Supplement

Activated L-arginine/nitric oxide pathway in pediatric cystic fibrosis and its association with pancreatic insufficiency, liver involvement and nourishment: overview and new results

Folke Brinkmann [1,†], Beatrice Hanusch [1,†,*], Manfred Ballmann [1,2], Sebene Mayorandan [3,4], Alexander Bollenbach [5], Kristine Chobanyan-Jürgens [3,6], Kathrin Jansen [1], Anjona Schmidt-Choudhury [1], Nico Derichs [3,7], Dimitrios Tsikas [5,‡] and Thomas Lücke [1,‡]

[1] University Children's Hospital, Ruhr University, 44791 Bochum, Germany; folke.brinkmann@rub.de (F.B.); manfred.ballmann@med.uni-rostock.de (M.B.); kathrin.jansen@rub.de (K.J.); a.schmidt-choudhury@klinikum-bochum.de (A.S.-C.); luecke.thomas@rub.de (T.L.)

[2] Current address: Paediatric Clinic, University Medicine Rostock, 18057 Rostock, Germany.

[3] Department of Paediatrics, Hannover Medical School, 30623 Hannover, Germany; Sebene.Mayorandan@ukmuenster.de (S.M.); Kristine.Chobanyan-Juergens@med.uni-heidelberg.de (K.C.-J.); arzt@kinderpneumologie-derichs.de (N.D.)

[4] Current address: Department of Paediatrics, University Clinic Münster, 48149 Münster, Germany.

[5] Institute of Toxicology, Core Unit Proteomics, Hannover Medical School, 30623 Hannover, Germany; bollenbach.alexander@mh-hannover.de (A.B.); tsikas.dimitros@mh-hannover.de (D.T.)

[6] Current address: Department of Clinical Pharmacology and Pharmacoepidemiology, Heidelberg University Hospital, 69120 Heidelberg, Germany, and Department of General Pediatrics, Neuropediatrics, Metabolism, Gastroenterology, Nephrology, Center for Pediatric and Adolescent Medicine, University Hospital Heidelberg, 69120 Heidelberg, Germany, and Pediatric Clinical-Pharmacological Trial Center (paedKliPS), Center for Pediatric and Adolescent Medicine, University Hospital Heidelberg, 69120 Heidelberg, Germany.

[7] Current address: KinderPneumologieDerichs, Pediatric Pneumology and Allergology, CFTR & Pulmonary Research Center, 30173 Hannover, Germany.

* Correspondence: beatrice.hanusch@rub.de; Tel.: +49-234-5092615
† Shared first authors
‡ Shared senior authors

Table S1. Anthropometric and clinical characteristics of cystic fibrosis (CF) patients

	CF patients
Number of subjects (*n*)	70
Height [percentile]	36.5 [13.9 – 60.9]
BMI [percentile]	41.8 ± 27.8
Shwachmann-Score	75.0 [70.0 – 75.0]
FEV1 [%]	90.5 ± 19.1
MEF25 [%]	59.5 [32.7 – 76.3]
Crispin-Norman-Score	4.5 [3.0 – 9.0]
FENO [ppb]	8.3 [5.9 – 12.9]
Fischer-Quotient	3.3 ± 0.6
Prothrombin time (%)	84.0 ± 13.2
Cholesterol [mM]	3.4 ± 0.7
Triglycerides [mM]	1.1 [0.9 – 1.6]
Urea [mM]	4.4 ± 1.1
Pancreas sufficient [*n* (%)]	10 (14.3)
P. aeruginosa negative [*n* (%)]	49 (70.0)
ABPA [*n* (%)]	6 (8.6)
Acute infect [*n* (%)]	24 (34.3)
Steroid treatment [*n* (%)]	16 (22.9)
Liver involvement [*n* (%)]	8 (11.4)

Abbreviations. BMI, body-mass-index; FEV1%, Forced expiratory volume in 1 s; MEF25%, mid expiratory flow 25%; FENO, fractional exhaled nitric oxide; ABPA, allergic bronchopulmonary aspergillosis.
Data are reported as median [25-75 percentile] (non-normal distribution) or as mean ± standard deviation (normal distribution).

