REVISTA ESPAÑOLA DE FISIOLOGIA, 50 (4), 229-238, 1994

Effect of Enriched Oil Diets on Some Cardiovascular Risk Factors in Rat's Recovery from Early Undernutrition

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(Received on July 27, 1994)

J. M. GUTIÉRREZ, A. FERNÁNDEZ, A. FUEYO, C. GONZÁLEZ, S. FERNÁNDEZ and A MENÉNDEZ-PATTERSON. *Effect of Enriched Oil Diets on Some Cardiovascular Risk Factors in Rat's Recovery from Early Undernutrition.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (4), 229-238, 1994.

This study evaluates the role of soybean and olive oil in the recovery and evolution of some cardiovascular risk factors in rats undernourished *in uterus* and during lactation. Beginning at 20 days of age, for a period of 130 days, control (C) and malnourished (M) animals were fed three different diets: standard diet (C, M), standard diet enriched with soybean oil (CS, MS) and standard diet enriched with olive oil (CO, MO). Body weight, systolic and diastolic blood pressure, total cholesterol, HDL-cholesterol and triglycerides levels of pups were measured at periods of 15, 30, 90 and 150 days of age. Malnutrition produced a decreased body weight and experimental diets were ineffective in body weight recovery. The controls, however, displayed overweight. Blood pressure values, taken at different ages and under different diets were within normal limits. Plasma triglycerides increased up to 30 days of age with early undernutrition returning, thereafter, to normal limits. Plasma cholesterol level decreased in all animals with age, except in the CS and CO groups, which showed a significant increase in this parameter at 5 months.

Key words: Early malnutrition, Cardiovascular risk factors, Enriched oil diets, Rat.

In recent years a number of investigations in both humans and animals have shown that malnutrition in early life may have profound effects on later, anatomical, biochemical, physiological and behavioral development (7, 8, 17, 24).

There is considerable evidence that early malnutrition significantly affects fat metabolism as a contributing factor of growth (3). Thus, low protein malnutrition in early development of the rat is

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associated with important deficiencies and alterations in the metabolism of essential fatty acids (EFAs) (14). In rats EFAs are essential for normal growth and development of the offspring (4, 6).

Inadequate intake of protein and energy during pregnancy and lactation in rats (13), adult dogs (I) and humans (37) produced a loss of cardiac muscle mass that was proportional to total body mass lost and induced changes in the cardiac function.

It has been previously commonly accepted that dietary saturated fatty acids (SFA) increase and polyunsaturated fatty acids (PUFA) decrease serum total cholesterol concentration (16). The results of studies on unsaturated fatty acids on serum lipoproteins in humans and experimental animals are controversial (19, 22, 35).

In spite of the extensive research in the last decades, the effects of dietary fats on blood pressure are uncertain. In some studies, an increased intake of PUFA has been found to be associated with a lowering of blood pressure (15), but in others no effects of PUFA on blood pressure could be demonstrated (31).

This study was designed to test the effect of rehabilitation in malnourished rats, with a malnutrition model developed in previous studies (8, 23), with a standard diet, varying the source of dietary lipids. The objetive in using different vegetable oils was: 1) to provide a high energetic diet; 2) to analyze the effects of these different diets on some cardiovascular risk factors.

Materials and Methods

Wistar rats from the Functional Biology Department (Physiology) of the University of Oviedo were used. The animals were kept under standard conditions of lighting and darkness (12 h each), temperature (23 ± 3 °C) and absolute humidity (65 ± 1 %). The animals had free access to water and were fed a nonpurified standard diet (Panlab S. L., Barcelona, Spain). The general composition of the diet was as follows: water, 12 %; protein, 18 %; fat, 3 %; fibre, 4.3 %; starch, 45 %; total sugar, 3.5 %; ashes, 8 %; phosphorus, 0.65 %; NaCl, 0.6 %; calcium, 1.28 %; vitamin A, 15,000 IU/kg; and cholecalciferol, 2,000 IU/kg; energetic value, 2,900 Cal/kg.

