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ORIGINAL ARTICLE

Effects of cholesterol and simvastatin treatment in patients with Smith–Lemli–Opitz syndrome (SLOS)

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Summary Smith–Lemli–Opitz syndrome (SLOS) is a malformation syndrome caused by deficiency of 7-dehydrocholesterol reductase catalysing the last step of cholesterol biosynthesis. This results in an accumulation of 7- and 8-dehydrocholesterol (7+8–DHC) and, in most patients, a deficiency of cholesterol. Current therapy consists of dietary cholesterol supplementation, which raises plasma cholesterol levels, but clinical effects have been reported in only a few patients. Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors were shown to reduce 7+8–DHC levels and increase cholesterol concentrations in two small trials with divergent clinical outcome. This retrolective study evaluates the effects of cholesterol only and of cholesterol plus the HMG-CoA reductase inhibitor simvastatin on plasma sterols in 39 SLOS patients and on anthropometric measures in 20 SLOS patients. Cholesterol as well as additional simvastatin decreased the plasma (7+8–DHC)/cholesterol ratio. However, the mechanism leading to the decreasing ratio was different. Whereas it was due to an increasing cholesterol concentration in the cholesterol-only cohort, a decreasing 7+8–DHC concentration was demonstrated in the cohort receiving additional simvastatin. We could not confirm a positive effect of simvastatin treatment on anthropometric measures or behaviour, as previously reported.

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Abbreviations

CK	creatine kinase
7-DHC	7-dehydrocholesterol
8-DHC	8-dehydrocholesterol
DHCR7	7-dehydrocholesterol reductase
GC-MS	gas chromatography-mass spectrometry
HMG-CoA	hydroxymethylglutaryl-coenzyme A
HR	cohort of patients with high (7+8–DHC)/cholesterol ratio
LR	cohort of patients with low (7+8–DHC)/cholesterol ratio
MAD	median absolute deviation
MSHFBA	*N*-methyl-*N*-trimethylsilylheptafluorobutyramide
SLOS	Smith–Lemli–Opitz syndrome

Introduction

Smith–Lemli–Opitz syndrome (SLOS, OMIM 270400) is an autosomal recessively inherited multiple malformation syndrome with an estimated prevalence of 1 in 60 000 (Kelley and Hennekam, 2000; Nowaczyk et al 2006) up to 1 in 10 000 (Ciara et al 2004). It is caused by a deficiency of microsomal 7-dehydrocholesterol reductase (DHCR7), converting 7-dehydrocholesterol (7–DHC) to cholesterol in the last step of cholesterol biosynthesis (Tint et al 1994). The enzyme deficiency results in an accumulation of 7–DHC and its isomer 8–DHC and, in most patients, a marked deficiency of cholesterol. The human *DHCR7* gene is located on chromosome 11q13 (Fitzky et al 1998; Wassif et al 1998; Waterham et al 1998) and more than 100 different mutations have been identified in SLOS patients so far. A correlation of the *DHCR7* genotype with the severity of the SLOS phenotype is confounded by the fact that most patients are compound heterozygotes. However, many of the most severely affected patients are homozygous or compound heterozygous for null mutations, the most common being IVS8–1G>C and W151X (Correa-Cerro et al 2005; Witsch-Baumgartner et al 2000). Clinical severity correlates best with the sum of all dehydrosterols expressed as a fraction of total sterols (Cunniff et al 1997; Kelley and Hennekam, 2000).

The clinical phenotype of SLOS is characterized by skeletal, genital and organ malformations, facial dysmorphism, failure to thrive, psychomotor retardation, behavioural abnormalities and photosensitivity. Less commonly reported problems include an increased frequency of infections and polyneuropathy. There is a wide clinical variability ranging from early death due to severe organ malformations to minimal facial abnormalities and near-normal mental development (Haas et al 2001).

With the discovery of the metabolic basis of SLOS, treatment strategies aiming to correct cholesterol deficiency by oral cholesterol supplementation were developed. Dietary cholesterol supplementation was found to increase serum cholesterol levels (Azurdia et al 2001; Irons et al 1997; Nwokoro and Mulvihill, 1997). A significant decrease of 7–DHC due to the inhibitory effect of cholesterol on HMG-CoA reductase was reported in one study (Sikora et al 2004). Observational studies suggest that cholesterol supplementation has beneficial effects on growth and behaviour, the frequency of infections (Elias et al 1997; Kelley and Hennekam, 2000; Nwokoro and Mulvihill, 1997), photosensitivity and polyneuropathy (Azurdia et al 2001; Starck et al 1999). There seems to be no effect of cholesterol supplementation on intrinsic cognitive abilities (Sikora et al 2004), most likely because cholesterol cannot be transported across the blood-brain barrier and because developmental impairment may partly be determined by irreversible prenatal cerebral maldevelopment.

As the accumulation of 7+8–DHC was thought to be the most harmful component in SLOS and was shown to be only marginally influenced by cholesterol supplementation, Jira and co-workers investigated the effect of exchange transfusions followed by treatment with simvastatin in two patients (Jira et al 2000). In both relatively mildly affected patients a rapid decrease of 7+8–DHC levels and an increase of cholesterol were observed. Mental, motor and social development as well as weight, length and head circumference also improved. However, in two more severely affected patients there was no clinical or biochemical benefit (Starck et al 2002). Instead, serious side-effects were observed. In one patient simvastatin had to be stopped because of an increase of aminotransferases and aggravated photosensitivity; the other patient showed a reversible moderate rise of creatine kinase (CK). Studies in fibroblasts demonstrated that simvastatin treatment decreased 7–DHC levels and increased cholesterol synthesis when residual DHCR7 activity was present. The increase of cholesterol synthesis was due to an increased expression of a hypomorphic *DHCR7* allele. However, in cells with two null alleles predicting no residual enzyme activity, simvastatin treatment resulted in rapid cell death (Wassif et al 2005). An increase of DHCR7 expression by simvastatin resulting in a decrease of 7+8–DHC was also demonstrated in a hypomorphic SLOS mouse model (Correa-Cerro et al 2006).

In this retrolective study (Schneider, 2001) we evaluate the effects of cholesterol supplementation and a combination of cholesterol supplementation plus simvastatin medication on biochemical parameters (plasma cholesterol, 7+8–DHC and the ratio (7+8–DHC)/cholesterol) in 39 patients and on anthropometric data in 20 patients with SLOS.

Patients and methods

Patients and treatment regimen

In all 39 patients (17 female, 22 male) SLOS was confirmed by GC-MS sterol analysis in serum (7–DHC>0.11 mg/dl, 7–DHC/cholesterol ratio>0.002).

