

# Lower body weight and BMI at birth were associated with early adiposity rebound in 21-hydroxylase deficiency patients

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**Abstract.** 21-hydroxylase deficiency (21-OHD) is the most common type of congenital adrenal hyperplasia. In addition to the clinical problems caused by adrenal insufficiency and androgen excess, a risk for obesity and metabolic syndrome during young adulthood is a major ramification of the disease. Although glucocorticoid therapy is very likely to be one of the contributory factors, the precise causes of the metabolic status of adult 21-OHD patients remain to be clarified. Previously we reported that 21-OHD patients developed early onset AR, a condition which might create a risk for obesity and metabolic syndrome in adulthood. In order to elucidate the association between the onset of AR and factors during the fetal period to early infancy, we conducted a retrospective longitudinal analysis of 29 21-OHD patients (male: 14 cases, female: 15 cases, salt wasting type: 16, simple virilizing type: 13), who were identified by newborn screening and followed up at least until the age 10 years. Body size at birth, lower body weight, and lower BMI were found to precipitate the timing of AR. On the other hand, no significant association was observed between the timing of AR and sex, gestational age, treatment regimen (including cumulative dose of HDC), and disease severity (the type of the disease, the value of DHEA-S and 17-OHP). There are two points to consider: first, in 21-OHD patients treated with glucocorticoid substitution therapy, the risk for early AR cannot be reduced by adjusting the dose of glucocorticoid; second, fetal factors might affect the metabolic status of 21-OHD patients.

**Key words:** 21-hydroxylase deficiency, Adiposity rebound, Glucocorticoid, Metabolic syndrome

**21-HYDROXYLASE DEFICIENCY (21-OHD)** is the most common type of congenital adrenal hyperplasia (CAH). Its major clinical manifestations are adrenal crisis, virilization of external genitalia, short stature, and infertility due to adrenal androgen excess. A major part of the treatment for 21-OHD is glucocorticoid therapy, which reduces the excessive production of androgen and its metabolites by the adrenal gland [1-3].

Obesity and metabolic syndrome during young adulthood are of serious concern for 21-OHD patients. Body mass index (BMI) is elevated in most 21-OHD patients [4, 5]. Furthermore, recent studies have shown

that adult 21-OHD patients tend to have metabolic syndrome [6, 7]. Although the precise factors affecting the metabolic status of 21-OHD adults have not been identified, glucocorticoid therapy is thought to play some role [8, 9]. 21-OHD patients require a supra-physiological dose of glucocorticoid in order to reduce the effects of androgen excess [10].

Previous reports including ours showed that the onset of adiposity rebound (AR), the point at which the BMI starts to increase after its nadir, occurred earlier in 21-OHD patients than in normal children [5, 11]. Premature AR has been identified as one of the possible contributory factors in adult obesity and metabolic syndrome [9, 7, 12-16]. We hypothesized that fetal and early infantile factors, including birth size and glucocorticoid therapy, could affect the timing of AR. We thus conducted a longitudinal retrospective analysis of 21-OHD patients to identify factors that might be associated with the timing of AR.

Submitted Apr. 18, 2016; Accepted Jul. 15, 2016 as EJ16-0194  
Released online in J-STAGE as advance publication Aug. 19, 2016

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## Materials and Methods

### Subjects

Since the introduction of newborn screening for CAH in Japan in 1989 [17, 18], 29 patients (14 males, 15 females) were diagnosed with the classical form of 21-OHD in the neonatal period, and were followed up continuously to the age of at least 10 years at Tokyo Medical and Dental University or Tokyo Metropolitan Children's Medical Center (Supplementary Table 1). All patients were identified by newborn screening, and the diagnosis of CAH was based on both clinical symptoms and hormonal analysis and most of patients were confirmed by genetic analysis. For all patients, treatment was started in the neonatal period.

