Study on Establishment of Congenic Strains and Screening of Characteristics in IRS-2 Deficient Mice to Support Translational Research on Type 2 Diabetes

Haruo HASHIMOTO

Central Institute for Experimental Animals, 1430 Nogawa, Miyamae, Kawasaki, Kanagawa 216-0001, Japan

Abstract: In research into type 2 diabetes, diet-based approaches, i.e., nutritional intake, are important approaches for therapeutic research. We would like to make the following two proposals from the standpoint of laboratory animal science for reproducible animal studies using type 2 diabetes mouse models. These include congenic strains of diabetes mouse models and improvement of diets used in daily care and management. In this research, the Irs2 homo-knockout mouse with both impaired glucose tolerance and insulin resistance, and thus type 2 diabetes, was established as a congenic strain. The effect of the genetic background on the onset of diabetes was examined. Next, we discussed which diets are appropriate for general care and management of mouse models in which the pathophysiology is controlled by nutritional conditions. Therefore, we prepared diets by converting the current Japanese and US diets to mice and adjusting the diet contents accordingly. We compared the insulin signals such as those of the liver, pancreas and white fat. We were thus able to establish an evaluation system closer to diabetes in the current population. Using this data as an example, we should consider the quality and ordinary diet of animals as important factors in animal experiments.

Key words: congenic strain, Irs2 homo-knockout mice, laboratory animal diet, type 2 diabetes

Introduction

Type 2 diabetes mellitus continues to increase rapidly all over the world and it is predicted to increase even more in the future, mainly in South America, Africa and Asia [40]. The number of patients with diabetes worldwide was 105 million in 2000 and by 2010, it is expected to rise sharply to 220 million [40]. Japan is no excepted from this increase in type 2 diabetes. The factors involved include the spread of automobiles, westernization of the diet and sedentary habits in work and daily activities since the end of the Second World War. In the Japanese National Health and Nutrition Survey conducted in 2008, the number of people suspected of having type 2 diabetes nationwide had increased the number of recorded to an estimated 18,700,000, which was 2.5 million more than in the survey 4 years previously. With this social background, both in vitro and in vivo research...
into treatments for type 2 diabetes is progressing rapidly. In the Central Institute for Experimental Animals, technology for microbiologic and genetic control has been under development for many years. In current research, we are approaching diabetes research from the aspect of the development of genetically uniform diabetes mouse models, mainly using genetic control techniques.

IRS-2

After binding to insulin receptors in the plasma membrane, insulin activates tyrosine kinase present in the cells and the insulin receptor substrate (IRS) family consisting of IRS-1 (pp185), IRS-2 (4PS/pp190) IRS-3 (pp60), and IRS-4 (pp160) undergo tyrosine phosphorylation [19] (Fig. 1). Tyrosine phosphorylated IRS binds to Src homology 2 (SH2) proteins such as PI3 kinase [3, 6, 20, 24], growth factor receptor-bound protein 2/Ash (GRB2/Ash) [34, 37], tyrosine phosphatase-2 (SHP-2) [25, 26, 30], and C-terminal Src kinase (Csk) [38]. These proteins are considered to be the key to the diverse and specific effects of insulin. IRS causes signal transmission failure by inhibition of tyrosine phosphorylation and serine phosphorylation through molecules causing insulin resistance via phosphoenolpyruvate carboxykinase (PCK) and IkappaB kinase (IKK) [28].

