Altered Carbon Dioxide Metabolism and Creatine Abnormalities in Rett Syndrome

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Abstract Despite their good appetite, many females with Rett syndrome (RTT) meet the criteria for moderate to severe malnutrition. Although feeding difficulties may play a part in this, other constitutional factors such as altered metabolic processes are suspected. Irregular breathing is a common clinical feature, leading to chronic respiratory alkalosis or acidosis. We assumed that these changes in intracellular pH cause disturbances in the metabolic

equilibrium, with important nutritional consequences. The study population consisted of a group of thirteen well-defined RTT girls with extended clinical, molecular and neurophysiological assessments. Despite normal levels of total dietary energy and protein intakes, malnutrition was confirmed based on significantly low fat-free mass index (FFMI) values. Biochemical screening of multiple metabolic pathways showed significantly elevated plasma creatine concentrations and increased urinary creatine/creatinine ratio in five RTT girls. Four girls, 10 years and older, were forceful breathers, one 13-year-old girl had an undetermined cardiorespiratory phenotype. An isolated increase of the urinary creatine/creatinine ratio was seen in two girls, a 9-year old forceful and a 4-year old feeble breather. Given that the young girls are feeble breathers and the older girls are forceful breathers, it is impossible to determine whether the elevated creatine concentrations are due to increasing age or cardiorespiratory phenotype. Furthermore, MeCP2 deficiency may cause epigenetic aberrations affecting the expression of the creatine-transporter gene, which is located at Xq28. Further studies are required to confirm these findings and to provide greater insight into the pathogenesis of the abnormal creatine metabolism in RTT.

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Abbreviations

BMI	Body mass index
FFM	Fat-free mass
FFMI	Fat-free mass index
ID	Intellectual disability
MECP2	Methyl CpG binding protein 2 gene
MeCP2	Methyl CpG binding protein 2
RTT	Rett syndrome
SD	Standard deviation
TBW	Total body water

Introduction

Rett syndrome (RTT; OMIM 312750) is a unique X-linked neurodevelopmental disorder. Affecting 1 in 10,000 females, RTT is a common genetic cause of severe intellectual disability in females (Smeets and Schrand-Stumpel 2005; Williamson and Christodoulou 2006). In up to 95% of all classical cases, RTT is caused by a mutation in the Methyl-CpG-binding protein 2 gene (*MECP2*) located on the long arm of the X chromosome (Xq28) (Amir et al. 1999; Williamson and Christodoulou 2006).

Despite their good appetite, the majority of the females with RTT meet the criteria for moderate to severe malnutrition. However, not all RTT females present in this manner (Rice and Haas 1988). The true prevalence of malnutrition in RTT is unknown, and the pathological mechanism underlying is barely understood. Although feeding difficulties may play a role, other constitutional factors such as altered metabolic processes are suspected (Motil et al. 1998; Oddy et al. 2007; Reilly and Cass 2001).

Irregular breathing is a common clinical feature of RTT, reflecting the immaturity of the brainstem in these females. The underlying pathophysiology involves a defective control mechanism of carbon dioxide exhalation, leading to chronic respiratory alkalosis or acidosis (Julu et al. 2008a; Julu et al. 2001; Smeets and Schrand-Stumpel 2005). This change in pH might cause disturbance of the metabolic equilibrium in RTT females, with potential consequences for their nutritional status.

Hitherto, no systematic studies on altered metabolic processes as a cause of impaired nutritional status in RTT have been carried out. The objective of this study was to examine whether chronic respiratory acidosis or alkalosis leads to disturbed metabolic profiles and enzyme activities in multiple metabolic pathways.

Patients and Methods

Ethical approval was obtained from the Medical Ethical Committee at the Maastricht University Medical Centre, and all parents provided informed written consent. The study was registered at *clinicaltrials.gov*.

Patient Population

The patient population consisted of a group of thirteen well-defined RTT girls with extended clinical, molecular and neurophysiological assessment. This assessment consisted of:

1. Clinical examination comprising a full physical examination including measurement of height, weight and Body Mass Index (BMI). In addition, clinical features

were scored uniformly using the International Scoring System (ISS) (Kerr et al. 2001). The ISS is used to determine clinical severity concerning 20 items (maximum score being 40), grouped into five functional domains (growth and development, musculoskeletal, movement, cortical and autonomic features).

