Universidade do Estado do Rio de Janeiro
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A ingestão precoce de dieta enriquecida com óleo de peixe reverte alterações bioquímicas, hepáticas e do tecido adiposo na prole de camundongos submetidos à restrição proteica

Rio de Janeiro
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Dissertação apresentada, como requisito parcial para obtenção do título de Mestre, ao Programa de Pós-graduação em Biologia Humana e Experimental da Universidade do Estado do Rio de Janeiro.

Orientadora: Prof[a]. Dra. Márcia Barbosa Águila

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Rio de Janeiro

2010

DEDICATÓRIA

Aos meus pais, à minha irmã e ao meu namorado que me apoiaram e incentivaram durante todo esse período de trabalho árduo.

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A Deus, por permitir que mais uma etapa tão importante da minha vida fosse concretizada.

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Albert Einstein

RESUMO

SARMENTO, Isabele Bringhenti. *Ingestão precoce de dieta enriquecida com óleo de peixe reverte alterações bioquímicas, hepáticas e do tecido adiposo na prole de camundongos submetidos à restrição protéica*. 2010. 61 f. Dissertação (Mestrado em Ciências - Biologia Humana e Experimental) – Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2010.

Estudos relacionam obesidade na vida adulta com baixo peso ao nascer (programação metabólica). O fígado é um dos órgãos mais afetados pela programação. O óleo de peixe é rico em ácidos graxos poli-insaturados (AGP) da família n-3: ácido eicosapentaenóico (EPA) e docosahexaenóico (DHA). O EPA e DHA são relacionados com redução da pressão arterial sistólica e ação anti-inflamatória. Testar a hipótese que a ingestão precoce de óleo de peixe (FO) pode reverter os efeitos deletérios da programação na prole adulta de camundongos. Fêmeas grávidas foram alimentadas com ração padrão (SC) ou dieta restrita em proteínas (LP) durante a gestação e lactação. Ao desmame, os seguintes grupos foram formados (de acordo com a suplementação com FO): SC-SC e SC-FO, LP-SC e LP-FO. Foram aferidas massa corporal, ingestão e eficiência alimentar, pressão arterial sistólica (PAS), insulina plasmática, glicose, fator de necrose tumoral (TNF)-alfa, colesterol total (CT), triglicerídeos (TG) e alanina aminotransferase (ALT), morfometria dos adipócitos, estereologia do fígado e expressão proteínas SREBP-1c e PPAR-alfa. A prole LP apresentou maior massa corporal, hipercolesterolemia e hiperglicemia Na idade adulta, os animais restritos tornaram-se hipertensos, com esteatose hepática e elevado nível da SREBP-1c. Entretanto, a prole LP com dieta suplementada com FO ocasionou menor ganho e menor massa corporal final. A dieta FO melhorou o metabolismo lipídico, diminuiu a concentração plasmática de CT e TG, reduziu a massa adiposa e o tamanho dos adipócitos. Além disso, LP-FO mostrou níveis reduzidos da ALT, redução da esteatose hepática, baixa expressão da SREBP-1c e aumento da expressão do PPAR-alfa, além de redução da PAS e dos níveis de TNF-alfa. A dieta com FO teve efeitos benéficos revertendo as respostas da programação sobre o metabolismo da glicose e lipídios, estrutura hepática e tecido adiposo na prole adulta programada.

Palavras-chave: Óleo de peixe. Programação. Fígado. Tecido adiposo.

ABSTRACT

Studies related obesity in adult life with malnutrition in early life, called “metabolic programming”. The liver seems to be one of the most affected by fetal programming. Fish oil is rich in polyunsaturated fatty acids (PUFA) of n-3 family: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are related with several biological reactions, like systolic blood pressure reduction and anti-inflammatory properties. We hypothesized that early fish oil (FO) intake would revert the programming responses in adult offspring. Pregnant mice were fed either standard chow (SC) or low-protein diet (LP) throughout pregnancy/lactation. At weaning, the following groups were formed (FO means supplemented with fish oil): SC-SC and SC-FO, LP-SC and LP-FO. We measured body mass, food intake, feed efficiency, blood pressure (BP), levels of plasma insulin, glucose, tumor necrosis factor (TNF-alpha), total cholesterol (TC), triglycerides (TG) and alanine aminotransferase (ALT). We also measured adipocyte morphometry, liver stereology and expression of the SREBP-1 and PPAR-alpha proteins.The LP offspring are predisposed to becoming fat, hypercholesterolemic and hyperglycemic. In addition, during adulthood, they become hypertensive with hepatic steatosis and had high level of SREBP-1. However, LP offspring that were fed FO-enriched diet had decreased body mass gain and lower final body mass. In addition, with FO diet, these mice have improved lipid metabolism with decrease in plasma TC and TG levels, reduced fat pad masses and adipocyte size. Furthermore, LP-FO offspring show low ALT level, reduced liver steatosis with low SREBP-1 protein expression and high PPAR-alpha expression, and improvement of BP and TNF-alpha level. Early fish oil intake has beneficial effects reversing the programming responses that control body mass, glucose and lipid metabolism, and liver and adipose tissue structure in adult programmed offspring.

Keywords: Fish oil. Programming. Liver. Adipose tissue.

LISTA DE ILUSTRAÇÕES

LISTA DE SIGLAS E ABREVIATURAS

AA	Ácido araquidônico
ALT	Alanine aminotransferase
AG	Ácido graxo
AGP	Ácidos graxos poli-insaturados
AUC	Area under the curve
BM	Body mass
BP	Blood pressure
ChREBP	Carbohydrate response element binding protein
DHA	Ácido docosahexaenóico
EPA	Ácido eicosapentaenóico
EFM	Epididymal fat mass
FA	Fatty acid
FE	Feed efficiency
FO	Fish oil
HOMA-IR	Homeostasis model assessment for insulin resistance index
LM	Liver mass
LP	Low protein chow
NAL	Naso-anal length
OGTT	Oral glucose tolerance test
P_P	Number of points that hit the structure
PPAR	Peroxisome Proliferator Activated Receptor
P_T	Total test-points
PUFA	Polyunsaturated fatty acid
RFM	Retroperitoneal fat mass
SC	Standard chow
SREBP-1c	Sterol Regulatory Element Binding Protein-1c
TC	Total cholesterol
TG	Triacylglycerids
TNF	Tumor necrosis factor
VLDL	Very low density lipoprotein

SUMÁRIO

INTRODUÇÃO

A hipótese da “Programação Fetal” propõe que um ambiente adverso intrauterino altera os meios metabólicos e hormonais do feto, resultando em adaptações de desenvolvimento para garantir a sobrevivência do mesmo (1). Se essas respostas adaptativas, projetadas para a sobrevivência em um ambiente fetal limitado persistirem na vida pós-natal, podem ocorrer distúrbios metabólicos e endócrinos (2). O baixo peso ao nascer está associado ao desenvolvimento de doenças crônico-degenerativas na fase adulta, como hipertensão arterial (3), resistência à insulina (4), disfunção vascular (5), obesidade (6), dislipidemia (7) e esteatose hepática (8, 9).

Este assunto foi primeiramente abordado em estudos com populações que foram expostas à fome, como aconteceu, por exemplo, durante a segunda guerra mundial na Holanda (10). Os filhos de mulheres holandesas que passaram fome durante a gestação desenvolveram obesidade, intolerância à glicose e hipertensão arterial (11), além de se tornarem mais suscetíveis ao desenvolvimento de diabetes mellitus na vida adulta (10).

No que tange à terapêutica das desordens metabólicas, a ingestão de óleo de peixe, rico em ácidos graxos poli-insaturados da família n-3 (AGP n-3), provou ser eficaz na redução tanto de triglicerídeos plasmáticos (TG), como nas concentrações de VLDL em animais experimentais (12). Em ratos, foi mostrado que, o óleo de peixe diminui os níveis de mRNAs que codificam várias enzimas envolvidas na lipogênese hepática *de novo* e aumenta a beta-oxidação dos ácidos graxos (AG) através de receptores ativados por proliferador de peroxissomos (PPAR)-alfa (13). Além disso, os AGP n-3 podem prevenir o desenvolvimento de esteatose hepática em camundongos com dieta hiperlipídica (14, 15). Estudos demonstram ainda que, a ingestão de óleo de peixe impede o acúmulo de gordura abdominal, em comparação com outros tipos de lipídios (16, 17).

