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André Brum Saccomori

# O EXERCÍCIO MATERNO ATIVA AS VIAS DE SINALIZAÇÃO MEDIADAS POR AKT/GSK-3β E SIRT1/3 NO CEREBELO DOS FILHOTES DE RATOS WISTAR

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Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Bacharel(a) em Biomedicina.

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## RESUMO

O ambiente intrauterino oferecido pelo estilo de vida materno influencia no desenvolvimento fetal pela programação do metabolismo. Um exemplo é a realização de exercício físico durante a gestação, a qual afeta positivamente o metabolismo cerebral da prole, podendo conferir resistência a condições adversas na vida pós-natal. No entanto os mecanismo adaptativos adjacentes promovidos pelo exercício materno ainda precisam ser determinados. Nós investigamos algumas vias de sinalização modificadas pelo exercício materno no encéfalo da prole no vigésimo dia embrionário e sétimo dia pós-natal. Ratas fêmeas nadaram uma semana antes do acasalamento e durante todo o período gestacional (5 dias por semana, durante 30 min/dia). Os cerebelos dos filhotes no vigésimo dia embrionário e sétimo dia pós-natal foram analizados. Análises por Western Blot revelaram que a razão entre pGSK-3β/GSK-3β e pAkt/Akt não foram modificadas pelo exercício materno no vigésimo dia embrionário. No dia sétimo dia pós-natal, tanto a razão pGSK-3β/GSK-3β como pAkt/Akt tiveram redução significativa no cerebelo, sugerindo aumento da atividade da GSK-3β como resultado da diminuição na fosforilação mediada por Akt. O imunoconteúdo de proteínas sensíveis ao metabolismo SIRT1 e SIRT3 foram aumentados no cerebelo devido ao exercício materno sem afetar os níveis de Mfn1, Drp1 e TFAM. Nossos resultados sugerem que as vias Akt/GSK-3β e SIRT1/3 no cerebelo dos filhotes são programadas pelo exercício materno indicando adaptações do metabolismo em resposta ao ambiente materno.



**Palavras-chave:** Programação Metabólica. Exercício Materno. Mitocôndria. Sirtuína 1, Sirtuína 3, GSK-3.

## ABSTRACT

The intrauterine milieu offered by maternal lifestyle influences the fetal development by programming the metabolism. In this context, physical exercise during pregnancy affects positively offspring's brain metabolism conferring resistance to adverse conditions in the postnatal life. However, the adaptive underlying mechanisms to maternal exercise are still unknown. In order to clarify this aspect, we set out to investigate some signaling pathways mediated by maternal exercise on offspring's cerebellum on embryonic day 20 and postnatal day 7. Female rats swam one week prior the mating and throughout pregnancy (5 days/week during 30 min/day). The cerebellum of pups on embryonic day 20 and on postnatal day 7 was analyzed. Western blot analyses revealed that pGSK-3β/GSK-3β and pAkt/Akt ratios were unchanged by maternal exercise in the cerebellum of pups on embryonic day 20. On postnatal day 7 both pGSK-3β/GSK-3β and pAkt/Akt ratios reduced significantly in the cerebellum, suggesting increased activity of GSK-3β as result of decreased phosphorylation by Akt. The cerebellar immunocontent of energy metabolism-sensing proteins SIRT1 and SIRT3 was increased by maternal exercise without affecting Mfn1 Drp1, and TFAM levels. Taken together, our findings show the Akt/GSK-3β and SIRT1/3 pathways are programmed by maternal exercise in the offspring's cerebellum indicating adaption of metabolism in response to maternal environment.



**Keywords:** Metabolic programming, maternal exercise, mitochondria, sirtuin 1, sirtuin 3, GSK-3

**SUMÁRIO**

# 1 INTRODUÇÃO COMPREENSIVA

O período embrionário, fetal e os primeiros meses de vida são cruciais para o desenvolvimento de um indivíduo, onde vários fatores podem exercer influência sobre o metabolismo e determinar a susceptibilidade para o desenvolvimento de doenças ao longo da vida, ditando o estado de saúde do indivíduo (Bale, Baram et al. 2010). Nesse contexto, diversas alterações no organismo do indivíduo adulto têm sido relacionadas com o estilo de vida da mãe durante o período gestacional (Bharathi, Natesh et al. 2012), o que é explicado pelo conceito de DOHaD, do inglês "Developmental Origin of Health and Disease" (Gluckman, Hanson et al. 2010). Doenças neurológicas na vida adulta, como as doenças de Alzheimer e Parkinson, têm sido relacionadas ao período de desenvolvimento do organismo através da programação metabólica (Bale, Baram et al. 2010). Nesse sentido, o exercício físico nesse período pode ser considerado uma estratégica capaz de promover adaptações metabólicas benéficas para auxiliar no crescimento saudável do feto e também proteger contra o desenvolvimento de doenças na idade adulta (Fidalgo, Falcao-Tebas et al. 2013).

## 1.1 PROGRAMAÇÃO METABÓLICA

A programação metabólica é caracterizada por alterações no metabolismo e epigenoma do feto que ocorrem durante o período intrauterino em resposta ao ambiente oferecido pela mãe (Bale, Baram et al. 2010). O período que um indivíduo passa no útero e o período neonatal têm sido reconhecidos como maiores responsáveis por ditar o risco de desenvolver determinadas doenças na vida adulta, como doenças neurológicas, diabetes, obesidade e doenças cardiovasculares (Dearden, Bouret et al. 2018). Um dos primeiros estudos que associou eventos de programação metabólica foi realizado por David Barker, o qual relacionou o surgimento de doenças cardiovasculares com a má nutrição das mães durante o período gestacional, dando origem à hipótese de Barker, ou hipótese do fenótipo econômico (Schulz 2010). Outro fato que ajudou a reforçar essa hipótese foi a análise das pessoas nascidas durante o período conhecido como "Dutch Hunger Winter" que ocorreu em parte da Holanda durante a segunda guerra mundial, onde as pessoas tinham pouco acesso a comida e ingeriam cerca de 400 quilocalorias por dia. As pessoas nascidas de mulheres grávidas durante esse período apresentaram maiores índices de doenças coronárias e desenvolvimento de diabetes do tipo II após os 50 anos de idade (de Rooij, Wouters et al. 2010).

Em contraste aos efeitos negativos nos filhos gerados por estressores durante o período gestacional, recentemente, estudos têm demonstrado que o metabolismo fetal pode ser programado positivamente por hábitos saudáveis da mãe durante a gestação (Bharathi, Natesh et al. 2012). Dessa forma, alguns estudos têm relacionado o exercício materno durante a gestação com a capacidade de promover efeitos benéficos sobre o metabolismo da prole (Fidalgo, Falcao-Tebas et al. 2013, Marcelino, Longoni et al. 2013). Dentre os benefícios pode-se destacar melhora nas fases de aquisição e retenção de memória, aumento no número de células hipocampais, prevenção da redução do número de neurônios hipocampais em modelo de hipóxia neonatal além de influência sobre parâmetros de crescimento fetoplacentário, peso no nascimento e teor de gordura ao nascer (Clapp, Kim et al. 2002, Akhavan, Emami-Abarghoie et al. 2008). Além disso, o grupo de pesquisa do laboratório de programação metabólica da Universidade Federal do Rio Grande do Sul, do qual faço parte, já demonstrou que a natação materna durante a gestação oferece benefícios para a prole relacionada a parâmetros mitocondriais, evidenciada pela maior biogênese mitocondrial e aumento da capacidade antioxidante em algumas regiões encefálicas (Marcelino, Longoni et al. 2013), além de melhor desempenho no teste de reconhecimento de objetos em filhotes machos na idade adulta (Marcelino, de Lemos Rodrigues et al. 2016).

## 1.2 EXERCÍCIO FÍSICO E NEUROPROTEÇÃO

O exercício físico já tem sido bastante estudado e empregado como estratégia de prevenção contra diversas doenças. Os benefícios para quem pratica exercício vão desde alterações na saúde física até modulação do metabolismo no sistema nervoso central (SNC) (Radak, Ihasz et al. 2014, Hamilton and Rhodes 2015). Estudos clínicos sugerem que o exercício físico pode ser eficaz em manter a integridade de algumas regiões encefálicas em indivíduos que possuem doenças neurodegenerativas como Esclerose Múltipla e Doença de Alzheimer (Prakash, Snook et al. 2010, Yu, Bronas et al. 2014), além de manter o volume de estruturas cerebrais relacionadas com o controle do movimento em indivíduos idosos (Nagamatsu, Weinstein et al. 2016). Os benefícios do exercício físico sobre o SNC não se limitam apenas às modificações geradas diretamente no cérebro, existem estudos relacionando o exercício com modificações cardiovasculares, o que culmina em benefícios para o SNC através do melhor aporte sanguíneo levando nutrientes para o cérebro (Getty, Wisdo et al. 2018). Apesar dos estudos clínicos demonstrarem resultados promissores em relação à prática de exercício físico, existem

limitações nesse tipo de estudo clínico que é a inviabilidade de investigação dos mecanismos celulares e moleculares pelos quais o exercício promove as adaptações no SNC. Tendo em vista esse fator, muitos estudos realizados em modelos animais buscam investigar os mecanismos responsáveis pelas adaptações geradas pelo exercício.

Em modelos animais, as adaptações benéficas no SNC promovidas pelo exercício físico incluem aumento nos depósitos de glicogênio (Dalsgaard 2006), aumento da captação de glicose pelas células, melhor capacidade oxidativa e aumento na atividade do sistema transportador de elétrons (STE) (Dishman, Berthoud et al. 2006, Gokbuget, Hartog et al. 2011). O aumento da atividade do STE, promovido pela prática de exercício, reflete o maior consumo de ATP necessário durante a atividade física, induzindo o organismo a produzir mais espécies reativas de oxigênio (EROs) (Gomes, Silva et al. 2012). EROs na literatura, até poucas décadas atrás, eram consideradas apenas prejudiciais para o organismo, atualmente já existem inúmeros trabalhos que evidenciam que as EROs apresentam papeis fisiológicos importantes para a sinalização e proteção celular. Níveis elevados de EROs resultantes do esforço físico podem proporcionar adaptações celulares benéficas devido à modulação de enzimas antioxidantes e enzimas de reparo do dano oxidativo, o que proporciona resistência ao estresse oxidativo (Gomes, Silva et al. 2012). Também é descrito que o aumento da capacidade oxidativa promovido pelo exercício é decorrente das modulações geradas na biogênese mitocôndrial (Vina, Gomez-Cabrera et al. 2009, Yan 2009, Zhang, Wu et al. 2012).