Additional molecular analysis was performed in 31 patients and revealed mutations in both alleles of the *DHCR7* gene (Table 1). Mean baseline serum cholesterol and 7+8–DHC concentrations were 86 mg/dl, (range 9–188 mg/dl) and 23.4 mg/dl (range 4.1–58.5 mg/dl), respectively. The clinical phenotype was characterized by the scoring system of Kelley and Hennekam (Kelley and Hennekam, 2000) as mild (<20, n=31), moderate (20–35, n=7) or severe (>35, n=1). Seven of 39 patients were not able to achieve sufficient caloric intake orally and had to be tube fed (HR 4; LR 1; SIMCHOL 2). Median age at diagnosis was 27 months (range 0.03–262 months). Cholesterol supplementation or cholesterol plus simvastatin was started within the next three months after diagnosis.

Thirty-seven patients initially received dietary cholesterol, given as crystalline cholesterol in 28 patients or as egg yolk in 7 patients. Cholesterol was purchased from FAGRON GmbH&Co KG, Barsbüttel, Germany. The product meets PhEur5 specificity. Two patients were excluded from the study because of noncompliance. When crystalline cholesterol was given, a dosage of 100 mg/kg per day was recommended for children; the actual dosage given to the patients ranged from 60 to 150 mg/kg per day. For adults > 18 years, a dosage of 40 mg/kg per day was recommended and given. When cholesterol was given as egg yolk, a cholesterol dosage of 40 mg/kg per day was recommended, based on an estimate of 220 mg cholesterol per egg yolk. The cholesterol dosage given to the patients ranged from 19 to 75 mg/kg per day. The lower dosage of cholesterol when applied as egg yolk compared to the application as crystalline cholesterol, followed studies reporting better resorption of egg yolk cholesterol and greater potency in raising cholesterol levels (Linck et al 2000; Sikora et al 2004).

In mildly affected patients the positive effect of simvastatin therapy was reported to be at least equal to that of cholesterol (Jira et al 2000; Starck et al 2002). Accordingly, patients diagnosed after 01.01.2003 with a mild clinical phenotype, a ratio of (7+8–DHC)/cholesterol<0.5 and no elevations of transaminases and CK were included in the present study and given additional simvastatin immediately after diagnosis (Cohort SIMCHOL). Patients nos. 14, 62 and 63 (Table 1) were treated with cholesterol plus simvastatin before this study started.

Simvastatin was not given to patients with elevated transaminases or CK (these parameters were routinely determined in all patients), because side-effects of simvastatin include reversible increases of these parameters (Starck et al 2002). Based on the strong correlation between clinical severity and the ratio of abnormal sterols to cholesterol we hypothesized that patients with a ratio of (7+8–DHC)/cholesterol of ≥0.5, supposed to have minimal residual DHCR7 activity (severe biochemical phenotype), should be at risk for cholesterol depletion under simvastatin medication. They therefore received cholesterol only until their (7+8–DHC)/cholesterol ratio fell below 0.5. Simvastatin was started with a dosage of 0.5 mg/kg per day and increased to 1.0 mg/kg per day after 4 weeks when transaminases and CK remained normal. In all patients, additional cholesterol supplementation at the dosages mentioned above was recommended. For better comparison with the SIMCHOL cohort, patients who initially received cholesterol only were assigned into subgroups according to a high (7+8–DHC)/cholesterol ratio (≥0.5, cohort HR) or a low ratio (<0.5, cohort LR). As only LR and SIMCHOL groups were compared, Table 2 gives the composition of these cohorts.

In some of the patients initially receiving cholesterol only, simvastatin was added to the medication later. In statistical analyses of LR and HR cohorts, only data during cholesterol monotherapy were included. The median follow-up time after diagnosis was 52 months (range 6–131 months).

Sterol analysis

Analysis of plasma sterols was performed by GC-MS using a slightly modified method as published previously (Kelley, 1995). In brief, 100 µl of EDTA plasma was hydrolysed at 60°C for 60 min with degassed 4% ethanolic KOH. 5α-Cholestane (Merck KGaA, Darmstadt, Germany) was added as an internal standard. The extraction was performed with *n*-hexane and the sample was derivatized with 50 µl MSHFBA to form trimethylsilyl derivatives. For GC-MS analysis the quadrupole mass spectrometer MSD 5972A (Agilent Technologies, Palo Alto, CA, USA) was run in the ion-selective monitoring mode.

The following characteristic mass fragments were used for quantification: *m/z* 217/357 (5α-cholestane, internal standard), *m/z* 329/368 (cholesterol), *m/z* 325/351 (7–DHC), and *m/z* 325/351 (8–DHC).

Gas chromatographic separation was achieved on a capillary column (DB-5MS, 30 m×0.25 mm; df 0.25; J & W Scientific, Folsom, CA, USA) using helium as carrier gas. The initial oven temperature of 100°C was raised after 2 min to 300°C at 35°C/min. The injector was held at 280°C and the transfer line at 290°C; 1 µl of the derivatized sample was injected in a splitless mode.

