Original Article

Multigenerations Assessment of Dietary Nucleotides Consumption in Weaned Rats

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With the multifaceted activities of nucleotides, there is a history of safe consumption of dietary nucleotides (NTs) in the human diet. This study investigated the multigenerations cumulative toxicity on rats' development after weaning. Weaning rats (F0) were fed with NTs at the dosage of 0.01, 0.04, 0.16, 0.64, and 1.28% (wt/wt) for 90 days and then mated for 1:1 pattern. The offspring was F1. F1 rats were fed with NTs for 90 days after weaning. Afterwards, F1 go on to the second reproductive part. We repeated the above process, until F3 rats were born. We observed the weight, food consumption in the whole experiment, and detected the blood indicators and organ pathology at the terminal. No abnormal reaction, behavior disorder, and organ pathology related to toxic symptom were observed in NTs-treated groups. Weight gain and diet utilization ratio of males in each NTs group had significant increase after weaning ($p < 0.05$). With the exception of decrease in uric acid ($p < 0.05$) of NTs male, there were no differences between the control and NTs groups in blood indicators. NTs could promote early growth and development of rats after weaning, especially in males. *Birth Defects Res (Part B)* 95:460–466, 2012. © 2012 Wiley Periodicals, Inc.

Key words: ***dietary nucleotides; development; after weaning; safety; Sprague-Dawley rat***

INTRODUCTION

Nucleotides and their metabolic products play key roles in many biological processes, as base units of nucleic acids, in transferring chemical energy, and in biosynthetic pathways as biological regulators and coenzyme components. They could modulate lipid metabolism, immune function, and intestinal microbiota and have a reparative effect in pathological conditions that demand intense nucleic acid and protein synthesis, such as intensive growth, maintaining some organ functions, and repair of certain tissues. Although nucleotides are synthesized endogenously, it has been showed that dietary supplementation with nucleotides (NTs), conditionally essential nutrients, may exert beneficial effects both in humans and animals, especially during their development (Lopez et al., 1996; Fontana et al., 1998; Iwasa et al., 2000; Sanchez and Gil, 2002; Jyonouchi et al., 2003; Yokoyama et al., 2004; Frankic et al., 2006; Sauer et al., 2010; Tahmasebi et al., 2011). As important human milk constituents, NTs are present in higher amounts in human milk than in cow milk and cow milk–based infant formulas (Schlimme et al., 2000). Aiming to provide health benefits attributed to human milk, such as immune function, growth, infectious morbidity, and reduced episodes of diarrhea, NTs have been fortified for many years (Boza, 1998; Maldonado et al., 2001; Yu, 2002; Aggett et al., 2003; Sauer et al., 2010).

At the same time, several studies have shown that adding NTs in infant formula is safe and confers benefits to the infant health (Carver and Stromquist, 2006; Singhal et al., 2008). Meanwhile, it is also suggested that the similar incidence of adverse events, between the human milk–fed and the formula-fed groups, provides presumptive evidence of the safety of NTs for preterm infants. However, most of the NTs studies were mainly focused on the lactation period. There are no available data, as far as our ability is concerned, on the safety of postweaning period in multigenerations.

The rat is considered to be a relevant model, for assessing potential reproductive dietary risks to humans from dietary supplementation of NTs (Sheng et al., 1998; Zhao et al., 2001). When rats are treated with NTs for their

Abbreviations: **NTs**, dietary nucleotides; **UA**, serum uric acid; **UMP**, uridine monophosphate.

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postweaning assessment throughout their reproduction and lactation period in multigenerational way, developmental effects should also be considered. Therefore, we were interested in whether NTs have multigeneration cumulative toxicity on rats after weaning. Furthermore, the effect of NTs on development, in postweaning period, was observed.

MATERIALS AND METHODS

Test Substance

NTs provided by Zhen-Ao Biotechnology (Dalian, China) were derived from brew yeast RNA, whose purity is more than 99%. The composition of NT mixture contained adenosine monophosphate (AMP), guanosine monophosphate (GMP), uridine monophosphate (UMP), and cytidine monophosphate (CMP) in the following ratio, 5′AMP (22.8), 5′CMP (26.6), 5′GMPNa_2 (30.2) 5′UMPNa_2 (20.4). The experimental diet was supplemented with "AIN93G" to give 10, 40, 160, 640, and 1280 mg of mixed NT kg^{-1} diet. Dietary ingredients were thoroughly mixed in a mixer, pellets were made, and air-dried at room temperature.

Experimental Animal and Housing Conditions

A total of 120 male and female Sprague-Dawley rats (4 weeks old), weighing 35 to 55 g, were obtained from the Animal Service of Health Science Center, Peking University, Beijing. Rats were housed two per plastic cages with free access to chow and tap water in a specific pathogen-free filter-protected air-conditioned room with controlled temperature (25 ± 28°C), relative air humidity (60 ± 5%), and 12-hr light/dark cycles (light on 07:30–19:30 hr). All animals were handled in accordance with the guidelines of the National Institutes of Health (NIH Publication No. 85-23, revised 1985) and the guidelines of the Peking University Animal Research Committee (www.lab.pku.edu.cn).

Experimental Design

After a 1-week acclimation period, rats were randomly assigned to one of six groups (10 animals per sex per group): one control group and five experimental groups. Control rats were fed with "AIN93G" as rodent diet (Vital River, Beijing). Rats in the five experimental groups were fed with 0.01, 0.04, 0.16, 0.64, or 1.28% (wt/wt) NTs in the diet (Vital River).