O grande número de benefícios resultantes do exercício físico faz com que guias de saúde como a *American College of Sports Medicine* (2014) e *European Guidelines on cardiovascular prevention in clinical pratice* (2016) indiquem a prática de atividade física por pelo menos 30 minutos diários para que os efeitos benéficos possam ser observados. Essas recomendações também são aplicadas a mulheres grávidas que não possuam complicações médicas (Committee on obstetric 2015). Quando aliamos a prática de exercício com programação metabólica, durante o período gestacional deve-se também levar em conta o tipo de exercício físico que está sendo realizado. A natação é considerada uma opção mais adequada, pois é um exercício de intensidade moderada e confere algumas vantagens para gestantes e comparação com outras modalidades exercício, oferecendo um ambiente livre de atritos, menores riscos de quedas, um ambiente termorregulador, diminuindo os riscos de estresse sobre o feto e tornando a sua prática segura e vantajosa (Katz 1996).

## 1.3 VIAS DE SINALIZAÇÃO CELULAR POTENCIALMENTE MODULADAS PELO EXERCÍCIO MATERNO

O estudo das vias de sinalização se faz necessário para entender os mecanismos moleculares de resposta a um estímulo que estão ocorrendo dentro das células para gerar um efeito no organismo. Uma das principais vias envolvidas na fase de desenvolvimento cerebral é a da glicogênio sintase cinase 3 (GSK-3), uma proteína cinase serina/treonina que fosforila e inativa a glicogênio sintase (Kim, Wang et al. 2009). O papel da GSK-3 no desenvolvimento cerebral envolve o controle de processos de neurogênese, polarização do neurônio e crescimento do axônio. Níveis de expressão aumentado anormal dessa proteína na vida adulta podem estar associados a desordens de desenvolvimento neuronal (Hur and Zhou 2010). GSK-3 também está envolvida com a via de sobrevivencia celular regulada pela Akt, importante nos períodos pós-desenvolvimento. A atividade da GSK-3 pode ser inibida pela fosforilação gerada pela Akt na Ser21 da GSK-3α ou Ser9 da GSK-3β (Srivastava and Pandey 1998). Estudos já demonstraram que no início da vida é importante que a atividade de GSK-3β esteja aumentada do mesmo modo que a atividade de Akt esteja diminuída para resultar no desenvolvimento neuronal adequado (Kim, Wang et al. 2009). Durante o período de desenvolvimento os níveis de β-catenina fosforilada estão aumentados, a GSK-3β é responsável por essa fosforilação, o que leva a degradação da β-catenina promovendo diminuição da proliferação neuronal e gerando estímulo para a diferenciação neuronal (Kim, Wang et al. 2009).

Como dito anteriormente, o exercício pode gerar uma série de modificações no organismo, alguns estudos buscam investigar quais moléculas de sinalização estão sendo moduladas pelo exercício. A mitocôndria é considerada o principal fator que promove adaptações metabólicas em resposta ao exercício físico. As propriedades de dinâmica mitocôndrial são responsáveis por gerar modificações de acordo com a diferente demanda energética necessária em cada compartimento de processos celulares (Jornayvaz and Shulman 2010, Kageyama, Zhang et al. 2011). Para que ocorram os processos de dinâmica mitocondrial é preciso mecanismos de fusão e fissão das mitocôndrias, tais processos são regulados por GTPases conhecidas como mitofusinas (MFN), responsáveis pela fusão mitocondrial e proteínas relacionadas a dinâmina (DRP), responsáveis pela fissão mitocondrial (Knott, Perkins et al. 2008).

Além desses processos, o exercício pode modificar o metabolismo energético de forma positiva através da indução de biogênese mitocondrial, processo que envolve a ação do co-ativador α do receptor γ ativado por proliferação de peroxissomos (PGC-1α) e reguladores de

transcrição mitocondrial como o fator de transcrição mitocondrial A (TFAM) (Ventura-Clapier, Garnier et al. 2008, Picca and Lezza 2015). A biogênese mitocondrial é o processo pelo qual há um aumento do número de mitocôndrias a partir de mitocôndrias já existentes (Gottlieb and Bernstein 2016). O principal fator responsável por ativar o PGC-1α é a sirtuína 1 (SIRT1), uma proteína pertencente a família das desacetilases dependentes de $NAD^+$, que também tem sua atividade aumentada em resposta ao exercício (Brenmoehl and Hoeflich 2013). A SIRT1 é localizada tanto no citosol quanto no núcleo das células, e assim como as outras sirtuínas, age através de mecanismos de desacetilação, além de ativar o PGC-1α que está relacionado a biogênese mitocondrial, a SIRT1 também ativa as proteínas *Forkhead box O* (FOXOs). As FOXOs são proteínas relacionadas com a transcrição de fatores envolvidos na resistência ao estresse oxidativo pela expressão das enzimas antioxidantes superóxido dismutase e catalase (Eijkelenboom and Burgering 2013). De forma similar, o exercício modula a ativação da SIRT3 que também está envolvida com o balanço redox (Tao, Coleman et al. 2010). A SIRT3 está presente majoritariamente na mitocôndria, e atua como ativador da enzima superóxido dismutase 2 promovendo proteção contra espécies reativas por meio da dismutação do superóxido mitocondrial (Brenmoehl and Hoeflich 2013).

O exercício físico também é capaz de induzir a expressão do Fator Neurotrófico Derivado do Encéfalo (BDNF), uma proteína responsável pela produção de neurotrofinas necessárias para o desenvolvimento neuronal (Schinder and Poo 2000, Lee, Kim et al. 2006). O BDNF é sintetizado na forma de pró-BDNF para ser convertido em BDNF maduro, essas duas formas de BDNF podem interagir com receptores diferentes e obter resposta celular diferentes, até mesmo opostas (Matsumoto, Rauskolb et al. 2008). Estudos sugerem que o BDNF está envolvido com a cognição, e que o exercício aumenta a expressão de mRNA podendo gerar efeitos de recuperação pós lesão traumática cerebral em ratos (Van Hoomissen, Chambliss et al. 2003, Griesbach, Hovda et al. 2004).

## 1.4 JUSTIFICATIVA

A área de programação metabólica é considerada nova no meio científico e pode proporcionar resultados de suma importância para a população. No entanto, os estudos relacionados a essa área ainda são incipientes. Nosso grupo já demonstrou que o modelo de natação em ratas prenhes é uma estratégia não farmacológica benéfica tanto para a rata que se exercita quanto para o filhote, modulando parâmetros de resistência ao estresse oxidativo e melhorando o desempenho cognitivo quando o filhote já alcançou a idade adulta. Por ser uma área nova no conhecimento, ainda não existem estudos que demonstrem os mecanismos celulares e moleculares pelos quais o exercício materno promove adaptação no metabolismo dos filhotes. Muitas doenças estão relacionadas com o período de desenvolvimento de um indivíduo e existem poucos estudos demonstrando a influência do estilo de vida materno durante a gestação em alterações benéficas nesse período de desenvolvimento. Portanto, esse trabalho faz-se necessário para contribuir com o conhecimento sobre a programação do metabolismo em resposta ao exercício materno.

## 1.5 OBJETIVOS

### 1.5.1 Objetivo geral

Investigar os efeitos do exercício materno de natação durante a gestação sobre vias de sinalização celulares no cérebro de filhotes em diferentes fases do desenvolvimento.

### 1.5.2 Objetivos específicos

a) Avaliar possíveis alterações na via de sinalização mediada por Akt/GSK-3β no cerebelo, córtex parietal, hipocampo e estriado dos filhotes, no vigésimo dia embrionário e sétimo dia pós-natal, provenientes de ratas submetidas ao protocolo de exercício durante a gestação;
b) Avaliar possíveis alterações nas vias de sinalização mediadas por SIRT1, SIRT3, MFN1, DRP1 e TFAM relacionadas à função mitocondrial e estado redox no

cerebelo dos filhotes, no dia pós-natal 7, provenientes de ratas submetidas ao protocolo de exercício durante a gestação;

c) Avaliar os níveis de BDNF no cerebelo dos filhotes, no dia pós-natal 7, provenientes de ratas submetidas ao protocolo de exercício durante a gestação.

## 2 ARTIGO CIENTÍFICO

O artigo científico intitulado “Exercise in Pregnancy Activates GSK-3β-downstream Akt and upregulates SIRT1/3 signaling in Wistar rats pups’ cerebellum” será submetido para o periódico *The Febbs Journal*, cujas normas para publicação estão descritas no ANEXO B.

# Exercise in Pregnancy Activates GSK-3β-downstream Akt and upregulates SIRT1/3 signaling in Wistar rats pups' cerebellum

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## Running title

Maternal exercise alters offspring's cerebellum signaling

## Abbreviations

AMPK, AMP-activated protein kinase; BDNF, brain-derived neurotropic factor; DOHaD, Developmental Origin of Health and Disease; DRP1, dynamin-related protein 1; ETC, electron transport chain; ED, embryonic day; FOXO, Forkhead box O; GD, gestational day; GSK-3, glycogen synthase kinase 3; TFAM, mitochondrial transcription factor A; MFN1, mitofusin 1; PGC-1α, peroxisome proliferator-activated receptor gamma co-activator 1α; PMSF, phenylmethanesulfonyl fluoride; PD, postnatal day; ROS, reactive oxygen species; SIRT, sirtuin; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase; TCA, tricarboxylic acid.

## Keywords

Metabolic programming, maternal exercise, mitochondria, sirtuin 1, sirtuin 3, GSK-3

## Compliance with Ethical Standards

## Conflict of Interest

The authors declare that they have no conflict of interest.

**Abstract**

The intrauterine milieu offered by maternal lifestyle influences the fetal development by programming the metabolism. In this sense, exercise during pregnancy affects positively offspring's brain metabolism conferring resistance to adverse conditions in postnatal life. However, the adaptive underlying mechanisms to maternal exercise have to be determined. We set out to investigate some signaling pathways mediated by maternal exercise on offspring's encephalon on embryonic day 20 and postnatal day 7. Female rats swam one week prior the mating and throughout pregnancy (5 days/week during 30 min). The brains of pups on embryonic day 20 and on postnatal day 7 were analyzed. Western blot analyses revealed that pGSK-3β/GSK-3β and pAkt/Akt ratios were unchanged by maternal exercise in the brain of pups on embryonic day 20. On postnatal day 7, both pGSK-3β/GSK-3β and pAkt/Akt ratios reduced significantly in the cerebellum, suggesting increased activity of GSK-3β as a result of decreased phosphorylation by Akt. The cerebellar immunocontent of energy metabolism-sensing proteins SIRT1/3 was increased by maternal exercise without affecting Mfn1, Drp1, and TFAM levels. Taken together, our findings show the Akt/GSK-3β and SIRT1/3 pathways are programmed by maternal exercise in the offspring's cerebellum indicating adaption of metabolism in response to maternal environment.

