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RATOS DESNUTRIDOS DURANTE O PERÍODO INTRAUTERINO OU APÓS O DESMAME APRSENTAM ALTERAÇÕES DA REATIVIDADE VASCULAR

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"Ratos desnutridos durante o período intrauterino ou após o desmame apresentam alterações da reatividade vascular"

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RESUMO

A desnutrição durante períodos críticos do desenvolvimento é uma das principais causas de várias alterações sistêmicas, predispondo o individuo ao desenvolvimento de diversas doenças, como a hipertensão arterial. Para avaliar os efeitos da desnutrição sobre alguns parâmetros hemodinâmicos e a reatividade vascular, ratos adultos machos, foram submetidos a dois tipos de dietas: padrão Labina® (23 % de proteína) e Dieta Básica Regional (DBR), hipoproteica (8% de proteína). Os animais foram divididos em 3 grupos: Controle (CT), alimentados com Labina®, em todas as fases de desenvolvimento; grupo desnutrido intra-útero (DIU), cuja mãe recebeu DBR, durante a gestação, nas demais fases do desenvolvimento, ela e sua prole foram alimentadas com Labina®; grupo desnutrido após o desmame (DPD), cuja matriz recebeu Labina®; nas fases de gestação e aleitamento e a prole, após desmame e idade adulta, DBR. Com três meses de idade foram mensurados *in vivo*: pressão artéria sistólica (PAS), pressão arterial diastólica (PAD), pressão arterial média (PAM) e a freqüência cardíaca (FC). A reatividade vascular foi avaliada *in vitro:* 1- no leito arterial caudal em resposta à fenilefrina (FE 0.001-300ug, *in bolus*), ao relaxamento a acetilcolina (ACh 10^{-10}-10^{-3} M) e ao nitroprussiato de sódio (NPS 10^{-9}-10^{-2} M), sob pré-contração com KCl (65 mM). 2- Em anéis de aorta, a reatividade à fenilefrina (FE, 10^{-10}- $3x10^{-4}$M) foi avaliada na presença e ausência do endotélio e para analisar os possíveis fatores endoteliais envolvidos no efeito da desnutrição, foram realizadas curvas de concentração-resposta à FE com: L-NAME (100 µM), Indometacina (INDO,10 µM), Tetraetilamônio (TEA, 2 mM) e Apocinina (APO, 0,3mM); também foram realizadas curvas concentração-resposta com ACh (10^{-11} – 10^{-4} M). A PAS, PAD, PAM foram maiores no grupo DPD quando comparado aos demais grupos. Na artéria caudal houve aumento da resposta máxima à PE e também aumento do relaxamento a ACh no grupo DPD quando comparado ao grupo CT, no grupo DIU essas respostas não foram modificadas. Em anéis de aorta a reatividade à FE não foi alterada no DPD e diminuiu no DIU. A remoção do endotélio aumentou a resposta à FE em todos os grupos sendo maior no DIU (E+ vc E- CT: 133,9 ± 28,19 vs DPD: 387,2 ± 68,02 vs DIU: 258,39 ± 61,91, $p<0,05$), O aumento da reatividade à FE após incubação com L-NAME foi maior nos grupos desnutridos (E+ vs L-NAME- CT: 91,00

± 14,22 vs DPD: 158,2 ± 23,93 vs DIU: 146 ± 43,58,p<0,05). A incubação com TEA aumentou a resposta à FE no DIU (%dAAC CT: 77,79±13,18 vs DPD: 88,95±24,86 vs DIU 156,27 ± 39,39, p<0,05). A APO reduziu a reatividade à FE em todos os grupos, embora o efeito tenha sido maior no grupo DPD (%dAAC CT: 15,03±6,32 vs DPD: 49,04±8,78 vs DIU: 41,60 ± 13,36,p<0,05). Nossos resultados demonstram que o aumento da pressão arterial (PA) no grupo DPD pode estar associado a um aumento de reatividade encontrado no leito arterial caudal. Embora esse efeito não tenha sido observado em segmentos isolados de aorta, encontrou-se um aumento de radicais livres que pode estar associado ao aumento da PA nesses animais. O aumento da liberação basal de NO na aorta e o aumento do relaxamento à ACh no leito caudal parecem ser mecanismos compensatórios ao aumento da PA e ao aumento de radicais livres. A desnutrição intra-uterina não é acompanhada de alterações da PA, mas promove aumento da liberação basal de NO e de um fator abridor para canais de potássio derivado do endotélio que conseqüentemente reduzem a reatividade à FE.

Palavras chaves: Desnutrição, pressão arterial, disfunção endotelial e estresse oxidativo.

ABSTRACT

Undernutrition during critical periods of development is a major cause of several systemic alterations, predisposing the individual to the development of various diseases such as hypertension. To evaluate the effects of undernutrition on some hemodynamic parameters and vascular reactivity, adult male rats, were submitted to two types of diets: Standard Labina® (protein content of 23%) and a Regional Basic Diet (RBD) (protein content 8%), that reproduces in the rat a type of protein undernutrition found in some humans of Northeastern Brazil. The animals were divided into 3 groups: Control (CT), fed with Labina®, in all stages of development; undernourished intrauterine (UIU), whose mother received DBR during pregnancy, and in the remaining stages of development, she and her offspring were fed Labina®; undernourished after weaning (UAW), whose mother received Labina® in stages of gestation and breastfeeding and the offspring after weaning and adulthood with RBD. After three months were measured directly *in vivo:* systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR).The vascular reactivity was evaluated *in vitro:* -1 in the tail arterial bed, in response to phenylephrine (PHE 0.001-300ug, in bolus) relaxation to acetylcholine (ACh 10^{-10} -10^{-3} M) and sodium nitroprusside (SNP 10^{-9} -10^{-2} M), under pre-contraction with potassium chloride (KCl 65 mM). 2- in aortic rings, where the reactivity to PHE (10^{-10}- $3x10^{-4}$M) was evaluated in the presence and absence of endothelium and to analyze the possible endothelial factors involved in the effect of underlnutrition, there were curve-response to FE with: L-NAME (100 μM), indomethacin (INDO 10 μM), tetraethylammonium (TEA, 2 mM), apocynin (APO 0.3mM). Concentration-response curves to ACh (10-11 – 10-4 M) were also carried out. The SBP, DBP, MAP and HR were higher in UAW when compared to other groups. In the study of vascular reactivity in the caudal artery, increased maximal response to PHE and also increased the a Ach relaxation in the UAW group when compared to CT .In the UIU group this response was not modified. In aortic rings the reactivity to PHE was not altered in the UAW and decreased in the UIU. Removal of endothelium increased the response to PHE in all groups was higher in UIU (E+ VS E- CT: 133.9 ± 28.19 vs UAW: 387.2 ±

68.02 vs UIU: 258.39 ± 61.91,p>0.05), The increase reactivity to PHE after incubation with L-NAME was higher in the undernourished groups.) (E+ vs L-NAME- CT: 91,00 ± 14.22 vs UAW: 158.2 ± 23.93 vs UIU: 146 ± 43.58;p<0.05). TEA increased the response to PHE in the UIU group (dAUC% CT: 77.79±13.8 vs UAW: 88.95±24.86 vs UIU 156.27 ± 39.39,p<0.05). APO reduced the reactivity to PHE in all groups, although the effect was greater in the UAW group (dAUC% CT: 15,03±6,32 vs UAW: 49.04±8.78 vs UIU: 41.60 ± 13.36; p<0.05). Our results demonstrate that the increase in the blood pressure (BP) UAW group may be associated with an increased reactivity found in the tail arterial bed. Although this effect was not observed in isolated segments of aorta, we found an increase of free radicals that may be associated with increased BP in these animals The increased basal release of NO in the aorta and increased relaxation to ACh in the flow bed appear to be compensatory mechanisms to increase BP and the increase in free radical. Intrauterine undernutrition is not accompanied by changes in BP, but further increase in basal release of NO and a factor for opening of potassium channels derived from the endothelium which consequently reduces the response to PHE.

Keywords: Undernutrition , blood pressure, endothelial dysfunction and oxidative stress.

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1. INTRODUÇÃO

A nutrição adequada, em qualidade e quantidade, é fundamental para o crescimento e desenvolvimento dos seres vivos inclusive do homem. Por outro lado, deficiências nutricionais, decorrentes do menor aporte ou ausência de macro e micronutrientes na alimentação, levam a estados de má nutrição e desnutrição cujos graus dependem do tipo da dieta, do período da vida em que foi consumida e da extensão temporal do consumo, o que pode causar efeitos lesivos, reversíveis ou não (Morgane *et al*.,1992). Segundo Andrade *(*1988*),* a desnutrição não se constitui em um fato isolado, mas no efeito da ação recíproca de fatores socioeconômicos, políticos, culturais e ambientais os quais atingem com maior intensidade as crianças que vivem em situações de extrema pobreza.

Existem dois conceitos quando se trata de quantidades diárias de energia e nutrientes considerados essenciais para o ser humano: “requerimentos mínimos” e “recomendações nutricionais”. Requerimentos mínimos representam as menores quantidades de um dado nutriente que devem ser consumidas diariamente através dos alimentos, quantidades estas suficientes para promover a saúde e prevenir as manifestações patológicas derivadas da carência deste nutriente, em particular. Já o conceito de recomendações nutricionais é bem mais abrangente, incluindo, as quantidades definidas como requerimentos mínimos e mais uma quantidade adicional, objetivando compensar as flutuações biológicas normais (Nóbrega, 1999).

Os riscos nutricionais de diferentes categorias e magnitudes permeiam todo o ciclo da vida humana, desde a concepção até a senectude do processo saúde/doença de cada população (MS, 2006). Desta forma, é importante planejar e iniciar estratégias, sobretudo para evitar e, também, para tratar a má nutrição e desnutrição. Para tal, as políticas de saúde alimentar devem adotar medidas direcionadas às prioridades regionais e à assistência da população, fundamentadas em resultados de pesquisa científica.

A desnutrição tem como causas diversos fatores, normalmente associados à pobreza e a falta de alimentos dela decorrente. Está relacionada a condições mínimas de existência. A desnutrição calórico-protéica se caracteriza pela existência de um desequilíbrio celular entre o fornecimento de nutrientes e energia por um lado, e por

outro, a demanda corporal para assegurar o crescimento, manutenção e funções específicas do organismo (Serapião, 1986).

Segundo a Organização das Nações Unidas, a má nutrição engloba um grupo de condições patológicas que resulta da falta concomitante de calorias e proteínas. A má nutrição calórico-protéica pode alterar a função de uma série de órgãos e tecidos (Cicogna *et al*., 1999 e 2001), tendo em vista que as proteínas são componentes importantes para a estrutura e função específicas das células do organismo (Jeor *et al*., 2001).

Dados da Organização Mundial de Saúde (OMS) revelam que, no mundo, aproximadamente 33% (182 milhões) de crianças com menos de cinco anos de idade estavam desnutridas no ano 2000. No Brasil, esse índice foi estimado em 10,5%. Já entre aquelas vivendo em áreas rurais no Nordeste, a taxa foi de 39,8%. A desnutrição energético-protéica (DEP) continua a ser a doença que mais causa mortes em crianças em todo o mundo.

Estudos realizados para avaliar os efeitos da má nutrição intra-uterina sobre os níveis de pressão arterial (PA), têm produzido resultados contraditórios. Langley-Evans *et al*., (1998), ao estudar ratos submetidos à dieta hipoprotéica, durante o desenvolvimento intra-uterino, demonstraram que os animais apresentavam baixo peso corporal ao nascimento e pressão arterial sistólica (PAS) elevada. Ratos filhos de mães normotensas, expostas a uma dieta de baixo teor protéico durante a gestação, apresentaram diminuição do peso corporal ao nascer, e elevação da PA, detectada na quarta semana de vida, o que permanecia até a vida adulta (Langley-Evans e Jackson, 1995; Woodall *et al*., 1996; Pérez *et al*., 2002). Entretanto, Tonkiss *et al*., (1998), observaram em ratos normotensos e hipertensos, submetidos à dieta hipoprotéica, durante a vida perinatal e vida adulta, que os níveis pressóricos do grupo normotenso não se alteraram, mas houve redução da PA no grupo hipertenso.

A má nutrição no período intra-uterino advém do aporte materno inadequado de nutrientes ou de fatores intrínsecos como deficiência no transporte destes nutrientes através da placenta, o que produz alterações observadas ao nascimento, bem como no desenvolvimento pós-natal, sugerindo que a má nutrição induz modificações na organização e no funcionamento de vários sistemas (Goldberg e Prentice, 1994).

Estudos realizados em humanos demonstraram a existência de uma relação entre a má nutrição fetal e incidência de doenças crônicas na vida adulta (Scrimshaw, 1997; Sloan *et al*., 2001), pontuando uma associação entre retardo do crescimento fetal, conseqüência da má nutrição no útero, com o desenvolvimento da hipertensão arterial sistêmica (HAS) (Eriksson *et al*., 2000) e doenças coronarianas na idade adulta (Leistikow, 1998).

1.1. Desnutrição e o Sistema Cardiovascular

As doenças cardiovasculares constituem um grave problema de saúde pública e atualmente vem se agravando. Estudos epidemiológicos associam as doenças cardiovasculares, a hipertensão arterial e o diabetes, ao retardo no desenvolvimento intra-uterino. Os experimentos com animais tem revelado mecanismos que programam estas doenças; a reatividade vascular vem emergindo como uma peça chave nessa programação. Vários estudos vem confirmando a relação entre doenças cardiovasculares e a restrição protéica *in útero* (Nwagwu, Cook e Langley-Evans, 2000).

Vários estudos demonstram que a restrição nutricional materna causa baixo peso no nascimento (Barker e Clark, 1997; Falkner, 2002; Lackland *et al*., 2003; Holemans *et al*., 2003), com desenvolvimento inadequado de órgãos como o pâncreas (Garofano *et al*., 1998) e o rim (Lucas *et al*., 1997), ao contrário do cérebro e pulmões que são relativamente protegidos (Desai e Hales, 1997). Essas alterações na organogênese predispõem a prole, adulta ao desenvolvimento de hipertensão (Falkner, 2002) e de doenças cardiovasculares (Barker *et al*., 1993).

A “Thrifty Phenotype Hypothesis”, proposta por Hales e Barker em 1992, postula que o diabetes tipo 2 e outras anormalidades metabólicas, como a síndrome X, possuem uma forte influência ambiental. A programação da organogênese é influenciada pelo ambiente nutricional fetal e pós-natal e pode determinar, como já foi relatada, a susceptibilidade do indivíduo ao desenvolvimento de alterações metabólicas e doenças cardiovasculares na vida adulta.

A desnutrição calórico-protéica pode desencadear diversas manifestações cardiovasculares dentre elas hipotermia, secundária ao baixo rendimento cardíaco pela diminuição do metabolismo total (Talner, 1990).

Investigação realizadas por Saraiva *et al.,* (1992) em crianças procedentes de favelas recifenses ou da Zona da Mata de Pernambuco com DCP, demonstraram que dos estudos eletrocardiográficos realizados, 95% dos enfermos apresentavam alteração na repolarização ventricular e baixa voltagem do QRS no plano frontal e o estudo ecocardiográfico apresentou diminuição da massa estimada para o ventrículo esquerdo entre outros achados. Cunha *et al.,* (1998),estudaram os efeitos da subnutrição energético-protéica na morfologia do miocárdio e observaram existir menor espessura dos cardiomiócitos nos indivíduos desnutridos.

A DEP também pode causar danos ao sistema cardiocirculatório em animais de experimentação (Cicogna *et al*., 1999). Pissaia *et al*., (1990) ao estudar os efeitos experimentais da desnutrição calórica protéica sobre o coração de ratos, concluíram que esta induz alterações morfológicas e eletrofisiológicas, havendo também aumento dos níveis circulantes de catecolaminas que têm importantes efeitos inotrópicos e metabólicos sobre o coração.

Segundo Langley-Evans *et al*., (1994) ratos submetidos a dietas hipoproteícas durante a gestação apresentavam alterações no crescimento e desenvolvimento fetal e, posteriormente, desenvolviam significativa hipertensão arterial.

O miocárdio ventricular tem sido um dos principais alvos das alterações provenientes da restrição alimentar. Alguns autores têm demonstrado que a restrição alimentar promove redução da distensibilidade ventricular e da função cardíaca (Cicogna *et al*., 1999 e 2001), diminuição da complacência do ventrículo esquerdo, redução da contratilidade do miocárdio e remodelamento ventricular excêntrica (Abel *et al*., 1979; Okoshi *et al*., 2002). Essas alterações provavelmente conduzem a um prejuízo da função sistólica do ventrículo esquerdo (Okoshi *et al*., 2002), disfunção miocárdica e alterações na função ventricular (Kyger *et al*., 1978; Mcknight *et al*., 1999). Entretanto, alguns autores observaram que, em preparação de coração isolado, a função sistólica permanece preservada (Klebanov *et al*., 1997; Nutter *et al*., 1979).

Estudos realizados em nosso laboratório com ratos desnutridos, mostraram alterações histológicas nos ventrículos, caracterizadas por células em apoptose,

necrose, e sinais de processos inflamatórios, esses achados histológicos também são observados na insuficiência cardíaca congestiva (Sant'Helena, 2009).

Embora as modificações miocárdicas tenham valor expressivo na função cardíaca, outras alterações tais como, o aumento da concentração de catecolaminas no plasma e na fibra miocárdica, encontradas em muitos estudos com protocolo de má nutrição, sugerem que, o estresse nutricional e a exposição continuada do coração aos níveis elevados de catecolaminas, causam danos à contratilidade miocárdica e às membranas celulares (Rossi *et al*., 1980; Pissaia *et al*., 1990; Davis e Johnston, 1990).