The roles of IRS-1, IRS-2, IRS-3, and IRS-4 are being verified by the establishment and analysis of knockout mice. IRS-1 deficient mice show inhibited growth and they are about 2/3 the size of wild-type mice. They also show insulin resistance [36]. However, they show hyperplasia of beta cells and maintain of insulin secretion, leading to hyperinsulinemia, but not impaired glucose tolerance [36]. In the liver, IRS-2 shows a high level of expression which compensates for the effects of IRS-1, but in the skeletal muscle, IRS-2 expression is low, indicating that the organ causing insulin resistance in the IRS-1 deficient mouse is the skeletal muscle. IRS-2 knockout mice show leptin resistance in the central nervous system and insulin resistance due to insulin transmission failure in the liver. IRS-2 deficient mice are utilized as a type 2 diabetes model because insufficient growth of beta cells leads to insulin secretion decrease resulting in type 2 diabetes [18]. The IRS-3 deficient mouse does not have growth disorders or impaired glucose tolerance and glucose uptake by the adipose tissue is not abnormal [21]. The IRS-4 deficient mouse shows only mild growth disorders and impaired glucose tolerance [5]. Therefore, at the Central Institute for Experimental Animals (CIEA), attention has been focused on the IRS-2 deficient mouse that has both impaired glucose tolerance and insulin resistance, i.e., type 2 diabetes. Strains have been established and their characteristics have been screened.

Breeding of the Irs2–/– Mouse Strain

The Irs2tm1Tka homozygote (Irs2homo-knockout or Irs2–/–) mouse was established by Kubota et al. (2000) [18] at the Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo and was introduced to CIEA in 2003. The genetic background of the Irs2–/– mouse at the time of introduction was a mixed background of C57BL/6J and CBA (B6J × CBA). We selected the C57BL/6Jc1 (B6J) strain and backcrossed it with the Irs2–/– mouse. Since C57BL/6J is a strain that is likely to develop hyperinsulinemia [1], when given a high lipid load [29] or due to glucose metabolic abnormalities [11, 12], it is considered that impaired glucose tolerance in the B6J strain likely occurs due to insulin resistance. Irs2–/– mice in the form of Irs2–/– knockout mice with C57BL/6Jc1 genetic backgrounds (B6J-Irs2–/– mouse) have currently reached the 18th generations at

Fig. 1. Structure and function of insulin receptor substrate (IRS).
CIEA, and the characteristics of the B6J-Irs2−/− mouse have been screened. Data for the B6J-Irs2−/− mouse are presented in this paper. The descriptions and data in the following sections are cited and abstracted from previous publications [2, 7–10].

**Basic Analysis of the Irs2−/− Mouse with a C57BL/6Jccl Genetic Background**

The male B6J × CBA-Irs2−/− mouse shows insulin resistance from 6 weeks of age and impaired glucose tolerance from 10 weeks of age. In the female B6J × CBA-Irs2−/− mouse, differences in impaired glucose tolerance from the wild type are very slight from 6 to 20 weeks of age. The Irs2−/− mouse, for which backcrossing with C57BL/6Jccl has reached the 10th generation, has become homozygous, and impaired glucose tolerance, insulin resistance, plasma metabolites, hepatic enzyme activity and skeletal muscle enzyme activity have been measured at 6, 14, and 24 weeks of age [9].

This earlier research included a glucose load study, an insulin load study and sampling every other day at each week of age. The research design was the same as that used in the research described below and the details were published in a previous publication [9].

