EDINÉIA GOEDERT

VARIAÇÃO NOS TEORES DE SÓDIO EM DIETA MULTICARENCIADA: EFEITOS SOBRE ALGUNS ASPECTOS DA FUNÇÃO RENAL

RECIFE-PE

2010

UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
MESTRADO EM BIOQUÍMICA E FISIOLOGIA

VARIAÇÃO NOS TEORES DE SÓDIO EM DIETA MULTICARENCIADA: EFEITOS SOBRE ALGUNS ASPECTOS DA FUNÇÃO RENAL

EDINÉIA GOEDERT

Orientadora: PROF. DRª. ANA DURCE OLIVEIRA DA PAIXÃO

Co-orientadora: PROFª. DRª. CARMEN DE CASTRO CHAVES

RECIFE - PE
2010

EDINÉIA GOEDERT

VARIAÇÃO NOS TEORES DE SÓDIO EM DIETA MULTICARENCIADA: EFEITOS SOBRE ALGUNS ASPECTOS DA FUNÇÃO RENAL

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

RECIFE-PE

2010

Goedert, Edinéia

Variação nos teores de sódio em dieta multicarenciada: efeitos sobre alguns aspectos da função renal / Edinéia Goedert. – Recife: O Autor, 2010.

49 folhas : il., fig., tab.

Orientador: Ana Durce Oliveira da Paixão

Dissertação (mestrado) – Universidade Federal de Pernambuco. CCB. Bioquímica e Fisiologia, 2010.

Inclui bibliografia.

1. Desnutrição 2. Conteúdo dietético de sódio 3. Filtração glomerular em ratos 4 Balanço de sódio 5 Concentração urinária I Título.

616.39 CDD (22.ed.) UFPE/CCB – 2010- 127

EDINÉIA GOEDERT

VARIAÇÃO NOS TEORES DE SÓDIO EM DIETA MULTICARENCIAL: EFEITOS SOBRE ALGUNS ASPECTOS DA FUNÇÃO RENAL

APROVADA PELA
COMISSÃO EXAMINADORA

Profª. Drª. Ana Durce Oliveira da Paixão
Universidade Federal de Pernambuco
Presidente da banca

Prof. Dr. Carlos José Eduardo das Dores Peres da Costa
Universidade Federal de Pernambuco
Avaliador

Profª. Drª. Ângela Amâncio dos Santos
Universidade Federal de Pernambuco
Avaliadora

DEDICATÓRIA

Com muito amor ao meu marido e aos meus filhos, Gabriela e Felipe, pela compreensão das muitas horas ausentes e a memória do meu pai com quem tive a honra de aprender e desfrutar de seus ensinamentos e, em especial, a minha querida mãe Eranilza, que sem ela não chegaria onde estou.

AGRADECIMENTOS

A Deus, que sempre esteve presente na minha vida e por me sustentar nos momentos mais difíceis. Acredito que sem ele, nada teria sentido.

A minha querida mãe Eranilza Maciel Goedert e a memória do meu pai Dionísio Goedert pela minha formação humana e pelos ensinamentos de valores eternos, onde somos todos diferentes, porém todos iguais. Aos meus irmãos que mesmo distante, sempre me apoiaram.

As orientadoras Prof[a]. Dr[a]. Ana Durce Oliveira da Paixão e Dr[a]. Carmen de Castro Chaves pelos ensinamentos grandiosos e confiança depositada para realização deste trabalho.

Aos queridos Mestres Dr[a]. Glória Isolina Buente Pinto Duarte, Dr. Carlos Peres da Costa Prof[a]. Dr[a]. Ângela Amâncio dos Santos e Prof. Dr. Claudio Gabriel Rodrigues pelo apoio e colaboração.

Em especial à Coordenadora do Programa de Pós-Graduação em Bioquímica e Fisiologia, Dr[a]. Vera Lúcia de Menezes Lima pela atenção e incentivo.

Ao Laboratório de Bioquímica, da UFRPE, em especial a Dr[a] Eneida e Telga Lucena Alves pelo apoio na realização do hemograma e ao Laboratório Marcelo Magalhães pela colaboração nas dosagens bioquímicas.

A querida amiga Aucelia Cristina Soares de Belchior pela grande e importante participação na realização deste sonho.

Aos colegas de mestrado, pelo convívio e amizade, tendo comigo compartilhado tantas alegrias, dificuldades e experiências.

Aos amigos Leucio, Djair, Valdilene, Geórgia, Samia, Naishe, Felipe, Aline, Deyse e Silvio, pela colaboração na realização dos experimentos e agradável convívio.

Aos amigos Jailson Honorato Pinto Júnior e Ana Virgínia Marinho, pelo apoio incondicional.

Aos professores do curso de mestrado em Bioquímica e Fisiologia pelos conhecimentos difundidos e a oportunidade de compartilhar novos conhecimentos.

A Nielson e Djalma pela amizade e apoio durante o curso.

A Isa Rocha de Lima e as demais pessoas que de alguma forma tenham contribuído para realização deste trabalho, os meus sinceros agradecimentos.

RESUMO

No presente trabalho, através de diferentes concentrações de sódio na dieta, investigamos se a natriurese observada nos ratos em DBR desde o desmame decorria do maior aporte de Na^+ ou da sua menor conservação pelo rim e o seu efeito sobre a concentração urinária. Para isso utilizamos uma dieta multicarenciada, localmente denominada dieta básica regional (DBR) e 60 ratos machos Wistar, tratados a partir do desmame com: i) dieta controle, nos padrões da American Institute of Nutrition (AIN), o grupo controle (C); ii) DBR suplementada com baixo teor de Na^+ (0,06%), o grupo DBRhipo; iii) DBR suplementada com teor normal de Na^+ (0,3%), o grupo DBRnormo; iv) DBR suplementada com teor alto de Na^+ (3,12%), o grupo DBRhiper. Foram avaliados: filtração glomerular (FG), reabsorção tubular de Na^+, capacidade de concentração urinária e níveis pressóricos arteriais. O balanço de Na^+ foi investigado através do uso de gaiolas metabólicas. A FG foi avaliada através do clearance de creatinina (C_{Cr}) e a reabsorção tubular de Na^+ foi avaliada a partir do clearance de Li^+. A capacidade de concentração urinária foi avaliada através da privação de água durante 12 h. O peso corporal (PC) apresentou-se comprometido ao longo de todo desenvolvimento. Aos 126 dias de idade, os grupos DBRhipo, DBRnormo, e DBRhiper apresentaram PC menor que o grupo C (200 ± 19; 229 ± 17; 164 ± 4 *vs* 439 ± 27 g, respectivamente, $P<0,05$). A FG e reabsorção tubular proximal de Na^+ apresentaram-se semelhantes entre todos os grupos. A reabsorção tubular distal de sódio apresentou-se maior no grupo DBRhipo em relação ao DBRnormo e C (99,15 ± 0,13 *vs* 92,90 ± 0,09 e 93,56±0,47 %, respectivamente, $P<0,05$) e menor no grupo DBRhiper em relação ao DBRnormo e C (87,54±0,68 *vs* 92,90 ± 0,09 e 93,56±0,47 %, respectivamente, $P<0,05$). Os níveis pressóricos arteriais apresentaram-se semelhantes entre os grupos. Diante da privação de água, o fluxo urinário apresentou-se mais elevado e a densidade urinária menor no grupo DBRnormo em ralação ao grupo C (1,25 ± 0,06 *vs* 1,13 ± 0,09 ml/100g e 1,051 *vs* 1,051 µg/ml, respectivamente, $P<0,05$). No entanto, a capacidade de excreção em relação ao ingerido de sódio apresentou-se similar entre estes dois grupos (99,01 ± 0,12 *vs* 99,02 ± 0,17 %). A desnutrição comprometeu o mecanismo de concentração urinária, e a excreção de sódio estava relacionada ao teor deste eletrólito na dieta.

Palavras-chave: Desnutrição, conteúdo dietético de Na^+, filtração glomerular em ratos, balanço de Na^+, concentração urinária.

ABSTRACT

The present study assessed whether the handling of Na^+ was altered in animals submitted to malnutrition multicarencial also known as DBR. Thereunto, we used male Wistar rats treated from weaning on with: i) control diet, AIN standards in the control group (CONc), ii) DBR supplemented with low-Na^+ (0.06%), the group DBRhipo iii) DBR supplemented with normal content of Na^+ (0.3%), the group DBRnormo iv) DBR supplemented with high content of Na^+ (3.12%), the group DBRhyper. Parameters such as glomerular filtration (GF), filtered load of Na^+ (CF_{NA+}), proximal tubular reabsorption of Na^+ ($RFrP_{Na}{}^+$) and distal tubular reabsorption of Na^+ ($RFrD_{Na+}$). The handling of Na^+ was investigated by using metabolic cages. FG was assessed by creatinine clearance (CCR) and ($RFrP_{Na}{}^+$) was calculated from the clearance of Li^+. Body weight (BW) maintained impaired throughout all development period. At 126 days, groups DBRhipo, DBRnormo and DBRhiper had BW lower than the CONC group (200.5 ± 18.60, 229.5 ± 17.57, 163.8 ± 3.89 vs 438.7 ± 27, 0, respectively, $P < 0.05$). The glomerular filtration rate, filtered load of Na^+, proximal tubular reabsorption of Na^+ were similar between groups DBRhipo, DBRnormo, and the group DBRhyper C. A ($RFrDNa^+$) was higher in group DBRhipo in relation to DBRnormo and CONc (99.14 ± 0.05 vs 93.07 ± 0.18 and 93.56 ± 0.47, respectively, $P < 0.05$) and lower DBRhiper group in relation to DBRnormo and C (88.12 ± 0.54 vs 93.07 ± 0.18 and 93.56 ± 0.47, respectively, $P < 0.05$). The handling of Na^+ did not appear altered in the group submitted to malnutrition multicarenciada, however, the group DBRhipo had a lower excretion of Na^+ and the group DBRhyper greater excretion of Na^+ relative to DBRnormo and the innovation because of the varied concentration of Na^+ containedin diet.

