

BUKTI KORESPONDENSI

Judul Artikel : Losartan Has a Comparable Effect to Human Recombinant ACE2 in Reducing Inflammation on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein

Jurnal : The Indonesian Biomedical Journal

Author : Hanestyha Oky Hermawan¹, Meity Ardiana^{1*}, I Gde Rurus Suryawan¹, Primasitha Maharany Harsoyo¹, Muhammad Rafli²

Corresponding: Meity Ardiana

No.	Perihal	Tanggal	Komentar Editor Jurnal	Komentar Penulis
1	Pengiriman Artikel	25 Juli 2023	Editor mengirim email konfirmasi dan invoice untuk submission fee. Email : Jul 25, 2023 at 8:56 AM	-
2	Pengiriman Revisi	2,6,7 Agustus 2023 13 September 2023	Editor memberitau bahwa naskah telah diberi kode sebagai M2023224. Sebelum naskah Anda dikirim ke reviewer kami, naskah telah diperiksa terlebih dahulu. Untuk daftar detail koreksinya, dapat menemukannya pada naskah terlampir. Mohon revisi naskah ini secara menyeluruh dan sesuai saran. Silakan kirimkan kepada kami email naskah a yang telah dikoreksi serta Formulir Kontribusi Penulis yang telah diisi sebelumnya 7 Agustus 2023 dan menandai/menyorot bagian naskah yang telah direvisi.	Penulis mengirimkan artikel yang telah diperbaiki (Lampiran). Email : Aug 6, 2023 at 11:30 AM

			<p>Email : Aug 2, 2023 at 11:15 AM</p> <p>Naskah Anda, nomor M2023224, sudah ada</p> <p>Lulus di pemeriksaan awal dan dikirim ke peninjau untuk proses peninjauan sejawat. Editor akan segera memberi tahu Anda setelah kami menerima tanggapan/komentar dari reviewer. Proses peninjauan biasanya memakan waktu sekitar 3-4 minggu.</p> <p>Email : Aug 7, 2023 at 2:46 PM</p>	
		4 s.d 20 September 2023	<p>Editor memberitau bahwa Artikel: Resubmit for Review.</p> <p>Email : Sep 4, 2023 at 11:27 AM</p> <p>Editor memberitau bahwa Artikel: Revisions Required</p> <p>Email : 13 September 2023 pukul 14.15</p>	<p>Penulis mengirim pesan akan merevisi segera.</p> <p>Email : Sep 5, 2023 at 9:40 AM</p> <p>Penulis mengirim revisi pertama.</p> <p>Email : Sep 11, 2023 at 11:56 PM</p> <p>Penulis mengirim revisi kedua.</p>

				Email : 20 September 2023 pukul 21.48
3	Penerimaan Artikel	21,28 September 2023	<p>Editor telah memutuskan bahwa naskah dapat diterima untuk diterbitkan, namun untuk menerima surat penerimaan, kami memerlukan Anda untuk melakukannya menyelesaikan pembayaran biaya publikasi.</p> <p>Berikut kami lampirkan invoice biaya penerbitan naskah M2023224. Silakan selesaikan pembayaran sebelumnya 29 September 2023</p> <p>Email : 21 September 2023 pukul 08.53</p> <p>Reminder APC. Email : 27 September 2023 pukul 14.20</p>	Penulis akan membayar APC. Email : 28 September 2023 pukul 06.33
			<p>Editor memberitau bahawa naskah : Accept Manuscript.</p> <p>Email : 2 Oktober 2023 pukul 16.45. Setifikat terlampir.</p>	



Hanestya Oky Hermawan <okyhermawan1@gmail.com>

[InaBJ] #2552 Submission Notification

Secretariat of InaBJ <secretariat@inabj.org>
To: okyhermawan1@gmail.com

Tue, Jul 25, 2023 at 8:56 AM

Dear Dr. Hanestya Oky Hermawan,

Good day. Thank you for your submission of the manuscript "**Losartan Does Not Trigger Cytokine Storms and Has Comparable Effect to Human Recombinant ACE2 on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein**" to The Indonesian Biomedical Journal.

Regarding the [regulation about submission and publication fee](#) for our journal, please notice that other than submission fee, once the manuscript is considered acceptable, authors need to also pay for the publication fee. For detailed information, please click the link provided above.

Hence, herein we attach the invoice for submission #2552. Please complete the payment before **July 31, 2023**. Please let us know once you have done with the payment process (make sure you send us the payment proof), so that we can proceed your manuscript to the initial check.

Thank you. If you have any questions, please contact us.

Best Regards,

--

Secretariat of The Indonesian Biomedical Journal

Prodia Tower 9th Floor

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Phone. +62-21-3144182 ext. 3872

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Hanestya Oky Hermawan <okyhermawan1@gmail.com>

[InaBJ] M2023224 Manuscript Initial Check

6 messages

Secretariat of InaBJ <secretariatinabj@gmail.com>
To: oky hermawan <okyhermawan1@gmail.com>

Wed, Aug 2, 2023 at 11:15 AM

Dear Dr. Hanestya Oky Hermawan,

Good day. Thank you for your submission of the manuscript "**Losartan Does Not Trigger Cytokine Storms and Has Comparable Effect to Human Recombinant ACE2 on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein**". Your manuscript has been coded as **M2023224**, please note this code for your reference to communicate with us regarding this manuscript in the future.

Before your manuscript is sent to our reviewers, it has been initially checked. For a detailed list of corrections, you can find it in the manuscript attached. Please revise this manuscript thoroughly and according to the suggestions. And also, if you have other unpublished data to be added to the manuscript, please do to enrich the quality of the manuscript.

