Title: Responses of salivary glands to intake of soft diet

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Abbreviations:

BrdU, 5-bromo-2'-deoxyuridine; Casp-3, cleaved caspase 3

ABSTRACT

Background: Modernization has made individuals prefer processed and cooked foods (soft food), but this eating habit may have negative effects on the oral cavity. However, laboratory animals fed with soft diet are commonly used in an attempt to clarify this issue, and various oral tissues, including the salivary glands have been examined. In this review, we summarize the findings of previous studies concerning the responses of salivary glands to daily intake of soft diet.

Highlight: The weight of the parotid glands decreased in rodents fed with soft diet (liquid or powder). In atrophic parotid glands, acinar cell shrinkage is histologically observed and the DNA content is reduced, showing that the atrophy is caused by a decrease in the size and number of acinar cells. Immunohistochemical examinations showed that the decrease in the acinar cell number was induced by suppression of acinar cell proliferation and acceleration of apoptosis. The atrophic parotid glands recovered following a change from soft to pellet diet. Other salivary glands, such as the submandibular, sublingual, and palatine glands, responded only slightly to the soft diet feeding.

Conclusion: Accumulated research data showed that a soft diet negatively affects the parotid glands much more than other salivary glands and that atrophic parotid glands are able to recover by switching to a hard diet. Therefore, it should be emphasized that good eating habits are important for not only digestion but also the health of oral tissues, including the salivary glands.

Keywords: Salivary glands, Liquid diet, Atrophy, Acinar cell

1. Introduction

With the rapid progress of food processing technology, processed and cooked foods have been common findings on dining tables in recent years. These foods are generally soft, and Yanagisawa et al. [1] reported that about 60% of the daily foods in Japan are sensed as being soft. This increase in soft foods has reduced the demand for extensive mastication in modern people. Therefore, the influence of soft food intake on various regions of the body, especially the oral maxillofacial region, has received much clinical attention. Experimental studies involving laboratory animals have shown that oral tissues such as the jaw bones [2-4], masseter muscle [5-7], temporomandibular joint [8-10], and periodontal tissue [11-13] are unfavorably affected by soft diet feeding.

Salivary glands not only function as digestive glands but also play important roles in supporting the environment in the oral cavity. Therefore, the responses of salivary glands to daily intake of a soft diet have been investigated as well. In this review, we summarize the results of former studies concerning the responses of salivary glands to soft diet feeding and introduce recent studies performed by our research group.

2. Responses of salivary glands to soft diet feeding

2.1. Parotid glands

The pioneering researchers who investigated the responses of salivary glands to a soft diet were Hall and Schneyer [14]. They fed a liquid diet prepared by mixing five parts water with one part ground laboratory pellet ration to rats aged 3 to 5 months. After 14 days

of feeding, the parotid glands became atrophic and their weight notably decreased. In the atrophic parotid glands, the acinar cells had histologically shrunk whereas the duct cells were normal. The biochemical analysis showed remarkable reductions in the amylase concentration of the glands and saliva and the total protein concentration of the secretions.

Thereafter, many researchers investigated the responses of the parotid glands to a soft diet. The reductions of amylase [15-21] and protein in parotid saliva [15] were biochemically confirmed. In addition, the composition of secretory protein was altered [16] and the parotid salivary flow rate decreased [22-24]. These data suggest that the function of the parotid glands was lowered by soft diet feeding. The parotid gland weight consistently decreased, although a powder diet or a liquid diet prepared with various mixing ratios was used as the soft diet in these studies [15, 17, 19-23, 25-42]. In the atrophic parotid glands, shrinkage of acinar cells was confirmed by counting the number of nuclei per field [17, 26, 43], performing histomorphometric measurements of the diameter and individual area of acinar cells [23, 33], and performing simple microscopic observations [40, 44, 45]. The DNA content of the parotid glands was also found to be reduced [15, 26, 31]. Based on these findings, parotid gland atrophy of experimental animals fed a soft diet appears to be caused by reduction of both acinar cell size and acinar cell number [31]. However, there is another opinion that much of the acinar tissue loss is due to acinar shrinkage [23, 31] because degenerative acinar cells are identified ultrastructurally [44, 45] but not histologically [23, 31].

