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***Effect of protein malnutrition
during gestation and lactation
on the physiology and liver structure in
adult life.***

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INTRODUCTION

- ✓ **Pregnancy and fetal development are periods of rapid growth and cell differentiation when mother and offspring are vulnerable to changes.**
- ✓ **Adverse events during development can be linked to an increased risk for developing metabolic diseases.**
- ✓ **Disease Nonalcoholic fatty liver disease (NAFLD, nonalcoholic fatty liver disease) and nonalcoholic steatohepatitis (NASH, nonalcoholic steatohepatitis), is a growing global health problem. The development of steatosis, liver fibrosis and cirrhosis often progresses to hepatocellular carcinogenesis, resulting in an indication for liver transplantation.**

✓The malnutrition suffered by pregnant women induced high susceptibility of their offspring to suffer in adult life metabolic diseases (type II diabetes, hypertension, cardiac disorders, dyslipidemia, fibrous liver cirrhosis and cancer).

✓This relationship has not been conclusively clarified. It has been suggested the hypothesis of the importance of the uterine environment during fetal development, "IUGR" (intrauterine growth restriction English) as the essential condition of metabolic diseases in adulthood.

The aim of this work

Is study

The effects of maternal protein deprivation during gestation-lactation on the offspring liver function and morphology.

Materials and methods

Animals

Female Wistar rats, 3 months of age, were maintained with food and water ad libitum. Controlled conditions of temperature and light were used. The beginning of pregnancy was determined by the finding of sperm in the vaginal lavage.

Pregnant Wistar rats of three months of age who were fed a diet hypoproteic, containing 8% of proteins (M) or 20% control (C).

The male offspring of mothers M, after weaning, were fed diet 8% of protein (MM) or control diet (reversed group: MC). In addition, male offspring from mothers C were feed with Diet Control (CC).

At day 60 post-birth the rats were slaughtered, bled by cardiac puncture and the liver was dissected.

Body weights and liver were lower in the MM group regarding CC and MC.

Diets were formulated according to The Experimental Animal Nutrition Committee, AIN-93. The hypoproteic diet was isocaloric.

Experimental Groups:

PERIOD	Mother during Gestacion/Lactation	After weaning until 60 d
EXPERIMENTAL GROUP	TYPE OF DIET	
CONTROL (C-C)	Control	Control
ALWAYS MALNOURISHED (M-M)	hypoproteic	hypoproteic
REVERSED (M-C)	hypoproteic	Control
MALNOURISHED AFTER WEANING (C-M)	Control	hypoproteic

samples Collection

60-day-old male offspring were sacrificed, bled by cardiac puncture and the liver was dissected. The following parameters were recorded:

LIVER PARAMETERS	LIVER DAMAGE (serum)	OXIDATIVE STRESS (liver)
Glycogen	Glutámic oxalacétic transaminase (SGOT)	ROS content
Triglycerides	Glutámic piruvic transaminase (SGPT)	Protein carbonylation
Cholesterol	Total protein	Lipid peroxidation
Total protein	Cholesterol	Total antioxidant capacity
Histology	Triglycerides	
	Glycemia	

Results

Liver Determinations

The liver cholesterol and triglyceride content was significantly higher in malnourished (CM and MM) for control (CC). TG reversed in MC. Glycogen decreased in malnourished (CM and MM). But does not reverse in MC.

EXPERIMENTAL GROUP (mg/ g de liver)	Glycogen	Triglycerides	Cholesterol	Total protein
CC	19.5±0.50	0.97±0.19	1.42±0.07	220,04±22
MM	0.34±0.02 d*	3.05±0.46*	4.17±0.08*	102,86±9*
MC	9.57±1.43 *	0.9±0.18	3.84±0.23 *	292,53±8*
CM	6.29±3.18 d*	3.69±0.22 *	3.56±0.06 *	143,94±9 d*

Liver damage

Serum determinations showed significant differences showing that malnourished animals (MM and MC) Lower values of total protein, cholesterol and triglycerides and higher for GOT and GPT compared to the control group.

