

RESEARCH LETTER

Reconstituted HDL infusion restores endothelial function in patients with type 2 diabetes mellitus

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Abbreviations

ApoA-I	apolipoprotein A-I
FAV	forearm tissue volume
FBF	forearm blood flow
L-NMMA	N^G monomethyl-L-arginine
M/C	ratio of flow in infused measurement and non-infused control arm
NO	nitric oxide
rHDL	reconstituted HDL
SNP	sodium nitroprusside

To the Editor: HDL-cholesterol is inversely correlated with cardiovascular events in all major epidemiological

studies [1]. HDL-increasing strategies have demonstrated that HDL-cholesterol increase is associated with decreased cardiovascular risk in high-risk individuals such as patients with type 2 diabetes [2]. Endothelial dysfunction, a hallmark of type 2 diabetes patients, has been shown to predict future cardiovascular events [3]. Therefore, we investigated the effect of reconstituted HDL (rHDL) on endothelial function measured both acutely (4 h after infusion) and 7 days after infusion in type 2 diabetes patients. Control volunteers were measured only at baseline and 4 h after infusion.

Seven non-smoking patients with type 2 diabetes (four men and three women, BMI 24.4 ± 1.6 kg/m²) and seven matched control volunteers (four men and three women, BMI 22.9 ± 1.8 kg/m²) were enrolled. Inclusion criteria for type 2 diabetes patients were: (1) fasting glucose >7.0 mmol/l; (2) no insulin therapy; and (3) triacylglycerol and LDL-cholesterol levels <2.0 and <3.5 mmol/l, respectively. Matched control individuals were volunteers who were recruited via advertisements. The presence of macrovascular disease (ECG abnormalities, abnormal ankle-brachial index or a history of cardiovascular events) served as exclusion criteria. Female participants were postmenopausal and not using hormone replacement therapy. The study protocol was performed at least 4 weeks after discontinuation of vasoactive medication, including ACE inhibitors, angiotensin receptor blockers and non-steroidal anti-inflammatory drugs. None of the patients or control volunteers used lipid-lowering medication. The Internal Review Board of the Academic Medical Center approved the study and all individuals gave written informed consent.

Vascular function was assessed using venous occlusion strain-gauge plethysmography (EC-4; Hokanson, Washington, DC, USA) [4]. Forearm blood flow (FBF), expressed as

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ml min⁻¹ 100 ml⁻¹ forearm tissue volume (FAV), was measured simultaneously in both arms. FBF responses to cumulative doses of the endothelium-dependent vasodilator serotonin (Sigma, Poole, UK; 0.6, 1.8 and 6 ng 100 ml⁻¹ FAV min⁻¹), the endothelium-independent vasodilator sodium nitroprusside (SNP; Spruyt Hillen, IJsselstein, the Netherlands; 6, 60, 180 and 600 ng 100 ml⁻¹ FAV min⁻¹), and the competitive inhibitor of endothelial nitric oxide (NO) synthase N^G-monomethyl-L-arginine (L-NMMA; Kordia, Leiden, the Netherlands; 50, 100, 200 and 400 µg 100 ml⁻¹ FAV min⁻¹) were measured. Agents were administered intra-arterially for 6, 4 and 8 min at each dose, respectively. Average FBF values of the measurement (cannulated) and control arm were obtained from the last six measurements of each measurement period. The three different infusion blocks proceeded after a 15 min rest period or until FBF had returned to baseline. The ratio of flow in the infused measurement (*M*) and non-infused control (*C*) arm was calculated for each recording (*M/C* ratio). The average value of the *M/C* ratio was calculated from these four to six *M/C* ratios, thus providing an internal control by excluding systemic factors from influencing the results [5]. Subsequently, a venous catheter was inserted in the contralateral arm for administration of rHDL (CSL-111; CSL Bioplasma, Parkville, VIC, Australia) at a dose of 80 mg/kg body weight over a period of 4 h. Subsequently, the infusion blocks were repeated. Blood samples were drawn from the individuals after a 12 h overnight fast, and at 4 h and 7 days after rHDL infusion. Descriptive statistics

between the two groups were compared by two-tailed independent Student's *t* tests or non-parametric tests, depending on skewedness of the data. Analysis of measurements for individuals between baseline and 4 h as well as baseline and 7 days after rHDL infusion was performed by two-way ANOVA for repeated measures with Bonferroni correction.