Keywords: Undernutrition, dietary Na^+, glomerular filtration rate, sodium balance, urinary concentration.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS

ADH - hormônio antidiurético

AD_{Na+} - aporte distal de Na^+

CONc - grupo controle e dieta controle

DBR – dieta básica regional

C_{cr} - *clearance* de creatinina

CF_{Na+} - carga filtrada de Na^+

C_{Li+} - *clearance* de lítio

D - densidade urinária

EDTA - ácido etilenodiamino tetra-acético

FSR - fluxo sanguíneo renal

GM - gaiola metabólica

Hct - hematócrito

IL - ingestão de água

IS - ingestão de sólido

Kf - coeficiente de ultrafiltração

LEC- líquido extracelular

min - minuto

PUF - pressão efetiva de ultrafiltração

RD - rim direito

RE - rim esquerdo

RFG - ritmo de filtração glomerular

$RFrD_{Na+}$ - reabsorção fracional distal de Na^+ I

$RFrP_{Na+}$ - reabsorção fracional proximal de Na^+

rpm - rotação por minuto

TP - túbulo proximal

$U_{cr}V$ - excreção urinária de creatinina

$U_{K+}V$ - excreção urinária de K^+

$U_{Na+}V$ - excreção urinária de Na^+

V - diurese

VS – versus

LISTA DE SÍMBOLOS

$[X]_u$ - concentração urinária

$[X]_p$ - concentração plasmática

Cl^- - cloro

g - grama

HCO_3 - bicarbonato

K^+ - potássio

Li^+ - lítio

LiCl - cloreto de lítio

Na^+ - sódio

NaCl - cloreto de sódio

ºC - graus Celsius

SUMÁRIO

1. INTRODUÇÃO

A Organização Mundial da Saúde (OMS) define a desnutrição como uma condição patológica causada por ingestão deficiente ou inadequada de calorias e/ou proteínas, sendo considerada a doença nutricional mais importante nos países em desenvolvimento. Sua prevalência nestes países está diretamente relacionada ao inadequado crescimento econômico e social, tendo como conseqüências graves a elevação das taxas de mortalidade infantil e prejuízo no desenvolvimento físico e mental de indivíduos (JANICKI *et al*, 2002). A nutrição adequada, em qualidade e quantidade, é fundamental para o crescimento e desenvolvimento dos seres vivos e do homem. Deficiências nutricionais, pelo menor aporte de macro e micronutrientes na alimentação, levam a desnutrição (MORGANE *et al*, 1992).

A desnutrição constitui um grave problema de saúde pública no Brasil, tem um elevado custo médico-social, sobretudo por sua contribuição no desenvolvimento e por doenças como acidente vascular cerebral, coronariopatias e insuficiência cardíaca e renal crônica. O número de internações no Sistema Único de Saúde no país, decorrente de doenças cardiovasculares vem aumentando e representa a principal causa de gastos em assistência médica (LESSA, 2006; AMARAL *et al*, 2004).

Apesar do aumento das taxas de obesidade infantil nos últimos anos, o número de crianças desnutridas é maior do que o de crianças obesas. Também, indivíduos com desnutrição no primeiro ano de vida apresentaram maior mortalidade por doenças cardiovasculares do que os que nasceram com baixo peso, mas não sofreram desnutrição pós-natal, sugerindo que a desnutrição na primeira infância é um importante prognóstico para doenças cardiovasculares (FERNANDES *et al*, 2003; SAWAYA *et al*, 2005). Estes estudos vem contribuindo para indicar que a desnutrição, especialmente na primeira infância, pode causar alterações bioquímicas, fisiológicas e cardiovasculares no individuo adulto.

A desnutrição tem sido associada à hipertensão arterial (HA). Estudos em crianças desnutridas de favelas na cidade de São Paulo, foi demonstrada a associação entre desnutrição, peso e altura na primeira infância e a HA, com aumento da pressão em crianças desnutridas com mais de 2 anos de idade e daquelas que se recuperaram da desnutrição após um período médio de 6 anos, bem como, há um aumento da pressão diastólica e sistólica em adolescentes de baixa estatura (SESSO *et al*, 2004).

Levantamentos alimentares e antropométricos na Zona da Mata Sul do Estado de Pernambuco, desde a década de 70, associaram a desnutrição severa com baixo peso,

sobretudo nas crianças (BATISTA *et al*, 1981; TORRES, 1975). Esses estudos orientaram a formulação da dieta experimental denominada Dieta Básica Regional (DBR) por TEODÓSIO *et al*, em 1990, multicarenciada e que reproduz as condições alimentares encontradas nos estudo acima.

O rim é muito importante na homeostase corporal e é o responsável pela manutenção do conteúdo corporal de Na^+, assegurando que a excreção de Na^+ seja precisamente igual a sua ingestão, num processo de ajuste denominado balanço de Na^+. Com excreção de Na^+ menor do que a ingestão ocorre um balanço positivo deste eletrólito, o Na^+ em excesso fica retido no organismo, sobretudo no extracelular (LEC), assim ocorre um aumento da ingestão de água levando a expansão do LEC, inclusive do volume sanguíneo, podendo haver elevação da PA e edema. Inversamente, se a excreção de Na^+ for maior do que sua ingestão, o balanço é negativo. Tem mecanismos que regulam separadamente as reabsorções de Na^+ e água. O Na^+ é filtrado, em seguida é reabsorvido cerca de 67% no túbulo proximal, 25% no ramo ascendente grosso da alça de Henle, 5% no túbulo convoluto distal e 3% no ducto coletor, este último o responsável pelo ajuste fino da reabsorção do Na^+, assegurando a precisão do balanço de Na^+ (AIRES, 2008).

Na diminuição do volume plasmático e/ou PA, as células granulares do aparelho justaglomerular segregam o hormônio renina, que ativa o angiotensinogênio transformando-o em angiotensina I. Ao passar pelos pulmões, a angiotensina I sofre ação da enzima conversora de angiotensina (ECA), sendo convertida em angiotensina II a qual, por sua vez, estimula a secreção de aldosterona no córtex adrenal. Assim, quando diminui a quantidade de Na^+ no organismo, a aldosterona aumenta a reabsorção deste íon no ducto coletor. A ação da aldosterona cria canais de Na^+ na membrana luminal e bombas sódio-potássio na membrana basolateral como no ducto coletor (CURY, 2009). Enquanto o sistema renina-angiotensina-aldosterona provoca a reabsorção de Na^+, o hormônio natriurético (HNA) e outros hormônios aumentam sua excreção de Na^+ na urina, inibindo sua reabsorção. O átrio cardíaco é um local de armazenamento do HNA em grânulos em células musculares. Na expansão do volume circulatório, como conseqüência, estas células são estiradas, o HNA é secretado, impedindo a reabsorção de Na^+ pela ação da aldosterona nas células principais do ducto coletor, aumentando a excreção de Na^+ e, assim, diminuindo a PA (AIRES, 2008).

Diante da importância do rim, pesquisadores vêm estudando efeitos da DBR sobre aspectos da função renal. Em 2003, PAIXÃO e colaboradores induziram desnutrição crônica desde o desmame com a DBR, observando vasodilatação e aumento do fluxo sanguíneo renal, além de

natriurese em ratos adultos, anestesiados. Em 2005, CASTRO-CHAVES e colaboradores, ratos adultos em desnutrição crônica com a DBR, e apresentavam aumentos da excreção de Na^+, filtração glomerular, reabsorção tubular proximal e dos pesos relativos do coração, fígado e testículos além de não concentrarem urina. Em 2007, CASTRO-CHAVES e colaboradores, ratos com o mesmo modelo de desnutrição crônica com a DBR apresentavam hipoproteinemia, aumento do peso seco do coração sugestivo de hipertrofia cardíaca e achatamento da borda em escova do túbulo proximal. Em 2009, no mesmo modelo, a não concentração de urina, os aumentos da filtração glomerular e da excreção de Na^+ foram reproduzidos, mas acompanhados de uma maior atividade da Na^+-ATPase, sugerindo ativação do Sistema-Renina-Angiotensina-Aldosterona. A Na^+-ATPase, situada na membrana basolateral do túbulo proximal, faz o ajuste fino do balanço corporal de Na^+, tendo a angiotensina II como um dos seus principais hormônios reguladores (LARA, 2010).

Neste trabalho foi utilizada uma dieta controle com caseína como fonte protéica e com teor normal e estável de Na^+. O presente trabalho objetiva investigar se a natriurese observada nos ratos em DBR desde o desmame decorria do maior aporte de Na^+ ou da sua menor conservação pelo rim, utilizando rações DBR com teores respectivamente baixos, normais e elevados de Na^+.

2. Objetivos

2.1. Geral

- Determinar se a natriurese nos ratos em DBR desde o desmame decorre do maior aporte de Na^+ ou da sua menor conservação pelo rim. Investigar se a desnutrição *per se* compromete o manuseio de Na^+ e/ou a capacidade de concentrar urina.

2.2. Específicos

- Investigar, através da utilização de uma dieta multicarenciada, com diferentes teores de sódio:

- a filtração glomerular e o transporte tubular proximal de sódio;
- o balanço de sódio e o volume plasmático;
- os níveis pressóricos
- a capacidade de concentrar urina

3. ARTIGO

NATRIURESIS DOES NOT ACCOUNT FOR URINARY CONCENTRATION INHABILITY IN THE CHRONICALLY UNDERNOURISHED RAT

Edineia Goedert [a], Ana DO Paixão [a], Carmen de Castro-Chaves [a]

[a] Departamento de Fisiologia e Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Recife, PE, Brazil.

