Journal of Cytology & Histology
ISSN: 2157-7099

Hurion et al., J Cytol Histol 2016, 7:4
DOI: 10.4172/2157-7099.1000439

Research Article Open Access

Changes in Pancreatic Islet Mass in Grey Mouse Lemurs (*Microcebus murinus*) Submitted to Caloric Restriction or Resveratrol Administration

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Abstract

Caloric restriction and resveratrol intake have beneficial effects on health, especially in the prevention and improvement of diabetes. Based on this evidence, this study has been conducted in order to examine the normal architecture of pancreatic islets and the long term effects on endocrine pancreas of standard diet, caloric restriction, or resveratrol in 19 adult grey mouse lemurs. Immunohistochemistry, with anti-insulin and anti-glucagon antibodies, and morphometric analysis were performed. The normal pancreatic islet was composed of an outer circle of α cells and a large inner body of β cells, the percentages of each cell types being 10.3 ± 3.3% and 69.7 ± 1.8%, respectively. A marked increase of the pancreatic islet mass was detected in 6 animals: 2 animals under standard diet,1 animal under calorie restricted diet, and 3 animals under standard diet supplemented with resveratrol. This lesion consisted mainly of an increase of β-cell mass in 4/6 animals, and seemed to appear in middle-aged adult primates (5.8 ± 0.8 years old). To our knowledge, there are no other studies which aim at describing the changes of the pancreatic islet mass in grey mouse lemurs.

Keywords: Caloric restriction; Grey mouse lemur; Immunohistochemistry; Insulin resistance; *Microcebus murinus*; Morphometric analysis; Pancreatic islets; Resveratrol

Abbreviation: CR: Caloric Restriction; CRF: Chronic Renal Failure; HES: Hematoxylin-Eosin-Saffron; RSV: Resveratrol Intake; SD: Standard Diet

Introduction

Microcebus murinus is a nocturnal prosimian primate belonging to the Lemuriforme infraorder and Cheirogaleidae family. It is one of the smallest lemurs (approximately 12 cm, 60-100 g) found in Madagascar [1]. This primate has a photoperiod-dependent seasonal cycle, which includes a pseudolethargic phase during which fattening occurs and all physiological and behavioral parameters are modified [2]. Over the past decades mouse lemurs have been bred in captivity and used as animal models to investigate age-related diseases [3]. In this regard, some studies have demonstrated the beneficial effects of caloric restriction (CR) on age-associated pathologies, such as diabetes mellitus, and lifespan [4]. Resveratrol, a dietary polyphenol present in numerous plants, could mimic the effect of CR [5]. The anti-diabetic properties of both treatments have been studied in various animal models, revealing that they can improve glucose homeostasis, decrease insulin resistance, and protect β cells, and, thereby, prevent or improve diabetes [5,6]. The main objectives of our project were to study the repartition and the proportion of α and β cells in normal pancreatic islet, and to analyze the impact of chronic moderate CR and resveratrol supplementation on endocrine pancreas of grey mouse lemurs.

Materials and Methods

Animals

All primates studied were males born in the French National Museum of Natural History (UMR 7179 CNRS/MNHN; European Institutions Agreement # E 91-114.1). The animals were caged in small social groups (3-5 individuals), while being kept under artificial light simulating the Malagasy photoperiod and fed with fresh fruits and a daily prepared mixture of ginger bread, cereals, milk, and eggs, a diet comprising 61% carbohydrates, 23% proteins, and 16% lipids.

Three young adult primates (young adults group: case Nos. 1-3) were used to analyze the normal pancreatic islet architecture. They were fed with the standard diet described above and were 3-year old at the time of death. The test group included 16 mouse lemurs (case Nos. 4-19). They were included in the study beginning at 3-year old. They were grouped according to the type of diet; that is, standard diet (6 animals, case Nos. 4-9, 105 Kj/day), CR diet (5 animals, case Nos. 10-14), 71Kj/day), and standard diet with resveratrol (5 animals, case Nos. 15-19, 200 mg/Kg.day^{-1}). Ages ranged from 4 to 9 years old at the time of death.

