BURNISTON et al., 2011).

A identificação dos mecanismos moleculares em resposta a dieta hiperlipídica por meio da comparação e análise diferencial da expressão proteica, denominada análise proteômica comparativa, pode fornecer informações valiosas para o diagnóstico; tratamento médico, bem como prognóstico. A análise proteômica tem sido amplamente aplicada em várias áreas da ciência como; no estudo de tumores, envelhecimento, exercício físico e recentemente nas doenças cardiovasculares (PETRIZ et al., 2015). Esta análise no VE da prole está na fronteira da ciência moderna e da tecnologia, fornecendo uma perspectiva global para a compreensão dos mecanismos moleculares complexos, que não são obtidas através da análise genômica (PETRIZ et al., 2015). Dessa maneira, a análise proteômica fornece informações mais abrangentes que podem esclarecer o fenótipo do VE da prole exposta a diferentes dietas.

1.5 Relação entre tendão calcâneo, obesidade e exercício físico

O tendão é constituído de tecido conjuntivo frouxo, denominado paratendão, que permite a livre circulação do tecido (MARQUETI et al., 2019). A segunda camada, é denominada epitendão, é contínua com o paratendão em sua superfície externa e, com o endotendão, na sua superfície interna, agrupando os endotendões de forma organizacional (MARQUETI et al., 2019). O endotendão envolve cada fibra individualmente e conduz vasos sanguíneos e nervos. Os tendões apresentam uma grande matriz extracelular (MEC), extremamente resistente a forças de tração e compressão (MARQUETI et al., 2019). A MEC é composta por aproximadamente 70% de água glicosaminoglicanos e proteínas importantes para a viscoelasticidade tendínea, como proteoglicanos, glicoproteínas e proteínas fibrosas, incluindo colágeno e elastina (MARQUETI et al., 2019).

Os movimentos articulares são provocados por meio da força de contração do tecido musculoesquelético, a qual é transmitida aos ossos pelos tendões. Esta junção mio-ósseo-tendinosa determina o nível de movimentação articular, sendo vital em todos os movimentos corpóreos (MARQUETI et al., 2019). Nesse processo, os tendões proporcionam armazenamento e transformação de energia elástica em mecânica, gerando economia de movimento e amplificação de potência durante o exercício físico

(MARQUETI et al., 2019). Os sinais mecânicos são traduzidos por meio das vias de sinalização molecular, que desencadeiam respostas adaptativas ao tendão (MARQUETI et al., 2019).

O tendão calcâneo é um dos mais importantes e resistentes tendões do corpo (PINGEL et al., 2013). Ele é formado pela união dos músculos gastrocnêmico e sóleo, com inserção no osso calcâneo (PINGEL et al., 2013). O tríceps sural apresenta função na articulação do joelho e tornozelo, participando dos movimentos de flexão do joelho e flexão plantar do tornozelo, apresentando importante função na marcha e em outras funções que envolvem o tornozelo e pé. Distúrbios agudos e crônicos neste tendão são responsáveis por até 50% de todas as lesões esportivas (BARIN et al., 2019).

Adicionalmente, vários estudos mostram que em indivíduos com sobrepeso e obesidade, os tendões frequentemente sofrem degeneração, podendo evoluir para um estágio sintomático, com dor e comprometimento funcional (ABATE et al., 2016). Os principais achados histopatológicos demonstram que estes indivíduos apresentam uma relativa escassez de colágeno, remodelamento prejudicado, e uma arquitetura da MEC desorganizada (ABATE et al., 2016). Tal comprometimento esta relacionado ao fato do tecido adiposo liberar vários peptídeos, hormônios bioativos e citocinas responsáveis por um estado de inflamação crônica no tendão (ABATE et al., 2016).

Por outro lado, o estilo de vida pode reduzir significativamente a degeneração do tendão e até mesmo melhorar a tendinopatia sintomática (WEARING et al., 2013). Portanto, o exercício físico é útil em indivíduos com sobrepeso ou obesidade (WEARING et al., 2013). Dados anteriores mostraram que o exercício físico promove respostas adaptativas fisiológicas, morfológicas, biomecânicas e bioquímicas (WEARING et al., 2013). Com relação à carga mecânica, é bem conhecido que o exercício exerce efeitos benéficos em regiões distintas dos tendões (MARQUETI et al., 2019). No entanto, a remodelação do tendão não é a mesma em diferentes regiões em relação à mesma aplicação de carga mecânica. Além disso, a intensidade da contração muscular é um elemento-chave nas respostas adaptativas do tendão (MARQUETI et al., 2019), o que justifica a importância do TF proposto na presente tese. O acúmulo de evidências oriundas de estudos em animais e humanos sugerem vários efeitos benéficos do exercício sobre o remodelamento do tendão, o que pode contribuir para as condições clínicas e desempenho tecidual (MARQUETI et al., 2019).

A renovação das proteínas é essencial para a saúde geral e manutenção do sistema musculoesquelético (BARIN et al., 2017). Nos tendões, estas moléculas também são essenciais na resposta ao exercício, tensão mecânica e remodelamento (BARIN et al., 2017). A proteômica integra as ferramentas ômicas nos levando a uma compreensão mais profunda das disfunções teciduais (BARIN et al., 2017). Nesse contexto, diversos atores moleculares e vias celulares foram incluídos na patogênese da obesidade e no tendão (ABATE et al., 2016). Ademais, o campo do exercício e das ciências do esporte também se beneficiou com o escopo das ômicas, especialmente com a proteômica.

O objetivo de construir uma biblioteca proteômica nos tendões da prole é auxiliar na descoberta de novas biotecnologias para o tratamento de doenças e lesões. A identificação e quantificação de proteínas-chave de diferentes organelas, processos biológicos e funções moleculares forneceriam novos *insights* sobre as vias moleculares envolvidas na herança intergeracional paterna. Uma compreensão mecanicista desses efeitos pode fornecer pistas cruciais para o desenvolvimento de abordagens terapêuticas para mitigar os distúrbios teciduais associados com a obesidade e sobrepeso.

1.6 Relação entretecido adiposo branco, obesidade e exercício físico

O tecido adiposo é o principal reservatório energético do organismo. As células deste tecido, os adipócitos, possuem a capacidade de armazenar triglicerídeos em quantidades correspondentes a 80- 95% de seu volume (KERSHAW et al., 2004). Devido a grande diferença de função e morfologia, os tecidos adiposos podem ser divididos em quatro tipos: tecido adiposo branco, marrom, bege e rosa (WRONSKA et al., 2012). O adipócito branco, quando totalmente desenvolvido, armazena os triglicerídeos em uma única e grande gota lipídica que ocupa a porção central da célula, deslocando o citoplasma, núcleo e demais organelas para a periferia (WRONSKA et al., 2012). Estes adipócitos brancos maduros são células que podem alterar acentuadamente seu tamanho em resposta a dieta hiperlipídica, conforme a quantidade de triglicerídeos acumulados (KERSHAW et al., 2004). A função deste adipócito é fornecer proteção mecânica, amenizando o impacto de choques e permitindo um adequado deslizamento de feixes musculares, uns sobre os outros, sem comprometer a sua integridade funcional (BJÖRNTORP, 1991). Além disso, por possuir distribuição mais abrangente, incluindo derme e tecido subcutâneo, é também considerado um excelente isolante térmico.

Os depósitos viscerais (intraperitoneais) compreendem principalmente: tecido adiposo epididimal (perigonadal), tecido adiposo mesentérico e o tecido adiposo retroperitoneal (WRONSKA et al., 2012). A utilização de modelos experimentais em roedores tem sido importante no estudo do tecido adiposo e obesidade. A grande maioria das investigações utilizam o tecido adiposo epididimal como referências para os tecidos viscerais (WRONSKA et al., 2012). O tecido adiposo epididimal é largamente usado como referência ao omento maior, contudo, é um depósito de gordura análogo ao tecido adiposo perigonadal em humanos (WRONSKA et al., 2012).

A obesidade está associada a alterações adversas no tecido adiposo epididimal que predispõem à desregulação metabólica (LEE et al., 2013). Essas alterações deletérias incluem, o acúmulo de macrófagos inflamatórios levando à ativação das vias de inflamação, redução do turnover de lipídeos e deposição de MEC em locais ectópicos (LEE et al., 2013). Essas alterações são precursoras do desenvolvimento de resistência à insulina e disfunção metabólica caracterizada na obesidade (LEE et al., 2013).

Nas últimas décadas, tornou-se cada vez mais claro que o tecido adiposo desempenha um papel significativo na regulação da sensibilidade à insulina e no metabolismo sistêmico (LEE et al., 2013). A distribuição do tecido adiposo, fibrose, estado inflamatório e a produção de adipocina, são todos prováveis determinantes do risco de doença metabólica e cardiovascular (LEE et al., 2013). Apesar do notável progresso recente neste campo, ainda existem muitas áreas que requerem uma investigação mais aprofundada, principalmente em relação a herança intergeracional. É necessária uma maior integração do papel das adipocinas com vias estabelecidas que regulam o metabolismo primário. A caracterização dessas vias mecanicistas é essencial não apenas para aumentar a compreensão da relação entre obesidade e tecido adiposo, mas também para desenvolver terapias eficazes que podem ter como alvo os eventos iniciais no desenvolvimento de distúrbios metabólicos e cardiovasculares na prole.

O exercício físico regular contribui para melhora da função mitocondrial e regulação das enzimas envolvidas no metabolismo dos ácidos graxos poli-insaturados (STANFORD et al., 2015). As mudanças induzidas pelo exercício no metabolismo dos adipócitos estão associadas a modificações na composição do tecido adiposo e remodelamento da MEC (STANFORD et al., 2015). O exercício afeta a liberação de adipocina do tecido adiposo e, portanto, pode atenuar a inflamação crônica e fibrose

relacionada a obesidade, além de melhorar a sensibilidade à insulina (TSILOULIS et al., 2015). Outra consequência do exercício é o fenômeno denominado "*beiging*", na qual é uma mudança fenotípica benéfica relacionadas a fatores termogênicos que melhoram a funcionalidade do tecido adiposo. Este processo é regulado pelas miocinas liberadas durante o exercício físico (STANFORD et al., 2016). Não obstante, o exercício proporciona muitos benefícios à saúde do tecido adiposo devido ao seu efeito no remodelamento da MEC (KAWANISHI et al., 2013). Recentemente, foi demonstrado que o efeito anti-fibrose no tecido adiposo mediado por exercício parece ser mais forte do que seu efeito na perda de peso, sugerindo que a inibição da fibrose não depende completamente do emagrecimento (LI et al., 2021).

2. OBJETIVOS

2.1. Geral

Investigar os efeitos de 8 semanas de treinamento de força realizado apenas pelo pai antes da fecundação sobre as adaptações moleculares no ventrículo esquerdo, tendão e tecido adiposo na prole exposta à dieta padrão e hiperlipídica.

2.2. Específicos

- Verificar os efeitos de diferentes modalidades de exercício paterno sobre os mecanismos epigenéticos no esperma, desenvolvimento do feto e da placenta, adaptações morfológicas, celulares e moleculares em vários sistemas fisiológicos da prole, bem como o desempenho físico, por meio de revisão narrativa.
- Avaliar os efeitos de 8 semanas de treinamento de força paterno sobre o perfil proteômico do ventrículo esquerdo da prole exposta à dieta padrão e hiperlipídica.
- Verificar os efeitos de 8 semanas de treinamento de força paterno sobre o perfil proteômico do tendão calcâneo da prole exposta à dieta padrão e hiperlipídica.
- Investigar os efeitos de 8 semanas de treinamento de força paterno sobre propriedades estruturais da MEC, perfil inflamatório e estado redox no tecido adiposo, bem como marcadores de estresse oxidativo e metabólicos na circulação da prole exposta à dieta padrão e hiperlipídica.

3. HIPÓTESE

O TF paterno pode modular uma “assinatura molecular” particular em cada tecido da prole exposta à dieta hiperlipídica. Além disso, esperamos encontrar diferenças nas adapatações moleculares da prole expostas a diferentes dietas.

4. MANUSCRITOS

4.1 Artigo científico publicado relacionado à tese:

“Impact of paternal exercise on physiological systems in the offspring”

Dados do periódico

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Impact of paternal exercise on physiological systems in the offspring

Ivo Vieira de Sousa Neto[1], Wagner Fontes[2], Jonato Prestes[3], Rita de Cassia Marqueti[1].

[1]Laboratory of Molecular Analysis, Graduate Program of Sciences and Technology of Health, Universidade de Brasília, Distrito Federal, Brazil
[2]Laboratory of Protein Chemistry and Biochemistry, Department of Cell Biology, Institute of Biological Sciences, Universidade de Brasília, Distrito Federal, Brazil
[3]Graduate Program of Physical Education, Universidade Católica de Brasília, Distrito Federal, Brazil

Corresponding authors
Ivo Vieira de Sousa Neto (ivoneto04@hotmail.com) and Rita de Cassia Marqueti (marqueti@unb.br) Campus Universitário, Centro Metropolitano 1, Conjunto A, Universidade de Brasília, UnB – Zip Code 72220-900. Tel.: +55 67 99871 4343.

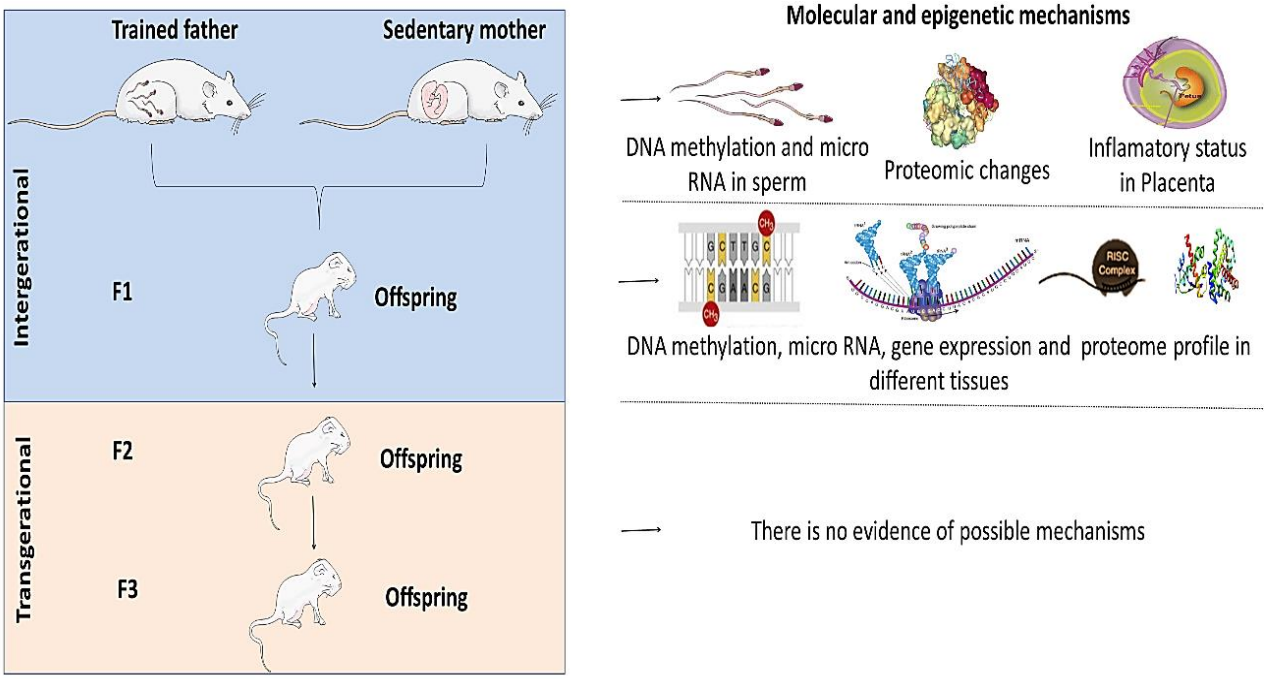
ABSTRACT

A significant number of studies have demonstrated that paternal exercise modulates future generations via effects on the sperm epigenome. However, comprehensive information regarding the effects of exercise performed by the father on different tissues and their clinical relevance has not yet been explored in detail. This narrative review is focused on the effects of paternal exercise training on various physiological systems of offspring. A detailed mechanistic understanding of these effects could provide crucial clues for the exercise physiology field and aid the development of therapeutic approaches to mitigate disorders in future generations. Non-coding RNA and DNA methylation are major routes for transmitting epigenetic information from parents to offspring. Resistance and treadmill exercise are the most frequently used modalities of planned and structured exercise in controlled experiments. Paternal exercise orchestrated protective effects over changes in fetus development and placenta inflammatory status. Moreover, paternal exercise promoted modifications in the ncRNA profiles, gene and protein expression in the hippocampus, left ventricle, skeletal muscle, tendon, liver, and pancreas in the offspring, while the transgenerational effects are unknown. Paternal exercise demonstrates clinical benefits to the offspring and provides a warning on the harmful effects of a paternal unhealthy lifestyle. Exercise in fathers is presented as one of the most logical and cost-effective ways of restoring health in the offspring and, consequently, modifying the phenotype. It is important to consider that paternal programming might have unique significance in the developmental origins of offspring diseases.

Keywords: physical activity**,** epigenetic inheritance, paternal transmission, offspring health.

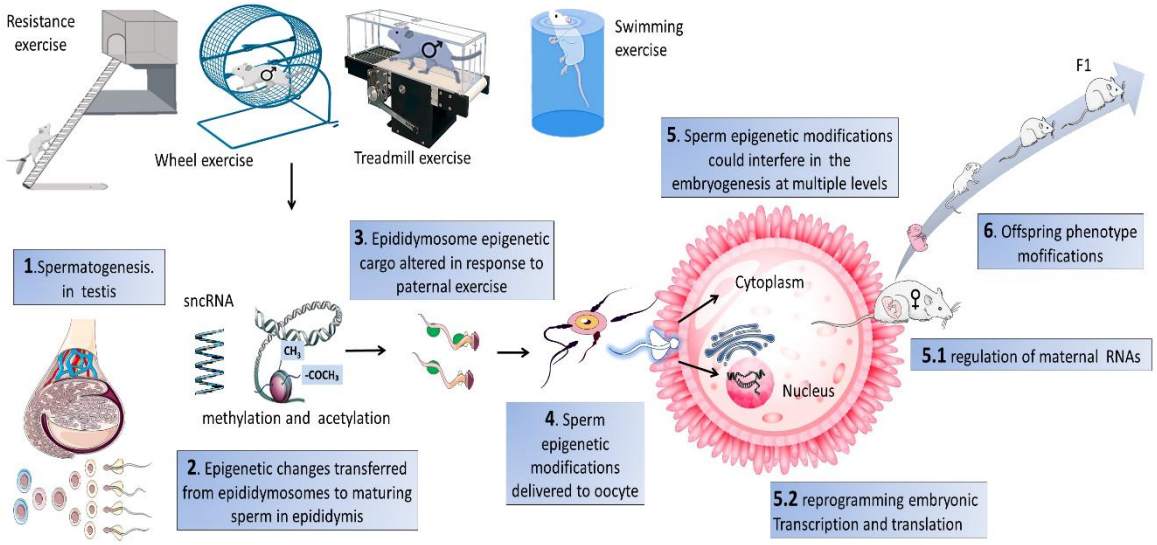
Paternal exercise on intergenerational or transgenerational programming

Figure 21: Propagation of paternal exercise effects to subsequent generations. Primary molecular and epigenetic responses to intergenerational and transgenerational inherence.



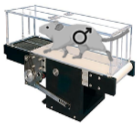
Paternal exercise modulates the offspring phenotype via changes in sperm epigenetic profile

Figure 2. Paternal exercise impacts sperm quality and epigenetic profile. Different exercise protocols in male mice (F0) can modify the sperm epigenome during the spermatogenesis process. Next, sperm epigenetic modifications are delivered to the oocyte, which could interfere in the embryogenesis process at multiple levels. Finally, these effects modulate offspring phenotype.



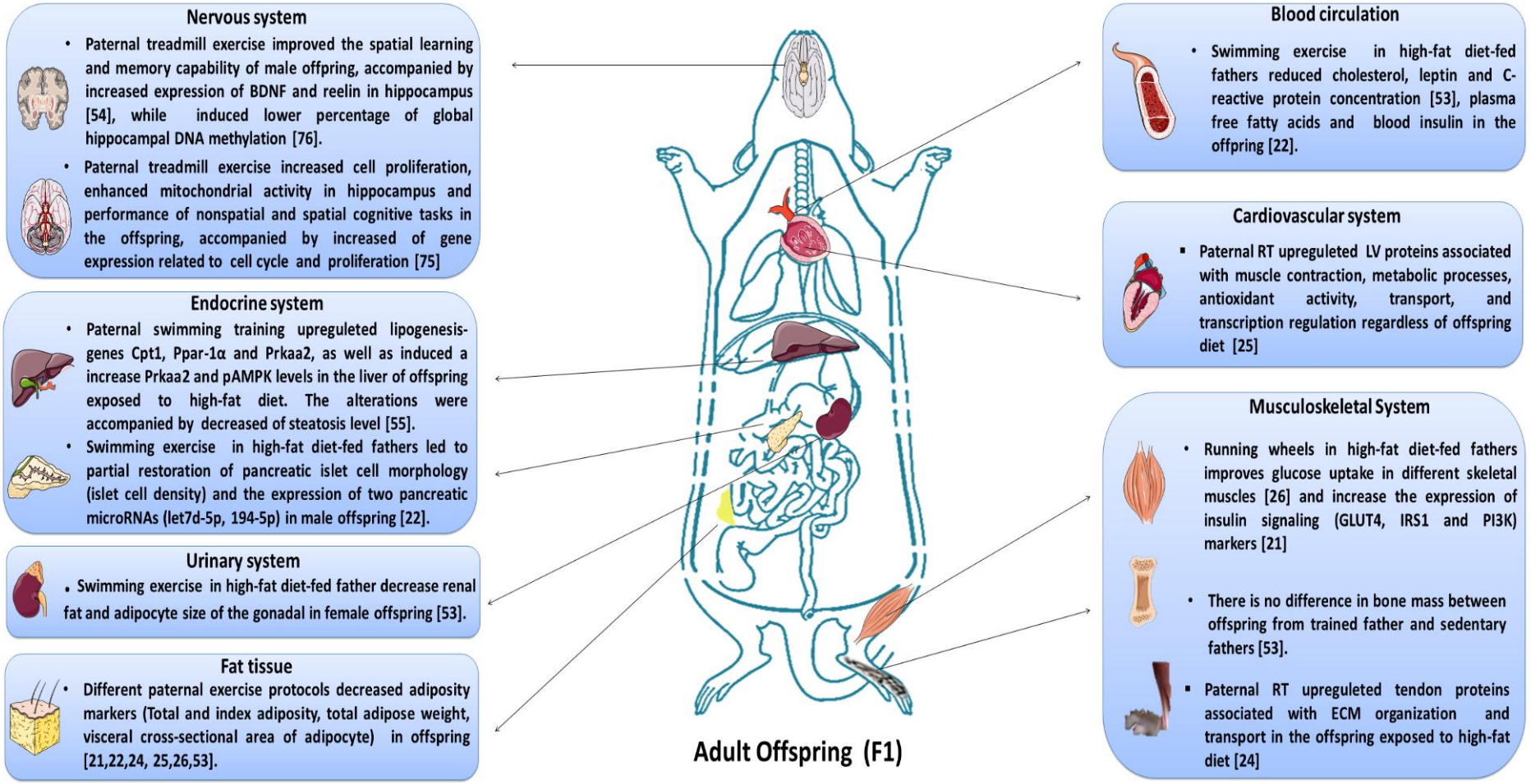
Classification of Paternal exercise protocols

Figure 3: Main paternal exercise protocols, research gaps, and future directions for new investigations.

	Resistance training	Voluntary wheel training	Treadmill training	Swimming training
				
Exercise Protocol	**Duration** : 8 weeks **Frequency** : 3 days/ week **Intensity** : 8 sets with 50, 75, 90 and 100% of maximal capacity; **Interval** : 120 s between sets	**Duration :** 3- 12 weeks **Frequency** : all day (5.8 – 7.0 km /day) **Intensity** :cannot be controlled	**Duration :** 3- 12 weeks **Frequency :** 5-6 days/ week **Intensity :** 12-15 m/min per 20-60 min/day **Slope** : 0°	**Duration** : 8 weeks **Frequency** : 3 days/ week **Intensity:** 30 min swim freely to simulate light exercise **Temperature**: 32 ± 1°C
Research Gaps	✓ Efficacy of short term protocols ✓ Effects on epigenetic markers	✓ Efficacy of different frequency and distance ✓ Optimal exercise dose	✓ Efficacy of HIIT vs. Continuous Cardiovascular Exercise ✓ Efficacy of diffrent slope	✓ Efficacy of Moderate and High intensity ✓ Efficacy of long term protocols
Future Directions	Assessments of epigenetic mechanism and effects of different types of RT	Establish the effectiveness of intensity, volume and recovery times	Access safety and efficacy of HIIT to improve offspring phenotype	News protocols with different intensity and durations

Effects of paternal exercise on physiological organ systems and different tissues in the offspring

Figure 4: Overview of the effects of paternal exercise on physiological systems in the offspring. Different exercise protocols might induce beneficial effects on distinct tissues and organs in the first generation (F1).



Conclusions and perspectives

The studies summarized in this review show that paternal exercise interventions can significantly alter offspring phenotype. Different exercise protocols might induce beneficial effects on distinct tissues and organs in the first generation, while the real transgenerational results are unknown. The lack of detailed understanding about the architecture and function of some tissues remains a challenge for further studies. Defining the specific mechanisms that mediate the positive effects of paternal exercise is an important step in developing optimized paternal exercise interventions to improve offspring health. An emerging body of compelling data demonstrates that paternal programming might have unique significance on the developmental origins of diseases. Creative approaches are still needed to identify specific epigenetic mechanisms over several generations in response to paternal exercise, and future human studies may be warranted to investigate this association. We believe that this review will pave the way for obtaining critical knowledge in this field. However, given the rapid development of research in this area, annual updates of this review are needed to keep pace with the latest findings regarding the relationship between paternal exercise and offspring.

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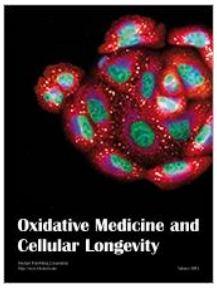
Author contributions

All authors contributed to article preparation; took part in drafting the article and critically reviewing for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

4.2 Artigo científico publicado relacionado à tese:



“Paternal resistance training induced modifications in left ventricle proteome independent of offspring diet”

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Paternal resistance training induced modifications in left ventricle proteome independent of offspring diet

Short Title: Paternal training and offspring heart proteome

Ivo Vieira de Sousa Neto[1], Ramires Alsamir Tibana[2,3], Jonato Prestes[2], Leonardo Gomes de Oliveira da Silva[1], Jeeser Alves Almeida[4,5], Octavio Luiz Franco[6,7], Edilamar Menezes de Oliveira[7], Fabricio Azevedo Voltarelli[2], João Luiz Quaglioti Durigan[1], Marcelo Valle de Sousa[8], Carlos André O. Ricart[8], Katyelle Ligia Rodrigues Botelho[8], Mariana S. Castro[8], Wagner Fontes[8], Rita de Cassia Marqueti[1].

[1]Graduate program of Sciences and Technology of Health, Universidade de Brasília, Distrito Federal, Brazil

[2]Graduate program of Physical Education, Universidade Católica de Brasília, Distrito Federal, Brazil

[3]Graduate program in Health Sciences, Universidade Federal do Mato Grosso, Cuiabá, Brazil

[4]Graduate Program in Health and Development in the Midwest Region, Faculty of Medicine, Universidade Federal do Mato Grosso do Sul, Campo Grande, Brazil.

[5]Research in Exercise and Nutrition in Health and Sports Performance - PENSARE, Graduate Program in Movement Sciences, Universidade Federal do Mato Grosso do Sul, Campo Grande, Brazil.

[6]Center for Proteomic and Biochemical Analyses, Graduate Program in Genomic Sciences and Biotechnology, Universidade Católica de Brasília, Distrito Federal, Brazil

[7]S-Inova Biotech, Graduate program in Biotechnology, Universidade Católica Dom Bosco, Campo Grande, Brazil;

[9] School of Physical Education and Sport, Universidade de São Paulo, São Paulo, Brazil

[8]Laboratory of Protein Chemistry and Biochemistry and Protein Chemistry, Department of Cell Biology, Institute of Biological Sciences, Universidade de Brasília, Distrito Federal, Brazil

Corresponding authors

Ivo Vieira de Sousa Neto (*ivoneto04@hotmail.com) and Rita de Cassia Marqueti (*marqueti@gmail.com) Campus Universitário, Centro Metropolitano 1, Conjunto A, Universidade de Brasília, UnB – Zip Code 72220-900. Tel.: +55 67 99871 4343.

ABSTRACT

Ancestral obesogenic exposure is able to trigger harmful effects in the offspring left ventricle (LV) which could lead to cardiovascular diseases. However, the impact of the father's lifestyle on offspring LV is largely unexplored. The aim of this study was to investigate the effects of 8 weeks of paternal resistance training (RT) on offspring left ventricle (LV) proteome exposed to control or high-fat (HF) diet. Wistar rats were randomly divided into two groups: sedentary fathers and trained fathers (8 weeks, 3 times per week with weights secured to the animals' tails). The offspring were obtained by mating with sedentary females. Upon weaning, male offspring were divided into 4 groups (5 animals per group): offspring from sedentary fathers, exposed to control diet (SFO-C), offspring from trained fathers, exposed to control diet (TFO-C), offspring from sedentary fathers, exposed to high-fat diet (SFO-HF) and offspring from trained fathers exposed to a high-fat diet (TFO-HF). The LC-MS/MS analysis revealed 537 regulated proteins among groups. Offspring exposure to HF diet caused reduction in the abundance levels of proteins related to cell component organization, metabolic processes and transport. Proteins related to antioxidant activity, transport and transcription regulation were increased in TFO-C and TFO-HF as compared with SFO-C and SFO-HF groups. Paternal RT demonstrated to be an important intervention capable of inducing significant effects on LV proteome regardless of offspring diet due to the increase of proteins involved into LV homeostasis maintenance. This study contributes to a better understanding of the molecular aspects involved in intergenerational inheritance.

Keywords: obesity, intergenerational, paternal programming, exercise training, heart proteome.

INTRODUCTION

Obesity is a chronic disease characterized by excessive fat deposition in adipocytes, while an interplay between many systems are implicated in obesity etiology, including unbalanced energy uptake, and energy expenditure, gene mutations, an aberrant gut microbiota and epigenetic factors [1]. Overweight and obesity rates have grown dramatically in most western countries, reaching more than 2 billion people worldwide [2]. Obesity has been associated with several deleterious effects, including functional disability, reduction in life expectancy and increased mortality [3,4]. Therefore, there is an urgent need to understand its causes in order to develop preventive strategies.

There is an alarming trend of overweight in very young children, including infants [5]. Several new lines of research explain that obesity and metabolic syndrome can be programmed before the uterine life of an individual through intergenerational inheritance [6]. Evidence from animal and human studies suggest that environmental perturbations, such as diet, stress, drugs before birth contribute to the development of permanent metabolic consequences in offspring, and result in negative effects on phenotype [7]. It is well established that paternal obesity is a risk factor for increased offspring adiposity, thereby predisposing to obesity [8]. The deleterious changes in metabolism are associated with glucose intolerance by dysfunction of pancreatic islets, with evident consequences in the first generation [9]. Offspring from obese fathers showed an increase of gonadal adiposity and the harmful effects of sperm metabolic function when compared to offspring from healthy fathers. These perturbed offspring phenotypes were associated with modifications of fathers´ sperm microRNA content [10].

Exercise training is a remarkable non-pharmacological strategy to prevent and treat weight gain, as well as contributing to reduce cardiovascular diseases risk, type 2 diabetes, cancers and general health parameters. Exercise is an important piece in the obesity puzzle, representing a potent regulator of intergenerational inheritance for health and disease risk [10]. Krout et al. [11] reported in mice that paternal preconceptional exercise (2 weeks of wheel running) prevented the increase of type 2 diabetes risk in the offspring after paternal HF diet. This protection may occur by increases in the expression of insulin signaling pathways in the skeletal muscle of the offspring. Moreover, McPherson et al.[10] demonstrated that aerobic paternal exercise (8weeks/swimming/3×week/30min) exerted positive effects on serum cholesterol levels, body composition and blood leptin concentration in offspring exposed to HF diet.

To note, ancestral obesogenic exposure is also able to trigger the onset and/or induce important changes in offspring left ventricle (LV) that could lead to cardiovascular diseases [12]. In rodent models of ancestral obesity, descendants showed an increased risk of myocardial dysfunction, such as LV hypertrophy and myocardial fibrosis [13]. Maternal HF diet exposure resulted in decreased cardiac function and compromised mitochondrial integrity in the LV, accompanied by increased lipid content in the next generation [14]. It has been demonstrated that children from obese female are at a greater risk for adverse cardiovascular outcomes and congenital heart defects [15] accompanied by increased risk of premature death [16]. However, the impact of the father's lifestyle on offspring LV adaptations remains poorly understood.

Although multiple studies determined that chronic exercise can be a crucial factor in intergenerational inheritance; the molecular mechanisms underlying cardiovascular benefits generated from exercise training remain to be investigated. In this sense, proteomics is an efficient technique with satisfactory precision that estimates a large number of proteins at the same time and represents an import method to clarify more profoundly the molecular networks behind physiological adaptations promoted by exercise training [17]. In a recent study, UPLC-MSE proteomics revealed modulation on LV proteome from rats submitted to resistance training (RT) [18]. The authors demonstrated that RT upregulates protein abundance levels related to metabolic processes, myofibril components, transporter and antioxidant activity on LV, which indicates that RT exerts important cardiac protective effects [18].

To date, we found no investigation regarding the effects of a paternal resistance training program in offspring proteomic profile. Furthermore, there is no proteomic study on LV in which offspring were exposed to HF diet. This information would be valuable to elucidate potential mechanisms induced by paternal training that could attenuate harmful alterations inherent to offspring high-fat diet. The global aim of this study was to investigate the effects of 8 weeks of paternal RT preconception on offspring LV proteome exposed to control and HF diet. We hypothesize that paternal RT upregulates protein abundance levels associated with myofibril components, metabolic processes, antioxidant activity, transport, nucleosome assembly, translation and transcription in the offspring. Moreover, we expect to find differences in offspring proteins abundance levels exposed to different diets.

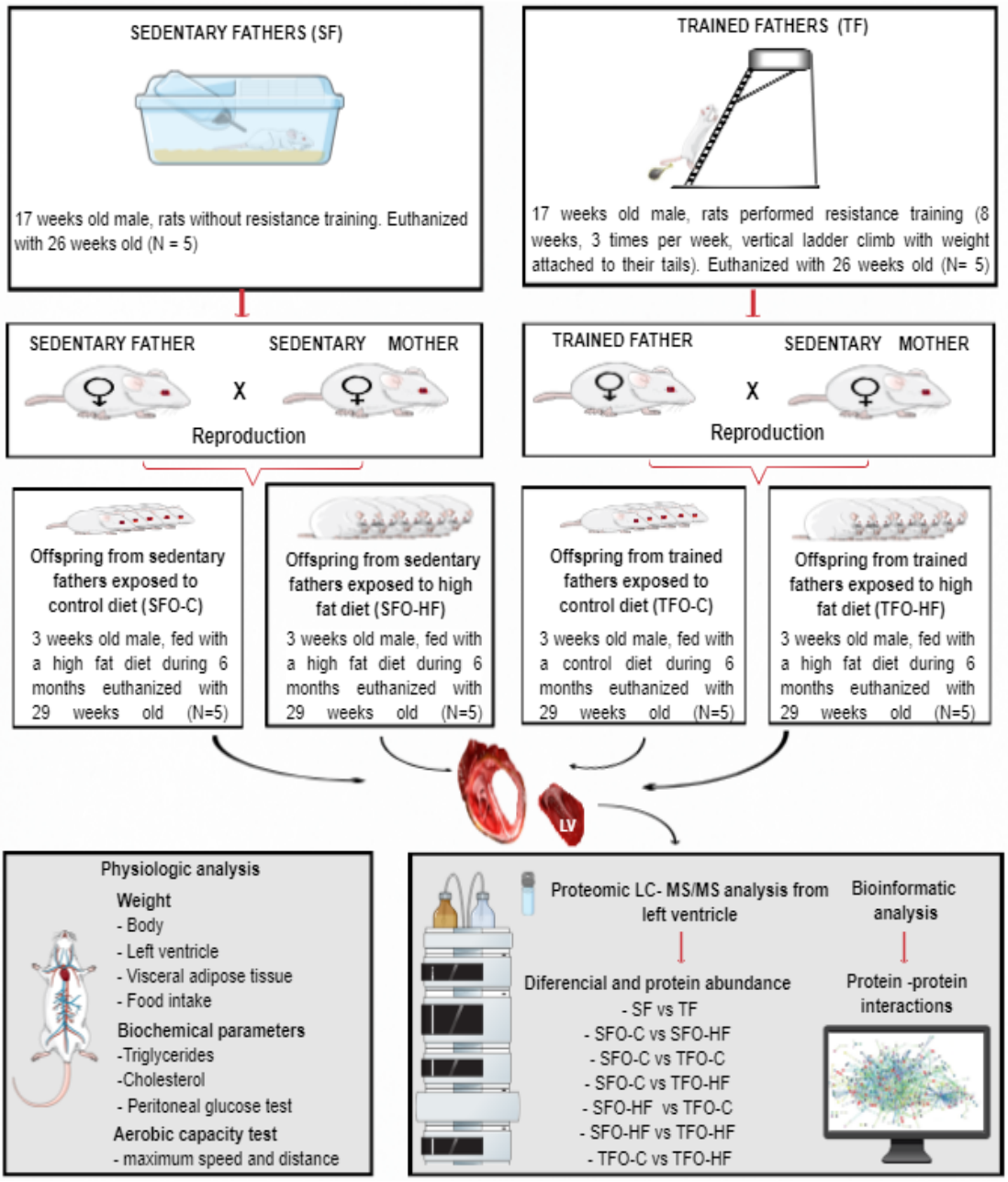
MATERIALS AND METHODS

Animals and grouping

All procedures were conducted in accordance with the USA Guide for care and use of laboratory animals [19]. The research protocol received approval from the Ethics Committee on Animal Experimentation from local University (protocol No. 010/13). Initially, 4-month-old male Wistar rats (*Ratus norvegicus albins*, weighing ± 376g) were placed in collective cages (maximal 4 rats per cage), and were randomly divided into two groups (5 animals per group): sedentary fathers (SF; did not perform RT) and trained fathers (TF; performed RT). The offspring were obtained by mating with sedentary females. After the 8 weeks of paternal RT, the estrous cycle of females was checked daily, and during proestrus phase, one male and one female were housed together for two consecutive days, during which they were allowed free access to a control diet (Purina®, Descalvado-SP, Brazil) and water. The experimental groups in this study were composed of 20 male pups. Litters were standardized among 5 pups each to avoid litters of disparate sizes, which were left together with their mothers until they were weaned at the age of twenty one days. Litters belonging to the same experimental group were offspring of different parents.

