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Characterization of Metabolic Effects of Energy Mal-Nutrition An Experimental Model for "*In Vivo*" Studies in Weaned and Adult Mice

Gauffin Cano Paola [1], Chaila Zulema[2], González Silvia[1, 3], and y P.de Ruiz Holgado Aída[1]
[1]Centro de Referencia para Lactobacilos (CERELA)-Chacabuco 145-Tucumán (4000)-Argentina
[2]Fundación ALAC (Asociación de Laboratorios Programa de Normas y de Alta Complejidad)
[3]Cátedra de Salud Pública de la Facultad de Bioquímica, Química y Farmacia de la Universidad Nacional de Tucumán

Abstract: Human or animal organisms respond against nutrients deficiency with a series of adaptive mechanisms in order to conserve the tissues. When mal-nutrition is mild, alterations are minimal and signs and symptoms are sub-clinic. The aims of this study were to establish a protein-energy mal-nutrition model and to characterize the early biochemical and hematological alterations related with the age and the sex. Swiss males and females mice of 3 weeks (weaned) and of 9 weeks (adults) were mal-nourished by restriction of balanced diet for 12 days. At 0, 4, 8 and 12 days of this mal-nutrition period we determined: daily body weight; thymus, spleen and liver weights; serum glucose, cholesterol, triglycerides, proteins and albumin; and hematological assays. white and red blood cells, hematocrit, and hemoglobin concentration. The mal-nutrition diet induced a lost of 10-25 % of body weight compared to well-nourished control, reaching a mild mal-nutrition condition. The thymus and spleen weights decreased by mal-nutrition diet and the liver weight did not suffer modification. In all the experimental groups we observed a significant descent of serum glucose, triglycerides and white blood cells number. The determination of serum parameters like serum glucose and triglyceride, leucocytes number and body weight, allow us to analyze the nutritional status in this experimental model. And it would also allow us to realize an effective and opportune nutritional intervention.

Key words: Mal-nutrition, Metabolic effects, Mice, Hematological parameters

Introduction

Protein-energy mal-nutrition (PEM) is characterized not only by an energy deficit due to a reduction in all macronutrients, but also by a deficit in many micronutrients. This syndrome is one example of the various levels of inadequate protein and/or energy intake between starvation (no food intake) and adequate nourishment. Although infants and children of some developing nations dramatically exemplify this type of mal-nutrition, it can occur in persons of any age in any country.

Clinically, PEM has three forms: dry (thin, desiccated), wet (edematous, swollen), and a combined form between the two extremes. The form depends on the balance of non-protein and protein sources of energy. Each of the three forms can be graded as mild, moderate, or severe. Grade is determined by calculating weight as a percentage of expected weight for length using international standards (normal: 90 to 110%; mild: 75 to 90%; moderate: 60 to 85%; severe: < 60%) (Gomez *et al.*, 1956).

The dry form of PEM, denominated marasmus, results from near starvation with deficiency of protein and non-protein nutrients. Marasmus is the predominant form of PEM in most developing countries. It is associated with the early abandonment or failure of breastfeeding and it are frequently associated with infections, mainly gastrointestinal infections (McLaren, 1966 and McLaren *et al.*, 1967).

The wet form of PEM is called kwashiorkor. The protein deficiency is usually more marked than the energy deficiency, and edema results. Kwashiorkor is less common and is usually manifested as marasmus-kwashiorkor. It tends to be confined to parts of the world (rural Africa, the Caribbean and Pacific islands).

The reasons for a progression of nutritional deficit into marasmus or kwashiorkor are unclear and cannot be solely explained by the composition of the deficient diet (ie, a diet deficient in energy for marasmus and a diet deficient in protein for kwashiorkor). Marasmus has been described as an adaptation to inadequate energy and protein intake and more recently, kwashiorkor has been considered a metabolic misadaptation of the organism to mal-nutrition, explaining the development of edema (Rao, 1974 and Pascal *et al.*, 2002). The study of these phenomena is considerably limited by the lack of an appropriate animal model. Understanding the physiological effects of mal-nutrition may aid in minimizing its adverse consequences.

Therefore, an urgent need exists to establish criteria and standards for assessing the nutritional status of animals in studies of experimental PEM. At present, it is difficult to assign most experimental animal systems to any particular category of human mal-nutrition, and this problem limits the relevance of experimental studies to apply in the human diseases. Two critical variables are stage of life and nature of the diet-induced disease (i.e. whether the form of PEM imposed is stunting or wasting and whether it should be classified as mild, moderate or severe) (Woodward, 1998; 2001).

