Research article

Fasting serum blood measures of bone and lipid metabolism in children with myelomeningocele for early detection of cardiovascular and bone fragility risk factors[†]

Alexander Van Speybroeck¹, Nicole M. Mueske², Steven D. Mittelman¹, Richard K. Kremer³, Deirdre D. Ryan^{1,2}, Tishya A. L. Wren²

¹Department of Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA,

²Children's Orthopaedic Center, Children's Hospital Los Angeles, Los Angeles, CA, USA, ³McGill University Health Centre, Montreal, Canada

Objective: This study examined serum levels in children with myelomeningocele to identify the prevalence of pre-clinical signs of disease.

Design: A prospective, cross-sectional study.

Setting: Patients were actively recruited from multidisciplinary care clinics at tertiary children's hospitals from 2010–2012. The control comparison group was recruited by word-of-mouth.

Patients: Twenty-eight children with myelomeningocele (93% Hispanic; 17 males; 10.0 ± 2.1 years) and 58 controls (84% Hispanic; 30 males; 10.4 ± 2.4 years) provided ≥ 8-hour fasting blood samples with concomitant dual-energy x-ray absorptiometry measurements of body fat.

Interventions: Not applicable.

Main Outcome Measures: The serum analysis included a lipid panel (cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein), insulin, glucose, leptin, aspartate aminotransferase, alanine transaminase, alkaline phosphatase, albumin, creatinine, calcium, phosphatase, parathyroid hormone, and vitamin D.

Results: Children with myelomeningocele had higher body fat (35.2% versus 29.9%, $p=0.01$) and altered lipid profiles (lower high-density lipoprotein levels, 43.9 mg/dL versus 51.6 mg/dL, $P=0.03$) suggesting elevated risk of metabolic syndrome. They also had a higher prevalence of vitamin D deficiency (43% versus 17%, $p=0.02$) and significantly lower levels of calcium (9.4 mg/dL versus 9.7 mg/dL, $P=0.003$) and alkaline phosphatase (187.0 U/L versus 237.0 U/L, $P=0.003$). Unexpectedly children with myelomeningocele had lower parathyroid hormone levels (14.5 pg/mL versus 18.4 pg/mL, $P=0.02$) than controls despite lower calcium, vitamin D and alkaline phosphatase levels. This suggests an alteration in the sensing mechanism or response of the parathyroid gland to normal physiological stimuli in patients with myelomeningocele.

Conclusions: Children with myelomeningocele have abnormal biochemical markers for cardiovascular disease, insulin resistance and bone and mineral metabolism. Early recognition and monitoring of these risk factors in patients with myelomeningocele may help prevent later complications.

Keywords: myelomeningocele, spina bifida, serum levels, metabolic syndrome, pediatrics

Introduction

Myelomeningocele (MM) is the most common and severe type of spina bifida occurring in approximately

60 of 100,000 live births.¹ MM affects the brain and spinal cord leading to a continuum of mental and functional impairments such as developmental delay, muscle weakness and paralysis, and loss of sensation.^{1–3} Prolonged periods of inactivity due to surgeries, pressure sores and urinary tract infections, and use of braces and assistive devices are common as these children develop^{2,3} leading to a

Correspondence to: Alexander Van Speybroeck, Children's Orthopaedic Center Children's Hospital Los Angeles 4650 Sunset Blvd., M/S 69 Los Angeles, CA 90027, USA. Email: avanspeybroeck@chla.usc.edu

[†]Study conducted at Children's Hospital Los Angeles, Los Angeles, California, USA.

higher incidence of obesity than those without disease and disability.⁴

Pediatric obesity has notably increased over the past two decades⁵ and this epidemic disproportionately affects those with disabilities such as MM.^{4,6–8} Obesity is a chief risk factor for multiple health issues including metabolic syndrome, type II diabetes, cardiovascular disease (CVD), cancer, and sleep apnea.^{9,10} As many as 80% of overweight youth in the general population will be overweight or obese as adults⁹ which is of particular concern for the MM population because they are experiencing an increase in life expectancy coupled with complex medical needs. Therefore it is important to detect signs of disease, particularly those related to obesity, as early as possible for prompt intervention, a greater possibility of prevention, and better treatment outcomes. Since children with MM have an increased risk of having abnormalities in lipid levels,^{11–13} metabolic profiles,¹³ bone metabolism markers,^{14,15} and vitamin D levels,¹⁵ all of which may be also affected by obesity, we endeavored to assess these serum levels within a single population and investigate differences among varying degrees of disease involvement.