In the present study, we defined the salt-wasting (SW) form of 21-OHD as 21-OHD with a plasma sodium value of less than 130 mEq/L and/or a plasma potassium value of more than 6 mEq/L at diagnosis. All other patients were classified as having the simple virilizing (SV) form of 21-OHD. Of the 29 patients, 16 were SW patients and 13 were SV patients. Patients with a non-classical form were not included in this study. In most patients, genetic analysis was performed to confirm the type of 21-OHD. After 1 year of age, none of the patients showed growth retardation, *i.e.*, the change of height (HT) standard deviation score (SDS) was more than -0.5/yr, suggesting that there was no evident overtreatment. The patients were continuously cared for with follow-up appointments every month during infancy and every 2-3 months during childhood.

The fitted BMI curve ' $y=f(x)$ ' was determined individually on each subject. To obtain the BMI curves, we used the four dimension polynomial functions with the natural logarithm where  $\text{Log}(\text{BMI}) = y = f(x)$ ,  $f(x) = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 + \varepsilon$ ,  $x$  was the age in months, and  $\beta_0, \beta_1, \beta_2, \beta_3$  and  $\varepsilon$  were determined by the curve fitting each subject. The timing of AR (age =  $a$ ) gave the minimal value of  $\text{Log}(\text{BMI})$ , which was calculated from derivatives of the given fitted  $\text{Log}(\text{BMI})$  curves as " $d/dx = f'(a) = 0$ " (Fig. 1) [19].

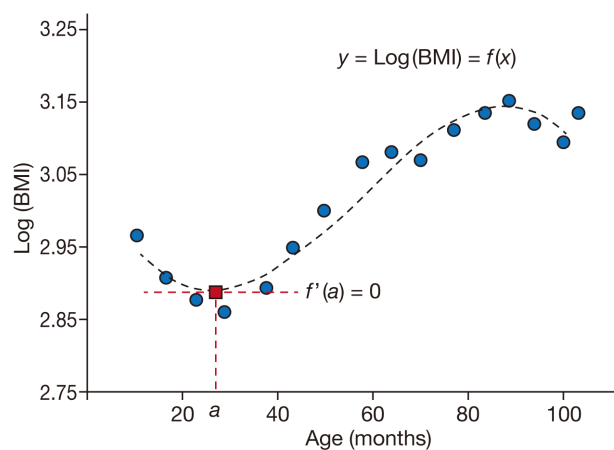
All patients received hydrocortisone (HDC) three times daily for glucocorticoid replacement until the age of 10 years. The patients were treated according to the clinical guidelines for 21-OHD in Japan (maintenance dose of HDC: 20~40 mg/m<sup>2</sup>) [20]. The HDC dose was adjusted based on auxological and hormonal data. The cumulative HDC dose was calculated by multiplying

the daily dose (mg/m<sup>2</sup>) for maintenance therapy by days of treatment. More precisely, on each visit, every maintenance therapy dose of daily HDC (mg/m<sup>2</sup>) was recalculated according to the auxological data obtained at the visit. We multiplied the each recalculated dose by the number of days from the day at the visit to the next visit (Table 1). We added the cumulative dose of each visit from the initiation of the therapy to 2 years of age. We did not take into account extra doses of HDC given in stressed conditions.

The present study was approved by the ethical committee of Tokyo Medical and Dental University and Tokyo Metropolitan Children's Medical Center.

### Statistical analysis

JMP® Ver10.0 (SAS Institute Inc.) was used for statistical analyses. The significance of the difference in AR values was evaluated by Wilcoxon analysis. The dependence of the parameters on the age at AR



**Fig. 1** An example of the BMI curve produced by fitting a cubic polynomial in age (the horizontal axis) to the natural log of the available observed BMI values (blue circles) for a representative subject. The logarithmic value of BMI was reconverted into BMI units. The minimum point on the fitted curve marks AR onset, the red square whose differentiated output becomes zero:  $f'(a) = 0$ .

