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*Full Length Research Paper*

# Effect of a hyper-protein diet on Wistar rats development and intestinal function

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**This study was designed to investigate the long-term effects of a high-protein diet on the functional and histological structure of the intestinal epithelium. Sixteen adult male Wistar rats (180 ± 2.27 g) were divided into two groups: 1) the control group, (n = 30) were fed a normal diet of 14% protein; 2) the P50-group (n = 30) were fed a 50% protein diet. The effects of a high-protein diet were studied over a period of 2 months. Functional and morphological differences between the high-protein and control groups were compared. Internal organs (liver, stomach, lungs, heart, kidneys, spleen, intestine, skin, surrenal glands, white and brown adipose tissues) were removed from each sacrificed animal. The organs were weighed, and histological studies were performed on jejunal fragments. The weight of the P50 group rats increased 79%, while the weight of the control-group increased 98% ($p < 0.01 \leq 0.05$). The weight of the white adipose tissue, the skeleton and the skin were significantly greater in control-group rats ($p < 0.01$). An important modification of the epithelial structure in the intestine was observed in rats of the P50 group. The average length of their villi was significantly reduced and there was a significant increase in their IEL ($p < 0.01$). Our results indicate that ingestion of a protein-rich diet over a long period leads to modification of the histological structure of the intestinal epithelium, as indicated by; pronounced atrophy of mucosa; marked inflammatory infiltration of lymphocytes in the chorion; and many intra-epithelial lymphocytes.**

**Key words:** Food intake, high-protein diet, intestine, intra-epithelial lymphocytes, milk proteins.

## INTRODUCTION

Unlike other classes of macronutrients, proteins are characterised by the presence of nitrogen (Potier de Courcy et al., 2003). These nutrients represent about 15% of the body mass but they vary constantly; they are at lower levels during fast and at higher levels after meal ingestion (Beaufrère, 2002). The long term consumption of protein rich food triggers various controversial debates, some studies showed that diets with high protein content can be a source of obesity (Toyomizu et al.,1989), while other findings suggested that they induce weight loss and can be used in slimming diets and as food complements for athletes (Lacroix et al., 2004). Moreover, these protein-rich diets were reported to be prescribed in cases of hyper insulinaemia associated with obesity (Baba et al., 1999; Storlien et al., 2000; Boden et al., 2005). Nevertheless, the long-term effect of such diets on the intestinal function and in particular, whether a high consumption of proteins would show any deleterious effect on the intestinal structure, remains unknown. We studied possible presence of anti milk-protein seric IgG induced by the protein rich diet. The aim of this experiment is to analyse the impact of a high-protein diet on the functional modifications in the intestine, the evolution of general body mass and the weight of certain organs in the adult rat.

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**Table 1.** Composition of the experimental diets P14 and P50.

| Nutrient (g/kg) | Normal –protein diet (P14) | High-protein diet (P50) |
|---|---|---|
| Total milk protein | 140 | 500 |
| Saccharose | 100.3 | 50 |
| Cornstarch | 622.4 | 312.7 |
| AIN 93 M mineral mix | 35 | 35 |
| Ain 93 V vitamin mix | 10 | 10 |
| Soybean oil | 40 | 40 |
| Cellulose | 50 | 50 |
| Choline | 2.3 | 2.3 |
| Metabolizable energy (kj/g) | 14.59 | 14.59 |

## MATERIAL AND METHODS

### Animals and diets

All animals used in these experiments were cared for in accordance with criteria in the european convention for the protection of vertebrate animals. Experiments were carried out on male Wistar rats weighing 180 ± 2.71 g at beginning of the experiment (n = 60). The animals were housed in individual stainless steel cages in a room with controlled temperature (22 ± 1°C) with food and water *ad libitum*.

After weaning, the animals were fed a normal diet during 15 days until they reached 180 g of body mass (young adult). The animals were then divided into two groups: the first group (n = 30, control group) received a diet containing 14% total milk protein content (composition: 80% casein, 10% SAB, and 10% of ß-lactoglobulin (ß-Lg) and α-lactalbumin (α-La) mix, while the second group (n = 30, experimental group) received a diet containing 50% protein content. The composition of the experimental diets produced by the Atelier de Production des Aliments Experimentaux (INRA, Jouy-en-Josas, France) is shown in Table 1. The amounts of energy, fat, cellulose, minerals, and vitamins were similar in both diets. The food thus had the same consistency in both groups. The two diets were offered *ad-libitum* during 60 days. The animals and the quantity of consumed food were weighed on a daily basis.