Table S2. Anthropometric and clinical characteristics of cystic fibrosis (CF) patients according to their pancreas sufficiency.

	CF patients		*p*
	Insufficient pancreas	Sufficient pancreas	
Number of subjects (*n*)	60	10	-
Height [percentile]	39.2 [15.0 – 65.7]	30.8 [11.8 – 44.2]	0.47
BMI [percentile]	39.6 ± 27.0	55.4 ± 30.2	0.10
Shwachmann-Score	75.0 [70.0 – 75.0]	75.0 [73.8 – 75.0]	0.25
FEV1 [%]	89.6 ± 19.9	95.8 ± 13.3	0.35
MEF25 [%]	59.0 [38.0 – 69.5]	76.0 [30.8 – 113]	0.24
Crispin-Norman-Score	4.50 [3.00 – 9.00]	4.50 [1.75 – 8.50]	0.58
FENO [ppb]	8.30 [5.90 – 13.0]	8.95 [5.33 – 14.7]	0.93
Fischer-Quotient	3.35 ± 0.59	3.08 ± 0.60	0.20
Prothrombin time (%)	**82.6 ± 13.0**	**92.3 ± 11.8**	**0.03**
Cholesterol [mM]	**3.30 ± 0.70**	**4.11 ± 0.50**	**0.01**
Triglycerides [mM]	1.05 [0.84 – 1.56]	1.19 [1.06 – 1.98]	0.22
Urea [mM]	4.45 ± 1.06	4.20 ± 1.52	0.49[#]
P. aeruginosa negative [n (%)]	40 (66.7)	9 (90.0)	0.26[+]
ABPA [*n* (%)]	5 (8.30)	1 (10.0)	1.00[+]
Acute infect [*n* (%)]	21 (35.0)	3 (30.0)	1.00[+]
Steroid treatment [*n* (%)]	14 (23.3)	2 (20.0)	1.00[+]

Abbreviations. BMI, body-mass-index; FENO, fractional exhaled nitric oxide; FEV1%, Forced expiratory volume in 1 s; MEF25%, mid expiratory flow 25%; ABPA, allergic bronchopulmonary aspergillosis.
Data are reported as median [25-75 percentile] (non-normal distribution) or as mean ± standard deviation (normal distribution). [#] Unequal variance t-test; [+] Fisher's exact test; Significant results are marked in bold.

Table S3. Anthropometric and clinical characteristics of cystic fibrosis (CF) patients according to the involvement of liver function.

	CF patients		p
	Liver involvement	No liver involvement	
Number of subjects (*n*)	8	62	-
Height [percentile]	17.5 [6.90 – 63.7]	38.8 [18.0 – 60.9]	0.30
BMI [percentile]	39.1 (± 26.6)	42.2 (± 28.2)	0.77
Shwachmann-Score	72.5 [70.0 – 75.0]	75.0 [70.0 – 75.0]	0.38
FEV1 [%]	83.4 ± 24.7	91.4 ± 18.4	0.27
MEF25 [%]	35.8 [18.0 – 64.3]	61.0 [38.0 – 80.5]	0.08
Crispin-Norman-Score	6.50 [3.00 – 16.0]	4.00 [2.25 – 9.00]	0.34
FENO [ppb]	6.15 [4.00 – 11.7]	8.50 [6.10 – 13.4]	0.11
Fischer-Quotient	3.31 ± 0.57	3.31 ± 0.60	1.00
CHE [kU/L]	**8.11 ± 1.95**	**9.67 ± 1.38**	**0.01**
Albumin [g/L]	41.0 [33.5– 43.0]	41.0 [39.0 – 43.0]	0.66
Prothrombin time (%)	**73.8 ± 20.4**	**85.34 ± 11.55**	**0.02**
Cholesterin [mM]	**2.78 ± 0.47**	**3.49 ± 0.72**	**0.02**
Triglyceride [mM]	1.14 [0.85 – 2.27]	1.07 [0.86 – 1.65]	0.67
Urea [mM]	3.70 ± 0.67	4.50 ± 1.15	0.06
P. aeruginosa negative [n (%)]	5 (62.5)	44 (71.0)	0.69[+]
ABPA [*n* (%)]	0 (0.00)	6 (9.70)	1.00[+]
Acute infect [*n* (%)]	4 (50.0)	20 (32.3)	0.43[+]
Steroid treatment [*n* (%)]	1 (12.5)	15 (24.2)	0.67[+]

Abbreviations. BMI, body-mass-index; FEV1%, Forced expiratory volume in 1 s; MEF25%, mid expiratory flow 25%; FENO, fractional exhaled nitric oxide; CHE, choline esterase; GFR, glomerular filtration rate; ABPA, allergic bronchopulmonary aspergillosis.