O sistema cardiovascular em situações de estresse, responde com um aumento da PA e da freqüência cardíaca (FC), pela estimulação do eixo-hipotálamo-hipofise-adrenal, e liberação hormonal de catecolaminas (Fazaa *et al.,* 1999). No entanto, os resultados das pesquisas realizadas sobre os efeitos da má nutrição sobre a biossíntese e liberação de catecolaminas são conflitantes. Alguns autores evidenciaram no período de má nutrição um aumento dos níveis de catecolaminas circulantes (Nutter *et al*., 1979; Pissaia *et al*., 1980; Kim *et al.,* 1994). Embora outros, em contraste, observaram uma diminuição na síntese de noradrenalina em ratos submetidos à má nutrição no período pré e pós-natal (Marichich *et al*., 1979)

A adaptação do coração, quando submetido à má nutrição crônica, recebe influências dos sistemas hormonal e autonômico. Além disso, alterações morfológicas, que podem estar associadas ou não a essas influências, também repercutem na sua função (Fazaa *et al*., 1999).

Hu *et al*., (2000), estudando ratos submetidos à privação protéica, no período intra-uterino, nos quais o comportamento hemodinâmico foi avaliado nos diferentes estágios de desenvolvimento, de adulto jovem à idade senil, demonstraram retardo no crescimento associado com aumento da pressão arterial diastólica (PAD), além de predisposição ao aparecimento de arritmias cardíacas, mais evidentes nos ratos mais velhos.

Em humanos, a alimentação pobre em teores protéicos e calóricos pode acarretar em perda de peso (Jeor *et al*., 2001), bradicardia, redução da contratilidade miocárdica, insuficiência cardíaca (Olubodun, 1992), e em animais, redução da

distensibilidade ventricular e da função cardíaca (Cicogna *et al*., 1999 e 2000; Okoshi *et al*., 2002).

1.2. Desnutrição e a Modulação Endotelial

O endotélio vascular é capaz de produzir e liberar substâncias vasodilatadoras e vasoconstritoras e por isso interfere diretamente no tônus vascular e, portanto, no controle da resistência vascular (Rubanyi, 1993). Dentre as substâncias que promovem o relaxamento do músculo liso vascular incluem a prostaciclina (PGI_2), o óxido nítrico (NO) e o fator hiperpolarizante derivado do endotélio (EDHF) (Moncada *et al*, 1977; Furchgott & Zawadski, 1980; Palmer *et al*, 1987; Félétou & Vanhoutte, 1988). Já entre aquelas capazes de promover contração do músculo liso vascular podemos citar a angiotensina II, a endotelina-1 e os metabólitos derivados da via do ácido araquidônico como tromboxano A_2 (TXA_2), prostaglandinas H_2 (PGH_2) e $F_{2\alpha}$ ($PGF_{2\alpha}$), além do ânion superoxido (O_2^-) (Rubanyi & Vanhoutte, 1986; Frolich & Forstermann, 1989; Vanhoutte, 1993).

Muitas dessas substâncias regulam o crescimento e a apoptose das células musculares lisas, além de interferirem também na agregação plaquetária e a adesão de leucócitos (Moncada *et al*, 1977; Moncada *et al*, 1991; Rubanyi, 1993). Deste modo, o endotélio possui uma participação essencial e ativa no controle e manutenção da PA. Nesse sentido, é fácil compreender que modificações na sua função, como, por exemplo, aumento da síntese e/ou liberação de fatores vasoconstritores ou fatores de crescimento podem promover aumento de resistência vascular e conseqüentemente, da PA. Modificações desse tipo já foram observadas por muitos pesquisadores nos processos hipertensivos (Taddei *et al*, 1993; Rizzoni *et al*, 1996; Rossi *et al*, 1997).

As alterações decorrentes da disfunção endotelial no processo hipertensivo são multifatoriais e em muitos casos, parecem depender do tipo de hipertensão desenvolvida bem como de sua duração e do leito vascular estudado.

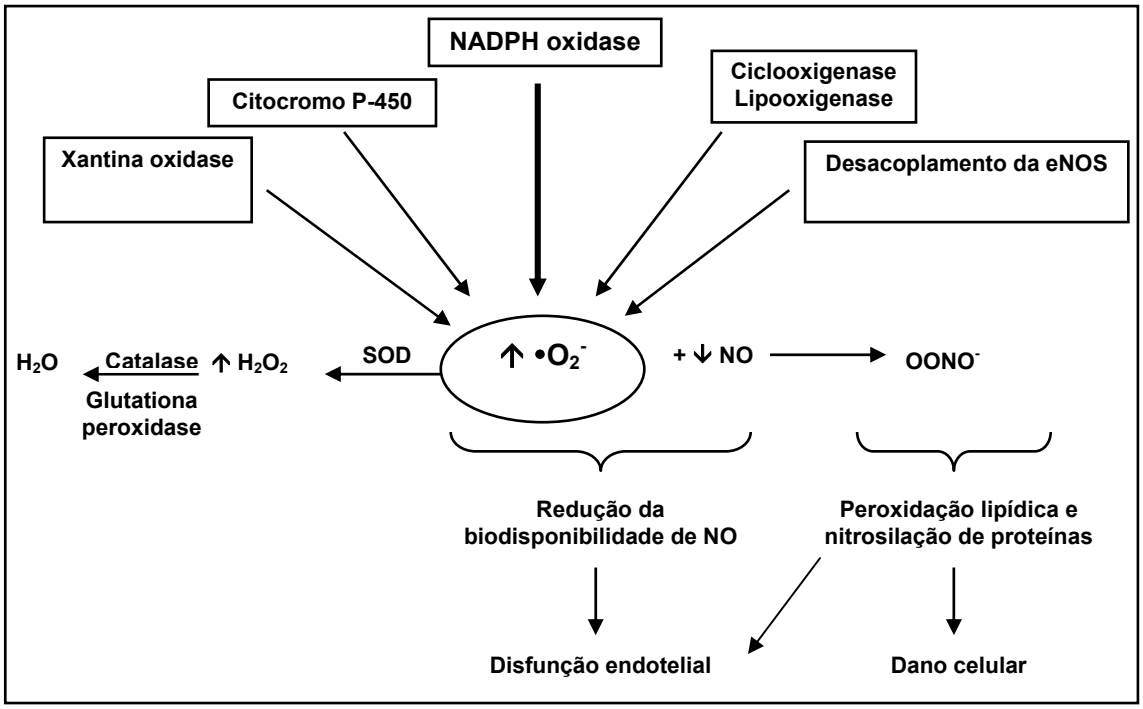
Um dos fatores que parece estar implicado são alterações no relaxamento dependente do endotélio. O NO tem se destacado com um dos mais importantes vasodilatadores liberado pelo endotélio, além de inibir fatores de crescimento e inflamatório, e atuar como anti-agregador plaquetário.

Franco *et al*., (2003), vem mostrando em seus trabalhos um aumento nas evidencias que sugerem uma associação entre o baixo peso ao nascer, o estresse oxidativo e a disfunção endotelial. E associa a redução do vasorelaxamento, dependente do endotélio, em parte devido à perda de NO (da biodisponibilidade do NO), ocorre na maioria dos casos de hipertensão crônica. Desse modo a privação nutricional intra-uterina tem sido associada com risco aumentado de hipertensão e acidente vascular cerebral, associada à disfunção vascular e a diminuição do relaxamento dependente do endotélio (Lamireau, *et al.,* 2002).

Vários pesquisadores tem demonstrado que a desnutrição é acompanhada de aumento da pressão arterial, e esse aumento se deve a modificação na função endotelial, (Goodfellow *et al*., 1998; Franco *et al*.,2003). Alguns trabalhos demonstram uma redução da síntese de NO na hipertensão, há também evidencias de aumento desse vasodilatador como mecanismo compensatório (Alexander *et al*, 1999; Rossoni *et al*, 2002b; Chang *et al*, 2002). No entanto, há pesquisadores que relatam que a síntese e liberação de NO parecem não ser alteradas na hipertensão, porém sua biodisponibilidade é reduzida devido ao aumento da formação de ânions superóxidos, que inativam o óxido nítrico, reduzindo sua ação vasorelaxante e promovendo vasoconstricção (Gryglewski *et al*, 1986; Suzuki *et al*, 1995). Entretanto, a disfunção endotelial resulta também de outras alterações além daquelas apresentadas na via do NO. O aumento do estresse oxidativo já está bem estabelecido como um fator presente na hipertensão arterial.

Na hipertensão arterial, o estresse oxidativo é caracterizado, por aumento de espécies reativas de oxigênio (ROS) principalmente, ânion superóxido ($^{\bullet}O_2^-$), originados a partir de NAD(P)H oxidases que catalizam a redução de 1 elétron de oxigênio usando NADH ou NAD(P)H como doadores de elétrons, além dos radicais hidroxila ($OH^{\cdot}$), peroxinitrito ($ONOO^-$), dentre outros, associado ainda com redução das defesas antioxidantes (Suzuki *et al*, 1995; Hamilton *et al*, 2001). O ânion superóxido ao se combinar com o NO, forma o peroxinitrito (esquema 1), que tem alta capacidade oxidativa (Beckman *et al*, 1990). Portanto, esse aumento de ROS poderia reduzir a biodisponibilidade de NO, contribuindo para a disfunção endotelial. Outros trabalhos que sustentam esse mecanismo são aqueles desenvolvidos com ratos hipertensos tratados com antioxidantes. Nesses ratos, o tratamento com análogos da

superóxido dismutase (SOD), tempol e vitaminas C e E, promovem redução da pressão arterial e melhora do relaxamento endotélio dependente (Chen *et al*, 2001; Yanes *et al*, 2005).



Esquema 1. Geração de espécies reativas de oxigênio (ROS). Um aumento na produção de ânions superóxidos ($^{\bullet}O_2^-$), principalmente devido à ativação da NAD(P)H oxidase, está envolvido na disfunção endotelial por redução da biodisponibilidade de óxido nítrico (NO) e aumento de peroxinitrito ($ONOO^-$). Adaptado de Fortuño *et al*, 2005.

Usando um modelo de desnutrição com restrição de 50% a ingestão dietética, Franco et al. (2002a, 2003) mostrou que a desnutrição intra-uterina além de retardar o crescimento fetal, induz à hipertensão e a disfunção endotelial, devido ao aumento do estresse oxidativo na prole, pois este é caracterizado por aumento na geração de superóxido devido à ativação da NADPH oxidase e redução das defesas antioxidantes. Estes fatores sugerem uma explicação potencial para a disfunção endotelial e o desenvolvimento de doenças cardiovasculares na vida adulta.

Em seus estudos do efeito a longo prazo da desnutrição intra-uterina sobre a função vascular na prole do sexo feminino, Franco, *et al.,* (2007) fornece evidencias de que após a desnutrição intra-uterina, a prole feminina apresenta um aumento da produção de superóxido o que é, pelo menos em parte, responsável pela disfunção endotelial observada nestes animais. Esses autores mostraram também, que a inibição da NADPH oxidase normaliza a geração de superóxido, melhorando o

relaxamento dependente do endotélio, sugerindo um importante papel desta enzima oxidativa nas alterações vasculares e, conseqüentemente, no desenvolvimento da hipertensão e outras doenças cardiovasculares.

Trabalhos demonstram que a angiotensina II, via receptores AT1, leva a produção de ROS na parede vascular em parte devido à ativação da NAD(P)H oxidase (Rajagopalan *et al*, 1996; Touyz & Schiffrin, 2001). Somado a isso, tanto as ROS quanto a angiotensina estimulam a produção de fatores de crescimento e outros agentes pró-inflamatórios que, em conjunto, promovem disfunção endotelial

Para Caravalo, *et al*., (2007) a privação severa de nutrientes *in utero* induz elevação nos níveis de pressão arterial e alterações da reatividade vascular como: prejuízo do relaxamento dependente do endotélio e aumento da resposta vascular à Angiotensina II. Vieira-Filho *et al*., (2009), usando um modelo de desnutrição idêntico ao usado no presente trabalho sugere uma correlação entre desnutrição materna, aumento do estresse oxidativo placentário, atividade anormal de transportadores renal de Na^+ e perturbação na sinalização da Angiotensina II, o que poderia contribuir mais tarde na programação de distúrbios na mobilização renal de Na^+ e no controle da PA como conseqüências específicas do crescimento intra-uterino prejudicado.

Lamireau, *et al.,* (2002), demonstram que o prejuízo na vasodilatação prejudicada, mediada pelo NO, em um modelo de programação fetal da hipertensão pela privação de proteína, não está relacionada com alterações na produção de NO, mas parece envolver disfunção das células musculares lisas, (como redução na expressão da guanilato ciclase solúvel e nos níveis de GMPc com expressão e função normal da NOS). É um mecanismo raramente descrito, associada com hipertensão arterial crônica e pode fornecer uma explicação fisiopatológica para a disfunção do relaxamento vascular em indivíduos previamente expostos à deficiência de nutrientes *in útero.*

Strufaldi *et al*., (2009), estudaram a associação inversa entre peso ao nascer e níveis de PA que parece ser programados durante a vida fetal, enquanto o ganho de peso durante a infância contribui para esse risco. Esse estudo veio confirmar e ampliar os resultados de vários estudos entre crianças, mostrando que os maiores níveis de pressão sanguínea foram encontrados em crianças que tinham baixo peso ao nascer, e as que na infância tinham ganho de peso acelerado aumenta este risco. Para Gupta

et al., (2004), a desnutrição intra-uterina está associada com o estresse oxidativo em recém-nascidos a termo de pequena idade gestacional de mães desnutridas.

Vários protocolos utilizando dietas quantitativas de composições diferentes têm sido propostos para o estudo dos efeitos da desnutrição ou da deficiência de determinados nutrientes na formação, desenvolvimento e manutenção das funções orgânicas (Hu *et al.,* 2000; Cicogna *et al*., 2001; Franco *et al*., 2002a, 200b, 2003, 2007).

Dados levantados mostram que em algumas áreas do Nordeste do Brasil, mais de 20% das crianças sofrem de segundo ou terceiro grau de desnutrição (Teodósio *et al*., 1990). Embora existam vários estudos sobre a desnutrição e o sistema cardiovascular, as repercussões da desnutrição prevalente no nordeste, sobre este sistema tem sido pouco estudado. Para estudar esse tipo de desnutrição Teodósio *et al* (1990) baseado em uma enquete alimentar desenvolveram um modelo experimental que reproduz no rato quadro muito semelhante ao da desnutrição protéico energética prevalente no Nordeste brasileiro.

Levando-se em consideração que a dieta básica regional (DBR), expressa o consumo básico diário de uma significativa parcela da população da zona da Mata de Pernambuco, a importância deste estudo, realizado em ratos desnutridos, será demonstrar possíveis relações entre essa dieta e alterações cardiovasculares. Assim, pretendemos avaliar no presente trabalho as repercussões da DBR em ratos submetidos a mesma, na vida intra-uterina e após o desmame, sobre alguns parâmetros hemodinâmicos e a reatividade vascular que foi estudada no leito arterial caudal, que se comporta como vaso de resistência e na aorta que tem características de vaso de condutância. Os resultados serão comparados com ratos normais submetidos a dieta padrão Labina®.

2. OBJETIVOS

2.1 – Gerais

Avaliar os efeitos da desnutrição (modelo DBR) em dois estágios de desenvolvimento (intra-útero e após o desmame) sobre a reatividade vascular e pressão arterial de ratos jovens.

2.2 – Específicos

- Avaliar o impacto da desnutrição sobre a reatividade vascular em vaso de resistência, no leito arterial caudal *in vitro*, de ratos jovens desnutridos comparando aos normonutridos
- Estudar a reatividade vascular e a modulação endotelial em vaso de condutância, aorta isolada de ratos jovens desnutridos, comparando aos normonutridos.
- Estudar a participação do NO, prostanóides, EDHF e espécies reativas do oxigênio na reatividade vascular de segmentos isolados de aorta de ratos desnutridos comparando aos controles.

3. ARTIGO A SER SUBMETIDO AO THE AMERICAN JOURNAL OF CLINICAL NUTRITION

UNDERNOURISHED RATS DURING THE INTRAUTERINE PERIOD OR AFTER WEANING APRSENTAM CHANGES IN VASCULAR REACTIVITY

ABSTRACT

Undernutrition during critical periods of development is a major cause of several systemic alterations, predisposing the individual to the development of various diseases such as hypertension. To evaluate the effects of undernutrition on some hemodynamic parameters and vascular reactivity, adult male rats, were submitted to two types of diets: Standard Labina® (protein content of 23%) and a Regional Basic Diet (RBD) (protein content 8%), that reproduces in the rat a type of protein undernutrition found in some humans of Northeastern Brazil. The animals were divided into 3 groups: Control (CT), fed with Labina®, in all stages of development; undernourished intrauterine (UIU), whose mother received DBR during pregnancy, and in the remaining stages of development, she and her offspring were fed Labina®; undernourished after weaning (UAW), whose mother received Labina® in stages of gestation and breastfeeding and the offspring after weaning and adulthood with RBD. After three months were measured directly *in vivo:* systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR).The vascular reactivity was evaluated *in vitro:* -1 in the tail arterial bed, in response to phenylephrine (PHE 0.001-300ug, in bolus) relaxation to acetylcholine (ACh 10^{-10} -10^{-3} M) and sodium nitroprusside (SNP 10^{-9} -10^{-2} M), under pre-contraction with potassium chloride (KCl 65 mM). 2- in aortic rings, where the reactivity to PHE (10^{-10}- $3x10^{-4}$M) was evaluated in the presence and absence of endothelium and to analyze the possible endothelial factors involved in the effect of undernutrition, there were curve-response to FE with: L-NAME (100 μM), indomethacin (INDO 10 μM), tetraethylammonium (TEA, 2 mM), apocynin (APO 0.3mM). Concentration-response curves to ACh (10-11 – 10-4 M) were also carried out. The SBP, DBP, MAP and HR were higher in UAW when compared to other groups. In the study of vascular reactivity in the caudal artery, increased maximal response to PHE and also increased the a Ach relaxation in the UAW group when compared to CT .In the UIU group this response was not modified. In aortic rings the reactivity to PHE was not altered in the UAW and decreased in the UIU. Removal of endothelium increased the response to PHE in all groups was higher in UIU (E+ VS E- CT: 133.9 ± 28.19 vs UAW: 387.2 ± 68.02 vs UIU: 258.39 ± 61.91,p>0.05), The increase reactivity to PHE after incubation

with L-NAME was higher in the undernourished groups.) (E+ vs L-NAME- CT: 91,00 ± 14.22 vs UAW: 158.2 ± 23.93 vs UIU: 146 ± 43.58;p<0.05). TEA increased the response to PHE in the UIU group (dAUC% CT: 77.79±13.8 vs UAW: 88.95±24.86 vs UIU 156.27 ± 39.39,p<0.05). APO reduced the reactivity to PHE in all groups, although the effect was greater in the UAW group (dAUC% CT: 15,03±6,32 vs UAW: 49.04±8.78 vs UIU: 41.60 ± 13.36; p<0.05). Our results demonstrate that the increase in the blood pressure (BP) UAW group may be associated with an increased reactivity found in the tail arterial bed. Although this effect was not observed in isolated segments of aorta, we found an increase of free radicals that may be associated with increased BP in these animals The increased basal release of NO in the aorta and increased relaxation to ACh in the flow bed appear to be compensatory mechanisms to increase BP and the increase in free radical. Intrauterine undernutrition is not accompanied by changes in BP, but further increase in basal release of NO and a factor for opening of potassium channels derived from the endothelium which consequently reduces the response to PHE.

