Dynamic Regulation of SARS-Cov-2 Binding and Cell Entry Mechanisms in Remodeled Human Ventricular Myocardium

Michael R. Bristow, MD, PhD,a,b,c Lawrence S. Zisman, MD,d Natasha L. Altman, MD,a,c Edward M. Gilbert, MD,e Brian D. Lowes, MD, PhD,f Wayne A. Minobe, BS,a Dobromir Slavov, PhD,a Jessica A. Schwisow, BS,a Erin M. Rodriguez, BA,a Ian A. Carroll, PhD,a,b Thomas A. Keuer, MS,b Peter M. Buttrick, MD,a,c David P. Kao, MD,a,c

VISUAL ABSTRACT

HIGHLIGHTS

- The CoV-2 cellular receptor ACE2 and 5 proteases implicated in fusion of virus and cell membranes that are vital to cell entry were expressed at the mRNA level in RNA extracted from septal EmBx of patients with F/NDC and NF control patients.
- ACE2 was up-regulated by 1.97 fold in 46 patients with F/NDC compared with NF control patients, but proteases showed similar degrees of expression.
- On LV reverse remodeling effected by beta-blocking agents, ACE2 expression, in the presence of unchanged doses of ACE inhibitors or ARBs, down-regulated into the normal range.
- ITGA5, which encodes an integrin that binds to ACE2 and to a motif in the CoV-2 spike protein binding domain, was expressed in both NF control subjects and subjects with F/NDC, was decreased in expression on reverse remodeling similar to ACE2, and is a candidate for facilitating CoV-2 binding and cell entry in LV myocardium.
Using serial analysis of myocardial gene expression employing endomyocardial biopsy starting material in a dilated cardiomyopathy cohort, we show that mRNA expression of the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) cardiac myocyte receptor ACE2 is up-regulated with remodeling and with reverse remodeling down-regulates into the normal range. The proteases responsible for virus-cell membrane fusion were expressed but not regulated with remodeling. In addition, a new candidate for SARS-CoV-2 cell binding and entry was identified, the integrin encoded by ITGAS. Up-regulation in ACE2 in remodeled left ventricles may explain worse outcomes in patients with coronavirus disease 2019 who have underlying myocardial disorders, and counteracting ACE2 up-regulation is a possible therapeutic approach to minimizing cardiac damage. (J Am Coll Cardiol Basic Trans Science 2020;5:871-83) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

SUMMARY

The 2020 coronavirus disease-19 (COVID-19) pandemic caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has many unique clinical features, including high infectivity, a protean clinical presentation and course, and life-threatening potential (1). Myocardial involvement is an important pathophysiologic component of some critically ill patients with CoV-2 infections (2-6), either due to myocarditis (5,6) or myocardial dysfunction without evidence of inflammation (2-4). The prevalence of cardiac complications in patients without underlying heart disease ranges from 20% to 30% (7-9), which when present worsens prognosis. In the initial report of patients receiving intensive care, evidence of cardiac injury was associated with a 50% mortality compared with a <10% mortality without such evidence (3). The prognosis worsens further when evidence of myocardial injury is superimposed on pre-existing cardiovascular disease, with mortality rising to above 60% (3).

