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JOSÉ LUIZ DE BRITO ALVES

Efeitos da desnutrição proteica perinatal sobre os mecanismos de controle da função cardiovascular e respiratória na prole de ratos acordados

RECIFE

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Dissertação apresentada ao Programa de Pós-Graduação em Nutrição do Centro de Ciências da Saúde da Universidade Federal de Pernambuco, para obtenção do título de Mestre em Nutrição.

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RECIFE

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“Os que encantam com a prática sem a ciência são como os timoneiros que entram no navio sem timão nem bússola, nunca tendo a certeza do seu destino”.

(Leonardo da Vinci)

RESUMO

No presente trabalho investigamos se o aumento na frequência respiratória e uma maior sensibilidade de quimiorreceptores centrais e periféricos são os mecanismos subjacentes ao desenvolvimento da hipertensão arterial na prole de ratos submetidos à desnutrição proteica durante a gestação e lactação (período perinatal). Ratas prenhes foram alimentadas com dieta normoprotéica (17% de proteína, grupo controle) ou com dieta hipoprotéica (8% de proteína, grupo experimental) durante a gestação e lactação. Todos os protocolos experimentais foram aprovados pelo comitê de ética em experimentação animal da UFPE (processo n° 23076.044454/2010-94). Após o desmame, a prole de ratos machos foram alimentados com dieta padrão de laboratório e os estudos funcionais realizados nos 30, 90 e 150 dias de vida. Foi verificado e peso e comprimento dos animais durante todo o experimento. Além disso, analisamos bioquimicamente a albumina, proteínas totais, ureia e creatinina séricas desses animais. O registro respiratório foi realizado por pletismografia de corpo inteiro e a pressão arterial foi aferida de forma direta pelo implante de cânula na artéria femoral. Os quimiorreceptores centrais foram ativados por hipercapnia (7% CO_2) e os periférico com cianeto de potássio (KCN – 0.05%). Os resultados mostraram que ratos submetidos à desnutrição proteica materna perinatal têm menor peso e comprimento ao nascer, que permanece até aos 90 dias. Aos 30 dias, os animais desnutridos expressaram redução dos níveis séricos de albuminas e proteínas totais, as quais foram normalizadas aos 90. Em contrapartida esses animais apresentaram creatinina e ureia aumentadas aos 30 e 90 dias. Também aos 30 dias de vida, a prole submetida à desnutrição proteica perinatal apresentou aumento na frequência respiratória e ventilação pulmonar de repouso, além de maior sensibilidade de quimiorreceptores centrais, no entanto sem modificações nos níveis pressóricos e de frequência cardíaca. Aos 90 dias, esses animais exibiram aumento de pressão arterial, como também uma maior resposta cardiovascular e respiratória à ativação do quimiorreflexo periférico. Resultados semelhantes foram obsevados também aos 150 dias de vida. Os resultados indicam que o aumento na frequência respiratória e a maior sensibilidade de quimiorreceptores centrais e periféricos podem estar envolvidos no desenvolvimento da hipertensão arterial na prole que passaram por desnutrição proteica na gestação e lactação.

Palavras-chave: Desnutrição. Plasticidade fenotípica. Hipertensão arterial. Respiração. Quimiorreflexo.

ABSTRACT

At this work we investigated if the respiratory frequency increased and greater sensitivity of peripheral and central chemoreceptors are mechanisms underlying to development of hypertension in offspring of rats submitted to perinatal protein undernutrition. Pregnant rats were fed with normoproteic (17% of protein) or hypoproteic (8% of protein) diet during pregnancy and lactation. All experimental protocols were approved by the ethics committee for animal experimentation of UFPE (process n° 23076.044454/2010-94). After weaning, the offspring males rats were fed with normoproteic diet and the functional studies performed at 30, 90 and 150 days of life. It was checked the weight and length of rats during all experiment. Furthermore, we analyzed biochemically the albumin, total protein, urea and creatinine serum from animals. The respiratory register was checked by whole body plethysmography and arterial pressure was measured directly by implant of the cannula in femoral artery. The centrals chemoreceptors were activated by hypercapnia (7%CO_2) and the periphery by potassium cyanide (KCN – 0.04%). The results showed that rats submitted to maternal perinatal protein undernutrition had lower birth weight and length, which remained at 90 days. At 30 days, the malnourished animals had reduction of serum levels of albumin, total protein, which was normalized at 90 days. On the other hand these animals showed increased urea and creatinine at 30 and 90 days. At 30 days, the offspring submitted perinatal protein undernutrition showed increase in respiratory frequency and ventilation baseline, beyond higher sensibility of centrals chemoreceptors, but no change in arterial pressure and heart rate. At 90 days, these animals showed increase in arterial pressure and increased response cardiovascular and respiratory to activation of peripheral chemoreflex. Similar results were seen at 150 days of life. These results indicate that the increase in respiratory frequency and higher sensibility of centrals and peripheral chemoreceptors can be involved in development of hypertension in offspring submitted to protein undernutrition during pregnancy and lactation.

Keyword: Undernutrition. Phenotypic plasticity. Hypertension. Breathing. Chemoreflex.

SUMÁRIO

APRESENTAÇÃO

O período da gestação e lactação (período perinatal) exerce uma importante sobre o status de saúde ou doença dos filhos. Um insulto durante este período, a exemplo da desnutrição, é um fator de risco para o desenvolvimento de doenças e agravos não transmissíveis, como diabetes mellitus tipo 2, obesidade e hipertensão arterial sistêmica (HAS) na vida adulta da progênie. Entre essas doenças, destaca-se a HAS, como o principal fator de risco de morte por doenças cardiovasculares. Entender a relação entre desnutrição durante a gestação e lactação e o desenvolvimento da HAS nos filhos é de real interesse e necessidade para a população mundial.

Estudos epidemiológicos e experimentais têm evidenciado que indivíduos ou animais submetidos à desnutrição durante gestação e/ou lactação desenvolvem HAS principalmente por danos no sistema renal e hiperatividade do eixo renina angiotensina. No entanto, sabe-se que a etiologia da HAS é multifatorial e envolve fatores genéticos e ambientais que podem favorecer o seu desenvolvimento. Entre estes fatores, a hiperativação do sistema nervoso simpático tem sido apontada como uma das principais causas desta afecção. A hiperativação simpática é caracterizada por um aumento na intensidade e na frequência das despolarizações elétricas do nervo simpático e também por um aumento nos níveis plasmáticos de catecolaminas, promovendo constrição dos vasos sanguíneos periféricos, aumento na resistência vascular periférica e consequente aumento nos níveis basais da pressão arterial.

Recentemente foi demonstrado que o sistema respiratório é capaz de modular o sistema nervoso simpático e predispor o aparecimento da HAS, isso porque um aumento de frequência respiratória induz hiperatividade simpática, qual pode reger o aparecimento desta patologia.

Outra questão relevante é que alterações na quimiossensibilidade periférica ou central podem estar envolvidas na gênese da hipertensão arterial. Os quimiorreceptores são células sensíveis à variações nas concentrações de O_2 e CO_2. A ativação desses quimiorreceptores por hipóxia induz hiperatividade simpática, taquipnéia, bradicardia e aumento da pressão arterial. Já foi demonstrado que uma maior sensibilidade de quimiorreceptores está envolvida na gênese da hipertensão arterial.

Sendo assim, nosso grupo de pesquisa tem investigado se o aumento na frequência respiratória e na quimiossensibilidade periférica ou central estão envolvidos

no desenvolvimento da HAS em ratos que passaram por desnutrição proteica durante a gestação e lactação.

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ARTIGO 1 – Artigo a ser submetido à Revista Brasileira de Nutrição**.**

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Origem da hipertensão arterial: um enfoque sobre a fisiologia respiratória e o ambiente perinatal

Origin of arterial blood pressure: an approach about the respiratory physiological and perinatal environment

Short title: Plasticidade fenotípica e o desenvolvimento da hipertensão

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RESUMO

Um insulto nutricional, a exemplo da desnutrição proteica, durante a gestação e/ou lactação, dito período crítico de desenvolvimento, tem sido associado com um maior risco do desenvolvimento de hipertensão arterial sistêmica na vida adulta. Um dos mecanismos propostos para o desenvolvimento da hipertensão são disfunções em neurônios geradores do ritmo respiratório e nos quimiorreceptores centrais e periféricos. Assim, esta revisão teve como objetivo discutir as repercussões da desnutrição proteica perinatal sobre o ritmo respiratório e quimiorreflexo, como mecanismos subjacentes ao desenvolvimento da hipertensão. Foram utilizadas as bases de dados MedLine/PubMed, Lilacs e Bireme, com publicações entre 2001 e 2012. Os termos de indexação utilizados foram: protein undernutrition, developmental plasticity, repiratory activity e chemoreflex. Conclui-se que a desnutrição proteica perinatal pode alterar o ritmo respiratório basal bem como a rede de quimiossensibilidade central e periférica da prole predispondo o aparecimento de hipertensão arterial sistêmica.

Termos de indexação: Desnutrição. Plasticidade fenotípica. Hipertensão arterial. Atividade respiratória. Quimiorreflexo.

INTRODUÇÃO

Atualmente, o mundo tem vivenciado um aumento de doenças e agravos não transmissíveis (DANT), a exemplo do diabetes mellitus, obesidade, dislipidemias e a hipertensão arterial. Compreender a origem e os aspectos fisiológicos subjacentes ao desenvolvimento dessas doenças é de real interesse e necessidade para população, bem como para o futuro da humanidade.

Entre as doenças acima citadas, destaca-se a hipertensão arterial sistêmica (HAS), como o principal desafio para políticas de saúde pública, além de ser um fator de risco para morte por doenças cardiovasculares(1).

Tem sido demonstrado por estudos experimentais e epidemiológicos que a origem ou gênese da hipertensão na vida adulta tem uma forte relação com a desnutrição durante o período de vida fetal e/ou pós-natal inicial(2-4). Estudos com ratos têm demonstrado que a redução de proteínas na dieta (6-9 % g/g) durante a gestação(5), lactação(6) ou após o desmame(7) leva ao aumento nos níveis basais de pressão arterial (PA) na prole.

Além disso, recentes estudos têm proposto que disfunções respiratórias e de quimiossensibilidade podem ser a causa do desenvolvimento da hipertensão, isso porque neurônios responsáveis por gerar a atividade respiratória são responsáveis por modular a despolarização de neurônios simpáticos, cuja maior atividade desses neurônios pode desencadear o aparecimento da HAS(8, 9).

Sendo assim, o objetivo desta revisão é discutir a origem da hipertensão arterial sistêmica fundamentada em alterações respiratórias e na história pregressa do ambiente fetal e pós-natal, relação esta denominada “Developmental Origin of Heath and Disease – DOHaD” ou Origem Desenvolvimentista da Saúde e da Doença.

MÉTODOS

Para revisão foram utilizadas as bases de dados Medline/Pubmed, Lilacs e Bireme, com os seguintes termos de indexação: protein undernutrition, developmental plasticity, repiratory activity e chemoreflex. Os artigos escolhidos foram selecionados de acordo com a relevância para os tópicos estudados. Para discussão sobre desnutrição proteica e o desenvolvimento da hipertensão arterial foram utilizados artigos entre os anos de 2001 e 2012 incorporados com artigos clássicos do tema pesquisado. Este estudo foi realizado entre os meses de agosto e novembro de 2012.

Ambiente perinatal e a origem das doenças

A afinidade entre o ambiente materno com o padrão de saúde ou doença da progênie já despertava em 1859 a curiosidade do Dr. Charles Darwin, em seu livro “The Origin of Species”, e persiste a despertar nos dias atuais a curiosidade de pesquisadores em várias partes do mundo. Isso porque, o ambiente pré-natal e pós-natal inicial são marcados por rápidas multiplicações e diferenciações celulares, estando suscetíveis ou propensos a alterações ambientais(10). Essa relação entre ambiente e modificações celulares é explicada pela epigenética, na qual o ambiente é uma peça fundamental e indutora de modificações de DNA, por processos de metilação e acetilação, podendo de maneira permanente alterar os nucleotídeos e predispor o aparecimento de doenças(11).

A hipótese de origem fetal da doença no adulto tem sido fundamentada no conceito de “fenótipo econômico” ou “thrifty phenotype”, na qual o feto é capaz de se adaptar e sobreviver em um ambiente de pobre nutrição, mesmo que para isso seja preciso reduzir seu crescimento somático(12). Essa flexibilidade de ajustar a trajetória de crescimento em resposta a diferentes condições ambientais é denominada plasticidade do desenvolvimento, situação na qual o genótipo pode produzir diferentes fenótipos de acordo com o ambiente no qual se desenvolve(13). Estas adaptações são benéficas a curto prazo, uma vez que garante sobrevivência da espécie, no entanto os custos dessa adaptação, pode ser expressa a longo prazo, por alterações fenotípicas e risco do aparecimento de doenças crônicas, como diabetes tipo 2, hipertensão e doença coronariana(14)

Para Gluckman, o feto se adapta e prediz o ambiente pós-natal no qual ele irá se desenvolver, através de um processo denominado de resposta adaptativa preditiva(15). Essa relação entre ambiente pré-natal e a previsão do ambiente futuro pós-natal tem como característica principal ajustar os fenótipos da prole para satisfazer otimamente os desafios impostos ao organismo em desenvolvimento(16). Extrapolando então para a desnutrição durante a gestação, os fenótipos da prole são ajustados para prever um ambiente pós-natal de insuficiência ou de carência nutricional. Quando o ambiente pré-natal é semelhante ao ambiente pós-natal, denominado por Gluckamn de “Match”, menor é o risco de aparecimento de doenças na vida adulta. No entanto, quando o ambiente pré-natal não coincide com o pós-natal, ou seja, desnutrição na gestação e fartura nutricional na vida pós-natal, também denominado de “Mismatch”, maior o risco de desenvolver doenças crônicas(17).

As teorias de Jonathan Wells destacam que o baixo peso ao nascer e a trajetória de crescimento são os principais fatores de risco para o desenvolvimento de doenças crônicas, ou seja, crianças com baixo peso ao nascer com uma rápida trajetória ou catch-up de crescimento, em outras palavras, um acelerado ganho de peso e gordura, resulta em uma alta carga metabólica para as funções vitais dessas crianças, sendo um fator de risco para o desenvolvimento de doenças crônicas(18).

Para corroborar com essa hipótese, Wells tem recentemente proposto um modelo conceitual entre desnutrição materna e o risco de doenças dos filhos, baseado em características fenotípicas. Para ele, os fenótipos apresentam uma capacidade metabólica (variedade de aspectos estruturais e funcionais de órgãos e sistemas que emergem da vida fetal e infância) e uma carga metabólica (carga imposta pelos tecidos e sua condição fisiológica sobre a capacidade metabólica homeostática)(19). A desnutrição durante a gestação e/ou lactação pode reduzir a capacidade metabólica fenotípica dos filhos e uma vez que estes são impostos a um ambiente de alta carga metabólica (dietas hiperlipídicas, hiperglicêmicas e sedentarismo) a relação capacidade x carga metabólica entraria em desequilíbrio, predispondo o aparecimento de doenças na progênie. No entanto, não se sabe o momento em que a balanço capacidade x carga desequilibra, visto que cada sistema detém uma

particularidade fisiológica, podendo esse desequilíbrio se expressar a curto ou em longo prazo(19).