Corresponding author: Carmen de Castro-Chaves, Departamento de Fisiologia e Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Av. Prof.Moraes Rego S/N, 50670-901, Recife, PE, Brazil. Phone: 21268530; Fax 21268976; e-mail: cchaves@ufpe.br

Abbreviations

BW; Body weight

$C_{cr;}$ creatinine clearance

CD; Control diet

C_{Li}^{+}; Li^{+} clearance

C_x ; clearance of x

DD; deficient diet

DD_{Na+}; distal Na^{+} delivery

FL_{Na+}; Na^{+} filtered load

GFR; glomerular filtration rate

HSDD; high Na^{+} DD

LSDD; low Na^{+} DD

NSDD; normal Na^{+} DD,

P_x; plasma concentration of x

$DFrR_{Na+}$; distal fractional Na+ reabsorption

$PFrR_{Na+}$; proximal fractional Na+ reabsorption

U_x; urinary concentration of x

V ; urine volume

vs; versus

ABSTRACT The nature of natriuresis, renal concentration inability and the plasma volume alteration in chronic undernutrition were all addressed in this study. Male Wistar rats were weaned on deficient diets (DD) variable in Na^+: 0.06%, LSDD; 0.3% NSDD; 3.12%, HSDD. Control diet (CD) was AIN 93 M. At weeks 8, 13 and 18 of age, 24 h Na^+ and H_2O balances were kept in metabolic cages. Urine concentration ability, glomerular filtration rate (GFR) and proximal tubular re-absorption were assessed in adult rats, respectively by 12 h water deprivation, creatinine (C_{cr}) and Li^+ clearances (C_{Li^+}) and Na^+ transport was calculated. Body weight (BW) was impaired in DD groups throughout the study. Diet intake was higher in HSDD and LSDD than in NSDD and CD groups ($P<0.05$). Na^+ excretion relative to intake was similar among NSDD, HSDD and CD. Water excretion relative to ingestion was similar among LSDD, NSDD and CD, been higher in HSDD than in CD group. Cl_{cr} and C_{Li^+} were both similar among all groups. To maintain Na^+ balance, fractional distal Na^+ re-absorption was increased in LSDD but lower in HSDD as to CD (99.1 ± 0.05; 87.5±0.7; 93.6 ± 0.5%, $P<0.05$). H_2O balance was also maintained, even at the HSDD group. NSDD rats were unable to concentrate urine contrasted to CD group. Thus, undernourished rats were able to maintain Na^+ balance, whereas they were unable to concentrate urine. This impairment seemed independent of Na^+, since its excretion was strictly correlated with the dietary intake.

Keywords: Undernutrition, dietary Na^+, glomerular filtration rate, sodium balance, urinary concentration.

Introduction

Natriuresis, defective water handling, impaired urine concentration,activation of renin-angiotensin-system, hypoproteinemia and metabolic alterations, were described in chronic undernutrition [1, 2]. Epidemiological studies in Pernambuco State, Brazil, found undernutrition mostly in children due to the diet consumed. These studies were the base for an experimental diet normal in carbohydrates but deficient in proteins, lipids, vitamins and minerals, and also in NaCl [3]. The effects of chronic undernutrition by this diet on renal function aspects have been addressed as follow. Adult rats with chronic undernutrition presented hypoproteinemia, renal vasodilatation and natriuresis leading to a negative balance, with unaltered GFR [4]. The intake of such a diet from weaning impaired renal Na^+ and H_2O conservation, but increased GFR and proximal tubule re-absorption in adult rats with unaltered levels of nitric oxide in blood and urine [5]. Juvenile and adult undernourished rats urinary Na^+-excretion was higher being twice the Na^+ intake a in control group) and the adult undernourished showed augmented fractional proximal Na^+ reabsorption (61.0 ± 0.3% vs 81.8 ± 2.2%) with a concomitant decrease in distal delivery (9.5 ± 0.5 lmol/min vs 14.0 ± 0.2 lmol/min per 100 g BW). At the molecular level, the lack angiotensin II sensibility could be due to Na^+-ATPase hyperactivity and the ATP-dependent Na^+ transporters were affected in opposite ways: the (Na^+-K^+)ATPase activity from undernourished rats fell by 30%, in parallel with a 20% decrease in its immunodetection, whereas the ouabain-insensitive Na^+-ATPase, which is responsible for the fine-tune control of Na^+ reabsorption, increased threefold [5].

The present work was designed to verify if the natriuresis seen on rats on chronic undernutrition was due to increased dietary Na^+ intake or to renal impairment to conserve

this electrolyte. Additionally, the effects of variable dietary Na^+ content and chronic undernutrition on renal concentrating mechanism were assessed. Plasma volume was measured in normal sodium content diets for its effect on effective circulatory volume.

2. Materials and methods

2.1. Materials

All reagents used here were of the highest purity available: creatinin kit Biosystem; Lithium chloride (Cloreto de lítio) P.A. VETEC, Sodium Cloride (Cloreto de sódio) P.A. VETEC.

2.2. Animals

Experiments were conducted in accordance with the Guide for the Care and Use for Laboratory Animals – EUA and were approved by the Ethics Committee for Animal Experimentation from the Federal University of Pernambuco, Brazil by report number N 029/06. Wistar rats were maintained in a room at 22±2^{o}C, with 12-h light–dark cycle, 50% humidity, and reared at four per collective cage with free access to food and water, except when indicated. Only male rats were used in all experiments. All rats were weighed weekly from weaning, and had tail blood collected at weaning and at three week intervals to measure hematocrit. 40 rats were previously adapted to individual metabolic cages, remaining there only during the experimental procedures [7]. At the completion of the 24h balance studies, renal concentration was assessed. At least 72 h later, the rats were treated with LiCl, as described below, to evaluate GFR and proximal tubule Na^+ re-absorption.

2.3. *Diets and experimental groups*

Control diet (CD) was AIN 93 M [8], modified by adding NaCl to obtain a Na^+ 0.3 g/100 g diet. The deficient diet (DD) was prepared according to Teodósio et al, 1990 [3] with

the following ingredients (g/100g): manioc flour (64.9), beans (18.5), sweet potatoes (12.9) and cured meat (3.7). All dietary components but manioc, were cooked, dehydrated at 60^{o} C, pulverized, weighed, mixed and had meat fat added (0.3 g/100g). The mixture was hydrated into a paste and dehydrated at 60^{o}C for 24 h to obtain pellets. Dietary modifications: i) the cured meat was repeatedly washed, the diet was prepared as above and the diet final sodium was 0,049 mg/100 g; ii) the diet was prepared as above employing cured meat washed as above, NaCl was added at the end, to obtain diets with low, normal and high Na^+ levels, respectively: 0.15 (LSDD), 0.3 (NSDD) or 0.6 (HSDD) g/100g [9]. Dietary composition (Table 1) was held at the Federal University of Pernambuco Nutrition Department. At the day 22nd day of life, age-matched rats were weaned and randomly assigned into CD or to one of the deficient diets LSDD, NSDD or HSDD until 18th week of age.

2.4. Sodium and water balances

Rats were housed in individual metabolic cages. As being previously adapted to the cages, after 1 day of acclimatization, diet and H_2O intake plus urine volume were measured for two 24 h periods at 8th, 13th and 18th weeks of age. Na^+ intake was calculated by the product of food intake and Na^+ dietary content. Data is a mean of the two measurements.

2.5. Renal concentration capacity

Renal concentration capacity was assessed by a 12 h overnight H_2O deprivation in rats from all groups but HSDD group, as previously done in humans [1] and in rats [10]. Urine volume, density and Na^+ concentrations were determined but Na^+ excretion was calculated. All values were corrected for 100g BW.

2.5. GRF and proximal tubule Na^+ re-absorption

GFR and proximal tubule Na^+ re-absorption were measured respectively by creatinine (Cl_{cr}) and lithium (Cl_{Li}) clearances while Na^+ tubular transport was calculated [11, 12]. General clearance formula (C_x) is: $C_x = U_x \times V / P_x$ (1)

Where, U_x is x concentration in urine, V is urine volume and P_x is plasma concentration of x.

The rats were given LiCl (0.06 mmol/100 g BW) by gavage and were maintained with H_2O but no food overnight (12 h). Then, they received a H_2O overload (5 mL/100 g BW) by gavage in two steps (3 and 2 mL/100 g BW), respectively 90 and 30 min before being housed in metabolic cages. At the end, blood was collected by decapitation in non-heparinized tubes. Then, major organs were removed and weighed. All values were corrected for 100 g of BW. Na tubular transport was assessed by the following formula:

Na^+ filtered load (FL_{Na+}) = $Cl_{cr} \times [Na^+]_{plasma}$, in µEq/min/100g (2);

Na^+ distal delivery (DD_{Na+}) = $Cl_{Li+} \times [Na^+]_{plasma}$, in µEq/min/100g (3);

Na^+ proximal tubule fractional reabsorption ($PTFr_{Na+}R$) =$[(FL_{Na+} - DD_{Na+})/FL_{Na+}] \times 100$(4);

Na^+ distal tubule fractional reabsorption ($DTFr_{Na+}R$) = $[(DD_{Na+})-(U_{Na+}V)/DD_{Na+}] \times 100$ (5).

2. 6. Plasma volume measurement

Plasma volume was assessed by Evans Blue dye, after anesthesia with sodium pentobarbital (60 mg/kg BW, i.p.). Briefly, a femoral artery was catheterized one 1 mL basal blood sample was collected in heparinized syringe to obtain plasma, after centrifugation and the catheter was filled with physiological saline. The dye (0.1% in physiological saline) was administered (100 µg/100 g BW) and the catheter was flushed with 200 µL of physiological saline. After 7.5 min, physiological saline in the catheter was discarded and another 1 mL

blood sample was collected as above. The plasmatic dye concentration was determined spectrophotometrically at 610 nm and compared to a standard curve constructed with known concentrations of Evans Blue dye and samples of basal plasma [13].

