

GROWTH HORMONE AND BODY COMPOSITION IN CHILDREN YOUNGER THAN 2 YEARS WITH PRADER-WILLI SYNDROME

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Objectives To assess body composition of infants with Prader-Willi syndrome (PWS) by using deuterium dilution and investigating the efficacy of early institution of growth hormone (GH) therapy in increasing lean mass (LM) and preventing massive obesity.

Study design One group of 11 children with PWS < 2 years before and during 30-month GH therapy (GH group) was compared with 6 infants administered only coenzyme Q₁₀ for 1 year (Q10 group). LM adjusted for height (LM_{Ht}) and relative fat mass (%FM_{Age}) standard deviation scores (SDS) were calculated from data of 95 healthy children.

Results Initially, LM_{Ht} of all patients was below the normal average. LM_{Ht} decreased by -0.46 ± 0.3 SD ($P = .03$) per year in the Q10 group but rose by 0.25 ± 0.3 SD ($P = .02$) per year during GH therapy, normalizing after 30 months (-0.70 ± 1.0 SD). Despite low to normal weight for height (WfH), %FM_{Age} was above the normal average (GH group, $31.0\% \pm 4.5\%$, Q10 group, $32.4\% \pm 9.5\%$). In the Q10 infants, %FM_{Age} increased by 0.71 ± 0.7 SD per year, whereas in the GH group, %FM_{Age} remained more stable up to 30 months.

Conclusions Diminished LM_{Ht} found in infants with PWS further declines during the early years. Early institution of GH therapy lifts LM_{Ht} into the normal range and delays fat tissue accumulation. (*J Pediatr* 2004;144:753-8)

Hypothalamic dysfunction appears to underlie many of the features of Prader-Willi syndrome (PWS), including short stature, hypogonadism, and the defective regulation of energy balance. A main feature of PWS is the disturbance of body composition, though in most cases, overt obesity sets in only after the 2nd year of life.^{1,2} Nevertheless, the shift to a reduced muscle mass has already been shown in young children (0.3 to 4.1 years of age).^{3,4}

At present, PWS is increasingly diagnosed as early as in infancy because pediatricians and neonatologists are more aware of the clinical picture of PWS (muscular hypotonia, feeding difficulties, and developmental delay).^{1,5} In addition, the genetic cause of PWS, the loss of function of imprinted genes on the long arm of chromosome 15, is well known, and methylation analysis is an efficient tool for early and reliable diagnosis of PWS.^{6,7}

In the infants with PWS, growth hormone (GH) therapy induced a catch-up growth only if weight for height (WfH) was normalized, that is, above the average in this group.⁸ The best point of time to initiate GH therapy remains unknown. The main aim of the current study was to clarify whether early institution of GH therapy results in increased muscle mass or prevents massive obesity.

It has been suggested that treatment with CoQ₁₀ enhances muscle tone and physical activity in infants with PWS (W. V. Judy, Southeastern Institute of Biomedical Research, United States, personal communication), because CoQ₁₀ enhances muscle function but is decreased in obesity.⁹ However, observations on positive effects of therapy so far have only come from uncontrolled studies and parents' reports. It was therefore another aim of this study to investigate the effect of CoQ₁₀ supplementation in infants with PWS.

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CoQ ₁₀	Coenzyme Q ₁₀	LM _{Ht}	Lean mass adjusted for height
FM	Fat mass	PWS	Prader-Willi syndrome
%FM _{Age}	Percentage of fat adjusted for age	SDS	Standard deviation score
GH	Growth hormone	WfH	Weight-for-height
LM	Lean mass		

Table. Clinical data in young children with PWS, treated with GH (n = 11) or with coenzyme Q10 (n = 6)

