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Resveratrol supplementation reduces ACE2 expression in human adipose tissue

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ABSTRACT

Angiotensin converting enzyme-2 (ACE2) is the cell-surface receptor enabling cellular entry of SARS-CoV-2. ACE2 is highly expressed in adipose tissue (AT), rendering AT a potential SARS-CoV-2 reservoir contributing to massive viral spread in COVID-19 patients with obesity. Although rodent and cell studies suggest that the polyphenol resveratrol alters ACE2, human studies are lacking. Here, we investigated the effects of 30-days resveratrol supplementation on RAS components in AT and skeletal muscle in men with obesity in a placebo-controlled cross-over study. Resveratrol markedly decreased ACE2 (~40%) and leptin (~30%), but did neither alter angiotensinogen, ACE and AT1R expression in AT nor skeletal muscle RAS components. These findings demonstrate that resveratrol supplementation reduces ACE2 in AT, which might dampen SARS-CoV-2 spread in COVID-19.

Introduction

Most severe acute respiratory coronaviruses (SARS-CoVs) use the hosts angiotensinogen-converting enzyme 2 (ACE2) as cell-surface receptor enabling viral entry [1]. This also applies to the novel coronavirus SARS-CoV-2, which binds to the membrane-bound form of ACE2 and thereby initiates the release of the viral genome intracellularly [2]. ACE2 is part of the renin-angiotensin-system (RAS), which not only regulates electrolyte homoeostasis and blood pressure, but also mediates pro-inflammatory signalling, thrombotic processes and fibrosis, thus contributing to pathological changes of organ structure and function[3]. Since RAS components are highly expressed in adipose tissue (AT), it is tempting to speculate that the excess AT in people with obesity may serve as a reservoir for SARS-CoV-2, thereby contributing to the high viral spread and the poor clinical outcomes of coronavirus disease 2019 (COVID-19) patients with obesity[4].

The ACE2 receptor and other components of the RAS have been suggested to play a pivotal role in the SARS-CoV-2 infection as well as COVID-19 progression and may provide a key target for the prevention and treatment of COVID-19 [2,3]. ACE2 *downregulation* might have beneficial effects *prior* to SARS-CoV-2

infection, since it may dampen cellular entry and replication of SARS-CoV-2. Once infected with SARS-CoV-2, however, the expression of ACE2 drops [5], which subsequently may result in elevated angiotensin II (Ang II) levels and worsened clinical outcomes. Indeed, circulating Ang II concentrations were markedly elevated in a small cohort of patients with COVID-19 compared to healthy controls, and were positively associated with viral load and lung injury[6]. Therefore, ACE2 *upregulation* might be beneficial *following* SARS-CoV-2 infection, since this may counteract the detrimental effects of high Ang II levels. To summarize, there is a clear necessity for compounds that block the ACE2 cellular receptor or reduce its expression to prevent SARS-CoV-2 entry in AT and viral spread to other organs, while agents that increase ACE2 expression and inhibit Ang II action may have beneficial effects on clinical outcomes in COVID-19 patients.

There is increasing evidence from rodent studies and *in vitro* experiments that the polyphenolic compound resveratrol may influence ACE2 expression. So far, studies have only investigated the effects of resveratrol on components of the RAS in rodents and cell lines [7–10]. Interestingly, these studies showed that *in vivo* resveratrol supplementation or *in vitro* incubation

with resveratrol increased the expression of ACE2 in several types of tissues and cell lines, including AT [7–10]. More specific, resveratrol decreased the expression of angiotensinogen (AGT) [10], angiotensin-converting enzyme (ACE) [7,9,10], and the Ang II type 1 receptor (AT1R) [8,10], and increased the expression of the AT2R [9,10] and Mas receptor (MAS-R) [9,10]. From these studies, it seems that resveratrol may stimulate the beneficial ACE2-Ang(1-7)-AT2R-MAS-R axis, while reducing the activity of the detrimental ACE-Ang II-AT1R axis. However, no human clinical trials have been performed so far to investigate if resveratrol impacts the expression of RAS component in humans *in vivo* as well.