The results indicated that the B6J-Irs2−/− mouse shows impaired glucose tolerance and insulin resistance in both males and females from 6 weeks of age (Fig. 2-[a], [d], [g], and [j]). When metabolites in the plasma were measured, hyperinsulinemia, hypertriglyceridemia and hyper-free fatty acidemia were observed. The activities of hepatic enzymes, fatty acid synthase (FAS), ATP citrate lyase (ACL) and malic enzyme, were exacerbated and lipid synthesis was increased. The mice tend to show characteristics of metabolic syndrome and the same tendencies are observed at each age. Therefore, it was judged that the B6J-Irs2−/− mouse could be used in research from 6 weeks of age. In this earlier research, two highly interesting phenomena were encountered. The first was that at 14 weeks of age, B6J-Irs2−/− mice with hypertriglyceridemia at 6 and 14 weeks of age showed significantly lower values of plasma metabolites than the wild type at 14 weeks of age, while FFA had decreased to the same level as in the wild type. Since the same results were reported in liver-specific insulin receptor knockout mice [22] around the same time, it was concluded that the pathophysiology of the B6J-Irs2−/− mouse became transiently worse at 14 weeks of age. This conclusion was basically the same as that reached in later studies. The second phenomenon was that in 20% of male B6J-Irs2−/− mice from 14 to 24 weeks of age, the mean blood glucose suddenly increased from 245.8 mg/dl to more than 500 mg/dl and the animals died within a short time with polydipsia/polyuria and loss of body weight. At 14 weeks of age, data could not be obtained because the mice had died before sampling, but at 24 weeks, samples could be obtained from two animals. In addition to hyperglycemia, insulin depletion and atrophy of the islets of Langerhans were marked. The number of cases increased thereafter and a detailed analysis was performed [2]. The results showed that the mice with a sudden increase of blood glucose showed similarities to humans with fulminant type 1 diabetes. The characteristics of fulminant type 1 diabetes as reported by Imagawa et al. are 1) sudden hyperglycemia, 2) wasting associated with polyuria in a short time, 3) C-peptide decrease and insulin depletion, 4) metabolic abnormalities associated with ketosis, 5) no onset due to autoantibodies, and 6) increase of extrapancreatic secretion [13, 14]. In our research, B6J-Irs2−/− mice with blood glucose increased to above 500 mg/dl were found to have characteristics 1)–5) by Imagawa et al. [Fig. 3 (insulin depletion) and Fig. 4 (clarification of no onset due to autoantibodies)] and resembled fulminant type 1 diabetes in humans. However, we have not yet determined why this phenomenon only occurs in some males.

**Comparison of B6J-Irs2−/− Mice Mated Naturally or Produced by Reproductive Engineering**

At present, many laboratory animal facilities and research facilities are using reproductive engineering methods with in vitro fertilization and embryo transfer to produce animals. The advantages of reproductive engineering include the ability to set the same delivery date in many animals, and the possibility of planned production and microbiologic screening by concomitant use of cesarean section in vinyl isolators for surgery. When research on the ontogenetic characteristics of B6J-Irs2−/− mice began, Kagohashi et al. of Shimane Univer-
sity reported that onset of type 1 diabetes in NOD mice produced by embryo transplants into ICR or DBA/2J recipients was later than that in NOD mice reared by foster mothers [17]. The B6I-Irs2−/− mice used in the investigation of ontogenetic characteristics [7] were prepared by in vitro fertilization and embryo transfer.

Fig. 2. Oral glucose tolerance test (GTT) and insulin tolerance test (ITT) results for male (GTT, a through c; ITT, g through i) and female (GTT, d through f; ITT, j through l) B6I-Irs2−/− and wild-type mice at 6 (a and d), 14 (b and e), and 24 (c and e) weeks of age. Data are presented as mean ± SE. *, P<0.05; +, P<0.01 (Student’s t-test) compared with value for wild-type controls. This Figure first appeared in Comparative Medicine 56: 176–187 [9].
We considered that the phenotypes, i.e., impaired glucose tolerance and insulin resistance, of B6J-*Irs2−/−* mice produced by pregnancies of parents of the same strains as reported by Kagohashi et al. [17] and B6J-*Irs2−/−* mice reared by embryo transplants into MCH(ICR) mice were probably different. Therefore, we compared B6J-*Irs2−/−* mice produced by natural mating or reproductive engineering. Detailed study conditions are shown in previous publication [8]. The results revealed that there were no differences in impaired glucose tolerance and insulin resistance between B6J-*Irs2−/−* mice produced by natural mating or reproductive engineering, i.e., the results showed that in the production of B6J-*Irs2−/−* mice, the same characteristics can be obtained through natural mating and/or embryo transfer.