Upon weaning, male offspring were divided into 4 groups (5 animals per group): offspring from sedentary father exposed to control diet (SFO-C), offspring from trained father exposed to control diet (TFO-C), offspring from sedentary father exposed to high-fat diet (SFO-HF) and offspring from trained father exposed to high-fat diet (TFO-HF). All animals came from the Central Vivarium of the Faculty of Physical Education of the Catholic University of Brasilia. The animals were housed in polypropylene cages at a temperature of 23 ± 2 °C with 12:12h dark: light cycle. The offspring were weighed in grams and evaluated weekly for 6 months using a digital scale (Filizola®, São Paulo, Brazil). Study experimental design is showed in Figure. 1.

Figure 1. Experimental design. Schematic illustration of the methodological steps in the following study.



Offspring Diet

The offspring exposed to control diets (66.00% carbohydrates, 24.00% protein, and 10.00% lipids, totaling 3.48 Kcal/g) were fed with standard feed (Labina Presence®, Paulinia, São Paulo, Brazil) and water ad libitum. The SFO-HF and TFO-HF groups were exposed to HF diets commercially purchased (20.27% carbohydrates, 19.89% protein, and 59.38% lipids, totaling 5.20 Kcal/g) (Prag solutions®, Biosciences, Jau, Brazil) and overload of 200 ml of soft drink per week (100 % carbohydrates - 21 g , sodium:10 mg totaling 0.85 Kcal/g), and water ad libitum after the 21 day of birth, during 6 months.

Compositions of the experimental diets are compiled in Supplementary data 1. Previous studies demonstrated the effectiveness of this HF diet on body weight gain, adipose tissue weight gain and plasma lipids in Winstar rats [20, 21]. Females were also kept on a control diet (Labina Presence®, Paulinia, São Paulo, Brazil) throughout gestation and lactation. The offspring food were weighed weekly in grams, using a digital scale (Filizola®, São Paulo, Brazil) and food intake (amount offered – amount remaining in the cage) was monitored**.**

Supplementary data 1

Composition of the experimental diets

Nutrients	**Diets**	
	Standard chow g%	High-fat diet g%
Proteins	24	19.89
Lipids	10	59.38
Carbohydrates	66	20.27
Fiber (Celulose)	5.0	6.46
Mineral mix	3.7	12.9
Vitamin mix	1.9	1.29
Choline bitartrate	0.2	2.60
Lard	0.0	3.16
Soy oil	0.0	3.23
Total energy (kCal/g)	3.48	5.2

Source: The calculations of chemical composition were based on nutritional information sent by the supplier of the products

Paternal resistance training protocol

The exercise protocol was designed according to Hornberger and Farrar [22]. Training procedures were also described elsewhere [23-25]. During the 8 weeks, climbing sessions were performed 3 times per week. Prior to the training period, a RT adaptation protocol was administered, which required the animals to climb a vertical ladder (1.1 m × 0.18 m, 2-cm grid, 80 ° incline) with loads attached to their tails. The size of the ladder required the animals to perform 8–12 movements. The load apparatus was secured to the tail by wrapping the proximal portion of the tail with a self-adhesive foam strip. If

necessary, a stimulus was applied, with tweezers, to the animal's tail to initiate the movement. At the top of the ladder, the rats reached a housing chamber, where they were allowed to rest for 120 s. The rats performed 3 sections with a 48-h interval. The first RT session started 3 days after the familiarization.

The initial climb consisted of a load that was 75 % of the animal's body mass. After this, an additional 30 g weight was added until the rat could not climb the entire ladder with such load. Failure was determined when the animal could not progress up the ladder after 3 successive stimuli to the tail. The highest load successfully carried was considered to be the rat's maximal carrying capacity. After maximum load capacity, training sessions for fathers consisted of 8 ladder climbs with 2 sets of each load 50, 75, 90 and 100 % of their maximal carrying capacity interspersed by 120 s of intervals between each set.

Offspring aerobic capacity

Incremental-speed treadmill running was used for assessing the maximal aerobic capacity in offspring and was completed in the last two weeks of diet exposure. The test was adapted from Almeida et al. [26]. In order to minimize stress related to physical exercise during the test, an adaptation protocol was adopted. Before the test, the animals were initially familiarized with running on a treadmill designed for small animals (li 870, Letica Scientifi, Barcelona, Espanha) during 3 days with a constant speed of 13 $m.min^{-1}$ during 10 min. The test session started 2 days after the familiarization period. During the incremental exercise, the rats started running at a speed of 13 $m.min^{-1}$, followed by speed increments of 3 $m.min^{-1}$ every 3 min until they reached fatigue. Volitional fatigue was defined as the point at which the animals were no longer able to maintain their pace with the treadmill, even when exposed to light electrical stimulation for 10 s. The maximal aerobic capacity was established by the antecedent stage.

Offspring blood sample collection and biochemical analysis

At the end of the 24-week exposure to diet, offspring were fasted overnight for the evaluation of blood glucose and lipid profile. The intraperitoneal glucose tolerance test was performed via a small incision on the distal end of the animal's tail. Animals received an injection of 20% D-glucose (2 g/kg body weight) and approximately 5 μl of

tail blood were collected to measure the blood glucose concentrations at 15, 30, 60 and 120 min following glucose injection. Blood glucose, triglycerides and cholesterol were measured using a glucometer (ACCU CHECK- Active, Roche®, Mannheim, Germany), and their respective reagent tapes, according to the manufacturer´s recommendations.

Euthanasia

To avoid the acute effects of RT, the fathers were euthanized with an intraperitoneal injection of xylazine solution (12mg/ kg of body weight) and ketamine (95 mg/ kg of body weight) 48h after the end of the training period. The offspring were euthanized using the same combination of solutions after 24 weeks of exposure to diet. The LV and visceral adipose tissue of the offspring were dissected and immediately washed with saline. The samples were weighed, and frozen in liquid nitrogen and stored in a freezer at − 84 °C.

Left ventricle protein extraction

Proteomic analysis and bioinformatics tools were adapted from Cury et al. [27] according to the needs of our research. Approximately 100 mg of LV from fathers and offspring were macerated in liquid nitrogen and mechanically homogenized using ~~a~~ mortar and pestle. Posteriorly, the sample was added to a lyses solution containing 10% (w/v) trichloroacetic acid and 0.07% (v/v) β-mercaptoethanol in cold acetone; the resulting suspension was thoroughly mixed by vortexing, and incubated for 3 h at 4 °C. Next incubation, samples were centrifuged (10,000 g for 20 min at 4 °C). The supernatant was removed, and the pellet was washed five times with 10% (w/v) trichloroacetic acid in acetone until the disappearance of pigments. The pellet was dried using a concentrator and resuspended in rehydration solution (7 M urea, 2 M thiourea, 250 mM TEAB, pH 8.5). The Protein concentration from each extraction was assayed using Qubit® 2.0 (Invitrogen, Carlsbad, USA). The purity of protein extracted was assessed in gel 10% SDS-PAGE.

Protein digestion

The extracted proteins from LV (200 μg) were prepared for proteomic analysis, as described in Cury et al. [27]. The sample was quantified by Qubit® 2.0 (Invitrogen,

Carlsbad, USA) for analysis by nanoscale liquid chromatography coupled to mass spectrometer.

Nano LC-MS/MS analysis

The chromatography and mass spectrometry analysis was adapted from Curry et al. [27] The tryptic peptides were applied to Dionex Ultimate 3000 liquid chromatographer (Sunnyvale, USA) for reversed phase nano-chromatography. One microgram from sample was injected into a column (2 cm x 100 μm, containing C18 5 μm particles), connected to the analytical column (32 cm x 75 μm, C18 3 μm), and eluted to the ionization source of the spectrometer. The elution solution was consisted of 0.1% (v/v) formic acid in water (solvent A), and 0.1% (v/v) formic acid in acetonitrile (solvent B), in a gradient of 2% to 35% solvent B for 180 min.

The eluted fractions were sprayed in the ionization source of the LTQ Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Germany). Mass spectrometry analysis analysis was performed in data-dependent acquisition mode, when peptides were applied to the Orbitrap analyzer in the range of 300-1650 m/z. with a resolution of 120000 FWHM. The fifteen more intense ions were automatically submitted to high-energy collision-induced dissociation fragmentation through a an insulation window of 2.0 m/z, gain control of 5×10^6, normalized collision energy of 35% and the threshold for the selection of 3000.

Database search and label-free quantification

The files of the mass spectrometer were analyzed by software Progenesis QI [28] for alignment of the MS1 peaks found in the chromatograms. The peptide peaks were quantified and grouped. Proteins identification was achieved using Peaks 7.0 software [29], to perform sequencing and PSM database search from the fragmentation information. The database was downloaded from UniProt, filtered by *Rattus spp*. taxonomy. Precursor ion mass error tolerance of 10 ppm, MS/MS mass tolerance of 0.05 Da, carbamidomethylation of cysteine residues (fixed modification) and deamidation and methionine oxidation (variable modifications) were used as search parameters. The peak area of each MS1 ion was calculated and these values were used for the intensity calculations. The identified proteins were filtered at 1% for false discovery rate, and a minimum of one peptide per protein was required for identification. The inclusion criteria for identified proteins were the presence in at least four of five animals from each group.

The proteins were grouped in biological process class according to Petriz et al. [17].**Protein interactions analysis**

Protein interactions analysis was adapted from Cury et al. [27] according to the requirements of our study. Upregulated proteins were investigated using bioinformatics tools such as STRING 10.0 (Search Tool for the Retrieval of Interacting Genes / Proteins) [30]. Protein–protein interactions were performed using the medium confidence score (0.400) filtered with Rattus norvegicus database.

Statistical analysis

The results from proteomic analysis were presented according Petriz et al. [17]. Kolmogorov - Smirnov and Levene's tests were used to analyze the homogeneity of the variance. A two-way mixed ANOVA was used to compare body weight evaluated weekly for six months and serum glucose levels in the intraperitoneal glucose tolerance test. The Mauchly test verified compound sphericity. When the assumption of sphericity was not met, the significance of F-ratios was adjusted according to the Greenhouse-Geisser procedure. Independent t-test was used for comparison of protein abundance levels between father's groups. A two-way independent ANOVA (training vs. diet as factors) was used to compare food intake, tissues weight, triglycerides, cholesterol, aerobic capacity, and protein abundance levels between offspring groups. Tukey post hoc test was applied to identify the differences. Two-way ANCOVA was applied to determine whether there was an interaction effect between offspring diet and paternal exercise on protein abundance levels while controlling for covariates (body weight and tissues weight). Simple main effects were performed with Bonferroni adjustment. For the inter-group comparative analysis of protein abundance levels were considered up-regulated and down-regulated only the proteins with a fold change of at least 2 in Log(e) ratio between the treatments. An alpha threshold of 0.05 was considered for significance. The statistical package for social sciences (SPSS, Inc., v. 21.0; IBM Corporation, Armonk, NY, USA) was used for statistical analysis and GraphPad Prism 6.0 (San Diego, CA, USA) was used for graphics design.

RESULTS

Body and tissues weights of offspring groups

There was a significant interaction between intervention groups and weeks on body weight for male offspring. The main effects showed that RT prevented body weight gain in the TFO-HF (398.8 ± 8.1) group as compared with the SFO-HF group (433.9 ± 34.7) after 24 weeks of high-fat diet exposure ($p = 0.001$). There was no difference between TFO-C and SFO-C groups on body weight (Fig 2A).

Offspring exposed to HF diet displayed higher left ventricle weight as compared with the offspring exposed to control diet ($p = 0.001$). Furthermore, offspring left ventricle weight was modified by paternal training, as shown by decreased left ventricle weight in TFO-C and TFO-HF as compared with SFO-C and SFO-HF groups, ($p= 0.02$ and $p = 0.001$, respectively). Also, the SFO-HF group displayed higher left ventricle weight when compared with the TFO-C group ($p = 0.001$) (Fig 2B).

There was a significant interaction between paternal training and offspring diet for visceral adipose tissue weight. The SFO-HF group showed increased visceral adipose tissue when compared with the SFO-C and TFO-C groups, ($p = 0.001$ and $p = 0.001$, respectively). Moreover, the TFO-HF group demonstrated increased visceral adipose tissue weight compared with the TFO-C group ($p = 0.020$), and lower visceral adipose tissue as compared with the SFO-HF ($p = 0.001$) (Fig 2C)

Overall food intake and Feed efficiency ratio (%)

Offspring exposed to the HF diet consumed fewer grams of food overall when compared with offspring exposed to the control diet ($p = 0.001$). However, animals consuming an HF diet had an increase in overall caloric consumption ($p = 0.001$). There were no differences in food intake between offspring from sedentary father and offspring from trained father in both diets ($p > 0.05$) (Fig 2D). Offspring exposed to the HF diet showed a higher feed efficiency ratio when compared with animals exposed to the control diet (22% vs. 10%) (Fig 2E). Regarding soft drink intake, the SFO-HF and TFO-HF groups consumed 200 ml offered per week.

Biochemical parameters

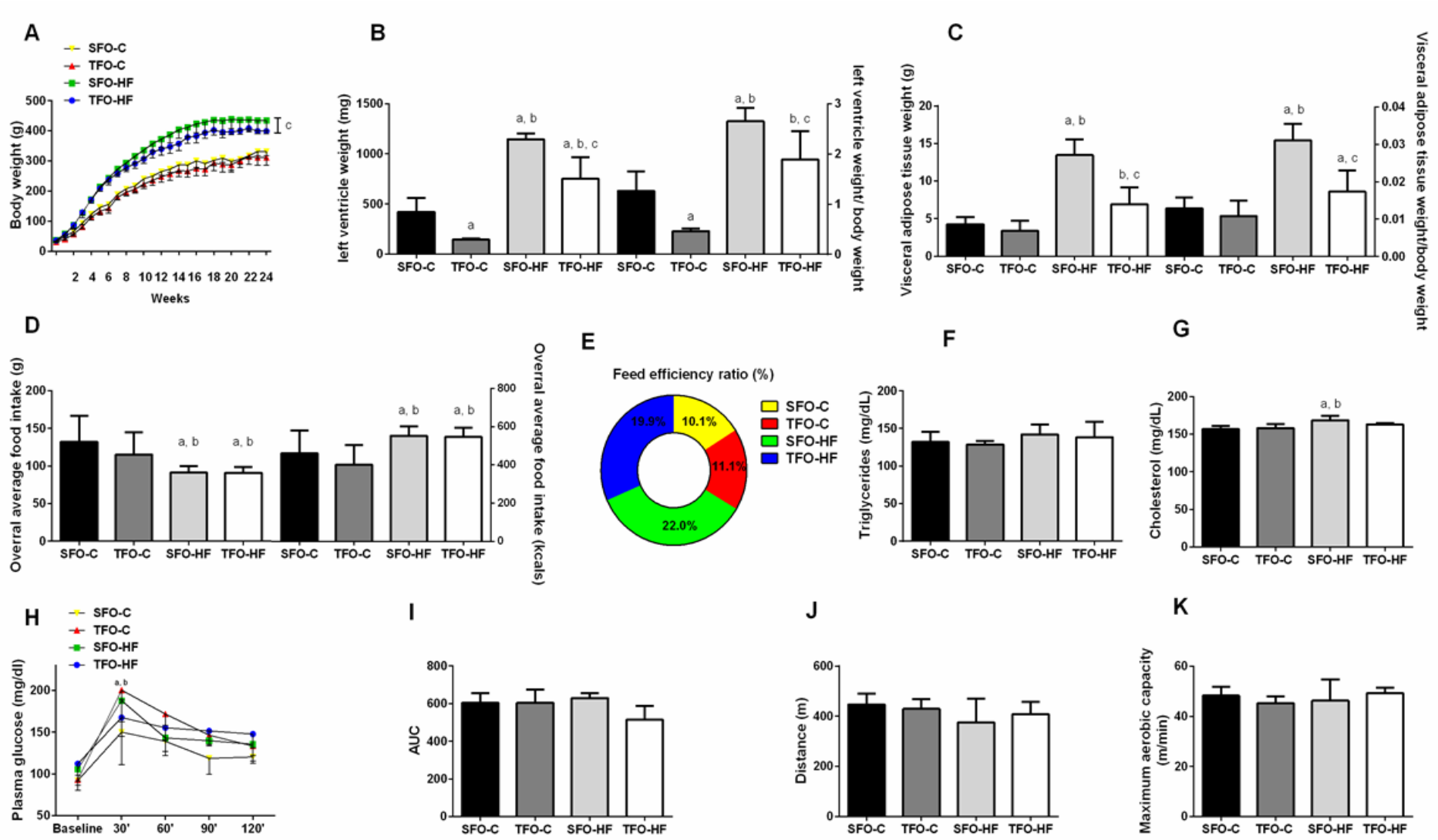
There was no difference between groups on serum triglycerides (Fig 2F). Serum cholesterol levels increased significantly in the SFO-HF group as compared with the SFO-C and TFO-C groups ($p = 0.001$ and $p = 0.01$, respectively), however there was no difference between SFO-HF and TFO-HF groups ($p > 0.05$) (Fig.2G).

The TFO-C group displayed higher levels of serum glucose after 30 min when compared with the SFO-C ($p = 0.001$). Besides, SFO-HF group showed increased levels of serum glucose after 30 min when compared with the SFO-C ($p = 0.001$) (Fig 2H). There were no differences in the area under the curve (AUC) of glucose response between groups ($p > 0.05$) (Fig 2I).

Offspring aerobic capacity

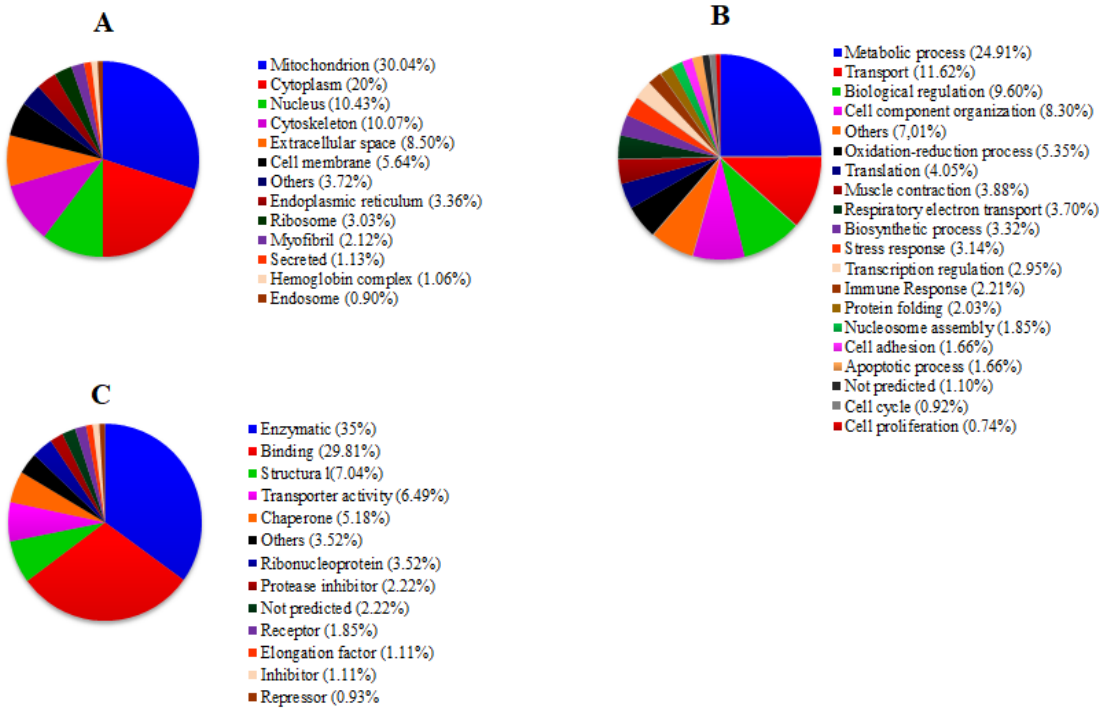
There was no change in maximal distance and aerobic capacity in the experimental groups ($p > 0.05$) (Fig. 2J-K).

Figure 2. Physiologic analysis in offspring exposed to control and high-fat diet.



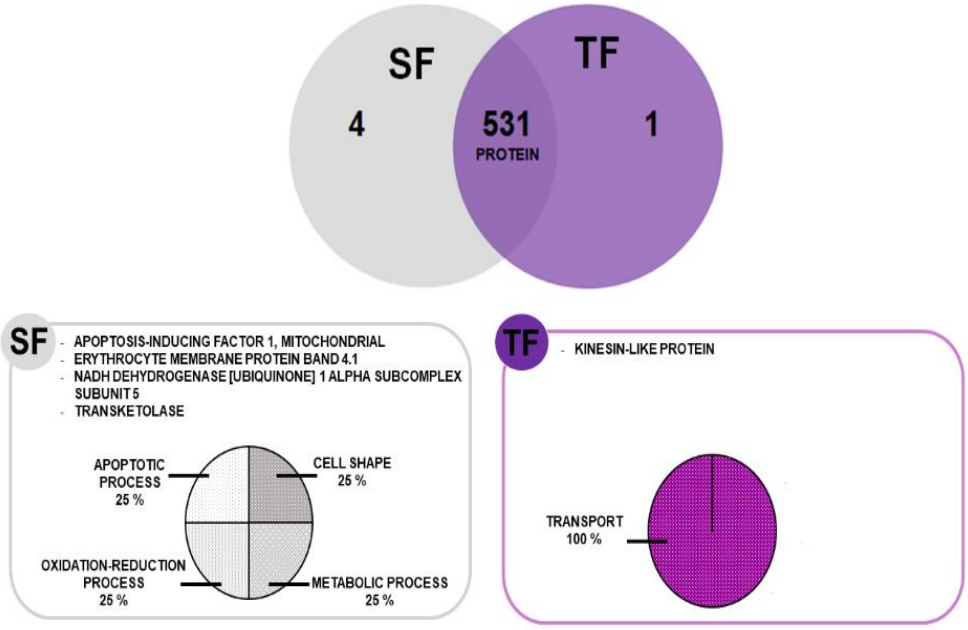
Functional proteome description

Data reported here revealed 557 proteins identified by LC-MS/MS analysis (supplementary data 2), however 536 proteins met the inclusion criteria and were classified according to UniProt database and Panther classification system in biologic process, molecular function and cellular localization. Supplementary data 3A shows the identified proteins according to their cellular localization (A), biologic process (B), and molecular function (C). In the present investigation, the majority of these proteins were derived from mitochondria (30.04%) followed by cytoplasm (20%), nucleus (10.43%) and cytoskeleton (10.07%) (Supplementary fig 1A) Additionally, the biological process classification indicated that these proteins were related to metabolic processes (24.91%), followed by transport (11.62%), biological regulation (9.60%), cell component organization (8.30%) and others (7.01%), (Supplementary fig 1B). Finally, the main molecular function activity observed was enzymatic (35%), followed by binding (29.81%), structural (7.04 %), transporter activity (6.49%), and chaperone activity (5.18%) (Supplementary fig 1C).

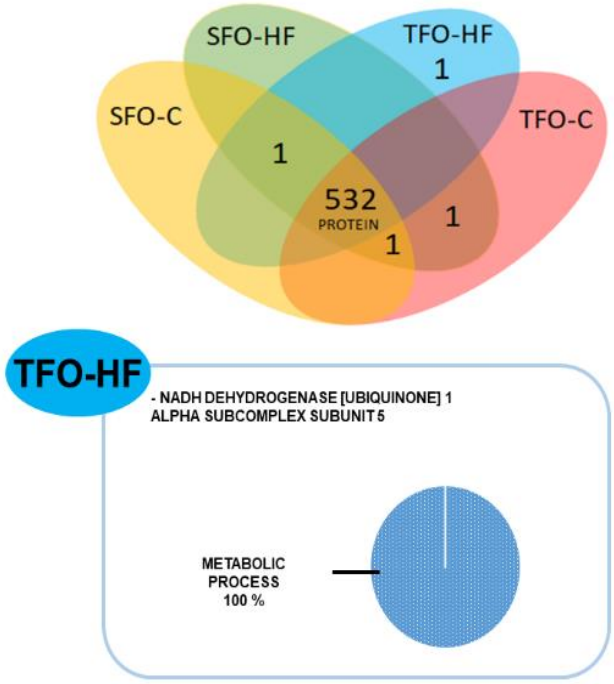


Venn diagram was used to show the distribution of the identified proteins into two experimental groups (Supplementary fig 2). From the 536 identified proteins, 531 were

common to all groups, while 4 proteins were identified only in the sedentary fathers group (SF) and 1 protein was identified only in the trained fathers group (TF). The identified proteins only in the SF group were mainly related to apoptotic process (apoptosis-inducing factor 1), cell shape (mitochondrial erythrocyte membrane protein band 4.1), oxidation-reduction process (NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5), and metabolic process (Transketolase). The protein identified only in the TF was associated with transport (kinesin-like protein).



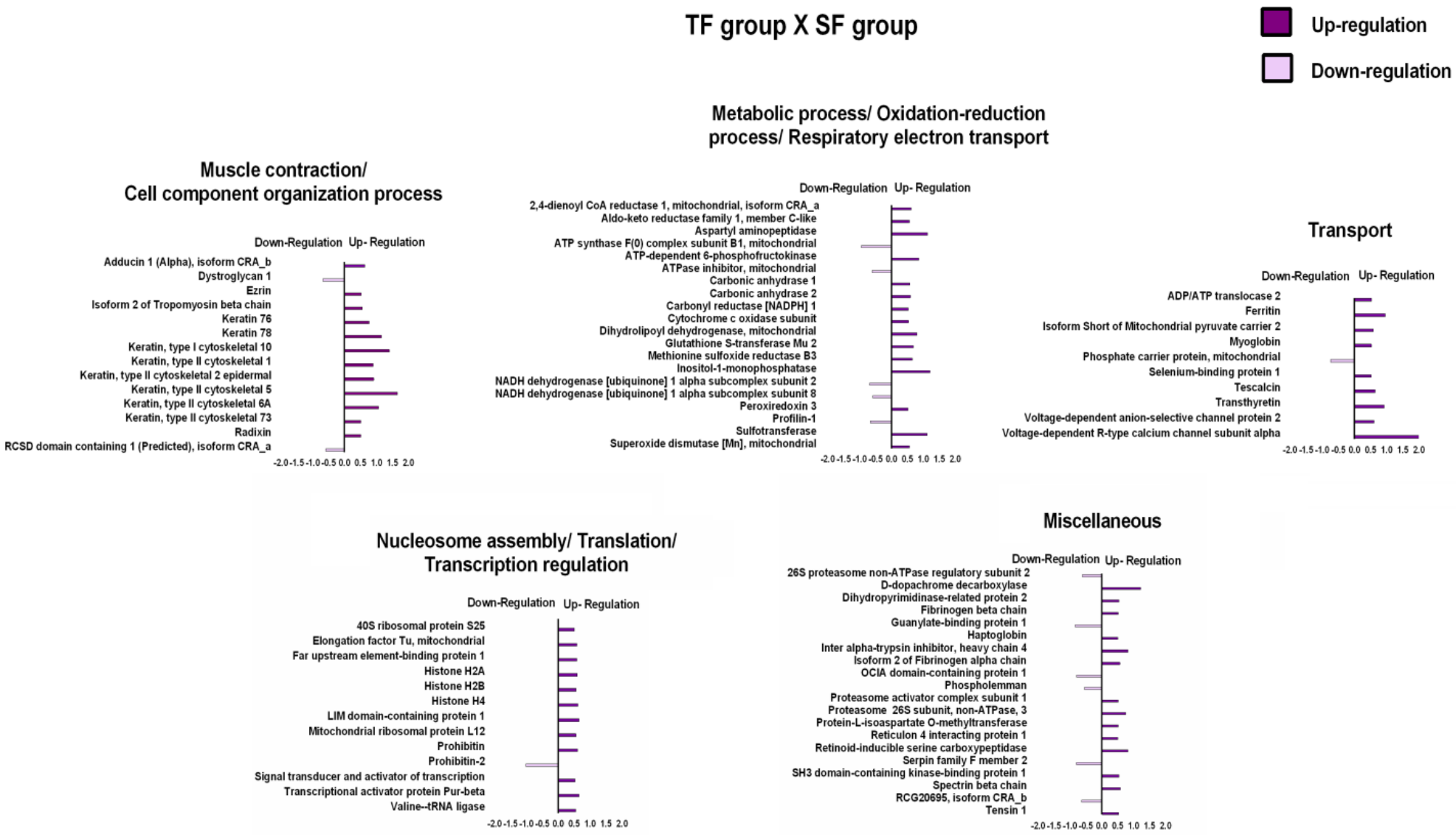
Regarding the four offspring groups, from the 536 identified proteins, 532 were common to all groups, with 1 protein identified only in the trained fathers' offspring exposed to high fat-diet group (TFO-HF). The identified protein exclusive to the TFO-HF group was related to metabolic process (NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5) (Supplementary figure 3).



Paternal left ventricle proteome

The effects of resistance training on paternal LV protein abundance levels were grouped according to their biological processes and are presented in Fig. 3. From TF group, abundance levels of 77 proteins were shown to be altered as compared with the SF group (62 proteins increased and 15 decreased). In this analysis (TF: SF), proteins were mainly related to muscle contraction and cell component organization (12 upregulated and 2 downregulated), metabolic process, respiratory electron transport and oxidation-reduction process (15 upregulated and 5 downregulated), transport (9 upregulated and 1 downregulated), nucleosome assembly, translation and transcription regulation (12 upregulated and 1 downregulated), and miscellaneous (14 upregulated and 6 downregulated).

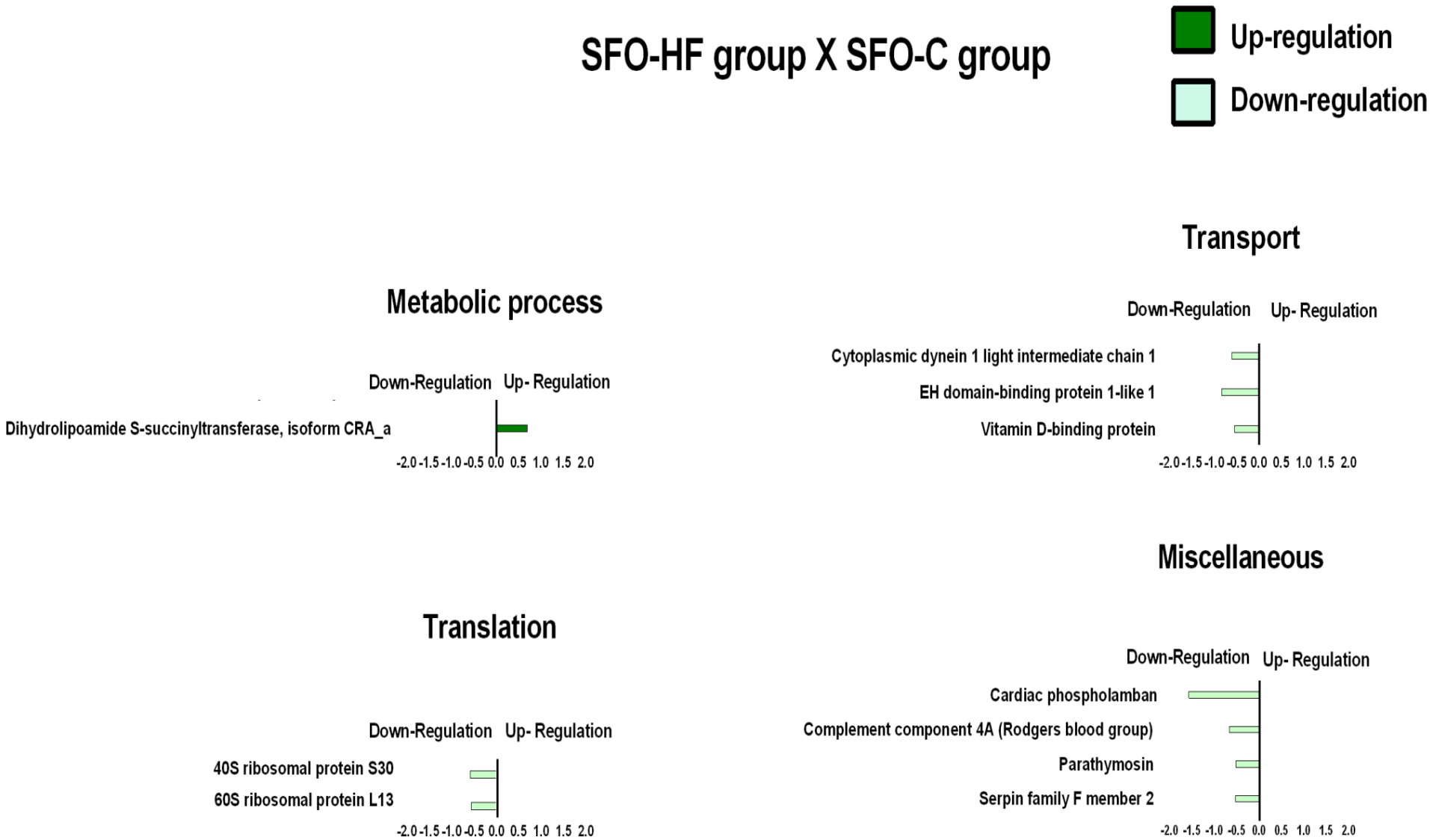
Figure 3. Effects of resistance training on LV proteome.



High-fat diet caused disturbance of proteins related to transport, translation and miscellaneous in the offspring

High-fat diet modifies an abundance of 10 proteins (1 protein increased and 9 decreased). In this analysis (SFO-HF: SFO-C), the proteins were mainly related to metabolic process, respiratory electron transport and oxidation-reduction process (1 upregulated), transport (3 downregulated), translation (2 downregulated), and miscellaneous (4 downregulated) (Fig 4).