Infusion of rHDL was well tolerated and no adverse events were recorded. Characteristics of type 2 diabetes patients and control volunteers during each measurement are listed in Table 1. Baseline FBFs were not significantly different between type 2 diabetes patients and control individuals (Table 1). Intra-arterial infusion of serotonin increased FBF in a dose-dependent manner in both groups (see Fig. 1a). At baseline, the FBF response to serotonin was attenuated in type 2 diabetes compared with control volunteers (*M/C* ratio in type 2 diabetes: 1.5±0.2 vs controls: 2.5±0.3; *p*<0.05). Four hours after rHDL infusion in type 2 diabetes, FBF response to serotonin increased significantly (*M/C* ratio to 1.9±0.2; *p*<0.05 compared with baseline). rHDL infusion had no significant effect on serotonin-induced vasodilation in control volunteers. In type 2 diabetes, 7 days after rHDL infusion serotonin responses had returned to baseline values (7 days, 1.5±0.2). At baseline, the maximal vasoconstrictor response to L-NMMA was blunted in type 2 diabetes compared with control volunteers (*M/C* ratio controls 0.6±0.1 vs type 2 diabetes 1.0±0.2; *p*<0.05, Fig. 1b). After rHDL infusion, the L-NMMA response improved in type 2 diabetes

Table 1 Clinical characteristics of type 2 diabetes patients and control volunteers

	Type 2 diabetes patients (<i>n</i> =7)			Control volunteers (<i>n</i> =7)	
	Baseline	4 h	7 days	Baseline	4 h
Waist circumference (cm)	101±5			90±6 [†]	
Metabolic syndrome	6/7			0/7	
Systolic BP (mmHg)	148±12	146±11	143±7	135±16	138±12
Diastolic BP (mmHg)	78±13	79±13	82±8	83±9	85±8
Heart rate (beats per min)	65±9	67±9	66±12	61±9	64±8
Total cholesterol (mmol/l)	5.6±0.4	6.5±1.4	5.4±1.3	5.3±0.4	6.7±1.3
LDL-cholesterol (mmol/l)	2.9±0.6	3.3±1.1	3.1±1.0	3.0±0.7	3.9±1.2
HDL-cholesterol (mmol/l)	1.1±0.2	2.7±0.7*	1.6±0.6	1.2±0.3	2.5±0.4*
ApoA-I (g/l)	1.2±0.1	2.8±0.4*	1.5±0.3	1.2±0.2	2.7±0.4*
Triacylglycerol (mmol/l)	1.5±0.4	1.6±0.6	1.6±0.5	0.8±0.3 [†]	1.8±1.3*
Glucose (mmol/l)	8.3±1.2	6.8±1.7	7.3±1.3	5.2±0.4 [†]	4.9±0.2
hsCRP (mg/l)	3.5±1.6	4.4±1.7	3.6±0.8	1.0±0.9 [†]	1.8±1.1
ASAT (U/l)	22.5±2.5	20.4±4.0	22.4±4.3	20.2±1.8	21.4±2.7
ALAT (U/l)	31.2±6.9	26.0±9.7	27.8±8.7	14.9±1.7 [†]	15.6±3.2
Basal FBF (ml 100 ml ⁻¹ FAV min ⁻¹)	4.1±2.0	3.7±0.8	3.9±1.3	2.6±0.9	2.8±0.7

Values are means±SD

**p*<0.05 compared with baseline within one group; [†]*p*<0.05 between type 2 diabetes and control volunteers ALT, alanine aminotransferase; ASAT, aspartate aminotransferase; hsCRP, high-sensitivity C-reactive protein