**Table 1** A sample case to illustrate the calculation method of cumulative HDC dose

Date of visit	A	B	C	D
BSA at each visit (m <sup>2</sup> )	a	b	c	
Dose of HDC (mg)	$\alpha$	$\beta$	$\gamma$	

We calculated the cumulative HDC dose of each visit between A and B:  $f(A) = (B-A)[\text{days}] \times \alpha[\text{mg}] / a[\text{m}^2]$ , and accumulated the HDC dose of each visit, *i.e.*, Total cumulative dose of HDC between A and D:  $y = f(A) + f(B) + f(C)$ . BSA, body surface area.

was evaluated with Pearson's product-moment correlation coefficients. To identify the independent factors associated with the onset of AR, sex, BMI at birth, low/high dose of induction HDC therapy, and the type of 21-OHD were included for the multivariate regression analysis. The significance level of all  $p$ -values was  $<0.05$ .

## Results

### *AR was not associated with the sex and the gestational ages of the patients*

The mean age of AR in our study was 36 months, and 20 of 29 patients developed AR before age 4 years. These results suggest that our patients developed AR earlier than normal subjects in whom AR usually occurs around 5-6 years of age [19, 21]. As no significant difference was observed in AR onset between male and female patients (Fig. 2A), we grouped the males and females together for further analyses. All patients were born at 37 to 41 weeks of gestational age, which did not show any association with the onset of AR (Fig. 2B).

### *The treatment regimen did not affect the onset of AR*

We hypothesized that treatment might have affected the timing of AR. In accordance with the guidelines for 21-OHD treatment in Japan, all patients received HDC induction therapy at a dosage of 100 mg/m<sup>2</sup> or more during the neonatal period. We then separately analyzed the effects of the induction and maintenance HDC therapies. For the induction therapy, we classified the patients into two groups, one treated with 100 mg/m<sup>2</sup> of HDC (the low dose or LD group) and the other treated with more than 100 mg/m<sup>2</sup> of HDC (the

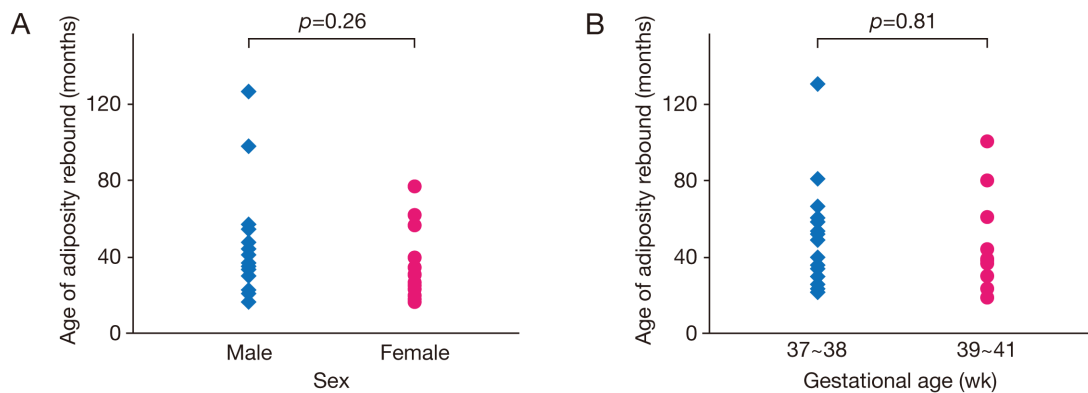
high dose or HD group). The two groups showed no significant difference in the onset of AR (Fig. 3A). Next, we examined the effect of the cumulative HDC dosage in maintenance therapy but similarly found no significant association between the cumulative amount of HDC and the timing of AR (Fig. 3B), suggesting that HDC therapy did not affect the onset of AR in 21-OHD patients. Further, we examined the association between fludrocortisone treatment and the age at AR. Fludrocortisone is normally not used for all patients during neonatal period, and in some cases, it is introduced later. Therefore, we tested for any relationship between fludrocortisone dosage at each stage and age at AR but failed to find any (Fig. 3C). Taken together, as long as the 21-OHD patients were treated according to the guidelines, the treatment regimen did not affect the onset of AR.