For the mating portion of the multigeneration reproduction feeding study, F0 female was paired with one F0 male from the same treatment group in cage, A vaginal smear examination was used to test whether female rats becoming pregnant at 7:00 hr for the next day. After confirmation of mating, the female was placed in an individual cage. We ensured that there were total 20 female rats that had been pregnant. The day parturition when initiated was designated as day 0 of lactation for the dams and postnatal day (PND) 0 for pups (F1). On PND 4, all pups were examined externally for abnormalities and culled to yield 4 pups/sex/litter. Culled pups were weighted and discarded. The dam and litter remained together and remained on the control or NTs diets until weaning on PND 21. After weaned, F1 were fed with control or NTs as the parental generations for 90 days. We randomly selected the healthy individuals from the number of rats in each group. Then, we repeated the multigeneration reproduction until F3 was born. When the rats were 90 days, eight of them from each group were executed at random. They were anesthetized by carbon dioxide inhalation and then sacrificed.

Animal Observations

Animals were observed daily and weekly for adverse clinical sign. A detailed physical examination was conducted at least once during the pretreatment period, once weekly during the test substance administration and on the day of necropsy. The animals were removed from their home cages and placed in a standard arena for observations. Clinical signs that were monitored include, but were not limited to, changes in skin, fur, eyes, mucous membranes; occurrence of secretions and excretions and autonomic activity. Individual body weights and food consumption for males and nonmated females were recorded individually on a weekly basis until sacrifice. The diet utilization ratio was calculated using the following formula:

$$\text{Diet utilization ratio} = [\text{weight gain (g)}/\text{food consumption (g)}] \times 100\%.$$

Hematology, Serum Biochemical Evaluation

Blood samples were collected from femoral artery for clinical pathology evaluations (hematology, serum chemistry) from rats (8 rats/sex/group) immediately before the scheduled necropsy on day 90. All animals were fasted overnight before blood collection.

The levels of alanine aminotransferase, aspartate aminotransferase, total protein, albumin, serum uric acid (UA), blood urea nitrogen, creatinine, total cholesterol, triglyceride, high-density lipoprotein cholesterol, and glucose in serum were detected by automatic biochemistry analyzer (Hitachi, Japan).

The following hematology parameters were analyzed using MLA Electra 1400 C^{TM} automated coagulation analyzer: white blood cell, lymphocyte (LY), monocytes (MO), neutrophilicgranulocyte (GR), lymphocyte count (LY#), monocytes count (MO#), neutrophilicgranulocyte count (GR#), red blood cell, hematocrit (HCT), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, blood platelet (PLT), mean platelet volume, plateletcrit (PCT), platelet distribution width, hemoglobin (HGB), eosinophil count (EO), eosinophil ratio (EO%), basophils count (BA), and basophils ratio (BA%).

Organ Pathology

All animals were euthanized by carbon dioxide inhalation followed by exsanguination. Animals were necropsied and the external surface, all orifices and the cranial, thoracic, abdominal, and pelvic cavities including viscera were examined. Organ weights were measured for the adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries with oviducts, spleen, testes, thymus, and thyroids/parathyroids from all animals. Approximately

40 tissues were collected from each animal in the study but were examined microscopically for the male and female and control male and female groups. Pathological examinations mainly focused on reproductive organ and kidney.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 19.0, SPSS, Chicago, IL). Variances in the measurement data were checked for homogeneity by Bartlett's test. When the data were homogeneous, the one-way analysis of variance test and Fisher's least significant difference t-test (LSD t-test) methods were used. Tamhane's T2 test is used after data are transformed to analyze data among multiple groups if variances are unequal. All reported p values were two-sided. A value of $p < 0.05$ was considered significant.

RESULTS

General Information, Body Weight, and Food Consumption

No significant difference was seen between control and NTs-treated groups in the incidence of clinical signs of toxicity and regular behaviors, in either male or female F1, F2, and F3 rats (data not shown).

Comparing with the controls, in 90 days after weaning, there were no significant differences on dietary intake of males and females in multigenerations ($p > 0.05$; data not shown).

As it is shown in Table 1, weight gain and diet utilization ratio of F1 male rats in each dose-dependent group, comparing with control, had significant increase in the whole 4 weeks after weaning whereas it was within 2 weeks after weaning ($p < 0.05$) in female rats in NTs groups . Moreover, from Table 2 it is suggested that there were remarkable increases of F2 NTs males in first 3 weeks, which are equivalent to the 1.28% male NTs group in the fourth week and females in each NTs group in the first 2 weeks after weaning ($p < 0.05$). Table 3 shows that the weight gain and diet utilization of F2 males were higher in all the 4 weeks similar to female rats in 1 and 2 weeks of NT-treated groups ($p < 0.05$).

Hematology, Serum Biochemical Evaluation

In regard to clinical pathology parameters, there were no differences in hematology parameters between control male and female rats and NTs-treated males and females in multigenerations ($p > 0.05$).

For serum biochemistry value, there were no differences between the control and NTs groups (Table 4) with the exception of statistically significant decrease in UA level of males in NTs ($p < 0.05$). Comparing the control, serum acid remarkably decreased in F1 males of all NTs-treated groups ($p < 0.01$). Statistically comparing the control, there was only 0.01% ($p < 0.01$), 0.04% ($p < 0.01$), and 0.16% ($p < 0.05$) NTs decrease in F2 males. And it showed that just UA level of 0.16% NTs of F3 rats was significantly lower than the control group. (Fig. 1).