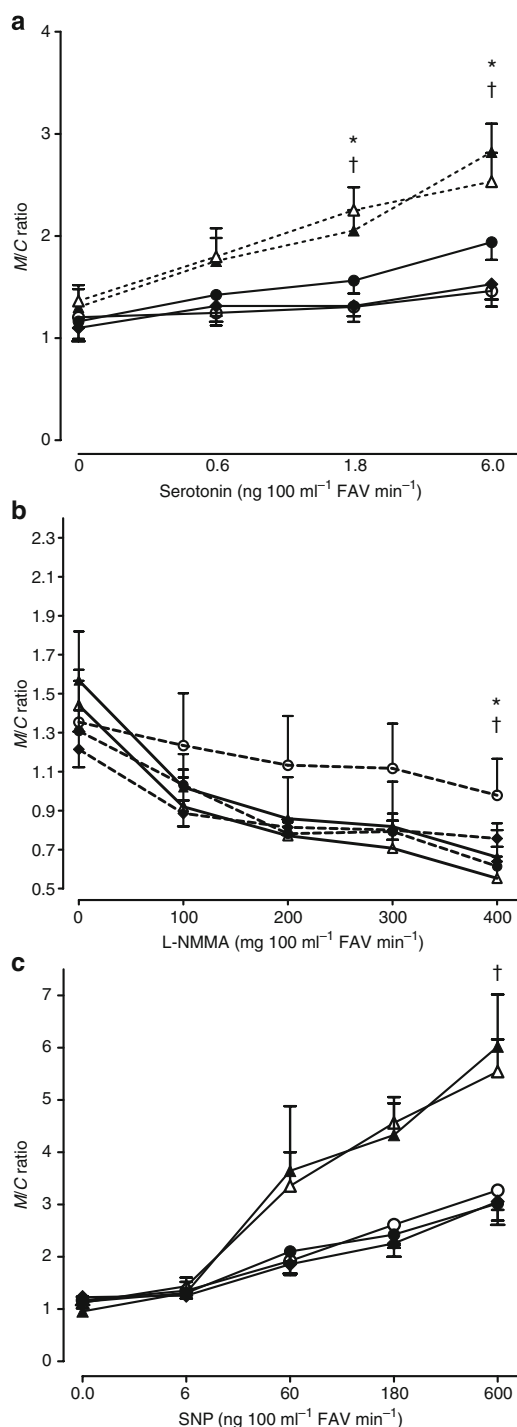


Fig. 1 Change in *M/C* ratio after stimulation with the endothelium-dependent vasodilator serotonin (**a**), NO inhibitor L-NMMA (**b**) and the endothelium-independent vasodilator SNP (**c**) before (white circles) and 4 h (black circles) and 7 days (diamonds) after rHDL infusion in type 2 diabetes patients as well as in control volunteers (before, white triangle; after 4 h, black triangle). ^b $p < 0.05$ for type 2 diabetes patients compared with control individuals and ^a $p < 0.05$ for changes after rHDL within type 2 diabetes patients. Data are means \pm SEM

compared with baseline (0.7 ± 0.1 ; $p < 0.05$). Although not significant, 7 days after rHDL infusion there still was a tendency towards improvement in type 2 diabetes (*M/C* ratio 0.8 ± 0.1 compared with baseline). rHDL infusion had no effect on L-NMMA response in control individuals. Finally, SNP responses were lower in type 2 diabetes vs control individuals at baseline (*M/C* ratio in type 2 diabetes, 3.0 ± 0.6 vs control individuals, 5.3 ± 0.7 ; $p < 0.05$, Fig. 1c) and rHDL infusion had no effect on SNP responses.

In conclusion, the present study confirms that basal and stimulated NO bio-availability is reduced in type 2 diabetes patients compared with control volunteers. Besides hyperglycaemia-induced reactive oxygen radical formation, other components of type 2 diabetes-associated dyslipidaemia such as small dense LDL are known to influence endothelial function in type 2 diabetes [3, 6]. Moreover, the high prevalence of metabolic syndrome in our patients is in line with previously published data [7]. rHDL resulted in a significant improvement of endothelial function within several hours in type 2 diabetes patients. More importantly, there was still a tendency towards improved NO availability 7 days after infusion, at a time when apolipoprotein A-I (ApoA-I) increase had largely disappeared. Acute HDL-increasing strategies are actively being pursued for further reducing cardiovascular burden [8], and thus far the lack of selective and potent HDL-increasing drugs has limited the success of the HDL-cholesterol increase concept. Our reported beneficial effects of ApoA-I infusion may lend further support to the development of ApoA-I-increasing strategies, also for patients with type 2 diabetes.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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