Author's response to reviews

Title: Regulation of vascular tone by adipocytes

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Author's response to reviews: see over
Changes MS: 2382175854814920 - Regulation of vascular tone by adipocytes

We would like to thank the reviewers and the editorial board for the critical review of our manuscript, and for the interesting remarks helping to improve the quality of the paper. The manuscript has been adapted based on the suggestions.

Comments referee 1:

- Comment #1: The authors might more clearly indicate that H2S is a novel, important candidate of “adipocyte-derived relaxing factor”, which produces endothelium-independent relaxations. There are two reports on H2S as important candidate of ADRF (Fang et al, Schleifenbaum et al., both in J. Hypertens.). It is also clear that removed endothelium had no effects (Fang et al., 2009).

We clearly indicated that H2S is a novel candidate for the ADRF as described on page 16:

“Also hydrogen sulfide has been proposed as a novel candidate of the ADRF or at least a mediator in the ADRF effect [115,118], which is consistent with inactivation of ADRF by heating (65°C, 10 min) [6]. Hydrogen sulfide has been recently described as a gasotransmitter generated by cystathionine gamma-lyase (CSE) in perivascular adipose tissue [119,120]. Blocking of CSE inhibits the vasorelaxing effect of perivascular adipose tissue in rat aorta and mice mesenteric arteries [115,118]. Moreover, hydrogen sulfide-induced vasorelaxation of rat aorta was inhibited by a particular ADRF-related potassium channel (KCNQ) blocker [115].”


- Comment #2: please cite the paper referenced by the reviewer and balance the discussion. The authors state that “it has been postulated that epoxyeicosatrienoic acids (EETs)/EDHF-dependent vasorelaxation might act as a back-up plan in case of reduced NO availability in vivo [36]”. To introduce a balanced discussion, it might be appropriate to discuss that EETs can also cause NO release to influence vascular tone (Hercule et al., ATVB, 2009).

“On the other hand, EETs are able to activate endothelial NO synthase and subsequently release NO to influence arterial tone [37].”

- Comment #3: please clarify. Fesüs et al. have studied adiponectin gene-deficient mice, but not adiponectin receptor 1 knock out mice.

“However, vasorelaxation induced by perivascular adipose tissue remained unchanged in adiponectin gene-deficient mice [91].”
• Comment #4: please clarify. At this place, the reference of Gao YJ, Lu C, Su LY, Sharma AM, Lee RM is misleading. Gao et al did not study adiponectin gene deficient mice. ADRF effects are endothelium-independent (see ad 1). Therefore, it is unlikely that hydrogen peroxide plays a role in the effects. Instead, it is possible that Gao’s work is more related to other adipokins, for example TNFalpha, which “impairs endothelium-dependent vasorelaxation in various vascular beds as a result of a decrease in endothelial NO release or an increase in NO-scavengers (ROS) [65]”. Of note, you also mentioned a recent study, which “has shown a reduced vasorelaxing effect of perivascular adipose tissue in response to TNF# and interleukin-6 (IL-6), which upregulate ROS [54]”.

We agree with the reviewer that Gao et al. did not study adiponectin gene deficient mice. It should however be noted that the ADRF might act through both endothelium-independent and -dependent mechanisms. (Cfr. “Whether nitric oxide (NO) formation and endothelium are involved the vasorelaxation effect by ADRF is still a matter of debate [6,92,112].”) We agree that perhaps not only hydrogen peroxide is responsible for the endothelium-independent pathway of the ADRF. Hydrogen peroxide relaxes arteries in an endothelium-dependent and independent way. (Cfr “Vasorelaxation is possibly induced by endothelium-dependent mechanisms, involving the release of vasodilating cyclooxygenase metabolites [23] and NO [24], and endothelium-independent mechanisms [21] mediated by activation of different potassium channels on smooth muscle cells [23,25,26].”). Therefore, we suggest to refer to Gao et al. but to omit the mentioning to the involvement of hydrogen peroxide.

