True Niacin Deficiency in Quinolinic Acid Phosphoribosyltransferase (QPRT) Knockout Mice

Katsumi SHIBATA
Department of Nutrition, School of Human Cultures, The University of Shiga Prefecture, 2500 Hassakacho, Hikone, Shiga 522–8533, Japan

Summary Pyridine nucleotide coenzymes (PNCs) are involved in over 500 enzyme reactions. PNCs are biosynthesized from the amino acid L-tryptophan (L-Trp), as well as the vitamin niacin. Hence, “true” niacin-deficient animals cannot be “created” using nutritional techniques. We wanted to establish a truly niacin-deficient model animal using a protocol that did not involve manipulating dietary L-Trp. We generated mice that are missing the quinolinic acid phosphoribosyltransferase (QPRT) gene. QPRT activity was not detected in qprt−/− mice. The qprt+/+, qprt+/− or qprt−/− mice (8 wk old) were fed a complete diet containing 30 mg nicotinic acid (NIA) and 2.3 g L-Trp/kg diet or an NIA-free diet containing 2.3 g L-Trp/kg diet for 23 d. When qprt−/− mice were fed a complete diet, food intake and body weight gain did not differ from those of the qprt+/+ and the qprt+/− mice. On the other hand, in the qprt−/− mice fed the NIA-free diet, food intake and body weight were reduced to 60% (p<0.01) and 70% (p<0.05) of the corresponding values for the qprt−/− mice fed the complete diet at day 23, respectively. The nutritional levels of niacin such as blood and liver NAD concentrations were also lower in the qprt−/− mice than in the qprt+/+ and the qprt+/− mice. Urinary excretion of quinolinic acid was greater in the qprt−/− mice than in the qprt+/+ and the qprt+/− mice (p<0.01). These data suggest that we generated truly niacin-deficient mice.

Key Words mice, niacin-deficient, pellagra, quinolinate phosphoribosyltransferase, tryptophan

We successfully generated QPRT-KO (quinolinic acid phosphoribosyltransferase knockout) mice. Here, we talk about establishment of true niacin deficiency in QPRT-KO mice. Humans need 13 kinds of specific vitamins. Rats and mice need 12 kinds of specific vitamins because they can synthesize vitamin C from glucose. However, this information is not correct since all three species can synthesize the necessary amount of nicotinamide (Nam) from dietary L-tryptophan (L-Trp). In the present symposium, we propose that Nam is a biofactor rather than a vitamin. For example, we habitually consume 80 g protein per day. Eighty grams of protein contains around 800 mg L-Trp. The conversion ratio of L-Trp to nicotinamide in humans is 1/60 on a weight basis (1). Thus, we can coin 13 mg Nam from 800 mg L-Trp, and it can provide us with the necessary amount of Nam and/or urocanic acid, and UDP-glucose 4-epimerase reaction. All enzymes exist in the liver. QPRT is located at the point on the connection between the L-Trp degradation pathway and the NAD cycle (5). The substrate quinolinic acid (QA) is synthesized from α-amino-β-carboxymuconate-γ-semialdehyde (ACMS) by a nonenzymic reaction. ACMS has another metabolic pathway, which leads to formation of acetyl-CoA via glutaryl-CoA and this is the main degradation pathway of L-Trp. Mammals such as mice, rats, and humans have alternative pathways of NAD biosynthesis. They includ-
ing humans synthesizing NAD from dietary nicotinic acid (NiA) and Nam even if the QPRT reaction is lacking because they have NiA phosphoribosyltransferase and Nam phosphoribosyltransferase. QPRT is the enzyme which connects the complete degradation pathway of L-Trp and the NAD synthesis. QPRT does not need ATP, unlike NiA phosphoribosyltransferase and Nam phosphoribosyltransferase.