Please send us an email of your corrected manuscript as well as the filled Author Contribution Form before **August 7, 2023** so that we can proceed with the peer-reviewing process. Do not forget to **mark/highlight the revised part of the manuscript**, so that the editor will notice the changes.

If you have any questions, do not hesitate to contact us. Thank you. We wish you a nice day.

Best Regards,

--

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Prodia Tower 9th Floor

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Fax. +62-21-3144181

<https://www.inabj.org>**2 attachments** **M2023224 Manuscript - Initial Check.docx**
258K **AUTHOR CONTRIBUTIONS FORM.doc**
222K**oky hermawan** <okyhermawan1@gmail.com>
To: Secretariat of InaBJ <secretariatinabj@gmail.com>

Wed, Aug 2, 2023 at 12:51 PM

Thank you, we will send a revised manuscript soon.

[Quoted text hidden]

oky hermawan <okyhermawan1@gmail.com>
To: muhammadraffi033.dr19@student.unusa.ac.id

Wed, Aug 2, 2023 at 12:54 PM

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oky hermawan <okyhermawan1@gmail.com>
To: Secretariat of InaBJ <secretariatnabj@gmail.com>

Sun, Aug 6, 2023 at 11:30 AM

Honorable Editor-in-Chief,
The Indonesian Biomedical Journal

On behalf of my co-authors, enclosed is a revised manuscript of 'Losartan Has a Comparable Effect to Human Recombinant ACE2 in Reducing Inflammation on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein' (M2023224) to be considered for publication as original research in The Indonesian Biomedical Journal.

It is our sincerest hope that these emendations align with your vision to provide the best content through your journal and that the findings in this study will be of interest to your readers.


We look forward to hearing from you in due course.

Sincerely,

Hanestya Oky Hermawan, MD, M.Biomed
Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga
47 Mayjend Prof. Dr. Moestopo Street
Surabaya 60132, Indonesia
Phone: +62-858-5555-7748
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3 attachments

 **Response Letter_Revised manuscript_M2023224.docx**
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Secretariat of InaBJ <secretariatnabj@gmail.com>
To: oky hermawan <okyhermawan1@gmail.com>

Mon, Aug 7, 2023 at 2:46 PM

Dear Dr. Hanestya Oky Hermawan,

Your manuscript, number M2023224, has passed our initial check and is being sent to reviewers for the peer reviewing process.

We will let you know immediately after we receive responses/comments from the reviewers. The review process usually takes around 3-4 weeks.

Thank you. We wish you a nice say.

Best Regards,
[Quoted text hidden]

Hanestya Oky Hermawan <okyhermawan1@gmail.com>
To: Secretariat of InaBJ <secretariatnabj@gmail.com>

Mon, Aug 7, 2023 at 6:13 PM

Thank you for the update.
[Quoted text hidden]



Hanestya Oky Hermawan <okyhermawan1@gmail.com>

[InaBJ] M2023224 Editor Decision - Resubmit for Review

5 messages

Secretariat of InaBJ <secretariat@inabj@gmail.com>
To: oky hermawan <okyhermawan1@gmail.com>

Mon, Sep 4, 2023 at 11:27 AM

Dear Dr. Hanestya Oky Hermawan,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**Losartan Has a Comparable Effect to Human Recombinant ACE2 in Reducing Inflammation on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein**".

Our decision is to: **Resubmit for Review.**

This manuscript is interesting, however it still needs some revisions before it can be published. Besides the comments our reviewers have given, please also pay attention to the use of English language, make sure you avoid grammatical and diction errors.

Find the file attached to detailed comments from Reviewer 1 (R1) and Reviewer 2 (R2), there are 2 files from R1 and 1 file from R2. Please revise this manuscript thoroughly and according to the suggestions before **September 11, 2023**. Mark/highlighted the revised part of the manuscript, so that the editor will notice the changes.

You are also obligated to provide a response letter with your response or the answer to reviewers' questions/comments. For an example on how to write a response letter, we also attach a response form template. Hopefully you find it well.

When you are done, you can upload it in: <https://inabj.org/index.php/ibj/author/submissionReview/2552>, or simply send us an email of your revised manuscript and response letter.





Please let us know when you have received this email. If you have any questions, do not hesitate to contact us. Thank you for your attention. We wish you a nice day.

Best Regards,

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4 attachments





-  **M2023224 F09 Manuscript Review Form (from R1).doc**
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Hanestya Oky Hermawan <okyhermawan1@gmail.com>
To: muhammadrafi033.dr19@student.unusa.ac.id

Mon, Sep 4, 2023 at 12:11 PM

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Hanestya Oky Hermawan <okyhermawan1@gmail.com>
To: Secretariat of InaBJ <secretariatnabj@gmail.com>

Tue, Sep 5, 2023 at 9:40 AM

Dear secretariat of InaBJ,
Thank you. We are currently working on the revision. We will send the revised manuscript as soon as possible.

Best regards,
Hanestya Oky Hermawan
[Quoted text hidden]

Secretariat of InaBJ <secretariatnabj@gmail.com>
To: Hanestya Oky Hermawan <okyhermawan1@gmail.com>

Mon, Sep 11, 2023 at 8:34 AM

Dear Dr. Hanestya Oky Hermawan,

Good day. A gentle reminder that we will be waiting for your revision of manuscript M2023224 until September 11, 2023.
Please let us know the progress of your revision. Please do not hesitate to contact us if you have any questions regarding the review results.

