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Original Article

New reference growth charts for Japanese girls with Turner syndrome

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Abstract ***Background***: Currently used growth charts for Japanese girls with Turner syndrome (TS) were constructed with auxological data obtained before the secular trend in growth reached a plateau. These charts were published in 1992 and may no longer be valid for the evaluation of stature and growth in girls with TS in clinical settings. Thus, we need to establish new clinical growth charts.

Methods: The samples for analysis were obtained by a retrospective cohort study. A total of 1867 Japanese girls with TS were registered between 1991 and 2004 for growth hormone (GH) treatment and their pretreatment anthropometric measurements were obtained. Reference growth charts were newly constructed using the LMS method from 1447 girls' cross-sectional data after exclusion of measurements derived from those with the presence of puberty, with previous growth-promoting treatment, or without cytogenetic evidence of TS.

Results: The new clinical reference growth charts differ from the old charts. Secular trends can be detected in both height and weight. Mean adult height on the new chart is 141.2 cm, 3.0 cm taller than the old data. This result seems attributable to the secular trend observed during the same period in Japanese women.

Conclusions: The newly constructed clinical reference growth charts for Japanese girls with TS seem to be better for the evaluation of growth in girls with TS born after approximately 1970, although selection bias and some other limitations in the present study should be kept in mind.

Key words growth chart, LMS method, secular trend, Turner syndrome.

Background

Turner syndrome (TS) is the most common chromosomal disorder in girls and affects about one in 1500 to 2500 live-born female infants.[1] One of the most significant features of the syndrome is short stature. Untreated girls are reported to be approximately 20 cm shorter than normal girls within their respective populations.[2] Growth hormone (GH) has been used to accelerate growth, and it is known to increase adult height.[3]

Growth patterns of girls with TS are different from those in normal populations mainly because of the short stature homeobox-containing gene on the X chromosome (SHOX) haploinsufficiency and their ovarian insufficiency. TS-specific growth curves have been published in various countries[4–11] including Japan,[12] and they have been clinically used for the evaluation of stature and growth. Those of the Japanese were constructed with data from subjects whose body measurements were obtained by sending questionnaires to their follow-up hospitals. The data consisted of 6255 measurements from 705 girls born between 1955 and 1989.

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Japan has experienced extremely rapid changes in eating habits together with vast socioeconomic changes since the end of the Second World War, and these changes have affected Japanese children's growth. The physical size of Japanese children has increased along with these environmental changes, and nutrition is thought to be the most important contributing factor. In Japan the food supply has been sufficient or even excessive since 1970. The acceleration of growth is reported to have been most prominent between 1955 and 1970,[13] but it has reached a plateau since around 1990, as discussed later. Thus the subjects analyzed in the currently used charts were born before the secular trend approached the recent plateau. Therefore, use of the presently available growth charts may be inadequate for the evaluation of recent cases of TS. In this context, construction of new reference charts and their validation have become necessary.

Methods

Population

The samples were obtained from a database compiled by the Foundation for Growth Science, Japan. The Foundation has been controlling the use of GH through its registration system in Japan, which judges candidates' eligibility for supplemental GH treatment according to the diagnostic criteria for GH deficiency established by the Ministry of Health, Labor and Welfare's Study

Table 1 Age distribution

Age (years)	Number
0	1
1	9
2	14
3	41
4	74
5	104
6	105
7	104
8	113
9	152
10	160
11	168
12	131
13	75
14	68
15	52
16	38
17	22
18	11
19	2
20	3
Total	1447

Group for Hypothalamo-Pituitary Disorders.[14] Medical doctors are encouraged to have each candidate registered for GH treatment at the Foundation using an application form that includes his/her pre-treatment anthropometric measurements, karyotype (in the case of TS), presence or absence of puberty, and evidence of informed consent from each subject regarding the use of the data for scientific purposes.

Between 1991 and 2004, 1867 girls were registered as TS subjects in this cohort. The diagnosis of TS was confirmed by reviewing all the reported karyotypes of cultured peripheral blood lymphocytes. In this study TS was defined as a karyotype that contains a cell line of monosomy lacking at least a distal major part in the short arm of the X chromosome. Subjects having no evidence of such karyotypic features, missing a description regarding puberty status, with secondary pubertal signs, with a history of previous growth-promoting therapy, or whose age was over 20 were excluded.

Statistical analysis

Data were cleaned in several stages. Bivariate plots of height and weight were used to identify gross disproportions. Data points were scrutinized, going back to the source data if necessary, and transcription errors were corrected. If a value was deemed highly unlikely (more than 5 standard deviation scores [SDS] from the mean), such a point was deleted, even in the absence of any evidence of a transcription error.

Reference growth charts were obtained using the LMS method,[15] which assumes that the data can be transformed to normality by a suitable power transformation (L); the distribution is then summarized by the median (M) and the coefficient of variation (S). The values of L, M, and S are constrained to change smoothly with age, and fitted values can be used to construct any required centile curves. The karyotypes of 45,X and non-45,X were compared for body height using analysis of covariance (ANCOVA) with covariates of age and age-karyotype interaction. This analysis was performed using JMP 6.0.3 (SAS Institute Inc., Cary, NC, USA.) and *P*-values less than 0.05 were considered statistically significant.

Results

In total, 420 subjects were excluded because of insufficient or inadequate cytogenetic evidence for the diagnosis (31 subjects), secondary pubertal signs (107 subjects), lack of records about puberty (14 subjects), previous growth-promoting treatment (264 subjects), age over 20 (one subject) and highly unlikely measurements (three subjects). The remaining 1447 subjects were analyzed. Table 1 lists the number of subjects according to age. Their birth years range from 1970 to 2002 (median: 1985). Perinatal information and their parents' anthropometric measurements were collected whenever possible. Gestational age is 39.6+/−1.6 weeks (n = 1268), birth length 46.8+/−2.7 cm (n = 633), birthweight 2.68+/−0.44 kg (n = 1322), and target height 157.6+/−7.2 cm (n = 1289). Target height was calculated by the formula adjusted for the Japanese before the secular trend reached a

Table 2 Karyotypes of 1447 subjects

	Non-Mosaic	Number of subjects	Mosaic	Number of subjects
Aneuploidy	45,X	432	45,X/46,XX	87
			45,X/47,XXX	91
			45,X/46,XY	16
			45,X/46,XX/47,XXX	6
		432		200
Structural abnormality	46,X,i(Xq)	128	45,X/46,X,i(Xq)	309
	46,X,del(Xp)	55	45,X/46,X,del(Xp)	22
	46,X,r(X)	3	45,X/46,X,r(X)	106
	others	10	45,X/46,X,+mar	109
			others	73
		196		619
Total		628		819

Table 3 LMS values of height and weight for the Japanese girls with Turner syndrome

Height				Weight			
Age (years)	L	M	S	Age (years)	L	M	S
1	1	66.75	0.024	1	1.63	6.92	0.094
1.5	1	71.25	0.025	1.5	1.37	8.02	0.094
2	1	75.44	0.026	2	1.11	9.10	0.094
2.5	1	79.1	0.026	2.5	0.86	10.06	0.095
3	1	82.39	0.027	3	0.64	10.90	0.096
3.5	1	85.46	0.028	3.5	0.44	11.65	0.097
4	1	88.38	0.028	4	0.24	12.37	0.099
4.5	1	91.11	0.029	4.5	0.06	13.04	0.102
5	1	93.68	0.029	5	−0.12	13.71	0.106
5.5	1	96.23	0.030	5.5	−0.32	14.44	0.111
6	1	98.75	0.030	6	−0.51	15.24	0.117
6.5	1	101.24	0.031	6.5	−0.69	16.10	0.124
7	1	103.81	0.031	7	−0.87	17.12	0.131
7.5	1	106.39	0.032	7.5	−1.03	18.32	0.139
8	1	108.79	0.032	8	−1.14	19.58	0.147
8.5	1	111.02	0.033	8.5	−1.19	20.83	0.154
9	1	113.18	0.033	9	−1.16	22.12	0.160
9.5	1	115.32	0.034	9.5	−1.04	23.52	0.165
10	1	117.53	0.034	10	−0.84	25.08	0.170
10.5	1	119.89	0.035	10.5	−0.60	26.76	0.176
11	1	122.35	0.035	11	−0.40	28.51	0.182
11.5	1	124.76	0.036	11.5	−0.28	30.26	0.187
12	1	127.03	0.036	12	−0.25	31.94	0.189
12.5	1	129.14	0.037	12.5	−0.26	33.50	0.190
13	1	131.03	0.037	13	−0.26	34.93	0.191
13.5	1	132.69	0.037	13.5	−0.23	36.23	0.191
14	1	134.14	0.038	14	−0.17	37.40	0.191
14.5	1	135.37	0.038	14.5	−0.10	38.43	0.191
15	1	136.38	0.038	15	−0.01	39.33	0.192
15.5	1	137.24	0.038	15.5	0.08	40.11	0.192
16	1	137.96	0.039	16	0.16	40.79	0.192
16.5	1	138.56	0.039	16.5	0.23	41.39	0.192
17	1	139.07	0.039	17	0.28	41.93	0.192
17.5	1	139.49	0.039	17.5	0.32	42.43	0.192
18	1	139.87	0.039	18	0.37	42.91	0.192
18.5	1	140.23	0.039	18.5	0.40	43.36	0.191
19	1	140.58	0.039	19	0.44	43.81	0.191
19.5	1	140.91	0.039	19.5	0.47	44.24	0.191
20	1	141.24	0.039	20	0.51	44.67	0.190

plateau.[16] Table 2 summarizes the number of subjects grouped by karyotype. There was no significant difference in height between 45,X and non-45,X subjects (regression coefficient: 0.19+/−0.14 cm, $P = 0.17$).