Methods

The study sample was comprised of 28 children with MM and 58 controls without physical disability ages 6–13 years; data was collected from December 2010 to December 2012. All participants were part of a larger observational study focusing on bone development. Those with MM were recruited from local hospitals and medical therapy units, while the control comparison group was recruited by word-of-mouth. Patients with MM were eligible for study enrollment if they were between 6–13 years old, possessed no other chronic conditions (other than hydrocephalus), were not on medications affecting growth or development, and did not have bilateral lower extremity metal implants. Typically developing children were eligible for this study if they were free from any chronic medical conditions, were not taking medications that would affect growth or development, and did not have any walking abnormalities. Written informed consent/assent was obtained from all participants and their guardian(s). All study procedures were approved by the hospital's institutional review board.

Height, body mass and other anthropometric measures were obtained by a physical therapist. Self-reported hours per week of television (TV) watching were recorded. Percent body fat and percent trunk fat were obtained from a whole body less head dual energy x-ray absorptiometry (DXA) scan. Retrospective chart review supplied blood pressure

measures within 6 months of the blood draw for patients with MM; blood pressure was not available for the control group. Using manual muscle testing, functional neurosegmental level was determined for the participants with MM based on the International Myelodysplasia Study Group (IMSG) criteria.¹⁶ The distribution of IMSG levels for the participants with MM was 8 sacral, 5 low lumbar, and 15 mid lumbar and above. Additionally, participants were classified as obese if their BMI percentile was at or greater than the 95th percentile in accordance with the Centers for Disease Control and Prevention criteria.¹⁷

Fasting (≥ 8 hours) blood samples were collected from all participants. The lipid panel measured cholesterol, triglycerides (TG), high-density lipoproteins (HDL) and low-density lipoproteins (LDL). Insulin resistance was calculated from insulin and glucose using the homeostatic model assessment of insulin resistance (HOMA-IR). Other measures in the serum analysis included: leptin, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase, albumin, creatinine, calcium, adjusted calcium, phosphate, intact parathyroid hormone (PTH) and total 25-hydroxy-vitamin D (25 OHD). Most serum levels were measured using Synchron LX20 Systems (Beckman Coulter, Fullerton, CA) with the exception of insulin (One Step Immunoenzymatic Sandwich Chemiluminescent Assay with DXI Beckman Coulter, Fullerton, CA), leptin (sandwich enzyme immunoassay cat #c PDLP00 R&D Systems, Minneapolis, MN), PTH (LIAISON® 1-84 PTH assay, a chemiluminescent immunoassay for the quantitative determination of PTH1-84 without cross-reaction to PTH7-84 fragment) and OHD (LIAISON® 25-OH Vitamin D TOTAL chemiluminescent assay using the Automated Chemiluminescent LIAISON® Instrument DiaSorin Inc., Stillwater, MN).

For the patient group, metabolic syndrome was defined as having 3 or more of the following risk factors: high blood pressure, excessive trunk adiposity, insulin resistance/glucose intolerance, high levels of TG, or low levels of HDL. Risk factors were defined based on criteria from Nelson *et al.*¹³ High blood pressure was defined as having systolic or diastolic blood pressure $\geq 90^{\text{th}}$ percentile for age, height and sex. Excessive trunk adiposity, as measured by DXA, was defined as having percent trunk fat $\geq 30\%$ for males and $\geq 35\%$ for females. Impaired glucose tolerance was defined as fasting glucose ≥ 100 mg/dL. High levels of TG were defined as ≥ 100 mg/dL, and low levels of HDL were defined as < 45 mg/dL for males and < 50 mg/dL for females. Metabolic syndrome was not defined for the control group as blood

pressure was not measured. For all participants, vitamin D status was classified as sufficient (≥ 30 ng/ml), insufficient (20–29 ng/ml), or deficient (< 20 ng/ml).¹⁸

Clinical characteristics and blood measures were compared between the MM and control groups using the two-sample Student's *t*-test for normally distributed continuous variables, the Mann-Whitney rank sum test for continuous variables not normally distributed, and the χ^2 test for categorical variables. Because of known relations between body fat and some blood serum measures such as vitamin D and the components of the lipid panel,^{18–20} this analysis was repeated using the generalized linear model including percent trunk fat as a covariate. For variables that were not normally distributed, medians, rather than means, are presented. Differences among the neurosegmental subgroups and controls were evaluated using analysis of variance (ANOVA) for normally distributed continuous data, Kruskal-Wallis tests for non-normal continuous data, and Chi-square tests for categorical data. Post-hoc analysis comparing each neurosegmental group to the control group was performed using 2-sample Student's *t*-tests for normal continuous data and Mann-Whitney rank sum tests for non-normal continuous data. Bonferroni adjustment was applied to adjust *p*-values for multiple comparisons. The level of significance was set at $P \leq 0.10$. All statistical analyses were performed using STATA (version 12.0, StataCorp LP, College Station, TX, USA).