### Blood sampling and anti milk anti proteins IgG determination

1 ml of blood was obtained from the tail of the animals (0, 7, 14, 21,… 54 days) according to the Waynforth method (1980). The blood sample was then centrifuged at 2500 rpm at 4°C during 15 min, the obtained serum was kept at -80°C for subsequent analyses of total plasma proteins and titles IgG were measured by ELISA method. Possible presence of anti-milk-protein seric IgG induced by the protein rich diet was investigated. The technique followed was adapted from the method described by Atbi et al. (2001).

### Organs

After a period of 60 days of diets, the rats were food deprived for one night. Then, they were sacrificed for body composition, weighed, anesthetized with pentobarbital via the intraperitoneal route at a dose of 30 mg/kg. The abdomen was opened, and the blood was removed with heparin from both the abdominal aorta and vena cava. The following organs were removed and weighed: intestine, liver, stomach, lungs, heart, spleen, surrenal glands, kidneys, white adipose tissue (mesenteric, epididimal, sub-cutaneous and retroperitoneal), the skin and the skeleton. A jejunal segment was also removed for the histological analysis, was cleaned with iced-Ringer at 140 mM and fixed with formol at 10% until utilisation.

### Histological analysis of the intestine

The aim of the histological analysis was to investigate any changes to the intestinal epithelium structure in rats ingesting a protein-rich diet. Special attention was directed towards checking if there has been any infiltration of lymphocytes or atrophy of the villi. The fragment was fixed in AFA (2% formalin, 5% acetic, 75% ethyl alcohol solution), embedded in paraffin and stained with hematoxylin-eosin for subsequent histological analyses for two days over which the nucleus and the cytoplasm were, respectively revealed. Briefly, paraffin was removed in toluene (56°C, 2 min) and the fragments were rehydrated in four successive ethanol-water solutions (100, 95, 90, 70°C) for 2 min and washed in water. The fragments were then incubated for 3 min in hematoxylin solution (5 g/l hematoxylin, 100 g/l potassium alun, 2.5 g/l mercuric oxide in water), incubated for 5 min in eosin solution (20 g/l eosin in ethanol), rinsed twice in 70% ethanol, incubated for 1 min in toluene, and mounted in strips for microscopy. Measurements of the height of villi at the jejunum were performed using an optical microscope equipped with a micrometer. For each tissue, three independent counts were completed, enumerating 100 enterocytes, so that intra-epithelial lymphocytes (IEL) values could be obtained for 100 enterocytes.

### Statistical methods

The results were analysed using a t-test and were presented as mean values ± SEM. A *p* value < 0.05 was considered as significant.

## RESULTS

### Body mass increase and daily average food consumption

At the beginning of the experiment body weights were the same in the two groups of rats (P14 = 180. 2 ± 1.14 g and P50 = 181. 2 ± 2.99 g). During the experiment, our results show a steady increase of body weights in the 2 groups of rats (Figure 1). This body weight stabilises from day 56 until the end of the experiment, however, from day 35, weight gain becomes significantly more important in rats of the P14 group. On day 60 of the experiment, the body weight gain was 144.8 ± 24.1 g for P50 group and 175.9 ± 22.9 g in the control group. It is worth noting that diminished daily food consumption in the P50 group rela-

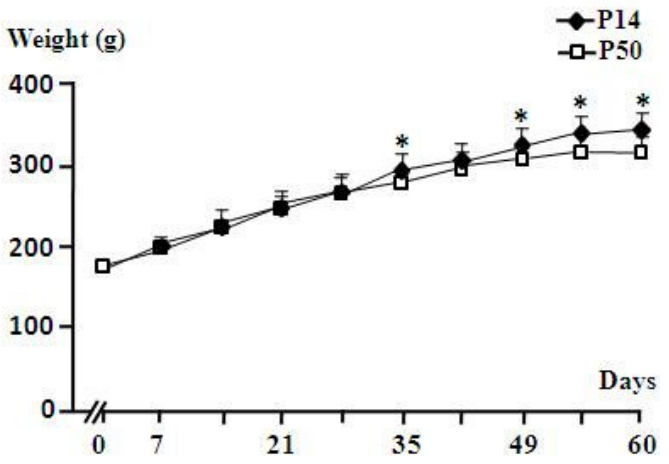


**Figure 1.** Average daily weight gain in rats fed on the normal (P14) (n = 30) and hyper protein diets (P50) (n = 30) during 2 months of experiment. Values are given as mean ± SD; *0.01 ≤ p ≤ 0.05.