## INTRODUCTION

Brain development encompasses sequential processes of proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis to reach mature functional neural circuitry [1-3]. Each one of these processes occurs differentially across various brain regions depending on regional and temporal emergence of developmental processes [2]. In both humans and rodents, the brain initiates to develop during embryonic and fetal periods and continues postnatally [2-5]. The activation of glycogen synthase kinase 3 (GSK-3) signaling pathway is crucial during brain development [6]. GSK-3 controls brain developmental processes such as neurogenesis, neuronal polarization, and axon growth; therefore, abnormal GSK-3 signaling can be associated with neurodevelopmental disorders [7].

The high sensitivity to endogenous and environmental cues during development can produce long-lasting consequences on brain function by programming cellular functions through metabolic and epigenomic adaptive modifications [4]. Changes in embryonic, fetal, and early neonatal metabolism and epigenome occurs due the plastic ability to respond adaptively to environmental stimuli [8]. The long-term environmental influence on offspring development are supported by the concept of Developmental Origin of Health and Disease (DOHaD) [9]. The temporal periods of developing brain region are critical, and they are recognized as windows of vulnerability [2]. Initial evidences of developmental brain vulnerability have been described after the exposure of animals or humans to neurotoxic compounds. Such compounds interfere with developmental processes and influence the susceptibility to diseases [10-12]. Lately, evidences have demonstrated that healthy maternal lifestyle across the vulnerable brain developmental windows emerged as beneficial stimuli, which can have long-term benefits to maternal and offspring's health conferring resistance to diseases [8]. Thinking in developmental processes of central nervous system development as opportunity instead susceptibility window, several studies demonstrated that maternal exercise before and during pregnancy promotes metabolic adaptations in the offspring protecting against hazardous stimuli [13-15]. These adaptations include modulation of antioxidant system in several brain

regions [16], increased mitochondrial function [16], induction of hippocampal neurogenesis [17], increased brain-derived neurotropic factor (BDNF) [18], and enhanced learning and memory [17, 19].

It is known that mitochondria are the main players to optimize metabolic adaptation in response to exercise [20]. Dynamic properties of mitochondria are essential to support high energy demand at long distances in neurons, thus controlling its distribution and function [21]. Therefore, in response to cellular energy requirement mitochondrial morphology dynamically change to ensure the proper energy support to different compartments in cellular processes [22]. Mitochondrial dynamic is determined by a balance between fusion and fission, which are orchestrated mainly by the GTPases mitofusin (Mfn) and dynamin-related protein (Drp), respectively [21]. Furthermore, energy metabolism is improved by inducing mitochondrial biogenesis, which is regulated by peroxisome proliferator-activated receptor–γ co-activator‑1α (PGC-1 α) and mitochondrial transcription factor A (TFAM) [23, 24]. PGC-1α is known as the master regulator of mitochondrial biogenesis by activating transcription factors, thus promoting the expression of TFAM [20], which in turn drives transcription initiation and replication of mitochondrial DNA (mtDNA) [24, 25]. Mitochondrial biogenesis and energy metabolism are under control of sirtuins (silent information regulators; SIRTs), SIRT1 and SIRT3, respectively [26]. SIRT1 have nuclear and cytosolic location, and it promotes mitochondria proliferation and oxidative phosphorylation by activating PGC-1 α. Conversely, SIRT3 is located in the mitochondria and it activates proteins engaged in the oxidative phosphorylation, tricarboxylic acid (TCA) cycle, and fatty-acid oxidation [26].

It is largely known that physical exercise induces mitochondrial biogenesis and improves mitochondrial bioenergetics in the brain [27-30]. However, little is known with regard to maternal exercise and its underlying mechanisms to promote benefits in offspring's brain metabolism. Park [31] showed that maternal running during pregnancy improves mitochondrial function in the hippocampus of neonates. In addition, our group demonstrated that maternal

swimming improves mitochondrial function and antioxidant defenses in the cerebellum, parietal cortex, hippocampus, and striatum of 7-day-old rats [16]. However, the molecular mechanisms underlying these benefits remain to be elucidated. Therefore, aiming to decipher the underlying adaptive processes responsible by offspring's metabolic benefits elicited by maternal exercise our goal was to investigate the effects of maternal exercise during pregnancy on signaling molecules levels in some brain regions (cerebellum, parietal cortex, hippocampus, and striatum) of pups on embryonic day 20 (E20) and on postnatal day 7 (PD7).

## RESULTS

### Effects of maternal swimming on Akt/GSK-3β signaling pathway in the fetus's encephalon on embryonic day 20 and postnatal day 7

GSK-3β is a downstream target of Akt, which phosphorylates GSK-3 on Ser9 inhibiting its activity [32]. Data analyses of Akt/GSK-3β pathway in the cerebellum, parietal cortex, hippocampus, and striatum obtained from fetuses on E20 indicated that Akt and GSK-3β activities remained unchanged in response to maternal swimming in comparison to control group in all encephalic regions (Fig. 1). The values of p-Akt/Akt ratio in the cerebellum [$t(10)=0.459$, $p=0.656$], parietal cortex [$t(10)=0.706$, $p=0.496$], hippocampus [$t(10)=0.759$, $p=0.465$], and striatum [$t(10)=1.874$, $p=0.090$] removed from fetuses of exercised group were similar to fetuses of control group. Similarly, the values of the ratio between p(Ser-9)-GSK-3β/GSK-3β in the cerebellum [$t(10)=0.778$, $p=0.454$], parietal cortex [$t(10)=0.793$, $p=0.446$], hippocampus [$t(10)=1.383$, $p=0.197$], and striatum [$t(9)=1.746$, $p=0.115$] of fetuses of exercised group were similar to fetuses of control group.

Interestingly, on PD7, we found a tottaly different profile in cerebellum. Akt phosphorylation was found significantly reduced in the cerebellum of 7-day-old pups born to exercised dams compared to control pups (Fig. 2A) [$t(14)=2.993$, $p=0.010$], without modifying total Akt levels [$t(14)=0.718$, $p=0.484$]. As shown in Fig. 2B, GSK-3β phosphorylation at Ser-9 was found reduced in the cerebellum of 7-day-old pups born to exercised dams compared to control pups

[t(14)=2.549, p=0.023], without modifying total GSK-3β levels [t(14)=0.0002, p=1.0]. Concerning other encephalic regions, maternal exercise did not modify p-Akt/Akt and p(Ser-9)-GSK-3β/GSK-3β ratios in the parietal cortex [t(14)=0.296, p=0.772; and t(14)=1.564, p=0.140, respectively], hippocampus [t(14)=0.841, p=0.414; and t(14)=0.932, p=0.367, respectively], and striatum [t(14)=2.096, p=0.053; and t(14)=2.050, p=0.059, respectively] of 7-day-old pups. These data indicate that maternal swimming reduces the kinase activity of Akt leading to increased levels of dephosphorylated GSK-3β, which becomes more active in the 7-day-old pup's cerebellum.

**Maternal swimming does not alter mature BDNF levels in the offspring's cerebellum on postnatal day 7**

The role of the neurotrophin brain-derived neurotrophic factor (BDNF) as intracellular signaling inducer is important for neuronal survival, morphology and plasticity in developing and mature central nervous system [33]. Mature BDNF levels were measured through ELISA in the offspring's cerebellum. It was observed that maternal exercise during pregnancy exerted no effect on this parameter, as demonstrated by similar values in the pups' cerebellum of exercised and control groups [t(14)=0.881, p=0.393].

**Maternal swimming elicits augmentation of Sirtuin 1 and 3 immunocontent in the offspring's cerebellum on postnatal day 7**

Next, we measured SIRTs 1 and 3 levels through Western blotting in pups' cerebellum. The SIRTs are deacetylases proteins playing essential role in energetic metabolism [34]. SIRT1 and SIRT3 immunocontent was found increased in the pups cerebellum born to exercised dams in comparison to control [t(18)=2.282, p=0.035; and t(12)=2.290, p=0.041, respectively].

**Effects of maternal swimming on mitochondrial parameters in the offspring's cerebellum on postnatal day 7**

Mitochondria proliferation and distribution in addition to its function of energy exchange are regulated by mitochondrial fusion and fission processes [21]. Mitochondria continuously undergo fusion and fission cycles [21], and this requires the transcription factor TFAM to ensure the stability of mitochondrial genome [35]. We assessed mitochondrial parameters such as the immunocontent of fusion protein, Mfn1, fission protein, Drp1, and mitochondrial transcription factor, TFAM. Mitochondrial dynamics proteins, Mfn1 and Drp1, were found unaltered in the cerebellum of pups [$t(14)=1.165$, $p=0.263$; and $t(14)=0.235$, $p=0.817$, respectively], indicating no effect of maternal exercise on this parameters on PD7. Similarly, it was found unchanged TFAM levels in the cerebellum of maternal exercised pups [$t(14)=0.284$, $p=0.780$] compared to control pups.

## DISCUSSION

It has been widely recognized that physical exercise during pregnancy promotes benefits to mother and fetus [36]. Exercising during pregnancy prevents maternal hypertension and excessive weight gain, prevents offspring's macrosomia at birth [37], and enhances cerebral maturation in newborn [38]. Despite available data from animal studies help to clarify whole body metabolic changes promoted by maternal exercise on offspring, the molecular basis on brain changes remains to be elucidated. Herein, we assessed key molecules involved in brain signaling that might be responsive to maternal exercise.

Setting up neural circuits during development relies on several morphogenetic steps, including neuronal migration and polarization, axon outgrowth and branching [39, 40]. These steps are orchestrated under influence of the signaling mediator GSK-3. GSK-3 transduces upstream signaling to reorganization of the axonal microtubules of cytoskeleton directed by extracellular cues [39]. As seen that GSK-3 is an important molecular regulator of neurogenesis, we initially sought to evaluate GSK-3β downstream Akt pathway. Our analyses of Akt/GSK-3β signaling demonstrate that increased activity of GSK-3β was present on 7-day-old pups' cerebellum (Figure 2A and 2B), which seems to be a result of intrauterine metabolic programming by maternal exercise. Interestingly, this signaling pathway was not affected on E20 (Figure 1),

despite the maternal exercise was performed up to delivery. The mechanism behind the switch effect must be investigated in future works. Here, the increased GSK-3β activity on 7-day-old pups' cerebellum (Figure 2B) was a result of decreased Akt activity. It has also been demonstrated that the control of GSK-3 activity during development occurs through activation of the ubiquitin–proteasome system [39]. Phosphorylated Akt is known to phosphorylate GSK-3β at serine 9, reducing GSK-3β activity [32]. During development phosphorylated Akt has been shown to be target of local protein degradation to ensure neuronal polarity [41]. Among the brain regions assessed in the present study, upregulation of GSK-3β was observed only in the cerebellum of 7-day-old pups. These findings might be explained by distinct temporal and regional maturation of brain regions, in which neurogenesis vary temporally within different regions [2]. Cerebellar neurogenesis is delayed in comparison to other brain developing regions, initiating early during embryonic stages and extending at postnatal days [2].