Results

Participant Characteristics

Participant demographic and anthropometric information are shown in Table 1. The control and MM groups did not differ significantly in terms of sex, ethnicity, age, Tanner stage of sexual maturity, body mass, BMI, obesity (according to CDC classifications) or percent trunk fat. The MM group had shorter stature, higher percent total body fat, and watched more TV compared to controls.

MM vs. Control

Lipid panel results showed that the MM group had significantly lower levels of HDL ($P = 0.03$), but did not differ from controls in levels of cholesterol, TG, or LDL ($P \geq 0.26$; Table 2). The MM group tended to have higher levels of insulin ($P = 0.10$), HOMA-IR ($P = 0.12$), and leptin ($P = 0.12$) though not all differences reached statistical significance. Glucose levels did not differ between the two groups ($P = 0.68$). The MM group had significantly lower levels of AST ($P = 0.03$), alkaline phosphatase ($P = 0.003$), albumin ($P = 0.04$), and creatinine ($P < 0.001$) but did not differ from controls on measures of ALT ($P = 0.91$). In terms of bone metabolism, the MM group had lower values of unadjusted ($P = 0.003$) and adjusted ($P = 0.09$) calcium, PTH ($P = 0.02$), and vitamin D ($P = 0.006$) and a trend towards higher levels of phosphate ($P = 0.11$; Table 2). Additionally, PTH and

Table 1 Comparison of demographic and clinical characteristics between the MM and control groups

Characteristic	Control (n = 58)	MM (n = 28)	P-value
Age in years, Mean (SD)	10.4 (2.4)	10.0 (2.1)	0.47
Sex, % (#) Male	52% (30)	61% (17)	0.49
Ethnicity, % (#) Hispanic	84% (49)	93% (26)	0.49
Tanner, % (#)			
1	45% (26)	54% (15)	0.59
2	14% (8)	18% (5)	
3	15% (9)	3% (1)	
4	12% (7)	11% (3)	
5	14% (8)	14% (4)	
TV in hours/week, Median (IR)	7.0 (10.0)	11.0 (8.0)	0.06
Height in cm, Mean (SD)	142.5 (15.4)	134.8 (16.0)	0.03
Body Mass in kg, Median (IR)	38.2 (25.9)	38.2 (17.4)	0.50
BMI in kg/m ² , Median (IR)	18.3 (7.3)	20.9 (8.0)	0.73
Height Percentile, Median (IR)	61.8 (59.9)	25.8 (59.1)	0.005
Body Mass Percentile, Median (IR)	72.6 (51.3)	72.5 (70.1)	0.46
BMI Percentile, Median (IR)	69.2 (57.3)	89.4 (52.9)	0.49
Obese ¹ , % (#)	28% (16)	36% (10)	0.46
% Total Body Fat, Mean (SD)	29.9 (8.8)	35.2 (9.4)	0.01
% Trunk Fat, Mean (SD)	25.8 (9.5)	29.3 (10.3)	0.12

Normally distributed continuous data are presented as Mean (Standard Deviation) and analyzed using the 2-sample Student's *t*-test. Continuous data that are not normally distributed are presented as Median (Interquartile Range) and analyzed using the Mann-Whitney Rank-Sum test. Categorical data are analyzed using the Chi-square test. ¹Obesity classified according to Centers for Disease Control and Prevention criteria, BMI ≥ 95 percentile of children at the same age and sex.

Table 2 Comparison of blood measures of lipid, insulin, liver, renal and bone metabolism between the control and MM groups