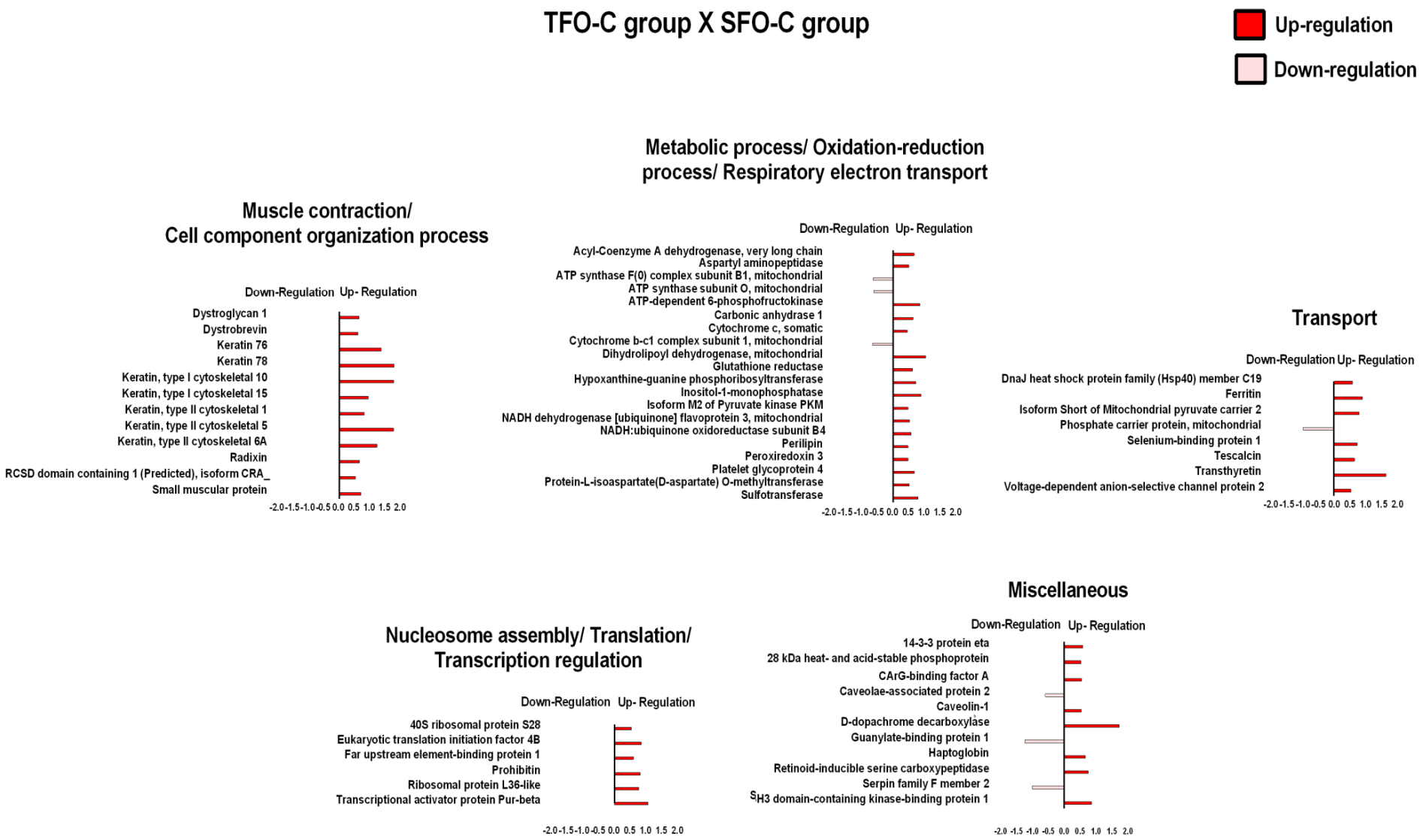
Figure 4. Effects of high-fat diet on LV proteome in the offspring.



Paternal RT modulates numerous biological pathways in left ventricle proteome of offspring exposed to control diet.

Paternal RT upregulates protein abundance related to myofibril components, antioxidant activity, transport, and transcription even when puppies are exposed to the standard diet. From the TFO-C group, abundance levels of 57 proteins were shown to be altered when compared with the SFO-C group (50 proteins increased and 7 decreased). In this analysis (TFO-C: SFO-C), proteins were mainly related to muscle contraction and cell component organization (12 upregulated), metabolic process, respiratory electron transport and oxidation-reduction process (17 upregulated and 3 downregulated), transport (7 upregulated and 1 downregulated), nucleosome assembly, translation, and transcription regulation (6 upregulated), and miscellaneous (8 upregulated and 3 downregulated) (Fig 5).

Figure 5. Effects of paternal resistance training on LV proteome in the offspring exposed to control diet



Paternal RT promoted the positive regulation of essential proteins associated to muscle contraction, antioxidant activity, transport, and transcription in the offspring exposed to a high-fat diet.

Most of the regulated proteins in TFO-HF presented an increased abundance rather than reduced when compared to other experimental groups. Most of the modulated proteins are associated with biological pathways related to the heart protection process.

The TFO-HF group displayed modified abundance levels of 70 proteins when compared with the SFO-HF group (67 proteins increased and 3 decreased) (Fig 6, table 1). Furthermore, the TFO-HF group, abundance levels of 36 proteins were shown to be altered when compared with the TFO-C group (31 proteins increased and 5 decreased), respectively. When the TFO-HF group was compared with the SFO-HF group, proteins were mainly related to muscle contraction, and cell component organization (5 upregulated), metabolic process, respiratory electron transport and oxidation-reduction process (23 upregulated and 3 downregulated), transport (8 upregulated), nucleosome assembly, translation, and transcription regulation (16 upregulated), and miscellaneous (15 upregulated) (table 1). There was statistically significant interaction between paternal training and offspring diet on 43 proteins whilst controlling for body and tissue weights (table 2). Moreover, when the TFO-HF group was compared with the TFO-C group, proteins were mainly related to muscle contraction and cell component organization (6 upregulated and 2 downregulated), respiratory electron transport and oxidation-reduction process (8 upregulated), transport (3 upregulated and 2 downregulated), nucleosome assembly, translation and transcription regulation (1 downregulated), and miscellaneous (14 upregulated and 1 downregulated). There was a statistically significant interaction between paternal training and offspring diet on 43 proteins while controlling for body and tissue weights (table 2). Values of the mean square, F, significance, partial eta squared, and observed the power of Two- way ANCOVA analyses are reported in Supplementary data 3.

Figure 6. Effects of paternal resistance training on LV proteome in the offspring exposed to high-fat diet.

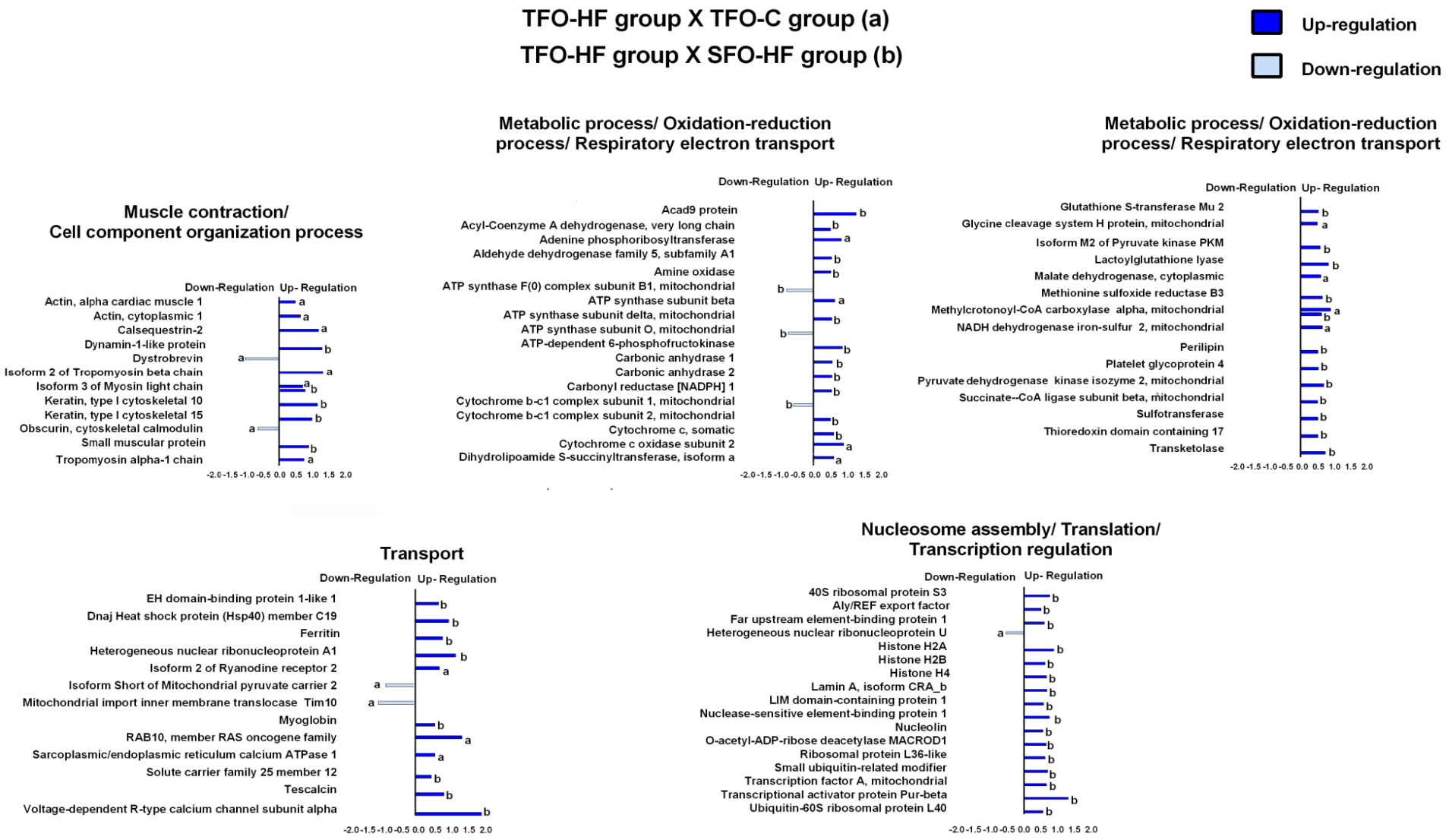


Table 1. Protein abundance levels related to miscellaneous functions from TFO-HF:TFO-C and TFO-HF: SFO-HF analysis considering only proteins with up-regulation and down-regulation ($p \leq 0.05$), with a delta (Δ) of at least ($\geq$) 0.5-fold change.

Protein description	Primary name	Biological process	p- value	Log (e) Fold change
TFO-HF:SFO-HF				
28 kDa heat and acid stable phosphoprotein	HAP28_RAT	Cell proliferation	0,0001	0,642709314
Annexin	ANXA1_RAT	Blood coagulation	0,0001	0,620131631
cAMP-dependent protein kinase inhibitor alpha	P63249|IPKA_RAT	Biological regulation	0,0001	0,551039992
Dynactin subunit 2	DCTN2_RAT	Cell cycle	<0,0001	0,518489876
Inter alpha-trypsin inhibitor, heavy chain 4	ITIH4_RAT	Inflammatory response	0,0003	0,782536172
Isoform 2 of G-protein-signaling modulator 1	Q9R080-2|GPSM1_RAT	Neurogenesis	0,0235	0,602697354
Kininogen-1	P08934|KNG1_RAT	Blood coagulation	0,0005	0,657253874
NSFL1 cofactor p47	NSF1C_RAT	Biological regulation	<0,0001	0,637752488
Protein disulfide-isomerase A6	PDIA6_RAT	Protein folding	0,0165	0,58285564
Protein-L-isoaspartate(D-aspartate) O-methyltransferase	PIMT_RAT	Protein repair	<0,0001	1,015385531
Protein phosphatase 2 , regulatory subunit A	Q5XI34_RAT	Cell cycle	0,0145	1,125946751
Retinoid-inducible serine carboxypeptidase	RISC_RAT	Biological regulation	0,0212	0,691632518
Serine proteinase inhibitor, clade H, member 1, isoform CRA_b	Q5RJR9_RAT	Biosynthetic process	0,0016	0,894924934
Serine/threonine-protein kinase mTOR	MTOR_RAT	Biological regulation	0,0027	0,774556009
Tensin 1	F1LN42_RAT	Cell adhesion	0,0157	0,517836179
TFO-HF:TFO-C				

Beta-2-microglobulin	B2MG_RAT	Immune Response	0,0028	-0,957888096
Bifunctional purine biosynthesis protein PURH	PUR9_RAT	Biosynthetic process	<0,0001	0,678632547
Cardiac phospholamban	PPLA_RAT	Biological regulation	<0,0001	-0,955260487
Caveolae-associated protein 3	CAVN3_RAT	Biological Rhythms	0,0002	-1,375582354
Eukaryotic translation initiation factor 4B	Q5RKG9|Q5RKG9_RAT	Biosynthetic process	0,011	-0,74609642
Fibrinogen beta chain	FIBB_RAT	Blood coagulation	0,0043	0,705823625
Guanylate-binding protein 1	GBP1_RAT	Immune Response	0,0291	1,253583879
Inter alpha-trypsin inhibitor, heavy chain 4	ITIH4_RAT	Inflammatory response	0,0007	0,620646553
Isoform 2 of Tyrosine-protein phosphatase non-receptor type 11	PTN11_RAT	Biological regulation	<0,0001	-0,612738686
Protein disulfide-isomerase A6	PDIA6_RAT	Protein folding	0,0137	0,617987716
Protein S100-A6	S10A6_RAT	Cell cycle	0,0025	-1,150239715
Reticulon 4 interacting protein 1	RT4I1_RAT	Neurogenesis	0,0114	0,747496638
Serine proteinase inhibitor, clade H, member 1, isoform CRA_b	Q5RJR9_RAT	Biosynthetic process	0,0009	1,181489284
Serine/threonine-protein kinase mTOR	MTOR_RAT	Biological regulation	0,0019	0,87608699

Table 2. P for interaction values between paternal training and offspring diet in protein abundance levels controlling body and tissues weights

Protein Description	Primary name	Biological process	p-value Interaction
Acad9 protein	B1WC61_RAT	Metabolic process	0.02
Amine oxidase	G3V9Z3_RAT	Metabolic process	0.01
Annexin	ANXA1_RAT	Blood coagulation	0.01
Aly/REF export factor	D3ZXH7_RAT	Transcription	0.02
ATP synthase subunit beta	G3V6D3_RAT	Metabolic process	0.03
ATP synthase subunit delta, mitochondrial	ATPD_RAT	Metabolic process	0.0001
Beta-enolase	ENOB_RAT	Metabolic process	0.003
Carbonyl reductase [NADPH] 1	CBR1_RAT	Metabolic process	0.0005
Cardiac phospholamban	PPLA_RAT	Biological regulation	0.001
CArG-binding factor A	Q9QX80_RAT	Biological regulation	0.002
Caveolae-associated protein 3	CAVN3_RAT	Biological Rhythms	0.01
Cytochrome b-c1 complex subunit 2, mitochondrial	QCR2_RAT	Respiratory electron transport	0.001
Dynamin-1-like protein	DNM1L_RAT	Cell component organization	0.01
Dystrobrevin	D4A772_RAT	Muscle contraction	0.03
EH domain-binding protein 1-like 1	A0A0G2K6R8_RAT	Transport	0.0003
Glutathione S-transferase Mu 2	GSTM2_RAT	Metabolic process	0.006
Guanylate-binding protein 1	GBP1_RAT	Immune Response	0.005

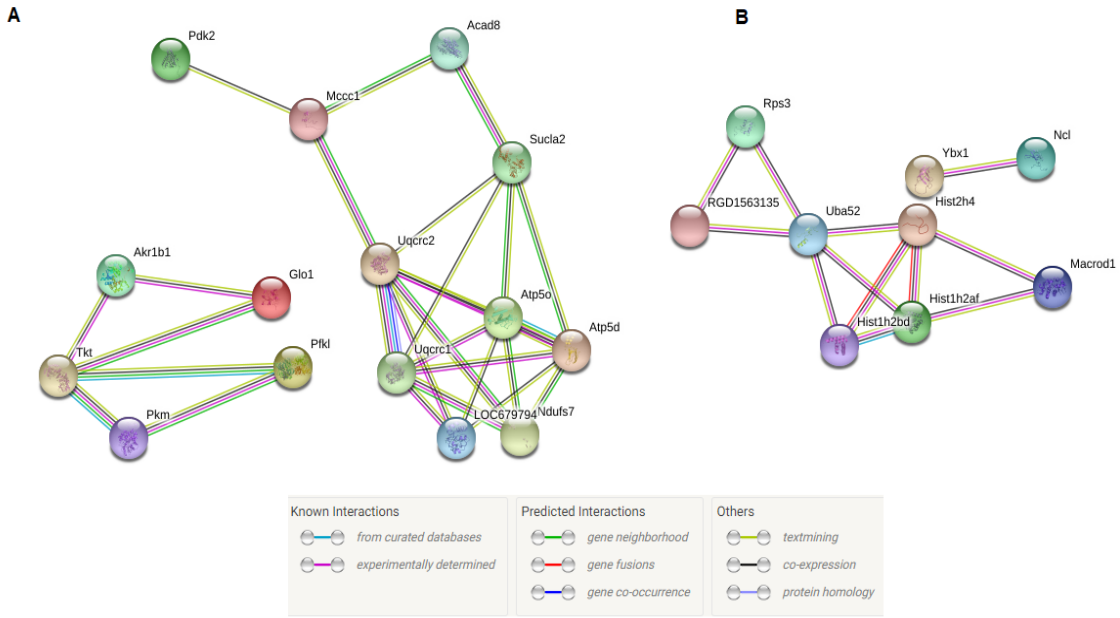
Heterogeneous nuclear ribonucleoprotein A1	ROA1_RAT	Transport	0.01
Histone H2A	D4ACV3_RAT	Nucleossome assembly	0.02
Histone H2B	H2B1_RAT	Nucleossome assembly	0.02
Inter alpha-trypsin inhibitor, heavy chain 4	ITIH4_RAT	Inflammatory response	0.003
Isoform 2 of G-protein-signaling modulator 1	GPSM1_RAT	Neurogenesis	0.04
Isoform 2 of Tyrosine-protein phosphatase non-receptor type 11	PTN11_RAT	Biological regulation	0.0005
Isoform Short of Mitochondrial pyruvate carrier 2	MPC2_RAT	Transport	0.04
Lactoylglutathione lyase	LGUL_RAT	Metabolic process	0.007
Lamin A, isoform CRA_b	G3V8L3_RAT	Chromatin organization	0.001
Methionine sulfoxide reductase B3	MSRB3_RAT	Oxidation-reduction process	0.003
Myoglobin	MYG_RAT	Transport	0.01
NSFL1 cofactor p47	NSF1C_RAT	Biological regulation	0.05
Phosphate carrier protein, mitochondrial	MPCP_RAT	Transport	0.03
Protein disulfide-isomerase A6	PDIA6_RAT	Protein folding	0.03
Protein-L-isoaspartate(D-aspartate) O-methyltransferase	PIMT_RAT	Protein repair	0.0002
Protein phosphatase 2 (Formerly 2A), regulatory subunit A (PR 65), alpha isoform,	Q5XI34_RAT	Cell cycle	0.001

Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 2, mitochondrial	PDK2_RAT	Metabolic process	0.04
RCSD domain containing 1 (Predicted), isoform CRA_a	A0A0G2K7I4_RAT	Muscle contraction	0.01
Ribosomal protein L10-like	RL10L_RAT	Translation	0.02
Ribosomal protein L36-like	RL36_RAT	Translation	0.01
Serine (Or cysteine) proteinase inhibitor, clade H, member 1, isoform CRA_b	Q5RJR9_RAT	Biosynthetic process	0.005
Serine/threonine-protein kinase mTOR	MTOR_RAT	Biological regulation	0.006
Succinate--CoA ligase [GDP-forming] subunit beta, mitochondrial	SUCB2_RAT	Metabolic process	0.001
Tescalcin	D3ZTN1_RAT	Transport	0.01
Transcription factor A, mitochondrial	TFAM_RAT	Transcription regulation	0.009
Tropomyosin alpha-1 chain	TPM1_RAT	Muscle contraction	0.05

Protein-protein interactions

STRING analysis revealed protein networks related to metabolic processes in TFO-HF:SFO-HF, showing high connectivity among oxidative stress protection, glycolysis, mitochondrial respiratory chain, and fatty acid metabolism proteins (Figure 7A). A prominent interaction network was found within proteins, such as methionine sulfoxide reductase B3, dihydrolipoyl dehydrogenase, glutathione S-transferase Mu 2, isoform M2 of pyruvate kinase PKM, and acyl-CoA dehydrogenase family member 9. We also found that upregulated proteins related to translation, nucleosome assembly, and transcription regulation interacted with each other, including histone H2A, histone H2B, histone H4, nucleolin, and 40S ribosomal protein S3 (Figure 7B). No significant protein networks were observed between the other groups and biological processes.

Figure 7. Protein-protein interaction analysis based on STRING with an interaction confidence score (0.400).



DISCUSSION

Pre-conceptional paternal RT upregulates protein abundance levels related to myofibril components, cell processes, metabolic processes, antioxidant activity, transport, nucleosome assembly, translation and transcription regulation on LV regardless of the offspring diet, which may be crucial for heart homeostasis and cellular function. In this context, overlapping proteins, which change in the same direction (i.e., up-regulation or down-regulation), were present in all of the three groups involved in RT (i.e., TF, TFO-C, and TFO-HF groups).

To our knowledge, decreased protein abundance of phospholemman and upregulation of spectrin beta chain in LV after RT are described for the first time in the present study. In the heart, phospholemman represents a direct molecular link between the regulation of Na^+/K^+ pump and $Na^+/Ca2^+$ exchanger activities in response to cellular signaling cascades mediated by kinases proteins [31]. When the heart is under stress, phospholemman enhances Na^+K^+ -ATPase activity minimizing risks of arrhythmogenesis, thereby at the expense of reduced inotropy [32]. Decreased phospholemman reduces Na^+K^+ pump activity, promoting contractile dysfunction [33]. The downregulation of this protein might represent an adaptation mechanism induced by RT to prevent inotropism reduction, likely as a compensatory process. Regarding the

spectrin beta chain, it has been reported as a crucial component in maintaining cardiac membrane excitability [34]. Spectrin beta chain downregulation is associated with heart failure and maladaptive cardiac remodeling [35]. However, this protein was upregulated by RT, which indicates regulatory effects on electrical functions in cardiomyocytes.

The RT provided significant structural and contractile proteins upregulation in fathers. The keratins overexpression can indicate a compensatory mechanism that maintained intercalated discs and epithelial tissue homeostasis [36]. The RT led to downregulation of respiratory electron transport chain proteins. Dantas et al. [18] found an overexpression of respiratory chain proteins on LV of rats after RT. However, used weighted aquatic jumps, which possibly has a more considerable aerobic component when compared to weighted stair climbing. This current result is similar to Schoepe et al. [37] that demonstrated decreased activity on respiratory chain complexes I and IV in the heart after 6 weeks of aerobic training. The authors demonstrated that cardiac oxidative capacity was further increased after 10 weeks of training, suggesting that this activity reduction is temporary. We might speculate that aerobic exercise can increase respiratory chain proteins in LV, while the RT beneficial effects would have a later onset.

In current study, both offspring from trained fathers showed a reduction in the abundance of three proteins related to respiratory electron transport chain (ATP synthase complex subunit B1, mitochondrial; ATP synthase subunit O, mitochondrial and Cytochrome b-c1 complex subunit 1) when compared with offspring from sedentary fathers, which suggests that paternal training modality may be crucial for a better understanding of the offspring adaptations. The lack of difference in distance and aerobic capacity in the incremental-speed treadmill running test might be due to the downregulation of these proteins. Future studies may wish to compare the effects of different exercise modalities (resistance, aerobic, and combined) on molecular mechanisms involved in intergenerational inheritance.

An important finding was that SFO-HF demonstrated a decrease on proteins related to muscle contraction, immune response and transport when compared with SFO-C group. The cardiac phospholamban protein plays an important role in cardiac contractility through calcium cycling, and is a crucial determinant of β-adrenergic stimulation [38]. Reduced of this protein might be related to myocardial dysfunction, increasing heart failure risk in the SFO-HF group. In addition, decreased complement component 4A and parathymosin proteins suggest a reduction in immune system

effectiveness, thus predisposing the animal to opportunistic infections [39, 40]. Finally, hypovitaminosis D plays an important role in the development of heart failure, myocardial infarction and arterial hypertension [41]. The decreased vitamin D-binding protein may indicate a transport deficit of vitamin D metabolites, leading to an increase of fatty acids polymerization, which could be detrimental in the circulatory system [42].

The TFO-HF group displayed higher protein abundance levels related to muscle contraction when compared to the TFO-C group. These findings suggest that the association between paternal resistance training and HF diet induces more significant LV structural adaptations than isolated HF diet. Although offspring exposure to HF diet may result in pathologic cardiac hypertrophy [43], paternal RT possibly normalize heart plasticity. Concomitantly, the proteins related to metabolic processes can be associated with elevated energy demands in cardiomyocyte due to paternal training and HF diet exposure. Otherwise, S100-A6 protein, junctophilin and cardiac phospholamban protein abundance levels were shown to be reduced in the TFO-HF group. Since these proteins play an import role is calcium homeostasis maintenance [44, 45].

It is worthy to point out that essential antioxidant proteins such as glutathione, methionine sulfoxide reductase, peroxiredoxin, ferritin, and albumin were upregulated in the three groups involved in exercise training (i.e., TF, TFO-C and TFO-HF groups) when compared with sedentary groups, which undergird the reliability and increase the importance of the findings. In other words, increased antioxidant defense induced by long-term paternal training can preserve cardiac mitochondria redox status, which is an important adaption in the first-line defense mechanisms against oxidative stress. Cellular reduction-oxidation balance plays a crucial role in heart function recovery and homeostasis. Reactive oxygen species (ROS) may contribute to the initiation and development of the pathological process, while an increase of anti-oxidants proteins can be important to maintain enzymatic operation in the heart metabolism, possibly preventing impairments in mitochondrial oxidative capacity [46].

The overproduction of ROS induced by HF diet, along with oxidant/antioxidant imbalance, can lead to oxidative stress and related tissue damage [46]. Previous studies have shown that RT is capable of upregulating nuclear factor erythroid-2-related factor 2 protein, the primary regulator of endogenous antioxidant defenses [47,48]. In this context, paternal RT can promote offspring cardioprotection via a redox-based mechanism against possible deleterious action of ROS in extreme stress situations. This fact would help to

prevent apoptosis, damage to cellular structures and cardiac injury. Considering these molecular findings, we can state that paternal RT can be used to produce greater key proteins regulation for cellular survival and energy metabolites homeostasis, which can minimize the deleterious effects of the HF diet and avoid major damages on LV. We hypothesize that these adaptations probably occur to prevent the cardiac dysfunction onset as an attempt to reinvigorate the heart. Relevantly, the TFO-HF group possibly has less vulnerable cardiomyocytes to cardiac insults and more capable of coping with oxidative stress due to an increase of several antioxidant and transport proteins involved in this protection process.

Paternal RT was also effective in preventing body and adipose tissue weight gain in the offspring exposed to HF diet. This result contrasts with Murashov et al. [49] that investigated the effects of voluntary wheel-running in C57BL/6J mice on their offspring's predisposition to insulin resistance and body weight. The authors showed that fathers subjected to wheel-running produced offspring that were susceptible to the unfavorable outcomes of an HF diet, displayed increased adiposity and impaired glucose tolerance. There are methodological explanations for discrepancy between the studies: different species, diet time exposure and training variables, which may attenuate beneficial effects. Another novelty was that paternal RT attenuated LV weight gain, which indicates that paternal RT can promote delay in pathologic hypertrophy.

Paternal RT did not result in decreased glucose AUC. Krout et al. [11] showed that paternal exercise was protective against insulin resistance by increasing the expression of insulin signaling markers in skeletal muscle resulting in normal T2D risk in offspring. The difference between the present study and Krout et al. [11], is the utilization of animals from different species. The C57Bl6/J lineage mice responds more pronouncedly to an HF diet, which can facilitate insulin resistance when compared to Wistar rats [50]. Also, a large amount of protein in an HF diet might delay the onset of insulin insensitivity [51]; however, the authors did not present the protein composition of the diet. Regarding the plasma lipid profile, paternal RT was not able to prevent any parameters. The effects of HF diet on lipid profile are controversial due to variations in the HF diet administered, moreover lipid profile are associated with the increase in unsaturated fatty acid and carbohydrate consumption and not only to HF diet [52].

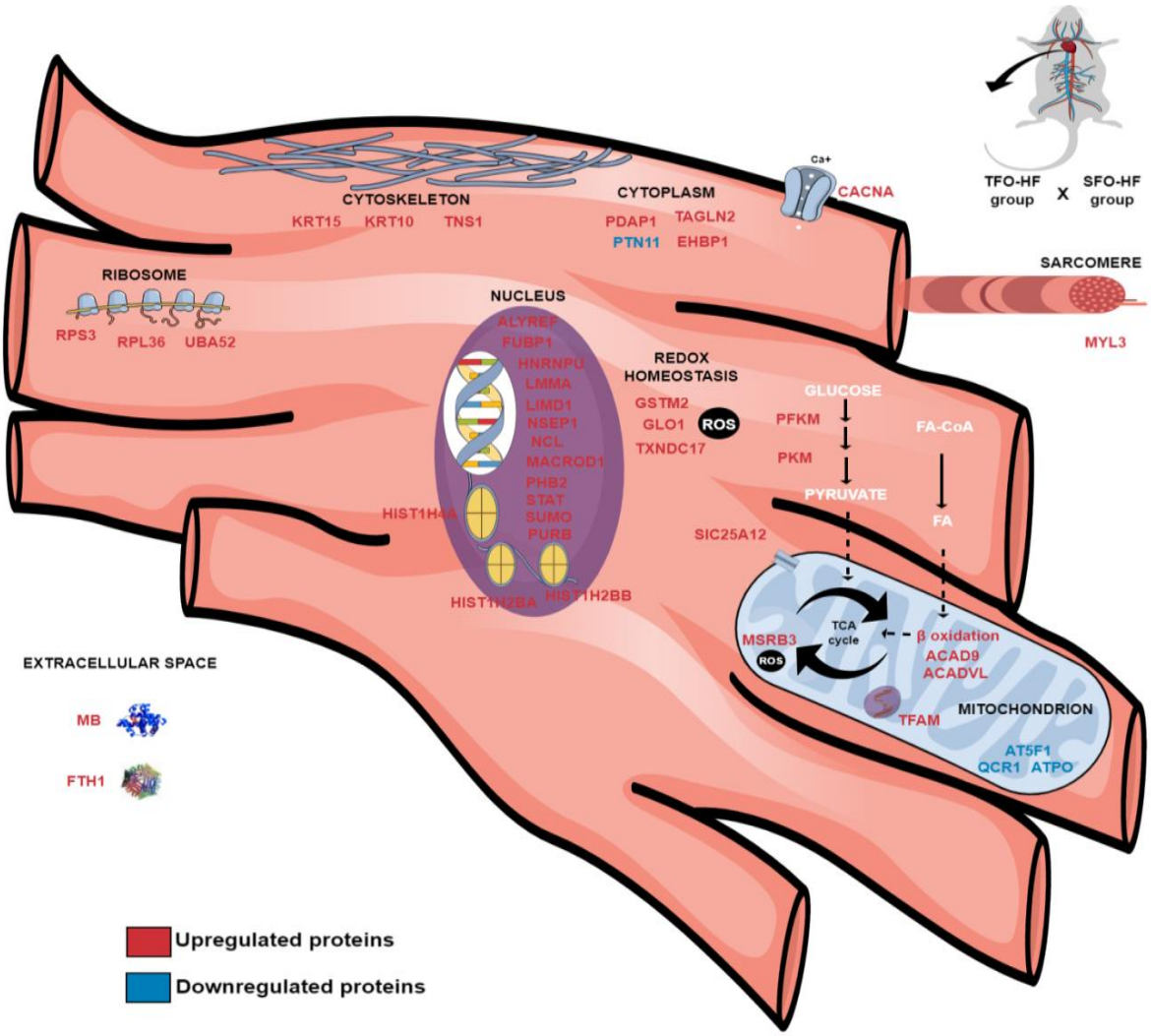
The Voltage-dependent R-type calcium channel (CACNA) increased in TFO-HF when compared with SFO-HF, which suggests that paternal RT can be potentially important for maintain normal contractility of the offspring heart. Molina-Navarro et al. [53] showed downregulation of CACNA in the patients with dilated cardiomyopathy when compared to healthy individuals, which suggests that a decrease of this channel may be related with pathological condition.

The TFO-HF: SFO-HF analysis detected more regulated proteins related to metabolic processes than any other comparison or biological process. In the normal heart, fatty acid oxidation accounts for 60% of myocardium energy demand, with the remaining provided by glucose and pyruvate metabolism [54]. The increased fatty acid uptake and concomitantly decreased glucose metabolism results in larger total production of ATP, but a reduction in the amount of ATP produced per mole of oxygen consumed [55]. In the heart failure, the metabolic switch towards favoring glucose oxidation over fatty acid oxidation had been considered a maladaptive change [56]. However, glucose-dependence is not, apparently, harmful in adult hearts and decreased glucose utilization seems to be deleterious in heart failure [56]. Our findings suggest that paternal RT might improve offspring cardiomyocytes energy efficiency.

Essential proteins related to transporting molecules involved in metabolism and ions bioavailability, were increased in TFO-HF group. The myoglobin regulates mitochondrial O_2 supply and diffusion, contributing to the protection of the respiratory chain, moreover balancing the nitric oxide level in the cardiomyocytes [57]. Ferritins contribute to maintenance of iron concentrates for cofactor syntheses and sequestration of iron from invading pathogens [58]. Solute carrier family 25 member 12 is important for calcium-binding mitochondrial carrier protein and reduced glucose-induced oxidative metabolism [59]. These molecular findings suggest that paternal RT can be relevant for cellular survival and energy metabolites homeostasis.

The offspring from trained fathers increased the histone proteins when compared with offspring from sedentary fathers. Paternal RT might be linked to a physiological status more prone to programming transcription [60]. Our findings demonstrated higher protein abundance levels associated with nucleosome assembly, translation and transcription regulation on TFO-HF group. An illustration of cardiomyocyte was used to clarify the location, the up and downregulation of main proteins in the TFO-HF:SFO-HF analysis (Fig. 8).

Figure 8. Modulation in cardiomyocyte proteome



Some limitations of the present study should be highlighted, such as the impossibility to analyze immunoblot analysis of key proteins, gene expression, morphology properties of LV, and ventricular functional assessments. In addition, future studies aim to define sperm epigenetic status of father. Lastly, microRNAs are epigenetic mechanisms capable of influencing offspring phenotype [10, 49].

CONCLUSIONS

It was identified that the father's lifestyle and offspring diet significantly modified the LV proteome, distinctly altering protein abundance levels. The offspring HF diet led to decreased protein abundance levels related to cell component organization, immune response, transport and translation, which may potentiate LV function worsening. On the

other hand, the beneficial effects of paternal RT on LV proteome are independent of offspring diet. Proteomic analysis demonstrated that paternal RT is a critical factor capable to reprogramming offspring LV proteins associated with muscle contraction, metabolic processes, antioxidant activity, transport, and transcription regulation. The present research provides valuable insights into the molecular mechanisms involved in paternal intergenerational inheritance.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Data in Supplementary Information Files

The Supplementary data 2 used to support the findings of this study are included within the article.

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FIGURE LEGENDS

Fig. 1 Experimental design. Schematic illustration of the methodological steps in the following study.

Fig. 2 Physiologic analysis in offspring exposed to control and high-fat diet. Body weight (A) , Left ventricle weight (B), Visceral adipose tissue weight (C),Overal average food intake in grams and Kcals (D), Feed efficiency ratio (E),Triglycerides (F), Cholesterol (G), Plasma glucose (H), The total area under the curve in the intraperitoneal glucose tolerance test (H), Distance (J), Maximum speed in the aerobic capacity test (K). Values are presented as means ± SD. SFO-C = offspring from sedentary fathers, exposed to control diet; TFO-C = offspring from trained fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. Statistically significant differences compared to: [a] SFO-C; [b] TFO-C; [c] SFO-HF, $p \leq 0.05$. (n = 5 per group).

Fig 3. Effects of resistance training on LV proteome. Histogram of protein abundance levels from intergroup analysis considering only proteins with down-regulation (light purple) and up-regulation (Purple) ($p \leq 0.05$), with a delta (Δ) of at least ($\geq$) 0.5-fold change. SF = sedentary fathers; TF = trained fathers. The X-axis represents the Log(e) ratio between the treatments (**TF:SF ratio**). All altered proteins are grouped according to their biologic process as noted in Gen Ontology (GO). Muscle contraction and cell component organization, Metabolic process, Oxidation-reduction process and respiratory electron transport, transport, nucleosome assembly, translation and transcription regulation and miscellaneous.

Fig 4. Effects of high-fat diet on LV proteome in the offspring. Histogram of protein abundance levels from intergroup analysis considering only proteins with down-regulation (light green) and up-regulation (green) ($p \leq 0.05$), with a delta (Δ) of at least ($\geq$) 0.5-fold change. SFO-C = offspring from sedentary fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet. The X-axis represents the Log(e) ratio between the treatments (**SFO-HF: SFO-C ratio**). All altered

proteins are grouped according to their biologic process as noted in Gen Ontology (GO). Metabolic process, transport, translation and miscellaneous.

Fig 5 Effects of paternal resistance training on LV proteome in the offspring exposed to control diet. Histogram of protein abundance levels from intergroup analysis considering only proteins with down-regulation (light red) and up-regulation (red) ($p \leq 0.05$), with a delta (Δ) of at least ($\geq$) 0.5-fold change. SFO-C = offspring from sedentary fathers exposed to control diet; TFO-C = offspring from trained fathers exposed to control diet. The X-axis represents the Log(e) ratio between the treatments (**TFO-C: SFO-C ratio**). All altered proteins are grouped according to their biologic process as noted in Gen Ontology (GO). Muscle contraction and cell component organization, Metabolic process, Oxidation-reduction process and respiratory electron transport, transport, nucleosome assembly, translation and transcription regulation, miscellaneous.