BDNF is known to play a role in neuron survival and differentiation during brain development [42]. In addition, physical exercise has long been shown to induce the expression of neurotrophic factors such as BDNF [43]. Several works have demonstrated that exercise increases BDNF mRNA expression and/or protein levels in the brain of adolescent [44, 45], adult [46], and aging rats [47, 48]. Furthermore, some studies have demonstrated that maternal exercise during pregnancy increases BDNF levels [18, 49, 50], while others studies have demonstrated that BDNF levels are transiently increased at early postnatal age [51] or even unchanged by maternal exercise during pregnancy [19]. Exercise modality employed and rodent strains used can account for these differences. Moreover, methodological variations are found among these studies, which measure mRNA expression or protein levels of BDNF. BDNF is synthesized as a precursor, pro-BDNF, which is converted to mature BDNF. Pro- and mature BDNF play distinct functions by interacting with different receptors and activating different intracellular pathways [52]. In the present study, mature BDNF levels were found unchanged in the cerebellum of pups by maternal exercise (Figure 3).

SIRTs are $NAD^+$-dependent deacetylases with key roles not only in histones deacetylation but also in transcriptional regulators deacetylation [53]. The role played by SIRTs controlling transcription factors activities allows the communication between metabolism and epigenetic regulation [54], highlighting the importance to study SIRTs during development in the metabolic programming field. SIRT1 and SIRT3 are known to control mitochondrial biogenesis and energy metabolism [26]. SIRT1 is known to deacetylate and activate PGC-1$\alpha$ and Forkhead box O (FOXO) transcription factor [55]. On activation, PGC-1 $\alpha$ mediates transcription of nuclear and mitochondrial genes involved in mitochondrial biogenesis and function [56], while FOXO mediates transcription of genes related to redox balance, such as antioxidant superoxide dismutase 2 (SOD2) and catalase genes, to increase the oxidative stress resistance [55]. The mitochondrial SIRT3 deacetylates several mitochondrial proteins, thus regulating mitochondrial functions [57]. SIRT3 acts as direct activator of oxidative phosphorylation, TCA cycle and fatty-acid oxidation proteins, and indirect activator of PGC-1$\alpha$ and AMP-activated protein kinase (AMPK) [26, 58] demonstrated that SOD2 deacetylation by SIRT3 is essential for the enzyme activity, evidencing the role of SIRT3 in sensing redox imbalance. Interestingly, [59] demonstrated that SIRT3 mediates adaptive responses of neurons to exercise. Here, we observed that maternal exercise induced an increase in SIRT1 and SIRT3 levels (Figure 4), indicating the exercise performed by mother promotes adaptive changes in the offspring`s cerebellar metabolism that remain evident at least on the first postnatal days. The present findings are complementary to our previous work [16]. In [16] we demonstrated significant increased activity of antioxidant enzymes, increased non-enzymatic antioxidant potential and reactivity, and increased mitochondrial mass and membrane potential in the cerebellum of pups. It seems that the enhanced mitochondrial function and antioxidant status underlying molecular mechanism is assigned to SIRT signaling in the cerebellum of 7-day-old pups. Our findings suggest that the increased levels of SIRT1 and SIRT3 mediates the beneficial adaptive effects of maternal environment promoted by physical exercise in the offspring`s cerebellum.

The central nervous system is highly plastic as it undergoes continuous remodeling not restricted to fetal and early postnatal development but also during adulthood [2]. Neuronal plasticity enables the adaptation to the environment by changing the structure and function of cells [60]. Mitochondria are fundamental organelles in the plasticity of neuronal circuits to supply the high metabolic requirement and to sustain vesicular neurotransmitter release [60, 61]. In addition, mitochondrial biogenesis, turnover, distribution, and morphogenesis are essential for brain development [62]. A balance between fusion and fission must be maintained for optimal mitochondrial function [63], and the nuclear encoded-TFAM is necessary for mitochondrial biogenesis. TFAM is translated in the cytosol and transported into the mitochondria to induce the transcription of essential mitochondrial enzymes encoded by mitochondrial genome [20]. The important role of mitochondrial dynamic in the development of central nervous system and synaptic plasticity is described by several authors [64-66]. Ishihara et al. [64] demonstrated that mitochondrial fission protein Drp1 is essential for embryonic development and synapse formation in mice. Kageyama et al. [65] demonstrated that mitochondrial division orchestrated by Drp1 is a mechanism of quality control to suppress oxidative damage and to promote neuronal survival. Here, we examined the Mfn1 and Drp1 levels, and we demonstrated that maternal exercise maintains the protein levels at control values (Figure 5). In addition, we observed that TFAM protein levels also remained unchanged by maternal exercise in 7-day-old pups' cerebellum. Taken together, the data obtained in the present study demonstrates that exercise during pregnancy programs the metabolism of offspring`s cerebellum by modulating activation of GSK-3β-downstream Akt pathway and SIRT1/3 signaling. So far, the present work offers insights to DOHaD and opens new avenues to promote preventive strategies to disease development. As temporal ontogeny of brain development extends beyond early postnatal days, it remains to be determined whether maternal exercise further induces time-dependent changes on brain metabolism at cellular and molecular levels that goes beyond PD7.

In summary, in the present study we found that maternal swimming during pregnancy differentially activated GSK-3β downstream Akt pathway in cerebellum. Moreover, further analyses in the 7-day-old pups' cerebellum indicated an upregulation of energy metabolism-sensing proteins, SIRT1 and SIRT3, associated to unchanged levels of TFAM, Mfn1, and Drp1.

## MATERIALS AND METHODS

### Animals

Adult male and female Wistar rats (80 days old) were housed with 12h dark/light cycle (lights on between 7:00h and 19:00h), controlled temperature (22 ± 2°C) and humidity (50-60%), and had free access to food and water. All experimental procedures and animals care were conducted in accordance with the National Institutes for Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996) and were approved by the local Ethics Commission of Universidade Federal do Rio Grande do Sul (CEUA/UFRGS; protocol number 32852). All efforts were made to minimize animal suffering, and to keep the number of animals at the minimum to demonstrate consistent effects.

### Maternal swimming protocol

Female rats were randomly assigned to sedentary (control) and exercised (involuntary swimming) groups. Rats swam one week prior the mating, to habituate to aquatic environment, and throughout pregnancy in a schedule of 5 days/week for 4 weeks lasting 30 min/day in a pool filled with 32±1 °C water, as previously described [16]. Control rats were immersed in water, carefully dried, and returned to the housing boxes in the same schedule of exercise group.

### Experimental design

During the mating two females were housed with one male. Pregnancy was confirmed by the presence of a vaginal plug and the embryonic day 0 (E0)/gestational day 0 (GD0) was established. Up to GD20, all pregnant females were housed individually. One subset of

pregnant females from sedentary and exercise group was euthanized by rapid decapitation without anesthesia and the fetuses on E0 were removed from the uteri of dams. Fetuses brain were dissected and fetal cerebellum, parietal cortex, hippocampus, and striatum were isolated. Although all fetuses were collected, only one per litter, randomly selected, was used for each analysis. Another subset of pregnant females was allowed to normal spontaneous vaginal delivery, and they were checked for birth twice a day (at 8 and 18 h) to allow the assignment of postnatal day (PD) 0. Littermates were housed up to PD7 with their mothers. The pups were euthanized on PD7 by decapitation; the brains were removed and pups cerebellum, parietal cortex, hippocampi, and striatum were dissected. Encephalic samples were frozen at -80 °C immediately after their dissection and it were stored until use.

**Mature BDNF measurement**

Mature BDNF protein content was measured in the cerebellar homogenate of pups at age of 7 days. Mature BDNF was measured through the E-Max ELISA kit (Promega) according to the manufacturer's recommendations. Briefly, cerebellum were individually homogenized (1:10 w:v) in lysis buffer containing: 137 mM NaCl, 20 mM Tris-HCl (pH 8.0), Igepal (1%), glycerol (10%), 1 mM phenylmethanesulfonyl fluoride (PMSF), 0.5 mM sodium vanadate, 0.1 mM EDTA, and 0.1 mM EGTA, and centrifuged for 3 min at 14,000 rpm at 4°C. Supernatant was diluted (1:5 v/v) in sample buffer and incubated on a 96-well flat-bottom plates previously coated with anti-BDNF monoclonal antibody and blocked with block and sample buffer. After sample incubation, plates were incubated with polyclonal anti-human antibody for 2 h and horseradish peroxidase for 1 h. Colorimetric reaction with tetramethylbenzidine was quantified in a plate reader at 450 nm. The standard BDNF curve, ranging from 0 to 500 pg/mL was assayed in each plate in parallel with the samples.

**Western blot assay**

Cerebellum, parietal cortex, hippocampus, and striatum samples from fetuses on E0 and from pups on PD7 were homogenized in ice-cold lysis buffer containing 4% sodium dodecyl sulfate

(SDS), 2 mM EDTA, 50 mM Tris, and 1% protease inhibitor cocktail. The homogenates were denatured 100°C for 5 min, and then centrifuged at 10,000 g for 30 min. After this, the supernatant containing the cytosolic fraction was collected, β-mercaptoethanol was added to a final concentration of 5%, and then, the samples were stored at -80 °C until use. Equal concentration of protein (50 µg) was loaded and immunodetected as previously described [67]. Membranes were incubated for 60 min at 4°C in blocking solution (Tris-buffered saline containing 5% non-fat milk and 0.1% Tween-20, pH 7.4) prior the incubation with the primary antibody. Membranes were incubated overnight at 4 °C in blocking solution containing one of the following primary antibodies: rabbit monoclonal anti-phospo(S473)-Akt (1:1000, Cell Signaling, catalog number #4058), anti-Akt (1:1000, Cell Signaling, catalog number #9272), anti-phospho(S9)-GSK-3β (1:1000, Cell Signaling, catalog number #9336), anti-GSK-3β (1:1000, Cell Signaling, catalog number #9315), anti-Mfn-1 (1:500, Abcam, catalog number #ab104274), anti-Drp-1 (1:500, Abcam, catalog number #ab154879), anti-SIRT1 (1:500, Santa Cruz Technologies, catalog number #sc-15404), anti-SIRT3 (1:500, Abcam, catalog number #ab189860), and rabbit monoclonal anti-β-actin (1:2000, Cell Signaling, catalog number #4967). After washing, the membranes were incubated with secondary antibody containing peroxidase-conjugated anti-rabbit IgG (1:2000, GE Healthcare Life Sciences, catalog number #NA934V) for 1 h. The chemiluminescence was detected using a digital imaging system (Image Quant LAS 4000, GE Healthcare Life Sciences) and analyzed using the Image J Software. The average optical density for the control group was designated as 100%.

