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DISSERTAÇÃO DE MESTRADO

A CARÊNCIA EM ÁCIDOS GRAXOS ESSENCIAIS NO PERÍODO PERINATAL RESULTA EM DISFUNÇÃO RENAL NA IDADE ADULTA

VALDILENE DA SILVA RIBEIRO

Orientadora: Profª. Drª. Ana Durce Oliveira da Paixão
Co-orientadora: Profª. Drª. Carmen de Castro Chaves

Recife
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Dissertação apresentada como um dos requisitos para obtenção do título de Mestre em Bioquímica e Fisiologia pela Universidade Federal de Pernambuco.

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Valdilene da Silva Ribeiro

A Carência em Ácidos Graxos Essenciais no Período Perinatal Resulta em Disfunção Renal na Idade Adulta

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

Aprovado em 28 / 07 / 2011 por:

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RESUMO

Na carência em ácidos graxos essenciais (CAGE) os níveis de ácidos graxos das famílias n-6 e n-3 estão diminuídos, estando aumentados os da família n-9. Em estudos anteriores, a CAGE do desmame a idade adulta alterou a hemodinâmica renal, incapacitou a excreção de expansão aguda de volume, aumentou a reabsorção de sódio no túbulo proximal e a altura da borda-em-escova. Embora a dieta materna possa afetar também o rim da prole, existem poucos dados renais resultantes da carência em ácidos graxos essenciais no período perinatal. Este trabalho investigou se a CAGE materna, das três semanas prévias à gravidez ao fim da lactação, altera a pressão arterial sistólica e/ou a função renal na prole adulta jovem, comparada com a prole de mães em dieta controle (CON). As duas proles receberam dieta padrão do desmame à 13ª semana. A evolução do ganho de peso corporal, desde o nascimento até as 13ª semana de vida, foi comprometida na CAGE. Nas 8ª e 13ª semanas de vida a ingestão de ração e água, o volume urinário, o balanço hídrico e a excreção de sódio durante 24h em gaiolas metabólicas não diferiram entre os animais CON e CAGE. Na 13ª semana, a pressão arterial sistólica (CON: 149±2 vs CAGE: 147±2 mm/Hg), a excreção de sódio (CON: 0,33±0,04 vs CAGE: 0,28±0,01 mmol/24h/100 g) em 24h, o *clearance* de creatinina (CON: 174,4±17,9 vs CAGE: 172,0±44,8 µl/min/100 g) e o índice renal (CON: 0,32±0,04 vs CAGE: 0,30±0,06 %) foram similares entre os animais CON e CAGE. Entretanto, a reabsorção fracional proximal de sódio foi menor nos animais CAGE (CON: 64,3±8,4%, vs. CAGE: 26,9±8,2 $p<0,05$) embora com maior *clearance* de lítio (CON: 56,5±7,6 vs. CAGE: 119,9±30,1 µl/min/100g). Os resultados sugerem que a CAGE perinatal retarda o desenvolvimento ponderal e imprime alterações funcionais no túbulo proximal na prole adulta jovem sem hipertensão arterial, que pode desenvolver-se em período posterior da vida.

Palavras-chaves: Ácidos graxos essenciais. Carência em ácidos graxos essenciais. Carência perinatal em ácidos graxos essenciais. Pressão arterial. Função glomerular. *Clearance* de creatinina. Função tubular proximal renal. *Clearance* de lítio. Reabsorção fracional proximal de sódio.

ABSTRACT

Essential fatty acid deficiency (EFAD) is characterized by decreased levels of n-6 and n-3 fatty acid families and accumulation of n-9 family. EFAD imposed from weaning until adult leads to altered renal hemodynamics, inability to excrete an acute volume expansion, increased sodium reabsorption at proximal tubule with prominent brush border. Since maternal diet may alter offspring development and perinatal EFAD data in kidney function is scarce, this study aimed to investigate whether maternal EFAD from 3 weeks before conception until weaning, alters systolic blood pressure and/or imprints an altered pattern on renal function in the young adult offspring. All pups were fed with a standard diet from weaning until week 13th. At 8th and 13th weeks of age, dietary and water intakes, urinary flow and water balance for 24h in metabolic cages were not different between EFAD and CON groups. In young adults offspring, systolic blood pressure (CON: 149±2 vs. EFAD: 147±2 mmHg), 24h sodium excretion, creatinine clearance (CON: 174.4±17.9 vs. EFAD: 172.0±44.8 µl/min/100g) and kidney wet weight index were all similar in both groups. However, sodium fractional proximal reabsorption was lower in the EFAD than in the CON group (26.9±8.2 vs. 64.3±8.4%, respectively, $p < 0.05$) but lithium clearance was higher in the EFAD (119.9±30.1 vs. 56.5±7.6 µl/min/100g, respectively, $p < 0.05$). These results suggest that perinatally imposed EFAD imprints alterations in proximal tubule renal function in the young adult offspring without hypertension, which could disclose later in life.

Keywords: Essential fatty acid. Essential fatty acid deficiency. Perinatal Essential fatty acid deficiency. Blood pressure. Glomerular Function. Creatinine clearance. Proximal Tubule Renal Function. Lithium Clearance. Sodium fractional proximal reabsorption.

LISTA DE ABREVIATURAS

ALA- ácido α-linolênico

EPA- ácido eicosapentaenóico

DHA- ácido docosahexaenóico

LA- ácido linoléico

AA- ácidos araquidônico

PUFA- ácidos graxos poliinsaturados

AGE- ácidos graxos essenciais

CAGE- carência em ácidos graxos essenciais

ON- óxido nítrico

SHR- ratos espontaneamente hipertensos

ECA- enzima conversora da angiotensina

GLA- ácido γ-linolênico

DGLA- ácido diomo- GLA

PGs- prostaglandinas

L-PK- L- piruvato quinase

ATP- adenosina trifosfato

COX- via das cicloxigenases

LOX- via das lipoxigenases

TXs- tromboxanos

LTs- leucotrienos

IL-13- interleucina - 13

SUMÁRIO

1 INTRODUÇÃO

1.1 O RIM

Os rins são órgãos pareados e situados em cada lado da coluna vertebral, atrás do peritônio. No adulto humano, cada rim pesa entre 115 e 170g, mede aproximadamente 11 cm de comprimento, 6 cm de largura e 3 cm de espessura (KOEPPEN & STATON, 2009).

1.2 NEFROGÊNESE

A organogênese do rim é um processo complexo, bem regulado e produz um órgão com grande variedade de funções. Há descrições detalhadas da nefrogênese por isso o rim tem sido um modelo para estudar o desenvolvimento de órgãos (BRENNER, 2000).

O desenvolvimento do rim ocorre em três fases morfologicamente distintas, todas derivadas do mesoderma intermediário: o pronefro sem função óbvia nos mamiferos; o mesonefro que funciona como um órgão excretor em toda a embriogênese e o metanefro ou rim permanente. Na embriogênese, o rim deriva do cume néfrico no mesoderma intermediário. O duto pronéfrico dá origem ao pronefro que se alonga formando o duto de Wolff que se desenvolve em broto ureteral, o qual é induzido pelo blastema metanéfrico a sair do duto mesonéfrico (BRENNER, 2000; RAMOS et al, 2006; SCHEDL & HASTIE, 2000). Ver figura 1. A invasão do ureter induz o blastema metanéfrico a sofrer nefrogênese, preparando o palco para o evento-chave da nefrogênese, quando o mesênquima metanéfrico é estimulado a provocar a condensação de células, resultando num epitélio mesenquimal de transição e formando o epitelio néfrico (BRENNER, 2000; RAMOS et al, 2006; SCHEDL & HASTIE, 2000).

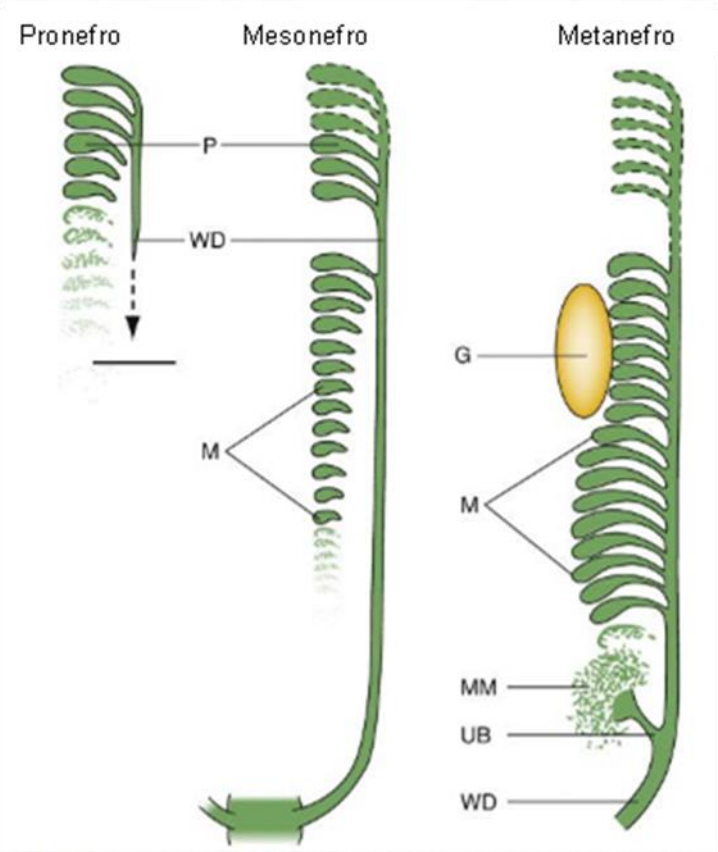


Figura 1. Três estágios de desenvolvimento do rim de mamíferos. O pronefro (P) e o mesonefro (M) desenvolvem-se numa direção rostral para caudal e os túbulos são alinhados adjacentes ao ducto de Wolff ou néfrico (WD). O metanefro desenvolve-se a partir de uma excentricidade da extremidade distal do ducto de Wolff, conhecido como broto ureteral epitélial (UB) em um conjunto de células conhecido como mesenquima metanéfrico (MM) (Fonte: BRENNER, 2000).

O blastema metanéfrico passa ainda por várias modificações celulares até formar o glomérulo e o complexo tridimensional do arranjo do nefro: o glomérulo com seus três tipos de células (endoteliais, mesangiais e podócitos), o aparelho justaglomerular e os túbulos proximal e distal (BRENNER, 2000; SCHEDL & HASTIE, 2000). Ver figura 2.