Organ Pathology

Organ weights relative to body weight were not different between the male and female control and NTs groups in multigenerations. Meanwhile, the macroscopic appearance of tissues for F1, F2, and F3 rats in NTs were within normal limits of this strain and age and similar to that of controls. Moreover, it is suggested that no abnormal tissues could be observed at terminal necropsy, though microscopically examined by paraffin section and H&E stain on F1, F2, and F3 rats of NTs (data not shown).

DISCUSSION

With its multifaceted activities, there is a history of safe consumption of NTs in the human diet. Moreover, the

Table 1
Weight Gain (g) and Diet Utilization in F1 Sprague-Dawley Rats Treated With Different Levels of Dietary Nucleotides

Sex	Weeks		Control	0.01% NTs	0.04% NTs	0.16% NTs	0.64% NTs	1.28% NTs
Male								
	1 week	Weight gain (g)	37.54 ± 4.56	40.72 ± 5.54*	41.40 ± 6.55**	43.02 ± 4.82**	40.97 ± 6.34*	43.13 ± 5.53**
		Diet utilization (%)	52.68 ± 2.28	56.40 ± 3.87**	56.34 ± 2.43**	56.88 ± 2.89**	56.74 ± 3.66**	56.89 ± 1.59**
	2 weeks	Weight gain (g)	53.87 ± 5.43	57.02 ± 10.11*	57.57 ± 5.62*	60.41 ± 6.73**	59.56 ± 6.56**	61.30 ± 7.71**
		Diet utilization (%)	48.58 ± 2.33	53.30 ± 7.62**	54.12 ± 1.29**	54.04 ± 1.69**	51.24 ± 2.08**	52.91 ± 2.56**
	3 weeks	Weight gain (g)	57.07 ± 5.98	64.84 ± 11.02**	64.52 ± 7.50**	63.29 ± 5.99**	65.43 ± 10.69**	66.18 ± 8.09**
		Diet utilization (%)	40.06 ± 1.75	44.73 ± 8.64**	45.76 ± 4.20**	44.98 ± 2.91**	45.75 ± 2.83**	45.80 ± 1.21**
	4 weeks	Weight gain (g)	46.64 ± 13.98	54.78 ± 12.40*	56.69 ± 16.70**	55.91 ± 9.04**	55.28 ± 14.90**	56.18 ± 13.45**
		Diet utilization (%)	33.81 ± 4.57	40.95 ± 5.95**	37.99 ± 5.78*	41.30 ± 1.74**	43.00 ± 2.83**	41.52 ± 3.41**
Female								
	1 week	Weight gain (g)	34.43 ± 4.13	36.87 ± 5.64*	37.84 ± 4.30*	38.21 ± 3.12**	38.23 ± 5.07**	37.38 ± 3.67*
		Diet utilization (%)	50.79 ± 2.26	54.07 ± 3.45*	53.28 ± 2.85*	53.05 ± 2.31*	52.38 ± 4.41*	53.95 ± 2.21*
	2 weeks	Weight gain (g)	42.05 ± 11.61	47.73 ± 6.97**	47.82 ± 5.14**	47.23 ± 4.23*	46.41 ± 2.55*	47.44 ± 6.31*
		Diet utilization (%)	43.01 ± 7.41	48.14 ± 5.07*	48.04 ± 2.35*	47.55 ± 3.07*	48.75 ± 2.53*	47.23 ± 6.15*
	3 weeks	Weight gain (g)	37.97 ± 13.47	34.39 ± 6.77	33.85 ± 7.45	39.14 ± 8.91	36.33 ± 8.44	33.67 ± 5.11
		Diet utilization (%)	30.95 ± 5.21	29.76 ± 4.44	28.75 ± 4.68	29.06 ± 2.28	31.47 ± 3.23	30.32 ± 3.99
	4 weeks	Weight gain (g)	29.57 ± 9.78	23.29 ± 6.40	25.90 ± 6.46	25.00 ± 8.97	24.78 ± 7.20	26.92 ± 6.41
		Diet utilization (%)	23.17 ± 4.05	22.03 ± 3.37	22.64 ± 3.10	23.29 ± 2.56	23.70 ± 4.96	22.39 ± 5.15

Data are mean ± SD values. Significant difference compared with the control group (by one-way analysis of variance): *$p < 0.05$; **$p < 0.01$.

Table 2
Weight Gain (g) and Diet Utilization in F2 Sprague-Dawley Rats Treated With Different Levels of Dietary Nucleotides