Cell proliferation and cell deletion are key factors in the regulation of cell numbers in tissues and organs. Since Kerr et al. [46] described apoptosis, research has demonstrated that

apoptosis plays an important role in cell deletion under physiological and pathological conditions [47]. Apoptotic cell disappearance has also been observed in the regressive process of salivary glands [48-54]. Accordingly, we investigated the involvement of proliferation and apoptosis of acinar cells in the atrophy of parotid glands induced by soft diet feeding using immunohistochemistry for 5-bromo-2′-deoxyuridine (BrdU), a marker of proliferating cells, and for cleaved caspase 3 (Casp-3), a marker of apoptotic cells [41]. In this experiment, control and experimental rats were given a pellet diet and a liquid diet, respectively, for up to 21 days. The experimental parotid glands weighed less than the control glands, and shrunken acinar cells were observed in the experimental glands (Fig. 1A, D), in agreement with previous studies. In the experimental parotid glands, the number of BrdU-positive acinar cells decreased (Fig. 1B, E) whereas the number of Casp-3-positive acinar cells increased (Fig. 1C, F). Under electron microscopy, typical apoptosis of acinar cells and apoptotic bodies phagocytosed by macrophages or adjacent epithelial cells were identified in the atrophic parotid glands (Fig. 2A, B), showing that the number of acinar cells decreased in the experimental glands [41]. In a later study, the participation of acinar cell apoptosis in atrophy of the parotid glands was also confirmed by ElGhamrawy [55]. Taken together, these findings indicate that reductions of both the size and number of acinar cells are important for atrophy of the parotid glands induced by soft diet feeding.

2.2. Submandibular and sublingual glands

Although many studies have examined the atrophy of the parotid glands induced by

soft diet as described above, fewer studies have focused on the submandibular and sublingual glands. Kuntsal et al. [56] reported that the weight of the submandibular glands in rats fed a liquid diet decreased similarly to that of the parotid glands and that the acinar cells exhibited a marked reduction of secretory granules, dilated granular endoplasmic reticulum, and swollen mitochondria. According to Scott and Gunn [33], a histometric analysis showed that the proportional volume, perimeter, and area of the acini in the submandibular glands of liquid-fed rats were smaller than those in pellet-fed rats, although there was no difference in the weight of the submandibular glands between the two groups. No atrophic alterations in the submandibular glands of animals fed a soft diet have been reported except for these studies [20, 25, 30, 32, 33, 39].

Examination of the sublingual glands has produced various findings and opinions. Some studies showed no difference in the weight of the sublingual glands between the liquid-diet group and pellet-diet group [20, 25, 39], and other studies showed that the sublingual glands weighed less in liquid-fed rats than in pellet-fed rats [19, 30, 32]. In contrast, Scott and Gunn [33] reported that the experimental sublingual glands weighed slightly more than the control glands. As mentioned above, the effects of a soft diet on the submandibular and sublingual glands seem to be inconsistent and controversial.

Accordingly, we investigated how a liquid diet affects these two salivary glands in the same way as for the parotid glands [57]. We found that the weights of the submandibular and sublingual glands of rats fed a liquid diet were not different from those of rats fed a pellet diet. Both of these glands of liquid-fed rats, in which no atrophic acinar cells were identified,

showed normal histology (Fig. 3A, D, G, J) and ultrastructure. Additionally, there were no differences in the immunohistochemical examination findings of BrdU (Fig. 3B, E, H, K) and Casp-3 (Fig. 3C, F, I, L). These findings demonstrate that a soft diet does not affect the submandibular and sublingual glands, in contrast to the remarkable influences of a soft diet on the parotid glands [57].