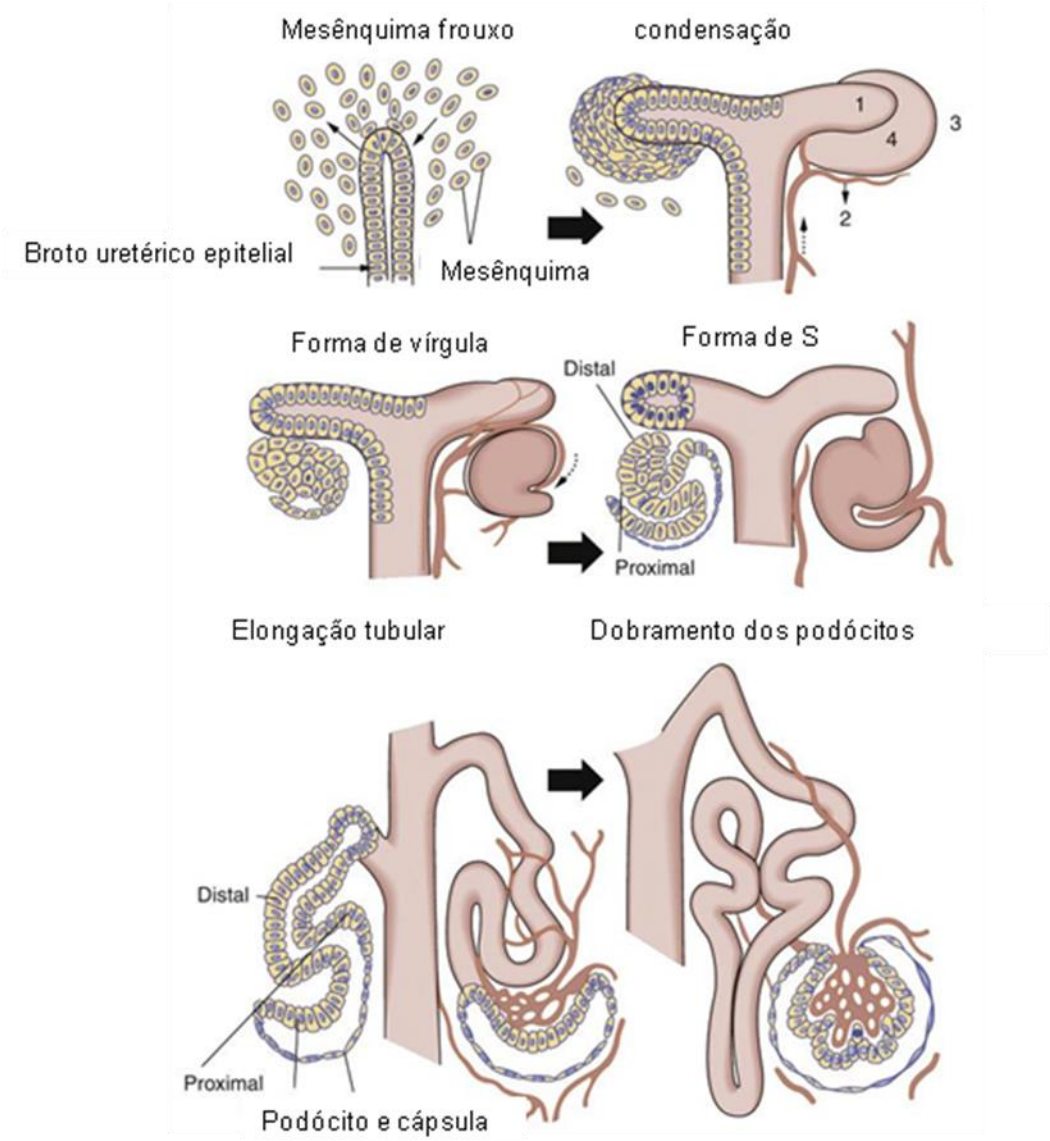


Figura 2. Diagrama esquemático do desenvolvimento de nephron. A interação recíproca entre o broto ureteral e o mesênquima metanefrico resulta em uma série de estágios morfológicos bem definidos, levando à formação do nefron (Fonte: BRENNER, 2000).

Em humanos, o desenvolvimento do rim metanéfrico ou rim permanente ocorre entre a quarta e quinta semanas de gestação, enquanto no rato a nefrogênese vai do 12° dia pós-concepção até ao término da segunda semana de vida pós-natal (NIGAM, APERIA, BRENNER, 1996). Neste período, o menor aporte ou a indisponibilidade de ácidos graxos poliinsaturados (PUFA) e de

ácidos graxos essenciais (AGE) pode ter conseqüências sobre a saúde do adulto (DEMMELMAIR, LARQUÉ, KOLETZO, 2007; INNIS, 2003).

Modelos de programação fetal mostram que o risco cardiovascular aumenta em animais expostos a desnutrição proteica no final da gestação, período em que há nefrogênese (ALEXANDER, 2007). Assim, a manipulação do estado nutricional no período pré-natal é crítico para a nefrogênese e pode levar a hipertensão arterial, redução no número de nefros e, também, do tempo de vida útil (ALEXANDER, 2006, 2007).

1.3 FUNÇÃO RENAL

Os rins realizam uma grande variedade de funções no organismo sendo a maioria essencial para vida. Os rins são responsáveis pela manutenção do volume e da composição do fluido extracelular ou meio interno do indivíduo em limites fisiológicos compatíveis com a vida, pela excreção de produtos metabólicos e de substâncias bioativas, como hormônios e fármacos entre outros e, ainda, regulam o equilíbrio ácido-básico, a pressão arterial bem como a gliconeogênese e a produção de hemácias e da vitamina D (AIRES, 2008; EATON & POOLER, 2009).

Os quatro processos renais básicos são a filtração glomerular, a reabsorção, a secreção e o metabolismo tubular (AIRES, 2008).

A filtração glomerular é a primeira etapa da formação da urina, num processo eminentemente circulatório, dependente da pressão arterial, do tônus das arteríolas aferente e eferente, bem como da permeabilidade do conjunto de camadas e da área dos capilares glomerulares (AIRES, 2008). Nesse processo 20% do plasma dos capilares glomerulares é ultrafiltrado sucessivamente através da barreira de filtração composta pelo endotélio dos capilares glomerulares, membrana basal e fendas do epitélio visceral da cápsula de Bowman, caindo no seu espaço (AIRES, 2008; EATON & POOLER, 2009).

A reabsorção e a secreção tubulares são processos renais que modificam a composição do fluido tubular. A passagem de solutos da luz tubular para o interstício e vice-versa envolve, sobretudo, o transporte ativo

primário, os secundários e, também, os passivos. Na reabsorção, os solutos e a água são atraídos pela elevada pressão oncótica dos capilares peritubulares e retornam à circulação sistêmica, enquanto na secreção, substâncias endógenas e exógenas difundem-se dos capilares peritubulares para a luz tubular para serem excretadas na urina (AIRES, 2008).

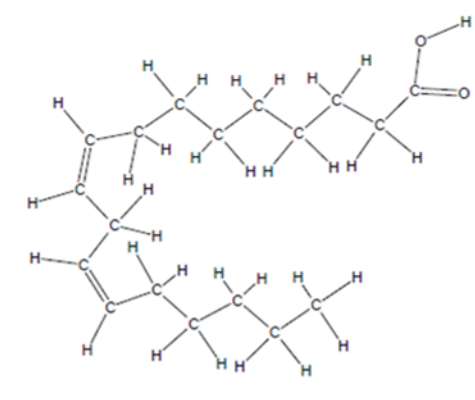
O túbulo proximal é o segmento do rim com maior capacidade transporte: reabsorve 2/3 da água, sódio, potássio e cloro; 100% da glicose, aminoácidos e bicarbonato; secreta ânions e cátions endógenos e exógenos; e, metaboliza substâncias. As microvilosidades de suas células formam a estrutura chamada borda-em-escova luminal, cuja função é aumentar a área de contato. Esse segmento contém transportadores específicos, que proporcionam ao epitélio uma alta capacidade de transporte (AIRES, 2008).

No metabolismo tubular, as células extraem nutrientes orgânicos e inorgânicos do fluido tubular ou do plasma dos capilares peritubulares e os metabolizam de acordo com as suas próprias necessidades. Um exemplo é a formação de bicarbonato no interior da célula tubular, a partir da reação entre CO_2 e H_2O catalisada pela enzima anidrase carbônica, formando o H_2CO_3 pela hidratação do CO_2 e, rapidamente, o H_2CO_3 dissocia-se em H^+ e HCO_3^- (AIRES, 2008). Em contraste, outras transformações metabólicas realizadas pelos rins não estão diretamente relacionadas às suas próprias necessidades nutricionais e sim com as do corpo, alterando a composição da urina e do plasma para retornar o corpo à homeostase (EATON & POOLER, 2009).

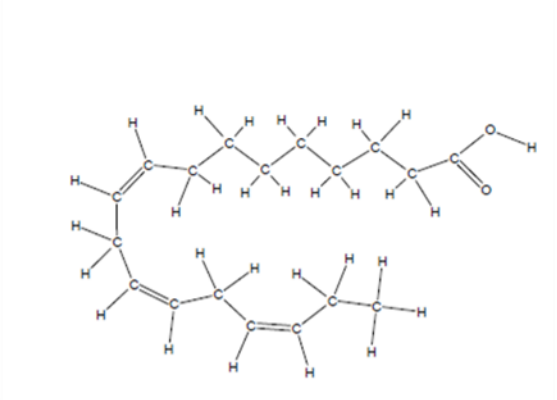
1.4 ÁCIDOS GRAXOS POLIINSATURADOS DE CADEIA LONGA

Os ácidos graxos poliinsaturados de cadeia longa (PUFA) pertencem quimicamente à classe dos lipídios simples. São constituídos por uma cadeia de hidrocarbonetos hidrofóbica que termina em um grupo carboxílico (DAS, 2006) e possuem duas ou mais duplas ligações em sua estrutura molecular (BENATTI et al, 2004). Os PUFA são classificados segundo uma nomenclatura abreviada que designa o número de átomos de carbono, o número de duplas ligações e a posição destas duplas ligações na cadeia carbônica bem como a posição das duplas ligações mais próximas ao grupamento metil terminal (DAS, 2006).

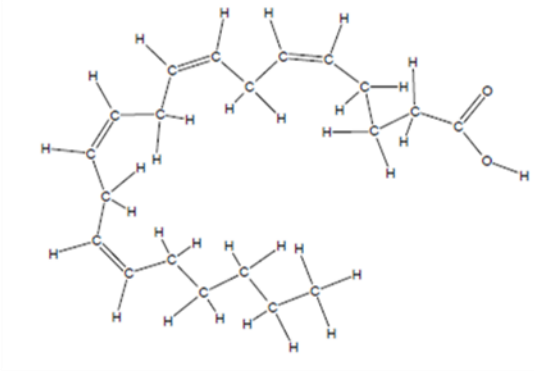
Existem duas famílias principais de PUFA, a n-3 e a n-6 (BENATTI et al, 2004; MUSKIET et al, 2006) originárias do ácido α-linolênico (ALA - 18:3, n-3) e seus derivados ácido docosahexaenóico (DHA, 22:6, n-3) e ácido eicosapentaenóico (EPA, 20:5, n-3) ou do ácido linoléico (LA- 18:2,n-6) e seu derivado ácido araquidônico (AA, 20:4, n-6). As estruturas químicas encontram-se na Figura 3. O ALA e o LA são AGE e, também, PUFA, mas nem todos os PUFA são AGE (DAS, 2006, 2008).



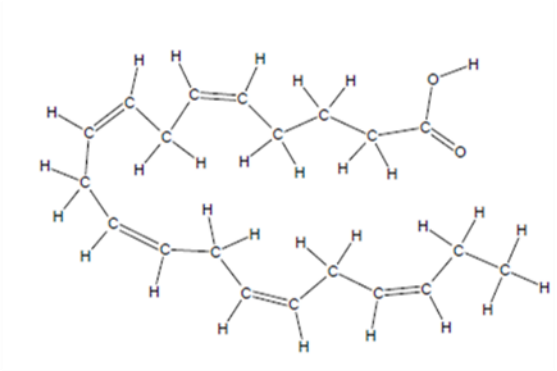
LA- Ácido linoléico (18:2,n-6)



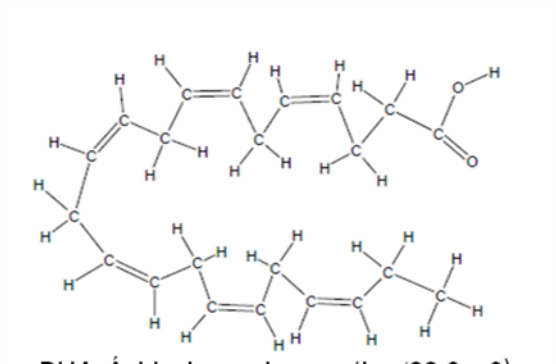
ALA- Ácido α-linolênico (18:3,n-3)



AA- Ácido araquidônico (20:4,n-6)



EPA- Ácido eicosapentaenóico (20:5,n-3)



DHA- Ácido docosahexaenóico (22:6,n-3)

Figura 3. Estruturas químicas do LA- Ácido linoléico, ALA- Ácido α-linolênico, AA- Ácido araquidônico, EPA- Ácido eicosapentaenóico e DHA- Ácido docosahexaenóico (Fonte: HOLUB et al, 2002).

O ALA e o LA são formados em plantas, mas não em células de mamíferos (INNIS, 2003) devido à ausência das enzimas dessaturases ∆12 e ∆15 necessárias para inserir a dupla ligação nas posições n-6 e n-3 da cadeia carbônica dos ácidos graxos (INNIS, 2003; STRIJBOSCH et al, 2008). O ALA e o LA não são sintetizados *de novo* pelos mamíferos e devem ser obtidos na dieta (DAS, 2006; STRIJBOSCH et al, 2008) sendo, assim, considerados AGE.