Data are reported as median [25-75 percentile] (non-normal distribution) or as mean ± standard deviation (normal distribution). [+] Fisher's exact test; Significant results are marked in bold.

Table S4. Anthropometric characteristics and biochemical parameters in plasma (P) and urine (U) of the cystic fibrosis patients according to the nutritional status [a]

	CF patients		*p*
	Nutritional failure[a]	Sufficiently nourished[b]	
Number of subjects (*n*)	11	59	-
Age [years]	13.2 [10.2 – 14.2]	11.1 [8.04 – 14.0]	0.18
Male [*n* (%)]	3 (27.3)	27 (45.8)	0.31[+]
Arg (P) [μM]	88.2 ± 16.8	90.7 ± 21.2	0.72
Citrulline (P) [μM]	33.0 [25.0 – 37.0]	37.0 [29.8 – 43.0]	0.22
ADMA (P) [μM]	0.68 ± 0.11	0.60 ± 0.12	0.06
Arg/ADMA (P)	**130 ± 19.1**	**151 ± 28.2**	**0.02**
Nitrate (P) [μM]	44.9 [37.3 – 58.4]	42.5 [37.5 – 51.6]	0.38
Nitrite (P) [μM]	2.03 [1.85 – 2.34]	2.08 [1.86 – 2.28]	0.57
$P_{NOx}R$	22.4 [19.4 – 25.7]	20.7 [16.8 – 24.3]	0.19
ADMA (U) [μM/mM creatinine]	8.93 [7.12 – 10.3]	7.74 [5.69 – 9.85]	0.21
DMA (U) [μM/mM creatinine]	56.5 [52.3 – 65.6]	58.3 [46.4 – 70.9]	0.93
DMA/ADMA (U)	7.31 [6.05 – 7.79]	7.69 [6.42 – 8.98]	0.19
Nitrat (U) [μM/mM creatinine]	175 [121 – 275]	146 [112 – 214]	0.34
Nitrit (U) [μM/mM creatinine]	0.21 [0.15 – 0.31]	0.20 [0.12 – 0.37]	0.93
$U_{NOx}R$	912 ± 404	764 ± 376	0.24

[a]Nutritional failure is defined as a BMI percentile < 10; [b]sufficient nourishment is defined as a BMI percentile ≥ 10

Abbreviations. Arg, arginine; ADMA, asymmetric dimethylarginine; DMA, dimethylamine; P, plasma; U, urine; PNOxR, plasma nitrate/nitrite ratio; UNOxR, urinary nitrate/nitrite ratio.

Data are reported as median [25-75 percentile] (non-normal distribution) or as mean ± standard deviation (normal distribution). Significant results are marked in bold.

Table S5. Anthropometric and clinical characteristics of the cystic fibrosis (CF) patients according to their nutritional status.