A well-established pathway by which the CoV-2 or the original SARS-CoV virus gains entry into cells includes membrane attachment by binding to angiotensin converting enzyme 2 (ACE2) (10,11), fusion of viral and cell surface membranes through the recruitment of host proteases (12-14), and virus internalization followed by assembly of cytoplasmic membranous structures into replication vesicles (15). In addition, there are other possible mechanisms of virus internalization such as binding to integrins (16,17), some of which also bind to ACE2 (18,19). Although CoV-2 or CoV cell internalization has been investigated in model systems (10-15) and is beginning to be evaluated in the human heart (20), it is unclear whether the virus enters human cardiac myocytes and whether the necessary biologic constituents are expressed in the heart. Of particular importance is the role of ACE2; in human ventricular myocardium, ACE2 is a highly functional enzyme present in cardiac myocytes (20) that breaks down angiotensin II to the counter-regulatory peptide angiotensin-(1-7) (21,22). In explanted human heart preparations from patients with end-stage heart failure with reduced (<0.50) left ventricular ejection fraction (HFrEF), ACE2 enzyme activity (22) as well as gene expression at the messenger ribonucleic acid (mRNA) (20,23) and protein (20,22) levels are up-regulated compared with organ donor control subjects. This is potentially important because up-regulated ACE2 might be a mechanism by which CoV-2 myocardial involvement is more prominent in patients with underlying heart muscle disease (20). However, it is unclear whether ACE2 is up-regulated in intact hearts with less severe pathologic...
remodeling/dysfunction than in explanted hearts from cardiac transplant recipients, who in contrast to most organ donor control subjects have been extensively treated with renin-angiotensin system (RAS) inhibitors that may affect ACE2 expression (24,25). In addition, it is not clear whether the proteases that prime and facilitate membrane fusion are expressed or regulated in the remodeled, failing human heart.

Finally, there is limited information on the status of integrins, particularly those that can bind to ACE2 or to the CoV-2 virus itself, to potentially effect virus-cell internalization (16). To obtain information on these mechanisms in nonfailing (NF) and remodeled intact human hearts, we analyzed data from a serial analysis of myocardial gene expression cohort study (26), where reverse remodeled left ventricles (LVs)

| TABLE 1 Baseline Characteristics |
|---------------------------------|
|                                | NF (n = 4) | F/NDC (n = 46) | p Value | F/NDC R (n = 30) | F/NDC NR (n = 16) | p Value |
| Age, yrs                        | 41.0 ± 17.1 | 45.6 ± 13.2 | 0.63    | 43.5 ± 13.2     | 49.7 ± 12.3     | 0.12    |
| Sex                             |            |              | 0.94    | 0.72             |                |
| Male                            | 3          | 33           | 21      | 12               |                |
| Female                          | 1          | 13           | 9       | 4                |                |
| Race, ethnicity                 |            |              | 0.15    | 0.35             |                |
| White                           | 4          | 30           | 21      | 9                |                |
| Black                           | 6          | 4            | 4       | 2                |                |
| Hispanic                        | 7          | 4            | 3       |                |                |
| Asian                           | 2          |              | 1       |                |                |
| Native American                 | 1          |              | 1       | 1                |                |
| Diabetes                        | 0 (0)      | 8 (17)       | 0.36    | 5 (17)           | 3 (19)         | 0.86    |
| Hypertension history            | 2 (50)     | 18 (39)      | 0.43    | 15 (50)          | 3 (19)         | 0.039   |
| BSA, m²                         | 2.20 ± 0.42| 1.97 ± 0.22  | 0.58    | 1.97 ± 0.22      | 1.96 ± 0.21    | 0.89    |
| BMI, kg/m²                      | 30.9 ± 8.1 | 28.9 ± 5.7   | 0.67    | 29.0 ± 6.3       | 28.9 ± 4.8     | 0.99    |
| Creatinine clearance, mg/dl     | 87.7 ± 7.9 | 80.2 ± 22.5  | 0.18    | 85.3 ± 18.8      | 71.0 ± 26.2    | 0.066   |
| HF failure duration, months     |            |              | 23.9 ± 45.9 | 7.0 ± 10.0 | 55.7 ± 67.0 | 0.011   |
| Atrial fibrillation             | 1 (25)     | 10 (22)      | 0.88    | 4 (13)           | 6 (38)         | 0.058   |
| NYHA functional class           | 0.87       |              | 0.55    |                |                |
| I                               | 2          |              |        |                |                |
| II                              | 2          | 25           | 16      | 10               |                |
| III                             | 21         |              | 14      | 6                |                |
| Heart rate, beats/min           | 77 ± 9     | 84 ± 21      | 0.24    | 87 ± 21          | 79 ± 20        | 0.21    |
| SBP, mm Hg                      | 118 ± 29   | 107 ± 14     | 0.51    | 106 ± 13         | 109 ± 17       | 0.51    |
| LVEF, %                         | 58.8 ± 7.4 | 26.6 ± 8.7   | 0.002   | 25.6 ± 8.2       | 27.8 ± 10.0    | 0.51    |
| RVEF, %                         | 38.3 ± 4.7 | 27.2 ± 9.0   | 0.032   | 27.2 ± 8.8       | 27.2 ± 9.7     | 0.99    |
| LV end-diastolic volume, ml     |            |              |        | 220 ± 83         | 255 ± 111      | 0.38    |
| PWP, mm Hg                      | 7 ± 6      | 12 ± 8       | 0.12    | 11.3 ± 8.7       | 14.5 ± 7.7     | 0.21    |
| PAP, mm Hg                      | 20 ± 2     | 24 ± 11      | 0.13    | 22.4 ± 9.9       | 27.1 ± 11.5    | 0.18    |
| Cardiac index, l/min/m²         | 2.8 ± 0.6  | 2.2 ± 0.6    | 0.10    | 2.3 ± 0.7        | 2.2 ± 0.6      | 0.74    |
| Mixed venous NE, pg/ml          | 372 ± 152  | 492 ± 331    | 0.44    | 452 ± 267        | 574 ± 438      | 0.39    |
| Mixed venous BNP, pg/ml         | 90 ± 46    | 234 ± 234    | 0.035   | 203 ± 240        | 334 ± 200      | 0.21    |
| On ACE inhibitors,              | 4          | 44           | 0.59    | 27               | 14             | 0.75    |
| Doses (enalapril equivalents), mg| 6.6 ± 9.0 | 9.3 ± 6.1    | 8.8 ± 5.0 | 8.2 ± 5.0     |                |
| On ARBs,                        | 1, 1 missing| 3            | 2       | 0.15             |                |
| Doses (losartan equivalents), mg| 50        | 46.9 ± 38.7  | 87.5 ± 17.7 |                |
| On beta-blockers                |            |              |        | 0.63             |                |
| Doses, mg/day                   |            |              |        | 0.33             |                |
| Carvedilol                      |            |              |        | 7                |                |
| Metoprolol succinate            |            |              |        | 21               |                |
| Doses, mg/day                   | 169 ± 55   | 176 ± 52     | 153 ± 62 |                |