Variable	Group	Baseline	6 mo	12 mo	18 mo	24 mo	30 mo
Age(y) [†]	GH	1.20 ± 0.5 [†]	1.72 ± 0.5	2.22 ± 0.5	2.72 ± 0.5	3.22 ± 0.5	3.79 ± 0.5
	Q10	0.42 ± 0.1	0.92 ± 0.1	1.40 ± 0.1	—	—	—
Height (SDS)	GH	-2.70 ± 1.8	-1.53 ± 1.5*	-1.13 ± 1.4*	-0.99 ± 1.3*	-0.77 ± 1.2*	-0.85 ± 1.0*
	Q10	-1.87 ± 0.8	-1.85 ± 1.3	-1.73 ± 0.9	—	—	—
WfH (SDS)	GH	-1.14 ± 1.0	-0.99 ± 1.3	0.02 ± 1.4*	0.61 ± 1.9*	1.47 ± 2.7*	2.05 ± 2.6*
	Q10	-0.64 ± 1.1	-0.24 ± 1.3	-0.22 ± 1.2	—	—	—
Lean mass for age (SDS)	GH	-2.99 ± 0.9	-2.30 ± 1.5*	-1.76 ± 1.2*	-1.85 ± 1.3*	-1.53 ± 1.2*	-1.39 ± 1.1*
	Q10	-2.20 ± 0.7	-1.56 ± 1.2	-2.12 ± 0.7	—	—	—

Values are mean ± SD.

* $P < .05$, $P < .01$ vs baseline, within groups.

[†]Significant difference ($P < .001$) between groups was found only for age but not for any other parameter, neither at the same point of time in the observation period nor at the same age, that is, the GH group at baseline and the Q10 group at 12 months.

METHODS

Study Subjects

The main group (GH group) consisted of 11 children (7 girls, 4 boys) with genetically confirmed PWS who were younger than 2 years of age at start of treatment (mean age, 1.20 years) (Table). They were treated with recombinant human GH (Genotropin, Pfizer, Duebendorf, Switzerland; 6 mg/m² per week, corresponding to 0.025 mg/kg per day). A second group (Q10 group) consisted of 6 infants (5 boys, 1 girl; mean age, 0.42 years). They were administered CoQ₁₀ at a dose of 2.5 mg/kg per day (Sanomit, a generous gift of MSE, Bad Homburg, Germany) but were not given GH. Genetic diagnosis of PWS had been established in the patients of this group. The study was approved by the Ethics Committee of the Children's University Hospital of Zurich, and informed consent was obtained from the parents.

Study Design

In all children with PWS, height and weight were measured by standard techniques¹⁰ at the start of the observation period and subsequently every 6 months. This included up to 30 months of GH therapy in the GH group and up to 12 months in the Q10 group.

The results are presented as standard deviation scores (SDS), using the first Zurich Longitudinal Study as reference.¹⁰ In the GH group, for one child, data were not assessed after 18 months, and for 4 children, body composition was not determined after 30 months. Parents were instructed to adapt food intake to ensure linear growth and stabilization of weight for height below the 90th percentile. Parents' compliance with these instructions was examined at every visit by semistructured interviews.

Measurement of Body Composition

Body composition was assessed by measuring total body water by stable isotope dilution, as described previously.^{11,12} ²H₂O (0.4 g per kg body weight; 99.9% ²H₂O, Cambridge Lab Isotopes, Innerberg, Switzerland) was administered in the

fasting state in the morning, and blood was taken 4 hours (range, 3 to 6 hours) later. ²H₂O enrichment was measured in serum samples prepared by vacuum dilution, with the use of a mass spectrometer (Mass Spec Model 20-20, PDZ Europe, Rudheath, Cheshire, United Kingdom). Lean mass (LM) was calculated from total body water, assuming an age-dependent water content and using coefficients as published earlier.¹¹ Fat mass (FM) was calculated by subtracting LM from total body weight and %FM by dividing fat mass × 100 by total body weight. To calculate normative values for LM and FM, data of 95 healthy children (47 boys and 48 girls) between the ages of 0 and 5 years were used.¹¹

Results of body composition of the study subjects are provided in SDS of the 95 healthy children, as described below.

Statistical Methods

REFERENCE DATA. In the group of the 95 healthy German children, LM was highly correlated with age and height, the relation being approximately linear for the latter (Fig 1, A). For this reason and because height was significantly reduced in the children with PWS at the start of the study, reference curves of LM adjusted for height (LM_H) were calculated. As the distribution of LM at each age was approximately normal in the reference group, a normal model with linear regression fit and constant standard deviation was used. Since the sex effect was not significant, identical reference curves were obtained for boys and girls. The fit explained 91.1% of the variability.

