Nutrición Hospitalaria

Nutr Hosp. 2015;32(3):1398-1399
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Cartas al director

Dietary intake of AIN-93 standard diet induces fatty liver with altered hepatic fatty acid profile in Wistar rats

La ingesta de la dieta estándar AIN-93 induce esteatosis hepática con aletración del perfil de ácidos grasos en ratones Wistar

To the editor:

As basic researchers in clinical nutrition area, it was with great interest that we read the recent Santos JF et al. article[1] describing hepatic steatosis associated to changes in liver fatty acids profile in Wistar rats fed *ad libitum* a standard diet AIN-93, at time points of one and four months. We recognize the value of such investigation because a standard nutritionally balanced diet is important for the welfare of laboratory rodents and to ensure that experimental results are not biased by unintended nutritional factors[2]. However, there are some major drawbacks in this study protocol that may limit the validity of its conclusions.

Current evidences support that *ad libitum* feeding is an uncontrolled variable in animal studies that may lead to metabolic disorders by accelerating body weight gain, decreasing glucose tolerance and peripheral insulin sensitivity, and increasing liver fat [3,4]. When comparing to dietary restriction, *ad libitum* feeding is also associated to an increase in size of different organs due to degenerative and proliferative injury, including liver [5,6]. Some of these alterations were observed in Santos JF et al. study using this kind of feeding and then may not be directly associated to the type of diet offered.

Moreover, Santos JF et al. compared nonindustrial manufactured AIN-93 diets to an industrialized commercial diet [1]. We do not feel comfortable with such approach because the industrial process enrolls standardized safety procedures to produce diets for laboratory animals that not only ensure the proper animal development, but also avoid contaminations by pathogenic microorganisms [6]. In this regard, diet pelletization requires appropriate well-controlled time, temperature and pressure of drying to avoid fungi fermentation and proliferation within the chow due to humidity, which can be better achieved industrially [6].

Unfortunately, Santos JF et al. did not detailed the mechanism underlying the pellet formation to support that potential fungi contamination did not occurs in provided AIN-93 diets and affected the hepatic response[1]. Furthermore, the American Institute of Nutrition states that AIN-93 diets should be stored at 4° C and its deterioration monitored periodically [7], but these authors also did not report if this specific refrigeration temperature and deterioration monitoring was properly attended [1].

Based on these observations, it would seem crucial to us to maintain consistency in using comparatives commercial diets or laboratory diets offered at standardized amounts and at fixed times to avoid bias within studies assessing the physiological and biochemical impact of the AIN-93 diet intake in rats.

References

1. Santos JF, Amaral MS, Oliveira SL, Barbosa JP, Cabral-Jr CR, Melo IS, Bueno NB, Freitas JD, Sant'ana AG, Ataíde TR. Dietary intake of AIN-93 standard diet induces fatty liver with altered hepatic fatty acid profile in Wistar rats. *Nutr Hosp*. 2015;31(5):2140-2146.
2. Hoevenaars FP, van Schothorst EM, Horakova O, Voigt A, Rossmeisl M, Pico C, Caimari A, Kopecky J, Klaus S, Keijer J. BIOCLAIMS standard diet (BIOsd): a reference diet for nutritional physiology. *Genes Nutr.* 2012;7(3):399-404.
3. Haseman JK, Ney E, Nyska A, Rao GN. Effect of diet and animal care/housing protocols on body weight, survival, tumor incidences, and nephropathy severity of F344 rats in chronic studies. *Toxicol Pathol*. 2003;31(6):674-81.
4. Keenan KP, Laroque P, Ballam GC, Soper KA, Dixit R, Mattson BA, Adams SP, Coleman JB. The effects of diet, ad libitum overfeeding, and moderate dietary restriction on the rodent bioassay: the uncontrolled variable in safety assessment. *Toxicol Pathol*. 1996;24(6):757-68.
5. Moraal M, Leenaars PP, Arnts H, Smeets K, Savenije BS, Curfs JH, Ritskes-Hoitinga M. The influence of food restriction versus ad libitum feeding of chow and purified diets on variation in body weight, growth and physiology of female Wistar rats. *Lab Anim*. 2012;46(2):101-7.
6. NATIONAL RESEARCH COUNCIL Nutrient *Requeriments of Laboratory Animals*. (Fourth Revised Edition, 1995). Washington, D.C.: National Academy Press, 1995, 173p.
7. Reeves PG, Nielsen FH, Fahey Jr GC. AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. *J. Nutr.* 1993;123(11):1939-51.