A relação entre desnutrição e o desenvolvimento de doenças crônicas no adulto, a exemplo de cardiovasculares, diabetes mellitus tipo 2 e obesidade tem sido evidenciada por estudos epidemiológicos (20, 21).

Além dos estudos epidemiológicos acima descritos, modelos animais têm sido utilizados para investigar os efeitos da desnutrição materna sobre o padrão de saúde ou doença da prole. A indução de uma desnutrição moderada em ratas durante a gestação e lactação (período perinatal) tem sido o modelo mais usado, o qual consiste em alimentar um grupo de fêmeas com dieta normoproteica (17% de proteína) e outro grupo com uma dieta hipoproteica (8%) durante o período perinatal e investigar as repercussões em longo prazo sobre o status de saúde ou doença da prole (22-24).

Desnutrição perinatal e a gênese da hipertensão arterial

Sabe-se que etiologia da hipertensão arterial é multifatorial e envolve fatores genéticos e ambientais que podem favorecer o seu desenvolvimento e ao que parece sua origem tem forte ligação com o ambiente fetal e pós-natal inicial.

Entender a relação entre desnutrição e hipertensão arterial é de fundamental importância mundial, nacional e, sobretudo para região Nordeste, visto que a desnutrição ainda é problema de saúde pública nesta região e a hipertensão um fator de risco para mortes por doenças cardiovasculares.

Estudos com humanos têm evidenciado que indivíduos com baixo peso ao nascer têm uma forte predisposição em desenvolver hipertensão arterial na vida adulta (25, 26). Um aumento significativo nos níveis plasmáticos de catecolaminas e insuficiência uteroplacental são os possíveis mecanismos subjacentes ao desenvolvimento da hipertensão (3, 27).

Além dos estudos epidemiológicos, pesquisas com ratos têm demonstrado que a redução no conteúdo de proteínas na dieta (6-9 % g/g) durante a gestação (5), lactação (6) ou após o desmame (7) leva ao aumento nos níveis basais de pressão arterial (PA) na prole, por uma diminuição de néfrons e glomérulos e hiperatividade do sistema renina-angiotensina. No entanto, sabe-se que o controle da pressão arterial não ocorre apenas em nível

do sistema renal e que o entendimento de outros mecanismos subjacentes ao desenvolvimento desta patologia é de fundamental importância.

Outros estudos têm demonstrado que a desnutrição durante o período fetal é um fator de risco para o desenvolvimento de hipertensão na prole, devido o contato excessivo do feto com glicocorticoides (28). Estudos com modelos experimentais de desnutrição tem observado uma redução da atividade da 11β-hidroxiesteróide desidrogenase (11βHD), enzima esta, indispensável na proteção do feto à elevações de glicocorticoides através da conversão de corticosterona em 11-dehidrocorticosterona (29, 30). Uma vez que a atividade dessa enzima encontra-se reduzida, maior é o contato do feto com os glicocorticóides, podendo levar a uma maior captação de sódio e cálcio no músculo cardíaco, além de prejudicar o desenvolvimento da glândula adrenal, por meio da supressão da secreção do hormônio adrenocortocotropina (ACTH). Isso resultaria em um prejuízo na comunicação do eixo hipotálamo-hipófise-adrenal e maior predisposição a quadros de elevação de pressão na prole (29). Essas comprovações iniciais servem de sustento para pesquisas mais atuais, as quais sustentam a hipótese de que a desnutrição na gestação altera a funcionabilidade da enzima 11βHD e aumento do contato do feto com glicocorticoides (27, 31).

Estudo utilizando modelos de restrição proteica em roedores durante a gestação tem demonstrado que ocorre redução na metilação de genes na prole. Bogdarina e colaboradores observaram uma reduzida metilação na região promotora proximal do receptor de angiotensina e aumento na expressão de receptor angiotensinérgico na glândula adrenal de ratos de mães submetidas à desnutrição proteica durante a gestação(32). Outro trabalho evidenciou um aumento na expressão de RNAm do angiotensinogênio e da enzima conversora de angiotensina e diminuição na expressão de RNAm do receptor de angiotensina II, na prole de ratos exposto a deficiência proteica pré-natal(33). Essas evidências epigenéticas são relevantes para melhor compreensão da gênese da hipertensão em modelos experimentais de desnutrição.

Nosso grupo de pesquisa tem se proposto a investigar a função respiratória e a participação dos quimiorreceptores centrais e periféricos como

mecanismos subjacentes ao desenvolvimento de hipertensão na prole de ratos submetidos à desnutrição proteica perinatal.

Função da respiração no controle da pressão arterial

A respiração é um complexo processo pelo qual o oxigênio (O_2) é captado do ar e é levado até as células para a obtenção da energia necessária para as funções vitais do organismo(9). Os movimentos respiratórios iniciam-se no útero e continua até o nascimento, momento no qual os circuitos neurais subjacentes à respiração deverão está adequadamente conectados aos pulmões e músculos respiratórios para enfrentar os desafios de oxigênio (O_2), gás carbônio (CO_2) e níveis de pH impostos pelo ambiente ao corpo(34).

Evidências experimentais com ratos tem demonstrado que o ritmo respiratório é gerado principalmente por neurônios localizados na área ventral do bulbo, também denominada de coluna respiratória ventral (CRV)(35). Nesse local encontram-se neurônios inspiratórios e expiratórios, os quais atuam conjuntamente para geração e modulação do ritmo respiratório(36). Além da CRV, algumas pesquisas também tem demonstrado que neurônios localizados na área dorsal do bulbo, sobretudo no núcleo do trato solitário (NTS), desempenham relevante função na modulação da atividade respiratória, a qual tem estreita conexão com neurônios da CRV(37) (**Figura 1**).

Na CRV, sobretudo na região rostral ventral lateral do bulbo (RVLM) e no NTS estão localizados neurônios geradores e moduladores da atividade simpática, os quais tem uma forte proximidade com os neurônios respiratórios. Estudos experimentais tem evidenciado a existência de um acoplamento entre neurônios simpático-respiratório, uma vez que a atividade simpática de repouso apresenta aumentos fásicos predominantemente durante a inspiração(8, 9).

A hiperativação simpática é caracterizada por um aumento na intensidade e na frequência das despolarizações elétricas do nervo simpático e também por um aumento nos níveis plasmáticos de catecolaminas(38, 39), promovendo constrição dos vasos sanguíneos periféricos, aumento na resistência vascular periférica e, consequentemente aumento nos níveis basais da pressão arterial.

Esses estudos experimentais acima descritos são de fundamental importância para compreensão da gênese da hipertensão arterial sistêmica, a qual parece está fortemente conectada com disfunções no padrão respiratório.

Função dos quimiorreceptores no controle da pressão arterial

A respiração é reflexamente controlada por células especiais sensíveis a mudanças na pressão parcial arterial (Pa) de O_2, de CO_2, ou na concentração de H^+ , e que agem para manter esses parâmetros em níveis ideais(40). Os órgãos sensórios responsáveis por esse controle homeostático são denominados de quimiorreceptores e estão localizados perifericamente, principalmente na bifurcação carotídea e arco aórtico e centralmente no bulbo (41) (**Figura 1**).

Em 1900 Kohn descreveu a organização do parênquima em ilhotas de células do corpo carotídeo e as denominou de células clusters ou células glomus, principal região onde estão localizados os quimiorreceptores periféricos(40). As células clusters são estruturas ricamente vascularizadas localizadas principalmente na bifurcação carotídea e arco aórtico, sendo formado por 2 tipos de células: células tipo I, principal, células glômica ou quimiorreceptores e tipo II ou sustentaculares(40). As células do tipo I estão em maior número do que as do tipo II e apresentam um grande número de mitocôndrias (12 a 15% do volume) e estão em contato com várias terminações nervosas(40). Quedas na pO_2 e pH e/ou elevação na pCO_2 (quadros de hipóxia) são detectadas pelos quimiorreceptores. Nas células glômus, a queda de oxigênio sanguíneo são transformados em sinais elétricos, os quais são enviados, via nervo glossofaríngeo e vago, ao sistema nervoso central, sobre tudo a nível bulbar (quimiorreceptores centrais), onde é originado respostas apropriadas para os órgãos efetores(42).

Os quimiorreceptores periféricos são ativados principalmente por hipóxia hipoxíca (mistura gasosa com 7% de O_2) ou por íons CN^- (hipóxia citotóxica)(43), enquanto os quimiorreceptores centrais são estimulados principalmente por hipercapnia (mistura gasosa com 7% CO_2)(44). A ativação dos quimiorreceptores centrais ou periféricos promove reflexamente hiperventilação, hiperatividade simpática, bradicardia e aumento da pressão

arterial(43, 45), desempenhando papel fundamental no controle ventilatório, bem como na homeostase pressórica.

Neste sentido, recentes estudos têm demonstrado que modificações no padrão respiratório, sobretudo na quimiossensibilidade ao CO_2, estão envolvidas na hiperativação do sistema nervoso simpático e no aparecimento da hipertensão arterial em ratos espontaneamente hipertensos(46, 47).

Outro estudo relevante e recém-publicado evidenciou a participação efetiva dos quimiorreceptores periféricos na gênese da hipertensão arterial sistêmica. Neste estudo foi demonstrado que a remoção destes quimiorreceptores periféricos no inicio da vida de ratos espontaneamente hipertensos (22-25 dias de vida) atenuou os valores pressóricos nestes animais na vida adulta(48).

Desnutrição perinatal, disfunções respiratórias e a origem da hipertensão

Foi evidenciado em humanos e em ratos que a desnutrição durante a gestação e/ou lactação é um fator de risco ao desenvolvimento de hipertensão na vida adulta da progênie. No entanto, os mecanismos ou as disfunções fisiológicas subjacentes ao desenvolvimento desta patologia ainda não estão totalmente esclarecidos. Nosso grupo de pesquisa tem investigado e comprovado que disfunções respiratórias e de quimiossensibilidade central e periférica (dados não publicados) podem ser a causa da gênese da hipertensão em ratos de mães que passam por desnutrição proteica durante a gestação e lactação.

Isso porque, a respiração e os quimiorreceptores exibem considerável plasticidade (neuroplasticidade respiratória) ou um “período critico” no desenvolvimento do controle respiratório(34, 49, 50) e alguns estudos têm evidenciado que os insultos ou stress, a exemplo de fumo, álcool, má nutrição, hipóxia, durante a vida fetal e pós-natal inicial podem comprometer a formação e maturação da rede neural respiratória e predispor o aparecimento de doenças (51, 52).

Em modelo de desnutrição proteica pós-natal já foi evidenciado que ratos alimentados com dieta baixa em proteína após o desmame tem aumentada resposta cardiovascular à ativação de quimiorreflexo(53). Outro estudo relevante e recém-publicado demonstrou que crianças nascidas com

baixo peso (<2500g) exibem um aumento na frequência respiratória de repouso em relação às crianças com peso normal(54).

Uma vez que a atividade respiratória modula a atividade simpática, hipotizamos que a desnutrição durante a gestação e lactação pode potencializar o acoplamento simpático respiratório no inicio da vida, o qual pode contribuir para o desenvolvimento da hipertensão na vida adulta (**Figura 2**).

Outra hipótese, investigada pelo nosso grupo de pesquisa é que esses animais submetidos à desnutrição proteica perinatal exibem maio sensibilidade de quimiorreceptores centrais e periféricos, os quais contribuem também para gênese da hipertensão (**Figura 3**).

CONCLUSÃO

Um feto é capaz de sobreviver em um ambiente de carência nutricional, no entanto essa sobrevivência gera um custo metabólico ao organismo. As repercussões desse custo podem ser expressas a curto ou em longo prazo. A desnutrição no inicio da vida induz danos ao sistema cardiovascular, o qual reflete com o aparecimento de hipertensão arterial sistêmica na vida adulta da prole. Os possíveis mecanismos subjacentes ao desenvolvimento da hipertensão são disfunções respiratórias e uma maior sensibilidade de quimiorreceptores centrais e periféricos.

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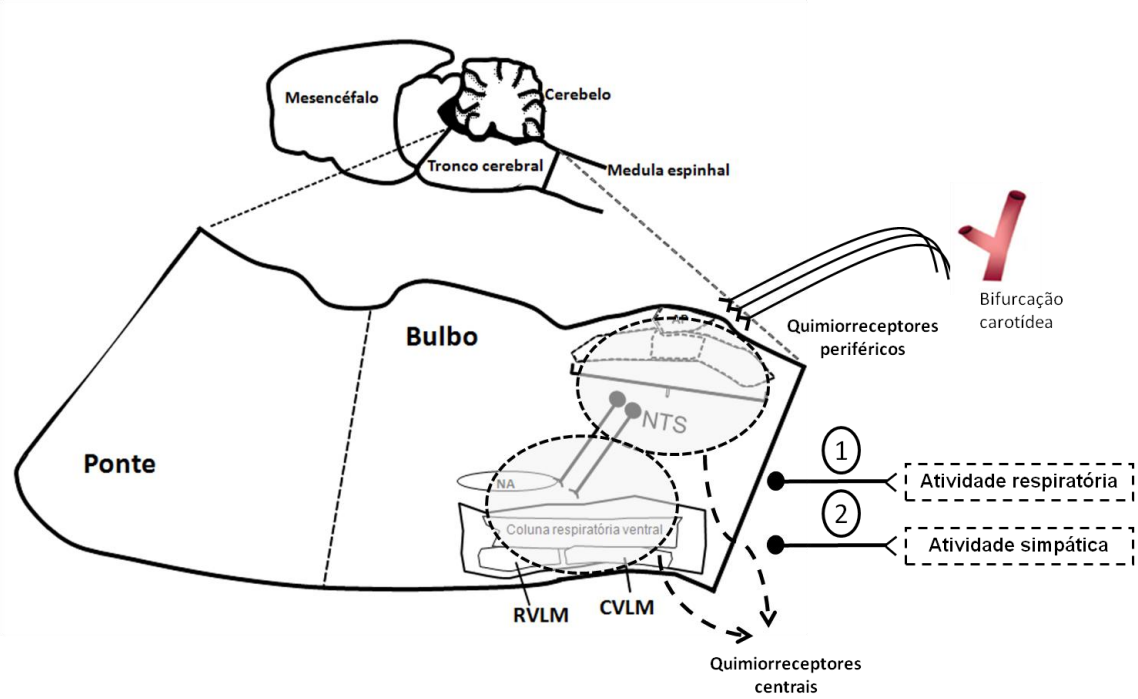

Figura 1. Representação esquemática do tronco cerebral, região onde estão localizados os neurônios geradores da atividade respiratória (1) e simpática (2), além dos quimiorreceptores centrais, os quais tem comunicação direta com os quimiorreceptores periféricos, localizados na bifurcação carotídea. Essas regiões desempenham papéis fundamentais na regulação da atividade respiratória e pressórica.