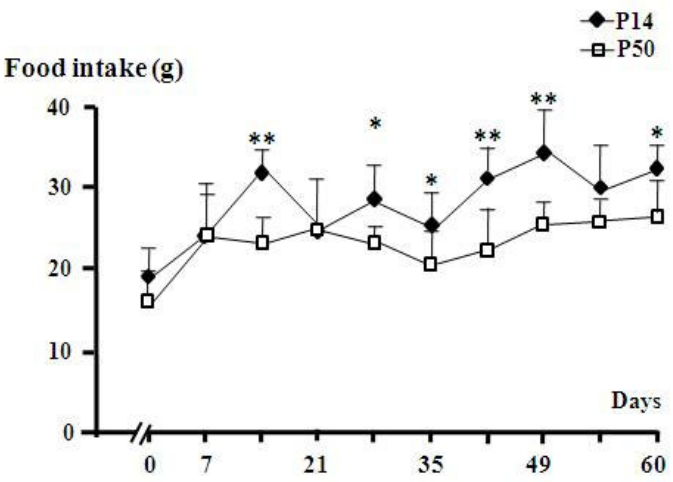


**Figure 2.** Average daily consumption in rats fed on the normal (P14) (n = 30) and hyper protein diets (P50) (n = 30). Values are given as mean ± SD; * 0.01≤ p ≤ 0.05; **0.01 ≤ p ≤ 0.05.

relative to the observed daily consumption in the P14 group was also observed (Figure 2). At the beginning of the experiment daily food consumption was not significantly different between the two groups, but on day 14 rats of P50 group reduced significantly their food intake when compared to P14 group. On day 60 of the experiment, food consumption was 17.56 ± 1.03 g for P50 group; and 21.90 ± 0.95 g for the control group (p ≤ 0.05).

### Organ weight

Weight of the white adipose tissue, the skeleton and the skin were significantly higher in rats fed with the 14% protein diet (p<0.01) (Table 2). However, there was no significant difference in the weight of the remaining organs analysed in this experiment.

### Anti-milk protein seric IgG titers

Seric IgG measurements obtained by ELISA indicate the presence of antibodies anti-casein, anti ß-Lg, anti α-Lac and anti total milk proteins. The titers vary with both the type of protein and the type of diet but the highest titers were obtained with whole milk (1/263). These results clearly show that the two diets with high protein content trigger a sensitisation by oral route against milk proteins in rats (Figure 3).

### Length of villi and lymphocyte infiltration

An important modification of the epithelial structure in the intestine was observed in rats of the P50 group when compared with the control group, the average length of villi was significantly reduced. The average height of the villi was 28.96 ± 6.19 µm (p < 0,01) (Figure 5) compared with 48.89 ± 6.96 µm in the control group; a reduction in length of approximately half, indicating marked atrophy originating from lymphocyte infiltration (IEL) (p < 0,01) (Figures 4 and 5) and the response of the gut-associated immune system. In the control group, the IEL count revealed an average lymphocyte of 17.9 ± 2.66 per 100 enterocytes, while the P50 the IEL count revealed 24.76 ± 3.44 per 100 enterocytes which was significantly higher (p < 0,01) (Figure 6). These results could possibly be the consequence of an interaction of dietary antigens with components of the immune system associated with the digestive tract, suggesting an oral sensitisation to the ingested milk protein in these animals.

## DISCUSSION

The aim of this study was to investigate the nutritional consequences of a long-term daily intake of a high protein diet; notably weight gain, (Morens et al., 2001) weight of various organs and the intestinal function. The results showed that the long term *ad libitum* consumption of a high-protein diet significantly reduced food intake and lowered WAT, in accordance with several midterm studies (Bensaid et al., 2003).