Table 1 Clinical phenotype and genotype of SLOS patients

Patient no.[a]	Sex	Severity score	Cohort	Tube feeding[b]	Cholesterol preparation[c]	Diagnostic plasma sterol levels				Genotype
						Cholesterol (mg/dl)	7–DHC (mg/dl)	8–DHC (mg/dl)	Ratio[d]	
17	M	20	HR		c	24	11.0	12.7	0.99	W151X/R352W
25	F	10	HR	x	c	38	18.8	27.8	1.23	W151X/T93M
27	F	moderate	HR		c	31	16.6	17.0	1.09	n.d.[e]
31	M	10	HR		ce	23	9.9	9.2	0.83	R443C/R352W
33	M	40	HR	x	c	9	12.9	8.8	2.44	IVS8–1G>C/IVS8–1G>C
38	M	15	HR		c	46	16.2	8.6	0.54	V236L/R352W
39	F	29	HR	x	c	15	5.2	6.3	0.74	n.d.
53	M	20	HR	x	c	29	20.9	24.9	1.59	n.d.
78	F	10	HR		c	34	13.1	15.9	0.85	IVS8–1G>C/Y234C
6	F	20	LR		c	100	15.0	11.7	0.27	IVS8–1G>C/R352W
10	M	20	LR		e	172	15.8	17.7	0.19	n.d.
19	F	10	LR		e	132	11.4	6.0	0.13	n.d.
32	F	5	LR	x	ce	130	27.8	21.8	0.38	n.d.
36	M	mild	LR		c	88	6.1	5.8	0.14	IVS8–1G>C/P51S
37	F	15	LR		e	81	10.4	8.1	0.23	V236L/V236L
45	M	15	LR		c	50	6.6	7.5	0.28	c.385_412+5del33bp/F255L
55	M	10	LR		ce	48	4.2	2.1	0.13	IVS8–1G>C/R352W
56	F	5	LR		ce	132	27.0	20.1	0.36	V273G/Y234C
66	F	5	LR		c	95	16.4	15.3	0.33	n.d.
67	M	5	LR		c	154	8.0	4.8	0.08	W151X/C380S
68	M	5	LR		c	97	11.7	6.9	0.19	W151X/C380S
71	F	12	LR		c	156	30.7	17.8	0.31	T93M/del16bp
79	F	8	LR		c	95	8.9	7.2	0.17	R404C/A247V
7	F	5	LR		c	57	10.0	7.8	0.31	W151X/Y432C
11	F	10	SIMCHOL		c	78	9.2	11.7	0.27	IVS8–1G>C/C380Y
12	F	5	SIMCHOL		c	140	12.1	18.6	0.22	IVS8–1G>C/C380Y
13	M	5	SIMCHOL		c	188	10.6	17.4	0.15	IVS8–1G>C/C380Y
14	M	20	SIMCHOL		c	79	12.8	8.7	0.27	IVS8–1G>C/W182L
28	M	15	SIMCHOL	x	c	56	11.3	9.3	0.37	IVS8–1G>C/T93M
82	M	5	SIMCHOL		ce	154	7.7	6.6	0.09	P51S/H299Y/R362C

63	M	10	SIMCHOL		c	47	7.0	6.0	0.28	IVS8–1G>C/C183Y
62	F	5	SIMCHOL		c	110	2.2	2.3	0.04	IVS8–1G>C/C183Y
64	F	10	SIMCHOL		c	70	19.5	15.2	0.50	n.d.
65	M	10	SIMCHOL		c	42	7.0	6.9	0.33	IVS8–1G>C/V326L
70	M	5	SIMCHOL		c	155	2.0	2.4	0.03	IVS8–1G>C/G30A
69	M	5	SIMCHOL		c	177	4.6	4.2	0.05	IVS8–1G>C/G30A
81	M	10	SIMCHOL	x	ce	53	14.5	10.2	0.46	Y432C/del A 421/422
23	M	5	SIM			134	4.3	4.0	0.06	IVS8–1G>C/C380Y
24	M	10	SIM			134	14.3	14.7	0.22	IVS8–1G>C/C380Y

LR, patients with a (7+8–DHC)/cholesterol ratio<0.5 receiving cholesterol; HR, patients with a (7+8–DHC)/cholesterol ratio≥0.5 receiving cholesterol; SIMCHOL, patients receiving cholesterol and simvastatin

[a] Patients 67 and 68; 11, 12 and 13; 62 and 63; 69 and 70 are siblings

[b] Ratio: (7+8–DHC)/cholesterol ratio

[c] Tube feeding: at least part of the nutrition was tube-fed

[d] Cholesterol preparation: c, crystalline cholesterol; e, egg yolk; ce, crystalline cholesterol plus egg yolk

[e] n.d.: not done

Table 2 Main characteristics of the LR and SIMCHOL groups

	LR	SIMCHOL
Median age at end of study (months)	123	129
Median time in this cohort (months)	36	36
Sex (f/m)	8/6	5/9
Mean plasma cholesterol (mg/dl)	109	100
Mean plasma 7+8–DHC (mg/dl)	25.2	18.4
Mean ratio (7+8–DHC)/cholesterol	0.23	0.18
Median cholesterol dosage (mg/kg per day)	90	80

