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Immunodetection of selected pancreatic hormones under intragastric administration of apelin-13, a novel endogenous ligand for an angiotensin-like orphan G-protein coupled receptor, in unweaned rats

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Abstract

Introduction: This study investigated the effects of intragastric administration of apelin-13 on the secretion of critical pancreatic hormones in a cohort of three-week-old Wistar rats. The research aimed to uncover apelin's modulatory roles in endocrine interactions dictating metabolic homeostasis during early life. **Material and Methods:** Rats were randomly assigned to control or experimental groups, receiving apelin-13 or saline for 14 days. The study population consisted of three-week-old Wistar rats of both sexes, weighing between 20 and 25 grams. Histological examination, analysis of variance and *t*-tests were employed to assess significant differences. **Results:** Distinctive alterations in large islet morphology were observed, indicating a notable reduction in size. Additionally, an increase in alpha- and beta-cell density within specific islet sizes was noted, suggesting significant changes in cell populations. The study found a substantial increase in mitotic activity and a decrease in apoptosis in small and medium-sized islets post apelin-13 administration, indicating its potential role in regulating cell survival and proliferation. **Conclusion:** The notable reduction in large islet size coupled with increased alpha and beta cell density implies a targeted impact of apelin-13 on pancreatic cell dynamics. Also, the observed increase in mitotic activity and decrease in apoptosis in small and medium-sized islets suggest its potential regulatory role in cell survival and proliferation within the pancreatic microenvironment.

Keywords: insulin, glucagon, pancreatic islets, diabetes, apoptosis.

Introduction

In 1998, Tatemoto *et al.* (30) utilised bovine stomach tissue samples to discover endogenous ligands for the orphan G-protein-coupled apelin receptor (APJ), leading to the isolation and purification of a novel peptide named apelin. This peptide, a member of the adipokines group, has since been recognised as an essential ligand for APJ. The subsequent exploration of this peptide has revealed that its 77-amino-acid pre-proprotein undergoes intricate processing, generating multiple biologically active apelin isoforms with varying lengths ranging from 12 to 36 amino acid residues, each exerting a spectrum of diverse pharmacological effects (28).

Through a series of experimental studies, it has been unequivocally demonstrated that all apelin isoforms, acting *via* the APJ receptor, are crucial in the regulation of a spectrum of pivotal physiological processes, those related to maturation and aging being among them (28). Investigations in mice have unveiled an age-dependent decline proportional to maturation (24) in the expression levels of apelin and its receptor in the kidneys, lungs and liver. Intriguingly, mice with knockout of either the apelin gene or the APJ receptor gene manifested accelerated

aging symptoms in the aforementioned organs. Significantly, upon restoration of normal apelin levels, a marked improvement in the overall health status of these individuals was observed, suggesting the potential of apelin as an anti-aging factor (24).

Building upon research conducted using animal disease models, it was postulated that apelin isoforms, particularly apelin-13, exert therapeutic effects in various disorders of the nervous system, cardiovascular system, respiratory system, gastrointestinal tract and kidneys, and in metabolic diseases associated with glucose metabolism (3, 28). In the course of most of these systemic diseases, the beneficial effects of apelin were dose-dependent and contingent on the mode of administration. These effects largely centred on the attenuation of caspase-3 levels and pro-inflammatory factors, leading to the mitigation of ongoing inflammation and reduction of apoptosis (3). However, the precise regulatory role of apelin remains enigmatic, a definition of that role not having been aided by the recently uncovered negative effect of this compound: under certain circumstances, apelin could also function as an activator of pro-inflammatory cytokines and promote tumour growth and angiogenesis (34). In sum, these observations suggest the potential dual nature of apelin, whereby its isoforms might exert harmful and detrimental effects under specific conditions.

The objective of this investigation was to elucidate the impact of intragastric administration of apelin-13 on the regulation of critical pancreatic hormones, namely insulin, glucagon, somatostatin and pancreatic polypeptide, in a cohort of unweaned rat subjects. The utilisation of unweaned rats as the model organism is of particular significance, given that this developmental stage is characterised by increased metabolic plasticity and endocrine adaptability. Such attributes render this age group especially relevant for the study of long-term physiological outcomes. This study aimed to delineate the alterations in secretion profiles of these pivotal pancreatic hormones after apelin-13 administration. By so doing, the research sought to shed light on the prospective modulatory effects that apelin signalling could exert within the complex network of endocrine interactions that dictate metabolic homeostasis during the formative phases of life. The insights derived from this investigation stand to augment our current understanding of apelin's roles in metabolic and developmental physiology.

