Influence of a GMO-containing diet on pancreatic acinar cells of adult mice: effects of a short-term diet reversion

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Summary
Previous studies on mice fed on a genetically modified (GM) soybean showed that changes in zymogen synthesis and processing as well as in cell nuclear activity take place in pancreatic acinar cells. In this study, we aimed at elucidating whether these modifications can be reversed. To do this, mice fed on GM soybean from their weaning to the third month of age were administered a diet containing control soybean for one additional month. In parallel, to investigate the influence of GM soybean on adult individuals, 3 month-old mice fed from their weaning on control soybean were administered a GM-containing diet for one month. Morphometry, cytochemistry and immunocytochemistry were used to analyse pancreatic acinar cells at light and electron microscopy. Our results demonstrate that a one month-diet reversion in adult mice can influence some morpho-functional features of pancreatic acinar cells, restoring in GM-fed mice some characteristics typical of controls and inducing in control mice modifications similar to those observed in animals fed on GM soybean from weaning. This implies that the modifications related to GM soybean are potentially reversible, but also that some modifications are inducible in adult organisms in relatively short time. Although the mechanisms responsible for such modifications still remain unidentified, these results confirm the need of further investigations to go deeper the possible consequences of GM food consumption.

Keywords: cell nucleus, diet, exocrine pancreas, genetically modified soybean, zymogen.

Introduction

The exocrine pancreas is an essential organ for food processing: its function consists in the synthesis, storage, and secretion of the different digestive enzymes, whose production depends on diet composition [1-4]. Both functional and structural modifications of pancreatic acinar cells have been described in relation to dietary changes [e.g. 5-7].

Genetically modified (GM) crops are the result of modern biotechnology and their introduction in human and animal diets is becoming more and more frequent [8]. Nowadays there is a general concern about the possible risks associated with their use, such as potential allergenity, toxicity and environmental side effects [9]. However, a limited number of studies have so far been made to assess the impact of GM-containing diets on animal and human health [e.g. 10-17], and very few data are available about the response of exocrine pancreas to GM food intake [13, 14, 17].

Previous studies on mice fed on a GM soybean showed that, although no structural modification occurs in pancreatic acinar cells, changes in zymogen synthesis and processing as well as in cell nuclear activity take place [13, 14].

In the present study, we aimed at elucidating whether these modifications can be reversed. To do this, mice fed on GM soybean from their weaning to the third month of age were administered a diet containing control soybean for one additional month. In parallel, to investigate the influence of GM soybean on adult individuals, 3 month-old mice fed from their weaning on control soybean were administered a GM-containing diet for one month. Morphometrical, cytochemical and immunocytochemical analyses at light and electron microscopy were carried out on pancreatic acinar cells, focus-

sing both on cellular organelles involved in zymogen pathways and on nuclear components responsible for RNA synthesis and maturation. The results obtained have been compared to the previously published ones on mice which always received either control or GM soybean [13, 14].

Materials and methods

Pregnant Swiss mice were fed ad libitum on a standard laboratory chow containing 14% GM soybean [12], a percentage corresponding to that usually present in the standard diet of this mouse strain and also included in the range (11-33%, [18]) generally used in the regulatory tests for GMOs. This GM soybean has been obtained by insertion of the bacterial CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) gene conferring a high level of tolerance to glyphosate, the active ingredient of the herbicide Roundup (GTS 40-3-2; [19]). In parallel, other pregnant mice were fed on the same diet with commercial, non GM soybean. Twelve female mice of the litter (six from each experimental group) were grown on the parental diet from weaning until the third month of age. Then, the diets were reversed: the control group was fed on GM soybean (named control-to-GM mice), while the GM-fed group was fed on control soybean (named GM-to-control mice). All animals were fed on the reversed diets for one month and then killed by cervical dislocation.

Samples of pancreas were quickly removed and processed for electron microscopy. They were fixed with 4% paraformaldehyde, dehydrated with ethanol and embedded in LRWhite resin as previously described [14]. Semithin sections (2 µm thick) were stained with 1% toluidine blue and observed with an Olimpus BX51 light microscope. Ultrathin sections were stained with either lead citrate or the EDTA method [20], for the visualisation of ribonucleoprotein constituents, and observed in a Philips CM 10 transmission electron microscope operating at 80 kV.