Statistical analysis

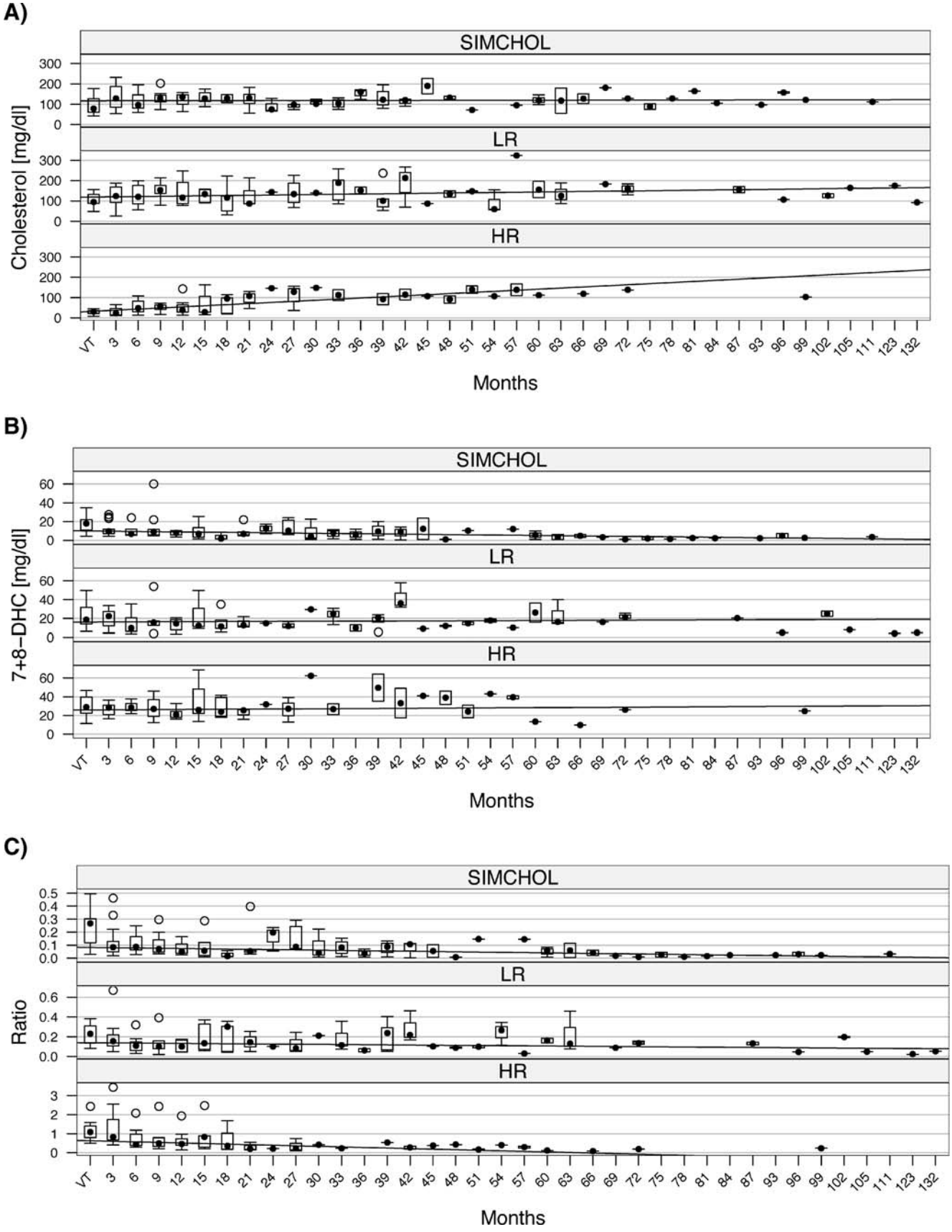
Biochemical data

Because of the retrolective nature of this study, the number of medical examinations as well as the amount of data available for cholesterol and 7+8–DHC concentrations and anthropometric data differed between patients. To achieve equidistant time intervals of measurements, we computed the averages for cholesterol and 7+8–DHC levels in 3-month intervals for each patient. To test a significant change of cholesterol, 7+8–DHC or the ratio of (7+8–DHC)/cholesterol over time, we calculated a nonparametric multiple median regression (Siegel, 1982). This approach is suitable for a repeated-measures design based on ordinal data. Nonparametric *U*-statistic was used to test significance. Although all models were computed with an intercept, we only report the slope of the regression line as an estimate for a change of cholesterol, 7+8–DHC or the ratio of (7+8–DHC)/cholesterol over time.

Anthropometric data

Standard deviation (SD) scores were calculated for weight (20 patients) and length/height (14 patients), according to the LMS method of Cole (1990) for patients ≤18 years old. Data for LMS values of weight and height of German children were kindly provided by K. Kromeyer-Hauschild (Institute for Human Genetics and Anthropology, University of Jena, Germany, personal communication) and calculated according to Kromeyer-Hauschild (Kromeyer-Hauschild et al 2001). SD scores for head circumference were calculated according to Prader (Prader et al 1988) for patients ≤15 years.

To characterize the gradient of the anthropometric data, a two-step procedure was applied. (1) A median-based linear regression was computed for each patient when at least three anthropometric measurements


A)
SIMCHOL
LR
HR
Cholesterol [mg/dl]
Months
B)
SIMCHOL
LR
HR
7+8–DHC [mg/dl]
Months
C)
SIMCHOL
LR
HR
Ratio
Months

◀**Fig. 1** Plasma concentrations of cholesterol (**A**), 7+8–DHC (**B**) and the ratio of (7+8–DHC)/cholesterol (**C**) over time in newly diagnosed patients. LR, patients with a (7+8–DHC)/cholesterol ratio<0.5 receiving cholesterol; HR, patients with a (7+8–DHC)/cholesterol ratio≥0.5 receiving cholesterol; SIMCHOL, patients receiving cholesterol and simvastatin; VT, before therapy. Sterol data were not included in this figure if patients changed from cholesterol only to a combination with simvastatin. Dots represent the median, boxes the 25th and 75th centiles. The whiskers represent 1.5 times the deviation from the 25th and 75th centiles. Values outside the whiskers are defined as outliers and are represented by open symbols. The line expresses a median regression. Cholesterol concentrations (**A**) increased significantly in LR patients (slope 1.25, $p<0.001$), but not in SIMCHOL patients (slope 0.14, $p=0.26$). A significant decrease of 7+8–DHC was seen in the SIMCHOL cohort (slope −0.24, $p<0.001$; (**B**) but not in the LR cohort (slope −0.08, $p=0.58$). Decrease of the fraction of abnormal sterols in relation to cholesterol (**C**) was significant in both LR and SIMCHOL cohorts (LR, slope −0.002, $p<0.001$, SIMCHOL, slope −0.002, $p<0.001$). In HR patients cholesterol concentration increased significantly (slope 5.44, $p<0.001$), but there was a slight, nonsignificant increase (slope 0.12, $p=0.11$) of 7+8–DHC. The ratio, however, decreased significantly (slope −0.029, $p<0.001$)

spanning more than six months were available. (2) According to the slope and p-value of the regression line, the change of anthropometric data was coded as follows: when the p-value was not significant, no change in anthropometric development was assumed; when the p-value was significant, a positive slope indicated an increase of anthropometric variables over time, whereas a negative slope defined a decrease of anthropometric variables over time. For each cohort, we report the frequency of positive, negative or no change over time. To assess whether changes of anthropometric parameters were significant between cohorts we used Fisher's exact test for count data.

In all statistical tests, a probability level of $p\leq0.05$ was taken as significant.

Results

Clinical severity, diagnostic sterol levels and genotypes of 39 SLOS patients are presented in Table 1. Of the 23 patients who received cholesterol, 9 were classified as HR, 14 as LR. Fourteen patients received a combination of cholesterol and simvastatin as primary treatment, forming the cohort SIMCHOL. Two patients (nos. 23 and 24) received simvastatin only, because of noncompliance, and therefore were excluded from statistical analyses.