Material and Methods

Animals. The experiments were sanctioned by the II Local Ethics Committee for Experiments on Animals in Lublin (Poland) under approval no. 49/2008. The study was conducted on a population of three-week-old Wistar rats of both sexes, weighing between 20 and 25 g (n = 12). These rats remained with their mothers from birth in standard housing conditions, with controlled temperature (21 ± 1°C) and humidity (60–70%). On day 10 of life, the rats were marked and randomly assigned into two different groups: a control group and an experimental group. Each group included six animals (n = 6), with two offspring from each mother allocated to each group. The allocation was chosen to ensure uniform exposure to apelin in breast milk and to maintain the heterogeneity of the study groups.

The experimental group rats were intragastrically administered Apelin-13 (Hokuriku University, Kanazawa, Japan) *via* a specialised cannula designed for mice (AgnTho's, Lidingö, Sweden), facilitating painless and stress-free hormone delivery. Apelin-13 was given at a dosage of 100 nmol/kg of body weight twice a day (9 a.m. and 9 p.m.) for 14 d. In parallel, the control group rats received physiological saline solution through appropriate means. The determination of the apelin-13 dosage was predicated on previous research findings (1, 13). Following a 12-h interval after the last apelin-13 administration, the rats were weighed and subsequently euthanised with carbon dioxide.

Sample collection and tissue processing. The experiment was conducted on non-weaned rats which remained with their mothers throughout the study to avoid stress; therefore, the animals were not subjected to fasting before slaughter. Immediately following euthanasia, the whole pancreas of approximately 2 g in weight was collected from each rat. These samples were fixed in 4% phosphate-buffered formaldehyde (pH 7.0), subsequently dehydrated through a graded series of ethyl alcohol, and then processed with Ottix Shaper and Ottix Plus nonpolar solvents (DiaPath, Martinengo, Italy). The samples were then embedded in Paraplast Plus (Sigma-Aldrich, St. Louis, MO, USA). Paraffin sections with a thickness of 4 µm were subsequently prepared using an HM 325 microtome (ThermoFisher Scientific, Waltham, MA, USA).

Immunohistochemistry. Immunohistochemical reactions were employed for the identification of selected pancreatic hormones and were conducted according to a previously described protocol (19). One modification implemented in this study was using a multicooker (RMC-PM381-E, Redmond/Power Point Inc, Shenzhen, China) to boil the samples in citrate buffer for 8 min. The antibodies used in the study are described in Table 1.

Morphometric analysis. The obtained images were subjected to analysis using ImageJ 1.52 image analysis software (27). The analyses were conducted on cross-sections of the pancreas at a magnification of 40×. Measurements were made of the pancreatic islet area (μm^2) and islet diameter (µm), determined using Feret's diameter function. The immunoreactive cells and the total cell count within the islet were also quantified. Subsequent analyses were performed based on these measurements. An islet's diameter classified it as small (50–100 µm), medium (100.01–150 µm) or large (>150 µm) based on the method described by Banaei-Bouchareb *et al*. (4).

Table 1. Primary and secondary antibodies used in the study

Antibody	Host	Catalogue number	Dilution	Source
Primary antibody				
Anti-glucagon	Mouse	14-9743-82	1:200	ThermoFisher, Waltham, MA, USA
Anti-insulin	Mouse	MA5-12037	1:200	ThermoFisher
Anti-somatostatin	Mouse	14-9751-82	1:5000	ThermoFisher
Anti-pancreatic polypeptide	Rabbit	ab272732	1:2000	Abcam, Cambridge,UK
Anti-cleaved caspase 3	Rabbit	AF7022	1:150	Affinity Biosciences, Cincinnati, OH, USA
Anti-proliferating-cell nuclear antigen	Rabbit	AF0239	1:150	Affinity Biosciences
Secondary antibody				
Anti-mouse/rabbit	Goat	DPVB-HRP	RTU	ImmunoLogic, Duiven, the Netherlands

RTU – ready to use

The distribution of each islet type varied, and the proportions of different types within the overall islet count were determined using contingency table functions. The counts of immunoreactive cells and the total cell count within the islet were used to calculate the percentage of immunoreactive cells. Additionally, based on the count of immunoreactive cells and the islet area, the density of immunoreactive cells per 1,000 μm^2 was calculated. The total cell count within the islet was divided by the islet area to obtain the islet density, which was calculated per 100 μm^2.