“It is possible that this vasorelaxing effect of perivascular adipose tissue in the adiponectin gene-deficient mice might be the result of an endothelium-independent pathway [92].”

• Comment #5: How is it possible that IL-6 produces vasorelaxation, but genetic deletion of IL-6 attenuates angiotensin II-induced hypertension in mice [87]. Please, provide a more critical discussion.

A different response of IL-6 is seen after a sustained increase or acute exposure of IL-6 as described on page 9:

“A sustained increase in pro-inflammatory cytokine interleukin-6 (IL-6) plasma levels is associated with high blood pressure [64,65]. On the other hand, acute exposure of IL-6 in vitro relaxes aortas [66].”

• Comment #6: “The angiotensinogen-deficient mice were normotensive, due to angiotensinogen expression.” What do you mean?

Described on page 18:

“When angiotensinogen expression was restricted to adipose tissue (on an angiotensin-deficient background), circulating angiotensinogen was detected and mice were normotensive.”
Comment #7: The authors state: “However, hydrogen sulfide generation and CSE expression in perivascular adipose tissue (but not in aorta) are shown to be increased in hypertensive rats [143], while the vasorelaxing effect of perivascular adipose tissue is shown to be impaired in hypertension [144].” This statement seems to be too broad and incorrect since different models of hypertension have been used. Please, note that hypertension in [143] has been induced by abdominal aortic banding. In this report, H2S generation and CSE protein expression were significantly increased in PAT of stenotic aortas but not in aortic tissues. Transplanting PAT into periadventitia of stenotic aortas ameliorated the elevated arterial blood pressure (Fang et al., J Hypertens 2009). In my view the data show that there seems to be a compensatory up-regulation of CSE in PAT of stenotic aortas, which could have been developed independently of hypertension? Please, note that CSE knockout mice are hypertensive (Yang et al., Science 2008). Please, also note that SHR rats have been used in [144] as model of hypertension, which is different to aortic banding.

The manuscript has been adapted according to the referees suggestion on page 16:

“However, hydrogen sulfide generation and CSE expression in perivascular adipose tissue of stenotic aortas (but not in aortic tissue) are shown to be increased in hypertensive rats induced by abdominal aortic banding [118], while the vasorelaxing effect of perivascular adipose tissue is shown to be impaired in spontaneously hypertensive rats [121]. This might indicate that other ADRF(s) besides hydrogen sulfide are impaired resulting in a reduced vasorelaxing effect of adipose tissue. On the other hand, it is difficult to compare both studies as different models of hypertension have been used. Furthermore, the up-regulation of CSE and hydrogen sulfide generation in perivascular adipose tissue of stenotic aortas may have been developed independently of hypertension as CSE knock-out mice are shown to be hypertensive [119].”

The fact that transplanting PAT into periadventitia of stenotic aortas ameliorated the elevated blood pressure (Fang et al., J Hypertens 2009), does not say anything about CSE expression. Furthermore, the reduced blood pressure by H2S from PAT could be the result of either stenosis and/or hypertension itself. Therefore, if the reviewer agrees, we prefer not to mention this statement in the manuscript.
Comments referee 2:

- **Comment #1:** A point to be addressed centers on page 18, lines 4-6, where it is not clear what the protein bands were. Were these ADRF bands?

  Described on page 16:

  “Furthermore, analyses of adipose tissue secrete in a recent electrophoresis study resulted in the visualization of different protein bands with different molecular masses (13.8 to 74.0 kDa) which may include ADRF [116].”

- **Comment #2:** somewhere in the discussion please mention the potential contribution of stretch, shear stress and mechanical factors. In the instances where obesity is associated with the production of an adipokine have the authors considered the influence of stretch or mechanical factors on adipokine production and endothelial dysfunction? The morphology of adipose tissue in obese individuals seems to warrant consideration of the influence of stretch or mechanical forces on blood vessel integrity and function.