Keywords: Undernutrition , blood pressure, endothelial dysfunction and oxidative stress.

INTRODUCTION

Undernutrition does not constitute an isolated fact, but the effect of the reciprocal action of socioeconomic, political, cultural and environmental factors which most strongly affect children living in extreme poverty, (Andrade, 1988).

Studies carried out to evaluate the effects of intrauterine malnutrition on the levels of blood pressure (BP) have produced conflicting results. Langley-Evans *et al* (1998) studied rats with a low protein diet during intrauterine development and demonstrated that the animals at birth had low body weight and high systolic blood pressure. Rats born to normotensive mothers exposed to a diet of low protein during pregnancy showed lower body weight at birth, and high blood pressure, detected in the fourth week of life, which remained until adulthood (Langley-Evans and Jackson, 1995; Woodall *et al*., 1996; Pérez *et al*., 2002). A reduction of endothelium-dependent vasorelaxation, partly due to loss of nitric oxide (NO) (the bioavailability of NO), occurs in most cases of chronic hypertension. The increased risk of hypertension and stroke associated with vascular dysfunction and decreased relaxation has been associated with nutritional deprivation in utero (Lamireau, *et al.,* 2002).

How fetal malnutrition can cause endothelial dysfunction remains unknown, as well as the mechanism "involved" in reducing the activity of nitric oxide (NO) , which may be due to reduction in its production or increased inactivation of it by oxygen free radicals. (Goodfellow *et al*., 1998).

Franco *et al.* (2002) found out that female pregnant Wistar rats fed either normal or 50% of the normal intake diets during the whole gestational period showed intrauterine undernutrition besides retarding fetal growth, also induced hypertension and endothelial dysfunction in the offspring, due to increased oxidative stress in the offspring, and this is characterized by increased generation of superoxide due to activation of NADPH oxidase and reduction of antioxidant defenses, suggesting a potential explanation for the endothelial dysfunction and development of diseases in adulthood.

Studying the long-term effects of intrauterine malnutrition on vascular function in offspring of female rats, fourteen weeks, Franco *et al.* (2007) examined the effects of the exogenous application of superoxide scavengers on the endothelium-dependent

vasorelaxation in the mesenteric microvessels. They also analyzed indicative parameters of oxidative stress by measuring superoxide anion concentration and the activity of superoxide dismutase as a marker of antioxidant defenses. The authors also found out that after intrauterine malnutrition, the female offspring has an increased production of superoxide which is at least partly responsible for endothelial dysfunction observed in these animals. They also showed that the inhibition of NADPH oxidase normalized superoxide generation and improved endothelium-dependent relaxation, suggesting an important role of this enzyme in oxidative function of vascular changes and, consequently, the development of hypertension and other cardiovascular diseases.

Strufaldi *et a*l (2009) studied the association between low birth weight and high blood pressure levels –which seems to be programmed during fetal life –and also noted that weight gain during childhood contributes to the increase the risk of high blood pressure. This served to confirm and extend the results of several studies among children, showing that the highest blood pressure levels were found in children who had low birth weight and this is already evident in the first decade of life. And the accelerated weight gain in childhood increases the risk. According to Gupta *et al.* (2004) the intrauterine malnutrition is associated with oxidative stress full term newborns small for gestational age of malnourished mothers.

Taking into account that the regional basic diet (RBD) expresses the basic daily consumption of a significant portion of the population in the state of Pernambuco (Teodósio *et al*., 1990), and the scarcity of studies of its effects on vascular reactivity in humans and animals, the importance of this study, in undernourished rats, is to show possible relationships between this diet and cardiovascular alterations. Therefore, we intend to evaluate in this study the impact of the DBR in these rats both in the intrauterine life and after their weaning, on some hemodynamic parameters and vascular reactivity. We studied vascular reactivity in the rat tail vascular bed (resistance vessels), and aortic rings (conductance vessels). The results will be compared with normal rats undergoing standard diet Labina®.

MATERIALS AND METHODS

Diets

Two types of diets were used for this work. The control diet was a commercial diet used in the Animal Facility of the Department of Physiology and Pharmacology, CCB-UFPE. The other diet , designated RBD, was prepared as recommended by Teodósio *et al.* (1990); Paixão, *et al*. (2003,2005); Vieira-Filho *et al*. (2009) consisting of beans – *Phaseolos vulgaris*, sweet *potato –ipomea batatas*, jerked beef and manioc *flour – Manihot esculenta*, macerated , formed in "pellets" and heated to 50^{o}C. The RBD pellets contain, per 100 g, 7.9 g protein, 69.3 g carbohydrate, 0.8 g lipid, 1.3 g of ash and 7.2 g fiber and provides 315.6 kcal (Teodósio *et al*., 1990). The RBD is hipocaloric by its carbohydrate content, but deficient in proteins, lipids, vitamins and minerals. The protein deficiency is both quantitative and qualitative, because the amino acid content is less than required.

Animals and experimental groups

Male Wistar rats from the Animal Facility of the Department of Physiology and Pharmacology of the Universidade Federal de Pernambuco . Were maintained at a controlled temperature of 23±2 ^{o}C, humidity 55±5% and light-dark cycles of 12h. and were divided in three experimental groups: Control Group Labina® (CT), RBD Intra-Uterine (UIU) and Post-Weaning (UAW), and attained 3 months of age, underwent the experimental protocol.

Control Group (CT) - The mothers were fed a chow diet during the pre-mating, mating, pregnancy and lactation. After weaning (21 days), pups had them same diet as their mothers(Labina®).

Group UIU – The mothers were fed a Labina® diet in the pre-mating phases. During the mating and pregnancy, the mothers were fed the RBD diet. After the birth of puppies the mother was fed Labina®. diet The offspring, after weaning, received Labina® until the period in which they were subjected to the experimental protocol.

Group UAW - The mothers were fed a diet Labina® during the phases pre-mating, mating, pregnancy and lactation. The offspring received the ration RBD, after weaning, up to 3 months.

Ethical considerations

All experimental procedures involving animals in this study were approved by the Ethics Committee for Animal Experimentation of the Biological Sciences Center of the Universidade Federal de Pernambuco, were carried out in accordance with the Committee's guidelines (Process Number 23076.008507/2007-16).

Hemodynamic measurements *in vivo.*

The rats were anesthetized with Urethane (1.2 g/kg, i.p.) and the left carotid artery was dissected carefully to avoid damage to the nerves located nearby. Subsequently, a polyethylene catheter (PE 50, Clay-Adams) filled with sterile heparinized saline (100 UI/ml) was introduced in this carotid artery. This catheter was connected to a pressure transducer (TSD104A) coupled to a preamplifier (DA100C Biopac Systems, Inc; CA), which was connected to a data acquisition system (MP100 Biopac Systems, Inc; CA). This last one, was connected to a computer .This system supplied continuous records of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR).

Study of vascular reactivity

Evaluation of vascular reactivity of the tail artery, *in vitro*

The *in vitro* perfusion technique of the tail arterial bed, as described by França *et al*. (1997) was used in the present study. After of the hemodynamic measurements, rats were heparinized (500 UI, ip). After 10 minutes, time to heparinization take effect, the tail artery was dissected and cannulated with a venous catheter (Safelet intracath 24G X ¾, NIPRO) filled with Krebs solution. Thereafter, the tail was separated from the animal body and the cannulated caudal artery was connected to a perfusion system which had its distal extremity removed so that the perfusate was not drained by the

venous system. The tail artery was perfused with Krebs-Henseleit composed solution (in mM): NaHCO3 27.2; NaCl 119; NaH2PO4 1; MgSO4 1.2; KCl 5.0; CaCl2 1.25; 11mM glucose; EDTA 0.03. This solution was maintained at a temperature of 36°C ± 0.5°C, bubbled with a carbonic mixture (95% of O2 and 5% of CO2), with pH stable at 7.4. The perfusion of the tail vascular bed was maintained under constant flow of 2.5 ml/min by a peristaltic pump (Milan, Colombo, Paraná, Brasil). The perfusion system was connected to a pressure transducer TSD104A - Biopac (connected to a preamplifier (DA 100C) that was connected to a system of data acquisition (MP 100 Biopac System, Inc; CA), records for continuous mean perfusion pressure (MPP) the tail vascular bed.

The preparation was subjected to a stabilization period of 40 minutes and, after which the experimental protocols started. Based on the relationship between pressure = flow x resistance, and as flow is a constant predetermined parameter, variations in pressure reflected in changes of vascular resistance.

Experimental protocols - After the stabilization period, administration of phenylephrine (PHE), an α_1- adrenergic agonist (1 μg, *in bolus*, in a volume of 100μl) was introduced in the perfusate, to verify the vascular responsiveness and discard the possibility of endothelial damage and vascular smooth muscle (VSM) by the cannulation technique. The functional integrity of endothelium and VSM were evaluated at the beginning of the experimental protocol. Next, the tail vascular bed was subjected to a pre-contraction by continuous infusion of PHE (10^{-7}M) and after the plateau, 5 μg/100 μl of acetylcholine (ACh) was administered *in bolus*. Soon after, the integrity of VSM was also investigated by the administration of 0.1 μg/100 μl, of sodium nitroprusside (SNP) *in bolus*. The vascular endothelium was considered intact when the relaxation to ACh was greater than or equal to 40%. The VSM was considered intact when the relaxation to SNP was greater than or equal to 50%. Afterwards, curves of PHE, ACh and SNP were performed, to verify if malnutrition had promoted changes in the responsiveness of the tail vascular bed.

To evaluate the vasoconstrictor response mediated by PHE, dose-response curve was done for PHE using increasing doses of this α_1- adrenergic agonist (0.001 - 300μg, *in bolus*, in a volume of 100 μl).

To evaluate the endothelium-dependent vasodilation, the tail artery was subjected to a pre-contraction with Krebs-Henseleit solution with 65 mM of KCl. After reaching the plateau, a curve of relaxation concentration-response in ACh concentrations 10^{-10} to 10^{-3} M was obtained. Perfusion was for a period of 4 minutes, thus obtaining, concentration-response curve to ACh.

To evaluate the endothelium-independent vasodilation, the tail artery was subjected to a pre-contraction with Krebs-Henseleit solution with 65 mM of KCl. After reaching the plateau, a curve of relaxation concentration-response was obtained for SNP concentrations of 10^{-9} to 10^{-2} M. perfusion was for a period of 3 minutes, thus obtaining, a concentration-response curve to the SNP.

Evaluation of aortic vascular reactivity *in vitro*

For the preparation of isolated rings, the descending thoracic aorta was carefully removed and quickly placed in a Petri dish and immersed in Krebs-Henseleit solution (in mmol/L: NaCl 118; KCl 4.7; $CaCl_2$.$2H_2O$ 2.5; $MgSO_4.7H_2O$ 1.2; KH_2PO_4 1.17; $NaHCO_3$ 25; EDTA 0.01 and 11 mM glucose). Next the adipose and conjunctive tissue adjacent to the artery was removed and the vessel was cut into rings of 4 to 5 mm in length. To obtain records of isometric tension, each vascular ring was placed in a glass chamber, containing 5 ml of Krebs-Henseleit solution, maintaind at 36± 0.5º C, continuously bubbled with a carbonic mixture, keeping the pH stable at 7.4. The rings were isolated and adapted to two metal wires in triangle shape that were put through the lumen of the vessel. One of the triangles was connected to a fixed stem attached to the and the other to a mobile stem connected to the force transducer. The rings were mounted so that the two metallic wires were parallel to the vessel lumen. Therefore, any change in diameter of the vessel was captured by the force transducer (GRASS® Force- displacement transducer FT03, Mass), connected to a system of data acquisition (MP 100 Biopac Systems, Inc; CA) for continuous records of tension imposed .

An initial resting tension of 1 g was applied to the rings and then the preparation was washed three times. The tension was adjusted if necessary every 15 minutes, during a period of 45 minutes of stabilization.

After stabilization, potassium chloride (KCl) 75 mM it was added to the bath to check the contractile activity of VSM induced depolarization and thus to evaluate the viability of the rings. After reaching a range of 1 g of force, the rings were washed three times with Krebs solution. Rings that did not contract 1g were discarded. After 30 minutes washing, a new concentration of KCl 75 mM was added to the bath and about 30 minutes was allowed until a plateau was attained in the record of the contraction. After the plateau, the rings were again washed three times and subjected to a 30 minute period of stabilization.If the tension of the ring did not return the baseline, it was subjected to a new wash. After 20 minutes, an evaluation the functional integrity of endothelium was carried out. The rings were precontracted with PHE 10^{-6} M. At the end of contraction, when the plateau was reached, a single dose of 10^{-5} M ACh was added. Rings that relaxed less than 90% of the plateau were discarded. Rings without endothelium relaxed up to 10% or even contracted.

Evaluation of endothelium-dependent relaxation - In some rings, concentration-response curves to ACh were done, with the aim of evaluating the endothelium-dependent relaxation through stimulation of nitric oxide (NO). After the plateau of contraction caused by PHE 10^{-6} M, increasing concentrations of ACh were added in the glass chamber (10^{-11} - 10^{-4} M).

Evaluation of the vasoconstrictor response induced by phenylephrine - After a period of stabilization and testing of the functional integrity of endothelium , the rings were incubated or not (control curves) with the drug of interest, and then, the concentration-response curves to PHE (10^{-10} – 3 x 10^{-4} M) were carried out.

Evaluation of the participation of nitric oxide in the vasoconstrictor response induced by phenylephrine - to evaluate the participation of NO in vascular reactivity to PHE, the rings were incubated for 30 minutes with *N*G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and then a concentration-response curve to PHE (10^{-10} - 3 x 10^{-4} M) was carried out.

Evaluation of the participation of prostanoids derived from cyclooxygenase enzyme in the vasoconstrictor response induced by phenylephrine – to evaluate the participation of the cyclooxygenase pathway in vascular reactivity to PHE, the rings were incubated for 30 minutes with 10 μM of

indomethacin, and then concentration-response curve to PHE (10^{-10} – 3 x 10^{-4} M) was done.

Evaluation of the participation of potassium channels sensitive to calcium in the vasoconstrictor response induced by phenylephrine - To evaluate the influence of potassium channels sensitive to calcium, on the contractile response induced by phenylephrine, arteries were incubated with tetraethylammonium (TEA, 2 mM), a blocker for potassium channels activated by calcium and then, a concentration curve response to PHE (10^{-10} – 3 x 10^{-4} M) was carried out.

Evaluation of the participation of reactive oxygen species in the vasoconstrictor response induced by phenylephrine - to evaluate the participation of free radicals derived from NADPH oxidase in vascular reactivity to PHE, the rings were incubated for 30 minutes with apocynin (APO, 0.3 mM) and then, a concentration-response curve to PHE (10^{-10} – 3 x 10^{-4} M) was carried out.

Evaluation of the participation of endothelium in vasoconstrictor response induced by phenylephrine - To carry this protocol the removal of the vascular endothelium was done mechanically with the use of metal wire. After a stabilization period, the preparations were pre-contracted with PHE and the absence of endothelium was confirmed by the inability to induce relaxation to ACh 10^{-5} M. After testing for the evaluation of endothelial integrity, the preparation was washed and concentration-response curve to PHE (10^{-10} – 3 x 10^{-4} M). was carried out.

Data presentation and statistical analysis

All data are expressed as mean ± standard error of mean (SEM). The statistical tests used were 1-way ANOVA for repeated measures and random ones, 2-way ANOVA for repeated measures and Student's t test unpaired. The post hoc Tukey test was used and $P<0.05$ was considered statistically significant. The software used for statistical analysis was GraphPad-Prism 5.0 (San Diego, CA, USA).

RESULTS

Body Weight

At birth, weight body of undernourished intra-uterine (UIU) group (3.65 ± 0.10g, n = 10) was lower than that observed in the control group (CT) (6.31 ± 0.07g, p <0, 01, n = 10) and undernourished post-weaning (UAW) one (6.45 ± 0.06g, P <0.01, n = 10). When weaning at 21st day after birth, there was recovery of body weight gain in the UIU group (48.02 ± 1.49g, P <0.01, n = 10), however it was lower than the control group (59.58 ± 2.9g, p <0, 01, n = 10) with no change in weight in the UAW group (58.15 ± 1.40g, P <0.01, n = 10). One hundred twenty days after birth, the UIU group (314.40 ± 8.58g, p <0.01, n = 10) still had lower body weight compared to the control group (376.25 ± 7.3g, p <0.01, n = 10), and the UAW group had a delay in weight gain (130.59 ± 9.7g, p <0.01, n = 10) (Table 2).