Next, the reproductive efficiency of B6J-*Irs2−/−* mice produced by both natural mating and reproductive engineering was investigated [10]. Five combinations of natural mating were employed: (1) female *Irs2−/−* mouse

---

**Investigation of the Reproductive Efficiency of the B6J-*Irs2−/−* Mouse**

Next, the reproductive efficiency of B6J-*Irs2−/−* mice produced by both natural mating and reproductive engineering was investigated [10]. Five combinations of natural mating were employed: (1) female *Irs2−/−* mouse...
In research to date, B6J-\(Irs2^{-/-}\) mice have been used to study diabetes from 6 weeks of age. These studies have screened their characteristics at each week of age, and a reproduction system for this mouse as a laboratory animal has been established. We reviewed the animal's diet when considering the research objectives since the diets used for laboratory animals, except for some special diets, consist mainly of various diets for ordinary nutritional management that are produced by several companies. However, the ingredients of these diets showed few differences and had not been reviewed for many years. Therefore, we prepared a general diet for laboratory animals based on the nutritional ingredients in the human postwar diets in Japan and modern diets in Japan and the US based on data obtained from the Japanese Health and Nutrition Surveys and the US Department of Agriculture (USDA). As a result, we unexpectedly found that diets for laboratory animals consisted of nutrients similar to those in the postwar Japanese diet (Table 1). Therefore, using the laboratory animal diet resembling the postwar Japanese diet (regular diet, Rd group) as a control, we fed a modern Japanese diet (Jd group) or modern American diet (Ad group) continuously to B6J-\(Irs2^{-/-}\) mice from the fetal stage to 6 weeks of age, and compared impaired glucose tolerance, insulin resistance, and insulin signaling in the liver, skeletal muscle, adipose tissue and pancreas. Detailed study conditions are given in previous publication [7]. The results showed that insulin resistance in the Jd group was worse than that in the animals given the conventional laboratory animal diet (Fig. 6[a]). In the Ad group, both impaired glucose tolerance and insulin resistance were worse (Fig. 6[a] and 6[b]). When metabolites in the blood were examined at that time, greater hyperglycemia and hyperinsulinemia were observed in the Jd and Ad groups than in the Rd group. However, triglycerides were significantly lower in the Jd and Ad groups than in the Rd group. These results supported the results and discussion on 14-week old B6J-\(Irs2^{-/-}\) mice described previously [9]. However, it is still not clear why worsening of the pathophysiology in B6J-\(Irs2^{-/-}\) mice causes a decrease in triglycerides. The liver, skeletal muscle, white adipose tissue and pancreas were analyzed at the same time. The results showed an increase in the activity of the malic enzyme that promotes lipid synthesis in the liver [32] of the Jd group. In the liver of the Ad group, expression of the sterol regulatory element binding protein (SREBP)-1c gene [4], and activities of ACL [32, 39] and malic enzyme increased. In the skeletal muscle, expression of the glucose transporter (GLUT) 4

