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Repercussão da desnutrição, durante a gestação, sobre o estresse oxidativo placentário e transportadores de sódio no rim da prole de ratos adultos

LEUCIO DUARTE VIEIRA FILHO

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Dissertação apresentada para a conclusão do Curso de Mestrado em Bioquímica e Fisiologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco.
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"Repercussão da desnutrição, durante a gestação, sobre o estresse oxidativo placentário e transportadores de sódio no rim da prole de ratos adultos"

Dissertação apresentada para o cumprimento parcial das exigências para obtenção do título de Mestre em Bioquímica e Fisiologia pela Universidade Federal de Pernambuco

Aprovado por: _____

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"Posso todas as coisas naquele que me fortalece".

Filipenses 4.13

SUMÁRIO

RESUMO

A desnutrição intra-uterina tem sido correlacionada com o desenvolvimento de doenças cardiovasculares e renais, que estão vinculadas ao balanço de Na+ alterado. No presente estudo, investigamos se a má-nutrição materna eleva o estresse oxidative placentário com impacto subseqüente nos transportadores renais de Na^+ dependentes de ATP, na prole. A má-nutrição materna foi induzida durante a gestação através de uma dieta multicarenciada, também denominada dieta básica regional. O estresse oxidativo foi avaliado pela mensuração de substâncias reativas ao ácido tiobarbitúrico, os quais estavam 35-40% maiores nas mães malnutridas. As bombas de sódio foram avaliadas nos ratos controle e intra-uterinamente malnutridos (MalN) (25 e 90 dias de vida). A atividade da $(Na^+ + K^+)$ATPase foi idêntica nos grupos aos 25 dias (~150 nmol $P_i \times mg^{-1} \times min^{-1}$); aumentou 40% com o desenvolvimento nos ratos controle, mas permaneceu constante na prole de mães malnutridas. Em contraste, nos ratos em idade juvenil, a atividade da Na^+-ATPase foi maior nos animais MalN do que nos controles (70 *vs* 25 nmol $P_i \times mg^{-1} \times min^{-1}$). Contudo, ela não acompanhou o desenvolvimento renal e corpóreo: aos 90 dias ela era 50% menor no MalN do que no controle. A estimulação máxima da Na^+-ATPase pela angiotensina II foi 35% menor no MalN do que nos ratos controle e foi deflagrada apenas com doses bem maiores do peptídeo (10^{-10}M), quando comparadas aos animais controles (10^{-14}M). A atividade da proteína kinase C, que é um mediadora dos efeitos da angiotensina II na Na^+-ATPase, atingiu um terço do valor normal. Podemos concluir que o estresse oxidativo placentário induzido má-nutrição altera o controle fino da manipulação renal de Na^+ na prole e contribui para a programação de distúrbios tardios da homeostase de Na^+.

Palavras-Chave: má-nutrição, desenvolvimento fetal, $(Na^+ + K^+)$-ATPase, Na^+-ATPase insensível à ouabaína

ABSTRACT

Intrauterine malnutrition has been linked to the development of adult cardiovascular and renal diseases, which are related to altered Na^+ balance**.** Here we investigated whether maternal malnutrition increases placental oxidative stress with subsequent impact on renal ATP-dependent Na^+ transporters in the offspring. Maternal malnutrition was induced in rats during pregnancy by using a basic regional diet available in Northeastern Brazil. Placental oxidative stress was evaluated by measuring thiobarbituric acid-reactive substances, which were 35-40% higher in malnourished dams. Na^+ pumps were evaluated in control and prenatally malnourished rats (25 and 90 days old). (Na^++K^+)ATPase activity was identical in both groups at 25 days (~150 nmol $P_i \times mg^{-1} \times min^{-1}$); it increased 40% with growth in control rats but remained constant in pups from malnourished dams (MalN). In contrast, in juvenile rats, the ouabain-insensitive Na^+-ATPase was higher in MalN than in controls (70 *vs* 25 nmol $P_i \times mg^{-1} \times min^{-1}$). Nevertheless, it did not accompany kidney and body growth: at 90 days it was 50% lower in MalN than in controls. The maximal stimulation of the Na^+-ATPase by angiotensin II was 35% lower in MalN than in control rats and was attained only with a much higher concentration of the peptide (10^{-10} M) than in controls (10^{-14} M). Protein kinase C activity, which mediates the effects of angiotensin II on Na^+-ATPase, reached one third of the normal value. It is concluded that placental oxidative stress induced by undernutrition disrupts the fine control of renal Na^+ handling in offspring and contributes to programming late disturbances of Na^+ homeostasis.

Key-words: undernutrition, fetal development, (Na^++K^+)-ATPase, ouabain-insensitive Na^+-ATPase

INTRODUÇÃO

Desenvolvimento Fetal e Programação de Doenças na Vida Adulta

A nutrição adequada durante a vida intra-uterina e os dois anos iniciais de vida tem sido apontada como fator essencial para a formação de capital humano. Crianças submetidas à desnutrição são mais suscetíveis a se tornarem adultos menores, com menor escolaridade e reduzida produtividade econômica (Victora *et al.* 2008).

A má-nutrição intra-uterina está relacionada com retardo do crescimento intra-uterino e risco aumentado de desenvolvimento de doenças cardiovasculares (Barker *et al.* 1993), renais (Hoy *et al.* 1999) e metabólicas (Desai & Hales, 1997), na vida adulta. A hipótese de programação fetal propõe que adaptações fetais *in utero* estariam relacionadas a essas alterações permanentes nas características do crescimento, metabolismo e fisiologia pós-natal (Hoy *et al.* 1999), mas que apresentariam benefícios à curto prazo no embrião e fetos para que o neonato se apresente melhor preparado para o ambiente adverso (Lau & Rogers, 2004). Baixo peso no nascimento, em particular, tem sido descrito como indicativo de retardo do crescimento intra-uterino devido à má-nutrição materna (Paixão *et al.* 2001; Barker *et al.* 1993; Falkner *et al.* 2002, Holemans *et al.* 2003).

A programação intra-uterina de doenças na idade adulta pode ser induzida por alterações na oxigenação fetal, oferta inadequada de nutrientes e ainda por hormônios, tais como os glicocorticóides maternos, todas podendo ocorrer isoladamente ou em conjunto (Fowden *et al.* 2006). Representam condições que programam doenças na idade adulta: dieta materna com alto teor de sódio, lipídios ou glicose, tabagismo, etanol e pré-eclampsia. Um fator comum a essas alterações das funções fisiológicas, como também as várias condições adversas citadas, é a elevação do estresse oxidativo.

Estresse Oxidativo

Um fator premente nas doenças crônico-degenerativas e que também influencia o desenvolvimento fetal é o estresse oxidativo, que pode ser definido como uma descompensação entre espécies reativas de oxigênio e a proteção anti-oxidante (extra e intra-celular) (Shoji & Koletzko, 2007) (Figura 1). Espécies reativas de oxigênio é um termo coletivo

para designar radicais livres derivados do oxigênio (superóxido, hidroxil) e também, não radicais derivados do oxigênio (peróxido de hidrogênio). Essas substâncias podem ser produzidas por diferentes mecanismos, como isquemia-reperfusão, ativação de neutrófilos e macrofágos, ácidos graxos livres, metabolismo das prostaglandinas e etc, podendo reagir com quase todo tipo de molécula presente em células vivas, como lipídios, proteínas, polissacarídeos e DNA (Shoji & Koletzko, 2007). Os radicais livres derivados do oxigênio também reagem com glicose produzindo compostos carbonila altamente reativos que, por sua vez, podem reagir com a porção amino-terminal da lisina, levando à formação de proteínas glicosiladas (Horie *et al.* 1997; Singh *et al.* 2000).