Morphometrical analyses were performed both at light and electron microscope. On semithin sections, 30 pancreatic acinar cells per animal were chosen from tele-insular regions; all cells contained both the nucleus and the zymogen granules [21]. The cellular, nuclear and total zymogen granule areas were measured on micrographs taken with a x100 oil-immersion lens by a computer-aided image analysis system (Olympus DP-Soft 3.0 for Windows 98). The cytoplasmic area, the nucleus/cytoplasm (N/C) ratio and the percentage of cytoplasmic area occupied by zymogen were then calculated.

Further morphometrical evaluations were made at the electron microscope on 15 pancreatic acinar cell nuclei per animal (final magnification: x15000). Nucleolar area, percentages of fibrillar centres (FCs), dense fibrillar component (DFC) and granular component (GC) per nucleolus, FC area, index of nuclear shape irregularity (the ratio between the measured perimeter and the circumference of the equivalent circle) and nuclear pore frequency (NP/µm of perimeter) were evaluated. Finally, the area of 100 zymogen granules per animal was measured.

In order to investigate the fine cellular distribution of α-amylase, a major pancreatic enzyme, immunocytochemical analyses were carried out by using a rabbit anti-α-amylase antibody (Sigma, Buchs, Switzerland). Ultrathin sections were floated for 3 min at room temperature in normal goat serum (NGS) diluted 1:100 in phosphate buffered saline (PBS) and then incubated for 17 h at 4°C with the primary antibody (Sigma, Buchs, Switzerland) diluted 1:1500 in a solution containing 0.1% bovine serum albumin (Fluka, Buchs, Switzerland) and 0.05% Tween 20 in PBS. After rinsing in PBS, sections were floated on NGS diluted 1:100 in PBS and then reacted for 20 min at room temperature with the goat anti-rabbit IgG secondary 12 nm gold-conjugated antibody (Jackson ImmunoResearch, Cardiff, UK) diluted 1:10 in PBS. Sections were then rinsed, air-dried and stained with lead citrate. As controls, some grids were treated with the incubation mixture without the primary antibody and then processed as described above.

The labelling density of α-amylase was evaluated in the rough endoplasmic reticulum (RER), the Golgi area (comprising the smooth vesicles, tubules, cisternae and the condensing vacuoles) and zymogen granules on 10 electron micrographs per animal (final magnification: x36000) by using a computerized image analysis system (Image Pro-Plus for Windows 95). The gold grains present over each compartment were counted and the labelling density was expressed as the number of gold grains per square micrometre.

All statistical comparisons were performed by the Kruskal-Wallis one way ANOVA test and the significance level was set at $p \leq 0.05$.

Results

Neither macroscopic alterations nor pathologic lesions were observed in the pancreatic gland of all animals at the time of death. Morphological observations carried out by light microscopy demonstrated similar structural features of the exocrine pancreas in the two experimental groups. In all mice the pancreatic acinar cells were organised in acini and showed the typical aspect of secretory polarized cells. The roundish nuclei occurred in the basal part of the cell, the surrounding cytoplasm was intensely stained because of the abundant RER and zymogen granules were grouped in large clusters located in the apical region, near the acinar lumen (Figure 1).

Accordingly, electron microscopy revealed the same intracellular organisation in all animals. The RER, arranged in parallel cisternae, was particularly abundant in the perinuclear region; the Golgi complex was located in the supranuclear region and showed numerous and well developed cisternae and vesicles; a large number of zymogen granules occurred in the apical region of the cell; the mitochondria showed many transversal cristae. In EDTA-stained samples the fine organisation of nuclear structural constituents was clearly observed. The pancreatic acinar cell nuclei of all animals contained large clumps of condensed chromatin distributed along the nuclear envelope and at the periphery of nucleoli; in the nucleoplasm, no difference between the two animal groups was observed in the distribution of perichromatin fibrils, perichromatin granules and interchromatin granules, i.e. those structures involved in pre-mRNA transcription and splicing [22]. On the other hand, the nucleoli of control-to-GM mice were generally smaller and contained larger FCs than GM-to-control animals (Figure 2).