	Control (n = 58)	MM (n = 28)	P-value	P-value adjusting for % trunk fat
Cholesterol (mg/dL)	165.9 (31.3)	162.8 (27.1)	0.45	0.90
Triglycerides (mg/dL)	65.5 (49.6)	67.8 (54.0)	0.26	0.91
HDL (mg/dL)	51.6 (14.3)	43.9 (17.4)	0.03	0.17
LDL (mg/dL)	99.8 (26.3)	99.0 (33.4)	0.84	0.71
Glucose (mg/dL)	81.1 (12.6)	81.1 (10.8)	0.68	0.63
Insulin (μ U/mL)	6.2 (5.6)	7.3 (7.8)	0.10	0.57
HOMA-IR	1.3 (1.3)	1.5 (1.3)	0.12	0.72
Leptin (ng/mL)	6.5 (12.5)	9.7 (14.4)	0.12	>0.999
AST (U/L)	28.0 (9.0)	23.5 (8.0)	0.03	0.21
ALT (U/L)	18.0 (6.0)	17.5 (8.0)	0.91	0.83
Alkaline Phosphatase (U/L)	237.0 (83.0)	187.0 (87.5)	0.003	0.002
Albumin (g/dL)	4.5 (0.3)	4.4 (0.4)	0.04	0.07
Creatinine (mg/dL)	0.51 (0.12)	0.36 (0.10)	<0.001	<0.001
Calcium (mg/dL)	9.7 (0.48)	9.4 (0.30)	0.003	0.004
Adjusted Calcium (mg/dL)	9.3 (0.32)	9.2 (0.44)	0.09	0.04
Phosphate (mg/dL)	4.9 (0.77)	5.1 (0.79)	0.11	0.19
PTH (pg/mL)	18.4 (10.6)	14.5 (8.9)	0.02	0.008
25 OHD (ng/mL)	26.4 (6.6)	22.2 (11.0)	0.006	0.009

Data are presented as Median (Interquartile Range). Univariate comparisons between groups were performed using the Mann-Whitney Rank-Sum test. Multivariate analysis adjusting for percent trunk fat was performed using the generalized linear model.

25OHD were negatively correlated for both the MM ($r = -0.43$, $P = 0.02$) and control ($r = -0.38$, $P = 0.003$) groups.

After including percent trunk fat as a covariate, the differences between groups for HDL ($P = 0.17$), insulin ($P = 0.57$), HOMA-IR ($P = 0.72$), leptin ($P \geq 0.999$) and AST ($P = 0.21$) were no longer seen (Table 2). However, all other significant differences persisted ($P \leq 0.07$).

Only 7% (2/28) of the MM and 22% (13/58) of the control group were classified as vitamin D sufficient (≥ 30 ng/mL; $P = 0.13$), while 50% (14/28) of the MM and 60% (35/58) of the control group were vitamin D insufficient (20–29 ng/mL; $P = 0.49$). Significantly more children with MM (43%, 12/28) were classified as vitamin D deficient (< 20 ng/mL) compared to the control group (17%, 10/58; $P = 0.02$).

Neurosegmental Levels

There were no differences among groups for sex or ethnic distribution, age, hours of TV watched per week, height, body mass, body mass percentile, or BMI (Table 3). However, the mid lumbar group had significantly lower height for age ($P = 0.03$) and more total body fat ($P = 0.004$) and trunk fat ($P = 0.06$) compared to the controls.

Although there were no significant differences among sub-groups in the lipid panel, levels of HDL tended to decrease with increasing neurosegmental level while levels of TG tended to increase (Table 4). The mid lumbar group had significantly higher measures than controls for leptin ($P = 0.02$) and phosphate ($P = 0.01$) and lower measures for AST ($P = 0.05$), alkaline

phosphatase ($P = 0.10$), creatinine ($P < 0.001$), unadjusted calcium ($P = 0.06$), PTH ($P = 0.09$), and vitamin D ($P = 0.02$). The low lumbar group only differed significantly from controls on measures of creatinine ($P = 0.002$), unadjusted calcium ($P = 0.006$), adjusted calcium ($P = 0.02$), and PTH ($P = 0.09$), while the sacral group did not differ significantly from controls on any measure.

Metabolic Syndrome

There were no significant differences between groups in the number of children classified as having each metabolic syndrome risk factor, though the percentage of participants with each risk factor was generally higher in the MM group. 31% of the control group compared to 43% of the MM group were positive for the truncal fat risk factor ($P = 0.34$). Only 4% and 0% for the control and MM groups respectively were positive for the glucose intolerance risk factor ($P \geq 0.99$). 19% of the control group and 25% of the MM group were positive for the high TG classification ($P = 0.58$) while 17% and 25% of the control and MM groups respectively were positive for the low HDL risk factor ($P = 0.40$). Blood pressure was not measured in the control group but 29% of the MM group was classified as having high blood pressure. In the MM group, 15% (4/28) of the children were classified as having metabolic syndrome with at least 3 of 5 risk factors. All of these children were obese (based on truncal adiposity) and had hypertension combined with high TG and/or low HDL. 32% had 0, 32% had 1 and 21% had 2 metabolic syndrome risk factors.