Statistical analysis. The outcomes were recorded as mean values with standard deviation. The normal distribution of each variable was evaluated using the Shapiro–Wilk normality test. In instances where the data followed a normal distribution, a two-way analysis of variance was executed, along with planned comparison assessments (Tukey). These evaluations were specifically designed to contrast the small pancreatic islets of the control group with those of the group that had undergone apelin treatment, to contrast the medium islets of one group with those of the other, and to do the same with the large islets. For datasets that did not conform to a normal distribution, multiple *t*-tests (Mann–Whitney) with appropriate comparison assessments (Dunn–Bonferroni) were applied. Any differences occurring at a probability with P-value of less than 0.05 were deemed significant. Statistical calculations were facilitated using the GraphPad Prism version 9.5.1 software suite running under the Windows operating system (GraphPad Software, San Diego, CA, USA).

Results

Analysis of pancreatic islets morphology. Notable changes were observed only in the case of the large islets' surface areas and islet diameters, in which significant size reductions were evident when comparing the cohort of control rats to those subjected to apelin treatment (Fig. 1 and Fig. 2A–D; P-value < 0.05). No noticeable differences were identified regarding the small or middle-sized islets across any of the evaluated parameters. An overall increase in islet area in relation to the total pancreas area was also observed (Fig. 1D; P-value < 0.001).

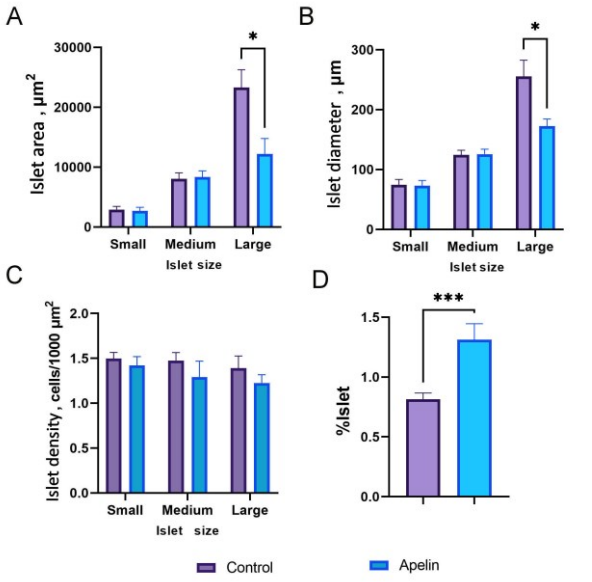

Fig. 1. (A) Islet area, (B) islet diameter and (C) islet density mean values (with standard deviation-whiskers) of small, medium and large islets in the control (n = 6) and apelin-treated (n = 6) rat groups and (D) percentage of islet area in the pancreas in the control and apelin-treated groups. Asterisks (*) indicate significant differences between control and apelin treated groups within like-sized subgroups of islets (* – P-value < 0.05; *** – P-value < 0.001)

Analysis of pancreatic alpha cells. In the alpha cells, a marked disparity became evident in medium-sized islets when comparing the control group with the apelin-treated group – specifically an increase in the percentage of stained cells and the cell density (Fig. 3; P-value < 0.001 and P-value < 0.01, respectively). No such distinctions, however, were discernible in the domains of small and large islets.

Analysis of pancreatic beta cells. In the beta cells, a significant increase was observed in the percentage of stained cells in large islets when comparing the apelin-treated group with the control group. No notable distinctions, however, were discernible in the domain of small and medium-sized islets (Fig. 4A; P-value < 0.05). Despite the lack of significant differences in stained cell percentages, a decrease in cell density was observed in small and medium-sized islets (Fig. 4B; P-value < 0.01).

Analysis of pancreatic delta cells. The only significant change observed was a decrease in delta-cell density in small islets in rats subjected to apelin treatment (Fig. 5B; P-value < 0.05).