Thank you. We wish you a nice day.

Best Regards,

[Quoted text hidden]

Hanestya Oky Hermawan <okyhermawan1@gmail.com>
To: Secretariat of InaBJ <secretariatnabj@gmail.com>

Mon, Sep 11, 2023 at 11:56 PM

Honorable Editor-in-Chief,
The Indonesian Biomedical Journal



On behalf of my co-authors, enclosed is a round 1 revised manuscript of 'Losartan Has a Comparable Effect to Human Recombinant ACE2 in Reducing Inflammation on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein' to be considered for publication as original research in The Indonesian Biomedical Journal.

It is our sincerest hope that these emendations align with your vision to provide the best content through your journal and that it is in our belief that the findings in this study will be of interest to your readers.

We look forward to hearing from you in due course.

Sincerely,
Meity Ardiana
Hanestya Oky Hermawan
[Quoted text hidden]

2 attachments

-  **Response Letter.xlsx**
14K
-  **Revision Losartan_ Round 1.docx**
319K



ardiana, Meity <meityardiana@fk.unair.ac.id>

[InaBJ] M2023224 Editor Decision Round 2 - Revisions Required

3 pesan

Secretariat of InaBJ <secretariatinabj@gmail.com>

13 September 2023 pukul 14.15

Kepada: oky hermawan <okyhermawan1@gmail.com>, meityardiana@fk.unair.ac.id

Dear Dr. Hanestya Oky Hermawan,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**Losartan Has a Comparable Effect to Human Recombinant ACE2 in Reducing Inflammation on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein**".

Our decision is to: **Revisions Required.**

Thank you for your revision in the previous round. However, this manuscript still needs some revisions as suggested by the editor. Please find the attached file to see the detailed comments.

Please revise this manuscript according to the suggestions before **September 20, 2023**. Mark/highlighted the revised part of the manuscript so that the editor will notice the changes. You are also obligated to provide a response letter with your response or the answer to each given comments. For an example on how to write a response letter, we also attach a response form template. Hopefully you find it well.

When you are done, you can upload it in: <https://inabj.org/index.php/ibj/author/submissionReview/2552>, or simply send us an email.

Please let us know when you have received this email. If you have any questions, do not hesitate to contact us. Thank you for your attention. We wish you a nice day.

Best Regards,

--

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Fax. +62-21-3144181

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**Response Form for Reviewer's Comments.xlsx**

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Secretariat of InaBJ <secretariatinabj@gmail.com>

18 September 2023 pukul 08.09

Kepada: oky hermawan <okyhermawan1@gmail.com>, meityardiana@fk.unair.ac.id

Dear Authors,

Good day. A gentle reminder that we will be waiting for your second round of revision for manuscript "**Losartan Has a Comparable Effect to Human Recombinant ACE2 in Reducing Inflammation on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein**" until **September 20, 2023**. Since we are planning to include your article in our next issue, an earlier response will be better to avoid further delay.

Please confirm whether you have read our previous email regarding the second round of review results. Thank you for your attention. We wish you a nice day.

Best Regards,

[Kutipan teks disembunyikan]

ardiana, Meity <meityardiana@fk.unair.ac.id>
Kepada: Secretariat of InaBJ <secretariatinabj@gmail.com>

20 September 2023 pukul 21.48

Honorable Editor-in-Chief,
The Indonesian Biomedical Journal

On behalf of my co-authors, enclosed is a second round revised manuscript of 'Losartan Has a Comparable Effect to Human Recombinant ACE2 in Reducing Inflammation on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein' (M2023224) to be considered for publication as original research in The Indonesian Biomedical Journal.

It is our sincerest hope that these emendations align with your vision to provide the best content through your journal and that the findings in this study will be of interest to your readers.

We look forward to hearing from you in due course.

Sincerely,

Dr. dr. Meity Ardiana, Sp.JP(K), FIHA

Department of Cardiology and Vascular Medicine, Airlangga University – Soetomo General Hospital, Surabaya, Indonesia
+62812-5980-8492

meityardiana@fk.unair.ac.id

[Kutipan teks disembunyikan]

2 lampiran



Response Form for Reviewer's Comments (1).xlsx

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ardiana, Meity <meityardiana@fk.unair.ac.id>

[InaBJ] M2023224 Publication Fee Invoice

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Secretariat of InaBJ <secretariatnabj@gmail.com>

21 September 2023 pukul 08.53

Kepada: oky hermawan <okyhermawan1@gmail.com>, meityardiana@fk.unair.ac.id

Dear Authors,

Good day. Thank you for your revision of manuscript M2023224 "**Losartan Has a Comparable Effect to Human Recombinant ACE2 in Reducing Interleukin-6 (IL-6) Levels on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein**".

Our editor has decided that your manuscript is acceptable for publication, but to receive a letter of acceptance, we need you to **complete the payment for publication fee**.

Herein we attach the invoice for publication fee of the manuscript M2023224. Please complete the payment before **September 29, 2023**. Let us know once you have done with the payment process (by sending us the payment proof).

Thank you. We will be waiting to hear from you.
We wish you a nice day.

Best Regards,

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Secretariat of InaBJ <secretariatnabj@gmail.com>

27 September 2023 pukul 14.20

Kepada: oky hermawan <okyhermawan1@gmail.com>, meityardiana@fk.unair.ac.id

Dear Dr. Meity Ardiana,

Good day. A gentle reminder that we will be waiting for your publication fee payment for manuscript M2023224 until **September 29, 2023**. Please send us a payment proof once you are done with the payment process.

Thank you.

