Online-Only Supplement

Vasculoprotective effects of dietary cocoa flavanols in hemodialysis patients: a double-blind, randomized, placebo-controlled trial

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Methods

For the determination of vascular functions (FMD and PWV) participants were required to fast at least 5 hours prior to the measurements. Comparisons using area-under the curve were conducted using Prism 5.0 (GraphPad) calculating the total peak area of FMD for each timepoint assessed (before, during and after HD) for the acute and acute-on chronic study, respectively.

Brachial blood pressure (BP) was measured in duplicate in the non-fistula arm by cuff and mercury sphygmomanometer after the participant had rested in a seated position for 10 min and the average of the 2 measurements was recorded.

After the participants had rested for 10 minutes we determined arterial stiffness by PWV. PWV was calculated from sequential recordings of electrocardiogram referenced carotid and femoral pressure waveforms obtained by using tonometry with the SphygmoCor device and transducer (AtCor Medical, Sydney, Australia). Wave transit-time was determined using the distance between carotid and femoral sites estimated from the distance between each artery location, the sternal notch and the R-wave of a simultaneously recorded electrocardiogram as reference frame (1).

Carotid intima-media thickness (IMT) was determined using high-resolution ultrasound (Vivid i ultrasound, GE Healthcare, Munich Germany). IMT was measured 1 cm distal the carotid bifurcation using end-diastolic (minimum dimension) images of the far wall of the distal common carotid artery.

Blood was drawn for clinical routine and the Institute of Clinical Chemistry and Laboratory Diagnostics, University Hospital Duesseldorf performed all analyses unless noted otherwise. (-)-Epicatechin and its related metabolites in plasama and dialysate fluid were determined by HPLC-FLD/UV and electrochemical detection using authentic standards provided by Mars Symbioscience, as described (2). Plasma nitrite and nitrate were

determinded by HPLC technique (Eicom, Eno-20), as described (3,4). The advanced glycation end product Carboxymethyl Lysine (CML, microcoat, Bernried, Germany), markers of inflammation hs-Interleukin 6 (IL-6, eBioscience, Vienna, Austria) and oxidative Stress oxidized LDL, OxLDL, Mercodia, Uppsala, Sweden) were measured by ELISAs following the manufacturers protocol, respectively.

Tables

Supplemental Table 1: Inclusion and Exclusion Criteria.

Inclusion Criteria:	**Exclusion Criteria**
end stage renal disease	acute renal failure
>18 years	acute infection
chronic intermittent hemodialysis	heart failure (NYHA IV)
	pregnancy
	anuria

Supplemental Table 2: Test drink composition and nutritional information.

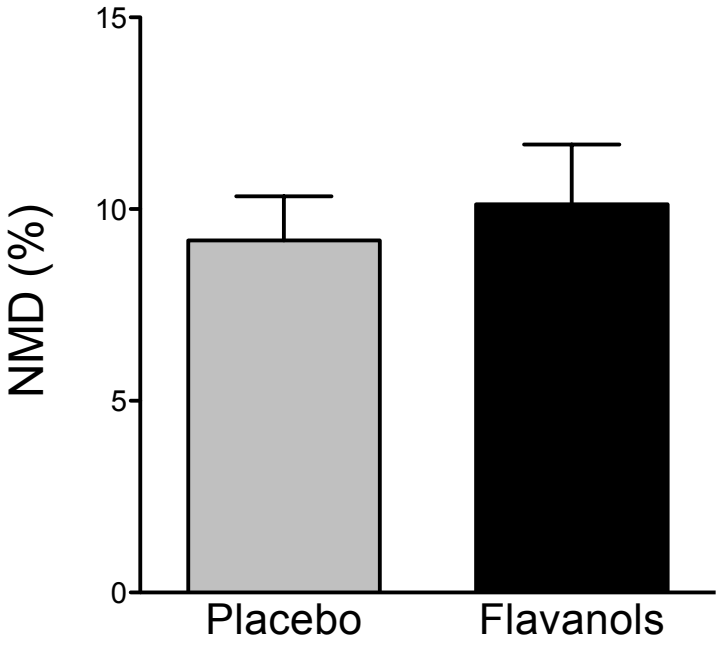
Total Daily Intake	**CF**	**Placebo**
Total cocoa flavanols (mg)	900	ND
(-)-Epicatechin	128	ND
(+)-Catechin	4	ND
(-)-Catechin	14	ND
(+)-Epicatechin	ND	ND
Total calories (kcal)	50	50
Total fat (g)	0	0
Stearic acid (g)	0	0
Total carbohydrates (g)	12	12
Sugars (g)	0.4	0.2
Protein (g)	0.2	0.2
Fiber (g)	1.2	0.8
Sodium (mg)	6	6
Potassium (mg)	190	170
Magnesium (mg)	2	2
Caffeine (mg)	20	12
Theobromine (mg)	88	92