2. Mutation analysis of *MECP2* by PCR, sequencing the coding regions and multiplex ligation-dependent probe amplification analysis (nomenclature according to the *MECP2A* isoform reference sequence AF158180, numbering starting at the A of the ATG translation initiation codon).
3. Detailed neurophysiological assessment using the Neuroscope, a technique for continuous and real-time assessment of brainstem function to define the cardiorespiratory phenotype (Julu and Engerström 2005). The different cardiorespiratory phenotypes (Apneustic, Feeble, and Forceful breathers) in RTT have been described in detail elsewhere (Julu et al. 2008a, b).

Diagnosis of RTT was based on the consensus diagnostic criteria for RTT and confirmed by mutation finding in *MECP2* gene (Hagberg et al. 2002). All girls were diagnosed as classical RTT.

Study Design

The RTT girls were recruited at the Clinical Genetics outpatient clinic at the Maastricht University Medical Centre, the Netherlands. All participants were clinically assessed by the same experienced clinician (Dr. E. Smeets). The nutritional status was assessed by a complete nutrition assessment and measurement of body composition. Blood and urine samples were collected for biochemical analysis and stored at -20°C until analysis after the appropriate workup.

Nutritional Status

Dietary intake was assessed using a standardized format including two 3-day food records. Parents were instructed to record all foods and beverages consumed on three subsequent days, including one weekend day. The timing of the first 3-day record period was prior to the blood sampling, and the second period was recorded approximately 6 months later. The amount of liquid and the size of food portions offered and actually consumed at each meal were reported in household measures. Energy and nutrient intake was calculated using the Dutch food composition table (NeVo, version 06) and the software Komeet[®] (BaS Nutrition Software, Arnhem).

In addition to the food records, a semistructured interview was administered to the parents to specify usual dietary intakes, eating abilities, and food preferences.

Body composition was measured using the Deuterium dilution method according to the Maastricht protocol (Westerterp et al. 1995). A random urine sample was used to determine the background isotope level before the administration of a D₂O mixture. At least 6 h after administration of a D₂O dose (per oz or through a PEG catheter), a urine sample was taken from the second voiding. Fat-free mass (FFM) was then calculated from total body water (TBW) using the child-specific hydration factors published by Lohman (Lohman 1989). Subsequently, FFM was divided by squared height to calculate the fat-free mass index (FFMI; kg/height(m)²). Malnutrition was defined as an FFMI below the fifth percentile (or −2 SD) for age and gender (VanItallie et al. 1990; Schutz et al. 2002).

Metabolic Measurements

Routine chemistry and hematology were carried out, as proposed by the Frösö Declaration and included full blood count, total protein, albumin, protein electrophoresis, urea, creatinine, electrolytes such as Na⁺/K⁺/Ca²⁺/Cl[−]/PO₄. Liver function tests were only done if specifically indicated (Julu et al. 2008b).

In addition, the metabolic screening program as shown in Table 1 was carried out to identify markers pointing toward possible consequences of an altered carbon dioxide metabolism. In view of the specific breathing irregularities in combination with feeding problems, one would expect changes in the “intermediate metabolism” including metabolism of glucose, glycogen, amino acids/proteins, and fatty acids. Short- and long-term changes due to, for example, a specific nutritional status are reflected in amino acid profiles in urine and plasma, acylcarnitine profiles in plasma (to also exclude a fatty acid oxidation defect or organic acidurias) and excretion of metabolites (organic acids) in urine (organic acid excretion profile).

Statistical Analysis

Using SPSS version 15.0, frequency tables including percentages were obtained for the variables “MECP2-mutation,” “cardiorespiratory phenotype,” and “anthropometry data.” Concerning age, mean values and standard deviation were calculated. Regarding ISS scores, both frequency tables and mean scores were calculated.

Results

Patient Characteristics

Patient characteristics are presented in Table 2.