Best Regards,

[Kutipan teks disembunyikan]

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[Kutipan teks disembunyikan]

ardiana, Meity <meityardiana@fk.unair.ac.id>

28 September 2023 pukul 06.33

Kepada: Secretariat of InaBJ <secretariatnabj@gmail.com>

Cc: oky hermawan <okyhermawan1@gmail.com>

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Metode Transfer BI Fast

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HANESTYA OKY H

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Secretariat of InaBJ <secretariatnabj@gmail.com>
Kepada: "ardiana, Meity" <meityardiana@fk.unair.ac.id>
Cc: oky hermawan <okyhermawan1@gmail.com>

29 September 2023 pukul 07.45

Dear Dr. Meity Ardiana,

Thank you for your publication fee payment for #2552 (M2023224). We will confirm it to our finance department. We will send you the Letter of Acceptance later soon after we receive the confirmation.

Thank you for your cooperation. We wish you a nice day.

Best Regards,

[Kutipan teks disembunyikan]



ardiana, Meity <meityardiana@fk.unair.ac.id>

[InaBJ] M2023224 Editor Decision - Manuscript Accepted

1 pesan

Secretariat of InaBJ <secretariatinabj@gmail.com>

2 Oktober 2023 pukul 16.45

Kepada: oky hermawan <okyhermawan1@gmail.com>, "ardiana, Meity" <meityardiana@fk.unair.ac.id>

Dear Authors,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**Losartan Has a Comparable Effect to Human Recombinant ACE2 in Reducing Interleukin-6 (IL-6) Levels on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein**".

Our decision is to: **Accept Manuscript.**

Your manuscript will be sent to our publisher for typesetting and you should receive the proofreading in due course.

Congratulations on your interesting research, and thank you for allowing us to publish this valuable material. Please let us know once you have read this email. We wish you a nice day.

Best Regards,

--

Secretariat of The Indonesian Biomedical Journal

Prodia Tower 9th Floor

[Jl. Kramat Raya No.150, Jakarta 10430, Indonesia](#)

Phone. +62-21-3144182 ext. 3872

Fax. +62-21-3144181

<https://www.inabj.org>**Certificate for Author #M2023224 - Hanestya Oky Hermawan [SIGNED].pdf**

285K

1 **Losartan Has a Comparable Effect to Human Recombinant ACE2 in**
2 **Reducing Interleukin-6 (IL-6) Levels on Human Adipocytes Exposed to**
3 **SARS-CoV-2 Spike Protein**
4

5 **ABSTRACT**

6 **Background:** High angiotensin-converting enzyme 2 (ACE2) expression in adipocyte cells
7 facilitates the initiation of SARS-CoV-2 infection and triggers a cytokine storm. This finding
8 suggests that obesity is an independent risk factor for the severity of the symptoms caused by
9 COVID-19. The use of cardiovascular medications that focus on ACE2, such as angiotensin II
10 receptor blockers, remains controversial, and their effects on inflammatory cytokine production
11 and ACE2 expression in cells, especially adipocytes, remain inconsistent.

12 **Methods:** The human adipocytes were isolated from obese donor subcutaneous adipose tissue
13 and infected with the subunit S1 spike protein from SARS-Cov-2. The adipocytes were later
14 treated with either hrsACE2 or losartan. The levels of ACE2 and inflammatory cytokines
15 interleukin (IL)-6, IL-1 β , and tumor necrosis factor (TNF)- α were measured using enzyme
16 linked immunosorbent assay (ELISA). ACE2 and S1 spike protein binding assays were also
17 performed.

18 **Results:** ACE2, Il-6, and TNF- α levels were significantly increased in human adipocyte cells
19 infected with SARS-Cov-2 but not IL-1 β . There was a statistically significant positive
20 correlation between ACE2 and IL-6 ($r=0.878$, $p<0.001$). Administration of losartan and
21 hrsACE2 was shown to reduce ACE2 levels and its binding to the SARS-CoV-2 S1 spike
22 protein, and IL-6 levels were statistically significant, but had no significant effect on IL-1 β or
23 TNF- α levels.

24 **Conclusion:** This study shows that the administration of losartan in COVID-19 may not be
25 harmful, but instead has a protective effect similar to that of HrsACE2 in preventing a cytokine
26 storm, especially IL-6.

27 **Keywords:** obesity, SARS-CoV-2, losartan, IL-6, ACE2

28

29 **Introduction**

30 The Coronavirus (COVID-19) pandemic has spread across the world becoming a global health
31 issue, leading to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) with
32 morbidity and mortality that cannot be underestimated. Currently, various types of vaccines
33 have been developed and are being used, which are key to reducing the transmission of
34 COVID-19. However, several factors are thought to reduce the protective effect, one of which
35 is obesity, which has many theories linking it to the immune system.[1] Obesity significantly
36 increases the likelihood of developing a serious case of COVID-19 and of experiencing severe
37 symptoms. People with obesity are strongly associated with a higher risk of requiring intensive
38 care treatment and facing a higher mortality risk in hospitals.[2–4] Obesity has long been
39 associated with adipocyte dysfunction that affects not only the metabolic homeostasis system
40 but also the body's overall homeostasis, particularly inflammation in immune system.[5]