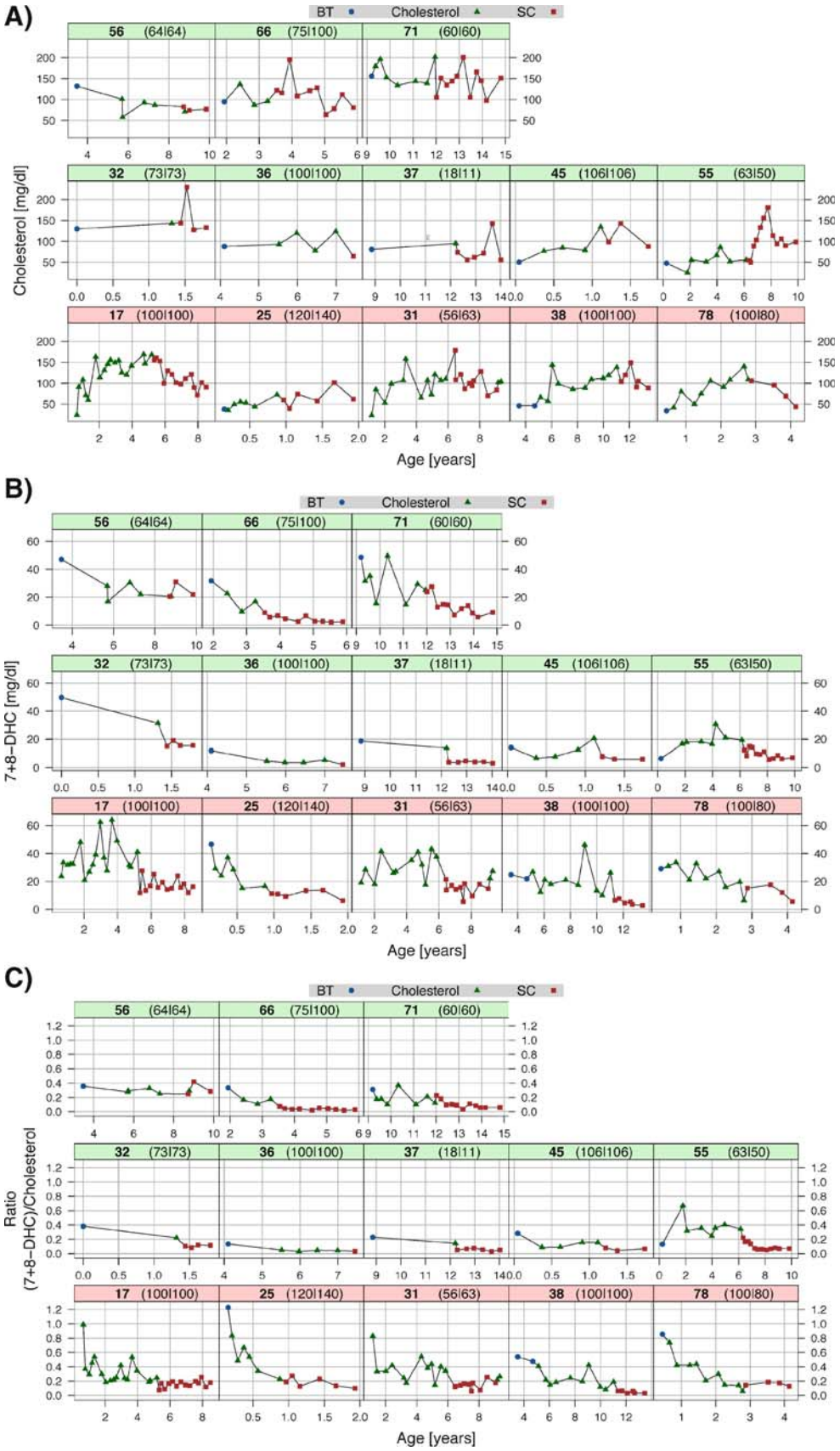
Because the dosage of cholesterol varied between patients, we calculated the mean cholesterol dosage in all cohorts, which was 77 mg/dl in the LR group (SD 30, range 20–120 mg/dl) and 79 mg/kg per day (SD 39, range 40–180 mg/dl) in the SIMCHOL group. There was no significant difference in the cholesterol dosage given to SIMCHOL patients compared to LR patients ($p=0.83$, Wilcoxon rank sum test with continuity correction). In the HR group, cholesterol dosage was higher (101 mg/kg per day, SD 17, range 70–130). This was marginally nonsignificant ($p=0.08$, Wilcoxon rank sum test with continuity correction). However, because this group is in many other ways different from the LR and SIMCHOL groups, only these cohorts were compared and the results of the HR patients are presented separately.

Biochemical data

Figure 1 demonstrates plasma concentrations of cholesterol and 7+8–DHC as well as their ratio over time of treatment in newly diagnosed patients. Cholesterol concentrations increased in all cohorts (Fig. 1A) and were significant in LR patients (slope 1.25, $p<0.001$), but not in SIMCHOL patients (slope 0.14, $p=0.26$). A significant decrease of 7+8–DHC was seen in the SIMCHOL cohort (slope −0.24, $p<0.001$, Fig. 1B) but not in the LR cohort (slope 0.08, $p=0.58$). Decrease of the fraction of abnormal sterols in relation to cholesterol was significant in all cohorts (Fig. 1C). The LR and SIMCHOL cohorts showed similar reductions (LR, slope −0.002, $p<0.001$; SIMCHOL, slope −0.002, $p<0.001$).

In HR patients, cholesterol supplementation significantly increased the cholesterol concentration (slope 5.44, $p<0.001$) but there was also a mild, nonsignificant increase of 7+8–DHC (slope 0.12, $p=0.11$). Reduction of the ratio was more prominent in HR patients (slope −0.029, $p<0.001$) than in the LR and SIMCHOL patients.

We then examined whether simvastatin therapy had any additional effect on sterol levels when it was given to patients who primarily received cholesterol only. Mean cholesterol doses were similar before and during simvastatin medication (80 mg/kg per day versus 81 mg/kg per day). The dosages for individual patients varied slightly, as shown in Fig. 2. These figures show the course of cholesterol, 7+8–DHC and their ratio in plasma of 13 patients who originally were assigned to the HR (5 patients) and LR (8 patients) cohorts and later switched to a combination of cholesterol and simvastatin. Statistical analysis of the whole group shows that addition of simvastatin resulted in a significant decrease of median 7+8–DHC and the ratio, but also of cholesterol (Table 3, 'Total'). However, the individual sterol profiles give the impression of a decrease of 7+8–DHC and their ratio


A)
BT Cholesterol SC
56 (64|64)
66 (75|100)
71 (60|60)
32 (73|73)
36 (100|100)
37 (18|11)
45 (106|106)
55 (63|50)
17 (100|100)
25 (120|140)
31 (56|63)
38 (100|100)
78 (100|80)
Cholesterol [mg/dl]
Age [years]
B)
BT Cholesterol SC
7+8−DHC [mg/dl]
Age [years]
C)
BT Cholesterol SC
Ratio (7+8−DHC)/Cholesterol
Age [years]

◀**Fig. 2** The course of plasma cholesterol (**A**) and 7+8–DHC concentration (**B**) as well as the ratio of (7+8–DHC)/cholesterol (**C**) over time in individual patients who first received cholesterol only (red: HR; green: LR) and later switched to a combination of cholesterol and simvastatin. Blue dots represent values before treatment was started (BT), green triangles represent cholesterol supplementation and red squares a combination of cholesterol and simvastatin (SC). Cholesterol doses of individual patients before and during simvastatin medication are given in the heading of each graph in parentheses after the patients' ID

mainly in LR patients and not in HR patients. We therefore did a separate statistical analysis for LR and HR patients (Table 3) and indeed found a significant decrease of 7+8–DHC and their ratio only in the LR cohort.