### *The onset of AR was not associated with the severity of the disease*

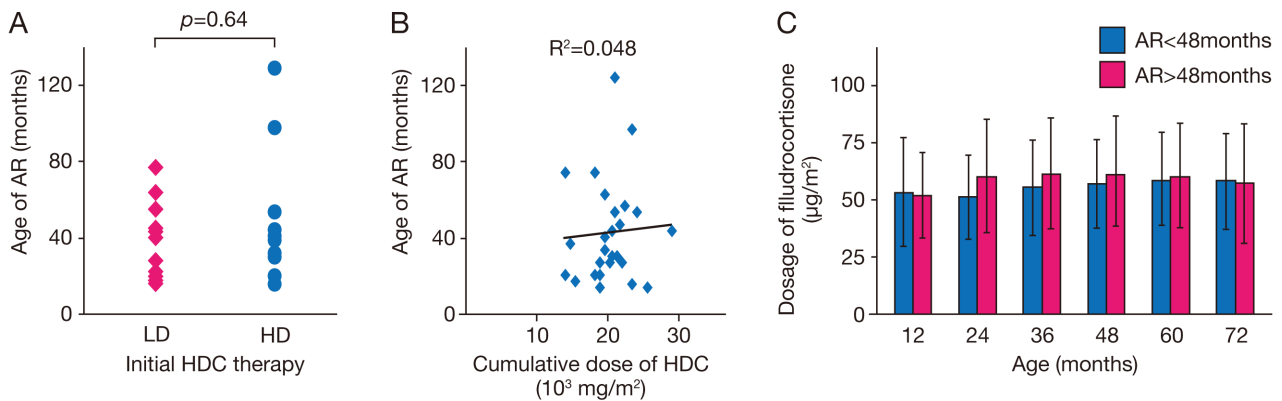
Next, we asked whether severity of the disease affect the age of AR onset. However, the serum levels of 17-OHP or DHEA-S sampled before HDC treatment, and the type of the disease, SV and SW, were not associated with AR onset (Fig. 4A, B, C).

### *Not body length, but body weight and BMI at birth associated with the timing of AR*

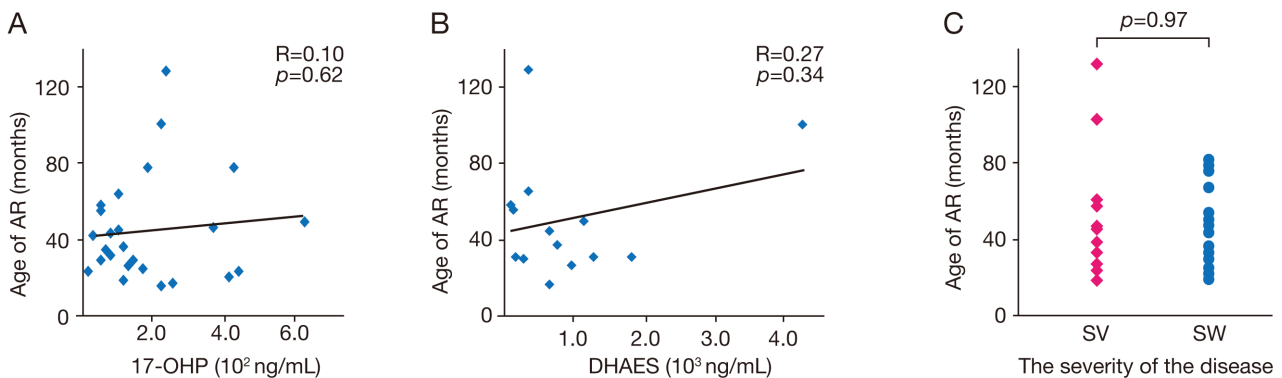
Finally, we analyzed the relationship between body size at birth and the timing of AR, and found that although body length was not associated with the age at AR (Fig. 5A), lower body weight and lower BMI at birth precipitated the timing of AR in 21-OHD patients (Fig. 5B, C).



