

Increases in Cholesterol Efflux Capacity and Changes in HDL Subfractions after Single Ascending Doses of MDCO-216 (apoA1-Milano/POPC)

Herman J Kempen, David G Kallend, Bela F. Asztalos*, James Otvos**,
S. Eralp Bellibas, Peter L Wijngaard

The Medicines Company Ltd

*Tufts University

**LipoScience LLC

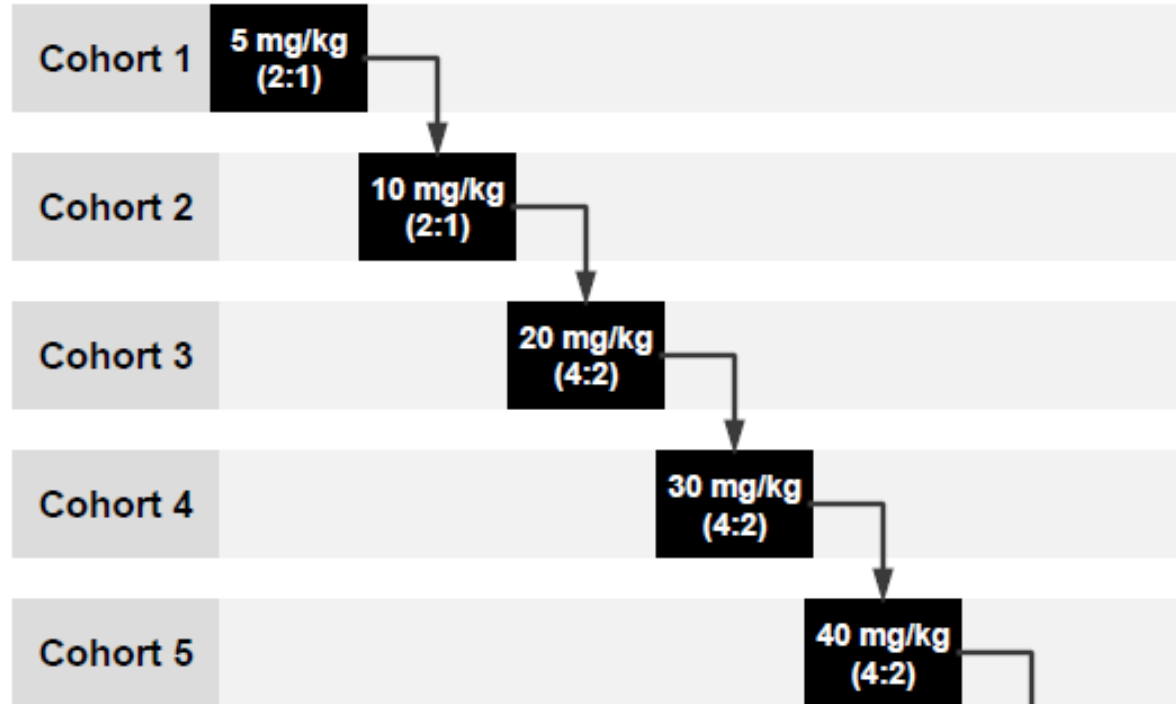
Disclosure potential conflicts of interest	
Research contracts:	-
Consulting:	The Medicines Company (Schweiz) GmbH: Herman Kempen
Employment in industry:	The Medicines Company : David Kallend, Eralp Bellibas, Peter Wijngaard
Stockholder of a healthcare company:	The Medicines Company, David Kallend, Eralp Bellibas, Peter Wijngaard
Owner of a healthcare company:	-
Other:	-



Phase I study MDCO-216

- Subjects were enrolled and dosed in single dose cohorts as described in the Figure
- Infusion of MDCO-216 or saline for 2 h.
- Blood sampling at baseline, 0.5, 2, 4, 8, 24, 48, 168 and 720 h
- Prior to escalation to the next dose all available data was reviewed by a Safety Review Committee and the decision taken whether to escalate to the next dose.
- Following the completion of the 5 cohorts of healthy volunteers, 4 cohorts of patients with stable CAD were enrolled.

Healthy Volunteers

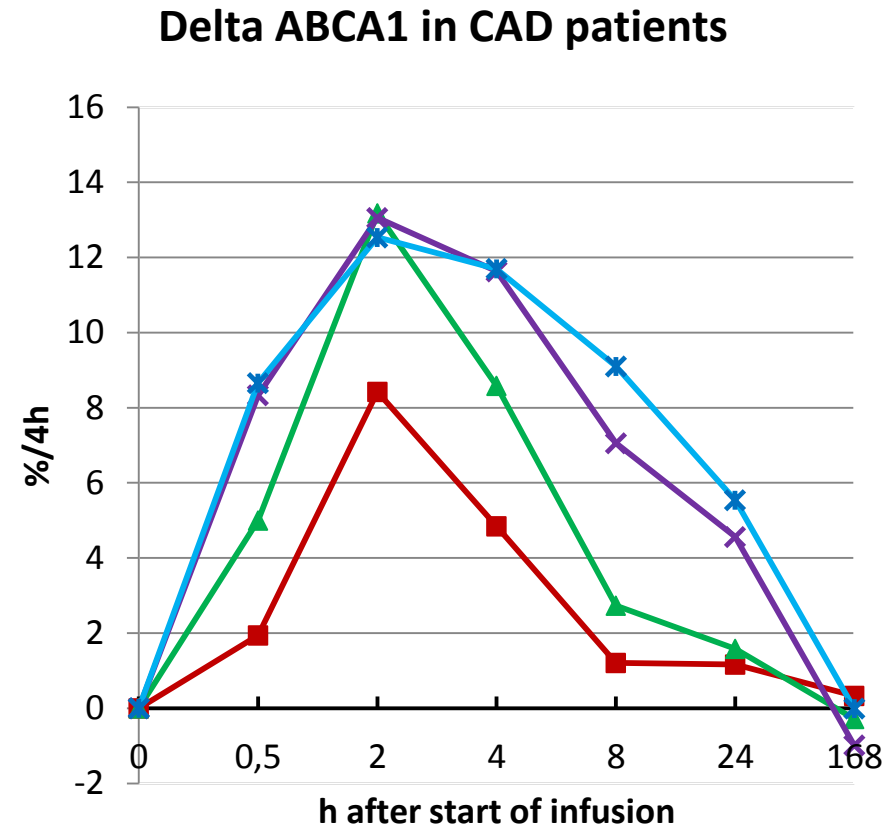
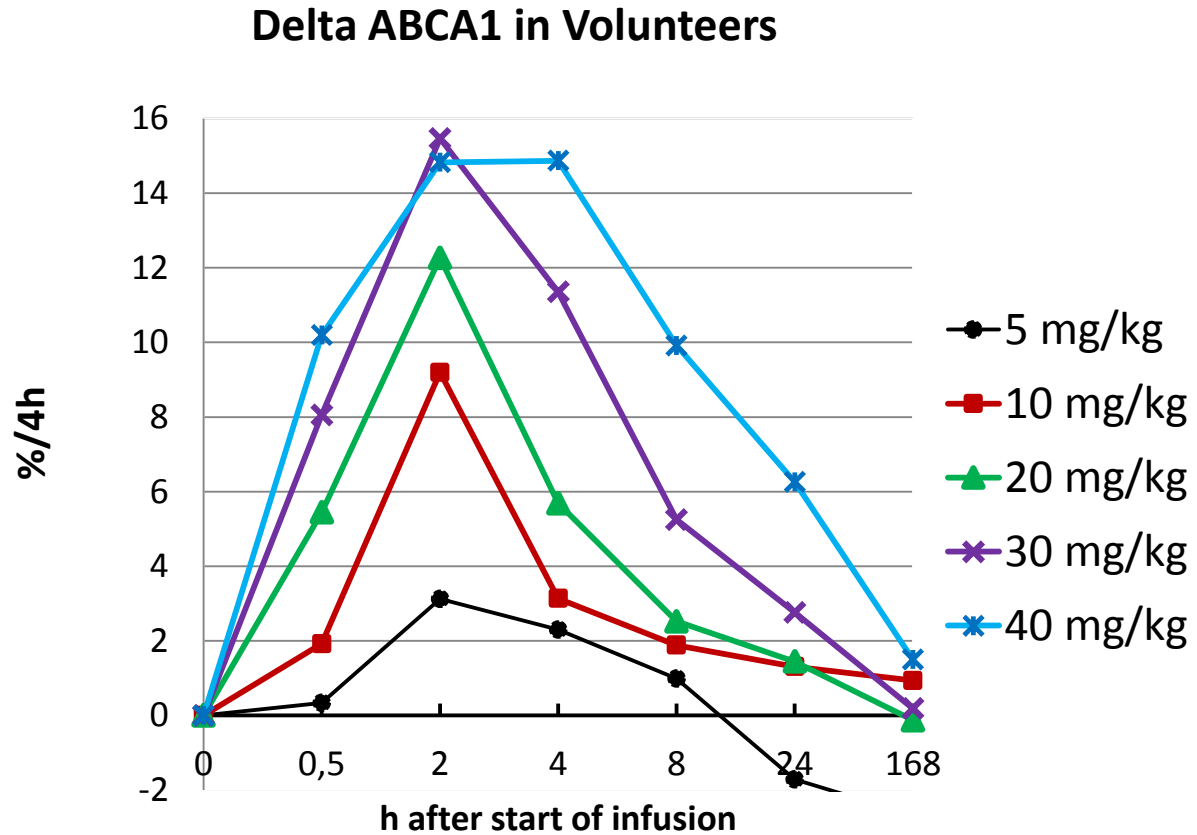