A nutrição tem suma importância no início da vida. A nutrição materna, durante a gestação e lactação assume papel crucial para o futuro da criança: ou ela terá uma vida sadia ou terá uma vida com uma doença crônico-degenerativa na fase adulta. E com base nos resultados deste trabalho, podemos propor que o consumo precoce de óleo de peixe pode ajudar a evitar os efeitos deletérios da programação metabólica.

Esta dissertação está dividida em quatro partes. “**Revisão da literatura**” que conceitua a programação metabólica e considera os efeitos potenciais da restrição proteica durante a fase de desenvolvimento fetal e os efeitos do óleo de peixe como possível tratamento para as alterações adversas causadas pela programação metabólica; em seguida estão os “**Objetivos**” do trabalho. A terceira parte, “**Resultados**”, está o artigo “*An early fish oil-enriched diet reverses biochemical, liver and adipose tissue alterations in offspring mice from maternal protein restriction*”, que foi submetido para a revista *Journal of Nutritional Biochemistry*. O artigo é o âmago da dissertação, pois descreve o trabalho propriamente dito: com descrição da metodologia, dos resultados e a discussão. E a parte final da dissertação consiste de um texto com as conclusões e perspectivas do trabalho.

1 **REVISÃO DA LITERATURA**

1.1 **Programação metabólica**

Durante a gestação, o desenvolvimento do feto é totalmente dependente da mãe e do ambiente intrauterino para um adequado suprimento nutricional (18). Uma alteração da qualidade da dieta materna durante períodos críticos do desenvolvimento pode alterar todo curso da gestação, “programando” o feto, e assim tornando-o susceptível ao surgimento de doenças cardiovasculares (DCV) na vida adulta (19).

Lucas em 1991 foi quem primeiro definiu o termo “programming” (programação), que ele descreveu ser *uma resposta permanente do organismo a insultos ou estímulos durante um período crítico do desenvolvimento* (20). Em termos simples, esta definição sugere que o feto ou neonato quando exposto a um ambiente atípico durante uma fase de crescimento rápido, o resultado são respostas adaptativas que podem tornar-se permanentes.

Um ano mais tarde Hales e Barker lançaram a hipótese do “thrifty phenotype” (fenótipo econômico) postulando que, durante os períodos de privação nutricional, o organismo do feto adotaria estratégias para maximizar sua sobrevivência (21).

Trabalhos sobre o período da fome holandesa de 1944-45 observaram incidência aumentada de obesidade entre adultos que sofreram desnutrição nos primeiros meses de vida intrauterina (10). Estudos epidemiológicos (11, 22, 23) e experimentais (24-26) sugerem que alterações metabólicas originam-se durante períodos embrionários. Dentre as alterações metabólicas, podemos citar hipertrigliceridemia, intolerância à glicose e resistência à insulina (27).

A diminuição do suprimento nutricional necessário para atingir a demanda adequada em um período crítico do desenvolvimento fetal pode alterar permanentemente a taxa de crescimento, assim como o desenvolvimento e a função de órgãos e sistemas fisiológicos (28). Posteriormente, foi descrita a hipótese da resposta adaptativa, a qual propõe que o feto interage dinamicamente com o ambiente e adapta-se para ganhar uma vantagem de sobrevivência no futuro (29). É imprescindível destacar ainda a influência do fator nutricional sobre o epigenótipo.

Crescentes indícios têm associado o desenvolvimento de doenças na vida adulta com modificações na expressão gênica (30).

E dentre os vários órgãos afetados pela programação metabólica, o fígado apresenta-se como um dos principais alvos. Evidências experimentais demonstram que animais submetidos à programação metabólica apresentam alterações enzimáticas que em associação com a diminuição das células beta-pancreáticas, progridem para um quadro de resistência à insulina (8, 31). Tais condições contribuem para um influxo de ácidos graxos (AG) (tanto para utilização como para lipogênese) que excedem sua liberação (degradação ou exportação) favorecendo a ocorrência de fígado gorduroso ou esteatose hepática (32, 33). A esteatose hepática não-alcoólica é considerada uma manifestação hepática da síndrome metabólica. Estudos mostram que 25% da população mundial apresenta esteatose hepática (34, 35). Embora a esteatose hepática não-alcoólica apresente um quadro inicial benigno, tal condição torna o fígado mais suscetível a um dano histológico mais intenso, podendo desta forma, progredir para um quadro de esteatohepatite, cirrose, carcinoma hepatocelular e até mesmo falência hepática (36, 37). A deficiência proteica durante a gestação e lactação não somente altera a atividade das enzimas hepáticas envolvidas na regulação da glicose (8, 38) e sensibilidade à insulina (39), como também, reduz a tolerância à glicose na prole adulta de ratos (40).

Um mecanismo relacionado às alterações hepáticas é a mudança na expressão de fatores de transcrição envolvidos na regulação do metabolismo de lipídios. Um desses fatores de transcrição é a proteína de ligação ao elemento de resposta esterol (SREBP), que regula as enzimas responsáveis pela síntese de colesterol, ácidos graxos e triglicerídeos. Foram caracterizadas e definidas três isoformas de SREBP, como a SREBP-1a, 1c e 2. Sendo que a isoforma SREBP-1c é predominante no fígado e no tecido adiposo e é a principal responsável pela regulação do metabolismo lipídico (41, 42). Além disso, ocorre aumento da expressão da SREBP-1c em resposta à hiperinsulinemia (43).

Animais adultos que receberam dieta hipoproteica durante a gestação/lactação apresentaram supressão das proteínas hepáticas relacionadas à lipogênese, como a SREBP-1c e proteína de ligação ao elemento de resposta carboidrato (ChREBP). Concomitantemente ocorre alteração do perfil lipídico e do metabolismo de carboidratos, além de hiperinsulinemia e esteatose hepática. Ademais, a expressão

de PPAR-alfa, relacionado à beta-oxidação, mostra-se diminuída nesses animais (44). Comprovando este fato, trabalhos em humanos revelam que, adultos que sofreram restrição do crescimento intrauterino apresentam aumento do tecido adiposo, principalmente tecido adiposo visceral na fase adulta (45). Tais achados sugerem que a lipogênese aumentada é um dos fatores que contribuem para o desenvolvimento da adiposidade visceral em modelo animal (46). Com o aumento da adiposidade, há aumento concomitante dos níveis de mRNA de TNF-alfa Este, por sua vez, desencadeia a resistência à insulina através da inativação de receptores de insulina (47). Outro mecanismo pelo qual TNF-alfa pode contribuir para a resistência à insulina é através do aumento dos níveis circulantes de AG livres devido à indução de lipólise e lipogênese hepática (48). Desta forma, a distribuição da gordura corporal é um fator importante na etiologia da resistência à insulina.

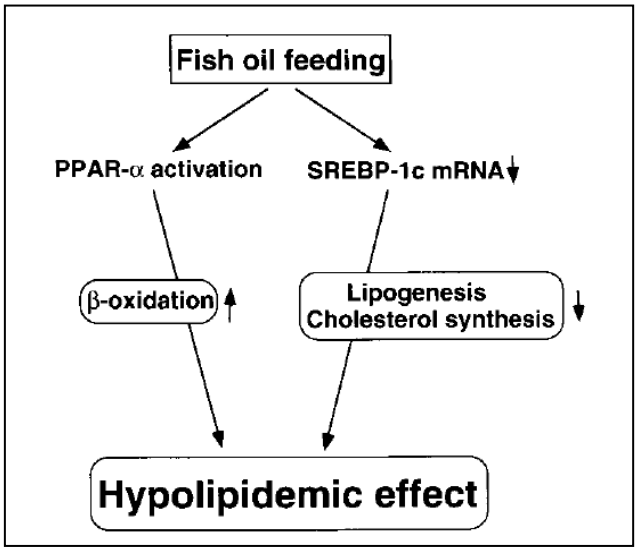
No que tange aos valores pressóricos, estudos experimentais evidenciam que a desnutrição favorece o desenvolvimento de hipertensão arterial sistêmica (HAS) na vida adulta (26, 49). A resistência à insulina e hiperinsulinemia são fatores determinantes no curso do desenvolvimento da HAS (50). Trabalhos também associam o desenvolvimento da HAS com alterações no desenvolvimento renal, como, por exemplo, diminuição do número de néfrons (51-53).

1.2 **Óleo de peixe**

Os estudos sobre óleo de peixe originaram-se na década de 1970, com esquimós da Groelândia que apresentavam pequena incidência de DCV. Descobriram depois que a baixa incidência de DCV estava associada ao elevado consumo de peixe (54-56).