3. Recovery of parotid gland atrophy by switching the diet

Research has clarified that the intake of soft food induces severe atrophy of the parotid glands. Thus, whether this parotid gland atrophy is recoverable by improvement of eating habits has attracted attention. In a series of studies by Schneyer et al. [34, 35, 42, 43, 58], rats were fed a liquid diet for a certain period and then fed a solid chow diet. The proliferative activity of acinar cells in the parotid glands increased and peaked on day 2 after switching the diet and decreased thereafter as confirmed by counting the number of mitoses and performing autoradiography with ^{3}H-thymidine. The reduced parotid gland weight, DNA content, RNA content, and acinar cell size gradually increased and reached normal levels. These findings suggest that the parotid gland atrophy induced by soft diet feeding is recoverable.

As previously mentioned, the balance between cell proliferation and apoptosis is important for regulation of cell populations. Apoptosis takes place in both regressive [48-54] and progressive alterations of salivary glands [59, 60]. Therefore, in our study [61], the changes in the weight, acinar cell size, acinar cell proliferation, and acinar cell apoptosis of

the parotid glands were investigated after switching from a liquid diet to a pellet diet. On the day on which the liquid diet was changed to the pellet diet (day 0), the parotid gland weight and individual acinar cell area of the experimental rats fed a liquid diet for 14 days were smaller than those of the control rats fed a pellet diet (Fig. 4A); these measurements then gradually increased over time (Fig. 4B) and reached the control level after 7 days (Fig. 4C). Although the number of BrdU-positive acinar cells was smaller in the experimental parotid glands than in the control glands on day 0 (Fig. 4D), there were many BrdU-positive cells (Fig. 4E) in addition to several mitotic figures (Fig. 4B) on days 1 and 3. These numbers then decreased over time (Fig. 4F). There was no difference in the number of Casp-3-positive acinar cells between the experimental and control rats except on day 0 [61]. Our data coincide with the reports of Schneyer et al. [34, 35, 42, 43, 58] and suggest that an increase in acinar cell size and proliferation but not apoptosis is important for recovery of the parotid glands. Similar phenomena were also observed in another study by Schneyer and Hall [62] in which the diet was switched from solid chow to a bulk diet requiring substantial chewing. Considering these studies, extensive mastication appears to be essential for the recovery of atrophic parotid glands.

4. Influences of soft diet on growth of salivary glands

4.1. Parotid glands

The preference of soft food over hard food, especially in children, as a modern dietary habit [63, 64] led us to consider how daily intake of soft food affects the growth of

salivary glands from a clinical aspect. However, no study has been performed to investigate the parotid glands of growing animals fed a soft diet immediately after weaning. Therefore, we designed a study to clarify this issue [65]. Rats were weaned on day 21 after birth and immediately fed a liquid or pellet diet in the experimental and control groups, respectively, for up to 8 weeks. The experimental rats never once ate a pellet diet after birth. The parotid gland weight in both groups increased as the rats grew, but the degree of this increase was much weaker in liquid-fed rats than in pellet-fed rats. The acinar cells in the parotid glands were histologically small in size on weaning day (Week 0) (Fig. 5A). They grew larger in the control glands (Fig. 5B) but remained small in the experimental glands over time (Fig. 5C). Many BrdU-positive acinar cells were observed at Week 0 (Fig. 5D) and decreased in both groups over time, whereas the labeling indices of BrdU were lower in the experimental glands (Fig. 5F) than in the control glands (Fig. 5E) throughout the experimental period. Casp-3-positive acinar cells were seldom identified in either group. Our data show that the growth of parotid glands is inhibited by a liquid diet because of suppression of acinar cell growth and proliferation [65].