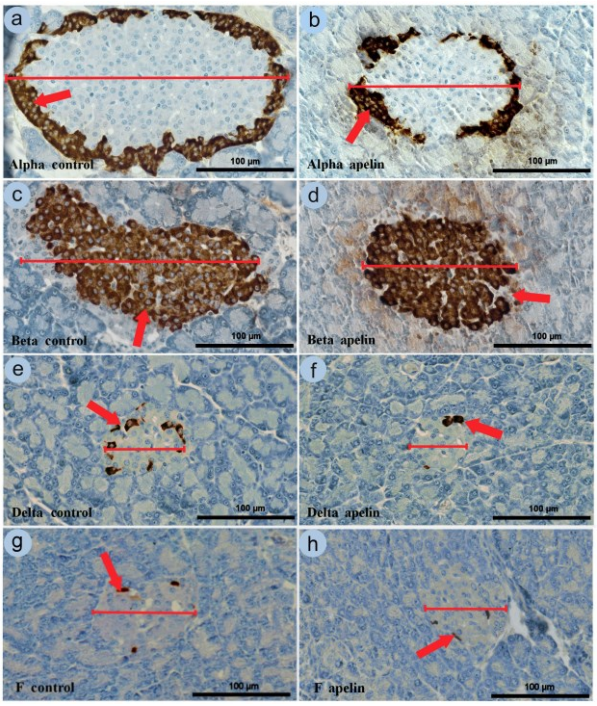

Fig. 2. (a and b) Immunoreactivity of glucagon, (c and d) insulin, (e and f) somatostatin and (g and h) pancreatic polypeptide in (a–d) large and (e–h) small pancreatic islets: a, c, e and g – control group; b, d, f and h – apelin group. Red lines indicate the diameter of the islet and red arrows indicate changes in immunoreactive cell density (cells/1,000 µm^2). Scale bar: 100 µm

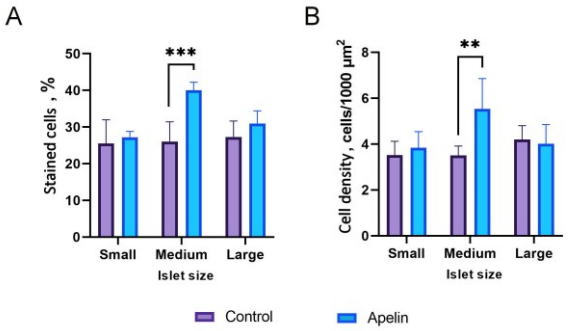

Fig. 3. (A) Stained alpha cell percentage and (B) alpha cell density mean values (with standard deviation-whiskers) in small, medium and large islets in the control (n = 6) and apelin-treated (n = 6) rat groups. Asterisks (*) indicate significant differences between control and apelin treated groups within like-sized subgroups of islets (** – P-value < 0.01; *** – P-value < 0.001)

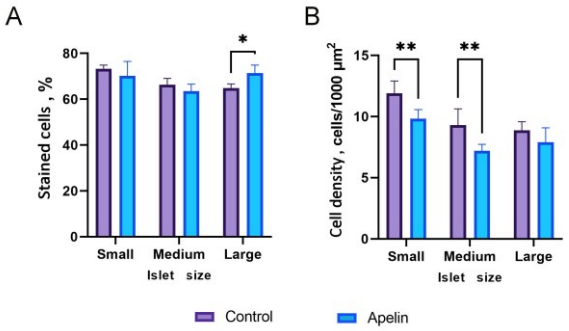

Fig. 4. (A) Stained beta cell percentage and (B) beta cell density mean values (with standard deviation-whiskers) in small, medium and large islets in the control (n = 6) and apelin-treated (n = 6) rat groups. Asterisks (*) indicate significant differences between the control and apelin-treated groups in like-sized subgroups of islets (* – P-value < 0.05; ** – P-value < 0.01)

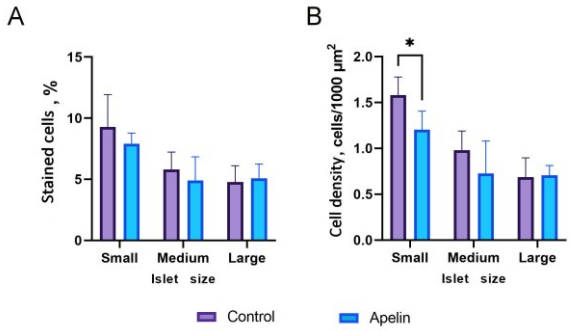

Fig. 5. (A) Stained delta-cell percentage and (B) delta cell density mean values (with standard deviation-whiskers) in small, medium and large islets in the control (n = 6) and apelin-treated (n = 6) rat groups. Asterisks (*) indicate significant differences between the control and apelin-treated groups in like-sized subgroups of islets (* – P-value < 0.05)