In contrast, FM and %FM in healthy children showed a much higher variability, with no simple relation to age or height. Reference curves for percentage of fat adjusted for age (%FM_{Age}) were obtained from a nonparametric regression fit of square root versus the logarithm of age. The square root transformation ensured the approximate normality of the data at each age, whereas the logarithm transformation accounted for the high increase of percentage of fat during the first year of life (Fig 2, B; back-transformed to linear age scale), as described earlier.^{13,14} Again, no significant sex differences

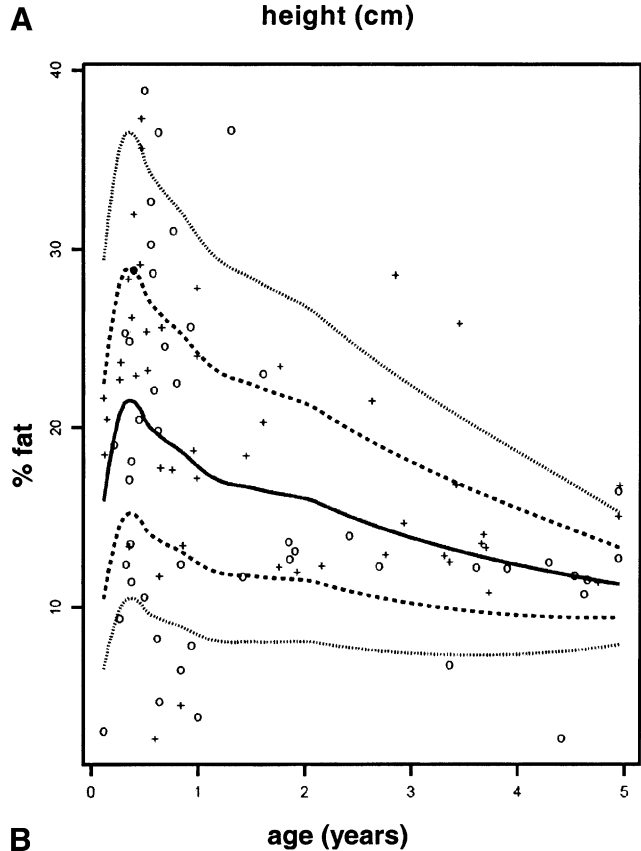
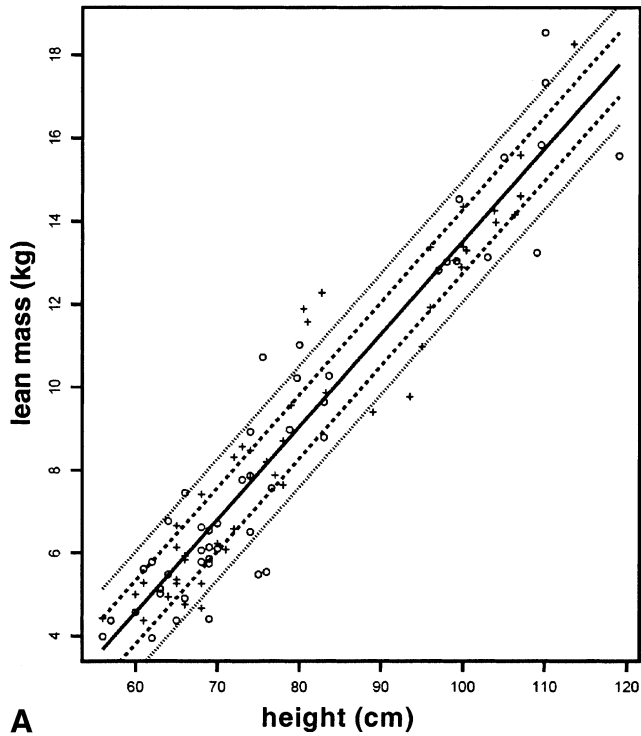


Fig 1. Reference curves ($n = 95$ healthy children): Boys are represented by *plus signs* and girls by *triangles*. *Solid lines* represent 50% quantiles; *dashed lines*, 25% and 75% quantiles; and *dotted lines*, 10% and 90% quantiles. **A**, linear plot of LM versus height; **B**, nonparametric regression fit of FM versus the logarithm of age, backtransformed to linear age scale.