All of the mouse lemurs enrolled in the present study died spontaneously or were euthanized due to advanced injuries, often in the context of a severe uremic syndrome. All experiments were performed in accordance with the Principles of Laboratory Animal Care (National Institutes of Health publication 86-23, revised 1985) and French national laws.

Preparation of samples and immunohistochemical analysis

Among other organs, the pancreas was systematically sampled, fixed for 48 hours in 4% paraformaldehyde and then stored in 70% ethanol at a temperature of 4 degrees Celsius. They were then post-fixed for 48 hours in 10% buffered formalin before being cut longitudinally. Each entire section was routinely processed, embedded in paraffin, and stained with hematoxylin-eosin-saffron (HES). Antibodies against

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Received October 06, 2016; **Accepted** November 12, 2016; **Published** November 22, 2016

Citation: Hurion M, Château-Joubert S, Servely JL, Pifferi F, Boyer C, et al. (2016) Changes in Pancreatic Islet Mass in Grey Mouse Lemurs (*Microcebus murinus*) Submitted to Caloric Restriction or Resveratrol Administration. J Cytol Histol 7: 439. doi: 10.4172/2157-7099.1000439

insulin and glucagon hormones were applied on two successive 3 µm sections using the Discovery XT Full System (Ventana Medical System, Inc) and a streptavidin-biotin detection system (DAB MAP, Roche Ventana). The primary antibodies used were a mix of two monoclonal mouse IgG antibodies directed against insulin (Insulin Ab-6 (INS04 + INS05), Thermo Scientific #MS-1379-R7, 0.5 µg/mL) and polyclonal rabbit IgG antibodies directed against glucagon (Cell Marque Corp Cat# 259A-18, 5.67 µg/mL).

Morphometric analysis

Observations and image acquisition were performed with an ImagerZ1 Zeiss microscope and an AxioCam HRc Zeiss camera. The total surface area for pancreas, pancreatic islets and α or β cells was quantified by using AxioVision 4.6.3 software. The threshold considered by the software as a specific cellular labeling for glucagon and insulin combines predefined values of hue, luminosity and saturation. As a lack of contrast between the endocrine and the exocrine pancreas was observed, we decided to use the insulin labelling in order to determine the outer delineation of the pancreatic islet. Therefore, there was a minimal and constant underestimation of the islet's total surface, and thus a constant overestimation of the α and β cell percentages. A summary of the results of morphometric analyses for control and test groups is provided in Table 1.

Results and Discussion

The normal endocrine pancreas, as observed for the young adults group (case Nos: 1-3), occupied 7.7 ± 4.5% of the total surface area of the pancreas. The pancreatic islets were circular in shape (200 to 300 micrometers in diameter). Immunostaining with insulin and glucagon antibodies and morphometric analysis highlighted their fine architecture. The α cells formed a discontinuous outer circle and occupied 10.3 ± 3.3% of the islet surface. The β cells were dispersed throughout the endocrine islet occupying 69.7 ± 1.8% of its surface (Figure 1).

In the test group, morphometric analysis revealed a marked increase of the pancreatic islet mass in 6 animals (percentage of total pancreatic surface area occupied by endocrine islets: 27.2 ± 15.6%; case Nos. 5, 8, 12, 15-17). This feature was detected preferentially in middle-aged adults (5.8 ± 0.8 years old). This increase of the endocrine mass appeared to be mainly caused by a major increase of β-cell mass in 4 of 6 animals (Figure 2); case Nos. 8, 15-17), by a major increase of α-cell mass in 1 of 6 animals (case No. 12), and by a major increase of both cell type mass in 1 out of 6 animals (case No. 5). One of these six animals (case No. 8) also exhibited a benign insulinoma, thus precluding assessment of the pancreatic surface area.

Under standard diet, 2 of 6 mouse lemurs (case No: 5, 8) presented an increase of pancreatic islet mass (19% for one; the percentage was not evaluated due to the presence of an insulinoma for the other). They were respectively 5 and 6 years old at the time of death. For the group of animals under a standard diet, the percentage of total pancreatic surface area occupied by endocrine islets was, on average, 10.0 ± 6.0%.