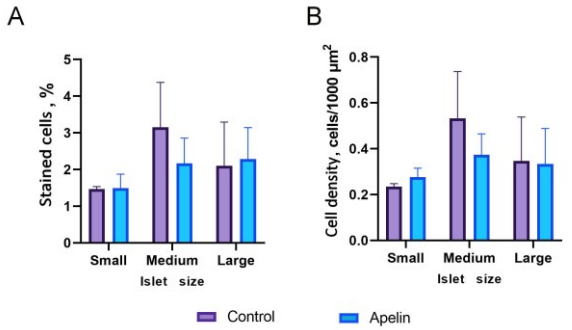

Fig. 6. (A) Stained F-cell percentage and (B) F-cell density mean values (with standard deviation-whiskers) in like-sized subgroups of islets in the control (n = 6) and apelin-treated (n = 6) rat groups

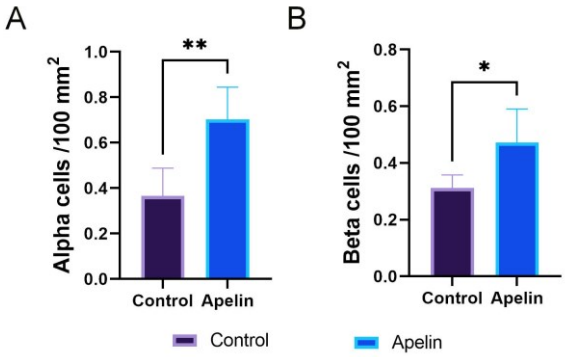

Fig. 7. (A) Alpha cell number per 100 µm^2 of pancreas and (B) beta cell number per 100 µm^2 of pancreas outside of the pancreatic islets mean values (with standard deviation-whiskers). Asterisks (*) indicate significant differences between the control (n = 6) and apelin-treated (n = 6) groups (* – P-value < 0.05; ** – P-value < 0.01)

Analysis of pancreatic F cells. No disparities which were statistically significant were detected in the F-cell populations between the control group rats and rats subjected to apelin treatment across all observed pancreatic islet size subgroups (Fig. 6).

Analysis of pancreatic non-islet alpha and beta cells. The count rose for both alpha and beta cells of those immunoreactive with insulin and glucagon and situated outside the pancreatic islets within the exocrine portion of the pancreas in apelin-treated rats (Fig. 7; P-value < 0.01 and P-value < 0.05, respectively).

Analysis of pancreatic cells mitosis and apoptosis. In a comprehensive examination of cell division (mitosis) and programmed cell death (apoptosis) in the exocrine portion of the pancreas, a substantial upsurge in the count of cells undergoing mitosis (Fig. 8A; P-value < 0.001) and a notable decrease in the count of cells undergoing apoptosis (Fig. 8B; P-value < 0.001) were observed after apelin administration. Specifically, within individual pancreatic islets, there was a marked increase in mitosis in small and medium-sized islets (Fig. 8C; P-value < 0.001) along with a reduction in apoptosis in small islets (Fig. 8D; P-value < 0.05) post apelin administration.