A total of 85 virgin females, aged 67-77 days, with a body weight of 230-240 g were placed in groups of three in separate cages with one male per cage. Copulation was verified by daily vaginal smears (10.00 A. M.) for the presence of sperm (day 0 = day of copulation). Pregnant females were randomly separated into two groups: one control (30 animals) and one malnourished (42 animals). During their pregnancy the latter were fed 14 g of diet daily while the former had free access to food. The diet of experimental females during the lactation period was 21 g per day.

The evolution of mothers (control and malnourished) as the model of malnutrition applied during gestation and lactation has been described elsewhere in detail (8, 23). Each dam was routinely given eight pups (preferably males).

At the end of the lactation period (20 d), ten controls (C) and ten malnourished (M) litters were given free access to the standard diet. Ten controls (CS) and ten malnourished (MS) litters were fed with the standard diet enriched with 7 % soybean oil. Another ten controls (CO) and ten malnourished (MO) litters were fed with the standard diet enriched with 7 % olive oil. The diets used were prepared by Panlab from the standard diet made up especially for the present study by addition of soybean oil (Koipesol trademark) and olive oil (Giralda trademark). Major

fatty acid components of the standard and experimental diets are shown in table I.

The pups were weighed at 15, 30, 90 and 150 days of age. At 15 days of age the male pups (preferably two animals per litter) were killed by decapitation at the same time of the day, trunk blood being collected and stored at –20 °C.

At 3 months of age 14 animals per group remained in metabolic cages (Tecniplast) during 10 days. Daily body weight and food intake were recorded.

After a 24 h fast, at the age of 30, 90 and 150 days, two male rats per litter (C, M, CS, CO, MS, MO), prior to being sacrificed, were anaesthetized with sodium pentobarbital (30 mg/kg i.p. and 30 mg/kg s.c.) and the right carotid artery was cannulated with a polyethylene catheter (PE-50 and PE-100).

Arterial blood pressure was measured with a pressure transducer Statham P23D (Gould Inc., Oxnard, Calif), connected to a Beckman polygraph Model 611-A (Beckman Instruments, Brea, Calif). Heart rate was calculated from phasic blood pressure recording (11).

Blood from the jugular vein was obtained and stored at –20 °C. Total cholesterol, HDL and triglycerides were measured by using an enzymatic colorimetric test (Boehringer Mannheim S.A. Biochemicals, Barcelona, Spain).

Table I. *Major fatty acid components (%) of standard and experimental diets.*

Fatty Acids	S	SS	SO
C16:0	19.71	12.90	13.20
C18:0	5.14	4.30	2.94
C16:1			1.05
C18:1	28.46	25.30	61.70
C18:2	46.65	51.70	20.90
C18:3		5.60	
SFA	24.85	17.20	16.14
MUFA	28.46	25.30	62.75
PUFA	46.65	57.30	20.90
MUFA/SFA	1.14	1.47	3.86
PUFA/SFA	1.87	3.33	1.29

S = Standard diet; SS = Standard diet enriched with soybean oil; SO = Standard diet enriched with olive oil.

Statistical analysis.– Snedecor's "F" (33) was used to determine the differences among dietary oils within each type of early nutritional group (control or malnourished), when the "F" revealed a significant value, multiple comparisons were carried out according to Tuckey's test (34). Student's *t* test with the FISHER and YATE correction (10) was used to determine the differences between the means of the following groups: C vs M; CS vs MS; CO vs MO.

Results

The body weight evolution of the offspring from 15 to 150 days of age are recorded in table II. Mean body weight of pups whose dam's food intake was restricted during pregnancy and lactation was always significantly lower for each time period in relation to pups whose dam was fed *ad libitum*. The experimental diets did not significantly increase the body weight of rats within the same model of early nutrition.