Table 3 Comparison of demographic and clinical characteristics among different neurosegmental levels in children with myelomeningocele and controls

Characteristic	Control (n = 58)	Sacral (n = 8)	Low Lumbar (n = 5)	Mid Lumbar (n = 15)	P-value
Age in year, Mean (SD)	10.4 (2.4)	11.3 (2.1)	9.1 (1.7)	9.7 (2.0)	0.24
Sex, % (#) Male	52% (30)	50% (4)	60% (3)	67% (10)	0.78
Ethnicity, % (#) Hispanic	85% (49)	75% (6)	100% (5)	100% (15)	0.24
Tanner, % (#)					
1	45% (26)	37.5% (3)	60% (3)	60% (9)	0.90
2	14% (8)	12.5% (1)	20% (1)	20% (3)	
3	15% (9)	12.5% (1)	0% (0)	0% (0)	
4	12% (7)	12.5% (1)	20% (1)	7% (4)	
5	14% (8)	25% (2)	0% (0)	13% (2)	
TV in hours/week, Median (IR)	7.0 (10.0)	14.5 (11.3)	10.0 (5.0)	12.0 (8.0)	0.20
Height in cm, Mean (SD)	142.5 (15.4)	141.0 (15.1)	130.3 (18.2)	133.0 (15.9)	0.10
Body Mass in kg, Median (IR)	38.2 (25.9)	33.3 (17.2)	45.5 (24.4)	40.2 (19.0)	0.77
BMI in kg/m ² , Median (IR)	18.3 (7.3)	16.4 (5.8)	19.6 (7.9)	21.3 (6.3)	0.36
Height Percentile, Median (IR)	61.8 (59.9)	36.7 (46.0)	27.4 (59.5)	8.1 (57.5)*	0.03
Body Mass Percentile, Median (IR)	72.6 (51.3)	34.8 (62.5)	90.3 (81.8)	78.8 (49.5)	0.65
BMI Percentile, Median (IR)	69.2 (57.3)	44.4 (59.9)	81.6 (43.2)	91.9 (36.4)	0.26
% Total Body Fat, Mean (SD)	29.9 (8.8)	29.1 (9.1)	33.0 (11.6)	39.2 (7.2)**	0.004
% Trunk Fat, Mean (SD)	25.8 (9.5)	23.6 (10.0)	27.4 (12.6)	33.0 (8.7)^	0.06

Normally distributed continuous data are presented as Mean (Standard Deviation) and analyzed using ANOVA with post-hoc 2-sample Student's *t*-tests. Continuous data that are not normally distributed are presented as Median (Interquartile Range) and analyzed using the Kruskal-Wallis test with post-hoc Mann-Whitney Rank Sum tests. Categorical data are analyzed using the χ^2 test.

** denotes significant difference from Control group at 0.01 level.

*denotes significant difference from Control group at 0.05 level.

^ denotes significant difference from Control group at 0.10 level.

Table 4 Comparison of blood serum measures between MM neurosegmental levels and controls

	Control (n = 58)	Sacral (n = 8)	Low Lumbar (n = 5)	Mid Lumbar (n = 15)	P-value
Cholesterol (mg/dL)	165.9 (31.3)	169.0 (18.6)	135.7 (113.7)	161.6 (25.5)	0.85
Triglycerides (mg/dL)	65.6 (49.6)	50.9 (31.0)	70.9 (46.9)	85.9 (77.1)	0.34
HDL (mg/dL)	51.6 (14.3)	47.4 (26.9)	44.1 (11.2)	40.6 (17.0)	0.12
LDL (mg/dL)	99.8 (26.3)	100.5 (32.5)	73.1 (66.9)	99.8 (24.0)	0.98
Glucose (mg/dL)	81.1 (12.6)	83.8 (8.1)	81.1 (3.6)	77.5 (23.4)	0.65
Insulin (uIU/mL)	6.2 (5.6)	7.0 (5.3)	6.3 (7.0)	8.4 (7.6)	0.27
HOMA-IR	1.3 (1.3)	1.6 (1.1)	1.3 (1.3)	1.6 (1.7)	0.42
Leptin (ng/mL)	6.5 (12.5)	4.5 (10.0)	5.0 (14.4)	15.3 (27.1)*	0.04
AST (U/L)	28.0 (9.0)	28.5 (8.5)	22.0 (5.0)	21.0 (9.0)*	0.06
ALT (U/L)	18.0 (6.0)	17.0 (6.5)	20.0 (7.0)	18.0 (10.0)	0.97
Alkaline Phosphatase (U/L)	237.0 (83.0)	180.5 (136.0)	182.0 (26.0)	204.0 (101.0)^	0.03
Albumin (g/dL)	4.5 (0.30)	4.4 (0.30)	4.5 (0.20)	4.3 (0.40)	0.21
Creatinine (mg/dL)	0.51 (0.12)	0.38 (0.18)	0.29 (0.079)**	0.32 (0.12)**	0.001
Calcium	9.7 (0.48)	9.6 (0.50)	9.4 (0.12)**	9.5 (0.40)	0.01
Adjusted Calcium	9.3 (0.32)	9.3 (0.52)	9.0 (0.24)*	9.2 (0.40)	0.04
Phosphate (mg/dL)	4.9 (0.77)	4.7 (0.85)	4.8 (0.25)	5.6 (0.99)*	0.007
PTH (pg/mL)	18.4 (10.6)	19.6 (6.7)	11.6 (2.2)^	14.2 (8.4)^	0.04
25 OHD (ng/mL)	26.4 (6.6)	20.1 (10.4)	29.4 (13.9)	22.3 (8.7)*	0.02