In the case of the animals under CR, only 1 of 5 mouse lemurs (case No: 12) had an increase of the endocrine pancreas (21%). It was 7-year old at the time of death, which made it the oldest animal out of the six harboring this lesion. For the group of animals under CR, the average percentage of total pancreatic surface area occupied by endocrine islets was 11.0 ± 6.7%. This value was not statistically different from that of animals under standard diet (Wilcoxon-Mann-Whitney, *p*: 1.00).

When standard diet was supplemented by resveratrol, 3 of 5 mouse lemurs (case Nos. 15-17) presented an increase of the endocrine pancreas (22%, 19%, 15%). Of these, one animal was 5-year old and the two others were 6-year old. The percentage of total pancreatic surface area occupied by endocrine islets for animals under this type of diet was, on average, 22.6 ± 19.1%, which was not statistically different from that of animals under standard diet (Wilcoxon-Mann-Whitney, *p*: 0.17), or that of animals under a diet supplemented with resveratrol (Wilcoxon-Mann-Whitney, *p*: 0.34). Endocrine cells of the pancreatic islets, other than α and β cells, were not analyzed by immunolabeling in this study. Regarding the normal endocrine islet architecture of young

Animal groups	Case No.	Diet	Age (years old)	Cause of death	% [Total endocrine islets surface/total pancreatic surface]	% [β cells surface/ endocrine islets surface]	% [α cells surface/ endocrine islets surface]	[% α cells surface]/[% β cells surface]
Young adults group	1	SD[a]	3	Anaplastic tumor	8	71	13	0,19
	2	SD	3	Trauma	2	70	11	0,15
	3	SD	3	Trauma	13	68	7	0,10
Test group	4	SD	4	CRF[d]	4	91	22	0,24
	5	SD	5	CRF	19	92	18	0,19
	6	SD	6	CRF	8	99	35	0,35
	7	SD	6	Multicentric lymphoma	6	77	22	0,29
	8	SD	6	Benign insulinoma	n/a	n/a	n/a	n/a
	9	SD	9	CRF	13	92	16	0,18
	10	CR[b]	4	Cardiac injury	3	97	23	0,24
	11	CR	5	Cardiac injury	7	70	20	0,28
	12	CR	7	CRF	21	74	23	0,31
	13	CR	7	Perianesthesia death	12	89	15	0,16
	14	CR	8	Trauma	12	83	23	0,28
	15	R[c]	5	CRF	22	82	5	0,06
	16	R	6	CRF	19	84	4	0,04
	17	R	6	CRF	55	68	2	0,03
	18	R	6	CRF	7	65	23	0,36
	19	R	8	CRF	10	55	10	0,19

[a]Standard diet; [b]Calorie restricted diet; [c]Standard diet with resveratrol; [d]Chronic renal failure.

Table 1: Morphometric analysis of pancreatic islets in 19 grey mouse lemurs according to age and type of diet.

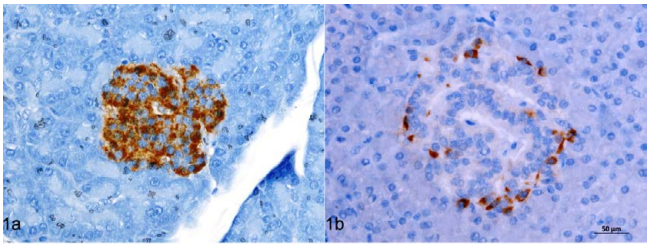

Figure 1: Pancreas, grey mouse lemur, No.3. Normal architecture of a pancreatic islet from a control animal composed of: (a) a large inner body of β cells. (b) A discontinuous outer row of α cells. Immunohistochemistry for (a) insulin and (b) glucagon. Streptavidin-biotin peroxidase detection system; hematoxylin counterstain.