2.7. Analytic technique

Urine density was assessed by refractometry (Atago), showing upper limit of 1.050. Na^+ and creatinine concentrations in serum and urine were determined respectively by a selective ion analyser 9180 (Roche) and colorimetry on a Beckman Coulter, CX9 ALX.

2.8. Statistical analysis

Data is presented as mean±SD. The statistical analysis was by *Student-Newman-Keuls* test. Significance values were set at 95% ($p<0.05$)

1. Results

As shown in Fig. 1, although at weaning all offspring had similar BW, the weight gain during development was decreased on the DD groups at all Na^+ levels. The adult LSDD rats weighed less than NSDD rats only at 18^{th} week of age, but the HSDD rats had the lowest weights at all weeks either compared to CD or to NSDD. The hematocrit increased with age similarly among groups: at weaning the value was 34±35 for all; at 8^{th} week (CD: 40±4, LSDD: 45±2, NSDD: 44±2, HSDD: 45 ± 2), at 13^{th} week (CD: 45±3, LSDD: 48±2, NSDD: 47±1, HSDD: 47±2) and at 18^{th} week of age (CD: 47±2, LSDD: 50±2, NSDD: 49±1, HSDD: 49±1). The ratio liver/body weight was higher only at HSDD compared to NSDD, while the relative heart weight (heart/body weight) was increased in HSDD either compared to CD or NSDD groups. The ratio testicles/body weight was augmented in all DD groups compared to CD, and in

HSDD also compared to NSDD. Undernutrion induced kidney atrophy as can be seen in groups LSDD and NSDD (Table 2), but the high dietary sodium led to kidney hypertrophy in the HSDD group. The renal index was similar between HSDD and CD.

As shown in Fig. 2, all rats on the DD from weaning had a higher diet intake throughout life than rats on CD at all study weeks but rats on LSDD and HSDD diet intake was greater than NSDD. The values of percent Na^+ excretion over intake were similar among NSDD, HSDD and CD (Fig. 2). Due to the low sodium intake, the levels of Na^+ urinary sodium concentration shown by LSDD were undetectable in the sodium electrolyte analyzer.

As can be seen in Fig. 3, at 8^{th} of age, HSDD exhibited H_2O intake 5 times all other groups but at 13^{th} and 18^{th} weeks of age, the difference was 3 times. The values of percent H_2O excretion in urine over intake decreased with age. At 8^{th} week the mean values were 40% for CD, LSDD and NSDD but 77% for HSDD; at 13^{th} week mean values were 31% for CD, LSDD and NSDD but 56% for HSDD; and at the 18^{th} week of age CD, LSDD and NSDD mean values were 23% and the rats from HSDD group excreted 55% percent of the H_2O intake. Dietary Na^+ influenced H_2O intake as expected, but H_2O balance was maintained independently of nutrition condition and Na^+ intake.

After 12 h overnight H_2O deprivation (Fig. 4), the decrement in urine volume was less than CD and lower urinary density on NSDD group points to incapacity to conserve H_2O..

Creatinin and lithium clearances were similar among rats from all groups (Table 3). The mean value of plasma lithium in all experimental groups was 0.30±0.09 µEq/mL, varying from 0.26 to 0.33, µEq/mL. Besides being measured after an acute administration, these values were well below the nephrotoxic levels observed in humans in chronic treatments [14]. Sodium tubular transport values (Table 4), assessed by filtered load, distal delivery plus

proximal tubule fractional re-absorption were similar among groups. However, fractional distal re-absorption was increased in LSDD and decreased in HSDD compared to CD and NSDD, due to Reinforcing data on maintained Na^+ balance in undernourished rats, the plasma volume was similar (3.4±0.41 vs 3.3±0.37mL) between NSDD and CD groups.

4. Discussion

The organ weight relative to body weight was adopted here due to great difference in rat weights from CD and the DD groups at 18th week of age. Rats on DD at different Na^+ levels weighed less than the ones on CD, due to deficient quality and quantity of nutrients but carbohydrates. Na^+ levels were the only difference among the DD, and NSDD rats weighed more than LSDD at 18th week but rats on HSDD group weighed less throughout the study, compared to CD and NSDD rats. The results on LSDD are different from the weight pattern seen on 12 week old rats, from weaning on normal protein diets but with low, normal or high s Na^+: the rats on low Na^+ diet weighed more than controls, and rats on the high Na^+ weighed less from the 8th week until adults [9], in agreement with the NSDD data here. Interestingly, the weight gain stabilized differently among the experimental groups: at the 13th week for the CD, at the 18th week for HSDD and LSDD but rats of NSDD group were still growing at the week 18th, since protein-malnourished rats grow more slowly but for longer durations [15] , although here the rats did not reach normal final size. Organ weights were more affected in the HSDD group. The higher testicles weights on all DD groups could be due to increased angiogenesis as previously been seen [16]. Higher heart weight in the HSDD rats might be by increased oxidative stress, as has been shown that a high-salt diet leads to increased generation of reactive oxygen species in striated muscle micro-vessels, which are responsible for decreased endothelium-dependent dilation [17]. This could lead to

cardiac hypertrophy by hemodynamic mechanisms. Furthermore, increased oxidative stress could lead also to hypertrophy in some tissue [18].

Regarding the sodium balance, the values of percent Na^+ excretion over ingestion shows that rats from all groups maintained their balances, However, previous results from this laboratory found natriuresis in DD rats from weaning: i) in adult rats in a high Na^+ control diet (807.5 mg/100g) or on a low Na^+ deficient diet (207.5 mg/100 g), although Na^+ intake was higher in control rats, Na^+ excretion ware similar but the percent excreted over the ingested was 3 times higher in the low sodium deficient diet rat, thus indicating a difficulty on sodium conservation [19]; ii) Urinary Na^+ excretion was increased and almost twice the Na^+ intake in juvenile and adult DD rats as to controls. At the molecular level, the ATP-dependent Na^+ transporters were affected in opposite ways. The (Na^+-K^+)ATPase activity from undernourished rats fell by 30%, in parallel with a 20% decrease in its immunodetection, whereas the ouabain-insensitive Na^+-ATPase, which is responsible for the fine-tune control of Na^+ reabsorption, increased threefold. Then, that early alterations in proximal tubule Na^+ pumps, together with an abnormally augmented urinary Na^+ excretion, might be the link between undernutrition and late renal dysfunction [6]. Also, anesthethized 3-month day old rats assigned from weaning to a multideficient diet, which was low in sodium, presented a high urinary sodium excretion, a negative sodium balance and renal vasodilatation as well [4]. In spite of the diverse dietary Na^+, balance was maintained at all weeks with Na^+ excretion paralleling ingestion in this study.

The present work was designed to shed some light on the nature of the natriuresis seen on rats on a DD since weaning. There were three major possibilities: increased ingestion, renal impairment to conserve Na^+ and comparison to a control diet low in Na^+. All rats on the DD from weaning had higher diet intake throughout life than rats on control. Na^+

intake is a product of diet intake and Na^+ dietary content, which was respectively low, normal and high on DD rats. The excretion relative to intake indicates that rats on DD at diverse Na^+ levels were able to maintain their respective Na^+ balances at all study weeks.

The capacity to concentrate urine is the finest and most complex of the renal functions, the last to be acquired in normal life and the first to be lost when functioning renal mass starts to decrease. Then, NSDD were unable to concentrate urine, corroborating literature data in humans [1, 2], in rats [2, 10] and our previous results [5, 6, 19]. The impairment of urinary concentration mechanisms was not correlated with increment in GFR, i. e, medullar washout seem unlikely in these undernourished animals, since plasma volume was unchanged in the normal sodium levels.

GFR is reduced both in humans with caloric protein malnutrition and experimentally in rats on low protein diets [2] contributing to the hydro-electrolytic alterations. Here, GFR was not different in adult conscious on DD, independently of dietary Na^+ content. Since plasma volume was similar between CD and DD rats, it may be suggested that effective circulatory volume might be unchanged. Measurement of GFR by creatinine clearance is a valid method in conscious animals, since GFR measured simultaneously by creatinine and by [^{3}H]inulin clearances, a significant correlation was shown between them [20]

The increased urinary volume in NSDD rats could not be attributed to reduction in Na^+ re-absorption in the proximal and distal tubules as can be seen in Table 4. Kudo and others [21] have shown that Na^+ re-absorption is reduced in the thick ascendant limb of Henle loop in undernourished rats and here, we cannot discard a reduced Na^+ re-absorption in the ascending limb of Henle's loop. Furthermore, the synthesis of urea is reduced in the liver of undernourished rats, consequently diminishing medullar osmolarity [22]. This

disturbance of urinary concentration leads to an increment of urea transporters in the inner medullary collecting ducts [23].

Acknowledgment

Financial support for this research was provided by CAPES through PROCAD 8052. We would like to thank Nielson Torres de Melo for his technical support, Laboratório Marcelo Magalhães for creatinin analysis and Marcelo CC Stabile for revising the English.

References

[1] Klahr S, Tripathy K, Garcia FT, Mayoral LG, Ghitis J, Bolanos O. On the nature of the renal concentrating defect in malnutrition. Am J Med 1967;43:84–96.

[2] Benabe JE, Martinez-Maldonado M. The impact of malnutrition on kidney function. Miner Electrolyte Metab 1998;24(1):20-6.

[3] Teodósio NR, Lago ES, Romani SAM, Guedes RCA. A regional basic diet from northeast Brazil as a dietary model of experimental malnutrition. Arch Latinoam Nutr 1990;40:533-47.