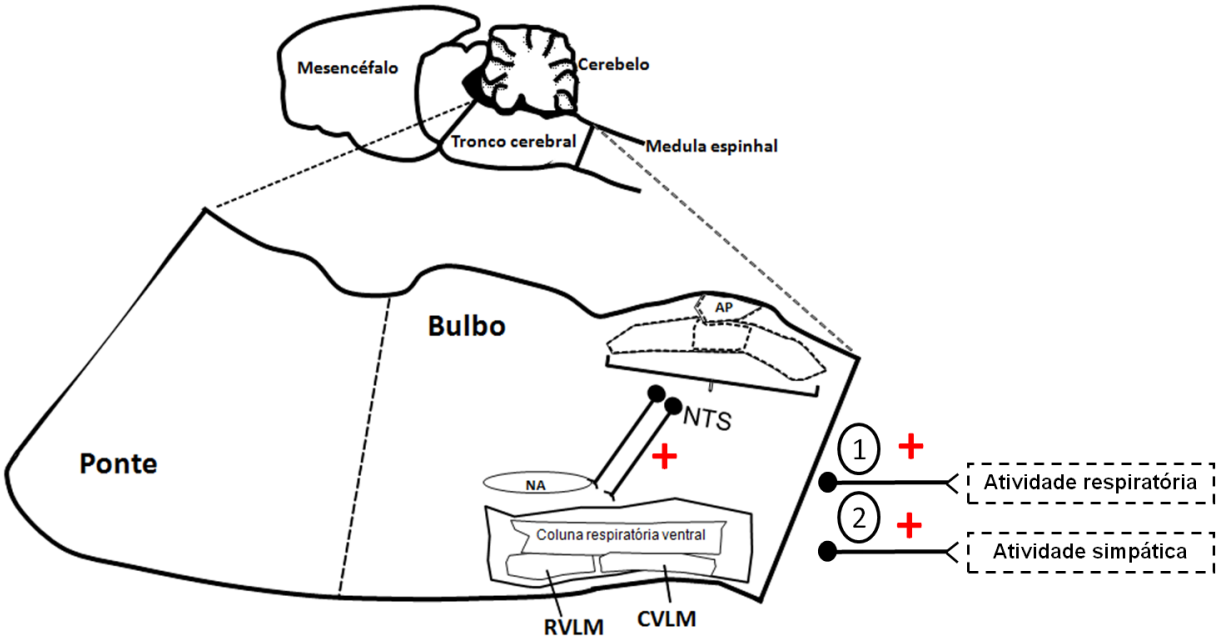

Figura 2. A desnutrição durante a gestação e lactação pode alterar a rede respiratória e simpática da prole e contribuir para o desenvolvimento da hipertensão arterial na vida adulta. **1** – neurônios geradores da atividade respiratória; **2** – neurônios geradores da atividade simpática.

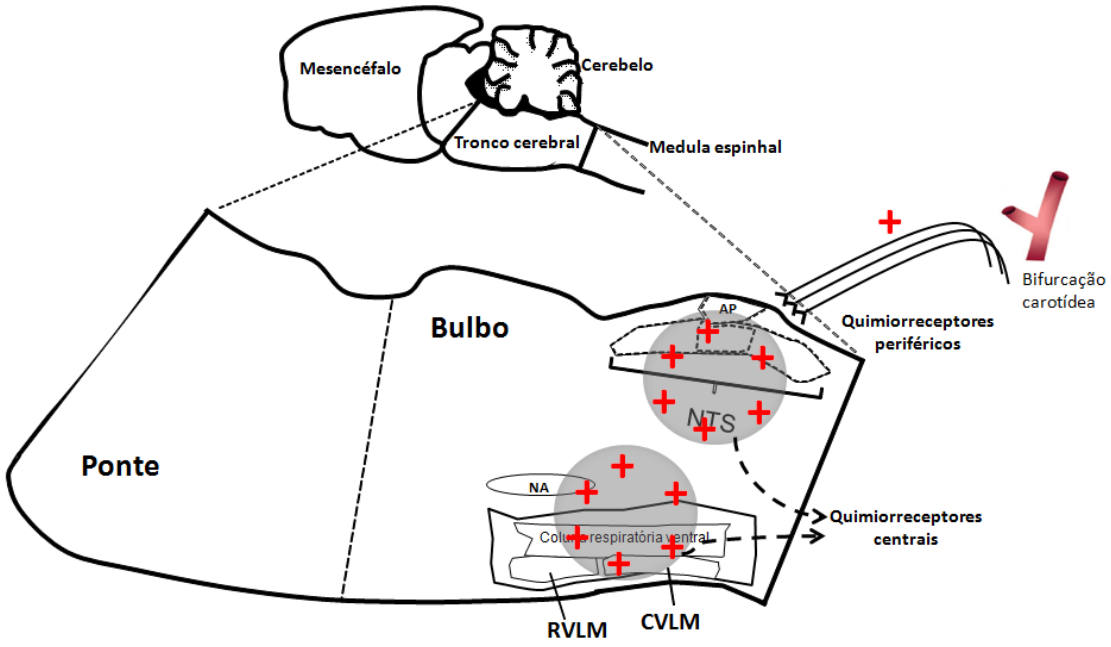

Figura 3. A desnutrição durante a gestação e lactação pode levar a um aumento na sensibilidade de quimiorreceptores centrais e periféricos na progênie. Essa maior sensibilidade pode estar envolvido na gênese da hipertensão.

MÉTODOS

Animais

Foram utilizados ratos machos Wistar do biotério do Centro Acadêmico de Vitória, procedentes de mães que passaram ou não por desnutrição proteica durante a gestação e lactação. Os animais foram mantidos em gaiolas de polipropileno (4 animais/gaiola), com água filtrada e ração *ad libitum*. Eles foram mantidos em ciclo claro escuro de 12h e com temperatura (22 a 25°C) e umidade (55 a 65 %) controladas. Todos os protocolos e procedimentos experimentais foram realizados de acordo com o Colégio Brasileiro de Experimentação Animal (COBEA) e aprovados pelo Comitê de Ética em Experimentação Animal (CEEA) do Centro de Ciências Biológicas da UFPE (processo n° 23076.044454/2010-94).

Dietas

Foram elaborados dois tipos de dietas a base de caseína: uma normoprotéica (17% de proteína) e outra hipoproteica (8% de proteína). Ambas as dietas foram produzidas no Departamento de Nutrição da Universidade Federal de Pernambuco de acordo com a AIN – 93 (55). As dietas são isocalóricas com alteração apenas no conteúdo proteico conforme a **tabela 1**.

Tabela 1 – Composição das dietas (g/100g de dieta)

Nutriente (g)	**Nomoprotéica (17% proteína)**	**Hipoprotéica (8% proteína)**
Caseína (85%)*	20	9,41
Amido dextrinisado	13	13,2
Celulose	5	5
Sacarose	10	10
Amido	39,74	50,34
Óleo de soja	7	7
Colina	0,25	0,25
Metinonina	0,3	0,3
Mix vitamínico	1	1
Mix mineral	3,5	3,5
Densidade energética (Kcal/g)	3,94	3,94

*A caseína utilizada continha 85% de pureza, analisada pelo método de Kjeldahl.

Indução da desnutrição proteica durante o período peri-natal (gestação e lactação)

Ratas Wistar com 90 - 100 dias de vida foram acasaladas com ratos machos férteis na proporção de 1:1. A observação da presença de espermatozoides no esfregaço vaginal foi utilizada para definir o 1° dia de prenhez.

Posteriormente, as ratas foram colocadas em gaiolas individuais e alocadas randomicamente em dois grupos: grupo normoprotéico (NP, recebeu dieta com 17% de proteína) e grupo hipoprotéico (HP, recebeu dieta com 8% de proteína) durante a gestação (21 dias) e lactação (21 dias). A prole proveniente destas fêmeas foi reduzida a oito ratos machos por ninhada. Ao 22º dia de vida, todos os filhotes receberam dieta normoprotéica (Labina, Purina Agribands).

Os estudos funcionais de medição da ventilação pulmonar, frequência cardíaca, da pressão arterial, bem como dosagens bioquímicas, foram realizados aos 30, 90 e 150 dias de vida da prole.

Avaliação do peso da prole

A partir do 19º dia de prenhez até o parto, as ratas foram observadas três vezes por dia (às 09:00, 14:00 e 18:00h), afim de registrar o momento do dia do nascimento dos filhotes e o peso ao nascer.

No 21º, 30º, 90 e 150º dias de vida foi registrado o peso e comprimento dos animais.

Determinação dos níveis séricos de proteínas, ureia e creatinina

Foram realizadas análises bioquímicas de proteínas e seus metabólitos com o propósito de investigar se a desnutrição proteica durante a gestação e lactação afeta o metabolismo proteico da prole. Para isso, aos 30º e 90° dias foram feitas as análises bioquímicas de albumina, proteínas totais, ureia e creatinina dos animais controle e experimentais. Os ratos foram anestesiados com ketamina (80 mg/kg, ip.) e xilazina (10 mg/kg, ip.) e, com o auxílio de um capilar de vidro heparinizado, foi coletado sangue (1-2 mL) através do rompimento do plexo retro-orbital. Após coagulação o sangue foi centrifugado a 3500 RPM por 10 minutos para obtenção do soro, o qual foi transferido para um tubo Eppendorf e armazenado a -20°C até a realização das análises bioquímicas através dos respectivos *kits* de reagentes e padrão (Labtest Diagnóstica, MG, Brasil) por

espectrofotometria. Os valores das globulinas foram obtidos pela diferença entre proteínas totais e albumina.

Medidas da ventilação pulmonar

As medidas de ventilação foram obtidas por pletismografia de corpo inteiro, em um sistema fechado (56). Durante a realização de cada medida de ventilação, o fluxo de ar foi interrompido e a câmara do animal permaneceu totalmente vedada por curtos períodos de tempo (~2 min). As oscilações de pressão causadas pela respiração do animal foram captadas por um dispositivo conectado à câmara que contém o transdutor diferencial de pressão e o amplificador de sinais (ML141 spirometer, PowerLab, ADInstruments). O sinal foi então enviado para o sistema de aquisição e análise dos dados (PowerLab, ADInstruments). A calibração do volume foi obtida durante cada experimento, injetando-se um de 1 mL dentro da câmara do animal com uma seringa graduada. Duas variáveis respiratórias foram medidas, a frequência respiratória (f) e o volume corrente (VT), o último calculado através da fórmula:

$$VT=PT/PK \times VK \times TA/Tamb \times (PB-PA)/PB-TA/TC\ (PB-PC),$$

onde VK: volume de ar injetado na câmara do animal para calibração; PT: deflexão de pressão associada com cada volume de ar corrente; PK: deflexão de pressão associada com cada volume de ar injetado para calibração, TC: temperatura corporal; Tamb: temperatura ambiente; TA: temperatura do ar dentro da câmara; PB: pressão barométrica; PC: pressão de vapor d'água à temperatura corporal; PA: pressão de vapor d'água à temperatura da câmara. A ventilação foi calculada pelo produto de f pelo VT. A ventilação e o VT estão apresentados nas condições de pressão barométrica ambiente, à Tc e saturados com vapor d'água (BTPS).

Procedimento cirúrgico e registro da frequência cardíaca e da pressão arterial

Um dia antes do estudo, os animais foram anestesiados com ketamina (80 mg/kg, ip.) e xilazina (10mg/kg, ip.) e foram inseridos os catéteres de polietileno na artéria e veia femoral, para registro da pressão arterial e infusão de drogas, respectivamente. As cânulas foram exteriorizadas pelo dorso do animal e estes receberam uma injeção de ketoprofeno (5 mg/kg ip). Após a cirurgia, os animais ficaram em recuperação por 18h até o início dos experimentos.

O registro da pressão arterial e da freqüência cardíaca foi realizado em animais não anestesiados por meio da conexão da cânula da arterial femoral com o transdutor

mecanoelétrico de pressão (ML866/P, ADInstruments, Power Lab, Bella Vista, NSW, Australia), cujo sinal foi devidamente amplificado, digitalizado por meio de uma interface analógico/digital e amostrado em 1000 Hz em um microcomputador equipado com um software apropriado (ChartTM Pro, ADInstruments, Bella Vista, NSW, Australia), para posterior análise.

A pressão arterial média (PAM) e freqüência cardíaca (FC) foram derivadas da pressão arterial pulsátil (PAP) por meio deste sistema de aquisição.

Ativação dos quimiorreceptores periféricos

Hipóxia citotóxica

Para estimular os quimiorreceptores periféricos e, consequentemente, ativar o quimiorreflexo foi utilizado o KCN 0,04 % (43), administrado por via endovenosa, através de um cateter venoso previamente implantado, e as respostas respiratórias e autonômicas foram registradas continuamente.

Ativação do quimiorreflexo central

O animal acordado e não restrito foi colocado na câmara pletismográfica, a qual esteve inicialmente ventilada com ar atmosférico umedecido (21% O_2) para aclimatação (~30-40 min.) e, logo após, foram realizadas medidas de ventilação pulmonar durante o repouso. Em seguida, os animais foram submetidos à hipercapnia, por 5 minutos. Para isso, a câmara foi ventilada com uma mistura gasosa padrão umedecido contendo 7% de CO_2 e as respostas respiratórias continuamente registradas.

Análise estatística

Os resultados estão expressos como média ± EPM (erro padrão da média). A comparação entre os dados do grupo normoprotéico (NP) e com baixa proteína (LP) foi realizado pelo teste "t" de Student não pareado. Já a comparação entre os dados do mesmo grupo foi avaliada pelo teste "t" de Student pareado. O nível de significância considerado foi $p<0,05$.

RESULTADOS

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Maternal low-protein diet increases ventilation and CO_2 chemoreception in early age: potential underlying mechanisms for hypertension in adulthood?

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Key words: Maternal undernutrition, developmental plasticity, chemosensitivity to CO_2, Hypertension**.**

SUMMARY

Maternal undernutrition increases the risk of adult chronic diseases, such as arterial hypertension. This study investigated the short and long-term effects of a maternal low-protein diet on arterial blood pressure (ABP), respiratory rhythm, CO_2 chemosensitivity, and biochemical markers of protein metabolism of offspring. Pregnant Wistar rats were fed either with normoproteic (NP group, 17% of protein) or low protein diet (LP group; 8% of protein) during pregnancy and lactation (perinatal). Direct measurements of ABP, respiratory frequency (RF), tidal volume (V_T) and ventilation (VE) and hypercapnia (7% CO_2) evoked respiratory responses were recorded in awake male rats at 30 and 90 d. Blood samples were collected for proteins, creatinine and urea serum analyzes. LP offspring had impaired growth, but increase percentage of body weight gain between 30 and 90. At 30 d, LP rats showed reduction in serum protein contents and increase in urea and creatinine serum levels, which were normalized at 90 d. ABP in LP was similar to NP rats at 30 d, but it was higher at 90 d. At 30 d, LP rats showed enhanced RF and VE, which was associated to increased respiratory response to CO_2. At 90 d, VE values CO_2 chemosensitivity of LP rats were restored to control range, but the RF was maintained increased. Thus, our data show that perinatal low-protein diet alters respiratory rhythm and CO_2 chemosensitivity in early age, which may be predisposing factors to increased arterial blood pressure at adulthood**.**

Key words: maternal undernutrition, developmental plasticity, chemosensitivity to CO_2, hypertension.

INTRODUCTION

Epidemiological and experimental studies have demonstrated that there is a tight relationship between undernutrition during perinatal life and subsequent development of adult diseases (57-59). This phenomenon is termed "developmental plasticity" or metabolic programming, and refers to the property of a given genotype to produce different phenotypes in response to distinct environmental conditions (13). The maternal low-protein diet model is one of the most extensively studied animal models of developmental plasticity (59). Exposure to a low-protein diet (8% casein) during gestation followed by the consumption of a normoproteic diet throughout the life-course was associated with growth restriction, slightly elevated systolic blood pressure and increased fasting plasma insulin concentration (59, 60). When maternal protein restriction is continued during lactation, there is long-lasting growth restriction, age-dependent loss of glucose tolerance, insulin resistance and hypertension, even when the offspring are weaned onto a control diet (23, 59, 61, 62).