---

**Evaluation of Diets Based on Current Japanese and American Diets Used in B6J-\(Irs2^{-/-}\) Mice**

In research to date, B6J-\(Irs2^{-/-}\) mice have been used to study diabetes from 6 weeks of age. These studies have screened their characteristics at each week of age, and a reproduction system for this mouse as a laboratory animal has been established. We reviewed the animal's diet when considering the research objectives since the diets used for laboratory animals, except for some special diets, consist mainly of various diets for ordinary nutritional management that are produced by several companies. However, the ingredients of these diets showed few differences and had not been reviewed for many years. Therefore, we prepared a general diet for laboratory animals based on the nutritional ingredients in the human postwar diets in Japan and modern diets in Japan and the US based on data obtained from the Japanese Health and Nutrition Surveys and the US Department of Agriculture (USDA). As a result, we unexpectedly found that diets for laboratory animals consisted of nutrients similar to those in the postwar Japanese diet (Table 1). Therefore, using the laboratory animal diet resembling the postwar Japanese diet (regular diet, Rd group) as a control, we fed a modern Japanese diet (Jd group) or modern American diet (Ad group) continuously to B6J-\(Irs2^{-/-}\) mice from the fetal stage to 6 weeks of age, and compared impaired glucose tolerance, insulin resistance, and insulin signaling in the liver, skeletal muscle, adipose tissue and pancreas. Detailed study conditions are given in previous publication [7]. The results showed that insulin resistance in the Jd group was worse than that in the animals given the conventional laboratory animal diet (Fig. 6[a]). In the Ad group, both impaired glucose tolerance and insulin resistance were worse (Fig. 6[a] and 6[b]). When metabolites in the blood were examined at that time, greater hyperglycemia and hyperinsulinemia were observed in the Jd and Ad groups than in the Rd group. However, triglycerides were significantly lower in the Jd and Ad groups than in the Rd group. These results supported the results and discussion on 14-week old B6J-\(Irs2^{-/-}\) mice described previously [9]. However, it is still not clear why worsening of the pathophysiology in B6J-\(Irs2^{-/-}\) mice causes a decrease in triglycerides. The liver, skeletal muscle, white adipose tissue and pancreas were analyzed at the same time. The results showed an increase in the activity of the malic enzyme that promotes lipid synthesis in the liver [32] of the Jd group. In the liver of the Ad group, expression of the sterol regulatory element binding protein (SREBP)-1c gene [4], and activities of ACL [32, 39] and malic enzyme increased. In the skeletal muscle, expression of the glucose transporter (GLUT) 4
Efficient reproductive method for B6J-Irs2<sup>−/−</sup> mice. [A] Repetition of IVF-ET in all female Irs2<sup>−/+</sup> mice used for IVF should make it possible to produce Irs2<sup>−/−</sup> infants. However, it is possible that the number of Irs2<sup>−/+</sup> females would decline because the reproduction index of the Irs2<sup>−/+</sup> infants were not stable. [B] Irs2<sup>−/−</sup> mice need to be produced by embryo transfer using Irs2<sup>−/−</sup> mice from a colony consisting of female Irs2<sup>−/+</sup> × male Irs2<sup>−/−</sup> mice to continue stable reproduction of the Irs2<sup>−/−</sup> mice, because the reproduction index of IVF-ET showed that the expansion of Irs2<sup>−/−</sup> mice using IVF-ET alone was impossible. In addition, use of vitrification in IVF-ET might produce better results (a), because the survival rate of embryos frozen by vitrification has been good in recent years. This Figure first appeared in *Experimental Animals* 57: 407–411 [10].

gene [23] decreased and FAS activity [32] increased in both the Jd and Ad groups. In the pancreas, hyperinsulinemia was more severe in the Jd and Ad groups than in the Rd group, but expression of the GLUT2 gene in the Ad group was decreased. Therefore, in the liver, skeletal muscle and pancreas of B6J-Irs2<sup>−/−</sup> mice, the modern Japanese diet and modern American diet caused an increase in enzymes related to lipid synthesis and glucose uptake was reduced. When B6J-Irs2<sup>−/−</sup> mice fed each of these diets were observed intraperitoneally using MRI, accumulation of intraperitoneal fat increased and hypertrophy of adipocytes occurred in the Jd group and Ad group when compared with the Rd control group (Fig. 7). At that time, expression of the lipid GLUT4 gene did not change in the Jd and Ad groups, but expression of the peroxisome proliferator activated receptor (PPAR) γ<sub>2</sub> gene and the plasma concentration of tumor necrosis factor (TNF) α increased. In both the Jd and Ad groups, plasma adiponectin concentration decreased. In the Ad group, plasma lecithin also increased. Based on these results, in the Jd group and Ad group, intraperitoneal fat increased and hypertrophy of adipocytes occurred due to lipogenic enzymes derived from the liver and skeletal muscle, and that TNFα and lecithin
secreted from hypertrophied adipose tissue increased, while adiponectin decreased, which caused worsening of insulin resistance. These conditions induced a decrease in glucose uptake in the skeletal muscle and this caused worsening of the impaired glucose tolerance. These results showed that the conventional diet for laboratory animals might cause over-estimation of the pathophysiology in not only the B6J–Irs2–/– mouse but also the other type 2 diabetes mouse models used to date.