[4] Paixão ADO, Nunes FA, Monteiro JC, Maciel CR. Low sodium chloride content in a multideficient diet induces renal vasodilatation in rats. Nutr Res 2003;23:85-9.

[5] Costa-Silva JH, Higa EM, Castro-Chaves C. A multideficient diet impairs renal H_2O and Na^+ conservation, increases glomerular and proximal tubule function. Abstract, Annals of World Congress of Nephrology, Singapore, 2005.

[6] Costa-Silva JH, Silva PA, Pedi N, Luzardo R, Lamas EM, Lara LS, Bezerra AM, Castro-Chaves CC, Vieyra A. Chronic undernutrition alters renal active Na^+ transport in young rats: potential hidden basis for pathophysiological alterations in adulthood? Eur J Nutr 2009;48:437–45.

[7] Mills DE, Huang YS, Narce M, Poisson JP. Psychosocial stress, catecholamines, and essential fatty acid metabolism in rats. Proc Soc Exper Biol Med 1994;205:56-61.

[8] Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A Diet. The Journal of Nutrition 1997;127:838S-41.

[9] Coelho MS, Passadore MD, Gasparetti AL, Bibancos T, Prada PO, Furukawa LL, et al. High- or low-salt diet from weaning to adulthood: Effect on body weight, food intake and energy balance in rats. Nutr Met Cardiovasc Dis 2006;16:148-55.

[10] Klein JD, Le Quach D, Cole JM, Disher K, Mongiu AK , Wang X, Bernstein KE, Sands JM. Impaired urine concentration and absence of tissue ACE: involvement of medullary transport proteins. Am J Physiol Renal Physiol 2002;283:517–24.

[11] Amaro CRPR, Padovani CR, Gontijo JAR, Figueiredo JF. Avaliação da função tubular proximal utilizando o *clearance* de lítio no tratamento pela Ciclosporina A em ratos. J Bras Nefrol 1997;19:369-75.

[12] Thomsen K. Lithium clearance as a measure of sodium and water delivery from proximal tubule. Kidney Int 1990;37(S28):S10-6.

[13] Farjanel J, Denis C, Chatard JC, Geyssant A. An accurate method of plasma volume measurement by direct analysis of Evans blue spectra in plasma without dye extraction: origins of albumin-space variations during maximal exercise. Eur J Appl Physiol Occup Physiol 1997;75:75-82.

[14] Raedler TJ, Wiedemann K. Lithium-induced nephropathies. Psychopharmacol Bull , 2007;40:134-49.

[15] Reichling TD, German RZ. Bones, muscles and visceral organs of protein-malnourished rats (*Rattus norvegicus*) grow more slowly but for longer durations to reach normal final size J. Nutr. 130: 2326–32, 2000.

[16] Carvalho M, Mateus L, Afonso F, Van Harten S, Cardoso LA, Redmer DA, Ferreira-Dias G. Testicular angiogenic activity in response to food restriction in rabbits. Reproduction 2009;137(3):509-15.

[17] Lenda DM, Sauls BA, Boegehold MA. Reactive oxygen species may contribute to reduced endothelium-dependent dilation in rats fed high salt. Am J Physiol Heart Circ Physiol 2000;279(1):H7-H14.

[18] Kitiyakara C, Chabrashvili T, Chen Y, Blau J, Karber A, Aslam S, Welch WJ, Wilcox CS. Salt intake, oxidative stress, and renal expression of NADPH oxidase and superoxide dismutase. J Am Soc Nephrol 2003;14(11):2775-82.

[19] Ribeiro VS, Silva PA, Lamas EM, Lara LS, Chaves CC, Vieyra A. A deficient diet from weaning affects renal structure, Na^+ conservation and blood pressure in the adult rat. Annals of the Covian Symposium, 2008. P. 12.

[20] Hoppe CC , Roger G, Moritz EKM, McEwen LAC, Fitzgerald SM, Dowling J, Bertram JF. Combined prenatal and postnatal protein restriction influences adult kidney structure, function, and arterial pressure. Am J Physiol 2007; 292:462–9.

[21] Kudo LH, Shimizu MHM, Seguro AC, Rocha AS. Renal concentrating defect in protein Malnutrition: the role of the thick ascending Limb of Henle and inner Medullary Collecting Duct. Nephron 1991;57:156-63.

[22] Aperia A, Broberger O, Larsson A, Snellman K. Studies of renal urea cycle enzymes. I. Renal concentrating ability and urea cycle enzymes in the rat during protein deprivation. Scand J Clin Lab Invest 1979;39(4):329-36.

[23] Isozaki T, Verlander JW, Sands JM. Low protein diet alters urea transport and cell structure in rat initial inner medullary collecting duct. J Clin Invest 1993;92(5):2448-57.

Table 1. Control (CD) and deficient diets (DD) composition (g/100g)

Diet composition	* CD (AIN 93 - M)	† DD
Protein	20.00	8.46
Lipíds	7.00	1.32
Carbohidrates	59.60	69.08
Mineral mix	3.50	-
Vitaminic complex	1.00	-
L-Cystine	0.30	-
Calories (Kcal)	381	315
NaCl	0.3	-
Na^+	0.06	-

*Values from AIN 93 M [8], modified by adding NaCl to obtain 0.3 g/100 g sodium; † DD = deficient diet, prepared with (g/100g) manioc flour (64.8), beans (18.3), sweet potatoes (12.8) and cured meat (3.7) as [3], modified by: 1) repeatedly washing the cured meat in order to obtain an almost null sodium diet (0,049 mg/100 g); 2) then adding NaCl (g/100 g) to obtain diets low (0.15-LSDD), normal (0.3-NSDD) or high (0.6-HSDD) in sodium.

Table 2. Effect of deficient diets with variable Na^+ content on body and relative organ weigth

WEIGHTS	**CD** (n=10)	**LSDD** (n=10)	**NSDD** (n= 18)	**HSDD** (n=9)
BODY (g)	425±21	202±11	229±18	164±4
LIVER	2.52±0.13	2.50±0.15	2.52±0.42	3.00±0.25**†**
HEART	0.35±0.04	0.36±0.09	0.35±0.03	0.47±0.05***†**
LUNGS	0.55±0.07	0.57±0.11	0.57±0.08	0.60±0.13
TESTICLES	0.73±0.23	1.40±0.11*****	1.12±0.16*****	1.50±0.16***†**
KIDNEYS	0.70±0.06	0.58±0.06*****	0.57±0.12*****	0.70±0.10**†**
SPLEEN	0.17±0.04	0.22±0.01	0.24±0.06	0.23±0.05

CD = Control diet was AIN 97 M [8], modified by adding NaCl to obtain 0.3 g/100 g sodium; DD = deficient diet, prepared with (g/100g) manioc flour (64.8), beans (18.3), sweet potatoes (12.8) and cured meat (3.7) as [3], modified by: 1) repeatedly washing the cured meat in order to obtain an almost null sodium diet (0,049 mg/100 g); 2) then adding NaCl (g/100 g) to obtain diets low (0.15-LSDD), normal (0.3-NSDD) or high (0.6-HSDD) in sodium. Measurements were held at age of 18 weeks. Values are in mean±SD. $P<0.05$: *vs CD; †vs NSDD by Student-*Newman-Keuls.*

Table 3. Effects of variable Na^+ content in deficient diets on glomerular (C_{cr}) and proximal tubule function (C_{Li+})

Group	N	V (µL/min/100g)	C_{cr} (µL/min/100g)	C_{Li}^+ (µL/min/100g)
CD	9	21.13±0.49	285.59±2.43	88.93±3.41
LSDD	10	20.79±2.02	278.74±14.74	83.20±13.77
NSDD	7	21.07±0.83	284.87±10.83	86.00±5.70
HSDD	10	21.43±1.44	296.01±20.00	86.89±6.14

Glomerular and proximal tubule function were measured respectively by creatinine (Cl_{cr}) and lithium (Cl_{Li}) clearances; General clearance formula is $C_x = U_x \times V / P_x$. CD = Control diet was AIN 93 M [8], modified by adding NaCl to obtain 0.3 g/100 g sodium; DD = deficient diet, prepared with (g/100g) manioc flour (64.8), beans (18.3), sweet potatoes (12.8) and cured meat (3.7) as [3], modified by: 1) repeatedly washing the cured meat in order to obtain an almost null sodium diet (0,049 mg/100 g); 2) then adding NaCl (g/100 g) to obtain diets low (0.15-LSDD), normal (0.3-NSDD) or high (0.6-HSDD) in sodium. Measurements were at of 18 week of age. Values are in mean±SD. $P<0.05$: *vs CD; †vs NSDD by Student-*Newman-Keuls.*

Table 4. Effects of variable Na^+ content in deficient diets on Na^+ tubular transport

iroup		FL_{Na+} (µEq/min/100g)	DD_{Na+} ıEq/min/100g)	FrR_{Na+} ⅙)	$DFrR_{Na+}$ (%)
CD		42.40±0.83	2.14±0.22	6.63±1.16	93.56±0.47
LSDD	0	39.85±2.35	1.91±1.22	0.03±5.65	99.15±0.13*†
NSDD		41.16±2.15	2.43±1.29	9.77±1.92	92.90±0.09
HSDD	0	43.05±2.92	2.64±0.99	0.56±2.37	87.54±0.68*†