Sex	Weeks		Control	0.01% NTs	0.04% NTs	0.16% NTs	0.64% NTs	1.28% NTs
Male								
	1 week	Weight gain (g)	30.30 ± 5.67	43.81 ± 4.25**	36.50 ± 7.16*	36.29 ± 9.40*	38.98 ± 6.26*	34.67 ± 5.75*
		Diet utilization (%)	47.26 ± 4.23	58.05 ± 4.27**	53.11 ± 4.34**	52.44 ± 8.99**	56.15 ± 2.26**	51.49 ± 6.39*
	2 weeks	Weight gain (g)	51.71 ± 6.13	58.86 ± 2.06**	55.04 ± 6.55*	58.79 ± 4.83**	55.49 ± 3.21*	60.29 ± 6.76*
		Diet utilization (%)	46.16 ± 7.08	50.17 ± 8.77*	50.00 ± 3.13*	50.14 ± 1.48*	50.66 ± 4.99*	52.83 ± 4.23**
	3 weeks	Weight gain (g)	50.08 ± 7.60	56.83 ± 12.26**	57.10 ± 7.81**	66.56 ± 11.33**	60.19 ± 6.28**	57.48 ± 11.69**
		Diet utilization (%)	36.94 ± 8.16	41.95 ± 8.04*	41.20 ± 5.11*	43.39 ± 7.43*	42.09 ± 1.68*	41.20 ± 5.00*
	4 weeks	Weight gain (g)	55.20 ± 15.02	71.46 ± 11.32**	62.08 ± 16.33**	64.77 ± 14.31**	70.84 ± 11.54**	72.05 ± 15.74**
		Diet utilization (%)	29.66 ± 7.87	39.42 ± 8.45*	33.55 ± 4.60*	37.14 ± 1.73*	38.25 ± 5.43*	39.52 ± 9.24*
Female								
	1 week	Weight gain (g)	33.86 ± 3.05	37.04 ± 6.61*	38.59 ± 3.08*	39.06 ± 6.38*	38.89 ± 6.94*	37.33 ± 4.97*
		Diet utilization (%)	47.27 ± 2.46	52.94 ± 7.45*	50.41 ± 2.46*	51.62 ± 7.77*	53.34 ± 3.41*	50.47 ± 5.89*
	2 weeks	Weight gain (g)	40.64 ± 3.81	47.35 ± 3.05**	45.44 ± 3.85*	43.64 ± 9.00*	47.88 ± 5.88**	47.74 ± 3.78**
		Diet utilization (%)	43.82 ± 3.41	48.91 ± 7.37*	46.13 ± 3.15*	46.76 ± 4.88*	48.95 ± 2.99*	47.75 ± 6.87*
	3 weeks	Weight gain (g)	38.20 ± 6.88	44.78 ± 8.16	37.56 ± 4.80	37.09 ± 11.57	42.62 ± 13.26	39.55 ± 9.02
		Diet utilization (%)	31.43 ± 2.84	35.22 ± 3.58	27.28 ± 10.06	28.94 ± 7.63	35.18 ± 12.61	32.02 ± 10.32
	4 weeks	Weight gain (g)	28.29 ± 5.12	29.62 ± 9.94	32.01 ± 14.90	29.40 ± 9.06	26.92 ± 9.06	32.81 ± 9.11
		Diet utilization (%)	21.29 ± 2.56	26.41 ± 12.70	24.69 ± 9.42	23.79 ± 2.74	21.28 ± 6.53	26.30 ± 5.28

Data are mean ± SD values. Significant difference compared with the control group (by one-way analysis of variance): *$p < 0.05$; **$p < 0.01$.

Table 3
Weight Gain (g) and Diet Utilization in F3 Sprague-Dawley Rats Treated With Different Levels of Dietary Nucleotides

Sex	Weeks		Control	0.01% NTs	0.04% NTs	0.16% NTs	0.64% NTs	1.28% NTs
Male								
	1 week	Weight gain (g)	34.31 ± 6.97	37.32 ± 9.39*	40.10 ± 4.14**	39.60 ± 5.52**	39.78 ± 5.49**	42.51 ± 5.47**
		Diet utilization (%)	48.17 ± 3.68	52.10 ± 6.38**	53.79 ± 2.62**	53.02 ± 1.74**	52.26 ± 5.69**	54.60 ± 2.22**
	2 weeks	Weight gain (g)	48.61 ± 8.20	54.36 ± 10.36**	55.04 ± 4.99**	51.37 ± 10.36*	59.50 ± 6.84**	54.88 ± 7.01**
		Diet utilization (%)	45.07 ± 6.57	49.15 ± 2.05*	53.06 ± 9.34**	46.41 ± 8.10	51.77 ± 7.69**	47.76 ± 3.89
	3 weeks	Weight gain (g)	60.56 ± 9.43	66.52 ± 9.23**	67.81 ± 9.53**	64.15 ± 6.45*	65.09 ± 9.58*	68.00 ± 9.58**
		Diet utilization (%)	42.79 ± 4.51	46.47 ± 6.88**	48.38 ± 4.36**	45.32 ± 4.07*	49.14 ± 5.76**	44.66 ± 8.80*
	4 weeks	Weight gain (g)	65.68 ± 8.84	67.99 ± 12.43	67.55 ± 6.42	63.48 ± 15.22	65.55 ± 10.87	72.61 ± 11.82*
		Diet utilization (%)	39.47 ± 4.29	36.99 ± 3.52	34.45 ± 3.49	40.32 ± 8.30	39.75 ± 2.38	42.77 ± 7.73*
Female								
	1 week	Weight gain (g)	30.27 ± 4.11	35.18 ± 4.73**	35.10 ± 3.19**	33.73 ± 4.64**	37.12 ± 4.51**	36.20 ± 4.62**
		Diet utilization (%)	46.25 ± 3.07	53.55 ± 1.58**	48.60 ± 5.61*	49.31 ± 2.43*	51.00 ± 2.42**	50.59 ± 1.64**
	2 weeks	Weight gain (g)	43.91 ± 5.58	47.37 ± 9.10*	46.13 ± 3.79*	47.95 ± 5.55*	49.21 ± 5.43**	47.39 ± 4.45*
		Diet utilization (%)	41.62 ± 3.18	46.74 ± 3.36**	47.77 ± 8.52**	47.44 ± 7.79**	47.00 ± 2.83**	45.90 ± 3.78*
	3 weeks	Weight gain (g)	38.78 ± 5.65	39.56 ± 6.77	37.83 ± 7.08	39.92 ± 7.11	38.78 ± 7.17	37.07 ± 4.58
		Diet utilization (%)	32.76 ± 3.39	27.90 ± 7.56	31.86 ± 2.76	30.62 ± 5.37	28.41 ± 2.17	31.21 ± 3.01
	4 weeks	Weight gain (g)	27.35 ± 8.38	27.24 ± 7.07	25.63 ± 4.86	33.15 ± 7.10*	30.33 ± 5.79	27.54 ± 6.66
		Diet utilization (%)	25.12 ± 6.40	26.98 ± 1.07	21.41 ± 2.03	26.18 ± 3.56	27.26 ± 6.28	24.16 ± 2.62

Data are mean ± SD values. Significant difference compared with the control group (by one-way analysis of variance): *$p < 0.05$; **$p < 0.01$.

compositional and effective data presented have already led to a recommendation to the US Food and Drug Administration (FDA) by an expert group to allow the fortification of breast milk substitutes with nucleotide levels consistent with total potentially available nucleosides methodology.