Hemodynamic Parameters

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were directly measured, as well as mean arterial pressure (MAP) and heart rate (HR) in anesthetized rats. Compared to the control group (102 ± 4.8 mmHg, n = 10), SBP was higher in the UAW group (118 ± 4 mmHg, p <0.05, n = 10), but the UIU group (100 ± 8 mmHg, n = 10) showed no difference. The DBP compared to control (54 ± 4 mmHg, n = 10) was higher in UAW (72 ± 5.8 mmHg, p <0.05, n = 10), but the UIU did not change. MAP was greater in the UAW (89 ± 5.9 mmHg p <0.05, n = 10) compared to CT (72 ± 4 mmHg, n = 10), but UIU (69 ± 8 mmHg, n = 10) did not change. The HR was higher in the UAW group (328 ± 11 bpm, p <0.05, n = 10) compared to control (302 ± 9 bpm, n = 10), in UIU (300 ± 12 bpm, n = 10) it was not modified. (Table 3).

Reactivity of the tail artery

When evaluating the effects of undernutrition on the tail vascular bed, we found an increase of the maximum response (R_{max}) to PHE in the UAW group (492.7 ± 29.5 mmHg, p <0.05, n = 10) when compared to CT group (295.3 ± 32 mmHg, p <0.05, n = 10). In the UIU group (249.5 ± 18 mmHg, p <0.05, n = 10), this response was not modified when compared to control (Figure 1 A and B, Table 4). In arteries previously

pre-contracted with PHE (10^{-7}M), the ACh induced a endothelium-dependent relaxation was greater in the UAW (86.5 ± 3.9%, mmHg, $p < 0.05$, n = 10), which was not observed in the UIU (72.4 ± 3.6%, mmHg, $p < 0.05$, n = 10) when compared to CT group (70.8 ± 4.2%, mmHg, $p < 0.05$, n = 10). (Figure 2 A and B, Table 5). The endothelium-independent relaxation induced by SNP was similar among the 3 groups. (Figure 3 A and B, Table 6).

Vascular reactivity of the aorta

Figures 6 to 17 (A and B, Table 7) show the dose-response curves to PHE (10-10 – 3 x 10-4 M) and the percentage of the difference area under the curve (dAUC), under the action of L-NAME, TEA, indomethacin, apocynin and after the endothelium removed. The reactivity to PHE in the aorta of animals UAW was unchanged when compared to the control group (Figure 6,). The UIU group showed reduced reactivity to PHE compared with the control group (Figure 7). The absence of the endothelium produced greater increases in reactivity to PHE in aortic rings of the undernourished UIU group when compared to control. The UAW group was not difference (dAUC% E + vs E- CT: 133.9 ± 28.19g versus UAW: 387.2 ± 68.02g versus UIU: 258.39 ± 61.91g, $p < 0.05$, n = 8). Increased reactivity to PHE was also observed after incubation with L-NAME (dAUC% E + vs L-NAME- CT: 91.00 ± 14.22g versus UAW: 158.2 ± 23.93g versus. UIU : 146 ± 43.58g, $p < 0.05$, n = 8). This increase in percentage (%) of dAUC was higher in UAW and UIU group, suggests increased release of NO in the undernourished groups UAW and UIU (Figures 8 - 11 A, B and C, Table 7). The effects of incubation with indomethacin in response to the PHE did not differ between the aortas of the undernourished group UAW and UIU and CT (Figure 12 and 13 A, B and C, Table 7). Incubation with TEA increased the response to PHE in the aorta of the undernourished UIU group compared with the control group, in the UAW group was not difference (dAUC% CT: 77.79 ± 13.18g versus. UAW: 24.86g ± 88.95 versus UIU:156.27 ± 39.39g, n = 8) (Figure 14 and 15 A, B and C, Table 7). Incubation with APO reduced the reactivity to PHE in all groups, although the effect was greater in the undernourished UAW group (dAUC% CT: 15.03 ± 6.32g versus UAW: 49.04 ± 8.78g versus UIU: 41.60 ± 13.36g, $p < 0.05$, n = 8), suggesting greater release of reactive oxygen species derived from NADPH oxidase. This was suggested by %dAUC of the

UAW and groups which showed a higher value under the condition of undernutrition (Figures 16 and 17 A, B and C, Table7).

DISCUSSION

In this study, we investigated the effects of undernutrition on vascular reactivity and some cardiovascular parameters at two important periods of development – in intrauterine life and after weaning – in order to elucidate some mechanisms that involve two relevant issues in public health, undernutrition and hypertension. Undernutrition was induced by a diet which reproduces a regional basic diet (RBD) (Teodósio *et al*., 1990), widely consumed by inhabitants living in an area of sugar cane plantations along the coast of the State of Pernambuco, Brazil. This diet has been associated both with lower weight gain in the malfunctioning of various organs, probably because of its low protein content and also due to deficiency of vitamins and minerals. It is important to mention that this diet is deficient in a qualitative rather than quantitative nature , the latter being used by most researchers, and represents the real consumption of this population . Depending on the region and season, other populations, not only in Northeast Brazil, as well as in other regions of the world can consume a similar diet. Therefore, the importance of knowing its effects on the organism, can help in the future to introduce corrective interventions, such as supplementation, in order to minimize the risk of diseases associated with undernutrition.

In this study, we used an experimental model of undernutrition induced by RBD, a multideficient diet (Table 1), with deficiency in protein, lipids, minerals and vitamins (Teodósio *et al*., 1990; Monteiro *et al*., 2001; Paixão *et al*., 2003, 2005; Vieira-Filho *et al*.,2009; Sant'Helena, 2009). Therefore, as a qualitative model, unlike other more common quantitative models, which consist of an isolated deficiency of protein (Langley e Jackson,1994; Langley-Evans *et al.,* 1998) or restriction in 50% of the diet (Franco *et al.,* 2002a, 2002b, 2003, 2007). The diet we utilized besides causing a slowing weight gain, increased blood pressure and changes in vascular reactivity in young rats subjected to this qualitative model of undernutrition at two important periods of development: in the intrauterine stage and after weaning.

Low birth weight (Table 2) presented by the animals submitted to intrauterine undernutrition cannnot be due to a reduced maternal caloric intake, however by a deficiency of important nutrients necessary for fetal development, Paixão *et al*.,(2003). At weaning (21 days old), a less weight gain was found in the control group. However, in adulthood (120 days old), animals submitted to undernutrition in utero partially recovered their weight, which was around 15% below compared to the control group a finding also reported by Paixão *et al*.,(2001). The animals submitted to undernutrition after weaning showed an early and marked reduction in weight gain, and this was maintained throughout the period of development. These results highlight the importance of nutritional status in the growth phase of the animal and have shown a greater impact of undernutrition in the post-weaning, since undernutrition was induced directly to offspring for a long time including the period of growth and development until adulthood. Similar results were observed by Monteiro (2001) and Sant'Helena, (2009) who used the same diet.

This reduction was also found in other studies using an experimental model in rats induced by dietary restriction of 50% (Sugizaki *et al*, 2005; Franco *et al.,* 2002a, 2002b, 2003, 2007), or low-protein diet with 6% protein (Tropia *et al*., 2001; Oliveira *et al*., 2004).

Alterations in body weight serve as an indicator of nutritional status of the individual and may be associated with alterations in the cardiovascular system. Many authors suggest that in humans and in animal models in which the inadequate supply of nutrients was induced during fetal development (in utero) and birth, cardiovascular disorders may be associated with, or predispose to the development of systemic hypertension arterial and cardiac dysfunction in adulthood (Langley-Evans e Jackson, 1994; Woodall *et al.,* 1996; Holemans *et al*., 1999, 2003; Hu *et al*., 2000; Pérez *et al*., 2002).

In our experiments, this qualitative model of undernutrition promoted changes in blood pressure and heart rate (Table 3), as well as, showed that the mean baseline values of SBP, DBP, MAP and HR were significantly higher in the UAW compared to CT, however the UIU didn't reveal any changes.

Several authors have studied undernutrition in quantitative models, Gardner *et al*., (1997) e Franco *et al*., (2002), Lamireau *et al.,* (2002), Ceravolo *et al*., (2007),

have showed that in rats where undernutrition was induced during pregnancy, besides producing a decrease in body weight can lead to hypertension in the offspring. Langley-Evans *et al*. (1998) showed , in rats deprived at an intrauterine, through a low protein (8% casein) and normosodic diet, an increase in blood pressure of the offspring at an young adulthood stage.

Nutter *et al.* (1979) and Fazza *et al.* (1999), evaluating the effects of undernutrition in rats chronically subjected to protein-calorie deprivation suggested that increased HR and blood pressure can be attributed to a greater hormonal response of the hypothalamic-pituitary-adrenal axis, by elevated levels of circulating catecholamines.

Study realized by Hu *et al*., (2000), in isolated rat hearts subjected to protein deprivation in the intrauterine period, and evaluated after 4, 9, 18 months of life, showed retardation of body growth, increased blood pressure and predisposition to cardiac arrhythmias, specially in aged animals, i.e., in their late stage of life.

In our results in the UIU group, we did not observe statistically significant alteration in blood pressure levels in relation to CT. These results corroborate the results found by Monteiro *et al.,* (2001), using rats deprived during lactation, through the DBR hyposodic diet fed to mothers. According to these authors, although the baroreflex sensitivity in these animals is increased in response to the hypotensive effect, the blood pressure levels were not modified.

In our studies, only the undernourished animals in the post-weaning exhibited an increase in HR, which corroborates with the findings of Monterio *et al*., (2001), which showed an increase in HR in animals subjected to chronic malnutrition.

The findings regarding the rise in blood pressure found in the UAW group may be related to increased vascular reactivity found as resistance vessel in the tail artery, where the UAW group presented increased vasoconstrictor response to PHE (Figure 1 A, Table 4), what would be causing this increase in blood pressure levels, probably due to increased peripheral vascular resistance.

Another factor contributing to the increase in blood pressure is a deficiency in the production (or bioavailability) of NO. Oxidative stress seems to favor a reduction in activity of superoxide dismutase enzyme and the consequent reduction in the synthesis of NO (Franco *et al*., 2002).

In the UAW group, there was an increase of relaxation to ACh in tail vascular bed (Figure 2 A, Table 5), which could be an adaptive mechanism to compensate for the increased reactivity. Holemans et al. (1999) using an experimental model applied in undernourished rats fed a normosodic diet and quantitative induced by a 50% restriction of food to the maternal rate during the second half of pregnancy. These authors have suggested that a slight disorder in vascular function caused by a compensatory response to tonic depletion of NO. In the UIU group, there were no changes in vascular reactivity (Figures 1 B and 2 B, Tables 4 and 5).

When considering reactivity in conductance vessels, undernutrition promoted changes in vascular reactivity in aortic rings, when studied *in vitro*. The UAW group did not alter the reactivity to PHE (Figure 6), however in the presence of L-name, a not specific inhibitor of eNOS, there was a greater vasoconstrictor response to PHE (Figure 10 A, B and C, Table7).This suggests a greater endothelial vasodilatory modulation in this group due to an increase in NO production. In the presence of COX inhibitor, indomethacin, there was no difference in the vasoconstrictor response to PHE in the UAW group compared with the controls (Figure 12 A, B and C, Table 7), probably due to the vasoactive prostanoids of this pathway seemed not to be altered. Rings of the DPW group, when incubated with TEA did not affect the reactivity in the control (Figure 14 A, B and C, Table 7), which leads to conclude that potassium channels seem to be not involved in the alterations in vascular reactivity found in the UAW. The blocking of NAPH oxidase, apocynin promoted a reduction of reactivity, and the vasoconstrictor response to PHE on CT and UAW, but greater magnitude in the DPW, suggesting increased release of reactive oxygen species derived from NADPHoxidase (Figure 16 A, B and C, Table 7). Therefore, the increase in free radicals derived from NADPH oxidase would be offset by the increase in production NO release in this model, UAW, resulting in no alteration in reactivity.

Supporting these results, Franco *et al*.(2007) studied the long-term effects of intrauterine undernutrition on vascular function in offspring of females and the implications of oxidative stress, found out that , after intrauterine undernutrition, the female offspring has an increased production of superoxide. This is at least partly responsible for endothelial dysfunction observed in these animals. They also observed that the inhibition of NADPH oxidase normalizes the generation of superoxide and

improves endothelium-dependent relaxation, suggesting an important role of oxidative enzyme in alteration vascular function and, consequently, the development of hypertension and other cardiovascular diseases.

The UIU group showed lower reactivity to PHE when compared with the controls (Figure 7). After endothelium removed , the vascular reactivity was increase in this group compared to the controls (Figure 9 A, B and C, Table 7), therefore, the endothelial modulation is increased in endothelial UIU. It suggests a greater release of relaxing factors, which may explain the decrease in reactivity found in this group. Incubation with L-NAME suggests that endothelial modulating factors seems to be in the NO, because the largest displacement of the curve to the left, caused by L-NAME was greater in the UIU when compared with control (Figure 11 A, B and C, Table7).

In the presence of a blocker for potassium channels activated by calcium, TEA, there was larger increase of the vasoconstrictor response to PHE in the UIU group, indicating increased activation of potassium channels. Thus, the endothelial derived hyperpolarizing factor in endothelium, which can be the NO, is increased in this group because the vasoconstrictor response was greater in its absence in this group compared with the controls (Figure 15 A, B and C, Table 7). That would inhibit the vasoconstrictor response to PHE already observed.

Franco *et al*. (2002a) have also shown that intrauterine undernutrition increases oxidative stress, suggesting a potential explanation for the endothelial dysfunction during development. Also, studying the effects of intrauterine undernutrition on the expression and activity of endothelial nitric oxide synthase (eNOS), Franco *et al*. (2002b) concluded that intrauterine undernutrition induces hypertension in both the offspring of male and female, and that hypertension may be more severe in males than in females. Undernutrition alters the endothelium-dependent responses and that endothelial dysfunction is associated with a decrease in activity / expression of eNOS in the aortas of the offspring.

Endothelial dysfunction can be a associated with a range of inter-related consequences of fetal undernutrition as with insulin resistance (which may also contribute to this dysfunction) and are interrelated preceding the development of hypertension, diabetes and ischemic heart disease. (Goodfellow *et al*., 1998). This author suggests that one aspect of this hypothesis is the dynamic nature of endothelial

function. How undernutrition can cause fetal endothelial dysfunction is still poorly understood, as well as the mechanism "involved" in reducing the activity of NO, which may be due to reduction in production or increased inactivation by oxygen free radicals.

Therefore, the results of our studies using a qualitative multideficient undernutritional model of low-protein, showed an increase in blood pressure in the DPW group, which was accompanied in vitro of increase in reactivity. It also revealed increased vascular reactivity to PHE in the tail artery and an increase in relaxation caused by ACh. The same was not observed in the UIU with this model of undernutrition. Undernutrition did not increase vascular reactivity to PHE in the aorta of the UAW group, but promoted decreased UIU. Moreover, we observed that undernutrition is accompanied by increased release of free radicals derived from NADPH oxidase, and an increase in basal release of NO, the latter could be a compensatory mechanism.

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FIGURES AND TABLES

Table 1. Proximate composition da Regional Basic Diet (RBD).

INGREDIENTS	**g (%)**	**COMPOSITION**					
		protein	Carbohydrate	Lipid	Minerals salts	Fibers	**Kcal**
Beans	18,34	3,99	10,66	0,24	0,57	1,09	60,76
Manioc *flour*	64,81	0,80	48,49	0,12	0,43	5,64	198,80
Jerked beef	3,74	2,74	0,43	0,06	0,06		14,57
Fat	0,35			0,35			3,15
Sweet potato	12,76	0,30	9,99	0,03	0,20	0,48	58,87
Total	100,00	7,83	69,57	0,80	3,96	7,21	336,15

Font: Modified of Teodósio *et al* (1990).

Table 2. Body weight of offspring.

Weight (g)	**CT (n=10)**	**UAW (n=10)**	**UIU (n=10)**
Birth	6.31 ± 0.7	6.45 ± 0.6	3.65 ± 0.10*
weaning	59.58 ±2.9	58.15 ± 1.40	48.02 ± 1.49*
21st day	376.25 ±7.3	130.59 ± 9.7*	314.40 ± 8.58*

Body weight (g). Control group (CT), undernourished group post-weaning (UAW) and intra-uterine (UIU). Values are expressed as mean ± SEM. The data were analyzed with 1-way ANOVA with Tukey post hoc test. (*p<0.05).

Table 3: Direct measurement of some cardiovascular parameters in *Wistar* rats.

Parâmetros	**CT (n=10)**	**UAW (n=10)**	**UIU (n=10)**
SBP(mmHg)	102 ± 4.8	118 ± 4*	100 ± 8
DBP (mmHg	54 ± 4	72 ± 5.8*	54 ± 7
MAP (mmHg)	72 ± 4	89 ± 5.9*	69 ± 8
HR (bpm)	302 ± 9	328 ± 11*	300 ± 12

Directly measured the blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR). Control group (CT), undernourished group post-weaning (UAW)) and intra-uterine (UIU). Values are expressed as mean ± SEM. The data were analyzed with 1-way ANOVA with Tukey post hoc test. (*p<0.05).