Experimental data in rats have documented that a reduction in diet protein content (6-9 % g/g) during pregnancy (5), lactation (6) or post-weaning period (7) induces increased levels of arterial blood pressure at adulthood. Moreover, studies also have showed that post-natal exposure to protein restriction can also lead to changes in lung parenchyma and structure, inducing impairment in ventilatory function of these animals (63). However, it is currently unclear the relationship between perinatal protein undernutrition and development of hypertension and respiratory dysfunction in the offspring as well as the underlying mechanisms responsible for those effects.

Arterial hypertension has a multifactorial etiology and involves genetic and environmental factors. Recently, studies have suggested that changes in the generation or modulation of respiratory function can also contribute to develop arterial hypertension (8, 46, 47, 64-67), suggesting that respiratory neurons located into brainstem could modulate cardiovascular system and that increased respiratory activity would lead to arterial hypertension by neural pathways. Furthermore, disorders in the mechanisms of control of breathing, such as chemoreflex, have also been pointed out as risk factor for the development of hypertension (68-70). Central respiratory chemoreflex plays important role in acid-base homeostasis (control of CO_2/H^+ levels in the body) and respiratory control, and contributes to modulation of cardiovascular function (71-73). It has been suggested that individuals who suffered malnutrition or had low birth weight develop hypertension (25, 74, 75). However, there are very few data reporting

the underlying mechanisms that could contribute to the emergence of those effects at adulthood. Thus, herein we evaluated the short (30 days)- and long (90 days)-term effects of a low protein diet during pregnancy and lactation on pulmonary ventilation during room air (basal) and during hypercapnia challenge (7% CO_2), and on the levels of ABP in the offspring. And we tested the hypothesis that a greater respiratory frequency and chemosensitivity to CO_2 in early age could be a potential hidden basis for an increased arterial blood pressure at adulthood.

MATERIALS AND METHODS

Ethical approval

The experimental protocol was approved by the Ethical Committee of the Biological Science Center (process nº 23076.044454/2010-94), Federal University of Pernambuco (UFPE), Brazil, and all experiments were performed in accordance with the recommendations of the Brazilian Committee of Animal Experimentation (COBEA).

Animals

Males Wistar rats were obtained from the Department of Physical Education and Sport Sciences, Federal University of Pernambuco. Animals were maintained in polypropylene cages (4 animals /cage) and remained in controlled laboratory conditions (12/12 h light/darkness cycle, temperature: 23–25 °C).

Diet Formulations

Normoproteic (17% of protein) and low protein (8% of protein) diets were elaborated at the Laboratory of Experimental Nutrition, Federal University of Pernambuco in concordance with the American Institute of Nutrition – 93 (55). The casein was previously analyzed and showed 85 % of purity (85g of protein for each 100g of casein). The diets were isocaloric, only the amount of protein was changed (Table 1).

Induction of protein undernutrition

Seven virgin females Wistar rats (90 d of life) were mated with male rats (100 at 150 days) in ratio 1:1 or 1:2 daily. The observation of sperm in the smear vaginal was used to define first day of pregnancy. After detection of pregnancy, the dams were placed in individual cages and randomly allocated in two groups: normoproteic group (NP, diet with 17% of protein) and low protein group (LP, diet with 8% of protein). The animals were fed during pregnancy (20-22 days) and lactation (21days) *ad libitum*. At weaning, the offspring of both groups were housed in collective cages (4 animals per cage) and received a standard diet (Labina, Agriband Purina, Sao Paulo, Brazil) *ad libitum*. The rats used for forming groups NP and LP were derived of three different dams. To investigate the effects of protein undernutrition the short- and long- term on the offspring, we used offspring at 30 and 90 days, respectively.

Evaluation of body weight, body length and percentage of body weight gain of offspring

From 19th day of pregnancy until birth of pups, the dams were observed three times a day (09:00 AM, 02:00 PM and 06:00 PM) to record birth weight of their pups. At day 3 (of life), litters were reduced to eight pups. Body weight and body length (nose-to-anus length) of the animals was recorded at the 30th and 90th days of life. The body weight and body length were used to determine the body mass index (BMI, grams/lengh^2) (24).

The percentage of Body weight gain was obtained by formula: % weight gain = weight 90th x 100/weight 30th.

Analysis of the serum levels of protein, urea and creatinine

Analyzes of proteins and its metabolites was performed for identify whether the protein undernutrition in pregnancy and lactation was affecting the proteic metabolism of offspring. Animals of both groups NP and LP groups (at 30th and 90th days of life) were fasted overnight (10-12h), anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) and the blood samples (about 1 - 2mL) were collected by plexus retro-orbital disruption after the registers of ventilatory parameters. Serum samples were obtained after centrifugation at 1096 *g* during 10 minutes. Serum was transferred to eppendorf tubes and stored -20 ºC until analysis of protein, urea and creatinine by commercial kits (Labtest Diagnostica, Minas Gerais, Brazil). The values of globulin were obtained by difference between total protein and albumin.

Basal respiratory parameters

At 30th and 90th days of life, measurements of ventilation (VE) were obtained using the whole body plethysmography method as described by Malan (1973). Before recording data, animals were placed into the Plexiglas chamber (5L) for a period of acclimatization (about 60 min) and the chamber was flushed with humidified room air and temperature at 25°C. After, in order to record VE the air flow was suspended for short periods (3 min) and the pressure oscillations caused by breathing of the animal were captured for an apparatus connected to chamber, which has the pressure differential transducer and the signal amplifier (ML141 spirometer, PowerLab, ADInstruments, Bella Vista, NSW, Australia). Then, the signal was fed into an

acquisition system and data analysis (PowerLab, ADInstruments, Bella Vista, NSW, Australia).

Activation of respiratory chemoreceptors by hypercapnia

After recording baseline VE, the respiratory responses to CO_2 were induced by flushing a hypercapnic gas mixture (7 % CO_2, 21 % O_2 and N_2 balance; Linde Gas, Barueri-SP, Brazil) into the plethysmographic chamber. This gas mixture was flushed into the chamber at a flow of 3 L/min, during 5 minutes, as previously standardized in our laboratory.

Analysis of respiratory parameters

Respiratory frequency (RF), tidal volume (V_T) and ventilation (VE) were determined in the room air condition (control baseline) and during hypercapnia challenge. All data were analyzed off-line by LabChart program (LabChart 7 Pro, ADInstruments, Bella Vista, NSW, Australia). It was selected a period of ten seconds for determination of mean RF. V_T was calculated from the pressure oscillation caused by the breathing of rats and using the formula previously described by Malan (76). VE was obtained by the product of RF and V_T, and it is presented at ambient barometric pressure and at body temperature, saturated with water vapour at this temperature (BTPS).

Evaluation of arterial blood pressure

Two days after the respiratory parameters were taken, NP and LP rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) for inserting a femoral arterial catheter (PE-50 connected to PE- 10) at 30 and 90 days old. The catheter was filled with heparinized saline (NaCl 0.9%) and exteriorized through the animal's back. After surgery, the animals received injection of ketoprofen (5 mg/kg ip) and remained for 18 hours of recovery until beginning of the recording experiments. After this period, the arterial blood pressure and heart rate were recorded in unanesthetized animals by connecting the femoral catheter in the pressure transducer. The signal was amplified (ML866/P, ADInstruments, Power Lab, Bella Vista, NSW, Australia), sampled at 2 kHz, digitalized and recorded in the microcomputer with appropriate software (LabChart7 Pro, ADInstruments, Bella Vista, NSW, Australia).

Analysis of arterial blood pressure and heart rate

Animal was placed into the recording chamber for a period of acclimatization (about 60 min) and pulsatile arterial pressure was recorded for 30 min at rest and the values of mean arterial pressure (MAP) and heart rate (HR) were calculated from this recording period. For determination of mean values of MAP and HR was selected a period of 5-10 min for each animal. All data were analyzed off-line in appropriate software (LabChart 7 Pro, ADInstruments, Bella Vista, NSW, Australia). At the end of experiments, the animals were killed by decapitation.

Statistical Analysis

Data were expressed as mean ± standard error (SE). Unpaired Student t–test was used for comparing NP and LP groups and paired Student t–test was used for analysis within NP or LP group. Significance level was fixed at $P<0.05$**.**

RESULTS

Effects of perinatal low-protein diet on the body weight, body length, percentage of body weight gain, body mass index and serum concentration of protein, urea and creatinine

Pups from mothers submitted to a low-protein had lower birth weight than normoproteic group (NP: 6.1±0.04 vs. LP: 5.2±0.05 g, P<0.0001). Likewise, LP group maintained a reduced body weight at 30 and 90 days (Table 2), but LP rats showed increase percentage of body weight gain between 30 and 90 days (NP: 374.4±13.7 vs. LP: 425.1±18.5, p=0.04). LP rats had less length at 30 and 90th, but no change in body mass index (Table 2). Biochemistry analysis of blood showed that offspring from LP dams had a decrease in serum concentrations of albumin, total protein and globulin at 30 d. However, at 90 d, these parameters were similar when compared with NP group. In addition, LP animals showed an increase in the urea and creatinine at 30 and 90 d (Table 3).

Effects of perinatal low-protein diet on the respiratory control and arterial blood pressure

Fig 1 illustrates breathing recordings of representative rats from NP and LP group, at 30 (Figure 1A) and 90 d (Figure 1B). At rest, it was observed that at 30 d, LP group had increased RF (NP: 106±3 vs. LP: 135±2 cycles/min, P<0.0001; Figure 2A) and VE values (NP: 1020±47 vs. LP: 1384±57 mL/kg/min, P<0.0001; Figure 2C) when compared to NP group, but no changes in VT (NP: 9.7±0.4 vs. LP: 10.3±0.5 mL/kg, P=0.325, Figure 2B). After activation of central chemoreceptors by hypercapnia, both NP and LP groups increased RF, VT and VE when compared to basal period (Fig 2). However, LP group exhibited higher values of RF during hypercapnia than NP (NP: 171±3 vs. LP: 181±3.5, P=0.024, Figure 2A). Likewise, LP group had higher values of VE during hypercapnia than NP, i.e. a larger respiratory response to CO2 (NP: 2271±85 vs. LP: 2770±152, P=0.0114, Figure 2C). No difference was noted in VT values during hypercapnia (NP: 14.3±0.7 vs. LP: 15.6±0.8, P=0.265, Figure 2B). Figure 3 illustrates pulsatile blood pressure recordings of representative rats from NP and LP group, at 30 (Figure 3A) and at 90 d (Figure 3B). At 30 days of life, NP and LP groups showed similar values of MAP (NP: 85.3±3.4 vs. LP: 89.1±9.5 mmHg, p= 0.773) and HR (NP: 454.1±26.5 vs. LP: 433.1±36.2 bpm, p= 0.661).

At 90 days old, the animals from LP group had again an augmented RF (NP: 92±3.9 vs. LP: 109.2±4.1, P=0.048; Figure 2A), but VT and VE values were similar to NP group. During hypercapnia, no differences were observed in RF, VT and VE values between NP and LP groups. At this age, LP showed higher values of MAP than NP animals (NP: 93.4±3.4 vs. LP: 111.5±1.8 mmHg, p=0.0005), but similar HR (NP:358±6.6 vs. LP:378.2±10.7 bpm, p=0.105).

Discussion

We observed that offspring from dams submitted to protein restriction during pregnancy and lactation had lower birth weight and impaired growth (up to 90 d) when compared to normoproteic group. Previous studies have already demonstrated that perinatal low protein diet exposure leads to lower weight in the offspring, suggesting that the equality in body weight between NP and LP animals may occur around 240 d (23, 77, 78). LP showed lower body weight, impaired growth rate, but similar BMI when compared to NP group. Our results are in accordance with previous studies that used the same model to induce short and long-last effects of maternal undernutrition (24, 78, 79). Indeed, at 30 days of life, LP rats group showed decreased serum concentration of albumin, total proteins and globulin (13.2, 24.2 and 36.4 %, respectively). Similarly, it was observed that at 21 days, offspring from protein-restricted dams during lactation have reduction of 21, 17 and 12% in serum values of albumin, total proteins and globulin, respectively, when compared with the rats of dams fed with 23% of protein in diet (80). The reduction in serum values of protein can be explained by two factors. First, the low supply of protein in maternal diet during pregnancy may induce a deficit on the maternal-fetal amino acid transport by placenta, which leads to restriction of fetal growth, mainly muscle and fat tissues, and produces a low birth weight (81, 82). Another factor might be related to protein restriction during lactation, which could reduce the levels of lactalbumina in the dams, reducing protein intake and hence protein synthesis by the pups (83).

LP animals presented increased levels of urea (about 17.7 %) and creatinine (about 36.2 %). As urea and creatinine are produced by protein metabolism, it is probable that protein catabolism may be enhanced in LP animals, which would reduce muscle mass and induce an increase in urea and creatinine serum levels. It is described that protein-restricted diet can cause protein wasting and metabolic acidosis and that acidosis should stimulate muscle protein degradation and increases the activity of branched-chain ketoacid dehydrogenase by an ubiquitin-dependent proteolytic pathway, reducing muscle mass in these animals (84, 85). In addition, it has been described that low-protein diet during pregnancy leads to increased levels of glucocorticoids in the mothers, which may induce to enhancement of protein catabolism in mothers and fetus/pups (31, 86, 87). Thus, we hypothesized that the effects observed in our biochemical analyses in the animals 30 days old might be due to increased protein catabolism. Furthermore, the increase in protein metabolism at 30 days may reflect an

adaptive mechanism of short-term survival, which will have a cost in long-term or adult life (18).

Increased baseline ventilation in animals from protein-restricted dams

In the present study, offspring from protein-restricted dams showed increased baseline respiratory frequency (up to 28 %) and ventilation (up to 40 %) at 30 days, when compared to normoproteic group. Further, our experimental data showed that low protein diet during pregnancy and lactation may induce permanent changes in mechanisms responsible for respiratory modulation and generation of offspring, affecting the control of respiratory frequency from these animals. In addition, recently, was showed that spontaneously hypertensive rats (SHR) before developing hypertension exhibited amplified respiratory-sympathetic coupling in early life and changes in baseline respiratory rhythm (47), suggesting that central mechanisms responsible for modulation and generation of respiration could be involved in the development of hypertension. Thus, we hypothesized that enhanced ventilation seen in FR and VE in early life of rats submitted to protein undernutrition during pregnancy and lactation, can leads to changes in respiratory-sympathetic coupling in early life, which would be contributing to raise arterial blood pressure in adult life. However, these hypotheses should be tested.

Increased CO_2/H^+ chemosensitivity in early age and risk factor for developing high levels of arterial pressure in adulthood

Central respiratory chemoreceptors play important role in acid-base homeostasis and respiratory control. These cells are sensitive to changes in CO_2/H^+ levels in the body and produces enhancement of breathing elicited by increases in CO_2/H^+ levels (71-73). Currently, it is believed that ventilatory responses elicited by their activation are due to their intrinsic sensitivity to CO_2/H^+ and inputs from specific regions of the central nervous system and carotid bodies, which lead to increased respiratory drive (71, 72, 88, 89). Besides the respiratory-related chemoreceptor responses, acute stimulation of central respiratory chemoreceptors by hypercapnia produces increase in sympathetic activity and arterial pressure (44, 90).

