October 30, 2013

Dear Jin-Lei Wang,

Please find enclosed the edited manuscript in Word format (file name: 5341-review.doc).

Title: HDL endocytosis in endothelial cells

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Name of Journal: *World Journal of Biological Chemistry*

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer
(1) SR-BI has been defined (p.6).
(2) Statistical analysis of Fig. 3C has been made. Statistical analyses have been added to the methods (p.10).
(3) The issue of paracellular transport of HDL has been addressed in the introduction (p.5,6).
(4) Quality of the reconstituted HDL particles has been discussed (p.11).
(5) The competable and temperature-sensitive manner of HDL uptake has been mentioned (p.12).

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Biological Chemistry*.

Sincerely yours,

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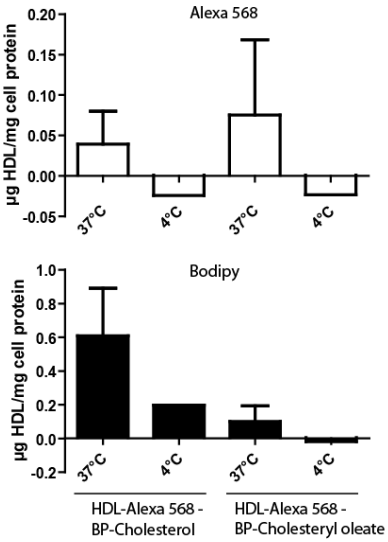
Additional information for the reviewers:

We found HDL uptake to be competed by addition of a 40-fold excess of unlabeled HDL in our experiments. This is the way how we measured unspecific cell association of HDL, which was used to calculate specific cell association (Fig. 3A).

We further analyzed the temperature sensitivity of the HDL uptake process. We used the double fluorescently labeled HDL particles HDL-Alexa 568-BP-Cholesterol and HDL-Alexa 568-BP-Cholesteryl oleate which were also used to analyze the remodelling of the particles during endocytosis (Fig. 3C). HUVECs were incubated with 50 µg/ml labeled HDL for 60 minutes either at 37°C (n=3) or at 4°C (n=1). Cells were then lyzed and fluorescence was measured. Specific cell association of HDL was again calculated by subtracting unspecific binding. We found no uptake of HDL-Alexa 568 (upper graph) at 4°C, indicating that holo-HDL particle uptake was inhibited at 4°C. BP-Cholesterol uptake from the double labeled particles was largely decreased at 4°C, BP-Cholesteryl oleate uptake was completely blocked, indicating that lipid transfer from HDL was also temperature dependent (lower graph).

Fig. HDL endocytosis is temperature-sensitive.



There was a legitimate concern raised about the composition and quality of the reconstituted HDL particles. In order to study the deliver of HDL and HDL-derived lipids in detail, we have to make use of cholesterol surrogates and other labels to follow HDL uptake. In order to assure that the reconstituted particles containing Bodipy-cholesterol or Bodipy-cholesteryl oleate resemble native HDL particles, we performed careful characterization of the particles in previous work. We found that size and shape of the reconstituted particles were comparable to native HDL and we claim therefore that they are suitable to provide physiological relevance.