O óleo de peixe é uma das principais fontes de AGP da família n-3, com quantidades significativas do ácido docosahexaenóico (DHA, 22:6n-3) e ácido eicosapentaenóico (EPA, 20:5n-3). A literatura revela, tanto em trabalhos epidemiológicos (57-59), como em trabalhos experimentais (60-63) que os AGP da família n-3 estão relacionados com diversas reações biológicas benéficas no organismo, como redução dos níveis pressóricos, ação anti-inflamatória e melhora do metabolismo lipídico.

Outra ação benéfica do EPA e DHA é seu efeito sobre a esteatose hepática não-alcoólica (64, 65). O desenvolvimento da esteatose hepática em animais submetidos à restrição proteica perinatal, deve-se principalmente ao aumento da lipogênese e da diminuição da beta-oxidação. O consumo do óleo de peixe suprime a expressão de proteínas relacionadas com a lipogênense, como a SREBP-1c e aumenta a expressão de PPAR-alfa, configurando assim um efeito hipolipidêmico (Esquema 1).



Esquema 1- Mecanismo do efeito hipolipemiante do consumo de óleo de peixe (66).

Os AGP n-3 são reguladores negativos da lipogênese hepática e sua suplementação na dieta protege o fígado contra deposição lipídica (64, 65). O DHA é um potente ativador do PPAR-alfa (67) que por sua vez ativa uma série de proteínas responsáveis pelo transporte de lipídios, pela beta-oxidação e pela termogênese (68). Baillie e colaboradores (69) relataram o efeito do óleo de peixe sobre a expressão da proteína mitocondrial desacopladora 2 e 3 (UCP-2 e UCP-3) e sobre o mecanismo de oxidação peroxissomal no fígado e músculo esquelético. Esses efeitos são possivelmente responsáveis pela redução do acúmulo lipídico no fígado e na redução dos depósitos de gordura.

O óleo de peixe também é eficaz na diminuição dos níveis de glicemia em camundongos e na melhora da resistência à insulina em roedores tratados com AGP n-3 (64, 70-72), assim como na melhora da sensibilidade à insulina em adultos

obesos (73). Pérez-Matute e colaboradores (74), sugerem que o aumento da sensibilidade à insulina induzida pelo óleo de peixe é devido à sua ação anti-inflamatória no tecido adiposo de ratos obesos. Outra ação benéfica dos AGP n-3 é o aumento dos níveis de adiponectina (74). A adiponectina é uma adipocina anti-inflamatória que diminui os níveis de glicemia, bem como aumenta a sensibilidade à insulina (75, 76).

Os AGP n-3 também podem originar a produção de novos mediadores lipídicos, conhecidos como resolvinas e protectinas. Esses mediadores lipídicos também possuem ações benéficas e protetoras (anti-inflamatórias, imunorregulatórias, antiesteatótica e melhora da sensibilidade à insulina) que justificam os efeitos benéficos dos AGP n-3 (65, 77, 78).

Em relação ao efeito hipotensor dos AGP n-3 foi bem evidenciado em humanos (79), em ratos espontaneamente hipertensos (61) e em ratos submetidos à programação metabólica (26). Os mecanismos envolvidos na ação anti-hipertensiva do óleo de peixe são ação vasodilatadora e antitrombótica através da competição do EPA com AA pelas enzimas cicloxigenase e lipoxigenase. Desta forma, a síntese de tromboxano A2 é inibida e a síntese de tromboxano A3 é ativada, ocasionando assim, efeitos vasodilatadores e antitrombóticos (80, 81). Além do mais, outros benefícios dos AGP n-3 sobre a função vascular podem estar relacionados à sua incorporação na membrana celular, o que ocasiona uma mudança na estrutura físico-química da mesma com alteração da fluidez, permeabilidade e função de suas proteínas. Por conseguinte, é possível que haja uma mudança da atividade enzimática, afinidade dos receptores e capacidade de transporte da célula, incluindo a síntese e a liberação de óxido nítrico (82).

Ainda com relação aos efeitos hipotensores dos AGP n-3, existe uma importante relação entre resistência à insulina e hipertensão arterial (50, 83). Sabe-se que o TNF-alfa é um importante indutor da resistência à insulina; no entanto, estudos revelam que o EPA e o DHA podem inibir a produção de citocinas inflamatórias, como o TNF-alfa, interleucina-1 (IL) e IL-2 (84, 85).

2 **OBJETIVOS**

2.1 **Objetivo geral**

Verificar se a ingestão precoce de dieta enriquecida com óleo de peixe é capaz de reverter os efeitos adversos provocados pela programação metabólica sobre a prole adulta de camundongos.

2.2 **Objetivos específicos**

Analisar em camundongos suíços machos os efeitos da dieta rica em óleo de peixe sobre:

- A evolução da massa corporal, depósitos de gordura visceral e pressão arterial;
- O metabolismo de carboidratos e de lipídios;
- Parâmetro morfológico-quantitativo para o fígado e tecido adiposo;
- Analisar a expressão de proteína lipogênica e proteína relacionada à beta-oxidação no fígado.

3 **RESULTADOS**

3.1 **Artigo Científico submetido *Journal of Nutritional Biochemistry***

Artigo Científico

ISABELE BRINGHENTI, ALINI SCHULTZ, TAMIRIS L RACHID, MARCO A BOMFIM, CARLOS A MANDARIM-DE-LACERDA, MARCIA B. AGUILA. An early fish oil-enriched diet reverses biochemical, liver and adipose tissue alterations in offspring mice from maternal protein restriction.

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Title: AN EARLY FISH OIL-ENRICHED DIET REVERSES BIOCHEMICAL, LIVER AND ADIPOSE TISSUE ALTERATIONS IN OFFSPRING MICE FROM MATERNAL PROTEIN RESTRICTION
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AN EARLY FISH OIL-ENRICHED DIET REVERSES BIOCHEMICAL, LIVER AND ADIPOSE TISSUE ALTERATIONS IN OFFSPRING MICE FROM MATERNAL PROTEIN RESTRICTION

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Running title: fish oil reverses alterations in mice

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Category of study: original research

Key words: fish oil; protein restriction; fatty liver; obesity; hypertension, insulin resistance

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ABSTRACT

Objective: Fetal programming is linked to adulthood metabolic and chronic diseases. We hypothesized that early fish oil (FO) intake would revert the programming responses in adult offspring. **Methods**: Pregnant mice were fed either standard chow (SC) or a low-protein diet (LP) throughout pregnancy/lactation. At weaning, the following groups were formed (FO means supplemented with fish oil): SC-SC and SC-FO, LP-SC and LP-FO. We measured body mass, food intake, feed efficiency and blood pressure (BP). In addition, we observed the levels of plasma insulin, glucose, tumor necrosis factor (TNF-alpha), total cholesterol (TC), triglycerides (TG) and alanine aminotransferase (ALT). Finally, we measured adipocyte morphometry, liver stereology and the expression of the SREBP-1c and PPAR-alpha proteins. **Results**: The LP offspring are predisposed to becoming fat, hypercholesterolemic and hyperglycemic. In addition, during adulthood, they become hypertensive with hepatic steatosis and have a high level of SREBP-1c. However, LP offspring that were fed an FO-enriched diet have decreased body mass gain and lower final body mass. In addition, with this diet, these mice have improved lipid metabolism with a decrease in plasma TC and TG levels, reduced fat pad masses and adipocyte size. Furthermore, these LP-FO offspring show low ALT level, reduced liver steatosis with low SREBP-1c protein expression and high PPAR-alpha expression, and improvement of BP and TNF-alpha level. **Conclusions**: Early fish oil intake has beneficial effects reversing the programming responses that control body mass, glucose and lipid metabolism, and liver and adipose tissue structure in adult programmed offspring.

INTRODUCTION

The fetal programming hypothesis proposes that an adverse intrauterine environment alters the metabolic and hormonal milieu of the fetus, resulting in developmental adaptations to ensure fetal survival (1). If these adaptive responses, designed for survival in a substrate-limited fetal environment, persist into postnatal life, metabolic and endocrine disorders may arise (2). Therefore, low birth weight has been linked to adulthood hypertension (3), insulin resistance (4), vascular dysfunction (5), obesity (6), dyslipidemia (7), and the liver appears to be highly affected by fetal programming (9).

Poor nutrition in early life and the type of diet utilized during the post-weaning period appear to be critical in the determination of obesity in adulthood. In addition, studies have suggested that inflammatory mediators such as tumor necrosis factor-alpha (TNF) and interleukins play a central role in the development of cardiovascular diseases. Elevated plasma levels of TNF-alpha have been found to be associated with obesity, insulin resistance and hypertriglyceridemia (86).