Nutrition screening identifies individuals who are malnourished or are at risk of mal-nutrition. The purpose of the nutritional screening is to determine whether a more detailed nutritional assessment is necessary. Objective and subjective data can facilitate early intervention and assist initiation of a formal nutrition intervention or supplementation. No single nutritional measurement can be considered 100 % sensitive and specific because non-nutritional responses to illness affect many nutrition indicators. A history of weight loss can be one of the most important pieces of information in the nutrition screening and assessment process (Sungurtekin *et al.*, 2004).
The goals of this study were to establish a mild protein-energy mal-nutrition model in mouse, and to characterize the early biochemical and hematological alterations related with the age and the sex. The identification of early signals caused by mal-nutrition would be of great utility to evaluate the effects of nutritional supplements, like probiotics, and therefore to select the appropriate re-nutrition diet. Such information is essential for design future studies to determine a marker for the early diagnosis of PEM and for the follow-up to treatment.

Material and Methods

Animals: Weaned (21 days) and adult (9 weeks), male and female Swiss mice from closed colony of the breeding unit kept at CERELA Institute were individually housed in metabolic cages on a 12:12 light cycle. The weaned mice were adapted, 2 days to eating a nutritionally complete pellet diet (balanced diet) providing 21 % of calories as protein, 66 % as carbohydrate and 13 % as fat. The adult mice received the balanced diet before starting the study.

Diet Groups: Following the adaptation to the balanced diet, weaned and adult mice were switched during 12 days to a restricted amount (approximately 25 %) of their normal food intake to match 10-25 % weights lost compared with the well-nourished control group. The well-nourished groups received the same diet administered *ad libitum*. The mal-nourished mice (MN) were separate into four diet groups. Two groups were the female and male weaned mal-nourished mice (WMN). And the other two groups were the female and male adult mal-nourished mice (AMN). On the morning of day 0, 4, 8, and 12 of mal-nutrition period the mice were killed by decapitation. Blood was recovery by cardiac puncture before the death of the animals. The blood samples for hematological parameters measures were collect into tubes with EDTA solution. The blood samples for serum biochemical measurements was collect into plastic centrifuge tubes and as kept on ice until centrifuged, and then the serum was separated and frozen at -20 °C. The thymus, spleen and liver organs were removed and kept in closed, saline-saturated dishes for subsequent cleaning and weighting.

Body Thymus Spleen and Liver Weights: To evaluate nutritional parameters such as body, thymus, spleen and liver weights, we performed this experiment in 10 mice of each group, to validate of the statistical results. These results were expressed in g as mean of $n = 10$. The body weights were monitored daily and the thymus, spleen and liver weights were measured at 0, 4, 8 and 12 days. These results were expressed as g for body weight and for organ weight as mg organ weight/100 mg body weight.

Biochemical Measurements: To evaluate biochemical parameters we performed the experiments in 6 mice of each group. Comparative assays between different groups were made under identical conditions.

Serum Analysis: Serum glucose, cholesterol, and triglyceride were determined with enzymatic methods. Serum total proteins an albumin were measured using colorimetric assays. These methods are applied by routine clinical biochemistry laboratory protocols.

Hematological Determinations: The hematocrit (HTO) and number of leukocytes and red blood cells were determined by hematocytometric methods. The leukocytes populations were differentiated on smears stained with Giemsa solution. The hemoglobin concentration was determined by colorimetric assays.

Statistics: The experimental data were expressed as means ± SD and were statistically evaluated by analysis of variance (ANOVA) with the SPSS computer programs. One-way ANOVA was used to determine the significant effects of mal-nutrition period on the nutritional and biochemical parameters. Differences were considered significant at $P < 0.05$.

Results and Discussion

The maintained decrease in proteins and energy intake cause cellular and metabolic adaptations in human and animal organisms. When this condition is present, the less food intake is used to the maintenance of the existent tissues, in detriment of the formation of new tissues and therefore affecting the organism growth. In mild mal-nutrition state there is a balance among nutrients contribution and demand, with minimum or null alterations of

common mal-nutrition markers (Ulibarri Perez *et al.*, 2002).The mild mal-nutrition is one of the most frequent in children, but generally it is one which less medical attention received. Obviously, when early it happens and more time it be prolonged, more damages it will cause.

In order to elucidate the early mechanisms involved in mal-nourished individuals, we considered necessary to development a mild mal-nutrition murine model, which could be relevant to human mal-nutrition in development countries. Although, the mouse has been extensively used in animal models of mal-nutrition, there is no standard murine model of mild protein-energy mal-nutrition (Anstead *et al.*, 2001). This model would allow us to know the early alterations that happen in this very frequent condition.

The body weight and linear growth characteristics of mal-nourished and well-nourished control mice are shown in Fig.s 1 and 2. The body weights of the adult control mice remain stable along the period of 12 days of the assay. The female control mice had a body weight significantly smaller than the male mice. When we analyzed this parameter in adult undernourished mice, we observed a lost of 13.07 % (males) and 12.54 % (females) of body weight compared with their respective well-nourished controls at the end of the mal-nutrition period (Fig. 1).

During the 12 days of study, the weaned control mice increased their corporal weight, and significantly smaller values were observed in the female mice. Likewise, in undernourished weaned mice, the body weight decreased, and male and female mice lost 25 % and 15 % of weight, respectively, in comparison to their respective well-nourished controls (Fig. 2).