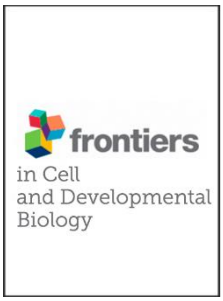
Fig 6. Effects of paternal resistance training on LV proteome in the offspring exposed to high-fat diet. Histogram of protein abundance levels from intergroup analysis considering only proteins with down-regulation (light blue) and up-regulation (blue) ($p \leq 0.05$), with a delta (Δ) of at least ($\geq$) 0.5-fold change. TFO-C = offspring from trained fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. The X-axis represents the Log(e) ratio between the treatments **(a: TFO-HF:TFO-C ratio, b: TFO-HF:SFO-HF ratio**). All altered proteins are grouped according to their biologic process as noted in Gen Ontology (GO). Muscle contraction and cell component organization, Metabolic process, Oxidation-reduction process and respiratory electron transport, transport, nucleosome assembly, translation and transcription regulation.

Fig 7. Protein-protein interaction analysis based on STRING with an interaction confidence score (0.400). SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet STRING analysis for differentially abundant proteins upregulated in the analysis (TFO-HF: SFO-HF). Red highlighted nodes are related to metabolic processes including oxidative stress protection,

glycolysis pathway, mitochondrial respiratory chain and fatty acid metabolism proteins (Figure 7A) and to translation, nucleosome assembly and transcription regulation (Figure 7B).

Fig. 8 Modulation in cardiomyocyte proteome. SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. The main proteins upregulated (red) and downregulated (blue) in the analysis (TFO-HF: SFO-HF). Cell membrane - Voltage-dependent R-type calcium channel subunit alpha (CACNA). Cytoplasm - 28 kDa heat and acid stable phosphoprotein (PDAP1), ATP-dependent 6-phosphofructokinase (PFKM), EH domain-binding protein 1-like 1 (EHBP1), Pyruvate Kinase (PKM). Cytoskeleton - Keratin, type I cytoskeletal 10 (KRT10), Keratin, type I cytoskeletal 15 (KRT15), Tensin 1 (TNS1). Extracellular space – Ferritin (FTH1), Myoglobin (MB). Isoform 2 of Tyrosine-protein phosphatase non-receptor type 11 (PTN11), Mitochondrion - Acyl-CoA dehydrogenase family member 9 (ACAD9), Acyl-CoA dehydrogenase, very long chain (ACADVL), ATP synthase F(0) complex subunit B1 (AT5F10, ATP synthase subunit O (ATPO), Cytochrome b-c1 complex subunit 1, mitochondrial (QCR1), Methionine sulfoxide reductase B3 (MSRB3), Succinate--CoA ligase (SUCLA2), Transcription factor A, mitochondrial (TFAM). Nucleus - Aly/REF export factor (ALYREF), Far upstream element-binding protein 1 (FUBP1), Heterogeneous nuclear ribonucleoprotein U (HNRNPU), Histone H2A (HIST1H2BA), Histone H2B (HIST1H2BB), Histone H4 (HIST1H4A), Lamin A, isoform CRA_b (LMMA), LIM domain-containing protein 1 (LIMD1), Nuclease-sensitive element-binding protein 1 (NSEP1), Nucleolin (NCL), O acetyl-ADP-ribose deacetylase MACROD1 (MACROD1), Prohibitin-2 (PHB2), Signal transducer and activator of transcription (STAT), Small ubiquitin-related modifier (SUMO), Transcriptional activator protein Pur-beta (PURB). Redox homeostasis – Glutathione S-transferase Mu 2 (GSTM2), Lactoylglutathione lyase (GLO1), Thioredoxin domain-containing protein 17 (TXNDC17). Ribosome - 40S ribosomal protein S3 (RPS3), Ribosomal protein L36-like (RPL36), Ubiquitin-60S ribosomal protein L40 (UBA52). Sarcomere- Isoform 3 of Myosin light chain (MYL3).

4.3 Artigo científico publicado relacionado à tese: "**Paternal resistance training modulates calcaneal tendon proteome in the offspring exposed to high-fat diet".**



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Paternal resistance training modulates calcaneal tendon proteome in the offspring exposed to high-fat diet

Short Title: paternal training and tendon proteome

Ivo Vieira de Sousa Neto[1], Ramires Alsamir Tibana[2], Leonardo Gomes de Oliveira da Silva[1], Eliene Martins de Lira[1], Gleyce Pires Gonçalves do Prado[1], Jeeser Alves de Almeida[3], Octavio Luís Franco[4,5], João Luiz Quaglioti Durigan[1], Adetola B. Adesida[6], Marcelo Valle de Sousa[7], Carlos André Ornelas Ricart[7], Hylane Luiz Damacena[7], Mariana S. Castro[7], Wagner Fontes[7], Jonato Prestes[2], Rita de Cassia Marqueti[1].

[1]Graduate program of Sciences and Technology of Health and Rehabilitation Sciences, University of Brasilia, Distrito Federal, Brazil

[2]Graduate program of Physical Education, Catholic University of Brasilia, Distrito Federal, Brazil

[3]Graduate program of Health and Development in the Central West Region, Federal University of Mato Grosso do Sul, Brazil

[4]Centro de Análises Bioquímicas e Proteômicas, Pós-graduação em Ciências Genômicas e Biotecnologia, Universidade Católica de Brasília, Brasília/DF, Brazil, 70790-160. 19

[5]S-Inova Biotech, Pós-graduação em Biotecnologia Universidade Católica Dom Bosco, Campo Grande/MS, Brazil, 79117-900

[6] University of Alberta, Divisions of Orthopaedic Surgery & Surgical Research,

Edmonton, Alberta, Canada

[7]Laboratory of Protein Chemistry and Biochemistry and Protein Chemistry, Department 24 of Cell Biology, Institute of Biological Sciences, University of Brasilia, Brasilia, DF, 25 Brazil

Corresponding authors

Ivo Vieira de Sousa Neto (ivoneto04@hotmail.com) and Rita de Cassia Marqueti (marqueti@gmail.com) Campus Universitário, Centro Metropolitano 1, Conjunto A, Universidade de Brasília, UnB – Zip Code 72220-900. Tel.: +55 67 99871 4343.

ABSTRACT

The increase in high-energy dietary intakes is a well-known risk factor for many diseases, and can also negatively impact the tendon. Ancestral lifestyle can mitigate the metabolic harmful effects of offspring exposed to high-fat diet (HF). However, the influence of paternal exercise on molecular pathways associated to offspring tendon remodelling remains to be determined. We investigated the effects of 8 weeks of paternal resistance training (RT) on offspring tendon proteome exposed to standard diet or HF diet. Wistar rats were randomly divided into two groups: sedentary fathers and trained fathers (8 weeks, three times per week, with 8–12 dynamic movements per climb in a stair climbing apparatus). The offspring were obtained by mating with sedentary females. Upon weaning, male offspring were divided into 4 groups (5 animals per group): offspring from sedentary fathers were exposed either to control diet (SFO-C), or to high-fat diet (SFO-HF); offspring from trained fathers were exposed to control diet (TFO-C) or to a high-fat diet (TFO-HF). The Nano-LC-MS/MS analysis revealed 383 regulated proteins among offspring groups. HF diet induced a decrease of abundance in tendon proteins related to extracellular matrix organization, transport, immune response and translation. On the other hand, the changes in the offspring tendon proteome in response to paternal RT were more pronounced when the offspring were exposed to HF diet, resulting in positive regulation of proteins essential for the maintenance of tendon integrity. Most of the modulated proteins are associated to biological pathways related to tendon protection and damage recovery, such as extracellular matrix organization and transport. The present study demonstrated that the father's lifestyle could be crucial for tendon homeostasis in the first generation. Our results provide important insights into the molecular mechanisms involved in paternal intergenerational effects and potential protective outcomes of paternal RT.

Keywords: overweight, intergenerational, paternal programming, exercise, tendon proteome.

INTRODUCTION

The global obesity disease is often causally linked to marked changes in lifestyle and diet, such as increases in high-energy dietary intakes and low levels of physical activity (Hruby and Hu, 2015). In animal models, consumption of high-fat (HF) diet promotes deleterious effects on morphological, biomechanical and biochemical properties of tendons (Biancalana et al., 2010; Boivin et al., 2013). Previous data have shown that rats exposed to HF diet displayed an increase of oxidized low-density lipoprotein deposition in extracellular matrix (ECM) as well a reduction of failure stress in the patellar tendon when compared with rats exposed to a standard diet (Grewal et al., 2014). Zucker rats displayed disorganized collagen fibril bundles and decreased average fibril diameter accompanied by adverse effects in displacement at maximum load and maximum strain, indicating a significant harmful ECM remodeling in deep digital flexor tendon (Biancalana et al., 2010). In this way, further investigations are necessary to determine strategies to attenuate these detrimental responses on tendons.

Ancestral environmental conditions can elicit lifelong effects on the offspring physiology, including increased susceptibility to the obesity and metabolic disorders development (Krout et al., 2018; Winther et al., 2018; Glendining and Jasoni, 2019). Although detrimental effects of maternal obesity during perinatal periods on adiposity and metabolic function in offspring are well established, paternal lifestyle may also influence offspring developmental outcomes. It has been reported that predisposing to obesity in adulthood for children increases around 83% if only one parent (father or mother) are obese (Whitaker et al., 1997; Dorosty et al., 2000). Paternal obesity is a risk factor for increased adiposity and glucose intolerance in the adult offspring (Ng et al., 2010; McPherson et al., 2015), besides harming the sperm metabolic function and fertility (McPherson et al., 2015). Therefore, paternal intergenerational inheritance has important implications in the offspring phenotype.

The protection of future unborn generations must be a paramount consideration, because it might contribute to a range of implications for offspring health. A recent study indicates that voluntary paternal exercise (running wheel) suppresses the detrimental effects of paternal HF diet on offspring, reducing the harmful effects on glucose tolerance, fat mass and glucose uptake in different skeletal muscles (Stanford et al., 2018). Krout et al. 2018 demonstrate that paternal exercise (2 weeks of wheel running) increases the

expression of insulin signaling pathways in offspring skeletal muscle, what can be an important tool to prevention type 2 diabetes. Although highly significant, the authors did not strictly manipulate training variables (duration, intensity, rest interval, training volume), which could amplify the understanding of exercise dose on metabolic variables, and tendon offspring. Considering the intimate relationship between the skeletal muscle and tendon unit, it might be reasonable that controlled paternal exercise may modulate the molecular pathways related to offspring tendon remodeling.

DNA, histone methylation, and small RNAs are the most thoroughly studied potential intergenerational mechanisms (Stanford et al., 2018), while other molecular processes can be relevant. This variety of modifications can change chromatin structure or recruit transcriptional cofactors to DNA in order to regulate gene expression and genome activity, which consequently might alter protein profile in a cell. Proteins are effectors of cellular mechanisms, and are critical players in the maintenance of cellular homeostasis (Malipatil et al., 2019). Proteomic analysis and bioinformatics tools allow an integrated view of the molecular pathways modulated by diet and exercise training (Kleinert et al., 2018). In a recent study, we examined the effects of resistance training (RT) on the protein profile of calcaneal tendon during aging. Barin et al. (Barin et al., 2017) demonstrated that RT (12 weeks, 3 times per week) up-regulates protein abundance levels related to ECM organization, the stability of the ciliary architecture and metabolic processes in old trained rats when compared with old sedentary rats. The authors demonstrated the relevance of RT as an intervention to attenuate the detrimental effects inherent to aging-associated changes (Barin et al., 2017). Relevantly, exercise programs can be often more successful than pharmacological interventions to protect against tendon weakening processes (Snedeker and Foolen, 2017). RT is considered an important non-pharmacologic agent capable of inducing significant effects on structural and mechanical properties and collagen synthesis in tendons (Marqueti et al., 2018), while other training modalities may have limited effects on EMC tendon remodeling (Buchanan and Marsh, 2001; Boivin et al., 2013).

The identification and quantification of key proteins from different organelles, biologic process, and molecular functions would provide new insights into the molecular pathways involved in paternal intergenerational inheritance. A mechanistic understanding of these effects can provide crucial clues for the development of therapeutic approaches to mitigate the tendon disorders associated with HF diet. The purpose of this study was to

investigate the effects of 8 weeks of paternal RT in offspring tendon proteome exposed to control and HF diet. We found evidence supporting the hypothesis that paternal RT regulates protein abundance levels directly related to the maintenance, organization, and integrity of the tendon ECM and that such regulation is enhanced by different diets.

MATERIALS AND METHODS

Animals and grouping

The proper care and use of laboratory animals in research were conducted following the USA Guide (National Research Council, 2011(Council, 2010)). The research protocol received approval from the Ethics Committee on Animal Experimentation from the Catholic University of Brasilia (Protocol No. 010/13).

First, 4-month-old male Wistar rats (*Ratus norvegicus albins*, weighing ± 376g) were placed in collective cages (3 or 2 rats per cage), and were randomly divided into two groups (5 animals per group): sedentary fathers (SF; did not perform RT, free to move around the cages) and trained fathers (TF; performed RT). The offspring were obtained by mating with sedentary females. After the eight weeks of paternal RT, the estrous cycle in female rats was verified daily, and during proestrus phase, one male and one female were housed together for two consecutive days for mating, during which they were allowed free access to a control diet (Purina®, Descalvado-SP, Brazil). The experimental groups in the current study were composed of 20 male pups. Litters were standardized among five pups each to avoid litters of different sizes, which were left together with their mothers until they were weaned. Litters belonging to the same experimental group were offspring of different parents.

Male offspring were weaned and divided into 4 groups (5 animals per group): offspring from sedentary father exposed to either control diet (SFO-C) or to high-fat diet (SFO-HF), and offspring from trained father exposed to either control diet (TFO-C), or to high-fat diet (TFO-HF). All animals came from the Central Vivarium of the Faculty of Physical Education of the Catholic University of Brasilia. The animals were housed in polypropylene cages (maximum 3 rats per cage) at a temperature of 23 ± 2 °C with 12:12h dark: light cycle. The offspring were weighed and evaluated weekly for six months using a digital scale (Filizola®, São Paulo, Brazil). Study experimental design is presented in Figure 1. Not including female offspring in present study has been justified due to the

variable nature of female data caused by hormonal fluctuations associated with the female reproductive cycle.

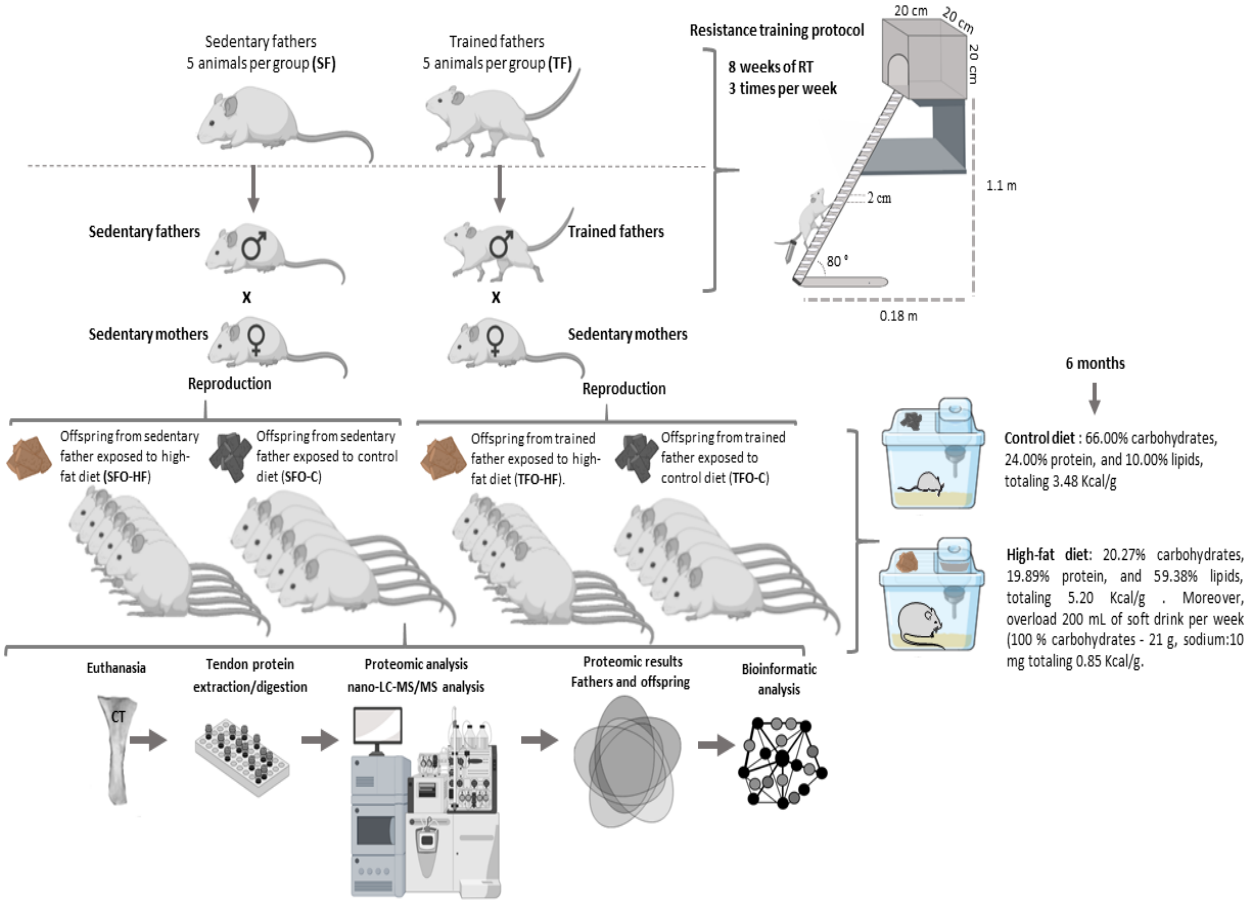


Figure 1. Experimental design. Schematic representation of the methodological sequence followed in

Table 1. Growth trajectory of the offspring

	SFO-C	TFO-C	SFO-HF	TFO-HF
Body weight				
Birth weight (g)	29.34 ± 1.46	27.83 ± 1.66	32.64 ± 1.47	31.92 ± 1.26
Weaning weight (g)	32.6 ± 1.62	30.92 ± 1.84	36.27 ± 1.64	35.4 ± 1.40
Final body weight (g)	331.36 ± 12.97	311.12 ± 22.81	433.9 ± 31.06[a,b]	398.84 ± 7.30[a,b,c]
Adiposity markers				
Total visceral adipose weight (mg)	4.26 ± 0.94	3.39 ± 1.34	13.46 ± 2.08[a,b]	6.91 ± 2.22[a,b.c]
Adiposity index (%)	1,29 ± 0.24	1,08 ± 0.36	4,75 ± 0.95	2,63 ± 0.68
Cross-Sectional Area of adipocyte (µm2)	3699.04 ± 548.27	3256.7 ± 306.59	5891.68 ± 839.35[a,b]	4473.6 ± 561.60[c]
Food intake				
Average caloric intake of feed (kcal)	459.51 ± 120.27	400.89 ± 103.75	551.74 ± 50.49[a,b]	546.63 ± 47.67[a,b]
Average caloric intake of soft drink (kcal)	20.4	20.4	20.4	20.4
Feed efficiency (%)	10.1 %	11.10 %	22.00 %	19.90 %
Metabolites				
Serum Glucose (mg/dl)	92.00 ± 9.79	93,33 ± 1.77	105.75 ± 16.48 [a,b]	112.25 ± 11.86 [a,b]
Serum Cholesterol (mg/dl)	156.50 ± 3.87	158.25 ± 4.79	168.40 ± 5.08[a,b]	163.00 ± 1.67

Serum Triglycerides (mg/dl)	132.0 ± 12.28	128.8 ± 3.96	142.0 ± 11.67	138.4 ± 18.24
Functional test				
Maximal velocity (m/min)	48.3 ± 3.16	45.3 ± 2.44	46.3 ± 7.48	49.3 ± 2.0
Maximal distance obtained in the test (m)	447.4 ± 38.45	429.0 ± 35.11	375.2 ± 84.41	407.8 ± 45,16

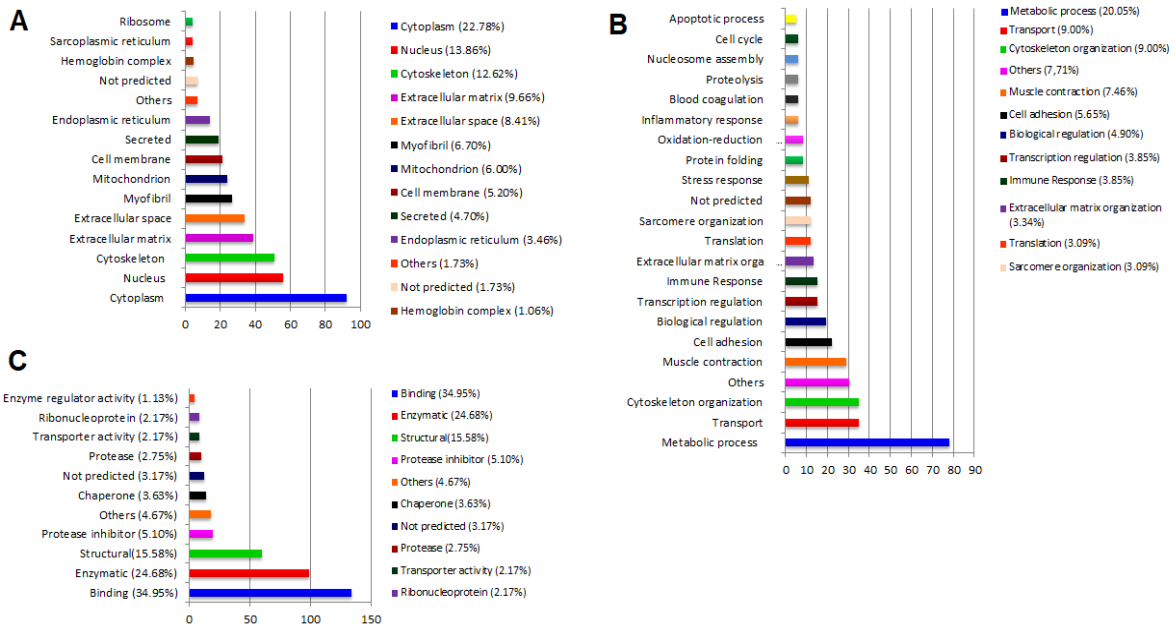
Values are presented as means ± SD. SFO-C = offspring from sedentary fathers, exposed to control diet; TFO-C = offspring from trained fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. Statistically significant differences compared to: a SFO-C; b TFO-C; c SFO-HF, $p \leq 0.05$. (n = 5 per group).

Functional proteome annotations

A qualitative overview of all identified proteins show 388 proteins identified by Nano- LC-MS/MS analysis (supplementary data 2), among them 384 (fathers) and 383 (offspring) proteins met the inclusion criterion and were classified according to UniProt database and Panther classification system in the biological process, molecular function and cellular localization.

Supplementary figure 1A shows the identified proteins according to their cellular localization (A), biological process (B), and molecular function (C). In the present investigation, most of these proteins were derived from the cytoplasm (22.78%) followed by the nucleus (13.86%), cytoskeleton (12.62%) and extracellular matrix (9.66%). Additionally, the biological process classification showed that 20.09% of these proteins were related to metabolic processes followed by transport (9%), cytoskeleton organization and others (7.71%), (Supplementary Fig 1B). Finally, the primary molecular function observed was binding (34.95%), followed by enzymatic (24.68%), structural (15.58 %), protease inhibitor (5.10%), and others (4.67%) (Supplementary Fig 1C).

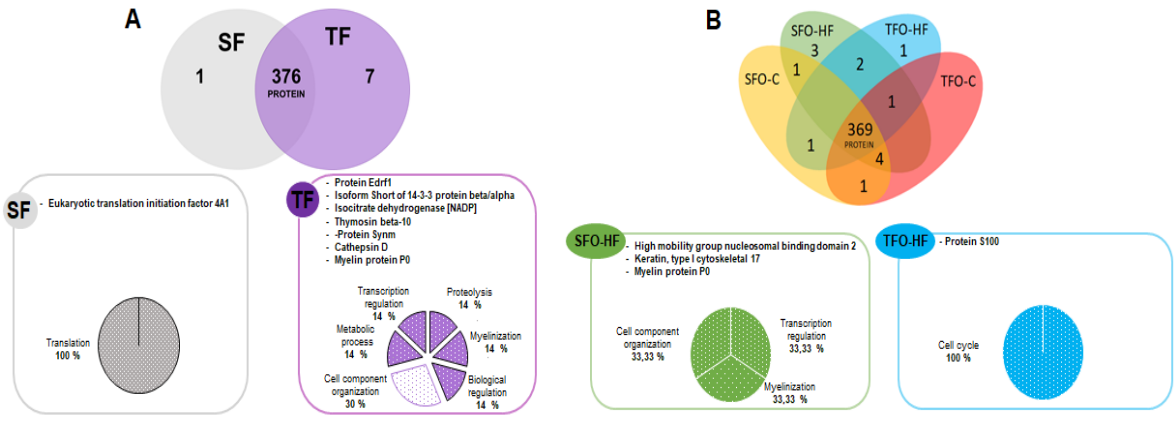
Supplementary figure 1: Classification of the identified proteins according to their cellular localization (A), biologic process (B), and molecular function (C).



Supplementary Figure 1. Classification of the identified proteins according to the GO terms: cellular localization (A), biologic process (B), and molecular function (C).

Venn diagram was used to show the distribution of the identified proteins into two experimental groups (Supplementary Fig 2A). From the 384 proteins identified in the fathers' samples, 376 were common to both groups, while 1 protein were identified only in the sedentary fathers´ group (SF) and 7 proteins were identified only in the trained fathers´ group (TF). The protein identified only in the SF group was mainly related to translation (Eukaryotic translation initiation factor 4A1). The proteins identified only in the TF were associated with cell component organization (Protein Synm and Thymosin beta-10), metabolic process (Isocitrate dehydrogenase [NADP]), proteolysis (Cathepsin D), myelinization (Myelin protein P0), biological regulation (Isoform Short of 14-3-3 protein beta/alpha) and transcription regulation (Protein Edrf1).

Regarding the four offspring groups, from the 383 identified proteins, 369 were common to all groups, while 3 proteins were identified only in the offspring from sedentary father exposed to high-fat diet (SFO-HF) and 1 protein was identified only in the offspring from trained father exposed to high-fat diet (TFO-HF). The high proportion of proteins identified in all groups confirms the consistency in the sample preparation. The proteins identified only in the SFO-HF group were mainly related to cell component organization (Keratin, type I cytoskeletal 17), myelinization (Myelin protein P0) and transcription regulation (High mobility group nucleosomal binding domain 2). The protein identified only in the TFO-HF was associated with the cell cycle (Protein S100) (Supplementary Fig 2B).



Supplementary figure 2 Venn diagram representation of the identified proteins by NanoUPLC-MS analysis in the fathers and offspring groups. Proteins exclusive to groups were classified by their biological process.

Principal Components Analyses (PCA) and Heatmaps

Results from the PCA in fathers and offspring are shown in Figure 2. The purpose of this analysis was to reveal patterns of protein abundance levels that may distinguish the experimental groups from one another. The 30 dots in the figure represent the five SF (grey), five TF (purple), five SFO-C (yellow), five TFO-C (red) five SFO-HF (green), and five TFO-HF (blue) samples. Notably, there is distinct clustering among the fathers groups, with the two sample groups occupying separable, non-overlapping, regions in the PCA plot. On the hand, overlapping was present among the SFO-C and TFO-C group. Finally, there were clear differences in the patterns of protein abundance levels among the others offspring groups (SFO-C, SFO-HF and TFO-HF), suggesting that paternal RT modulates the protein profile of the calcaneal tendon, especially noticeable in offspring exposed to HF diet. The variability in data captured by PCA along PC1 and PC2 were 15.7% and 12.7%, respectively (Fig 2A) Figure 3 displays a heat map normalized abundance levels from each father and offspring. As expected, there were notable differences in the protein levels among fathers (Fig 3A). The SFO-C and TFO-HF groups showed majority high abundance levels when compared with TFO-C and SFO-HF groups, respectively (Fig 3B). Considering the criteria for down-regulation and up-regulation, the abundance of 79 proteins was shown to be altered in fathers after RT (78 protein upregulated and 1downregulated). The abundance of 25 proteins was shown to be altered by HF diet (8 protein upregulated and 17 downregulated). Paternal RT modified 14 proteins in offspring exposed to control diet (3 proteins upregulated and 11 downregulated) and 33 proteins in offspring exposed to HF diet (27 proteins upregulated and 6 downregulated), respectively (Fig 3C).

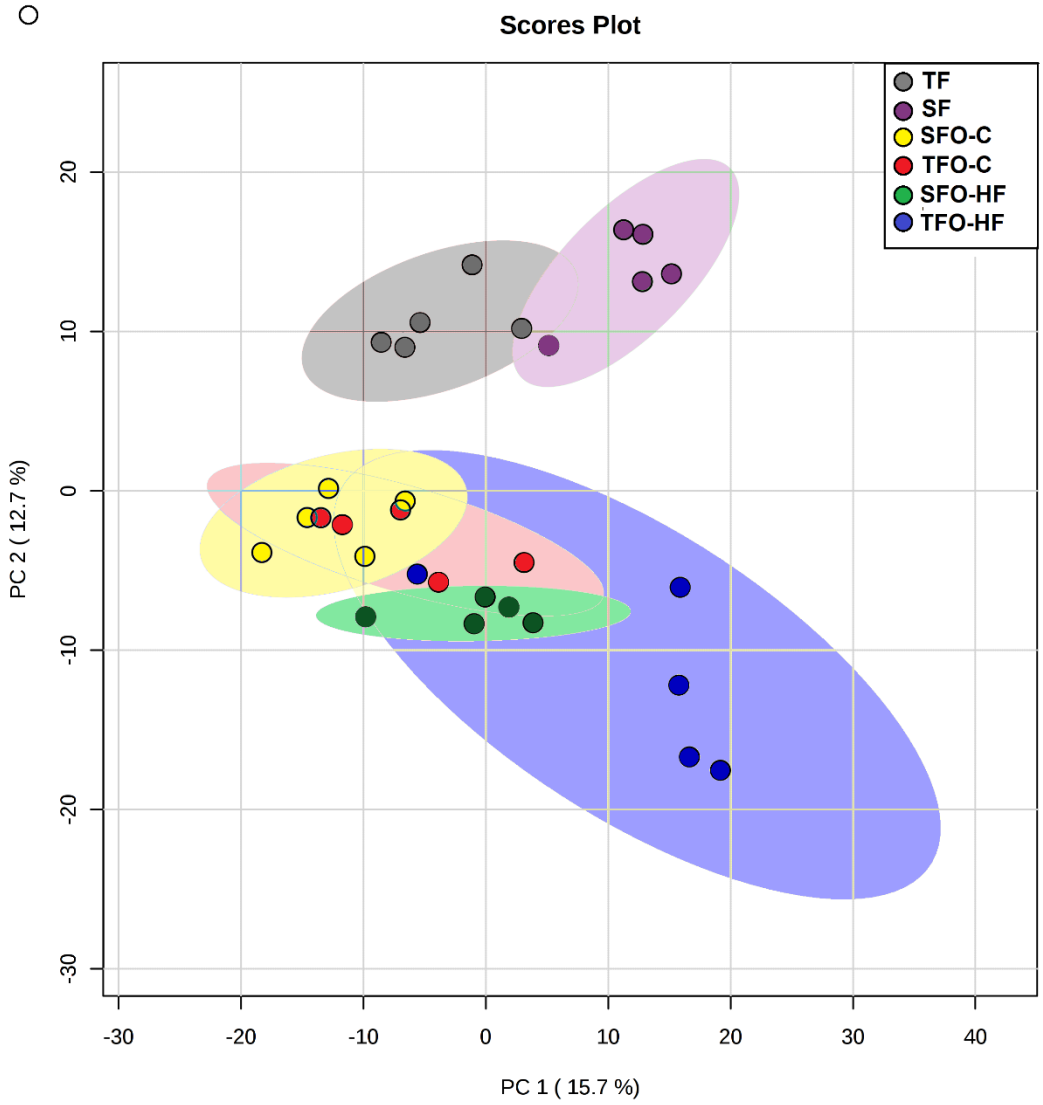


Figure 2. The results of Principal Components Analyses (PCA). Score plot of proteomic data set acquired by Nano-LC-MS/MS of the six biological groups: SF = sedentary fathers (gray) ; TF trained fathers (purple) .; SFO-C = offspring from sedentary fathers , exposed to control diet (yellow); TFO-C = offspring from trained fathers exposed to control diet (red); SFO-HF = offspring from sedentary fathers exposed to high-fat diet (green); TFO-HF = offspring from trained fathers exposed to a high-fat diet (blue). Each animal is represented by individual points.

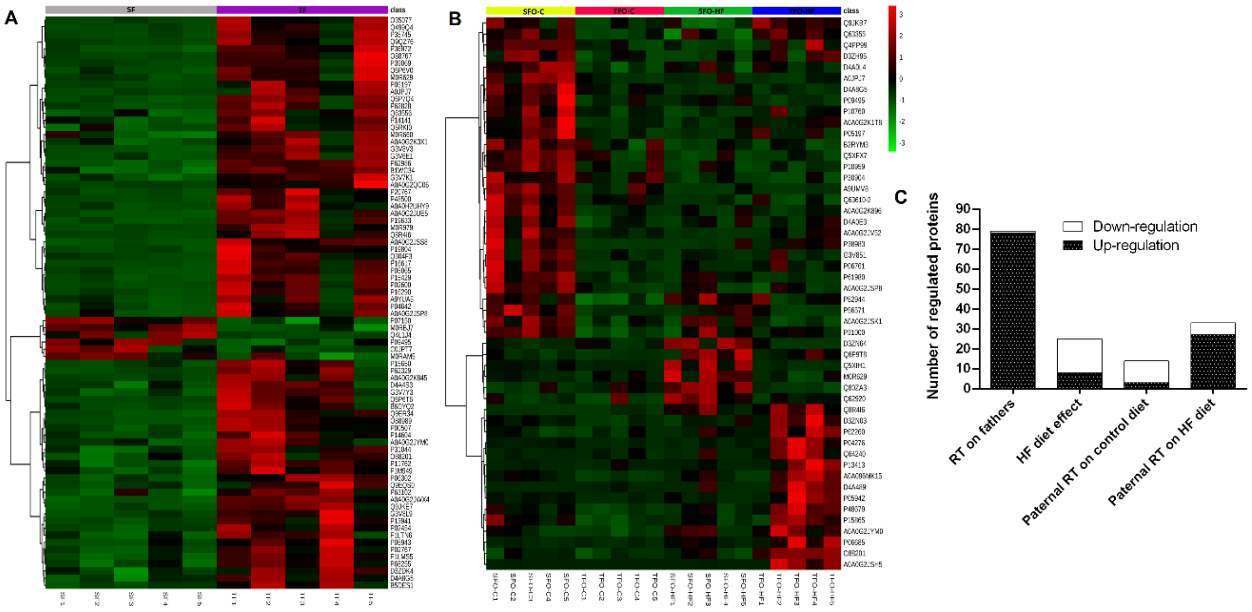


Figure 3. Heat map of the changes for each animals in the fathers (A) and offspring groups (B). Each horizontal line represents an individual protein. The top 80 proteins regarding fathers and 50 from offspring, respectively. SF = sedentary fathers; TF trained fathers; SFO-C = offspring from sedentary fathers, exposed to control diet; TFO-C = offspring from trained fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. Read counts for each animal has been plotted as log2. Green and red indicate a decrease and increase of protein abundance levels, respectively. Overall number of regulated (down-regulation or up-regulation) protein for training and diet as factors (C).

Father's tendon proteome

The effects of resistance training on paternal tendon protein abundance levels were grouped according to their biological processes and are presented in Figure 4 and Table 2. Supplementary data 3 shows fold-change, p-values t test related to regulated proteins. From TF group, abundance levels of 79 proteins were shown to be altered as compared with the SF group (78 proteins increased and 1 decreased). In this analysis (TF:SF), proteins were mainly related to muscle contraction and sarcomere organization (12 upregulated), cell adhesion, cytoskeleton organization/ extracellular matrix

organization (9 upregulated and 1 downregulated); metabolic process, respiratory electron transport and oxidation-reduction process (34 upregulated), transport (8 upregulated), inflammatory response/ immune response/stress response (5 upregulated), translation and transcription regulation, cell cycle (4 upregulated) and miscellaneous (6 upregulated).