Os AGE são importantes componentes de todas as membranas celulares (DAS, 2008) onde atuam em processos metabólicos e fisiológicos através de três mecanismos: influem nas propriedades das membranas, receptores, sistemas de transporte e canais iônicos; atuam sobre a sinalização celular por serem precursores de eicosanóides; e, tem efeito direto sobre a expressão gênica (INNIS, 2003).

Os estudos revelam que AGE/PUFA desempenham um papel significativo em condições clínico-patológicas como inflamação, síndrome metabólica e aterosclerose (DAS, 2006) pela formação de precursores pró- e anti-inflamatórios (DAS, 2008) além de possuírem ações renais diurética, anti-hipertensora e β–bloqueadora (DAS, 2008; FUNK, 2001).

Os AGE tem um papel na prevenção e no tratamento de doenças cardiovasculares, por terem efeitos antiarrítmico (BENATTI et al, 2004) e cardioprotetor. Estas funções estão relacionadas no fato de que a ingestão de n-3 reduz a mortalidade por doenças coronarianas (OGAWA et al, 2009; MORI, 2006). Um estudo indica que o DHA e o EPA têm diferentes propriedades hemodinâmicas e anti-aterogênicas, sendo o primeiro mais eficiente na redução da pressão arterial, freqüência cardíaca e melhora da função vascular (MORI, 2006).

Mori et al, (2000) mostraram que o efeito dos ácidos graxos n-3 sobre a diminuição da pressão arterial era relacionado ao seu benefício sobre as funções vasculares, cardíacas e autonômicas pelo seguinte mecanismo: o n-3 aumenta a liberação de óxido nítrico (ON) alterando a função vascular e modifica a liberação de adenosina difosfato, de prostánoides vasoativos como

o tromboxano A2 e a prostaciclina I2 e, possivelmente, de fatores hiperpolarizantes derivados do endotélio.

Outro benefício do n-3 na função vascular está na sua incorporação ao plasma e às membranas celulares, alterando a estrutura físico-química da membrana e levando a mudanças na fluidez, permeabilidade e função das membranas e de proteínas a ela ligadas. Conseqüentemente, é provável que a atividade enzimática, a afinidade dos receptores bem como a capacidade de transporte da célula, incluindo a síntese ou liberação de ON sejam afetadas (MORI, 2006).

A dieta rica em ALA tem efeito anti-hipertensivo em ratos espontaneamente hipertensos (SHR), com seis semanas de vida, diminuindo a atividade da enzima conversora da angiotensina (ECA) e a expressão do RNA mensageiro na aorta (OGAWA et al, 2009).

Os AGE também estão envolvidos na prevenção de alguns cânceres como os de mama e coloretal (BOURRE, 2007). Estudos mostram que a diminuição do consumo de óleo de peixe em mulheres correlaciona-se com a alta incidência de câncer de mama (KAIZER et al, 1989). Dois constituintes principais do óleo de peixe, o DHA e o EPA suprimem o crescimento de células de câncer de mama in vitro e em modelos animais (CHAJÈS et al, 1995; ROSE & CONNOLLY 1999; SCHLEY et al, 2005), como também impede a formação de lesões osteolíticas no osso em mulheres com câncer de mama, indicando supressão de Metástase de células cancerígenas da mama para os ossos (MANDAL et al, 2010). Vários outros estudos têm mostrado que as pessoas que comem mais peixes estão com menor risco de desenvolver câncer do cólon retal (CAYGILL & HILL, 1995), esta situação foi confirmada em vários estudos de caso-controle (WILLETT et al, 1990; KATO et al, 1997). Pessoas que comeram 80 gramas ou mais peixe por dia tinham uma chance 40% menor de desenvolver câncer cólon retal do que aqueles que comiam menos de 10 gramas por dia (NORAT et al, 2005).

Os PUFA formam precursores (AA, EPA e DHA) de potentes moléculas anti-inflamatórias: lipoxinas, resolvinas e protectinas que são

essenciais para suprimir o processo inflamatório e melhorar a cicatrização (DAS, 2006; 2008).

Evidências indicam um potencial uso terapêutico de PUFA em várias doenças psiquiátricas (FERRAZ et al, 2008) e neuro-degenerativas (MACNAMARA & CARLSON, 2006). O consumo semanal de uma ou mais porções de peixes ricos em n-3 protege do declínio cognitivo do envelhecimento (MORRIS et al, 2005).

Os ácidos graxos são muito mais do que fontes metabólicas e energéticas e contribuem com o crescimento, o desenvolvimento e a saúde humana (INNIS, 2011).

1.5 METABOLISMO DOS ÁCIDOS GRAXOS ESSENCIAIS

O LA é convertido em ácido γ-linolênico (GLA, 18:3, n-6) pela ação da dessaturase Δ6, sendo elongado e formando o dihomo-GLA (DGLA, 20:3, n-6), precursor de uma série de prostaglandinas (PGs). O DGLA também pode ser convertido a ácido araquidônico (AA, 20:4, n-6) pela ação da dessaturase Δ5. O AA é a forma precursora de duas séries de prostaglandinas, de tromboxanos bem como de quatro séries de leucotrienos. O ALA é convertido em ácido eicosapentaenóico (EPA, 20:5, n-3) pelas Δ5 e Δ6. O EPA é o precursor de três séries de prostaglandinas e de cinco séries de leucotrienos e, depois de elongado e dessaturado, origina o ácido docosahexaenóico (DHA, 22:6, n-3), (DAS, 2006, 2008; KOLETZKO et al, 2008; INNIS, 2003). Os LA, GLA, DGLA, AA, ALA, EPA e o DHA são todos PUFA, mas apenas LA e ALA são AGE (DAS, 2006, 2008). A cascata de metabolisação encontram-se na Figura 4.

O LA, ALA e o ácido oléico, para serem metabolizados, competem pelas mesmas dessaturases e elongases. O aumento da concentracão desse último indica deficiência em AGE (DAS, 2006). A atividade das Δ5 e Δ6 diminui no *diabetes melittus* e na hipertensão arterial e é inibida pela ingestão de gorduras saturadas e *trans,* alcóol e colesterol (DAS, 2006; JUMP, 2008). Dietas ricas em gorduras saturadas modificam o metabolismo dos AGE, reduzem o número de metabólitos com ações antiinflamatórias, aumentam os níveis de citocinas

pro-inflamatórias e, assim, iniciam e aceleram a aterosclerose devido à inflamação persistente (DAS, 2006). Além da alimentação, vários outros fatores podem interferir no seu metabolismo tais como exercício físico, resistência à insulina, deficiência de vitaminas, estresse e drogas (BENATTI et al, 2004).

O fígado tem um papel chave no metabolismo lipídico corporal influindo sobre a composição da gordura. A glicólise hepática e a lipogênese *de novo* são reguladas pelos PUFA da dieta, sendo vias metabólicas para a utilização de glicose e armazenamento de combustível sob a forma de glicogênio e triglicerídeos e, assim, a presença de n-3 na dieta regula a expressão gênica hepática (JUMP, 2008).

Os PUFAs suprimem ao menos uma enzima glicolítica, a L-piruvato quinase (L-PK) e, ainda, várias enzimas envolvidas na síntese de ácidos graxos monoinsaturados e na lipogênese *de novo*, incluindo ATP-citrato-liase, acetil CoA carboxilase, ácido graxo sintase (JUMP, 2008).

O AA é metabolizado através de três vias enzimáticas: a via das cicloxigenases (COX) forma tromboxano A2, prostaglandina E2 e prostaciclina, metabólitos que contribuem na regulação do fluxo sanguíneo renal e no ritmo de filtração glomerular; a via das lipoxigenases (LOX) forma leucotrienos e lipoxinas, que possuem ações inflamatórias, sobre o crescimento celular no músculo liso vascular e na hemodinâmica renal, contraindo vasos e células mesangiais glomerulares; e, finalmente, a via da citocromo P450 forma os ácidos epoxieicozatrienóico e 20-hidroxieicosatetraenóico (IMIG, 2000; 2006). O ácido 20-hidroxieicosatetraenóico é vasoconstritor renal (IMIG, 2000; 2006) e induz natriurese por inibição da Na^+, K^+-ATPase no túbulo proximal e do co-transportador Na^+-K^+-$2Cl^-$ no ramo ascendente espesso da alça de Henle (MCGIFF & QUILLEY, 1999; ROMAN et al, 2000; ROMAN, 2002).

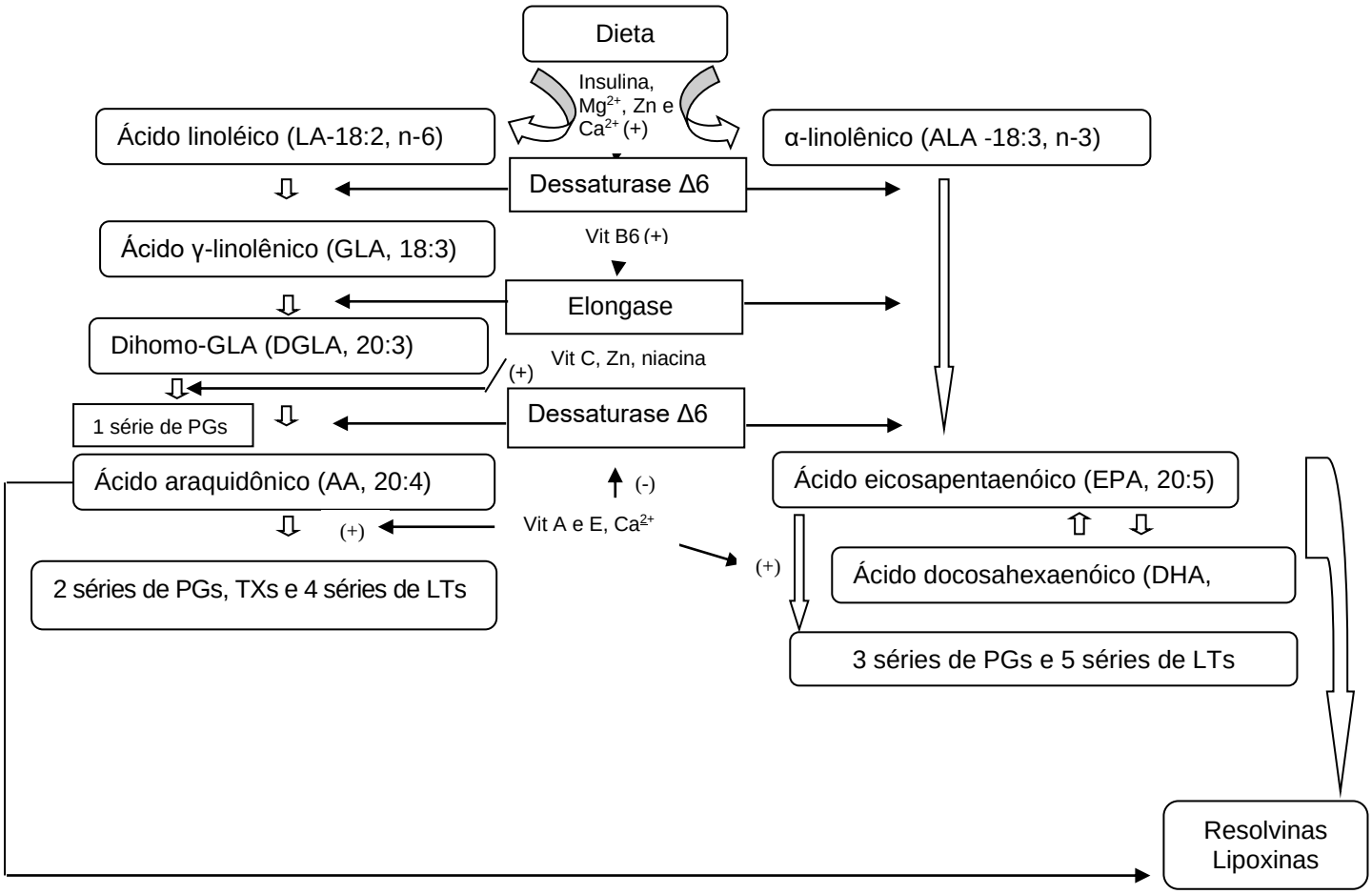


Figura 4. Representação esquemática do metabolismo dos AGE com os co-fatores que aumentam a atividade das dessaturases Δ5 e Δ6, das elongases e a formação de prostaglandinas (PGs), tromboxanos (TXs) e leucotrienos (LTs) .
(+) significa aumento da atividade da enzima ou aumento da formação do produto. (-) significa inibição da atividade da enzima ou diminuição na formação do produto (DAS, 2006).