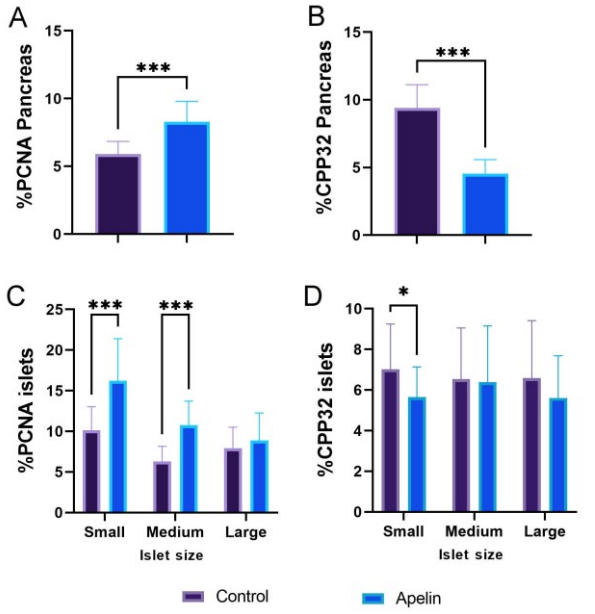

Fig. 8. (A) Proliferating cell nuclear antigen percentage (%PCNA) of positive exocrine pancreatic cells, (B) proenzyme form of cysteine protease, 32 kDa percentage (%CPP32) of positive exocrine pancreatic cells, (C) %PCNA of positive cells in individual pancreatic islets and (D) %CPP32 of positive cells in individual pancreatic islets mean values (with standard deviation-whiskers) for the control (n = 6) and the apelin-treated (n = 6) rat groups. Asterisks (*) indicate significant differences between control and apelin treated groups (* – P-value < 0.05; *** – P-value < 0.001)

Discussion

The primary objective of our study was to evaluate the effects of intragastric administration of apelin-13 on the expression of key pancreatic hormones in unweaned rats. Our results revealed significant alterations in pancreatic islet architecture, along with modulatory effects on alpha and beta cells. However, it is crucial to contextualise these findings within the broader scientific discourse on apelin's role in pancreatic physiology.

Extensive research has established that apelin acts as an inhibitor of insulin secretion. This finding is further supported by studies on incubated insulinoma cells (11) and genetically modified mice lacking the apelin gene, in which the subjects showed not only elevated levels of insulin secretion but also symptoms of glucose intolerance (8). Interestingly, work by Kapica *et al.* (13) identified apelin in pancreatic vesicles but not in islets, setting apelin in contrast to the APJ receptor, which is present in both islets and pancreatic ducts. Complementing these observations, research carried out by Ringström *et al.* (25) confirmed the presence of apelin in specific pancreatic islet cells, namely the alpha and beta cells of rats. The biological impact of apelin appears to be modulated by both the administration route and the age of the test subjects. To illustrate, a pair of experiments by Antushevich *et al.* (1) employed intragastric and intraperitoneal administration of apelin, and although their study focused on the exocrine part of the pancreas, their results should be mentioned. These studies revealed age-related differences in physiological responses: young weaned rats displayed an uptick in lipase activity without any concomitant changes in overall protein levels or the activities of trypsin and amylase within the pancreatic homogenate, while adult rats experienced an increase in both total protein content and enzymatic activities, including those of lipase, trypsin and amylase.

The conducted study revealed a significant reduction in the pancreatic islet area in rats treated with apelin-13, this decreasing by 47.52% compared to the control group. Additionally, the average islet diameter decreased by 32.43%. Importantly, islet density remained unchanged, suggesting that the reduction in islet size was likely due to a decrease in cell numbers rather than cell size. Further analyses of cells secreting the key pancreatic hormones insulin and glucagon revealed an increase in the proportion of glucagon-secreting cells (alpha cells) in medium-sized islets and insulin-secreting cells (beta cells) in large-sized islets compared to the control group. This was accompanied by an increase in the density of alpha cells in these islet size subgroups and a decrease of beta cell density in small and medium-sized islets. This increase contributed to the absence of a significant reduction in overall islet density, despite a notable decline in the density of the cells which typically dominate the islets, *i.e.* beta cells. Nevertheless, it is noteworthy that the overall islet density displayed indications of a discernible trend, which means that its values pointed to differences which were very close to statistically significant between the control group and the experimental group (P-value = 0.056–0.097). While other variables did not demonstrate significant changes (except for small decreases in the density of delta cells), the morphometric measurements implied that the constancy of the proportion of cells secreting somatostatin and pancreatic polypeptide in the medium and large islets may be misleading. This can be attributed to the reduced islet area, leading to the plausible assumption that despite stable proportional cell numbers, there may have been a concurrent reduction in the absolute number of cells secreting these hormones.