We previously conducted a well-controlled clinical trial in which healthy males with obesity received resveratrol supplementation for 30 days (150 mg/day) in a randomized, placebo-controlled, cross-over study [11]. In the present study, we examined for the first time if resveratrol supplementation impacts the expression of RAS components in AT and skeletal muscle compared to placebo in people with obesity.

Methods

The current study was part of a previously published randomized, double-blind, placebo-controlled cross-over trial[11]. The original study protocol was reviewed by the Medical Ethics Committee of Maastricht University (clinicaltrial.gov: NCT00998504). Eleven males with obesity who were otherwise healthy (no family history of diabetes or any other endocrine disorder and not using any kind of medication throughout the present study) participated in this study. Participant characteristics are presented in Table 1. Further details can be found in Timmers et al. 2011[11]. The study had two treatment conditions: 30 days of placebo and 30 days of 150 mg/day *trans*-resveratrol (99.9%; DSM Nutritional Products Ltd.). All participant adhered to

the protocol, which was verified by analyses of free and conjugated resveratrol in plasma. Both compounds were present in plasma during the resveratrol treatment period, while no resveratrol could be detected during placebo treatment. At the end of each treatment period, an abdominal subcutaneous adipose tissue biopsy (6 hours after ingestion of a high-fat liquid meal) as well as a skeletal muscle biopsy (m. vastus lateralis; under fasted conditions) were collected by needle biopsy, as previously described [11,12]. Total RNA was extracted from the biopsies and stored at −80 degrees Celsius until analyses. Next, total RNA was reverse transcribed using the High-Capacity-RNA-to-cDNA-kit (Applied Biosystems) and qRT-PCR was performed in the CFX384 Real-Time System (Biorad) (for details see **Supplementary Methods qRT-PCR**, including primer sequences). Gene expression was defined using a derivative of the $\Delta\Delta C_t$ method[13]. The expression of the housekeeping genes were presented as $2^{-\Delta Ct}$. Data are presented as mean ± SEM. Differences between resveratrol and placebo treatment were investigated by Student's paired t-test using SPSS 24.0 (IBM USA). The significance threshold was set at $p < 0.05$.
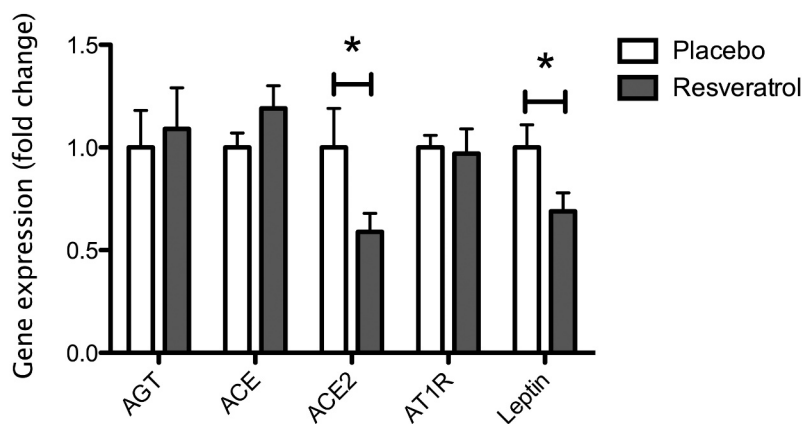
Results

Resveratrol supplementation significantly decreased gene expression of ACE2 and leptin in abdominal subcutaneous AT compared to placebo (Figure 1; p = 0.029 and p = 0.041, respectively). No significant differences were found in AT gene expression of AGT, ACE and AT1R between resveratrol and placebo (Figure 1; p > 0.05). Furthermore, no detectable differences were observed in the gene expression of AGT (fold change: 1.11 ± 0.17 versus placebo, p = 0.461) and ACE (fold change: 1.26 ± 0.08 versus placebo, p = 0.203) in skeletal muscle (data not shown). Expression of ACE2, AT1R and leptin could not be assessed reliably in skeletal muscle.