Effects of undernutrition on the vascular reactivity of the tail artery bed of *Wistar* rats

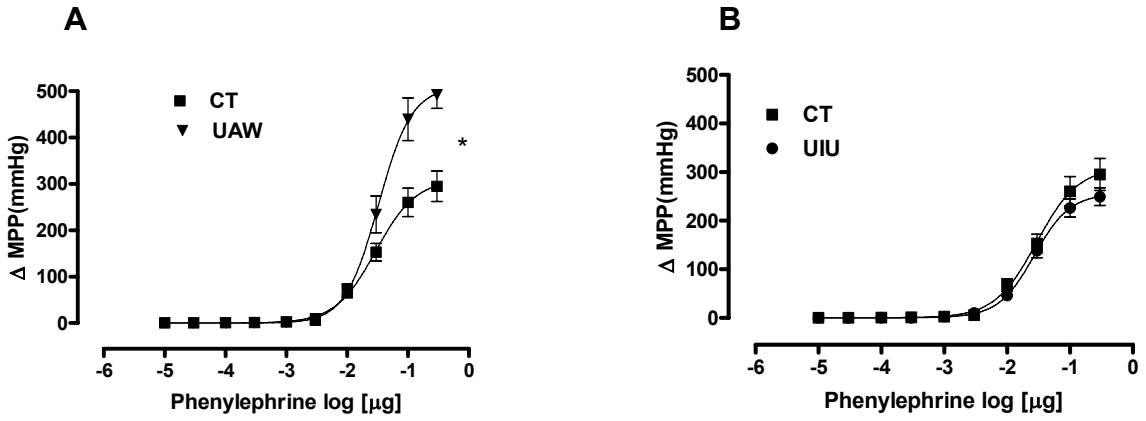

Figure 1- Dose-response curve to phenylephrine (PHE) in the tail artery bed *Wistar* rats, control group (CT, N=8), undernourished group post-weaning (UAW), N=8) and intra-uterine (UIU, N=8). Mean perfusion pressure (ΔMPP) in relation to increasing doses of PHE. *p<0.05 CT vs UAW). p > 0.05 CT vs UIU. Values are expressed as mean ± SEM. The data were analyzed with 2-way ANOVA for repeated measures with Tukey post hoc test and Student's t test unpaired.

Table 4: Maximum response (R_{max}, Δ PPM mmHg) and sensitivity pD2 to phenylephrine in the tail arterial bed *Wistar* rats control group (CT, N=8), undernourished group post-weaning (UAW), N=8) and intra-uterine (UIU, N=8).

Groups (n=8)	R_{max} (Δ PPM mmHg)	pD2
CT	295.3 ± 32.8	1.53 ± 0.09
UAW	492.7 ± 29.5*	1.49 ± 0.06
UIU	249.5 ± 18.1	1.57 ± 0.05

Values are expressed as mean ± SEM. "N" indicates the number of animals used in each group; Student's t test unpaired, *p<0.05.

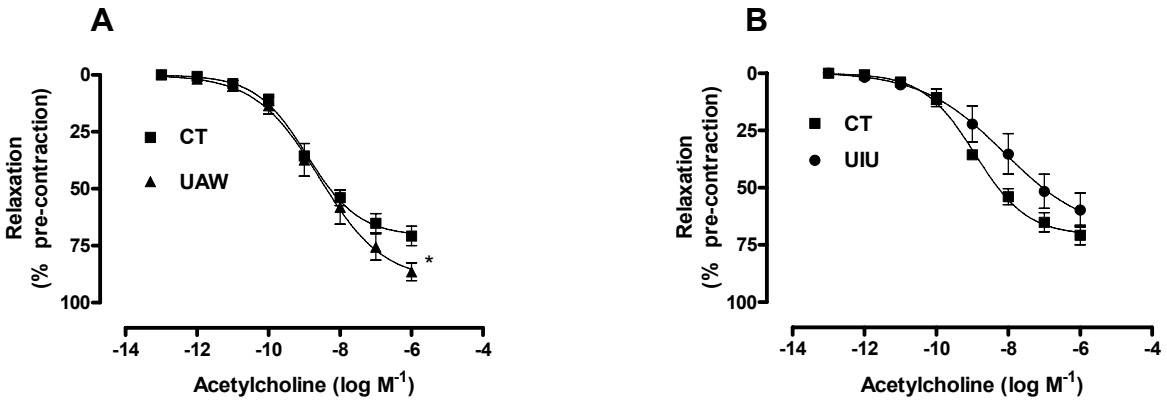

Figure 2- Concentration-response curves to endothelium-dependent relaxation produced by acetylcholine (ACh) in the tail artery bed *Wistar* rats Control group (CT, N=8), undernourished group post-weaning (UAW), N=8) and intra-uterine (UIU, N=8). % relaxation produced in the tail artery bed previously contracted with phenylephrine in relation to increasing concentration of ACh.*p<0.05 CT vs UAW), p>0.05 CT vs UIU. Values are expressed as mean ± SEM. The data were analyzed with 2-way ANOVA for repeated measures with Tukey post hoc test and Student's t test unpaired.

Table 5: Maximum response ($R_{max,}$ % relaxation produced in the tail artery bed previously contracted with phenylephrine) and sensitivity pD2 to acetylcholine in the tail arterial bed *Wistar* rats. Control group (CT), undernourished group post-weaning (UAW)) and intra-uterine (UIU).

Grupos (n=8)	**R_{max} (% relaxation)**	**pD2**
CT	70.8 ± 4.2	8.9 ± 0.1
UAW	86.5 ± 3.9*	8.6 ± 0.2
UIU	72.4 ± 3.6	8.1 ± 0.7

Values are expressed as mean ± SEM. "N" indicates the number of animals used in each group; Student's t test unpaired, *p<0.05.

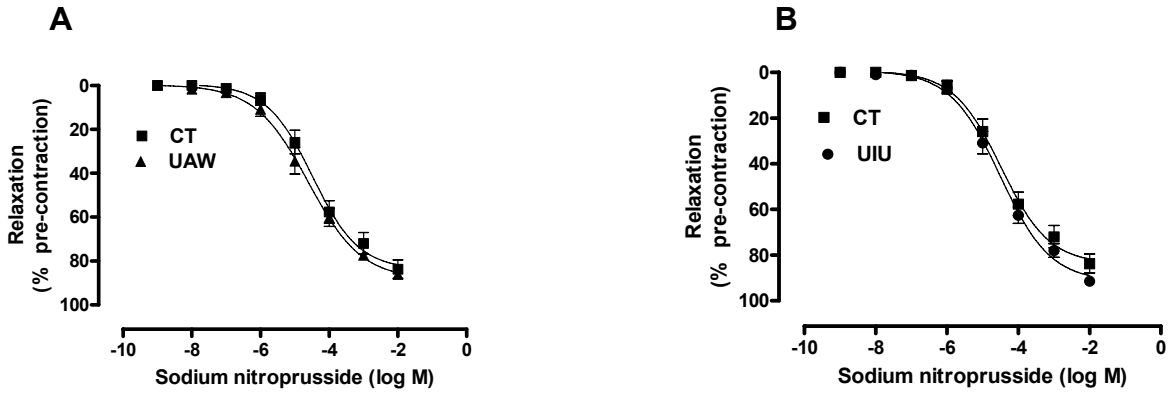

Figure 3- Concentration-response curve for endothelium-independent relaxation produced by sodium nitroprusside (SNP) in the tail artery bed *Wistar* rats Control group (CT, N=8), undernourished group post-weaning (UAW), N=8) and intra-uterine (UIU, N=8). % relaxation produced in the tail artery bed previously contracted with phenylephrine in relation to increasing concentration of SNP. CT vs UAW), CT vs UIU. Values are expressed as mean ± SEM. The data were analyzed with 2-way ANOVA for repeated measures with Tukey post hoc test and Student's t test unpaired.

Table 6: Maximum response (R_{max}, % relaxation produced in the tail artery bed previously contracted with phenylephrine) and sensitivity pD2 to sodium nitroprusside in the tail arterial bed *Wistar* rats. Control group (CT), undernourished group post-weaning (UAW)) and intra-uterine (UIU).

Grupos (n=8)	**R_{max} % relaxation)**	**pD2**
CT	83.7 ± 4.2	4.5 ± 0.1
UAW	86.1 ± 1.6	4.6 ± 0.1
UIU	91.4 ± 1.8	4.5 ± 0.1

Values are expressed as mean ± SEM. "N" indicates the number of animals used in each group; Student's t test unpaired.

Effects of undernutrition on the vascular reactivity to phenylephrine in aortic isolated rings of *Wistar* rats

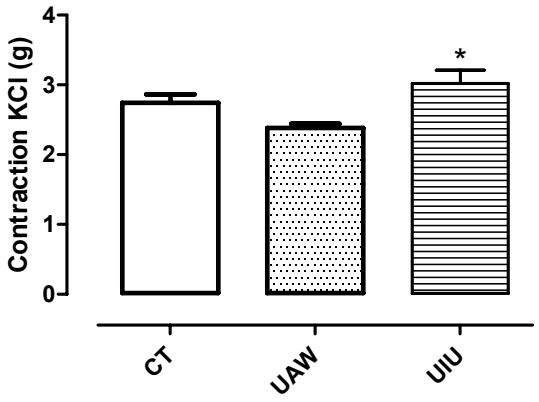

Figure 4- Maximum contraction produced by KCl (75mM) in isolated aortic rings with endothelium of Wistar rats. Control group (CT, N=8), undernourished group post-weaning (UAW), N=8) and intra-uterine (UIU, N=8). Values are expressed as mean ± SEM. The data were analyzed with 1-way ANOVA with Tukey post hoc test. *p<0.025 vs UAW).

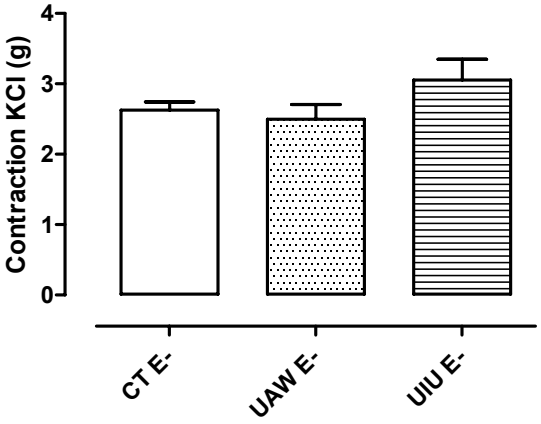

Figure 5- Maximum contraction produced by KCl (75mM) in isolated aortic rings without endothelium of Wistar rats. Control group (CT, N=8), undernourished group post-weaning (UAW), N=8) and intra-uterine (UIU, N=8). Values are expressed as mean ± SEM. The data were analyzed with 1-way ANOVA with Tukey post hoc test.

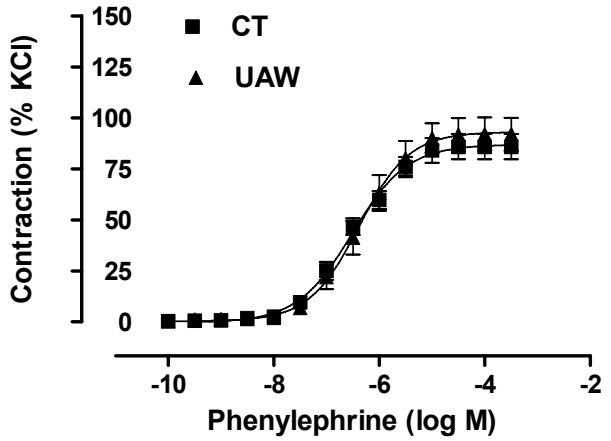

Figure 6- Concentration-response curve to phenylephrine in isolated aortic rings of Wistar rats. Control (CT, n=8) and undernourished after weaning (UAW), n=8). Values are expressed as mean ± SEM; Student's t test unpaired.

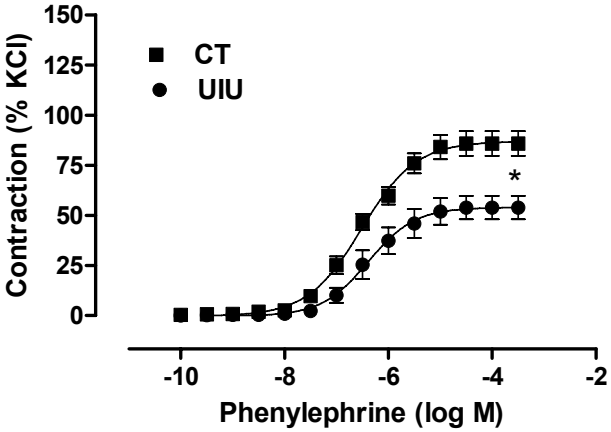

Figure 7- Concentration-response curve to phenylephrine in isolated aortic rings of Wistar rats. Control (CT, n=8) and undernourished intra-uterine (UIU, n=8). Values are expressed as mean ± SEM; Student's t test unpaired. *p< 0.05 for pD_2 and R_{max}: UIU *vs* CT.

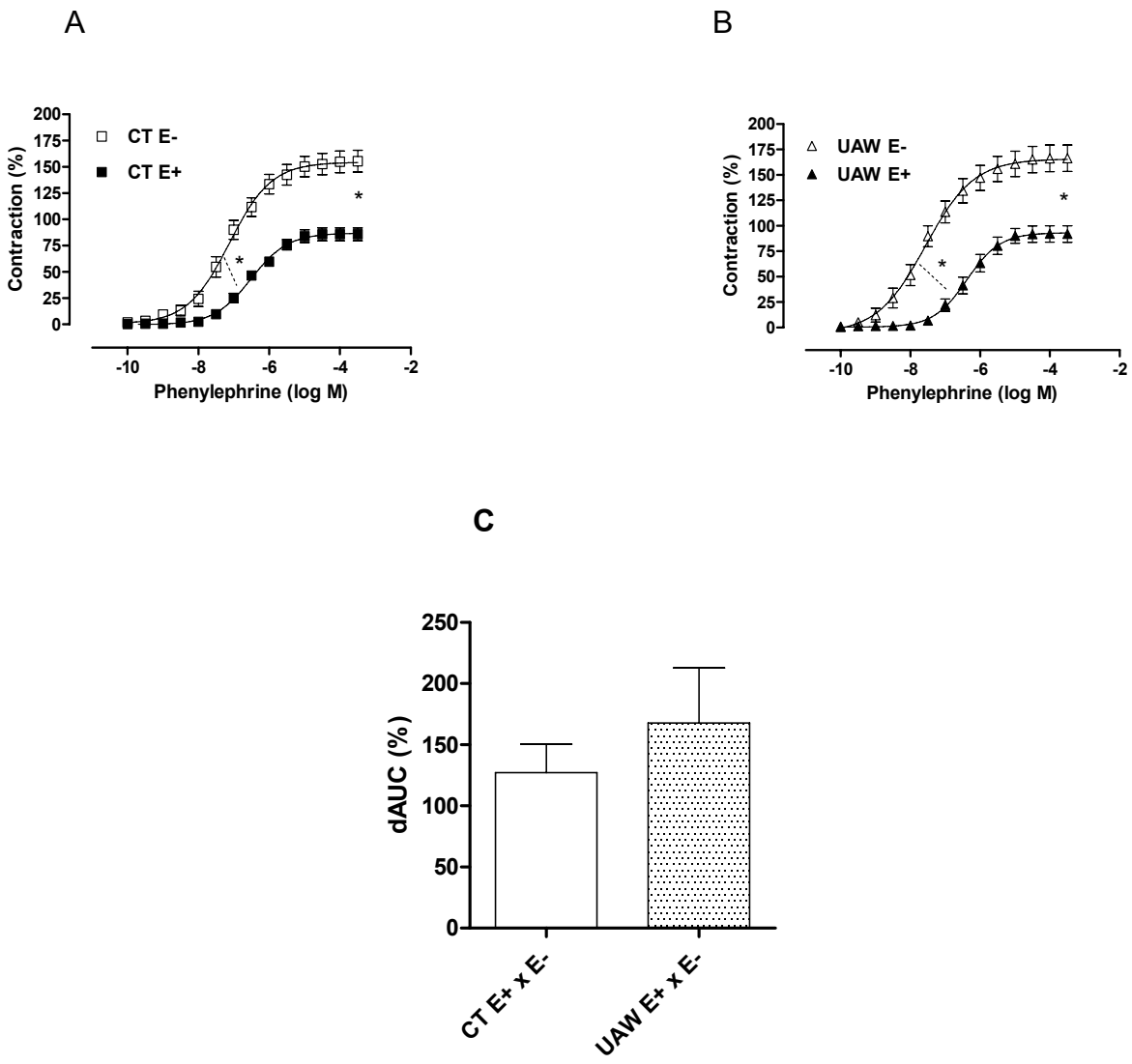

Figure 8- Concentration-response curves to phenylephrine in isolated aortic rings of *Wistar* rats: (A) before (CT E+, n=8) and after removal of endothelium (CT E-, n=8)); (B) Effect of undernutrition after weaning, UAW), (UAW) E+, n=8) before and after removal of the endothelium (UAW) E-, n=8); (C) Percentage difference of area under the curve in vessels with endothelium intact and denuded. Values are expressed as mean ± SEM; Student's t test unpaired. *$p < 0.05$ for pD_2 and R_{max}: CT E+ *vs* Ct E-; pD_2: UAW E+ *vs* UAW E- and dAUC% - CT E+ and E- *vs* UAW E+ and E-.