ND=Not detectable

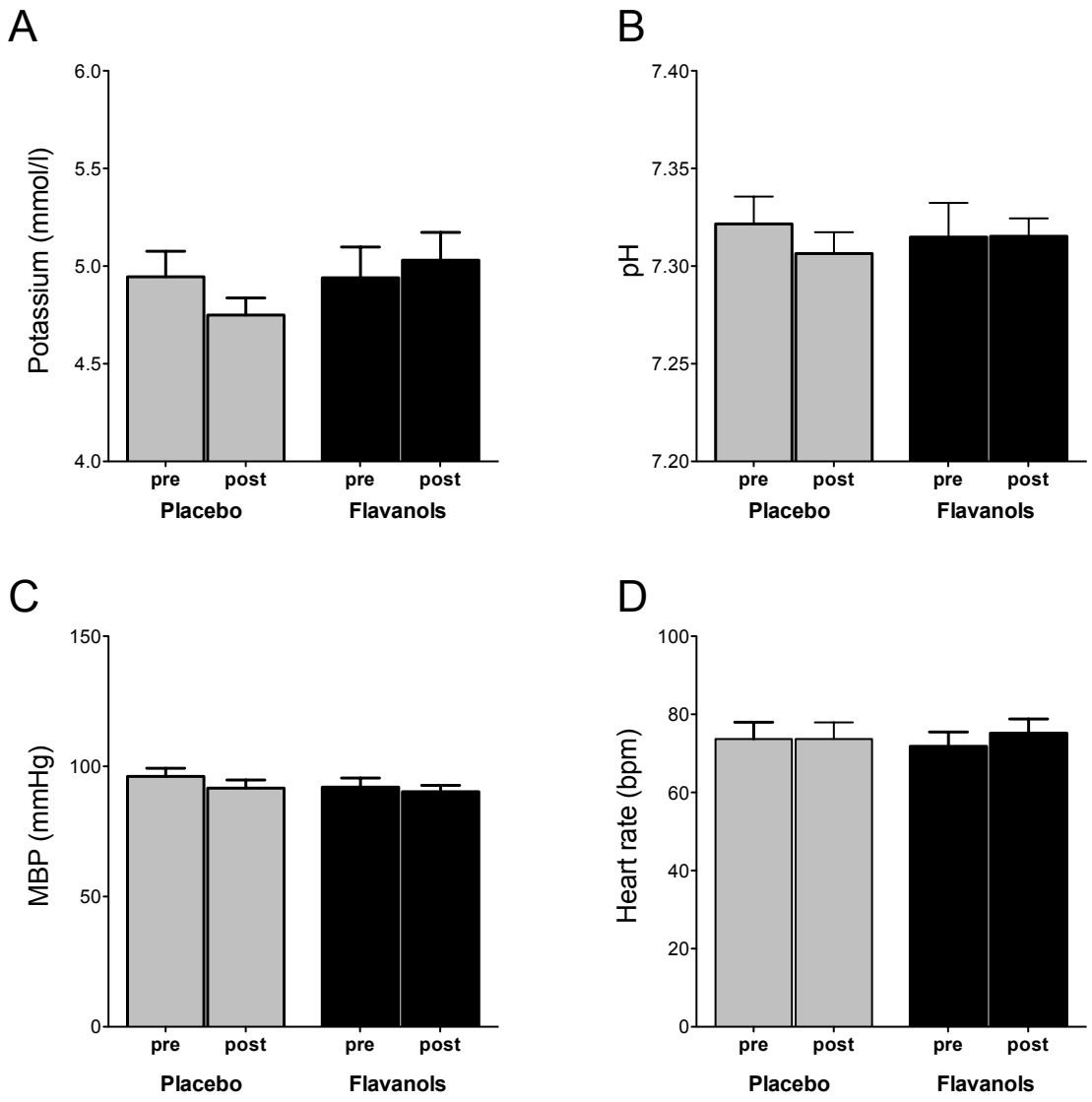
Supplemental Table 3: Baseline patient characteristics of the acute study.