Our studies about body weight allowed to determinate that, a period of 12 days with a restriction of 25 % of the daily portion of food intake induced a mild mal-nutrition (loss of 10 % to 25 % of body weight compared with the well-nourished control) (Gomez *et al.*, 1956).

The results obtained from thymus, spleen and liver weights are shown in Tables I and 2. The thymus of the adult mice is significantly smaller than that of the weaned mice, as it happens in human organism. The thymus and spleen weights of adult mal-nourished mice decreased significantly from day 8 of mal-nutrition diet (Table I). In weaned mal-nourished mice, the thymus and spleen weights decreased significantly at day 12 of mal-nutrition diet (Table 2).

Among the alterations observed in both post-mortem undernourished children's studies and in experimental models in animals, the most frequent characteristic was the appearance of thymic atrophy like a critical and premature parameter of the nutritional state and promoter of a serious immunologic deficiency as consequence of a "nutritional thymectomy ". The mal-nourished individual becomes in an immunocompromised host, fact that makes it susceptible to infections, as much in frequency as in the duration of the same ones (Dourov, 1986).

Likewise, we observed in a moderate mal-nutrition murine model a significantly decrease in the thymus weight, in concordance with alterations in functionally and maturation of lymphocytes, and it was associated with an increase risk of infections (Gauffin Cano and Perdigon, 2003). In our mild mal-nutrition model we observed that lymphoid organs like thymus and spleen are already affected by this process. However, it is broadly well-known that in these organisms the infections are less frequent than severe and moderate mal-nutrition organisms. Therefore, in this level of mal-nutrition, the immune system functionality would be not significantly affected.

The liver weight of weaned and adult animals did not suffer modifications in this mal-nutrition model (Table I and2) No significantly differences related with the sex were observed for thymus, spleen and liver weights in all mice groups studied.

The results of glucose, cholesterol, triglyceride, protein and albumin measurements are shown in Fig.s 3 and 4. As it was expected, in adult and weaned mice, serum glucose began to decrease progressively from the day 4 and thereafter. In day 12 the values were significantly smaller in comparison to well-nourished control (Fig. 3).

The hypoglycemia can cause serious damages in an organism, particularly in those organs like the nervous system, whose metabolism uses glucose as principal energy source. However, in children suffering chronic mal-nutrition this clinical situation is overcome by adaptation mechanisms (Gussinyé *et al.*, 2000).

Several studies indicate that the mal-nutrition induces a diminution of the plasma total cholesterol, which was not observed in our model (Etukudo *et al.*, 1999). Though, the mal-nutrition diet induced significantly reduction of triglycerides values at day 4 in adults and weaned mice. Furthermore, the serum cholesterol and triglycerides values in female mice were higher than male mice (Fig. 3 and 4).

As it was already mentioned the mal-nutrition induces adaptive mechanisms that involve the endocrine system. Usually, reduced food intake was associated with hypoglycemia, which conduce to a hypoinsulinemia, and enhance of growth hormone secretion. The hypoinsulinemia and the increase of growth hormone level induce a redistribution of body energy from body fat and could be the cause of low triglycerides values.

Serum proteins and albumin concentrations did not significantly change in any of studied animals regarding to the controls. Despite mal-nutrition is associated with a decreased in the rates of whole-body protein synthesis and breakdown, as an adaptive mechanism to conserve energy and amino acid when they are in scarce supply (Manary *et al.*, 1998 and Covinsky *et al.*, 2002). In this mild mal-nutrition model, the serum proteins concentrations did not

Table 1: Thymus, spleen and liver weights of mal-nourished and well-nourished adult mice.

		mg organ weight/100 mg body weight					
		Thymus		Spleen		Liver	
		Male	Female	Male	Female	Male	Female
d0	Malnourished	0,21 ± 0,04	0,26 ± 0,04	0,34 ± 0,04	0,36 ± 0,10	5,2 ± 1,2	4,7 ± 0,5
	W-N Control	0,20 ± 0,03	0,28 ± 0,02	0,35 ± 0,06	0,36 ± 0,10	4,3 ± 0,4	4,6 ± 0,4
d4	Malnourished	0,16 ± 0,03	0,21 ± 0,02	0,28 ± 0,02	0,32 ± 0,03	4,3 ± 0,1	4.0 ± 0,5
	W-N Control	0,20 ± 0,03	0,27 ± 0,01	0,28 ± 0,01	0,37 ± 0,01	4,8 ± 0,6	4,3 ± 0,4
d8	Malnourished	0,14 ± 0,03a	0,19 ± 0,03a	0,23 ± 0,03a	0,29 ± 0,02a	3,8 ± 0,3	4,0 ± 0,2
	W-N Control	0,23 ± 0,05	0,27 ± 0,03	0,32 ± 0,05	0,38 ± 0,10	4,7 ± 0,5	4,6 ± 0,2
d12	Malnourished	0,09 ± 0,03ab	0,15 ± 0,02ab	0,22 ± 0,04ab	0,28 ± 0,03ab	4,0 ± 0,3	3,8 ± 0,5
	W-N Control	0,22 ± 0,04	0,25 ± 0,05	0,31 ± 0,02	0,36 ± 0,03	4,2 ± 0,2	4,3 ± 0,4

The thymus, spleen and liver weights were measured at 0, 4, 8 and 12 days of mal-nutrition period.
W-N: well-nourished control
Values are means ± SD of 10 mice per group. One way ANOVA Test.
a- Values significantly different from well-nourished mice of the same age.
b- Values significantly different to the beginning of the period of treatment inside oneself group.