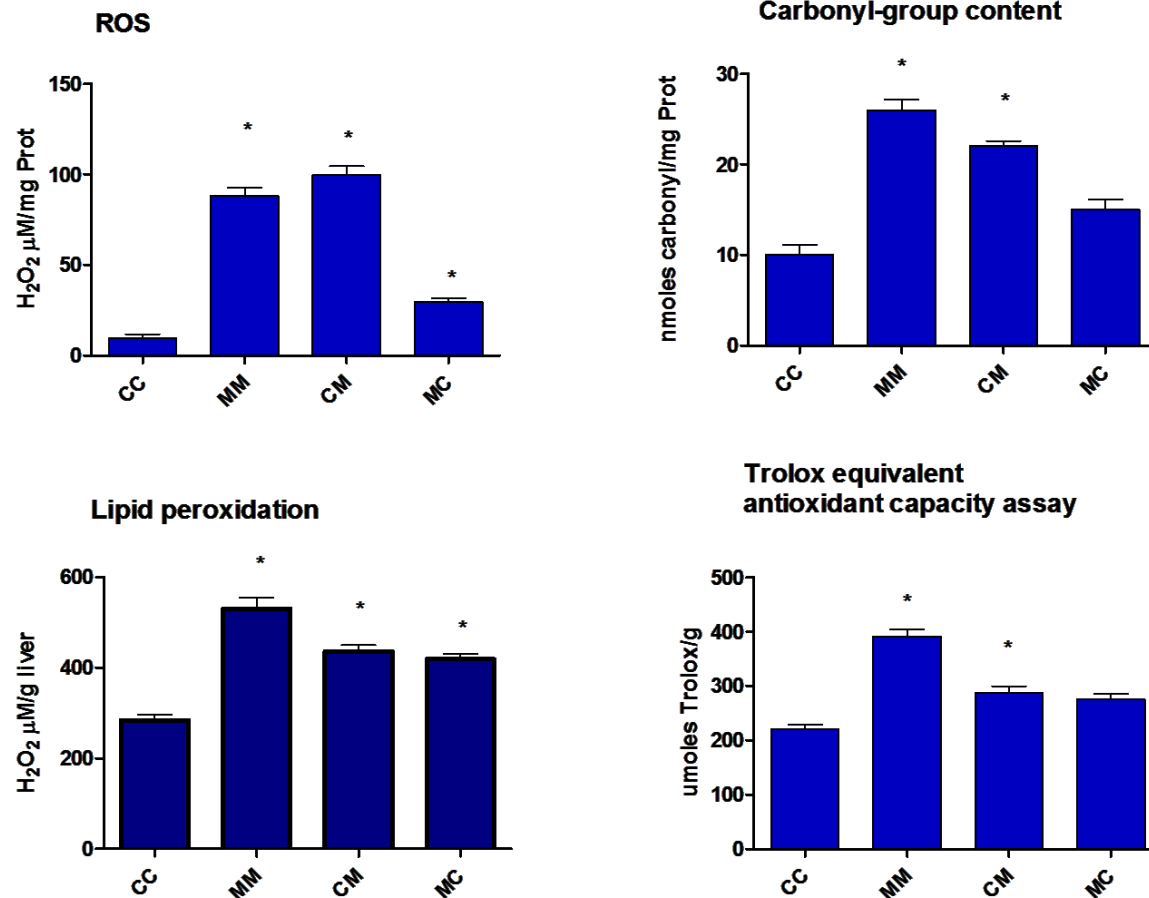
The reversed group (MC) showed increased levels of GPT and cholesterol decreased relative to control.

Groups	Cholesterol (mg/dl)	Total protein (g/dl)	Triglycerides (mg/dl)	SGOT (u/l)	SGPT (u/l)
CC	112,6±7	6,2±03	150,3±9	220±18	92±7
MM	58,3±4 *	4,7±03 *	73,6±5 *	391±25 *	217,3±11 *
MC	89±8 *	5,9±02	140,3±11	187±21	95±7
CM	61±4 *	4,5±03 *	82±6 *	275±22 *	202±13 *