In many studies on diabetes, a high lipid load is applied to mouse strains or type 2 diabetes mouse models, and impaired glucose tolerance, insulin resistance and insulin signaling in the organs are evaluated. However, the lipid content of high fat diets for laboratory animals is 40–60% [16, 35]. In human diets, the lipid content is 27.2% in modern Japanese diets and 34.6% in American diets. The typical modern Japanese and American diets that we developed for this study had lipid contents of 24.8 and 35.4% respectively. Therefore, the impaired glucose tolerance, insulin resistance and insulin signaling in the organs of type 2 diabetes mouse models are considered to be excessive due to the high fat diets which have been used in studies to date. We compared the results of these earlier studies with those of our study. In the comparison of the American diet group with the

Table 1. Conversions used for converting human nutritional contents to those for laboratory mice

(a)

<table>
<thead>
<tr>
<th></th>
<th>Modern American diet (a)</th>
<th>Modern Japanese diet (b)</th>
<th>Japanese diet after WWII (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intake weight (kcal/day)</td>
<td>Relative value (%)</td>
<td>Intake weight (kcal/day)</td>
</tr>
<tr>
<td>Calories</td>
<td>2,409.1 → 462.9d</td>
<td>118.7</td>
<td>1,985.1 → 384.9</td>
</tr>
<tr>
<td></td>
<td>Intake weight (g/day)</td>
<td>Relative value (%)</td>
<td>Intake weight (g/day)</td>
</tr>
<tr>
<td>Protein</td>
<td>94.9 → 18.2</td>
<td>15.8</td>
<td>81.5 → 15.8</td>
</tr>
<tr>
<td>Fat</td>
<td>92.7 → 17.8</td>
<td>34.6</td>
<td>59.9 → 11.6</td>
</tr>
<tr>
<td>Fatty acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total weight</td>
<td>85.5 → 16.4</td>
<td>31.9</td>
<td>56.9 → 11.0</td>
</tr>
<tr>
<td>SFA</td>
<td>31.3 → 6.0</td>
<td>11.7</td>
<td>16.0 → 3.1</td>
</tr>
<tr>
<td>MUFA</td>
<td>35.8 → 6.9</td>
<td>13.4</td>
<td>20.5 → 4.0</td>
</tr>
<tr>
<td>PUFA</td>
<td>18.4 → 3.5</td>
<td>6.9</td>
<td>20.3 → 3.9</td>
</tr>
<tr>
<td>Moisture</td>
<td>298.8 → 57.4</td>
<td>49.6</td>
<td>280.0 → 54.3</td>
</tr>
</tbody>
</table>

(b)

<table>
<thead>
<tr>
<th></th>
<th>Modern American diet</th>
<th>Modern Japanese diet</th>
<th>Japanese diet after WWII</th>
<th>CA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>8.8</td>
<td>10.0</td>
<td>11.1</td>
<td>8.3</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>24.3</td>
<td>22.5</td>
<td>20.2</td>
<td>26.8</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>15.5</td>
<td>10.1</td>
<td>3.9</td>
<td>5.0</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>5.4</td>
<td>5.2</td>
<td>5.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Crude ash (%)</td>
<td>6.4</td>
<td>6.1</td>
<td>6.2</td>
<td>7.6</td>
</tr>
<tr>
<td>NFE (%)</td>
<td>39.6</td>
<td>46.2</td>
<td>53.2</td>
<td>48.9</td>
</tr>
<tr>
<td>Calories (kcal/100g)</td>
<td>395.1</td>
<td>365.0</td>
<td>328.9</td>
<td>347.4</td>
</tr>
<tr>
<td>Fat energy (%)</td>
<td>35.4</td>
<td>24.8</td>
<td>10.7</td>
<td>—</td>
</tr>
<tr>
<td>CP energy (%)</td>
<td>24.6</td>
<td>24.6</td>
<td>24.5</td>
<td>—</td>
</tr>
</tbody>
</table>