Centile curves were fitted to the data of all subjects together using the LMS method. For height, the distribution was assumed to be normal, while for weight there was appreciable skewness, to which the age-varying power transformation was adjusted. Table 3 provides values for L, M and S of height and weight by age. Clinical growth references for height and weight are shown in Figures 1 and 2, respectively. References for height and weight expressed as SDS are superimposed on those that are currently used in Figures 3 and 4.

Discussions

We produced new clinical reference growth charts for Japanese girls with TS who did not present with puberty. The charts were constructed using the LMS method, which we believe is one of the most widely applied approaches.[17] The LMS method is often used to construct age-related references not only of normal populations[18] but also of Down syndrome[19] and Williams syndrome[20] disease-specific populations. The number of subjects analyzed in this study was sufficient, being comparable to numbers analyzed in the construction of other TS-specific charts. All subjects were confirmed by chromosomal analyses to meet the definition of TS and were properly selected, excluding subjects who had undergone pubertal development or previous growth-promoting treatment or both. Although these charts were not derived from a totally unbiased TS population, they can be presumed to represent growth in girls with TS who are ordinarily seen in clinical practice, because the charts were constructed using adjacent data before GH treatment. We believe that these charts have been adequately and successfully produced taking these points into consideration.

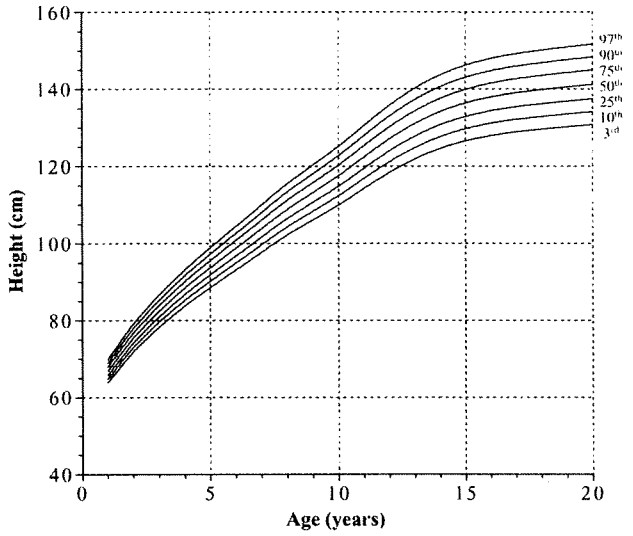

Fig. 1 Height chart for Japanese girls with Turner syndrome without puberty.

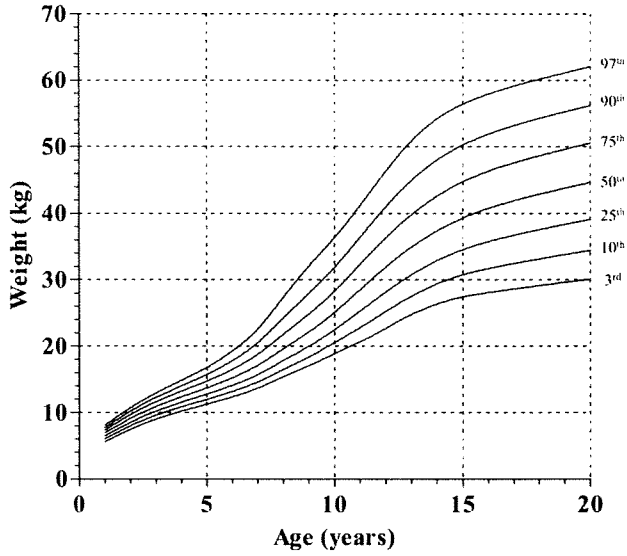

Fig. 2 Weight chart for Japanese girls with Turner syndrome without puberty.

Differences can be detected between the two charts in both height and weight growth (Figs 3 and 4). For example, the adult height from the new chart is 141.2 cm, which is 3.0 cm taller than the previous height when it is defined as the mean height at the age of 20 years. In the previous study, birth years ranged from 1955 to 1989 (median unknown). Given the year of publication, the adult height in the study had to be derived from subjects born before 1972. The standard adult heights of Japanese women in 1970, 1975, 1980, 1985, 1990, 1995, 2000 and 2005 were 155.6 cm, 156.3 cm, 157.0 cm, 157.6 cm, 157.9 cm, 158.0 cm, 158.1 cm and 158.0 cm, respectively.[21] This indicates that the secular trend in adult height has reached a plateau since approximately 1990 in Japan. Judging from the birth-year distribution, we know therefore that the old Japanese charts for TS were constructed with data from subjects the majority of whom were born before growth in height reached a plateau. On the other hand, the birth years in the present study ranged from 1970 to 2002 and 85.2% of the subjects were born after 1980. The new TS-specific growth charts therefore differ because they were constructed with data from a generation in which appreciable advances in secular height growth had disappeared. Secular growth trends in TS subjects have also been noted and studied in other countries.[9,22] In countries where secular trends

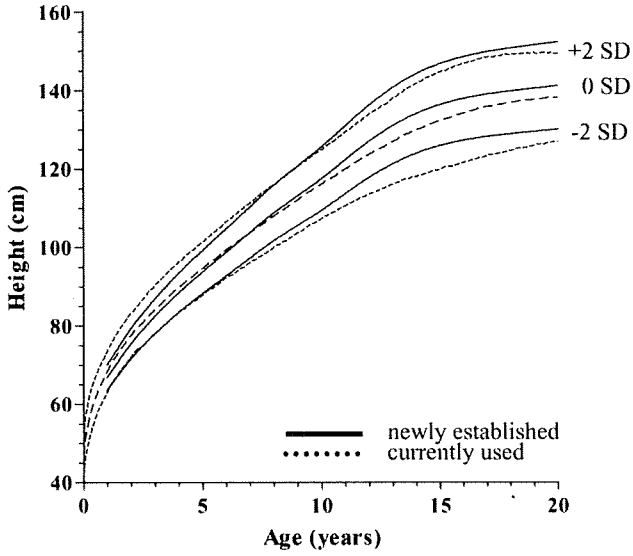

Fig. 3 Height chart for Japanese girls with Turner syndrome without puberty in comparison with the currently used one.[12] Solid line, newly established; dotted line, currently used.

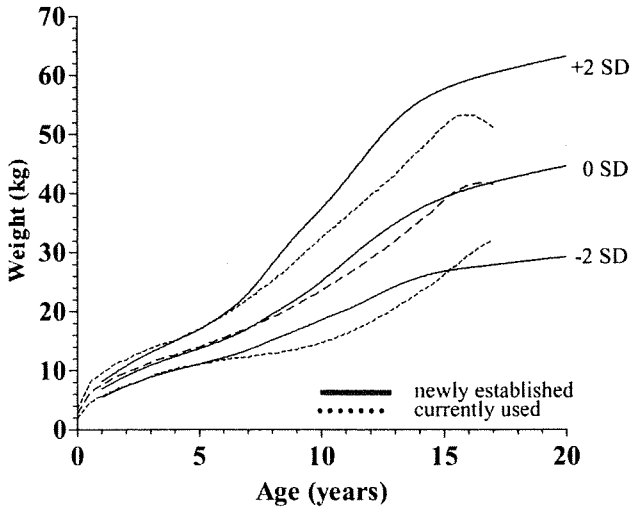

Fig. 4 Weight chart for Japanese girls with Turner syndrome without puberty in comparison with the currently used one.[12] Solid line, newly established; dotted line, currently used.

have reached a plateau, there is little need to construct newer charts. Taken together, we conclude that the new charts can be used hereafter as the more adequate reference for the evaluation of the growth of girls with TS born after approximately 1970.

There are three limitations to the present study. The first is a selection bias. This retrospective cohort consists of those diagnosed with TS in medical centers, a subpopulation from which subjects who are not significantly smaller than the normal population are more easily omitted. More specifically, physicians do not usually register girls with TS if they are taller than −2 SDS of the female standard, because the registry is designed primarily to designate candidates for GH treatment. It is of note that in Japan the indication of GH for TS is limited to subjects shorter than −2 SDS. The heights of the majority of girls with TS usually drop below the fifth percentile of the normal girl growth curve only after the subjects are from two to five years old.[1] This implies that this kind of bias more severely affects subjects younger than approximately three years of age. Before general application of these reference charts for TS, further validation is rewarding, especially in younger ages. The second limitation is the number of study samples. The numbers of infants and older children are small, so at these ages (especially under three years and over sixteen years) the charts may not be sufficiently reliable. This limitation is shared by the other reference charts, including currently used Japanese charts. With regard to older girls, it has become more difficult to obtain height data from subjects without previous growth-promoting treatment, because GH treatment for girls with TS has become very common in Japan and, what's more, the starting age has been decreasing. Despite this limitation, the adult height in this study is −3.3 SDS of the normal population[2,3] and is considered to be valid by comparison to adult heights in other countries (−4.2 to −2.5 SDS).[2] The third limitation is derived from the fact that this study is a cross-sectional study. We do not know whether subjects without puberty at the time of registration will or will not develop spontaneous puberty later. It is reported that those with spontaneous puberty are significantly taller than those without puberty from 12 years of age onward, although pubertal development and growth spurt do not seem to affect final adult height.[7] Theoretically, two types of growth charts may be needed during the peripubertal period, but we produced one specific for girls without pubertal signs because of the limited number of pubertal subjects. However, when we plotted the data from all 107 subjects with pubertal development on the new chart, with only one exception they were distributed within +/−2 SDS of the other subjects' data (data not shown). Accordingly, the presence of puberty does not influence the major difference in the pubertal height, though further investigation is necessary.