1.6 FONTES DE ÁCIDOS GRAXOS ESSENCIAIS

As principais fontes de ácidos graxos n-3 são os peixes e os óleos vegetais e, estes últimos, são a maior fonte de ALA. O ALA é encontrado no cloroplasto de folhas de vegetais verdes, como o espinafre e em sementes de linho, linhaça, nozes, canola e outros (BENATTTI et al, 2004; MCCUSKER & GRANT-KELS, 2010). As principais fontes de ácido eicosapentanoico (C20:5n-3, EPA) e ácido docosahexanoico (C22: 6n-3, DHA) são sardinha, arenque, atum, cavala, óleo de figado de bacalhau (CALDER & GRIMBLE, 2002), e, ainda, moluscos, algas e crustáceos (SILVA et al, 2007).

Os vegetais são a principal fonte dos ácidos graxos n-6, o LA é encontrado em grande quantidade na dieta ocidental no óleo de milho, cártamo, girassol e soja (CALDER & GRIMBLE, 2002; BENATTI et al, 2004) bem como em óleos de cânhamo, semente de uva, germe de trigo e algodão (MCCUSKER & GRANT-KELS, 2010).

1.7 EVOLUÇÃO HUMANA E MUDANÇAS NOS HÁBITOS ALIMENTARES

Os seres humanos evoluiram numa dieta com níveis elevados de ácidos graxos n-3 e n-6, com proporção n-6/n-3 de 1/2:1 e com baixa quantidade de gordura saturada até há aproximadamente 10.000 anos (SIMOPOULOS et al, 1999; BENATTI et al, 2004; CANDELA et al, 2011). Após isso, com o desenvolvimento da agricultura, começaram a ocorrer mudanças no comportamento alimentar.

Entretanto, nos últimos 100-150 anos aumentou a ingestão de gorduras saturadas, de ácidos graxos trans à partir da hidrogenação de óleos vegetais bem como de ácidos graxos n-6. O aumento deste foi considerável e a proporção n-6/n-3 passou para 20-30:1 foi alterada devido à produção de óleos de sementes vegetais de milho, açafrão e algodão (SIMOPOULOS et al, 1999; BENATTI et al, 2004; CANDELA, LÓPEZ, KOHEN, 2011) na “dieta moderna”. A ênfase da agricultura atual na produção, diminuiu o teor de ácidos graxos n-3 em alimentos tais como vegetais de folhas verdes, carnes de animais, ovos e, até mesmo, peixes (SIMOPOULOS et al, 1999).

A quantidade de n-3 na dieta moderna vem diminuindo, sobretudo nos alimentos provenientes de fontes marinhas. Além disso, a quantidade de ácidos graxos n-6 na dieta moderna aumentou, podendo interferir no metabolismo e síntese de DHA e EPA (SIMOPOULOS et al, 1999). Assim, a diminuição relativa e a absoluta na quantidade de ácidos graxos n-3 na dieta levaram a um desequilíbrio e ao aumento na proporção n-6/n-3 (BENATTI et al, 2004).

1.8 ÁCIDOS GRAXOS ESSENCIAIS E DESENVOLVIMENTO PERINATAL

O prejuizo do crescimento fetal é uma das principais causas de morbidade perinatal, traz conseqüências clínicas a longo prazo, aumenta o risco de doenças no desenvolvimento e no adulto, inclusve diabetes tipo 2, hipertensão e doenças cardiovasculares (BARKER, 1994; MARTYN & BARKER, 1994).

O ambiente intra-uterino adverso altera o metabolismo fetal e resulta em adaptações no desenvolvimento para garantir a sobrevivência do feto. Tais alterações podem ter seqüelas significativas para a saúde dos filhos a longo prazo na vida adulta (VICKERS et al, 2006).

A mãe desempenha um papel importante na oferta de AGE para os filhos e deve consumir uma dieta rica em alimentos-fontes para atender suas necessidades e as da criança (SILVA et al, 2007).

A dieta materna adequada é importante desde antes da concepção, durante a vida intra-uterina e lactação, pois o desenvolvimento do feto depende dos nutrientes maternos, via placenta e leite (BENEDETTO et al, 2007; BOURRE, 2007) que determina o tipo de AGE que se acumula nos tecidos fetais (SCHMEITS et al, 1999).

O leite materno é a maior fonte de energia, AGE e de vitaminas para o lactante. Os AGE das famílias n-3 e n-6 correspondem de 15 a 20% respectivamente dos ácidos graxos presentes no leite (INNIS, 2003; SILVA et al, 2007). Alguns fatores podem afetar a composição lipídica do leite: dieta materna, estilo de vida (KOLETZKO et al, 2008), pós-parto, período do dia,

paridade, situação sócio econômica, desordens metabólicas e infecções (SILVA et al, 2007).

A concentração adequada de AGE no leite materno modula o sistema auditivo dos filhos e garante a conservação da audição ao longo da vida (BOURRE, 2007). A carência dos ácidos graxos n-3 danifica a audição e levando ao envelhecimento prematuro do sistema nervoso auditivo (BOURRE et al, 1999; BOURRE, 2007).

A quantidade de substratos que atinge a placenta depende estritamente da dieta e do metabolismo materno. A placenta desempenha um papel importante pela sua capacidade de transferência conjugada ao seu metabolismo, determinando a quantidade de nutrientes na circulação fetal (CETIN et al, 2005; CETIN; ALVINO; CARDELLICCHIO, 2009) e o crescimento intrauterino (FOWDEN et al, 2006).

O abastecimento adequado de AGE é necessário, também, ao longo do desenvolvimento e no adulto para manter as funções normais como a do cérebro, da retina, dos sistemas imune e inflamatório e a cardiovascular (BENATTI et al, 2004).

A suplementação com ácidos graxos n-3 na gestação reduz a incidência de asma na infância e os níveis da interleucina IL-13 no sangue do cordão umbilical, indicando uma possível modulação pré-natal do sistema imunológico, resposta que pode levar a doenças alérgicas na infância (KLEMENS, BERMAM, MOZURKEWISH, 2010). Há evidências que a deficiência perinatal em ácidos graxos n-3 leva a desenvolver hipertensão no adulto. Ratos submetidos à carência em ácidos graxos essenciais (CAGE) nos períodos pré e pós-natal até adulto apresentaram elevação da pressão arterial em até 17 mmHg (ARMITAGE et al, 2003).

A CAGE perinatal está associada à diminuição dos níveis séricos do hormônio leptina na mãe e na prole (KOROTKOVA et al, 2001; KOROTKOVA et al, 2002; KOROTKOVA et al, 2005) e à alterações no peso corporal, mineralização e massa óssea, sugerindo que a ingestão materna de AGE pode ser um dos fatores de risco para o desenvolvimento de osteoporose na vida adulta (KOROTIKOVA et al, 2005).

1.9 OS AGEs, E OS FOSFOLIPÍDIOS DE MEMBRANA

Os principais componentes estruturais da membrana são os lipídios e as proteínas, os glicídios estão em quantidade mínimas e coexistem com várias enzimas importantes. A membrana celular, além de separar a célula do meio extracelular, desempenha um papel importante no transporte de substâncias entre os dois meios (CAMPBELL, 2000).

Os lipídios estão agrupados em bicamadas, contendo proteínas integrais e periféricas que se movimentam livremente neste plano constituindo, assim, o modelo do mosaico fluido (SINGER & NICHOLSON, 1972). A superfície da bicamada polar contém grupamentos carregados, o interior da bicamada de hidrocarbonetos apolares consiste de cadeias saturadas e insaturadas de ácidos graxos e de um sistema de anéis de colesterol (CAMPBELL, 2000). Foi demonstrada, também, a existência de microdomínios regulatórios na bicamada lipídica, chamados *rafts* lipídicos, que concentram várias proteínas com importantes funções na transdução do sinal hormonal (EDIDIN, 2003; VEREB et al, 2003).

Os fosfolipídios são os lipídios mais abundantes da membrana celular, sua região polar contém um glicerol ligado a um fosfato conectado a grupos colina, etanolamina e serina que originam os principais fosfolipídios de membrana dos mamíferos: fosfatidilcolina, fosfatidilserina, fosfatidiletanolamina e esfingomielina (CAMPBELL, 2000).

Os AGE são importantes constituintes das membranas celulares, com um grande impacto na sua fluidez e função (DAS, 2006; DAS, 2008; SCHMITZ & ECKER, 2008; STRIJBOSCH et al, 2008). Os PUFA de cadeia longa (AA, EPA, DHA) são incorporados nas membranas celulares, particularmente nos fosfolipídios da bicamada lipídica. Dependendo da proporção de cada um na membrana, a mesma sofre alterações na sua fluidez (DAS, 2006) e, portanto, em sua capacidade para a alojar diferentes enzimas, receptores, canais e poros, permitindo assim uma melhor adaptação das suas funções fisiológicas de acordo com sua necessidade (ARTERBURN; BAILEY; OKEN, 2006).

Os lipídios da dieta, inclusive os ácidos graxos, têm funções que se estendem muito além de fonte de energia metabólica e de armazenamento

(INNIS, 2011). Desempenham papéis fundamentais na regulação do crescimento e coordenação celular, na comunicação inter e intracelular e atuam como moduladores da expressão de genes que integram o fornecimento de nutrientes e substratos ao ambiente metabólico (JUMP, 2008; INNIS, 2011).

A composição dos PUFA da membrana celular depende da dieta (SIMOPOULOS, 1999) e, assim, a variação no aporte lipídico dietético pode modificar a composição de ácidos graxos dos fosfolipídios das membranas.

No rim, os glicerofosfolipídios mais abundantes são as fosfatidilcolinas com 72% (NEALON et al, 2008) ou 57% (MITCHELL et al, 2007), as fosfatidiletanolaminas com 19% (NEALON et al, 2008) ou 23% (MITCHELL et al, 2007) e as fosfatidilserinas com 5% (NEALON et al, 2008) ou 4,9 a 8,4% (MITCHELL et al, 2007) cuja meia-vida varia respectivamente de 23, a 32 e a 58 dias (DEMAR et al, 2004).

Evidências mostram que a CAGE altera o perfil de fosfolipídio das membranas celulares e dos tecidos dos órgãos como um todo: em eritrócitos, fígado, coração, músculo esquelético e rim. Neste último, aumentou os níveis de ácidos graxos saturados das famílias n-9 em contraste com o decréscimo nos níveis dos AGE das famílias n-3 e n-6. (MOUSSA et al, 2005). Os mesmos autores observaram ainda que cada órgão responde especificamente à deficiência, fato justificado pelas diferentes atividades das elongases e dessaturases presentes nesses tecidos.

A CAGE, durante oito semanas em camundongos adultos, alterou o perfil fosfolipídico dos eritrócitos, com uma severa diminuição dos níveis de AGE das famílias n-3 e n-6, acompanhada de aumento nos níveis de ácidos graxos saturados das famílias n-7 e n-9. Além disso, a CAGE também provocou alterações na absorção intestinal, diminuindo a absorção total de gordura, a atividade da lactase e, portanto, a capacidade de digestão da lactose (LUKOVAC et al, 2008).