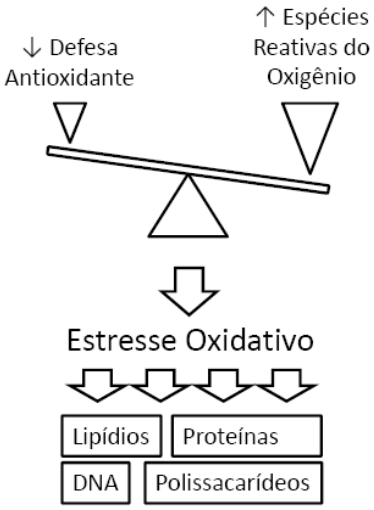

Figura 1. O estresse oxidativo é originado a partir do desbalanço entre a produção de espécies reativas do oxigênio e a defesa antioxidante do organismo. O estresse oxidativo pode reagir com lipídos, proteínas, polissacarídeos e até, mesmo o DNA, induzindo alterações funcionais e estruturais.

Desde que a peroxidação lipídica induzida por radicais livres do oxigênio é uma das mais importantes causas de lesão celular, enzimas antioxidantes podem prevenir o inicio de reações lesivas. Quando o mecanismo antioxidante é exaurido, as membranas celulares ficam irreversivelmente danificadas (Karowicz-Bilinska *et al.* 2002).

Glutationa peroxidase, catalase e a superóxido dismutase são as principais enzimas antioxidantes. O radical superóxido é convertido a peróxido de hidrogênio pela superóxido

dismutase, e este, é, então, removido pela glutationa peroxidase ou pela catalase (Biri *et al.* 2007).

Estresse oxidativo parece estar implicado em diversos processos patológicos. Na vida adulta o estresse oxidativo está implicado em diversas patologias programadas pela desnutrição intra-uterina, como disfunção vascular e hipertensão (Franco *et al.* 2007), estresse oxidativo renal elevado (Magalhães *et al.* 2006), diabetes (Horie *et al.* 1997).

Gestação e o Estresse Oxidativo

A gestação é um estado fisiológico associado com estresse oxidativo aumentado devido ao aumento da "taxa" metabólica e a demanda elevada por oxigenação tecidual (Shoji & Koletzko, 2007). Níveis de marcadores de peroxidação, como hidroperoxidos lipídicos e malonildialdeído, apresentam-se elevados em gestantes comparadas a mulheres não-gestantes (Morris *et al.* 1998). O estresse oxidativo elevado no cordão umbilical de crianças prematuras tem sido apontado como um importante determinante de mortalidade e morbidade (Weinberger *et al.* 2006; Granot e Kohen, 2004; Shoji & Koletzko, 2007). Durante a gestação, as possíveis fontes de produtos da oxidação seriam a mãe, a placenta ou o feto, no entanto há evidências de que o feto metaboliza mais do que produz radicais livres (Weinberger *et al.* 2006), apesar de apresentarem mecanismo de defesa anti-oxidante deficiente (Sullivan & Newton, 1998) . Apesar de a placenta ser uma importante fonte de peróxidos lipídicos (Mutlu-Turkoglu *et al.* 1998; Klingler *et al.* 2003), ela também é uma fonte de enzimas anti-oxidantes que seriam suficientes para o controle da peroxidação lipidica em gestações normais (Mueller *et al.* 2005; Gitto *et al.* 2002).

Estresse Oxidativo e a Programação Intra-uterina

Da mesma maneira que o estresse oxidativo parace estar implicado em patologias durante a vida adulta, ele tem sido apontado como importante fator na programação de doenças durante a vida intra-uterina. Níveis elevados de estresse oxidativo têm sido observado em situações adversas correlacionadas com retardo do crescimento intrauterino, como pré-eclampsia (Roberts e Lain, 2002), diabetes (Peuchant *et al.* 2004) e exposição ao etanol (Kay *et al.* 2006). Placentas obtidas de mulheres com pré-eclampsia apresentam níveis de espécies reativas ao oxigênio aumentados e níveis de enzimas anti-oxidantes diminuídos (Atamer *et al.* 2005; Serdar *et al.* 2005; Walsh *et al.* 2000; Vanderlelie *et al.* 2005). Gestantes diabéticas

apresentam status oxidante aumentado e sistema anti-oxidante diminuído (Kinalski *et al.* 2001). Finalmente, a ingesta crônica de etanol induz diminuição de redutases hepáticas e elevação do estresse oxidativo hepático (Hoek & Pastorino, 2004). A morbidade induzida pelo álcool, durante o desenvolvimento fetal, é parcialmente atribuida ao elevado estresse oxidativo. Kay *et al.* 2006, mostraram na placenta, *in vitro*, um aumento do estresse oxidativo induzido pelo etanol. Hipercolesterolemia, tabagismo, processos inflamatórios e infecciosos também promovem aumento do estresse oxidativo e estão relacionados com baixo peso ao nascimento (Luo *et al.* 2006).

A má-nutrição, uma causa freqüente de retardo do crescimento intra-uterino, envolve deficiências de proteínas e/ou micronutrientes, que podem prejudicar e promover elevação do estresse oxidativo, por diminuição da produção de enzimas de defesa antioxidante, como a albumina e a glutationa (Block *et al.* 2002; Willcox *et al.* 2004).

O mecanismo pelo qual o estresse oxidativo induz retardo do crescimento intrauterino parece estar relacionado à estimulação da síntese de tromboxano (Walsh *et al.*1993; Walsh, 2004) e ao mesmo tempo, à inibição d a síntese de prostaciclina (Walsh, 2004) (Figura 2). Essa mudança na relação entre prostaciclina e tromboxano, provoca vasoconstrição placentária e comprometimento da nutrição fetal. Além do mais, ânions peróxidos aumentados em células de vilosidades placentárias humanas expostas ao etanol diminuem a disponibilidade do óxido

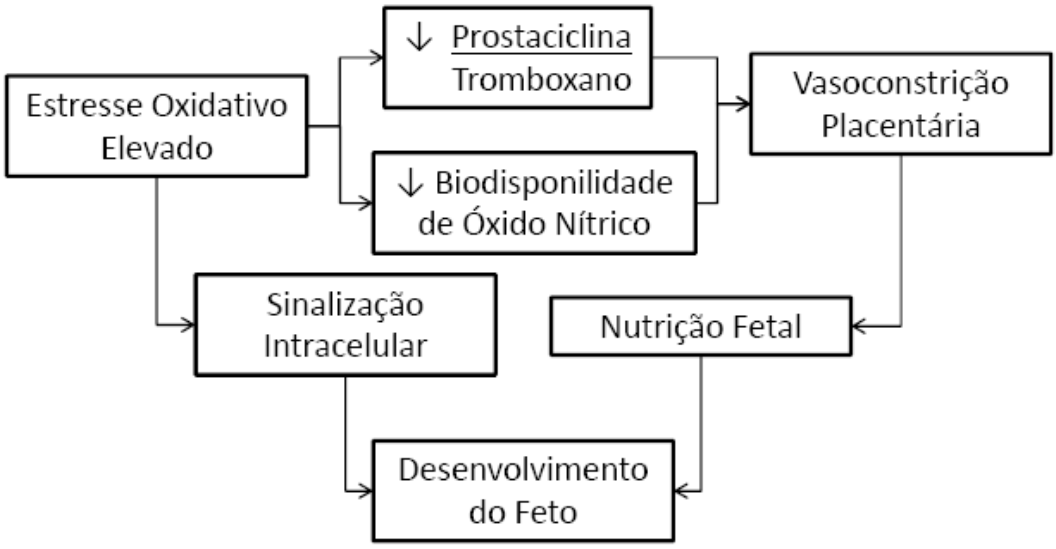

Figura 2. O estresse oxidativo elevado pode afetar o desenvolvimento do feto através de alteração da nutrição fetal. A diminuição da produção de prostaciclina, paralela ao aumenta da produção de tromboxano, e a diminuição da biodisponbilidade de óxido nítrico podem induzir vasoconstrição placentária, levando a comprometimento do aporte de nutrientes ao feto. Por outro lado, pode haver reação direta de substância pró-oxidantes com componentes sinalizadores intracelulares ou com o próprio DNA celular, pertubando, assim, o funcionamento adequado da maquinaria celular.