41 Adipocytes are believed to play a significant role in the mechanism underlying SARS-
42 CoV-2 infection by facilitating transmission, replication, and release of the virus. The infection
43 process of SARS-CoV-2 begins with the binding of viral glycoprotein molecules to the
44 angiotensin-converting enzyme 2 (ACE2) receptor on the membrane of host cells. It is
45 important to note that SARS-CoV-2 can only infect cells that express the ACE2 receptor.[6,7]
46 Adipocytes, which are known to express high levels of ACE2 receptors, have been found to
47 exhibit elevated ACE2 gene expression in various tissues such as the small intestine, testis,
48 kidneys, heart, thyroid, and adipose tissue. Surprisingly, gene bank data analysis revealed that

49 ACE2 gene expression in adipose tissue surpassed that in the lungs. Furthermore, individuals
50 with obesity tend to have adipocytes that produce higher levels of proinflammatory cytokines
51 like interleukin (IL)-6 even without any external stimuli, in comparison to non-obese
52 individuals.[8–10] IL-6 is known to be the cause of cytokine storms that led to multiple organ
53 damage in COVID-19.[11,12] This evidence could be an explanation of how obesity can
54 develop severer clinical manifestations of COVID-19.[13,14]

55 ACE2 is a glycoprotein that is firmly embedded in the cell membranes and is widely
56 expressed in various tissues. It serves as a vital natural regulator of the renin–angiotensin
57 system (RAS). One of its key functions is the conversion of angiotensin II (Ang II) into
58 angiotensin-(1-7), as well as the conversion of angiotensin I (Ang I) into angiotensin (1-9).[15]
59 Raising concern about the use of ACE inhibitors (ACEIs) and angiotensin II receptor blockers
60 (ARBs) have increased since the identification of ACE2 as a SARS-CoV-2 receptor. Several
61 animal experiments have shown that the administration of ACEI and ARB causes ACE2
62 receptor overexpression, especially in the cardiovascular system. ACE2 overexpression is
63 hypothesized to increase susceptibility to SARS-CoV-2 infection and aggravate the severity of
64 COVID-19.[16][17]

65 However, the impact of ACEI and ARB on ACE2 receptor expression is not uniform
66 across all types [16]. Additionally, no existing studies have examined this effect in cases where
67 baseline ACE2 levels are already elevated in obese individuals. To address this, we conducted
68 a previous study to evaluate the influence of losartan, a commonly used ARB. We specifically
69 examined the effect of losartan on ACE2 expression and production of proinflammatory
70 cytokines in SARS-CoV-2 infected adipocytes, which simulate obesity conditions *in vitro*.

71

72 **Methods**

73 ***Primary Culture of Adipocytes***

74 Adipocytes were isolated from subcutaneous adipose tissue taken from a donor. The donor was
75 an individual with obesity (BMI >30) without any significant history of disease indicated with
76 normal blood count, normal renal and liver function, and without cardiac structure and function
77 abnormality assessed by echocardiography. The donor also never tested positive for COVID-
78 19 or received any COVID-19 vaccines. This study was conducted under the approval of the
79 Health Research Ethic Committee Faculty of Medicine, Universitas Brawijaya, Malang,
80 Indonesia (No. 198/EC/KEPK/07/2021).

81 Adipose tissue was obtained from subcutaneous in the abdomen region by surgical
82 resection. The donor was obtained from a person who had signed a consent form for a surgical
83 resection of the abdominal area to remove subcutaneous adipose tissue for research purposes.
84 Adipose tissue was then isolated enzymatically.[18] Briefly, adipocytes were finely minced
85 and digested using type 1 Collagenase (Cat. # 17018029, Thermo Fisher Scientific, Waltham,
86 MA, USA) for 30 min in a 37°C water bath with shaking at 100 rpm. Cells were passed through
87 a nylon mesh filter and washed with culture media three times. Cells were resuspended and
88 were cultured in growth media composed of Dulbecco's modified Eagle's medium/F-12 (Cat.
89 #11330032, Thermo Fisher Scientific) with the addition FBS (Cat. # F2442, MilliporeSigma,
90 Burlington, MA, USA) and 1% penicillin-streptomycin (Cat. #15070-063, Thermo Fisher).
91 Adipocyte culture was established in an incubator at 37°C and 5% CO₂ and then treated at 90%.
92 Adipocytes that have been cultured are divided into three groups: positive control, negative
93 control, and treatment. The negative control consists of adipocytes that have not received any
94 treatment. The positive control includes adipocytes exposed only to SARS-CoV-2 without any
95 additional treatment. The treatment group consists of SARS-CoV-2-exposed adipocytes and
96 further divided into two subgroups: one receiving Losartan, and the other receiving hrsACE2.
97 ***SARS-CoV-2 Subunit S1 Spike Protein Exposure to Adipocytes***

98 Adipocytes were infected with the S1 subunit spike protein of SARS-Cov-2 (Cat. #230-30162-
99 100, RayBiotech, Peachtree Corners, GA, USA,) using a modified direct exposure
100 approach.[19] Adipocytes were streaked on 96-well plates at a density of 1×10^4 cells per well,
101 followed by treatment with 10 nM SARS-CoV-2 subunit S1 spike protein and incubation at
102 room temperature for 30 min.

103 ***Treatment with Losartan***

104 As much as 0.7 μ M losartan (Merck, Cat. No BP867) was added 30 minutes after SARS-CoV-
105 2 S1 spike protein exposure to adipocytes [20]. The effect of losartan with human recombinant
106 soluble ACE2 (hrsACE2) was also compared. One-hundred μ g/mL hrsACE2 was added to
107 another group of adipocytes culture treated with SARS-CoV-2 subunit S1 spike protein [21].