Uma dieta equilibrada é importante ao longo de toda vida, fornecendo os lipídios necessários e contendo AGE em níveis adequados. Por outro lado, dietas com baixo teor lipídico e várias patologias podem acarretar uma carência em AGE. Os estudos bem como as confirmações epidemiológicas e experimentais da programação intra-uterina de doenças cardiovasculares, endócrinas e renais no adulto (Barker, 1994) motivaram este projeto de carência materna sobre a prole. Assim, aspectos funcionais renais serão estudados em proles de mães em dieta carente em AGE desde sessenta dias antes do acasalamento até o fim da lactação.

2 OBJETIVO

2.1 OBJETIVO GERAL

Investigar se a CAGE materna perinatal afeta aspectos funcionais no rim da prole.

2.2 OBJETIVOS ESPECÍFICOS

Em ratos em crescimento e adultos, descendentes de mães em dieta com teores normais ou CAGE desde sessenta dias prévios ao acasalamento ao fim da lactação, mantidos em dieta padrão desde o desmame:

a) Acompanhar o peso corporal;
b) Medir hematócrito, colesterol e proteína no plasma;
c) Avaliar as ingestões de dieta e água, o balanço de água, as excreções urinárias de sódio, proteína e uréia no animal em crescimento e adulto;
d) Avaliar a pressão arterial; a filtração glomerular; a função tubular proximal; os pesos do coração, pulmão, fígado, baço, rim e testículo no animal adulto.

3 ARTIGO

Artigo a ser submetido ao periódico Lipids

Perinatally imposed essential fatty acid deficiency imprints alterations in renal function of the young adult rat

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Running head: Programming adult renal function alteration by perinatal essential fatty acid deficiency

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Essential fatty acid deficiency (EFAD) is characterized by decreased levels of n-6 and n-3 fatty acid families and accumulation of n-9 family. EFAD imposed from weaning until adult leads to altered renal hemodynamics, inability to excrete an acute volume expansion, increased sodium reabsorption at proximal tubule with prominent brush border. Since maternal diet may alter offspring development and perinatal EFAD data in kidney function is scarce, this study aimed to investigate whether maternal EFAD from 3 weeks before conception until weaning, alters systolic blood pressure and/or imprints an altered pattern on renal function in the young adult offspring. All pups were on a standard diet from weaning until week 13th. At 8th and 13th weeks of age, dietary and water intakes, urinary flow and water balance for 24h in metabolic cages were not different between EFAD and CON groups. At young adults offspring, systolic blood pressure (CON: 149±2 vs. EFAD: 147±2 mmHg), 24h sodium excretion, creatinine clearance (CON: 174.4±17.9 vs. EFAD: 172.0±44.8 µl/min/100g) and kidney wet weight index were all similar at both groups. However, sodium fractional proximal reabsorption was lower in the EFAD than in the CON group (26.9±8.2 vs. 64.3±8.4%, respectively, $p < 0.05$) but lithium clearance was higher in the EFAD (119.9±30.1 vs. 56.5±7.6 µl/min/100g, respectively, $p < 0.05$). These results suggest that perinatally imposed EFAD imprints alterations in proximal tubule renal function in the young adult offspring without hypertension, which could disclose later in life.

Keywords: Essential fatty acid, Essential fatty acid deficiency, Perinatal Essential fatty acid deficiency, Blood pressure, Glomerular Function, Creatinine clearance, Proximal Tubule Renal Function, Lithium Clearance, Sodium fractional proximal reabsorption.

Introduction

Features of essential fatty acid deficiency (EFAD) are decreased levels of the n-6 and n-3 fatty acid (FA) families and an accumulation of the n-9 FA family. Linoleic acid (LA; C18:2n-6) and α-linolenic acid (ALA; C18:3n-3) are essential

FAs (EFAs) from the n-6 and n-3 FA series, respectively, which cannot be synthesized *de novo* by animals and has to be obtained from dietary sources. LA can be converted to n-6 long-chain polyunsaturated fatty acids (n-6 PUFA) while ALA is a substrate for biosynthesis of n-3 long-chain polyunsaturated fatty acids (n-3 PUFA) [see the review 1]. All of them are membrane constituents and play several biological roles. For instance, arachidonic acid (C20:4n-6) is a precursor of second messengers which play an important role in increasing vascular resistance and, in the kidney, are inhibitors of tubular sodium reabsorption [2], docosahexaenoic acid (C22:6n-3) is particularly necessary for brain development and its deficiency leads to cognitive impairment [3].

EFAD imposed from weaning until adult age leads to changes in renal hemodynamics and inability to excrete an acute volume expansion [4], increases proximal tubule sodium reabsorption and shows a prominent proximal tubule brush border [5]. Specific deficiency in dietary n-3 PUFA spanning all life including during perinatal life, leads to increment in blood pressure [6]. Evidence regarding repercussion on adult renal function from perinatal EFAD is scarce, though there are evidences that general dietary deficiency such as in multiple nutrients [7, 8], low protein [9, 10] or intake restriction [11] during prenatal life change irreversibly renal function.

Considering our previous reports that chronic EFAD affects sodium homeostasis [4, 5] and that maternal diet may alter offspring development, this study aimed to investigate whether EFAD from 3 weeks before conception until weaning, imprints an altered pattern of certain renal function parameters in the adult offspring.

Material and Methods

Ethical considerations

Experimental procedure was approved by the Committee for Experimental and Animal Ethics at the Federal University of Pernambuco and performed in accordance with its rules.

Animals

30 day old female Wistar rats, maintained in a room at 22 ± 3 ºC with a 12-h light–dark cycles, were randomly assigned for control (CON, n = 6) or EFAD (n = 6) diet. Dams were maintained onto these diets throughout 3 weeks before mating at mating, pregnancy and lactation. Briefly, after 3 weeks on their respective diets, groups of 3 females and 1 male mated in collective cages. Pregnancy was confirmed by at least 10 g of weight gain in the first 10 days of mating. Then, dams were housed in individual cages until weaning, at offspring age of 21 days. After weaning, grousp of 4 male pups (CON, n = 15 and EFAD, n = 13) were housed in collective cages, in accordance with perinatal maternal dietary treatment and all of them were given a standard diet until 13 weeks of age. Body weight was taken at birth, weaning and weekly till weaning. Some renal function parameters were measured at age of 8 weeks. At 13 weeks animals were assigned for blood pressure measurement and creatinine clearance evaluation.

After measurement of functional parameters, the animals were exsanguinated by decapitation. Furthermore, several other organs were collected to obtain their weights.

Diets

Diets, prepared according to AIN 93 M [12], differed only by the lipid composition: 5% of soy oil for CON and 5% of hydrogenated copra oil for EFAD diet (Table 1), in accordance with previous reports of experimental EFAD [13, 4, 5]. After weaning all animals were maintained on a balanced commercial rodent chow (Purina Agribands).

Evaluation of blood pressure and renal function

At 8 and 13 weeks animals were housed in metabolic cages (Tecniplast Gazzada S.a r.l., Buguggiate, Italy) for a period of 24 hours in order to measure diet and water intake, urinary flow and density, urinary sodium ($U_{Na^+}V$), urinary urea and water balance.

Blood pressure was measured in conscious 13 weeks old rats by tail-cuff plethysmography (IITC Life Science B60-7/16", Life Science Instruments, Woodland Wills, USA).

Glomerular filtration rate (GFR) and sodium fractional proximal tubule reabsorption ($FPT_{Na}R$) were measured by evaluating endogenous creatinine and administered lithium clearances [14, 5] respectively. The animals received lithium chloride (0.06 mEq/100 g, by gavage) and were maintained for 12 h deprived from food but with free access to water In order to inhibit antidiuretic hormone secretion, they received 5 ml/100 g water overload, divided 3 and 2 ml/100 g respectively at 60 an 20 min before being housed in metabolic cage for 3 h with continous urine collection. Blood samples were withdrawn at the end of this period.

The following expressions were used to calculate the renal physiological parameters: Creatinine clearance = U_{cr} x V/P_{cr}, where V is the urinary volume (in µl/min) and U_{cr} and P_{cr} are the urinary and plasma creatinine concentrations, respectively (in mmol/l); Na^+ filtered load = creatinine clearance (in µl/min) x P_{Na} (Serum Na^+ concentration in µmol/ml); $FPT_{Na}R$ = [(Na^+ filtered load – Na^+ distal delivery)/Na^+ filtered load] x 100; Na^+ distal delivery = Li^+ clearance x P_{Na}; Li^+ clearance = U_{Li} x V/P_{Li}, where V is the urinary volume (in µl/min) and U_{Li} and P_{Li} are the urinary and plasma lithium concentrations, respectively (in mmol/l). All parameters were corrected to 100 g body weight, when appropriate.

Analytical methods

Serum protein was measured by the Folin phenol method [15]. Urinary protein was measured by precipitation with 3% sulfosalicylic acid [16]. Urinary urea, serum cholesterol, total membrane cholesterol of the whole kidney, urinary and serum creatinine were measured employing commercial kits (Labtest, Lagoa Santa, MG, Brazil). Serum and urinary Na^+, K^+ and Li^+ were measured by an electrolyte analyzer (AVL 9180, Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Data are expressed as means ± SE. Statistical significance of differences ($P < 0.05$) was assessed using two-tailed unpaired Student's *t*-test.

Results

General parameters

At ages of 8 and 13 weeks, 24h diet and water intake, urinary flow, water balance, as well as urinary density (Table 2) were not different between EFAD and CON groups. Erythrocyte plasma membrane could be affected by EFAD on course [17], however early EFAD did not affect hematocrit levels at weaning (CON: 31 ± 3 and EFAD: 31 ± 3), neither at adult age (CON: 47 ± 2 and EFAD: 46 ± 2). At age of 13 weeks, the wet weight index (Table 3) of kidney, heart, testis, lungs, liver and spleen were also unaffected, although the body weight gain evolution, from birth to 13 week old, was significantly compromised in the EFAD group (Fig. 1). From weaning to 7 weeks old, the body weight of EFAD was 17.9 to 9.6% ($p < 0.05$) lower than the CON group. From
8 to 13 weeks, the differences between groups were of 8.1 to 5.4% ($p < 0.05$).

Metabolic parameters, renal function and blood pressure in rats aged 13 weeks

Plasma cholesterol was the same for both groups (CON: 1.97 ± 0.06 and 1.99 ± 0.07 mmol/l). Serum protein was higher in EFAD than in CON group (74 ± 1 vs. 81 ± 2 mg/l, respectively, $p < 0.05$), while systolic blood pressure was the same for both groups (CON: 149 ± 2 and EFAD: 147 ± 2 mmHg).

EFAD rats, aged 8 weeks or 13 weeks, do not present any change in urinary sodium excretion (Fig. 2). It is noteworthy to emphasize that $U_{Na^+}V$ decreases along development.

Table 4 shows parameters during evaluation of creatinine and lithium clearances. Serum sodium and creatinine, urinary creatinine, lithium filtered load and urinary flow were the same for both groups, while serum lithium in EFAD was lower ($p < 0.05$) and its fractional excretion was higher in EFAD ($p < 0.05$) than in the CON group.

In Fig. 3, it may be seen that GFR, measured by creatinine clearance, was similar for both groups, as was the sodium filtered load. Differently, $FPT_{Na}R$ was lower ($p < 0.05$) in EFAD than in CON group. Reduced $FPT_{Na}R$ by its turn

leds to increased ($p < 0.05$) distal sodium delivery, as well as increased ($p < 0.05$) lithium clearance in EFAD compared to CON group.

Table 1 Composition of control (CON) and EFAD diet

Diet	**Weight %**
Casein	20.7
Starch	46.8
Sucrose	21.0
Cellulose	1.8
Oil[a]	5.0
Vitamin (AIN-93 mix)[b]	0.9
Minerals (AIN-93 mix)[c]	3.7
D,L-Cystine	0.1
Butyl hydroxytoluene	0.0001

[a] CON diet contains soy oil with 52.84% and 7.26 % of C18:2n-6 and of C18:3n-3, respectively [18], while EFAD diet contains hydrogenated copra oil with 0.2% of C18:2n-6 and lacks C18:3n-3 [5,13].

[b] (Rhoster Ind. Com. LTDA, VG Paulista, SP, Brazil) containing (mg%): folic acid 20, niacin 300, biotin 2, calcium pentothenate 160, pyridoxine 70, riboflavin 60, thiamine chloride 60, vitamin B_{12} 0.25, vitamin K_1 7.5. Additionally containing (UI%): vitamin A 40,000; vitamin D_3 10,000; vitamin E 750.