nítrico, o que corrobora com vasoconstrição placentária (Kay *et al.* 2000). Tendo em vista que o estresse oxidativo elevado nos trofoblastos pode afetar adversamente hormônios que são aí produzidos, como estrógeno e progesterona, a vasoconstrição seria agravada, pois estes hormônios são parcialmente responsáveis pela manutenção do fluxo placentário (Ahluwalia *et al.* 1992). Por outro lado, o estresse oxidativo ainda pode danificar a estrutura e função de proteínas celulares envolvidas em vias de regulação de transdução de sinais e expressão gênica (Barford, 2004). Esses achados indicam que o desequilíbrio entre a produção de substâncias pró-oxidantes e a defesa antioxidante pode induzir alterações no ambiente fetal, ou no próprio feto, que podem repercutir com programação de doenças na vida adulta, inclusive repercussões na função renal.

Programação Intra-uterina e Fisiopatologia Renal

No que concerne ao rim especificamente, ratos mal-nutridos intra-uterinamente apresentam oligonefrenia (Langley-Evans *et al.* 1999; Paixão *et al.* 2001), atividade de renina aumentada (Langley-Evans & Jackson, 1995), expressão aumentada de receptores AT1 da angiotensina II (Sahajpal & Ashton, 2003) e *upregulation* de transportadores de Na^+ apicais (co-transportes Na^+-K^+-$2Cl^-$ e Na^+-Cl^-) (Manning *et al.* 2002), na vida adulta. Tais alterações podem induzir balanço positivo de sódio (Guyton, 1989). Mesmo que o controle final da reabsorção de sódio renal não ocorra no túbulo proximal, evidências apontam para reabsorção proximal de sódio aumentada em indivíduos hipertensos (Burnier *et al.* 1994), e também em ratos espontaneamente hipertensos (Biollaz *et al.* 1986).

Reabsorção Tubular Proximal

No epitélio tubular renal, a (Na^++K^+)ATPase é a principal enzima, que utiliza energia derivada da hidrólise do ATP, para o transporte de Na^+ através do membrana basolateral (Féraille & Doucet, 2001), gerando assim o gradiente de energia necessário para a reabsorção de sódio através dos transportadores de sódio apicais.

Uma segunda bomba de sódio, a Na^+-ATPase, tem sido associada com o controle fino da reabsorção de sódio no segmento proximal (Rangel *et al.* 2005; Beltowski *et al.* 2007; Lara *et al.* 2008). Esta enzima, diferentemente da (Na^++$K^{+)}$ATPase, é insensível à ouabaína e sensível a furosemida, e tem sido descrita numa diversidade de tecidos animais (Moretti *et al.*

1991; Caruso-Neves *et al.* 1997). Nas células do túbulo proximal, esta enzima é localizada no membrana basolateral e está envolvida na extrusão de sódio paralela ao cloreto e água (Proverbio *et al.* 1989). Esta bomba de sódio é regulada por uma complexa cascata de sinalização ligada à membrana no qual a proteína kinase C desempenha papel crucial (Rangel *et al.* 2005; Lara *et al,.* 2008). Esta kinase também é considerada um importante sensor biológico de estresse oxidativo em diferentes tecidos (Sugden & Clerk, 2006; Nitti *et al.*2008) e faz parte de algumas das vias de sinalização intracelular do sistema renina-angiotensina.

Sistema Renina-Angiotensina

No sistema renina-angiotensina, a angiotensina II é produzida sob a forma de pró-hormônio, o angiotensinogênio. Este pró-hormônio é produzido principalmente no fígado, e em menores quantidades em outros tecidos, especialmente rim. A renina é uma aspartilpeptidase, que apresenta uma ação altamente específica de clivagem do angiotensinogênio em angiotensina I, em pH neutro (Misono *et al.* 1982). A angiotensina I, principalmente no pulmão, sofre ação da enzima conversora de angiotensina (ECA), dando origem a angiotensina II. A angiotensina II exerce efeitos fisiológicos diversos e complexos. Isso acontece devido à diversidade de receptores de angiotensina II, suas cascatas de sinalização e padrão complexo de expressão e distribuição tempo-espacial (Timmermans *et al*, 1993; Inagami *et al.* 1994). A angiontensina II exerce seu efeito através de um grupo de receptores, os receptores AT1 (AT1R), AT2 (AT2R) e outros ainda em caracterização (Inagami *et al.* 1993), tendo os receptores AT1 e AT2 efeitos fisiológicos mais importantes. Estudos de acoplamento de ligantes e autoradiografia mostraram que a maioria dos tecidos, inclusive o rim, apresentam uma mistura de ambos subtipos de receptores (Edwards & Aiyar, 1993).

Os inumeráveis efeitos da angiotensina II dependem do tempo (agudo vs. crônico) e do tecido no qual atua. A via clássica de sinalização do AT1R é mediada pela proteína Gq que leva a liberação de Ca^{+2} e ativação dos sistemas mediados pela proteína kinase C (Booz *et al.* 1994), enquanto do AT2R se dá via uma proteína Giα (Kang *et al.* 1994; Zhang e Pratt, 1996). Adicionalmente à ativação das vias de sinalização da proteína G, a angiotensina II, via AT1R, exerce sua função via *mitogen activated protein kinases* (ERK 1/2, JNK, p38MAPK (Molloy *et al.* 1993) e também levando à ativação da NADPH oxidase, estimulando a geração de espécies

reativas do oxigênio, amplamente implicadas na inflamação vascular e fibrose (Mehta & Griendling, 2007).

SRA e Pressão Arterial

O SRA tem papel central no balanço de Na^+ e água pelo organismo, através da modulação da excreção renal de sódio, e também exerce importante papel no controle da pressão arterial, através da ação vasoconstritora da angiotensina II. Dessa forma, esse sistema desempenha importante papel na regulação à longo prazo da pressão arterial, e no balanço de fluídos e eletrólitos (Jagadeesh, 1998), inclusive através de modulação direta na reabsorção proximal. Esta modulação ocorre através de diversos mecanismos, os quais incluem regulação das ATPases e co-transporte $Na^{+/}HCO3^-$ basolaterais proximais e trocador Na^+/H^+ e a H^+-ATPase apical (Liu and Cogan, 1988; Garvin, 1991; Mitchell *et al.* 1992; Eiam-Ong *et al.* 1993; Wang & Geibisch, 1996). Uma ação bifásica da angiotensina II no túbulo proximal têm sido descrita em estudos que utilizaram técnicas de micropunção e microperfusão tubular(Ploth & Navar, 1979; Wang & Chan, 1990). O efeito estimulatório da angiotensina II na reabsorção de Na^+ no túbulo proximal têm sido associada com atividade aumentada do antiporte Na^+/H^+ na membrana luminal e do contransporte $Na^+/HCO3^-$ e da $(Na^+ + K^+)$ATPase da membrana basolateral (Amlal *et al*. 1998). Por outro lado, o efeito natriurético da angiotensina II, está associado com aumento na pressão arterial renal, que diminui a reabsorção fracional de sódio nos túbulos proximais e distais e podem aumentar a entrega de Na^+ aos túbulos (Alkhunaizi *et al*. 1996). Adicionalmente, foi observado que concentrações altas de angiotensina II inibem a atividade do antiporte Na^+/H^+, da $(Na^+ + K^+)$ATPase e da Na^+-ATPase da membrana basolateral das células do túbulo proximal (Amlal *et al.* 1998; Ammar *et al.* 1991; Rangel *et al.* 2005).