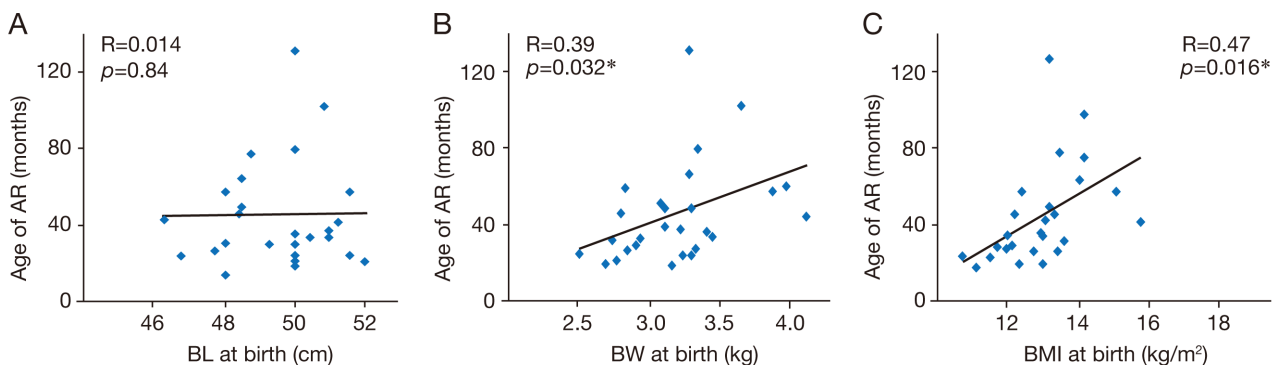
**Fig. 2** The onset of AR was not associated with sex (A) and the gestational age (B) of the patients. Wilcoxon test was used for statistical analysis.



**Fig. 3** The treatment regimen did not affect the timing of AR in 21-OHD patients. (A): A scatter diagram showing the AR in the lower dose (LD) and higher dose (HD) groups, during the induction therapy. The patients in the LD and HD groups were treated with  $100 \text{ mg/m}^2$  HDC and with more than  $100 \text{ mg/m}^2$  of HDC, respectively. The Wilcoxon test was used for the statistical analysis. (B): A scatter diagram showing the cumulative HDC dosage in maintenance therapy during the first 2 years of life. Least squares method was used for the statistical analysis. (C): The dosage of fludrocortisone treatment at each age, respectively. The patients were classified into two groups, the early AR (blue bar) and late AR groups (red bar). The Early AR and Late AR groups were defined by the occurrence of AR before 48 months of age and after 48 months of age, respectively.



**Fig. 4** The onset of AR was not associated with hormonal data or the severity of the disease. The scatter diagram indicates the relationship between the onset of AR and the serum level of 17-OHP (A) or DHAES (B). Pearson's product-moment correlation coefficient was used for analysis. (C) The Scatter diagrams of the onset of AR in SV and SW patients. The Wilcoxon test was used for statistical analysis.



**Fig. 5** The scatter diagram showing the relationship between the onset of AR and body length (A), body weight (B), or BMI (C) at birth. Pearson's product-moment correlation coefficient was used for analysis. \*  $p < 0.05$ .

### ***Lower BMI at birth is an independent risk for early AR in 21-OHD patients***

In order to examine whether a lower BMI at birth is an independent risk factor for early AR in 21-OHD, we performed multivariate statistics excluding the effect of confounding factors. Our present study has limited number of samples, so for the sake of efficient analysis, we chose representative factors that could affect the body size at birth and growth after birth, *i.e.*, the cumulative dose of HDC, the type of the disease, and sex. The analyses revealed that a lower BMI was indeed an independent risk factor with the corrected *p* value of 0.041 (Table 2).

## **Discussion**

Two observations can be made regarding the precipitation of AR in this study. First, glucocorticoid therapy, provided that it is administered according to the guidelines, does not have an effect on the timing of AR in 21-OHD patients. Second, BMI and body weight at birth were found to be associated with the timing of AR, implying the involvement of some fetal factors.