Data are presented as Median (Interquartile Range) and analyzed using the Kruskal-Wallis test. Post-hoc analysis comparing each neurosegmental group to the control group was performed using Mann-Whitney Rank Sum tests with Bonferroni adjustment for multiple comparisons.

** denotes significant difference from Control group at 0.01 level.

* denotes significant difference from Control group at 0.05 level.

^ denotes significant difference from Control group at 0.10 level.

Discussion

This study showed that children with MM have elevated and/or adverse serum levels revealed in blood panels. Some of these abnormalities, such as HDL, insulin, and AST, were related to increased adiposity in these

patients, while others, such as alkaline phosphatase, albumin, creatinine, calcium, PTH and vitamin D occurred independent of trunk fat. Further, this study found for the first time that the degree of these abnormalities was associated with neurosegmental level;

namely, youth with only sacral involvement had values similar to healthy controls while lumbar involvement was associated with abnormalities in the lipid profile, bone metabolism markers, and metabolic syndrome markers.

These findings highlight the importance of screening tests for comorbidities in youth with MM. Outside of vitamin D levels, 68% of our MM participants had one or more abnormalities, compared to 53% of the control group. Since some of these abnormalities did not appear to be related to obesity, they might not have been discovered during routine health care visits in the absence of specific screening for MM comorbidities. Our findings highlight the greatly increased risk MM youth have of complications such as CVD and osteoporosis. Since both of these conditions are largely preventable and treatable, routine serum screening could be an important component of care for youth with MM, particularly those with abnormalities at higher segmental levels. On the other hand, we did not uncover any subclinical renal dysfunction in the present study, and so the utility of routine creatinine monitoring is not supported by our results.

Children with MM had adverse lipid profiles placing them at greater risk for future CVD with significantly lower HDL levels than controls. Notably lower HDL levels were seen with increased disease severity, though the differences were not statistically significant. These results agree with those reported in past studies,^{12,13} which all report mean HDL values for young people with MM that are considered borderline as a CVD risk factor.²¹ Because adverse lipid profiles are being seen in young people with MM and levels of HDL have been inversely associated with artery thickness in adolescent females indicating low levels of HDL as a considerable CVD risk factor even in youth without disability,²² these profiles are clearly important to consider due to the often complex medical care these children need and the high incidence of obesity. Furthermore, in our study, youth with MM had significantly more truncal fat than controls despite similar body mass and BMI, particularly those with higher level lesions. Therefore, the risk of developing CVD would be expected to be even greater compared to children of healthy body mass.

Children with MM are at an increased risk of osteoporosis and pathological fracture^{23,24} for many reasons, including decreased mobility and weight bearing, higher incidence of renal disease, increased body fat, lack of dietary vitamin D intake, and poor exposure to sunlight. This serum level analysis showed children with MM to have an extremely high rate of

vitamin D insufficiency or deficiency (93%), which is similar to what has been reported in past studies,¹⁵ though our control population also had an increased rate (78%); the higher fat mass in the MM group and the inverse relationship previously reported between 25OHD levels and body fat may partially explain this group difference.^{18,20} The rates of vitamin D deficiency also seen in this control group of primarily Hispanic children were similar to rates reported in other studies.^{25,26} Interestingly, despite the low levels of 25OH vitamin D, our MM subjects had lower levels of alkaline phosphatase and PTH compared to healthy controls. It is unclear why these levels, which are generally elevated in vitamin D deficiency, were in fact low. It is possible that these patients were resistant to the normal PTH response to low vitamin D, though such a phenomenon has not to our knowledge been reported. As we did not measure 1,25 OH vitamin D levels, we cannot rule out the possibility that a compensatory increase in this active form of vitamin D prevented the elevation in PTH and alkaline phosphatase. In any case, routine screening for vitamin D deficiency in this population may uncover a potentially treatable risk factor for osteoporosis and fracture in these patients.