The dietary intake of fish oil (FO), rich in n-3 polyunsaturated fatty acid (PUFA), has proven to be effective in lowering both plasma triglyceride (TG) and very low density lipoprotein (VLDL) concentrations in experimental animals (12). In rats, it has been shown that FO decreases the level of mRNAs that encode several enzymes involved in *de novo* hepatic lipogenesis and enhances fatty acid (FA) oxidation [a peroxisome proliferator activity receptor (PPAR)-alpha stimulated this process] (13). In addition, n-3 PUFA can prevent steatosis in mice with dietary-induced hepatic steatosis (87, 88). FO feeding prevents abdominal fat accumulation compared to the ingestion of other types of dietary oils (16, 17).

Based on these data, we hypothesized that early FO intake would help or revert the programming responses on body composition, carbohydrate and lipid metabolism, and liver and adipose tissue structure in programmed adult offspring.

MATERIALS AND METHODS

Sampling and diet protocols

All procedures with animals were performed according to the guidelines of the animal ethics committee of the State University of Rio de Janeiro and were carried out in accordance with conventional guidelines for experimentation with animals (NIH Publication Nº. 85-23, revised 1996). Animals were maintained under controlled conditions (21±2°C, humidity 60±10%, 12:12 h dark-light cycle) with free access to food and water.

Virgin Swiss female mice were caged with males overnight. Beginning at the first day of pregnancy, mice were housed individually in cages and randomly assigned to either a group fed standard chow (SC) with 19% protein content or a group fed a 5% low-protein chow (LP) throughout the pregnancy and lactation period (21 days after birth). At weaning, one male pup per litter was randomly assigned to form the groups, which were maintained until the age of four months. The offspring were divided based on the diet they were fed before and after weaning. For the offspring, one group continued on a SC diet (SC-SC or LP-SC) and the other group was switched to an FO-enriched diet (SC-FO or LP-FO). The mineral and vitamin contents in the experimental diets were identical to all groups, in accordance with the AIN-93G recommendation (89). From 3-mo of life until euthanasia, offspring received the maintenance diet (AIN-93M) (Table 1).

Table 1 – Composition of experimental diets, low-protein chow (LP), standard chow (SC) and fish oil-enriched standard chow (FO), prepared according to the AIN-93 (the first number refers to the growth diet, AIN-93G, and the number in parentheses refers to the maintenance diet, AIN-93M, when its composition differed from the growth diet).

Ingredients	**Diet (g/Kg)**		
	LP	**SC**	**FO**
Casein	50.0	200.0	200.0
L-Cystine	1.5	3.0 (1.8)	3.0 (1.8)
Cornstarch	680	530 (620)	530 (620)
Sucrose	100.0	100.0	100.0
Soybean oil	70.0	70.0 (40.0)	7.0 (4.0)
Fish oil	-	-	63.0 (36.0)
Fiber	50.0	50.0	50.0
Vitamin	10.0	10.0	10.0
Mineral mixture	35.0	35.0	35.0
Choline	2.5	2.5	2.5
Antioxidant	0.014	0.014	0.014
Total (g)	1,000.	1,000.0	1,000.0
Energy (kJ/g)	16.6	16.6 (15.9)	16.6 (15.9)
Carbohydrate	78	64 (76)	64 (76)
Protein (%)	5	19 (14)	19 (14)
Lipid (%)	17	17 (10)	17 (10)

Blood pressure

Systolic blood pressure (BP) was measured every week in 3- to 4-mo-old conscious mice through the non-invasive method of tail-cuff plethysmography (Letica LE5100, Panlab, Barcelona, Spain). Animals went through a 2-week period of adaptation before the beginning of the measurement of BP.

Blood and tissue sampling

At 3 mo of age, an oral glucose tolerance test was performed with 25% glucose in sterile saline (0.9% NaCl) at a dose of 1 g/kg and was administered by orogastric gavage after a 6h fasting period. The plasma glucose concentration was measured prior to glucose administration at 0, 15, 30, 60 and 120 minutes after glucose. On the day before euthanasia, animals were food-deprived for 6 hours, then anesthetized (intraperitoneal sodium pentobarbital), and blood samples were obtained by cardiac puncture for further analyses. Fat deposits (retroperitoneal and epididymal fat masses) were carefully dissected from both sides of the animal and measured.

Biochemical analyses and serum hormone concentrations

Serum was obtained by centrifugation (120 g/15 min) at room temperature. Total cholesterol (TC), triacylglycerides (TG) and alanine aminotransferase (ALT) were measured by a kinetic-colorimetric method according to the manufacturer's instructions (Bioclin System II, Quibasa, Belo Horizonte, MG, Brazil). Fasting glucose concentrations were determined at euthanasia read by a glucometer (Accu-chek, Roche Diagnostic, Germany). The fasting insulin concentration was measured by a radioimmunoassay (Cat. RI-13K for insulin, intra-assay coefficient of variation was 1.4 %). The homeostasis model assessment for insulin resistance index was calculated as (fasting glucose x fasting insulin)/22.5 (90)). Mice serum analysis of

TNF-alpha was performed using commercially available Elisa kits (Human/Mouse TNF-alpha ELISA Ready-SET-go, San Diego, CA, USA).

Adipocyte morphometry

Adipose tissue was fixed (freshly prepared 1.27 mol/L formaldehyde, 0.1 M phosphate-buffered, pH 7.2), embedded in Paraplast plus (Sigma-Aldrich Co., St. Louis, MO, USA), sectioned (5 µm of thickness) and stained with hematoxylin-eosin. The sectional area of the adipocytes was measured on digital images acquired at random (50 adipocytes/animal, TIFF format, 36-bit color, 1280x1024 pixels) with the LC Evolution camera and the Olympus BX51 microscope, and analyzed with Image-Pro Plus version 7.0 software (Media Cybernetics, Silver Spring, MD, USA) (91).

Liver stereology

The liver was sliced into several minor fragments; some pieces were kept for 48 h at room temperature in the fixative (the same fixative used previously). Random liver fragments were embedded in Paraplast plus (Sigma-Aldrich Co., St. Louis, MO, USA), sectioned to 5µm, and then stained with hematoxilin-eosin. Several slices were cut per fragment and ten microscopic fields per animal were analyzed at random (blind analysis) using a video-microscopic system and a test-system composed of 36 test-points (P_T) (92). Briefly, the volume density (Vv) was estimated by point counting fat droplets on hepatic tissue (steatosis): Vv[steatosis,liver]: = P_P[steatosis]/ P_T[liver], (P_P is the number of points that hit the structure and P_T is the total test-points) (93, 94).

Western Blotting analysis

Liver tissue not used in microscopy had been frozen and homogenized in lysis buffer and supplemented with a protease inhibitor cocktail. The tissue homogenates were centrifuged at 3,000 g for 20 min twice at 4°C, and the supernatants were used for Western blotting analysis. Briefly, protein concentrations were determined using

the BCA Protein Assay kit (Thermo Scientific). Ten micrograms of protein extract were loaded onto a 10% polyacrylamide gel (Amersham Bio Science) and separated proteins were transferred to a nitrocellulose membrane (Amersham Hybond-P). The membranes were blocked for 1 h, 30 min at room temperature with 5% non-fat dry milk in Tris-buffered saline (TBS) (Amershan Bio Science) containing 0.05% Tween-20 (Bio Rad). Then, the membranes were incubated overnight at 4°C with anti- sterol regulatory element binding protein (SREBP-1c) or anti-PPAR-alpha at 1:1000 dilutions (Rabbit, Polyclonal-Santa Cruz, Biotechnology). After incubation, the membranes were washed with TBS containing 0.05% Tween-20 and then incubated with horseradish peroxidase-conjugated secondary antibodies (at 1:8000 dilution for SREBP-1c and 1:5000 dilution for PPAR-alpha). Visualization was performed using the ECL kit according to the manufacturer's protocol (GE Healthcare Bio-Sciences). SREBP-1c and PPAR-alpha protein bands were visualized on Amershan Hyperfilm, scanned and quantified by image analysis.

Data Analysis

Data are expressed as mean and standard error of the mean (SEM). All data were analyzed using two-way ANOVA as appropriated, to consider effects of maternal LP and post-weaning FO. One-way ANOVA, when used, was followed by post-hoc Tukey test. Differences in the same group at different times were tested with a paired *t*-test (Statistica version 8.0, Statsoft, Tulsa, OK, USA). A *P*-value of ≤0.05 was considered statistically significant.