Conclusions

We have constructed new clinical reference growth charts for Japanese girls with TS using data from 1447 subjects who did not present with puberty. As they are assumed to belong to the generation beyond the secular trend in Japan, these charts are expected to be widely used in various clinical settings with all the limitations in mind and await further validation.

Acknowledgement

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References

1 Saenger P. Turner's syndrome. *N. Engl. J. Med.* 1996; **335**: 1749–54.
2 Ranke MB, Grauer ML. Adult height in Turner syndrome: Results of multinational survey 1993. *Horm. Res.* 1994; **42**: 90–4.
3 Baxter L, Bryant J, Cave CB, Milne R. Recombinant growth hormone for children and adolescents with Turner syndrome. *Cochrane Database Syst. Rev.* 2007; Jan 24; (**1**): CD003887.
4 Bernasconi S, Larizza D, Benso L *et al.* Turner's syndrome in Italy: Familial characteristics, neonatal data, standards for birth weight and for height and weight from infancy to adulthood. *Acta Paediatr.* 1994; **83**: 292–8.
5 Haeusler G, Schemper M, Frisch H, Blümel P, Schmitt K, Plöchl E. Spontaneous growth in Turner syndrome: Evidence for a minor pubertal growth spurt. *Eur. J. Pediatr.* 1992; **151**: 283–7.
6 Lyon AJ, Preece MA, Grant DB. Growth curve for girls with Turner syndrome. *Arch. Dis. Child.* 1985; **60**: 932–5.
7 Massa G, Vanderschueren-Lodeweyckx M, Malvaux P. Linear growth in patients with Turner syndrome: Influence of spontaneous puberty and parental height. *Eur. J. Pediatr.* 1990; **149**: 240–50.
8 Naeraa RW, Nielsen J. Standards for growth and final height in Turner's syndrome. *Acta Paediatr. Scand.* 1990; **79**: 182–90.
9 Ranke MB, Pflüger H, Rosendahl W *et al.* Turner syndrome: Spontaneous growth in 150 cases and review of the literature. *Eur. J. Pediatr.* 1983; **141**: 81–8.
10 Rongen-Westerlaken C, Corel L, Broeck JVD *et al.* Reference values for height, height velocity and weight in Turner's syndrome. *Acta Paediatr.* 1997; **86**: 937–42.
11 Sempé M, Hansson BC, Limoni C. Growth curves in untreated Ullrich-Turner syndrome: French reference standards 1–22 years. *Eur. J. Pediatr.* 1996; **155**: 862–9.
12 Suwa S. Standards for growth and growth velocity in Turner's syndrome. *Acta Paediatr. Jpn.* 1992; 206–21.
13 Murata M, Hibi I. Nutrition and the secular trend of growth. *Horm. Res.* 1992; **38** (Suppl. 1): 89–96.
14 Tanaka T, Takano K, Hanew K *et al.* Registration system for growth hormone (GH) treatment with standardized immunoreactive GH values in Japan. *Endocr. J.* 1998; **45**: 459–65.
15 Cole TJ, Green PJ. Smoothing reference centile curves: The LMS method and penalized likelihood. *Stat. Med.* 1992; **11**: 1305–19.
16 Ogata T, Matsuo N, Tamai S, Osano M, Tango T. Target height and target range for the Japanese (in Japanese). *Jpn. J. Pediatr.* 1990; **94**: 1535–40.
17 Wright EM, Royston P. A comparison of statistical method for age-related reference intervals. *J. R. Stat. Soc. Ser. A* 1997; **160**: 47–69.
18 Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumstance fitted by maximum penalized likelihood. *Stat. Med.* 1998; **17**: 407–29.
19 Styles ME, Cole TJ, Dennis J, Preece MA. New cross sectional stature, weight, and head circumference references for Down's

syndrome in the UK and Republic of Ireland. *Arch. Dis. Child.* 2002; **87**: 104–8.
20 Martin NDT, Smith WR, Cole TJ, Preece MA. New height, weight and head circumference charts for British children with Williams syndrome. *Arch. Dis. Child.* 2007; **92**: 598–601.
21 Ministry of Education. *Annual Report of School Health Statistics*. The Printing Office, The Ministry of Finance, Tokyo, 2005 (in Japanese).
22 Ranke MB, Stubbe P, Majewski F, Bierich JR. Spontaneous growth in Turner's syndrome. *Acta Paediatr. Scand.* 1988; **343** (Suppl.): 22–30.
23 Suwa S, Tachibana K. Standard growth charts for height and weight of Japanese children from birth to 17 years based on a cross-sectional survey of national data. *Clin. Pediatr. Endocrinol.* 1993; **2**: 87–97.

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REGULAR ARTICLE

Inconsistent determination of overweight by two anthropometric indices in girls with Turner syndrome

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Keywords
Body mass index, Obesity, Turner syndrome, Weight for height

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Abstract

Aim: To evaluate the prevalence of overweight in girls with Turner syndrome (TS) as classified by the two major anthropometric indices, body mass index (BMI) and weight-for-height (WFH) and to make growth reference charts of them for comparison with those of the normal population.

Method: The samples for analysis were obtained from a retrospective cohort. In total, 1447 girls' cross-sectional data were analysed. Subjects were divided into four groups by ages: group A (0–5.99 years), B (6–10.99 years), C (11–15.99 years) and D (16–20.99 years). The cut-off values of overweight by BMI and WFH were those of the 90th percentile and 120 percent, respectively and the prevalence was calculated. For constructing growth reference charts, the LMS method was used.

Results: The prevalence of overweight differed between the two indices. The proportions of the coincidental classification in all subjects, group A, B, C and D were 82.53%, 89.96%, 91.79%, 69.98% and 60.61%, respectively. These differences corresponded to the difference of age-dependent patterns of the two indices from those of the normal population, as judged from the growth charts constructed with all subjects.

Conclusion: A discrepancy in the prevalence of overweight as classified by BMI and WFH for girls with TS was detected.

INTRODUCTION

Turner syndrome (TS) is the most common chromosomal abnormality in females and affects about one in 1500 to 2500 live-born female infants (1). A cardinal clinical feature of TS is linear growth failure resulting in extreme short stature. Growth patterns of girls with TS are different from those of the normal population mainly because of haploinsufficiency of the short stature homeobox-containing gene on the X chromosome (SHOX) and ovarian insufficiency. Moreover, girls with TS are reported to frequently become overweight as they grow up (2–5). Many problems of females with TS in adult life are compounded by obesity (6). Therefore, it seems very important to pay attention to overweight in clinical practice.

There is a growing global epidemic of childhood obesity, with a large variation in secular trends across countries (7,8). At present, there is still no widely agreed standard for classifying overweight in children and adolescents (7). Previously, many researchers chose to use weight-for-height (WFH) for this purpose, especially for children under 10 years of age (7). In recent years, body mass index (BMI) has been more often accepted as a valid indirect measure of adipose tissue in both children and adolescents for survey purposes (9,10), although there are several reports that many pediatricians do not use BMI in clinical situations (11,12). For girls with TS, BMI is sometimes applied as one of the surrogate markers of adiposity (13,14). However, it is unknown whether BMI can be adequately used for this group of people whose growth patterns are different from the normal population.

In this study, we compared the prevalence of overweight determined by two major anthropometric indices, BMI and WFH, in girls with TS and made growth reference curves of both BMI and WFH to compare them with those of the normal population.

Abbreviations
TS, Turner syndrome; GH, growth hormone; BMI, body mass index; WFH, weight for height; SDS, standard deviation score; EDF, equivalent degrees of freedom.

METHODS

Population

The samples were obtained from a database registered at the Foundation for Growth Science, Japan. The Foundation has been controlling the use of growth hormone (GH) by its registration system in Japan through judging eligibility for GH treatment (15). Medical doctors are encouraged to have each candidate registered for GH treatment at the Foundation using an application form which includes his/her pretreatment anthropometric measurements, karyotypes (in the case of TS), presence or absence of puberty and evidence of informed consent from each subject regarding the use of the data for scientific purposes.

Between 1991 and 2004, 1867 girls were registered as TS subjects in this cohort. The diagnosis of TS was confirmed by reviewing all the reported karyotypes of cultured peripheral blood lymphocytes. In this study, TS was defined as a karyotype, which contains a cell line of monosomy lacking at least a distal major part in the short arm of the X chromosome. Subjects having no evidence of such karyotypic features, missing a description regarding puberty status, with pubertal signs, with a history of previous growth-promoting therapy or whose age was over 20 were excluded.

Classification of overweight

Japan Society for the study of obesity recommends that children who have 120% or more of the standard weight are classified as overweight (16). WFH is one of the most available and useful standard weights in Japan (17). Therefore, in this study we calculated percent overweight using WFH. The calculation formula was 100 × (weight value − WFH)/WFH. With regard to BMI, the cut-off values of overweight in Japanese children have been reported to be those above the 90th percentile of normal standards (18,19). In this study, the values of the 90th percentile for normal Japanese sex-specific BMI-for-age (20) (which was established by the LMS method) were used for the cut-off values of overweight. BMI was calculated as weight in kilograms divided by square of height in meters.