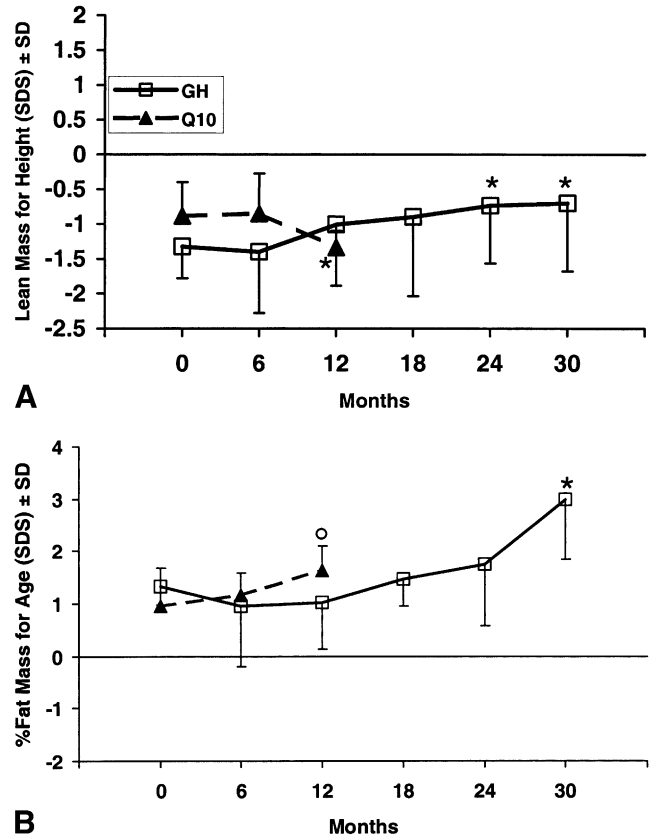


Fig 2. Body composition in children with PWS. **A**, LM adjusted for height; **B**, relative fat, adjusted for age, expressed as SDS of a group of 95 healthy control subjects. Differences between the groups are not significant, and significant differences within the groups are marked by $*P < .05$ vs baseline and by $^{\circ}P < .05$ vs 6 months of observation.

were detected, and the fit explained only 13.6% of the variability.

Using these reference curves, we were able to obtain SDS (= difference between patient's data and mean of the reference fit, divided by the SD of the reference fit) of lean mass adjusted for height (LM_{Hr}) and of $\%FM_{Age}$ for each child with PWS at each measurement. For every child, the annual increase of SDS was determined as the total increase of SDS between first and last measurement, divided by the time of observation. A similar methodology was applied to obtain SDS of LM adjusted for age (LM_{Age}), of relative fat adjusted for height, and of FM adjusted for both height and age. To allow for comparison, results based on SDS of LM_{Age} are also provided.

PATIENT RESULTS. All data were processed by GAS 4.4 of the Institute for Medical Informatics (IMI, Zurich, Switzerland). Data are shown as means and standard deviations. In each group, it was possible to test for a significant change of SDS from baseline up to 30 months by using the nonparametric Wilcoxon signed rank test for paired samples. Differences between the groups were identified by the Mann-Whitney U test, both at the start of the study and after 12 months, as well

as at the same age, that is, at 0 months in the GH group and at 12 months in the Q10 group. The annual changes were also compared between the groups by the Mann-Whitney *U* test. Correlations were tested by Spearman rank correlation. *P* values < .05 were considered to be significant.

RESULTS

Anthropometry

At start of the study, short stature was present in both groups of children with PWS (Table). In the GH group, 8 of 11 children presented with height below 2 SD; the corresponding proportion in the Q10 group was 2 of 6 children. WfH SDS was less markedly decreased than height (Table). In the GH group, baseline WfH SDS was below 2 SD in 3 children and, in the Q10 group, in 1 child. Height SDS rose into the normal range (mean, -0.85 SD) after 30 months of GH treatment but remained unchanged in the Q10 group. In the GH group, WfH started to rise after the first 6 months of GH treatment, whereas the WfH increase observed in the Q10 group was not significant. There was no significant difference between WfH at the same age between the groups (GH group, baseline, and Q10 group, 12 months).