Table 1 General overview of selective metabolic screening including matrix and methodology

Analysis	Matrix	Methodology
Amino acid analysis	Urine, plasma	LC–MS/MS
Organic acid analysis	Urine, plasma	GC–MS, silylated
Purines and pyrimidines	Urine, plasma	LC–MS/MS
Guanidinoacetate and creatine	Urine, plasma	LC–MS/MS
Phenolic compounds	Urine	HPLC-fluorescence
Acylcarnitine profiling	Plasma	MS/MS
Very-long chain fatty acids	Plasma	LC–MS/MS
Sialotransferrines	Plasma	Isoelectric focusing
Methylmalonate	Plasma	LC–MS/MS
Homocysteine	Plasma	LC–MS/MS
Adenosine deaminase activity	Erythrocytes	HPLC–UV
Adenine phosphoribosyltransferase activity	Erythrocytes	HPLC–UV
Hypoxanthine phosphoribosyltransferase activity	Erythrocytes	HPLC–UV
Inosine triphosphatase activity	Erythrocytes	HPLC–UV
Nucleotide profile	Erythrocytes	HPLC–UV
α-Galactosidase A activity	Leukocytes	Fluorescence

LC–MS/MS liquid chromatography with tandem mass spectrometry, *GC–MS* gas chromatography-mass spectrometry, *HPLC* high-performance liquid chromatography, *MS/MS* tandem mass spectrometers, *HPLC–UV* high-pressure liquid chromatography with UV detector

Age

The ages of RTT girls ranged from 2 years and 4 months to 20 years and 3 months (mean age 9 years and 5 months, SD = 5 years and 11 months).

Anthropometry

Mean height, weight, BMI, and head circumference scores of RTT girls were below that of their age group. The height of 54% of the RTT girls was below the fifth percentile, of which 86% were 9 years or older. Regarding BMI, 31% of the girls were below the fifth percentile and two girls had a BMI over the 50th percentile. Regarding age, low BMI was present in 75% of those 9 years and older.

ISS Score

Mean ISS scores for the RTT girls was 18.8 (range: 8–27, SD = 6.78). Severe RTT (score 25–29) was present in two

Table 2 The clinical characteristics of the RTT girls

RTT girl	Age (yr. month)	Height (cm + p)	Weight (kg + p)	Head circumference (cm + p)	BMI (kg/m ² + p)	ISS	MECP2 mutation ^a	CRPh	tcpCO ₂ (mmHg)
1	2.4	85 (p2)	11.3 (p10)	45 (p2)	15.6 (p25)	14	p.R255X	FeB	43.3
2	3.11	98 (p50)	16 (p25)	48.5 (p25)	16.7 (p50)	8	p.T158M	FeB	48.2
3	4.0	107 (p70)	14.5 (p10)	50.5 (p50)	12.7 (p2)	8	p.R294X	FeB	52.3
4	4.3	103 (p50)	20.5 (p98)	47 (p2)	19.3 (p98)	21	fs710dupG	FeB	50.6
5	5.3	112 (p50)	19 (p50)	49 (p25)	15.2 (p50)	20	p.R270X	FeB	40.9
6	6.2	113 (p25)	18 (p25)	49 (p25)	14.3 (p25)	9	p.R306C	FoB	22.3
7	9.8	120 (<p2)	20.5 (p2)	51 (p25)	14.2 (p10)	20	fs705delG	FoB	15.5
8	9.8	132 (p2)	26.4 (p10)	49.5 (p2)	15.2 (p25)	20	p.P152R	FoB	25.8–42.0
9	10.11	127 (<p2)	24.5 (p2)	50.5 (p25)	15.2 (<p2)	26	p.R106W	FoB	15.5
10	13.3	148 (p2)	31.7 (p2)	50 (<p2)	14.5 (p2)	23	p.R294X	FoB	10.5–22.5
11	13.11	148 (<p2)	54 (p75)	54 (p50)	24.7 (p98)	27	c.1158del55	Und	35.2
12	19.3	171 (p50)	65 (p75)	52 (p3)	22.3 (p50)	24	p.R306C	FoB	20.8
13	20.3	150 (<p2)	43 (p2)	53 (p25)	19.1 (p2)	24	p.R168X	FoB	N.A.