Figure 3 shows the changes of anthropometric variables. Two patients in the LR cohort showed a significant increase of the weight-SD score, whereas this was only seen in one of the SIMCHOL patients. A significant decrease of the weight-SD score was seen in two of the SIMCHOL patients, but not in any patient in the LR cohort. There were two patients with an increasing and one with a decreasing growth-SD score in the LR cohort, whereas the growth-SD score did not increase in any SIMCHOL patient and decreased in two. Three LR patients showed an increase of the SD score for head circumference, whereas there was no significant change of head circumference in the SIMCHOL cohort. In the HR group, changes of weight and growth-SD scores were similar to those in the LR cohort. However, the described changes of anthropometric parameters were not significant between cohorts (weight p=0.44, height p=0.52, head circumference p=0.20; Fisher's exact test for count data, two-sided).

Side-effects

There were no side-effects reported in the cohorts receiving cholesterol monotherapy (cohorts LR and HR).

Six patients who received additional simvastatin reported side-effects, which necessitated a reduction or discontinuation of the medication. In patient 10, elevated aminotransferases (AST 200 U/L, normal<35) were seen after 4 weeks of simvastatin treatment and returned to normal when simvastatin was discontinued.

Three patients (nos. 19, 64, 69) developed severe sleep disturbance under simvastatin treatment at a dosage of 1 mg/kg per day. In patient 64, sleep became worse when simvastatin was increased from 0.5 to 1 mg/kg per day. Discontinuation of simvastatin resolved the sleeping problem. Reintroduction of simvastatin 4 months later at a dosage of 0.5 mg/kg per day was well tolerated, but increase to 1 mg/kg per day resulted in insomnia up to 48 h. Shortly after discontinuation of simvastatin, sleep clearly improved and parents refused another reintroduction. One week after starting simvastatin at a dosage of 0.5 mg/kg per day, patient 19 became increasingly anxious and developed dysphylaxia. Because there was no improvement during the next two weeks, simvastatin was discontinued, resulting in a resolution of sleep and behavioural problems. Parents did not wish a reintroduction of simvastatin. In patient 69, mild sleep problems had been known for a long time. They deteriorated after introduction of simvastatin and did not respond to benzodiazepines. An improvement was seen when simvastatin was discontinued and promethazine was added. A reintroduction of simvastatin was well tolerated.

After 4 years of cholesterol supplementation, patient 31 became increasingly autoaggressive and showed self-mutilating behaviour (head banging, pulling of his ear lobes, picking his eyes). Under the hypothesis that the elevation of cholesterol precursors might contribute to behavioural abnormalities, simvastatin therapy was started. Clinically there was no persistent improvement of the autoaggressiveness and in the further course periods with severe self mutilating behaviour became longer and more severe. After 3 years simvastatin therapy was discontinued during a period of severe autoaggressiveness and an immediate improvement of behaviour was observed. Although self-mutilation did not stop completely, his handling was facilitated and he appeared to be more alert.

Patient 65, diagnosed at the age of 21 years, lived in a nursing home, where his handling became increasingly difficult because of self-mutilating behaviour. Feeding was very problematic and he was massively underweight (BMI 13.9). After starting a combined medication of simvastatin at a dosage of 0.3 mg/kg per day and cholesterol, he showed more social interaction and self-mutilation was clearly reduced. In addition, he showed significant weight gain (BMI 18.3 18 months after start of the medication). When simvastatin was increased to 0.5 mg/kg per day, the autoaggressive behaviour recurred but immediately improved after reducing simvastatin to 0.25 mg/kg per day.

Discussion

This is the first study analysing the effects of cholesterol monotherapy versus a combined therapy of cholesterol plus simvastatin in a large group of patients. Biochemically, cholesterol supplementation as well as additional simvastatin resulted in a decrease

Table 3 Concentration of cholesterol and 7+8–DHC in mg/dl as well as the ratio of (7+8–DHC)/cholesterol in patients who primarily had cholesterol only (classified as HR or LR) and later received additional simvastatin

Metabolite	Treatment	Cohort					
		Total (n=13)		HR (n=5)		LR (n=8)	
Cholesterol (mg/dl)	C	110	p=0.008	110	p=0.06	110	p=0.08
	SC	84		84		84	
7+8–DHC (mg/dl)	C	20.6	p<0.001	26.2	p=0.06	20.0	p=0.008
	SC	6.2		6.2		6.4	
Ratio	C	0.19	p=0.005	0.23	p=0.19	0.16	p=0.02
	SC	0.07		0.13		0.06	

We compared sterol concentrations determined at the last medical examination during cholesterol only (C) and before the end of the study during simvastatin and cholesterol (SC). p-Values are given for each comparison (Wilcoxon signed rank test with continuity correction)

of the plasma (7+8–DHC)/cholesterol ratio when the medication was started immediately after diagnosis. The effect on the ratio in the LR cohort was identical to that in the SIMCHOL cohort, indicating that initially additional simvastatin does not improve the ratio more than cholesterol monotherapy in mildly affected patients. However, the mechanism leading to the decreasing ratio was different in the LR and the