Our results show that the daily intake is reduced with a high-protein diet. This decrease is reflected in a general weight loss in the animals. These findings are in agreement with previous work (Jean et al., 2002a, b). We also demonstrated that a protein-rich diet leads to a modification of the histological structure of the intestinal epithelium, which is manifested by an atrophy of the villiae and an important increase of intra-epithelial lymphocytes. These modifications represent the manifes-

**Table 2.** Weight of organs in rats after 60 days of normal (14%) (n =30) and high (50%) (n=30) protein diets

| Weight (g) | Rats group ( 14%) | Rats group (50%) |
|---|---|---|
| Body weight | 356 ± 23.06 | 323.93 ± 20.42** |
| Liver | 10.75 ± 1.39 | 10.09 ± 0.36 |
| Spleen | 0.70 ± 0.13 | 0.63 ± 0.09 |
| Stomach | 2.23 ± 0.0.47 | 1.94 ± 0.22 |
| Intestine | 10.06 ± 1..10 | 9.97 ± 0.48 |
| Lung | 1.33 ± 0.42 | 1.4 ± 0.09 |
| Heart | 0.95 ± 0.16 | 0.95 ± 0.14 |
| Kidney | 2.20 ± 0.09 | 2.21 ± 0.18 |
| Adrenals | 0.08 ± 0.01* | 0.07 ± 0.04 |
| White adipose tissue | 41.75 ± 5.05 | 33.34 ± 3.26** |
| Skin | 54.07 ± 6.05 | 44.45 ± 3.53** |
| Skeleton | 186.60 ± 10.16 | 146.67 ± 7.52** |

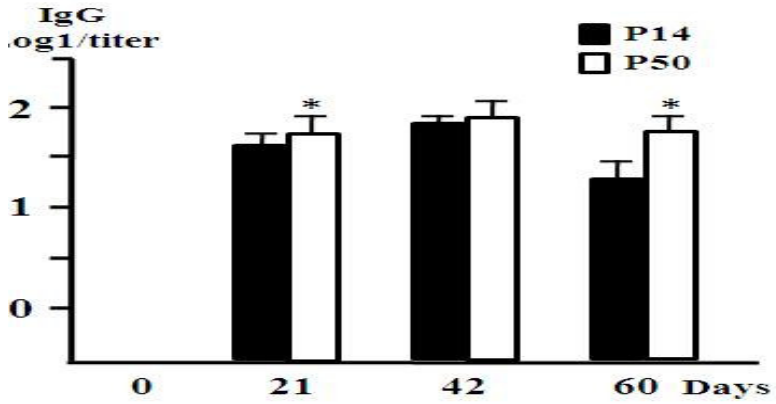


**Figure 3.** Anti-milk protein seric IgG titers for rats fed on a P14 and a P50 diets measured by enzyme-linked immunosorbent assay (ELISA) and expressed as $\log_{10}$ of 1/titer. Values are given as mean ± SD; *p< 0.001.