In our previous multigenerations study, we observed the gestational, puerperal, and lactational period in parent rats (including average weight, number of living fetuses per litter, average body weight and sex ratio), while the indexes of physiological (including auricular appendage, incisor eruption, eyes opening, vaginal opening, testicular descent, and foreskin separation) and neural (including forelimb positioning, hindlimb positioning, surface righting, cliff escape, negative gravitropism and air righting) development in new-born rats during their lactational period. The above indexes do not have remarkable differences compared with the controls.

This study, on safety of NTs supplementation in postweaning period, aims at augmenting the currently available safety data in multigenerations. It indicated that up to 1.28%, NTs was without adverse effects. There was no evidence of target organ toxicity. In addition, the results of our study showed that NTs may

Table 4
The Effect of NTs on the Serum Biochemistry Parameters of Sprague-Dawley Rats: Multigeneration Data

Sex	Parameter	Control	0.01% NTs	0.04% NTs	0.16% NTs	0.64% NTs	1.28% NTs
F1 male							
	TP (g/l)	65.60 ± 2.32	64.09 ± 8.64	65.08 ± 13.82	64.76 ± 20.92	61.30 ± 5.36	58.34 ± 7.95
	ALB (g/l)	26.90 ± 2.64	26.34 ± 4.14	26.00 ± 6.34	23.91 ± 2.42	23.94 ± 1.91	26.59 ± 3.74
	GLB (g/l)	38.70 ± 0.89	37.75 ± 4.93	34.08 ± 7.82	37.70 ± 2.73	37.36 ± 4.18	35.75 ± 4.79
	A/G	0.70 ± 0.08	0.70 ± 0.06	0.61 ± 0.08	0.64 ± 0.06	0.64 ± 0.06	0.63 ± 0.07
	ALT (U/l)	44.51 ± 7.18	42.12 ± 12.44	35.62 ± 10.21	55.97 ± 55.26	41.05 ± 13.03	31.27 ± 4.73
	AST (U/l)	204.27 ± 30.46	181.61 ± 26.45	176.22 ± 45.89	176.24 ± 32.17	171.41 ± 32.22	185.55 ± 32.72
	BUN (mmol/l)	4.84 ± 0.38	4.60 ± 0.71	4.74 ± 1.33	5.32 ± 0.98	4.85 ± 0.63	4.40 ± 0.41
	TB (μmol/l)						
	CR (μmol/l)	54.85 ± 4.64	49.49 ± 10.76	40.46 ± 13.40	46.24 ± 10.09	55.07 ± 5.90	49.66 ± 15.96
	UA (μmol/l)	96.12 ± 19.58	77.59 ± 19.05*	65.46 ± 19.14**	64.55 ± 20.80**	67.74 ± 12.10**	70.87 ± 7.97**
	TC (mmol/l)	1.69 ± 0.24	1.68 ± 0.22	1.35 ± 0.40	1.61 ± 0.31	1.69 ± 0.36	1.38 ± 0.34
	TG (mmol/l)	0.59 ± 0.20	0.47 ± 0.24	0.43 ± 0.22	0.71 ± 0.32	0.61 ± 0.31	0.62 ± 0.34
	HDL (mmol/l)	0.61 ± 0.06	0.64 ± 0.08	0.52 ± 0.15	0.57 ± 0.11	0.60 ± 0.12	0.49 ± 0.10
	GLU (mmol/l)	4.12 ± 0.32	3.63 ± 0.81	3.37 ± 0.68	5.42 ± 1.35	4.14 ± 0.25	4.00 ± 0.30
F1 female							
	TP (g/l)	70.76 ± 6.22	67.16 ± 4.71	59.92 ± 13.12	64.66 ± 8.71	65.10 ± 4.16	61.20 ± 4.84
	ALB (g/l)	29.86 ± 3.86	26.86 ± 2.78	24.57 ± 6.03	25.55 ± 5.16	26.47 ± 2.90	23.71 ± 2.01
	GLB (g/l)	40.90 ± 2.66	40.30 ± 3.57	36.35 ± 7.47	37.16 ± 4.01	38.62 ± 3.43	36.49 ± 4.16
	A/G	0.73 ± 0.06	0.67 ± 0.09	0.71 ± 0.07	0.68 ± 0.09	0.69 ± 0.11	0.67 ± 0.08
	ALT (U/l)	44.57 ± 16.27	33.17 ± 6.44	31.86 ± 10.44	38.30 ± 27.38	37.12 ± 12.99	29.74 ± 16.72
	AST (U/l)	192.55 ± 29.03	171.54 ± 15.71	171.80 ± 40.04	173.70 ± 24.58	174.56 ± 25.61	166.55 ± 18.36
	BUN (mmol/l)	4.80 ± 1.18	4.36 ± 0.61	4.09 ± 0.97	3.95 ± 0.62	6.29 ± 4.16	3.72 ± 0.74
	TB (μmol/l)						