Priscila Garla, Raquel Susana Torrinhas, Alweyd Tesser, Felipe Aprobato, Ronaldo Oliveira Filho, Márcia Antunes and Dan Linetzky Waitzberg

Nutrition Laboratory and Metabolic Surgery Digestive - Medical Research Laboratory (LIM 35), Faculty of Medicine - University of São Paulo.

Correspondence: Priscila Garla.
E-mail: prigarla@gmail.com

Recibido: 11-V-2015.
Aceptado: 18-VI-2015.

The critical reflection brought by the letter from Garla et al. about our findings on the effects of AIN-93 standard diet in Wistar rats[1] presents interesting elements.

Concerning the arguments about the *ad libittum* regime in the diet offer, although well founded and indeed a possible triggering factor of the observed hepatic steatosis, we would like to remind that this procedure was similarly adopted for all groups and even then, the groups fed with the commercial diet did not exhibited (commercial diet group – 1 month) or exhibited a more modest change in the histological pattern (commercial diet group – 4 months), when compared to the groups fed with the AIN-93 diet; it means different metabolic effects of the diets and a worse impact caused by the AIN-93. Therefore, the argument that the AIN-93 formulation promoted hepatic steatosis, unlike the commercial diet and regardless of the offering period, becomes relevant.

It is pertinent to indicate, in addition to the group's own experience in previous experimental protocols[2], that liver and even kidney changes were associated with the use of AIN-93 diet, according to reports from other authors[3,4], corroborating the findings of our investigation.

Regarding the handling of the formulation, an equally important concern, we recognize that the use of industrialized formulas offers greater standardization assurance of planned dietary treatments, but we take the opportunity to point out that besides the cautious control of AIN-93 diet preparation and storage (4° C for 15 days at maximum) in the study under discussion, similar liver abnormalities results were found in a recent study conducted by the group[5] in which this diet was purchased ready to use from a specialized commercial laboratory, leading us to confirm consistent the data.

References

1. Santos JF, Amaral MS, Oliveira SL, Barbosa JP, Cabral-Jr CR, Melo IS, Bueno NB, Freitas JD, Santana AG, Ataíde TR. Dietary intake of AIN-93 standard diet induces fatty liver with altered hepatic fatty acid profile in Wistar rats. Nutr Hosp. 2015;31(5):2140-2146.
2. Ataíde TR, Oliveira SL, Silva FM, Vitorino Filha LGC, Tavares MCN, Sant'Ana AEG. Toxicological analysis of the chronic consumption of diheptanoin and triheptanoin in rats. *Int J Food Sci & Tech*. 2009; 44(3):484-492.
3. Lien EL, Boyle FG, Wrenn JM, Perry RW, Thompson CA, Borzelleca JF. Comparison of AIN-76A and AIN-93G diets: a 13-week study in rats. *Food Chem Toxicol*. 2001;39(4):385-92.
4. Klurfeld DM. Kidney and bladder stones in rodents fed purified diets. *J Nutr*. 2002; 132(12):3784.
5. Santos JCF, Araújo ORP, Valentim IB, Andrade KQ, Moura FA, Smaniotto SS, Marques dos Santos JM, Gasparotto J, Gelain DP, Goulart MOF. Choline and Cystine Deficient Diets in Animal Models with Hepatocellular Injury: Evaluation of Oxidative Stress and Expression of RAGE, TNF-α, and IL-1β**.** Oxid Med Cell Longev. 2015; Article ID 121925.

Juliana Farias Santos[1], Monique Suruagy Amaral[1], Suzana Lima Oliveira[1], Júnia Porto Barbosa[1], Cyro Rego Cabral-Jr[1], Ingrid Sofia Melo[1], Nassib Bezerra Bueno[1], Johnatan Duarte Freitas[2], Antônio Goulart Sant'ana[3] and Terezinha Rocha Ataíde[1]

Faculdade de Nutrição - Universidade Federal de Alagoas. [2]Coordenadoria de Química - Instituto Federal de Alagoas. [3]Instituto de Química e Biotecnologia - Universidade Federal de Alagoas, Brasil.

DOI:10.3305/nh.2015.32.3.9225