Stable CAD Patients

Pharmacodynamic parameters measured

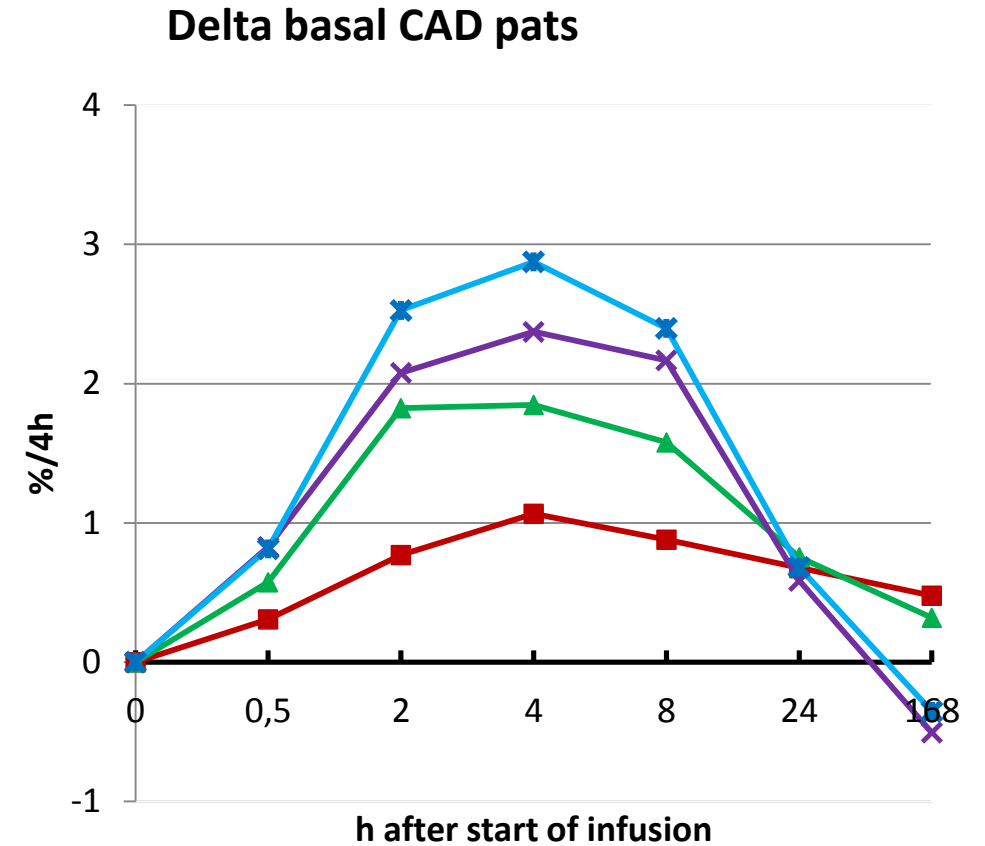
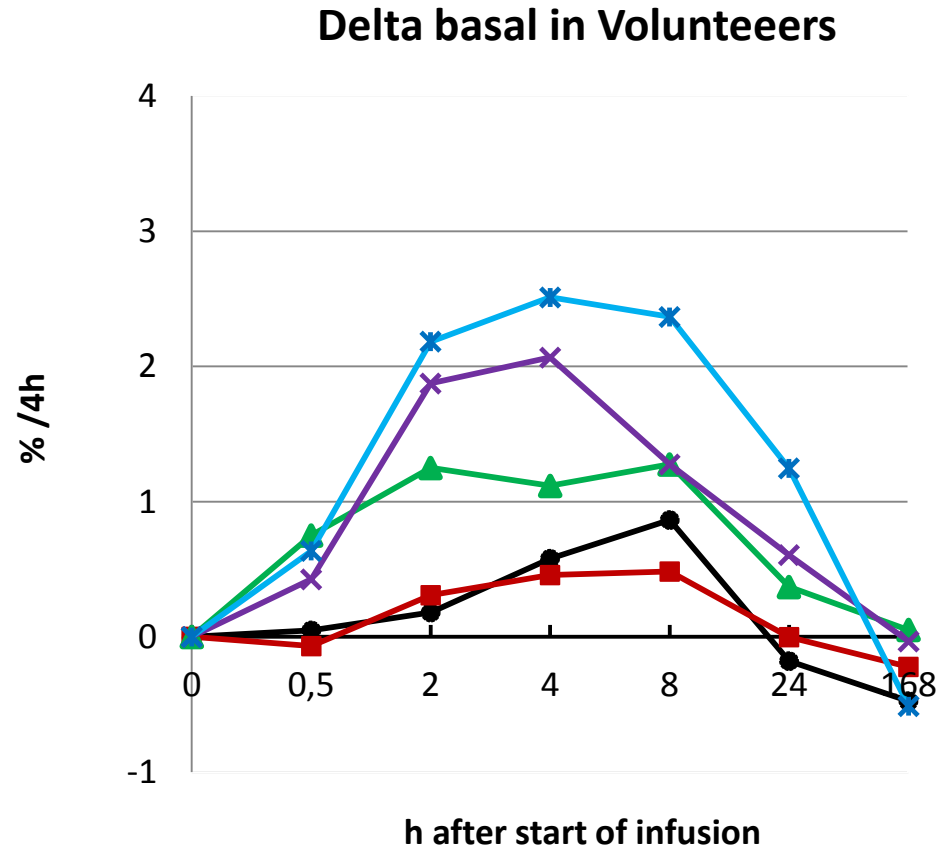
Lipid Profile	Cholesterol efflux capacities	Lipoprotein analysis	HDL subfractions
(AMC-Clin-chem)	(Vascular Strategies)	(Calabresi lab/LipoScience)	(Asztalos Lab/Pacbio)
Total cholesterol	Basal (J774)	FPLC-TC/FC/TG/PL	2D-PAGGE reacted
Free cholesterol	ABCA1 (J774 +cAMP)	FPLC-ApoAI/ApoA-IM/ ApoB	for total apoA-I or
Triglycerides	ABCG1 (BHK)	CER activity	apoA-IMilano
Phospholipids	SRB1 (Fu5AH)		
HDL-cholesterol		Lipoprotein particle numbers	Prebeta-1 HDL ELISA
LDL-cholesterol		and sizes	
Apo A-I (total)			
Apo A-II			
Apo B			
Apo E			

Increase in ABCA1 mediated cholesterol efflux capacity after infusion of MDCO-216



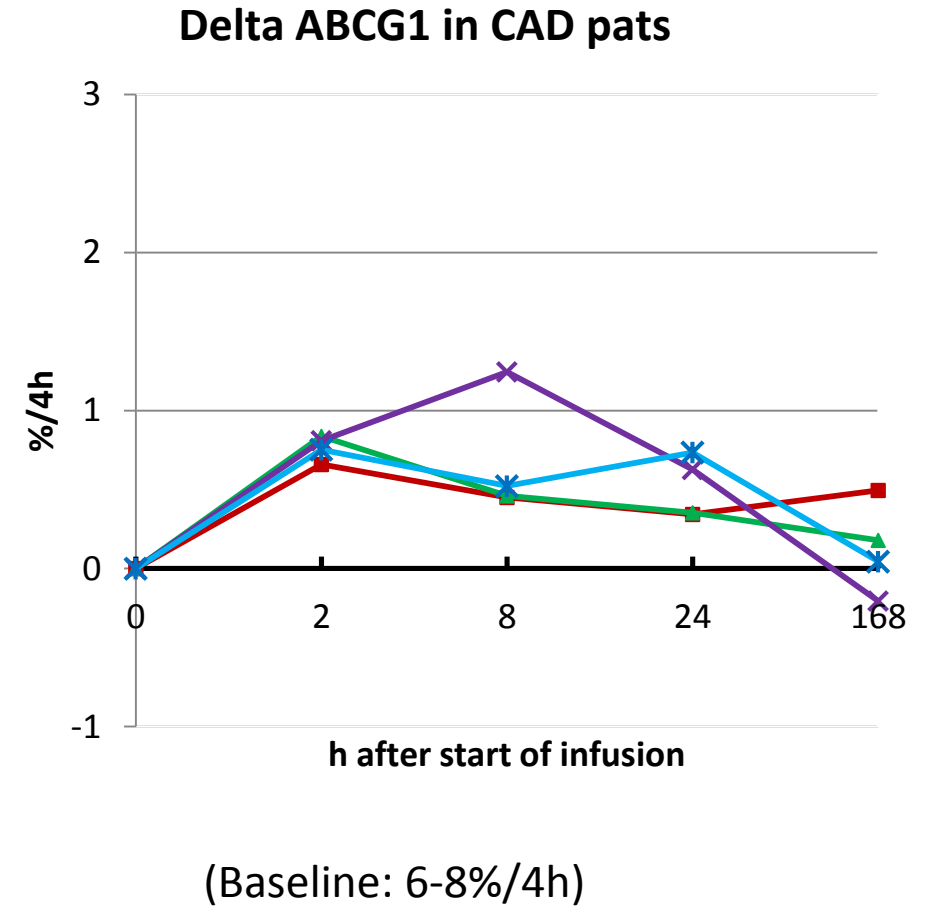
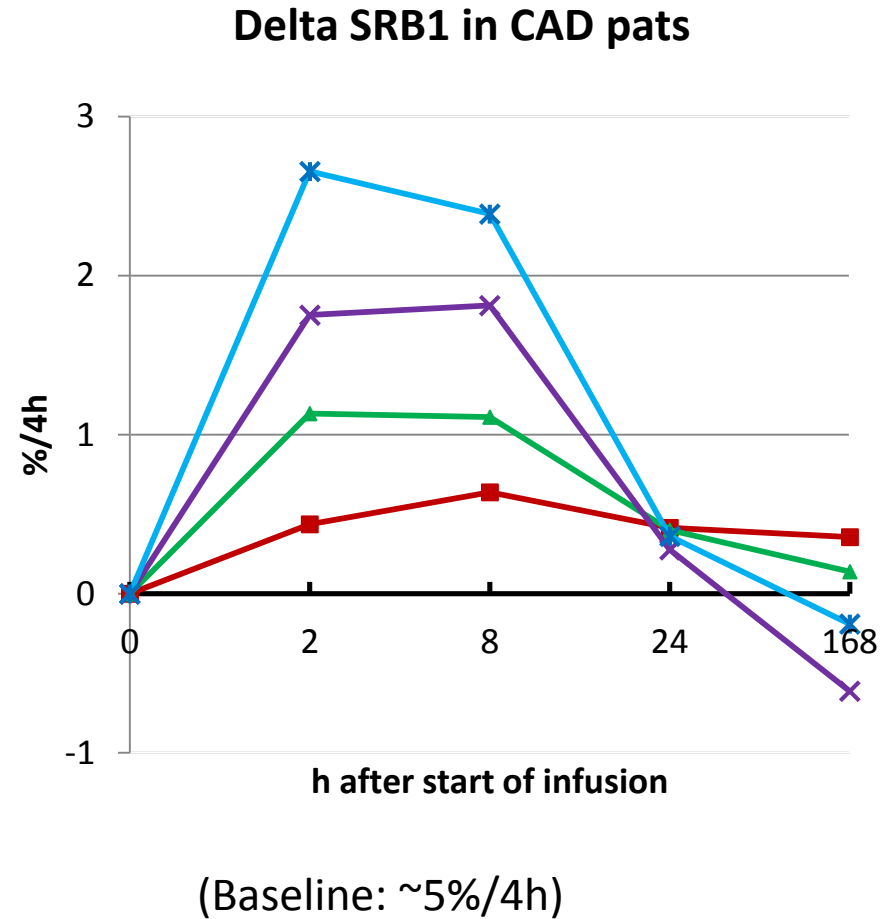
(Baseline: 4 - 8 %/4h)