**Statistical analyses**

Statistical analyses were performed using GraphPad Prism, version 6 (La Jolla, CA, USA). Data were analyzed using two-tailed *t* test. Differences was considered when P value was $<0.05$.

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**Author Contributions**

CPK and ABS designed research, conducted experiments, analyzed the results, formatted the graphs, and wrote the paper, JBH, conducted experiments and assisted the writing of the manuscript, PMA, TBM, conducted the experiments, PNL and CGS assisted the research design, CM designed research, conducted the experiments, obtained financial support, and assisted the manuscript preparation. All authors read and approved the final manuscript.

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## FIGURE CAPTIONS

**Figure 1:** Effect of maternal swimming on Akt/GSK-3β signaling pathway in the brain of fetuses on embryonic day 20. Ratio of p-Akt/Akt and p-GSK-3β/GSK-3β immunocontent in the cerebellum (A-B), parietal cortex (C-D), hippocampus (E-F), and striatum (G-H). Data are presented as mean ± standard mean error (n=6), and data were analyzed through Student's *t* test. Representative immunoblot is shown in the bottom of each graph.

**Figure 2:** Effect of maternal swimming on Akt/GSK-3β signaling pathway in the brain of pups on postnatal day 7. Ratio of p-Akt/Akt and p-GSK-3β/GSK-3β immunocontent in the cerebellum (A-B), parietal cortex (C-D), hippocampus (E-F), and striatum (G-H). Data are presented as mean ± standard mean error (n=8). Student's *t* test showed a significant difference in the cerebellar Akt/GSK-3β activities between maternal swimming and control group. Representative immunoblot is shown in the bottom of each graph. *$p<0.05$ and **$p<0.01$ compared to control.

**Figure 3:** Effect of maternal swimming on mature BDNF levels in the pup's cerebellum on postnatal day 7. Data are presented as mean ± standard mean error (n=8), and data were analyzed through Student's *t* test.

**Figure 4:** Effect of maternal swimming on sirtuins 1 and 3 immunocontent in the pup's cerebellum on postnatal day 7. Immunocontent of Sirtuin 1 (A) and Sirtuin 3 (B). Sirtuin 1 and Sirtuin 3 levels are expressed as the average percentage of control. Representative immunoblot (normalized to b-actin protein) is shown in the bottom of each graph. Data are presented as mean ± standard mean error (n=8). Student's *t* test showed a significant difference in the cerebellar sirtuin 1 and 3 immunocontent between maternal swimming and control group. *$p<0.05$ compared to control.

**Figure 5:** Effect of maternal swimming on mitochondrial parameters in the pup's cerebellum on postnatal day 7. Immunocontent of TFAM (A), mitofusin (B), and dynamin-related protein (C). TFAM, mitofusin, and dynamin-related protein levels are expressed as the average

percentage of control. Representative immunoblot (normalized to b-actin protein) is shown in the bottom of each graph. Data are presented as mean ± standard mean error (n=8). Student's *t* test showed no significant difference between maternal swimming and control group.

**Figure 1**

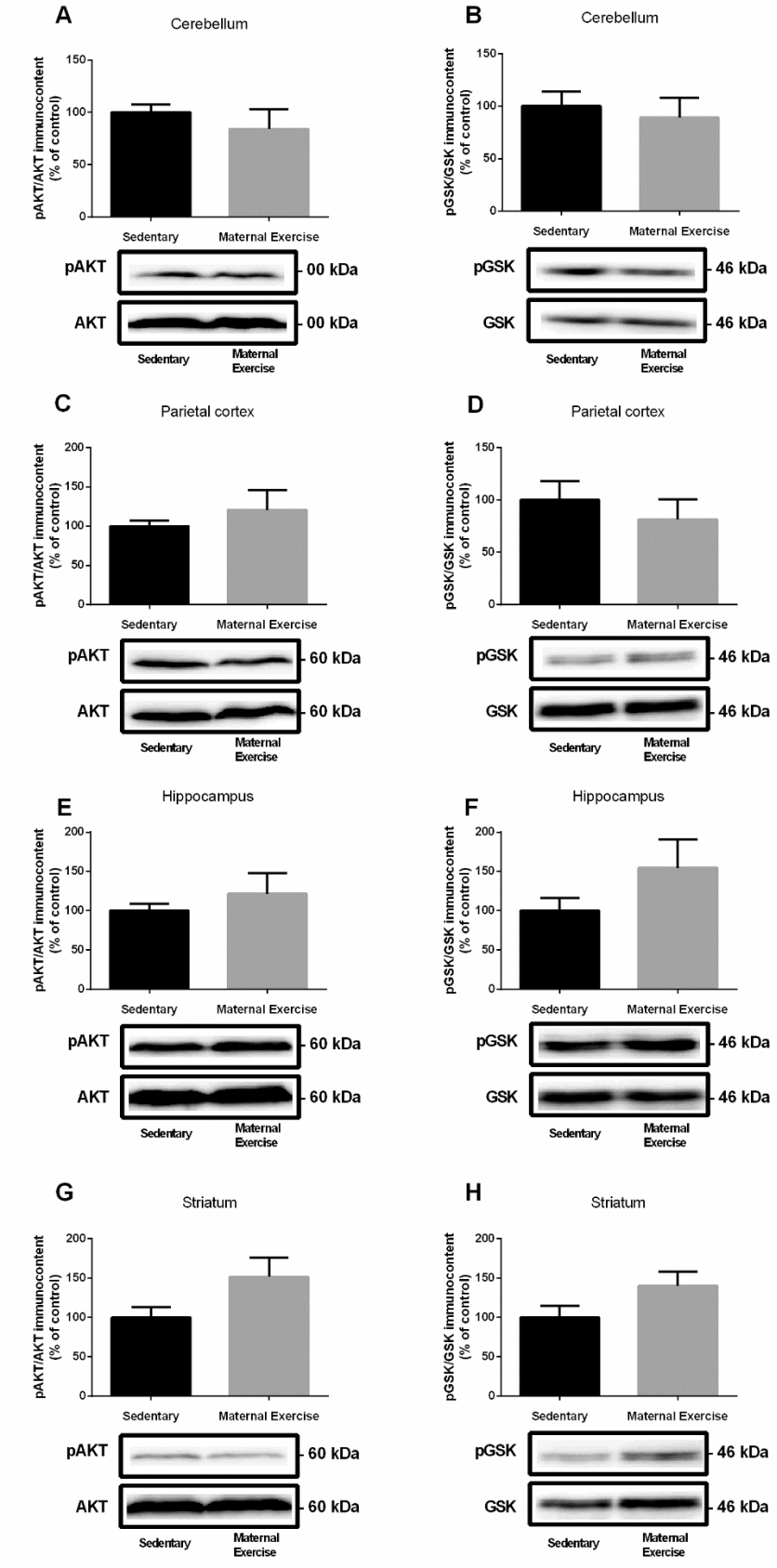

A
Cerebellum
pAKT/AKT immunocontent (% of control)
150
100
50
0
Sedentary
Maternal Exercise
pAKT
00 kDa
AKT
00 kDa
Sedentary
Maternal Exercise
B
Cerebellum
pGSK/GSK immunocontent (% of control)
150
100
50
0
Sedentary
Maternal Exercise
pGSK
46 kDa
GSK
46 kDa
Sedentary
Maternal Exercise
C
Parietal cortex
pAKT/AKT immunocontent (% of control)
200
150
100
50
0
Sedentary
Maternal Exercise
pAKT
60 kDa
AKT
60 kDa
Sedentary
Maternal Exercise
D
Parietal cortex
pGSK/GSK immunocontent (% of control)
150
100
50
0
Sedentary
Maternal Exercise
pGSK
46 kDa
GSK
46 kDa
Sedentary
Maternal Exercise
E
Hippocampus
pAKT/AKT immunocontent (% of control)
200
150
100
50
0
Sedentary
Maternal Exercise
pAKT
60 kDa
AKT
60 kDa
Sedentary
Maternal Exercise
F
Hippocampus
pGSK/GSK immunocontent (% of control)
200
150
100
50
0
Sedentary
Maternal Exercise
pGSK
46 kDa
GSK
46 kDa
Sedentary
Maternal Exercise
G
Striatum
pAKT/AKT immunocontent (% of control)
200
150
100
50
0
Sedentary
Maternal Exercise
pAKT
60 kDa
AKT
60 kDa
Sedentary
Maternal Exercise
H
Striatum
pGSK/GSK immunocontent (% of control)
200
150
100
50
0
Sedentary
Maternal Exercise
pGSK
46 kDa
GSK
46 kDa
Sedentary
Maternal Exercise