Food intake, weight gain and feed efficiency are shown in table III. The values of food intake (Kcal) were significantly higher in control animals than experimental animals, whereas the weigth gain was significantly lower in control animals than experimental ones. The early malnourished animals showed a greater feed efficiency than the controls, and the experimental diets increased this parameter.

Table IV contains the blood pressure values (systolic and diastolic) in rats of different ages and on different diets. The data for these parameters were within normal values. At 30 days of age, the val-

Table II. *Effect of enriched oil diets (soybean and olive) on body weight (g) in controls and malnourished male rats recovering from early undernutrition.* Mean ± S.E. In parentheses the number of animals studied.

Age	STANDARD DIET		STANDARD DIET + SOYBEAN OIL		STANDARD DIET + OLIVE OIL	
Days	C	M	C	M	C	M
15	29.10 ± 0.76 (17)	12.89 ± 0.50 (26)[a]				
30	85.40 ± 2.42 (8)	54.60 ± 6.33 (10)[a]	86.40 ± 65 (10)	48.75 ± 9.76 (8)[a]	84.40 ± 3.28 (10)	49.05 ± 5.83 (8)[a]
90	330.38 ± 13.26 (8)	280.50 ± 5.25 (8)[a]	350.90 ± 9.45 (8)	289.00 ± 11.53 (8)[a]	356.50 ± 12.85 (10)	281.00 ± 9.64 (10)[a]
150	398.00 ± 14.07 (8)	353.75 ± 11.18 (8)[b]	440.70 ± 10.07 (10)	321.20 ± 12.00 (10)[a]	440.90 ± 10.17 (10)	339.40 ± 9.66 (10)[a]

Only the statistically significant differences are shown; C = Controls; M= Malnourished; C vs M: a = p<0.01; b = p<0.05

Table III. *Food intake (Kcal) (A), weight (g) gain (B) and feed efficiency, as measured in total weight gain/total feed during ten days in matabolic cages in control and malnourished male rats at 3 months of age eating standard diet and enriched oil diets (soybean and olive).* Legend as in table II. b = p<0.01; c = p<0.05

	STANDARD DIET		STANDARD DIET + SOYBEAN OIL		STANDARD DIET + OLIVE OIL	
	C	M	C	M	C	M
A	716.11 ± 18.0 (9)	664.51 ± 17.0 (12)[c]	739.10 ± 17.42 (12)	642.59 ± 20.69 (11)[b]	715.69 ± 17.23 (12)	602.63 ± 18.78 (9)[a]
B	27.11 ± 2.54 (9)	35.33 ± 2.0 (13)[c]	28.99 ± 3.70 (9)	38.90 ± 2.60 (11)[c]	31.43 ± 3.33 (11)	37.42 ± 3.00 (9)
A/B	28.44 ± 2.29 (9)	19.79 ± 1.5 (12)[b]	25.29 ± 3.94 (9)	16.37 ± 1.45 (11)[c]	25.21 ± 2.50 (11)	17.11 ± 1.39 (9)[c]
Taking «C» as 100	100	143.71	112.45	173.73	112.82	166.22
Taking «M» as 100		100		120.89		115.66

Table IV. *Effect of enriched oil diets (soybean and olive) on systolic and diastolic blood pressure (S.B.P., D.B.P mm Hg) in controls and malnourished male rats recovering from early undernutrition.* Legend as in table II. ** p<0.01; * p<0.05 vs C eating standard diet:

Age		STANDARD DIET		STANDARD DIET + SOYBEAN OIL		STANDARD DIET + OLIVE OIL	
days		C	M	C	M	C	M
30	S.B.P.	105.63 ± 7.28 (8)	100.63 ± 15.45 (8)	122.00 ± 3.80 (10)	106.88 ± 9.98 (8)	119.00 ± 2.5 (10)	91.25 ± 7.91 (8)[a]
	D.B.P.	75.00 ± 8.02 (8)	75.63 ± 13.74 (8)	88.67 ± 2.50 (9)	83.13 ± 8.84 (8)	87.00 ± 2.71 (10)	67.50 ± 7.07 (8)[b]
90	S.B.P.	147.50 ± 3.50 (8)	136.87 ± 4.71 (8)	145.00 ± 1.83 (10)	147.50 ± 6.47 (8)	150.50 ± 4.62 (10)	141.00 ± 6.09 (10)
	D.B.P.	106.25 ± 3.46 (8)	96.87 ± 5.08 (8)	97.5 ± 1.86 (9)	104.37 ± 4.75 (8)	107.00 ± 3.27 (10)	99.50 ± 4.62 (10)
120	S.B.P.	133.75 ± 3.60 (8)	137.50 ± 3.40 (8)	144.00 ± 3.06 (10)*	144.00 ± 7.53 (10)	149.44 ± 2.12 (9)**	150.50 ± 2.16 (10)
	D.B.P.	94.37 ± 3.69 (8)	100.62 ± 2.7 (8)	106.00 ± 3.32 (10)	99.00 ± 5.71 (10)	103.33 ± 1.18 (9)*	104.00 ± 1.24 (10)

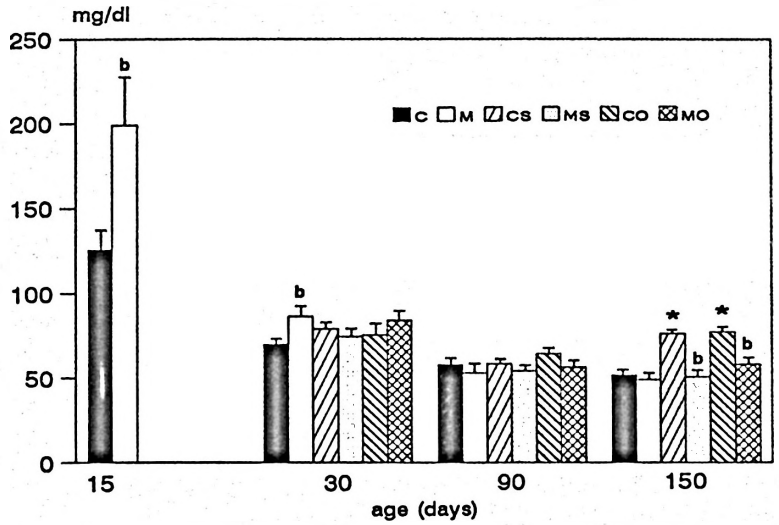

Fig. 1. *Effect of enrich oil diets (soybean and olive) on total cholesterol in control and malnourished rats recovering from early malnutrition.*
C = Control eating standard diet; M = Malnourished eating standard diet; CO = C eating standard diet enriched with olive oil; CS = C eating standard diet enriched with soybean oil; MO = M eating standard diet enriched with olive oil; MS = M eating standard diet enriched with soybean oil.
C vs M, CS vs MS and CO vs MO: $^{b}p \leq 0.01$; C vs CS and C vs CO: $^{*}\ p \leq 0.01$.