Statistical analysis

Data were cleaned in several stages. Bivariate plots of height and weight were used to identify gross disproportions. Data points were scrutinized, going back to the source data if necessary and transcription errors were corrected. If a value was deemed highly unlikely (more than 5 standard deviation scores [SDS] from the mean), such a point was deleted, even in the absence of any evidence of a transcription error.

Populations were divided arbitrarily into four groups according to age: group A (age of 0–5.99 years), B (age of 6–10.99 years), C (age of 11–15.99 years) and D (age of 16–20.99 years). The anthropometric data were calculated to BMI and percent overweight if there were standard data of normal Japanese values corresponding to the same ages.

Reference growth charts were obtained by the LMS method (21). This assumes that the data can be transformed to normality by a suitable power transformation (L) and the distribution is then summarized by the median (M) and coefficient of variation (S). Using penalized likelihood, three curves (L, M and S) can be fitted as cubic splines by nonlinear regression and the extent of smoothing was controlled by equivalent degrees of freedom (EDF). Fitting and smoothing were done with lmsChartMaker Pro ver.2.3 (Medical Research Council, London, UK).

RESULTS

In total, 420 subjects were excluded because of insufficient or inadequate cytogenetic basis of diagnosis (31 subjects), presence of pubertal signs (107 subjects), lack of records about puberty (14 subjects), previous growth-promoting treatment (264 subjects), age over 20 (one subject) or highly unlikely measurements (three subjects). The remaining 1447 subjects were analysed for constructing reference curves. Table 1 lists the number of the subjects by age. It is to be noted that none of the data from the girls with TS was collected after GH administration. Their birth years ranged from 1970 to 2002 (median: 1985). Perinatal information and their parents' anthropometric measurements were collected whenever possible. Average birth length was 46.8 ± 2.7 cm (n = 633), birth weight 2.68 ± 0.44 kg (n = 1322) and target height 157.6 ± 7.2 cm (n = 1289), which was very similar to the average adult height for Japanese females (157.9 cm) in 1990 (22). Target height was calculated by the formula adjusted for Japanese before the secular trend had

Table 1 Age distribution

Age (years)	Number
0	1
1	9
2	14
3	41
4	74
5	104
6	105
7	104
8	113
9	152
10	160
11	168
12	131
13	75
14	68
15	52
16	38
17	22
18	11
19	2
20	3
Total	1447

Table 2 Karyotypes of 1447 subjects

	Non-mosaic	Number of subjects	Mosaic	Number of subjects
Aneuploidy	45,X	432	45,X/46,XX	87
			45,X/47,XXX	91
			45,X/46,XY	16
			45,X/46,XX/47,XXX	6
		432		200
Structural Abnormality	46,X,i(Xq)	128	45,X/46,X,i(Xq)	309
	46,X,del(Xp)	55	45,X/46,X,del(Xp)	22
	46,X,r(X)	3	45,X/46,X,r(X)	106
	Others	10	45,X/46,X, + mar	109
			Others	73
		196		619
Total		628		819

Table 3 Prevalence of overweight

Group	BMI (+)/ WFH (+)	BMI (−)/ WFH (+)	BMI (+)/ WFH (−)	BMI (−)/ WFH (−)	The number of the same classification /Total number	The number of the same classification* /Total number
A	9 (3.77%)	4 (1.67%)	20 (8.37%)	206 (86.19%)	215/239 (89.96%)	213/233 (91.42%)
B	184 (29.07%)	44 (6.95%)	8 (1.26%)	397 (67.72%)	581/ 633 (91.79%)	554/633 (87.52%)
C	176 (35.70%)	148 (30.02%)	0 (0%)	169 (34.28%)	345/ 493 (69.98%)	314/493 (63.69%)
D	16 (24.24%)	26 (39.39%)	0 (0%)	24 (36.36%)	40/66 (60.61%)	39/66 (59.09%)
All Subjects	385 (26.90%)	222 (15.51%)	28 (1.96%)	796 (55.63%)	1181/1431 (82.53%)	1120/1425 (78.60%)

Definition of each group is written in the text.

BMI (+) indicates overweight subjects defined by BMI; (−) no overweight subjects.

WFH (+) indicates overweight subjects defined by WFH; (−) no overweight subjects.

Numbers in parentheses show percentages of the total number in each group.

*Figures in this particular column indicate the same classification of overweight determined by BMI and WFH when International cut-off values of BMI (10) instead of Japanese cut-off values were used. It is of note that the numbers of the subjects analysed are different, because International cut-off values can be obtained only for girls older than two years of age.

reached a plateau (23). Table 2 summarizes the number of the subjects grouped by karyotypes.

BMI-for-age can be obtained for girls older than 1.5 years of age, and WFH can be given for the heights taller than 70 cm in Japan. Therefore, we could not have the 90th percentile value of BMI and/or percent overweight in 16 subjects. Finally, 1431 subjects were evaluated for the difference of prevalence of overweight between the two indices, BMI and WFH. Prevalence of overweight by the two definitions of each group is shown in Table 3.

Centile curves were fitted to the data all together using the LMS method. For both BMI and WFH there were appreciable skewness and the age-varying power transformation were adjusted for them. EDF for (L, M, S) of BMI and WFH are (3,7,4) with age transformed and (2,5,4) with age rescaled, respectively. Growth references for BMI and WFH are shown in Figures 1 and 2, respectively. These references are superimposed on those of the normal population in Figures 3 and 4.

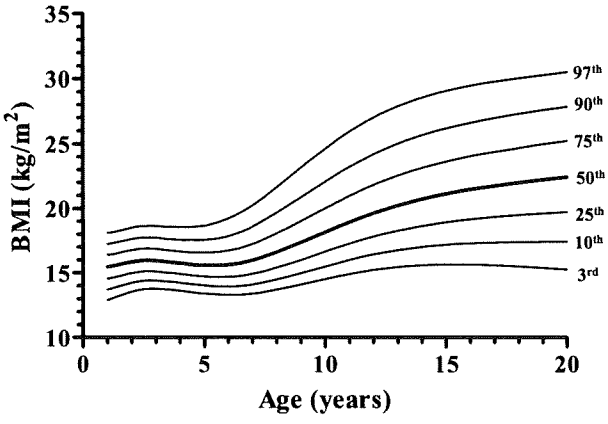

Figure 1 BMI chart for Japanese girls with Turner syndrome without puberty.

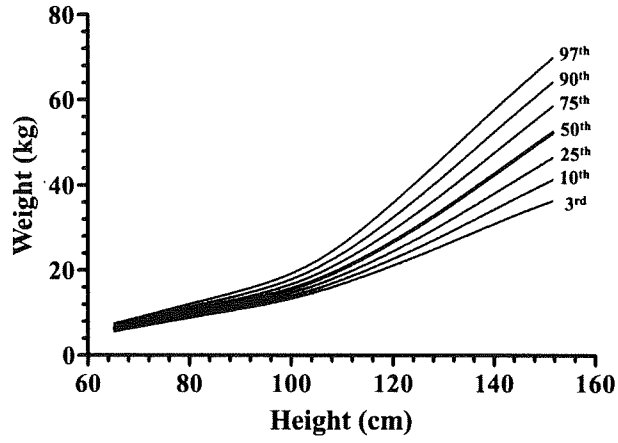

Figure 2 WFH chart for Japanese girls with Turner syndrome without puberty.

DISCUSSIONS

Evaluation of overweight is usually made with anthropometry for practical reasons. Among the various anthropometric indices, BMI is the most widely used both clinically and academically, especially after the International Obesity Task Force recommended BMI as a valid surrogate marker of adiposity (7). However, different measures and references have been used in each country for classification of overweight, as is the case in Japan, where percent overweight has been used in preference to BMI. It is also reported that in normal children aged 2–19 years, no differences were found between BMI and WFH in detecting overweight in terms of percentage body fat or total fat mass as determined by

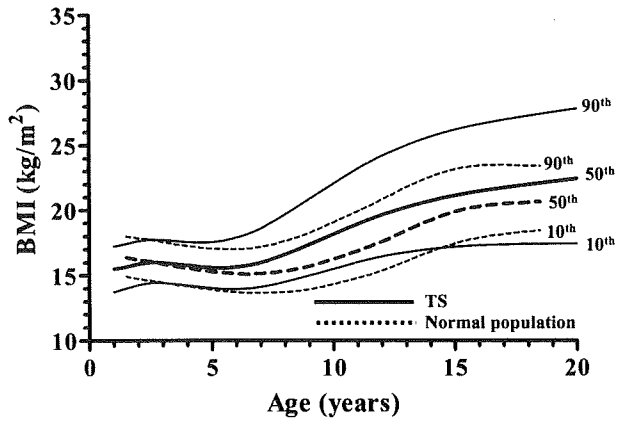

Figure 3 BMI chart for Japanese girls with Turner syndrome without puberty in comparison with normal population (20).