p percentile, ISS total ISS score, CRPh cardiorespiratory phenotype, FeB feeble breather, FoB forceful breather, Und undetermined, tcpCO₂ transcutaneous pCO₂ (reference values 38–44 mmHg), N.A. not acquired

^a Nomenclature according to the MECP2A isoform reference sequence AF158180, numbering starting at the A of the ATG translation initiation codon

girls, mild to less severe RTT (score 10–24) in eight and very mild RTT (score below 10) in three girls. Severe RTT was only seen in girls 9 years and older and very mild scores only in those younger than 9 years. Mean score of the girls younger than 9 years was 13.3 (range: 8–21, SD = 5.99), compared to 23.4 (range: 20–27, SD = 2.70) in those 9 years and older.

Mutation Analysis

Both missense and nonsense mutations in *MECP2* were each observed in five girls. Of these mutations, four were located in the Methyl-CpG binding domain and six in the transcription repression domain. Two girls had a frameshift mutation and only one girl had a CTS deletion. In view of the variety in mutations, no differences concerning other patient characteristics could be observed.

Cardiorespiratory Phenotype

Of the 13 girls included in this study, five girls were feeble breathers, seven girls were forceful breathers, and one girl had an undetermined cardiorespiratory phenotype. Remarkably, all feeble breathers were below 5 years of age and all forceful breathers were 6 years and older. Furthermore, Table 2 shows the tcpCO₂ values measured during the Neuroscope assessment. As one can see, overall feeble breathers show high tcpCO₂ levels in contrast to forceful breathers who show low tcpCO₂ levels.

Table 3 Nutritional status of the RTT girls including age-specific and lifestyle adjusted recommended/reference values

RTT girl	Dietary energy (kcal/day)	Protein (gm/kg/day)	FFMI (kg/l ²)
1	1,179 (800)	3.0 (>0.9)	11.94 (unknown)
2	1,222 (800)	2.4 (>0.9)	12.71 (unknown)
3	1,920 (1,120)	4.5 (>0.9)	N.A.
4	1,150 (1,120)	2.3 (>0.9)	9.62 (unknown)
5	1,189 (1,120)	2.1 (>0.9)	9.12^a (11.7–15.7)
6	1,010^b (1,120)	1.3 (>0.9)	11.48^a (11.7–15.7)
7	1,137 (1,120)	2.4 (>0.9)	11.60^a (11.7–15.7)
8	1,222 (1,120)	1.9 (>0.9)	10.34^a (11.7–15.7)
9	1,710 (1,680)	2.2 (>0.9)	12.05^a (11.7–15.7)
10	1,828 (1,680)	1.6 (>0.9)	10.46^a (13.2–17.2)
11	895^b (1,680)	1.2 (>0.9)	11.23^a (13.2–17.2)
12	1,893 (1,680)	0.9 (>0.8)	12.29^a (13.8–17.6)
13	1,630^b (1,680)	1.5 (>0.8)	12.18^a (13.8–17.6)

FFMI fat-free mass index, N.A. not acquired

^a Low dietary energy compared to the recommended values

^b FFMI value < 2 SD or p5 (Freedman et al. 2005; Schutz et al. 2002)

Nutritional Status

Results on dietary intakes and body composition are presented in Table 3

Dietary Intakes

Dietary intakes were evaluated and compared to age-specific standards for an inactive lifestyle, by which the normal values were adjusted to 80% of the recommended values for healthy girls. Overall, the total daily dietary energy was reasonably normal in the RTT girls, as the mean dietary intake was 117% of the adjusted recommended values. Almost 77% of the RTT girls met these adjusted recommended values. Only three girls had a dietary energy intake below these values, respectively, of 90%, 53%, and 97% of the recommended values. The RTT girl having a dietary energy intake of only 53% of the recommended values had a BMI at the 98th percentile and she was on a strict diet.

Protein intake in all patients was above the age-specific recommended values.

Prior to blood and urine sampling, no specific nutrients were consumed which could influence the metabolic investigations as has been described by Arias and colleagues (Arias et al. 2007).

Body Composition

As one can see in Table 3, 9 RTT girls confirm the definition of malnutrition, defined as an FFMI below the fifth percentile (or -2 SD) for age and gender. Since reference values for girls younger than 5 years of age are not available, one cannot confirm malnutrition in these younger girls.