At 30 days, LP rats had a high ventilatory response to CO_2 exposure, suggesting that neural pathway responsible for CO_2 chemoreception may be altered in early life of the offspring from protein-restricted dams.

It is described that protein-restricted diet can stimulate proteolytic pathways, which could lead to chemoreceptor sensitization (84, 85). Thus, we suggest that protein-restricted dams during pregnancy and lactation and their offspring may develop changes in protein catabolism and an enhanced CO_2 sensitivity. This hypothesis is strongly supported at least in part by our findings, showing that animals from protein-restricted dams had low serum levels of proteins and increase in indicators of protein catabolism, which were associated to enhanced respiratory rhythm and ventilatory responses to CO_2 at early life. In agreement with this hypothesis, it was observed that at 90 d, HP animals restored their levels of protein and showed normalized ventilatory responsiveness to CO_2. Furthermore, these findings are the first demonstration in vivo and in unanesthetized animals showing that the increase in respiratory frequency and chemosensitivity to CO_2 in early life may contribute for the increase of blood pressure in experimental models of proteic undernutrition.

Recently, studies have shown that central chemoreception is a widely distributed property in the brain (91). Putative areas shown to act as CO_2/pH chemosensors, have been tested both *in vivo* and *in vitro* conditions, such as the medullary raphe, retrotrapezoid nucleus, locus coeruleus and the nucleus of solitary tract (72). The results of present study suggest that LP rats have increased respiratory rhythm and CO_2 sensitivity, but neither unveil which of these putative chemoreceptor sites could be affected, nor the long-term changes that may occur in each of these sites due to perinatal low protein treatment. This remains an interesting issue to be further investigated.

It is well described that critical period to development of respiratory system is during all fetal period and, after birth, until postnatal day 15 in rats (49, 89, 92). After birth, there are an intense development and maturation of chemoreceptors and respiratory network, and any environmental perturbations in that critical period, such as acid-base imbalance, hypoxia or hypercapnia, can alter the development of the respiratory system (49).

Presently, we verified that hypercapnia, as expected, induced an increased respiratory drive to breathing, resulting in increases in RF, V_T and consequently VE. Moreover, it was observed that animals from protein-restricted dams had higher RF and VE values than control animals at 30, but not at 90 days old, suggesting that in earlier

age, the effects of perinatal proteic undernutrition are more intense. In fact, among all periods studied (30 and 90 days), 30-day old animals seemed to have greater side effects, presenting low serum levels of proteins, increase in urea and creatinine values, enhanced basal RF and respiratory responses to hypercapnia, but no changes in arterial blood pressure. At 90 days, those animals still presented increased respiratory frequency, but no more changes in ventilation at rest and during hypercapnia, showing that at this age there was a normalization of CO_2/H^+ chemosensitivity. Thus, our data suggest that the changes in CO_2/H^+ chemosensitivity in early age induced by perinatal proteic undernutrition were restored at 90 days old. However, the effects on the respiratory rhythm seem to be unchangeable and correlated to increased arterial pressure at this age and, could be related to changes in gene expression of the chemosensory transducers, modifying cellular phenotype in the chemosensitive cells. These effects could induce permanent modifications in the neuronal activity and respiratory pattern generator as well as in CO_2 chemosensitivity in the brainstem. It is described that changes in respiratory rhythm generation may be associated to development of hypertension in some experimental models (38, 47, 93). In addition, studies have showed a sympathetic overactivity in those conditions, which seems to have a strict relationship between respiratory dysfunctions and development of hypertension. Thus, the hypothesis that animals submitted to perinatal protein undernutrition would present changes CO_2/H^+ chemosensitivity and in respiratory rhythm were supported by our experimental data and thereby creates new insights into the comprehension of the underlying mechanisms involved in the development of arterial hypertension in perinatal protein-restricted individuals.

In conclusion, our data showed that side effects induced by protein-restricted diet during perinatal period increased respiratory rhythm in early age, probably by changes in CO_2/H^+ chemosensitivity, which was correlated to increased arterial pressure in adulthood and may determine a risk factor to the development of arterial hypertension.

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Conflit of Interest:

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Figure Captions

Fig. 1 Representative tracings of rats at rest and during hypercapnia at 30 and 90 days old. Representative tracing of ventilation before and at the last minute of hypercapnia exposure at 30 (a) and 90 days old (b) of rats from dams submitted to normoproteic (NP, 17 % of protein) or low protein diet (LP, 8 % of protein) during pregnancy and lactation. All pups were fed standard chow from weaning.

Fig. 2 Perinatal protein undernutrition increased ventilation at rest and during hypercapnia at 30 days and respiratory frequency at 90 days. Evaluation of respiratory frequency (RF, a), tidal volume (VT, b) and ventilation (VE, c) before (Basal) and after hypercapnia (7% CO2) in 30 and 90 days old rats from dams submitted to normoproteic (NP, 17 % of protein, black bars, n=14) or low protein diet (LP, 8 % of protein, gray bars, n=16) during pregnancy and lactation. All pups were fed standard chow from weaning. *different from its respective NP group at specific age, assessed by unpaired Student's t–test ($P<0.05$) and # difference within group at different age, assessed by paired Student's t–test ($P<0.05$).

Fig. 3 Representative tracings blood pressure baseline of rats at 30 and 90 days old. Representative tracing of pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) baseline at 30 (a) and 90 days old (b) of rats from dams submitted to normoproteic (NP, 17 % of protein) or low protein diet (LP, 8 % of protein) during pregnancy and lactation. All pups were fed standard chow from weaning).

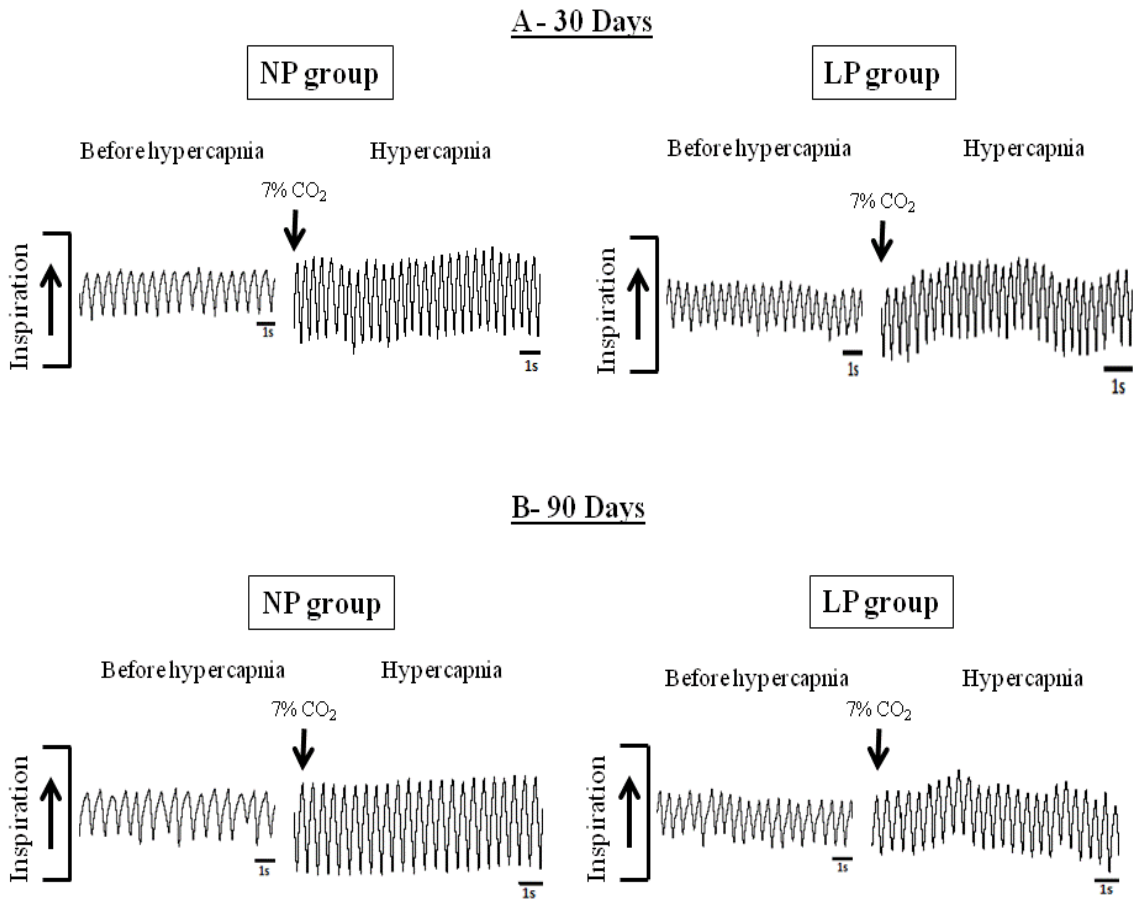
A - 30 Days
NP group
LP group
Before hypercapnia
Hypercapnia
7% CO2
Inspiration
1s
B- 90 Days
NP group
LP group
Before hypercapnia
Hypercapnia
7% CO2
Inspiration
1s

Figure 1.

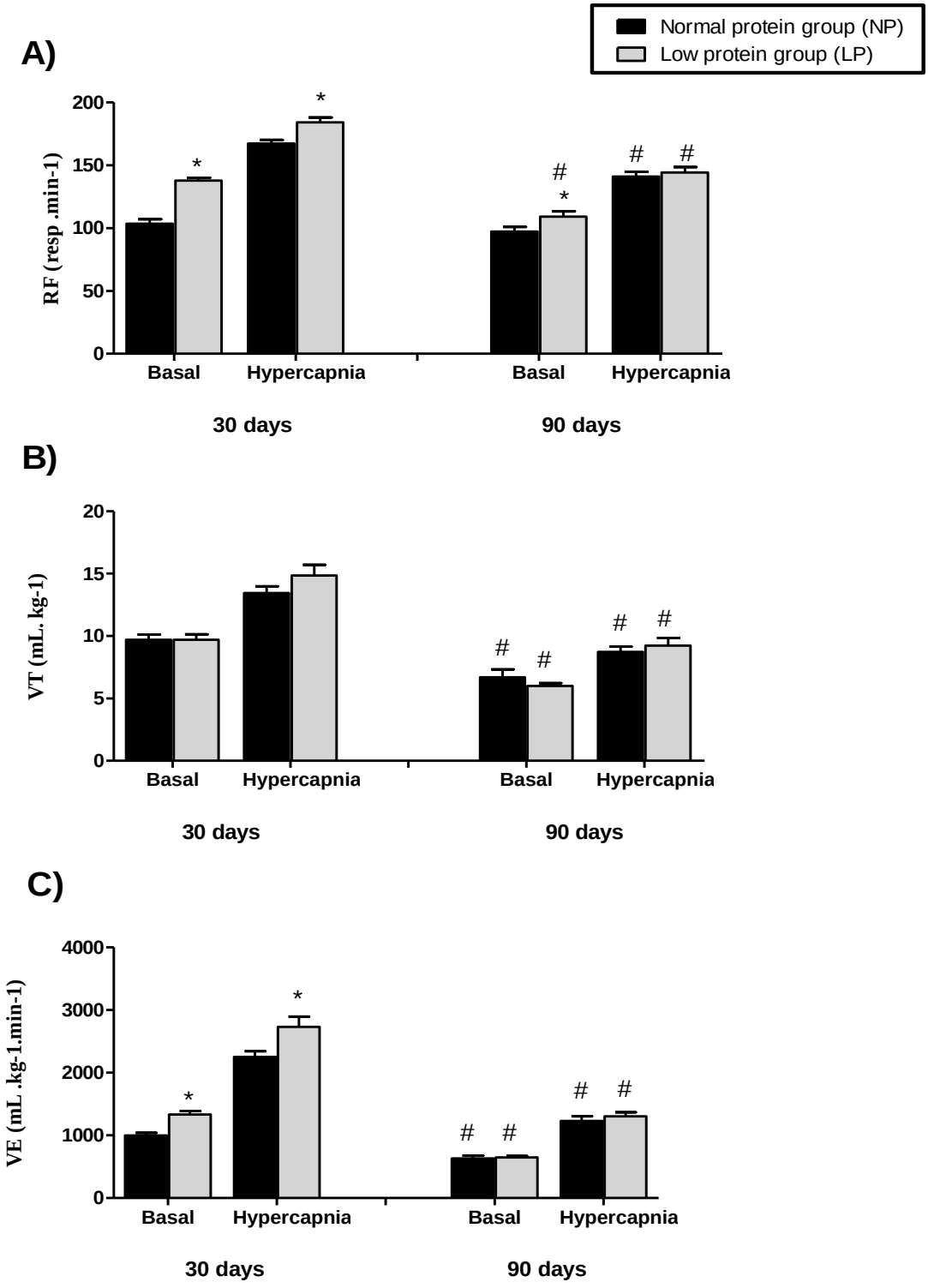
A)
Normal protein group (NP)
Low protein group (LP)
RF (resp .min-1)
200
150
100
50
0
*
*
*
#
#
#
Basal
Hypercapnia
Basal
Hypercapnia
30 days
90 days
B)
VT (mL. kg-1)
20
15
10
5
0
#
#
#
#
Basal
Hypercapnia
Basal
Hypercapnia
30 days
90 days
C)
VE (mL .kg-1.min-1)
4000
3000
2000
1000
0
*
*
#
#
#
#
Basal
Hypercapnia
Basal
Hypercapnia
30 days
90 days

Figure 2.