  Whether mechanical forces exerted by blood flow, such as shear stress, stretch and pressure at the endothelial surface, is altered in obesity, affecting blood vessel integrity and function, is still a matter of debate. For example, in brachial arteries some studies give evidence for a maintained local shear stress during vascular remodelling in obesity (Chung et al. 2009: “The brachial artery remodels to maintain local shear stress despite the presence of cardiovascular risk factors”), while other studies demonstrated a lower shear stress (Hamburg et al. 2010: “Maladaptive enlargement of the brachial artery in severe obesity is reversed with weight loss.”). Unfortunately as yet no information is available whether altered shear stress on blood vessels influences adipokine production by adipose tissue. Therefore, if the reviewer agrees, we would not like to mention this discussion into the manuscript in order to simplify the text.
Comments referee 3:

- Comment #1: “surroundings” not surrounding; “pathways” not pathway

This has been adapted in the manuscript on page 3 (surroundings) & 6 (pathways).

- Comment #2: Table 1: “angiogenesis” not angiogenese

This has been adapted in the manuscript.

- Comment #3: It would be helpful to include a hierarchy regarding effects of different factors in vasoconstriction/relaxation – if you think this is feasible of course.

We are afraid it is not feasible to put a hierarchy of the vasoactive effects of the adipokines described in the manuscript due to the complexity of these effects.
Abstract page 2 line 9: “Adipokines like adiponectin, omentin and visfatin are vasorelaxants, even as the as yet unidentified “adipocyte-derived relaxing factor” (ADRF).” Incomplete sentence? Do you mean that ADRF is suspected to be a vasorelaxant too?

The sentence is changed into:

“For example the unidentified “adipocyte-derived relaxing factor” (ADRF) released from adipose tissue has shown to relax arteries. Besides the ADRF, other adipokines like adiponectin, omentin and visfatin are vasorelaxants.”

Abstract page 2 line 15: “Dysregulated synthesis of these vasoactive adipokines may underlie the compromised vascular reactivity in obesity and obesity-related disorders.”

How does the vasoactive characteristic relate to the pro/anti-inflammatory functions mentioned at the beginning of the abstract – perhaps draw both concepts together in the final sentence of the abstract, for clarity.

“Dysregulated synthesis of the vasoactive and pro-inflammatory adipokines may underlie the compromised vascular reactivity in obesity and obesity-related disorders.”

Please lead into the next section by briefly mentioning the importance of the vasoactive and vasoconstrictive activities of the adipokines, and hint that inflammation is also a key process. This will set the reader up to expect the extra detail that you give in the next section.

“Maintenance of a normal amount of adipose tissue is essential as imbalance can cause serious health problems, since dysregulated release of adipokines may lead to vascular disturbances and inflammation.”

Finish this section by introducing the fact that you’ll now describe each adipokine and its effects in physiological and obesity-related environments. Also give some higher-order subheadings in this section to group the vasorelaxors, the vasoconstrictors, and the dual function vasoregulators.

Page 5: “The vasoactive adipokines and their role in physiological conditions, in obesity and obesity-related disorders are described further in more detail.”

Subheadings are added in the adapted version of the manuscript.

In the conclusions it would also be interesting to our readers if you could talk about what latest information there is about targeting adipokines as potential therapeutics to tackle obesity – any other related information about adipokines and obesity are being linked in the clinic?
This has been described on page 20:

“One therapeutic strategy to counter the progression of obesity-related vascular diseases is elevating adiponectin and omentin levels. Adiponectin levels are already elevated when using thiazolidinediones, telmisartan, angiotensin converting enzyme inhibitors, rimonabant and taranabant [88]. On the other hand, development of specific agonists to target adiponectin and omentin receptors or inhibiting detrimental adipokines signaling pathways may be new and promising to attenuate the pro-inflammatory effects and ultimately to reduce the progression of obesity-related vascular diseases.”