**Figure 2**

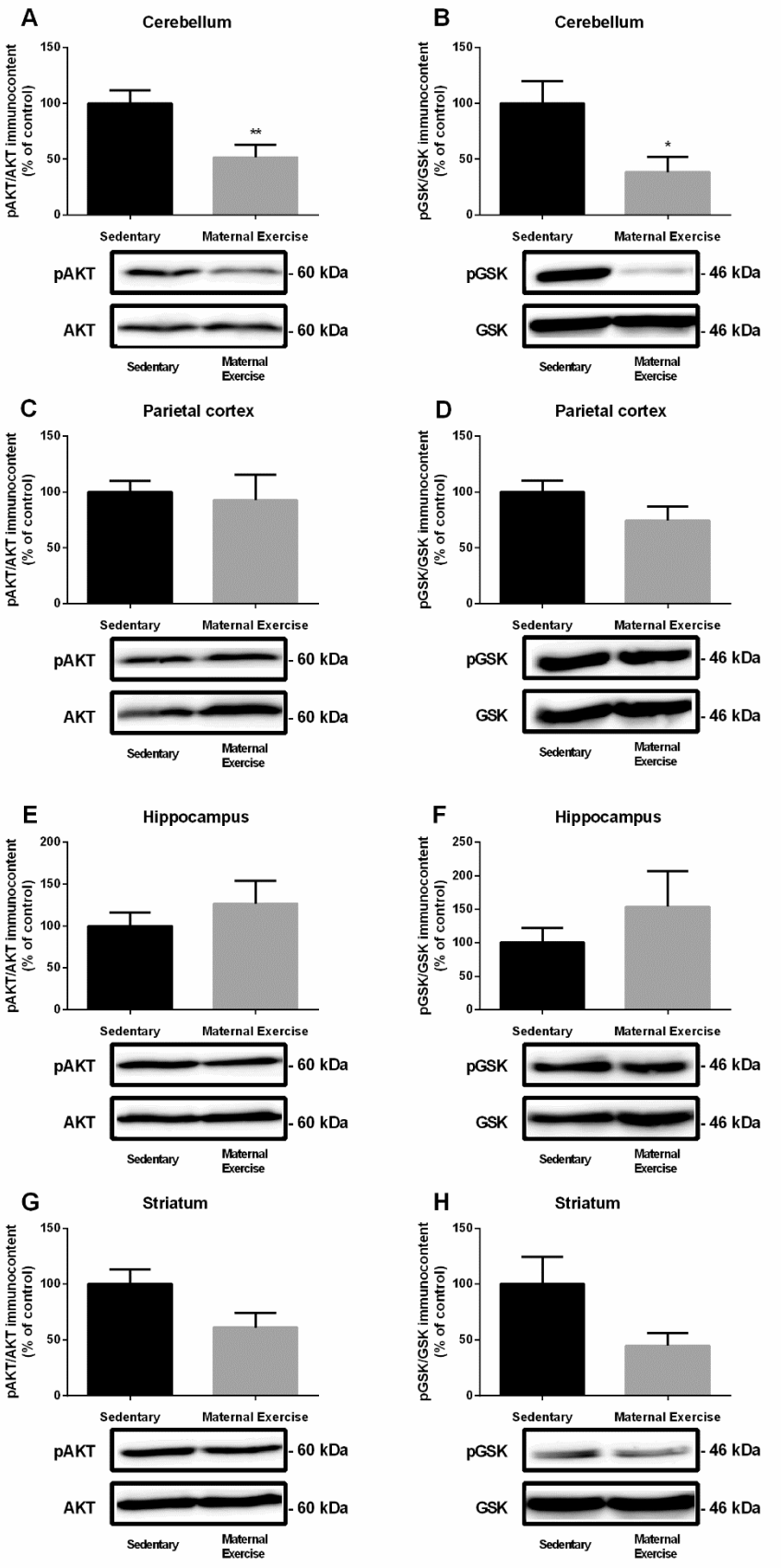

A
Cerebellum
pAKT/AKT immunocontent (% of control)
150
100
50
0
**
Sedentary
Maternal Exercise
pAKT
60 kDa
AKT
60 kDa
Sedentary
Maternal Exercise
B
Cerebellum
pGSK/GSK immunocontent (% of control)
150
100
50
0
*
Sedentary
Maternal Exercise
pGSK
46 kDa
GSK
46 kDa
Sedentary
Maternal Exercise
C
Parietal cortex
pAKT/AKT immunocontent (% of control)
150
100
50
0
Sedentary
Maternal Exercise
pAKT
60 kDa
AKT
60 kDa
Sedentary
Maternal Exercise
D
Parietal cortex
pGSK/GSK immunocontent (% of control)
150
100
50
0
Sedentary
Maternal Exercise
pGSK
46 kDa
GSK
46 kDa
Sedentary
Maternal Exercise
E
Hippocampus
pAKT/AKT immunocontent (% of control)
200
150
100
50
0
Sedentary
Maternal Exercise
pAKT
60 kDa
AKT
60 kDa
Sedentary
Maternal Exercise
F
Hippocampus
pGSK/GSK immunocontent (% of control)
250
200
150
100
50
0
Sedentary
Maternal Exercise
pGSK
46 kDa
GSK
46 kDa
Sedentary
Maternal Exercise
G
Striatum
pAKT/AKT immunocontent (% of control)
150
100
50
0
Sedentary
Maternal Exercise
pAKT
60 kDa
AKT
60 kDa
Sedentary
Maternal Exercise
H
Striatum
pGSK/GSK immunocontent (% of control)
150
100
50
0
Sedentary
Maternal Exercise
pGSK
46 kDa
GSK
46 kDa
Sedentary
Maternal Exercise

**Figure 3**

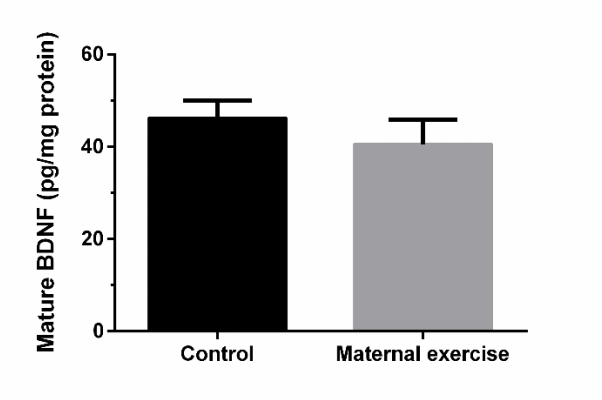



**Figure 4**

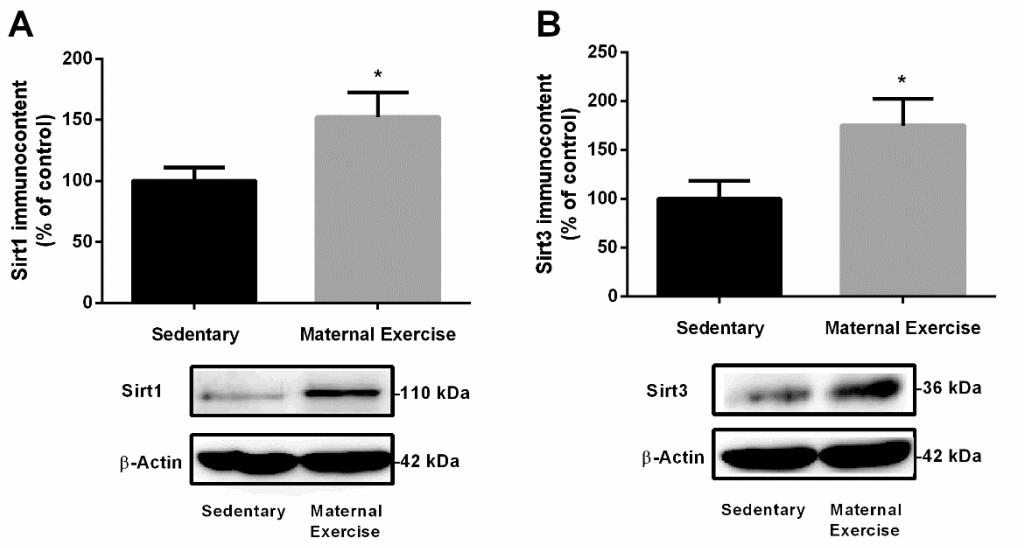
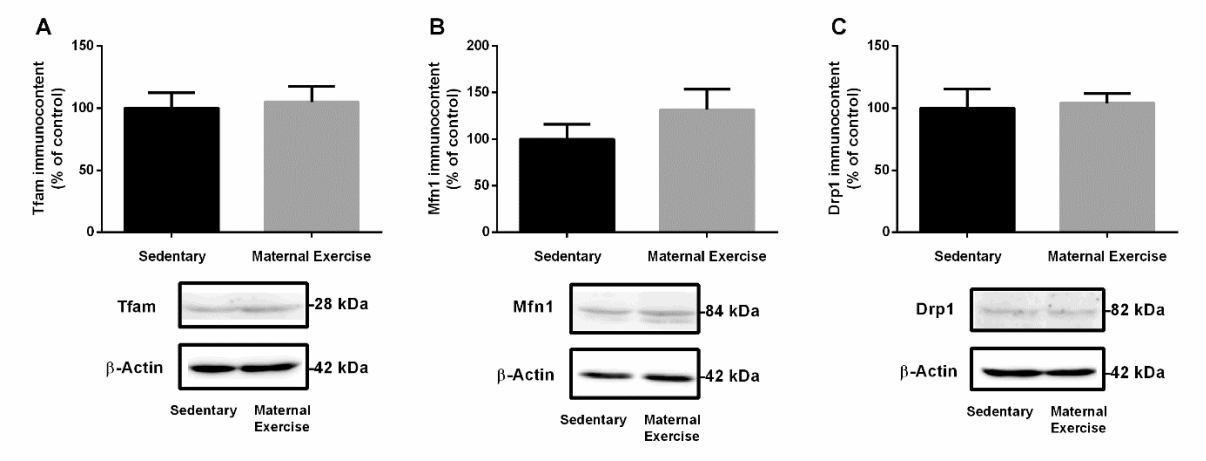


**Figure 5**


A
Tfam immunocontent (% of control)
150
100
50
0
Sedentary
Maternal Exercise
Tfam
28 kDa
β-Actin
42 kDa
Sedentary
Maternal Exercise
B
Mfn1 immunocontent (% of control)
200
150
100
50
0
Sedentary
Maternal Exercise
Mfn1
84 kDa
β-Actin
42 kDa
Sedentary
Maternal Exercise
C
Drp1 immunocontent (% of control)
150
100
50
0
Sedentary
Maternal Exercise
Drp1
82 kDa
β-Actin
42 kDa
Sedentary
Maternal Exercise

# 3 CONCLUSÕES E PERSPECTIVAS

Podemos concluir com esse trabalho que as modificações que ocorrem durante o período de desenvolvimento intrauterino em resposta à natação materna durante a gestação são evidentes no cerebelo da prole no sétimo dia pós-natal enquanto que as modificações não são observadas no córtex parietal, hipocampo e estriado. A atividade reduzida da Akt e, consequente aumento da atividade da GSK-3β no cerebelo parece ser decorrente da diferenciação neuronal, o que é considerado benéfico durante esse período de desenvolvimento e pode acarretar em futura neuroproteção. Os níveis de sirtuinas observados no presente estudo explicam os resultados previamente publicados pelo nosso grupo de pesquisa. A SIRT1 está relacionada com diversos fatores, dentre eles o PGC-1α, que é o principal regulador da biogênese mitocondrial e que pode estar mais ativo em decorrência da sinalização aumentada de SIRT1, o mesmo vale para as proteínas FOXO, que podem estar com sua atividade aumentada devido a sinalização da SIRT1, resultando em aumento da expressão de enzimas antioxidantes. A SIRT3 aumentada também pode estar relacionada com as defesas antioxidantes, pois ela está presente na mitocôndria, e seu aumento pode resultar na ativação da superoxido dismutase 2, a qual é responsável por combater um dos principais agentes causadores do estresse oxidativo.