Evaluation of metabolic syndrome components showed that children with MM tended to have greater rates of obesity, elevated TG, and low HDL compared to controls. This study found the MM group also had a high prevalence of hypertension (29%), much higher than what has been reported in children without spina bifida (2.6–10.7% depending on BMI).⁶ Based on these risk factors, 15% of the children with MM in this study were classified as having metabolic syndrome. While this rate is quite high, it is lower than the rate of 32.4% for patients with spina bifida studied by Nelson *et al.*¹³ The difference between studies is likely due to the younger age range in the current study.

Abnormalities in TG, HDL, leptin, AST, creatinine, calcium, phosphate, PTH and vitamin D levels in patients with MM compared to controls were greatest in the highest neurosegmental group (mid lumbar and above); though, likely due to the small sample size, many differences did not reach statistical significance. Thus, not surprisingly, the risk factors for obesity, CVD, insulin resistance, and osteoporosis were higher in patients with higher neurosegmental involvement. It is possible that some of these differences are largely tied to body composition – larger amounts of fat and lower amounts of muscle. Hydrocephalus and shunts, tethered cord, orthopaedic surgeries, bladder/bowel issues, seizures, spasticity and joint contractures, and pressure sores are more common or exacerbated in

those with higher level lesions.²⁷ The severity of these secondary health impairments in the children with higher level lesions may have systemic effects that are being seen in this serum analysis. This would justify increased monitoring, nutritional support, and medical and therapeutic exercise interventions in spina bifida patients with higher level involvement.

One limitation of this study was the absence of blood pressure values for the control group; therefore, metabolic syndrome could not be investigated in its entirety. Additionally, the method of assessing blood pressure in the patient group was not ideal, as it was not reassessed on multiple occasions for confirmation. However, the high prevalence of high blood pressure readings, along with the increased incidence of other components of the metabolic syndrome, highlight the importance for routine screening in the population. Another weakness of the present study is that our sample consisted primarily of Hispanics. While further work should be done on other ethnicities, it is important to study Hispanics because MM has a higher prevalence in Hispanic populations.^{28–30} Lastly, due to the limited sample size when patients were subdivided into neurosegmental groups, differences were not as apparent, and the results for that sub analysis should be considered preliminary.

Conclusions

Elevated CVD and metabolic syndrome risk factors, and possibly lower vitamin D levels in the MM group are primarily associated with greater obesity, while the musculoskeletal risk factors may be reflective of MM. The biochemical abnormalities observed are also suggestive of dysregulated parathyroid function which warrants further investigation. The issues evaluated in this study are likely exacerbated in children with higher lesion levels.