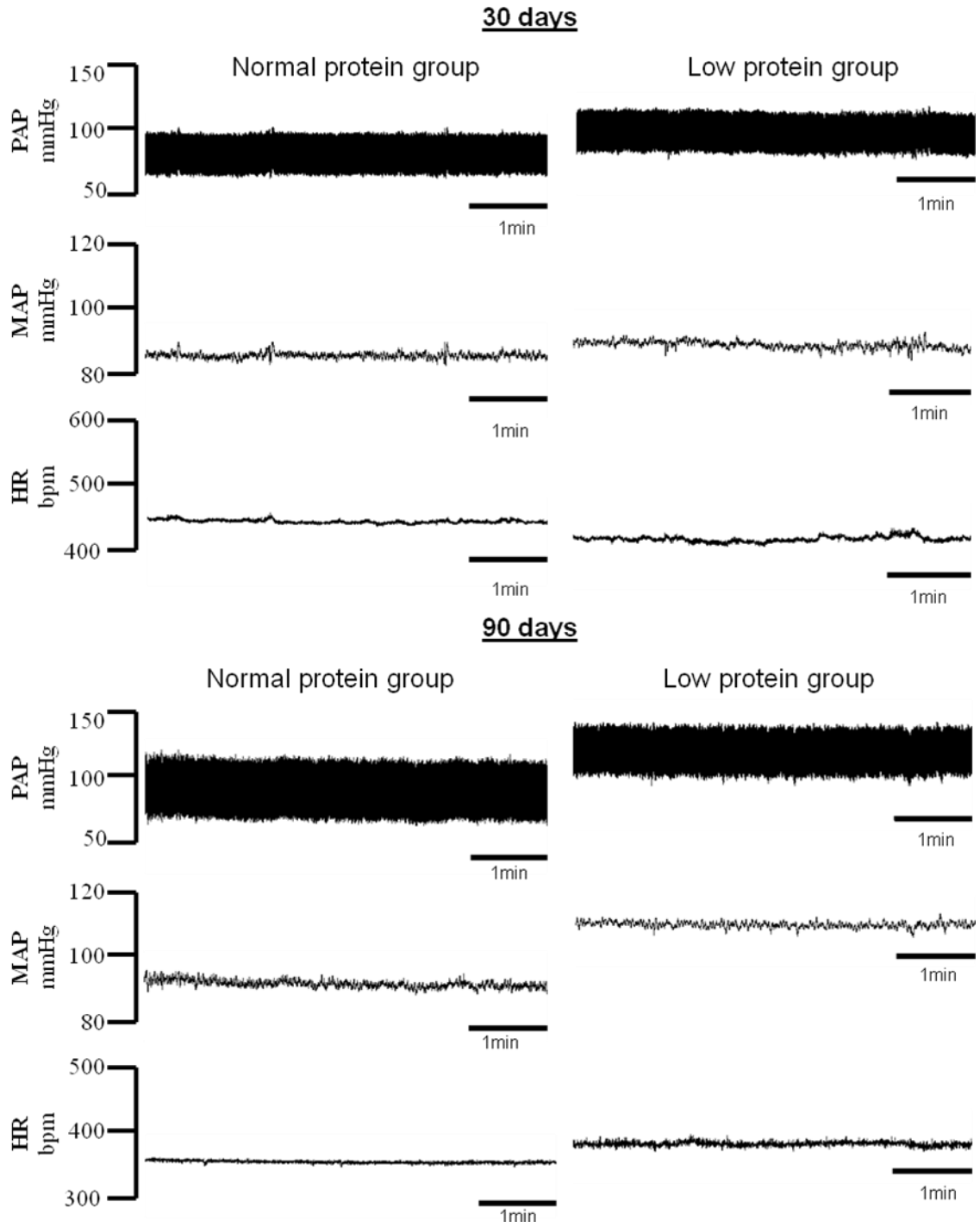
30 days
Normal protein group
Low protein group
PAP
mmHg
150
100
50
1min
MAP
mmHg
120
100
80
1min
HR
bpm
600
500
400
1min
90 days
Normal protein group
Low protein group
PAP
mmHg
150
100
50
1min
MAP
mmHg
120
100
80
1min
HR
bpm
500
400
300
1min

Figure 3.

Table 1 – Nutritional composition of diets (g/100g diet)

Nutrient	Normal protein (17% protein)	Low protein (8% protein)
Casein (85%)*	20	9,41
Dextrin cornstarch	13	13,2
Cellulose	5	5
Sucrose	10	10
Cornstarch	39,74	50,34
Soyben oil	7	7
Colin	0,25	0,25
Metinonin	0,3	0,3
Vitamin mix	1	1
Mineral mix	3,5	3,5
Energy density (Kcal/g)	3,94	3,94

* The casein used in preparation of diet had 85% purity.

Table 2 - Body weight, body length (nose-to-anus length), and body mass index (BMI, grams/ length^2) of rats from dams submitted to normoproteic (NP, 17 % of protein, n=14) or low protein diet (LP, 8 % of protein, n=16) during pregnancy and lactation.

Parameters	Birth		30 days		90 days	
	NP	LP	NP	LP	NP	LP
Body weight	6.1±0.04	5.2±0.05*	83.1±3.17	65.7±2.7*	306.2±5.89	273.8±7.92*
Body lenght	5.0±0.1	4.6±0.1*	13.2±0.16	11.9±0.14*	22.7±0.17	22.1±0.21*
BMI	0.27±0.007	0.24±0.009*	0.47±0.02	0.46±0.02	0.59±0.01	0.56±0.01

Data are presented as means ± S.E.M. *$p < 0.05$ by Student t –test.

Table 3 – Biochemistry parameters obtained at 30 and 90 days of rats from dams submitted to normoproteic (NP, 17 % of protein, n=14) or low protein diet (LP, 8 % of protein, n=16) during pregnancy and lactation.

Biochemistry parameters	30 days			90 days		
	NP	LP	p-value	NP	LP	p-value
Albumin (g/dL)	3.34±0.08	2.90±0.07	0.0006	3.85±0.15	3.74±0.23	0.690
Proteins totals (g/dL)	6.44±0.16	4.88±0.17	<0.0001	6.80±0.23	6.85±0.24	0.529
Globulin (g/dL)	3.10±0.21	1.97±0.20	0.0005	3.13±0.27	3.07±0.31	0.900
Urea (mg/dL)	48.8±1.12	57.4±2.24	<0.0001	40.9±1.60	49.8±2.31	0.004
Creatinin (mg/dL)	0.47±0.01	0.64±0.03	0.0005	0.35±0.02	0.47±0.05	0.04

Data are presented as means ± S.E.M.

ARTIGO 3 – Artigo em processo de elaboração para ser submetido ao British Journal of Nutrition

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Pressor and ventilatory responses to hypoxia are enhanced in animals submitted to perinatal protein undernutrition.

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Introduction

The prevalence of chronic noncommunicable disease, such as diabetes mellitus, dyslipidemias and hypertension is growing in world population. Researchers have directed toward the importance of nutrition during early life in the development of those diseases (20, 94). Studies have suggested that a given genotype can be modulated by different environmental conditions and produce distinct phenotypes, which have been named as "developmental plasticity" or developmental programming(13).

Several animals models have been used in order to investigate the effects of the undernutrition in early life on the pattern of health or disease in long-term (95). Induction of mild undernutrition in rats during pregnancy and lactation (perinatal period) has been the most used model, which is usually characterized by submitting female rats to low protein diet (8%) during perinatal period and investigate the long-term repercussions on the offspring (22-24). Some experimental data have documented that a reduction in diet protein during pregnancy (4), lactation (6) or post-weaning period (7) induces increased levels of arterial blood pressure at adulthood. Thus, the comprehension of the relationship between nutritional insults in early life and developmental programming of hypertension have interested public authorities, since actually the systemic arterial hypertension (SAH) represents a major public health problem and is an important risk factor for the development of coronary heart disease, stroke and death (96, 97).

Arterial hypertension has a multifactorial etiology and involves genetic and environmental factors. Studies have revealed that one of the main factors in the generation of SAH in both human and animal models is the increase in sympathetic tone (48, 98). In this way, recently it is known that one of the main factor to increase sympathetic tone is the dysfunction in the mechanisms involved in respiratory control, such as peripheral chemoreceptors, which when stimulated produce hyperventilation, bradycardia, sympathoexcitation and hypertension (9, 99).

Peripheral chemoreceptors are mainly located in a small organ in the carotid artery bifurcation, named carotid body. At this site, the cells are chemosensitivity to change in PaO_2, $PaCO_2$ and pH in blood (40). The carotid body chemoreceptors play a main function in respiratory and cardiovascular homeostatic control and during hypoxia, the chemoreceptors are stimulated and the chemoreflex is activated, leading to hyperventilation, bradycardia, sympathoexcitation and hypertension (34). Those chemoreceptors are stimulated naturally, for anemia, metabolic and respiratory acidosis

, as well as pharmacologically by cyanide (CN^-), which induces activation of those chemoreceptors, producing the same respiratory and cardiovascular responses described before (40) .

In perspective of developmental programming, studies have showed that early-life experiences can disrupt and alter developmental trajectory and lead to maladaptive changes in respiratory control, being a potential for pathological conditions (100-102). Furthermore, have been demonstrated that critic period for maturation of peripheral chemoreceptors occurs after birth. Thus, insults during fetal and postnatal early life could affect the morphological and functional maturation process of the peripheral chemoreceptors (50, 52).

Thus, herein we evaluated the effects of a low protein diet during pregnancy and lactation on cardiovascular and respiratory response to peripheral chemoreflex activation by hypoxia. We tested the hypothesis that an increase in cardiovascular and respiratory response during activation of chemoreceptors could be a potential hidden basis for an increased arterial blood pressure at adulthood of rats submitted to perinatal protein undernutrition.

Material and Methods

Ethical approval

The experimental protocol was approved by the Ethical Committee of the Biological Science Center (process nº 23076.044454/2010-94), Federal University of Pernambuco (UFPE), Brazil, and all experiments were performed in accordance with the recommendations of the Brazilian Committee of Animal Experimentation (COBEA).

Animals

Males Wistar rats were obtained from the Department of Physical Education and Sport Sciences, Federal University of Pernambuco. Animals were maintained in polypropylene cages (4 animals /cage) and remained in controlled laboratory conditions (12/12 h light/darkness cycle, temperature: 23–25 °C).

Diet Formulations

Normoproteic (17% of protein) and low protein (8% of protein) diets were elaborated at the Laboratory of Experimental Nutrition, Federal University of Pernambuco in concordance with the American Institute of Nutrition – 93 (55). The diets were isocaloric, only the amount of protein was changed (**Table 1**).

Induction of protein undernutrition

Virgin females Wistar rats (90 d of life) were mated with male rats in ratio 1:1 or 1:2 daily. The observation of sperm in the smear vaginal was used to define first day of pregnancy. After detection of pregnancy, the dams were placed in individual cages and randomly allocated in two groups: normoproteic group (NP, diet with 17% of protein) and low protein group (LP, diet with 8% of protein). The animals were fed during pregnancy (20-22 days) and lactation (21days). At weaning, the offspring of both groups were housed in collective cages (4 animals per cage) and received a standard diet (Labina, Agriband Purina, Sao Paulo, Brazil). Dams were killed with an overdose of anesthetic.

Basal arterial blood pressure

One day before experiments NP and LP rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) for inserting a femoral arterial (for cardiovascular measurements) and vein (for drug infusion) catheter (PE-50 connected to PE- 10) at 90

and 150 days old. The catheter was filled with heparinized saline (NaCl 0.9%) and exteriorized through the animal's back. After surgery, the animals received injection of ketoprofen (5 mg/kg ip) and remained for 18 hours of recovery until beginning of the recording experiments. On the experimental day, the arterial blood pressure and heart rate were recorded in unanesthetized animals by connecting the femoral catheter in the pressure transducer. The signal was amplified (ML866/P, ADInstruments, Power Lab, Bella Vista, NSW, Australia), sampled at 2 kHz, digitalized and recorded in the microcomputer with appropriate software (ChartTM Pro, ADInstruments, Bella Vista, NSW, Australia).

Basal respiratory parameters

After 60 min of cardiovascular baseline records, measurements of ventilation (VE) were obtained using the whole body plethysmography method as described by Malan (1973). Were recorded two VE register with the animal completely at rest. For this, the air flow was suspended for short periods (3 min) and the pressure oscillations caused by breathing of the animal were captured for an apparatus connected to chamber, which has the pressure differential transducer and the signal amplifier (ML141 spirometer, PowerLab, ADInstruments, Bella Vista, NSW, Australia). Then, the signal was fed into an acquisition system and data analysis (PowerLab, ADInstruments, Bella Vista, NSW, Australia).

Chemoreflex activation

After recording ventilatory and pressoric baseline response, the peripheral chemoreceptors was stimulated by intravenous administration of potassium cyanide (KCN, 0.05%, 100 µL), previously described (43). The peripheral chemoreceptors activation was considered effective by increase in pressure, ventilatory and bradicardic response.

Analysis of arterial blood pressure and heart rate

Pulsatile arterial pressure was recorded for 60 min at rest and the values of mean arterial pressure (MAP) and heart rate (HR) were calculated from this recording period. For determination of mean values of MAP and HR was selected a withdrawal of 5-10 min for each animal. All data were analyzed off-line in appropriate software (LabChart 7 Pro, ADInstruments, Bella Vista, NSW, Australia).

Analysis of respiratory parameters

Respiratory frequency (RF) was analyzed off-line by Lab Chart program (LabChart 7 Pro, ADInstruments, Bella Vista, NSW, Australia). It was selected a withdraw of ten seconds at rest and after chemoreflex activation for determination of mean RF.

Analysis of cardiovascular and respiratory response to chemoreflex activation

The changes in MAP were quantified using the high peak response values and under valleys for HR in a maximum interval of 10s after infusion of KCN.

For analyze of RF during chemoreflex activation was quantified the number of breath in withdraw of five seconds at the moment of intravenous administration of KCN. We estimate the RF in period of 60 seconds or respiration/min.

The delta values (Δ) of MAP, HR and RF were obtained by difference between the response during chemoreflex activation and basal moment.

Statistical Analysis

Data were expressed as mean ± standard error (SE). Unpaired Student t–test was used for comparing NP and LP groups and paired Student t–test was used for analysis within NP or LP group. Significance level was fixed at $P<0.05$**.**

Results

Effects of low-protein diet during pregnancy and lactation on the cardiovascular respiratory baseline parameters and in responses to chemoreflex activation at 30, 90 and 150 days

Figure 1 illustrates representative recordings of pulsatile arterial pressure (PAP), MAP and HR baseline and during chemoreflex activation from NP and LP groups at 30 days. At 30 days blood pressure (NP: 78.2±6.6 vs. LP: 72.8±5.7 mmHg, p=0.0.553, figure 3, panel A) and heart rate (NP: 458.6±44.2 vs. LP: 426.8±46.4 bpm, p=0.634, figure 2, panel B) baseline were similar between groups, but rats LP showed increased respiratory frequency (NP: 121±6 vs. LP: 137±3 resp.min^{-1}, p=0.03, figure 2, panel C). During chemoreflex activation no change was seen in blood pressure (ΔMAP-NP: 22.4±4.1 vs. ΔMAP-LP: 29.4±9.5 mmHg, p=0.519, Fig. 2, panel A) and heart rate (ΔHR-NP: 375±44 vs. ΔHR-LP: 323±27 bpm, p=0.340, figure 2, panel B), but LP rats showed greater respiratory response (ΔRF-NP: 79.7±9.25 vs. ΔRF-LP: 138.8±2.6 resp.min^{-1}, p=0.0003, figure 2, panel C).

Figure 3 illustrates representative recordings of pulsatile arterial pressure (PAP), MAP and HR baseline and during chemoreflex activation from NP and LP groups at 90 days.

At 90 days LP rats have increases in arterial blood pressure (NP: 94.3±3.5 vs. LP: 111.8±3.8 mmHg, p<0.0001, figure 4, panel A) and respiratory frequency (NP: 102.8±2 vs. LP: 116.1±3.6 resp.min^{-1}, p<0.0001, figure 4, panel C), but no changes in heart rate baseline (NP: 349.7±8.3 vs. LP: 349.7±13.1 bpm, p=0.997, figure 4, panel B). During chemoreflex activation LP rats showed greater pressoric response (ΔMAP-NP: 37.9±6 vs. ΔMAP-LP: 55.2±4.2 mmHg, p<0.0001, Fig. 4, panel A) and respiratory (ΔRF-NP: 102.2±8.6 vs. ΔRF-LP: 131.2±8 resp.min^{-1}, p<0.0001, figure 4, panel C). No change was seen in heart rate (ΔHR-NP: 270.2±7 vs. ΔHR-LP: 263.8±12.6 bpm, p=0.214, figure 4, panel B).

Figure 5 illustrates representative recordings of pulsatile arterial pressure (PAP), MAP and HR baseline and during chemoreflex activation from NP and LP groups at 150 days.