The results of the morphometric evaluations are described in Tables 1 and 2. Briefly, cell, cytoplasm and zymogen granule area, nuclear shape index, pore frequency, percentage of FC, DFC and GC were similar in the two experimental groups. Conversely, nuclear area, N/C ratio, zymogen percentage, FC area were significantly higher and nucleolar area significantly lower in control-to-GM mice in comparison to GM-to-control animals.

As shown in Figure 3, in pancreatic acinar cells the α-amylase labelling was distributed over the RER, the Golgi complex area and the zymogen granules. As expected, the mitochondria and nuclei appeared devoid of gold grains. The labelling density over the zymogen granules (Table 3) was significantly higher in control-to-GM than in GM-to-control mice.

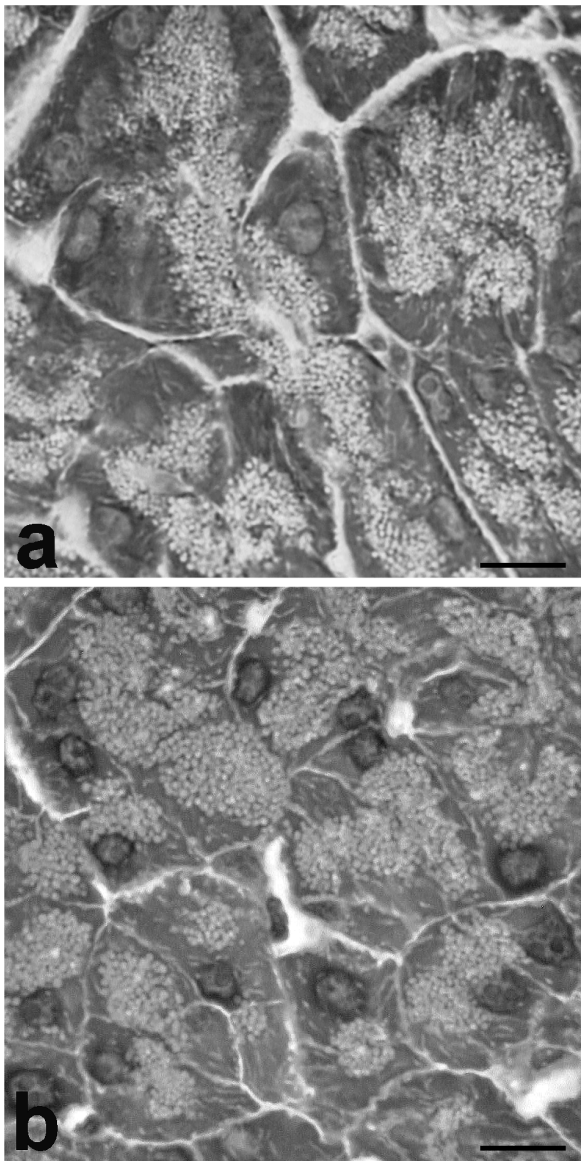

Figure 1. Exocrine pancreas from a control-to-GM (a) and a GM-to-control mouse (b); LRWhite-embedded, toluidine blue-stained samples. Zymogen area appears as an aggregate of small granules located in the apical region of the cytoplasm, while roundish nuclei occur in the basal part of the cell. Bars: 10 μm.