Os dois receptores da angiotensina estão relacionados com o desenvolvimento e manutenção da hipertensão essencial e renovascular, como também na progressão de patologias renais. Inibição da ação da angiotensina II, através do uso de inibidores da enzima conversora de angiotensina (ECA) e antagonistas AT1R, tem mostrado diminuir proteinúria, microalbuminúria, glomeruloesclerose, e nefroesclerose em diversos modelos experimentais e ensaios clínicos (Sandberg *et al.* 2000). Além disso, estudos têm demonstrado que os receptores desse sistema apresentam alteração do padrão de expressão em animais que

apresentam desenvolvimento de hipertensão induzida por desnutrição intrauterina (Sahajpal & Ashton, 2003).

Justificativa

Com base nos dados acima expostos, nos propomos investigar se a elevação do estresse oxidativo no ambiente fetal está participando do mecanismo de programação intrauterina induzida pela má nutrição multifatorial durante a gestação, em ratos. Além disso, nos propomos avaliar se essa má nutrição pode induzir alteração da atividade dos transportadores de sódio dependentes de ATP da membrana tubular proximal da prole, na vida juvenil e adulta, bem como sua regulação pelo SRA, na vida adulta, visto que essas enzimas são importantes para regulação do transporte de água e sal, e estão envolvidas na gênese da hipertensão.

OBJETIVOS

Objetivo Geral

Avaliar se a desnutrição multifatorial influencia o estresse oxidativo placentário, bem como a atividade dos transportadores de sódio da membrana basolateral proximal da prole adulta, sob condições basais e sob estimulação da angiotensina II.

Objetivos Específicos

1) Avaliar em fêmeas submetidas à normonutrição ou má nutrição durante acasalamento e prenhez:
    a. O ganho de peso e consumo dietético;
    b. O estresse oxidativo hepático e placentário.
2) Avaliar em ratos macho, obtidos de fêmeas submetidas à normo ou má nutrição durante acasalamento e prenhez:
    a. O peso corpóreo e renal no 20ª dia fetal e aos 25 e 90 dias de vida;
    b. A atividade da $(Na^+ + K^+)$-ATPase e da Na^+-ATPase aos 25 e 90 dias de vida;
    c. A resposta da Na^+-ATPase a concentrações crescentes de angiotensina II aos 90 dias de vida;
    d. A atividade da proteína kinase C aos 90 dias de vida.

ARTIGO

Placental oxidative stress in malnourished rats and changes in kidney proximal tubule sodium ATPases in the offspring

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Short title: Sodium ATPases in prenatally malnourished rats

Summary

1) Intrauterine malnutrition has been linked to the development of adult cardiovascular and renal diseases, which are related to altered Na^+ balance**.** Here we investigated whether maternal malnutrition increases placental oxidative stress with subsequent impact on renal ATP-dependent Na^+ transporters in the offspring.
2) Maternal malnutrition was induced in rats during pregnancy by using a basic regional diet available in Northeastern Brazil. Placental oxidative stress was evaluated by measuring thiobarbituric acid-reactive substances, which were 35-40% higher in malnourished dams (MalN). Na^+ pumps were evaluated in control and prenatally malnourished rats (25 and 90 days old).
3) $(Na^+ + K^+)$ATPase activity was identical in both groups at 25 days (~150 nmol $P_i \times mg^{-1} \times min^{-1}$); it increased 40% with growth in control rats but remained constant in pups from MalN.
4) In contrast, in juvenile rats, the ouabain-insensitive Na^+-ATPase was higher in MalN than in controls (70 *vs* 25 nmol $P_i \times mg^{-1} \times min^{-1}$). Nevertheless, it did not accompany kidney and body growth: at 90 days it was 50% lower in MalN than in controls. The maximal stimulation of the Na^+-ATPase by angiotensin II was 35% lower in MalN than in control rats and was attained only with a much higher concentration of the peptide (10^{-10} M) than in controls (10^{-14} M).
5) Protein kinase C activity, which mediates the effects of angiotensin II on Na^+-ATPase, reached one third of the normal value.
6) These results indicate that the placental oxidative stress may contribute to fetal undernutrition, which leads to later disturbances in Na^+ pumps from proximal tubule cells.

Key-words: undernutrition, fetal development, $(Na^+ + K^+)$ATPase, ouabain-insensitive Na^+-ATPase

Introduction

Intrauterine malnutrition has been linked to growth retardation and increased risk of developing cardiovascular[1,2], renal[3] and metabolic diseases[4] in adult life. Increased maternal oxidative stress, specifically in the plasma and erythrocytes, has been seen in adverse conditions, such as diabetes[5]. It has been hypothesized that oxidative stress, through increase of thromboxane and decrease of nitric oxide levels, influences the placenta-fetus relationship[6,7] compromising fetal nutrition. Furthermore, oxidative stress may impair the structure and function of cellular proteins involved in regulating signal transduction pathways and gene expression[8]. Recently, Proverbio and coworkers showed that placental hypoxia led to increased formation of reactive oxygen species, which can affect active ion transporters[9]. Kidneys of offspring from malnourished rat mothers also show elevated production of reactive oxygen species[10] and, in addition, they can develop oligonephroenia[11-13], increased renin activity[14], increased angiotensin II AT_1 receptor expression[15] and up-regulation of Na^+ transporters[16]. All these alterations may induce a positive Na^+ balance and lead to hypertension and its consequences in adult life[1,2].

Even though the final control of renal Na^+ reabsorption does not occur in the proximal tubule, there is evidence of increased proximal tubule sodium reabsorption in hypertensive subjects[17] and also in spontaneously hypertensive rats[18]. In the basolateral membranes of tubule cells, (Na^++K^+)ATPase is the molecular machinery that couples energy derived from ATP hydrolysis to bulk active Na^+ fluxes across the epithelium[19]. A second Na^+ pump, the ouabain-insensitive Na^+-ATPase, has been associated with the fine tuning of proximal Na^+ reabsorption[20-22] and it is strongly stimulated by administration of a superoxide-generating mixture[21]. This second Na^+ pump is regulated by a complex membrane-bound signaling cascade in which the renin-angiotensin system (RAS) and protein kinase C (PKC) play a crucial role[20,22]. This kinase is also considered an important biological sensor of oxidative stress in different tissues[23,24].

The aim of this study was threefold: (i) to investigate whether maternal malnutrition increases placental oxidative stress, (ii) to investigate whether possible changes in the formation of reactive oxygen species at a placental level are accompanied by changes in (Na^++K^+)ATPase, Na^+-ATPase and PKC activities in the proximal tubule cells of offspring, (iii) to evaluate whether maternal undernutrion affects the response of the Na^+-ATPase to angiotensin II (Ang II).

Materials and methods

Ethical considerations

All experimental procedures involving the animals were approved by the Committee for Ethics in Animal Experimentation of the Federal Universities of Pernambuco and Rio de Janeiro, and they were carried out in accordance with the Committee's guidelines.