	CR (μmol/l)	60.61 ± 11.11	50.64 ± 3.47	42.00 ± 12.18	45.85 ± 11.35	75.01 ± 62.47	45.85 ± 7.85
	UA (μmol/l)	92.61 ± 18.19	93.05 ± 22.89	78.20 ± 24.73	68.21 ± 17.03	74.76 ± 16.02	74.66 ± 12.14
	TC (mmol/l)	1.97 ± 0.34	1.83 ± 0.40	1.86 ± 0.36	1.69 ± 0.46	1.72 ± 0.34	1.67 ± 0.44
	TG (mmol/l)	0.43 ± 0.24	0.55 ± 0.55	0.33 ± 0.23	0.27 ± 0.12	0.60 ± 0.31	0.30 ± 0.15
	HDL (mmol/l)	0.72 ± 0.09	0.68 ± 0.10	0.60 ± 0.13	0.61 ± 0.15	0.62 ± 0.10	0.64 ± 0.15
	GLU (mmol/l)	4.01 ± 0.65	3.71 ± 0.46	3.43 ± 0.76	4.36 ± 1.15	3.87 ± 1.44	3.50 ± 0.58
F2 male							
	TP (g/l)	74.70 ± 1.81	75.99 ± 4.42	75.89 ± 2.19	72.55 ± 7.14	75.24 ± 4.76	78.06 ± 5.07
	ALB (g/l)	47.58 ± 1.71	47.87 ± 1.78	49.06 ± 1.64	45.93 ± 3.55	46.99 ± 2.47	49.21 ± 2.56
	GLB (g/l)	27.13 ± 2.24	28.12 ± 3.22	26.83 ± 2.04	26.63 ± 3.93	28.25 ± 3.32	28.85 ± 3.06
	A/G	1.76 ± 0.21	1.72 ± 0.19	1.84 ± 0.18	1.74 ± 0.17	1.69 ± 0.18	1.71 ± 0.14
	ALT (U/l)	46.50 ± 3.74	51.33 ± 8.34	50.88 ± 6.66	49.25 ± 9.25	51.00 ± 10.11	52.00 ± 14.46
	AST (U/l)	161.88 ± 21.54	150.11 ± 36.72	161.13 ± 13.32	143.00 ± 23.62	178.25 ± 30.99	157.38 ± 24.13
	BUN (mmol/l)	6.63 ± 0.98	7.32 ± 0.41	7.42 ± 1.25	7.36 ± 1.42	6.62 ± 0.72	7.42 ± 1.13
	TB (μmol/l)	3.91 ± 0.82	3.76 ± 0.53	3.26 ± 0.45	3.08 ± 1.20	3.58 ± 0.58	3.75 ± 0.80
	CR (μmol/l)	50.25 ± 15.51	48.00 ± 4.66	46.38 ± 5.34	43.38 ± 7.91	43.00 ± 3.74	51.50 ± 10.70
	UA (μmol/l)	71.38 ± 14.30	57.56 ± 8.90*	57.00 ± 7.58*	52.13 ± 9.98**	65.75 ± 11.36	62.38 ± 7.09
	TC (mmol/l)	1.04 ± 0.22	1.22 ± 0.59	1.30 ± 0.32	1.38 ± 0.53	1.40 ± 0.62	1.12 ± 0.46
	TG (mmol/l)	1.87 ± 0.20	2.33 ± 0.45	2.10 ± 0.30	1.74 ± 0.44	2.16 ± 0.50	1.64 ± 0.34
	HDL (mmol/l)	1.26 ± 0.17	1.57 ± 0.26	1.41 ± 0.15	1.14 ± 0.35	1.46 ± 0.29	1.11 ± 0.23
	GLU (mmol/l)	5.26 ± 0.39	5.23 ± 0.83	5.91 ± 0.64	5.33 ± 0.34	5.10 ± 0.33	4.86 ± 0.36
F2 female							
	TP (g/l)	86.73 ± 4.76	83.06 ± 5.10	85.59 ± 6.73	82.26 ± 5.63	85.00 ± 3.61	80.39 ± 3.44
	ALB (g/l)	59.46 ± 3.30	58.61 ± 3.22	60.50 ± 5.83	56.04 ± 4.61	59.14 ± 2.71	55.46 ± 3.09
	GLB (g/l)	27.27 ± 2.86	24.45 ± 3.11	25.09 ± 3.34	26.23 ± 1.97	25.86 ± 2.20	23.93 ± 1.32
	A/G	2.20 ± 0.20	2.45 ± 0.30	2.15 ± 0.21	2.33 ± 0.18	2.33 ± 0.18	0.67 ± 0.08
	ALT (U/l)	43.14 ± 7.40	45.25 ± 8.26	49.13 ± 8.06	42.63 ± 13.67	48.50 ± 12.92	53.25 ± 13.22
	AST (U/l)	147.00 ± 17.71	129.63 ± 27.66	178.25 ± 22.79	139.38 ± 25.47	175.38 ± 46.93	190.00 ± 59.78
	BUN (mmol/l)	7.46 ± 1.04	7.74 ± 1.24	7.33 ± 0.52	6.63 ± 0.75	6.64 ± 0.84	7.02 ± 1.28
	TB (μmol/l)	2.73 ± 0.89	3.67 ± 0.69	3.69 ± 0.63	3.40 ± 1.05	3.40 ± 0.60	3.57 ± 0.53
	CR (μmol/l)	52.43 ± 7.59	55.00 ± 4.75	52.13 ± 2.53	49.13 ± 2.95	50.50 ± 7.05	55.38 ± 7.31
	UA (μmol/l)	62.29 ± 8.94	51.75 ± 8.36	69.50 ± 12.06	51.50 ± 9.78	63.00 ± 12.58	71.25 ± 15.33
	TC (mmol/l)	0.81 ± 0.33	0.98 ± 0.64	0.73 ± 0.21	0.85 ± 0.32	0.79 ± 0.19	0.58 ± 0.10
	TG (mmol/l)	1.86 ± 0.33	2.22 ± 0.32	2.06 ± 0.33	1.95 ± 0.33	2.38 ± 0.56	1.53 ± 0.57
	HDL (mmol/l)	1.47 ± 0.27	1.70 ± 0.24	1.60 ± 0.26	1.50 ± 0.22	1.85 ± 0.37	1.17 ± 0.42
	GLU (mmol/l)	5.74 ± 0.75	6.00 ± 0.63	5.74 ± 0.71	5.39 ± 0.52	5.77 ± 0.78	5.60 ± 1.10