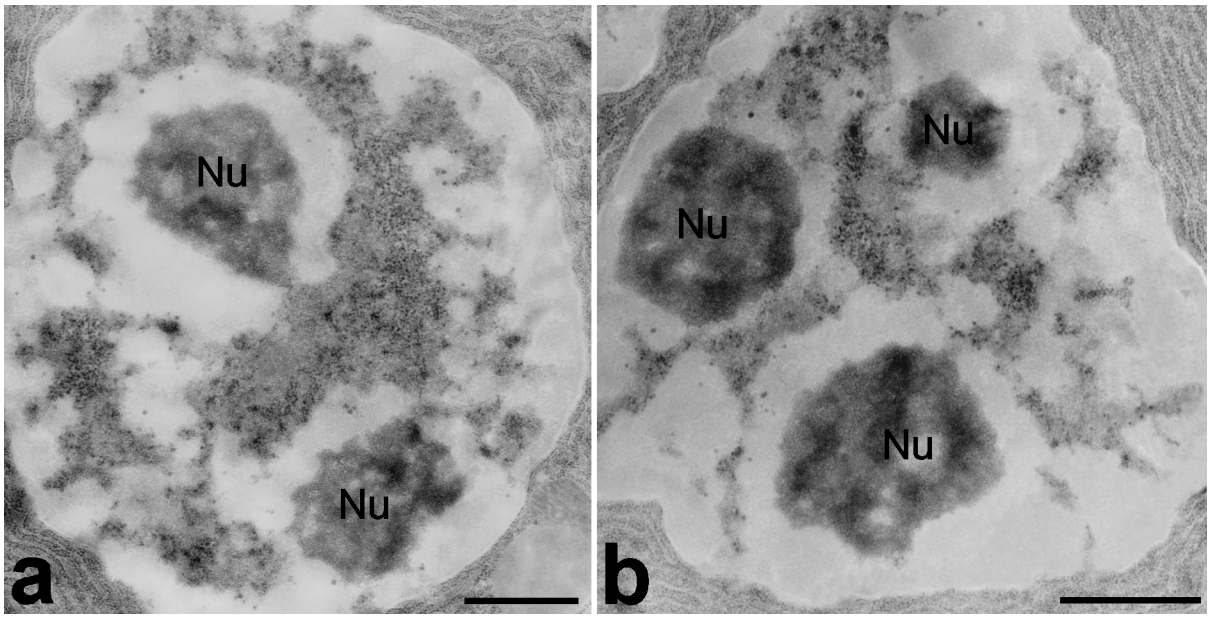

Figure 2. Pancreatic acinar cell nuclei from a control-to-GM (a) and a GM-to-control mouse (b); LRWhite-embedded, EDTA-stained samples. The general aspect of the nucleus is similar; however, the nucleoli (Nu) appear generally larger in GM-to-control mice. Bars: 1 μm.