most serious diabetes among the three groups and reports on high fat diets given to mouse strains to date, the expressions of both PPARγ2 and SREBP-1c, that are the keys to increased lipids and hypertrophy, increased in, and the same results as in previous reports were obtained [15, 16, 27]. However, the results for glucokinase, phosphoenol pyruvate carboxy kinase (PEPCK) and GLUT4 in this study differed from those from reports on high fat loads with a calorie ratio of more than 40% [31]. In the diet comparison, these values did not change across the Rd, Jd and Ad groups and the values for liver glucokinase, PEPCK and lipid GLUT4 differed from those of the other reports. The result of a study on high lipid loads with a calorie ratio of more than 40% [16] showed excessive responses with respect to these points. In a previous report showing high PPARγ in the same way as in the diet comparison study [15], fatty liver was caused by a high-fat diet, but even though aspartate aminotransferase (AST) in the Ad group was higher than that in the Rd group, there were no signs of fatty liver in the liver tissue. Since young mice at 6 weeks of age were used in the diet comparison study, it was unlikely that fatty liver would have been observed, and these mice were unlikely to have developed fatty livers. In the B6J-Irs2–/– mouse model in which the phenotype changes to the fulminant type, discovered by Hashimoto et al. (2006) [9], fat droplets were observed (Fig. 8). When fatty liver is observed in this model, the condition is terminal.

The Japanese and American diets developed for the study showed a specific pathophysiology with inhibition of the excessive response of insulin signals when compared with the high-fat diets used in ordinary care and management to date. Therefore, the results for the B6J-Irs2–/– mouse due to the Japanese and American diets appear to be close to those for humans and it is recommended that these diets should be used in ordinary care and management of laboratory animals.

**Conclusion**

We have established the B6J-Irs2–/– mouse strain with a C57BL/6J background and an efficient production method for this strain, as well as a system for rearing this strain as a laboratory animal. However, the mechanism of the onset of diabetes related to the IRS family is still unclear in many aspects and we plan to screen new genes and compounds related to modification of diabetes. At CIEA, a basic structure is being established for animal models of not only diabetes but also other human diseases using technologies for microbiologic and genetic control based on original concepts. It is
Hoped that this research will provide translational support for medical research to develop treatments for patients with these diseases.

**Acknowledgments**

The author wishes to express his gratitude to Professor Emeritus Kinori Kosaka and Professor Takashi Kadowaki of the Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo for their valuable guidance in these studies on the Irs2<sup>−/−</sup> mouse. I also wish to thank Professor Toshiro Arai of Nippon Veterinary and Life Science University for the measurements used in this research. Thanks are also due to the staffs of the Central Institute for Experimental Animals.
STUDY ON ESTABLISHMENT OF CONGENIC STRAINS IN IRS-2 DEFICIENT MICE

Nippon Veterinary and Life Science University and Keio University for their assistance. Finally, I am grateful to Dr. Tatsuji Nomura, the director and Kyoji Hioki, Yasuyuki Ohnishi and Mamoru Ito of the Central Institute for Experimental Animals for providing me with the opportunity to study diabetes.

These studies were partially supported by a Grant-in-Aid for Young Scientists (B) (No. 17700380) and Challenging Exploratory Research (No. 22650095) to Haruo Hashimoto, a Grant-in-Aid for Scientific Research (A) (No. 17200029) to Toshiro Arai from the Ministry of Education, Culture, Sports, Science and Technology, of Japan.

The author received the 22th JALAS young investigator award in 2010.

References