Filtered load (FL_{Na+}), distal delivery (DD_{Na+}), proximal ($PFrR_{Na+}$) and distal fractional reabsorption ($DfrR_{Na+}$). CD = Control diet was AIN 93 M [8], modified by adding NaCl to obtain 0.3 g/100 g sodium; DD = deficient diet, prepared with (g/100g) manioc flour (64.8), beans (18.3), sweet potatoes (12.8) and cured meat (3.7) as [3], modified by: 1) repeatedly washing the cured meat in order to obtain an almost null sodium diet (0,049 mg/100 g); 2) then adding NaCl (g/100 g) to obtain diets low (0.15-LSDD), normal (0.3-NSDD) or high (0.6-HSDD) in sodium. Measurements were at week 18 of age. Values are in mean±SD. $P<0.05$: *vs CD; †vs NSDD by Student-*Newman-Keuls.*

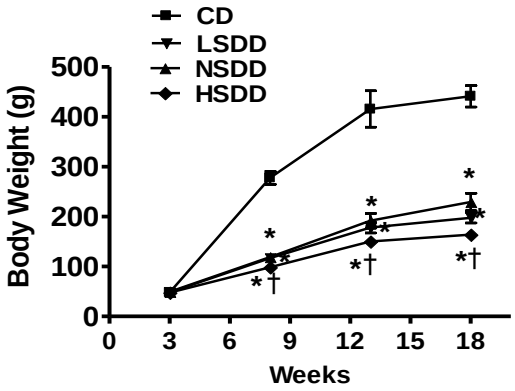
CD
LSDD
NSDD
HSDD
Body Weight (g)
500
400
300
200
100
0
0
3
6
9
12
15
18
Weeks

Fig. 1

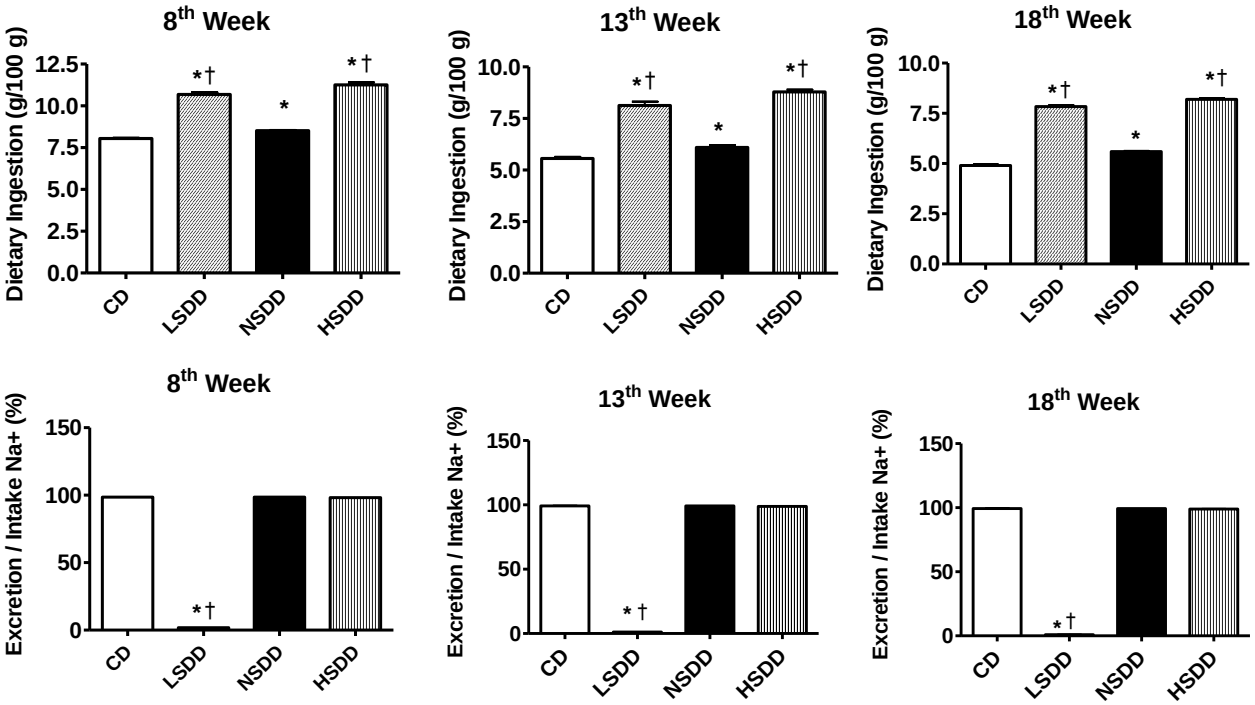
8th Week
13th Week
18th Week
Dietary Ingestion (g/100 g)
Excretion / Intake Na+ (%)
CD
LSDD
NSDD
HSDD

Fig.2

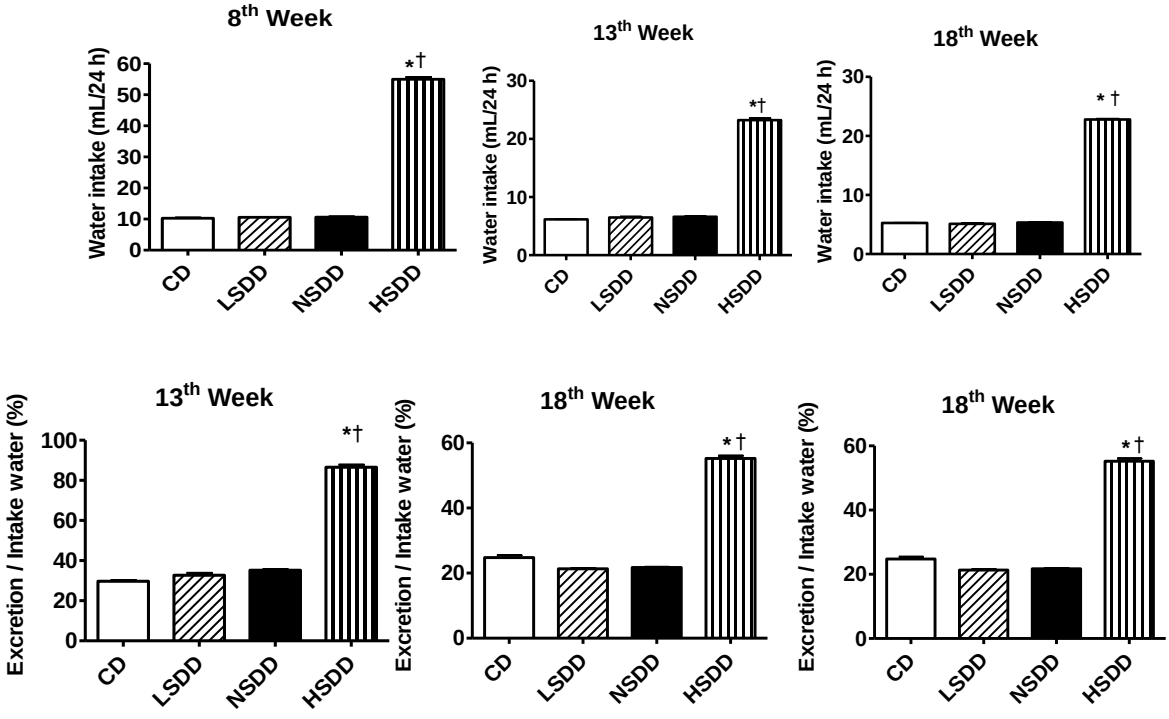
8th Week
13th Week
18th Week
Water intake (mL/24 h)
Excretion / Intake water (%)
CD
LSDD
NSDD
HSDD
*†

Fig. 3

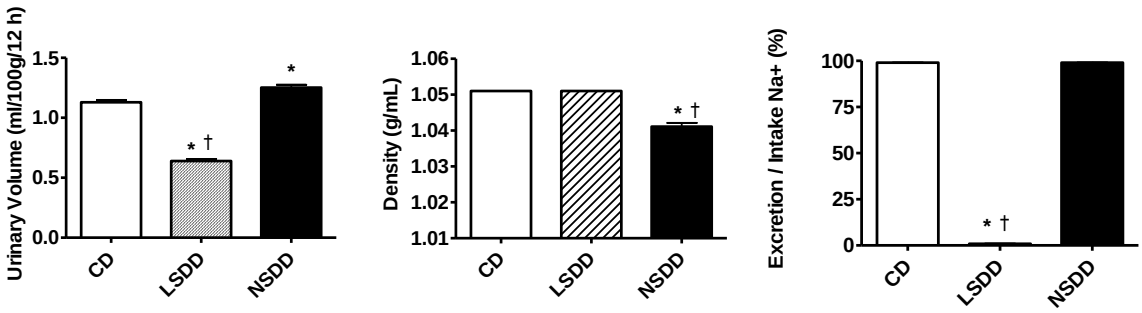
Urinary Volume (ml/100g/12 h)
Density (g/mL)
Excretion / Intake Na+ (%)
CD
LSDD
NSDD
*
* †

Fig. 4

Fig.1. Effect of deficient diets low (LSDD), normal (NSDD) or high (HSDD) in Na^+ on rat body weight. Male rats were fed, from weaning, a control Na^+ sodium diet (CD) or deficient diets either low (0.06 g/100 g), normal (0.3 g/100 g) or high (3.0 g/100 g) in Na^+. All rats were weighed weekly from weaning to week 18 of age. Values are in mean±SD. $P<0.05$: *vs CD; †vs NSDD by Student-*Newman-Keuls.*

Fig.2. Effects of deficient diets low (LSDD), normal (NSDD) or high (HSDD) in Na^+ on 24h diet intake (A) and percent Na^+ excretion over ingestion (B). Male rats, from weaning, were fed a control normal Na^+ diet (CD) or deficient diets either low (0.06 g/100 g), normal (0.3 g/100 g) or high (3.0 g/100 g) in Na^+. Studies were held at weeks 8, 13 and 18 of age. Values are in mean±SD. $P<0.05$: *vs CD; †vs NSDD by Student-*Newman-Keuls.*

Fig.3. Effects of deficient diets low (LSDD), normal (NSDD) or high (HSDD) in Na^+ on 24h H_2O intake (A) and percent H_2O excretion over intake (B). Male rats, from weaning, were fed a control normal Na^+ diet (CD) or deficient diets either low (0.06 g/100 g), normal (0.3 g/100 g) or high (3.0 g/100 g) in Na^+. Studies were held at weeks 8, 13 and 18 of age. Values are in mean±SD. $P<0.05$: *vs CD; †vs NSDD by Student-*Newman-Keuls.*

Fig.4. Effect of deficient diets low (LSDD) or normal (NSDD) in Na^+ on rats deprived of H_2O for 12 h. Male rats, from weaning, were fed a control normal Na^+ diet (CD) or deficient diets either low (0.06 g/100 g) or normal (0.3 g/100 g) in Na^+. The study was held at week 18 of age, the rats had free access to diets but not H_2O overnight, the more active period. The HSDD rats were not included, due to the elevated H_2O intake. Values are in mean±SD. $P<0.05$: *vs CD; †vs NSDD by Student-*Newman-Keuls.*

4. CONCLUSÕES

A desnutrição e o teor de sódio não influenciaram: a filtração glomerular; transporte tubular proximal de sódio; balanço de sódio; volume plasmático; níveis pressóricos.