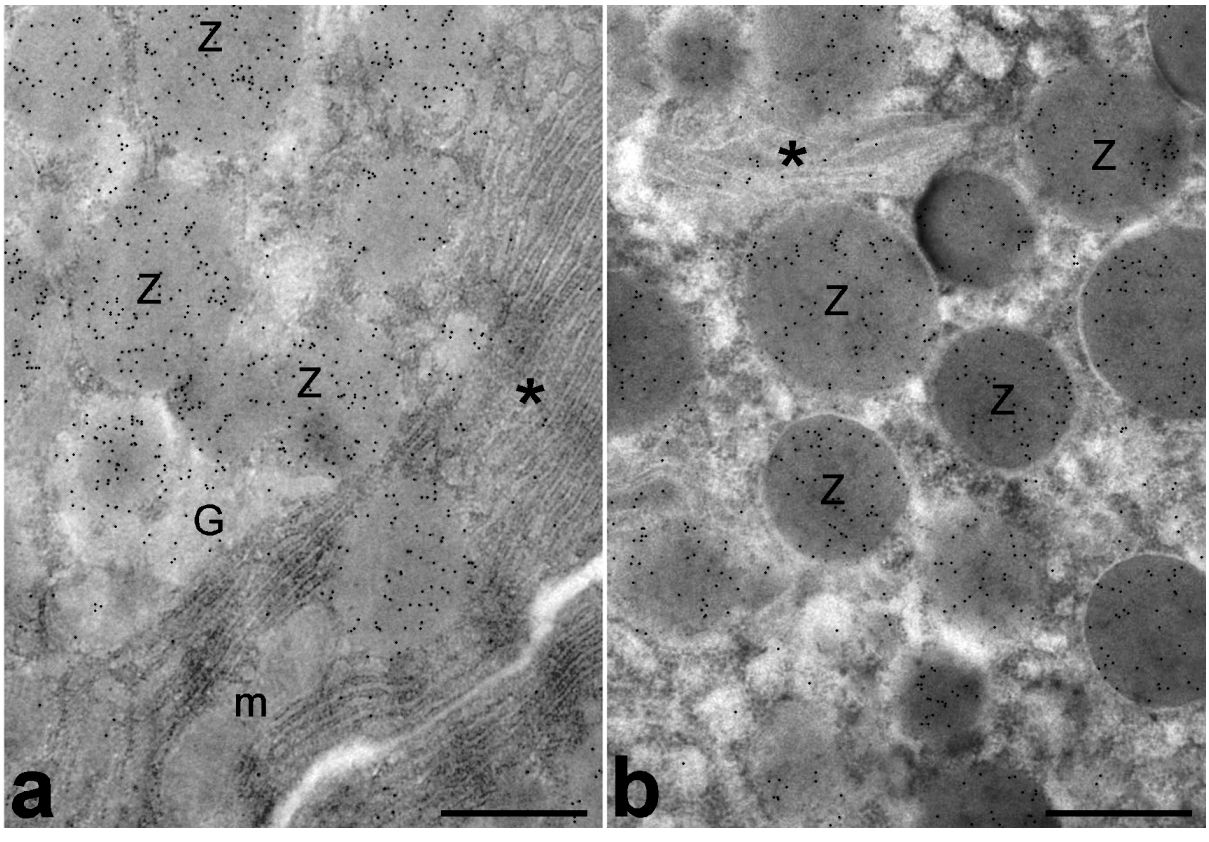

Figure 3. Distribution of α-amylase in pancreatic acinar cells from a control-to-GM (a) and a GM-to-control mouse (b); LRWhite-embedded, lead citrate-stained samples. RER (asterisks), Golgi complexes (G) and zymogen granules (z) are specifically labelled, whereas mitochondria (m) are devoid of gold grains. The labelling over zymogen granules appears stronger in a than in b. Gold grain contrast was digitally enhanced by Adobe Photoshop. Bars: 0.5 μm.

Discussion

In the present study, we further investigated the role of GM soybean intake in the induction of morpho-functional modifications in pancreatic acinar cells of adult mice. In fact, in previous reports [13-14] we described both cytoplasmic and nuclear changes in pancreatic acinar cells of mice fed on a GM soybean from their weaning to the eighth month of age; however, we did not established whether such changes were permanent or strictly dependent on the GM food intake.

According to our previous observations on control and GM-fed mice [13-14], here we demonstrate that the general organization and the fine morphology of pancreatic acinar cells are similar in control-to-GM and GM-to-control mice, thus confirming that GM soybean intake does not induce marked modifications in the cell structural constituents. Similarly, the absence of statistically significant differences in cellular and cytoplasmic area, as well as in the nucleolar percentage of DFC and GC between control-to-GM and GM-to-control mice confirms previously published data on pancreatic acinar cells from control and GM-fed mice, thus demonstrating that these cellular constituents are not affected by the GM soybean-containing diet.

The nuclear area, the N/C ratio and the zymogen-containing area are higher in control-to-GM than in GM-to-control mice; taking into account that these values were observed to be lower in controls than in GM-fed mice [13], it may be suggested that a GM-containing diet can have an influence on these features of pancreatic acinar cells.

Other variables analysed in the present study, namely zymogen granule area, nuclear shape index, pore frequency, nucleolar percentage of FC and α-amylase density in RER and Golgi apparatus do not show significant differences between control-to-GM and GM-to-control mice, although they were found to markedly differ between control and GM-fed mice [13-14]. We may hypothesize that a one-month diet reversion is able to induce only a partial reversion of these features, and that a more prolonged period could be necessary to obtain a complete restoration of the original differences.

Some variables such as FC area and α-amylase density in zymogen granules maintain a difference between control-to-GM and GM-to-control mice similar to that found between control and GM-fed mice [13-14]. In this case, the one-month diet reversion does not influence these features, which therefore seem to need longer period to undergo modifications.