4.2. Submandibular and sublingual glands

Next, we investigated the effects of a liquid diet on the growth of the submandibular and sublingual glands in the same way, and the obtained data were totally different from those in the parotid glands [66]. Although the individual area and BrdU-labeling index of acinar cells were lower in the experimental than control sublingual glands at Week 8, there were no

differences in the other parameters of the sublingual glands or in any parameters of the submandibular glands, including the gland weight, individual acinar cell area, BrdU, Casp-3, and ultrastructure, between the two groups. These findings demonstrate that there is no influence or only a very slight influence of a soft diet on the growth of the submandibular and sublingual glands in growing rats [66]. However, our results were inconsistent with the only other paper concerning the responses of the submandibular glands to a soft diet by Kim [67]. In that study, mice were fed a liquid diet prepared by mixing four parts water to one part powdered diet after weaning, and the submandibular gland weight was lower in the liquid-fed mice than in the pellet-fed mice. The differentiation of acinar cells and convoluted tubule cells was retarded, and diminution of total DNA, total RNA, and amylase was biochemically observed in the experimental submandibular glands. The reason for the difference between the study by Kim [67] and our study is unclear, but it might have been caused by the different preparations of the liquid diet. Because the liquid diet used in the study by Kim [67] was more watery, the submandibular glands might have reacted more strongly.

5. Physiological mechanism of atrophic alterations of parotid glands to intake of soft food

How parotid gland atrophy is induced by a soft diet has also been a topic of interest. Hall and Schneyer [14] speculated that liquid diet feeding reduced the extent of mastication and decreased the degree to which the parotid glands were subjected to reflex stimulation, with the result that parotid gland atrophy represented atrophy of disuse. They also reported

that atrophic alterations in the parotid glands of liquid-fed rats were similar to those in parotid glands that had undergone parasympathectomy [14] because reductions in their weights, acinar cell size, and amylase concentrations were observed in the parasympathectomized parotid glands as well [68]. In the atrophic parotid glands of animals fed a soft diet, the following substances are reported to be down-regulated: acetylcholine, a neurotransmitter involved in parasympathetic nerve stimulation [39]; choline acetyltransferase, which is required for acetylcholine synthesis [25]; and neuropeptides such as substance P and vasoactive intestinal peptide, which are involved in secretion in response to parasympathetic stimulation [30]. Taking these facts into consideration, it could be speculated that parasympathetic nerves play important roles in the atrophic alterations of the parotid glands after intake of a soft diet.

This idea might also explain the different sensitivity to a soft diet between the parotid glands and the submandibular and sublingual glands because the innervation of the parotid glands is different from that of the other two glands. The secretory center of the parotid glands is the inferior salivary nucleus in the brainstem, from which the preganglionic parasympathetic fibers of the parotid glands arise and accompany the glossopharyngeal nerve. In contrast, the secretory center of the submandibular and sublingual glands is the superior salivary nucleus, from which the preganglionic parasympathetic nerves of these glands arise and accompany the facial nerve [57]. We studied the responses of the palatine glands to liquid diet feeding. The palatine glands have the same innervation as the submandibular and sublingual glands. This suggests that the palatine glands should show no or little response to a

liquid diet. The results of our study met our expectations and strongly support this idea [69, 70]. Although the above-described facts suggest a close relationship between parasympathetic nerves and the reactions of salivary glands, the evidence is indirect. Further research is necessary to clarify this issue.

6. Conclusion

The research data accumulated in this review show that the parotid glands unfavorably respond to a soft diet much more strongly than do other salivary glands and that the parotid gland atrophy induced by a soft diet is able to recover by switching to a hard diet. Further research is necessary to clarify why the response of the parotid glands to a soft diet is different from that of other salivary glands.

Fletcher, an American nutritionist nicknamed “The Great Masticator,” stated that all food must be deliberately masticated and not swallowed until it turned to liquid, a concept that became known as “Fletcherism” [71]. This is the first argument pointing out the importance of mastication. Many studies have scientifically verified that good eating habits are important for not only digestion but also the health of oral tissues, including the salivary glands. The findings of our review indicate that both dental scientists and clinical dentists should emphasize this recommendation to the general public.

Ethical statement

The animal experimentation in this study was approved by the Laboratory Animal Committee of Hokkaido University (Approval Nos. 09-0009, 10-0126, 13-0206, 14-0108, and 15-0094) and complied with the Guide for the Care and Use of Laboratory Animals of Hokkaido University.

Conflict of interest

The authors declare no conflict of interest.