At 150 days, LP rats exhibited higher arterial blood pressure (NP: 97.4±3.9 vs. LP: 106.7±3.3 mmHg, p=0.0008, figure 6, panel A), heart rate (NP: 349.1±6.5 vs. LP: 366.5±9.8 bpm, p=0.002, figure 6, panel B) and respiratory frequency (NP: 89.9±3.8 vs. LP: 98.5±9 resp.min^{-1}, p=0.04, figure 6, panel C) at rest. During chemoreflex activation

LP rats showed greater response pressoric (ΔMAP-NP: 51.5±3.9 vs. ΔMAP-LP: 60.4±6.9 mmHg, p=0.02, Fig. 6, panel A) and respiratory (ΔRF-NP: 84.9±8.4 vs. ΔRF-LP: 112.9±18.9 resp.min^{-1}, p=0.004, figure 6, panel C). No change was seen in heart rate (ΔHR-NP: 280.3±11.9 vs. ΔHR-LP: 291.6±5.7 bpm, p=0.058, figure 6, panel B).

Discussion

Our results showed that at 90 and 150 days, rats submitted to protein undernutrition during pregnancy and lactation exhibited increase in mean arterial pressure (about 19% and 10% respectively). Previous experimental studies have showed that protein undernutrition in pregnancy (103, 104), lactation (6) or postnatal (7) induces arterial hypertension in offspring. The findings of those studies have suggested damage in renal system as main cause of appearance of hypertension. Furthermore, a recent review about the fetal origin of hypertension in experimental models revealed that the results are strongly dependent on applied measurement techniques and animal models (105). Our research group has investigated the changes in cardiovascular and respiratory system as triggering mechanism of increase in arterial pressure in malnourished rats during fetal and postnatal life. The present study showed that a high peripheral chemosensivity may be involved in the short and long-term effects leading to the development of hypertension.

The respiratory activity is generated and modulated for neurons primarily located in ventral and dorsal region of medulla (35, 36). Furthermore has been proposed that changes in respiratory activity are risk factor for development of arterial hypertension (8, 106). Furthermore, studies have showed that the development of respiratory system during fetal and early postnatal life is highly plastic to environmental perturbations (49, 89). Thus, we hypothesized that the protein undernutrition during pregnancy and lactation can affect permanently the respiratory rhythm baseline of offspring and predispose sympathetic hyperactivity and development of arterial hypertension in LP rats.

The first synapses of peripheral chemoreceptors in the central nervous system occur at the nucleus of tractus solitarius (NTS) (107, 108). This nucleus contains a complex neuronal circuitry responsible for integration and transmission of chemosensory information to other brain nuclei that control efferent respiratory and sympathetic reflex responses (109, 110). Then, peripheral chemoreceptors play an important role on cardiovascular and respiratory homeostasis (9) and enhanced CB activity may contribute to the development of hypertension (38, 111). It is widely known that activation of peripheral chemoreceptors by hypoxia produces: i) increase in arterial blood pressure by sympathetic activation; ii) bradycardia, by parasympathetic stimulation; and iii) tachypnea by strong stimulation of respiratory network (Haibara et al. 1995; Braga et al. 2007; Costa-Silva et al., 2010).

Previous studies demonstrated that rats fed with low protein diet (6%) in the post weaning had changes in the activity of peripheral chemoreceptors (53), which suggested that changes in protein content may induce modifications in O2 chemosensitivity in those animals. However, the effects produced by low protein diet during pregnancy and lactation on the offspring had not been tested. . Thus, our research brings unprecedented results about of cardiovascular and respiratory coupling, which are important for understanding the ontogenesis of hypertension in malnourished individuals. Here, we showed that animals submitted to maternal low protein diet had important changes in sensitivity of peripheral chemoreceptors from childhood to adulthood, mainly higher sympathetic and respiratory pathways of the chemoreflex, suggesting an enhancement of neural processing of that respiratory reflex in the brainstem. Then, we hypothesized that a chronic activation and a high chemosensitivity of those chemoreceptors may induce to chronic stimulation of respiratory network and increase in efferent sympathetic pathways, contributing to the development of arterial hypertension in those animals.

Studies have demonstrated that insults, as hypoxia, smoke, drugs during fetal life and early postnatal can harm the morpho-functional maturation of peripheral chemoreceptors and predispose the appearance of pathologies in offspring (52, 102). In spontaneously hypertensive rats was demonstrated that the changes in chemoreceptors sensitivity is attributed to a decrease in the lumen of the carotid body arteries and reduced blood flow (112). We believe that the protein undernutrition during pregnancy and lactation can also affect the maturation of CB chemoreceptors, once they become physiologically functional in early life postnatal.

Previous studies showed that human patients with systemic hypertension have enhanced activity of carotid body (CB) chemoreceptors (113, 114). Similar results also were observed in clinical and experimental chronic heart failure (115). Furthermore, recently was demonstrated that the appearance of hypertension in spontaneously hypertensive rats is critically dependent of activity of CB chemoreceptors (48). Thus being, we hypothesized that the increase in arterial blood pressure at 90 and 150 days old in rats submitted to protein undernutrition in pregnancy and lactation is due a greater chemosensivity of CB chemoreceptors in early and adult life, which possibly reflect in changes in sympathetic-respiratory coupling and leads to hypertension.

The hypothesis that animals submitted to perinatal protein undernutrition would present increase in peripheral chemosensitivity were supported by our experimental data

and thereby creates new insights into the comprehension of the underlying mechanisms involved in the development of arterial hypertension.

In conclusion, our data showed that rats from dams fed with low protein diet during pregnancy and lactation have increased respiratory rhythm and peripheral chemoreceptors activity, which was correlated to increased arterial pressure in adult life.

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Table 1 – Nutritional composition of diets (g/100g diet)

Nutrient	**Normal protein (17% protein)**	**Low protein (8% protein)**
Casein (85%)*	20	9,41
Dextrin cornstarch	13	13,2
Cellulose	5	5
Sucrose	10	10
Cornstarch	39,74	50,34
Soyben oil	7	7
Colin	0,25	0,25
Metinonin	0,3	0,3
Vitamin mix	1	1
Mineral mix	3,5	3,5
Energy density (Kcal/g)	3,89	3,89

* The casein used in preparation of diet had 85% purity.

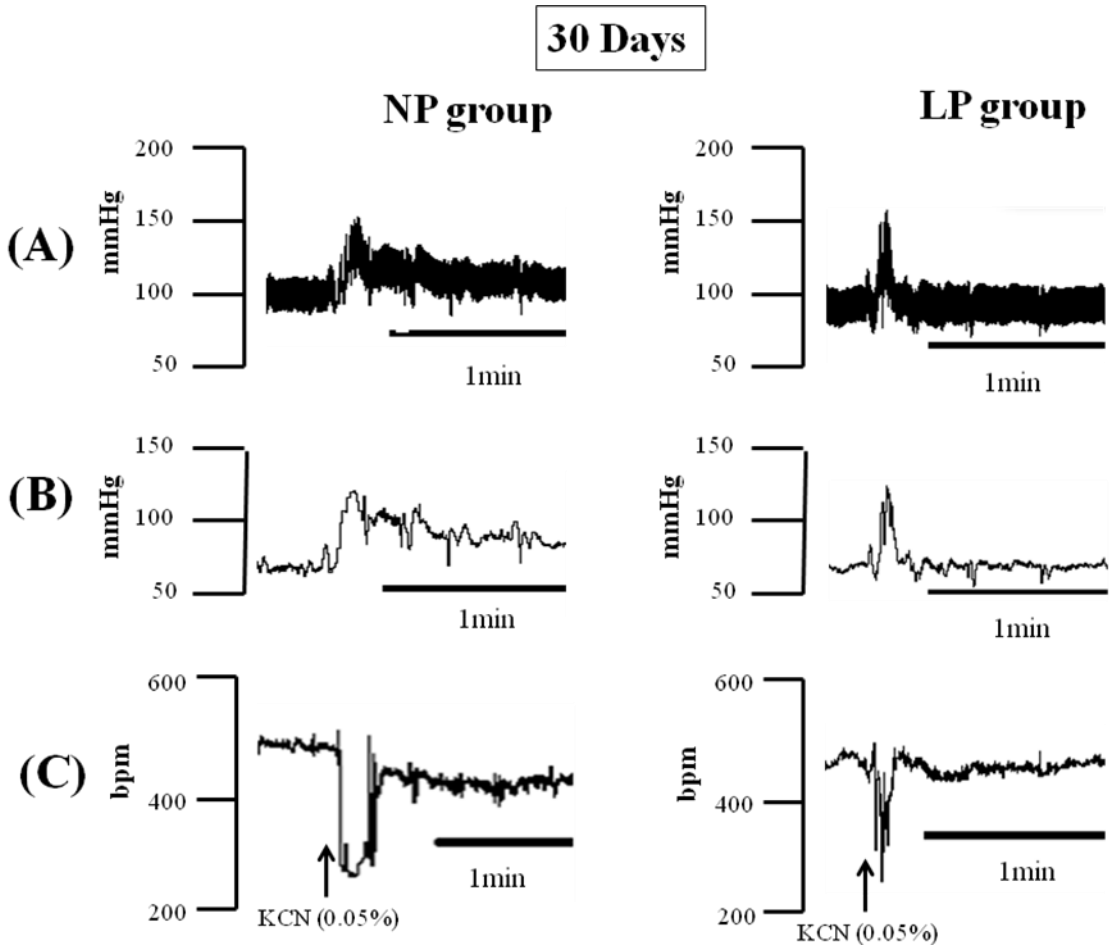

Figure 1. Representative tracings of rats at rest and during chemoreflex activation at 30 days old. Representative tracing of pulsate arterial pressure (PAP, panel A), mean arterial pressure (MAP, panel B) and heart rate (HR, panel C) at 30 days old of rats from dams submitted to normoproteic (NP, 17 % of protein) or low protein diet (LP, 8 % of protein) during pregnancy and lactation. All pups were fed standard chow from weaning.

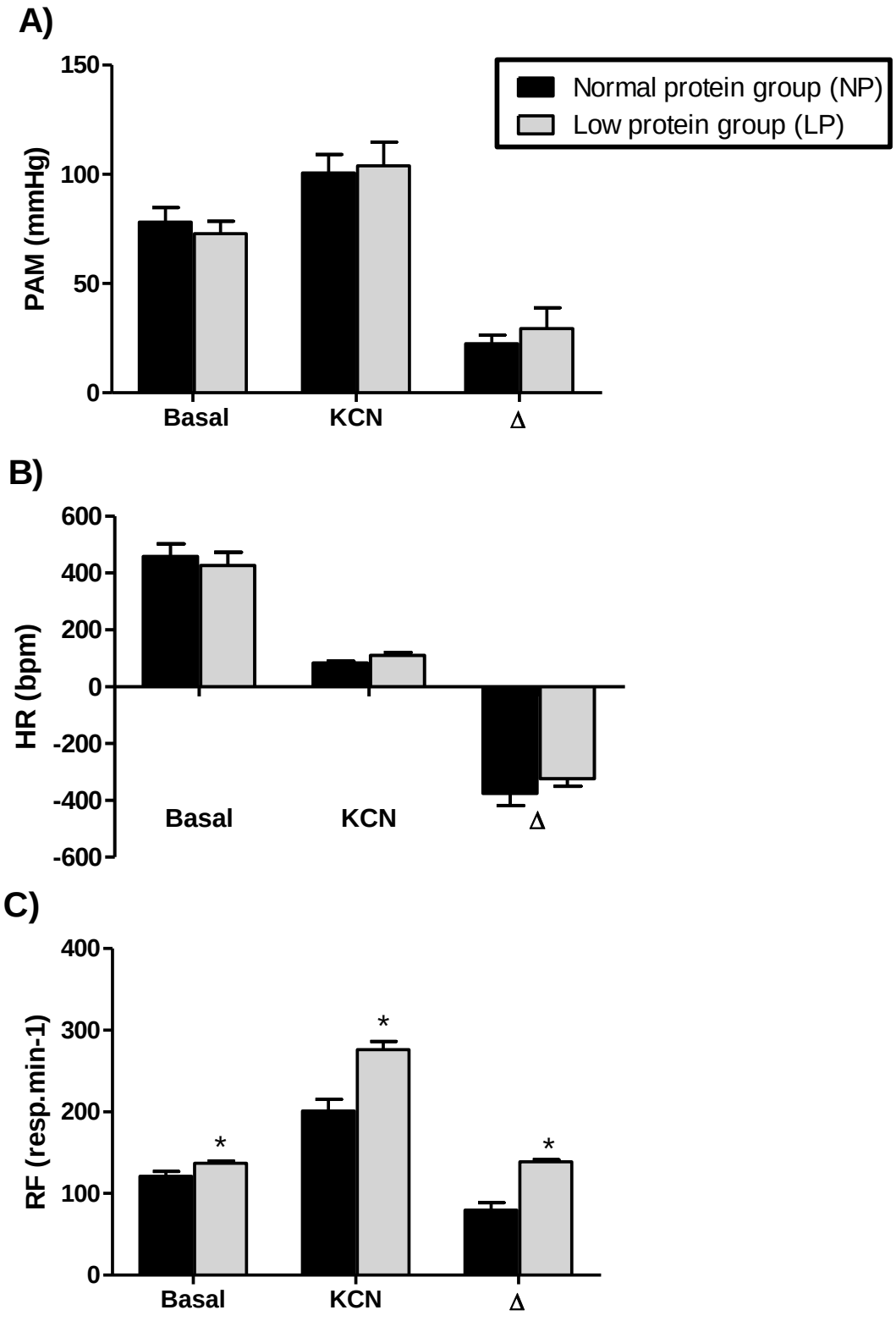

Figure 2. Low protein diet during pregnancy and lactation increased respiratory frequency during chemoreflex activation at 30 days old. Evaluation of mean arterial pressure (MAP, panel A), heart rate (HR, panel B) and respiratory frequency (RF, panel C) before (Basal) and during chemoreflex activation (KCN, 0.04%) in 30 days old rats

from dams submitted to normoproteic (NP, 17 % of protein, black bars, n=5) or low protein diet (LP, 8 % of protein, gray bars, n=5) during pregnancy and lactation. All pups were fed standard chow from weaning. *Statistically significant difference (P<0.05) assessed by Student's t–test.