Oxidative Stress

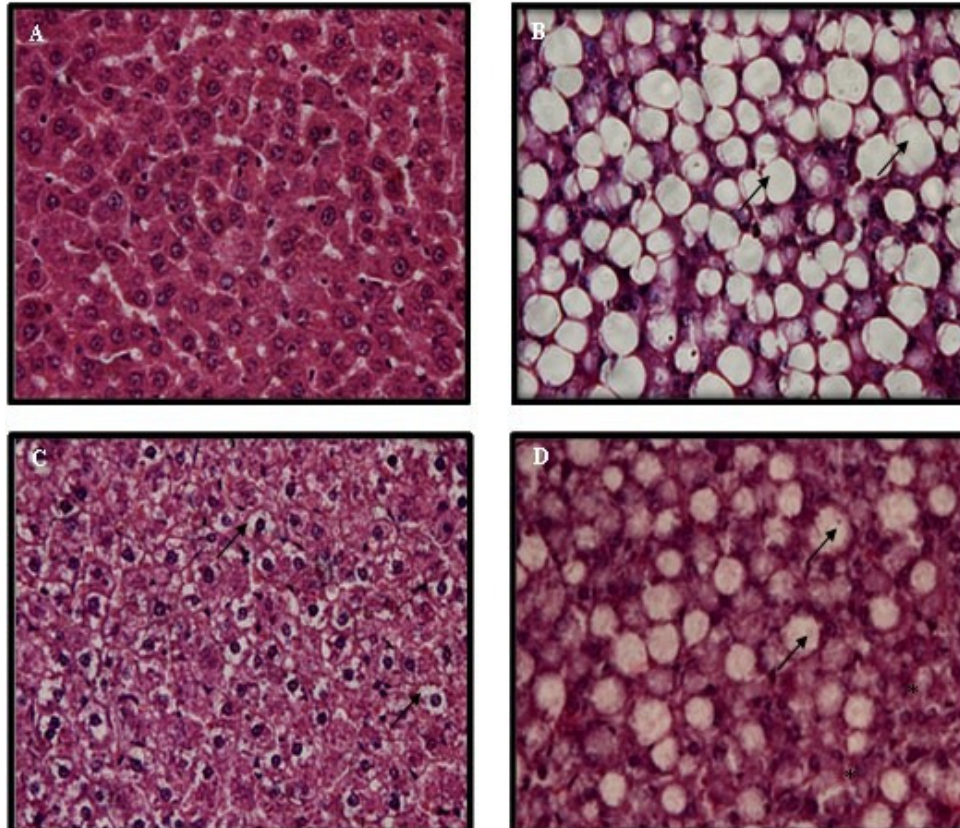
ROS content, total antioxidant capacity, protein carbonylation and lipid peroxidation in liver in malnourished rats (CM and MM) were higher than the control (CC).

The oxidation state correlates with liver damage.



The hepatic structure

Figure 1:

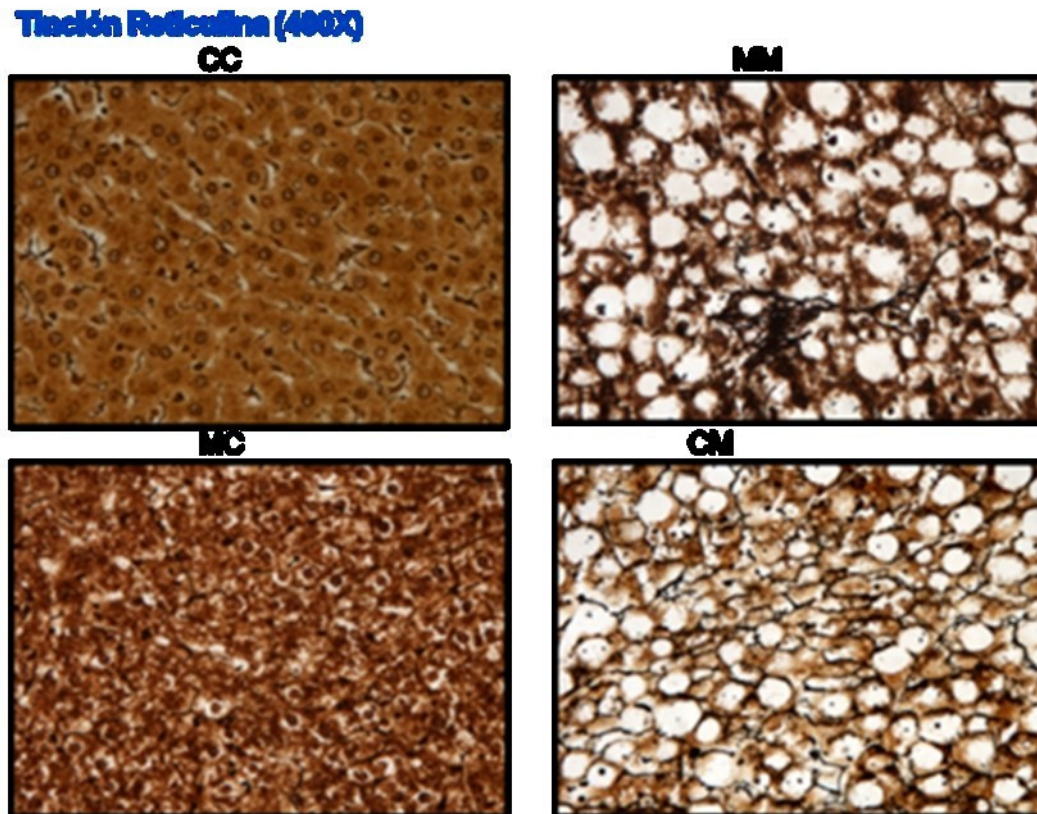


(H / E, 400 X) Staining

- CC, normal liver histology.
- MM, the hepatic architecture is completely altered; hepatocytes are displaced by large lipid vacuoles (arrowheads).
- MC, reversed animals, liver structure is partially preserved; the presence of cell swelling was detected (arrowheads).
- CM, a large number of lipid vacuoles (arrowheads) is observed, however you can see a large number of normal hepatocytes.

Integrity and distribution of reticular fibers

Figure 2



Reticulin Staining

CC, the plot of reticular fibers is observed and kept tidy.

MM network showed discontinuous fibers characterized by the presence of agglomerates thereof.

MC, the organization of reticular fibers resulted similar to that of controls with an array.

CM, the network of fibers is observed partially preserved and broken by the presence of broken fibers.

CONCLUSIONS

- ✓ **In physiological signaling, ROS can modify redox-sensitive amino acids in a variety of proteins, including phosphatases, ion channels, and transcription factors .**
- ✓ **The regulation of physiological responses by free radicals is embedded in these basic mechanisms of redox homeostasis.**
- ✓ **Oxidative stress responses provide some of the best-studied examples of redox-responsive signaling pathways.**

- ✓ **When ROS generation is within the limits of the buffering capacity of the cell, it helps in maintaining a proper intracellular redox environment.**
- ✓ **Excess generation of ROS may result in cell death or different pathological processes.**
- ✓ **Thus, a balancing act determines the role of ROS in the cell. In a life form it is a constant battle between pro- and anti-oxidants and 'healthy' survival depends on a tightrope walk.**

- ✓ **This work examines the evidence of the metabolic disease process involving the liver's oxidative stress induced by protein malnutrition in pregnant and lactating mothers, manifested in the adult lives of their offspring.**
- ✓ **Protein malnutrition in pregnant mothers induced liver changes in the oxidative status of their offspring with marked damaged liver function and morphology.**

In summary.

- ✓ **we found male rats showing high liver injury, high hydrogen peroxide production and less effective antioxidant machinery.**
- ✓ **Malnourished male offsprings have an increased risk of organ dysfunction**
- ✓ **High susceptibility to suffer metabolic diseases, obesity, diabetes, heart damage, among others, that are related to diet in early life and that manifest in adulthood.**
- ✓ **While the link between impaired fetal growth and the risk for developing chronic disease in adulthood is undoubtedly strong, the underlying mechanisms are still being elucidated.**

- ✓ **These results contribute support to the hypothesis of "fetal programming-IUGR."**
- ✓ **Finding relevant results that help explain that malnutrition during development induce a vulnerability to developing metabolic diseases in adulthood, like, NASH and NAFLD, closely related to insulin resistance, type 2 diabetes, cirrhosis and hepatocellular carcinoma.**

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