108 ***ACE2-spike Protein Binding Assay***

109 The impact of losartan and hrsACE2 on the interaction between the SARS-CoV-2 spike protein
110 and ACE2 was evaluated using a binding assay kit (Cat. #CoV-SACE2-1, RayBiotech)
111 following the manufacturer's instructions. To prepare the test reagents, losartan or hrsACE2
112 was mixed with 1.25 μ L of 100x ACE2 protein concentrate, resulting in a final volume of 100
113 μ L. Each test reagent was added to the appropriate wells coated with SARS-CoV-2 spike
114 protein and incubated overnight at 4°C with gentle shaking. The wells were then washed four
115 times using 300 μ L of wash buffer and subsequently incubated with 100 μ L of a detection
116 antibody for 1 hour at room temperature. Following additional washing steps, 100 μ L of HRP-
117 Conjugated anti-IgG, 100 μ L of TMB substrate, and 50 μ L of stop solution were sequentially
118 added to each well. The absorbance of the solution was immediately measured at 450 nm.

119 ***Measurement of ACE2 and Proinflammatory Cytokines Levels***

120 ACE2, IL-6, Interleukin-1 β , and tumor necrosis factor (TNF)- α levels were measured using
121 ELISA kits according to the manufacturer's manual (Cat. #Ab235649, Abcam, Cambridge,
122 UK; Cat. #E-EL-H0102, Elabscience, Houston, TX, USA; Cat. #E0143Hu, BT Lab,

123 Birmingham, UK; Cat. #E0082Hu, BT Lab, respectively). Adipocyte culture supernatant was
124 added to each primary antibody-coated well and incubated. After washing, secondary detection
125 antibody was added to each well and incubated. Following additional washing, substrate was
126 added, and the reaction was stopped using a stop solution. Each well's optical density was
127 determined using a microplate reader.

128 ***Statistical Analysis***

129 One-Way ANOVA and was followed by Tukey's Post Hoc were used to analyze and compare
130 the difference between groups. Data were first tested for normality with the Shapiro-Wilk test.
131 Using 5% alpha, data were analyzed using SPSS version 26.0 (IBM Corporation, Armonk, NY,
132 USA) and were considered significant if the p -value <0.05 .

133

134 **Result**

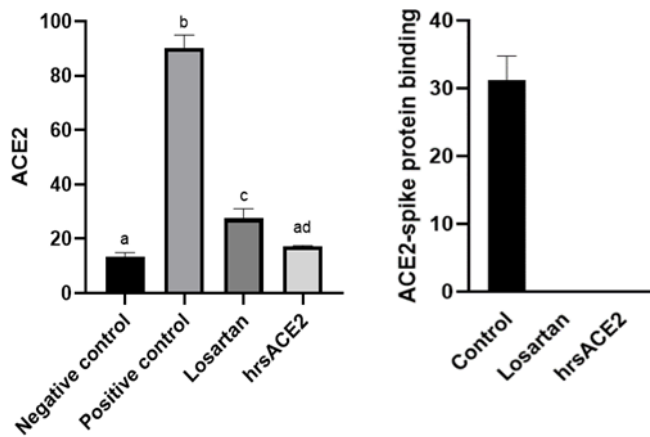
135 ***SARS-CoV-2 S1 Spike Protein Exposure Increased ACE2, IL-6, and TNF- α Level***

136 The effect of SARS-CoV-2 S1 spike protein on adipocytes was the first to be investigated.
137 Significant elevation of ACE2 was seen after 30 minutes of incubation of spike protein
138 compared to a negative control (90.22 ± 4.72 vs 13.33 ± 1.51 ng/mL, $p<0.001$). Spike protein
139 exposure also significantly increased the level of proinflammatory cytokines IL-6 (60.00 ± 1.33
140 vs 21.34 ± 2.56 ng/mL, $p=0.000$) and TNF- α (284.91 ± 34.82 vs 138.00 ± 55.92 ng/mL, $p=0.007$),
141 but not in IL-1 β (1171.66 ± 198.10 vs 895.33 ± 46.23 pg/mL, $p=0.109$).

142 ***Losartan and hrsACE2 Reduced the Level of ACE2 and Inhibited the Binding of ACE2- 143 spike Protein***

144 The effect of losartan and hrsACE2 on ACE2 level and its consequences on the binding of
145 ACE2 with SARS-CoV-2 spike protein was also evaluated. ACE2 levels significantly reduced
146 following the addition of losartan (27.51 ± 3.48 ng/mL) and hrsACE2 (17.33 ± 0.18 ng/mL) into
147 adipocyte culture exposed to SARS-CoV-2 spike compared to a positive control (90.22 ± 4.72

148 ng/mL, $p < 0.001$). ACE2 reduction level was slightly better in the hrsACE2 group (Figure 1).
 149 There was no ACE2-spike protein binding detected in losartan and hrsACE2 group, as was
 150 observed in the control group (31.23 ± 3.53 ng/mL) (Figure 1).