Table 1. Baseline characteristics of study participants*.

	Placebo	Resveratrol	P-value
Age, years	53 ± 2[#]	53 ± 2[#]	N/A
Body weight, kg	100.1 ± 3.5	99.6 ± 3.7	0.50
BMI, kg/m^{2}	31.6 ± 0.7	31.4 ± 0.8	0.48
Body fat, %	26.4 ± 0.53[#]	26.4 ± 0.53[#]	N/A
Systolic blood pressure, mmHg	131 ± 3.1	132 ± 3.0	0.22
Diastolic blood pressure, mmHg	82 ± 2.5	83 ± 2.6	0.20
Glucose, mmol/l	5.44 ± 0.10	5.44 ± 0.13	0.96
Insulin, mU/l	16.37 ± 1.76	15.38 ± 2.05	0.67
Triglycerides, mmol/l	1.86 ± 0.19	1.92 ± 0.21	0.80
Nonesterified fatty acids, μmol/l	357 ± 69	320 ± 31	0.56

Characteristics of participants measured at the start of each intervention period (day 0). Values are given as mean ± SEM. *Data obtained from Timmers et. al 2011[11]; #Only measured once, at the start of the first intervention period by dual-energy X-ray absorptiometry; N/A, not applicable

Figure 1. Effect of 30-days resveratrol supplementation on gene expression of RAS components in human adipose tissue. Resveratrol treatment significantly decreased the expression of ACE2 and leptin in adipose tissue but did not alter the expression of AGT, ACE and AT1R compared to placebo (n = 10). Values are presented as fold change, mean ± SEM. *p < 0.05, by Student's paired t-test. AGT, angiotensinogen; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; AT1R, angiotensin II type 1 receptor.

Discussion

Here, we report that 30-days of resveratrol supplementation significantly decreased the expression of ACE2 with about 41%, and leptin with about 31% in abdominal subcutaneous AT in males with obesity. SARS-CoV-2 uses the hosts ACE2 as cell-surface receptor for entering the cells, which is followed by the release of the viral load and lysosomal breakdown of ACE2. Hence, ACE2 expression and/or activity in target tissues is an attractive target for prevention or reduction of SAR-CoV-2 host cell infection and viral shedding. People with obesity often display increased activation of the RAS, systemically as well as in AT [3,14]. It has thus been proposed that ACE2 in AT provides an important link between obesity and the increased susceptibility to and severity of SARS-CoV-2 infection[3]. Hence, lowering expression of ACE2 by resveratrol supplementation could be an interesting strategy to lower the entry of SARS-CoV-2 into AT.

In the present study, we also found that resveratrol decreased the expression of leptin in AT, which was functionally reflected in lower serum leptin levels, as previously described[11]. Leptin is a well-characterized pro-inflammatory adipokine that is produced by AT. Leptin has been suggested to be involved in the onset of several local and systemic effects reported in critically ill patients with COVID-19[15]. Significantly higher levels of serum leptin have recently been reported by in COVID-19 patients requiring mechanical ventilation compared to control critically ill non-infected patients with similar BMI[15]. Moreover, Van der Voort et al [15]. suggested that excessive AT and leptin production could contribute to the development of respiratory failure in COVID-19 patients[15]. Thus, it can be speculated that the reduced leptin expression upon resveratrol supplementation in people with obesity might have beneficial effects on COVID-19 outcome. Effects of resveratrol on RAS were not seen in skeletal muscle, which may imply that resveratrol has no effects on the expression of AGT and ACE in skeletal muscle or the dose and/or bioavailability of resveratrol was too low to render effects in skeletal muscle. Future studies are warranted to further investigate the effects of resveratrol supplementation on protein expression (e.g. Western Blot/immunohistochemical assays) or activity of RAS components in human adipose tissue.