RESULTS

Biometry

Maternal LP yielded low birth weight in LP offspring compared to SC offspring (SC=1.86±0.05 g *versus* LP=1.54±0.03 g; less 17%, $p<0.0001$). This difference was maintained at 7-days old (SC=5.31±0.12 g *versus* LP=4.22±0.12 g; less 21%, $p<0.0003$), at 14-days old (SC=9.28±0.11 g *versus* LP=8.49±0.23 g; less 9%, $p<0.0001$) and at 21-days old (SC=11.89±0.14 g *versus* LP=10.12±0.17 g; less 15%, $p<0.001$).

A significant attenuation of BM gain (less 19%, $p<0.01$) in association with a reduction in fat mass, was observed in LP-FO offspring, not in LP-SC offspring (Table 2). However, no significant difference was observed in the naso-anal length. We did not discover any significant difference in FE (body mass gain in grams/kilojoules of food consumed per animal) among the groups during the analysis.

FO offspring had significantly reduced fat pads. The retroperitoneal fat pads ($p<0.008$) and epididymal fat pads ($p<0.0005$) of the LP-FO offspring were 60% smaller than the pads of the LP-SC group. The epididymal fat pads of the SC-FO group were 62% smaller than the pads of the SC-SC group ($p<0.006$). The retroperitoneal fat mass and epididymal fat mass/body mass ratios were also attenuated in LP-FO offspring than the other groups.

The LP-SC offspring had the highest BP levels. Compared to NP-SC offspring, LP-SC offspring had 10% higher BP levels at the third week ($p<0.05$) and 23% higher levels at the fourth week ($p<0.05$). The FO intake was able to lower BP values in LP-FO offspring in comparison to the LP-SC offspring by the second week (a 10% decrease, $p<0.01$), by the third week it was reduced by 14% ($p<0.01$) and by the fourth week it was reduced by 20% ($p<0.0002$). In addition, there was a statistical difference in BP levels between NP-FO and NP-SC offspring at the fourth week. Moreover, there was interaction between maternal LP and post-weaning FO, showing that FO controlled BP levels ($p<0.001$, two-way ANOVA) (Fig. 1).

Table 2 – Biometry of groups studied. Legend: SC (standard chow), LP (low-protein chow), FO (fish oil-enriched standard chow), BM (body mass), NAL (naso-anal length), RFM (retroperitoneal fat mass), EFM (epididymal fat mass), FE (feed efficiency), LM (liver mass). Data are expressed as mean±standard error of the mean (SEM). NS: no significance.

Groups	**Parameters**							
	Final BM (g)	NAL (cm)	RFM (g)	EFM (g)	RFM/BM	EFM/BM	FE (g/kJ)	LM (g)
SC-SC	45.55±1.40	10.27±0.03	0.72±0.09	2.29±0.15	0.014±0.002	0.05±0.003	6.1±2.1	1.48±0.09
SC-FO	39.35±1.42	10.33±0.03	0.27±0.08	0.86±0.25	0.006±0.001	0.03±0.003	6.6±2.6	1.19±0.05
LP-SC	44.38±1.56	10.28±0.01	1.39±0.22	3.24±0.24	0.028±0.004	0.06±0.004	5.6±2.0	1.61±0.05
LP-FO	35.86±2.45	10.28±0.05	0.56±0.15	1.36±0.26	0.013±0.002	0.03±0.002	5.3±3.1	1.47±0.09
ANOVA and Tukey test								
SC-SC *vs*. SC-FO	NS	NS	NS	0.006	NS	0.006	NS	NS
SC-SC *vs*. LP-SC	NS	NS	NS	NS	NS	0.01	NS	NS
SC-FO *vs*. LP-FO	NS	NS	NS	NS	NS	NS	NS	NS
LP-SC *vs*. LP-FO	0.01	NS	0.008	0.0005	0.01	0.0001	NS	NS

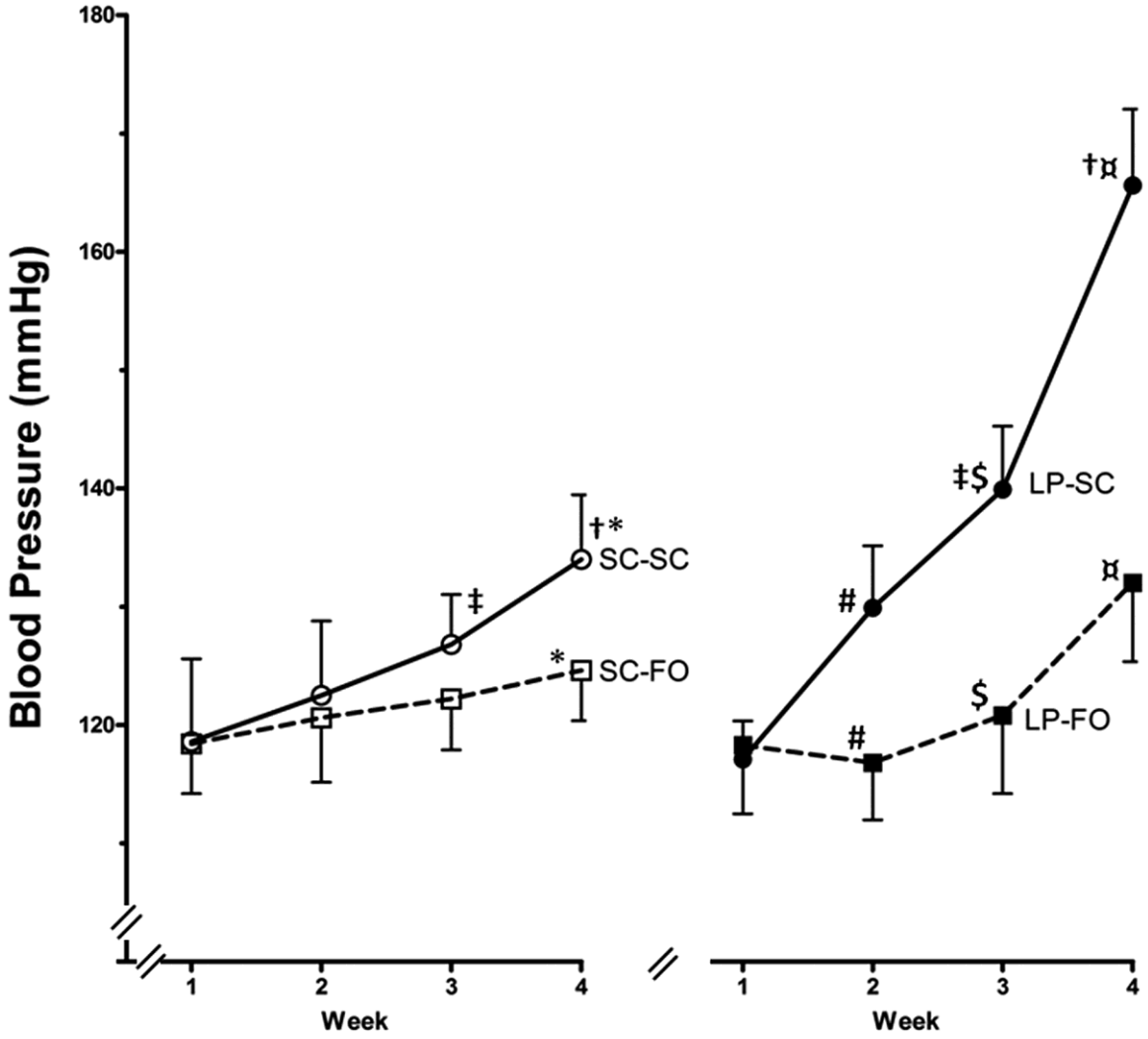

Figure 1 - Systolic blood pressure evolution throughout the experiment. (○) SC-SC; (□) LP-SC; (●) SC-FO; (■) LP-FO. Values are means with their standard error shown by vertical bars for n=5 mice per group. Mean values with superscript symbols were significantly different.

Biochemical data

The biochemical data are shown in Table 3. The total cholesterol (TC) concentration was 24% higher in LP-SC compared to SC-SC offspring (p<0.0003). FO lowered these values in LP-FO mice in comparison to LP-SC offspring (reduced by 31%, p<0.0001). Interaction in TC was observed between maternal LP and post-weaning FO (p<0.0001, two-way ANOVA), emphasizing that FO had a beneficial effect on TC levels. Both SC-FO and LP-FO offspring had a significant decrease in plasma TG levels compared to their counterparts, SC-SC *versus* SC-FO (minus 21%, p<0.005) and LP-SC *versus* LP-FO (minus 16%, p<0.04).