TF group X SF group
Up-regulation
Down-regulation
Muscle contraction/
Sarcomere organization
Down-Regulation
Up- Regulation
Actinin alpha 3, isoform CRA_a
Calsequestrin-1
Myomesin 2
Myosin binding protein C, fast-type
Myosin binding protein C, slow-type
Myosin light chain 1/3, skeletal muscle isoform
Myosin regulatory light chain 2
Parvalbumin alpha
Protein Myh1
Protein Tnnc2
Troponin I type 2 (Skeletal, fast), isoform CRA_a
Troponin T, fast skeletal muscle
-2 -1 0 1 2
Cell adhesion, Cytoskeleton organization/
Extracellular matrix organization
Down-Regulation
Up- Regulation
Collagen alpha-1(III) chain
Keratin, type II cytoskeletal 2 epidermal
Membrane-associated guanylate kinase
Myocilin
Procollagen C-endopeptidase enhancer 1
Protein Col4a2
Protein Ldb3
Protein Thbs1
Thrombospondin-4
Thymosin beta-4
-2 -1 0 1 2
Metabolic process/ Oxidation-reduction
process/ Respiratory electron transport
Down-Regulation
Up- Regulation
Aconitate hydratase, mitochondrial
Acylphosphatase-2
Adenine phosphoribosyltransferase
Adenylate kinase isoenzyme 1
Adenylosuccinate synthetase isozyme 1
Alpha-1,4 glucan phosphorylase
Amylo-1, 6-glucosidase, 4-alpha-glucanotransferase
Aspartate aminotransferase, mitochondrial
ATP synthase subunit delta, mitochondrial
ATP-dependent 6-phosphofructokinase, muscle type
Beta-enolase
Carbonic anhydrase 3
Enoyl-CoA hydratase, mitochondrial
Fructose-bisphosphate aldolase A
Glucose-6-phosphate isomerase
Glutathione S-transferase Mu 1
Glutathione S-transferase Mu 2
-2 -1 0 1 2
Down-Regulation
Up- Regulation
Glyceraldehyde-3-phosphate dehydrogenase
Glycerol-3-phosphate dehydrogenase NAD, cytoplasmic
L-lactate dehydrogenase A chain
Lactoylglutathione lyase
Long-chain specific acyl-CoA dehydrogenase, mitochondrial
Malate dehydrogenase, cytoplasmic
Nucleoside diphosphate kinase B
Obg-like ATPase 1
Peroxiredoxin 5, isoform CRA_c
Peroxiredoxin-6
Phosphoglucomutase 1
Phosphoglycerate kinase 1
Phosphoglycerate mutase 2
Protein Adipoq
Succinate dehydrogenase [ubiquinone] flavoprotein subunit
Transaldolase
Triosephosphate isomerase
-2 -1 0 1 2
Transport
Down-Regulation
Up- Regulation
Acyl-CoA-binding protein
Electron transfer flavoprotein subunit alpha
GTP-binding nuclear protein Ran
Myoglobin
Selenium-binding protein 1
Serotransferrin
Serum albumin
Transthyretin
-2 -1 0 1 2
Inflamatory response/ Imune response/
Stress response
Down-Regulation
Up- Regulation
Ig lambda-2 chain C region
Protein deglycase DJ-1
Protein Igkv6-20
Prothymosin alpha
T-kininogen 2
-2 -1 0 1 2
Translation/ Transcription regulation/ Cell cycle
Down-Regulation
Up- Regulation
60S ribosomal protein L23a
Polymerase I and transcript release factor
Protein Ckap5
Protein S100-A10
-2 -1 0 1 2

Figure 4. Effects of resistance training on father tendon proteome. Analysis of proteins from the tendons of the trained versus control animals (fathers). Histogram of protein abundance levels from intergroup analysis considering only proteins with down-regulation and up-regulation ($p \leq 0.05$), with a ln(fold change) of at least (≥) 0.5. SF = sedentary fathers; TF = trained fathers. The X-axis represents the natural logarithm of the ratio between the treatments (purple : **TF:SF ratio**). All altered proteins are grouped according to their biologic process as noted in Gene Ontology (GO). The proteins were considered reliably identified only if presenting an FDR<1% and at least two matching peptides. Supplementary data 3 shows detailed information about each protein outlined in this figure.

High-fat diet cause a reduction in the abundance of proteins related to ECM tendon remodeling

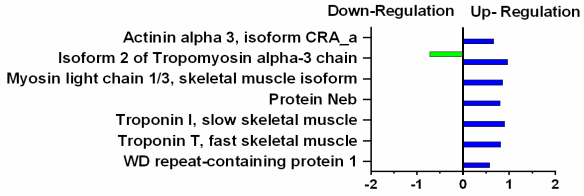
Supplementary data 3 shows fold-change, p-values of factors (diet and training) and p-values of Tukey's multiple comparisons test related to regulated proteins. The effects of high-fat diet on the offspring´s tendon proteome were grouped according to their biological processes and are presented in Figure 5. Regarding SFO-HF group, the abundance of 25 proteins was shown to be altered when compared with SFO-C (8 protein increased and 17 decreased). In this analysis (SFO-HF: SFO-C), proteins were mainly related to muscle contraction and sarcomere organization (1 downregulated); cell adhesion, cytoskeleton organization/ extracellular matrix organization (3 upregulated and 1 downregulated); metabolic process, respiratory electron transport and oxidation-reduction process (2 upregulated and 3 downregulated), transport (2 downregulated); inflammatory response/immune response/stress response (1 upregulated and 3 downregulated); transcription regulation/translation/cell cycle (2 downregulated), and miscellaneous (2 upregulated and 5 downregulated).

Additionally, from TFO-HF group, abundance levels of 34 proteins were shown to be altered when compared with the TFO-C group (34 proteins increased), proteins were mostly related to muscle contraction and sarcomere organization (7 upregulated); cell adhesion, cytoskeleton organization/extracellular matrix organization (5 upregulated); metabolic process, respiratory electron transport and oxidation-reduction process (4 upregulated), transport (5 upregulated); inflammatory response/imune response/stress

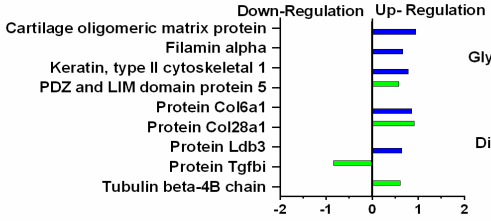
response (3 upregulated); transcription regulation/translation/cell cycle (5 upregulated), and miscellaneous (5 upregulated).

SFO-HF group X SFO-C group
TFO-HF group X TFO-C group

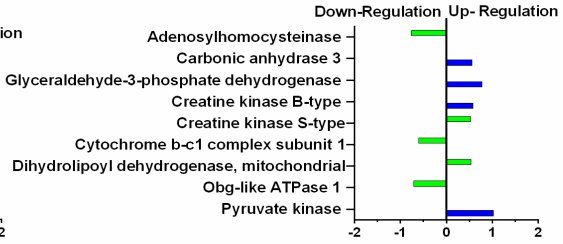
Muscle contraction/ Sarcomere organization



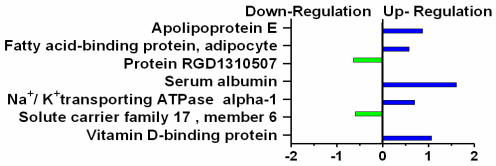
Cell adhesion, Cytoskeleton organization/ Extracellular matrix organization



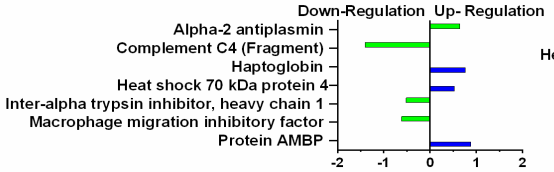
Metabolic process/ Oxidation-reduction process/ Respiratory electron transport



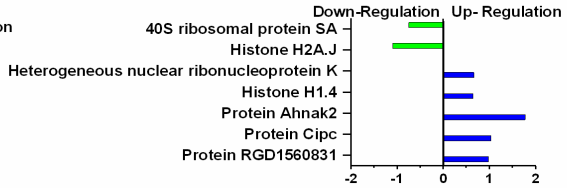
Transport



Inflamatory response/ Imune response/ Stress response



Transcription/Translation regulation/ Cell cycle



Miscellaneous

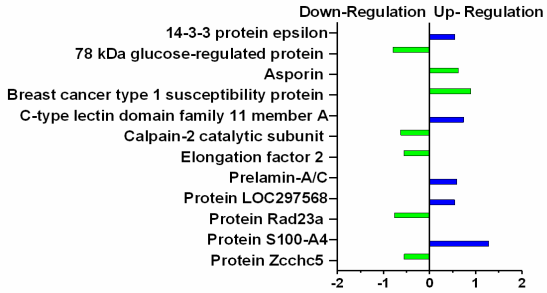


Figure 5. High-fat diet effects on tendon proteome of the offspring. Histogram of protein abundance levels from intergroup analysis considering only proteins with down-regulation and up-regulation ($p \leq 0.05$), with a ln(fold change) of at least (≥) 0.5. SFO-HF = offspring from sedentary fathers exposed to high-fat diet; SFO-C = offspring from sedentary fathers exposed to control diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet; TFO-C = offspring from trained fathers exposed to control diet. The X-axis represents the natural logarithm of the ratio between the treatments (green : SFO-HF:SFO-C ratio; blue : TFO-HF:TFO-C ratio). All altered proteins are grouped according to their biologic process as noted in Gen Ontology (GO). The proteins were considered reliably identified only if presenting an FDR<1% and at least two matching peptides. Supplementary data 3 shows detailed information about each protein outlined in this figure.

Paternal RT promoted up-regulation of essential proteins associated to muscle contraction, ECM organization, transport, and transcription in the offspring exposed to a high-fat diet.

The effects of paternal resistance training on offspring tendon protein abundance levels exposed to control and high-fat diet were grouped according to their biological processes and are presented in Figure 6 and table 3. From TFO-C group, abundance levels of 14 proteins were shown to be altered when compared with SFO-C group (3 proteins increased and 11 decreased). In this analysis (TFO-C:SFO-C), proteins were mainly related to cell adhesion, cytoskeleton organization/extracellular matrix organization (2 downregulated) ; metabolic process, respiratory electron transport and oxidation-reduction process (1 upregulated and 2 downregulated), transport (1 downregulated), inflammatory response/ immune response/ stress response (2 downregulated), translation (1 upregulated and 1 downregulated) and miscellaneous (1 upregulated and 3 downregulated).

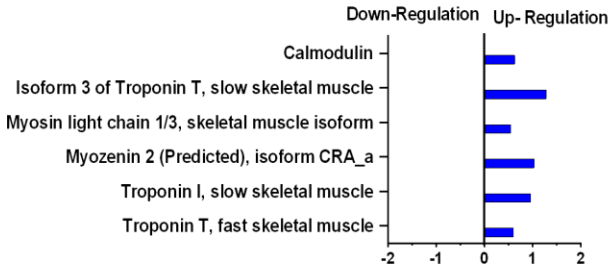
Finally, from TFO-HF group, abundance levels of 41 proteins were shown to be altered when compared with the SFO-HF group (25 proteins increased and 8 decreased) (Fig 5, table 2). In this analysis (TFO-HF:SFO-HF), proteins were mainly related to muscle contraction and sarcomere organization (7 upregulated); cell adhesion, cytoskeleton organization/extracellular matrix organization (6 upregulated and 2

downregulated); metabolic process, respiratory electron transport and oxidation-reduction process (1 upregulated and 3 downregulated), transport (3 upregulated and 1 downregulated); inflammatory response/immune response/stress response (2 upregulated and 2 downregulated); ttranscription regulation/translation/cell cycle (4 upregulated) and miscellaneous (2 upregulated).

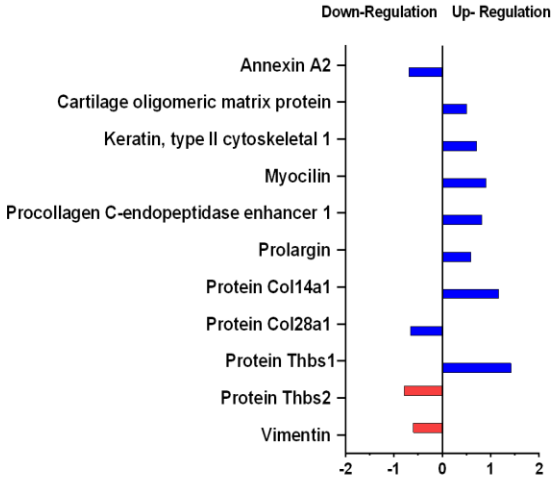
TFO-C group X SFO-C group

TFO-HF group X SFO-HFgroup

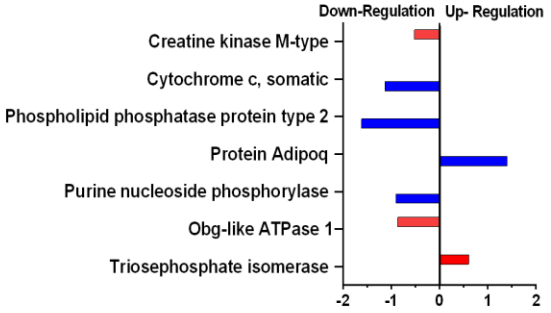
Muscle contraction/ Sarcomere organization



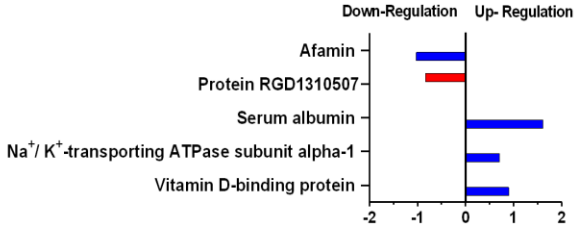
Cell adhesion/ Cytoskeleton organization/ Extracellular matrix organization



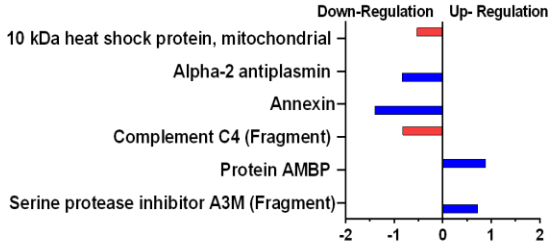
Metabolic process/ Oxidation-reduction process/ Respiratory electron transport



Transport



Inflamatory response/ Imune response/ Stress response



Transcription/Translation regulation/ Cell cycle

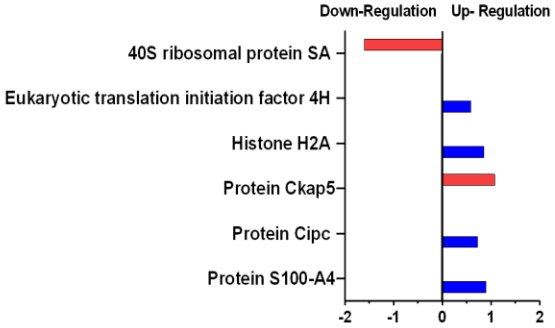


Figure 6. Effects of paternal resistance training on tendon proteome in the offspring exposed to control and high-fat diet. Histogram of protein abundance levels from intergroup analysis considering only proteins with down-regulation and up-regulation ($p \leq 0.05$), with a ln(fold change) of at least (≥) 0.5. TFO-C = offspring from trained fathers exposed to control diet; SFO-C = offspring from sedentary fathers exposed to control diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; The X-axis represents the natural logarithm of the ratio between the treatments **(red: TFO-C:SFO-C ratio, blue: TFO-HF:SFO-HF ratio**). All altered proteins are grouped according to their biologic process as noted in Gen Ontology (GO). The proteins were considered reliably identified only if presenting an FDR<1% and at least two matching peptides. Supplementary data 3 shows detailed information about each protein outlined in this figure.

Protein-protein interactions (PPI)

The PPI network of the significantly regulated proteins related to metabolic processes in TF: SF was composed by 33 nodes and 187 edges with an average node degree of 11.13, it indicated high connectivity among oxidative stress protection, glycolysis, citric acid cycle, and fatty acid metabolism proteins (Fig 7A). Upregulated proteins related to sarcomere organization, muscle contraction, ECM organization, cell adhesion, and cytoskeleton organization in TF:SF displayed an interaction network composed of 21 nodes and 58 edges with an average node degree of 5.5. Figure 7B shows the main components of network constructed, such as, troponin T, fast skeletal muscle, actinin alpha 3 and troponin I, type 2, col 1 and col 4.

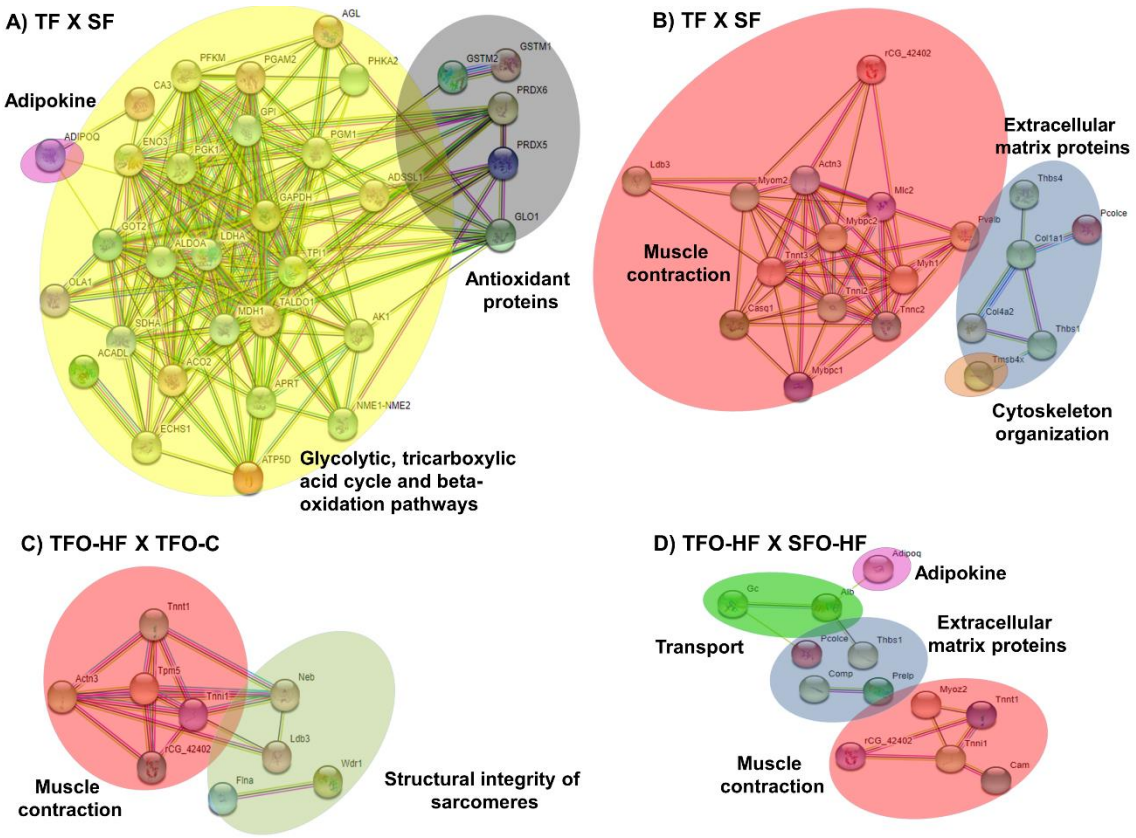


Figure 7. Protein-protein interaction analysis based on STRING network analysis with an interaction confidence score of 0.4. SF = sedentary fathers; TF = trained fathers; TFO-C = offspring from trained fathers exposed to control diet ; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. STRING analysis for differentially abundant proteins in the fathers (A and B) and offspring groups (C and D). All altered proteins are grouped according to their biologic process as noted in Gen Ontology (GO).Primary metabolism (yellow), adipokine (pink), oxidation-reduction process, (dark grey) and muscle contraction (red), sarcomere organization (light grey), cytoskeleton organization (orange) and extracellular matrix organization (blue), transport (green)

Regarding offspring, the PPI network of the specific upregulated proteins related to sarcomere organization and muscle contraction in TFO-HF:TFO-C groups displayed 12 nodes and 18 edges with an average node degree of 3.00 (Fig 7C). Curiously, a prominent interaction network was found within, myosin light chain 1/3, skeletal muscle isoform, isoform 2 of tropomyosin alpha-3 chain, actinin alpha 3, troponin T, fast skeletal

muscle, troponin I, slow skeletal muscle and LIM domain-binding protein. Upregulated proteins related to sarcomere organization, muscle contraction, ECM organization, transport and adipokine in TFO-HF:SFO-HF groups displayed 16 nodes and 11 edges with an average node degree of 1.38 (Fig 7D). No significant protein networks were observed between downregulated proteins, other groups and biological processes. Finally, upregulated proteins related to cell adhesion, extracellular matrix structural and cytoskeleton organization from TF and TFO-HF group were involved in the same interaction network (Figure 8).

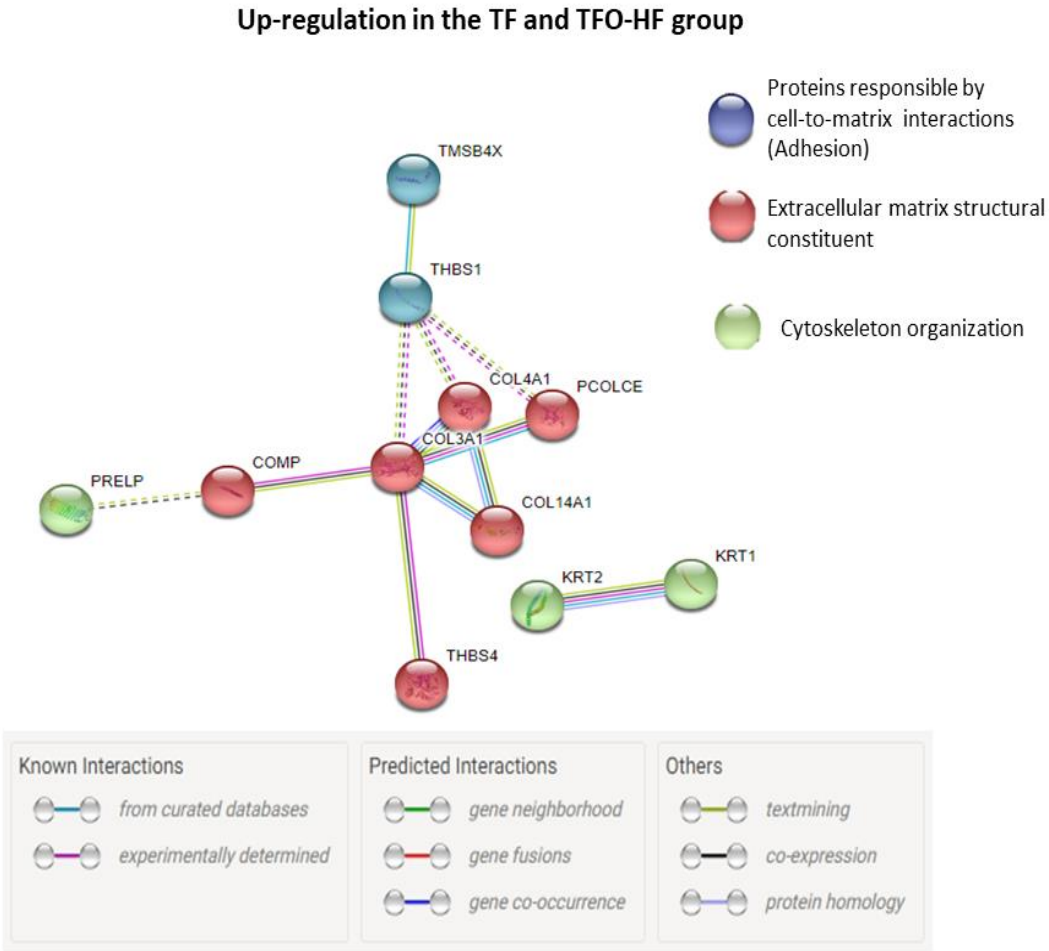


Figure 8. Proteins involved in the same interaction network regarding TF and TFO-HF group. Protein-protein interaction analysis based on STRING network analysis with an interaction confidence score of 0.4. TF = trained fathers; TFO-HF = offspring from trained fathers exposed to a high-fat diet. Blue highlighted nodes are proteins responsible

by cell-to-matrix interactions. Red highlighted nodes are extracellular matrix structural constituent. Green highlighted nodes are Proteins responsible by cytoskeleton organization. Upregulated proteins are grouped according to their biologic process as noted in Gen Ontology (GO).

Discussion

The current study was conducted to determine the effects of paternal RT on offspring tendon proteome exposed to different diets. Our results support the initial hypothesis, showing that SFO-HF group presented downregulation of significant proteins related to muscle contraction, ECM organization, transport, immune response, and translation when compared with SFO-C. On the other hand, the effects of paternal RT on tendon proteome were more pronounced when the offspring were exposed to HF diet and most of the regulated proteins presented an increased abundance rather than reduced. Moreover, paternal RT was effective to prevent adiposity gain in the TFO-HF group, representing a phenomenon of major clinical significance. To our knowledge, the present study demonstrated for the first time that paternal exercise modulates the abundance of proteins attributed to the maintenance of tendon functional integrity suggesting that paternal RT can prevent the detrimental effects related to an offspring unhealthy diet. Our findings provide valuable insights into the molecular mechanisms involved in intergenerational inheritance and this approach present innovation in the current knowledge on tendons.

The present proteomic analysis comparing the TF group versus SF group (fathers) demonstrated that 80 proteins were upregulated, while only 1 protein was downregulated. We found that RT increased key antioxidant proteins (i.e., peroxiredoxin 5, lactoylglutathione lyase, glutathione S-transferase mu 2, peroxiredoxin-6 and glutathione S-transferase Mu 1). Cellular reduction-oxidation balance plays a crucial role in the homeostasis of connective tissues such as enthesis and tendon (Bestwick and Maffulli, 2004). Recent evidences suggest that oxidative stress plays a crucial role on tendinopathic changes (Lim et al., 2012; Lee et al., 2017; Fu et al., 2018). Morikawa et al. (2014) reported that the antioxidant enzyme superoxide dismutase 1 deficiency in mice induced several histopathological changes in the supraspinatus tendon enthesis, including decreased type I collagen, formation of misaligned collagen fibers and misaligned 4-

layered structure (tendon proper, nonmineralized fibrocartilage, mineralized fibrocartilage and bone) compared with the wild type mice. Taken together, the antioxidant enzymes upregulation in the TF group might play a crucial role in the detoxification of reactive oxygen species following RT, possibly preventing impairments in oxidative capacity. We might speculate that these adaptations may reflect a protection mechanism vital for the maintenance of tendon homeostasis, including appropriate collagen fibers organization.

The RT protocol led to an upregulation of a large number of metabolic proteins (i.e., glycolytic, tricarboxylic acid cycle and beta-oxidation) in the TF group compared to the SF group, probably due to increased energy demand and the need to maintain the enzymatic operation in the primary metabolism. These proteins present in different metabolic pathway are key enzymes in the conversion of substrates and in ATP production. These changes may contribute to fibroblast survival and ECM turnover in response to RT (Kjaer et al., 2005). Furthermore, the upregulated proteins displayed a protein interaction network with high connectivity (Fig 6A). On the other hand, Kaux et al. 2017 demonstrated that proteomic analysis in tendons of rats exposed to eccentric training (treadmill run set at a 15° inclination, 1 hour, 3 times per week for 5 weeks) after acute lesions presented a significant decrease in the abundance of glucose metabolism enzymes compared to untrained groups. The discrepancy between these results may be due to differences in intensity, volume, type, and duration of the training protocols employed, as well as acute lesions. Besides, these authors have not addressed the period between last exercise session and euthanasia, which obscure the chronic effects of exercise training on proteomic profile.

Surprisingly, no prominent proteomic adaptations were seen in offspring exposed to control diets whose fathers underwent RT (TFO-C) compared to SFO-C, suggesting limited effects of paternal exercise. Krout et al. 2018 reported that the benefits of paternal exercise on body fat and offspring insulin resistance in skeletal muscle were more noticeably observed when the offspring were exposed to an HF diet. The paternal RT effects on offspring tendon proteomic profile were more evident in the presence of HF diet than in low-fat diet fed to the offspring. The evaluation of only single point in time is a considerable limitation of this study. A more effective strategy would involve displaying protein abundances in different time-points since the beginning of dietary intake in order to clarify the time-course effects of standard diet on offspring tendon proteome.

On the other hand, an important finding of the current study was that SFO-HF demonstrated a decrease of proteins involved in tendon remodeling when compared with SFO-C group. Our results corroborate with transcriptome study which demonstrated that $ApoE^{-/-}$ associated with HF diet, promotes mRNA-level dysregulation of signaling pathways related with ECM synthesis, and repair in the tendon, suggesting mechanism of hypercholesterolemia-induced tendinopathy (Li et al 2019). TGF-β superfamily orchestrates essential cellular processes that include proliferation, differentiation, and growth of the ECM (Klein et al., 2002). Downregulation of the protein TGFβ1 in present study might indicate a panel of unfavorable remodeling processes inherent to HF diet**.** This result substantiates the decreased abundance of 40S ribosomal protein in the present study. Ribosomal proteins are responsible for ribosome assembly and protein translation, which is crucial for cell survival (Zhou et al., 2015). Impairment of any of these two cellular processes can severely retard cell growth (Zhou et al., 2015). The SFO-HF displayed a reduced abundance of macrophage migration inhibitory factor (MIF). Although MIF is a proinflammatory cytokine involved in many inflammatory reactions and disorders, recent studies indicated that MIF may be a mediator of anti-inflammatory and immunosuppressive macrophage functions (Yaddanapudi et al., 2013; Peng et al., 2018). Furthermore, the decrease of MIF can inhibit cell proliferation due to decreased expression of cell cycle regulators (Denz et al., 2010) and autophagy suppression (Lee et al., 2016).

Additionally, our data indicated that asporin abundance levels were upregulated in rats exposed to HF diet. Asporin, a class I small leucine-rich proteoglycan, regulates chondrogenesis, and inhibits TGFβ1-induced expression of matrix genes (Nakajima et al., 2007), acting as a critical regulator of joint diseases, including osteoarthritis (Coburn, 2005). Moreover, asporin regulates collagen mineralization, which is a common mechanism of tendinopathy (Xu et al., 2015). Thus, one possibility that should not be ruled out is that asporin overexpression might promote tendon mineralization and possibly contribute to disability and mechanical dysfunctions (Kalamajski et al., 2009; Houari et al., 2014). Further, HF diet promotes downregulation of proteins with multiples metabolic activity functions and responsible by chemical signalizing in primary metabolism (adenosylhomocysteinase cytochrome complex, obg-like ATPase 1, solute carrier family 17 and 78 kDa glucose protein). Reduced abundance of these proteins might compromises tissue microenvironment, metabolism homeostasis and consequently cellular longevity.

Previous data reported detrimental effects of HF diet on tendon biomechanical and morphological properties (Boivin et al., 2013; Grewal et al., 2014). Diet switch from HF diet to low-fat diet seems to resolve metabolic dysfunction, but it was not able to reverse tendinopathic changes (Studentsova et al., 2018). Here, we observed the increase of several proteins related to structural organization of ECM components (cartilage oligomeric matrix protein, filamin alpha, keratin type II cytoskeletal, protein Col6a1) in TFO-HF group when compared with the TFO-C group, indicating that apparently the progression towards a more degenerative phenotype would have a later onset with paternal RT. Despite highly significant, it remains to be determined whether these adaptations at the protein level are enough to attenuate the disorganized collagen and deterioration of tendon biomechanical properties related to HF diet. Moreover, there was no significant reduction of proteins related to tissue damage, repair, ECM structural stability (figure 5), suggesting that possibly there may be a delay in maladaptive effects inherent to our HF diet rodent model.

Regarding plausible mechanisms, Stanford et al. 2018 described that paternal exercise training significantly regulates multiple classes of small RNAs in sperm, suppressing some harmful paternal dietary effects on small RNA levels, and thus implicating these small RNAs as potential mediators in the transmission of paternal environmental information to the next generation. Considering these findings, and a possible mechanistic explanation, we speculate that offspring proteome would be more influenced when the offspring were exposed to a HF diet. This brief explanation would be a reasonable description to the minimal changes seen in TFO-C compared with SFO-C, and might clarify the relevant proteomic changes seen in TFO-HF compared with SFO-HF.

It is worth noticing that paternal RT promotes a significant increase of proteins related to muscle contraction and sarcomere organization when compared to offspring from sedentary fathers in both high-fat diet (TFO-HF: SFO-HF) and those not exposed to HF diet (TFO-C: SFO-C). According to Subramanian and Schilling, 2015, tendon morphogenesis is mediated by interactions with skeletal muscle and bidirectional communication between tissues is essential for tendon integrity maintenance. Moreover, such relationship is potentially accentuated in response to the exercise training (Lidstone et al., 2016). It is reasonable to suspect that cross-talk between tenocytes and muscle cells occur to maintain connectivity and normal tendon remodeling, as well as strengthen

attachments under tension (Barin et al., 2019). Possibly, healthy heterotypic interactions between tissues might help mitigate the deleterious effects inherent to HF diet.

It has been widely shown that the tendon structure adapts in response to mechanical loading modifying mainly the ECM architecture and composition (Marqueti et al., 2018). An important outcome of the present study was a considerable increase in abundance of several proteins related to cell adhesion, cytoskeleton organization and ECM organization in the TFO-HF group when compared to the SFO-HF group. Type XIV collagen up-regulation is associated with regulation of assembly and linear fibril growth, which can be important for stabilization of immature fibrils (Ansorge et al., 2009). Moreover, this protein is often present in areas of high mechanical stress, indicating it potentially has a role in maintaining mechanical properties of tissues (Ansorge et al., 2009). Procollagen C-endopeptidase enhancer 1 is a crucial enzyme for accurate and efficient conversion of fibrillar procollagens to their self-assembling monomers (Vadon-Le Goff et al., 2011). Upregulation of this protein might represent an adaptation mechanism to maintain enzymatic operation in the tendon remodeling (Bourhis et al., 2013). At the same time, TFO-HF group presented increased abundance levels of glycoproteins responsible for cell–matrix interactions. The enhancement in the cartilage oligomeric matrix protein and thrombospondin 1 (Thbs1) could facilitate ECM structural support, mechanical stability, and adhesion between cells (Sodersten et al., 2006; Wang et al., 2017). It is worth mentioning that Procollagen C-endopeptidase enhancer 1 and Protein Thbs1 changed in the same direction (i.e up-regulation) in the two groups involved with exercise training (i.e TF and TFO-HF group), representing a relevant overlapping between fathers and the offspring. The upregulated proteins in these two groups generated a protein network that showed relevant connectivity (Figure 8). Considering these molecular findings, we suggest that paternal RT can be a relevant intervention capable of inducing significant effects on offspring proteins involved in ECM necessary for structural arrangement and mechanical function.

A novelty of this work was the finding of protein col28a1, increased after HF diet exposure, but paternal HF significantly prevents such increase. The collagen XXVIII is widely detected in the sciatic nerve at the basement membrane of specific Schwann cells surrounding the nerve fibers (Veit et al., 2006). However, the discussion of our findings is not an easy task, because there is no evidence of their specific functions in the tendon (Gebauer et al., 2016). Moreover, RT-PCR revealed a broader expression of collagen

XXVIII in newborn than in adult mice (Gebauer et al., 2016), which limits the interpretation of results up to now described.

Another finding from this study was that TFO-HF presented high abundance levels of adiponectin when compared with SFO-HF group. In human clinical studies, adiponectin has shown the ability to improve insulin sensitivity, tenocyte progenitor cells proliferation and differentiation, which makes it a therapeutic agent in treating diabetic tendinopathy (Rothan et al., 2013). Presumably, adiponectin might represent a promising biological target to prevent tendon abnormalities related to HF-diet. In animals models, adiponectin-deficient mice demonstrated a near-normal insulin sensitivity when fed with control diet, however, developed insulin resistance in skeletal muscle after two weeks of exposure to HF diet (Maeda et al., 2002). In the same experimental model, the area under the curve of blood glucose did not reveal significant differences between offspring groups (data not published yet), which may be related to Wistar rats not responding pronouncedly to an HF diet when compared to other mouse strains. Future studies will be required to evaluate the link between changes of adiponectin and glucose uptake by tenocytes besides glucose transporters.

The present study demonstrated an increase of proteins for transporting molecules involved in tendon metabolism and ions bioavailability in TFO-HF group when compared with SFO-HF group. It has been demonstrated that vitamin D binding protein has a role in maintaining the total levels and the amount of free vitamin D (Bikle and Schwartz, 2019). In this context, Angeline et al. (Angeline et al., 2014) found that decreased levels of vitamin D influence early healing after rotator cuff repair, resulting in decreased fibrocartilage formation and disorganized collagen. The authors suggest that one possible beneficial function of vitamin D in the healing process is to decrease tendon inflammation (Angeline et al., 2014) by downregulating the cellular response to tumor necrosis factor-α, and by regulating matrix metalloproteinase 9 (Bahar-Shany et al., 2010; Dougherty et al., 2016). In the present study, we speculate that the upregulation vitamin D binding protein herein described may help to control the inflammation in order to maintain tendon morphology. Another protein, sodium/potassium-transporting ATPase catalyzes the hydrolysis of ATP coupled with the exchange of sodium and potassium ions across the membrane (Xie and Cai, 2003). An increase of this protein can contribute to create a proper electrochemical gradient, providing the energy for active transport of ions (Xie and Xie, 2005). This regulatory response becomes increasingly important in muscle-tendon junction when it is required a strongest excitation in response to exercise

(Gundersen, 2011). A schematic representation of fibroblast was used to clarify the location, the up and downregulation, and the role of the main proteins identified in this study (TFO-HF:SFO-HF) (Fig. 9). Proteins that are differentially regulated by paternal RT are particularly promising candidate proteins to transduce exercise-induced tendon health benefits and they represent specific targets for the development of future biomarkers and therapeutics.