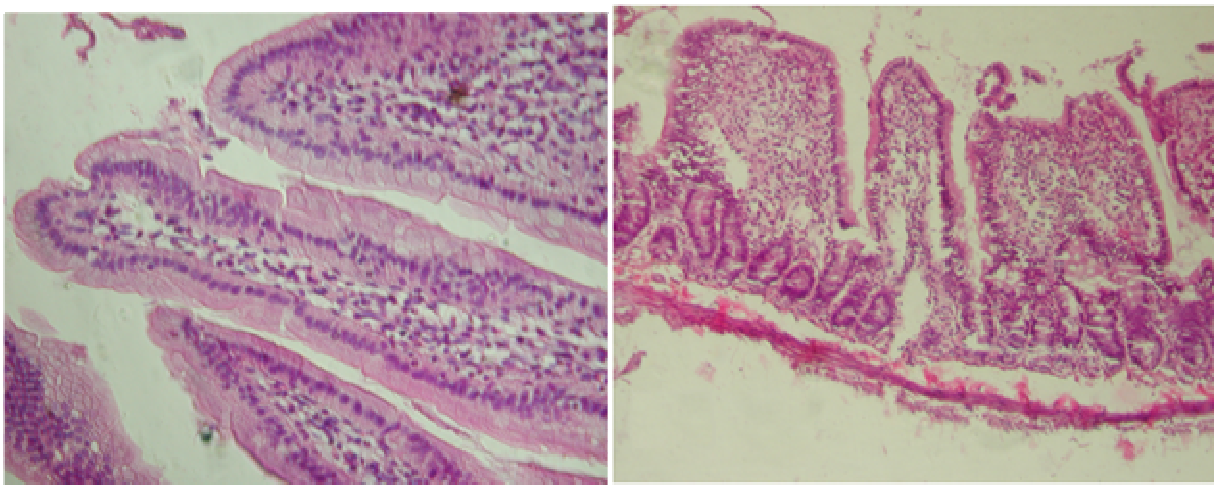


**Figure 4.** Optical microscopy observation (G x20) of a jejunal fragment of the rat intestine under the P14 and P50 diets respectively. **a**: Normal villi; **b**: Shortened villi, epithelial pseudostratification and an important lymphocyte infiltration.

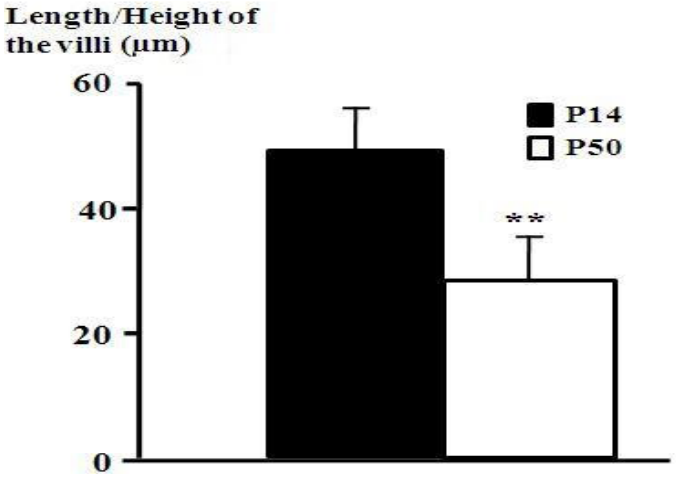


**Figure 5.** Villi length measured on jejunal fragments in rats fed on a P14 and a P50 diets. Values are given as mean ± SD; **p < 0.01.

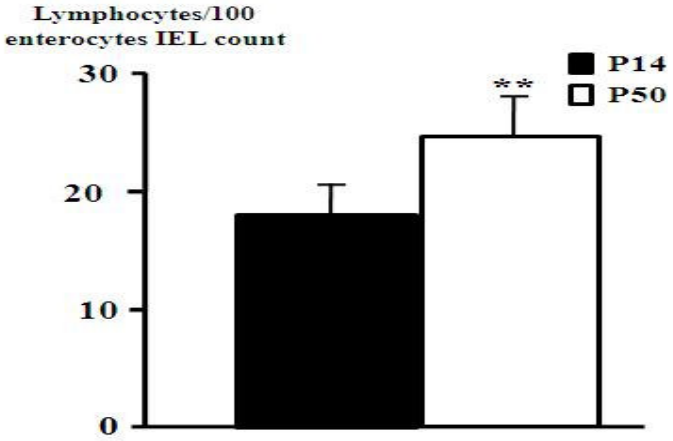


**Figure 6.** Number of intraepithelial lymphocytes (IEL) observed on the intestinal villi of the jejunal fragments in rats fed on the P14 and P50 diets. Values are given as mean ± SD; **p< 0.01.

tation of abnormal phenomena induced by a chronic exposure of the intestinal epithelium to high levels of proteins. Moreover, regular administration of diets high in protein content seems to trigger the appearance of IgG type seric antibodies against the ingested proteins (McCarty et al., 2000). This phenomenon suggests a sensitisation of the animals by oral route which can be explained by an immune response associated with the digestive tract as reflected by higher concentrations of intra-epithelial lymphocytes (Addou-Benounan et al., 2004).

## Conclusion

The results of this study show that a high consumption of proteins could affect several organs and alter the function of the intestine; therefore, caution should be exercised when administrating long term hyper-protein diets for humans. Reactivity to the antigen by the intestine has been observed with both ß-Lg and α-La sensitized animals, thus suggesting that a local immune response is not specifically an important step in the potential milk protein antigens to induce oral sensitisation and reactivity.

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