Our data suggest that it would be difficult to prevent early AR in 21-OHD patients by adjusting dosages of HDC. Recent studies have shown that 21-OHD patients have an increased risk for obesity and metabolic syndrome during young adulthood [7, 12]. Glucocorticoid therapy using supra-physiological dosages has been proposed as a possible contributory factor [13, 22]. Our study revealed that the HDC maintenance therapy did not affect the timing of AR. Another factor that could affect the timing of onset is induction therapy with a high dose HDC. Because all patients in the present study received high dose induction therapy, it was difficult to evaluate the effect of this therapy. Alternatively, we compared the two groups that received an initial high or low dose HDC treatment. Based on the results, we assumed that the initial high dose of HDC did not profoundly affect the timing of AR. Thus our study showed that neither the induction nor maintenance therapy using HDC affected the timing of AR. Corroborating our data is a recent study from the UK, which reported that 30% of CAH patients started to develop obesity before steroid therapy, suggesting the disease process influences weight gain independently of glucocorticoid therapy [23]. Taken together, although our data did not exclude the possibility that a supra-physiolog-

**Table 2** Multivariate statistics of representative factors

	Corrected <i>p</i> value
Form (SV, SW)	0.917
BMI at birth	0.041 *
HDC cumulative dose	0.358
Sex (Male, Female)	0.549

Multivariate statistical analysis revealed that a lower BMI was an independent risk factor in 21-OHD patients. \* *p*<0.05.

ical dose of glucocorticoid might be a risk factor for metabolic syndrome in 21-OHD patients, our study revealed that the timing of AR onset did not depend on the dosage of glucocorticoid administered according to the guidelines.

The association between body size at birth and age at AR implies that early AR is caused by fetal factors, either environmental or genetic. Fetal factors are also thought to affect the risk for developing type-2 diabetes in adulthood. One meta-analysis demonstrated that a birth weight of less than 2,500 g might pose a risk for developing type-2 diabetes [24]. Considering that early AR onset has been identified as a risk factor for obesity and metabolic syndrome in adulthood, an association between body size at birth and age at AR is plausible. However, our results require more careful interpretation. First, 21-OHD patients are rarely born smaller than the norm for their gestational age [25], and in our study none of the patients had a birth weight of less than 2,500 g. Further, few studies have examined the direct association between body size at birth and the age at AR onset in normal healthy subjects without intrauterine growth failure, and one birth cohort reported the absence of any association between them [26].

Although it is beyond the scope of our study to identify the environmental factors that affect the timing of AR, we hypothesize that excess androgen during the fetal and perinatal periods may be one contributory factor. In several animal models, intrauterine androgen exposure has been linked to metabolic derangement in adolescence and adulthood [27-30]. For instance, in rhesus monkeys, exposure to excess androgen during the fetal period leads to the development of insulin resistance with visceral adiposity, impaired glucose metabolism, and dyslipidemia [29, 31].

Selecting a method of estimating age at AR through a retrospective epidemiological analysis like the present study is difficult. Generally, there are two



approaches: one is a visual evaluation of the individual, or the examination of serial BMI values by which AR is identified by the lowest point on the BMI curve before the onset of weight increase. The other method consists of the application of 2nd or 3rd order polynomial equations to the serial BMI data of individuals [32]. In our present study, the latter approach was employed. Our study was performed at two different hospitals, and our anthropometric data were not prospectively obtained by using strict protocols. Indeed, the BMI curve in some cases showed a plateau with repeated minor increases and decreases. In such cases the visual evaluation could have been biased by each measurement.

Our present study has some limitations. Firstly, the timing of AR onset would be influenced by other factors, such as the genetic and postnatal environmental factors. For example, the BMI of the parents reportedly affects the timing of AR [26, 33], suggesting that the genetic and postnatal environmental factors affect

the timing of onset. However, due to the unavailability of information, we could not include these factors in our analysis. The retrospective element is another limitation. In order to elucidate the mechanisms of AR in 21-OHD patients, a prospective cohort study using the same treatment regimen is necessary. In addition, a long-term study is required to show an association between the age at AR and metabolic status during adulthood.