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CRediT authorship contribution statement

Shigeru Takahashi: Conceptualization, Investigation, Supervision, Writing-original draft, Funding acquisition. **Akihiro Nezu:** Conceptualization, Investigation, Writing-review and editing. **Akihiko Tanimura:** Conceptualization, Writing-review and editing. **Yoshiyuki Nakamichi:** Investigation, Writing-review and editing. **Tsuneyuki Yamamoto:** Conceptualization, Writing-review and editing.

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Figure legends

Fig. 1. Parotid glands of rats fed **(A–C)** a pellet diet (PG-C) and **(D–F)** a liquid diet (PG-E). **(A, D)** HE on day 21, **(B, E)** BrdU on day 14, and **(C, F)** Casp-3 on day 3. Scale bars = 10 μm. The size of the acinar cells was smaller in the **(D)** liquid-fed rats than in the **(A)** pellet-fed rats. Fewer BrdU-positive acinar cells and more Casp-3-positive cells (arrows) were identified in the **(E, F)** liquid-fed rats than in the **(B, C)** pellet-fed rats.

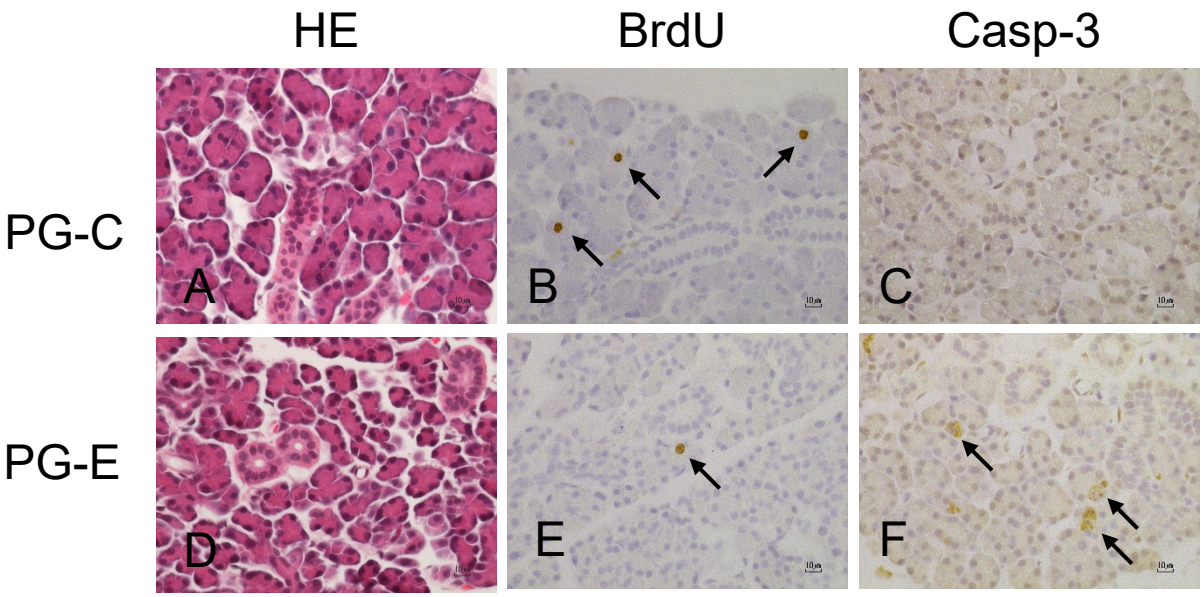
Fig. 2. Ultrastructure of acinar cells in parotid glands of rats fed a liquid diet for 14 days. Scale bars = 2 μm. **(A)** The characteristic nuclear fragments and whorls of rough endoplasmic reticulum in the acinar cells are observed. **(B)** Apoptotic bodies (arrows) are phagocytosed by adjacent acinar cells.