[c] (Rhoster Ind. Com. LTDA, VG Paulista, SP, Brazil) containing (mg%): B 1.426, Ca 1.429, Cl 4.49, Cu 17.241, Cr 2.865, S 0.086, Fe 100, F 2.872, P 0.569, I 0.0593, Li 0.285, Mg 1.448, Mn 30, Mo 0.432, Ni 1.431, K 10.287, Se 0.428, Si 14.326, Na 2.938, Vn 0.287, Zn 86.

Table 2 Effects of perinatal EFAD on general parameters evaluated for 24h in metabolic cages

	Age, 8 weeks		**Age, 13 weeks**	
	CON (n = 11)	**EFAD (n = 15)**	**CON (n = 11)**	**EFAD (n = 15)**
Diet intake (g/100 g/24 h)	10 ± 1	9 ± 1	7 ± 1	7 ± 1
Water intake (ml/100 g/24 h)	17 ± 1	15 ± 1	11 ± 1	11 ± 1
Urinary flow (ml/100 g/24h)	6 ± 1	5 ± 1	5 ± 1	5 ± 1
Urinary density (g/ml)	1.048 ± 001	1.049 ± 0.005	1.046 ± 0.001	1.049±0.005
Water balance(ml/100g/24 h)	11 ± 1	10 ± 1	7 ± 2	6 ± 2
Urinary urea (mmol/100g/24 h)	109.4± 11.3	82.1 ± 3.0	95.00 ± 5.6	100.7 ± 6.3

Values are mean ± SE.

Table 3 Effects of perinatal EFAD on wet organ mass index in rats aged 13 weeks

	CON (n= 11)	**EFAD (n = 10)**
Spleen, %	0.11±0.05	0.14±0.06
Heart, %	0.30±0.05	0.29±0.06
Liver, %	2.50±0.23	2.60±0.21
Lungs, %	0.39±0.03	0.46±0.13
Left kidney, %	0.32±0.04	0.30±0.06
Testis, %	0.43±0.04	0.42±0.02

Values are mean ± SE.

Table 4 Parameters after lithium administration (0.06 mEq/100 g, by gavage) and 5% (v/w) water overload, in rats aged 13 weeks

	CON	**EFAD**
Serum Na^+ (mmol/l)	144 ± 2	144 ± 2
Serum creatinine (µmol /l)	56.29 ± 3.58	70.37 ±7.22
Urinary creatinine (µmol /l)	591.2 ±170.5	541.1 ± 180.5
Serum Li^+ (mmol/l)	0.44 ± 0.03	0.35 ± 0.03*
Li^+ filtered load (µmol/min/100 g)	66.17 ± 4.37	60.58 ± 15.69
Li^+ Fractional excretion	0.36 ± 0.08	0.73 ± 0.08*
Urinary flow (µl/min/100 g)	20.8 ± 0.9	22.8 ± 1.4

Li^+ filtered load = creatinine clearance (in µl/min) x Serum lithium; Fractional excretion of Li^+ = Urinary lithium excretion/Li^+ filtered load. Parameters were corrected to 100 g body weight when appropriate. Values are mean ± SE. * $p < 0.05$ vs. CON.

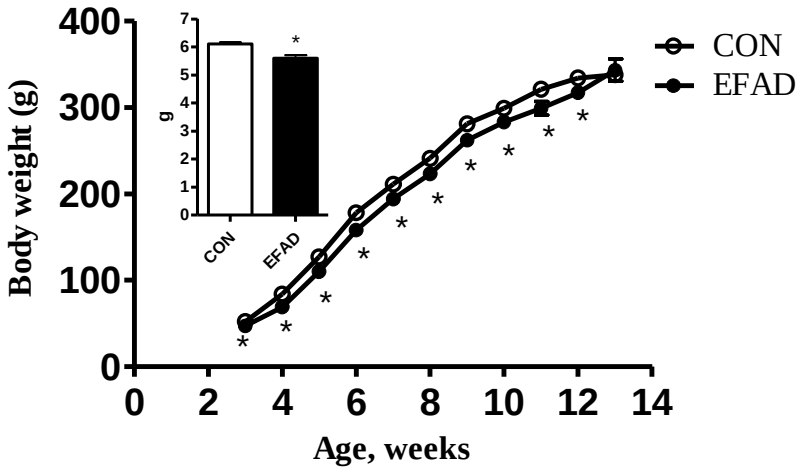

Fig. 1 Effects of perinataly imposed essential fatty acid deficiency (EFAD) on body weight evolution. Control (CON) group (n = 36, from birth to weaning and 15 from weaning until age of 13 weeks) comprises offspring of dams maintained from 3 weeks before pregnancy until weaning in a balanced diet prepared according to AIN 93 M, containing soy oil, while EFAD group (n = 36, from birth to weaning and 13 from weaning until age of 13 weeks) comprises offspring of dams maintained in the same balanced diet, except for the copra oil here, but during the same period as CON group. Values are means ± SE. SE bars are very small to appear in the graph scale. *$P < 0.05$ with respect to CON group.

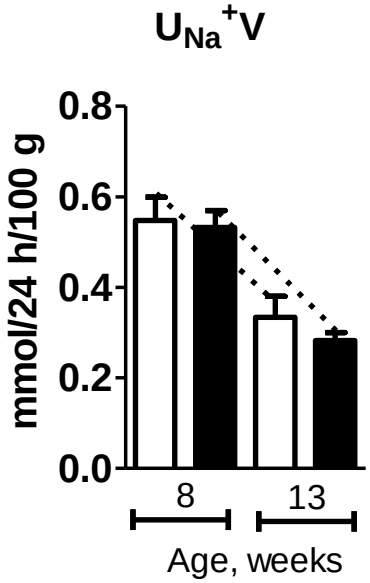

Fig. 2 Effects of perinatally imposed essential fatty acid deficiency (EFAD) on urinary sodium excretion ($U_{Na}^{+}V$). Dotted lines show the evolution of urinary sodium excretion in accordance with age. Results are mean ± SE.

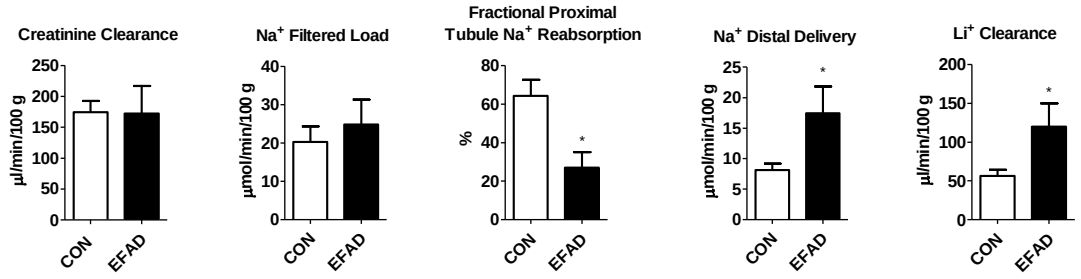

Fig. 3 Effects of perinatally imposed essential fatty acid deficiency (EFAD) on glomerular and proximal tubule function in rats aged 13 weeks. See group description in Fig. 1 and details for parameters calculations in Material and Methods. Results are mean ± SE. **P* < 0.05 with respect to CON group.

Discussion

In this study it was shown that essential fatty acid deficiency imposed to mothers from 3 weeks before pregnancy until weaning leads to reduced fractional proximal tubule sodium reabsorption in the adult offspring.

EFAD-induced increased basal metabolism that leads to low body weight is known since 1931 [19, 20], although its actual mechanism is not yet known. There is evidence that respiratory frequency is increased in EFAD rats [20], but chain enzymes activity in the mitochondria are changed in heart and skeletal muscle [19]. Undernutrition during lactation normally affects more severely the body weight development [21] than undernutrition restricted to fetal life. In the present work, the EFAD during prenatal and lactation periods compromised irreversibly the body weight gain. However, the lessened difference of body between CON and EFAD at adult age, compared with post-weaning, suggests that the catch up could happens at later age. It has been shown that EFAD during lactation depresses leptin levels in the offspring [22]. By its turn, lowered leptin during perinatal period could lead to hyperleptinemia and obesity at later life [23, 24]. Different from our study, in which EFAD begun before pregnancy, in a study by Korotkova and coworkers [25], EFAD begun at the last 10 days of pregnancy, body weights of EFAD rats were higher that of control rats. Together, these reports indicate that repercussion of EFAD depends likely on maternal essential fatty acid reserve. In our study, tough body weight evolution indicates that metabolic rate is higher in EFAD rats than in CON group, the serum protein and urinary urea levels are evidence that protein synthesis and degradation were reduced.

The striking reduction of $FPT_{Na}R$ (CON: 64% vs. EFAD: 27%) shown by EFAD group indicates that proximal tubule membrane constituents were changed, once this alteration was not due to GFR increment or to increment in sodium filtered load, because reduction in $FPT_{Na}R$ is a expected response to sodium overload in the proximal tubule [26]. Further, the reduction in $FPT_{Na}R$ was likely compensated for distal tubule reabsorption, since urinary sodium excretion and systolic blood pressure were unchanged in EFAD group. However, reduced $FPT_{Na}R$ leads to sodium overload in juxtaglomerular apparatus that recruits tubuloglomerular feedback and afferent arteriolar

vasoconstriction, though GFR did not seem yet to be affected. In contrast to our findings, EFAD spanning all life including in the perinatal period, causes hypertension in rats [6, 27].

In conclusion, perinatal EFAD imprints irreversible alterations in the proximal tubule function. Even that at adult juvenile rats this was not correlated with a pathological pattern, this possibility is not discarded to happen in later life.

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4 CONCLUSÕES

A carência perinatal em ácidos graxos essenciais resulta em alterações na função tubular proximal de ratos adultos jovens, embora sem apresentar hipertensão ou outra patologia aparente, que pode surgir mais tardiamente.

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ANEXO A – CARTA DE AUTORIZAÇÃO DA COMISSÃO DE ÉTICA EM EXPERIMENTAÇÃO ANIMAL (CEEA) DA UFPE

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Ofício nº 245/10

Recife, 01 de março de 2010

Comissão de Ética em Experimentação Animal (CEEA) da UFPE
Para.: **Profa. Ana Durce Oliveira da Paixão**
Departamento: Fisiologia e Farmacologia/ UFPE
Processo nº 23076.028019/2009-89

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: ***"O PERFIL DE INGESTÃO DE ÁCIDOS GRAXOS ESSENCIAIS NO PERÍODO PERINATAL PODE RESULTAR EM DISFUNÇÃO RENAL NA IDADE ADULTA."***

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,

Observação: Origem dos animais: Biotério do Departamento de Fisiologia e Farmacologia da UFPE; Animais: Ratos, Linhagem: Wistar; Sexo: Machos e Fêmeas; Idade:Crescimento e adultos; Nº de Animais previsto no projeto: 48(quarenta e oito) animais.

Profa. Maria Teresa Jansem
Presidente do CEEA
UFPE

CCB: Integrar para desenvolver

ANEXO B – ARTIGO PUBLICADO NA FOOD AND NUTRITION SCIENCES

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Scientific Research

Perinatally Imposed Essential Fatty Acid Deficiency Changes Renal Function of the Adult Rat

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Abstract

This study was designed to investigate whether essential fatty acid deficiency early during development could change the content of phospholipids and cholesterol in whole membranes of the kidney and renal function at adult life. For this, female Wistar rats were maintained on a standard diet or on an essential fatty acid deficient diet (EFAD) from the age of 30 days, throughout the pregnancy, at age of 90 days and until the weaning, for evaluation of their offspring. Weanling rats were maintained on a standard diet until the age of 13 weeks. Systolic blood pressure (SBP), glomerular filtration rate (GFR), urinary sodium excretion (UNa^+V), positive cells for angiotensin II (Ang II) and cholesterol and phospholipids in whole membranes of the kidney were evaluated. Cholesterol, total phospholipids and the relative content of classes of phospholipids were unaltered in the cortex and medullary kidney. SBP, GFR and UNa^+V were also unaltered in the EFAD group. However, the number of positive cells for Ang II in the tubulointerstitial area of the renal cortex was higher in the EFAD group. Therefore, these findings indicated that although cholesterol and phospholipids were unaltered and urinary sodium excretion was unchanged, Ang II expression in the kidney was erroneously programmed and later hindering of renal function was not ruled out.