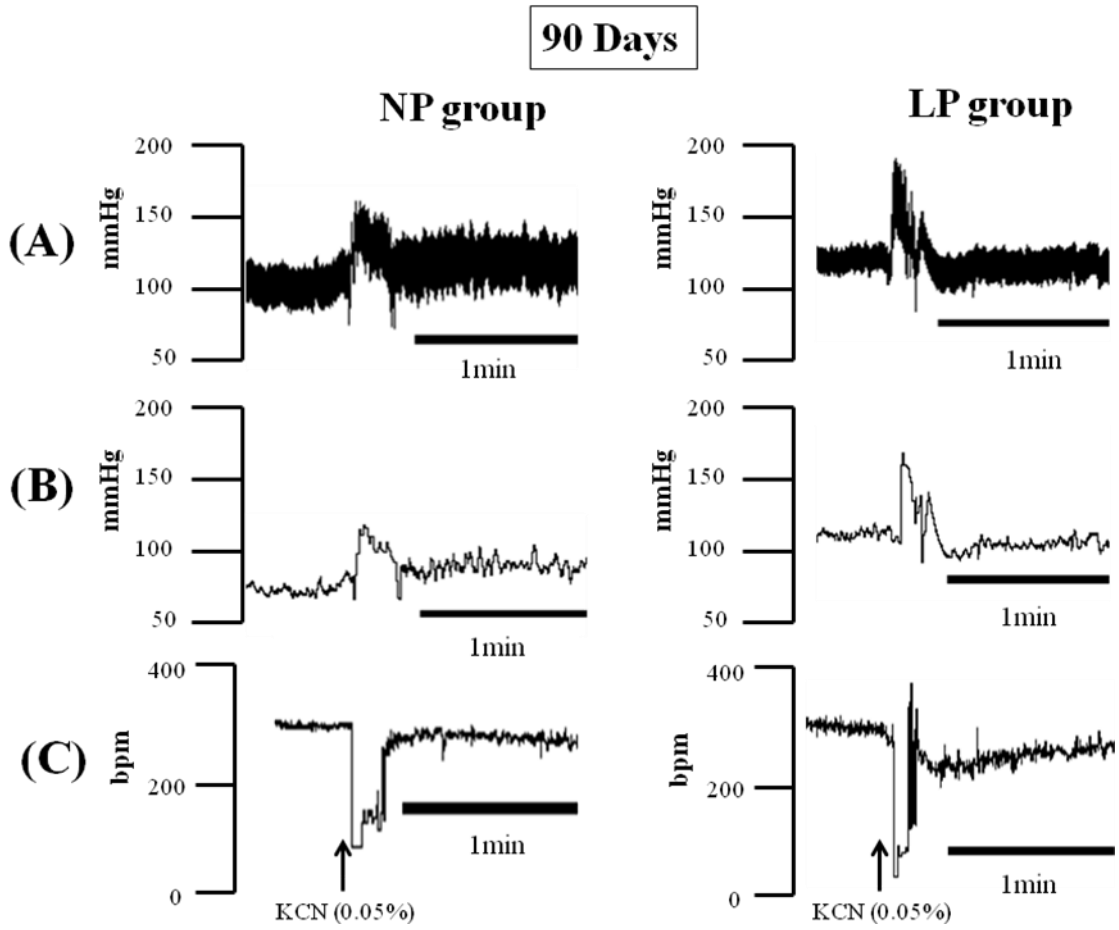

Figure 3. Representative tracings of rats at rest and during chemoreflex activation at 90 days old. Representative tracing of pulsate arterial pressure (PAP, panel A), mean arterial pressure (MAP, panel B) and heart rate (HR, panel C) at 90 days old of rats from dams submitted to normoproteic (NP, 17 % of protein) or low protein diet (LP, 8 % of protein) during pregnancy and lactation. All pups were fed standard chow from weaning.

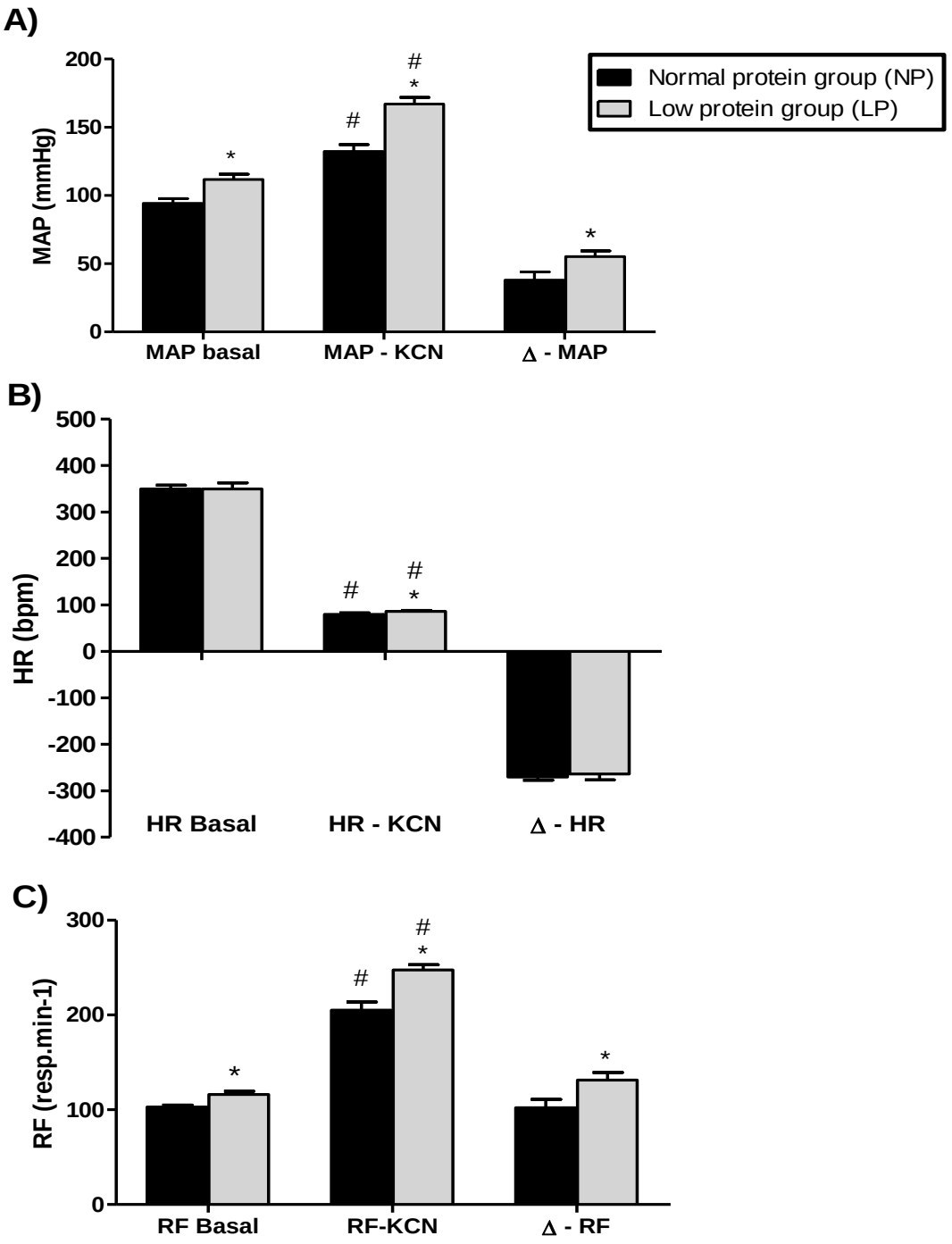

Figure 4. Low protein diet during pregnancy and lactation increases arterial blood pressure and respiratory frequency at rest and during chemoreflex activation, but change no change heart rate at 90 days old. Evaluation of mean arterial pressure (MAP, panel A), heart rate (HR, panel B) and respiratory frequency (RF, panel C) before (Basal) and during chemoreflex activation (KCN, 0.05%) in 90 days old rats from dams submitted to normoproteic (NP, 17 % of protein, black bars, n=8) or low protein diet (LP, 8 % of protein, gray bars, n=8) during pregnancy and lactation. All pups were fed standard chow from weaning. *Statistically significant difference

(P<0.05) assessed by Student's t–test and # indicates difference within group, assessed by paired Student's t-test.

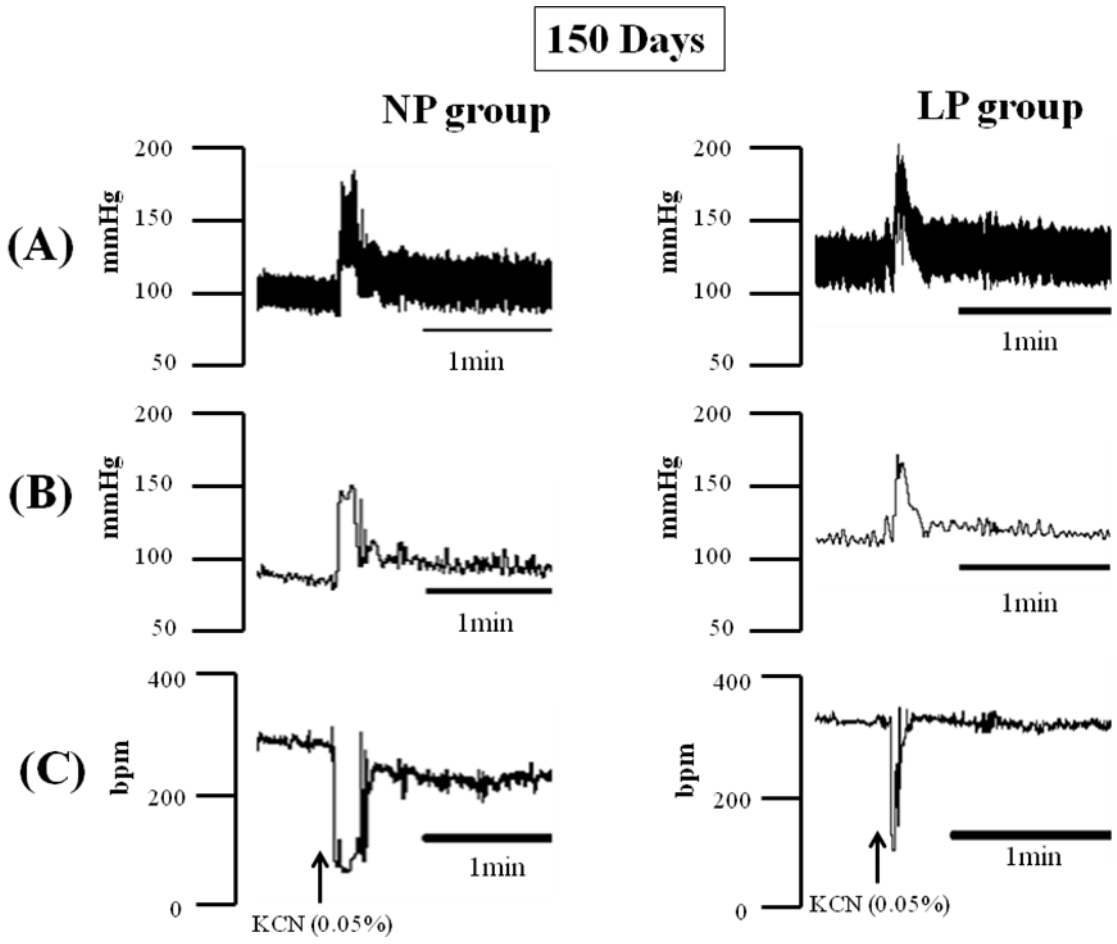

Figure 5. Representative tracings of rats at rest and during chemoreflex activation at 150 days old. Representative tracing of pulsate arterial pressure (PAP, panel A), mean arterial pressure (MAP, panel B) and heart rate (HR, panel C) at 150 days old of rats from dams submitted to normoproteic (NP, 17 % of protein) or low protein diet (LP, 8 % of protein) during pregnancy and lactation. All pups were fed standard chow from weaning.

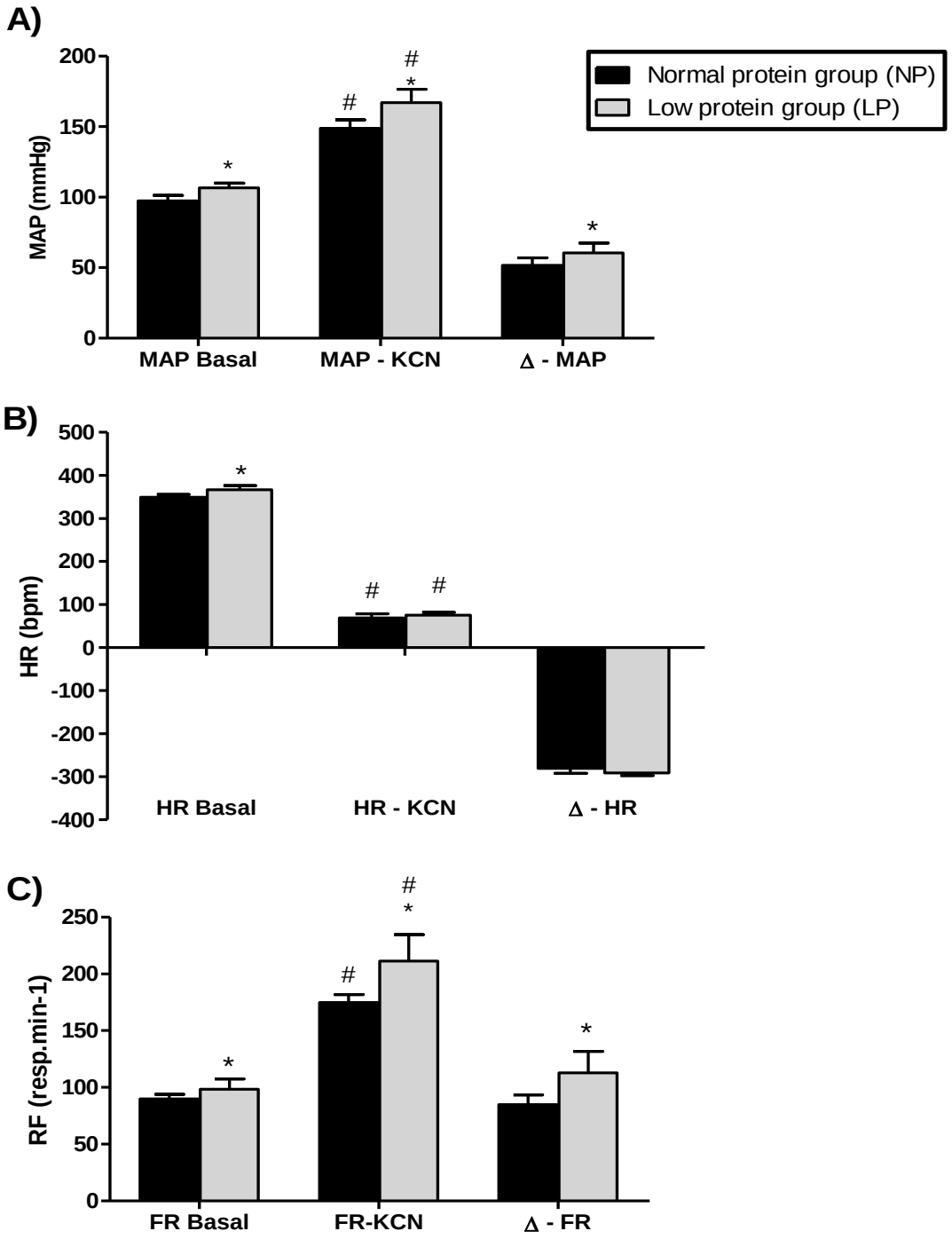

Figure 6. Low protein diet during pregnancy and lactation increases arterial blood pressure, respiratory frequency and heart rate at rest and during chemoreflex activation at 150 days old. Evaluation of mean arterial pressure (MAP, panel A), heart rate (HR, panel B) and respiratory frequency (RF, panel C) before (Basal) and during chemoreflex activation (KCN, 0.04%) in 150 days old rats from dams submitted to normoproteic (NP, 17 % of protein, black bars, n=7) or low protein diet (LP, 8 % of protein, gray bars, n=6) during pregnancy and lactation. All pups were fed standard

chow from weaning. *Statistically significant difference ($P<0.05$) assessed by Student's t–test and # indicates difference within group, assessed by paired Student's t-test.

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