Experimental animals

Female Wistar rats weighing 200-250 g were randomly mated and the presence of spermatozoids in their vaginal plugs designated the first day of gestation. Dams were given standard pellet chow (control group, n = 10), or a deficient diet that mimics that one widely used in Northeast Brazil (prenatally malnourished group, MalN group, n = 11), throughout mating and pregnancy. In some dams in each group (Control, n = 6; MalN, n = 7) gestation was interrupted at the 20^{th} day to evaluate placental and hepatic oxidative stress. Fetal body weight and kidney weight were recorded and the dams were killed under anesthesia by lesion of the diaphragm. Four dams of each group (Control and MalN) carried to term and at the day of birth, each litter was culled to 8 pups and the males were weighed (13 in Control and 16 in MalN). Therefore the further studies represent programming of 4 dams. At 25 days of age, the male offspring were weaned on standard diet. To study Na^+ transporters, they were sacrificed by cervical rupture at 25 days (Control, n = 5; MalN, n = 4) or 90 days (Control, n = 8; MalN, n = 12).

Diet

Malnutrition was induced through a deficient diet as previously described[10,25]. The ingredients of the diet (g/g%) comprised beans (18.3), manioc flour (64.8), jerked meat (3.7) and sweet potato (12.8), which were cooked, dehydrated at 60°C and pulverized. All components were mixed with water. Meat fat (0.35%) was then added and the mixture was shaped into balls that were dehydrated at 60°C for 24 h. The content of main dietary nutrients is shown in Table 1.

Evaluation of placental oxidative stress

On day 20 of gestation, dams were anesthetized with sodium pentobarbitone (Cristália Produtos Químicos Farmacêuticos), 60 mg/kg ip, to remove the placentas and liver. Placental and hepatic oxidative stress was evaluated using levels of thiobarbituric acid reactive substances (TBARS) according to the method of Buege and

Aust[26]. The tissue was macerated in 5 ml of 1.15% KCl per gram in an ice bath. Subsequently, 1 ml of 0.375% (w/v) thiobarbituric acid (Sigma-Aldrich) in 75% (w/v) trichloroacetic acid (Vetec Química Fina Ltda.) was added to each milliliter of tissue homogenate. The tubes were sealed and heated in a water bath at 100ºC for 15 min. After cooling, the protein precipitate was centrifuged for 10 min, the supernatant separated and the absorbance measured at 535 nm.

Preparation of isolated membranes

Isolated membranes were used to measure the two Na^+-stimulated ATPase activities and the PKC activity. The membranes were prepared as previously described[27] from the outer cortex (*cortex corticis*) of kidneys of animals aged 25 and 90 days. In this region of renal tissue, more than 90% of the cell population corresponds to proximal tubules[28,29]. The kidneys were collected after sacrifice and maintained in a cold isotonic buffer containing 250 mM sucrose, 10 mM Hepes-Tris (pH 7.4), 2 mM EDTA and 0.15 mg/ml trypsin inhibitor (Sigma-Aldrich; type II-S). Thin transverse slices of the *cortex corticis* (0.5 mm thick) were removed using a Stadie-Riggs microtome and carefully dissected using iridectomy scissors to avoid contamination with the rest of the tissue. The suspension of fragments was homogenized in the same cold solution (4 ml/g) using a Teflon/glass homogenizer. The homogenate was centrifuged at 10,000×*g* for 15 min at 4ºC in a Sorvall RC-5B centrifuge using a SS-34 rotor, and the resulting supernatant was centrifuged at 15,000×g for 20 min. Finally, another centrifugation was performed in a Beckman L5-50B ultracentrifuge at 35000×*g* for 45 min using a 70 Ti rotor; the pellet was resuspended in 250 mM sucrose to a final concentration of 15-30 mg protein/ml, aliquoted into tubes and stored at -20ºC. Protein concentration was determined by the Folin phenol method[30] using bovine serum albumin as a standard; 2.5% (w/v) SDS was added to solubilize the integral membrane proteins.

Measurement of ATPase activities

Activities of the $(Na^+ + K^+)$ATPase and ouabain-insensitive Na^+-ATPase were measured colorimetrically using unlabelled ATP[31] or [γ-^{32}P]ATP[32], respectively. In $(Na^+ + K^+)$ATPase assays the membranes (0.1 mg/ml final concentration) were preincubated at 37^{o}C for 20 min with or without 2 mM ouabain (Sigma-Aldrich). Except when the effects of varying Na^+ and K^+ concentrations were examined (Fig. 2B), the assay mixtures were then supplemented with 50 mM Bis-Tris-propane (pH 7.4), 0.2 mM EDTA, 5 mM $MgCl_2$ and 120 mM NaCl. The hydrolysis reaction was started by adding ATP (5 mM) and KCl (24 mM) and stopped after 10 min by adding two vol of 0.1 M HCl-activated charcoal. The $(Na^+ + K^+)$ATPase activity was calculated as the

difference between the P_i released in the absence and presence of ouabain. The released P_i was spectrophotometrically measured in an aliquot of 0.2 ml of the supernatant obtained after centrifugation of the charcoal suspension at 1500×*g* for 5 min.

The ouabain-insensitive Na^+-ATPase activity was calculated from the difference between the [^{32}P]P_i released in the absence and the presence of 2 mM furosemide (Sigma-Aldrich). The hydrolysis reaction was started by adding [γ-^{32}P]ATP (5 mM, specific activity ~1.7×10^6 cpm/nmol) to the membranes (0.2 mg/ml) preincubated with 2 mM ouabain, as described above, in the presence of 20 mM Hepes-Tris (pH 7.0), 10 mM $MgCl_2$ and 120 mM NaCl. After 10 min the reaction was stopped by adding two vol 0.1 M HCl-activated charcoal. The released [^{32}P]P_i was measured by liquid scintillation counting (Packard) in an aliquot of 0.2 ml of the supernatant obtained after centrifugation of the charcoal suspension (1500×g for 5 min).

PKC activity

The PKC activity was analyzed by measuring the incorporation of the γ-phosphoryl group of [γ^{32}-P]ATP (specific activity ~6.6×10^7 cpm/nmol) into histone in the absence and presence of 10 nM calphostin C (Sigma-Aldrich), an inhibitor of diacylglycerol-activated PKCs. The reaction was started by adding [γ-^{32}P]ATP (10 μM) to a reaction medium (0.1 ml) containing 20 mM Hepes-Tris (pH 7.0), 4 mM $MgCl_2$, 1.5 mg/ml histone and 0.7 mg/ml membrane protein. After 10 min, the reaction was stopped with 0.1 ml 40% (w/v) TCA and the sample was immediately placed on ice. An aliquot (0.1 ml) was removed immediately after vigorous stirring, filtered through a Millipore filter (0.45 μm pore size) and washed with ice-cold 20% (w/v) TCA and 0.1 M phosphate buffer (pH 7.0). The radioactivity was quantified in a liquid scintillation counter (Packard).

Statistical analysis

The data are presented as means ± S.E.M. Differences between groups were analyzed using an unpaired Student's *t*-test, while one-way ANOVA, followed by Tukey post-test, was used to verify differences among experimental groups. The differences were considered significant at $p < 0.05$.

Results

General data on dams and offspring

Maternal data are shown in Table 2. The total weight gain and dietary intake during 20 days of pregnancy were lower ($p < 0.01$) in MalN than in control dams, although the energy intake was similar in the two groups because the deficient diet is hypercaloric. MalN dams also showed lower placental weight and poorer reproductive outcome than control dams. On the other hand, TBARS levels in the placenta were significantly higher in the MalN group than the controls (Fig. 1); this difference was also found in liver, the control organ for induced oxidative stress (inset to Fig. 1). The fetuses from MalN had lower body weights than those from controls. Fetal kidney weight was also lower in the MalN than the control group but the kidney weight/body weight ratio was similar in the two groups. The lower body weight of the MalN group fetuses persisted at birth and also at weaning, but recovered to a normal value at 90 days after birth, presumably because of the normal diet. MalN animals presented birth weight lower than that shown by control group, either those designated for the protocol at age of 25 days (5.98 ± 0.11 vs. 6.82 ± 0.06, respectively, $p < 0.05$) or those designated for the protocol at age of 90 days (5.21 ± 0.23 vs. 6.06 ± 0.15 g, respectively, $p < 0.05$). Irrespective of age, the kidney weight/body weight ratio remained similar in the two groups (Table 3).