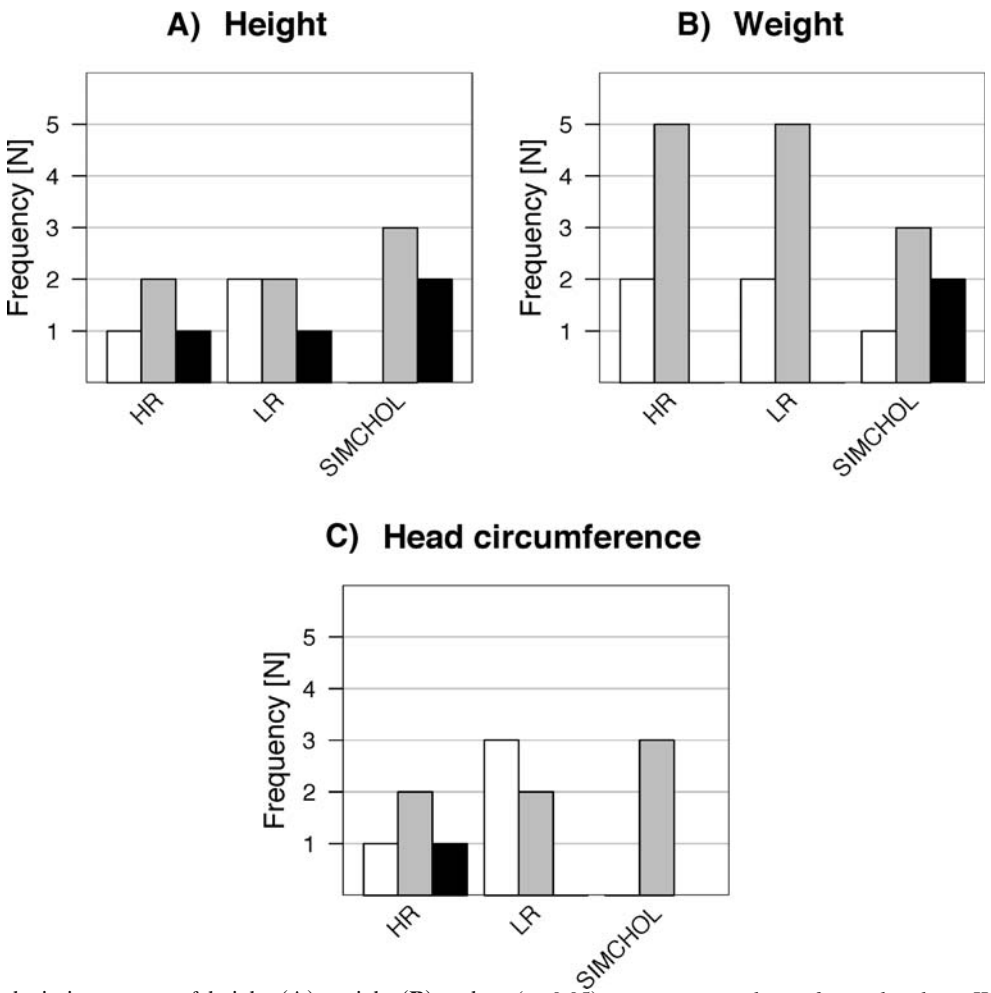


Fig. 3 Standard deviation scores of height (**A**), weight (**B**) and head circumference (**C**) were calculated and the frequency of patients in whom significant changes of anthropometric data ($p \leq 0.05$) were seen are shown for each cohort. White bars show a significant increase, grey bars represent no change and black bars show a significant decrease of the anthropometric parameter

SIMCHOL cohorts. In the LR cohort it was based on an increasing cholesterol concentration, whereas in the SIMCHOL cohort it was due to a decreasing 7+8–DHC concentration. It is also remarkable that additional simvastatin given to patients who already received cholesterol for a longer period (previously classified as HR or LR patients) resulted in a further significant reduction of the ratio, which was mediated by a clear reduction of 7+8–DHC even though the cholesterol concentration also significantly declined. It seems likely that the reduction of the ratio caused by cholesterol monotherapy is limited because, once normal cholesterol concentrations are achieved, a further decrease of the ratio can only be obtained by reducing 7+8–DHC.

The observation that cholesterol concentration did not increase significantly in the SIMCHOL cohort and even decreased in patients switching from cholesterol-only to additional simvastatin is somewhat surprising. It was previously reported that simvastatin increases *DHCR7* expression (Wassif et al 2005), which leads to a rise of cholesterol concentrations in plasma and CSF in mildly affected SLO patients (Jira et al 2000). Augmented residual cholesterol synthesis was assumed to be mediated by sterol regulatory element binding proteins (SREBP). The slight and nonsignificant increase of cholesterol concentration in our SIMCHOL cohort could be explained by the fact that it was already within the normal range before simvastatin was administered. Therefore, the activation of SREBP by simvastatin could be reversed by feedback inhibition. In addition, the SIMCHOL patients who showed the least increase of cholesterol concentration had the lowest levels of 7+8–DHC; therefore, activation of DHCR7 would have a lesser impact in those than in patients with higher levels of abnormal sterols. The significant decrease of cholesterol concentration in patients switching from cholesterol monotherapy to a combination with simvastatin could be due to the selection of patients. Five of them (38%) were HR patients, who initially were not eligible for simvastatin therapy. After improvement of their sterol pattern during cholesterol supplementation, we decided to start them on simvastatin medication. As is shown in Fig. 2, cholesterol concentration decreased in all HR patients, whereas this was not the case in LR patients. However, there was great variability, so that the decrease of median cholesterol concentration was not significant and similar in both groups. In the HR patients the reduction of 7+8–DHC and the ratio was also not significant, whereas it was significant in LR patients. It is likely that the sterol pattern after cholesterol supplementation does not reflect the true capacity of DHCR7 in HR patients and they probably did not have enough residual enzyme activity to achieve relevant upregulation. This highlights that there is no improvement of sterol pattern in patients with a severe biochemical phenotype and they are even at risk of cholesterol depletion on simvastatin therapy.

Although the cohorts are in many ways different and cannot be compared directly, it is worth mentioning that biochemically more severely affected patients (cohort HR) showed a greater increase of cholesterol and a greater improvement of the ratio than mildly affected patients (cohort LR). The most obvious reason would be that this group received higher cholesterol doses, but this was not significant. It can also be speculated that a near-normal cholesterol concentration, as seen in our LR patients, would downregulate SREBP activation, resulting in decreased endogenous cholesterol synthesis; the effect of exogenous cholesterol would therefore partly be nullified by this mechanism. In the HR patients, cholesterol synthesis remains maximally upregulated owing to the persistent cholesterol deficiency. Although their biochemical phenotype is more severe than that of the LR patients, most of them still have some residual DHCR7 activity, so that the combination of exogenous cholesterol and upregulation of the biosynthetic pathway results in an increased cholesterol concentration.

We could not confirm a positive effect of simvastatin treatment on anthropometric measurements, previously reported in two patients treated with simvastatin for 14 and 23 months (Jira et al 2000). Indeed, development of height, weight and head circumference on average was less favourable in the SIMCHOL cohort than in the LR cohort. Although the differences between the two cohorts were not nearly significant, we cannot exclude that simvastatin treatment has a negative rather than a positive effect on physical development in SLOS patients with a mild biochemical phenotype. In addition, several patients on simvastatin treatment showed severe sleeping or behavioural problems that improved after discontinuation of simvastatin. As the presence of these effects was not ascertained in a systematic fashion, we cannot state with certainty that they represent true side-effects of simvastatin treatment in SLOS patients. Future prospective studies should use standardized instruments to document behavioural changes in the different treatment cohorts.

Several limitations of the current study are worthy of discussion, most importantly the varying dosage of cholesterol, which is due to the retrolective nature of a

study conducted in different centres. In addition, we chose different dosages for cholesterol given as egg yolk or as crystalline cholesterol. When we designed the study in 2002 we assumed a better resorption and a higher potency in raising cholesterol levels of egg yolk cholesterol (Linck et al 2000; Sikora et al 2004). Three years later, Lin and colleagues found no relevant difference between the absorption of cholesterol in the two different preparations (Lin et al 2005), so that our six patients receiving egg yolk cholesterol in lower dosage might have had a disadvantage. However, the mean cholesterol dosages used in the LR cohort and in the SIMCHOL cohort were not significantly different, allowing a comparison between these groups.