Increase in basal cholesterol efflux capacity after infusion of MDCO-216

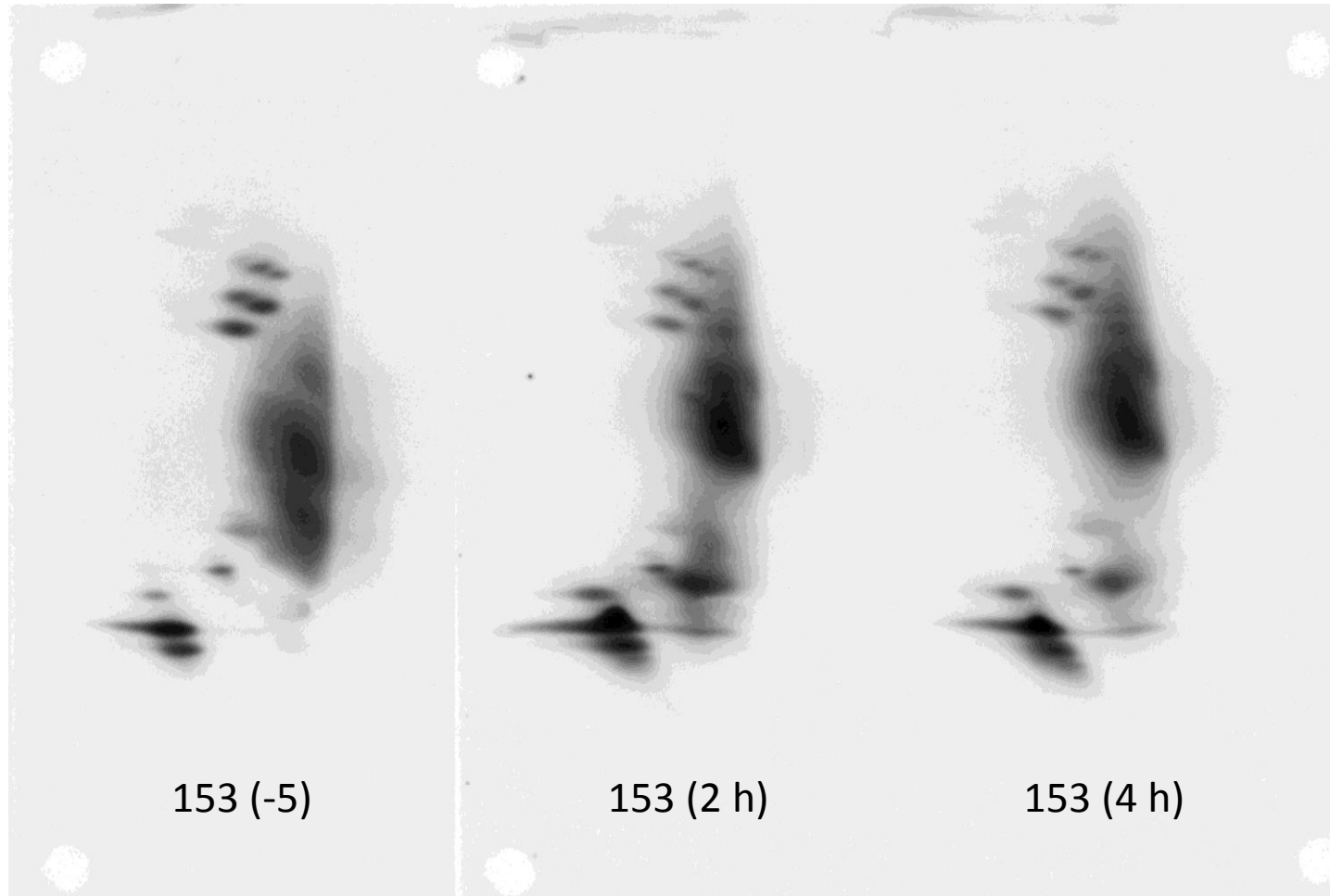


(Baseline: ~7 %/4h)

Increase in SRB1 and ABCG1-mediated cholesterol efflux capacity after infusion of MDCO-216.



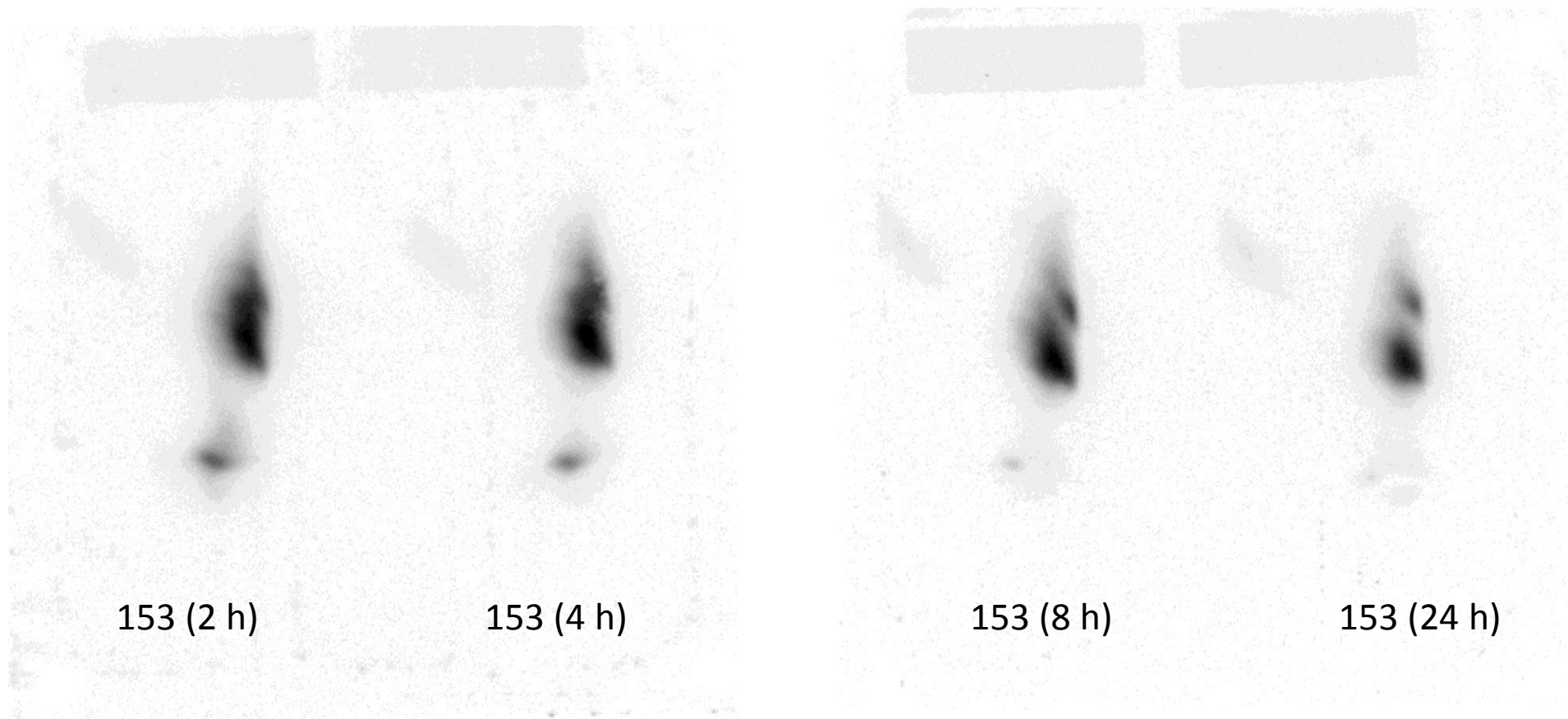
Changes in HDL-subfractions after 40 mg/kg MDCO-216
2D-PAGGE of whole plasma; membranes were reacted for
total ApoA-I



- α -1
- α -2
- α -3
- α -4
- prebeta-1

Changes in HDL-subfractions after 40 mg/kg MDCO-216

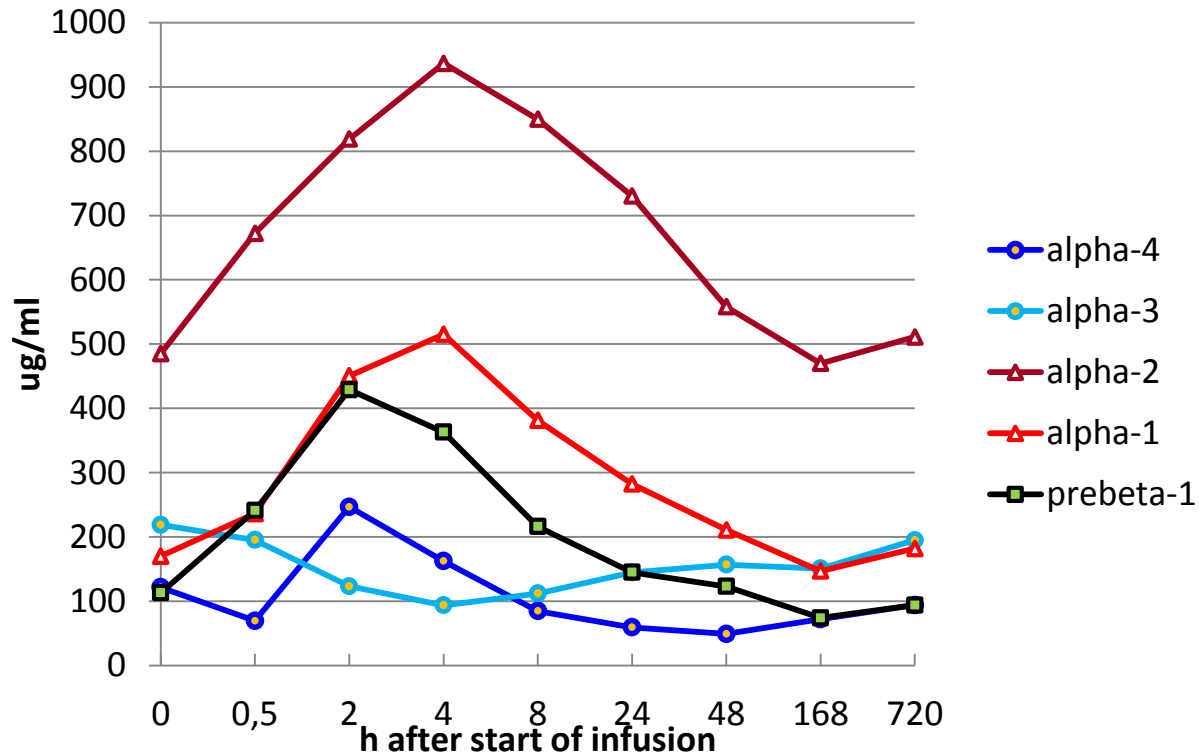
2D-PAGGE of whole plasma; membranes were reacted for ApoA-I Milano