Visto que os trabalhos relacionados à sinalização celular na programação metabólica são poucos, ainda há muito que ser investigado. Portanto, nossas perspectivas são avaliar a expressão das proteínas PGC-1α, FOXOs e SIRT1 em frações nucleares do cerebelo da prole cujas mães se exercitaram durante a gestação a fim de reforçar a nossa teoria sobre as vias de sinalização afetadas.

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# ANEXO A – CARTA DE APROVAÇÃO DA CEUA

U F R G S
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**PRÓ-REITORIA DE PESQUISA**

**Comissão De Ética No Uso De Animais**

CEUA UFRGS

## *CARTA DE APROVAÇÃO*

**Comissão De Ética No Uso De Animais analisou o projeto:**

**Número:** 32852
**Título:** INVESTIGAÇÃO DAS VIAS DE SINALIZAÇÃO QUE CONTRIBUEM PARA OS EFEITOS ADAPTATIVOS PROMOVIDOS PELO EXERCÍCIO MATERNO DURANTE A GESTAÇÃO SOBRE O CEREBELO DA PROLE

**Vigência:** 01/05/2017 à 30/04/2018

**Pesquisadores:**

**Equipe UFRGS:**

CRISTIANE MATTE - coordenador desde 01/05/2017
ANDRÉ BRUM SACCOMORI - Aluno de Mestrado desde 01/05/2017

***Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 08/05/2017 - SALA 330 DO ANEXO I DO PRÉDIO DA REITORIA - CAMPUS CENTRO - UFRGS-PAULO DA GAMA,110 BAIRRO FARROUPILHA -, em seus aspectos éticos e metodológicos, para a utilização de 46 fêmeas ratas wistar adultas, 23 ratos machos adultos e 32 filhotes machos, provenientes do CREAL-Bioquímica, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.***

Porto Alegre, Sexta-Feira, 19 de Maio de 2017

MARCELO MELLER ALIEVI
Coordenador da comissão de ética

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# ANEXO B – NORMAS DE PUBLICAÇÃO DA REVISTA "THE FEBS JOURNAL"

## Online submission

Online submission of manuscripts is via **https://mc.manuscriptcentral.com/febsj/**

Step-by-step instructions on how to submit your manuscript are available online during the submission process, which does not need to be completed in one session. Any queries can be sent to the Editorial Office (**febsj@febs.org**).

### *Initial submission*

At initial submission, we recommend that authors submit their text, figures and tables as a single PDF file. Alternatively, the following files will be automatically converted into a single PDF:

- The manuscript (including title page, abstract, main text, references and tables) saved as a PDF, .doc or .rtf file. Tables should be included after the references.
- Figures can either be included in the main text file for reviewing purposes, or be provided as separate files (see **Preparation of electronic artwork for publication** for details of recommended file formats). All figures must be given a figure number.
- Supplementary material (e.g. large datasets or raw data supporting an existing figure; see **Supplementary material**).

**NB** Any unpublished papers that are cited must be uploaded as Supporting Documents for referees to access. An electronic copy of any related paper under consideration or in press elsewhere must also be submitted as a Supporting Document; failure to do so may delay the review process.

The following information must be provided during the submission stage:

- Names, institutions and email addresses of all the co-authors.
- The names and email addresses of four recommended referees, together with their institutions. Please do not suggest anyone whom you have collaborated or published with in the last 3 years, or scientists based at your own institution, as they will not be approached. Authors are encouraged to suggest a **Member of the Editorial Advisory Board** as a preferred referee, if appropriate.
- Evidence of database submission (see **Structural data**, **Sequence and proteomics data** and **Transcriptomic and functional genomics data**).
- Approval of citation of any personal communications.
- If the manuscript is a resubmission, please upload a letter containing point-by-point responses to the referees.

On successful submission, you will receive onscreen acknowledgement with a manuscript reference number. All authors will receive an email acknowledgement.

### *Submission of a revised manuscript*

Please follow the instructions provided in the editorial decision letter. You will need to:

- Respond to the referees' comments online.
- Upload a revised version of the text, including any tables, as a .doc file. Alterations to the text should be highlighted by using track changes in Word. Alternatively, changes

can be highlighted in **BOLD TYPE**. Please ensure that only ONE set of changes is visible.

- Upload an unmarked copy of the manuscript text as a Supporting Document. (If your paper is accepted for publication, an unmarked copy of the document will be required for displaying in the 'Recently Published' articles section of the Journal's homepage.)
- Upload separate print-quality figure files in PDF, TIFF or EPS format. It is essential to follow the instructions described below in **Preparation of electronic artwork for publication**.
- Upload a short abstract of no more than 60 words and, in a separate file, a small square figure (see **Graphical abstracts** for more information).

***Editorial policy***

Submission of a manuscript implies: (1) that the work described has not been published before (except in the form of an abstract or as part of a lecture, review or thesis); (2) that it is not under consideration for publication elsewhere; (3) that its publication in the present form has been approved by all authors and by the responsible authorities in the institutions where the work was carried out; and (4) that, if accepted, it will not be published elsewhere in the same form, in any language, without the consent of FEBS, the licence holder. Prior or concurrent submission of the manuscript to an institutional repository or a not-for-profit subject-based preprint server does not constitute prior publication (see **Preprints**). Previously published abstracts, etc. should be referred to in the Introduction.

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*The FEBS Journal* endorses the **San Francisco Declaration on Research Assessment**.

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*The FEBS Journal* supports rapid and open scientific communication. Authors are free to upload their work to their personal website, their company's/institution's repository or archive, or a not-for-profit subject-based preprint server or repository ahead of or concurrently with submission to the Journal. Posting a manuscript to a recognised preprint server does not constitute prior publication.

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Submitted manuscripts are assigned to a handling Editor who is responsible for its evaluation. The Editor-in-Chief's decision regarding publication is based on the reports of referees and the handling Editor's recommendation, which will, at the Editorin-Chief's discretion, be transmitted to the authors. Authors will be informed of the editorial decision, on average, within 4 weeks of submission of a Regular Paper. The status of your manuscript can be checked via your 'Author Centre' at: **https://mc.manuscriptcentral.com/febsj/**. Enquiries should be addressed to the Editorial Office (**febsj@febs.org**).

This Journal works together with **FEBS Open Bio** to enable rapid publication of good-quality research that cannot be accepted for publication by *The FEBS Journal*. Authors may be offered the option of transferring the paper, along with any related peer reviews, to *FEBS Open Bio* for consideration by its Editors.

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Authors are required to meet the criteria for authorship as recommended by the International Committee of Medical Journal Editors (**ICMJE**). The specific contributions of each author must be described in an Author Contribution statement.

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Authors should consider and follow the ethical standards described below. The processing of a paper may be delayed if there is any doubt about it conforming to these ethical standards.

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Any breach of research or publication ethics including plagiarism, submission of fraudulent results/data including doctored figures, dual publication and false or incomplete attribution of authorship will not be tolerated. It will also be considered malpractice for an author to make inappropriate contact with a referee/Editor during the review process with the aim of influencing the outcome. *The FEBS Journal* will take action where misconduct is suspected, along the lines of the general principles outlined in Guidelines on Good Publication Practice, produced by the Committee on Publication Ethics (COPE). The Guidelines are available from the COPE website (**www.publicationethics.org**).

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*The FEBS Journal* endorses the **ARRIVE Guidelines** for reporting *in vivo* animal experiments. Whenever appropriate, authors should include in the Materials and Methods (Experimental Procedures) section:

- A statement indicating that the experiments were performed in accordance with *named* national legislation, where it exists, or, in its absence, with the *named* institutional/local body concerned with the ethics of experimentation (e.g. the National Research Council, or NIH in the USA). Experiments should be carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) or with the Guidelines laid down by the NIH in the USA regarding the care and use of animals for experimental procedures.
- A full description of the anaesthetic and surgical procedures used, and of peri-operative care.
- Evidence that authors took adequate steps to ensure that animals did not suffer unnecessarily at any stage of an experiment, whether acute or chronic.

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Research involving human subjects should comply with the Code of Ethics of the World Medical Association (Declaration of Helsinki).
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If human subjects are used, manuscripts must be accompanied by a statement in the Materials and Methods (Experimental Procedures) section, indicating that:

- The experiments were undertaken with the understanding and written consent of each subject.
- The study methodologies conformed to the standards set by the Declaration of Helsinki.
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Authors should ensure that all risks are minimised and the subjects are not injured and do not feel they have been abused as a result of participating in the study. Fully informed consent should always be sought.

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The Editor reserves the right to reject a paper if there is doubt as to whether appropriate procedures have been used.

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## Types of papers

***Regular paper***

Regular papers are the main form of publication of new research results in *The FEBS Journal*. There is no formal limitation on length but a regular paper rarely exceeds about 7,500 words (39,000 characters, without spaces). Shorter papers are, of course, welcome and the Editors will make recommendations for shortening any paper if that appears appropriate without loss of essential content. A concise, well-written paper is easier for the Editor and referees to evaluate, which can help to speed up publication.

We have no formal limit on the number of references, again subject to readability, and strongly encourage citation of primary literature, where appropriate, instead of reviews in research articles. It is important to ensure the original authors are given due credit.

***Reviews***

*The FEBS Journal* publishes reviews in all areas of the molecular life sciences. Reviews should appeal to a broad readership, including non-specialists. Ideally, reviews should convey new ideas, rather than simply a collation of information on a topic.

Reviews are commissioned by the Editorial team and are subject to peer review; therefore an invitation to contribute a review does not guarantee acceptance. Authors are encouraged to provide the details of three preferred referees at submission.

To maximise their exposure, all reviews and minireviews are made freely available immediately on publication in *The FEBS Journal*. Authors of reviews and coordinators of minireviews are also encouraged to record a podcast to accompany their article.

Unsolicited reviews are not considered but, if authors wish to submit a review proposal, they may do so. Pre-submission enquiries about timely contributions can be addressed to Professor Seamus Martin (Editor-in-Chief; **martin@febs.org**), and should include a 250-word summary, an overview of the article structure, and a list of key recent references.