Body Composition

At the beginning of the study, all 17 young children with PWS of both groups presented with an LM below the normal average, whether adjusted for height (LM_{Ht} , Fig 2, A) or for age (Table). LM_{Ht} was slightly but not significantly higher in the Q10 group than in the GH group before the start of therapy. During 1 year of observation, LM_{Ht} significantly decreased by -0.46 ± 0.34 SD ($P = .03$) in the Q10 infants to -1.33 ± 0.24 SD, reaching the same level as in the GH group before start of therapy (Table). LM_{Ht} in the Q10 group was negatively correlated with the duration of observation (Spearman correlation, -0.49). In contrast, LM_{Ht} rose significantly during GH therapy, (average annual increase 0.25 ± 0.31 SD, $P = .02$). In fact, in 9 of 11 children, LM_{Ht} improved during GH therapy, whereas all Q10 infants had lost LM_{Ht} . It needs to be pointed out that the changes in LM were more pronounced when related to age (Table): Initially, all children had a more decreased LM, which increased to a more significant degree, by 0.67 ± 0.34 SD per year ($P = .001$). Such presentation, however, does not indicate the effective gain in LM, or rather muscle mass, because it does not take into account that GH accelerates longitudinal growth and therefore increases LM, as evident from Figure 1, A.

Although baseline WfH was below the normal mean, percentage of fat mass was increased (GH group, $31.0\% \pm 4.5\%$; Q10 group, $32.4\% \pm 9.5\%$), and $\%FM_{Age}$ was found to be above the normal average in all 17 children with PWS, without significant differences between the groups (Fig 2, B). In 5 of 6 of the Q10 infants, $\%FM_{Age}$ increased during the year of observation, resulting in a mean annual elevation of 0.71 ± 0.74 SD ($P = .09$); this trend was mainly due to an increase during the second half of the observation period

(Table). In contrast, the modest annual increase of $\%FM_{Age}$ (0.32 ± 0.6 SD) was not significant in the GH group and, in fact, during the first 6 months of GH therapy, $\%FM_{Age}$ had decreased in 9 of 11 children (Fig 2, B). Nevertheless, a significant development of adiposity was observed after 30 months, and $\%FM_{Age}$ became significantly higher than before the start of therapy (Fig 2, B).

Even though other measures describing the fat compartment were taken into account in the form of $\%FM$ SDS adjusted for height or absolute FM adjusted for height or age (data not shown), the current results on fat gain in PWS did not differ in any substantial way from the results mentioned above.

In healthy, normal-weight children, there was a negative correlation between FM and LM adjusted for height ($r = -0.35$, $P = .0007$); children with higher LM therefore had lower FM. Likewise, at start of the study, the correlation coefficient of LM_{Ht} versus FM_{Ht} was negative ($r = -0.53$, $P = .03$) in the combined group encompassing all children with PWS, but it was not significant in the GH group before GH therapy. In contrast, after 24 and 30 months of therapy, the relation turned positive in the GH group: r LM_{Ht} versus $FM_{Ht} = 0.36$ (not significant), and $r = 0.79$, $P = .03$, respectively; therefore, the greater FM was, the higher LM became.

The evaluation of the semistructured interviews showed that the children with PWS did not change their eating habits before and during GH therapy, nor did they exhibit overt hyperphagia during the same periods.

DISCUSSION

Direct measurements of total body water demonstrate that LM is significantly reduced in infants and young children with PWS, even when corrected for short stature. The most relevant finding of the current study is that GH therapy promotes an important gain in lean tissue mass adjusted for height, if treatment is instituted in early childhood.

Our results indicate that the natural course in PWS is to lose lean mass during early childhood: First, in the infants of the Q10 group, there was a significant decrease of LM_{Ht} from -0.88 SD at the age of 0.4 years to -1.33 SD at the age of 1.4 years. Second, this finding was corroborated by our previous study in children with PWS 7 years,¹⁵ that showed LM reduction to -3 SD adjusted for height. Third, as inferred from the results of our earlier anthropometric studies,³ LM also appears to diminish from infancy to childhood. In contrast, in older children and adolescents with PWS, it is known that LM is reduced^{15,16} and that the ratio of LM to FM remains stable.¹⁶