Table 2: Thymus, spleen and liver weights of mal-nourished and well-nourished weaned mice.

		mg organ weight/100 mg body weight					
		Thymus		Spleen		Liver	
		Male	Female	Male	Female	Male	Female
d0	Malnourished	0,54 ± 0,08	0,57 ± 0,07	0,56 ± 0,09	0,69 ± 0,05	4,5 ± 1	4,2 ± 1,4
	W-N Control	0,67 ± 0,09	0,57 ± 0,08	0,58 ± 0,06	0,53 ± 0,06	4,9 ± 1	4,6 ± 1,0
d4	Malnourished	0,53 ± 0,04	0,56 ± 0,06	0,59 ± 0,16	0,46 ± 0,07	4,4 ± 0,8	4,6 ± 0,8
	W-N Control	0,54 ± 0,11	0,58 ± 0,10	0,69 ± 0,12	0,59 ± 0,08	4,5 ± 0,7	4,8 ± 1,4
d8	Malnourished	0,46 ± 0,16	0,45 ± 0,07	0,31 ± 0,03a	0,39 ± 0,05a	5,7 ± 1	4,2 ± 0,7
	W-N Control	0,51 ± 0,15	0,55 ± 0,12	0,65 ± 0,09	0,59 ± 0,06	5,0 ± 1,2	4,0 ± 1,1
d12	Malnourished	0,27 ± 0,03ab	0,37 ± 0,01ab	0,34 ± 0,01ab	0,34 ± 0,01ab	5,3 ± 0,6	4,1 ± 0,1
	W-N Control	0,46 ± 0,12	0,56 ± 0,08	0,75 ± 0,01b	0,65 ± 0,11b	5,6 ± 0,5	4,3 ± 0,1

The thymus, spleen and liver weights were measured at 0, 4, 8 and 12 days of mal-nutrition period.
W-N: well-nourished control
Values are means ± SD of 10 mice per group. One way ANOVA Test.
a- Values significantly different from well-nourished mice of the same age.
b- Values significantly different to the beginning of the period of treatment inside oneself group

decrease and we estimate that it occurs due to the energy requirements are satisfy only from the body fat. Furthermore, since in mild mal-nutrition model liver weight was no affected, the hepatic synthesis of proteins is not expected to be altered (Tables I and2). Contrary, in severe mal-nutrition, where hepatic damage was present, dramatically decrease in hepatic synthesis of proteins like transthyretin and albumin has been seen observed (Ingenbleek and Bernstein, 1999). The hematological determinations of mal-nourished and well-nourished mice are shown in Tables3 and4. In adult mal-nourished group the leukocytes number diminished abruptly from the day 4. The red blood cells number in this group did not suffer alterations by mal-nutrition diet compared with well-nourished control; we observed a moderate normochromic or slightly hypochromic anemia with normal red blood cell size. The hematocrit and hemoglobin concentration decreased progressively during the mal-nutrition period, but the descent was most significant at days 8 and 12 (Table 3). In weaned mice group the leukocytes number decrease significantly from day 8, compared to well-nourished control. During the 12 days of study the red blood cells number and hematocrit values augmented progressively in the weaned well-nourished mice (control) and the same response was observed in the weaned mal-nourished groups.
hemoglobin concentration did not modify with the mal-nutrition diet in comparison with the control (Table 4). In none of the hematological parameters we observed a significantly influence of the gender (Table 3 and 4).
Differential leukocytes recount showed a bigger percent of lymphocytes than neutrophiles in both, adult and weaned well-nourished mice. Likewise, the differential leukocytes recount in mal-nourished mice exhibited the same pattern (data not shown). Although the mal-nutrition is mild the number of leukocytes was significantly decreased, but not the ratio of each type of white blood cell. Therefore, it is necessary to determine if the functionally of the leukocytes is really affected.

Table 3: Hematological determinations of mal-nourished and well-nourished adult mice.

