Endocrine responses during CPAP-withdrawal in obstructive sleep apnoea: data from two randomised controlled trials

Sira Thiel[1] · Sarah R Haile[2] · Mirko Peitzsch[3] · Esther I Schwarz[1] · Noriane A Sievi[1] · Salome Kurth[1] · Felix Beuschlein[4,5] · Malcolm Kohler[1,6*] · Thomas Gaisl[1*]

[1] Department of Pulmonology and Sleep Disorders Centre, University Hospital Zurich, Zurich, Switzerland
[2] Department of Epidemiology, Biostatistics, and Prevention Institute, University of Zurich, Zurich, Switzerland
[3] Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany.
[4] Department of Endocrinology, Diabetology and Nutrition, University Hospital Zurich, Zurich, Switzerland.
[5] Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Munich, Germany
[6] Centre for Integrative Human Physiology, University of Zurich, Zurich, Switzerland
*Shared senior authors

Significance and rationale of multi-steroid panels.

Multi-steroid analysis can also be used to investigate adrenocortical function in detail. Currently, these diagnostic approaches are mainly used to evaluate inborn errors of steroidogenesis or for differential diagnosis of patients with adrenal tumors.[1] While short-term changes in adrenocortical steroid levels may not have been detectable, the expected increases to DHPG and NE levels indicate that blood collection was performed at a time point when overall endocrine responses were present. As catecholamines are both, a chemical neurotransmitter and a hormone.[1] While plasma epinephrine levels primarily reflect adronomedullary secretion of the hormone, plasma NE stems mainly from the exocytoc release of NE from sympathethic noradrenergic nerves.[2] Levels of DHPG, the main neural metabolite of NE, and plasma catechols could further provide information about CPAP treatment, although not routinely measured stressors, such as the tilt-table test, and drugs such as yohimbine can produce increased plasma levels of both NE and DHPG, providing clinical information about sympathetic function.[2 3] Elevated NE and DHPG but normal epinephrine levels in OSA, emphasize the relevance of sympathetic activation to high BP levels and long-term cardiovascular risks.

Analytics

Sleep studies were followed by blood sampling. Fasting blood samples were drawn between 7 and 9 AM in the morning into ethylenediaminetetraacetic acid, lithium heparin, and serum tubes. Samples were stored -80°C in polypropylene tubes (Mirco tube 2ml with cap, SARSTEDT AG & Co. KG, Numbrecht, Germany). Concentrations of the plasma steroid hormones aldosterone, corticosterone, cortisol, cortisone, 11-deoxycorticosterone, 11-deoxycortisol, 21-deoxycortisol, 18-hydroxycortisol, and 18-oxo-cortisol were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) and exact methods are described elsewhere.[4] Plasma concentrations of

norepinephrine (NE), epinephrine, and 3, 4-dihydroxyphenylglycol (DHPG) were analyzed by high pressure liquid chromatography electrochemical detection (HPLC-ECD).[5] Further plasma parameters such as renin were measured at the central laboratory of the Institute of Clinicial Chemistry and Laboratory Medicine at the University Hospital Carl Gustav Carus in Dresden (Germany) using the Cobas 8000 from Roche, iSYS multiple discipline automated system from immunodiagnosticsystem (IDS), and the Architect from Abbott Laboratories, respectively.

Statistical methods and data analysis

Continuous variables have been described as mean (SD) and compared using a t-test, while categorical variables have been described as n (%) and compared using the $\chi 2$ test. Comparison of lab parameters at follow-up by treatment has been performed using linear regression to estimate a treatment effect, while also adjusting for the same lab parameter at baseline as well as which study the patient came from. The coefficients for the treatment effect should be interpreted as the mean outcome with subtherapeutic CPAP (sham) minus the mean outcome with therapeutic CPAP. A second model further adjusted for sex, age, body-mass-index, and presence of hypertension (i.e. clinical diagnosis). The entire complete cases analysis was repeated after multiple imputation (10 imputations) to account for missing data in the primary outcome. No corrections have been made for multiple testing, but only p-values <0.005 were considered statistically significant, while p-values between 0.005 and 0.05 have been considered suggestive.[6] The statistical analysis was performed in R (R version 3.4.4. (2018-03-15), R Core Team 2018), using the *mice* package to perform the multiple imputation analysis.