Because of the younger age and the coenzyme Q10 treatment, the Q10 group differs from an ideal control group. The GH group mainly includes children during the second year of life. This was a result of the fact that during 1997 to 2000, PWS was rarely diagnosed during the first year of life. The children of the Q10 group were diagnosed in 2001, at a younger age. However, we did not want to start GH therapy during the

first year of life, a period of increased general risk for sudden infant death.¹⁷⁻¹⁹ Instead, the recently diagnosed infants were treated with Q10, since, at the same time, the reduction of Q10 levels in obesity was first published.⁹ After 1 year of treatment, the children of the Q10 group had the same age and an equally reduced lean mass, LM_{Ht}, as the children of the GH group at the initiation of treatment. Therefore, it was concluded that Q10 has no influence on LM_{Ht}. In fact, the data of children of both groups before GH treatment appear to represent adjacent sections of the same slope, indicating the spontaneous decline of LM_{Ht} in PWS. At this age, however, fat mass was slightly but not significantly higher in the Q10 group, possibly because of improved nutritional support²⁰ and not as a consequence of Q10 treatment. However, the typical reduction in lean mass was still found in the infants of the Q10 group.

To compensate for differences in age when comparing between the Q10 and GH groups, mainly the gradients of the annual changes of body composition were analyzed.

As to the effects of GH therapy on body composition in this study, LM improved independent of the changes related to longitudinal growth because LM was adjusted for height. Therefore, it may be concluded that if treatment is instituted early, the deficit of muscle mass in PWS is improved by GH therapy. The finding that LM_{Ht} reaches -0.7 SD, within the normal range, is even more important, as in former studies in older children with PWS, LM_{Ht} was markedly lower after more than 3 years of GH therapy,²¹ for example, -1.77 ± 2.3 SD.¹⁵

However, the effect of physical activity to increase muscle mass may affect the efficacy of GH.^{22,23} Though physical training has recently been shown to enhance physical activity and LM in PWS,²⁴ reduced physical activity remains one of the central features of PWS.²⁴⁻²⁶ Nevertheless, GH is a prerequisite for normal muscle growth, and GH therapy therefore adds to improving muscle and lean mass and as such also physical strength and activity.²⁴⁻²⁸ Because hypoactivity is not remedied entirely in patients with PWS,²⁴ this might explain why, even during early GH therapy, LM remains slightly below the normal average.

From this perspective, energy balance in PWS is disturbed in several respects: First, resting energy expenditure is reduced because of decreased LM.^{4,29,30} Second, activity-related energy expenditure is also reduced due to the low level of physical activity.^{23,24,31} These defects are combined with decreased satiety and enhanced food intake³²⁻³⁴ and result in a positive energy balance. The disturbance of energy balance combined with the decrease in LM and physical activity results in a progressive accumulation of FM in this syndrome. The level of relative fat mass in the children of the current study, amounting to an average 32% at the age of 11.4 months, matches the results of American infants and toddlers with PWS, in whom 35% of FM was found at the age of 12.4 months, also assessed by deuterium dilution.⁴

As shown in the current study, GH therapy may delay and reduce but not prevent the accretion of fat in PWS after the second year of treatment. According to the observations in the present work and in other studies, food intake was not

influenced by GH therapy.^{26,35} The increase in adiposity during GH treatment in children with PWS has also been noticed by us¹⁵ and others.^{26,36}

It is important to note that the continuous increase of fat tissue in PWS without GH therapy is not accompanied by a comparable increase of LM, as observed in children with nonsyndromal obesity³⁷ or in other obese individuals, in whom increased FM is highly correlated with elevated LM.³⁸ In young children with PWS, in contrast, LM is reduced despite fat accumulation. The pathologic relation between lean and fat mass in PWS may be the consequence of GH deficiency to some extent because it is normalized during GH therapy and the corresponding correlation even turns positive, as seen in otherwise healthy obese individuals.

Further investigations are needed to clarify a potential long-term benefit for children with PWS to have more LM and metabolically active tissue, even though the increase of fat mass cannot be entirely prevented. Nevertheless, some suggestions about the optimal time to initiate GH treatment can be deduced from the current study as well as from earlier studies in older children with PWS. In our experience, the improvement of lean mass is the most important aim in limiting obesity in children with PWS. In accordance with current data, we do not recommend to start GH therapy in PWS in the first year of life because of an increased risk of sudden infant death during this period. Providing enough food intake is most important for normal growth during infancy.¹⁵ The start of GH therapy in the second year of life appears to be more appropriate than beginning later in childhood.^{8,15}

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