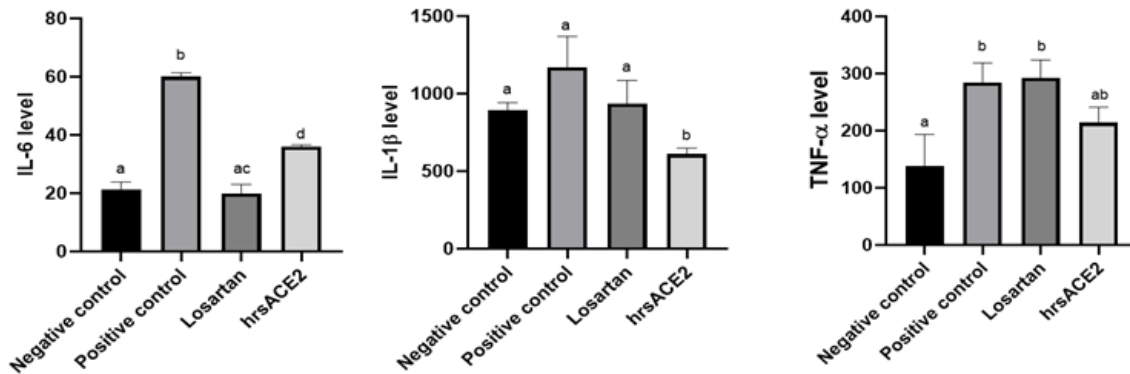
151

152 **Figure 1. ACE2 levels and ACE2-spike protein binding levels in all experiment groups.**

153 ^{a,b,c,d}Different annotations indicate statistically significant differences between groups (Post-
 154 Hoc Test).

155 *Losartan and hrsACE2 Lowered IL-6 Levels*

156 Measurement of proinflammatory cytokines levels showed that only IL-6 was significantly
 157 lowered after losartan (19.96 ± 3.05 ng/mL) and hrsACE2 (36.11 ± 0.53 ng/mL) treatment
 158 compared to a positive control (60.00 ± 1.32 ng/mL, $p < 0.001$). Losartan has a larger reduction
 159 of IL-6 than hrsACE2 (Figure 2). The hrsACE2 also decreased IL-1 β significantly compared
 160 to the positive control (611.00 ± 38.43 pg/mL vs 1171.66 ± 198.10 pg/mL, $p < 0.05$), but this
 161 finding was not observed in the losartan group (Figure 2). Both losartan and hrsACE2 had no
 162 significant effect on TNF- α levels (Figure 2). Pearson correlation measurement showed that
 163 IL-6 was positively correlated with ACE2 ($r = 0.878$, $p < 0.001$) (Figure 3).

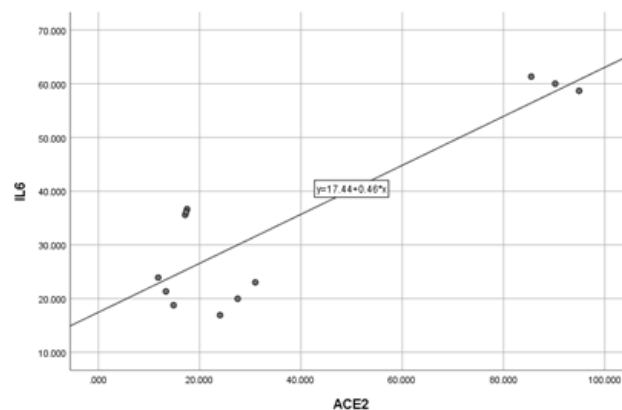


164

165 **Figure 2. Proinflammatory cytokines (IL-6, IL-1 β , and TNF- α) levels in all experiment**
 166 **groups.** ^{a,b,c,d}Different annotations indicate statistically significant differences between groups
 167 (Post Hoc Test).

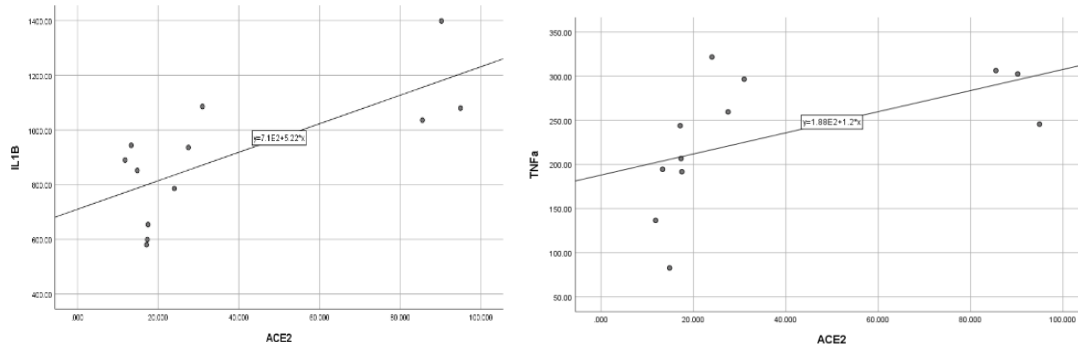
168 *ACE-2 levels correlated with IL-6 levels but not with IL-1 β and TNF- α*

169 In this study, the results also showed that ACE2 levels had a strong correlation with IL-6. This
 170 means that the higher the ACE2 levels in SARS-CoV-2 infection, the higher the IL-6 levels
 171 ($r=0.878$, $p<0.001$). However, there was no significant correlation between ACE2 with IL-1 β
 172 and TNF- α (Figure 3, Figure 4).



173

174 **Figure 3. The Scatterplot graph showed a strong positive correlation between ACE2 and**
 175 **IL-6 ($r=0.878$, $p<0.001$).**



176
177

178
179

180 **Figure 4. The Scatterplot graph (a,b) showed no significant correlation between ACE2**
181 **with IL-1 β and TNF- α ($p > 0.001$).**

182 Discussion

183 ACE2 plays a key role in developing SARS-CoV-2 infection (COVID-19) related
184 cytokine storm, characterized by a surge of interleukin(IL)-6 and IL-1 β . [22] It has been shown
185 that in acute respiratory distress syndrome (ARDS), ACE2 is a significant regulator of
186 inflammatory responses. [23] Recently, a phase II trial on hrsACE2 has shown promise in
187 attenuating acute lung injury in ARDS while establishing a safety profile. [24] The hrsACE2
188 may also be beneficial in treating COVID-19 and its complication by acting as a decoy for
189 circulating SARS-CoV-2 virus and converting Ang II to angiotensin-(1-7). [25]

190 This study successfully identified the presence of ACE2 expression in adipose tissue,
191 which has supported previous literature that gene expression databases show ACE2 expression
192 is present in subcutaneous adipose and human visceral adipose tissue, where levels are en
193 higher than those in human lung tissue. [26] Hence, in obese patients, high levels of adipose
194 tissue indicate high levels of ACE2, compared to patients without obesity. High levels of
195 adipose tissues lead to an increase of pro-inflammatory cytokines in SARS-CoV-2 infection.