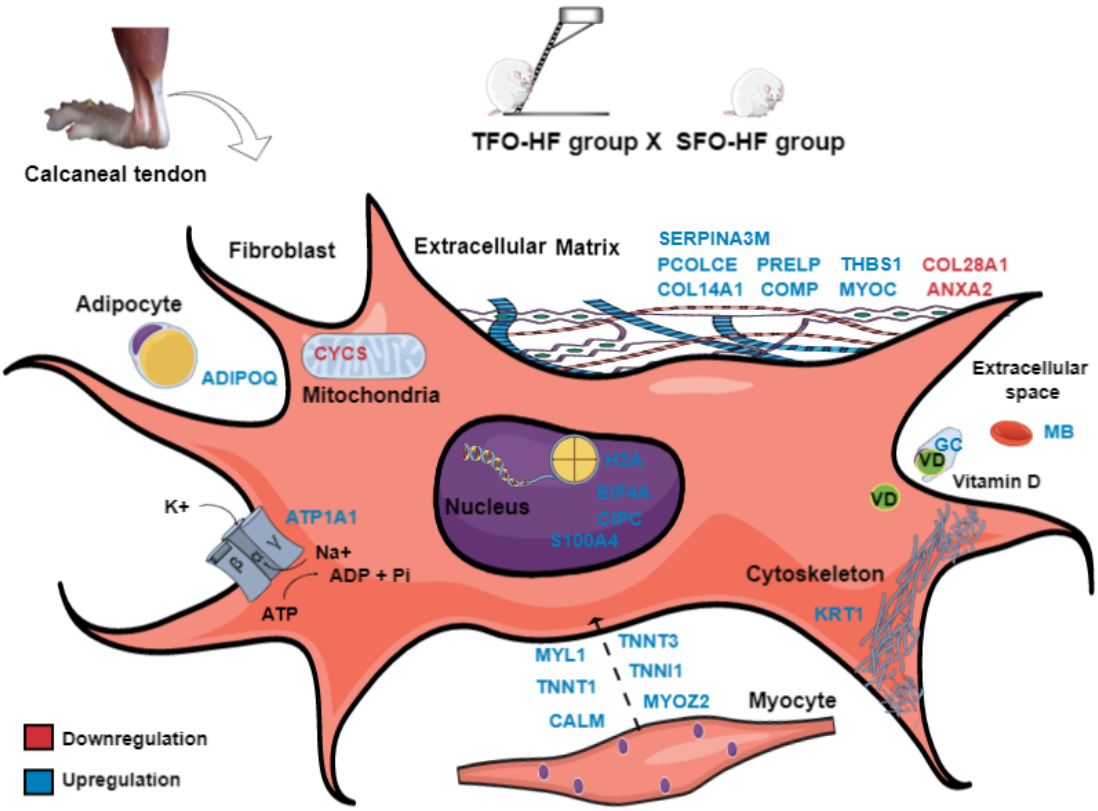


Figure 9. Paternal resistance training affects offspring calcaneal tendon proteome exposed to high-fat diet. SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. The main upregulated (red) and downregulated (blue) proteins in the analysis (TFO-HF: SFO-HF). Adipocyte = Protein Adipoq (ADIPOQ). Cell membrane = Sodium/potassium-transporting ATPase subunit alpha-1 (ATP1A1). Cytoskeleton = Keratin, type II cytoskeletal 1 (KRT1). *Extracellular space = Vitamin D-binding protein (GC), Serum albumin (MB).* Extracellular matrix = Annexin A2 (ANXA2), Cartilage oligomeric matrix protein (COMP), Myocilin (MYOC), Procollagen C-endopeptidase enhancer 1

(PCOLCE), Prolargin (PRELP), Protein Col14a1 (COL14A1), Protein Col28a1 (COL28A1), Serine protease inhibitor A3M (SERPINA3M), Thrombospondin-1 (THBS1). Mitochondrion = Cytochrome c, somatic (CYCS). Myocyte = Calmodulin (CALM), Isoform 3 of Troponin T, slow skeletal muscle (TNNT3), Myosin light chain 1/3, skeletal muscle isoform (MYL1), Myozenin 2 (MYOZ2), Troponin I, slow skeletal muscle (TNNI1), Troponin T, fast skeletal muscle (TNNT1). Nucleus = Eukaryotic translation initiation factor 4H (EIF4A), Histone H2A (H2A), Protein Cipc (CIPC), Protein S100-A4 (S100A4).

To our knowledge, this is the first study that used the RT modality in fathers and we elucidated the importance of this type of training in future generations. This study contributes to better understanding of tendon biology and clarifies more profoundly the molecular networks behind physiological adaptations promoted by paternal RT. Our results open new perspectives for studies based on transcriptomics, metabolomics, and functional assays. Despite all the proteomic profiles, the lack of other analyses, such as morphological properties, tendon functional assessments would be relevant to clarify adjacent mechanisms involved in intergenerational inheritance. The size of the tendon was small (~80 ug of tissue weight) and only allowed us to obtain an adequate amount of proteins for proteomic analysis. Due to the specific procedures performed we cannot use the processed sample to other analyzes that require different methods of extraction. Modifications in sperm provide a potential molecular basis to explain the contribution of the father's lifestyle on the offspring phenotypes and non-coding RNAs are probabilistic epigenetic mechanisms. Further investigations are required to evaluate the link between sperm epigenetic status and tendon proteome.

CONCLUSION

Paternal RT affect the abundance of numerous proteins in the tendon and HF diet results in disturbance of important proteins which might explain tendon disorders associated to an unhealthy diet. Most interestingly, paternal RT modulates pathways in the tendon of the offspring, being such modulation more evident when the offspring is subjected to HF diet. Most of the modulated proteins are associated to biological pathways related to tendon protection and damage recovery, such as ECM organization, transport and inflammatory mediators, suggesting a protective effect of paternal exercise

against potential harmful effects of a HF diet. Tendency to tendon diseases and higher prevalence of tendon injuries in the offspring could be partially explained by the father behavior.

Author Contributions:

IVSN, RAT, JP and RCM: conceived and planned the design of the experiments; IVSN, RAT, LGOS, EML, GPGP, JAA, performed the experiments and analyzed the data; MVS, CAOR, H L D, MSC, WF: performed methods of label-free quantification and bioinformatics. IVSN, RAT, JP, OLF, JLQD, WF and RCM interpreted the results and worked in the writing of manuscript; OLF, JLQD, ABA, WF and RCM involved in planning and supervised the work, also contributed to the design and implementation of the research and provided critical feedback and analysis of the manuscript. All authors discussed the results and contributed to the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Data in Supplementary Information Files

The Supplementary data 1 and 2 were used to support the findings of this study and are included within the article.

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4.4 Artigo científico relacionado à tese (em fase final de redação):

“**Protective role of intergenerational paternal resistance training on fibrosis, inflammatory profile and redox profile in the adipose tissue of offspring fed with high-fat diet.”**

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Protective role of intergenerational paternal resistance training on fibrosis, inflammatory profile and redox status in the adipose tissue of male offspring fed a high-fat diet.

Ivo Vieira de Sousa Neto[1,2], Jonato Prestes[3], Graciele Vieira Ramos[1], Guilherme, Borges Pereira[4], Jesser Alves de Almeida[5], Fabiane Hiratsuka Veiga de Souza[6], Paulo de Souza[7], Ramires Alsamir Tibana[8], Octavio Luis Franco[9,10], João Luiz Quaglioti Durigan[11], Thiago dos Santos Rosa[3], Rodrigo Vanerson Passos Neves[3], Wagner Fontes[6], Rita de Cassia Marqueti[1,2,11].

[1]Laboratory of Molecular Analysis, Faculty of Ceilândia, Universidade de Brasília, Distrito Federal, Brazil; [2]Graduate Program of Sciences and Technology of Health,Universidade de Brasília, Distrito Federal, Brazil; [3]Graduate program of Physical Education, Catholic University of Brasilia, Distrito Federal, Brazil; [4]Department of Physiological Sciences, Universidade Federal de São Carlos, São Paulo, Brazil; [5]Graduate program of Health and Development in the Central West Region, Federal University of Mato Grosso do Sul, Brazil; [6]Laboratory of Protein Chemistry and Biochemistry and Protein Chemistry, Department 24 of Cell Biology, Institute of Biological Sciences, University of Brasilia, Brasilia, DF, 25 Brazil; [7]Laboratory of Electron Paramagnetic Resonance, Institute of Physics, University of Brasilia, Brasília, DF, Brazil; [8]Graduate Program in Health Sciences, Faculdade de Medicine, Universidade Federal do Mato Grosso (UFTM), Cuiabá, Brazil; [9]Graduate Program in Genomics Science and Biotechnology, Universidade Católica de Brasília, Brasília/DF, Brazil,
[10]S-Inova Biotech, Graduate Program in Biotechnology,Universidade Católica Dom Bosco, Campo Grande/MS, Brazil, [11]Graduate Program in Rehabilitation Sciences, Universidade de Brasília, Distrito Federal, Brazil.

Corresponding authors

Ivo Vieira de Sousa Neto (ivoneto04@hotmail.com) and Rita de Cassia Marqueti (marqueti@gmail.com) Campus Universitário, Centro Metropolitano 1, Conjunto A, Universidade de Brasília, UnB – Zip Code 72220-900. Tel.: +55 67 99871 4343.

ABSTRACT

The increase in high-energy dietary intakes is a well-known risk factor for obesity and negatively impacts adipose tissue function. Ancestral lifestyle can alleviate the detrimental metabolic effects of offspring fed a high-fat diet (HFD). However, this mechanism is still unclear. Here, we evaluate the role of the intergenerational paternal exercise model on fibrosis, inflammatory profile, and redox status in the adipose tissue of male offspring fed a high-fat diet and explored to what extent potential programming effects affect metabolic profile and blood circulating markers. Wistar rats were randomly divided into two groups: sedentary fathers and trained fathers (8 weeks of resistance training, three times per week, with 8–12 dynamic movements per climb in a stair climbing apparatus). The offspring were obtained by mating with sedentary females. Upon weaning, male offspring were divided into four groups (7 animals per group): offspring from sedentary fathers were exposed either to control diet (SFO-C) or to a high-fat diet (SFO-HF); offspring from trained fathers were exposed to control diet (TFO-C) or to a high-fat diet (TFO-HF)**.** Paternal RT was highly effective in attenuating adipocyte size, collagen deposition, besides down-regulating genes (CTGF, VEGF, C/EBPα SREBP1, MCP-1, and NF-kB), pro-inflammatory cytokines levels (Tnf-α, and IL1-β), MMP-2 activity, ROS production, H_2O_2 and TBARS levels in the epididymal adipose tissue of offspring fed an HFD. (TFO-HF vs. SFO-HF). Moreover, paternal RT increases adiponectin levels and SOD activity in the TFO-HF group. Interestingly, these findings were accompanied by substantial improved antioxidants enzymes (SOD and α-Klotho) and decreased metabolic markers (insulin and leptin levels) in the blood circulation. Taken together, our results indicate potential protective role of intergenerational paternal resistance training on adipose tissue remodeling pathways and metabolic health of first generation fed an HFD.

Keywords: offspring adipose tissue, metabolic dysfunction, intergenerational, paternal programming, paternal exercise.

INTRODUCTION

Adipose tissue (AT) highlights out among metabolically essential tissues due to its high plasticity, multicellularity, and multifunctional functions (Kajimura, 2017). Under conditions of overnutrition, increased energy storage in the form of triacylglycerides occurs in AT, triggering a massive tissue expansion, which delineates overweight and obesity conditions (Lewis, Carpentier, Adeli, & Giacca, 2002). It has been established that a high-fat diet (HFD) induces hypertrophic adipocytes, accompanied by tissue hypoxia, and chronic low-grade inflammation, which leads to tissue and systemic insulin resistance onset (M. Q. He et al., 2020; Kuipers et al., 2019; Poret et al., 2018).

Orchestrating adipocytes expansion requires extracellular matrix (ECM) remodeling and a high degree of interaction between cells and ECM (Mariman & Wang, 2010; Ruiz-Ojeda, Mendez-Gutierrez, Aguilera, & Plaza-Diaz, 2019). Adipose fibrosis is characterized as an excessive accumulation of ECM components, including collagen I and IV deposition (Datta, Podolsky, & Atabai, 2018). The ECM enlargement is related to complex interplay between different immune cells, pro-inflammatory mediators and matrix metalloproteinases (MMPs), which may strong influence the redox state (Castro, Grune, & Speckmann, 2016). Among the relevant signaling pathways, growth factors (CTGF, IGF-1 VEGF, and TGF-β) (Chun et al., 2019) and transcription factors (C/EBP alpha, SREBP1, and NF-κB) (White & Stephens, 2010) are linked to the adipogenesis process, fibrosis appearance and inflammatory cascade, possibly by pro-inducing oxidants agents (8-hydroxyguanosine, F2-isoprostanes, lipid peroxidation, nitric oxide, and protein carbonyl) (Castro, Grune, & Speckmann, 2016) and cytokines (TWEAK, TNF-α, IL-1β, IL-6 and MCP1 protein) (Kang et al., 2016). Certainly, AT secretes many bioactive molecules, which partly overlaps with blood circulation, according to metabolic signals and energy homeostasis (Coelho, Oliveira, & Fernandes, 2013). Hence, mutual crosstalk between ECM turnover, oxidative stress, and inflammatory state modulates adipocyte functioning and aggravated glucose metabolism.

Exercise training is a potent mediator of lipolysis while preventing rapid ECM expansion in mice fed with an HFD (Kawanishi, Niihara, Mizokami, Yano, & Suzuki, 2013; Li et al., 2021) and consequently attenuates inflammatory response and reactive oxygen species (ROS) production in parallel with improved primary metabolism. Some studies suggest that exercise is a promising non-pharmacological approach for retards

ongoing adipose tissue fibrosis and adipogenesis process, linked to the PPARγ-mediated mechanism(Li et al., 2021). Currently, resistance training (RT) has a prophylactic impact by suppressing pro-inflammatory factors (i.e., TNF-α, IL-6, IL1-β, and MCP-1) and enhancing antioxidant enzymes (superoxide dismutase, catalase) and adiponectin (Medeiros et al., 2021), which may have crucial roles on redox state, adipose tissue homeostasis maintenance, and consequently ameliorates metabolic dysfunction.

Evidence suggests that future generation might inherit beneficial exercise-mediated effects via intergenerational epigenetics inheritance (Vieira de Sousa Neto, Fontes, Prestes, & de Cassia Marqueti, 2021). Current advances reported that active molecules in the sperm epigenome clarify the contribution of the father's lifestyle, in which offspring phenotype reprogramming is established (Kusuyama, Alves-Wagner, Makarewicz, & Goodyear, 2020; Stanford et al., 2018; Vieira de Sousa Neto et al., 2021). In rodent models, exercise as an intervention in fathers before conception orchestrated fetus development and diminished placenta inflammatory status (Claycombe-Larson, Bundy, & Roemmich, 2020). Furthermore, paternal exercise promoted adjustments in the ncRNA profiles, besides modulates key genes and proteins expression in the hippocampus (Yin et al., 2013), left ventricle (de Sousa Neto, Tibana, Prestes, et al., 2020), skeletal muscle (Krout et al., 2018), tendon (de Sousa Neto, Tibana, da Silva, et al., 2020), liver (Batista et al., 2020), and pancreas (McPherson et., 2002) responsible by tissues remodeling in the first offspring and consequently improve holistic health. Although studies have demonstrated that the father’s behavior may modulate molecular players responsible for several tissues remodeling, the underlying molecular mechanisms at adipocyte level in the first offspring exposed to HPD remain unclear. Advances to map key paternal exercise-regulated pathways in offspring adipose tissue is a valuable issue for health, treatment guidance, and design interventions, and elucidate possible adaptive mechanisms regarding the fraction of this heritability. Combined functional assessments, morphological, biochemical, and molecular approaches might help understandthe underlying mechanistic basis of HPD-associated metabolic disease in the offspring.

Using an intergenerational paternal exercise model, we investigated whether eight weeks of RT performed by father before pregnancy could induce protective effect against harmful effects of HPD on fibrosis, inflammatory profile, and redox status in the adipose tissue of offspring. We explored to what extent potential programming effects affect metabolic and oxidative stress biomarkers in blood circulation. The novel and clinically

relevant hypothesis are that paternal RT could minimize responses at the transcriptional level (adipogenic and growth factors, inflammatory pathways), ECM protein turnover, proinflammatory cytokines levels, ROS production, and pro-oxidant agents in epididymal adipose tissue induced by HFD and that such regulation displays an overlapping with blood circulation.

MATERIALS AND METHODS

Animals and experimental groups

All procedures were conducted following the Guide for the Care and Use of Laboratory Animals (U.S. National Research Council, 1996) in compliance with the ARRIVE guidelines. The research protocol received approval from the Ethics Committee on Animal Experimentation from the Universidade Católica de Brasília, Brasília (protocol No. 010/13). Initially, fourteen (n=14) four-month-old *Wistar* rats (*Rattus norvegicus albinus*, weighing 359g ± 32.4) were obtained from the Central Vivarium of the Faculty of Physical Education of the Universidade Católica de Brasilia. The animals were housed in collective cages (maximum of four rats per cage) and received water and standard feed for rodents *ad libitum* during the experimental period. The animals were randomly distributed into two subgroups: sedentary fathers (SF; n=7; did not perform RT) and trained fathers (TF; n=7; performed RT).

Paternal Resistance Training:

The RT protocol used in this study was adapted from Hornberger and Farrar (2004). The procedures have been described in previous intergenerational studies (de Sousa Neto et al., 2017; de Sousa Neto, Tibana, da Silva, et al., 2020). The RT protocol was carried out for eight weeks, with the climbs performed three times a week (Monday, Wednesday, and Friday) in the afternoon (between 2 pm and 4 pm). Initially, the rats were adapted to the resistance exercise protocol, which required the animals to climb a vertical ladder (1.1 x 0.18 m, 2cm step, 80% climb angle) with the load apparatus fixed to their tail, via a self-adhesive foam strip wrapped around the proximal portion. The size of the steps meant the animals performed 8 to 12 movements per climb. In the familiarization

phase, the rats were placed at the bottom of the stairs and were stimulated to climb with a weight attached to the tail. If necessary, a stimulus through finger tweezers was applied to the animal's tail to start the movement. The rats reached a housing chamber at the top of the stairs (20 x 20 x 20cm), where they rested for 2 minutes. This procedure was repeated until the animals could voluntarily climb the subsequent set without stimulation (de Sousa Neto et al., 2021; de Sousa Neto et al., 2018). The familiarization sessions were performed three times with an interval of 48 hours.

Three days after familiarization, each animal was evaluated to determine its maximum load capacity, which consisted of 4 to 8 ladder climbs with gradually heavier loads. The initial climb consisted of carrying a load corresponding to 75% of the animal's body weight. After that, weights of 30g were added progressively until the rat was unable to complete the climb. Failure to climb was determined when the animal was unable to complete the climb after three successive tail stimuli. The highest load carried to the top of the stairs was considered the maximum load capacity of the rat for that specific training session. After defining the maximum load capacity, the training sessions comprised eight climbs (TF), two sets with each load of 50%, 75%, 90%, and 100% of the animal's maximum load capacity. Average feed intake was measured for each experimental group 24h and 48h after the first RT session and 72h after the second RT session.

In each week, respecting the progressive overload principle of the training, 30g (approximately 13% of the animal's body weight) were added, referring to the weight of the previous week with 100% of the maximum loading capacity, in order to guarantee the desired stress resulting from the exercise.

Offspring groups

The offspring came from mating with sedentary females. The estrous cycle of the females was checked daily after completing the RT, and during the proestrus phase, a male and a female were housed together for two consecutive days with free access to a control diet (Purina®, Descalvado-SP, Brazil) and water. After birth, the experimental groups in this study consisted of 28 male pups. The litters were standardized to 7 pups each to avoid litters of different sizes and were left with their mothers for 21 days of breastfeeding. The litters belonging to the same experimental group were descended from different parents.

After weaning, male pups were divided into 4 groups (n=7 per group): offspring from sedentary father submitted either to control diet (SFO-C) or high-fat diet (SFO-HF)

and offspring from trained father submitted to control diet (TFO-C) or high-fat diet (TFO-HF). The animals were housed in polypropylene cages with a temperature of 23 ± 2°C and light:dark cycle of 12:12h. The pups were weighed, in grams, on a digital scale, weekly for six months (Filizola®, São Paulo, Brazil).

Offspring Diet

The offspring submitted to the control diet (SFO-C and TFO-C) were fed with standard feed (66.00% carbohydrates, 24.00% protein, and 10.00% lipids, totaling 3.48 Kcal/g, Labina Presence®, Paulínia, São Paulo, Brazil) and water *ad libitum*. Females were also kept on the control diet during gestation and lactation periods. The SFO-HF and TFO-HF groups were submitted to the commercially acquired HF diet (20.27% carbohydrates, 19.89% protein, and 59.38% lipids, totaling 5.20 Kcal/g) (Prag® solutions, Biosciences, Jaú, Brazil) and an overload of 200 mL of soft drink (high-fructose corn syrup, caramel coloring, caffeine and phosphoric acid) per week (100% carbohydrates - 21g, sodium: 10mg totaling 0.85 Kcal/g), in addition to having free access to water from the 21st day after birth for 6 months. Detailed compositions of the experimental diets were reported previously (de Sousa Neto et al., 2020b).

Some studies have demonstrated the HFD efficacy on body weight gain and adiposity in *Wistar* rats (de Sousa Neto, Tibana, da Silva, et al., 2020; de Sousa Neto, Tibana, Prestes, et al., 2020; Goncalves et al., 2018). Soft drinks for rats are used as a complement and characterization of the HFD and are an effective strategy to total energy intake increase and body weight gain in the long-term HPD conditions (Kjaergaard et al., 2017; Lozano et al., 2016; Swithers, Martin, Clark, Laboy, & Davidson, 2010).

2.4 Euthanasia:

First, fathers groups were euthanized with an intraperitoneal injection of xylazine solution (12 mg/kg body weight) and ketamine (95 mg/kg body weight), 48 hours after the resistance training period to avoid the acute effects of exercise. The offspring were weighed on a digital scale (Filizola, São Paulo, Brazil) before euthanasia. The offspring groups were euthanized with the same combination of solutions after six months of exposure to the HFD. Afterward, the epididymal adipose tissues were immediately dissected, and one part was fixed in 4% buffered paraformaldehyde for histological analysis and other frozen in RNase-free tubes using liquid nitrogen (for qPCR, cytokines,

zymography, and redox state analysis) and then stored at -80°C for further molecular analysis. An experienced researcher dissected epididymal adipose tissue to prevent contamination of other tissues. The adiposity index was calculated as (total body fat/final BW) × 100. Furthermore, blood samples were drawn from the left internal jugular vein by venipuncture and collected in lithium heparin-coated vacutainer tubes without EDTA. Blood plasma was centrifuged at 4 500 rpm for 20 min and immediately frozen and stored at − 80 °C in small volume aliquots until used for the assays.

Metabolic analysis and offspring aerobic capacity

Twenty-two weeks in the diet exposure period, animals were fasted for 6 h for the glucose tolerance test ipGTT (2g/kg; glucose 50%) and monitored for two hours after the glucose infusion. Glucose concentrations were measured in duplicate (Accu-Check Advantage. Roche®) to the determined area under the curve (AUC, [mg/dL x 120min]) according to Medeiros et al. 2021. All analyzes were performed using whole blood drawn from the tail. Plasma insulin and leptin levels were determined by ELISA assays using commercial kitst (mouse/rat Quantikine & Immuno Assay kit, R&D Systems, Minneapolis, MN, USA) and then analyzed at 450 nm using a plate reader (Spectra Max 250; Molecular Devices, San Jose, CA, USA).

Incremental-speed treadmill running was used for assessing the aerobic capacity in offspring groups and was performed in the 8 and 22 weeks in the diet exposure period. All tests were adapted from Almeida et al. 2014. An familiarization protocol was adopted to reduce stress linked to exercise,. The animals were familiarized with running on a treadmill designed for Wistar rats (li 870, Letica Scientifi, Barcelona, Espanha) for 3 days with a constant speed of 13 m.min-1 during 10 min. The test session started 2 days after the familiarization section. During the incremental exercise, the animals started running at a speed of 13 m.min-1, followed by speed increments of 3 m.min-1 every 3 min until they reached fatigue. Fatigue was definite as the point at which the animals could no longer maintain their pace in the exercise, even when exposed to light electrical stimulation for 10 s. The antecedent stage established the maximal aerobic capacity.

Histological and Immunohistochemical analysis

Epididymal adipose tissue was fixed in 10% neutral-buffered formalin (VWR, Mississauga, Ontario, Canada), dehydrated in xylene (Fisher Scientific), and embedded in paraffin at 60ºC. The sections of 6 mm cut on the microtome (Leica, Wetzlar, Germany) and then placed onto glass microscope slides. Paraffin was removed with xylene, and sections were stained with hematoxylin and eosin (HE, Sigma Aldrich, St. Louis, MO, USA) and Masson's trichrome dye for determining adipocyte cross-sectional area (CSA) and collagen deposition, respectively. One hundred fifty cells from 5 animals per group were used to determine the cross-sectional area (ImageJ software; National Institute of Mental Health, Bethesda, MD) proposed by Macpherson et al. 2015. Collagen fibers were quantified using these steps: image acquisition and processing, setting the scale, deconvolution of the color images, and quantification of the collagen fibers. The entire protocol was performed as described previously by Li et al. 2021. Pictures were acquired using an Olympus BV51 microscope equipped with an SV Micro Sound Vision digital camera (Preston South, Australia) at 20x magnification. The adipocyte CSA and Masson-positive area were conducted by a blinded researcher, attenuating possible bias related to this process.

For immunohistochemical analysis of collagen type I, slides were incubated with a solution containing the primary antibodies in 1% bovine serum albumin in 0.1 M PBS at 4°C. The primary antibodies used in this study were collagen type I (Mouse Anti-Collagen I Monoclonal Antibody, Unconjugated, Clone COL-1, 1:1000; Abcam, Cambridge, MA, USA). The slides were rinsed, dehydrated, cleared in xylene, and covers lipped with Permanent Mounting Medium (Thermo Fisher Scientific). Estimation of collagen I was performed qualitatively by experienced research using visual gradation for each microscopic field.

RNA extraction from epididymal adipose tissue samples

RNA isolation from adipose tissue was performed using the TRIzol method according to Chomczynski & Sacchi, (1987). Epididymal adipose tissue samples (28 animals -7 per group) were homogenized in a tube containing five stainless steel balls (diameter, 2.3 mm) (BioSpec Products, Bartlesville, OK, USA) and three silicon-carbide sharp particles (1 mm) (BioSpec Products) containing 1 mL of trizol. Samples were shaken in a FastPrep-24 instrument (MP Biomedicals, Solon, OH, USA) This process was repeated ten times with ice cooling between each shaking step to avoid RNA

degradation. . A NanoDrop® spectrophotometer (ND-1000; NanoDrop Technologies Inc., Wilmington, DE, USA) was used to quantify RNA concentrations in each sample by determining the absorbance ratio 260/280 and 260/230 for assessing the purity of the samples. TURBO DNA-free kit (Ambion - Life Technologies - 1907M) was used for DNA digestion, according to the manufacturer's recommendations. Next, samples were frozen at -80ºC.

qRT-PCRReverse Transcription

To evaluate epididymal adipose tissue gene expression, a total of 1 μg of RNA extracted from each sample were converted into cDNA (final volume 20 μL) using SuperScript ™ VILO ™ MasterMix reverse transcriptase (Invitrogen-Cat. 11755-010) according to the manufacturer's protocol. To perform reverse transcription, the samples were incubated at 25°C for 10 minutes, at 42°C for 60 minutes, and at 85°C for 5 minutes before being stored at -20°C in a freezer.

Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCR was performed using TaqMan Universal PCR Master Mix system (Applied Biosystems, CA, USA - Cat. 4304437). Ten μL of GoTaq Probe qPCR Master Mix (Promega - A6102) were homogenized and combined with 1 μL of the primer 20x, an amount of cDNA determined according to the standard curve, and water for a final volume of 20 μL. The amplification reaction was performed by QuantStudioTM3 (Applied Biossystems) according to the manufacturer's instructions. qRT-PCR was performed using a QuantStudio 3 Real-Time PCR System (Applied Biosystems) for the following genes: CCAAT/enhancer binding protein alpha (*Cebpa*); Type I collagen alpha 1 chain (*Col1a1*), Type III collagen alpha 1 chain (*Col3a1*), Connective tissue growth factor (*Ctgf*), Fatty acid-binding protein 4 (*Fabp4*);Pro-insulin like growth factor IA (*Igf1a*), monocyte chemoattractant protein-1 (*Mcp1*); Matrix Metalloproteinase-2 (*Mmp2*), nuclear factor E2-related factor 2 (*Nrf2*), nuclear factor kappa b (*Nfkb*); peroxisome proliferator-activated receptor alpha (*Ppara*), ribosomal protein lateral stalk subunit P0 (*Rplp0*); Sterol regulatory element-binding protein 1 (*Srbp1*); transforming growth factor, beta 1 (*Tgfb1*); Tissue inhibitor of matrix metalloproteinase-2 (*Timp2*),

tumor necrosis factor alpha (*Tnfa*); TNF superfamily member 12 (*Tweak*) and Vascular endothelial growth factor (*Vegf*) (Table 1).

Table 1. List of tested genes:

Table 1. List of tested genes associated with growth factors, adipogenic and factors regulators of lipid metabolism, extracellular matrix, inflammation and antioxidant response:

mRNA	Code (Life technologies)	mRNA	Code (Life technologies)
Cebpa	rn00560963	***Ppara***	rn00566193
Col1a1	rn01463848	***Srebp1***	rn01495769
Col3a1	rn01437681	***Rplp0***	rn03302271
Ctgf	rn01537279	***Tgfb1***	rn00572010
Fabp4	rn04219585	***Timp-2***	rn00573232
Igf1a	rn00710306	***Tnfa***	dr03126850
Mcp1	rn00580555	***Tweak***	rn01461586
Mmp2	rn01538170	***Vegf***	rn01511602
Nrf2	rn01767215		
Nfkb	rn00595794		

CCAAT/enhancer binding protein alpha (*Cebpa*); Type I collagen alpha 1 chain (*Col1a1*), Type III collagen alpha 1 chain (*Col3a1*), Connective tissue growth factor (*Ctgf*), Fatty acid-binding protein 4 (*Fabp4*); Pro-insulin like growth factor IA (*Igf1a*), Matrix Metalloproteinase-2 (*Mmp2*), monocyte chemoattractant protein-1 (*Mcp1*), nuclear factor E2-related factor 2 (*Nrf2*), nuclear factor kappa b (*Nfkb*); peroxisome proliferator-activated receptor alpha (*Ppara*), ribosomal protein lateral stalk subunit P0 (*Rplp0*); Sterol regulatory element-binding protein 1 (*Srbp1*); transforming growth factor, beta 1 (*Tgfb1*); Tissue inhibitor of matrix metalloproteinase-2 (*Timp2*), tumor necrosis factor alpha (*Tnfa*); TNF superfamily member 12 (*Tweak*) and Vascular endothelial growth factor (*Vegf*).

For each gene, all samples were amplified simultaneously with technical duplicates from the same cDNA in a single run. The expression of each target gene was normalized based on the expression of the constitutive RPLPO gene, which was used as

the control of endogenous RNA, due to lower intra and intergroup variability compared to the other housekeeping genes tested (GAPDH and β-actin). The ΔCt values of the samples were determined by subtracting the mean Ct value of the target gene from the mean Ct value of the housekeeping gene. Subsequently, the ΔΔCt values were calculated by subtracting the ΔCt value of the condition of interest from the ΔCt of the control condition. Finally, 2-ΔΔCt values were computed for presentation of relative expression data. The amount of sample and the efficiency of the reaction of each gene analyzed in the present study were determined from a standardization curve, having slope reference parameters equal to -3.3, R2 = 0.9-1.0 and efficiency above 90%.

Cytokine levels in epididymal adipose tissue

Total proteins were extracted from tissues using phosphate buffer saline (PBS 1%, pH 7.4) supplemented with protease inhibitor cocktail (Roche, Germany). Next, the samples were centrifuged (14.000g for 30 minutes at 4°C) and supernatant was transferred to a new tube. The total quantification of proteins were performed in a NanoDrop 2000 spectrophotometer (Thermo Scientific) using a 260/280 nm relation. Multiple analysis kit was obtained from Linco Research Inc. (St. Charles, MO). Millipore multiscreen 96 well filter plates (Bedford, MA) were used for all multiplex kit. Adiponectin, IL-6, MCP-1, IL-1β, and TNF-α levels were determined by enzyme-linked immunosorbent assay (ELISA), using the respective rat development kit (RADPCMAG-82K-05). Cytokine levels were expressed as picogram per milliliter (pg/mL), after comparison with the standard curve proposed by the ELISA kit used in accordance with the manufacturer's specifications. Standard curves for each cytokine were generated using serial dilutions of the mediators supplied, with each sample titrated by linear interpolation. All samples were determined in duplicate to guarantee reliability. The minimum detectable level was 0.30 pg/mL. The intra-assay coefficient of variation was 0.30–1.02 %, and the inter-assay coefficient of variation was 0.06–3.82 %. Data was collected using the Luminex-200 system Version 1.7 (Luminex, Austin, TX).

Gelatin zymography

The zymography technique was used to measure MMP-9 and MMP-2 activity. Tissue: The epididymal adipose tissue extracts from fathers and offspring were homogenized and incubated in extraction buffer [10 mM cacodylic acid, pH 5.0; 0,15 M NaCl; 1μM $ZnCl_2$; 20mM $CaCl_2$; 1.5 mM NaN_3 and 0.01% Triton X-100] with five

stainless steel balls (diameter, 2.3 mm) (BioSpec Products, Bartlesville, OK, USA) and three silicon-carbide sharp particles (1 mm) (BioSpec Products) by being shaken in a FastPrep-24 instrument (MP Biomedicals, Solon, OH, USA). Next, the solution was centrifuged for 30 min (13.000g at 4°C), and the supernatant was reserved. A NanoDrop® spectrophotometer (ND-1000; NanoDrop Technologies Inc., Wilmington, DE, USA) was used to quantify protein concentrations.

Thirty mg of total protein were loaded into each lane. The samples were concentrated in 30µg of protein and 10µl of sample buffer without β-mercaptoethanol (reducing agent) and were resolved by electrophoresis on polyacrylamide gel with SDS and gelatin, in the final concentration of 1mg/mL. After the run, the gel was washed twice for 20 minutes in 2.5% Triton X-100 solution to remove the SDS. Next, the gel was incubated in the substrate buffer (Tris- HCl 50mM pH 8.0, CaCl 2.5mM; NaN_3 0.02% and $ZnCl_2$ 10mM), at 37°C, for 18 hours.

Blood samples: Samples containing 0.5 µL of plasma were added to 0.5 µl of SDS (8 %) (v: v). Samples were then vortexed and added to 10 µL of sample buffer without β-mercaptoethanol (reducing agent), containing SDS (8 %).

Subsequently, the gel was stained with Coomassie Brilliant Blue for 60 min. Afterward, the gel was washed in a solution of methanol 30% and acetic acid 10%, according to Sousa Neto et al. 2017. Pro and active isoform bands were identified via standard techniques using molecular weight criteria. The averages of the band intensities were measured using Image Master 2D Platinum 7.0 software and conducted by a blinded researcher, attenuating possible bias related to this process. The bands found in all groups were 92–70 kDa, as proposed by previous studies that evaluated MMPs in the adipose tissue and blood circulation (de Sousa Neto et al., 2017).

Electron paramagnetic resonance (ROS production)

The ROS production in the epididymal adipose tissue was assessed by electron paramagnetic resonance (EPR) using the spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH) according to Gomes et al. 2018. Small pieces (approximately 100 mg) of the tissues were placed in separate tubes and washed three times with Krebs HEPES buffer KHB each. It was added 700 µL of working solution containing 200 µM 1-hydroxy-3-methoxycarbonyl -2,2,5,5- tetramethylpyrrolidine (CMH), 25 µM DF, 5 µM DETC, and heparin sodium (50 IU·mL-1). The tubes were

incubated under gentle shaking at 37 ºC. After 60 min, 450 µL of the sample was transferred to a 1 mL de-capped syringe and snap-frozen in liquid nitrogen. All the samples were stored at –80 ºC until the EPR measurements were performed.

EPR spectroscopy EPR measurements were performed in a Bruker spectrometer (Bruker EMXplus, Germany), equipped with an X-band (9 GHz) high sensitivity cavity (Bruker ER 4119HS, Germany). For ROS detection, the samples were transferred to a liquid nitrogen dewar (Noxygen, Germany), and the spectra were recorded at 77 K. The instrumental settings were 2 mW microwave power, 5G amplitude modulation, 100 kHz modulation frequency, and 200G sweep width. The peak height, meaning the distance between the lowest and the highest points in the first derivative spectrum, was used to detect the signal. A calibration curve was obtained using the nitroxide radical (CP•) diluted in KHB to the following concentrations: 0, 5, 10, 50, and 100 µM. A linear calibration curve was obtained in this concentration range, and all the recorded data were within this calibration range. The results are reports in the CM levels.

Redox state in epididymal adipose tissue and blood circulation

The NO was measured using Griess reaction (Miranda et al., 2001), according to the following protocol: homogenate of adipose tissue samples were deproteinized with zinc sulfate (20%) in PBS; 100 µL of each sample were disposed in duplicates in a 96-well plate, a solution of 100 µL of vanadium chloride, 50 µL sulfanilamide and 50 µL of N-(1-Naphthyl) ethylenediamine dihydrochloride, were added, a standard nitrite curve were also added. After, the plate was homogenized and incubated for 40 min at 37◦C. The samples were read in a spectrophotometer at 540 nm.