Finally, the nucleolus shows a larger size in GM-

Table 1. Mean ±SE values of variables considered in pancreatic acinar cells of the two animal groups.

	Cell area (μm^2)	*Nuclear area (μm^2)*	*Cytoplasm area (μm^2)*	*N/C ratio*	*Zymogen area %*	*Zymogen granule area (μm^2)*
Control-to-GM	136.83±5.95	22.55±0.79	114.28±5.42	0.22±0.01	22.74±1.19	0.15±0.01
GM-to-control	143.29±4.85	19.67±0.69	123.61±4.48	0.17±0.01	19.10±1.04	0.16±0.01
p	0.40	0.007	0.19	<0.001	0.02	0.75

Table 2. Mean ±SE values of variables considered in pancreatic acinar cell nuclei of the two animal groups.

	Nuclear shape index	*Chromatin %*	*Pore frequency (pore nr/μm^2)*	*Nucleolar area (μm^2)*	*FC area (μm^2)*	*FC %*	*DFC %*	*GC %*
Control-to-GM	1.20±0.02	22.64± 1.03	1.06± 0.09	1.18± 0.13	0.03± 0.004	2.32± 0.58	23.47± 1.93	74.20± 1.90
GM-to-control	1.22±0.01	24.20± 1.74	0.92± 0.05	1.73± 0.20	0.02± 0.001	1.64± 0.35	21.98± 1.92	76.38± 1.99
p	0.42	0.45	0.23	0.03	0.04	0.31	0.58	0.43

Table 3. Mean±SE values of labelling densities obtained with the anti-α-amylase antibody in pancreatic acinar cells (gold particles/μm²).

	RER	*Golgi complex*	*Zymogen*
Control-to-GM	26.07±2.69	64.99±7.86	192.21±6.09
GM-to-control	35.62±4.83	55.99±7.48	151.61±4.29
p	0.10	0.42	<0.001

to-control mice than in control-to-GM ones, while no difference was found between control and GM fed mice [13, 14]. The nucleolus is a very dynamic structure able to rapidly change its architecture in response to various stimuli, including diet [23-26]. Since larger nucleoli are generally found in cells with a higher metabolic rate [24], a more intense nucleolar activity could be hypothesised in GM-to-control mice. Accordingly, the same animals show a smaller FC size, which is also an index of increased rRNA synthetic rate [24].

In summary, our results demonstrate that a one month-diet reversion in adult mice can influence some morpho-functional features of pancreatic acinar cells, restoring in GM-fed mice some characteristics typical of controls and inducing in control mice modifications similar to those observed in animals fed on GM soybean from weaning. This implies that the modifications related to GM soybean are potentially reversible, but also that some modifications are inducible in adult organisms in relatively short time.

Interestingly, most of the effects of the diet reversion observed in pancreatic acinar cells are comparable to those previously reported for hepatocytes of mice submitted to the same experimental treatment [27], indicating that GM soybean intake can exert a similar influence –mainly on both RNA and protein synthesis/processing - on different tissues. It is likely that the exocrine pancreas and the liver are especially sensitive to a change in the diet due to their central role in the digestion and processing of food [5-7, 23, 25, 26].

At present, no conclusive evidence exists as to the factor(s) which can induce the modifications observed. The GM soybean used in our studies has been treated in the field with the herbicide Roundup and, although the treatment conditions were not specified by the manufacturer, it is worth considering the possible presence in the crops of traces of Roundup or its metabolites [28, 29]. Interestingly, Roundup has been demonstrated to interfere with nuclear functions [30-32] as well as to alter many metabolic pathways [e.g. 33-36]. However, we cannot exclude that some other factors than the presence of herbicide residues could be responsible for the cellular modifications described in GM-fed mice. For instance, this GM soybean has been reported to contain amounts of phytoestrogens lower than the non-GM ones [37] and, taking into account the influence these compounds exert at multiple levels of cell activity [38, 39], a difference in their daily intake could play a role in the tissue modifications in mice fed on GM soybean.