In conclusion, we cannot confirm a positive effect of simvastatin on behaviour in SLOS patients with a mild biochemical phenotype. Planned prospective studies are needed to evaluate the safety and efficacy of simvastatin in this condition.

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References

Azurdia RM, Anstey AV, Rhodes LE (2001) Cholesterol supplementation objectively reduces photosensitivity in the Smith–Lemli–Opitz syndrome. *Br J Dermatol* **144**: 143–145.

Ciara E, Nowaczyk MJ, Witsch-Baumgartner M, et al (2004) DHCR7 mutations and genotype–phenotype correlation in 37 Polish patients with Smith–Lemli–Opitz syndrome. *Clin Genet* **66**: 517–524.

Cole TJ (1990) The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* **44**: 45–60.

Correa-Cerro LS, Wassif CA, Waye JS, et al (2005) DHCR7 nonsense mutations and characterisation of mRNA nonsense mediated decay in Smith–Lemli–Opitz syndrome. *J Med Genet* **42**: 350–357.

Correa-Cerro LS, Wassif CA, Kratz L et al (2006) Development and characterization of a hypomorphic Smith-Lemli-Opitz syndrome mouse model and efficacy of simvastatin therapy. *Hum Mol Genet* **15**: 839-851.

Cunniff C, Kratz LE, Moser A, Natowicz MR, Kelley RI (1997) Clinical and biochemical spectrum of patients with RSH/Smith–Lemli–Opitz syndrome and abnormal cholesterol metabolism. *Am J Med Genet* **68**: 263–269.

Elias ER, Irons MB, Hurley AD, Tint GS, Salen G (1997) Clinical effects of cholesterol supplementation in six patients with the Smith–Lemli–Opitz syndrome (SLOS). *Am J Med Genet* **68**: 305–310.

Fitzky BU, Witsch-Baumgartner M, Erdel M, et al (1998) Mutations in the Delta7-sterol reductase gene in patients with the Smith–Lemli–Opitz syndrome. *Proc Natl Acad Sci USA* **95**: 8181–8186.

Haas D, Kelley RI, Hoffmann GF (2001) Inherited disorders of cholesterol biosynthesis. *Neuropediatrics* **32**: 113–122.

Irons M, Elias ER, Abuelo D, et al (1997) Treatment of Smith–Lemli–Opitz syndrome: results of a multicenter trial. *Am J Med Genet* **68**: 311–314.

Jira PE, Wevers RA, de Jong J, et al (2000) Simvastatin. A new therapeutic approach for Smith–Lemli–Opitz syndrome. *J Lipid Res* **41**: 1339–1346.

Kelley RI (1995) Diagnosis of Smith–Lemli–Opitz syndrome by gas chromatography/mass spectrometry of 7-dehydrocholesterol in plasma, amniotic fluid and cultured skin fibroblasts. *Clin Chim Acta* **236**: 45–58.

Kelley RI, Hennekam RC (2000) The Smith–Lemli–Opitz syndrome. *J Med Genet* **37**: 321–335.

Kromeyer-Hauschild K, Wabitsch M, Kunze D, et al (2001) Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Monatsschr Kinderheilkd* **149**: 807–818.

Lin DS, Steiner RD, Flavell DP, Connor WE (2005) Intestinal absorption of cholesterol by patients with Smith-Lemli-Opitz syndrome. *Pediatr Res* **57**: 765-770.

Linck LM, Lin DS, Flavell D, Connor WE, Steiner RD (2000) Cholesterol supplementation with egg yolk increases plasma cholesterol and decreases plasma 7-dehydrocholesterol in Smith–Lemli–Opitz syndrome. *Am J Med Genet* **93**: 360–365.

Nowaczyk MJ, Waye JS, Douketis JD (2006) DHCR7 mutation carrier rates and prevalence of the RSH/Smith–Lemli–Opitz syndrome: where are the patients? *Am J Med Genet A* **140**: 2057–2062.

Nwokoro NA, Mulvihill JJ (1997) Cholesterol and bile acid replacement therapy in children and adults with Smith–Lemli–Opitz (SLO/RSH) syndrome. *Am J Med Genet* **68**: 315–321.

Prader A, Largo RH, Molinari L, Issler C (1988) Physical growth of Swiss children from birth to 20 years of age. *Helv Paediatr Acta* **43**: 58–59.

Schneider B (2001) Analysis of therapeutic efficacy in observational cohort studies. *Cancer Chemother Pharmacol* **47**(Suppl): S35–S37.

Siegel AF (1982) Robust regression using repeated medians. *Biometrika* **69**: 242–244.

Sikora DM, Ruggiero M, Petit-Kekel K, Merkens LS, Connor WE, Steiner RD (2004) Cholesterol supplementation does not improve developmental progress in Smith–Lemli–Opitz syndrome. *J Pediatr* **144**: 783–791.

Starck L, Bjorkhem I, Ritzen EM, Nilsson BY, von Dobeln U (1999) Beneficial effects of dietary supplementation in a disorder with defective synthesis of cholesterol. A case report of a girl with Smith–Lemli–Opitz syndrome, polyneuropathy and precocious puberty. *Acta Paediatr* **88**: 729–733.

Starck L, Lovgren-Sandblom A, Bjorkhem I (2002) Simvastatin treatment in the SLO syndrome: a safe approach? *Am J Med Genet* **113**: 183–189.

Tint GS, Irons M, Elias ER, et al (1994) Defective cholesterol biosynthesis associated with the Smith–Lemli–Opitz syndrome. *N Engl J Med* **330**: 107–113.

Wassif CA, Maslen C, Kachilele-Linjewile S, et al (1998) Mutations in the human sterol delta7-reductase gene at 11q12–13 cause Smith–Lemli–Opitz syndrome. *Am J Hum Genet* **63**: 55–62.

Wassif CA, Krakowiak PA, Wright BS, et al (2005) Residual cholesterol synthesis and simvastatin induction of cholesterol synthesis in Smith–Lemli–Opitz syndrome fibroblasts. *Mol Genet Metab* **85**: 96–107.

Waterham HR, Wijburg FA, Hennekam RC, et al (1998) Smith–Lemli–Opitz syndrome is caused by mutations in the 7-dehydrocholesterol reductase gene. *Am J Hum Genet* **63**: 329–338.

Witsch-Baumgartner M, Fitzky BU, Ogorelkova M, et al (2000) Mutational spectrum in the Delta7-sterol reductase gene and genotype–phenotype correlation in 84 patients with Smith–Lemli–Opitz syndrome. *Am J Hum Genet* **66**: 402–412.