For thiobarbituric acid-reactive substances (TBARS) measure, homogenate adipose tissue samples were diluted in 320 µL MiliQ H2O (1:5) and 1 mL of trichloroacetic acid (TCA) 17.5%, pH 2.0 was added, following the addition of 1 mL of thiobarbituric acid (TBA) 0.6%, pH 2.0. After the homogenization, the samples were kept in a water bath for 30 min at 95◦C. Subsequently, samples were immersed in ice and 1 mL of TCA 70%, pH 2.0 were added, and another incubation of 20 min at room temperature were done. The samples were centrifuged (3000 g for 15 min) and the supernatant were read in a spectrophotometer at 540 nm. The concentration of lipid peroxidation products were calculated using the molar extinction coefficient equivalent to malondialdehyde (MDA equivalent = 1.56 × 105 M−1 cm−1) according to Ohkawa et

al . 1979. For detection of intercellular H_2O_2, samples were incubated with 20 µM H2DCFDA for 15 min. The DCF level was measured using a flow cytometer (Cytomics FC500, Beckman Coulter, Brea, CA, USA).

The antioxidant chemicals were measured using commercial kits following the manufacturer's protocol. The SOD activity was measured using the SOD assay kit (Sigma-Aldrich R, CA, United States), read at 450 nm. Catalase activity was measured using the AmplexTM Red catalase assay kit (Thermo Fisher Scientific R, MA, United States) at 560 nm. Serum levels of F2-isoprostanes, protein carbonyl, 8-OHDG and α-Klotho, (IBL Co., Ltd, Japan, and Immutopics Inc., USA) was determined in duplicate using a specific rat enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, Inc., San Diego, USA). The overall intra- and inter-assays CVs for markers were in a range of 2 to 15 %.

Statistical analysis

The results are expressed as mean ± standard deviation (SD). The Shapiro-Wilk test was used to determine the normality of data, and the Levene test was used to analyze the homogeneity of the variance. Two-way ANOVA (diet x paternal training) was used to compare the dependent variables between the offspring groups . When a significant difference was detected, the Tukey post hoc test was applied to identify where the difference occurred. Independent t-test was used for comparison between father's groups. Simple Pearson's r correlations were utilized to determine the associations between adipocyte size, collagen deposition, redox balance, and cytokines. following the effect size ratings: small, r = 0.2 to 0.49, moderate, r = 0.5 to 0.79, and large, r > 0.8. An alpha level of $p \leq 0.05$ was considered significant. The software GraphPad Prism 8.3 (San Diego, CA, USA) was used for statistical analysis and graphics design.

RESULTS

Paternal RT modulates adiposity index and metabolic markers of offspring submitted to a high-fat diet

The offspring submitted to the HFD demonstrated a higher final body weight and adiposity index when compared with offspring exposed to the standard diet ($p = 0.001$; Fig 1A-B) following 24 weeks of diet exposure. Paternal RT reduced the body weight,

epididymal adipose tissue weight, and adiposity index in the offspring submitted to an HFD (p= 0.01 and p = 0.01, respectively). The 24-week HFD-fed offspring showed a greater increase in plasma glucose, insulin and leptin levels (p = 0.001). No significant difference regarding the Ip GTT AUC was observed between groups (p > 0.05; Fig 1E). However, paternal RT mitigates the increase in plasma insulin and leptin levels in TFO-HF compared to the SFO-HF group (p = 0.004 and p = 0.03; Fig 1F-G). There were no differences in the maximum speed at 8 weeks (p > 0.05). Regarding the time effect, we observed improved performance at 24 weeks compared to 8 weeks (p = 0.001), but no differences between offspring (p > 0.05; Fig 1H).

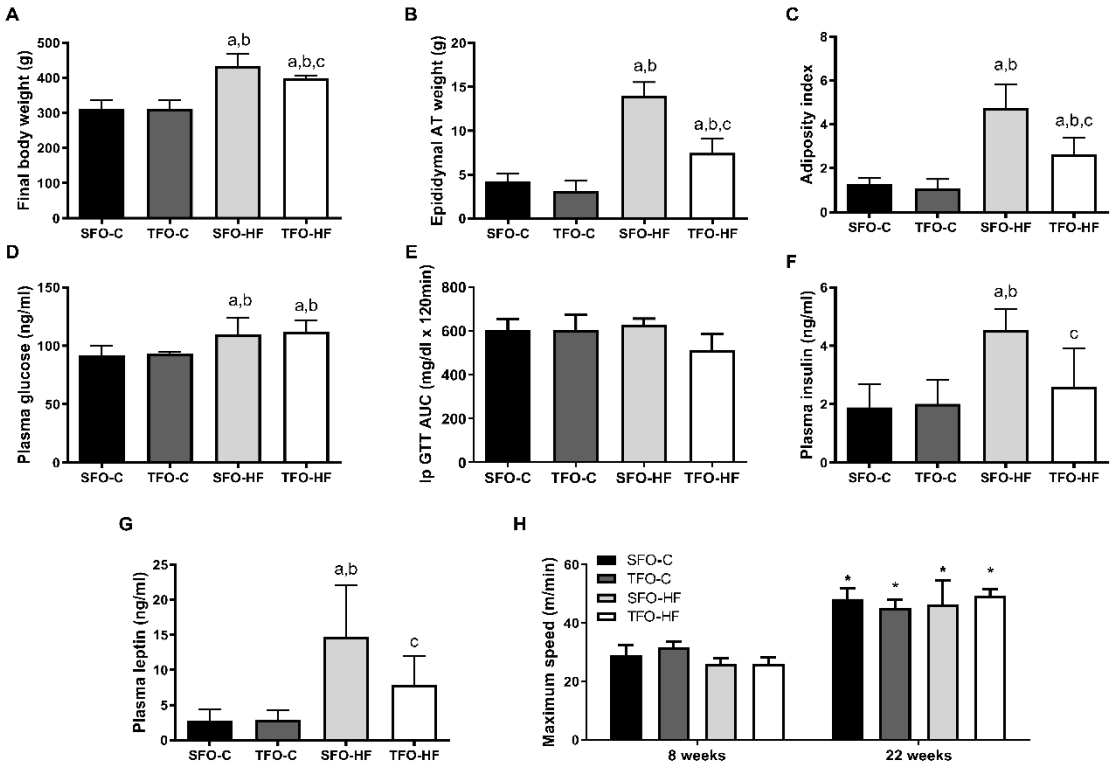

Figure 1. Effects of HPD and paternal RT on body and tissue wight, metabolic parameters and aerobic capacity in the offspring. Final Body weight (A), epidydimal adipose tissue weight (B), adiposity index (C), plasma glucose (D), Ip GTT AUC (E), plasma insulin (F), plasma leptin (G), Maximum speed (H). Values are presented as means ± SD. SFO-C = offspring from sedentary fathers, exposed to control diet; TFO-C = offspring from trained fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. Statistically significant differences compared to: [a] SFO-C; [b] TFO-C; [c] SFO-HF [*]8 weeks, $p \leq 0.05$. (n = 7 per group).

Paternal RT reduces adipocyte size and existing collagen deposition in epididymal adipose tissue of offspring submitted to a high-fat diet

Histological sections of epididymal adipose tissue stained with HE (Fig 2A) showed that adipocyte cross-sectional area (CSA) was significantly increased in SFO-HF when compared with the SFO-C group ($p = 0.01$; Fig 2D). Larger adipocytes in the relative frequency (22000 to> 6000 μm^2) were observed in the SFO-HF group compared to other groups (Fig 2E). Furthermore, the 24-week HFD-fed offspring showed a greater increase in Masson,s trichrome staining area (Fig 2B) than the control diet (SFO-HF vs. SFO-C; $p = 0.0001$). Fewer collagen 1 fibers around adipocytes in epididymal adipose tissue were observed in the TFO-HF compared to the SFO-HF group (indicated in the figure by the black arrow) (Fig. 2C). However, paternal RT retards adipocyte size and ongoing fibrosis in TFO-HF compared to SFO-HF ($p = 0.01$; Fig. 2D and F).

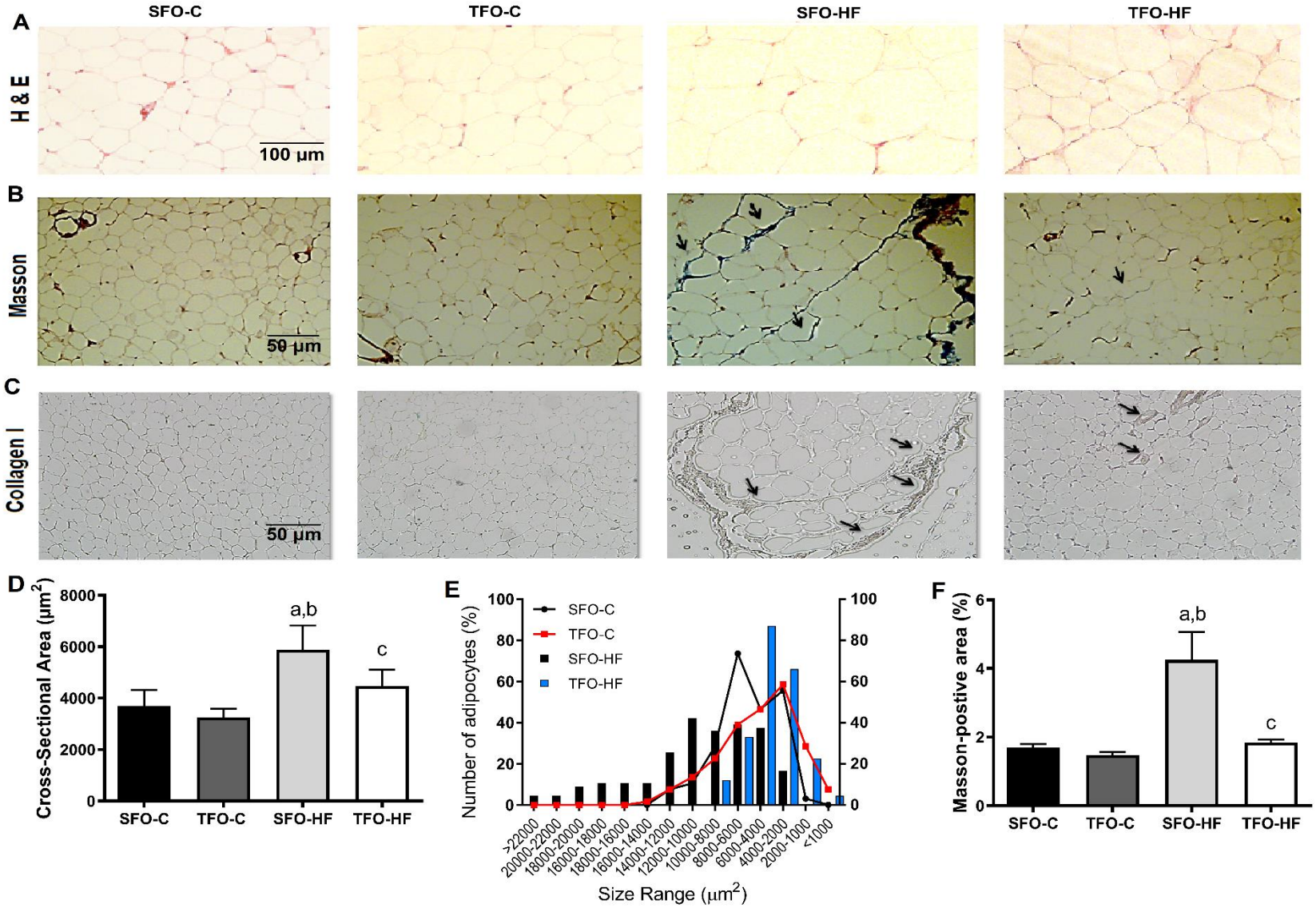
SFO-C
TFO-C
SFO-HF
TFO-HF
A
H & E
100 μm
B
Masson
50 μm
C
Collagen I
50 μm
D
Cross-Sectional Area (μm2)
8000
6000
4000
2000
0
a,b
c
SFO-C
TFO-C
SFO-HF
TFO-HF
E
Number of adipocytes (%)
100
80
60
40
20
0
SFO-C
TFO-C
SFO-HF
TFO-HF
>22000
20000-22000
18000-20000
16000-18000
18000-16000
16000-14000
14000-12000
12000-10000
10000-8000
8000-6000
6000-4000
4000-2000
2000-1000
<1000
Size Range (μm2)
F
Masson-postive area (%)
6
4
2
0
a,b
c
SFO-C
TFO-C
SFO-HF
TFO-HF

Figure 2. Effects of HPD and paternal RT on epididymal adipocyte size and fibrosis in the offspring. Cross-sectional area (A), adipocyte size distribution (B), Masson-positive area (C). Values are presented as means ± SD. SFO-C = offspring from sedentary fathers, exposed to control diet; TFO-C = offspring from trained fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. Statistically significant differences compared to: [a] SFO-C; [b] TFO-C; [c] SFO-HF, $p \leq 0.0.5$; (n = 5 per group).

Effects of HFD and paternal RT on growth factors mRNA levels in the epididymal adipose tissue

C*tgf, Tgfβ1* and *Vegf* mRNA levels were significantly upregulated with HFD (SFO-HF vs. SFO-C; p = 0.0001; p = 0.008 and p = 0.004) (Fig 3A-D). Paternal RT downregulated *Ctgf* and *Vegf* mRNA levels in the TFO-HF group compared to the SFO-HF group (p= 0.003 and p= 0.0001). No changes were observed in mRNA levels of *Igf1* between offspring groups (p> 0.05; Fig 3B).

Effects of the HFD and paternal RT on adipogenic and factors regulators of lipid metabolism mRNA levels in the epididymal adipose tissue

Cebpa, *Fabp4* and *Srebp1* mRNA levels were increased with HFD (SFO-HF vs. SFO-C; p = 0.03, p =0.02 and p = 0.01 (Fig 3J-L). Paternal RT downregulated *Cebpa* and *Srebp1* mRNA levels in the TFO-HF group compared to the SFO-HF group (p= 0.03 and p= 0.001). No changes were observed in mRNA levels of *Ppara* between offspring groups (p> 0.05; Fig 3H).

Effects of HFD and paternal RT on structural matrix proteins and remodeling, enzymes mRNA levels in the epididymal adipose tissue

HFD and paternal RT did not modulate *Col1a1*, *Mmp-2* and *Timp-2* (p>0.05; Fig 3 I-L). However, *Col3a1* mRNA levels were significantly upregulated with HFD (SFO-HF vs. SFO-C; p=0.04) (Fig 3J).

Effects of the HFD and paternal RT on inflammatory pathways and antioxidant response mRNA levels in the epididymal adipose tissue

HFD did not modulate *Mcp*1 and *Nfkb* (SFO-HF vs. SFO-C; p>0.05). Paternal RT downregulated *Mcp*1 mRNA levels in the TFO-HF group when compared to the SFO-HF group (p= 0.03, Fig 3M). Additionally, paternal RT downregulated *Nfkb* mRNA levels independent of offspring diet (TFO-C vs. SFO-C; p= 0.004 and TFO-HF vs. SFO-HF; p = 0.0005) (Fig 3N). No changes were observed in mRNA levels of *Tnfa*, *Tweak* and *Nrf2* between offspring groups (p> 0.05; Fig 3M-H).

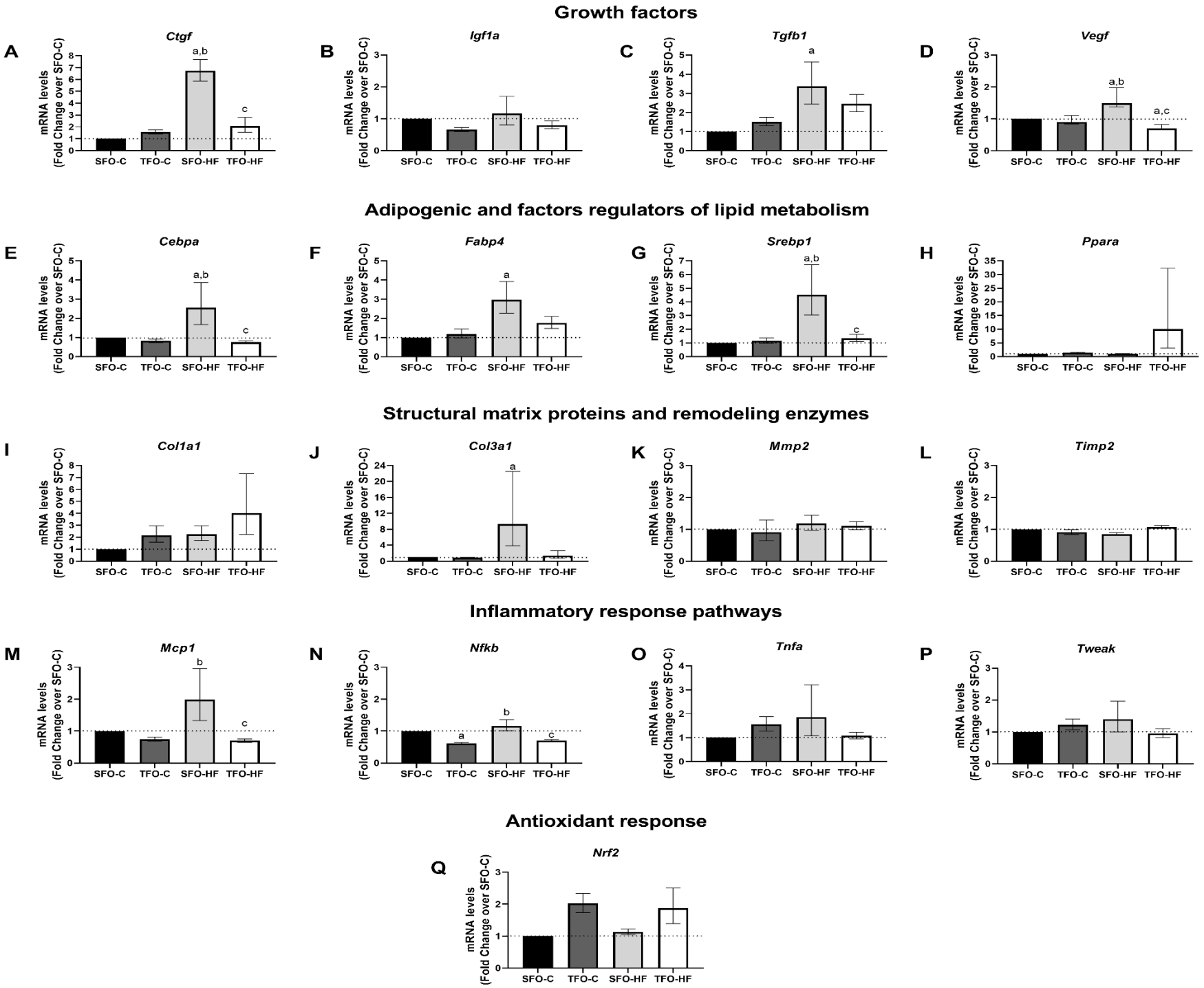
Growth factors
A
Ctgf
a,b
c
B
Igf1a
C
Tgfb1
a
D
Vegf
a,b
a,c
Adipogenic and factors regulators of lipid metabolism
E
Cebpa
a,b
c
F
Fabp4
a
G
Srebp1
a,b
c
H
Ppara
Structural matrix proteins and remodeling enzymes
I
Col1a1
J
Col3a1
a
K
Mmp2
L
Timp2
Inflammatory response pathways
M
Mcp1
b
c
N
Nfkb
a
b
c
O
Tnfa
P
Tweak
Antioxidant response
Q
Nrf2
mRNA levels
(Fold Change over SFO-C)
SFO-C
TFO-C
SFO-HF
TFO-HF

Figure 3. Effects of HPD and paternal RT on mRNA levels in the epididymal adipose tissue of offspring. Growth factors (A-D), adipogenic and factors regulators of lipid metabolism (E-H), Structural matrix proteins and remodeling enzymes (M-P), Antioxidant response (Q). The expression level is represented by the $2^{-\Delta\Delta CT}$. Values are presented as geometric means and back-transformed SEM. SFO-C = offspring from sedentary fathers, exposed to control diet; TFO-C = offspring from trained fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. Statistically significant differences compared to: [a] SFO-C; [b] TFO-C; [c] SFO-HF, $p \leq 0.05$. (n = 7 per group).

Paternal RT decrease cytokines levels in the epididymal adipose tissue of offspring submitted to a high-fat diet

As expected, the Tnf-α, IL1-β and Mcp-1 levels were increased with HFD (SFO-HF vs. SFO-C; p = 0.004 and p = 0.004 and p = 0.007. However, paternal RT mitigated the HFD-associated enhance in the Tnf-α and IL1-β levels (TFO-HF vs. SFO-HF; p= 0.01 and p = 0.004) (Fig 4A-B). Moreover, paternal RT increased adiponectin levels in the offspring exposed to HFD (TFO-HF vs. SFO-HF; p = 0.0002; Fig 4E). No changes were observed in Mcp-1 levels in response to paternal RT (p>0.05; Fig 4C).

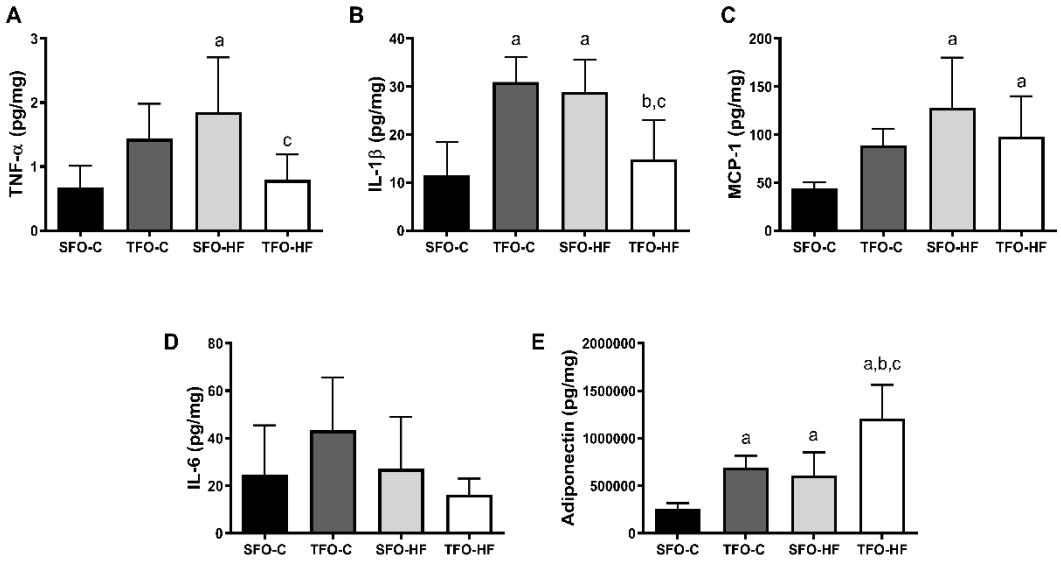

Figure 4. Effects of HPD and paternal RT on cytokines levels in the epididymal adipose tissue of offspring. Tnf-α (A), IL1-β (B), MCP-1 (C), Adiponectin (D). Values are presented as means ± SD. SFO-C = offspring from sedentary fathers, exposed to control diet; TFO-C = offspring from trained fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. Statistically significant differences compared to: [a] SFO-C; [b] TFO-C; [c] SFO-HF, $p \leq 0.05$. (n = 7 per group).

Paternal RT downregulates MMPs activity in the epididymal adipose tissue and blood circulation of offspring submitted to a high-fat diet.

The pro and active MMP-2 activity decreased in the epididymal adipose tissue of fathers in response to RT (TF vs. SF; p=0.001 and p=0.002, respectively) (Fig 5A-C). The TFO-C and SFO-HF groups showed higher pro MMP-2 activity compared to SFO-C (p=0.04 and p=0.0001, respectively. Furthermore, TFO-HF presented lower pro and active MMP-2 activity compared to the SFO-HF group (p=0.001 and p=0.009, respectively) (Fig 5B-D). MMP-9 in the epididymal adipose tissue activity was not detected by zymography.

Regarding MMP-9 activity in blood circulation, the SFO-HF group showed the highest activity values when compared to the offspring exposed to the control diet (p = 0.001) (Fig. 5F). Pro and active MMP-9 activity changed similarly in fathers and offspring, showing down-regulation in the groups under the RT regimen (TF vs. SF; p = 0.02 and TFO-HF vs. SFO-HF; p=0;0001; Fig 5E-H). Concerning MMP-2 activity, similar results were found. Both isoforms of MMP-2 were reduced (p = 0.004 and p =0.01) in the TF group compared to the SF group. Pro and active MMP-2 activity were significantly upregulated with HFD (SFO-HF vs. SFO-C; p=0.001). However, the TFO-HF group reduced pro and active MMP-2 activity in the plasma compared to SFO-HF (p = 0.001) (Fig. 5J and L).

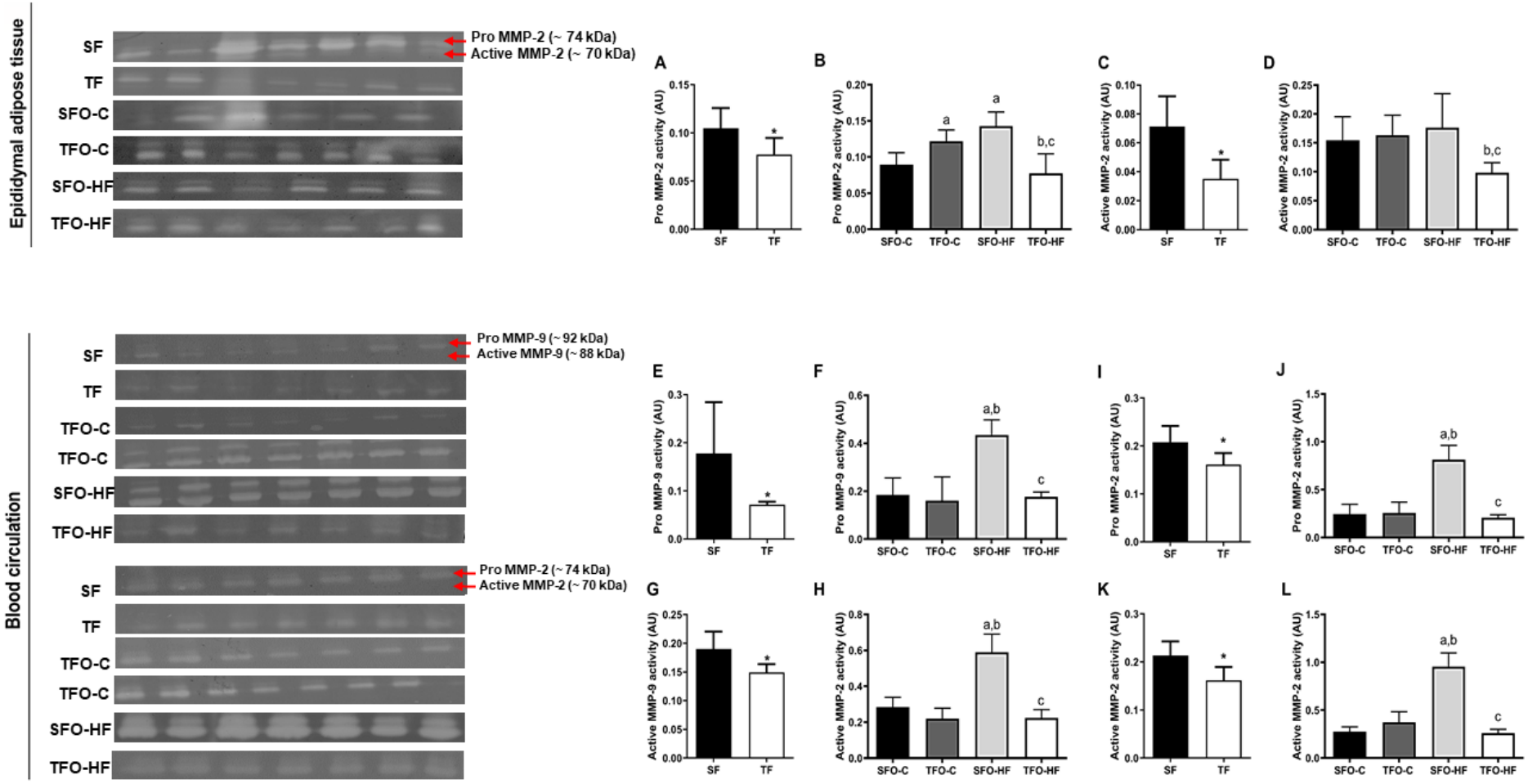
Epididymal adipose tissue
SF
TF
SFO-C
TFO-C
SFO-HF
TFO-HF
Pro MMP-2 (~ 74 kDa)
Active MMP-2 (~ 70 kDa)
A
B
C
D
Pro MMP-2 activity (AU)
Active MMP-2 activity (AU)
Blood circulation
Pro MMP-9 (~ 92 kDa)
Active MMP-9 (~ 88 kDa)
E
F
I
J
Pro MMP-9 activity (AU)
Pro MMP-2 activity (AU)
G
H
K
L
Active MMP-9 activity (AU)
Active MMP-2 activity (AU)

Figure 5. Effects of HPD and paternal RT on MMPs in the epididymal adipose tissue and blood circulation of offspring. Pro MMP-2 in the adipose tissue (A-B), Active MMP-2 in the adipose tissue (C-D), Pro MMP-9 in the blood circulation (E-F), Active MMP-2 in the blood circulation (G-H), Pro MMP-2 in the blood circulation (I-J), Active MMP-2 in the blood circulation (K-L). Values are presented as means ± SD. SFO-C = offspring from sedentary fathers, exposed to control diet; TFO-C = offspring from trained fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. Statistically significant differences compared to: *SF; [a] SFO-C; [b] TFO-C; [c] SFO-HF, $p \leq 0.05$. (n = 7 per group).

Paternal RT improved redox status in the epididymal adipose tissue and blood circulation of offspring submitted to a high-fat diet.

The $CM^{\bullet}$ amount in the epididymal adipose tissue was higher in the SF compared to the TF group (p=0.004; Fig 6A), indicating a stronger production rate of ROS. The HPD induced an increase in the ROS production (SFO-HF vs. SFO-C; p = 0.005) (Fig 6B), meanwhile paternal RT counterbalance ROS generation (TFO-HF vs. SFO-HF; p = 0.006). Consistent with this notion, SFO-HF showed marked increases in the hydrogen peroxide concentrations (H_2O_2) by DCF-RFU (p = 0.0001). In contrast, paternal RT significantly reduced H_2O_2 production (p = 0.001) and TBARS (p = 0.003) levels in the HFD-treated offspring (Fig. 6C and F). The TFO-C and SFO-HF showed lower NO bioavailability when compared to SFO-C group (p = 0.001; Fig 6D). SOD activity decreased with HFD (SFO-HF vs. SFO-C; p = 0.001), while paternal RT induced a significant increase in TFO-HF group when compared to SFO-HF group (p = 0.02; Fig 6E)

Regarding blood circulation, SFO-HF exhibited increases in the F2-isoprostanes levels compared to the SFO-C group (p= 0.0001; Fig 6G), whereas paternal RT significantly reduced this pro-oxidant agent (p = 0.002) and blood protein carbonyls levels (p = 0.01) levels in the HFD-treated offspring (Fig 6G-H). NO and α-Klotho levels were decreased with HFD (SFO-HF vs. SFO-C; p = 0.0001 and p = 0.004 (Fig 6J and M). Paternal RT increased NO bioavailability regardless of offspring diet (TFO-C vs. SFO-C; p= 0.0001 and TFO-HF vs. SFO-HF; p = 0.0001) (Fig 6J). Additionally, paternal RT

increased SOD activity and α-Klotho levels in TFO-HF compared to the SFO-HF group ($p = 0.04$ and $p = 0.02$). No changes were observed in 8-OH-2-deoxyguanosine levels and catalase activity levels between offspring groups ($p>0.05$; Fig 6I and L).

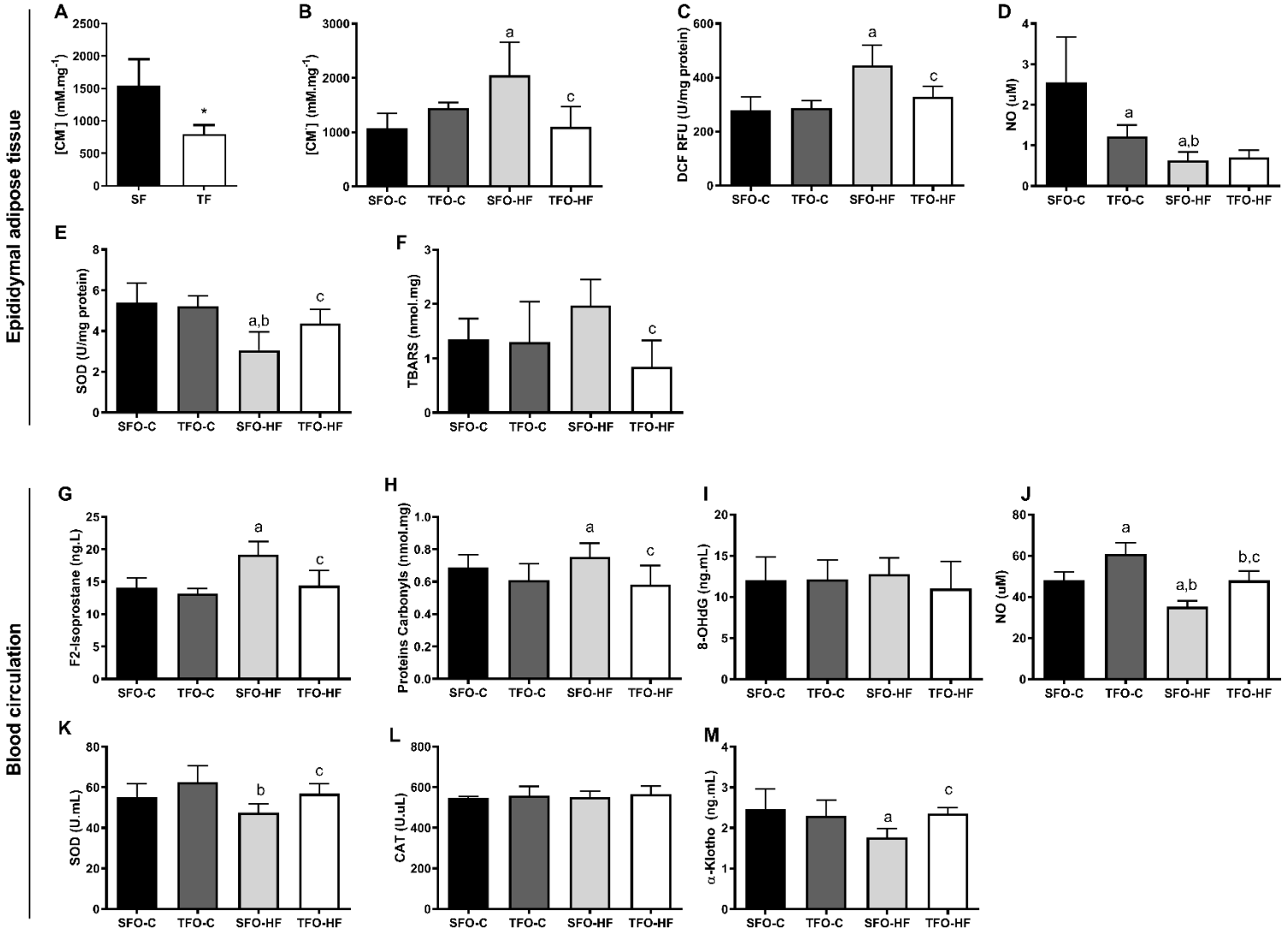
Epididymal adipose tissue
Blood circulation
A
[CM] (mM.mg-1)
SF
TF
*
B
[CM] (mM.mg-1)
SFO-C
TFO-C
SFO-HF
TFO-HF
a
c
C
DCF RFU (U/mg protein)
D
NO (uM)
a
a,b
E
SOD (U/mg protein)
a,b
c
F
TBARS (nmol.mg)
G
F2-Isoprostane (ng.L)
H
Proteins Carbonyls (nmol.mg)
I
8-OHdG (ng.mL)
J
NO (uM)
b,c
K
SOD (U.mL)
b
L
CAT (U.uL)
M
α-Klotho (ng.mL)

Figure 6. Effects of HPD and paternal RT on balance redox in the epididymal adipose tissue and blood circulation of offspring. ROS production in fathers and offspring (A-B), DCF-RFU (C), NO bioavailability (D), SOD activity (E), TBARS levels (F). Blood F2-isoprostanes (G), Blood proteins carbonyls (H), Blood 8-OH-Dg (I), Blood NO (J), Blood SOD (K), Blood CAT (L), Blood α-Klotho (M). Values are presented as means ± SD. SFO-C = offspring from sedentary fathers, exposed to control diet; TFO-C = offspring from trained fathers exposed to control diet; SFO-HF = offspring from sedentary fathers exposed to high-fat diet; TFO-HF = offspring from trained fathers exposed to a high-fat diet. Statistically significant differences compared to: *SF; [a] SFO-C; [b] TFO-C; [c] SFO-HF, $p \leq 0.05$. (n = 7 per group).