The present work provides additional data on the role of GM soybean in the induction of morpho-functional modifications in pancreatic acinar cells in adult mice; although the mechanisms responsible for such modifications still remain unidentified, these results confirm the need of further investigations to go deeper the possible consequences of GM food consumption.

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References

1. Wicker C, Puigserver A. Effects of inverse changes in dietary lipid and carbohydrate on the synthesis of some pancreatic secretory proteins. Eur J Biochem 1987;162:25-30.
2. Schick J, Verspohi R, Kern H, Sheele G. Two distinct adaptive responses in the synthesis of exocrine pancreatic enzymes to inverse changes in protein and carbohydrate in the diet. Am J Physiol 1984;247: G611-G616.
3. Gingras D, Bendayan M. Evaluation of pancreatic amylase mRNA upon cholinergic stimulation of secretion. J Histochem Cytochem 2005;53:93-103.
4. Lee KY, Ahn HC, Kim C, Kim SH, Kim DK, Park HS. Pancreatic exocrine response to long-term high-fat diets in rats. J Pancreas 2006;7:397-404.
5. Brown RA, Thompson AG, McArdle AH, Gurd FN.

Alteration of exocrine pancreatic storage enzymes by feeding on an elemental diet: a biochemical and ultrastructural study. Surg Forum 1970;21:391-393.
6. The TL, Maxwell WL, Thirumalai C. Light microscopic and ultrastructural changes of the exocrine pancreas in magnesium deficient rats. Exp Pathol 1980;18:245-253.
7. Brannon PM. Adaptation of the exocrine pancreas to diet. Annu Rev Nutr 1990;10:85-105.
8. Sanvido O, Romeis J, Bigler F. Ecological impacts of genetically modified crops: ten years of field research and commercial cultivation. Adv Biochem Eng Biotechnol 2007;107:235-278.
9. Celec P, Kukuchovà M, Renczésovà V, Natarajan S, Pàlffy R, Gardlik R, Hodosy J, Behuliak M, Vlkovà B, Szemes S, Turna J. Biological and biomedical aspects of genetically modified food. Biomed Pharmacother 2005;59:531-540.
10. Ewen SW, Pustzai A. Effects of diets containing genetically modified potatoes expressing Galanthus nivalis lecitin on rat small intestine. Lancet 1999;354:1353-1354.
11. Chiter A, Forbes JM, Blair GE. DNA stability in plant tissues: implications for the possibile transfer of genes from genetically modified food. FEBS Lett 2000;481:164-168.
12. Malatesta M, Caporaloni C, Gavaudan S, Rocchi MBL, Tiberi C, Gazzanelli G. Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. Cell Struct Funct 2002;27:173-180.
13. Malatesta M, Caporaloni C, Rossi L, Battistelli S, Rocchi MBL, Tonucci F, Gazzanelli G. Ultrastructural analysis of pancreatic acinar cells from mice fed on genetically modified soybean. J Anat 2002;201:409-416.
14. Malatesta M, Biggiogera M, Manuali E, Rocchi MBL, Baldelli B, Gazzanelli G. Fine structural analyses of pancreatic acinar cell nuclei from mice fed on GM soybean. Eur J Histochem 2003;47:385-388.
15. Vecchio L, Cisterna B, Malatesta M, Martin TE, Biggiogera M. Ultrastructural analysis of testes from mice fed on genetically modified soybean. Eur J Histochem 2004;48:449-453.
16. Malatesta M, Boraldi F, Annovi G, Baldelli B, Battistelli S, Biggiogera M, Quaglino D.: A long-term study on female mice fed on a genetically modified soybean: effects on liver ageing. Histochem Cell Biol 2008 (in press).
17. Trabalza-Marinucci M, Brandi G, Rondini C, Avellini L, Giammarini C, Costarelli S, Acuti G, Orlandi C, Filippini G, Chiaradia E, Malatesta M, Crotti S, Antonini C, Amagliani G, Manuali E, Mastrogiacomo AR, Moscati L, Haouet MN, Gaiti A, Magnani M. A three year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance on sheep. Livestock Sci 2008;113:178-190.