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References

- Boulet SL, Yang Q, Mai C, Kirby RS, Collins JS, Robbins JM, *et al.* Trends in the postfortification prevalence of spina bifida and anencephaly in the United States. *Birth Defects Res A Clin Mol Teratol* 2008;82(7):527–32.
- Liptak GS, Dosa NP. Myelomeningocele. *Pediatr Rev* 2010;31(11):443–50.
- Mitchell LE, Adzick NS, Melchionne J, Pasquariello PS, Sutton LN, Whitehead AS. Spina bifida. *Lancet* 2004;364(9448):1885–95.
- Finding Balance Obesity and Children with Special Needs. Ability Path; 2011.
- Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999–2004. *JAMA* 2006;295(13):1549–55.
- Sorof JM, Lai D, Turner J, Poffenbarger T, Portman RJ. Overweight, ethnicity, and the prevalence of hypertension in school-aged children. *Pediatrics* 2004;113(3 Pt 1):475–82.
- Mueske NM, Ryan DD, Van Speybroeck AL, Chan LS, Wren TAL. Fat Distribution in Children and Adolescents with Myelomeningocele. *Dev Med Child Neurol* 2015;57(3):273–8.
- Shurtleff DB, Walker WO, Duguay S, Peterson D, Cardenas D. Obesity and myelomeningocele: anthropometric measures. *J Spinal Cord Med* 2010;33(4):410–9.
- Daniels SR, Arnett DK, Eckel RH, Gidding SS, Hayman LL, Kumanyika S, *et al.* Overweight in children and adolescents: pathophysiology, consequences, prevention, and treatment. *Circulation* 2005;111(15):1999–2012.
- Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, *et al.* Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2006;113(6):898–918.
- Buffart LM, van den Berg-Emons RJ, Burdorf A, Janssen WG, Stam HJ, Roebroek ME. Cardiovascular disease risk factors and the relationships with physical activity, aerobic fitness, and body fat in adolescents and young adults with myelomeningocele. *Arch Phys Med Rehabil* 2008;89(11):2167–73.
- Rendeli C, Ausili E, Castorina M, Antuzzi D, Tabacco F, Caldarelli M. Homocysteine, folate, lipid profile and MTHFR genotype and disability in children with myelomeningocele. *Childs Nerv Syst* 2006;22(10):1316–21.
- Nelson MD, Widman LM, Abresch RT, Stanhope K, Havel PJ, Styne DM, *et al.* Metabolic syndrome in adolescents with spinal cord dysfunction. *J Spinal Cord Med* 2007;30:S127–39.
- Okurowska-Zawada B, Konstantynowicz J, Kulak W, Kaczmarek M, Piotrowska-Jastrzebska J, Sienkiewicz D, *et al.* Assessment of risk factors for osteoporosis and fractures in children with meningocele. *Adv Med Sci* 2009;54(2):247–52.
- Okurowska-Zawada B, Kozerska A, Zelazowska B, Kulak W, Wasilewska A, Wysocka J. Serum 25-Hydroxyvitamin D, osteocalcin, and parathormone status in children with meningocele. *Neuropediatrics* 2012;43(6):314–9.
- Wright JG. Neurosegmental level and functional status. In: Sarwark JF, Lubicky JD, eds. *Caring for the Child with Spina Bifida*. 2003/05/15 ed. Rosemont, IL: American Academy of Orthopaedic Surgeons; 2001:67–78.
- Barlow SE, Committee E. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. *Pediatrics* 2007;120(Suppl 4):S164–92.
- Gilsanz V, Kremer A, Wren TAL, Kremer R. Vitamin D status and its relation to muscle mass and muscle fat in young women. *J Clin Endocrinol Metab* 2010;95(4):1595–601.
- Daniels SR, Morrison JA, Sprecher DL, Khoury P, Kimball TR. Association of body fat distribution and cardiovascular risk factors in children and adolescents. *Circulation* 1999;99(4):541–5.
- Kremer R, Campbell PP, Reinhardt T, Gilsanz V. Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. *J Clin Endocrinol Metab* 2009;94(1):67–73.
- Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics* 2011;128(Suppl 5):S213–56.
- Mittelman SD, Gilsanz P, Mo AO, Wood J, Dorey F, Gilsanz V. Adiposity predicts carotid intima-media thickness in healthy children and adolescents. *J Pediatr* 2010;156(4):592–7.
- Ausili EI, Focarelli B, Tabacco F, Fortunelli G, Caradonna P, Massimi L, *et al.* Bone mineral density and body composition in

- a myelomenigocele population: effects of walking ability and sport activity. *Eur Rev Med Pharmacol Sci* **2008**;12:349–54.
- 24 Quan A, Adams R, Ekmark E, Baum M. Bone mineral density in children with myelomeningocele. *Pediatrics* **1998**;102(3):e34.
- 25 Au LE, Harris SS, Dwyer JT, Jacques PF, Sacheck JM. Association of serum 25-hydroxyvitamin D with race/ethnicity and constitutive skin color in urban schoolchildren. *J Pediatr Endocrinol Metab* **2014**;27(11-12):1095–100.
- 26 Kumar J, Muntner P, Kaskel FJ, Hailpern SM, Melamed ML. Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004. *Pediatrics* **2009**;124(3):e362–70.
- 27 Verhoef M, Barf HA, Post MW, van Asbeck FW, Gooskens RH, Prevo AJ. Secondary impairments in young adults with spina bifida. *Dev Med Child Neurol* **2004**;46(6):420–7.
- 28 Williams LJ, Rasmussen SA, Flores A, Kirby RS, Edmonds LD. Decline in the prevalence of spina bifida and anencephaly by race/ethnicity: 1995-2002. *Pediatrics* **2005**;116(3):580–6.
- 29 Shin M, Besser LM, Siffel C, Kucik JE, Shaw GM, Lu C, *et al*. Prevalence of spina bifida among children and adolescents in 10 regions in the United States. *Pediatrics* **2010**;126(2):274–9.
- 30 Berry JG, Bloom S, Foley S, Palfrey JS. Health inequity in children and youth with chronic health conditions. *Pediatrics* **2010**; 126(Suppl 3):S111–9.