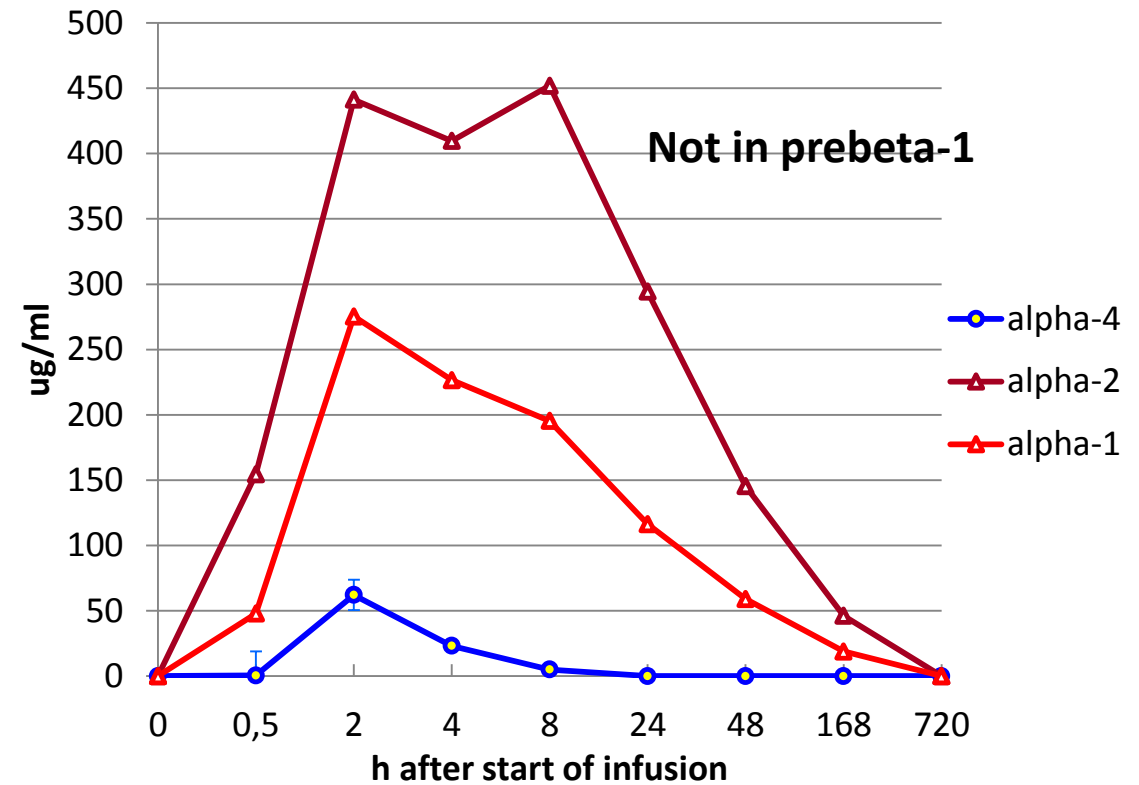
- α -1
- α -2
- α -3
- α -4,
- prebeta-1

Changes in HDL subfractions (2D PAGGE) after infusion of 40 mg/kg MDCO-216 (volunteers)

Distribution total apoA-I

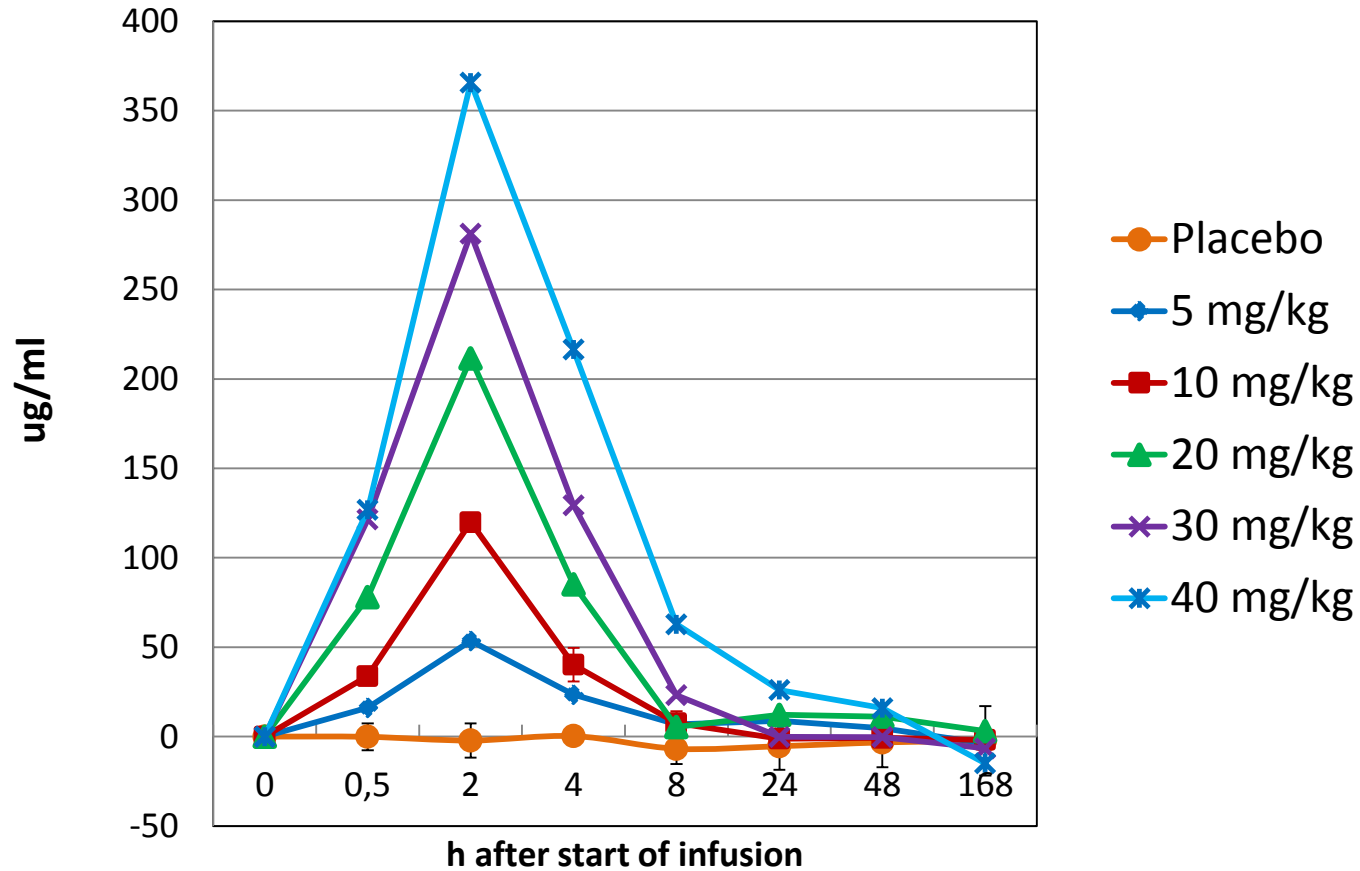


Distribution A-I Milano

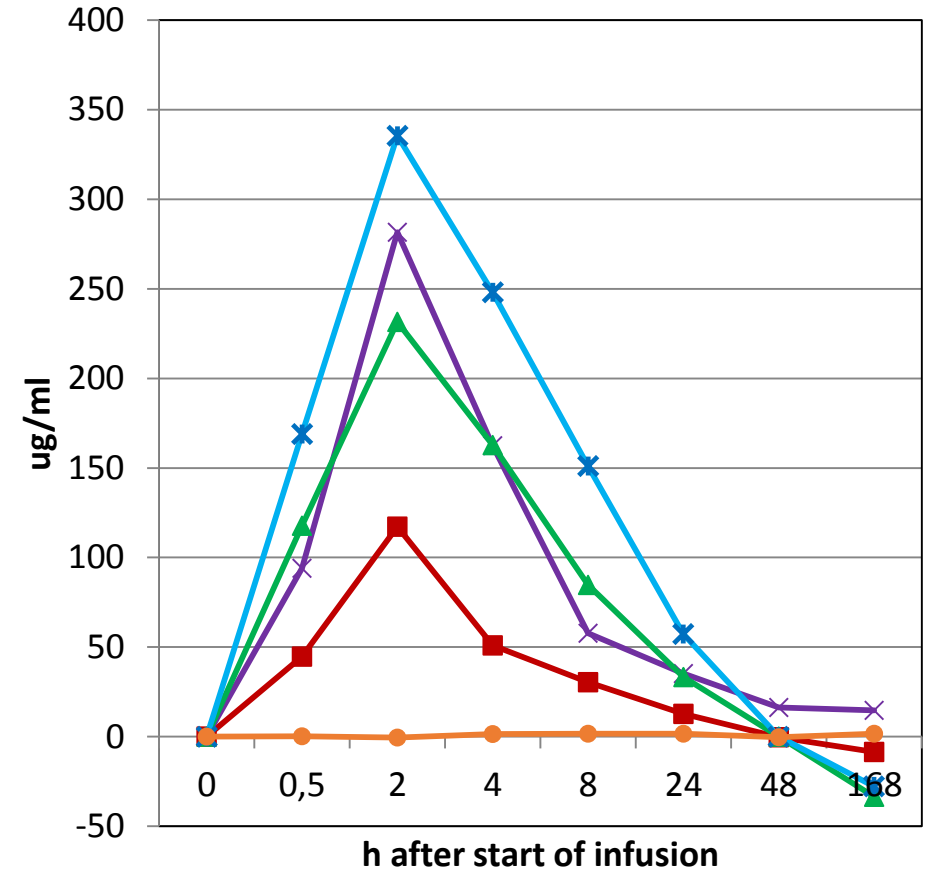


Change in prebeta-1 HDL (ELISA) after infusion of MDCO-216 (Sandwich ELISA using Dai-ichi Sekisui kit)