		Hematological determinations Adults mice			
		Mal-nourished		W-N Control	
		Male	Female	Male	Female
d0	Leucocytes ($10^{3/mm3}$)	6.7 ± 0.6	6.8 ± 0.3	6.1 ± 1.8	7.7 ± 1.2
	Red cells (106/mm3)	8.9 ± 1.9	8.3 ± 2.7	11.0 ± 1	11.2 ± 2
	Hemoglobin (g/dl)	13.9 ± 2	12.7 ± 1.1	16.8 ± 2	15.9 ± 8.4
	Hematocrito (%)	53.6 ± 2.5	50.1 ± 6.4	53.1 ± 4	55.5 ± 3
d4	Leucocytes (103/mm3)	2.2 ± 0.4a	3.1 ± 0.4a	8.7 ± 0.9	6.1 ± 1
	Red cells(106/mm3)	9.0 ± 0.9	9.8 ± 0.2	10.6 ± 0.3	10.2 ± 1.5
	Hemoglobin (g/dl)	14.1 ± 0.9	13.3 ± 1	15.6 ± 2	15.2 ± 1.6
	Hematocrito (%)	56.1 ± 3.3	49.3 ± 2	50.6 ± 1.9	51.6 ± 4.3
d8	Leucocytes (103/mm3)	2.7 ± 0.3a	2.9 0.2a	7.1 ± 0.63	6.6 ± 1
	Red cells(106/mm3)	11.49 ± 2	9.1 ± 1.1	11.2 ± 1	10.8 ± 1.6
	Hemoglobin (g/dl)	12.1 ± 1.3a	11.3 ± 1.3a	15.1 ± 1.6	15.1 ± 1.1
	Hematocrito (%)	37.8 ± 4a	47.0 ± 3a	56.8 ± 3.7	52 ± 5
d12	Leucocytes (103/mm3)	2.7 ± 0.3ab	2.9 ± 0.2ab	7.1 ± 0.63	6.6 ± 1
	Red cells(106/mm3)	11.5 ± 2	9.1 ± 1.1	11.2 ± 1	10.8 ± 1.6
	Hemoglobin (g/dl)	12.1 ± 1.3a	11.3 ± 1.2a	15.1 ± 1.6	15.1 ± 1.1
	Hematocrito (%)	37.8 ± 4a	37.0 ± 3.5a	50 ± 3.7	52 ± 5

The hematological determinations were measured at 0, 4, 8 and 12 days of mal-nutrition period. W-N: well-nourished control. Values are means ± SD of 6 mice per group. One way ANOVA Test. a- Values significantly different from well-nourished mice of the same age. b- Values significantly different to the beginning of the period of treatment inside oneself group.

Table 4: Hematological determinations of weaned mal-nourished and well-nourished mice.

		Hematological determinations Weaned mice			
		Malnourished		Control	
		Male	Female	Male	Female
d0	Leucocytes ($10^{3/mm3}$)	6.1 ± 0.7	5.8 ± 1	4.9 ± 0.4	5.7 ± 0.8
	Red cells(106/mm3)	6.3 ± 1	6.4 ± 1.5	6.3 ± 0.7	6.4 ± 1.2
	Hemoglobin (g/dl)	11.2 ± 1	9.9 ± 1.2	10.6 ± 1.7	9.9 ± 1.3
	Hematocrito (%)	33.3 ± 1.5	32.3 ± 3	33 ± 3.2	32.3 ± 4
d4	Leucocytes (103/mm3)	5.3 ± 0.8	5.1 ± 0.8	5.4 ± 0.7	6.3 ± 0.3
	Red cells(106/mm3)	7.3 ± 0.4	7.3 ± 0.2	7.7 ± 1	8.8 ± 1.4
	Hemoglobin (g/dl)	12.5 ± 1.2	11.9 ± 0.6	12.8 ± 2	11 ± 1.8
	Hematocrito (%)	39.7 ± 2.9	37.4 ± 1.2	42.3 ± 2.8	41.7 ± 2.6
d8	Leucocytes (103/mm3)	3.8 ± 0.3a	4.1 ± 0.7a	7 ± 0.52	5.9 ± 0.9
	Red cells(106/mm3)	7.8 ± 0.8	6.8 ± 0.7	6.7 ± 0.7	6.4 ± 0.8
	Hemoglobin (g/dl)	12.2 ± 1	11.7 ± 2	10.8 ± 1.7	8.8 ± 1.2
	Hematocrito (%)	41.2 ± 1.2	38.1 ± 1.8	39.7 ± 1.5	37.4 ± 3.9
d12	Leucocytes (103/mm3)	5.4 ± 1.3a	5.6 ± 0.5a	8.3 ± 1.3b	7.9 ± 1.2b
	Red cells(106/mm3)	11 ± 0.2b	13.9 ± 2b	12.3 ± 1.2b	13.9 ± 2b
	Hemoglobin (g/dl)	12.8 ± 2.1b	12.1 ± 1.3b	11.4 ± 2.3b	10.9 ± 1.9b
	Hematocrito (%)	53.6 ± 4	57 ± 2.7	41 ± 6.3	58.1 ± 4

The hematological determinations were measured at 0, 4, 8 and 12 days of mal-nutrition period. W-N: well-nourished control. Values are means ± SD of 6 mice per group. One way ANOVA Test. a- Values significantly different from well-nourished mice of the same age. b- Values significantly different to the beginning of the period of treatment inside oneself group.