Table 1.var	term	estimate	std.error	statistic	p.value	conf.low	conf.high	Multiple imputation
dhpg	trtsham	124.73	45.25	2.76	0.0076	34.20	215.26	no
dhpg	trtsham	123.32	45.46	2.71	0.0087	32.28	214.37	yes
ne	trtsham	71.32	26.22	2.72	0.0085	18.86	123.79	no
ne	trtsham	76.37	26.91	2.84	0.0062	22.48	130.27	yes
cortisol	trtsham	25.20	10.57	2.38	0.0203	4.03	46.36	no
cortisol	trtsham	24.19	10.80	2.24	0.0289	2.55	45.83	yes
dhea	trtsham	0.87	0.34	2.54	0.0135	0.19	1.56	no
dhea	trtsham	0.86	0.35	2.50	0.0153	0.17	1.56	yes

Comparison of raw data with multiple imputation data.

References:

1. Eisenhofer G, Kopin IJ, Goldstein DS. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacological reviews* 2004;56(3):331-49. doi: 10.1124/pr.56.3.1 [published Online First: 2004/08/20]
2. Goldstein DS, Eisenhofer G, Stull R, et al. Plasma dihydroxyphenylglycol and the intraneuronal disposition of norepinephrine in humans. *The Journal of clinical investigation* 1988;81(1):213-20. doi: 10.1172/jci113298 [published Online First: 1988/01/01]
3. Goldstein DS, Cheshire WP. Roles of catechol neurochemistry in autonomic function testing. *Clinical autonomic research : official journal of the Clinical Autonomic Research Society* 2018;28(3):273-88. doi: 10.1007/s10286-018-0528-9 [published Online First: 2018/05/01]
4. Peitzsch M, Dekkers T, Haase M, et al. An LC-MS/MS method for steroid profiling during adrenal venous sampling for investigation of primary aldosteronism. *The Journal of steroid biochemistry and molecular biology* 2015;145:75-84. doi: 10.1016/j.jsbmb.2014.10.006 [published Online First: 2014/10/15]
5. Eisenhofer G, Masjkur J, Peitzsch M, et al. Plasma Steroid Metabolome Profiling for Diagnosis and Subtyping Patients with Cushing Syndrome. *Clinical chemistry* 2018;64(3):586-96. doi: 10.1373/clinchem.2017.282582 [published Online First: 2017/12/07]
6. Benjamin DJ, Berger JO, Johannesson M, et al. Redefine statistical significance. *Nature Human Behaviour* 2018;2(1):6-10. doi: 10.1038/s41562-017-0189-z

Figure 1. CONSORT flow diagram.