n		10
Male n		9
Age (years)		64.1 ± 9.5
Weight (kg)		87.9 ± 9.9
Body mass index (kg/m^2)		28.9 ± 1.4
Renal diagnosis (n)		
	Hypertensive/large vessel	2
	Diabetic nephropathy	3
	Polycystic kidney disease	1
	Glomerulonephritis	2
	Other/miscellaneous	2
Dialysis vintage (m)		16 (12, 46)
Hypertension (n)		10
Diabetes (n)		4
Current smoker (n)		3
Hypercholesterinaemia (n)		3
CVD (n)		2
Medication (n)	ASS	5
	Statin	6
	AT blocker	3
	ACE-I	4
	b blocker	6
	Ca-channel blocker	2
	Diuretics	10

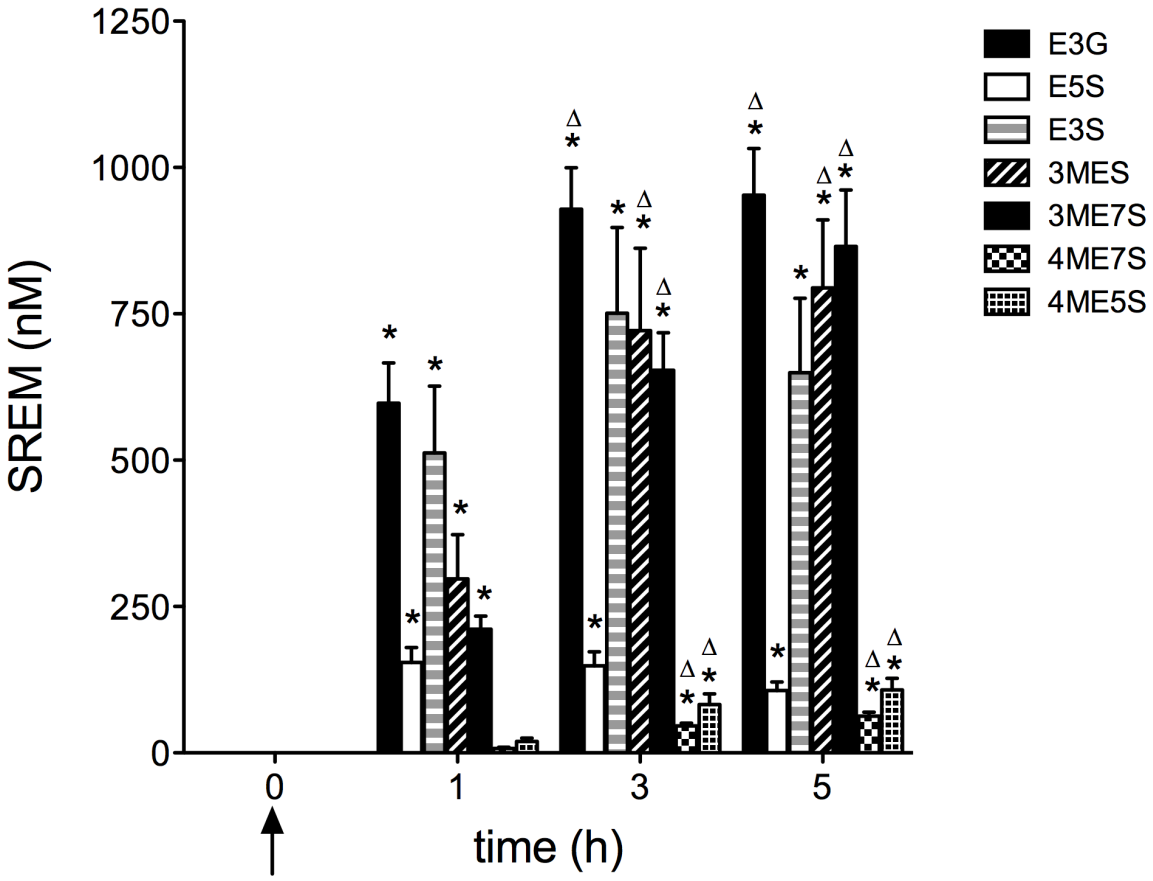
Supplemental Figure 1



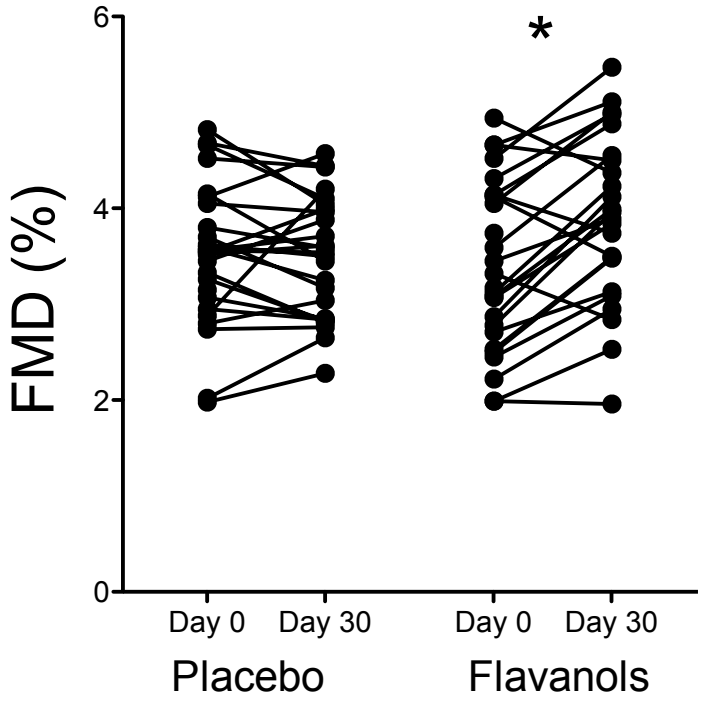
Supplemental Figure 3.



Supplemental Figure 3.



Supplemental Figure 4.



Supplemental Figure 5.

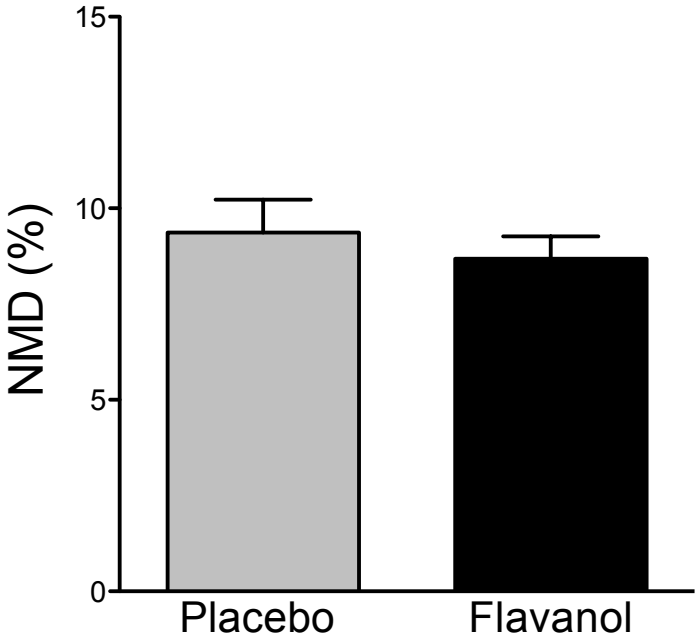


Figure Legends

Supplemental Figure 1. Acute study: Endothelial independent function in patients with ESRD.

No effect for Nitroglycerin-mediated vasodilation (NMD) i.e., endothelium-independent vasodilation in ESRD patients following acute ingestion of CF or placebo, n=5.

Supplemental Figure 2. Baseline acute study: Acute effect of dietary flavanols on clinical routine and hemodynamics in patients with ESRD.

No effect on potassium and pH values (A) and (B) or mean blood pressure (MBP) and heart rate (C) and (D) in CF group *vs*. placebo. *$p<0.05$, n=10, Data are given as mean ± SEM.