Proximal tubule (Na^++K^+)ATPase and Na^+-ATPase activities

(Na^++K^+)ATPase activity was the same in the control and MalN groups at 25 days after birth. It increased by 35-40% in the control group but not in the MalN group at 90 days after birth (Fig. 2A). Thus, at 90 days (Na^++K^+)ATPase activity was lower in the MalN than in the control group, even over a broad range of Na^+ and K^+ concentrations (sum constant and equal to 150 mM; Fig. 2B). For the ouabain-insensitive Na^+-ATPase, the abnormal growth trajectory gave the opposite picture: the activity of this enzyme increased rapidly to a value well above of the control at 25 days and then stopped (Fig. 3A), so it did not follow body growth, which showed normal mass at 90 days (Table 3). In contrast, the activity of this second Na^+ pump increased more than four times between 25 and 90 days in the control group, in parallel with body weight (Table 3). Thus, at 90 days, the Na^+-ATPase activity in MalN was significantly lower than that in controls, despite the initial burst at 25 days.

Effects of angiotensin II on proximal tubule Na^+-ATPase activity

At the age of 90 days, the Na^+-ATPase activity in the control group responded biphasically to a range of angiotensin II (Ang II) concentrations (Fig. 3B): (i) between 10^{-14} and 10^{-12} M Ang II it increased by 50%; (ii) at higher concentrations it returned to baseline values. In contrast, the Ang II dependence of Na^+-ATPase activity in MalN showed a flattened bell-like curve with maximum stimulation at 10^{-10} M, though the activity under these conditions was only about the same as the unstimulated activity in the control group.

PKC activity in membranes from proximal tubule cells

Since membrane-associated PKC is a key mediator of the Ang II effects on the ouabain-insensitive Na^+-ATPase in kidney cells[22], its activity was measured at 90 days after birth, when the pump was inhibited in the MalN group (Fig. 3). Figure 4 shows that PKC activity, like Na^+-ATPase activity, was lower in MalN than in the control group.

Discussion

In the present work we studied the influence of maternal undernutrition on placental oxidative stress and its consequences for renal active Na^+ transport. Na^+ fluxes across the tubular epithelium account for much of the metabolic expenditure in kidney tissue and are responsible for water and salt conservation in mammals in both early and adult life[19]. Here, maternal malnutrition was induced by using a diet that mimics a basic regional diet widely consumed in an area of sugarcane cultivation along the coast of Pernambuco State, Brazil[10,25]. This diet has been linked with both lower weight gain and maternal dietary intake, probably because of its low protein content and hypercaloric features, respectively. MalN mothers had fewer fetuses than controls, and the nodular and cystic formations found during placenta withdrawal (not shown) indicate fetal reabsorption, which could be a consequence of the very low calcium content of the diet (Table 1). Deficiency of calcium as well as other micronutrients has been implicated in different pregnancy complications, including fetal wastage[33]. It is important to mention that, depending on the region and on the season, the mineral composition of the deficient Northeastern Brazilian diet can vary, as in other regions worldwide[34].

The MalN body weight at the 20th day of fetal life and at birth indicates intrauterine growth retardation, which persisted at weaning but was completely recovered during the growth trajectory between 25 and 90 days. The metabolic acceleration that

supports the more rapid growth observed in this study may be pertinent to the fact that prenatal undernutrition often leads to overweight offspring[35]. The lowered fetal weight in the MalN group coexists with lowered placental weight, as has been shown in other intrauterine growth retardation studies[36]. More importantly, the MalN dams showed elevated placental oxidative stress, probably because of undernutrition-associated hypoxia[9,37], thus explaining the reduced body weight at birth and in the early stages of growth (Table 2).

It may be considered, therefore, the possibility that placental production of reactive oxygen species in MalN rats (Fig. 1) are correlated with the huge increase in ouabain-insensitive Na^+-ATPase in young (25 day old) rats (Fig. 3A) as well as in the abnormal response to Ang II (Fig. 3B) and in the down-regulation of PKC (Fig. 4). In a recent paper, Beltowski and coworkers[21] demonstrated that elevation of renal ouabain-insensitive Na^+-ATPase is related to an increase in reactive oxygen species. These peroxide intermediates lead to high Na^+-ATPase activities by scavenging NO and limiting its inhibition of the pump. The view above is supported by the recent observation from our laboratory that production of TBARS is increased in prenatally malnourished juvenile rats[10]. Other factors such as circulating cortisol[38] and altered RAS[14,15,39] can also contribute to the production of reactive oxygen species. Altogether, these stressful conditions that affect the maternal intrauterine environment and fetal organs, including the kidney and its Na^+ transporters, may lead to renal and cardiovascular alterations in the adult offspring[1-3].

Comparison of Figs. 2A and 3A indicates, however, that the two active Na^+ transporters in the proximal tubules of the offspring are affected by maternal undernutrition in opposite ways during the growth trajectory from 25 to 90 days, *i.e.* during the period of kidney maturation[40]. The $(Na^+ + K^+)$ATPase activity is identical in both groups at 25 days, but does not increase concomitantly with body weight in MalN. Since this pump is considered to be responsible for most Na^+ (and water) reabsorption[19] in both infants and adults, its decreased activity in adulthood might indicate a global impairment of kidney development. At the end of this period, the ouabain-insensitive Na^+-ATPase activity, which is strongly stimulated in early life (probably as a consequence of the placental oxidative stress[21], shows no further change, also reflecting compromised renal growth and fewer tubules[11-13] despite the recovery of normal kidney and body weight (Table 3).

Hypertension in weaning rats from undernourished mothers has been associated with RAS[14]. Although the possible link between enhanced Na^+-ATPase activity in MalN offspring and Ang II levels has not yet been studied, other models of prenatal malnutrition have shown increased plasma angiotensin converting enzyme

(ACE)[14] and increased plasma renin activity[41] in juvenile animals. However at an early age of four weeks, prenatal malnourished rats have not developed hypertension[42], despite these enzyme modifications. Thus, the early activation of Na^+-ATPase at 25 days, provides the hypothesis that the fine-tunned Na^+ reabsorption is inappropriately elevated in these rats before development of hypertension. Current experiments in a model of perinatal undernutrition show an early increase in Na^+-ATPase which is not accompanied by altered blood pressure (unpublished data from our laboratory). These observations and those from Vehaskari and coworkers[42] add support to the view that an early disturbed Na^+ handling precedes late arterial pressure alterations.

It has been proposed that the post-weaning period could be a critical window for changing blood pressures during adulthood by modulating RAS activity when animals are submitted to prenatal undernutrition[41]. A question emerges from this reasoning. Maternal malnutrition has been related to increased Na^+ and fluid reabsorption during the early stages of hypertension[41], so how can this fact be reconciled with the reduced (Na^++K^+)ATPase and Na^+-ATPase activities in the proximal tubules of adult MalN rats? (Figs. 2 and 3). If RAS, and therefore aldosterone production, is activated in young rats from undernourished mothers[43] and this stimulation persists as a programmed effect, a compensatory augment in Na^+ may occur in aldosterone-responsive distal nephron segments such as the convoluted distal tubule[44]. This view is supported by the observation of Bertram and coworkers[45] that increased mRNA for the (Na^++K^+)ATPase α-subunit, which is responsive to adrenal hormones, is found in the kidneys of prenatally undernourished rats. Furthermore, it has been shown that GFR is increased at age of 90 days, however Na^+ excretion has not been increased[13], suggesting that increased delivery of Na^+ to distal nephron occurs followed by increased Na^+ reabsorption in this nephron segment[16].