Similar findings were reported in neonate rats with streptozotocin-induced diabetes. Gallego *et al.* (9) documented a reduced islet area and an increased proportion of alpha cells. However, the percentage of

beta cells differed from ours, as a decrease was observed in their study. These outcomes are likely attributable to beta cell necrosis induced by streptozotocin, an antibiotic with a selective affinity for beta cells commonly used for diabetes induction (7). Interestingly, in our study, no discernible changes were found in F cells and barely any in delta cells upon apelin-13 treatment across all types of pancreatic islets. This observation contrasts with the suggestion of a recent study that apelins, including apelin-13, may exert some modulatory influence on the secretion of somatostatin and pancreatic polypeptide (25); however, because in our case only a decrease in delta cell density in small islets was observed, our results are inconclusive. Several factors could contribute to this divergence in results. One particularly noteworthy consideration is the age of the rats used in our investigation. It is important to recognise that the metabolic system of the adopted unweaned rat model is still in a formative stage of development. This nascent state could render these systems less responsive or sensitive to changes induced by apelin-13. Furthermore, the specific developmental timing of the rats, which were not fully mature, may have acted as a significant limiting factor on the observed physiological changes. It is plausible that the delta and F cells in these juvenile rats had not yet reached a developmental stage where they could interact effectively with apelin-13. It could also be hypothesised that there may have been differences in receptor expression or availability at this developmental stage, leading to reduced or altered functionality of apelin-13 with respect to these cell types. However, we opted for unweaned rats as our experimental subjects for several compelling reasons. Firstly, this developmental stage allowed the stabilisation of apelin levels, as it is a period where the concentration of apelin acquired from maternal milk begins to diminish. Maintaining a consistent level of apelin was crucial for the integrity of our study design. Secondly, the existing literature suggests that the weaning stage serves as a pivotal moment for the maturation of pancreatic cells (29). We sought to examine whether supplying exogenous apelin in the dosage applied during the period leading up to weaning would expedite the maturation and stabilisation of pancreatic cells, potentially influencing insulin levels in the following stages of development. As the study was conducted on young, unweaned rats suckling maternal milk, the intragastric administration of apelin in this study was the most physiological method of delivering the hormone during this period of life.

To gain the advantage of a model lending itself to stabilisation of apelin levels, it was necessary to accept a limitation this imposed on the study. The use of very young and small animals limited the volume of blood obtained during sample collection and made it impossible to measure hormone or glucose levels in the blood. The insufficient quantity of blood precluded accurate measurements of hormonal and glucose concentrations, impeding a comprehensive analysis of physiological responses.

Our study proved a remarkable increase in mitotic activity within small and medium-sized islets – specifically, a 102.78% increase in small islets and a 125.50% increase in medium-sized islets – after apelin administration. Furthermore, our findings demonstrated a 32.47% decrease in apoptosis in small islets after apelin administration. This substantial augmentation in mitotic rates suggests that apelin may serve as a regulatory molecule, bolstering mitotic activity while inhibiting apoptotic pathways in the pancreatic islet cells. *In vitro* studies revealed that apelin could have divergent effects on cell proliferation, by either promoting it (14) or inhibiting it (2), with the outcome depending on the specific cell line. Apelin also had a significant impact on cell proliferation and death processes in other tissues, as it inhibited apoptosis and stimulated proliferation (2). The reduction in apoptosis aligns with the research by Liu *et al.* (15), who highlighted the anti-apoptotic role of the apelin/APJ system. This could potentially confer a protective role on apelin, particularly in the context of metabolic diseases where islet cell dysfunction is a hallmark, such as type 2 diabetes. The study by Chen *et al.* (6) suggested that long-term treatment with apelin had a protective effect on pancreatic islet beta cells in rats with type 1 and 2 diabetes.

The reduction in apoptosis and an increase in mitosis particularly in small islets, suggested that apelin played a pivotal role in the genesis of new islet cells, which could indicate a specific mechanism by which the apelin/APJ system contributed to cellular survival, thereby maintaining the integrity of pancreatic islets. The smaller the size of the islet, and *ipso facto* the newer it is, the higher the likelihood that it contains beta cells: the smallest islets are often made up almost entirely of these cells. A more potent protective effect on new islet cells may be imputed to apelin on the basis of its protection of beta cells as Chen *et al*. (6) suggested. Given the consistent distribution of both small and large pancreatic islets, it seems likely that the smaller islets serve as a resource for regenerating the larger ones, especially when significant damage occurs from inflammation or other harmful events. Lower beta cell viability in small islets corroborates the hypothesis advanced by Tomita (31), who indicated that apoptosis in pancreatic beta cells plays a critical role in the pathogenesis of type 2 diabetes. This notion was also confirmed by our findings demonstrating a significant increase in the percentage of alpha and beta cells following apelin treatment. This correlation underlined apelin's crucial role in cell proliferation in the pancreatic islets, thereby expanding what is understood of its multifaceted influence on pancreatic physiology. Interestingly, the observed increase in the number of small clusters and single cells expressing insulin and glucagon located outside the pancreatic islets in rats