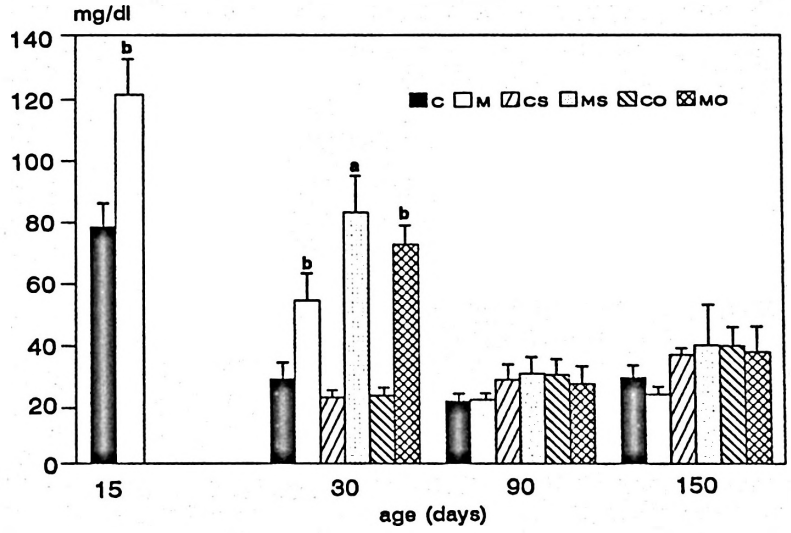

Fig. 2. *Effect of enrich oil diets (soybean and olive) on total triglycerides in control and malnourished rats recovering from early malnutrition.*
C = Control eating standard diet; M = Malnourished eating standard diet; CO = C eating standard diet enriched with olive oil; CS = C eating standard diet enriched with soybean oil; MO = M eating standard diet enriched with olive oil; MS = M eating standard diet enriched with soybean oil.
CS vs MS: $^{a}p \leq 0.01$; C vs M and CO vs MO: $^{b}p \leq 0.05$.

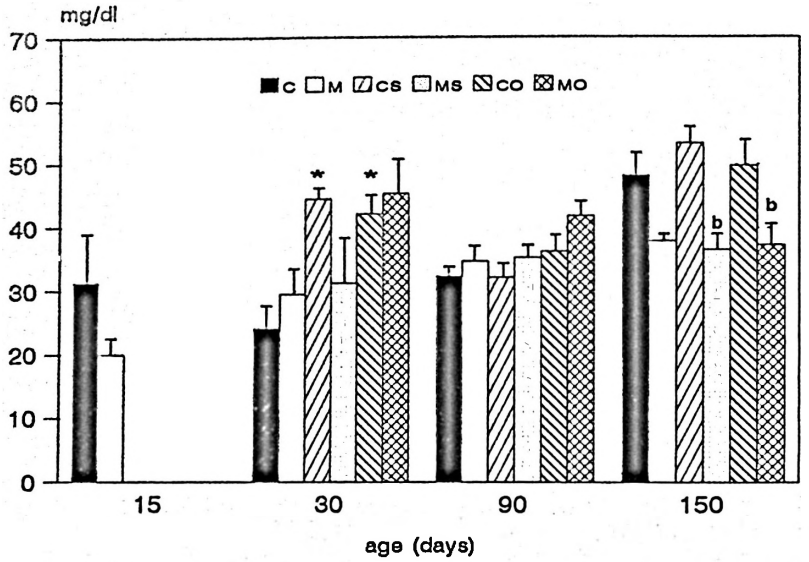

Fig. 3. *Effect of enrich oil diets (soybean and olive) on total HDL-cholesterol in control and malnourished rats recovering from early malnutrition.*
C = Control eating standard diet; M = Malnourished eating standard diet; CO = C eating standard diet enriched with olive oil; CS = C eating standard diet enriched with soybean oil; MO = M eating standard diet enriched with olive oil; MS = M eating standard diet enriched with soybean oil. C vs CS and C vs CO: * $p \leq 0.01$; CS vs MS and CO vs MO: $^{b}p \leq 0.01$.

ues of systolic and diastolic blood pressure in MO were significantly lower ($p < 0.01$ and $p < 0.05$) than those for CO. At 3 months of age the results were similar in all the groups studied. Significant statistical differences were found between CO and CS when compared with C at 5 months of age, since in controls the continuous ingestion of the oil diets increased both systolic and diastolic pressure values.

Plasma cholesterol level decreased in all animals from 15 to 150 days of age (fig. 1). At 150 days of age CS and CO significantly increased this parameter compared with the other groups. At 15 and 30 days, the values of total cholesterol and triglycerides (fig. 2) were significantly higher in the early malnourished animals (M) than in the controls (C). There were no differences in these parameters between these two groups at 3 and 5 months of age. The control animals eating oil diets (CS and CO) significantly increased the values of HDL-cholesterol at 30 days of age compared with the controls (C) (fig. 3). These differences disappear at 90 days. Nevertheless, at 150 days of age these animals once again significantly increased the values of this parameter in comparison to malnourished animals eating oil diets.