To conclude, our data demonstrate that resveratrol supplementation significantly reduces the expression of ACE2 and leptin in human adipose tissue. Our findings could have implications for individuals with obesity, since resveratrol supplementation might render them less susceptible for SARS-CoV-2 via lower ACE2 receptor expression in AT. Moreover, resveratrol decreased AT leptin expression, which might have beneficial effects on COVID-19 outcome. It should be noted that ACE2 expression is suppressed following SARS-CoV-2 entry, which may result in elevated Ang II levels and may worsen clinical outcomes in patients with COVID-19. Hence, studying the impact of resveratrol on ACE2 gene expression and SARS-CoV-2 clinical outcomes during infection is important. In addition, the effects of resveratrol supplementation on viral load, viral shedding and COVID-19 outcome in people with obesity and other risk populations are also worth examining. Finally, future studies should investigate which dose of resveratrol has the most pronounced effect on ACE2 expression.

Acknowledgments

Disclosure statement

No potential conflict of interest was reported by the authors.

AUTHOR CONTRIBUTIONS

MKCH, EEB and GHG conceived and designed research; JJ and NH performed experiments; ML analyzed data; ML, MKCH, and GHG interpreted results of experiments; ML prepared figures and drafted manuscript; MKCH and GHG edited and revised manuscript; all authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (GHG) upon reasonable request.

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References

[1] Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426(6965):450–454.

[2] Gheblawi M, Wang K, Viveiros A, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. Circ Res. 2020;126(10):1456–1474.

[3] Goossens GH, Dicker D, Farpour-Lambert NJ, et al. Obesity and COVID-19: a perspective from the european association for the study of obesity on immunological perturbations, therapeutic challenges, and opportunities in obesity. Obes Facts. 2020;13(4):439–452.

[4] Ryan PM, Caplice NM. Is adipose tissue a reservoir for viral spread, immune activation, and cytokine amplification in coronavirus disease 2019? Obesity. 2020;28 (7):1191–1194.

[5] Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med. 2005;11 (8):875–879.

[6] Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci. 2020;63(3):364–374.

[7] Oliveira Andrade JM, Paraiso AF, Garcia ZM, et al. Cross talk between angiotensin-(1-7)/Mas axis and sirtuins in adipose tissue and metabolism of high-fat feed mice. Peptides. 2014;55:158–165.

[8] Moran CS, Biros E, Krishna SM, et al. Resveratrol inhibits growth of experimental abdominal aortic aneurysm associated with upregulation of angiotensin-converting enzyme 2. Arterioscler Thromb Vasc Biol. 2017;37(11):2195–2203.

[9] Kim EN, Kim MY, Lim JH, et al. The protective effect of resveratrol on vascular aging by modulation of the renin-angiotensin system. Atherosclerosis. 2018;270:123–131.

[10] Tiao MM, Lin YJ, Yu HR, et al. Resveratrol ameliorates maternal and post-weaning high-fat diet-induced non-alcoholic fatty liver disease via renin-angiotensin system. Lipids Health Dis. 2018;17(1):178.

[11] Timmers S, Konings E, Bilet L, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab. 2011;14 (5):612–622.

[12] Konings E, Timmers S, Boekschoten MV, et al. The effects of 30 days resveratrol supplementation on adipose tissue morphology and gene expression patterns in obese men. Int J Obes (Lond). 2014;38 (3):470–473.

[13] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25 (4):402–408.

[14] Goossens GH, Blaak EE, van Baak MA. Possible involvement of the adipose tissue renin-angiotensin system in the pathophysiology of obesity and obesity-related disorders. Obes Rev. 2003;4(1):43–55.

[15] van der Voort PHJ, Moser J, Zandstra DF, et al. Leptin levels in SARS-CoV-2 infection related respiratory failure: a cross-sectional study and a pathophysiological framework on the role of fat tissue. Heliyon. 2020;6(8):e04696.