A desnutrição comprometeu o mecanismo de concentração urinária, no entanto a excreção de sódio não parece contribuir com este distúrbio, uma vez que esta se manteve estreitamente correlacionada com a concentração deste eletrólito na dieta.

5. REFERÊNCIAS BIBLIOGRÁFICAS

ALLEYNE, G. A. O. The effect of severe protein calorie malnutrition on the renal function of Jamaican children. **Pediatrics**, v. 39, p. 400–411, 1967.

AIRES, M. M. **Fisiologia**. 3.ed. Rio de Janeiro: Guanabara Koogan, 2008.

AMARAL, A. C. S.; COELI, C. M.; DA COSTA, M. C. E.; DA SILVA CARDOSO, V.; DE TOLEDO, A. L. A.; FERNANDES, C. R. Perfil de morbidade e de mortalidade de pacientes idosos hospitalizados. **Cad Saúde Pública** [online], v. 20, n. 6, p. 1617-1626, 2004.

AMARO, C. R. P. R.; PADOVANI, C. R.,; GONTIJO, J. A. R.; FIGUEIREDO, J. F. Avaliação da função tubular proximal utilizando o clearance de lítio no tratamento pela Ciclosporina A em ratos. **J Bras Nefrol**, v.19, p.369-375, 1997.

BANKIR, L. & ROUFFIGNA, C. C. Urinary concentrating ability: insights from comparative anatomy**. Am J Physiol Regul Integr Comp Physiol,** v. 249, p. 643-666, 1985.

BARKER, D. J. & CLARK, P. M. Fetal undernutrition and disease in later life. **Reproduction,** v. 2, p. 105-112, 1997.

BATISTA FILHO, M. Desnutrição protéico-energética em 3 capitais brasileiras: São Luís, Recife e São Paulo. Rio de Janeiro, **Bol Ofic Sanit Panamer**, v.90, p.48-58, 1981.

BION, F. M.; PESSOA, D. C. N. P.; LAPA, M. A. G.; CARVALHO, M. J. Uso de uma multimistura como suplementação alimentar: estudo em ratos. **Arch Lat Amer Nutr**, v. 47, n. 3, p. 242-247, 1997.

CARVALHO, M.; MATEUS, L.; AFONSO, F.; VAN HARTEN, S.; CARDOSO, L.A.; REDMER, D.A.; FERREIRA-DIAS, G. Testicular angiogenic activity in response to food restriction in rabbits. **Reproduction**, v. 137, n. 3, p.509-15, 2009.

CASTRO-CHAVES, C.; COSTA-SILVA, J. H.; SUEMITSU, E. H. A multideficient diet impairs renal H_2O and Na^+ conservation but increases glomerular and proximal. In: 3RD WORLD CONGRESS OF NEPHROLOGY, 2005, Cingapura. Nephrology, 2005, v. 10. p. 241-241.

CASTRO-CHAVES, C.; PEDI, N.; COSTA-SILVA, J. H.; PAZ, A. M. R.; DA FONTE FILHO, L.; SILVA, P. A.; LARA, L.; VIEYRA, A. Anemia, Hipoprotenemia, Ventricle Hypertrophy, increased Na+-Atpase activity & lack of sensibility to Angiotensin II in the Undernourished Rat. In: 7RD WORLD CONGRESS OF NEPHROLOGY. Rio de Janeiro, **Abstract**, p. 66, 2007.

COELHO, M. S.; PASSADORE, M. D.; GASPARETTI, A. L.; BIBANCOS, T.; PRADA, P. O.; FURUKAWA, L. L.; FURUKAWA, L. N. S.; FUKUI, R. T.; CASARINI, D. E. ; SAAD, M. J.A.; LUZ, J.; CHIAVEGATTO, S.; DOLNIKOFF , M. S.; HEIMANN, J. C. High- or Low- salt diet fron weaning to adulthood: effect on body weight, food intake and chergy balance in rats. **Nut Metab Cardiovasc Dis**, p. 148-155, 2006.

COELHO, M. S. **Obesidade induzida por restrição crônica no consumo de sal na dieta: avaliação do perfil hormonal e do apetite em ratos wistar**. Dissertação, Universidade de Sao Paulo: COELHO, M. S., 2001.

COLES, H. **Patologia Clínica Veterinária**, 3ªed. São Paulo: Manole, 1984. p. 91-98.

COSTA, E. et al. Desnutrição recente, crônica e pregressa em quatro localidades do Estado de Pernambuco, **Saúde Pública**, v. 15, p. 211 – 20, 1981.

COSTA-SILVA, J. H.; SILVA, P. A.; PEDI, N.; LUZARDO, R.; EINICKER-LAMAS, M.; LARA, L. S.; BEZERRA, A. M.; CASTRO-CHAVES, C.; VIEYRA, A. Chronic undernutrition alters renal active Na+ transport in young rats: potential hidden basis for pathophysiological alterations in adulthood? **Eur J Nutr**, v. 48, p. 437-445, 2009.

CURI, R. & JOAQUIM, P. **Fisiologia Básica,** Rio de Janeiro: Guanabara Koogan, 2009.

DA SILVA, A. A.; DE NORONHA, I. L.; DE OLIVEIRA, I. B.; MALHEIROS, D. M.; HEIMANN, J. C. Renin-angiotensin system function and blood pressure in adult rats after perinatal salt overload. **Nutr Metab Cardiovasc Dis**, Jun, v. 13, n. 3, p. 133-9, 2003.

FAILACE, R. **Blood cell count. Manual of interpretation**. Porto Alegre: Artmed, 2003, p.298.

FARINI, M.; COSTA, P. V.; CHIAPPINI, C. J.; SOUSA, S.; PONTES, S. Z. B. Indices hematológicos de ratos em uso de multimistura básica do município de Quissamã- RJ. In: CONGRESSO NACIONAL DA SOCIEDADE BRASILEIRA DE ALIMENTAÇÃO E NUTRIÇÃO. São Paulo. **Resumos v**4, 1996.

FARJANEL, J.; DENIS, C.; CHATARD, J. C.; GEYSSANT, A. An accurate method of plasma volume measurement by direct analysis of Evans blue spectra in plasma without dye extraction: origins of albumin-space variations during maximal exercise. **Eur J Applied Physiol Occupatl Physiol**, v. 75, n. 1, p. 75-82, 1997.

FERNANDES, M. T.; SESSO, R.; MARTINS, P. A.; SAWAYA, A. L. Increased blood pressure in adolescents of low socioeconomic status with short stature. **Pediatr Nephrol,** v. 18, p. 435-439, 2003.

FLORÊNCIO, T. T.; FERREIRA, H. S,; CAVALCANTE, J. C.; SAWAYA, A. L. Short stature, obesity and arterial hypertension in a very low income population in North-eastern Brazil. **Nutr Metab Cardiovasc Dis**, v. 14, n. 1, p. 26-33, 2004.

GARCIA, W. E.; GONTIJO, J. A. R.; FIGUEIREDO, J. F. Clearance de lítio como método de avaliação do manuseio tubular de sódio em ratos acordados, estudados em gaiolas metabólicas. **J Bras Nefrol,** v. 13, p. 89-94, 1991.

HAWKINS, P.; STEYN, C.; OZAKI, T.; SAITO, T.; NOAKES, D. E.; HANSON, M. A. Effect of maternal undernutrition in early gestation on ovine fetal blood pressure and cardiovascular reflexes. **Am J Physiol Regul Integr Comp Physiol,** v. 279, p. 340-348, 2000.

HENDERSON, C. M.; ROBINSON, L.M.; DAVIDSON, P. W.; HAVEMAN, M.; JANICKI, M. P.; ALBERTINI, G. Overweight Status, Obesity, and Risk Factors for Coronary Heart Disease in Adults With Intellectual Disability. **J Policy Pract Intellec Disabil,** v. 5, n. 3, p.174–177, 2008.

HOFFMAN, D. J.; ROBERTS, S. B.; VERRESCHI, I.; MARTINS, P. A.; DE, N. C.; TUCKER, K. L.; SAWAYA, A. L. Regulation of energy intake may be impaired in

nutritionally stunted children from the shantytowns of Sao Paulo, Brazil. **J Nutr,** v. 130, p. 2265-2270, 2000.

HOFFMAN, D. J.; SAWAYA, A. L.; COWARD, W. A.; WRIGHT, A.; MARTINS, P. A. DE, N. C.; TUCKER, K. L.; ROBERTS, S. B. Energy expenditure of stunted and nonstunted boys and girls living in the shantytowns of Sao Paulo, Brazil. **Am J Clin Nutr,** v. 72, p. 1025-1031, 2000.