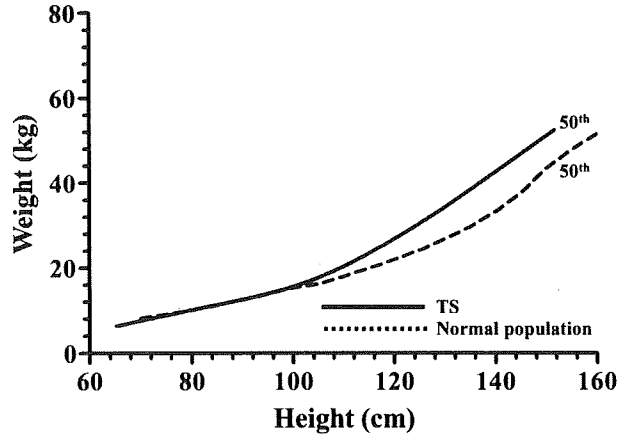

Figure 4 WFH chart for Japanese girls with Turner syndrome without puberty in comparison with normal population (17). Only the 50th percentile curves are shown, because WFH for the Japanese normal population has only values of the 50th percentile.

dual-energy X-ray absorptiometry (24). In this study, we found that in TS, percentages of overweight differed between the two methods and the ratio of discordance became larger with age (Table 3). Considering the consistency of the two indices in the normal population, it is surprising that more than 30% of subjects with TS older than 10 years of age were classified differently. When International cut-off values of BMI (10) instead of Japanese cut-off values were used for classification of overweight, we obtained the similar results (Table 3). These results indicate specific difficulty in defining overweight for girls with TS by anthropometric indices. The TS consensus study group recommends females with TS should aim to have a BMI less than 25 kg/m^2 in an updated clinical practice guideline (6). And the clinical report of the American Academy of Pediatrics states that diet and exercise for weight control should be discussed for girls with TS, because obesity may be a particular problem for them (25). Although it is reported that BMI is a good marker of obesity and associated cardiovascular risk in adult females with TS (26), it is still unknown whether or not BMI should be used as a surrogate marker of overweight for girls with TS in clinical practice. The answer to this question is beyond the scope of our present study. Nevertheless, discrepancies of classification of overweight by the two indices were shown in this study and therefore attention should be paid to the determination of overweight for girls with TS by anthropometric indices.

In an attempt to uncover the reason for the discrepancies discussed above, we thought that it would give us new insight to compare the growth patterns of the two indices between girls with TS and normal subjects. As the first step, we produced clinical reference charts of BMI and WFH for Japanese girls with TS who did not develop puberty, because these charts had not been produced before. They were constructed by the LMS method, which is thought to be one of the most widely applied approaches (27). In addition, diagnoses of all the subjects were confirmed by the definition of TS based on the chromosomal analyses and properly selected by excluding the cases of pubertal development and/or previous growth-promoting treatment. From this perspective, we believe that these charts have been adequately and successfully produced and can be used as appropriate standards in clinical practice. To our knowledge, these are the first charts of BMI and WFH for TS girls in an Asian population.

The newly constructed reference growth chart for BMI of girls with TS in comparison with that of the normal population (Fig. 3) shows that the difference of the 50th percentile values between girls with TS and the normal population increases towards approximately 11 years of age, and then tends to decrease with age. This phenomenon is also seen in another TS-specific BMI chart (5). It is of note that peak growth velocity of Japanese girls occurs around 11 years of age (28). This coincidence of the age may suggest that the different BMI growth pattern is associated with lack of puberty in TS girls. However, the difference cannot be explained only by pubertal development, because growth patterns of girls with TS differ from the normal population also in the prepubertal stage (2–5). In addition, when we investigated the appropriate power (p) of the weight/height^p index according to ages, the optimal value of p was not appreciably different from that in the normal girls (data not shown), which was approximately two in pre-school children, increased gradually to around three at age 11 and fell back to the level of two thereafter (29,30). As for the WFH reference, we find that WFH is quite normal below the height of 100 cm, but above 120 cm there is a more rapid increase of the WFH in TS girls (Fig. 4). This finding is consistent with observations, which have been reported in western countries (2,4,5). Through comparison of TS-specific reference growth charts for BMI and WFH with those of the normal population, we could illustrate the different degrees of distinction of growth patterns between girls with TS and normal girls in each index. Therefore, our finding of discrepancy in the prevalence of overweight as classified by the two indices for girls with TS probably corresponds to these differences of growth patterns

in the two indices from the normal population, although the nature of the differences remains unclear.

This study has two limitations. The first one is a selection bias. This retrospective cohort consists of those diagnosed as TS in medical institutes, which means that subjects who are not significantly smaller than the normal population are probably missing. More specifically, physicians do not usually register girls with TS if they are taller than −2SDS of the female standard, because the registry is primarily for candidates of GH treatment. It is of note that indication of GH for TS is limited to subjects shorter than −2SDS in Japan. The height of the majority of girls with TS usually drops below the fifth percentile of the normal girl growth curve only after an age between two and five years (1). This implies that a selection bias occurs more severely in subjects younger than approximately three years of age. Therefore, values under three years of age in the BMI reference chart for girls with TS are not very reliable. The second limitation is the fact that we cannot know which indices are better for identifying overweight for girls with TS from this study. Our present study only illustrated a discrepancy in the classification of overweight between the two indices, although medical practitioners are eager to know which index is better for evaluating weights in clinical settings. This research question needs to be answered. However, our study does not make any proposals, because there were no other clinical data on obesity than the anthropometric measurements in this retrospective cohort. Further investigation is needed to understand the characteristics of overweight of girls with TS in relation to obesity and metabolic syndrome in adulthood, and to develop an adequate anthropometric screening method for possible obesity in TS.

CONCLUSIONS

A discrepancy in the prevalence of overweight as classified by BMI and WFH for girls with TS was detected and it became larger with age. This specific discordance corresponded to the different degrees of distinction of growth patterns in two indices compared with the normal population. Careful interpretation of the anthropometric indices is essential for the determination of overweight for girls with TS. Further investigation is required to reveal a better method for evaluating obesity by anthropometric measurements.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors have no conflict of interest.

References

1. Saenger P. Turner's syndrome. *N Engl J med* 1996; 335: 1749–54.
2. Ranke MB, Pflüger H, Rosendahl W, Stubbe P, Enders H, Bierich JR, et al. Turner syndrome: spontaneous growth in 150 cases and review of the literature. *Eur J Pediatr* 1983; 141: 81–8.
3. Suwa S. Standards for growth and growth velocity in Turner's Syndrome. *Acta Paediatr Jpn* 1992; 34: 206–21.
4. Bernasconi S, Larizza D, Benso L, Volta C, Vannelli S, Milani S, et al. Turner's syndrome in Italy: familial characteristics, neonatal data, standards for birth weight and for height and weight from infancy to adulthood. *Acta Paediatr* 1994; 83: 292–8.
5. Rongen-Westerlaken C, Corel L, Broeck JVD, Massa G, Karlberg J, Albertsson-Wikland K, et al. Reference values for height, height velocity and weight in Turner's Syndrome. *Acta Paediatr* 1997; 86: 937–42.
6. Bondy CA for The Turner Syndrome Consensus Study Group. Care of girls and women with Turner syndrome: a guideline of the Turner syndrome study group. *J Clin Endocrinol Metab* 2007; 92: 10–25.
7. Lobstein T, Baur L, Uauy R, IASO International Obesity TaskForce. Obesity in children and young people: crisis in public health. *Obes Rev* 2004; 5(Suppl 1): 4–104.
8. Janssen I, Katzmarzyk PT, Boyce WF, Vereecken C, Mulvihill C, Roberts C, et al. Health behavior in school-aged children obesity working group. Comparison of overweight and obesity prevalence in school-aged youth from 34 countries and their relationships with physical activity and dietary patterns. *Obes Rev* 2005; 6: 123–32.
9. Dietz WH, Robinson TN. Use of the body mass index (BMI) as a measure of overweight in children and adolescents. *J Pediatr* 1998; 132: 191–3.
10. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *Brit Med J* 2000; 320: 1240–3.
11. Voelker R. Improved use of BMI needed to screen children for overweight. *JAMA* 2007; 297: 2684–5.
12. Barlow SE, Dietz WH, Klish WJ, Trowbridge FL. Medical evaluation of overweight children and adolescents: reports from pediatricians, pediatric nurse practitioners, and registered dietitians. *Pediatrics* 2002; 110: 222–8.
13. Corel LJA, Van Den Broeck J, Rongen-Westerlaken C, Massa G, Wit JM. Body weight in children with Turner syndrome treated with growth hormone. *Int J Obesity* 1996; 20: 957–62.
14. Gravholt CH, Naeraa RW, Brixen K, Kastrup KW, Mosekilde L, Jøgensen JOL, et al. Short-term growth hormone treatment in girls with Turner syndrome decreases fat mass and insulin sensitivity: a randomized, double-blind, placebo-controlled, crossover study. *Pediatrics* 2002; 110: 889–96.
15. Tanaka T, Takano K, Hanew K, Nishi Y, Igarashi Y, Hirano T, et al. Registration system for growth hormone (GH) treatment with standardized immunoreactive GH values in Japan. *Endocr J* 1998; 45: 459–65.
16. Asayama K, Ohzeki T, Sugihara S, Ito K, Okada T, Tamai H, et al. Criteria for medical intervention in obese children: a new definition of 'obesity disease' in Japanese children. *Pediatr Int* 2003; 45: 642–6.
17. Ito Y, Fujieda K. Obesity (in Japanese). *Shoonikashinryo* 2003; 66: 1913–19.
18. Japan Society for the Study of Obesity. *Manual for the treatment of obesity disease in children (in Japanese).* Tokyo, Ishiyaku Publishers Inc, 2004.
19. Guillaume M. Defining obesity in childhood: current practice. *Am J Clin Nutr* 1999; 70(Suppl 1): 126S–30S.
20. Inokuchi M, Hasegawa T, Anzo M, Matsuo N. Standardized centile curves of body mass index for Japanese children and