Keywords

Angiotensin II, Phospholipids, Glomerular Filtration Rate

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1. Introduction

Some features of essential fatty acid deficiency (EFAD) are the decreased levels of the n-6 and n-3 fatty acid (FA) families and an accumulation of the n-9 FA family. Linoleic acid (LA; C18:2n-6) and α-linolenic acid (ALA; C18:3n-3) are essential FAs (EFAs) from the n-6 and n-3 FA series, respectively, which cannot be synthesized *de novo* by animals and have to be obtained from dietary sources. LA can be converted to n-6 long-chain polyunsaturated fatty acids (n-6 PUFA), while ALA is a substrate for biosynthesis of n-3 long-chain polyunsaturated fatty acids (n-3 PUFA) [1]. All of them are membrane constituents and play several biological roles. For instance, arachidonic acid (ARA, C20:4n-6) is a precursor of second messengers which play an important role in increasing vascular resistance and, in the kidney, are inhibitors of tubular sodium reabsorption [2]. Docosahexaenoic acid (DHA, C22:6n-3) is particularly necessary for brain development and its deficiency leads to cognitive impairment [3] and other neurodegenerative diseases [4].

n-3 PUFA deficiency, in particular, during pregnancy and up to the time of weaning has been associated with a mild increase in blood pressure when the rats reach the age of 8 months [5]. When EFAD is imposed from weaning until adult age, changes in renal hemodynamics and inability to excrete an acute volume expansion [6] have been observed, as well as an increment in proximal tubule sodium reabsorption [7]. On the other hand, a multideficient diet-induced lifelong undernutrition, including in the perinatal period, where fat content provides only 4.6% of energy contrasting with 13.3% in the standard diet, leads to lowered cholesterol and phospholipids, lessened $(Na^+ + K^+)$ATPase activity in basolateral membranes of the renal tubules, increased fractional Na^+ excretion and unchanged blood pressure in young rats [8]. Furthermore, it is known that EFAD can reduce the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis, to reduce the cholesterol synthesis in the liver [9].

Considering that malnutrition during development may imprint irreversible functional changes in the kidney, the present study investigated the hypothesis that perinatally imposed EFAD could change cholesterol and phospholipids in whole membranes of the adult rat kidney, and also whether positive cells for angiotensin II (Ang II) in the kidney, renal Na^+ excretion and blood pressure were changed.

2. Material and Methods

2.1. Ethical Considerations

The experimental procedure was approved by the Committee for Experimental and Animal Ethics at the Federal University of Pernambuco and performed in accordance with its rules.

2.2. Animals

Thirty day old female Wistar rats maintained in a room at 22˚C ± 3˚C with a 12-h light-dark cycle, were randomly assigned to a standard (C group, n = 6) or an EFAD (EFAD group, n = 6) diet. At age of 90 days, these rats were breeding, and were maintained on their respective diets during pregnancy and lactation until weaning. Therefore, dams were submitted to a total of 102 to 112 days of either a C or an EFAD diet, with a maximal variation of 10 days for breeding, when pregnancy was confirmed by at least 10 g of body weight gain. Pregnant dams were housed in individual cages until weaning, at offspring age of 21 days. After weaning, male pups (C, n = 15 and EFAD, n = 13) were housed in collective cages with 4 animals, in accordance with perinatal dietary treatment and all of them were given a standard diet (Purina Agribands) until the age of 13 weeks. Body weight was taken at birth, weaning and weekly after weaning. Some renal function parameters were measured at age of 8 weeks. At 13 weeks animals were assigned for blood pressure measurement and creatinine clearance evaluation. After measurement of functional parameters, the animals were exsanguinated by decapitation for kidneys withdrawal to evaluate membrane cholesterol and phospholipids. Furthermore, several other organs were collected to obtain their weights.

2.3. Diets

The formulation of diets, prepared according to AIN 93 M [10] differed only by the lipid composition: 5% of soy oil for the C diet and 5% of babassu oil for the EFAD diet (**Table 1**). The soy oil shows 52.8% and 7.3% of C18:2n-6 and of C18:3n-3, respectively [11], while the babassu oil shows 1.4% - 6.6% of C18:2n-6 and lacks C18:3n-3, according to the manufacturer (Rhoster Ind. Com. LTDA, VG Paulista, SP, Brazil).

Table 1. Composition of control (C) and essential fatty acid deficient (EFAD) diets.

Diet	wt%
Casein	20.7
Starch	46.8
Sucrose	21.0
Cellulose	1.8
Oil[a]	5.0
Vitamin (AIN-93 mix)[b]	0.9
Minerals (AIN-93 mix)[c]	3.7
D, L-cystine	0.1
Butyl hydroxytoluene	0.0001

[a]The C diet contains soy oil that shows 52.84% and 7.26% of C18:2n-6 and of C18:3n-3, respectively [11], while the EFAD diet contains babassu oil that shows 1.4% - 6.6% of C18:2n-6 and lacks C18:3n-3, according to the manufacturer (Rhoster Ind. Com. LTDA, VG Paulista, SP, Brazil). [b](Rhoster Ind. Com. LTDA) containing (mg%): folic acid 20, niacin 300, biotin 2, calcium pentothenate 160, pyridoxine 70, riboflavin 60, thiamine chloride 60, vitamin B12 0.25, vitamin K1 7.5. Additionally containing (UI%): vitamin A 40,000; vitamin D3 10,000; vitamin E 750. [c](Rhoster Ind. Com. LTDA) containing (mg%): B 1.426, Ca 1.429, Cl 4.49, Cu 17.241, Cr 2.865, S 0.086, Fe 100, F 2.872, 10.593, Li 0.285, Mg 1.448, Mn 30, Mo 0.432, Ni 1.431, K 10.287, Se 0.428, Si 14.326, Na 2.938, Vn 0.287, Zn 86.

2.4. Evaluation of Blood Pressure and Renal Function

At 8 and 13 weeks animals were housed in metabolic cages (Tecniplast Gazzada, Buguggiate, Italy) for a period of 24 hours in order to measure diet and water intake, urinary flow and urinary sodium ($UNa^{+}V$). The systolic blood pressure (SBP) was measured in conscious 13 week old rats by tail-cuff plethysmography (IITC Life Science B60-7/16, Life Science Instruments, Woodland Wills, USA).

Glomerular filtration rate (GFR) was measured by evaluating endogenous creatinine clearance [7]. For this, the animals were housed in metabolic cages for 3 h with continuous urine collection. Blood samples were withdrawn at the end of this period.

The following expressions were used to calculate the renal physiological parameters: Creatinine clearance = Ucr × V/Pcr, where V is the urinary volume (in µl/min) and Ucr and Pcr are the urinary and plasma creatinine concentrations, respectively (in mmol/l). Renal function parameters were corrected to 100 g body weight, when appropriate.

2.5. Evaluation of Phospholipids in Membranes of the Kidney

One of the kidneys was collected after the rats had been killed by decapitation and was maintained in cold isotonic buffer containing 250 mmol/l sucrose, 10 mmol/l HEPES-Tris (pH 7.4), 2 mmol/l EDTA and 0.15 mg/ml trypsin inhibitor (Type II-S) supplemented with 1 mmol/l PMSF. Cortex was separated from medulla on an ice pad. The fragments were separately homogenized using a teflon/glass homogenizer. To obtain total membranes, the homogenate was centrifuged at 17,000 g for 60 min; the resulting sediment was resuspended in 250 mM sucrose, aliquoted into tubes and stored at −20˚C. Lipids were extracted from total kidney membranes as described by [12] [13]. Total membrane phospholipids (TPL), and phosphatidylcholine (PC), sphingomyelin (Spm), phosphatidylethanolamine (PE) and phosphatidylserine (PS), were separated using bi-dimensional thin-layer chromatography with silica gel H containing 2.5% of magnesium acetate. The first dimension consisted of chloroform:methanol:aqueous ammonia (65:35:5), and the second dimension consisted of chloroform:acetone:methanol:acetic acid:water (50:20:10:10:5). Iodine vapor was used to visualize the spots of individual phospholipids that were marked according to the relative mobilities of chosen standards. Individual phospholipid spots were scraped and the samples were digested with 0.3 ml of 99.9% sulfuric acid by heating at 180˚C, using a heater plate for 2 h. After the tubes were chilled, one drop of 30% H_2O_2 was added to the samples. To ensure optically clear samples the tubes were heated on a heater plate for 2 h. The phosphorus measurement to determine the TPL was performed as described previously [13] [14]. Protein concentration was determined using the Folin phenol method [15] with bovine serum albumin as the standard; 2.5% (w/v) sodium dodecyl sulphate was added to solubilize integral membrane proteins.

Evaluation of positive cells to Ang II in the kidney the immunohistochemical evaluation for Ang II positiv cells in renal cortical cells was carried out as previously described [16]. Transverse slices of kidneys (3 mm were fixed in 10% neutral-buffered formalin until being encapsulated in paraffin. After appropriate embedmer in paraffin, 6-µm sections were used for incubation with antibody against Ang II (1:200 dilution) overnight a 4˚C. Sequentially, they were exposed for 1 hour to the conjugated biotin secondary antibody against rabb (1:400 dilution), followed by 1-h incubation with avidin-biotin-peroxidase complex in a humid chamber, a room temperature and visualized by using diaminobenzidine (DAB). The sections were counter-stained by usin 0.5% methyl green to count positive cells for Ang II in 60 fields, measuring 166,000 µm^2, throughout the tubu lointerstitial region and in 60 glomeruli.

Analytical methods serum cholesterol, total membrane cholesterol of renal cortex and medulla, and urinar and serum creatinine were measured employing commercial kits (Labtest, Lagoa Santa, MG, Brazil). Serum an urinary Na^+ were measured by an electrolyte analyzer (AVL 9180, Roche Diagnostics GmbH, Mannheim, Ger many).

Statistical analysis data is expressed as means ± SE. Statistical significance of differences ($P < 0.05$) was as sessed using two-tailed unpaired Student's t-test.

3. Results

From birth to 13 weeks of age, body weight development was significantly compromised in the EFAD grou (**Figure 1**). From weaning to 7 weeks of age, the body weight of EFAD was 17.9 and 9.6% ($P < 0.05$) lowe respectively, than the C group. From 8 to 13 weeks, the differences between groups were, respectively, of 8.1 t 5.4% ($P < 0.05$). At age of 13 weeks, the wet weight index (**Table 2**) of kidney, heart, testis, lungs, liver an spleen were unaffected. At ages of 8 and 13 weeks, 24 h diet and water intake, urinary flow, water balance, an the urinary density and urinary urea (**Table 3**) did not differ between the EFAD and C groups.

The EFAD did not change the levels of cholesterol or the levels of TPL in renal membranes, neither in th cortical region nor in the medullary region. The relative content of PC, PS, PE and Spm also did not change wit the EFAD (**Figure 2**). Regarding blood pressure and renal function, systolic blood pressure (SBP) and GFR measured as creatinine clearance and urinary sodium excretion (UNa^+V), were unchanged (**Figure 3**). Th number of positive cells for Ang II in the glomeruli was unaltered in the EFAD group. However, the number o positive cells for Ang II in the tubule-interstitial area increased in the EFAD group (**Figure 4**).

Table 2. Effects of perinatal EFAD on wet organ mass index in 13-week-old rats.

	CON (n = 11)	EFAD (n = 10)
Spleen, %	0.11 ± 0.05	0.14 ± 0.06
Heart, %	0.30 ± 0.05	0.29 ± 0.06
Liver, %	2.50 ± 0.23	2.60 ± 0.21
Lungs, %	0.39 ± 0.03	0.46 ± 0.13
Left kidney, %	0.32 ± 0.04	0.30 ± 0.06
Testis, %	0.43 ± 0.04	0.42 ± 0.02

Values are mean ± SE.