The smaller number of nephrons, the reduced capacity to reabsorb Na^+ and the increased intrarenal vascular resistance in the pups may elicit abnormal RAS responses at the macula densa level; ultimately, these could lead to the onset of hypertension[11-13,46]. At an adult age when they show increased blood pressure[46], the reduced capacity to reabsorb Na^+ in the proximal tubule due to pressoric natriuresis[47] may also elicit abnormal responses at the macula densa level. Ultimately, these responses could lead to an increased intrarenal vascular resistance[13] and to a decrease in plasma renin activity[41].

An early alteration in RAS could also explain the abnormal response of the ouabain-insensitive Na^+-ATPase to Ang II in adult MalN (Fig. 3), since increased RAS activity has been observed in prenatally malnourished adult rat kidneys[48]. Whereas the control group exhibited the well-documented biphasic behavior with 40-50% stimulation

at physiological Ang II concentrations followed by a progressive decrease, the weaker stimulation in MalN animals was additionally shifted to right (Fig. 3B). Clearly, this response indicates that MalN rats are hyporesponsive to Ang II in vitro. It can be hypothesized that if higher Ang II concentrations are required for adequate modulation of fluid reabsorption in the proximal tubules and body fluid balance in the whole animal, this might lead to the development of a long-term hypertensive response.

The cellular signaling event related to the hyporesponsiveness of Na^+-ATPase to Ang II appears to be down-regulation of PKC (Fig. 4), which participates in the signaling cascade that links Ang II, AT_1R and the Na^+-ATPase[20]. It can be proposed that the accentuated decrease in PKC activity results from reactive oxygen species[10], since this enzyme is sensitive to alterations in cellular redox state[24]. Moderate oxidant concentrations can activate PKC, whereas intense and persistent production of reactive oxygen species, as found in the kidneys from pups[10] after maternal undernutrition, probably promotes inactivation of the kinase rather than activation, as shown in liver[24]. Furthermore, decrease in PKC activity may result from RAS programmation.

In conclusion, the present findings suggest a correlation between maternal malnutrition, increased placental oxidative stress, abnormal activity of renal Na^+ transporters and disrupted Ang II signaling, which could contribute to programming[49] late disturbances in renal Na^+ handling and arterial pressure control as specific consequences of impaired intrauterine growth.

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Table 1. Diet composition (g/g%)

	Control [1]	Deficient [2]
Protein	23	8
Carbohydrates	41	78
Ether extract	2.5	1.7
Vitamin suplement	Yes	No
Sodium	0.28	0.16
Potassium	0.9	0.3
Calcium	1.8	0.04
Iron	0.018	0.007
Moisture	13	11
kcal/100 g	278	356

[1] As indicated by the manufacturer (Purina Agriband, Paulínia, SP, Brazil). [2] According to the Laboratory of Experimentation and Analysis of Food (LEEAL), Nutrition Department, Federal University of Pernambuco.

Table 2. Maternal data.

	Control (n = 6)	MalN (n = 7)
Body weight at 20th gestation day (g)	347 ± 9	242 ± 9 *
Total weight gain (g)	112 ± 5.5	4.9 ± 10.8 *
Total dietary intake during gestation (g)	394 ± 15	302 ± 6.5 *
Total energy intake (kcal)	1096 ± 41	1136 ± 25
Number of fetuses	12.8 ± 0.8	8.14 ± 1.1 *
Placenta weight (g)	0.40 ± 0.01	0.34 ± 0.02 *

Maternal data were obtained at 20th day of gestation in dams fed with standard (control) or deficient (MalN) diet during pregnancy. Results are mean ± SEM. *: $p < 0.001$ *vs*. control.

Table 3. Fetuses, newborn and offspring: general data.

	Control	MalN
Fetuses and newborn pups		
Fetal BW (g) at 20 days	2.27 ± 0.04 (7)	2.02 ± 0.05 *(8)
Fetal KW (mg)	15.3 ± 0.9 (7)	12.2 ± 0.6 *(8)
KW/BW at 20th day of fetal life (%)	0.66 ± 0.05 (7)	0.60 ± 0.03 (8)
25-90 day pups		
BW at birth (g)	6.4 ± 0.14 (13)	5.4 ± 0.19 *(16)
BW at weaning (g)	66.2 ± 1.6 (5)	56.9 ± 1.2 *(4)
KW at weaning (g)	0.70 ± 0.02 (5)	0.64 ± 0.01 (4)
KW/BW at weaning (%)	1.06 ± 0.02 (5)	1.12 ± 0.03 (4)
BW at age of 90 days (g)	332 ± 9 (8)	323 ± 7 (12)
KW at age of 90 days (g)	2.5 ± 0.1 (8)	2.5 ± 0.01 (12)
KW/BW at age of 90 days (%)	0.75 ± 0.02 (8)	0.77 ± 0.01 (12)

Male fetuses and offspring of dams were fed with standard (Control) or deficient (MalN) diet during pregnancy. BW, body weight; KW, kidney weight. Results are mean ± SEM. *: $p < 0.05$ *vs.* control. Kidney weights representative of pups weaned at 25 days were obtained from 4-5 randomly selected pups that were sacrificed the same day for ATPase studies (n = 5 pups from Control dams; n = 4 pups from MalN dams). The number of animals is indicated in parentheses. The results therefore represent programming from 4 MalN dams and 4 control dams studied in parallel.

Figure Legends

Figure 1. Levels of thiobarbituric acid reactive substances (TBARS) in the placenta of control (empty bar; n = 6) and malnourished (filled bar; n = 7) dams at the 20th day of gestation. MDA = malondialdehyde. Results are mean ± SEM. Statistical difference (* $P < 0.05$) with respect to Control group. Inset: TBARS in liver. Bars, number of experiments and statistical difference are the same as for the placenta data.

Figure 2. (Na^++K^+)ATPase activity in kidney proximal tubules of control (empty bars) and prenatally malnourished (filled bars) rats at 25 (Control, n = 5; MalN, n = 4) and 90 (Control, n = 8; MalN, n = 12) days measured with 120 mM Na^+ and 24 mM K^+. *: $P < 0.05$ *vs*. Control; #: $P < 0.05$ *vs*. 25 days (A). (Na^++K^+)ATPase activity in proximal tubules of control (empty circles, n = 8) and prenatally malnourished (filled circles, n = 12) rats measured at 90 days, using the concentrations of Na^+ and K^+ shown on the abscissa. P_i = inorganic phosphate released from ATP in the medium. Results are mean ± SEM from at least four assays. *: $P < 0.05$ *vs*. Control (B).

Figure 3. Ouabain-insensitive Na^+-ATPase activity in kidney proximal tubules of control (empty bar) and prenatally malnourished (filled bars) rats at 25 (Control, n = 5; MalN, n = 4) and 90 (Control, n = 8; MalN, n = 12) days. *: $P < 0.01$ *vs.* the corresponding age-matched Control; †: $P < 0.01$ *vs.* 25 days in Control (A). Na^+-ATPase activity in proximal tubules of control (empty circles, n = 8) and malnourished (filled circles, n = 12) rats at age of 90 days, measured in the absence and presence of the Ang II concentrations shown on the abscissa. P_i = inorganic phosphate released from ATP in the medium. Results are mean ± SEM from at least four assays (B). *: $P < 0.01$ *vs.* Control at the same Ang II concentration; ‡: $P < 0.05$ vs. the corresponding diet-matched value without Ang II.