Interplay between adipocyte size, metabolic blood markers, collagen deposition, ROS production, and cytokines

Interestingly, there was a significant positive correlation between adipocyte size and blood glucose levels ($r = 0.64$; $p = 0.001$), blood insulin levels ($r = 0.60$; $p = 0.004$) and blood leptin levels ($r = 0.65$; $p = 0.001$) (Fig 7 A-C). Also, there was a significant positive correlation between adipocyte size with ROS production ($r = 0.62$; $p = 0.003$), Tnf-α ($r = 0.52$; $p = 0.01$), IL1-β ($r = 0.62$; $p = 0.003$) and Mcp1 levels ($r = 0.65$; $p = 0.001$) (Fig 7 D-G).

Similarly, we found a significant positive correlation between Masson-positive area and ROS production ($r = 0.84$; $p = 0.001$), Tnf-α ($r = 0.65$; $p = 0.001$), IL1-β ($r = 0.58$; $p = 0.006$) and Mcp1 levels ($r = 0.70$; $p = 0.006$) (Fig 7 H-K). Finally, there was a significant correlation between Tnf-α ($r = 0.62$; $p = 0.003$), IL1-β ($r = 0.74$; $p = 0.001$) and Mcp1 levels ($r = 0.72$; $p = 0.0001$) with ROS production (Fig 7 L-N). No correlations were observed between other cytokines and pro-oxidant or antioxidant agents ($p>0.05$)

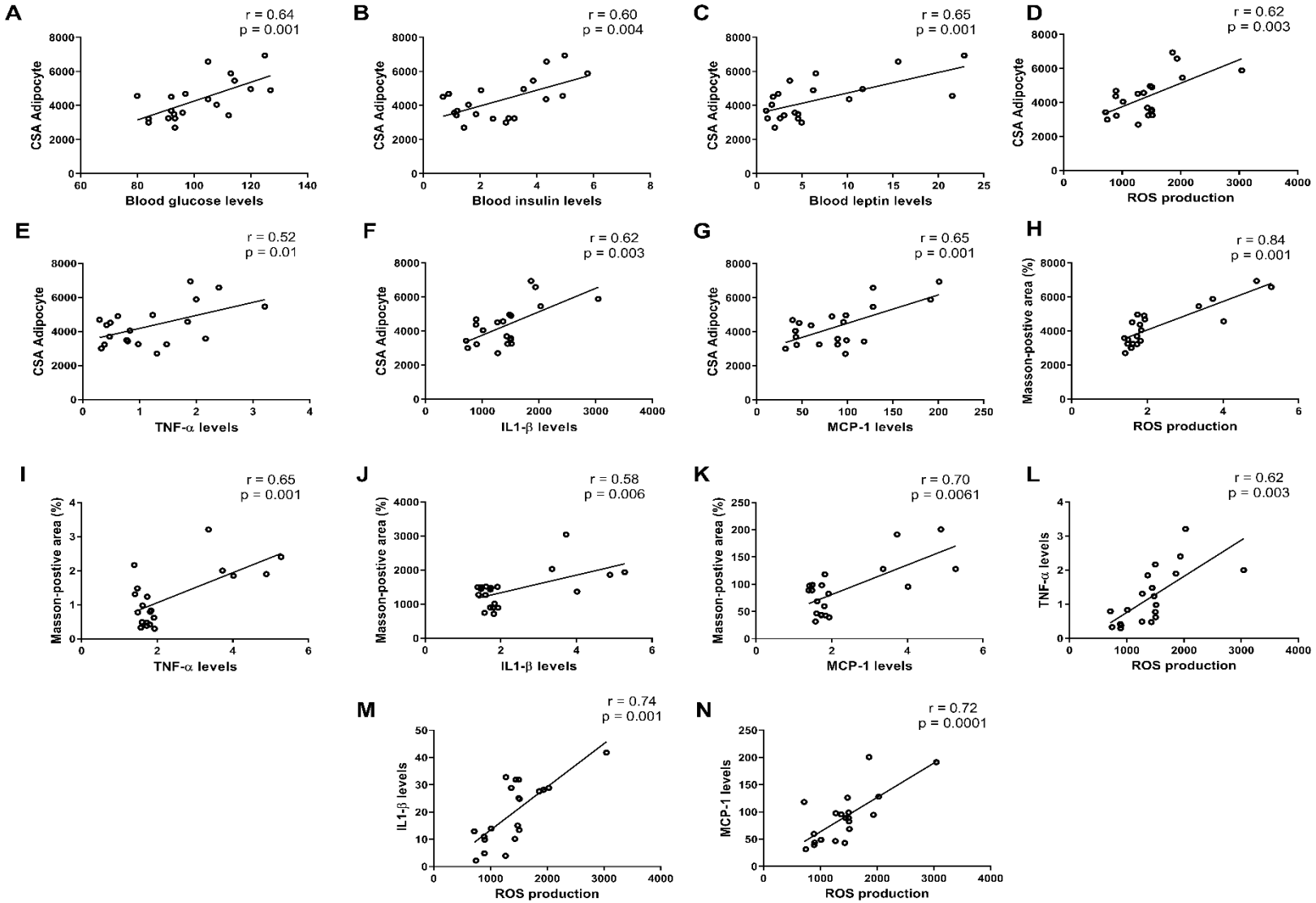
A
r = 0.64
p = 0.001
CSA Adipocyte
Blood glucose levels
B
r = 0.60
p = 0.004
CSA Adipocyte
Blood insulin levels
C
r = 0.65
p = 0.001
CSA Adipocyte
Blood leptin levels
D
r = 0.62
p = 0.003
CSA Adipocyte
ROS production
E
r = 0.52
p = 0.01
CSA Adipocyte
TNF-α levels
F
r = 0.62
p = 0.003
CSA Adipocyte
IL1-β levels
G
r = 0.65
p = 0.001
CSA Adipocyte
MCP-1 levels
H
r = 0.84
p = 0.001
Masson-postive area (%)
ROS production
I
r = 0.65
p = 0.001
Masson-postive area (%)
TNF-α levels
J
r = 0.58
p = 0.006
Masson-postive area (%)
IL1-β levels
K
r = 0.70
p = 0.0061
Masson-postive area (%)
MCP-1 levels
L
r = 0.62
p = 0.003
TNF-α levels
ROS production
M
r = 0.74
p = 0.001
IL1-β levels
ROS production
N
r = 0.72
p = 0.0001
MCP-1 levels
ROS production

Figure 7. Correlations between CSA adipocyte with metabolic blood markers (A-C); CSA adipocyte with ROS production (D); CSA adipocyte with cytokines (E-G); collagen deposition with ROS production (H); collagen deposition with cytokines (I-K); cytokines with ROS (L-N).

DISCUSSION

This study confirmed that HPD induces adipocyte hypertrophy and collagen deposition while upregulating adipogenic factors, growth factors, and inflammatory pathways mRNA levels, besides increases local proinflammatory cytokines and ROS production in the offspring epididymal adipose tissue. These findings combined may have contributed to the impaired metabolic parameters, elevated pro-oxidant agents, and inflammatory mediators (MMPs) in the blood circulation. In contrast, paternal RT was highly effective in down-regulating genes, enzymes, and structural compounds in the male offspring fed with an HFD, which are potentially involved in chronic inflammation, oxidative stress, and adipose tissue dysfunction. Interestingly, specific changes in biomarkers were reflected in blood circulation. For the first time, to our knowledge, the molecular and cellular bases inherent to the protective role of intergenerational paternal RT were investigated in the adipose tissue. The current study brings a new approach to understanding the biology of adipose tissue, it clarifies the key mechanisms involved in intergenerational epigenetic inheritance and improved the comprehension of remodeling and clinical implications.

Long-term of HFD increase adipocytes size and collagen I deposition into the visceral adipose tissue and conducts to development of fibrosis in rodents (Kawanishi et al., 2013; Li et al., 2021) and humans (Divoux et al., 2010; Guglielmi et al., 2015), which also starts to metabolic dysfunction (Li et al., 2021). Our findings confirm that our HFD model has considerable harmful effects on adipocyte structure linked to adipogenic and ECM remodeling. Probably, cross-regulation of C/EBPα and FABP4 and SREBP1, TGF-β, and CTGF control the transcriptional pathway of adipogenesis and niche-specific composition and structure of the ECM microenvironment (Pellegrinelli, Carobbio, & Vidal-Puig, 2016; Velotta, Jones, Wolf, & Cheviron, 2016). Collectively, up-regulation

of mRNA levels might clarify adipocyte growth and increase positive Masson staining in the SFO-HF group.

An exciting result was that paternal RT might have an anti-fibrotic effect on adipose tissue, partially explained by downregulated CTGF mRNA levels and lower active MMP-2 activity in TFO-HF group. A study conducted by Battula et al. (2013) revealed that CTGF regulates adipocyte differentiation, collagen I deposition and facilitates MMP2 activation to modulate ECM turnover. This mutual crosstalk suggests a regulatory role of paternal RT supported by growth factors for adipose structure maintenance. Li et al. (2021), also demonstrated that exercise attenuates exacerbated collagen deposition and decreased fibrosis-related gene expression (*Col3a1*, *Col6a1*, *Lox* and *fibronectin*) in adipose tissue of HFD-fed in mice. Regarding plausible mechanisms, the authors suggest that PPARγ activation has been known to decrease Hypoxia-inducible factor 1 (HIF-1α) expression and lessen adipose tissue fibrosis. Considering that HIF-1α is a major transcriptional activator for vascular endothelial growth factor (VEGF) gene (Forsythe et al., 1996) this axis provides a mechanistic explanation for the protective effect of exercise against HFD-induced adipose fibrosis in the current study.

It has been widely shown that adipose tissue fibrosis occurred in obesity and inflammation, which in turn is mediated by the NF-kB signaling pathway (Watanabe et al., 2016). NF-κB is activated by HPD and regulates thousands of primary and secondary response genes, including cytokines and transcription factors (Carlsen et al., 2009). In the current study, paternal RT down-regulates NF-kB mRNA levels, which may have contributed to decrease of classic pro-inflammatory cytokines levels, such as TNF-α and IL-1β, while increase adiponectin in epididymal adipose tissue of the TFO-HF group. Moreover, this result is in agreement with the decrease in ROS production in the TFO-HF group since it has been demonstrated that ROS production (H_2O_2) modulates IKK-dependent NF-kB activation by promoting the redox-sensitive activation of the PI3K/PTEN/Akt and NIK/IKK pathways (J. H. Kim et al., 2008; Morgan & Liu, 2011). These new findings reveal that paternal RT is a helpful tool to control the inflammatory profile and consequently healthy adipose tissue phenotype in the offspring exposed to HFD. Additionally, the anti-inflammatory effect inherent to paternal RT is reinforced by lower plasma pro and active MMP-2 and MMP-9 activityThe -up-regulation of MMPs activity in the blood circulation is linked to macrophage and neutrophils activation, immune-resident cells, tumor progression, and apoptosis process (Chen et al., 2013;

Fingleton, 2017). These results argue that adipose tissue synthesizes and releases many bioactive molecules, including MMP-2 and MMP-9, in the blood circulation, which may also have influenced the cytokines bioavailability.

Low-grade chronic inflammation is an important cause of abnormal generation of ROS in adipose dysfunction. Remarkably, Hauck et al. (2019) reveal that pro-inflammatory cytokine, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and IL-1β, secreted from activated M1 macrophages increase ROS production in adipose tissue of obese individuals, which confirm our correlations (Figure 7). Our study's most significant novelty was the possible interplay between adipocyte size, metabolic blood markers, collagen deposition, ROS production, and cytokines in the intergenerational model. This information will provide clues for the development of therapeutic agents that target adipose function and cell-cell bidirectional crosstalk mechanism. Interestingly, collagen deposition had the strongest relationship with ROS production (large effect). We posited that this could be attributed to NF-kB signaling pathways as previously demonstrated in other tissues (Lijnen, van Pelt, & Fagard, 2012). Nevertheless, it is important to emphasize that no major molecular changes were seen in offspring exposed to control diets whose fathers underwent RT (TFO-C) compared to SFO-C, suggesting limited effects of paternal RT under offspring healthier diet.

The current study addresses several significant outcomes. Paternal RT upregulated adiponectin levels while decrease insulin and leptin levels in the offspring exposed to HFD, supporting the critical role of adipokines in metabolism homeostasis. Adiponectin is an adipose tissue-secreted molecule, which acts as an important modulator of insulin sensitivity and lipid metabolism and has pronounced anti-inflammatory and anti-atherosclerotic effects (Ruan & Dong, 2016). Furthermore, adiponectin can control body weight maintenance and maintains healthy adipose tissue expansion while rescuing ectopic lipid accumulation in obese animal models (Milan et al., 2002). A possible explanation is that a decrease of insulin and leptin levels enhanced by paternal RT may be, at least in part, supported by regulation of adiponectin signaling pathway (Ruan & Dong, 2016), as well as by adipocyte size decreases. According to Matsuda et al. (2011), ROS also suppresses adiponectin production in adipocytes; howeverrecent studies have demonstrated that adiponectin protects against oxidative stress-induced damage in the adipose tissue (Fruhbeck, Catalan, Rodriguez, Ramirez, Becerril, Portincasa, et al., 2017; Fruhbeck, Catalan, Rodriguez, Ramirez, Becerril, Salvador, et al., 2017). In the same

way, the leptin levels may influence ROS production and the expression of MCP1, which clarifies our findings. However, we found that MCP-1 mRNA levels in the TFO-HF group were not reflected at the level of proteins at the time-point analyzed, probably due to delayed synthesis between mRNA or protein during state transition, besides proteins disconnected from the transcripts.

Under the pathological conditions, ROS production induce cellular dysfunction and tissue damage by direct oxidative adjustment of biomolecules, and modulate redox-sensitive signal transduction pathways (Zhou et al., 2021). Subsequently, adipocytes operate in a new, greater redox equilibrium, limiting the discharge of pivotal antioxidant defenses under nutritional overload (Taherkhani, Suzuki, & Ruhee, 2021), which might explain lower SOD activity in the SFO-HF compared to the SFO-C group. We observed an overlapping of SOD activity in the epididymal adipose tissue and blood circulation, which change in the same direction (i.e. down-regulation) in the TFO-HF group compared to the SFO-HF group, implying a protective factor against the undesired effects of HFD. Moreover, the paternal exercise program was effective in decreasing TBARS levels in adipose tissue under HFD. These adaptations could be important to maintain enzymatic operation in adipose tissue metabolism, possibly preventing impairments in mitochondrial oxidative capacity and DNA damage. Of note, SOD catalyzes the dismutation of O_2 into oxygen and H_2O_2, serving as a key antioxidant (Okuno et al., 2018). The overexpression of SOD in rodents adipocytes showed enlargement of these cells and decreased ectopic fat accumulation and improved insulin sensitivity (Han et al., 2016), which corroborates our findings (Han et al., 2016). Another study reported that ROS production inhibits healthy adipose expansion by suppressing the SREBP1-mediated lipogenic pathway and lysine-specific histone demethylase 1A (KDM1A) protein expression, which is considered a key epigenetic developmental regulator (Okuno et al., 2018). Thus, further studies using an intergenerational paternal exercise model will be required to evaluate the involvement of KDM1A protein expression on balance redox in the offspring.

Exercise training conferred protection against ROS production by augmentation of Nrf2-antioxidant signaling, the primary regulator of endogenous antioxidant defenses that regulates 200 cytoprotective genes (Tonelli, Chio, & Tuveson, 2018). However, paternal RT did not modulate Nrf2 mRNA levels, suggesting that other adjacent molecular pathways or post-translational regulation were involved in mediating redox

homeostasis. Therefore, it is important to highlight that cellular redox homeostasis in response to intergenerational epigenetic inheritance is achieved by a balance between multiple pathways required to carry out complex physiological processes. In contrast to the current study, Lehnig et al. (2019) showed that voluntary wheel running (152 ± 7km over a 3 wk) training increased the expression of Nrf1 and Nrf2 mRNA levels in the mice perigonadal white adipose tissue. The discrepancy between these outcomes may be due to differences in adipose tissue depots, besides type, intensity, volume, and training duration of the protocols employed.

Moreover, there is substantial evidence that suggests heterogeneous molecular signatures in different adipose depots, as well as exercise training results in individual responses, which likely explain, different gene expressions between studies. Additionally, the lack of difference in aerobic capacity between offspring groups might be due to the absence of the Nrf2 change, since this transcription factor has been involved in training adaptation to improve aerobic performance (Z. He et al., 2008; Toledo-Arruda et al., 2020) through the availability of substrates for respiration, and ATP production, as well mitochondrial biogenesis improved (Done & Traustadottir, 2016; Oh et al., 2017). Thus, one possibility that should not be ruled out is that offspring maintain adherence to regular exercise training independent of the father's lifestyle for physical performance maintenance.

Conventionally, elevated F2-isoprostanes and proteins carbonyls systemic levels are interpreted as indicators of harmful oxidative stress, resulting in subsequent damage to cellular proteins, lipids, and nucleic acids (Il'yasova, Wong, Waterstone, Kinev, & Okosun, 2017). We identified that pre-conceptional paternal RT alleviates F2-isoprostanes and proteins carbonyls in blood circulation in the offspring fed-HPD, which is an important adaption in the first-line defense mechanisms against oxidative stress. Possible mechanisms linking RT with these components included shear stress, hemo-concentration, and signaling pathways involving osmoregulation (Georgescu et al., 2017). Additionally, Paternal RT increases NO bioavailability in blood circulation regardless of offspring diet. Such effects may contribute to the vascular tone homeostasis and arterial stiffness, while improves O2 supply and functional sympatholytic (Hoiland, 2015; Nosarev, Smagliy, Anfinogenova, Popov, & Kapilevich, 2014). NO also participates in the heart contractility control and calcium cycling, besides contributes to

the protective effect of ischemic pre- and postconditioning (Rastaldo et al., 2007). These hypotheses are supported by Sousa Neto et al. 2020, which revealed that paternal RT upregulates protein abundance levels related to calcium channel, myofibril components, metabolic processes, antioxidant activity on the left ventricle regardless of the offspring diet. It is important to mention that NO changes in blood circulation may not accurately reflect of adipose tissue adaptations (Figure 6). A plausible explanation is a distinct diffusivity, biological activities, and metabolic demands differences between the cell compartments (Kozlov et al., 2001).

A study elucidated that α-Klotho-treated obese mice experienced reduced adipocyte size and elevated energy expenditure, despite no changes in blood glucose levels (Rao et al., 2019), corroborating our outcomes in the TFO-HF group. This encouraging therapeutic potential of this protein is supported by previous studies, which observed that α-Klotho overexpression attenuates diabetes progression through improved insulin release and ROS buffering (Lin & Sun, 2015). Other studies demonstrated that α-Klotho, a known anti-aging protein, exerts varied beneficial physiological effects, includinghyperglycemia attenuation, calcium, and phosphate homeostasis maintenance, and enhanced ROS production buffering enzymes, such as SOD (Assimos, 2012; Huang, 2010; Lin & Sun, 2015). Thus, the paternal sedentary lifestyle may contribute to the obesity epidemic in modern societies. The molecules analyzed here are a potential target for optimizing the metabolic health of offspring born to fathers with the typical sedentary lifestyle, and provide insights gor guide the development of future pharmacotherapies. An illustration of adipocyte and blood circulation was used to clarify the location, the up and downregulation of main molecules in the TFO-HF:SFO-HF analysis (Fig. 8).

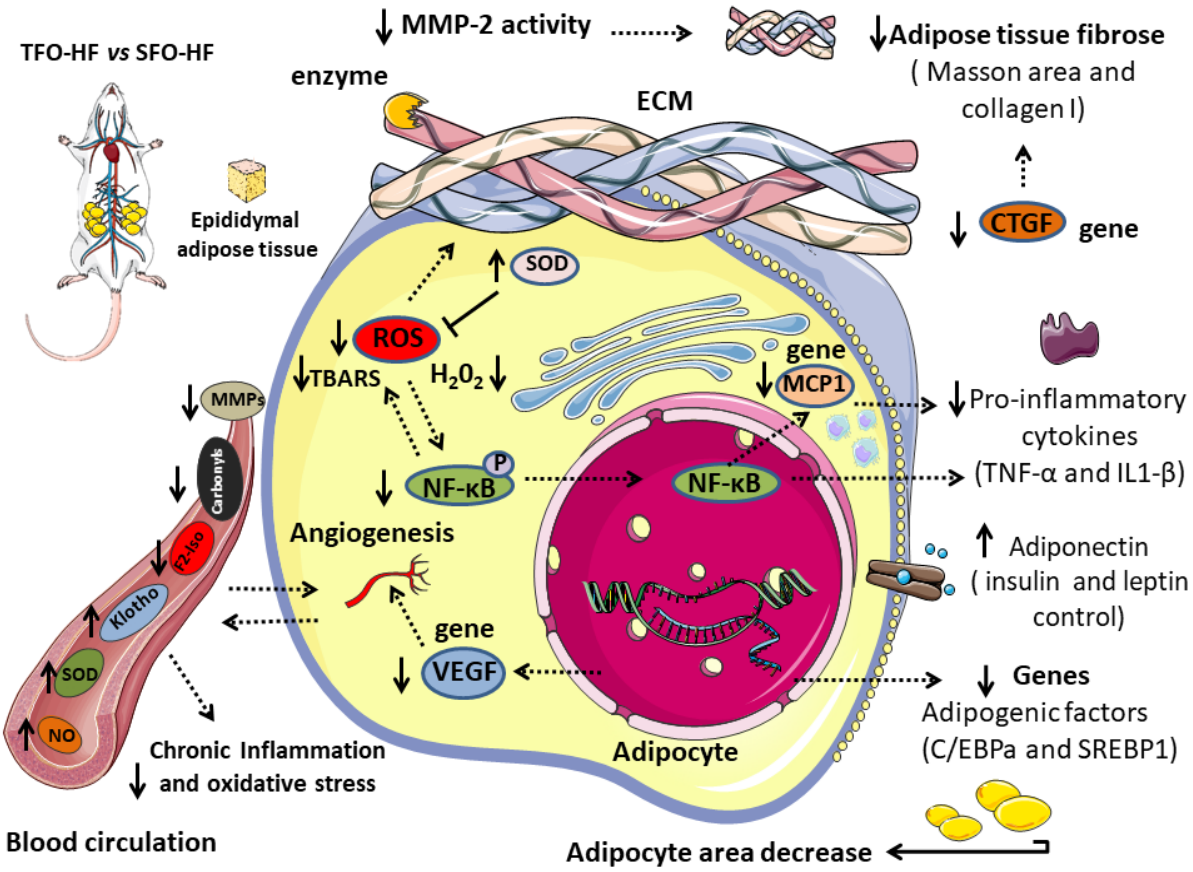

Figure 8: Overview of adipose tissue remodeling pathways in the TFO-HF *vs*. SFO-HF analysis. (↑) upregulated and (↓) downregulated. The figure was created using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Some limitations should be mentioned, such as the impossibility to analyze immunoblot of key proteins and offspring muscle strength. The evaluation of only a depot and specific time-point also are substantial limitations. Different time points from the beginning of dietary consumption would be valuable to elucidate the HFD time-course effects on molecular mechanisms. tRNA-derived small RNAs, miARN, DNA methylation/ acetylation, and histone modification during the spermatogenesis process are the main paths, which are probably responsible for transmitting the epigenetic inheritance landscape from fathers to first offspring (Kusuyama et al., 2020; Vieira de Sousa Neto et al., 2021). Thus, further investigations are necessary to assess the relationship between sperm epigenetic marks and adult offspring adipose tissue

remodeling pathways. The intrinsic or extrinsic divergences between rodents and human physiology present another set of obstacles in analogizing human and animal research; however, blood circulation markers identified here could be potentially useful as predictive factors to evaluate the effects of paternal exercise in human applications.

CONCLUSION

The results of this study demonstrated for the first time that both paternal exercise and offspring diet seem to have a high impact on adipose tissue remodeling. Paternal RT compensated the detrimental effects of offspring HFD by downregulation of genes linked to adipogenesis and chronic inflammation, besides decrease cytokines levels, ROS production, pro-oxidants agents, MMP-2, and ECM structural compounds (collagen). These findings were accompanied by the substantial improved antioxidants enzymes and decreased metabolic markers in the blood circulation. Of relevance, these molecular mechanisms can contribute towards an adipose tissue health phenotype and impact cellular longevity, even when offspring were exposed to an unhealthy diet. The present research provides valuable insights into the molecular mechanisms involved in adipose tissue biology and intergenerational inheritance via paternal lineage.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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5. CONSIDERAÇÕES FINAIS

O estilo de vida do pai e a dieta da prole modificaram significativamente o perfil proteômico do ventrículo esquerdo e do tendão calacâneo , alterando distintamente os níveis de abundância de diversas proteínas. Os efeitos do TF paterno sobre o proteoma do ventrículo esquerdo são independentes da dieta da prole, enquanto no tendão essa modulação foi mais evidente quando a prole é exposta a dieta hiperlipídica. A maioria das proteínas moduladas está associada à ativação de vias biológicas relacionadas à proteção tecidual, processos metabólicos, transporte e regulação da transcrição celular. No tecido adiposo, o TF paterno atenuou os efeitos deletérios da dieta hiperlipídica na prole, incluindo a diminuição do tamanho do adipócito, redução de fatores indutores de fibrose e produção de espécies reativas de oxigênio. Ademais, diminiu as citocinas pró-inflamatórias, bem como agentes pró-oxidantes, e atividade de metaloproteinase 2 e por fim, acarretou na regulação negativa de genes associados a adipogênese e inflamação. Estes achados foram acompanhados por aumento de enzimas antioxidantes chaves e diminuição de marcadores metabólicos importantes (insulina e leptina) na circulação sanguínea. Estes eventos moleculares sugerem que as possíveis disfunções ou danos nestes tecidos na primeira geração podem ser parcialmente explicadas pelo estilo de vida do pai.

O TF paterno parece ser capaz de modular uma “assinatura molecular” própria em cada tecido da prole e este conhecimento é importante para desvendar as especificidades fenotípicas teciduais da prole inerentes ao comportamento do pai. O conjunto dos efeitos protetores do exercício paterno sobre os diversos sistemas fisiológicos podem potencialmente melhorar a saúde da primeira geração. Considerando as epidemias de doenças crônicas na sociedade, essas descobertas destaca a importância do exercício físico ancestral sobre o risco metabólico propagado através das gerações. Por fim, a presente pesquisa fornece informações valiosas sobre os mecanismos moleculares envolvidos na herança intergeracional por meio da linhagem paterna, e consequentemente amplia as possibilidades de geração de novos alvos terapêuticos e aplicações clínicas nas doenças crônicas não transmissíveis, como a obesidade e diabetes.

6. PERSPECTIVAS FUTURAS

O perfil epigenético do adipócito é constituído também pelas modificações pós-tradução (PTMs) de proteínas que controlam a expressão gênica (XU et al., 2015). Esses mecanismos regulatórios são extremamente importantes, pois fornecem uma identidade a cada célula e, podem transportar informações dos pais para os filhos que estão envolvidas na manutenção e na herança de assinaturas epigenéticas adquiridas (KRISHNA et al., 1993). As PTMs constituem uma forma de regulação dinâmica da funcionalidade das proteínas, sua atividade, e sua localização subcelular, bem como modulação da expressão diferencial de genes que permitem a uma célula adaptar em resposta a estímulos, como exercício físico e dieta (MELO-BRAGA et al., 2015). A fosforilação, glicolisação, metilação e acetilação podem alterar a biossíntese e função das proteínas, bem como sua conformação e, assim, controlar sua capacidade de se associar com outras moléculas (MELO-BRAGA et al., 2015). Neste contexto, ferramentas de análises destas PTMs e bioinformática permitem uma visão integrada das vias moleculares moduladas pela dieta e pelo treinamento físico e uma compreensão mecanicista detalhada desses efeitos é interessante para o entendimento do remodelamento no tecido adiposo (MELO-BRAGA et al., 2015). A caracterização das vias de sinalização expande a gama de informações biológicas e ajuda obter uma visão mecanicista aprofunda dos eventos moleculares regulatórios que são essenciais para integridade do tecido adiposo da prole.

Um dos mecanismos subjacentes mais importantes envolvidos na regulação das modificações pós-tradução da proteína é microRNA (miRNA), cujo é considerado uma classe de pequenos reguladores de RNA não codificantes (LAM et al., 2015). Os miRNAs podem modular a expressão gênica pós-transcricional por ligação a região 3′ não traduzida de seus mRNAs alvos, e por conseguinte modular sua expressão. Tal efeito promove sua degradação ou repressão translacional, o que consequentemente altera o perfil proteômico. Recentemente foi demostrado que os miRNAs são os moduladores potenciais da proteína quinase, fosfatase, acetiltransferase, desacetilase, bem como fatores de splicing (BAZRGAR et al., 2020). Desse modo, a desregulação de miRNAs pode causar modificações pós-tradução como fosforilação e acetilação

Os miRNAs representam um dos principais mecanismos candidatos dos quais a fisiologia paterna modula o desenvolvimento do fenótipo da prole por meio de seu amplo potencial na regulação de expressão gênica e proteica (MCPHERSON et al., 2015). Assim, compreender o crosstalk entre PTMs e miRNAs que interferem na adipogênese é a chave para identificação de estratégias terapêuticos para obesidade programada pela herança intergeracional paterna. Interessantemente, os miRNAs possuem um papel chave na modulação da adipogênese e angiogênese, além de influenciar a expressão de adiponectinas e hormônios ligados a resistência à insulina (KLÖTING et al., 2009). Uma análise da expressão global de miRNAs em tecido adiposo subcutâneo e visceral, demonstrou, que a expressão de miR-17-5p, miR-132, miR-134, miR-181a, miR-27a, miR-30e, miR-21, miR-20 a, miR -17, miR -204ª, miR -128 possuem um papel na disfunção do tecido adiposo e no desenvolvimento da obesidade. Adicionalmente, a expressão de miR-17-5-p, miR-132, miR-99a, miR-134, miR-181a, miR-145 e miR-26b-5p, miR-27a-3p, miR-186-5p, miR-155 estavam associados tanto com a morfologia do tecido adiposo quanto à parâmetros metabólicos, incluindo hemoglobina glicada, leptina, adiponectina e interleucina-6 (KLÖTING et al., 2009).

Sendo assim, ainda referente a esse projeto, pretende-se fazer as modificações pós-traducionais de proteínas (fosforilação, glicolisação, metilação e acetilação) e os miRNAs no tecido adiposo da prole exposta à dieta controle e hiperlipídica. A hipótese desse trabalho é de que a prole exposta à dieta hiperlipídica demonstra uma série de alterações de fosforilação em várias enzimas chave envolvidas no metabolismo dos lipídios e homeostase da glicose, acompanhado com o aumento da expressão de miRNAs associados a funções pró-adipogênicas quando comparado à prole exposta à dieta controle. No entanto, esperamos que o treinamento paterno resulte em fatores de proteção na prole independentemente do tipo de exposição à dieta. Tais fatores estariam relacionados as modificações pós-tradução de proteínas (fosforilação, glicolisação, metilação e acetilação), as quais podem causar a diminuição da expressão de micro RNAs pró-adipogênicos, além de aumentar a expressão de moléculas anti-adipogênicos. Acreditamos que uma extensa modificação pós-tradução de proteínas do tecido adiposo fornece uma visão holística dos aspectos moleculares que fundamentam distúrbios metabólicos complexos. O *cross-talk* entre as adaptações pós-tradução de proteínas e os miRNAs correspondentes que regulam estas vias poder um grande avanço na biologia do tecido adiposo e facilitara o aparecimento de novos marcadores moleculares diagnósticos.

Por fim, essas informações serão importantes, visto que, estudos intergeracionais que abordam a influência do exercício físico, podem contribuir como ferramenta no monitoramento e prevenção de possíveis doenças e fatores de risco nas gerações futuras, além de esclarecer os possíveis efeitos deletérios no tecido adiposo.

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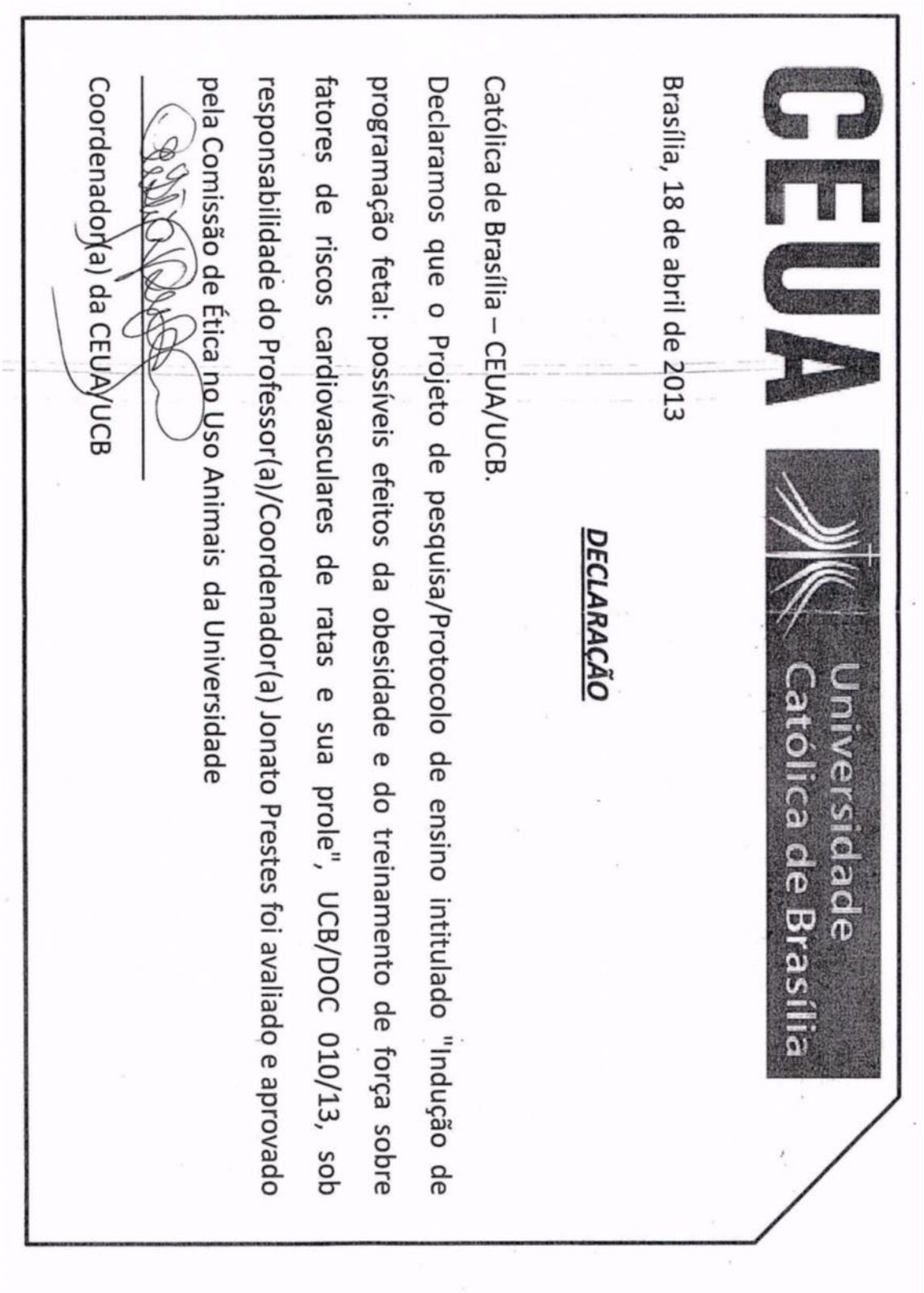
8. ANEXOS

8.1 Certificado do Comitê de Ética no Uso Animal



CEUA

Universidade Católica de Brasília

Brasília, 18 de abril de 2013

DECLARAÇÃO

Católica de Brasília – CEUA/UCB.

Declaramos que o Projeto de pesquisa/Protocolo de ensino intitulado "Indução de programação fetal: possíveis efeitos da obesidade e do treinamento de força sobre fatores de riscos cardiovasculares de ratas e sua prole", UCB/DOC 010/13, sob responsabilidade do Professor(a)/Coordenador(a) Jonato Prestes foi avaliado e aprovado pela Comissão de Ética no Uso Animais da Universidade

Coordenador(a) da CEUA/UCB

8.2 Demais produções científicas durante o processo de doutorado (2018-2021)

1. DE SOUSA NETO, IVO VIEIRA; DURIGAN, JOÃO LUIZ QUAGLIOTI; CARREIRO DE FARIAS JUNIOR, GONÇALO ; BOGNI, FABIO HENRIQUE ; RUIVO, AMANDA LIMA ; ARAÚJO, JULIANA OLIVEIRA DE ; NONAKA, KEICO OKINO ; SELISTRE-DE-ARAÚJO, HELOÍSA ; MARQUETI, RITA DE CÁSSIA . Resistance Training Modulates the Matrix Metalloproteinase-2 Activity in Different Trabecular Bones in Aged Rats. Clinical Interventions in Aging, v. Volume 16, p. 71-81, 2021.

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