Discussion

The oil diets employed in our rehabilitation model did not normalize the body weight of early malnourished male rats. Animals achieve their normal genetically determined body size depending on the stage of development during which the deprivation is introduced and on its degree and duration (5). In our experimental model deprivation was applied during a critical period in the rat's development. So that these animals never acquired the body size of the controls.

However, our data (table III) indicate that malnourished rats showed (at 90 days of age) an accelerated growth response, as they gained more weight and had a greater feed efficiency than control rats during the ten days stay in the metabolic cages. These findings are in agreement with the results of OCKEN and GRUNEWALD (27), and they showed a rapid rate of *catch-up* growth that the undernourished animals experiment in an attempt to normalize body size.

Control rats on oil diets increased the body weight gain significantly during the experimental period compared to the controls (C = 313.5 g; CS = 354.3 g; CO = 356.5 g). These results are consistent with other investigations which show that high-fat diets reliably produce overweight and obesity in laboratory animals (28). Early malnourished rats on oil diets did not significantly increase the body weight gain compared with early malnourished rats on standard diet (M = 299.15 g; MS =272.45 g; MO = 290.4 g). Pre- or postnatal undernutrition retards growth and functionality of the gastrointestinal tract (21) and reduces lipid and protein uptake by the enterocytes (32). Postnatal undernutrition causes a decrease in antral gastrin levels, a situation that could not be normalized by nutritional rehabilitation even when the body weight and gastrointestinal tract was corrected (20). The antral gastrin hormone has an effect on all major gastrointestinal activities including secretion, motility and absorption. High levels of cholescystokinine (CCK) produced by increased fat ingestion competitively inhibit the gastrin-stimulated acid (37). Malnourished animals on oil diets could have their gastrin secretion diminished and CCK secretion increased, both factors producing a decreased food ingestion and reducing the capacity of the whole intestine to absorb these nutrients.

In men, increased amounts of monounsaturated (MUFA) and PUFA in the diet do not show any effect on blood pressure (24, 32). However, in normal animals, the effect of dietary fat on this parameter is controversial. In our study, the blood pressure values (diastolic and systolic) measured at different ages and for different diets were within normality, whereas the same parameter in CS and CO groups, compared with C, at five months of age, were slightly significantly increased. This result might be due to these animals being overweight, and there being as in humans (31) a close relationship between body weight and blood pressure.

Early undernutrition increased plasma cholesterol and triglycerides in the malnourished pups, which maintained increased levels of these parameters up to 30 days of age (figs. 1 and 2). These results are in accordance with those of other authors (18) who have shown that undernourishment of the mother alters the lipidic metabolism in the offspring, as the activity of hepatic lipoprotein lipase (LPL) increased in the pups and the temporal appearance of LPL activity in the liver modifies the role of this organ in the overall lipoprotein metabolism.

At three and five months of age, after 10 and 18 weeks of feeding on oil diets, the triglyceride level was similar in all groups (table IV), which suggests that there is an adaptation to a longer feeding period or to an introduction of the diet at a young age (26).

The ingestion of oil diets did not affect cholesterol values in three month old rats. However, at five months control animals eating oil diets (CS and CO) showed statistically significant increased cholesterol levels compared with the other groups. These results are not in agreement with those in other works. In this sense, the literature abounds with clinical trials which demonstrate that decreasing SFA intake

and/or increasing both PUFA (19) and MUFA (12) in the diet in humans result in a decrease in plasma cholesterol levels. However, rat studies have demonstrated different results in relation to dietary fat and cholesterol level (9). Reductions in plasma cholesterol concentration vary greatly in studies, due to differing genetic backgrounds, initial plasma lipid levels, age and sex of subjects, relative restrictiveness of the experimental diet, and variation in the baseline diet to which the modified diet is compared. Whether the diets are prepared and given to the subjects in a controlled environment or dietary modification is achieved by dietary counselling and this may in itself also influence the magnitude of the change.