196 Two mechanisms have been proposed to explain this phenomenon. First, leptin, which
197 is secreted by adipose tissue, is a pleiotropic molecule that functions to coordinate a person's

198 immunity, specifically host innate immunity and adaptive responses and subsequently affects
199 the increased secretion of pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6.[27]
200 However, in this study, the results showed that SARS-COV-2 infection in adipocyte cells only
201 significantly increased TNF- α , IL-6, not IL-1 β . This is because only 12% of those cytokines
202 are produced by adipocytes. The primary source of these cytokines is non-fat cells in adipose
203 tissue. In the context of IL-1 β production, it is one of the cytokines produced the least by
204 adipocytes, compared to TNF and IL-6.[28,29] Second mechanism is the fact that obese
205 patients have higher levels of ACE2, which is the main route of entry of SARS-CoV-2
206 indirectly leads to increased viral replication and reproduction in the patient's body. This
207 explains other studies that suggest that elevated plasma ACE2 has been associated with poor
208 outcomes in patients with COVID-19.[31]

209 This study also found that exposure to SARS-CoV-2 protein spikes increased ACE2
210 levels. The mechanism by which ACE2 is upregulated is thought to be at the transcriptional
211 level by interferon which also appears to be elevated in SARS-CoV-2 infection.[32] This study
212 also found that the SARS-Cov-2 protein spike increased the levels of pro-inflammatory
213 cytokines. It is suspected that there is a disruption in this cytolytic activity, leading to the
214 prolonged activation of innate immunity cells, which then many pro-inflammatory cytokines
215 have increased secretion in undue pathways and cause cytokine storms.[33–35]

216 Losartan administration in this study showed the effect of reducing ACE2 levels in
217 SARS-CoV-2 Infection. Losartan has a high affinity for ACE2, which results in its direct
218 binding to ACE2, then prevents the virus from penetrating such that infection does not begin.
219 This study showed that losartan also decreased the binding between the SARS-Cov-2 spike
220 protein and ACE2. Previous *in-silico* studies supported this finding, showing that losartan can
221 reduce the affinity of the virus to ACE2 by distorting the receptor binding domain (RBD) on
222 SARS-CoV2 to attach to ACE2.[36] This study also showed that losartan administration can

223 reduce IL-6 levels in SARS-Cov-2 Infection. The results of this study showed that hrsACE2
224 administration had similar effects to losartan administration. Previous studies have indeed
225 shown that hrsACE2 has therapeutic benefits in COVID-19. In addition to inhibiting the
226 binding of SARS-CoV-2 with ACE2, hrsACE2 also minimizes multiple organ damage. [37].
227 HrsACE2 has been shown to effectively protect mice from SARS-CoV-2 Infection as
228 evidenced by reduced virus replication, histologic changes and decreased inflammation in the
229 lungs.[38]

230 Thus, it can be concluded that losartan has a beneficial effect on SARS-CoV-2
231 infection, by reducing the binding of SARS-CoV-2 with ACE2, which directly and indirectly
232 reduces pro-inflammatory cytokines, especially IL-6, which has been shown to cause various
233 kinds of severe clinical manifestations of COVID-19. These findings provide additional
234 support for the safety of losartan usage in obese patients with COVID-19 infections. Since
235 inflammation is also a fundamental part of the pathophysiology of severe COVID-19, even in
236 non-obese patients, the results may also be applicable to non-obese patients with severe
237 COVID-19. Furthermore, additional discoveries, like the connection between losartan, ACE2,
238 and IL-6, hold the potential to provide valuable insights into a range of medical conditions
239 marked by involvement in the RAS system pathway and inflammation, including conditions
240 like cardiovascular disease.

241 However, the specimens used in this study are only viral protein spikes, not whole
242 viruses, which are expected to be sufficiently representative of the actual condition of COVID-
243 19 infection. In addition, this study also did not measure other parameters related to ACE2,
244 such as Angiotensin-(1-7), and also did not measure other inflammatory pathways that may be
245 related to ACE2 and adipokines. Therefore, further research is essential to conduct a thorough
246 examination, leading to comprehensive results when evaluating the impact of losartan on the
247 RAS system, encompassing both SARS-CoV-2 infection and various other scenarios.

248

249 Conclusions

250 This study provides evidence that losartan reduced ACE2 and IL-6 levels indicating that
251 losartan might not be harmful when given to COVID-19 patients, especially in patients with
252 obesity. Contrarily, losartan has a similar protective effect to human recombinant ACE2 in
253 preventing cytokine storms, mainly due to IL-6.

254

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258 Authors Contribution

259 HOM, MA, IGRS, and PMH conceptualized and designed the research, collected data,
260 analyzed and interpreted the results. HOM and MR developed data analysis and research results
261 to prepare manuscripts, and revised manuscripts. All authors reviewed the results and approved
262 the final version of the manuscript.

263

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