Supplemental Figure 3. Baseline acute study: Plasma epicatechin metabolites in ESRD after a singular ingestion of flavanols.

Plasma concentration of structurally-related epicatechin metabolites (SREM) in end-stage renal disease patients, with CF ingestion at 0 hr. SREM quantified corresponds to epicatechin-3′-b-D-glucuronide (E3G), (–)-epicatechin-3′-sulfate (E3S), (–)-epicatechin-5-sulfate (E5S), 3′-O-methyl(–)-epicatechin-5-sulfate (3MES), 3′-O-methyl(–)-epicatechin-7-sulfate (3ME7S), 4′-O-methyl(–)-epicatechin-5-sulfate (4ME5S) and 4′-O-methyl(–)-epicatechin-7-sulfate (4ME7S). Data are expressed in nM (nmol of SREM/L of plasma). *denotes $p<0.05$ compared to baseline, $^{\Delta}$compared to 1 hr value after CF ingestion, n=10.

Supplemental Figure 4. Chronic study: Chronic effects of dietary flavanols in patients with ESRD.

Chronic effect on endothelial function following a 30-day ingestion period of CF or placebo (FMD, flow-mediated dilation). *denotes $p<0.05$

Supplemental Figure 5. Chronic study: Endothelial independent function in patients with ESRD.

No effect for Nitroglycerin-mediated vasodilation (NMD) i.e., endothelium-independent vasodilation in ESRD patients following chronic ingestion of CF or placebo, n=7-9.

References

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