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 lemuris. 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 lemuris.

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

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.

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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 photo-period-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 lemuris 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 anti-diabetic properties of CRF: Chronic Renal Failure; HES: Hematoxylin-Eosin-Saffron; RSV: Resveratrol Intake; SD: Standard Diet

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 lemuris (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*). Ages ranged from 4 to 9 years old at the time of death.

All of the mouse lemuris 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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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 lemur (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 lemur (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 lemur (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

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

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Case No.</th>
<th>Diet</th>
<th>Age (years old)</th>
<th>Cause of death</th>
<th>% [Total endocrine islets surface/total pancreatic surface]</th>
<th>% [β cells surface/ endocrine islets surface]</th>
<th>% [α cells surface/ endocrine islets surface]</th>
<th>[% α cells surface]/(% β cells surface)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young adults group</td>
<td>1</td>
<td>SD*</td>
<td>3</td>
<td>Anaplastic tumor</td>
<td>8</td>
<td>71</td>
<td>13</td>
<td>0,19</td>
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<tr>
<td></td>
<td>2</td>
<td>SD</td>
<td>3</td>
<td>Trauma</td>
<td>2</td>
<td>70</td>
<td>7</td>
<td>0,10</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>SD</td>
<td>3</td>
<td>Trauma</td>
<td>13</td>
<td>68</td>
<td>7</td>
<td>0,10</td>
</tr>
<tr>
<td>Test group</td>
<td>4</td>
<td>SD</td>
<td>4</td>
<td>CRF#</td>
<td>4</td>
<td>91</td>
<td>22</td>
<td>0,24</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>SD</td>
<td>5</td>
<td>CRF</td>
<td>19</td>
<td>92</td>
<td>18</td>
<td>0,19</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>SD</td>
<td>6</td>
<td>CRF</td>
<td>8</td>
<td>99</td>
<td>35</td>
<td>0,35</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>SD</td>
<td>6</td>
<td>Multicentric lymphoma</td>
<td>6</td>
<td>77</td>
<td>22</td>
<td>0,29</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>SD</td>
<td>6</td>
<td>Benign insulinoma</td>
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<td>n/a</td>
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<td>n/a</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>SD</td>
<td>9</td>
<td>CRF</td>
<td>13</td>
<td>92</td>
<td>16</td>
<td>0,18</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>CR#</td>
<td>4</td>
<td>Cardiac injury</td>
<td>3</td>
<td>97</td>
<td>23</td>
<td>0,24</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>CR</td>
<td>5</td>
<td>Cardiac injury</td>
<td>7</td>
<td>70</td>
<td>20</td>
<td>0,28</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>CR</td>
<td>7</td>
<td>CRF</td>
<td>21</td>
<td>74</td>
<td>23</td>
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<tr>
<td></td>
<td>13</td>
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<td>Perianesthesia death</td>
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<tr>
<td></td>
<td>14</td>
<td>CR</td>
<td>8</td>
<td>Trauma</td>
<td>12</td>
<td>83</td>
<td>23</td>
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<td>16</td>
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<td>6</td>
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<tr>
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<td>R</td>
<td>6</td>
<td>CRF</td>
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<td>68</td>
<td>2</td>
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</tr>
<tr>
<td></td>
<td>18</td>
<td>R</td>
<td>6</td>
<td>CRF</td>
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<td>65</td>
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<tr>
<td></td>
<td>19</td>
<td>R</td>
<td>8</td>
<td>CRF</td>
<td>10</td>
<td>55</td>
<td>10</td>
<td>0,19</td>
</tr>
</tbody>
</table>

*Standard diet; #Calorie restricted diet; §Standard diet with resveratrol; CR: Chronic renal failure.
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, an increase of α-cell mass. Immunohistochemistry for (a) insulin and (b) glucagon. Streptavidin-biotin peroxidase detection system; hematoxylin counterstain. A discontinuous outer row of α cells. Immunohistochemistry for (a) insulin and (b) glucagon. Streptavidin-biotin peroxidase detection system; hematoxylin counterstain.

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.

References