In summary, we carried out a retrospective analysis of 21-OHD patients, examining the factors accelerating the onset of AR. Our data revealed that lower body weight and BMI at birth were significantly associated with early AR, suggesting that the risk for early AR might depend on fetal factors.

## Acknowledgement

We are indebted to Dr. James Robert Valera for his help in editing this English manuscript.

**Supplementary Table 1** Clinical details of 29 cases in the present study

Case	Sex	Type	GA (wk)	Body size at birth			Endocrinological data		Initial HDC Tx (mg/m <sup>2</sup> )	Cumulative dose of HDC (mg/m <sup>2</sup> )		AR (months)
				BW (g)	Ht (cm)	BMI (kg/m <sup>2</sup> )	17OHP (ng/mL)	DHEAS (ng/mL)		0~1 yr	0~2 yr	
1	M	SW	39	2,850	51.5	10.75	446	ND	100	11,464	17,967	24.0
2	M	SW	41	3,136	51.0	12.06	114	726	200	11,684	19,389.3	36.5
3	M	SW	41	3,674	50.8	14.24	226	4,217	200	14,596	23,426	100.0
4	M	SW	38	3,300	52.0	12.20	370	ND	200	10,965	20,456.2	45.6
5	M	SW	37	3,300	50.0	13.20	235	311	200	11,390	20,599.3	129.0
6	M	SW	39	3,890	ND	ND	53	114	200	11,266	20,897.5	55.4
7	M	SW	38	2,716	48.0	11.79	258	ND	100	10,667	18,306.4	17.2
8	M	SW	38	2,946	50.0	12.26	55	1,238	50	10,553	20,104.4	30.5
9	M	SW	38	2,822	46.3	13.16	73	624	150	10,861	19,179.4	43.3
10	M	SW	41	3,986	51.5	15.03	55	ND	100	11,876	21,933.1	57.8
11	M	SV	41	3,410	51.0	13.11	63	ND	100	11,518	20,206.9	34.3
12	M	SV	39	3,315	52.0	12.26	410	ND	100	9,071	14,905.8	20.6
13	M	SV	39	3,100	48.5	13.18	626	1,102	100	11,780	21,137.7	49.0
14	M	SV	36	3,126	48.5	13.29	100	ND	100	20,065	29,211	45.9
15	F	SW	37	2,852	48.0	12.38	57	76	100	12,276	24,287.5	57.5
16	F	SW	38	3,342	50.0	13.37	173	ND	50	6,778	13,978.6	24.6
17	F	SW	39	3,240	50.0	12.96	ND	ND	ND	ND	ND	36.1
18	F	SW	41	3,174	ND	ND	222	617	200	14,594	25,430.9	16.1
19	F	SW	39	2,760	48.0	11.98	47	239	175	12,207	21,641	29.5
20	F	SW	41	3,250	50.0	13.00	ND	ND	400	13,467	ND	21.1
21	F	SV	37	2,510	46.8	11.46	18	ND	100	11,221	18,516.4	23.5
22	F	SV	39	3,300	48.5	14.03	104	356	100	12,136	19,321.4	64.2
23	F	SV	41	3,358	48.8	14.10	190	ND	100	10,299	17,685.1	77.2
24	F	SV	39	2,948	49.3	12.13	141	149	100	8,898	18,058.8	30.2
25	F	SV	38	3,362	50.0	13.45	420	ND	80	6,030	13,293.5	77.4
26	F	SV	40	4,130	51.2	15.75	26	ND	100	7,029	14,302.1	41.9
27	F	SV	42	2,912	47.8	12.74	130	946	ND	ND	ND	26.4
28	F	SV	37	2,780	50.0	11.12	120	ND	100	13,856	23,339.5	18.4
29	F	SV	39	3,468	50.5	13.60	86	1,807	100	12,372	21,092.9	32.1

AR, Adiposity rebound; GA, gestational age; BW, body weight; Ht, height; BMI, body mass index; 17OHP, 17-hydroxyprogesterone; DHEAS, dehydro-epiandrosterone sulfate; HDC, hydrocortisone; SV, simple virilizing form; SW, salt wasting form.

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