adolescents based on the 1978–1981 national survey data. *Ann Hum Biol* 2006; 33: 444–53.
21. Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med* 1992; 11: 1305–19.
22. Ministry of Education: *Annual report of school health statistics (in Japanese)*. The Printing Office, The Ministry of Finance, Tokyo, 1990.
23. Ogata T, Matsuo N, Tamai S, Osano M, Tango T. Target height and target range for the Japanese (in Japanese). *Jpn J Paediatr* 1990; 94: 1535–40.
24. Mei Z, Grummer-Strawn LM, Pietrobelli A, Goulding A, Goran MI, Diez WH. Validity of body mass index compared with other body-composition screening indexes for the assessment of body fatness in children and adolescents. *Am J Clin Nutr* 2002; 75: 978–85.
25. Frías JL, Davenport ML, Committee on Genetics and Section on Endocrinology. Health supervision for children with Turner syndrome. *Pediatrics* 2003; 111: 692–702.
26. Ostberg JE, Attar JH, Mohamed-Ali V, Conway GS. Adipokine dysregulation in Turner syndrome: comparison of circulating interleukin-6 and leptin concentrations with measure of adiposity and c-reactive protein. *J Clin Endocrinol Metab* 2005; 90: 2948–53.
27. Wright EM, Royston P. A comparison of statistical method for age-related reference intervals. *J R Statist Soc A* 1997; 160: 47–69.
28. Suwa S, Tachibana K, Tanaka T, Maesaka H, Yokoya S. Longitudinal standards for height and height velocity for Japanese children from birth to maturity. *Clin Pediatr Endocrinol* 1992; 1: 5–13.
29. Cole TJ. Weight/height^p compared to weight/height^2 for assessing adiposity in childhood: influence of age and bone age on p during puberty. *Ann Hum Biol* 1986; 13: 433–51.
30. Hattori K, Hirohata T. Age change of power in weight/height^p indices used as indicators of adiposity in Japanese. *Am J Hum Biol* 2002; 14: 275–9.

早産児の成長・発達の異常とその予防

—早産児のNICU退院後の成長とその異常—

早産児の二次性徴，生殖機能

堀川玲子

はじめに—SGA児とAGA児

低出生体重児では，二次性徴の発来が早いことが知られている。しかし，本当に二次性徴発来が早いというデータは意外に少ない。同じ低出生体重児でも早産児で月例相当の出生体重であった児(AGA児)と月例に比し低出生体重児(SGA児)では二次性徴の問題は異なっている可能性がある。一方で，在胎30週未満の早産児では，AGA児であってもSGA児と同様の二次性徴や生殖機能に関する問題が起こる可能性があり，逆に週数が十分であってもSGA児の場合は同様の問題が起こる可能性が高いと考えられる。また，二次性徴の構成要素であるアドレナーキと呼ばれる副腎の成熟は，子宮内発育不全のあったSGA児では早い傾向にあり，いわゆるアンドロゲン過剰の状態が作られやすいとされている[1]。アドレナーキは正常な性成熟のプロセスであるが，近年早発アドレナーキがインスリン抵抗性と関連しており，このことが成人後のメタボリック症候群のリスクを高める可能性につながることが示されている[1]。本稿では，特に二次性徴と生殖機能の問題が起こりやすいとされる在胎30週未満のAGA児を含めた低出生体重児について概説し，アドレナーキの特徴とその問題点についても述べる。

子宮内環境と思春期

「思春期」は，性成熟・成長の加速・副腎の成熟を認める時期である。性成熟の開始を規定するのは性腺刺激ホルモン分泌刺激ホルモン(gonadotropin releasing hormone：GnRH)の脈動的分泌上昇である。GnRHは間脳視床下部より分泌され，下垂体における性腺刺激ホルモン[ゴナドトロピン，Gn：黄体化ホルモン(luteinizing hormone：LH)と卵胞刺激ホルモン(follicle-stimulating hormone：FSH)]の脈動的分泌回数と分泌頂値の上昇を促し，LHとFSHは性腺に働いて性ホルモン分泌と胚細胞の成熟を促進する(HPG系)[2]。この結果，男児では精巣容積の増大と外性器の成熟が起こり，女児では乳房発達(テラルキ：thelarche)と月経発来(メナーキ：menarche)が起こる。また，精子・卵子の形成が進行する。GnRHの思春期レベルの脈動的分泌上昇をきたすアラームの役を担うのが“ゴナドスタット”であり，その上流にある“developmental clock”である。このゴナドスタットや，HPG系全体に影響を及ぼすのは，さまざまな環境因子である。この環境因子の一つに，胎生期の栄養もあげられる。動物実験では，胎生期の低栄養による子宮内発育遅延があると，雌ラットでは思春期早発が，雄ラットでは思春期遅発が誘発されるが，胎生期の低栄養がなくて生後の低栄養のみでは，雌では思春期開始時期に影響せず雄では思春期遅発となる。

ヒトの場合，社会経済の進歩による生後の栄養改善は，男女ともに思春期のタイミングを早めることが知られている[3]。ヒトの胎生期の栄養と発育が思春期のタイミングに及ぼす影響については，さまざまな報告がされており，一定の見解に

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は至っていない。これについては後述する。

HPG系の目覚めと同時あるいは先行して，副腎における副腎性アンドロゲン分泌の上昇が認められる。これが次に述べるアドレナーキ(adrenarche)である。アドレナーキは，内分泌的にはdehydroepiandrosterone-sulfate(DHEA-S)の年齢に伴う上昇で示される。

アドレナーキと関連した用語として，性毛発生(プバーキ：pubarche)がある。性毛は陰毛と腋毛があるが，多くは陰毛が先行するためプバーキは陰毛発生とほぼ等しいと考えられる。陰毛は，女児では通常大陰唇上やその内側に現れ，徐々に恥骨上方に拡大していく。男児では陰嚢から発毛が始まる。HPG系が保たれていても，副腎アンドロゲンが著しく低値であるような疾患では，プバーキは全く認められないかあっても不完全なため，プバーキはアドレナーキによる副腎アンドロゲン分泌上昇によるものと考えられる。プバーキ開始時期には，人種，肥満度などで差が認められる。

DHEA-Sの標準範囲は幅が広く，個人差が大きい[4]。

思春期発来時期には個人差，男女差がある。日本人女児の乳房腫大開始年齢は平均9～9.5歳，初経年齢は平均約12歳3カ月と考えられる。また女性は男性よりも約1年早く思春期の変化が起こるが，成長曲線の変化(成長加速の開始とピーク時期)には約2年の差がある。男女ともに思春期発来時期の2.5 SDの範囲は±2～3年であり，これを超えた時に思春期早発症，および思春期遅発症と診断される。早発陰毛(premature pubarche)は，男児で10歳(海外では9歳)，女児で8歳未満での陰毛発生と定義され，陰毛以外の思春期徴候は伴わず骨年齢促進も認めないとされる。

思春期の異常，あるいはバリエーションは，暦年齢における二次性徴開始時期の異常だけではない。後述するように，SGA性低身長症では思春期開始時期は暦年齢相当でも，その後の進行が著しく早い，低身長のうちに思春期発来したために成人身長が低下してしまう，といったことも問題である。

アドレナーキの機序

副腎アンドロゲンの緩徐な分泌上昇を「アドレナーキ」といい，男児では7歳頃から，女児では6歳頃から開始される。

副腎アンドロゲン上昇の機序は，副腎皮質ステロイド合成酵素の活性と発現量の変化に伴いACTH分泌に反応して産生されるステロイド分画が変化するためと考えられる[7]。すなわち，ACTH依存性にΔ5ステロイド(17OHプレグネノロン，デヒドロエピアンドロステロンDHEA)がΔ4ステロイド(17OHプロゲステロン，アンドロステンジオン)より相対的に多く上昇する。この結果として，DHEA-sulfate(DHEA-S)が血中の17ケトステロイドの多くの部分を占めるようになる。DHEA-Sは，アドレナーキのマーカーとなる。この時，体表面積当たりのコルチゾール産生は不変であるので，コルチゾールに対するDHEA産生も相対的に上昇する。このような17OHプロゲステロン，DHEA，DHEA-Sの産生は副腎皮質網状層で行われ，主に網状層におけるステロイド合成酵素の変化が起こることによりアドレナーキのホルモン変化が起こる[8]。

アドレナーキがなぜ起こるのか，その機序は解明されているわけではない。一時，副腎の独立した成熟説があったが，現在は下垂体の成熟に伴うものと考えるのが主流である。下垂体からのACTHの前駆物質であるPOMC，プロラクチン，副腎皮質アンドロゲン分泌促進ホルモン(cortex androgen-stimulating hormone：CASH)などの刺激が，アドレナーキの原因と推測されている[9,10]。このほか，インターロイキン6も副腎皮質網状層に強く発現し，DHEA産生を促進する[11]。また，皮下脂肪の蓄積とレプチン値の上昇が17，20ライエース活性を上昇させてDHEA-S産生を促進することも明らかとなってきた[12]。インスリンやIGF-I，成長ホルモンの関与も考えられるが明らか

ではない。これらの刺激により，胎児副腎の遺残を起源とする，独自の酵素特性をもった副腎網状層の前駆細胞が分化・成長して，副腎アンドロゲンの産生が亢進するのであろうと考えられる。