Volunteers

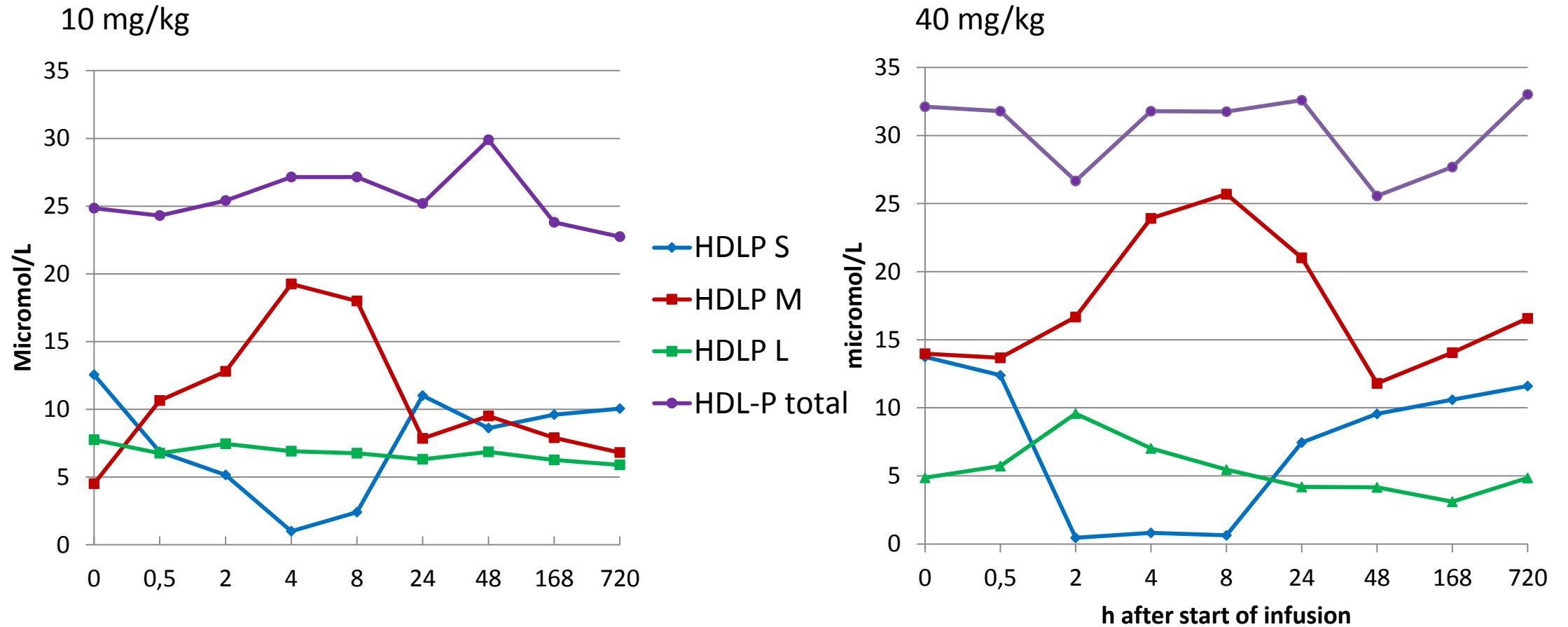


CAD patients

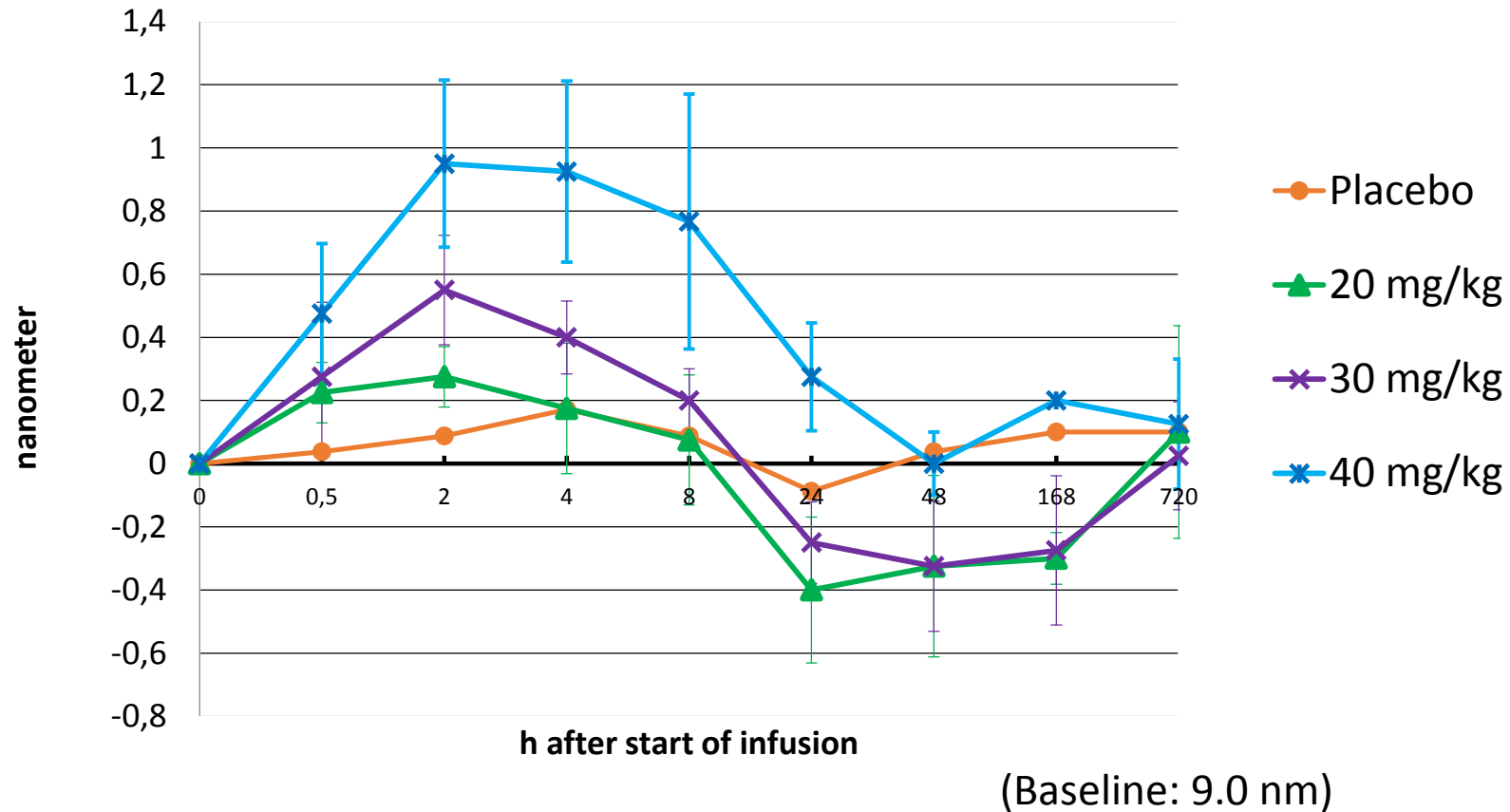


(Baseline: 42-84 ug/ml)

Changes in HDL particle concentrations (1H-NMR) after infusion of MDCO-216 in volunteers.



Changes in HDL diameter (1H-NMR) after infusion of MDCO-216 in volunteers.