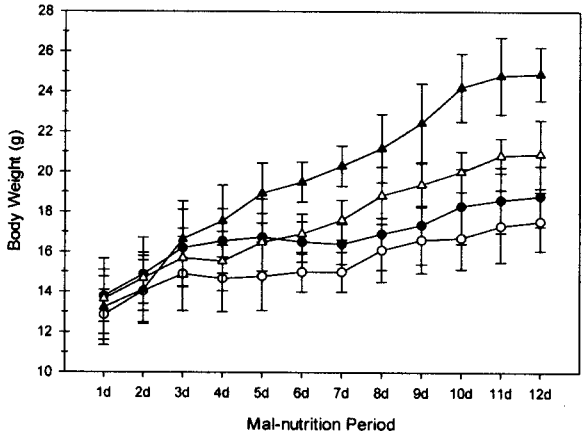

Fig. 1: Body weight of male and female mal-nourished and well-nourished adult mice. The body weights were monitored daily for a period of 12 days until reaching a lost of weight of 10-25% in comparison with the well-nourished control mice. Values are means ± SD of 10 mice per group. * significantly different of well-nourished control mice. One way ANOVA Test. M-N: mal-nourished mice - W-N: well-nourished mice

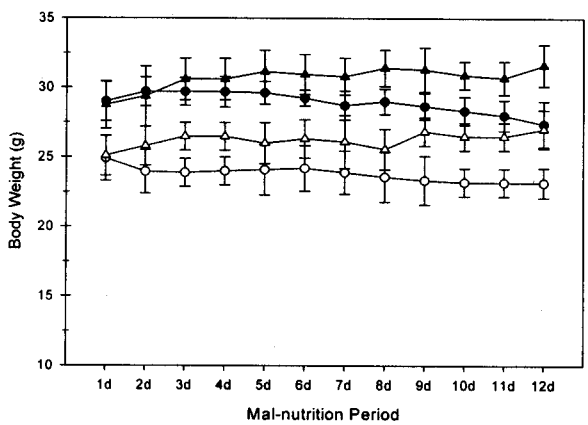

Fig. 2: Body weight of male and female weaned mal-nourished and well-nourished mice. The body weights were monitored daily for a period of 12 days until reaching a lost of weight of 10-25% in comparison with the well-nourished control mice. Values are means ± SD of 10 mice per group. * significantly different of well-nourished control mice. One way ANOVA Test. M-N: mal-nourished mice - W-N: well-nourished mice

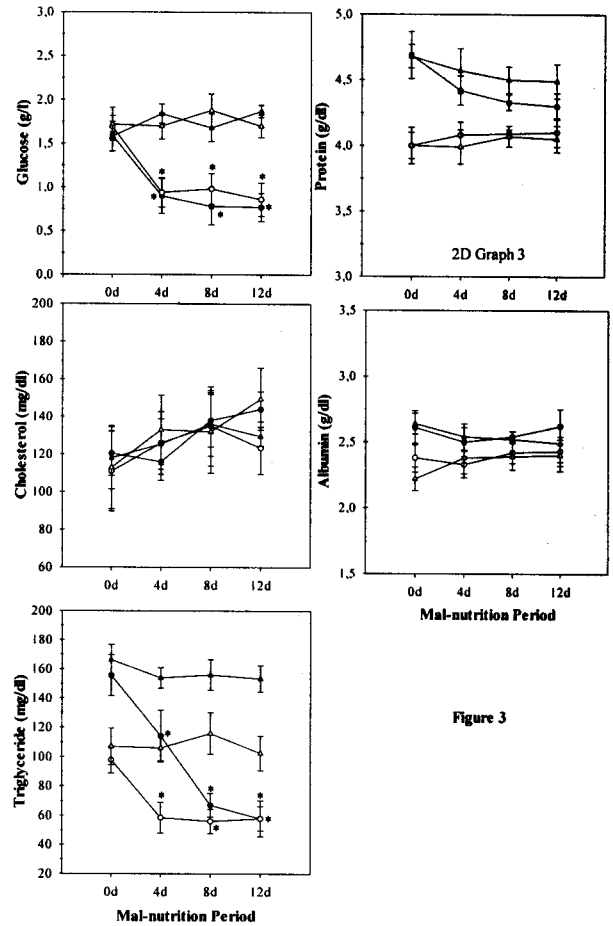

Figure 3

Fig. 3: Biochemical determinations of male and female adult mal-nourished and well-nourished mice. The serum glucose, cholesterol, triglyceride, proteins and albumin determinations were measured at 0, 4, 8 and 12 days of mal-nutrition period. Values are means ± SD of 6 mice per group. * Significantly different from well-nourished control mice. One way ANOVA Test. M-N: mal-nourished mice - W-N: well-nourished mice

Similar hematological results were obtained with a murine moderate mal-nutrition model, in which the immune system was also significantly affected (Gauffin Cano and Perdigón , 2003; Gauffin Cano *et al.*, 2002a,b).