Metabolic Investigations

As shown in Table 4, significantly elevated plasma creatine concentrations and increased urinary creatine/creatinine ratios were observed in five RTT girls. An isolated increase of the urinary creatine/creatinine ratio was seen in two girls. The creatinine plasma concentrations were relatively low in all RTT girls. The slightly elevated guanidinoacetate concentrations observed in three females are not clinically relevant, since they do not meet the criteria of a guanidinoacetate methyltransferase deficiency (Stromberger et al. 2003). An elevated CK concentration was seen in a girl with normal creatine concentrations. Other metabolic investigations (Table 1) showed no abnormalities.

Elevated creatine concentrations were only seen in those 9 years and older, except for one 4-year-old girl with an isolated increased urinary creatine/creatinine ratio. Mean age of the girls having an elevated plasma creatine was 14.54 years (range: 9.6–20.3, SD = 4.33), compared to 5.09 years (range: 2.3–9.7, SD = 2.34) of the girls having a normal plasma creatine. Mean age of the girls having an increased urinary creatine/creatinine ratio was 13.07 years (range: 4.3–20.3, SD = 2.34), compared to 5.47 years (range: 2.3–9.7, SD = 2.75) in the normal urinary creatine/creatinine ratio group.

Anthropometric characteristics between the two groups were roughly equal, except for height. Height was below the fifth percentile in 83% of the girls with elevated creatine concentrations, and greater than the 50th percentile in 57% of the girls with normal creatine concentrations.

Table 4 Creatine metabolism in RTT girls including age-specific reference values

RTT girl	Cr plasma ($\mu\text{mol/l}$)	Cr/Crn urine (mmol/mol)	Crn plasma ($\mu\text{mol/l}$)	CK (U/L)	GAA plasma ($\mu\text{mol/l}$)	GAA/Crn urine (mmol/mol)
1	75.7 (17–109)	490.6 (6–1,200)	29.7^a (35–62)	73 (<140)	1.1 (0.35–1.8)	116.2 (4–220)
2	79.5 (17–109)	421.3 (6–1,200)	27.0^a (35–62)	79 (<140)	2.1^b (0.35–1.8)	102.3 (4–220)
3	88.1 (17–109)	N.A.	30.7^a (44–71)	77 (<140)	1.6 (0.35–1.8)	N.A.
4	81.0 (17–109)	828.8^b (17–720)	28.0^a (44–71)	49 (<140)	1.2 (0.35–1.8)	76.8 (4–220)
5	83.5 (17–109)	28.6 (17–720)	34.4^a (44–71)	44 (<140)	1.4 (0.35–1.8)	26.8 (4–220)
6	63.0 (17–109)	629.7 (17–720)	28.8^a (44–71)	285^b (<140)	1.2 (0.35–1.8)	56.7 (4–220)
7	52.9 (17–109)	756.2^b (17–720)	43.6^a (44–80)	104 (<140)	1.4 (0.35–1.8)	73.2 (4–220)
8	68.7 (17–109)	124.3 (17–720)	29.2^a (44–80)	66 (<140)	0.8 (0.35–1.8)	45.0 (4–220)
9	70.4^b (6–50)	1,305.3^b (17–720)	31.7^a (53–88)	65 (<140)	1.6 (0.35–1.8)	72.7 (4–220)
10	73.5^b (6–50)	448.0^b (11–240)	42.3^a (62–97)	64 (<140)	1.6 (0.35–1.8)	64.4 (4–220)
11	101.5^b (6–50)	1,040.3^b (11–240)	40.3^a (62–97)	26 (<140)	1.4 (0.35–1.8)	59.2 (4–220)
12	88.5^b (6–50)	403.7^b (11–240)	36.7^a (50–100)	53 (<140)	1.3 (1.0–3.8)	106.7^b (3–78)
13	65.0^b (6–50)	538.3^b (11–240)	38.3^a (50–100)	19 (<140)	1.9 (1.0–3.8)	82.7^b (3–78)

Cr creatine, Crn creatinine, CK creatine kinase, GAA guanidinoacetate, N.A. not acquired