Fig. 3. Submandibular glands of rats fed **(A–C)** a pellet diet (SMG-C) and **(D–F)** a liquid diet (SMG-E). Sublingual glands of rats fed **(G–I)** a pellet diet (SLG-C) and **(J–L)** a liquid diet (SLG-E). HE on **(A, D)** day 21 and **(G, J)** day 14. **(B, E, H, K)** BrdU on day 21. **(C, F, I, L)** Casp-3 on day 7. Scale bars = 10 μm. There are no differences in morphology, BrdU, or Casp-3 in either the submandibular glands or sublingual glands of the two groups. Arrows, immunopositive acinar cells.

Fig. 4. Histological alterations of rat parotid glands atrophied by liquid diet feeding for 14 days after a change to pellet diet feeding. HE on **(A)** day 0, **(B)** day 3, and **(C)** day 14. BrdU

on **(D)** day 0, **(E)** day 1, and **(F)** day 14. Scale bars = 10 μm. On day 0, the acinar cells are **(A)** small in size and **(D)** rarely BrdU-positive. **(B)** The acinar cells become larger and some mitotic acinar cells (arrow) appear on days 1 and 3. **(C)** The acinar cells recover to a normal size after 7 days. **(E)** Many BrdU-positive acinar cells (arrows) are observed on days 1 and 3.