Table 4
Continued

Sex	Parameter	Control	0.01% NTs	0.04% NTs	0.16% NTs	0.64% NTs	1.28% NTs
F3 male							
	TP (g/l)	70.89 ± 4.15	69.16 ± 2.69	71.64 ± 3.50	68.99 ± 3.03	71.91 ± 2.86	71.04 ± 2.38
	ALB (g/l)	47.75 ± 1.66	46.74 ± 2.11	47.89 ± 1.94	46.33 ± 2.46	47.39 ± 1.36	47.49 ± 1.59
	GLB (g/l)	23.14 ± 3.65	22.43 ± 2.62	23.75 ± 3.27	22.66 ± 2.65	24.53 ± 2.52	23.55 ± 1.23
	A/G	2.13 ± 0.39	2.11 ± 0.27	2.06 ± 0.28	2.09 ± 0.31	1.95 ± 0.23	2.03 ± 0.10
	ALT (U/l)	68.25 ± 12.26	61.38 ± 7.78	60.75 ± 12.69	61.88 ± 5.99	59.25 ± 3.66	65.25 ± 5.97
	AST (U/l)	197.33 ± 38.93	175.25 ± 31.30	194.00 ± 30.46	209.76 ± 37.11	158.71 ± 39.72	195.91 ± 18.43
	BUN (mmol/l)	5.03 ± 0.86	4.83 ± 0.60	4.91 ± 0.50	5.08 ± 0.83	4.37 ± 0.56	4.97 ± 0.53
	TB (μmol/l)	1.98 ± 0.78	2.55 ± 0.58	2.47 ± 0.63	2.28 ± 0.57	2.29 ± 0.55	2.69 ± 0.46
	CR (μmol/l)	53.88 ± 3.23	47.13 ± 3.87	52.88 ± 5.79	49.63 ± 4.84	48.88 ± 3.31	52.38 ± 5.04
	UA (μmol/l)	66.08 ± 12.50	62.79 ± 9.95	68.90 ± 10.57	51.39 ± 7.91*	71.09 ± 3.99	67.95 ± 7.83
	TC (mmol/l)	1.03 ± 0.28	0.94 ± 0.25	0.98 ± 0.27	1.31 ± 0.39	1.08 ± 0.30	1.07 ± 0.24
	TG (mmol/l)	1.65 ± 0.19	1.71 ± 0.39	1.64 ± 0.21	1.54 ± 0.32	1.85 ± 0.47	1.70 ± 0.13
	HDL (mmol/l)	1.11 ± 0.13	1.14 ± 0.25	1.05 ± 0.13	0.97 ± 0.27	1.22 ± 0.28	1.09 ± 0.11
	GLU (mmol/l)	4.58 ± 0.39	4.80 ± 0.58	4.26 ± 0.48	4.64 ± 0.75	5.11 ± 0.71	5.04 ± 0.45
F3 female							
	TP (g/l)	76.89 ± 6.13	77.49 ± 4.00	78.19 ± 3.99	76.68 ± 5.77	78.50 ± 6.44	77.76 ± 7.28
	ALB (g/l)	54.43 ± 4.46	56.33 ± 3.95	56.18 ± 5.17	56.43 ± 3.55	57.20 ± 5.10	56.09 ± 6.58
	GLB (g/l)	22.46 ± 2.96	21.16 ± 1.98	22.01 ± 2.52	20.25 ± 2.81	21.30 ± 2.64	21.68 ± 2.02
	A/G	2.46 ± 0.31	2.68 ± 0.35	2.59 ± 0.48	2.84 ± 0.33	2.71 ± 0.34	2.60 ± 0.35
	ALT (U/l)	45.00 ± 3.46	43.50 ± 6.78	42.25 ± 10.63	47.50 ± 11.87	53.00 ± 9.87	47.50 ± 11.82
	AST (U/l)	207.13 ± 34.53	166.64 ± 34.47	156.54 ± 21.77	192.84 ± 35.36	207.53 ± 91.30	256.79 ± 40.34
	BUN (mmol/l)	5.25 ± 0.66	5.37 ± 0.71	5.14 ± 0.33	5.49 ± 0.69	6.09 ± 2.89	6.12 ± 1.08
	TB (μmol/l)	2.73 ± 1.27	3.01 ± 0.56	2.25 ± 0.84	2.90 ± 0.41	2.51 ± 1.10	2.52 ± 0.73
	CR (μmol/l)	63.50 ± 5.83	59.88 ± 2.30	59.00 ± 4.78	59.13 ± 5.08	58.75 ± 6.18	64.00 ± 7.98
	UA (μmol/l)	75.48 ± 10.47	76.94 ± 10.88	74.08 ± 11.13	79.15 ± 5.98	74.43 ± 15.24	80.08 ± 16.04
	TC (mmol/l)	0.88 ± 0.16	0.94 ± 0.33	0.98 ± 0.27	1.05 ± 0.18	1.36 ± 0.74	1.01 ± 0.36
	TG (mmol/l)	1.76 ± 0.19	1.79 ± 0.35	2.01 ± 0.57	1.64 ± 0.32	1.85 ± 0.42	1.83 ± 0.32
	HDL (mmol/l)	1.29 ± 0.11	1.34 ± 0.23	1.47 ± 0.41	1.25 ± 0.27	1.36 ± 0.32	1.37 ± 0.25
	GLU (mmol/l)	4.82 ± 0.46	5.03 ± 0.55	4.93 ± 0.69	4.95 ± 0.44	5.05 ± 1.02	5.26 ± 1.38