^a Elevated value

^b Low value of creatinine

Furthermore, only the girls with an elevated creatine concentration in plasma and urine had a severe ISS score, and the girls with a normal creatine concentration in plasma and urine had a very mild ISS score. The mean score of the girls in the elevated plasma creatine group was 24.0 (range: 20–27, SD = 2.45) versus 14.3 (range: 8–21, SD = 6.02) in the normal plasma creatine group. The mean score of the girls in the increased urinary creatine/creatinine ratio group was 23.6 (range: 20–27, SD = 2.51) compared to 14.2 (range: 8–20, SD = 5.76) in the normal urinary creatine/creatinine ratio group. Except for ISS domain four (mental/cortical function), in which the scores were equally distributed in both groups, girls with an elevated creatine showed high scores in all domains.

In view of the variety in mutations, no differences concerning other patient characteristics could be observed.

Concerning the cardiorespiratory phenotype, in the elevated creatine group, four girls were forceful breathers and one girl had an undetermined cardiorespiratory phenotype. The girls with an isolated increased urinary creatine/creatinine ratio were a forceful and a feeble breather.

Finally, all RTT girls wherefore reference values of FFMI were available met the criteria for malnutrition. Therefore, a possible relationship between elevated creatine concentrations and an impaired nutritional status could not be investigated.

Discussion

This study is the first attempt to correlate metabolic alterations as a possible explanation of impaired nutritional status in RTT. Despite normal levels of total dietary energy and protein intakes, malnutrition was confirmed in all RTT girls wherefore reference values of FFMI were available. Although the loss of skeletal muscle mass could be partly due to physical inactivity, these low values are certainly an indication for impaired nutritional status in these RTT girls.

Blood and urine samples were collected for biochemical screening of multiple metabolic pathways. We observed significantly elevated plasma creatine concentrations and increased urinary creatine/creatinine ratios in half of the RTT girls. The creatinine plasma concentrations were relatively low, as one would expect in view of the low FFMI values. Nonetheless, the elevated creatine/creatinine ratios are most likely primarily due to the elevated creatine concentrations, considering the high plasma creatine concentrations at the same time.

Given that in this study the young girls were feeble breathers and the older girls were forceful breathers, it is impossible to determine whether the elevated creatine concentrations are age related or associated with the cardiorespiratory phenotype. This age distribution is likely

coincidental since previous studies showed no significant age difference between the different cardiorespiratory phenotypes (Julu and Engerström 2005). At present, it is uncertain whether an altered carbon dioxide metabolism affects the creatine metabolism in females with RTT.

It is difficult to explain why creatine concentrations should increase with age in the RTT cohort. According to the reference values for creatine, creatine concentrations normally decrease with increasing age (Salomons et al. 2003). Furthermore, Horská and colleagues reported stable creatine concentrations with increasing age in RTT girls using proton magnetic resonance spectroscopy (Horská et al. 2009). Consequently, it seems highly unlikely that the altered creatine concentrations can be explained by age.

Regarding the cardiorespiratory phenotype, two possible explanations can be given. First of all, as has been described by Julu and colleagues, a major difference in the cardiorespiratory phenotypes is the blood pH value (Julu et al. 2008a, b). Females with a forceful breathing type tend to be alkalotic due to excessive loss of CO₂ via respiration, in contrast to females with a feeble breathing pattern who tend to be acidotic. The pH differences between these phenotypes can be as much as 0.4–0.5 pH-units (personal observation Dr. P.O.O. Julu). This pH difference may affect the creatine metabolism in RTT girls. Second, it can be speculated that the elevated creatine concentrations might be due to the increased energy expenditure as described in forceful breathers (Julu et al. 2008b). Phospho-creatine (P-creatine) together with ATP makes up the phosphagen energy system. P-creatine contains the high-energy phosphate bond, which is 3–8 times as abundant as ATP. As soon as more ATP is needed, P-creatine transfers its high energy phosphate to ATP (Guyton and Hall 2000). Following this suggestion, it may be speculated that in forceful breathers, the elevated creatine concentrations are indirectly caused by higher P-creatine needs.