Figure 4. Protein kinase C activity in proximal tubules of control (empty bar) and prenatally malnourished (filled bar) rats at age of 90 days (Control, n = 8; MalN, n = 12). ~P = esterified phosphate. Results are mean ± SEM. *: $P < 0.01$ vs. Control.

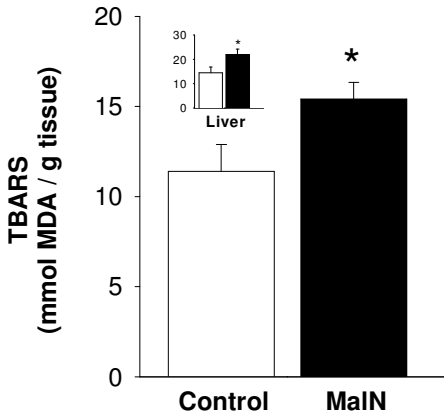

Fig. 1. Vieira-Filho *et al.*

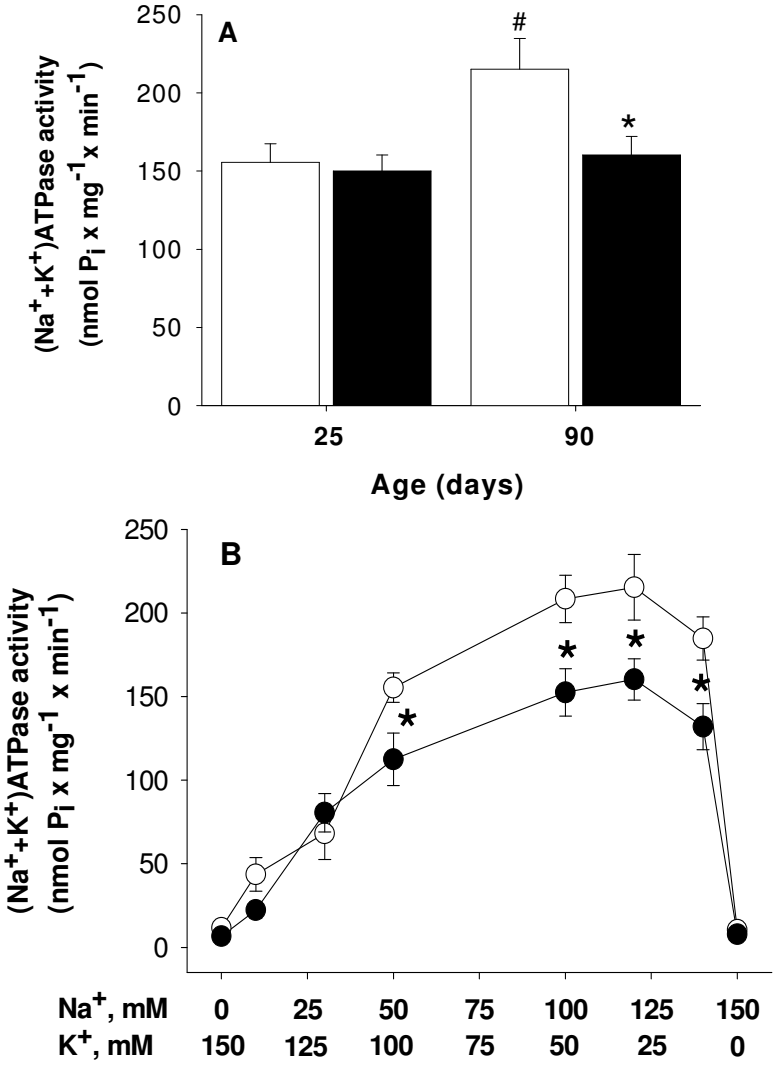

Fig. 2. Vieira-Filho *et al.*

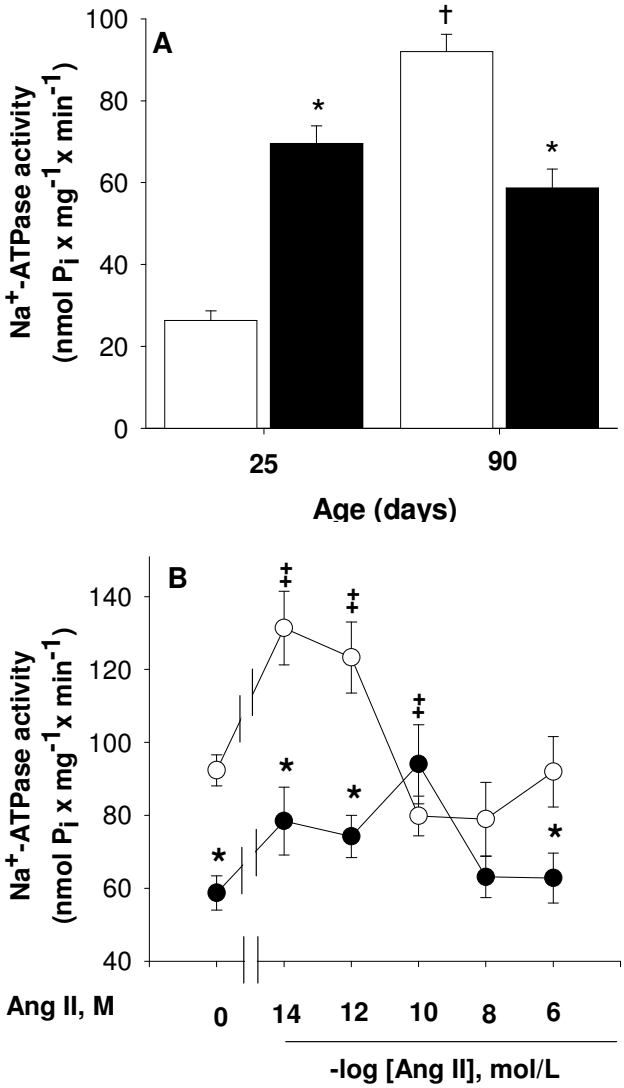

Fig. 3. Vieira-Filho *et al.*

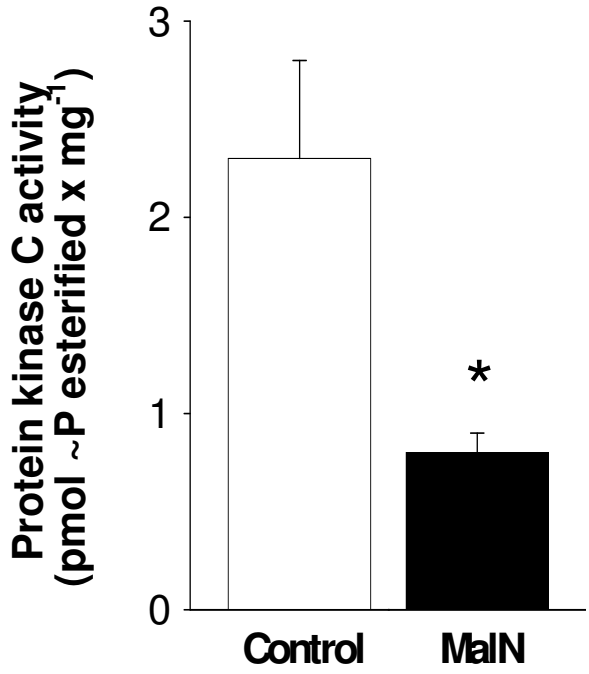

Fig. 4. Vieira-Filho *et al.*

CONCLUSÃO

Os achados do presente trabalho sugerem uma correlação entre má-nutrição maternal, estresse oxidativo placentário aumentado, atividade anormal dos transportadores renais de sódio e sinalização da angiotensina II alterada, que podem contribuir para a programação de distúrbios tardios na homeostase do Na^+ e controle da pressão arterial, como conseqüências específicas do crescimento intra-uterino prejudicado.

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