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin/globulin; UA, serum uric acid; BUN, blood urea nitrogen; CR, creatinine; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; GLU, glucose. Data are mean ± SD values. Significant difference compared with the control group (by one-way analysis of variance): *$p < 0.05$; **$P < 0.01$.

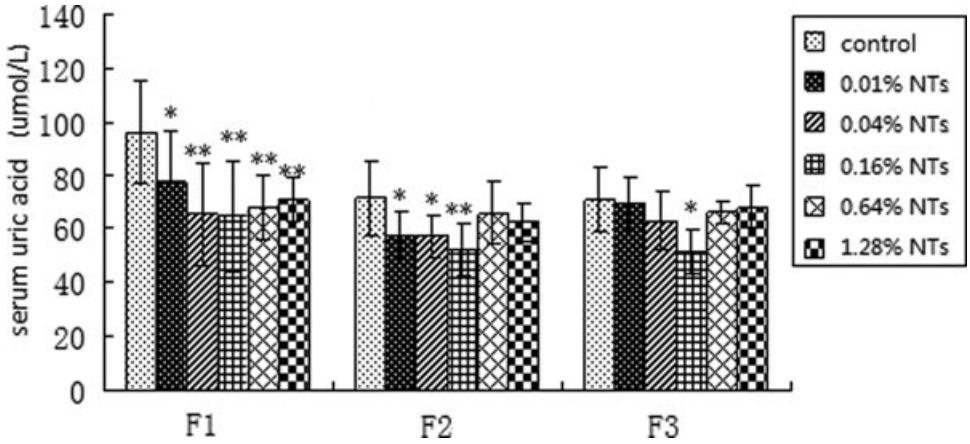

Fig. 1. Serum uric acid levels in male F1, F2 and F3 rats 90 days after weaning. Compared with control group: *$p < 0.05$; **$p < 0.01$.

play roles in the promotion of early growth and development after weaning, especially to male filial rats. To the best of our knowledge, this is the first nucleotide study of multigeneration safety and the effects of NTs on development in postweaning period.

As a whole, it can be seen in this study that NTs played an active role in accelerating growth and development in male rats 3 to 4 weeks after weaning, which can be indicated by faster weight gain and higher diet utilization ratio. In a similar way, it was higher diet utilization ratio that can be inferred that NTs may promote development, maturation, and function perfection of filial rats' intestine, leading to a better digestion, absorption, and utilization of diet. Effect of NTs on the growth and development of

female rats also existed, despite an inferior effect comparing with male rats. This may be related to the development character and hormone level of female rats (Hak and Choi, 2008; Sumino et al., 1999). Furthermore, no clinical adverse effect changes were revealed on liver and kidney function of NTs-treated groups throughout the experiment.

We analyzed the whole hematology parameters; compared with the control groups, the significant changes were not noted in NTs-treated groups. Additionally, compared with the control, there were no remarkable changes of serum biochemical data in NTs groups with the exception of statistically significant decrease in UA level.

The study showed that NTs decreased UA in male rats. UA is a product of purine nucleotide catabolism, which was mainly excreted by the kidneys. Nevertheless, no abnormal kidney was observed, at terminal necropsy, microscopically in multigeneration rats of NTs. The possible reason is that rodents, such as rats, do not have same purine metabolism as human being. In humans, it may lead to an increase in UA when too much diet containing purine NT is consumed, or there is abnormal purine metabolic disorder. However, there is uricase in rodents' body, which may decompose UA into allantoin expelled from the kidneys. And our results may be decreased owing to the fact that consumption of NTs increased the UA level and activate uricase, speeding up the decomposition and excretion of UA. Furthermore, NTs may reduce or inhibit the de novo synthesis of NTs in liver throughout feedback system. In addition, the result that NTs had no relationship with UA of female rats may be related to hormone. Estrogen itself could promote the excretion of UA, which may avoid the accumulation of UA and weaken the activation of uricase (Hak and Choi, 2008; Yahyaoui et al., 2008).

Whether the observed increased weight gain is attributable to increased lean body mass or increased fat deposition is the drawback of experimental design, which we did not test. So, it could not be speculated whether the males were getting fatter, which can be a topic for future research.

CONCLUSION

A multigeneration assessment of NTs is reported in the postweaning period, in which NTs were administered in the diet of Sprague-Dawley rats to provide dose up to 1.28%. In addition, NTs may play roles in the promotion of early growth and development after weaning, especially to male filial rats. Complement of NTs decreased UA level in males instead of increasing it, which may be related to the activation of uricase or inhibition of NTs de novo synthesis throughout feedback system caused by exogenous NTs.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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