早発アドレナーキにおいては，アドレナーキのマーカーであるDHEA-Sの血中濃度は，およそ40 μg/dL以上となるが，DHEA-S値には個人差が大きいため，この値はあくまで参考値と考える。DHEA-S値は通常，思春期早期のレベルを超えることはない。130 μg/dLを超える場合は，過剰アドレナーキ(exaggerated adrenarche)と考えられ，代謝予後に影響する。また，テストステロンは40 ng/dL，アンドロステンジオンは75 ng/dLが上限の上昇を示す[13]。早発アドレナーキの病因は明らかではないが，単に副腎皮質網状層の成熟が低年齢にシフトしただけではなく，何らかのステロイド産生機構の調節障害が考えられる[13]。

低出生体重児と思春期・アドレナーキと生殖機能

1．思春期の進行と成長

出生時体重の小さい児は，思春期早発傾向にあると報告されている[14]。臨床的にも，SGA性低身長症では二次性徴発来年齢が低いという印象があるが，実際の研究では，必ずしも思春期早発傾向が実証されてはいない。

スウェーデンのコホートスタディでは，男児においてはSGAグループとAGAグループで思春期発来年齢に有意差はなかったが，女児ではSGAグループのほうが初潮年齢が5カ月早かった[14]。別の報告ではSGAの女児では，二次性徴発来年齢自体はAGAと有意差は認めないが，二次性徴の進行が早く初潮年齢が約1年早かった[1]。一方フランスからの報告では，女児の思春期開始年齢，初潮年齢はAGA児と有意差はなかった[3]。しかしながら，全体としての年齢の有意差は認めなくても，思春期早発を示す児の割合はSGAのほうが高く，多重解析で思春期発来時期予測に影響を及ぼす要素を検討してみると，出生時体重SDSが最も相関が高かった。

このように，思春期発来時期については結論の分かれているところであるが，男女差については多くの報告で認められている。男児では，ほとんどの報告で思春期早発傾向は認めておらず，一部，急激な体重増加をきたした例や，キャッチアップが不良な例に限り思春期早発を認めるという報告がある。一方，胎生期の環境が劣悪な栄養状態にあるなど著しく不良な場合，特に男児で性腺機能低下をきたして思春期遅発や永続性の性腺機能低下症となる可能性もある。一部の症例では，停留精巣，尿道下裂の頻度上昇も認める[14]。

年齢的には思春期早発ではなくても，低身長であるのに思春期が発来する，いわゆる身長に対して相対的な思春期早発(低身長思春期発来)を認めることも多く，これが成人身長の低下をもたらす要因となっている。先に述べたスウェーデンのデータでも，思春期発来時の身長がSGAではAGAよりも5 cm低かった。そのために成人身長が低下するとも考えられる。

SGAで生後キャッチアップのみられなかったSGA性低身長症に対し，成長ホルモン(GH)治療が行われている。GH(IGF-I)は性腺に対し，*in vitro*ではゴナドトロピンに対する感受性を亢進する作用を有するため[16]，GH治療，特に高用量のGH投与は二次性徴の時期と進行を促進することが予想されたが，これまでの報告では用量にかかわらず，思春期発来時期の早発は認めず，進行もGH治療を行わなかった群と同様であった[3,17]。このことから，現行のGH治療はSGA性低身長症における思春期を修飾することはないと考えられる。

2．内分泌学的特徴

内分泌学的には，SGA児では男女ともにAGA児よりも血中FSH値が高値であり，その傾向は若年男児により顕著である。LH，インヒビンBはSGAとAGAでは差がないことから，胎生期の発

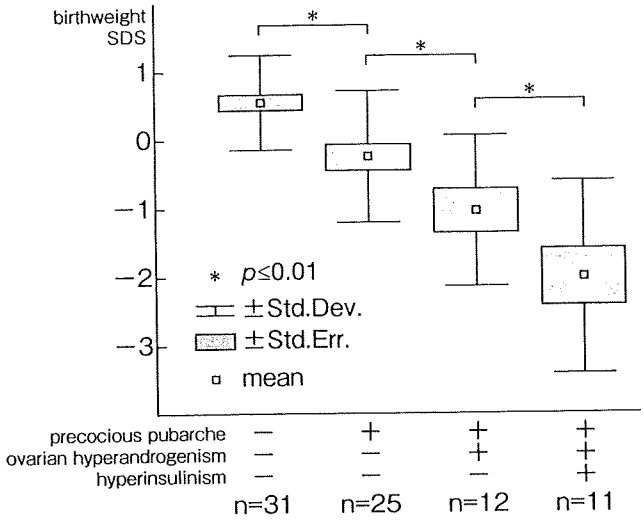

図1　早発プバーキ，卵巣性高アンドロゲン血症，高インスリン血症と出生時体重 SDS の関連(Ibanez ら，1998)[16]

育障害は FSH-インヒビン B のループのリセットをもたらし，同じインヒビン B のレベルでも FSH の反応性が過大となっていると考えられる[1]。また，SGA/AGA に限らず，低出生体重児は思春期前の LH，FSH 値が高く，インヒビン B が低値であることも報告されている[14]。この理由は明らかではないが，胎生期の環境がセルトリ細胞と顆粒膜細胞の発育不全をもたらすためではないかと考えられ，このことは女児での原始卵胞数の減少，男児での精巣容積と精子数の減少を介して妊孕性の低下につながることが予想される。

また，SGA 児では出生時体重が小さくて 8 歳時の BMI 値が高いほど，DHEA-S 値が高値である[1]。Veening ら[18]は思春期前の SGA 児は AGA 児よりも DHEA-S 値が高値であると報告しているが，一方で Boonstra ら[19]は有意差がないと報告している。

3. アドレナーキと生殖機能

SGA の児では，早発アドレナーキや早発陰毛が起こりやすいと考えられているが，否定的な報告もある[1]。多くの報告では，SGA 児において先に述べた DHEA/DHEA-S 高値の傾向を認め，特に生後乳幼児期早期に体重増加が著しい例ではより DHEA-S の高値を認めている[14]。早発アドレナーキは，高アンドロゲン血症を伴いやすく，高アンドロゲン血症はインスリン抵抗性を亢進して，成人後のメタボリック症候群のリスクを高めると考えられているが，Ibanez ら[16]は，SGA 児と早発アドレナーキの関連を検討し，出生時体重が低下するに従いこれらの代謝異常と多嚢胞性卵巣のリスクが上昇するとした(**図1**)。Rosenfield らは早発アドレナーキを認めた女児の 20%に，メタボリック症候群のリスクファクターである多嚢胞性卵巣を認めると報告し[13]，これは SGA 児における早発アドレナーキと多嚢胞性卵巣の関連に合致する。一般的に PCOS は，女性不妊の原因の一角を占める。PCOS のリスクが高いということを，将来不妊となるリスクとイコールとすることは飛躍が過ぎるかもしれないが，可能性は示唆される。

SGA におけるこれらの変化は，Barker 仮説や DOHAD 仮説(別稿参照)から説明される。Ibanez らは，胎生期の発達可塑性によるプログラミングが関与して高インスリン血症や CRH 上昇が起こり，高インスリン血症は SHBG 低下や free IGF-I の上昇を惹起し，これらが高アンドロゲン血症・早発アドレナーキを誘発した結果，PCOS の頻度が上昇するとした[1,20](**図2**)。一方，急激な体重増加を伴わない SGA 女児のグループでは，BMI をマッチさせた AGA グループと DHEA-S 値やアンドロゲン値は有意差がなく，血中レプチン濃度の高値，思春期早期のインスリン抵抗性が認められるとの報告もあり[14]，必ずしも高アンドロゲン血症の介在が必須ではない可能性も示唆される。最近の報告では，IGF-I 受容体の多型と早発アドレナーキの関連が報告されている[21]。SGA の病因として IGF-I 受容体異常があげられることから，SGA における早発アドレナーキと IGF-I 系との関連が示唆される。

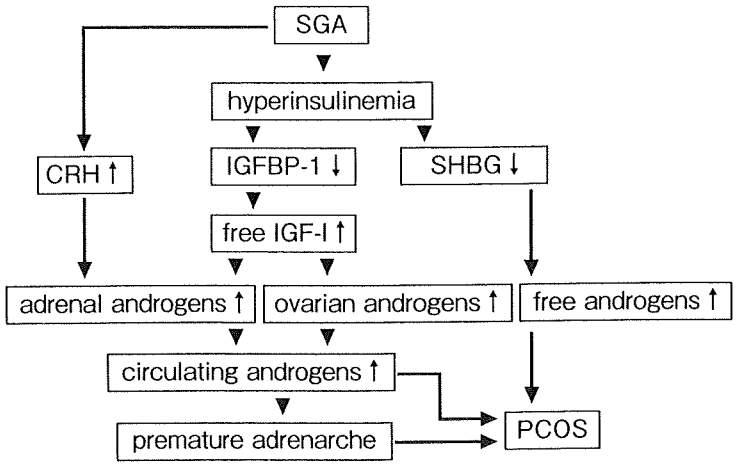

図2 SGAと高インスリン血症，高アンドロゲン血症，多嚢胞性卵巣症候群のカスケード
(Ibanez, Silfen らのデータより Garcia らが改変，2004)[20]

おわりに

低出生体重児，特にSGA児と早産AGA児における思春期発来のタイミング，二次性徴進行，アドレナーキとPCOSやメタボリック症候群のリスク，生殖機能について述べた。多くの報告が後方視的研究であり，対象症例数もさまざまであることから，結論は議論のあるところもあるが，いずれにしても特にSGA児では思春期の異常と生殖機能の低下，成人後の代謝異常のリスクを有することは明らかであり，長期のフォローアップが必要と思われる。