treated with apelin suggested a neogenetic basis. A study by Mezza *et al.* (18) reported increased expression of single cells and small clusters expressing insulin in pancreatic tissue in individuals with impaired glucose tolerance. These results are consistent with the hypothesis that apelin serves as a key modulatory factor in the generation of new cells in pancreatic islets.

The regeneration of pancreatic islet cells occurs *via* the self-renewal of existing beta cells and through intra-islet progenitors. Additionally, centroacinar/terminal ductal cells expressing stem cell markers may also contribute to islet regeneration. Separate studies suggested neogenesis *via* the transdifferentiation of acinar cells post treatment with activin or glucagon-like peptide (21), giving rise to cells that produce insulin and glucagon. The co-expression of pivotal factors – specifically Pdx1, Ngn3 and MafA – within the acinar portion initiated the transformation of acinar cells into fully functional beta cells in mice, indicating the potential for generating islet cells (20). Notably, pancreatic duct epithelial cells express the pro-endocrine marker Ngn3. The robust regenerative capacity of alpha cells is particularly noteworthy, especially concerning changes observed in the glucagon signalling pathway (26).

Numerous studies have explored pregnancy's impact on regulating beta cell mass in rodents and humans. During rodent pregnancy, primary compensation mechanisms seemed to involve existing beta cell proliferation and enhancements in their functionality, likely including beta cell neogenesis (23). Maternal apelinaemia is more pronounced during pregnancy, with the placenta releasing significant apelin amounts around the 17th gestational day in rats – this period marks the highest apelin release in both the mother and the foetus, the latter exhibiting double the apelin levels of the mother. By delivery day (the 21st day), apelin levels have halved in both the foetus and the mother, suggesting a placental source of prenatal apelin aimed at augmenting nutrient transfer, especially glucose, from mother to foetus. In newborns, tissue distribution of apelin and APJ mirrored that in adults (22). Apelin is also richly secreted in colostrum, albeit less in milk, impacting food intake and energy balance and stimulating gastrointestinal tract development.

In rats, apelin and APJ expression levels in the stomach, duodenum and colon peak at birth but significantly decline postnatally. The elevated apelin mRNA levels during birth hint at apelin's role in postnatal gastrointestinal (GI) tract development. Notably, apelin-containing cells in the rat stomach only emerge around the weaning period and are absent in neonates. It was theorised that the GI tract's intrinsic apelin production might be dispensable in the early postnatal phase, given the significant apelin levels detected in breast milk (32). The rise in gastric apelin-containing cells post weaning suggested that weaning prompted the synthesis of apelin, stimulated by the shift from breast milk to solid food (33). In our study, administering apelin aimed to restore its GI tract levels to match the natural peak concentration in maternal milk, thus extending its duration of action in the GI tract.

Conclusion

The observed increase in mitosis and decrease in apoptosis following apelin administration have intriguing implications, particularly in the context of tumour growth (5) It is well-established that uncontrolled cell division, represented by increased mitosis and reduced apoptosis, is a hallmark of tumour proliferation. The substantial augmentation in mitotic activity, notably in small and medium-sized islets, underscores apelin's potential role as a regulatory molecule bolstering cell proliferation (10). This heightened mitotic activity could constitute a crucial mechanism through which apelin influences cell survival, thus maintaining the integrity of pancreatic islets, but also causing uncontrolled cell growth leading to the formation of neoplastic growth. While our study focused on the pancreas, it is conceivable that apelin may exert similar effects in other tissues (16). Consequently, the knowledge gleaned from apelin research could contribute to the development of targeted apelin-blocking therapies aimed at inhibiting tumour growth (12). Understanding the delicate balance of cell division and apoptosis under apelin's influence is paramount, as this information may be leveraged to develop innovative approaches in cancer research.

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