	CF patients		*p*
	Nutritional failure[a]	Sufficiently nourished[b]	
Number of subjects (*n*)	11	59	-
Height [percentile]	32.9 [10.5 – 53.6]	40.3 [17.1 – 67.2]	0.46
Shwachmann-Score	**70.0 [65.0 – 75.0]**	**75.0 [70.0 – 75.0]**	**< 0.001**
FEV1 [%]	**73.0 ± 22.3**	**93.7 ± 16.8**	**0.00**
MEF25 [%]	**29.0 [21.0 – 63.0]**	**61.0 [38.2 – 82.0]**	**0.04**
Crispin-Norman-Score	**10.0 [5.00 – 16.0]**	**4.00 [2.00 – 7.50]**	**<0.001**
FENO [ppb]	8.20 [4.70 – 15.7]	8.30 [5.90 – 12.8]	0.91
Fischer-Quotient	3.50 ± 0.85	3.27 ± 0.53	0.24
Prothrombin time (%)	81.8 ± 9.38	84.4 ± 13.8	0.55
Cholesterol [mM]	3.39 ± 0.57	3.40 ± 0.76	0.94
Triglycerides [mM]	1.05 [0.77 – 1.37]	1.10 [0.86 – 1.85]	0.47
Urea [mM]	3.95 ± 0.79	4.50 ± 1.17	0.15
Pancreas sufficiency [*n* (%)]	1 (9.10)	9 (15.2)	1.00[+]
P. aeruginosa negative [*n* (%)]	5 (45.5)	43 (74.6)	0.08[+]
ABPA [*n* (%)]	**3 (27.3)**	**3 (5.10)**	**0.045[+]**
Acute infect [*n* (%)]	6 (54.5)	18 (30.5)	0.17[+]
Steroid treatment [*n* (%)]	6 (54.5)	11 (18.6)	0.11[+]
Liver involvement [*n* (%)]	1 (9.1)	7 (11.9)	1.00

[a] Nutritional failure is defined as a BMI percentile < 10; [b] sufficient nourishment is defined as a BMI percentile ≥ 10 Abbreviations. BMIp, BMI percentile; FEV1%, Forced expiratory volume in 1 s; MEF25%, mid expiratory flow 25%;ABPA, allergic bronchopulmonary aspergillosis.

Data are reported as median [25-75 percentile] (non-normal distribution) or as mean ± standard deviation (normal distribution). [+] Fisher's exact test. Significant results are marked in bold.

Table S6. Summary of the results of Spearman correlation analyses between members of the Arg/NO pathway in plasma (P) and urine (U) in the cystic fibrosis patients of the study.

Correlation pair	Correlation coefficient	*p* value	Data pairs (*n*)
Arg (P) vs. Cit (P)	0.465	0.000	68
Arg (P) vs. ADMA (P)	0.636	0.000	68
Arg (P) vs. $P_{NOx}R$ (P)	0.240	0.049	68
Arg/ADMA (P) vs. Cit (P)	0.345	0.004	68
Arg (P) vs. ADMA (U)	0.302	0.014	68
Arg (P) vs. DMA/ADMA (U)	-0.469	0.000	66
ADMA (P) vs. Cit (P)	0.267	0.028	68
ADMA (P) vs. Nitrite (P)	0.438	0.000	66
ADMA (P) vs. $P_{NOx}R$ (P)	0.300	0.013	68
ADMA (U) vs. ADMA (P)	0.438	0.000	66
DMA/ADMA (U) vs. ADMA (P)	-0.446	0.000	66
DMA (U) vs. $P_{NOx}R$	0.250	0.043	66
DMA (U) vs. Arg/ADMA (P)	-0.272	0.027	66
DMA (U) vs. ADMA (U)	0.661	0.000	68
Nitrate (U) vs. Nitrate (P)	0.585	0.000	66
Nitrate (U) vs. ADMA (U)	0.561	0.000	68
Nitrate (U) vs. DMA (U)	0.618	0.000	68
Nitrite (U) vs. ADMA (U)	0.480	0.000	68
Nitrite (U) vs. DMA (U)	0.449	0.000	68
Nitrate (U) vs. $P_{NOx}R$	0.439	0.000	66
Nitrate (U) vs. Nitrite (U)	0.658	0.000	68
$U_{NOx}R$ vs. $P_{NOx}R$	0.386	0.001	66
GFR vs. $P_{NOx}R$	-0.023	0.853	68
GRF vs. $U_{NOx}R$	-0.099	0.423	67
Shwachman-Score vs. Nitrite (P)	0.255	0.040	65
Shwachman-Score vs. Arg/ADMA (P)	0.279	0.024	65

Abbreviations. Arg, arginine; ADMA, asymmetric dimethylarginine; DMA, dimethylamine; P, plasma; U, urine; $P_{NOx}R$, plasma nitrate/nitrite ratio; $U_{NOx}R$, urinary nitrate/nitrite ratio; GFR, glomerular filtration rate.