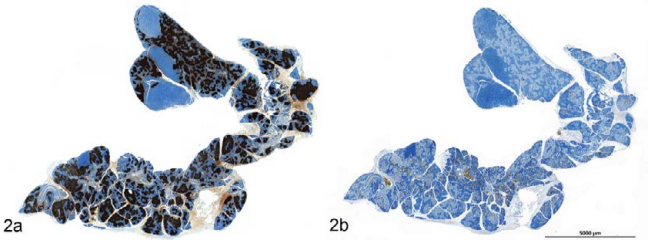

Figure 2: Pancreas, grey mouse lemur, No. 17. (a) diffuse severe increase in pancreatic islet mass from an animal under standard diet supplemented with resveratrol showing a severe increase of β-cell mass. (b) not showing an increase of α-cell mass. Immunohistochemistry for (a) insulin and (b) glucagon. Streptavidin-biotin peroxidase detection system; hematoxylin counterstain.

adult mouse lemurs, and in contrast to other non-human primates such as the cynomolgus monkey, α cells form in grey mouse lemurs islets a discontinuous outer circle and β cells form the large inner body of the endocrine islet. In humans, both cell types are widely distributed over the entire surface of the islet. The distribution of α and β cells in this prosimian primates is thus very similar to that of rodents. The percentage of total pancreatic surface area occupied by α and β cells was identical to that previously described in other mammals [7].

In our cohort, a marked increase of the pancreatic islet mass was observed in 6 of the 16 animals, in part explained by an increase in the β-cell mass. This seemed to have preferentially affected middle-aged adults, and was interpreted as a reactive change. This observation is consistent with the results of an another study on β-cell proliferation: following prolonged and repeated glucose infusion in adult rats, which mimics chronic hyperglycemia, an increase in the total mass of β cells through either hyperplasia or hypertrophy was observed [8]. Pancreatic β cells are able to adapt the production of insulin according to metabolic demands. When an insulin resistant state develops, both hyperplasia and hypertrophy of pancreatic β cells, and individual β-cell activity also increases so as to try to maintain glucose homeostasis [9]. As no destructive lesions were observed in the pancreas of the mouse lemurs, a state of peripheral insulin resistance could explain, at least in part, this observation. Unfortunately, as data regarding the serum concentration of insulin and glucose at the time of death were not available for these animals, the possible existence of a metabolic syndrome similar to the pre-diabetic stage in human type 2 diabetes mellitus could not be confirmed.

In the case of animals under CR, only 1 of 5 animals presented an increase of pancreatic islet mass. It was the oldest animal exhibiting this lesion (7-year old). The onset of this lesion seems to have been delayed by this type of diet, that may have had a protective effect against the establishment of an insulin resistant sate. The recent study of Marchal et al. concerning the same lemur colony, supports this hypothesis, demonstrating a positive effect of a CR on insulin sensitivity. With this diet, an improvement of insulin sensitivity prevented a compensatory hypersecretion of insulin, and therefore any change in pancreatic islet structures [10].

Under a standard diet supplemented with resveratrol, 3 of 5 animals presented an increase in the pancreatic islet mass, partly explained by an increase in the β-cell mass. The intake of resveratrol did not appear to have delayed the development of these lesions, and may even have favored their onset. Data from the literature indicate that in the case of other species resveratrol may exert a direct impact on β cells. On normal pancreatic islets from male Wistar rats, one of the effects of resveratrol was a decline in insulin secretion. This observation was explained in part by the induction of metabolic disturbances in β cells, and in particular a shift in islet glucose metabolism from mitochondrial oxidation to anaerobic glycolysis [11]. Thus, in the context of insulin decrease, compensatory increase in pancreatic islet mass may have occurred in these mouse lemurs.

Conclusion

We observed that the normal architecture of lemur endocrine islets was more similar to that of rodents than that of primates, including humans. The proportion of α and β cells was identical to that of other mammals. An increase in the pancreatic islet mass was observed in the case of 6 middle-aged animals: 2 animals under standard diet, 1 animal under CR, and 3 animals under resveratrol supplemented diet. This could partially be explained due to an increase of the β-cell mass. To our knowledge, this would be the first description of an increase in the pancreatic islet mass for this species. The influence of CR or resveratrol on the onset or delay of this lesion remains to be further investigated. A study including longitudinal blood sampling, greater sample size, and immunolabeling of all islet cell types is warranted to determine their impact on pathology in greater detail and give statistical strength to the observations. Nevertheless, our study highlights the aging mouse lemur as a promising spontaneous model for the study of type 2 diabetes mellitus with potential utility for the evaluation of new preventive or therapeutic procedures.

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