We first disrupted the qprt gene using homologous recombination. The targeting vector was constructed in the genomic qprt exon 2 and 3 with the PGK-neo cassette. The constructed targeting vector was introduced into a 129 Svj mouse ES cell line. The positive clone was injected into C57BL/6 mouse blastocysts to obtain chimeric mice that transmitted the mutation through the germline. Heterogenous mice were crossed with C57BL/6 mice for one generation. Interbreeding the resulting heterozygotes produced wild-type (qprt<sup>+/+</sup>), heterozygote (qprt<sup>+/−</sup>) and homozygote (qprt<sup>−/−</sup>) mice. The disruption of qprt was verified by the absence of the QPRT gene indicated by Southern blot.

Niacin-deficient animals cannot be created by merely removing preformed niacin from a complete diet (6). Therefore, researchers have been attempting to create animal models that cannot biosynthesize Nam or NiA from L-Trp.

We first checked whether or not the qprt<sup>−/−</sup> mice became truly niacin deficient when they were fed a diet without preformed niacin. Table 1 shows the compositions of the diets. Two types of purified diets were made. One was a complete purified diet containing 30 mg NiA and 2.3 g L-Trp/kg diet. The other was a purified NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

The 8-wk-old mice were placed in individual metabolic cages and divided into 6 groups: three genotypes and two diets; qprt<sup>++/+</sup>, qprt<sup>+/−</sup> or qprt<sup>−/−</sup> mice were fed the purified complete diet or the purified NiA-free diet. Each group consisted of 2 or 3 males and 3 females. They were fed the complete diet or NiA-free diet ad libitum for 23 d. As shown in Fig. 2, the complete diet did not have adverse effects on body weight gain in any of the genotypes. The NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

Fig. 1. Trp metabolic pathway. ACMS, α-amino-β-carboxymuconate-ε-semialdehyde; ACMSD, α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase; 2-AMA, 2-aminomuconic acid; AMS, α-amino-α-carboxymuconate-ε-semialdehyde; 5-HIAA, 5-hydroxyindole-3-acetic acid; MNA, N<sup>1</sup>-methylnicotinamide; NaMN, nicotinic acid mononucleotide; NaAD, nicotinic acid adenine dinucleotide; NiA, nicotinic acid; NMN, nicotinamide mononucleotide; 2-OAA, 2-oxoadipic acid; 2-Py, N<sup>1</sup>-methyl-2-pyrrolidone-5-carboxamide; 4-Py, N<sup>1</sup>-methyl-4-pyrrolidone-3-carboxamide; QA, quinolinic acid; QPRT, quinolinic acid phosphoribosyltransferase.

We first disrupted the qprt gene using homologous recombination. The targeting vector was constructed in the genomic qprt exon 2 and 3 with the PGK-neo cassette. The constructed targeting vector was introduced into a 129 Svj mouse ES cell line. The positive clone was injected into C57BL/6 mouse blastocysts to obtain chimeric mice that transmitted the mutation through the germline. Heterogenous mice were crossed with C57BL/6 mice for one generation. Interbreeding the resulting heterozygotes produced wild-type (qprt<sup>+/+</sup>), heterozygote (qprt<sup>+/−</sup>) and homozygote (qprt<sup>−/−</sup>) mice. The disruption of qprt was verified by the absence of the QPRT gene indicated by Southern blot.

Niacin-deficient animals cannot be created by merely removing preformed niacin from a complete diet (6). Therefore, researchers have been attempting to create animal models that cannot biosynthesize Nam or NiA from L-Trp.