We described a murine model of mild mal-nutrition based on weight, sex and age. The importance of creating a model of mild mal-nutrition resides in the possibility of studying markers for a precocious diagnosis of this pathology. Serum and hematological parameters measured in this work are biologically relevant indices of mal-nutrition, and some of them are

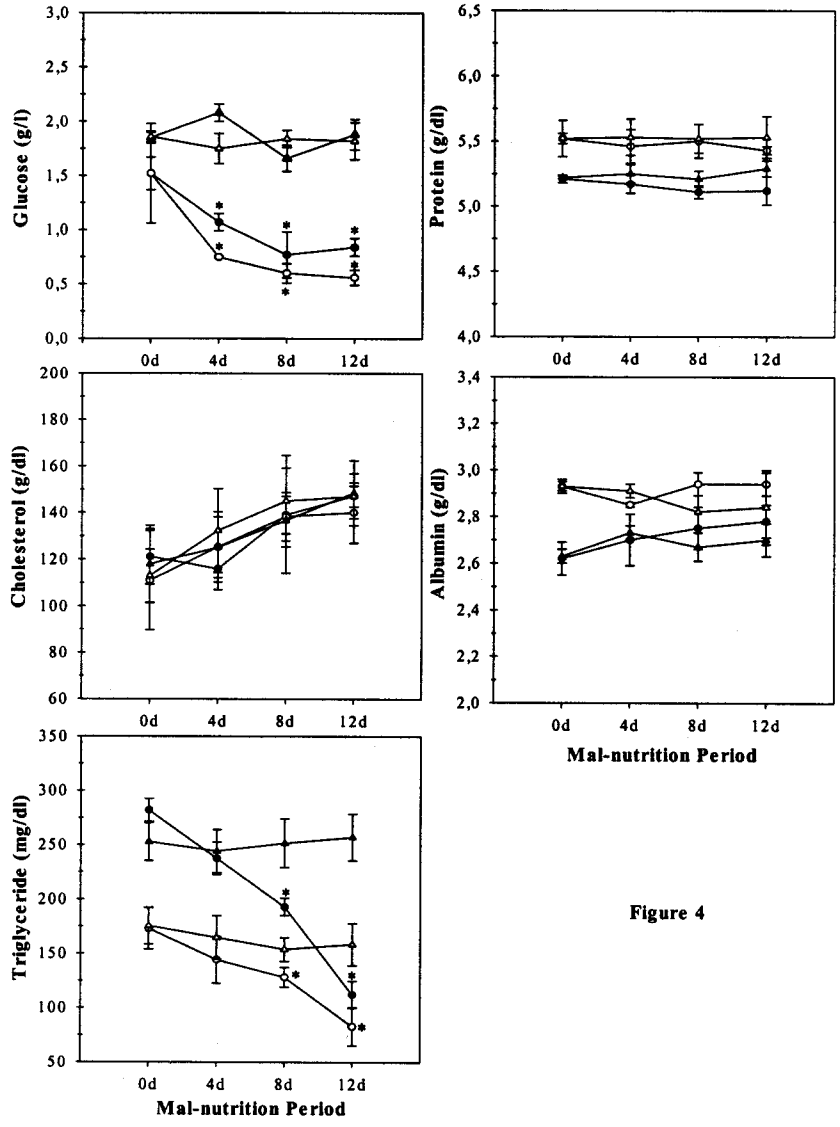

Figure 4

Fig. 4: Biochemical determinations of male and female weaned mal-nourished and well-nourished mice. The serum glucose, cholesterol, triglyceride, proteins and albumin determinations were measured at 0, 4, 8 and 12 days of mal-nutrition period.

already affected in a mild mal-nutrition. Actually, glucose, triglyceride and leukocytes number result useful tools to evaluate mild mal-nutrition, but we considered that is necessary to analyze other metabolic parameters like insulin, leptin, growth hormone, and IGF-I.

The early diagnosis is necessary for an effective and opportune nutritional intervention. The prevention of the causes that lead to the mal-nutrition, more than the treatment should be our basic objective.

Several studies about probiotics have shown that it can be used as innovative tools for treating dysfunctions caused by mal-nutrition. The spectrum of activity of probiotics can be divided into nutritional, physiological, and antimicrobial effects. Various effects are summarized as follows: improvement of the nutritional quality of food and feed; stimulation of vitamin synthesis and enzyme production; stabilization of gut microflora and competitive exclusion of enteric pathogens; enhancement of innate host defenses by production of antimicrobial substances; reduction of serum cholesterol by assimilation mechanisms; decreased risk of colon cancer by detoxification of carcinogens and tumor suppression by modulation of cell-mediated immunity. However, it was demonstrated that lactic acid bacteria and fermented products administered at severe mal-nourished mice were not able to increase the protective intestinal mechanisms to the same levels found in the well-nourished host (Perdigón and Oliver, 2000). But, in a moderate mal-nutrition animal model the positive effects of probiotics were demonstrate after a

short re-nutrition period (Perdigón *et al.*, 1994; Naidu *et al.*, 1999). Therefore, we considered that our experimental model of mild mal-nutrition is appropriate to study the beneficial effects of probiotics, and would be necessary to analyze different doses of administration for an effective response.