Furthermore, as the creatine-transporter gene (*SLC6A8*) is located adjacent to MECP2 at Xq28, MeCP2 deficiency may cause epigenetic aberrations affecting the expression of the *SLC6A8*. The clinical phenotypes of RTT and an X-linked creatine transporter deficiency overlap regarding mental retardation, epilepsy, and language delay (Salomons et al. 2001). However, few studies have examined the brain metabolites of RTT females using proton magnetic resonance spectroscopy (Gökçay et al. 2002; Hashimoto et al. 1998; Horská et al. 2000, 2009; Khong et al. 2002; Pan et al. 1999). Overall, *N*-acetylaspartate/total creatine was significantly decreased compared with age-matched controls, primarily reflecting reduced *N*-acetylaspartate levels. Even so, an 8% greater mean creatine concentration was previously found in the frontal white matter in the RTT group. This difference was not significantly different,

because of a high variability in creatine levels in both RTT and control groups (Horská et al. 2000, 2009). Nevertheless, in case of a decreased expression of *SLC6A8*, less creatine would be present in the brain, making this diagnosis highly unlikely in our patients (Salomons et al. 2001).

Clinically, the girls with an elevated creatine concentration differed from girls with a normal creatine concentration regarding height and ISS score. Girls with an elevated creatine concentration were substantially shorter and a more severe phenotype was observed. This severe phenotype reflected several functional domains, that is growth and development, musculoskeletal, movement, and autonomic features.

In summary, this is the first study to report abnormalities in creatine concentrations in RTT girls. Hitherto, only Freilinger and colleagues performed metabolic screening including urinary creatine/creatinine ratio (Freilinger et al. 2007). They did not report any abnormalities in creatine/creatinine ratios in 29 Rett females with a mean age of 13 years. Reference values were not defined. However, as can clearly be seen in that article, three females have a highly elevated creatine/creatinine ratio. Furthermore, an *MECP2* mutation was identified in 76% of the females, which is rather low compared to previously published data (Williamson and Christodoulou 2006). Cardiorespiratory data were not available. So, the discrepancy may be because of difference in reference values or patient populations, as the diagnosis of RTT in the previous study was mainly based on clinical criteria alone and a possibly lower percentage of forceful breathers. The strength of our study was the use of a well-defined group of RTT girls. Only due to these strict inclusion criteria, our study is hampered by the small sample size, which does not allow us to understand the observed creatine abnormalities fully. At this moment, we are not able to confirm our hypothesis regarding metabolic alterations as a possible explanation of impaired nutritional status in RTT, since impaired nutritional status was seen in all RTT girls wherefore reference values of FFMI were available. Different etiologies can explain the observed elevated creatine concentrations in the RTT girls. Except for creatine transporter defects, no other neurological disease ever showed elevated creatine values. Further studies concerning the creatine metabolism in relation to the nutritional requirements and cardiorespiratory status or phenotype of RTT girls are important in order to provide appropriate and effective management.

Conclusions

Despite normal levels of total dietary energy and protein intakes, malnutrition was confirmed in all RTT girls wherefore reference values of FFMI were available. An

important percentage of RTT girls showed creatine concentrations above the reference values. Currently, it is undetermined how the creatine metabolism is affected in females with RTT, and how this may affect their nutritional status. Further studies are required to confirm these findings and to provide greater insight into the pathogenesis of the abnormal creatine metabolism in RTT.

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Synopsis

A striking increase in creatine concentration was observed in the body fluids of females with Rett syndrome, which may reflect the altered carbon dioxide metabolism of these girls or their impaired nutritional status or both.

Contributor's Statement Page

The article has not been and will not be published elsewhere in substantially the same form. The article has been circulated and final approval of the version to be peer-reviewed was secured from all co-authors prior to article submission. This includes confirmation of absence of previous similar or simultaneous publications their inspection of the manuscript, their substantial contribution to the work, and their agreement to submission.

Details of the Contributions of Individual Authors

Nicky S.J. Halbach: involvement in conception and design analysis and interpretation of data and drafting the article.

Eric E.J. Smeets: involvement in conception and design analysis and interpretation of data and revising the article critically for important intellectual content.

Jörgen Bierau: involvement in conception and design analysis and interpretation of data and revising the article critically for important intellectual content.

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