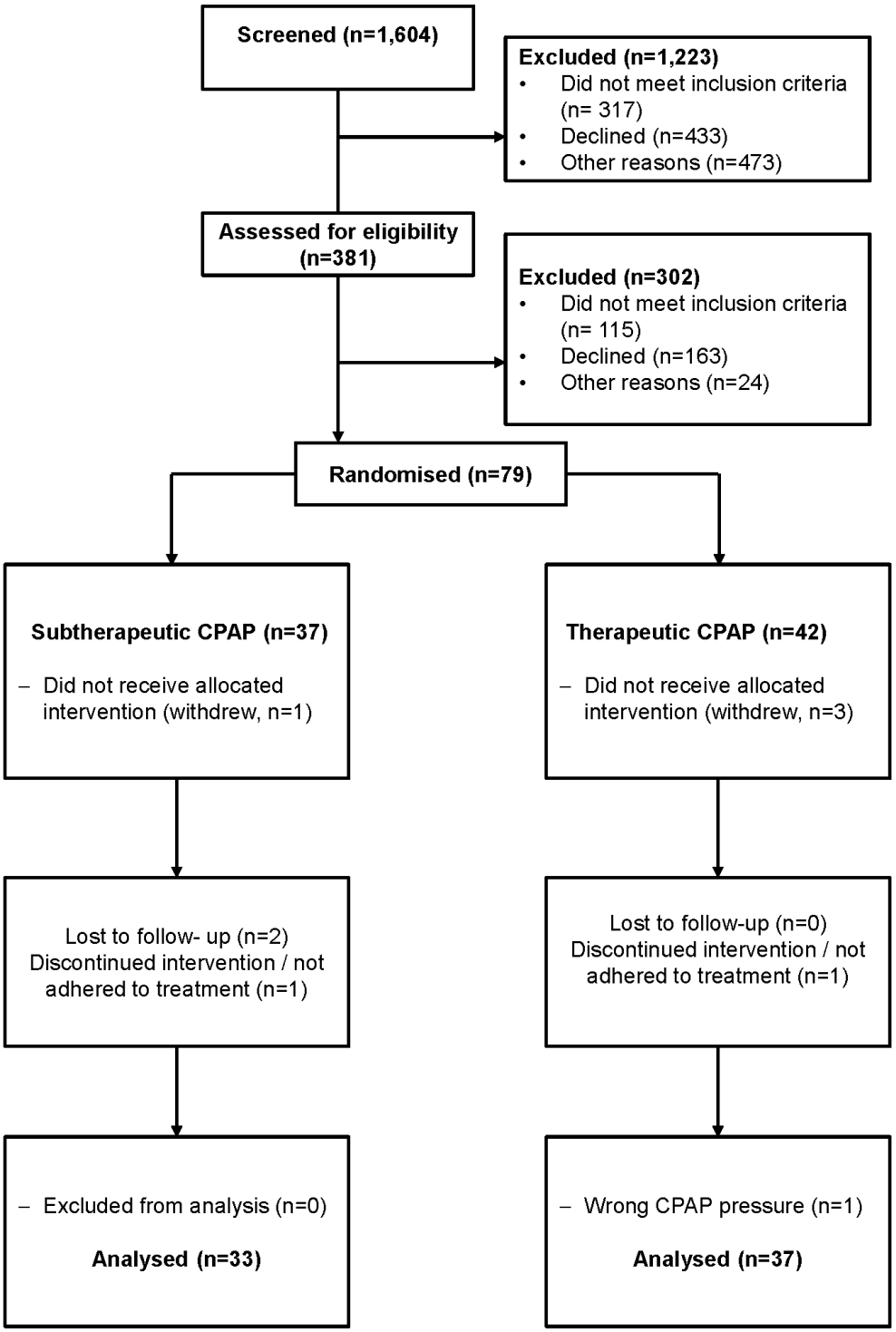

Table 1. Effect of CPAP withdrawal.

	Therapeutic CPAP (n=37)		**CPAP-withdrawal (n=33)**		**Treatment effect**		
	Baseline	Follow-up	Baseline	Follow-up	Difference in change	95% CI	p-value
AHI, events per hour	3.9 ± 2.7	5.3 ± 6.5	6.4 ± 4.9	40.9 ± 25.2	+31.8	+22.8 to +40.6	<0.001
ODI, events per hour	2.8 ± 2.7	4.4 ± 6.5	4.0 ± 4.9	40.4 ± 25.2	+31.5	+22.4 to +40.5	<0.001
ESS, points	6.7 ± 3.7	8.7 ± 4.8	8.0 ± 4.1	10.5 ± 5.5	+1.9	+0.2 to 3.5	0.02
Lowest saturation during night, %	85.9 ± 12.4	88.0 ± 4.9	85.1 ± 4.6	74.6 ± 8.8	-11.7	-28.4 to -5.1	<0.001
Average saturation during night, %	94.0 ± 2.3	94.2 ± 1.6	93.7 ± 2.0	91.6 ± 2.6	-5.05	-13.2 to -2.7	<0.001
Percent of total sleep time below 90% saturation, %	2.9 ± 1.2	0.8 ± 1.3	6.0 ± 13.8	23.8 ± 7.3	+15.2	+13.0 to +24.1	<0.001

AHI=Apnoea Hypopnea Index; CPAP=Continuous Positive Airway Pressure; ODI=Oxygen Desaturation Index; ESS=Epworth Sleepiness Scale