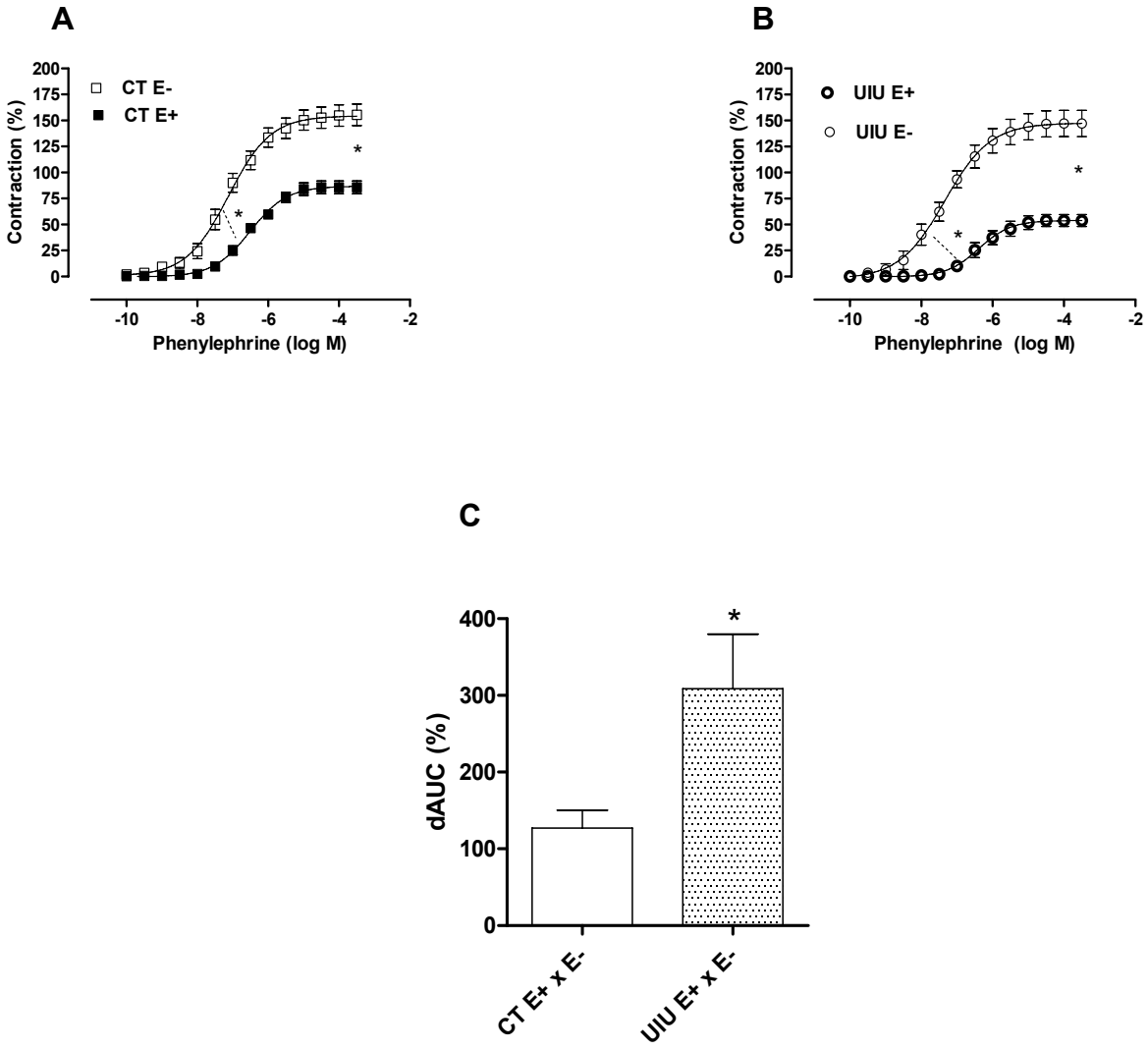

Figure 9- Concentration-response curves to phenylephrine in isolated aortic rings of Wistar rats: (A) before (CT E +, n=8) and after removal of endothelium (CT E-, n=8); (B) The effect of intra-uterine undernutrition, UIU, before (UIU E +, n=8) and after removal of endothelium (UIU E-, n=8); (C) Percentage difference of increase of area under the curve in vessels with endothelium intact and denuded. Values are expressed as mean ± SEM; Student's t test unpaired. *$p < 0.05$ for pD_2 and R_{max}: CT E+ *vs* CT E-; pD_2: UIUE+ *vs* UIU E- and dAUC% E + VS E- CT: 133.9 ± 28.19g vs UIU: 258.39 ± 61.91g.

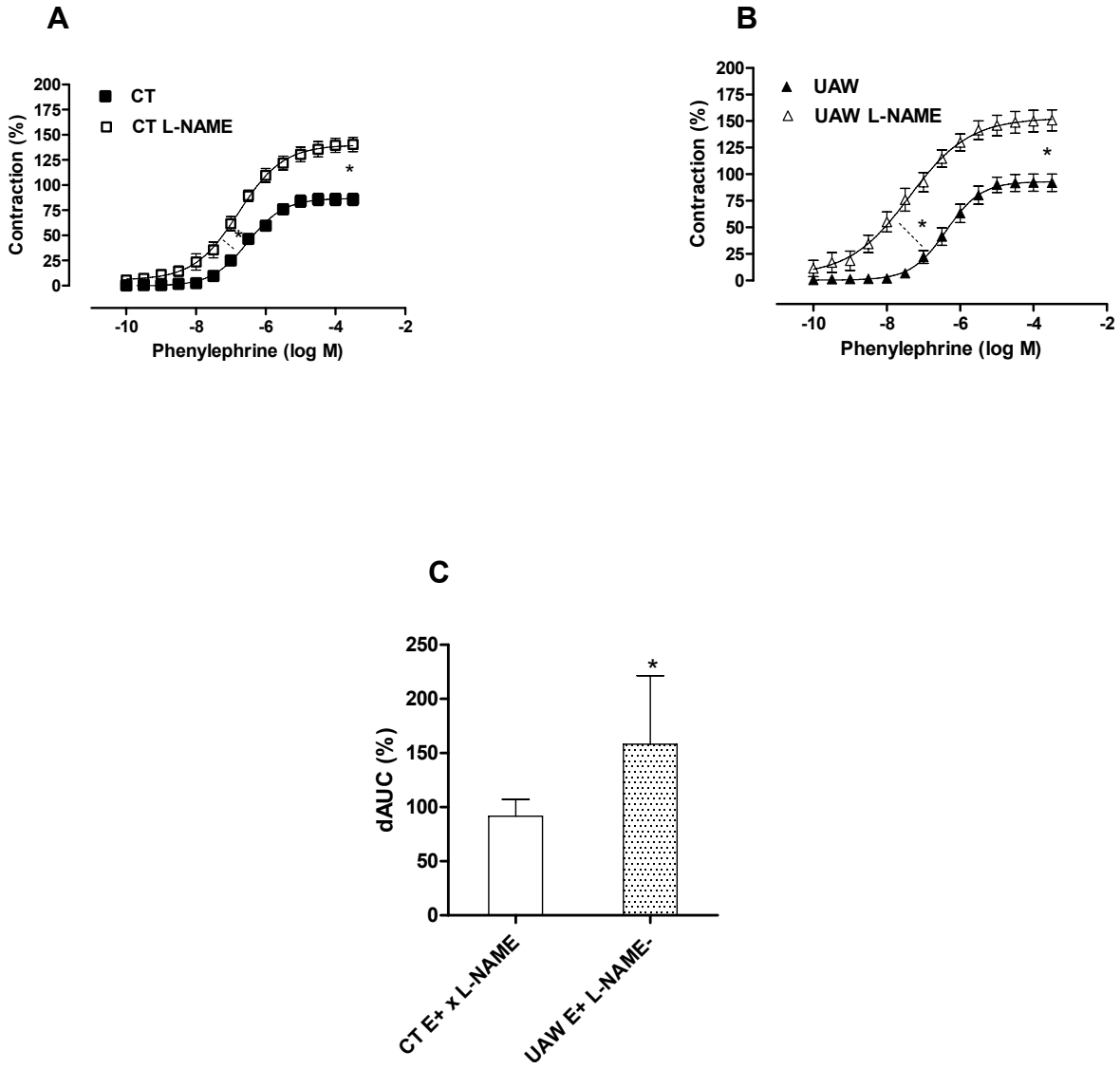

Figure 10- Concentration-response curves to phenylephrine in isolated aortic rings of Wistar rats: (A) before (CT E +,n=8) and after incubation with L-NAME (CT L-NAME, n=8); (b) Effect of undernutrition after weaning before, UAW, (UAW + E, n=8) and after incubation with L-NAME (UAW L-NAME, n=8); (C) Comparison of the percentage difference of area under the curve in control and undernourished. Values are expressed as mean ± SEM; Student's t test unpaired. *p < 0.05 for pD2 and R_{max}: CT E+ vs CT L-NAME; pD2 and R_{max}: UAW E+ vs UAW L-NAME and dAUC% E + vs L-NAME- CT: 91.00 ± 14.22g vs UAW: 158.2 ± 23.93g.

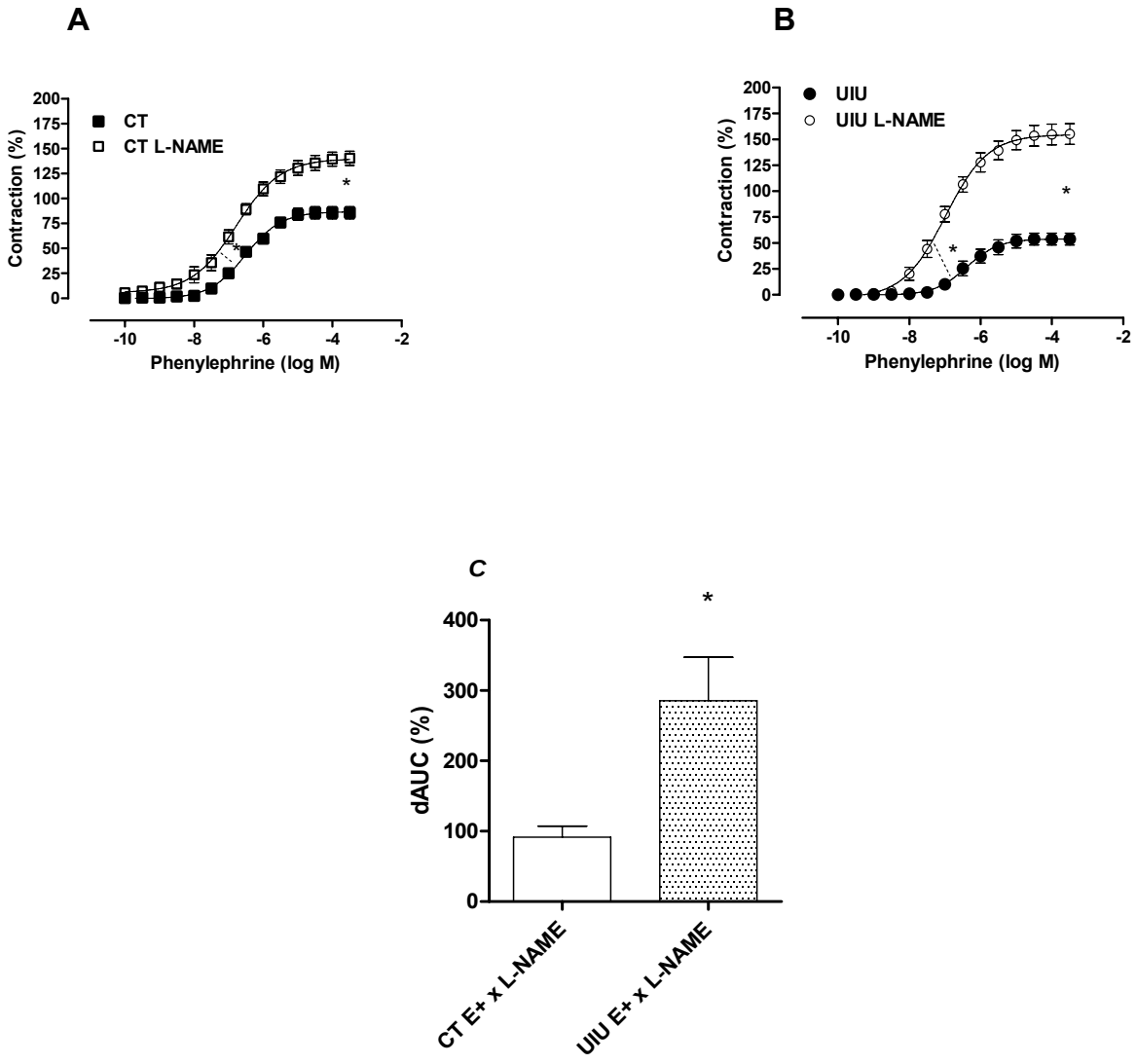

Figure 11- Concentration-response curves to phenylephrine in isolated aortic rings of Wistar rats: (A) before (CT E+,n=8) and after incubation with L-NAME (CT L-NAME n=8); (B) The effect of intra-uterine undernutrition before (UIU E +, n=8) and after incubation with L-NAME (UIU L-NAME, n=8); (C) Comparison of the percentage difference of area under the curve in control and undernourished. Values are expressed as mean ± SEM; Student's t test unpaired. *$p < 0.05$ for pD_2 and R_{max}: CT E+ *vs* CT L-NAME; pD_2 and R_{max}: UIU E+ *vs* UIU L-NAME and dAUC% E + vs L-NAME- CT: 91.00 ± 14.22g vs UIU : 146 ± 43.58g.

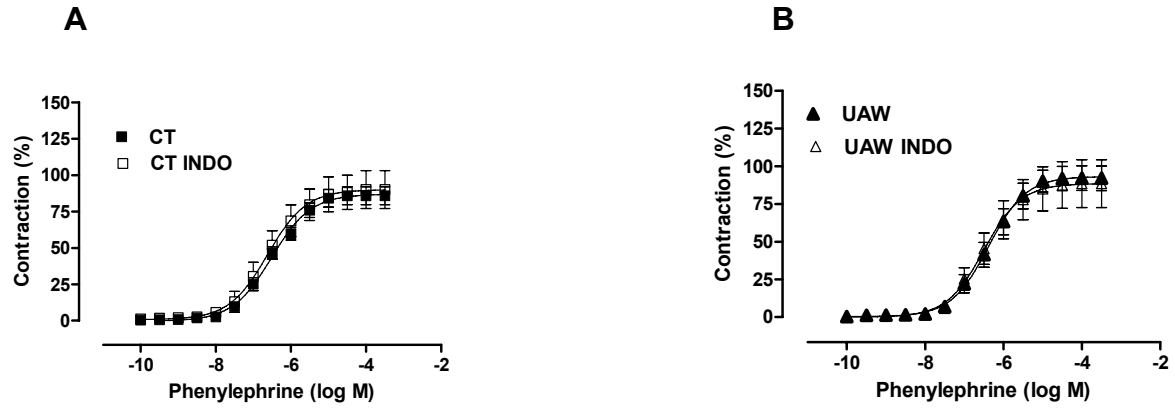

Figure 12- Concentration-response curves to phenylephrine in isolated aortic rings of Wistar rats: (A) before (CT E+, n=8) and after incubation with indomethacin (CT INDO,n=8); (B) Effect of undernutrition after weaning before, UAW, (UAW E+, n=8) and after incubation with indomethacin (UAW INDO). Values are expressed as mean ± SEM; Student's t test unpaired. $p>0.05$, for R_{max} and $pD_{2:}$ CT *vs* CT INDO and ; R_{max} and pD_2: UAW *vs* UAW INDO.

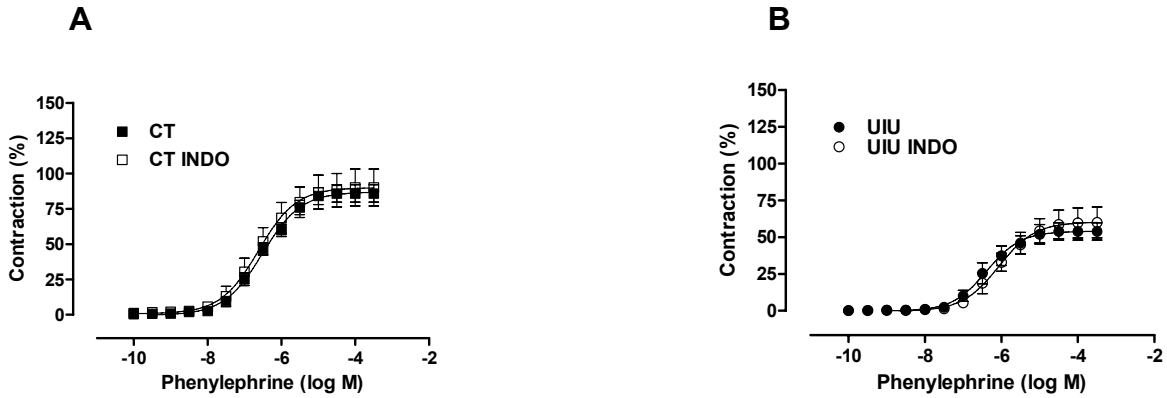

Figure 13- Concentration-response curves to phenylephrine in isolated aortic rings of Wistar rats: (A) before (CTE +,n=8) and after incubation with indomethacin (CT INDO, n=8); (B) The effect of intra-uterine undernutrition before (UIU E+, n=8) and after incubation with indomethacin (UIU INDO, n=8). Values are expressed as mean ± SEM; Student's t test unpaired. $p>0.05$, for R_{max}: CTE+ *vs* CT INDO; R_{max} and pD_2: UIU E+ *vs* UIU INDO and % dAUC% - Control *vs* UIU.