We first checked whether or not the qprt<sup>−/−</sup> mice became truly niacin deficient when they were fed a diet without preformed niacin. Table 1 shows the compositions of the diets. Two types of purified diets were made. One was a complete purified diet containing 30 mg NiA and 2.3 g L-Trp/kg diet. The other was a purified NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

The 8-wk-old mice were placed in individual metabolic cages and divided into 6 groups: three genotypes and two diets; qprt<sup>++/+</sup>, qprt<sup>+/−</sup> or qprt<sup>−/−</sup> mice were fed the purified complete diet or the purified NiA-free diet. Each group consisted of 2 or 3 males and 3 females. They were fed the complete diet or NiA-free diet ad libitum for 23 d. As shown in Fig. 2, the complete diet did not have adverse effects on body weight gain in any of the genotypes. The NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

The 8-wk-old mice were placed in individual metabolic cages and divided into 6 groups: three genotypes and two diets; qprt<sup>++/+</sup>, qprt<sup>+/−</sup> or qprt<sup>−/−</sup> mice were fed the purified complete diet or the purified NiA-free diet. Each group consisted of 2 or 3 males and 3 females. They were fed the complete diet or NiA-free diet ad libitum for 23 d. As shown in Fig. 2, the complete diet did not have adverse effects on body weight gain in any of the genotypes. The NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

We first checked whether or not the qprt<sup>−/−</sup> mice became truly niacin deficient when they were fed a diet without preformed niacin. Table 1 shows the compositions of the diets. Two types of purified diets were made. One was a complete purified diet containing 30 mg NiA and 2.3 g L-Trp/kg diet. The other was a purified NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

The 8-wk-old mice were placed in individual metabolic cages and divided into 6 groups: three genotypes and two diets; qprt<sup>++/+</sup>, qprt<sup>+/−</sup> or qprt<sup>−/−</sup> mice were fed the purified complete diet or the purified NiA-free diet. Each group consisted of 2 or 3 males and 3 females. They were fed the complete diet or NiA-free diet ad libitum for 23 d. As shown in Fig. 2, the complete diet did not have adverse effects on body weight gain in any of the genotypes. The NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

The 8-wk-old mice were placed in individual metabolic cages and divided into 6 groups: three genotypes and two diets; qprt<sup>++/+</sup>, qprt<sup>+/−</sup> or qprt<sup>−/−</sup> mice were fed the purified complete diet or the purified NiA-free diet. Each group consisted of 2 or 3 males and 3 females. They were fed the complete diet or NiA-free diet ad libitum for 23 d. As shown in Fig. 2, the complete diet did not have adverse effects on body weight gain in any of the genotypes. The NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

The 8-wk-old mice were placed in individual metabolic cages and divided into 6 groups: three genotypes and two diets; qprt<sup>++/+</sup>, qprt<sup>+/−</sup> or qprt<sup>−/−</sup> mice were fed the purified complete diet or the purified NiA-free diet. Each group consisted of 2 or 3 males and 3 females. They were fed the complete diet or NiA-free diet ad libitum for 23 d. As shown in Fig. 2, the complete diet did not have adverse effects on body weight gain in any of the genotypes. The NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

The 8-wk-old mice were placed in individual metabolic cages and divided into 6 groups: three genotypes and two diets; qprt<sup>++/+</sup>, qprt<sup>+/−</sup> or qprt<sup>−/−</sup> mice were fed the purified complete diet or the purified NiA-free diet. Each group consisted of 2 or 3 males and 3 females. They were fed the complete diet or NiA-free diet ad libitum for 23 d. As shown in Fig. 2, the complete diet did not have adverse effects on body weight gain in any of the genotypes. The NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

The 8-wk-old mice were placed in individual metabolic cages and divided into 6 groups: three genotypes and two diets; qprt<sup>++/+</sup>, qprt<sup>+/−</sup> or qprt<sup>−/−</sup> mice were fed the purified complete diet or the purified NiA-free diet. Each group consisted of 2 or 3 males and 3 females. They were fed the complete diet or NiA-free diet ad libitum for 23 d. As shown in Fig. 2, the complete diet did not have adverse effects on body weight gain in any of the genotypes. The NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

The 8-wk-old mice were placed in individual metabolic cages and divided into 6 groups: three genotypes and two diets; qprt<sup>++/+</sup>, qprt<sup>+/−</sup> or qprt<sup>−/−</sup> mice were fed the purified complete diet or the purified NiA-free diet. Each group consisted of 2 or 3 males and 3 females. They were fed the complete diet or NiA-free diet ad libitum for 23 d. As shown in Fig. 2, the complete diet did not have adverse effects on body weight gain in any of the genotypes. The NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.