There is considerable diversity in the baseline diets used by the animals before the dietary modification was introduced and the time period necessary for the stabilization of the changes of plasma lipids in response to diets varied greatly in the literature. The high cholesterol level shown by our control animals eating oil diets (CS and CO) at five months of age is a consequence of our dietary pattern, as these animals remained on these diets from weaning, and this nutritional pattern has not been used by other authors. Moreover, decreasing SFA intake by increasing PUFA in the diet produced the same effect on cholesterol levels as that of decreasing SFA intake by increasing MUFA.

Somewhat controversial is the effect of diets high in MUFA or PUFA in the level of HDL-cholesterol in plasma. Most of the work indicates that high PUFA diets result in a decrease in HDL-cholesterol (30), although the effect is not always consistent. Some studies support the neutrality of MUFA with respect to this parameter, and other evidences propose that MUFA may be as effective as PUFA in lowering LDL-cholesterol without simultaneously reducing those of HDL-cholesterol (25). What is apparent from our data is that our monounsaturated enriched diet (olive oil) increased plasma HDL-cholesterol concentrations to a comparable extent as did our polyunsaturated enriched diet (soybean oil) in control animals at 30 and 150 days of age. Although these data are very controversial with other results shown in the literature, it should be emphasized that our diets and the time of application were quite different from those used in other studies.

Early malnourished animals on oil diets (MS and MO) at five months of age showed plasma cholesterol levels similar to malnourised and control animals on standard diet; this may be due to the fact that early undernutrition reduces lipid uptake by the enterocytes (32). Malnutrition can alter the enzymatic activities depending on the age period in which it is imposed, and in this experiment it was imposed during a critical period for intestinal differentiation. On the other hand, data from BROWN *et al.* (2) indicates that the initial adaptive response in lipid transport to a common metabolic challenge during adulthood differs between animals born to dams fed different diets, concluding thus that the expression of genes regulating cholesterol metabolism can be modulated by maternal diets in the rat.

J. M. GUTIÉRREZ, A. FERNÁNDEZ, A. FUEYO, C. GONZÁLEZ, S. FERNÁNDEZ y A. MENÉNDEZ-PATTERSON. *Efecto de dietas enriquecidas con aceites sobre algunos factores de riesgo cardiovascular durante la recuperación de ratas subnutridas tempranamente.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (4), 229-238, 1994.

Se evalúa el papel de los aceites de soja y de oliva en la recuperación y evolución de algunos factores de riesgo cardiovascular en ratas subnutridas *in utero y* durante la lactancia. A los

20 días de edad y durante 130, los animales controles (C) y los malnutridos (M) se alimentan con tres tipos de dietas: estándar (C, M), estándar enriquecida con aceite de oliva (CO, MO) y estándar enriquecida con aceite de soja (CS, MS). A los 15, 30, 90 y 150 días de edad se determina el peso corporal, la presión arterial sistólica y diastólica y los niveles séricos de colesterol total, HDL-colesterol y triglicéridos. La subnutrición produce una disminución del peso corporal, mostrándose ineficaces las dietas enriquecidas en aceites para su recuperación, sin embargo, en los animales controles producen sobrepeso. Los valores de presión arterial obtenidos en todas las edades y con las distintas dietas se encuentran dentro de los límites normales. Los triglicéridos aumentan en los animales subnutridos tempranamente, a los 30 días de edad, volviendo después a la normalidad. El colesterol plasmático disminuye con la edad en todos los grupos de animales, a excepción de CS y CO que presentan un aumento significativo a los 5 meses.

Palabras clave: Malnutrición temprana, Factores de riesgo cardiovascular, Dietas enriquecidas con aceite, Rata.

Acknowledgements

This work was supported by a Grant from the "Investigación de la Seguridad Social" (Spain) (No 91/0667).

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