Table 3. Effects of perinatal EFAD on general parameters evaluated for 24 h in metabolic cages.

	Age, 8 weeks		Age, 13 weeks	
	CON (n = 11)	EFAD (n = 15)	CON (n = 11)	EFAD (n = 15)
Diet intake (g/100g/24h)	10 ± 1	9 ± 1	7 ± 1	7 ± 1
Water intake (ml/100g/24h)	17 ± 1	15 ± 1	11 ± 1	11 ± 1
Urinary flow (ml/100g/24h)	6 ± 1	5 ± 1	5 ± 1	5 ± 1
Urinary density (g/ml)	1.048 ± 0.001	1.049 ± 0.005	1.046 ± 0.001	1.049 ± 0.005
Water balance (ml/100g/24h)	11 ± 1	10 ± 1	7 ± 2	6 ± 2
Urinary urea (mmol/100g/24h)	109.4 ± 11.3	82.1 ± 3.0	95.00 ± 5.6	100.7 ± 6.3

Values are mean ± SE.

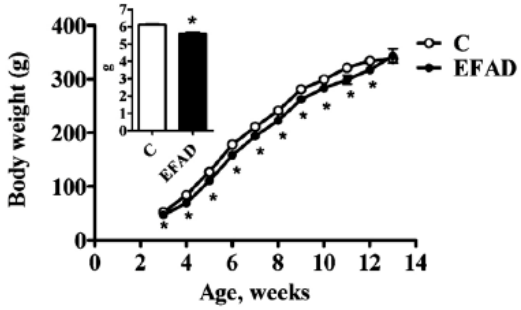

Figure 1. Effects of perinataly imposed EFAD on body weight evolution. The control (C) group (n = 36, from birth to weaning and 15 from weaning until age of 13 weeks) comprises offspring of dams maintained from age of 30 days and throughout pregnancy until weaning in a balanced diet prepared according to AIN 93 M, containing soy oil; while the EFAD group (n = 36, from birth to weaning and 13 from weaning until age of 13 weeks) comprises offspring of dams maintained in the same balanced diet, except for the replacement of babassu oil for soy oil, during the same period as the C group. Values are means ± SE. SE bars are very small to appear in the graph scale. *P < 0.05 with respect to the C group.

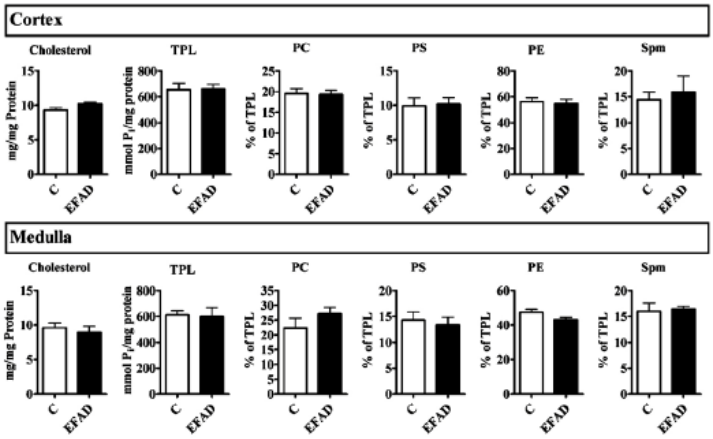

Figure 2. Effects of perinatally imposed EFAD on cholesterol and phospholipids in whole membranes of the kidney. See group description in Figure 1. The graphs are showing total phospholipids (TPL) and the relative amounts of phospholipids classes, phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE) and sphingomyelin (Spm). Results are mean ± SE of 6 essays.

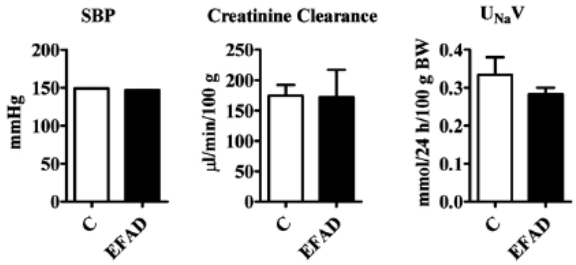

Figure 3. Effects of perinatally imposed EFAD on blood pressure and renal function. The parameters are systolic blood pressure (SBP), creatinine clearance and urinary sodium excretion (UNa^+V). See group description in Figure 1 and details for parameters calculations in Material and Methods. Results are mean ± SE of 8 animals in each group.

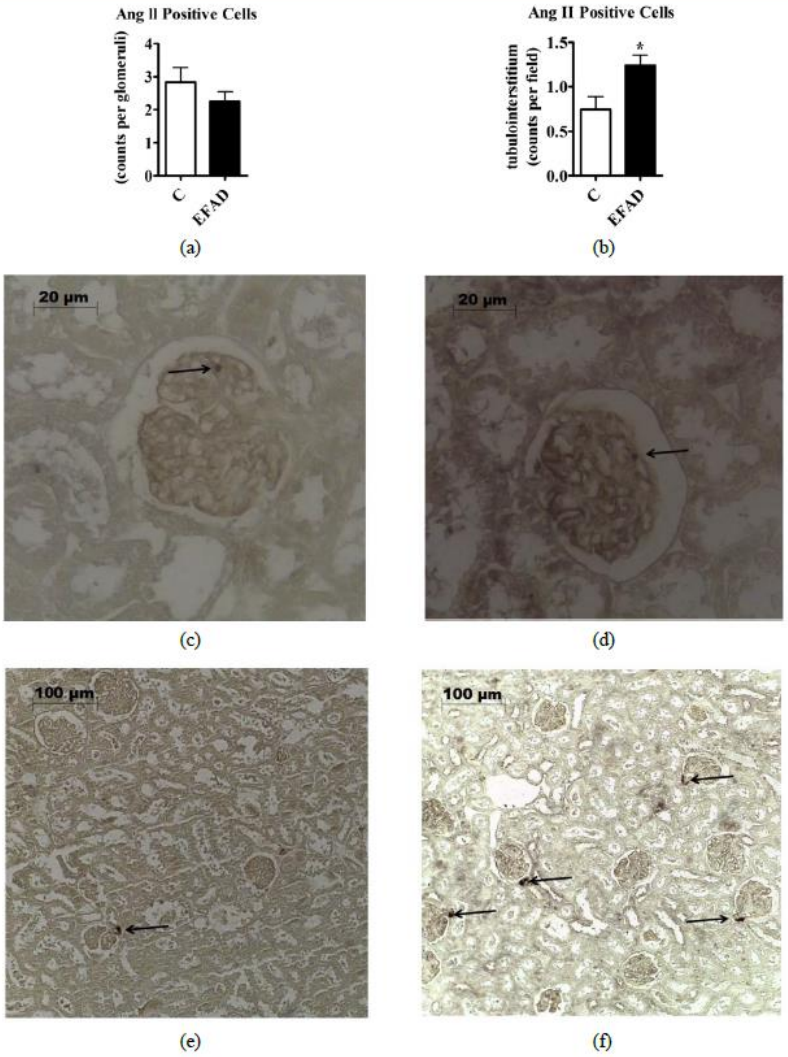

Figure 4. Effects of perinatally imposed EFAD on the number of positive cells for Ang II in the kidney. See group description in Figure 1. (a) The average number of cells showing Ang II per glomerulus in 60 glomeruli; (b) The average number of cells showing Ang II, counted in 60 fields measuring 166,000 μm^2. Results are mean ± SE of 6 slides in each group; (c) Representativeimmunolocalization for positive cells to Ang II, pointed by arrows, in glomeruli of C group; (d) Representative immunolocalization for positive cells to Ang II, pointed by arrows, in glomeruli of EFAD group; (e) Representative immunolocalization for positive cells to Ang II, pointed by arrows, in the tubulointerstial region of the C group; (f) Representative immunolocalization for positive cells to Ang II, pointed by arrows, in the tubulointerstial region of the EFAD group. $^{*}P < 0.05$ with respect to the C group.

4. Discussion

The hypothesis that perinatally imposed EFAD could change cholesterol and phospholipids in whole membranes of the adult rat kidney was not supported. However, the tubule-interstitial area in the kidney presented an increased number of positive cells for Ang II, even though the renal sodium excretion and GFR were unchanged. These findings indicate that the Ang II expression in the kidney was erroneously programmed and that later hindering of renal function is not ruled out.

Taking into account that the mothers were submitted to EFAD for 60 to 70 days before the first day of pregnancy, the offspring was effectively subjected to lower levels of n-6 and n-3 PUFA, from the conception until the weaning. ARA and DHA, respectively, products of linoleic and α-linolenic acids, essential fatty acids, are drastically reduced in plasma [17] [18] and in tissues as the kidney [17] after 8 weeks of treatment.

The reduced birth weight and the lower body weight gain during development were a characteristic effect of EFAD [19] [20]. To reduce body weight, there is evidence that EFAD leads to increased basal metabolism [21] [22], although its actual mechanism is not yet known. Respiratory frequency is increased in EFAD rats [22], but chain enzymes activity in the mitochondria are changed in the heart and skeletal muscle [21]. Undernutrition during lactation normally affects body weight development [23] more severely than undernutrition restricted to fetal life. Under EFAD, particularly during lactation, the plasma levels of IGF-I are reduced [20] contributing to the reduction in body weight. In the present study, the EFAD during prenatal and lactation periods compromised body weight gain irreversibly. However, the lessened difference of body weight between C and EFAD at adult age, compared with post-weaning, suggests that the catch up could happen at a later age. This is likely due to the fact that EFAD during lactation depresses leptin levels in the offspring [20] [24] during the early stages of development. However, lowered leptin during the perinatal period could lead to hyperleptinemia and obesity later in life [25] [26].

Considering that cholesterol was unchanged in the membranes of the kidney, the first assumption that may be taken is that HMG-CoA reductase activity was not programmed during the perinatal period, at least in the kidney. HMG-CoA reductase is the rate-limiting enzyme for cholesterol synthesis. There is evidence that EFAD decreases HMG-CoA reductase activity [9], when the animals are evaluated immediately after the diet was imposed. It is worthy to emphasize that in the present study the essential fatty acid replenishment began after the weaning, at age of 21 days, and that the animals were evaluated at age of 90 days. Regarding phospholipids, the present data does not ensure that specific PUFA, such as ARA and DHA, were recovered, something that may be considered one limitation of this study. However, the present data determines that total phospholipids are not changed in the membranes of the kidney. Increased activity of delta 9 desaturase, responsible for synthesis of monounsaturated FA, is one of the effects of EFAD [27]. The activity of this enzymeis recovered in the liver after perinatal (n-3) PUFA deficiency is followed by its repletion after the weaning [28]. However, there is evidence that in the hypothalamusan imbalance between (n-6) and (n-3) PUFA early in life is not recovered at adult age [29].

Aside from the unaltered cholesterol and phospholipids in the kidney, the urinary sodium excretion was also unchanged, as well as the glomerular filtration rate. Therefore, fractional sodium excretion was not evaluated. However, the increased number of cells positive for Ang II in the tubule-interstitial area, suggests that changes in the renin angiotensin system were caused by EFAD. The expression of Ang II in the kidney is one marker of renal development during nephrogenesis. The presence of Ang II during kidney development leads to an increase in the glial cell-derived neurotrophic factor (GDNF) [30], which is a crucial growth factor for ureteric bud proliferation [31]. Increased at adult life in the kidney, Ang II has been correlated with increased oxidative stress and increased sodium reabsorption [32], or even increased blood pressure [33]. However, in the present study the EFAD group did not show increased SBP. A previous research study showed that maintenance of an imbalance spanning the whole life of the rat, until the age of 33 weeks, leads to elevated blood pressure, while the replacement of the diet at the age of 12 weeks leads to a reduction in the levels of blood pressure, even though the animals had higher blood pressure than control rats [5]. Thus, together, this previous evidence allied to an increased number of Ang II cells in the kidney, may indicate that renal function and hypertension may occur later in life.

5. Conclusion

In summary, essential fatty acid deficiency imposed during perinatal period programmed an increase in the number of cells positive for Ang II in the kidney.

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