The 8-wk-old mice were placed in individual metabolic cages and divided into 6 groups: three genotypes and two diets; qprt<sup>++/+</sup>, qprt<sup>+/−</sup> or qprt<sup>−/−</sup> mice were fed the purified complete diet or the purified NiA-free diet. Each group consisted of 2 or 3 males and 3 females. They were fed the complete diet or NiA-free diet ad libitum for 23 d. As shown in Fig. 2, the complete diet did not have adverse effects on body weight gain in any of the genotypes. The NiA-free diet containing 0 mg NiA and 2.3 g L-Trp/kg diet.
Table 1. Compositions of the diets.

| Ingredient                          | Normal diet | NiA-free diet |
|-------------------------------------|-------------|---------------|
| Vitamin-free milk casein            | 200         | 200           |
| L-Methionine                        | 2           | 2             |
| Gelatinized cornstarch              | 469         | 469           |
| Sucrose                             | 234         | 234           |
| Corn oil                            | 50          | 50            |
| Mineral mixture (AIN-93G-MX)        | 35          | 35            |
| Vitamin mixture (AIN-93-VX)         | 10          | 0             |
| NiA-free vitamin mixture (AIN-93-VX)| 0           | 10            |

1 Normal diet contains 30 mg (=244 μmol) NiA/kg diet and 2.3 g (=11.3 mmol) of Trp/kg diet.
2 NiA-free diet contains 0 mg NiA/kg diet and 2.3 g (=11.3 mmol) of Trp/kg diet.
3 AIN-93G-MX and AIN-93-VX.
4 NiA was removed from the AIN-93-VX.

had disappeared in the small intestine of the qprt−/− mice fed the NiA-free diet.

In conclusion, the data presented here indicate that we successfully generated a truly niacin-deficient mouse (8). This mouse could be used to study various biochemical conversions in mammals. Understanding the mechanism of action of NAD may shed light on this link.

REFERENCES

1) Fukuwatari T, Ohta M, Kimura N, Sasaki R, Shibata K. 2004. Conversion ratio of tryptophan to niacin in Japanese women fed on a purified diet conforming to the Japanese Dietary Reference Intakes. J Nutr Sci Vitaminol 50: 385–391.
2) World Health Organization. 2002. Pellagra and its prevention and control in major emergencies. Geneva, Switzerland: World Health Organization.
3) Seal AJ, Greene PI, Dibari F, Cheung E, Kyroussis E, Smedo P, van den Briel T. 2007. Low and deficient niacin status and pellagra are endemic in postwar Angola. Am J Clin Nutr 85: 218–224.
4) Miller DE. 1978. Pellagra deaths in the United States. Am J Clin Nutr 31: 558–559.
5) Nishizuka Y, Hayashi O. 1963. Studies on the biosynthesis of nicotinamide adenine dinucleotide. I. Enzymic synthesis of niacin ribonucleotides from 3-hydroxyanthranilic acid in mammalian tissues. J Biol Chem 238: 3369–3377.
6) Shibata K, Tanaka K, Murakata K. 1986. Efficiency of exogenous quinolinic acid as niacin in rats. Agric Biol Chem 50: 2025–2032.
7) Beal MF, Kowall NW, Ellison DW, Mazurek ME, Swartz KJ, Martin JB. 1986. Replication of the neurochemical characteristics of Huntington’s disease by quinolinic acid. Nature 321: 168–171.
8) Terakata M, Fukuwatari T, Sano M, Nakao N, Sasaki R, Fukunaka S, Shibata K. 2012. Establishment of true niacin deficiency in quinolinic acid phosphoribosyltransferase knockout mice. J Nutr 142: 2148–2155.