HOFFMAN, D. J.; SAWAYA, A. L.; VERRESCHI, I.; TUCKER, K. L.; ROBERTS, S. B. Why are nutritionally stunted children at increased risk of obesity? Studies of metabolic rate and fat oxidation in shantytown children from Sao Paulo, Brazil. **Am J Clin Nutr**, v. 72, p. 702-707, 2000.

HOUGHTON, B.L.; HUANG, C.; JOHNS, E. J. Influence of dietary sodium on the blood pressure and renal sympathetic nerve activity responses to intracerebroventricular angiotensin II and angiotensin III in anaesthetized rats. **Exp Physiol,** v. 95, n. 2, p. 282-95, 2010.

JANICKI, M. P.; DAVIDSON, P.W.; HENDERSON, C. M.; MCCALLION, P.; TAETS, J. D.; FORCE, L. T.Health characteristics and health services utilization in older adults with intellectual disability living in community residences. **J Intellect Disabil Res,** 46, 287– 298, 2002.

KUDO, L. H.; SHIMIZU, M. H. M.; SEGURO, A. C.; ROCHA, A. S. Renal concentrating defect in protein malnutrition: the role of the thick ascending limb of Henle and inner medullary collecting duct. **Nephron**, v. 57, p. 156–163, 1991.

LARA, L. S; VIVES, D.; CORREA, J. S.; CARDOZO, F. P; MARQUES-FERNADES, M. F.; LOPES, A. G.; CARUSO-NEVES, C. PKA-mediated effect of MAS receptor in counteracting angiotensin II-stimulated renal Na(+)-ATPase. **Arch Biochem Biophys**, v 12, 2010.

LESSA, Í.; MAGALHÃES, L.; ARAUJO, M. J.; DE ALMEIDA FILHO, N.; AQUINO, E.; OLIVEIRA, M.M. Arterial hypertension in the adult population of Salvador (BA) - Brazil. **Arq Bras Cardiov,** v. 87, n. 6, p. 747-756, 2006.

MAGALHAES, J. C. G.; SILVEIRA, A. B.; MOTA, D. L.; PAIXÃO, A. D. O. Renal function in juvenile rats subjected to prenatal malnutrition and chronic salt overload. **Exp Physiol**, v. 91, p. 611-619, 2006.

MEDEIROS, M. C.; TEODÓSIO, N. R.; PESSOA, D. C. N.; BION, F. M.; SOARES, J. K. B.; BARRETO, E. M. F.; NASCIMENTO, E. History of Regional Basic Diet (RBD) - a dietary model of experimental malnutrition, **Neurobiologia**, v. 71, n. 4, p. 53-70, 2008.

MILLS, D. E., HUANG, Y. S., NARCE, M.; POISSON, J. P. Psychosocial stress, catecholamines, and essential fatty acid metabolism in rats. **Proc Soc Exp Biol Med**, v. 205, p.56-61, 1994.

MORGANE, P. J., MILLER, M., KEMPER, T., STEM, W. C., FORBES, W. B., HALL, R., BRONZINO, J. D., KISSANE, J., HAWRYLEWICZ, E. &.RESNICK, O. The effects of protein malnutrition on the developing nervous system in the rat. **Neurosci Biobehav Rev**, v. 2, p.137-230, 1978.

MORGANE, P. J.; AUSTIN-LAFRANCE, R. J.; BRONZINO, J. D.; TONKISS, J.; GALLER, J. R. Malnutrition and the developing central nervous system. In: Isaacson RL, Jensen KF, editors. The vulnerable brain: nutrition and toxins. New York: Plenum Publishing Corporation; 1992. p. 3–44.

PAIXÃO, A. D. O.; MACIEL, C. R.; TELES, M. B. B. ; FIGUEIREDO-SILVA, J. . Regional Brazilian Diet-Induced Low Birth Weight Is Correlated with Changes in Renal Hemodynamics and Glomerular Morphometry in Adult Age. **Biol of The Neonate**, Basel, v. 80, n. 3, p. 239-246, 2001.

PAIXÃO, A. D. O.; MACIEL, C. R.; MONTEIRO, J. C. Low sodium chloride content in a multideficient diet induces renal vasodilatation in rats. **Nutr Res** (New York), v. 23, n. 1, p. 85-89, 2003.

PENNELL, J. P.; SANJANA, V.; FREY, N. R.; JAMISON, R. L. The Effect of Urea Infusion on the Urinary Concentrating Mechanism in Protein-Depleted Rats, **J Clin Invest**, v. 55, p. 399-409, 1975.

PINHEIRO, D. C. S. N.; FAVALI, C. B. F.; SOUSA FILHO, A. A.; COSTA, P. V. Parâmetros hematológicos de camundongos e ratos do Biotério Central da Universidade Federal do Ceará. **Bol Inf Cobea**, n. 3, p. 6-9, 1998.

PRADA, P. O.; OKAMOTO, M. M.; FURUKAWA, L. N. S.; MACHADO, U. F.; HEIMANN J. C.; DOLNIKOFF, M. S. High- or Low-Salt Diet From Weaning to Adulthood: Effect on Insulin Sensitivity in Wistar Rats. **Hypertension**, v. 35, p. 424-429, 2000.

RANGEL, L. B. A. ; CARUSO-NEVES, C. ; LARA, L. S. ; LOPES, A. G. Angiotensin II stimulates renal proximal tubule Na_+-ATPase activity through the activation of protein kinase C , **Bioch Biophys Acta Biomemb**, v. 1564, n. 2, p. 310-316, 2002.

REEVES, P.G. Componentes of the AIN-93 Diets as Improvements in the AIN-76A Diet. **J Nutr.** v. 127, p. 838S-841S, 1997.

SAWAYA, A. L.; MARTINS, P.; HOFFMAN, D.; ROBERTS, S. B. The link between childhood undernutrition and risk of chronic diseases in adulthood: a case study of Brazil. **Nutr Rev**, v. 61 n. 5, p. 168-75, 2003.

SAWAYA, A. L.; SESSO, R.; FLORÊNCIO, T. M.; FERNANDES, M. T.; MARTINS, P. A. Association between chronic undernutrition and hypertension. **Matern Child Nutr**, v. 1, n. 3, p.155-63, 2005.

SESSO, R.; BARRETO, G. P.; NEVES, J.; SAWAYA A. L. Malnutrition is associates with increased blood pressure in childhood. **Neprhon Clin Pract**, v. 97, p. 61-66, 2004.

SWENSON, S. J.; SPETH, R. C.; PORTER, J. P. Effect of a perinatal high-salt diet on blood pressure control mechanisms in young Sprague-Dawley rats. **Am J Physiol Regul Integr Comp Physiol,** v. 286, p. R764–R770, 2004.

TEODÓSIO, N. R.; LAGO, E. S.; ROMANI, S. A. M.; GUEDES, R. C. A. A regional basic diet from northeast Brazil as a dietary model of experimental malnutrition. **Arch. Latinoam de Nutr**, v. 40, p. 533-547, 1990.

THOMSEN, K. Lithium clearance as a measure of sodium and water delivery from proximal tubule. **Kidney Int**., v. 37, p. 10-16, 1990.

TORRES, M. A. **Estado nutricional e aspectos sócio-econômicos de famílias rurais do trópico semi-árido**. Universidade Federal de Pernambuco, Recife: Torres M. A. p. 155, 1975.

VALDEZ, R.; ATHENS, M. A.; THOMPSON, G. H.; BRADSHAW, B. S. & STERN, M. P. Birthweight and adult health outcomes in a biethnic population in the USA. **Diabetologia**, v. 37, p. 624-631, 1994.

VALERO, V. B. **Manual para Técnicos de Biotério**. 1. ed. São Paulo-SP: Escola Paulista de Medicina. 1990.

VIDONHO, A. F. JR; DA SILVA, A. A.; CATANOZI, S. ; ROCHA, J. C; BEUTEL, A.; CARILLO, B. A.; FURUKAWA, L. N.; CAMPOS, R. R.; DE TOLEDO BERGAMASCHI, C. M.; CARPINELLI, A. R.; QUINTÃO, E. C.; DOLNIKOFF, M. S.; HEIMANN, J. C. Perinatal salt restriction: a new pathway to programming insulin resistance and dyslipidemia in adult Wistar rats. **Pediat Res**, v. 56, p. 842-848, 2004.

6 - ANEXOS

Universidade Federal de Pernambuco
Centro de Ciências Biológicas

Av. Prof. Nelson Chaves, s/n
50670-420 / Recife - PE - Brasil
fones: (55 81) 2126 8840 | 2126 8351
fax: (55 81) 2126 8350
www.ccb.ufpe.br

Ofício nº 029/06

Recife, 16 de junho de 2006

Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
Para: Profa. Carmen Castro Chaves
LAFIRE - UFPE

Os membros da Comissão de Ética em Experimentação Animal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEEA-UFPE) avaliaram seu projeto de pesquisa intitulado **"Avaliação da desnutrição induzida por uma dieta multicarente (Dieta Básica Regional) sobre aspectos funcionais, estruturais, celulares e moleculares do rim".**

Concluímos que os procedimentos descritos para a utilização experimental dos animais encontram-se de acordo com as normas sugeridas pelo Colégio Brasileiro para Experimentação Animal e com as normas internacionais estabelecidas pelo National Institute of Health Guide for Care and Use of Laboratory Animals as quais são adotadas como critérios de avaliação e julgamento pela CEEA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 9.605 – art. 32 e Decreto 3.179-art 17, de 21/09/1999, que trata da questão do uso de animais para fins científicos.

Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais realizados.

Atenciosamente,

Silene Carneiro

Prof. Silene Carneiro do Nascimento
Presidente CEEA