18. Séralini GE, Cellier D, Spiroux de Vendomois J. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Arch Environ Contam Toxicol 2007;52:596-602.
19. Padgette SR, Kolacz KH, Delannay X, Re DB, LaVallee BJ, Tinius CN, Rhodes WK, Otero YI, Barry GF, Eichholtz DA, Peschke VM, Nida DL, Taylor NB, Kishore GM. Development, identification and characterization of a glyphosate-tolerant soybean line. Crop Sci 1995;35:1451-1461.
20. Bernhard W. A new staining procedure for electron microscopical cytology. J Ultrastruct Res 1969; 27: 250-265.
21. Gingras D, Bendayan M. Differences in secretory granule content in pancreatic acinar cells from peri-insular and tele-insular regions. Pancreas 1992; 7:477-485.
22. Fakan S. Ultrastructural cytochemical analyses of nuclear functional architecture. Eur J Histochem 2004;48:5-14.
23. Bailey RP, Vrooman MJ, Sawai Y, Tsukada K, Short J, Lieberman I. Amino acids and control of nucleolar size, the activity of RNA polymerase I, and DNA synthesis in liver. Proc Natl Acad Sci USA 1976;73:3201-3205.
24. Schwarzacher HG, Wachtler F. The nucleolus. Anat Embryol 1993;188:515-536.
25. Lopez-Navarro AT, Bueno JD, Gil A, Sanchez-Pozo A. Morphological changes in hepatocytes of rats deprived of dietary nucleotides. Br J Nutr 1996;76:579-589.
26. Lopez-Navarro AT, Ortega MA, Peragon J, Bueno JD, Gil A, Sanchez-Pozo A. Deprivation of dietary nucleotides decreases protein synthesis in the liver and small intestine in rats. Gastroenterology 1996; 110:1760-1769.
27. Malatesta M, Tiberi C, Baldelli B, Battistelli S, Manuali E, Biggiogera B. Reversibility of hepatocyte nuclear modifications in mice fed on genetically modified soybean. Eur J Histochem 2005;49:237-242.
28. Granby K, Johannesen S, Vahl M. Analysis of glyphosate residues in cereals using liquid chromatography-mass spectrometry. Food Addit Contam 2003; 20:692-698.
29. Arregui MC, Lenardón A, Sanchez D, Maitre MI, Scotta R, Enrique S. Monitoring glyphosate residues in transgenic glyphosate-resistant soybean. Pest Managment Science 2004;60:163-166.
30. Marc J, Mulner-Lorillon O, Boulben S, Hureau D, Durand G, Bellé R. Pesticide Roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation. Chem Res Toxicol. 2002;15:326-331.
31. Marc J, Mulner-Lorillon O, Bellé R. Glyphosate-based pesticides affect cell cycle regulation. Biol Cell 2004;96:245-249.

32. Marc J, Le Breton M, Cormier P, Morales J, Bellé R, Mulner-Lorillon O. A glyphosate-based pesticide impinges on transcription. Toxicol Appl Pharmacol 2005;203:1-8.
33. Walsh LP, McCormick C, Martin C, Stocco DM. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environ Health Perspect 2000;108:769-776.
34. Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P. Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (Oreochromis niloticus). Environ Toxicol 2003;18:260-267.
35. Peixoto F. Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. Chemosphere 2005;61:1115-1122.
36. Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE. Differential effects of glyphosate and roundup on human placental cells and aromatase. Environ Health Perspect 2005;113:716-720.
37. Lappé MA, Bailey EB, Childress C, Setchell KDR. Alterations in clinically important phytoestrogens in genetically modified, herbicide-tolerant soybeans. J Med Food 1998;1:241-245.
38. Cassidy A. Potential risks and benefits of phytoestrogen-rich diets. Int J Vitam Nutr Res 2003;73:120-126.
39. Plessow D, Waldschlager J, Richter DU, Jeschke U, Bruer G, Briese V, Friese K. Effects of phytoestrogens on the trophoblast tumour cell lines BeWo and Jeg3. Anticancer Res 2003;23:1081-1086.