Insulin plasma levels were within the normal range, but in SC-FO offspring, this value was reduced in comparison to SC-SC offspring (minus 44%, p<0.01). Moreover, the insulin plasma levels were 40% lower in LP-FO offspring versus LP-SC offspring (p<0.01).

In LP-SC offspring, there was a significant increase in the plasma glucose level. The LP-SC group had a 20% higher plasma glucose level than SC-SC offspring (p<0.01). The plasma glucose level was lower in LP-FO than LP-SC offspring (minus 34%, p<0.0001). Interaction was observed between maternal LP and post-weaning FO (p<0.01, two-way ANOVA), emphasizing that FO had a beneficial effect on glycemia. The area under the curve (AUC) of OGTT analysis confirmed reduction of this parameter in LP-FO mice compared to LP-SC offspring (minus 37%, p<0.003). In contrast, the AUC of OGTT was 38% larger in LP-SC offspring than SC-SC offspring (p<0.02). These results corroborated with HOMA-IR index analysis and demonstrated that FO has beneficial effects on glucose intolerance. The HOMA-IR index was 54% higher in LP-SC than SC-SC offspring (p<0.004). FO also lowered this parameter in LP-FO compared to LP-SC offspring (minus 35%, p<0.004) and in the SC-FO group compared to SC-SC offspring (minus 48%, p<0.009). Nevertheless, LP-FO animals showed increased values of plasma glucose compared to SC-FO offspring (plus 94%, p<0.003).

Finally, the TNF-alpha level was higher in LP-SC mice compared to SC-SC offspring (plus 35%, $p<0.02$) and FO significantly lowered the TNF-alpha level as shown by a comparison of LP-FO offspring to LP-SC offspring (minus 39%, $p<0.003$). Maternal LP and post-weaning FO had an additive effect in increasing TNF-alpha levels and an interaction existed between these parameters ($p<0.01$, two-way ANOVA).

LP-SC had higher values of ALT than SC-SC offspring (plus 66%, $p<0.01$). Conversely, LP-FO showed lower values of ALT in comparison with LP-SC offspring (minus 37%, $p<0.01$).

Table 3 – Biochemical parameters of groups studied. Legend: SC (Standard chow), LP (low-protein chow), FO (fish oil-enriched standard chow), TC (total cholesterol), TG (triglycerides), HOMA (homeostasis model assessment for insulin resistance), AUC (area under the curve), OGTT (oral glucose tolerance test), TNF (tumor necrosis factor), ALT (alanine aminotransferase). Data are expressed as mean±standard error of the mean (SEM). NS: no significance. Two-way ANOVA disclosed significant interactions between maternal LP and post-weaning FO (p<0.0001 for TC; p<0.01 for glucose; p<0.01 for TNF- alpha).

Groups	**Parameters**							
	TC (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	Insulin (µU/mL)	HOMA-IR	AUC OGTT (arbitrary units)	TNF-alpha (µU/mL)	ALT (mg/dL)
SC-SC	120.4±2.1	51.2±2.0	173.8±4.0	9.6±0.5	67.4±4.3	24,339.0±1,329.0	65.2±6.7	23.6±3.1
SC-FO	111.4±3.2	40.6±2.4	153.0±8.5	5.4±0.5	35.0±3.9	20,607.0±1,182.0	66.8±3.2	19.5±4.4
LP-SC	149.6±1.7	47.2±1.3	207.6±9.8	11.2±1.3	103.7±5.8	33,521.0±3,072.0	88.1±3.3	39.2±2.4
LP-FO	103.0±6.0	39.6±1.5	137.0±3.8	9.5±0.6	67.9±7.5	21,207.0±2,134.0	54.2±3.6	24.6±2.6
	ANOVA and Tukey test							
SC-SC *vs*. SC-FO	NS	0.005	NS	0.01	0.009	NS	NS	NS
SC-SC *vs*. LP-SC	0.0003	NS	0.01	NS	0.004	0.02	0.02	0.01
SC-FO *vs*. LP-FO	NS	NS	NS	0.01	0.003	NS	NS	NS
LP-SC *vs*. LP-FO	0.0001	0.04	0.0001	NS	0.004	0.003	0.003	0.01

Quantitative microscopic analyses

In the LP-SC group, adipocyte hypertrophy was evident; it was 17% higher than in SC-SC offspring (p<0.005). FO significantly lowered adipocyte size by 20% in LP-FO mice compared to LP-SC offspring (p<0.001). This result was also observed in SC-FO mice compared to SC-SC offspring (p<0.002) (Fig. 2).

There were no significant differences in liver mass among the groups (Table 2). However, large accumulation of lipid content in LP-SC offspring was observed. LP-SC offspring showed greater hepatic steatosis than SC-SC offspring (more 80%, p<0.02). LP-FO mice had reduced steatosis in comparison to LP-SC offspring (less 50%, p<0.01). We observed lipid vacuoles of various sizes in hepatocytes of the LP-SC offspring (Fig. 3).

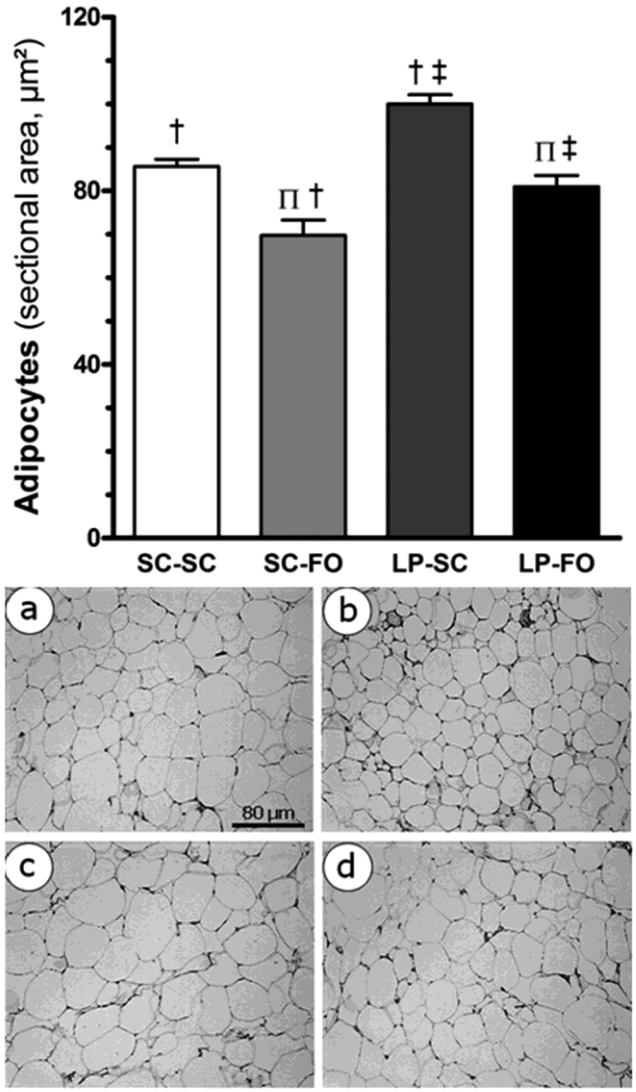

Figure 2– Mean sectional area of adipocytes. Values are means with their standard error shown by vertical bars for n=5 mice per group. Mean values with superscript symbols were significantly different. Photomicrographs of the adipose tissue (Hematoxylin-Eosin, same magnification): (**a**) SC-SC and (**b**) SC-FO offspring, with normal adipocytes; (**c**) LP-SC offspring with adipocyte hypertrophy, and (**d**) LP-FO offspring, with intermediary adipocyte size.