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Please see key points for authors of reviews above, but note the following specific guidance:

- Main text: this should normally be 3,000–5,000 words.
- References: 50–75 references. If appropriate, please include the other minireviews in the series. The series coordinator will be able to provide authors' names and titles, and the publisher will insert pagination at proof stage.
- Figures and tables: up to 5 figures and tables can be included.

***State-of-the-Art Reviews***

A State-of-the-Art Review is an invited contribution wherein authors summarise the current state of play in their research area, and highlight where the major unresolved questions lie.

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A Discovery-in-Context Review is an invited contribution that aims to convey the context in which a major scientific advance was made.

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A Structural Snapshot is an invited contribution that presents a succinct discussion of a particular structure recently published by the authors' laboratory.

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A Viewpoint is an invited contribution that aims to convey new ideas or controversial perspectives on cutting-edge topics.

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A Commentary is an invited contribution that discusses and contextualises recently published research.

## Types of data

***Papers with three-dimensional models of proteins***

If your manuscript describes a three-dimensional model of a protein that has been manually built, you should consider depositing it in the **PMDB database** (see also ***NAR* (2006) 34, 306–309**). The database will return a unique identifier that you can include in your manuscript, thereby allowing readers to access your model. The accession number should be included in the manuscript on the first page, in the format: 'Database: model data are available in the PMDB database under the accession number XXXX'. The model can be stored either as a full model with 3D coordinates in PDB format, or as an alignment to a known structure in the CASP format. You may keep your model on hold (i.e. not public) for up to three months after deposition.

***Structural data***

Authors of manuscripts describing macromolecular structures must deposit the atomic coordinates and related experimental data (structure factor amplitudes/intensities and/or NMR restraints and chemical shifts) in the **RCSB Protein Data Bank**.

The PDB ID must be included in the manuscript. Authors must also submit the PDB Summary Validation Report that includes a PDB ID, provided after annotation by the wwPDB, for review at the time of submission. A validation report is not necessary if the coordinates discussed in the manuscript have already been released by the PDB.

Authors must agree to release the atomic coordinates and experimental data when the associated article is published.

Electron microscopy-derived density maps (all averaging methods, including sub-tomogram averaging) must be deposited in EM Data Bank (**EMDB**). For electron tomographic studies with no averaging, deposition of one or more representative tomograms in EMDB is strongly

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Please include PDB and/or EMDB accession codes in the manuscript on the title page in the following format:

Database: structural data are available in XXX database(s) under the accession number(s) XXX.

If requested by the Editor and/or referees, the authors must make the atomic coordinates and related experimental data (structure factor amplitudes/intensities and/or NMR restraints and chemical shifts) available for review purposes only.

***Sequence and proteomics data***

Sequences should be treated as follows:

- Protein sequences, which have been determined by direct sequencing of the protein, must be submitted to **UniProt**. Please note that accession numbers are not provided in advance for protein sequences that are the result of translation of nucleic acid sequences. These translations will automatically be forwarded from the EMBL nucleotide database and are assigned UniProt accession numbers.
- Results from characterisation experiments should also be submitted to UniProt, including such information as function, subcellular location, subunit composition, etc. See **http://www.uniprot.org/update**.
- Proteomics data with protein identifications by mass spectrometry should be submitted to the **PRIDE database** at the EMBL Outstation The European Bioinformatics Institute website.
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- Novel nucleotide sequence data (including predicted translations) must be submitted to one of the partner databases of the **International Nucleotide Sequence Database Collaboration**, the **European Nucleotide Archive**, **GenBank** or the **DNA Databank of Japan**. Submission to any one of the three collaborating databanks is sufficient to ensure data entry in all.

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***Transcriptomic and functional genomics data***

Functional genomics data, in particular gene expression (microarray or RNAseq), chromatin binding and modification (Chip-seq) and similar must be submitted to **ArrayExpress** at EBI or **Gene Expression Omnibus** at NCBI and should be compliant with MIAME or MINSEQE standards.

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***Enzyme activity data***

For papers reporting kinetic and thermodynamic data concerning biological catalysts (enzymes, other catalytic proteins and nucleic acids), authors must include the identity of the catalyst, its origin (e.g. species, tissue) and the nature of any post-translational modification. The method of preparation and criteria of purity, assay conditions, methodology, activity and any other information relevant to judging the reproducibility of the results must also be reported. Authors are advised to consult the **Beilstein Institut STRENDA Commission website** for more details and suggestions.

Enzyme activity (steady-state) should be reported in terms of $V_{max}$ (nmol or µmol product formed per amount ((protein)) per time) or, preferably, as $k_{cat}$ ($V_{max}$ divided by molar enzyme concentration), in $min^{-1}$ or $s^{-1}$. $K_m$ units are given in molarity. Values of $k_{cat}$ ($V_{max}$) and $K_m$ should be estimated using nonlinear fitting. The software system used in nonlinear fitting should be cited.

Parameters should include estimates of error (e.g. SE). The use of linear transformation for Michaelis−Menten parameters is discouraged. Only in selected cases should linear graphical data be shown (e.g. graphical presentation of inhibition to inform on mechanism).

A lack of activity should be defined in terms of a limit of detection. In a series of comparisons to a basal or 'control' level of activity (e.g. set as unity or '100%'), this activity should be indicated, in the units mentioned above, along with estimates of error.

The inclusion of examples of some of the raw data is encouraged, at least as part of a Supplemental Data section. Please refer to the **STRENDA website** regarding enzyme inhibition. $K_i$ values are preferred to $IC_{50}$.

***Hyperlinking to databases***

For the benefit of readers, the online version of a manuscript in *The FEBS Journal* provides direct hyperlink access to entries in the databases listed below.

Accession numbers referred to within the body text can also be hyperlinked in the online version. Please ensure that accession numbers (IDs) are complete and in an accurate format in bold in the manuscript in the following format: Database ID: XXXX

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4. UniProt Protein Database
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7. GEO
8. ArrayExpress
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Where appropriate, authors are requested to include accession numbers for these databases in their manuscripts.

## Data Sharing

In accordance with our **Author Guidelines**, authors must deposit all 'structured' data sets (e.g. gene sequences, protein structures, microarray data, etc.) in the appropriate public databases and include the accession number in their paper.

However, we recognize that authors might also wish to share the raw data underlying other figures and tables included in their paper. To facilitate data sharing, Wiley have partnered with the **figshare** repository. Authors wishing to deposit raw data files in figshare should upload them as 'Data Files' during the submission process. Data Files will be available to the editors and reviewers, but will not be formally peer reviewed. Upon acceptance of an article, the Data Files will be deposited free of charge to figshare on the author's behalf, with a CC-0 license (no rights reserved) applied. The data will be assigned a single DOI, which will be added to a Data Accessibility section in the article. The data on figshare will be linked back to the original article in The FEBS Journal.

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Authors must not submit data that is sensitive in nature or should not be made publicly available due to privacy, security, and/or safety concerns, such as human subject data or the location of endangered species.

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Please use standard fonts (Times, Times New Roman, Arial or Helvetica for general text; Courier or Courier New for sequence alignments; Symbol font for Greek characters and other non-standard characters such as the degree symbol) in your document. Asian-based font sets may produce uncertain results.

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Manuscripts must contain the following:

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**Introduction**

**Results**

**Discussion**

**Materials and Methods or Experimental Procedures**

**Acknowledgements**

**Author Contributions**

**References**

**Tables**

**Schema**

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**Figures**

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***Title page***

*Title.* This should be concise but informative.
See **http://olabout.wiley.com/WileyCDA/Section/id-828012.html** for suggestions on how to optimise your title and abstract for search engine discoverability. Avoid abbreviations.

*Authors' names*. These should appear below the title, with the first or middle name of each author given in full. Given names should appear first.

*Addresses.* The laboratories where the work was carried out should be given below the authors' names. If the work was carried out at more than one laboratory, the names of the authors should be followed by superscript numbers, which should also precede the names of the appropriate laboratories.

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*Abbreviations.* These should be defined unless included in the **table of accepted abbreviations**. They should be introduced only if essential owing to frequent repetition or excessive length of the full name. For further details, see the **Nomenclature, symbols, units and abbreviations** section below.

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*Databases*. Please provide accession codes for new deposits, if appropriate.

*Keywords.* Please provide up to 5 keywords for Regular papers and up to 10 for Reviews. These keywords will be printed alongside the summary [abstract??] and are indexed by PubMed.

*Conflicts of Interest.* Any real or perceived conflicts must be disclosed on the title page.

***Abstract***

The abstract should give a concise statement of the problem, the experimental approach, and the major findings and conclusions. The abstract should be carefully worded to reflect search terms on PubMed. It should not contain more than 250 words and should be intelligible without reference to other parts of the paper. References, if cited, should be given in full (without the title of the paper). Abbreviations should be avoided.

***Introduction***

The Introduction should provide the necessary context to support the importance of the discovery being reported. A brief summary of the major findings should be provided at the end of the Introduction.

***Results***

The Results section should describe the experiments performed and results obtained therein. The Results should be subdivided with topical subheadings.

***Discussion***

The Discussion can be subdivided with topical subheadings. It is permissible to combine Results and Discussion if a clearer, shorter paper is produced.

***Materials and Methods or Experimental Procedures***

It is essential to provide sufficient detail in this section to allow others to reproduce the work. There is no word limit and authors are strongly encouraged to report their experimental approaches in detail.

***Acknowledgements***

Authors must ensure that they include all funding grant numbers in the Acknowledgments section of the manuscript.

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A short description of the contribution of each author (initials only) should be provided in this section. Authors are required to meet the criteria for authorship as recommended by the International Committee of Medical Journal Editors (**ICMJE**). Specific categories of contribution include: planned experiments; performed experiments; analysed data; contributed reagents or other essential material; wrote the paper; other.

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- SI units and quantities should be used (see **http://www.bipm.fr/enus/3_SI/si.html**) but Å, cal, p.p.m. can be used where appropriate.
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Reference to articles cited as 'in press' should include the title and the name of the journal. Reference to unpublished work, including papers in preparation, should be kept to a minimum and should be mentioned in parentheses in the text as unpublished work, not in the reference list. The names of all contributors to the work should be given. Personal communications should be mentioned only in the text. Permission must be sought and obtained from the relevant person.

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5. Langer T & Neupert W (1994) Chaperoning mitochondrial biogenesis. In *The Biology of Heat Shock Proteins and Molecular Chaperones* (Morimoto RI, Tissières A & Georgopoulos C, eds), pp. 53-83. Cold Spring Harbor Laboratory Press, Plainview, NY.
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