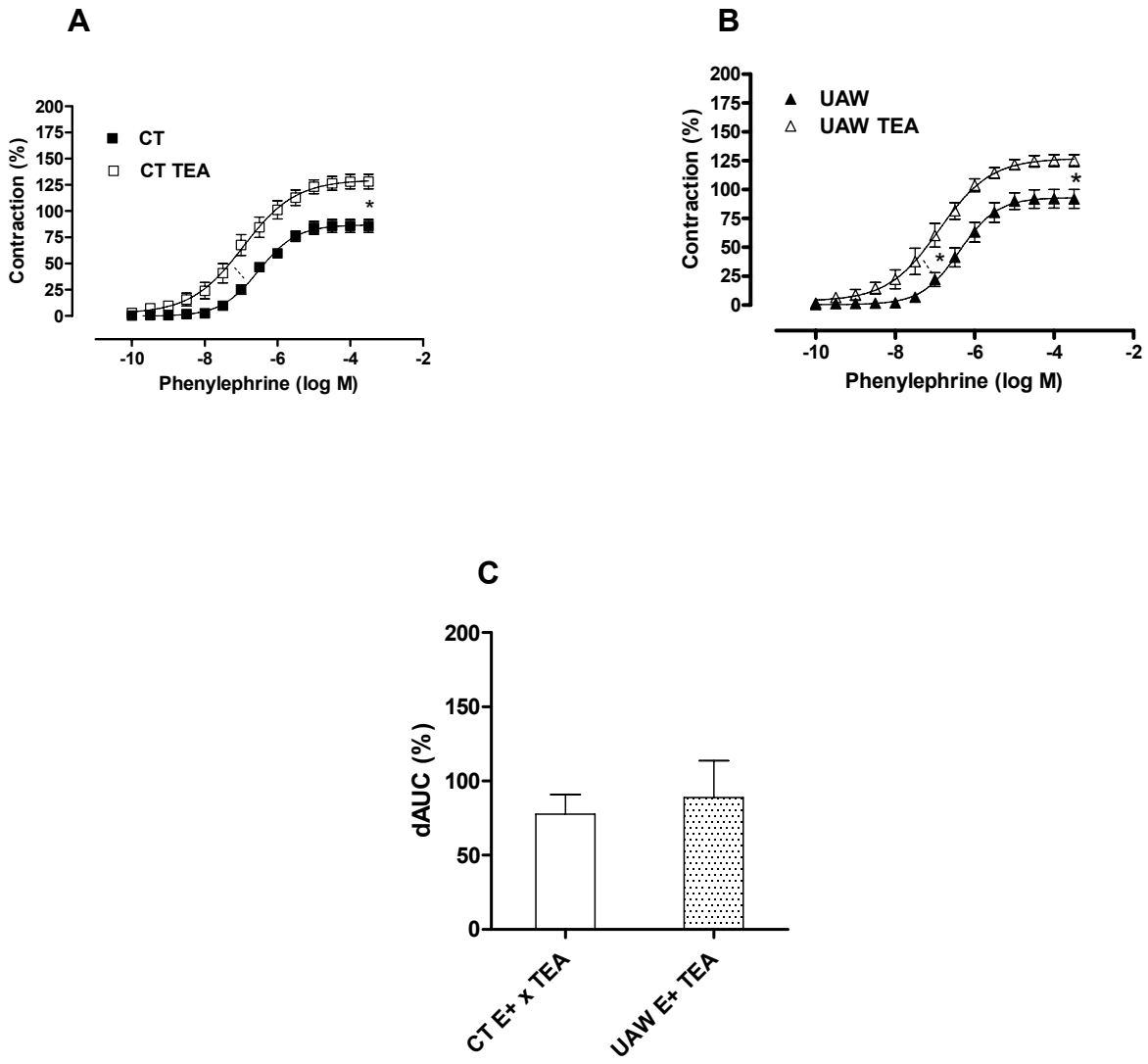

Figure 14- Concentration-response curves to phenylephrine in isolated aortic rings of Wistar rats: (A) before (CT E+,n=8) and after incubation with tetraetilamonia (CT TEA, n=8); (B) undernourished after weaning before (UAW E +, n=8) and after incubation with tetraetilamônio (UAW TEA, n=8); (C) Comparison of the percentage difference of area under the curve in control and undernourished. Values are expressed as mean ± SEM; Student's t test unpaired**.** *p < 0,05 for R_{max}: CT E+ *vs* CT TEA; R_{max}: UAW E+ *vs* UAW +TEA and dAUC% - Control *vs* UAW.

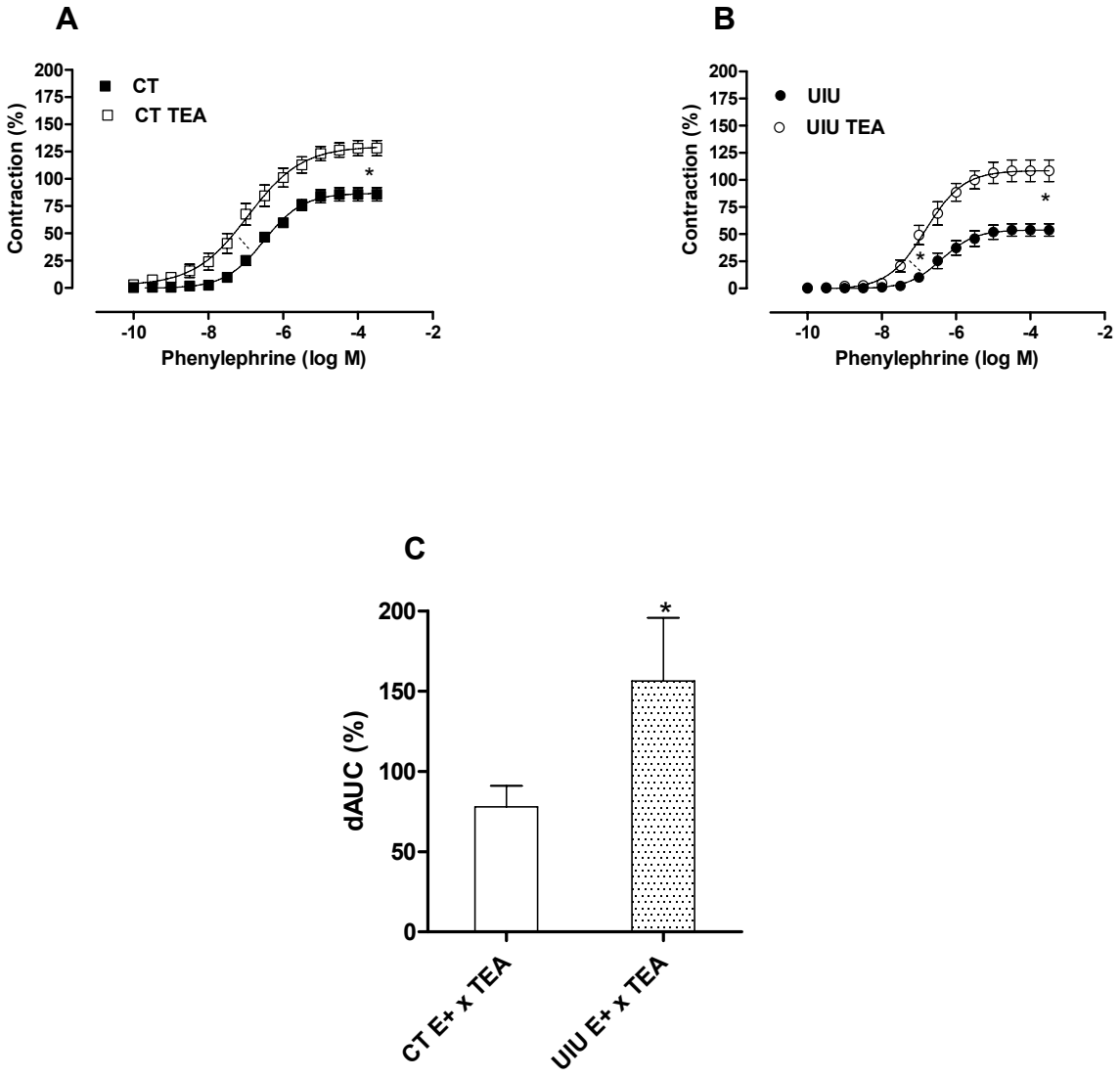

Figure 15- Concentration-response curves to phenylephrine in isolated aortic rings of Wistar rats: (A) before (CT E+,n=8) and after incubation with tetraetilamônio (CT TEA, n=8); (B) undernourished intra-uterine before (UIU E+, n=8) and after incubation with tetraetilamonia (UIU TEA, n=8); (C) Comparison of the percentage difference of area under the curve in control and undernourished. Values are expressed as mean ± SEM; Student's t test unpaired**.** *p < 0,05 for R_{max}: CT E+ *vs* CT TEA; R_{max}: UIU E+ *vs* UIU +TEA and dAUC% CT: 77.79 ± 13.18g vs UIU:156.27 ± 39.39g.

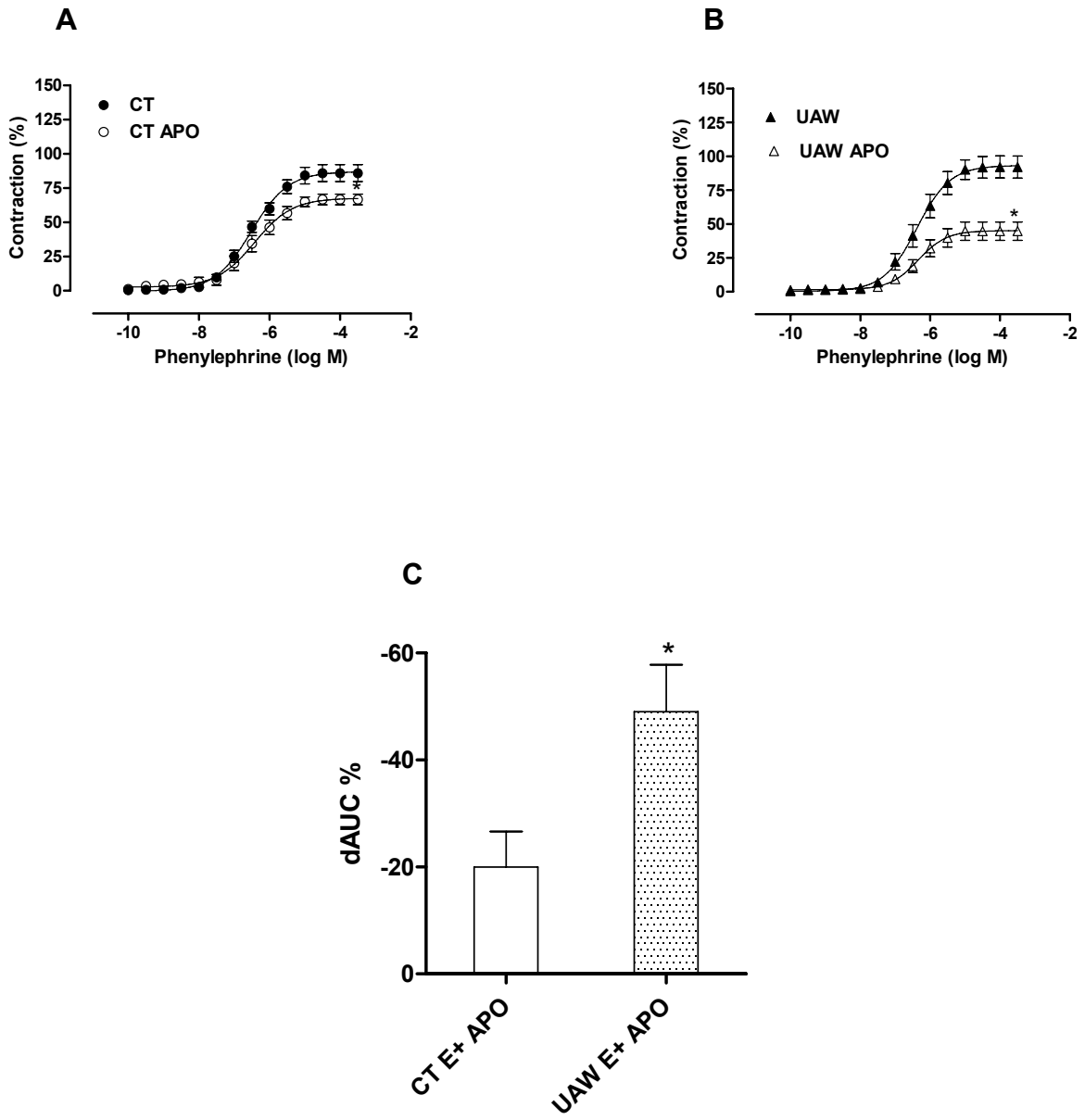

Figure 16- Concentration-response curves to phenylephrine in isolated aortic rings of Wistar rats : (A) before (CT E+,n=8) and after incubation with apocynin (CT APO, n=8); (B) Undernourished after weaning before (UAW E+, n=8) and after incubation with apocynin (UAW APO, n=8); C) Comparison of the percentage difference of area under the curve in control and undernourished. Values are expressed as mean ± SEM; Student's t test unpaired. *$p < 0.05$ for R_{max}: CT E+ *vs* CT APO; R_{max}: UAW E+ *vs* UAW +APO and dAUC% CT: 15.03 ± 6.32g vs UAW: 49.04 ± 8.78g.

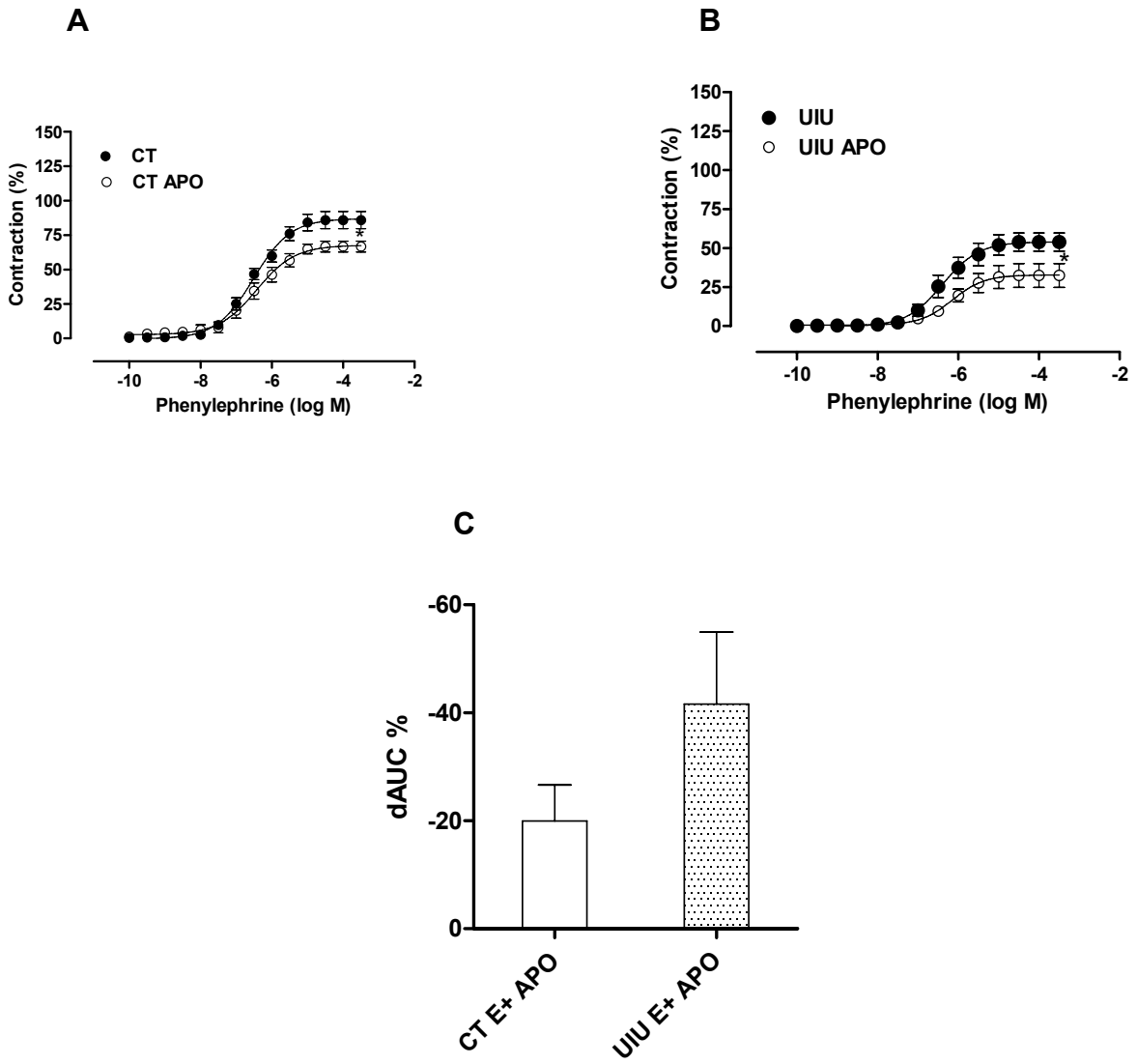

Figure 17- Concentration-response curves to phenylephrine in isolated aortic rings of Wistar rats: (A) before (CT E+, n=8) and after incubation with apocynin (CT APO, n=8), (B) undernourished intra-uterine before (UIU E+,n=8) and after incubation with apocynin (UIU APO n=8); (C) Comparison of the percentage difference of area under the curve in control and undernourished. Values are expressed as mean ± SEM; Student's t test unpaired. $p > 0.05$ for R_{max}: CT E+ *vs* CT APO; R_{max}: UIU E+ *vs* DIU +APO and dAUC% CT: 15.03 ± 6.32g vs UIU: 41.60 ± 13.36g.

Table 7- Maximum response (R_{max}, g) and sensitivity (pD2) to phenylephrine in isolated aortic rings of control rats (CT) and undernourished intra-uterine (UIU) and after weaning (UAW) with and without endothelium, in the presence of L-NAME, tetraetilamonium (TEA), apocynin (APO) and indomethacin (INDO).