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Reference

Anstead, G., B. Chandrasekar, W. Zhao, J. Yang, L. Perez and P. Melby, 2001. Malnutrition alters the innate immune response and increase early visceralization following Leishmania donovani infection. Infec. Immun. 69 : 4709-4718.

Covinsky, K., M. Covinsky, R. Palmer and A. Sehgal, 2002. Serum albumin concentration and clinical assessments of nutritional status in hospitalized older people: Different sides of different coins?. J. Am. Geriatr. Soc., 50 : 631.

Dourov, N., 1986. Thymic atrophy and Immune Deficiency in Malnutrition, in H.K. Muller-Hermelink (Ed), The Human Thymus. Histophysiology and Pathology. Springer Verlag-Berlin, Heidelberg, Germany, pp 127-150.

Etukudo, M., E. Agbedana, O. Akinyinka and B. Osifo, 1999. Plasma electrolytes, total cholesterol, liver enzymes, and selected antioxidant status in protein energy malnutrition. Afr. J. Med. Med. Sci., 28 : 81-85.

Gauffin, Cano P. and G. Perdigón, 2003. Probiotics induce resistance to enteropathogens in a re-nourished mice model. J. Dairy Res. 69 : 303-216.

Gauffin, Cano P., G. Agüero and G. Perdigón, 2002a. Immunologic effects of yogurt addition to a re-nutrition diet in a malnutrition experimental model. J. Dairy Res., 69 : 303-316.

Gauffin, Cano P., G. Agüero and G. Perdigón, 2002b. Adjuvant effects of Lactobacillus casei added to a renutrition diet in a malnourished mouse model. Biocell, 26 : 35-48.

Gomez, F., R. Galvan, S. Frerenk, J. Munoz, R. Chavez and J. Vasquez, 1956. Mortality in second and three degree malnutrition. J. Trop. Pediatr., 2 77-83.

Gussinyé, M., N. Torán and A. Carrascosa, 2000. Hipoglucemia, in: Argente Oliver E., Carrascosa Lezcano A., Gracia Bouthelier R. and Rodríguez Hierro F. (Ed), Tratatado de Endocrinologia Pediatrica y de la Adolescencia. Ediciones Doyma SA, Barcelona, pp 927-940.

Ingenbleek, Y. and L. Bernstein, 1999. The stressful condition as a nutritionally dependent adaptive dichotomy. Nutrition, 15 : 305-320.

Manary, M., R. Broadhead and K. Yarasheski, 1998. Whole-body protein kinetics in marasmus and kwashiorkor during acute infection. Am. J. Clin. Nutr., 67 : 1205-1209.

McLaren, D., 1966. A fresh look at protein-calorie malnutrition. Lancet, 27 : 485-488.

McLaren, D., P. Pellett and W. Read, 1967. A simple scoring system for classifying the severe forms of protein-calorie malnutrition of early childhood. Lancet, 11 : 533-535.

Naidu, A.,. W. Bidlack and R. Clemens, 1999. Probiotic spectra of lactic acid bacteria (LAB). Crit Rev. Food Sci. Nutr., 38 : 13-126.

Pascal, N., E. Amouzou, A. Sanni, F. Namour, I. Abdelmouttaleb, M. Vidailhet and J. Guéant, 2002. Serum concentrations of sex hormone biding globuline are elevated in kwashiorkor and anorexia nervosa but not in marasmus. Am. J. Clin. Nutr., 76 : 239-244.

Perdigón, G. and G. Oliver, 2000. Modulation of the immune response of the immunosuppressed host by probiotics, in: Fuller R. and Perdigón G. (Eds), Probiotics 3: Immunomodulation by the gut microflora and probiotics. Kluwer Academic Publisher. London, pp. 148-175.

Perdigón, G., M. Rachid, M. De Budeguer and J. Valdez, 1994. Effect of yogurt feeding on the small and large intestine associated lymphoid cells in mice. J. Dairy Res., 61 : 553-562.

Rao, K., 1974. Evolution of kwashiorkor and marasmus, Lancet, 20 : 709-711.

Sungurtekin, H, U. Sungurtekin, V. Hanci and E. Erdem, 2004. Comparison of two nutrition assessment techniques in hospitalized patients. Nutrition. 20 : 428-432.

Ulibarri, J. Perez, M. Picon, E. García Benavent and A. ÄLvarez-Estrada, 2002. Early detection and control of undernourishment in hospitals. Nutr. Hosp., 17 : 139-146.

Woodward, B., 1998. Protein, calories, and immune defenses. Nutr Rev. 56-1-Pt 2 S84-92.

Woodward, B., 2001. The effect of protein-energy malnutrition on immune competence, in: R.M. Suskin and K. Tontisirin (Ed), Nutrition, immunity, and infection in infants and children (Nestlé Nutrition Workshop Serie, Pedriatic Program, Nestec Ltd). Philadelphia: Vevey/Lippincott W and W pp. 89-120.