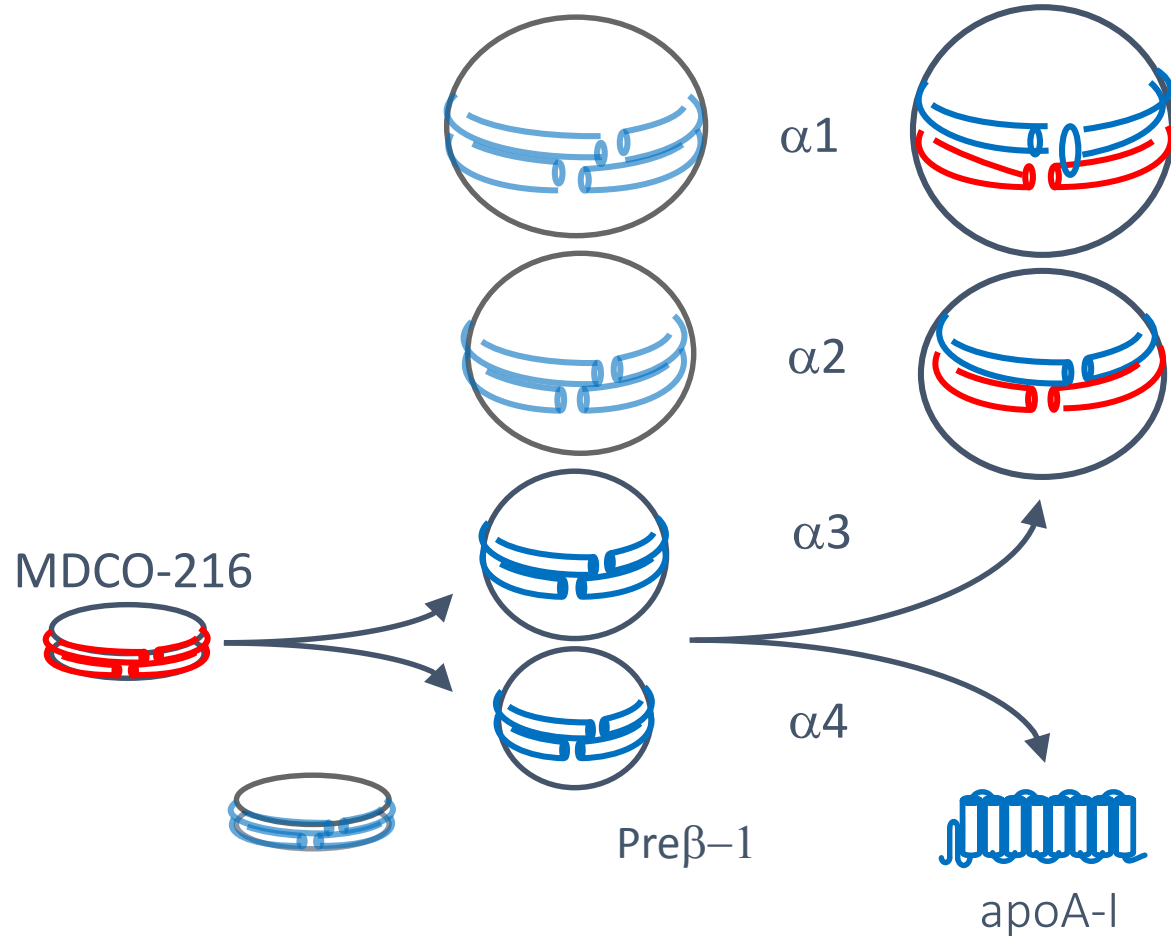


Conclusions 1

1. Rapid and very pronounced increase in ABCA1-mediated chol efflux
 - peak at 2-4 h, back to baseline at 24 h except after high dose
2. Smaller increase in basal, SRB1-mediated and ABCG1-mediated effluxes
 - Peak at 4-8 h, nearly back to baseline at 24 h
3. Rapid and pronounced increase in prebeta-1 HDL, containing only „wild-type“ apoA-I, no apoA-IMilano
 - peak at 2 h, back to baseline at 24 h at all doses
4. Rapid loss of smaller (α -3 and α -4) HDL and rise of apoA-IMilano in α -1 and α -2 HDL
 - Peak/trough at 4-8 h, back to baseline at 24 h-48 h
5. Rapid increase in HDL-size (shift from small to medium-sized HDL)
 - Peak at 2-4 h, back to or below baseline at 24 h
6. No increase in total HDL particle concentration

Hypothetical initial events:

MDCO-216 fuses with small HDL, generating novel α -1 and α -2 HDL containing apoA-IMilano and displacing endogenous apoA-I to become prebeta-1 HDL (this happens also in vitro upon incubation with plasma: Kempen et al, J Lipids 2014)



- α -1 with both A-I WT and A-IMilano. Long half-life
- α -2 with both A-I WT and A-IMilano. Long half life
- Rapid decrease of α -3
- Rapid decrease of endogenous α -4
- Rapid increase in free apoA-I (prebeta-1 HDL). Short half-life.

Question: which changes in HDL subfractions „explain“ the increase in basal and ABCA1 mediated cholesterol efflux capacities?

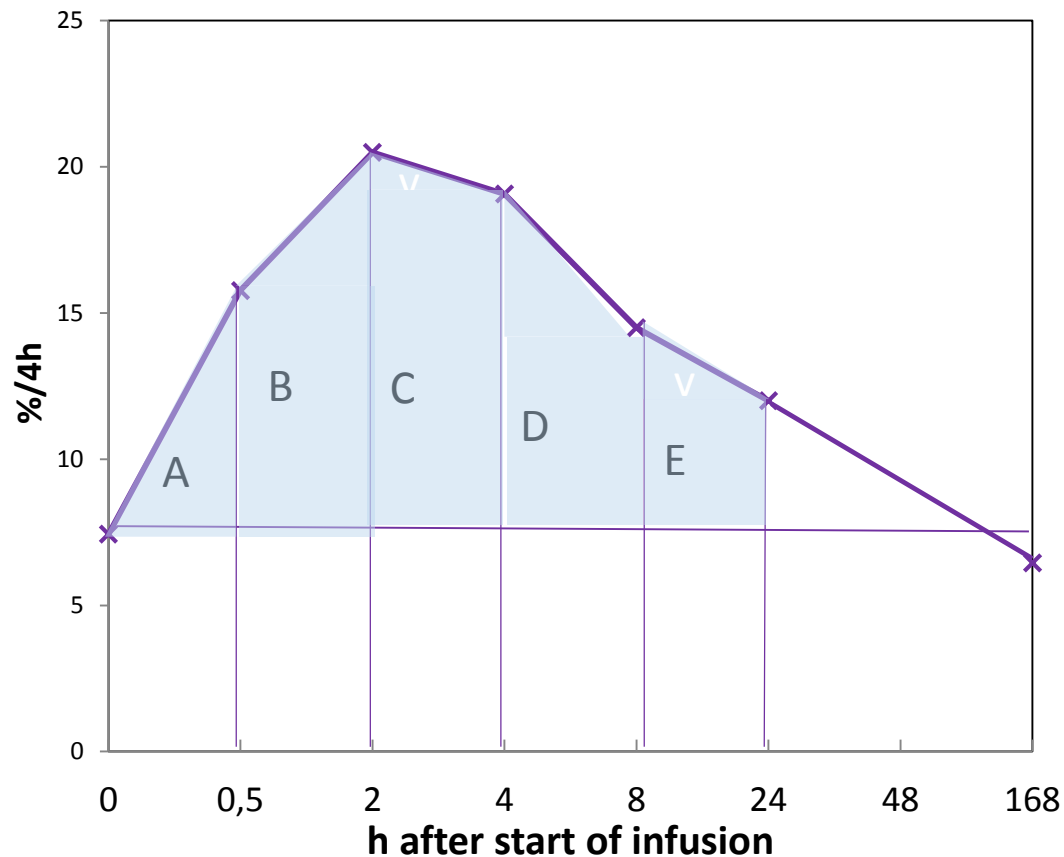
Correlations between ABCA1-mediated efflux and HDL subfractions in healthy males: Asztalos et al, J Lipid Res 46: 2246-53, 2005

TABLE 2. Univariate and multivariate regression analyses of ABCA1-mediated cholesterol efflux from J774 macrophages on apoA-I-containing HDL subpopulations

Subpopulation	Univariate Analysis				Multivariate Analysis			
	B	se(B)	t Value	P Value	B	se(B)	t Value	P Value
Pre β -1	0.0617	0.0174	3.54	0.0006	0.0686	0.0218	3.15	0.0022
Pre β -2	0.1039	0.0987	1.05	0.2953	0.0229	0.1145	0.20	0.8422
α -1	0.0157	0.0115	1.36	0.1758	-0.0131	0.0274	-0.48	0.6341
α -2	0.0359	0.0125	2.88	0.0048	0.0402	0.0200	2.01	0.0477
α -3	0.0068	0.0101	0.67	0.5050	-0.0188	0.0173	-1.08	0.2810
Pre α -1	0.0185	0.0300	0.62	0.5384	0.0703	0.0736	0.95	0.3420
Pre α -2	0.0044	0.0427	0.10	0.9190	-0.0818	0.0931	-0.88	0.3815
Pre α -3	-0.0620	0.0777	-0.80	0.4265	0.0484	0.0947	0.51	0.6103

B, regression coefficient; se(B), standard error (B); t, t-test [B/se(B)].

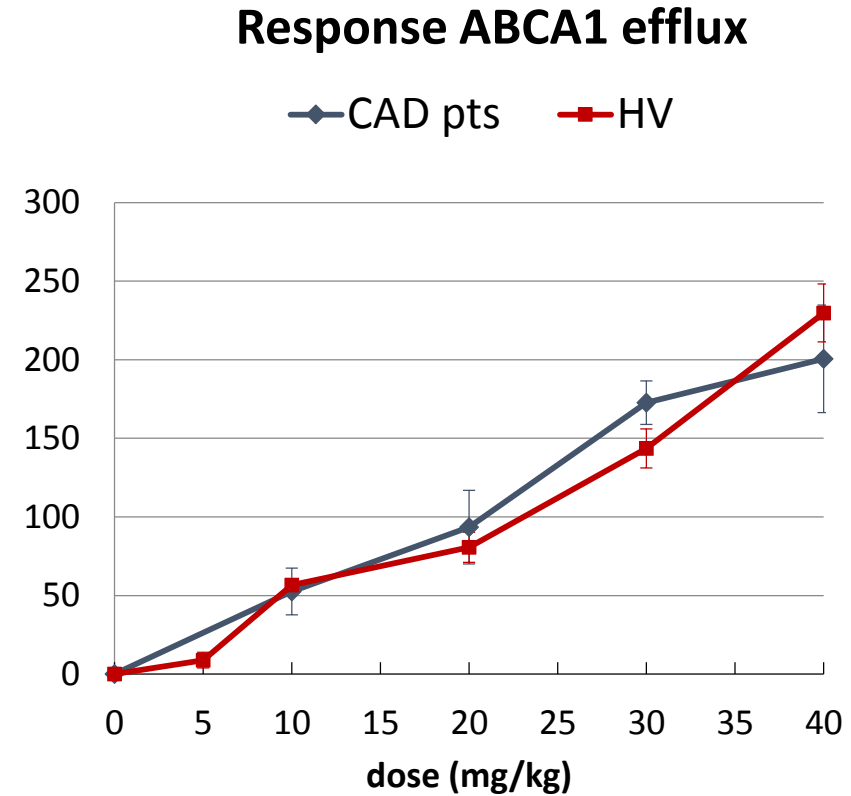
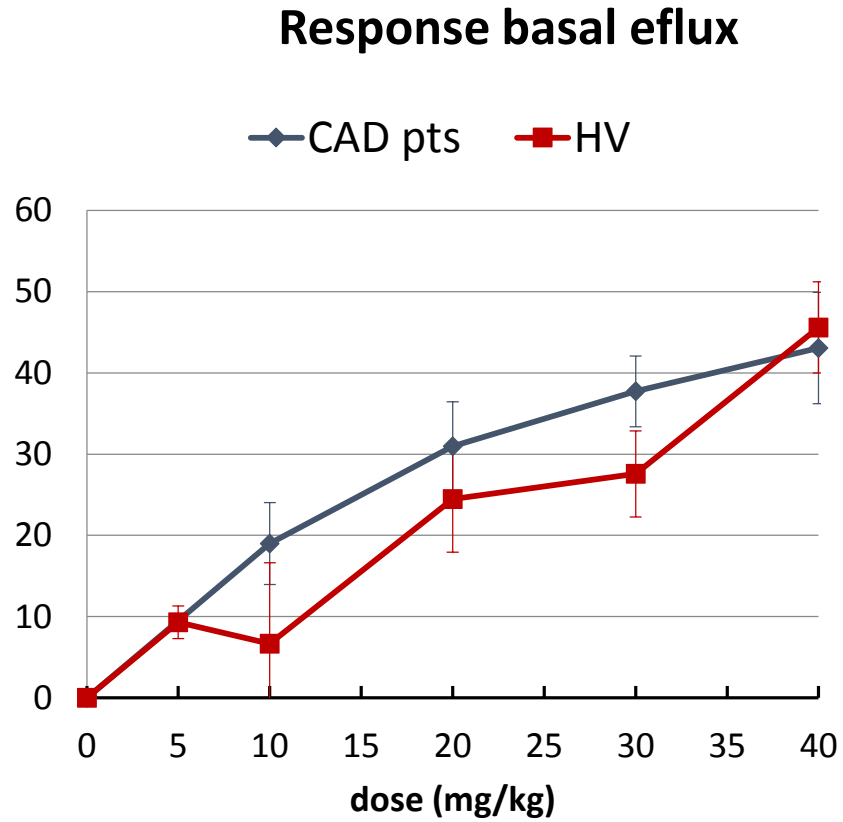
„Response“: Area Under the Effect Curve for 24 h after start of infusion, calculated for each subject and each parameter