文献

1) Ibanez L, Dimartino-Nardi J, Potau N, et al : Premature adrenarche—normal variant or forerunner of adult disease? Endocr Rev **21** : 671, 2000
2) Bourginigon JP : Chapter 19 Control of the onset of puberty. Pediatric Endocrinology, Lippincott Williams & Wilkins, Philadelphia, pp285–298, 2004
3) Hernandez MI, Mericq V : Impact of being born small for gestational age on onset and progression of puberty. Best Practice Res Clin Endocrinol Metab **22** : 463–476, 2008
4) 小児基準値研究班 編：日本人小児の臨床検査基準値，日本公衆衛生協会，pp 441，453，457，1997
5) 堀川玲子：思春期の身体の発達・性の発達—最新の知見．小児科診療 **5** : 68，2005
6) Rich BH, Rosenfield R, Lucky A, et al : Adrenarche : Changing adrenal response to adrenocorticotropin. J Clin Endocrinol Metab **52** : 1129, 1981
7) Gell JS, Carr BR, Sasano H, et al : Adrenarche results from development of a 3 β -hydroxysteroid dehydrogenase-deficient adrenal reticularis. J Clin Endocrinol Metab **83** : 3695, 1998
8) Parker LN : Adrenarche. Endocrinol Metab Clin North Am **20** : 71, 1991
9) Taha D, Mullis PE, Ibanez L, et al : Absent or delayed adrenarche in Pit-1/POU1F1 deficiency. Horm Res **64** : 175, 2005
10) Ehrhart-Bornstein M, Hinson JP, Bornstein SR, et al : Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. Endocr Rev **19** : 101, 1998
11) Biason-Lauber A, Zachmann M, Schoenle EJ : Effect of leptin on CYP17 enzymatic activities in human adrenal cells : new insight in the onset of adrenarche. Endocrinology **141** : 1446, 2000
12) Rosenfield RL : Clinical review : Identifying children at risk for polycystic ovary syndrome. J Clin Endocrinol Metab **92** : 787, 2007
13) Preece MA : Puberty in children with intrauterine growth retardation. Horm Res **48**(Suppl 1) : 30, 1997
14) Hernandez MI, Martinez A, Capurro T, et al : Comparison of clinical, ultrasonographic, and biochemical differences at the beginning of puberty in healthy girls born either small for gestational age or appropriate for gestational age : preliminary results. J Clin Endocrinol Metab **91** : 3377, 2006
15) Horikawa R, Asakawa K, Hizuka N, et al : Growth hormone and insulin-like growth factor I stimulate Leydig cell steroidogenesis. Eur J Pharmacol **166** : 87–94, 1989
16) Ibanez L, Potau N, Francois I, et al : Precocious pubarche, hyperinsulinism, and ovarian hyperandrogenism in girls : relation to reduced fetal

growth. J Clin Endocrinol Metab 83 : 3558–3562, 1998
17) Horikawa R, Tanaka T : Growth hormone treatment in short Japanese children born small for gestational age. Horm Res 62(suppl 3) : 128–136, 2004
18) Veening MA, van Weissenbruch MM, Roord JJ, et al : Pubertal development in children born small for gestational age. J Pediatr Endocrinol Metab 17 : 1497–1505, 2004
19) Boonstra V, van Pareren Y, Mulder P, et al : Puberty in growth hormone-treated children born small for gestationa age(SGA). J Clin Endocrinol Metab 88 (12) : 5753–5758, 2003
20) Garcia MH, Muzumdar RH, Saenger P : Chapter 14 Endocrinopathies in infants who are small for gestational age. Pediatric Endocrinology Mechanisms, Manifestations, and Management, Lippincott Williams & Wilkins, Philadelphia, pp224–230, 2004
21) Roldan MB, White C, Witchel SF : Association of the GAA1013 → GAG polymorphism of the insulin-like growth factor-1(IGF1R)gene with premature pubarche. Fertil Steril 88 : 410–417, 2007

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Original Article

Wide Range of Biotin (Vitamin H) Content in Foodstuffs and Powdered Milks as Assessed by High-performance Affinity Chromatography

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Abstract. The biotin (vitamin H) contents of various foodstuffs were determined by using a newly developed high-performance affinity chromatography with a trypsin-treated avidin-bound column. Biotin was derivatized with 9-anthryldiazomethane (ADAM) to fluorescent biotin-ADAM ester. A wide range of biotin contents were found in various foodstuffs depending upon the species (strain), season, organ (of plants and animals), geography, freshness, preparation method and storage method. Among the foodstuffs and fermented foods tested, it was found that wide distributions of biotin content were observed in powdered milk, natto, sake (rice wine), beer, edible oil and sea weed. Since powdered milk is important for child health and development, 14 kinds of powdered and special milks for use in children's diseases were intensively measured. We found that several special milk powders for children with allergies contained low levels of free biotin. Use of these powdered milks caused skin diseases and alopecia in some patients possessing thermolabile serum biotinidase, and administration of free biotin improved their symptoms dramatically. Therefore, it is essential to estimate the total and free biotin contents on each foodstuff in order to improve effective biotin intake and support better health and quality of life for people.

Key words: total biotin, free biotin, wide distribution, foodstuffs, powdered milk

Introduction

Determination of biotin, especially free-form biotin, in foodstuffs is important, because appropriate biotin intake is beneficial in attaining a good quality of life (QOL), better health and development of children and adults, improved physical mechanisms that combat aging and disease and efficient mental capacity.

Recently, we developed a new high-performance affinity chromatographic (HPAC) determination method for biotin using a trypsin-

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Dedication: In memory of the kind encouragement of my beloved daughter, Reiko Hayakawa (21 November 1979 – 1 February 2007).

treated avidin-bound column (1). In this new method, biotin is derivatized by 9-anthryldiazomethane (ADAM) to an ester of fluorimetric biotin-ADAM and detected fluorimetrically at an excitation wavelength of 365 nm and emission wavelength of 412 nm (1–3). This is a simple chromatographic method using the affinity of avidin for biotin. We recently found that avidin is a bifunctional binding protein; i.e., avidin (a well-known biotin-binding protein) can also strongly recognize lipoic acid (4). However, biotin and lipoic acid can be separated and measured safely using this new chromatographic technology. It is a rapid (analysis requires one day per sample), reliable and sensitive fluorometric detection method that makes use of the linear calibration line through the origin. Furthermore, other nutrients and antibiotics do not interfere with this chemical method; i.e., other biological biotin assay methods are sensitive to nutrients and antibiotics in serum samples.

Herein, we describe the wide ranges of biotin contents detected among various foodstuffs depending on the species (strain), season, organ (of plants and of animals), geographical area, freshness and preparation and storage methods. The importance of the free biotin content in powdered milk in relation to babies, who have unstable biotinidase and exhibit biotin deficiency symptoms, is also discussed.

Materials and Methods

Chemicals and reagents

Highly pure form of methanol, acetonitrile, ethanol and ethyl acetate (>99.8%), D-biotin, activated charcoal (acid washed; for column chromatography; P/N 035-18081), 2-propanol (HPLC grade), ethylene glycol (amino acid analysis grade), 25% ammonia water (metal analysis grade), sulfuric acid, sodium chloride, lithium chloride (anhydrous; amino acid analysis grade; >97%) and sodium dihydrogen phosphate dihydrate were purchased from Wako Pure Chemical Industries (Osaka, Japan). D-Desthiobiotin (5-methyl-2-oxo-4-imidazolidine hexanoic acid; D 1411), biocytin (ε-N-biotinyl-L-lysine, Mr 372.5; B 4261) and biotin methyl ester (B 7883) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and 9-anthryldiazomethane (ADAM) was purchased from Funakoshi Pharmaceutical (Tokyo, Japan). A 0.25% (w/v) trypsin-EDTA solution was purchased from Invitrogen Corporation (Grand Island, NY, USA).

A light-intercepting microtube with a cap (2 ml; P/N 72.693.018) and a microtube with cap (2 mL; P/N 72.694.007) were obtained from Sarstedt Aktiengesellschaft & Co. (Nümbrecht, Germany). Microcentrifuge tubes (1.5 mL, polypropylene, lock-cap; P/N 96.8668.9.01) were obtained from Treff AG (Degersheim, Switzerland). Ekicrodisc 13 CR (0.2 μm; PTFE; P/N E135), Ekicrodisc 13 (0.2 μm; Versapor; P/N E134) and Ekicrodisc 25 membrane filters (0.2 μm; Versapor; P/N E254) were obtained from Nihon Pall Ltd. (Tokyo, Japan). Paper pH indicator (pH 6.4–8.0, narrow range) were obtained from Whatman Ltd. (Maidstone, Kent, England). Blades and disposable scalpels were obtained from Feather Safety Razor Co. (Osaka, Japan).

An affinity column, Bioptic AV-1 (250 × 4.6 mm I.D.; with chicken egg-white avidin bound to a 5 μm diameter silica gel), was purchased from GL Sciences Inc. (Tokyo, Japan). The contents of the column were removed using an HPLC pump. Bioptic AV-1 affinity gels (5 μm diameter silica gel) are now available (1 g and/or 10 g) from GL Sciences Inc.

Trypsin-treated avidin-bound gel was prepared as described previously (1). A trypsin-treated avidin-bound column (33 × 4.6 mm I.D.) was then prepared.

Ten types of natto (a Japanese food made from fermented soybeans), thirteen sakes (rice wines), ten beers, four coffees, three red wines, four breads, four cheeses, three vinegars, four bananas (three from the Philippines and one