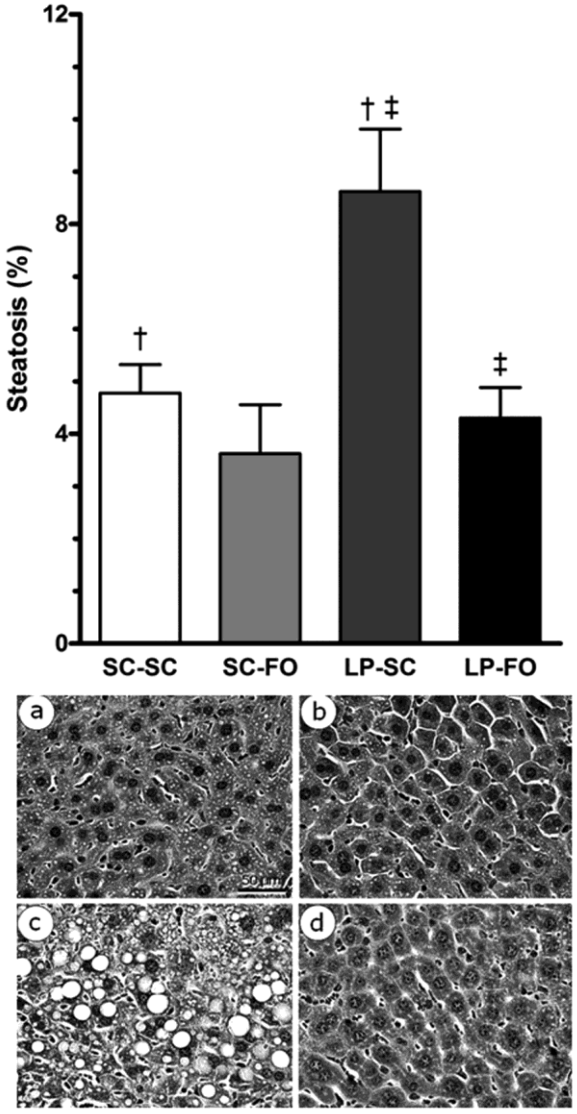

Figure 3 – Volume density of hepatic steatosis (Vv[st]). Values are means with their standard error shown by vertical bars for n=5 mice per group. Mean values with superscript symbols were significantly different. Photomicrographs of the liver (hematoxylin and eosin stain). The normal appearance in SC-SC and SC-FO offspring are shown in (**a)** and **(b)**, respectively; macro and microvesicular steatosis in the LP-SC offspring in (**c**); and improved steatosis in LP-FO offspring in (**d**).

Western Blotting

The hepatic expression of SREBP-1c protein increased in the LP-SC offspring in comparison to SC-SC (p<0.0001). In contrast, in LP-FO offspring, the expression of SREBP-1c was approximately 50% lower than in LP-SC offspring (p<0.0001) (Fig. 4). The hepatic expression of PPAR-alpha protein was more than 60% higher in LP-FO than in LP-SC offspring (p<0.001). Indeed, LP-FO offspring showed increased values of hepatic expression of PPAR-alpha protein than SC-FO offspring (plus 100%, p<0.001) (Fig. 5).

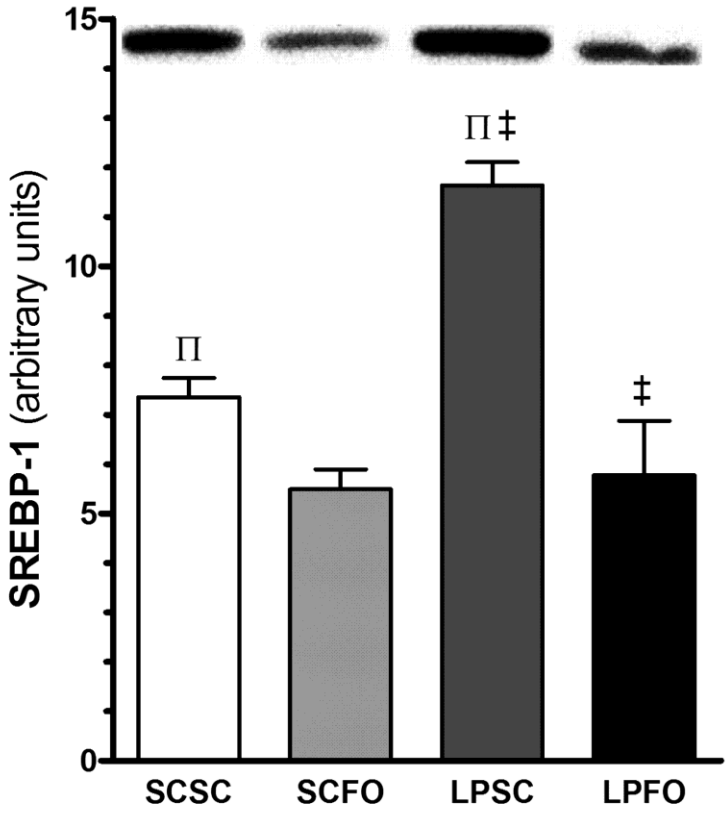

Figure 4 – Hepatic expression of sterol response element binding protein (SREBP-1c). In the bottom, representative Western blots with bands corresponding to groups in the order SC-SC, SC-FO, LP-SC, and LP-FO. Values are means with their standard error shown by vertical bars for n=5 mice per group. Mean values with superscript symbols were significantly different.

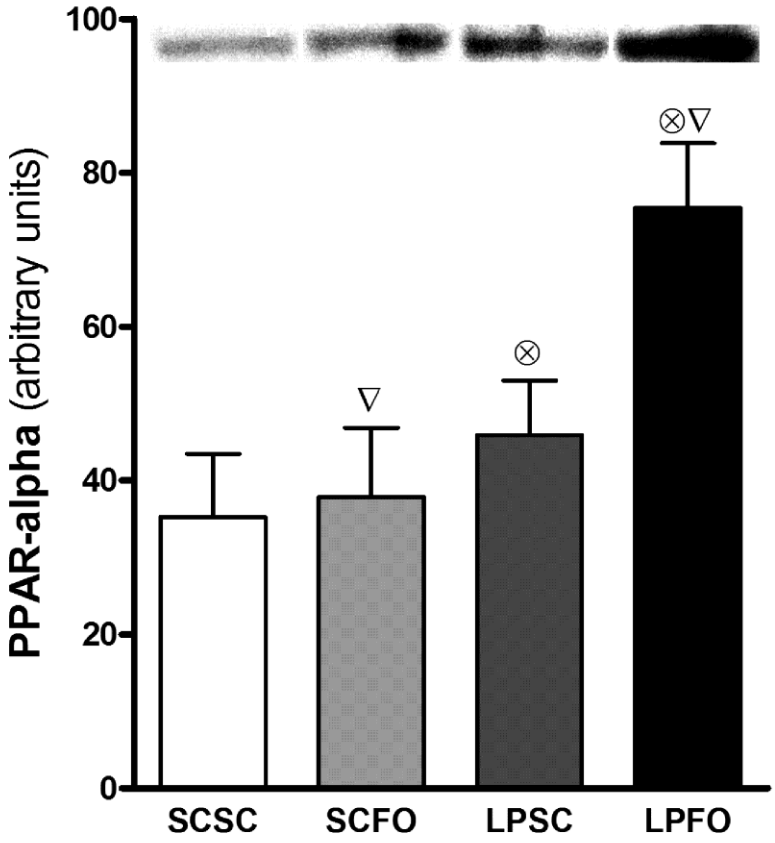

Figure 5 – Hepatic expression of peroxisome proliferator-activated receptor (PPAR)-alpha. Representative Western blots with bands corresponding to groups in the order SC-SC, SC-FO, LP-SC, and LP-FO. In the bottom, values are means with their standard error shown by vertical bars for n=5 mice per group. Mean values with superscript symbols were significantly different.

DISCUSSION

The effect of early FO administration was evaluated in adult mice, which were subjected to maternal protein restriction during gestation and lactation. Protein restriction during development results in low birth weight and insults to several organs as documented in previous studies from our group (26, 51). The present study indicated that LP offspring are predisposed to becoming obese, hypercholesterolemic, hyperglycemic, and hypertensive in adulthood. The discovery that hepatic steatosis developed in the mice was an important finding in this study. Alternatively, LP offspring fed FO had reduced final body mass, improved lipid metabolism (reduction in plasma TC and TG levels), reduced fat pad masses and adipocyte size, reduced liver steatosis, and reduced BP and TNF-alpha levels.

Present findings showed a significant increase in the size of epididymal and retroperitoneal fat pads occurred in LP mice offspring. Restriction of pregnant rats to 30% of *ad libitum* intakes produced gross adiposity in their mature offspring (95), which resulted in the increased expression of genes encoding a number of lipogenic enzymes, suggesting that enhanced lipogenesis is one of the factors that lead to visceral adiposity in this animal model (46). Importantly, we observed that LP offspring that were fed FO just after weaning had lower fat body deposition. The FO was effective in reducing the proportion of fat deposits, although the animals had proportionally eaten the same amount of food as the other groups.