GRUPOS (N=8)	R_{max} (g)	pD_2
CT E+	85.83 ± 6.19	6.54 ± 0.08
CT E-	155.38 ± 10.37#	7.166 ± 0.10
CT L-NAME	140.40 ± 7.12*#	6.859 ± 0.13
CT INDO	89.25 ± 12.53	6.659 ± 0.14
CT TEA	129.00 ± 6.66#	6.999 ±0.22
CT APO	68.21 ± 4.24*	6.497 ± 0.14
UAW E+	92.73 ± 8.03	6.349 ± 0.14
UAW E-	165.42 ± 13.41	7.641 ± 0.15
UAW L-NAME	151.41 ± 10.10*	7.494 ± 0.15
UAW INDO	95.95 ±1 6.41	6.413 ± 0.10
UAW TEA	125.92 ± 5.03	6.996 ± 0.19
UAW APO	44.97 ± 6.79*	6.289 ± 0.09
UIU E+	54.25 ± 5.60	6.288 ± 0.15
UIU E-	147.84±13.44#	7.413 ± 0.14
UIU L-NAME	154.78 ± 9.96#	7.005 ± 0.06
UIU INDO	60.20 ± 10.50	6.095 ± 0.14
UIU TEA	107.92 ± 9.57#	6.828 ± 0.12
UIU APO	32.73 ± 7.74	6.22 ± 0.14

Values are expressed as mean ± SEM. “N" indicates the number of animals used in each group; Student’s t test unpaired, *#p<0.05.

Table 8- Maximum response (R_{max}, g) and sensitivity (pD2) to acetylcholine in isolated aortic rings of *Wistar* rats control group (CT) and undernourished intra-uterine group(UIU) and after weaning (UAW).

GRUPOS (N=8)	R_{max} (g)	pD_2
CT	93.86 ± 2.7	6.15 ± 0.60
UAW	100.05 ± 1.12	6.88 ± 0.72
UIU	97.58 ± 2.31	6.23 ± 0.33

Values are expressed as mean ± SEM. “N" indicates the number of animals used in each group; Student’s t test unpaired.

4. CONCLUSÕES

O modelo de desnutrição qualitativo empregado nos períodos intra-útero (DIU) e após-desmame (DPD) vs normonutrido mostrou :

- Redução no peso corporal em ambos os grupos, sendo mais severa no grupo DPD.

- A desnutrição pós-demame promove elevação de PA, que pode ,em parte, ser divido ao aumento da reatividade vascular *in vitro*, no leito arterial caudal, no entanto foi observado um aumento do relaxamento a ACh , o que provavelmente estaria atuando como um mecanismo compensatório. Em seguimentos isolados de aorta, a desnutrição pós-desmame não é acompanhada de alterações na reatividade vascular à FE. Porém, há um aumento da liberação de NO que é contrabalanceado pelo aumento de radicais livres derivados da NADPHoxidae, sugerindo que nesse modelo há uma disfunção endotelial.

- A desnutrição intra-uterina não promoveu alterações da PA, nem alterações na reatividade vascular caudal a agentes vasoconstritores e vasodilatadores, em contrapartida , em seguimentos isolados de aorta esse modelo promoveu queda de reatividade vascular à FE que pode estar associada ao aumento da liberação basal de NO e da liberação de um fator hiperpolarizante derivado do endotélio abridor de canais para potássio sensíveis ao cálcio.

- Portanto, a desnutrição dependendo do período do desenvolvimento, afeta diferentemente os níveis pressóricos e a resposta vascular em ratos.

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ANEXOS

ESTUDO DA REATIVIDADE VASCULAR NO LEITO CAUDAL DE RATOS JOVENS SUBMETIDOS A DESNUTRIÇÃO EM DIFERENTES MOMENTOS DO DESENVOLVIMENTO

[1]Belchior ACS, [2]Faria TO, [2]Padilha AS, [2]Angeli JK, [2]Siman FDM, [2]Meira EF, [2]Siveira EA, [2]Vassallo DV, [1]daCosta CJEDP.
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Objetivo: Avaliar se a desnutrição em dois diferentes momentos do desenvolvimento - pós-desmame (PD) e intra-útero (IU) - modifica a reatividade do leito arterial caudal (LAC) de ratos jovens.
Métodos: Após o desmame, ratos Wistar foram submetidos ou não a uma dieta multicarencial (dieta básica regional) até o período da idade adulta (~ 3 meses). Dois grupos se formaram: ratos controle (CT, n=9) e PD (n=6). No grupo IU(n=9), as matrizes foram desnutridas apenas durante o período gestacional. Após atingir a idade adulta, os ratos foram anestesiados e o LAC foi cateterizado, removido e perfundido sob fluxo constante (2,5 mL/min), com solução de Krebs-Henseleit (gaseificada com 5% CO_2 e 95%O_2, à 36ºC). Alterações na pressão de perfusão média (PPM, mmHg) foram produzidas por doses crescentes de fenilefrina (PE 0.001-300ug, *in bolus*), ou por perfusões com concentrações crescentes de acetilcolina (ACh 10^{-10}-10^{-3} M) e nitroprussiato de sódio (NPS 10^{-9}-10^{-2} M), sob pré-contração com KCl (65 mM). Resultados expressos como média ± EPM, análise estatística teste-t de Student, significante p<0,05.
Resultados: No grupo PD houve aumento da resposta máxima à PE (492,7±29,5 mmHg) quando comparado ao grupo CT (295,3 ± 32,8 mmHg). No grupo IU (249,5±18 mmHg; p<0.05), essa resposta não foi modificada, e não ocorreu alterações na sensibilidade (pD2) a PE. O relaxamento a ACh aumentou no grupo PD (86,5±3,8%), quando comparado ao grupo CT (70,8±4,2%). Já no IU não houve alterações nem na resposta máxima (72,4 ± 3,6%, p<0.05), nem na pD2 a PE. O relaxamento induzido pelo NPS foi similar entre os grupos.
Conclusão: A desnutrição PD promove aumento da reatividade à PE no LAC de ratos jovens. Ao mesmo tempo, aumenta o relaxamento à ACh, sem modificar, no entanto, o relaxamento ao NPS. Esses resultados sugerem que a liberação estimulada de óxido nítrico, avaliada pelo relaxamento à ACh, está incrementada, possivelmente agindo como um mecanismo compensatório ao aumento da reatividade vascular à PE no grupo PD. A desnutrição IU não modifica essas respostas.

Apoio Financeiro: FAPES/FUNCITEC; CAPES; CNPq; FACEPE.

A DESNUTRIÇÃO APÓS O DESMAME E NA FASE INTRA-UTERINA ALTERA A MODULAÇÃO ENDOTELIAL EM AORTA DE RATOS JOVENS

[1]Angeli JK,[1]Padilha AS,[2]Belchior ACS,[1]Faria TO,[1]Siman FDM,[1]Meira EF, [1]Siveira EA,[1]Vassallo DV,[2]daCosta CJEDP-[1]Universidade Federal do Espírito Santo, Vitória - [2]Universidade Federal de Pernambuco, Recife.

Objetivo: Avaliar a reatividade vascular e a modulação endotelial em aorta isolada de ratos jovens desnutridos após o desmame (DBRPD) e no período intra-uterino (DBRIU)
Materiais e Métodos: Foram usados anéis de aorta de ratos Wistar controle (CT n=10), desnutridos DBRPD (n=10) e DBRIU (n=10). A reatividade à fenilefrina (FE, 10^{-10}-$3x10^{-4}$M) foi avaliada na presença e ausência do endotélio e após incubação com L-NAME, indomentacina, tetraetilamônio e apocinina. Resultados expressos média±EPM e diferença da área abaixo da curva (dAUC%). Análise estatística: teste-t de Student (significância $p<0.05$). Protocolo de desnutrição: Ratos DBRPD foram submetidos a uma dieta multicarencial (dieta básica regional) até a idade adulta (3 meses). Já os DBRIU, a dieta multicarencial foi dada a mãe no período de acasalamento e durante a gravidez
Resultados: A reatividade à FE não alterou nos DBRPD, mas reduziu nos DBRIU. A ausência do endotélio ou a incubação com L-NAME aumentou a reatividade à FE de ambos os grupos desnutridos quando comparados ao CT (dAUC% - E+ vs E- CT: 133.9±28.19 vs DBRPD: 387.2±68.02 vs DBRIU: 308.7±71.1; E+ vs L-NAME CT: 91.00±14.22 vs DBRPD: 158.2±23.93 vs DBRIU: 285.3±61.9). A incubação com indometacina não modificou a resposta à FE em nenhum dos grupos. Já a incubação com tetraetilamônio reduziu a resposta à FE apenas nos DBRIU quando comparados aos CT (dAUC% CT: 77.7±13.1 vs DBRIU: 156.2±39.9). A incubação com APO reduziu a reatividade à FE em todos os grupos, mas esse efeito foi significativo apenas entre os DBRPD e CT (dAUC% CT: 15.03±6.32 vs DBRPD: 49.04±8.78)
Conclusões: A desnutrição promove diferentes tipos de disfunção endotelial, dependentes do período do seu estabelecimento. A desnutrição após o desmame aumenta a liberação de óxido nítrico (NO) e espécies reativas de oxigênio (ROS) derivadas da atividade da NADPH oxidase. Esses fatores se contrapõem, não modificando a reatividade à FE. Já a desnutrição intra-uterina não é acompanhada de aumento da produção de ROS, mas apresenta maior liberação de NO e fator hiperpolarizante derivado do endotélio, que por sua vez, reduzem a reatividade à FE

Auxílio Financeiro: CNPq, FAPES/FUNCITEC, FACEPE.

ESTUDO DA REATIVIDADE VASCULAR NO LEITO CAUDAL DE RATOS JOVENS SUBMETIDOS A DESNUTRIÇÃO EM DIFERENTES MOMENTOS DO DESENVOLVIMENTO

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Objetivo: Avaliar se a desnutrição em dois diferentes momentos do desenvolvimento - pós-desmame (PD) e intra-utero (IU) - modifica a reatividade do leito arterial caudal (LAC) de ratos jovens.
Métodos: Após o desmame, ratos Wistar foram submetidos ou não a uma dieta multicarencial (dieta básica regional) até o período da idade adulta (~ 3 meses). Dois grupos se formaram: ratos controle (CT, n=9) e PD (n=6). No grupo IU as matrizes foram desnutridas apenas durante o periodo gestacional Após atingir a idade adulta, os ratos foram anestesiados e o LAC foi cateterizado, removido e perfundido sob fluxo constante (2,5 mL/min), com solução de Krebs-Henseleit (gaseificada com 5% CO_2 e 95%O_2, à 36ºC). Alterações na pressão de perfusão média (PPM, mmHg) foram produzidas por doses crescentes de fenilefrina (PE 0.001-300ug, *in bolus*), ou por perfusões com concentrações crescentes de acetilcolina (ACh0^{-10}-10^{-3} M) e nitroprussiato de sódio (NPS10^{-9}-10^{-2} M), sob pré-contração com KCl (65 mM). Resultados expressos como média ± EPM, análise estatística teste-t de Student, significante $p<0,05$.
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Conclusão: A desnutrição PD promove aumento da reatividade à PE no LAC de ratos jovens. Ao mesmo tempo, aumenta o relaxamento à ACh, sem modificar, no entanto, o relaxamento ao NPS. Esses resultados sugerem que a liberação estimulada de óxido nítrico, avaliada pelo relaxamento à ACh, está incrementada, possivelmente agindo como um mecanismo compensatório ao aumento da reatividade vascular à PE no grupo PD. A
desnutrição IU não modifica essas respostas.

Apoio Financeiro: FAPES/FUNCITEC; CAPES; CNPq; FACEPE.

A DESNUTRIÇÃO APÓS O DESMAME E NA FASE INTRA-UTERINA ALTERA A MODULAÇÃO ENDOTELIAL EM AORTA DE RATOS JOVENS

[1]Angeli JK,[1]Padilha AS,[2]Belchior ACS,[1]Faria TO,[1]Siman FDM,[1]Meira EF, [1]Siveira EA,[1]Vassallo DV,[2]daCosta CJEDP-[1]Universidade Federal do Espírito Santo, Vitória - [2]Universidade Federal de Pernambuco, Recife.

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Auxílio Financeiro: CNPq, FAPES/FUNCITEC

Trabalho apresentado oralmente no IV Congresso da SBC-ES (Sociedade Brasileira de Cardiologia- Espírito Santo)-2009

A desnutrição após o desmame na fase intra-uterina altera a modulação endotelial em aorta de ratos jovens.

FARIA, T O, ANGELI, J K, PADILHA, A S, BELCHIOR, A C S, SIMAN, F D M, MEIRA, E F, ALMEIDA, E A S, VASSALLO, D V e COSTA, C J E D P.

Universidade Federal do Espírito Santo, Vitória, ES, BRASIL e Universidade Federal de Pernambuco, Pernambuco, PE, BRASIL.

Introdução: Langley-Evans et al. (1994) mostraram em animais que, a desnutrição pode ser responsável por alterações no sistema cardiovascular, como o aumento da pressão arterial.
Objetivos: Avaliar a reatividade vascular e a modulação endotelial em aorta isolada de ratos jovens desnutridos após o desmame (DBRPD) e no período intra-uterino (DBRIU).
Métodos: Foram usados anéis de aorta de ratos Wistar controle (CT n=10), desnutridos (DBRPD n=10) e (DBRIU n=10). A reatividade à fenilefrina (FE) foi avaliada na presença e ausência do endotélio e após incubação com L-NAME, indomentacina, tetraetilamônio e apocinina. Os ratos DBRPD foram submetidos a uma dieta multicarencial até a idade adulta. Já os DBRIU, a dieta foi dada a mãe no período de acasalamento e durante a gravidez. Resultados expressos média±EPM e diferença da área abaixo da curva (dAUC%). Análise estatística: teste-t de Student (significância $p<0.05$).
Resultados:A reatividade à FE não alterou nos DBRPD, mas reduziu nos DBRIU. A ausência do endotélio ou a incubação com L-NAME aumentou a reatividade à FE de ambos os grupos desnutridos quando comparados ao CT (dAUC% - E+ vs E- CT: 133.9±28.19 vs DBRPD: 387.2±68.02 vs DBRIU: 308.7±71.1; E+ vs L-NAME CT: 91.00±14.22 vs DBRPD: 158.2±23.93 vs DBRIU: 285.3±61.9). A incubação com tetraetilamônio reduziu a resposta à FE apenas nos DBRIU quando comparados aos CT (dAUC% CT: 77.7±13.1 vs DBRIU: 156.2±39.9). A incubação com apocinina reduziu a reatividade à FE apenas entre os DBRPD e CT (dAUC% CT: 15.03±6.32 vs DBRPD: 49.04±8.78).
Conclusões: A desnutrição após o desmame aumenta a liberação de óxido nítrico (NO) e espécies reativas de oxigênio (ROS) derivadas da atividade da NADPH oxidase. Esses fatores se contrapõem, não modificando a reatividade à FE. Já a desnutrição intra-uterina não é acompanhada de aumento da produção de ROS, mas apresenta maior liberação de NO e fator hiperpolarizante derivado do endotélio, que por sua vez, reduzem a reatividade à FE.
Auxílio Financeiro: CNPq, CAPES, FAPES/FUNCITEC, FACEPE.

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ESTUDO DA REATIVIDADE VASCULAR NO LEITO CAUDAL DE RATOS JOVENS SUBMETIDOS A DESNUTRIÇÃO EM DIFERENTES MOMENTOS DO DESENVOLVIMENTO

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Objetivo: Avaliar se a desnutrição em dois diferentes momentos do desenvolvimento - pós-desmame (PD) e intra-utero (IU) - modifica a reatividade do leito arterial caudal (LAC) de ratos jovens.

Métodos: Após o desmame, ratos Wistar foram submetidos ou não a uma dieta multicarencial (dieta básica regional) até o período da idade adulta (~ 3 meses). Dois grupos se formaram: ratos controle (CT, n=9) e PD (n=6). No grupo IU as matrizes foram desnutridas apenas durante o periodo gestacional Após atingir a idade adulta, os ratos foram anestesiados e o LAC foi cateterizado, removido e perfundido sob fluxo constante (2,5 mL/min), com solução de Krebs-Henseleit (gaseificada com 5% CO_2 e 95%O_2, à 36ºC). Alterações na pressão de perfusão média (PPM, mmHg) foram produzidas por doses crescentes de fenilefrina (PE 0.001-300ug, *in bolus*), ou por perfusões com concentrações crescentes de acetilcolina (ACh0^{-10}-10^{-3} M) e nitroprussiato de sódio (NPS10^{-9}-10^{-2} M), sob pré-contração com KCl (65 mM). Resultados expressos como média ± EPM, análise estatística teste-t de Student, significante $p<0,05$.

Resultados: No grupo PD houve aumento nas pressões sistólica (PAS), diastólica (PAD) e na média (PAM), assim como aumento da resposta máxima à PE (492,7 ± 29,5 mmHg) quando comparado ao grupo CT (295,3 ± 32,8 mmHg). No grupo IU (249,5±18 mmHg; $p<0.05$), essa resposta não foi modificada, e não ocorreu alterações na sensibilidade (pD2) a PE. O relaxamento a ACh aumentou no grupo PD (86,5 ± 3,8%), quando comparado ao grupo CT (70,8 ± 4,2%). Já no IU não houve alterações nem na resposta máxima (72,4± 3,6%, $p<0.05$), nem na pD2 a PE. O relaxamento induzido pelo NPS foi similar entre os grupos.

Conclusão:

- A desnutrição pós-desmame provocou aumento das PAS, PAD e da PAM.
- Promoveu aumento da reatividade à FE no LAC de ratos jovens.
- Aumentou o relaxamento à ACh.
- Não modificou o relaxamento ao NPS
- Sugerindo que esse aumento na Pressão Arterial está associado ao aumento da reatividade vascular à FE no grupo DPD.
- Não foi possível avaliar se a desnutrição no grupo DIU modifica essas respostas

Apoio Financeiro: FAPES/FUNCITEC; CAPES; CNPq; FACEPE.