Example: increase in ABCA1-mediated efflux above baseline after 30 mg/kg MDCO-216 in subject 153

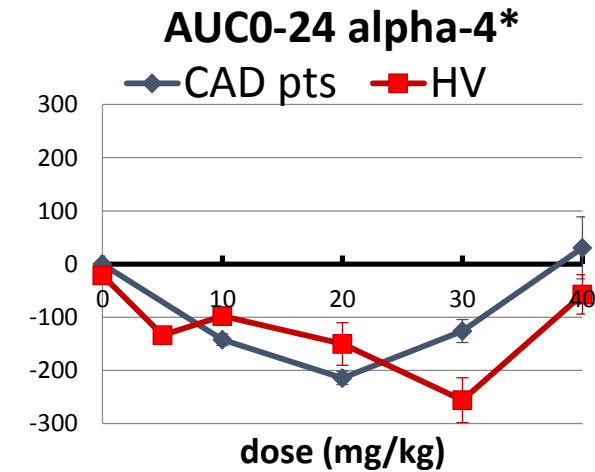
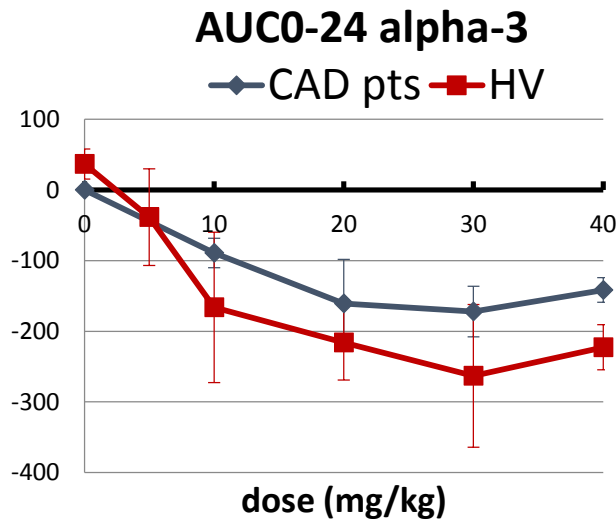
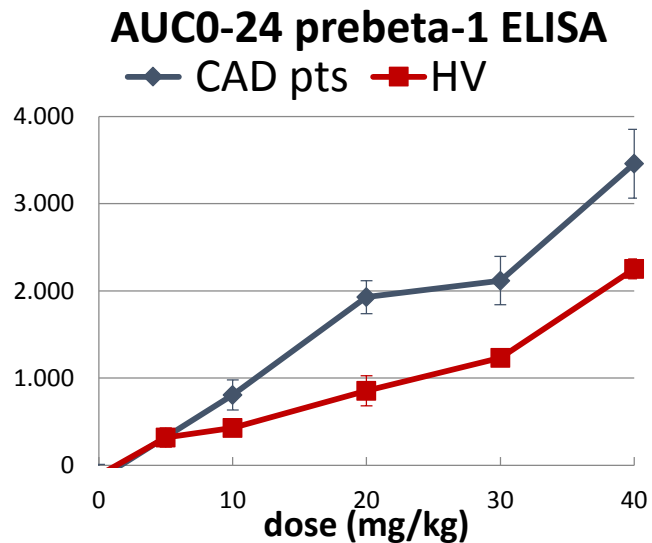
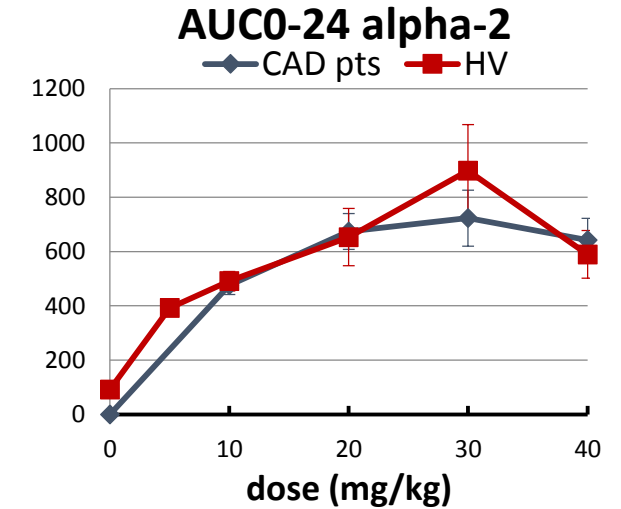
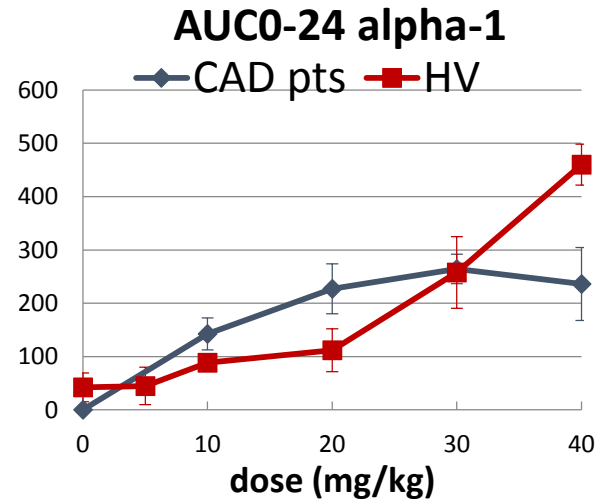
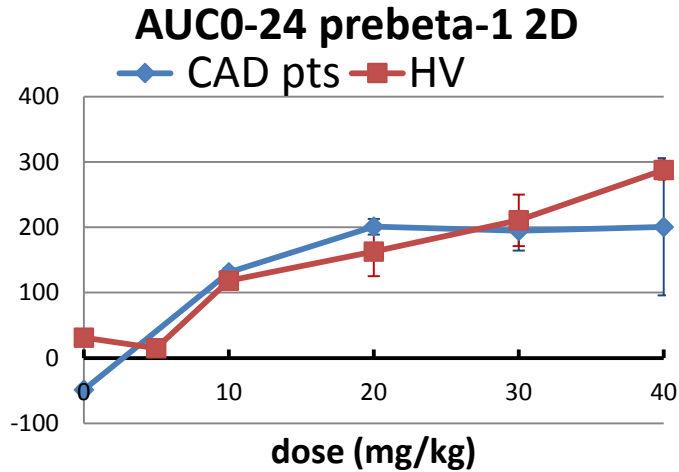
AUEC0-24 = Sum (areas A-E)

Dose-response curves for basal and ABCA1-mediated efflux



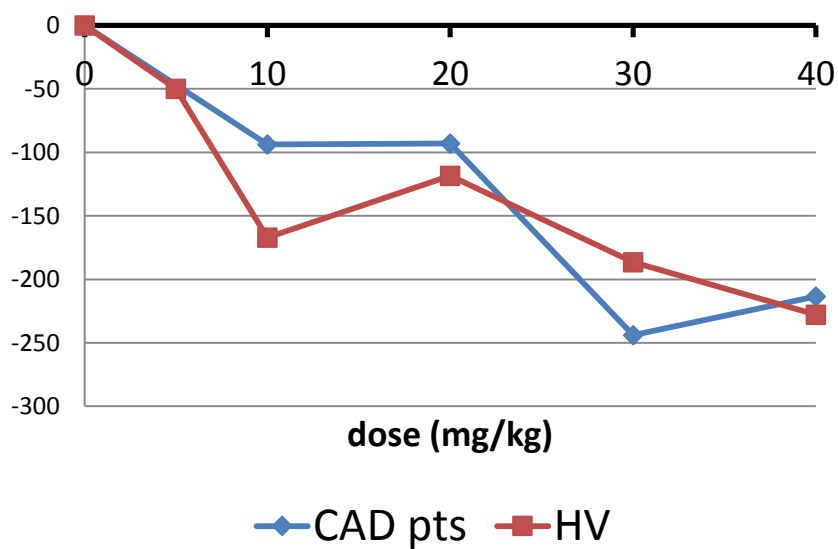
(means +/- SEM)

Dose-response curves for HDL-subfractions (total apoA-I after 2D-PAGGE)

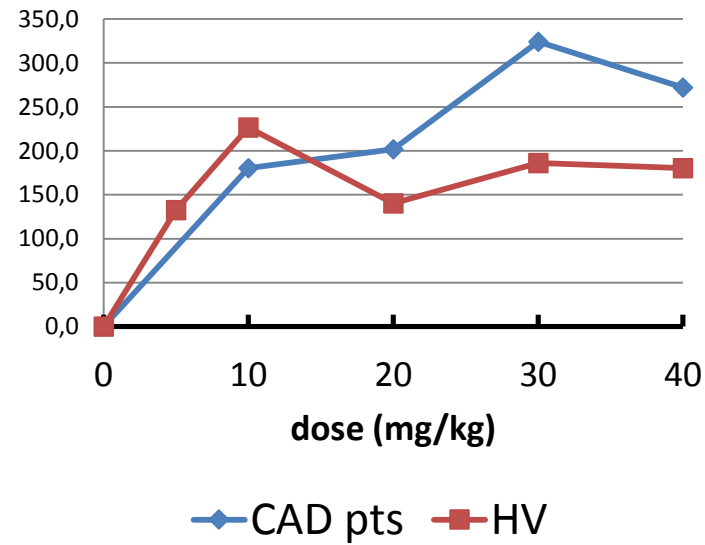


Dose-response curves for HDL-subfraction particle concentrations (1H-NMR)