Fig. 5. Growing parotid glands of rats **(A, D)** at the start of the experiment (0w), **(B, E)** fed a pellet diet (PG-C), and **(C, F)** fed a liquid diet (PG-E). HE at **(A)** week 0 and **(B, C)** week 4. BrdU at **(D)** week 0 and **(E, F)** week 1. Scale bars = 10 μm. Acinar cells are **(A)** small in size and **(D)** commonly BrdU-positive in parotid glands of 3-week-old rats just weaned. **(B)** Although the acinar cells become larger in the pellet-fed-rats over time, **(C)** those in the liquid-fed rats remain small. **(E)** Many acinar cells in the pellet-fed rats but **(F)** only some in the liquid-fed rats are BrdU-positive.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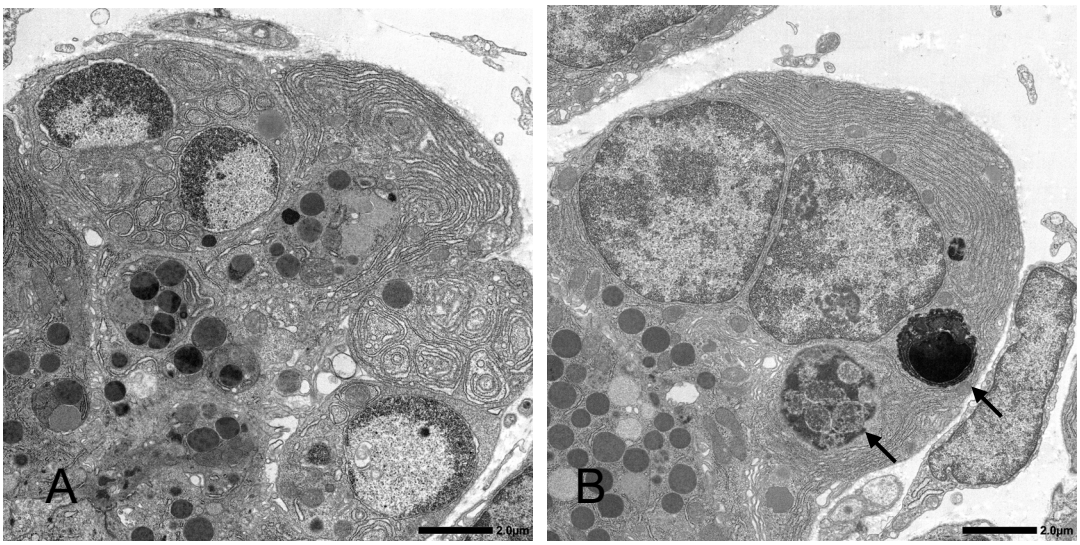
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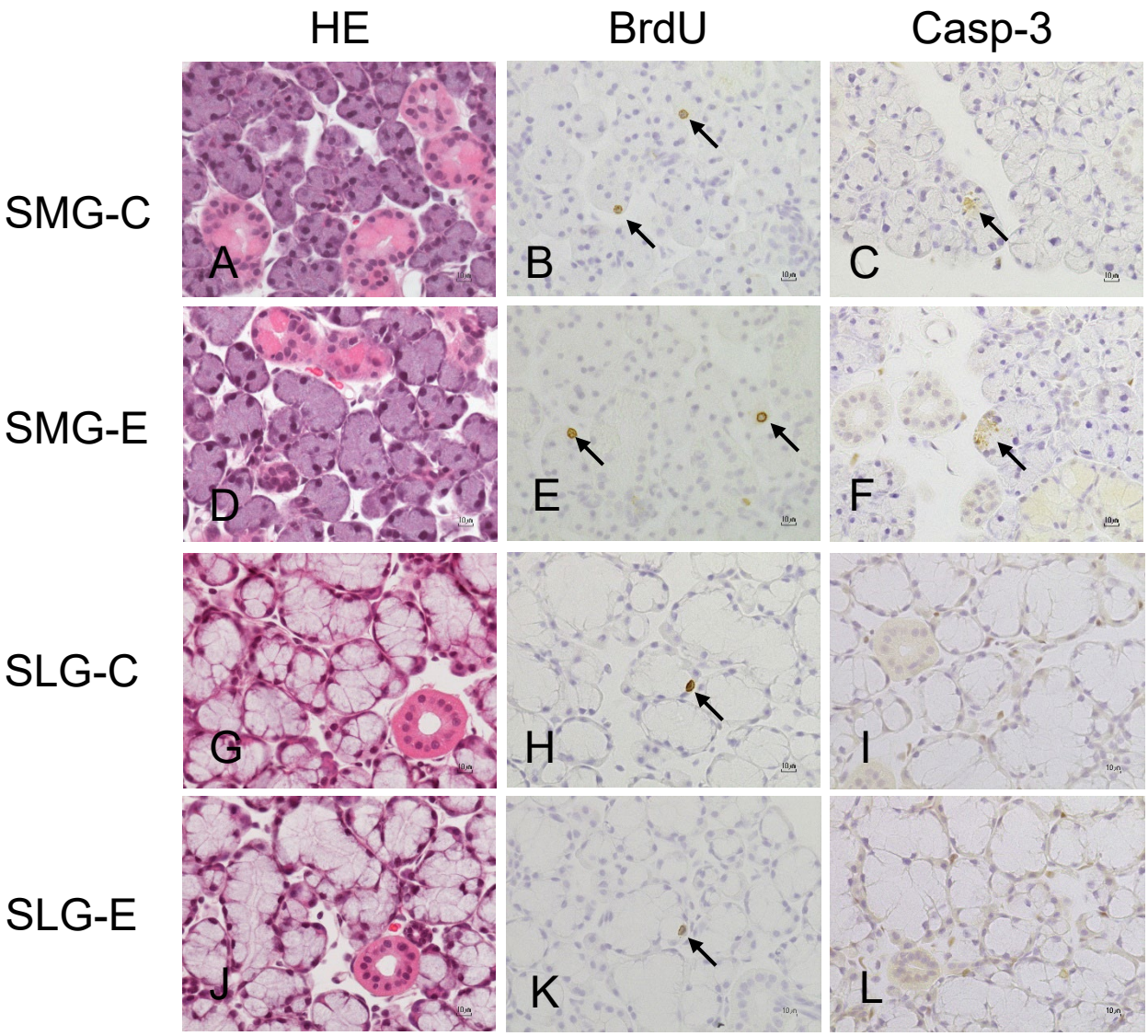
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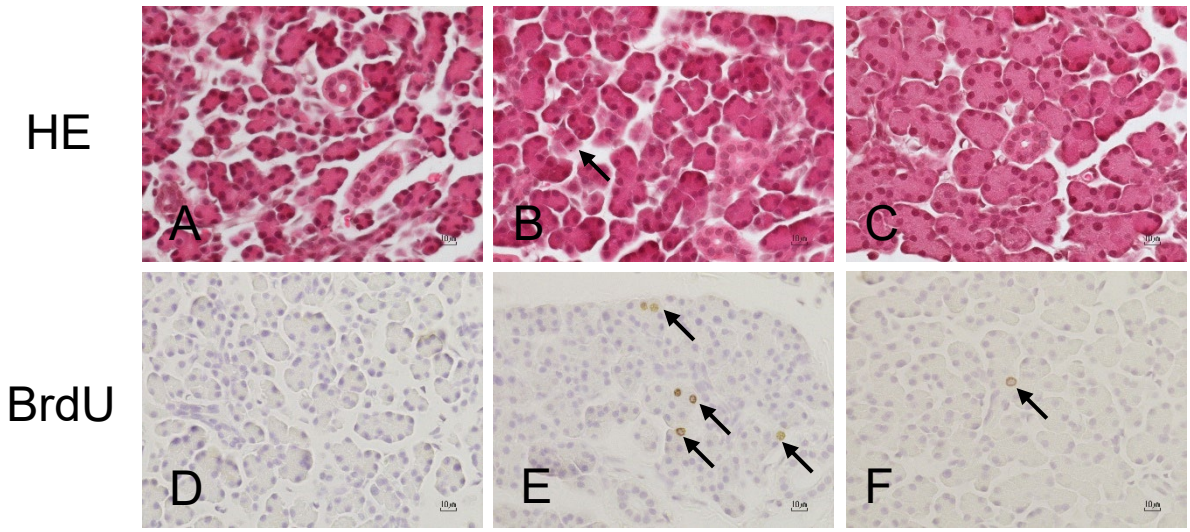
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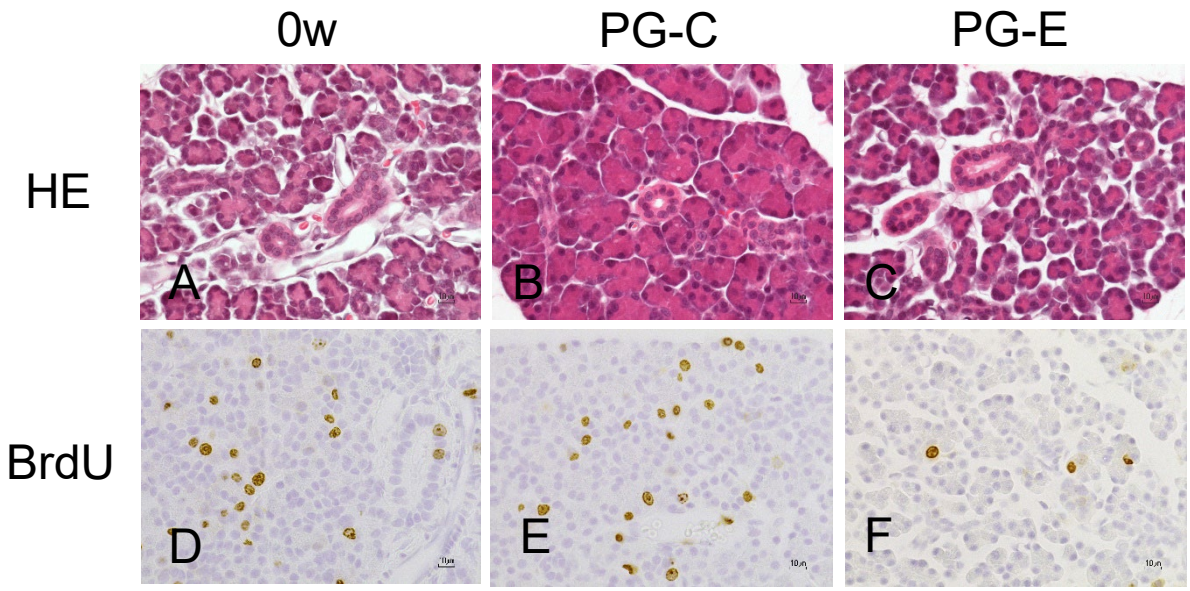
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