Dietary n-3 PUFA has been found to induce the expression of mitochondrial uncoupling protein-2 and -3 in skeletal muscles and increases the less efficient peroxisomal FA oxidation pathway in liver and skeletal muscle (69). Dietary n-3 PUFA induces the genes of the FA oxidation pathway and also reduces body fat deposition in animals and humans. This reduction in body fat occurs by inducing the expression of genes involved in thermogenesis; therefore, total body heat production increases (69, 96). These effects on thermogenesis suggest that dietary FO may cause the reduction of body mass and fat deposition found in LP-FO offspring.

As supported by previous work, plasma lipids varied, but animals that received LP during gestation and/or lactation showed altered lipid metabolism as shown by elevated plasma TC and TG levels. These changes in LP offspring may be a consequence of the altered expression of key genes involved in the regulation of fat metabolism and insulin signaling, and the features emerged with aging (44). LP-FO offspring showed significantly reduced plasma TC and TG levels. Furthermore, ingestion of enriched FO has been associated with increased beta-oxidation and down-regulation of genes encoding for lipogenic enzymes (13). LP-SC offspring had high glycemia, an impaired oral glucose tolerance test and high HOMA-IR index, and borderline high values of insulinemia, indicating that insulin action on peripheral target tissues resulted in insulin resistance. These data agree with recent literature that has shown glucose impairment and insulin resistance in animals subjected to LP during the gestation/lactation periods (97). In contrast, LP-FO offspring showed a reduction in all these parameters. In addition, in response to n-3 PUFA ingestion, fat oxidation increases in skeletal muscle, which is associated with a reduction in TG droplets and an improvement in glucose uptake and glycogen storage in other animals in addition to human (69, 98). The hypolipidemic effect of FO decreases the availability of lipid fuel within the skeletal muscle and may, in turn, restore glucose oxidation and help normalize insulin resistance (12).

TNF-alpha appears to play a role in metabolic syndrome development. The increased expression of TNF-alpha has been associated with insulin resistance, obesity, hypertriglyceridemia, and glucose intolerance (86, 99), suggesting that TNF-alpha interferes with insulin action by altering the catalytic activity of the insulin receptor. In the present study, TNF-alpha plasma levels increased in LP-SC offspring, but LP-FO offspring showed a significant decrease in TNF-alpha plasma levels. In addition, this decrease in TNF-alpha plasma levels was accompanied by a decrease in plasma lipids, glucose levels and body adiposity.

BP was significantly augmented in LP-SC animals. Evidence links insulin resistance and hyperinsulinemia to the development of hypertension. Furthermore, creating a state of insulin resistance in experimental animals led to elevate BP (50). In addition, hypertension is also programmed by maternal protein restriction

(52, 100). Maternal LP is associated with reduced nephron number at birth through impaired renal development (51, 52), which predisposes the development of hypertension later in life (101). Previous studies from our laboratory have shown that FO reduces BP in experimental animals (16, 60, 61). The anti-hypertensive effect of FO has been attributed to altered biosynthesis of eicosanoids (102), suppression of the synthesis and release of TNF-alpha (103), reduction of plasma TG and viscosity (104), and a decrease intracellular sodium concentrations (105). In the present study, as mentioned, LP-FO offspring showed significantly reduced plasma TNF-alpha, TC, TG, insulin and glucose levels; therefore, this reduction may, in part, be responsible for the lowering effect of FO on BP.

Adipose tissue metabolism correlates with the size of the adipocytes in both rodents and humans (106). LP-SC offspring had bigger adipocytes than the other groups. In contrast, FO administered to LP offspring was able to limit adipocyte hypertrophy. It is well know that n-3 PUFA activate PPARs and that the expression of the adipocyte PPAR-gama isoform controls the expression of genes involved in adipogenesis as well as lipid and glucose metabolism (107).

Fatty liver, caused by chronic hepatocyte accumulation of lipids, can ultimately lead to inflammation and scarring, with the potential to progress to cirrhosis and liver failure (108). It is well documented that fatty liver is related to the high expression of SREBP-1c, which is elevated in response to hyperinsulinemia (109). Moreover, insulin resistance is a strong driver of fat accumulation in the liver (110). Interestingly, FO intake was accompanied by a significant decrease of hepatic lipid accumulation in LP-FO offspring, which is in agreement with previous findings (87). Additionally, a FO enriched diet was capable of reducing SREBP-1c protein expression in offspring submitted to protein restriction. Alternatively, PPAR-alpha protein expression was higher in LP-FO offspring. Insulin and glucose stimulate lipogenesis by activating the transcription factors, sterol regulatory element binding protein (SREBP)-1c and carbohydrate response element binding protein (ChREBP), respectively (111, 112). N-3 PUFA inhibits both the expression and nuclear translocation of SREBP-1c and ChREBP, which suppresses lipogenesis (113, 114). The beneficial effects of FO ingestion during hepatic steatosis may be attributed to the suppression of lipid synthesis in the liver and the up regulation of

FA oxidation thought PPAR-alpha activation (115, 116). Fish oil, which contains docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), is traditionally used as a functional food against metabolic diseases. These beneficial health effects of DHA and EPA are thought to arise from their binding and activating of PPARs (117, 118). This idea can explain the mechanism involved in the reduction of hepatic steatosis in animals fed FO.

In conclusion, the present findings show compelling evidence that early FO intake by offspring from protein-restricted mothers can revert the negative responses on BP, body adiposity, glucose and lipid metabolisms, and liver structure by adulthood.

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4 **CONCLUSÃO E PERSPESCTIVAS**

O óleo de peixe possui ações biológicas benéficas sobre os efeitos adversos induzidos pela restrição proteica materna durante a gestação e a lactação. Constatou-se melhora do metabolismo de carboidratos, com diminuição dos níveis glicêmicos, bem como da insulinemia. O óleo de peixe também exerce efeitos sobre os depósitos de gordura corporal, com diminuição da massa adiposa e do tamanho dos adipócitos. Houve também preservação da arquitetura hepática, diminuição dos níveis de colesterol e triglicerídeos séricos e diminuição da expressão de proteínas lipogênicas concomitante ao aumento da beta-oxidação.

Um problema que afeta os países do Terceiro Mundo como o Brasil, é a desnutrição crônica. Apesar do quadro desfavorável, é preciso reconhecer que houve avanços no conhecimento dos efeitos da desnutrição intrauterina e lactação sobre a programação metabólica e suas repercussões na vida adulta. Este conhecimento se baseia em estudos epidemiológicos, mas, também em grande parte, em pesquisas experimentais. Por exemplo, o estudo do efeito da desnutrição intrauterina experimental, como observamos, é um dos modelos mais utilizados na avaliação das respostas de adaptação e posterior surgimento de doenças crônicas na prole.

A grande questão do trabalho era se o tratamento precoce com óleo de peixe em filhotes que sofreram restrição proteica durante a gestação poderia amenizar, ou mesmo evitar, o desenvolvimento de doenças crônicas na fase adulta. Nesta linha de pensamento, os óleos dietéticos podem ser entendidos como fatores a mais que podem vir a evitar o processo de programação metabólica. E conseguimos, pelo menos em parte, responder esta questão. A restrição proteica intrauterina provocou aumento da massa de gordura corporal e favoreceu o desenvolvimento de síndrome metabólica com esteatose hepática nesses animais, que foi significativamente atenuada pela suplementação precoce com óleo de peixe na dieta.

Sem dúvida alguma, uma nutrição adequada é fundamental para o crescimento e desenvolvimento normal durante os períodos pré e pós-natais. Tais achados experimentais sugerem que a suplementação alimentar com óleo de peixe, fonte

de EPA e DHA, pode ser útil num país como o nosso onde a obesidade e a síndrome metabólica atingem grande parte de nossa população.

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ANEXO – Resultado do Comitê de Ética

UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO
INSTITUTO DE BIOLOGIA ROBERTO ALCÂNTARA GOMES
COMISSÃO DE ÉTICA PARA O CUIDADO E USO DE ANIMAIS EXPERIMENTAIS

CERTIFICADO

Certificamos que o Protocolo nº **CEA/217/2008** sobre ***"Efeito da ingestão de dietas com gordura do leite de cabra e óleo de peixes do Pantanal em animais que sofreram restrição protéica neonatal"***, sob a responsabilidade de **Márcia Barbosa Águila,** está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética Para o Cuidado e Uso de Animais Experimentais do Instituto de Biologia Roberto Alcântara Gomes da UERJ (CEA), em **25/03/2008.** Este certificado expira em **25/03/2010.**

Rio de Janeiro, 25 de março de 2008.

Prof. Antonio Carlos da Silva
Coordenador – CEA/IBRAG/UERJ

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