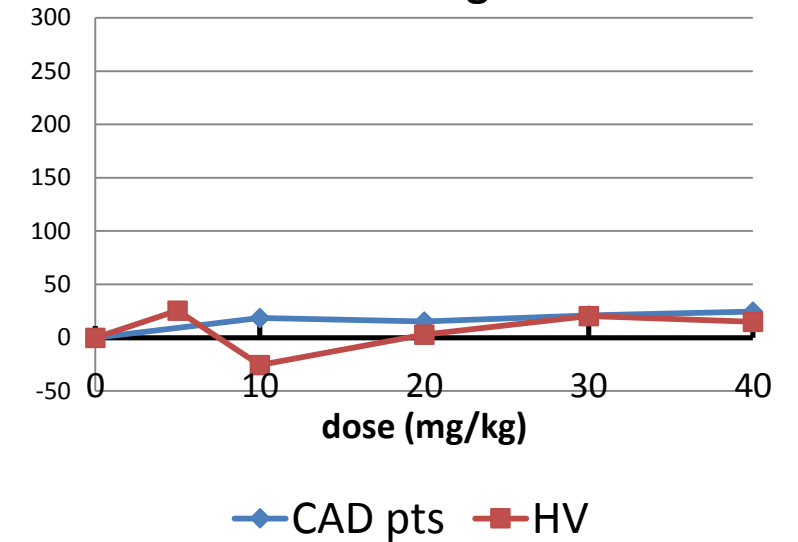
AUEC0-24 small HDL



AUEC0-24 medium HDL



AUEC0-24 large HDL



Conclusions 2

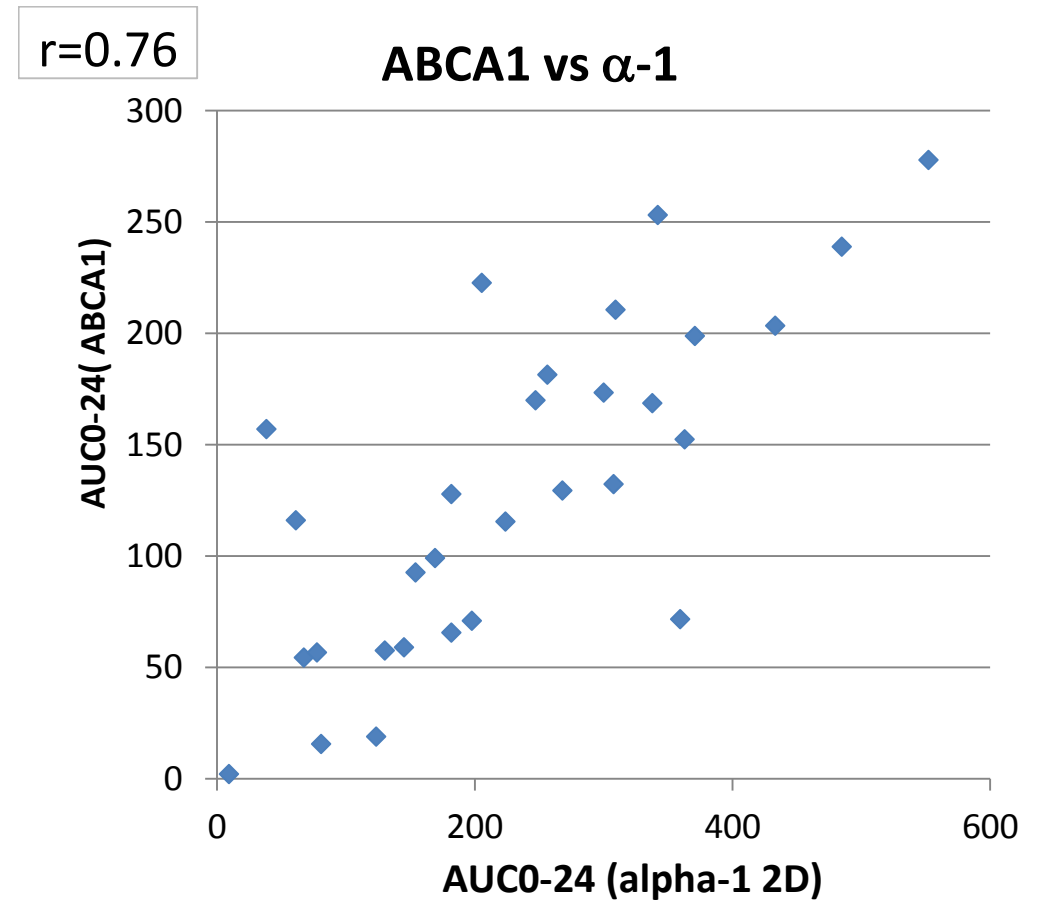
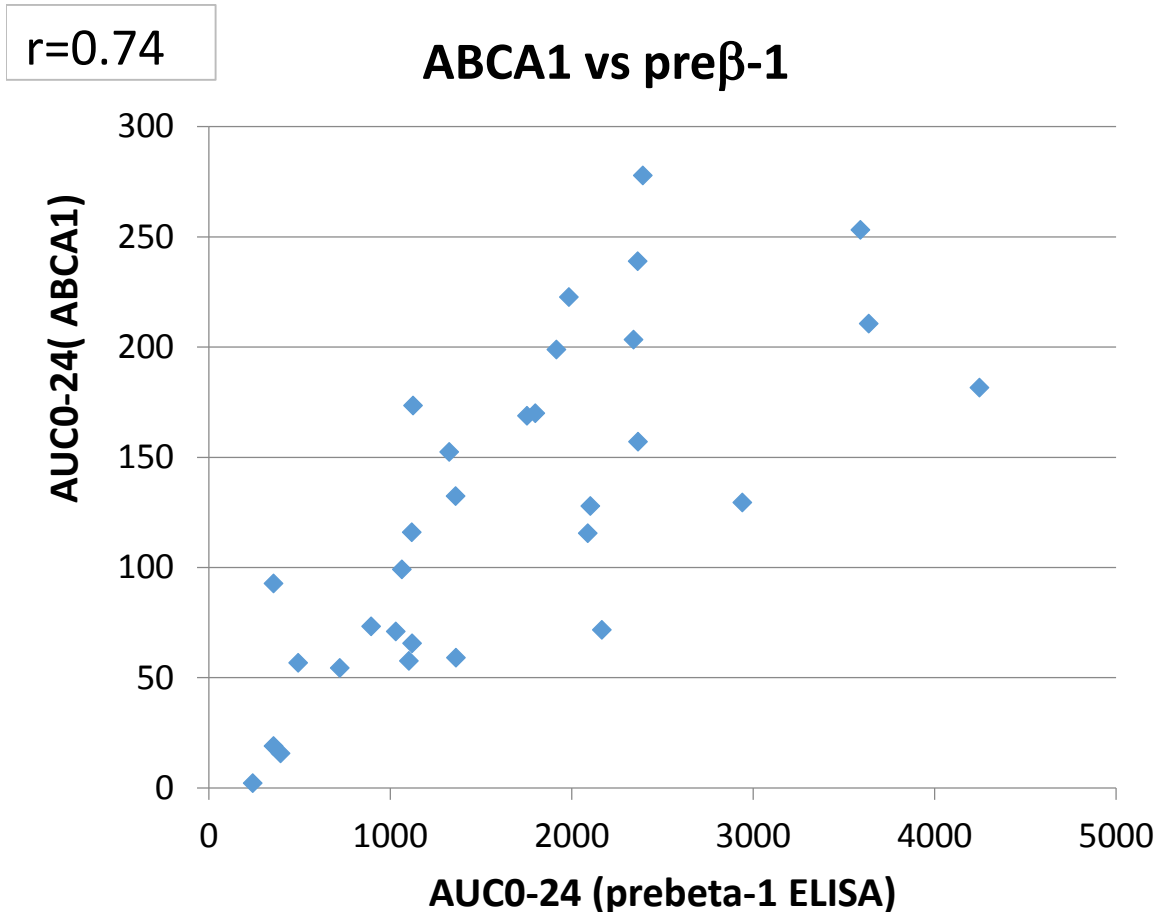
- Responses for basal and ABCA1-mediated cholesterol efflux keep increasing nearly linearly over the tested dose-range
- Responses for HDL subfractions reach plateau, except response for prebeta-1 HDL ELISA and α -1 HDL in volunteers which were linear
- Responses in apoA-I in α -1 and α -2 HDL correspond with response in medium-sized, not large-sized HDL (may explain lack of effect on ABCG1-mediated efflux)

Correlation of responses for basal and ABCA1-mediated cholesterol efflux with responses for HDL-subfractions (n=32)

Yellow: significant at $p < 0.05$

Response in:	<u>basal efflux</u>	<u>ABCA1 mediated efflux</u>
Pre β -1 Elisa	0.72	0.74
Pre β -1 2D	NS	0.47
α -1 2D	0.68	0.76
α -2 2D	NS	NS
α -3 2D	-0.41	NS
α -4 2D	NS	NS
large HDL-P	0.55	NS
medium HDL-P	NS	NS
small HDL-P	-0.53	-0.55

Response of ABCA1-efflux correlates strongly with response of pre β -1 HDL and α -1 HDL



Multiple regressions for basal and ABCA1-mediated efflux responses

Response in basal efflux

	Coefficient	SE	p
Intercept	7.57	3.59	0.043
α -1	0.043	0.01	0.008
Pre β -1 (E)	0.0075	0.0021	0.001

Response in ABCA1 mediated efflux

	Coefficient	SE	p
Intercept	13.2	15.4	0.39805
α -1	0.27	0.06	0.0002
Pre β -1 (E)	0.033	0.009	0.0008

Conclusions 3

- Responses of basal and ABCA1 mediated effluxes correlate positively with responses of pre β -1 HDL and α -1 HDL, and inversely with that of small HDL
- In multiple regression both pre β -1HDL and α -1 HDL responses remain significantly related with basal and ABCA1-mediated efflux responses
- α -1 HDL loaded with apoA-I Milano may drive cholesterol effluxes even when pre β -1 HDL is back to baseline

More on this Phase I study:

- Xchange Session 15 “HDL functionality”: Monday 14:45 - 16:30 (presentation by C Sirtori)
- Clinical Breakthroughs: modifying lipids, recent developments Tuesday 14.59 – 16.29 (presentations by SE Bellibas and D Kallend)
- **Further Q&A:** Poster Session 6.4: “Therapy” Tuesday from 13.45 - 17.00 (posters by Bellibas, Kallend, Kempen)

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