Values are mean ± SD, n (%), or n. All doses are in mg/day. For definitions of responder, nonresponder see the Methods section.

ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; BMI = body mass index; BNP = B-type natriuretic peptide; BSA = body surface area; F/NDC = nonischemic dilated cardiomyopathy with heart failure group; HF = heart failure; LV = left ventricle; LVEF = left ventricular ejection fraction; NE = norepinephrine; NF = nonfailing group; NR = nonresponder; NYHA = New York Heart Association; PAP = pulmonary artery pressure; PWP = pulmonary wedge pressure; R = responder; RVEF = right ventricular ejection fraction; SBP = systolic blood pressure.
were compared with unchanged ventricles exposed to the same pharmacologic regimen.

**METHODS**

**PATIENT MATERIAL AND PROTOCOL.** Control subjects were 4 individuals with left ventricular ejection fractions (LVEFs) ≥50% (mean: 59 ± 7%) who had endomyocardial biopsies (EmBx) to rule out myocarditis or other infiltrative processes and had no histopathologic abnormalities. Patients with HFrEF and pathologic eccentric remodeling consisted of 47 patients with LVEFs ≤40% and New York Heart Association functional class II or III heart failure from nonischemic dilated cardiomyopathy (F/NDC) of uncertain etiology. These patients had right ventricular (RV) mid-distal interventricular septum EmBx performed at baseline for diagnostic and research purposes, and then after 3 and 12 months of beta-blocker therapy for research material only, as previously described (26,27) in the BORG (Effect of Beta-blockers on Structural Remodeling and Gene Expression in the Failing Human Heart) study (NCT01798992). The 4 subjects with normal LV function and 46 of the 47 patients with NDC (mean LVEF: 26.6 ± 8.7%) had technically adequate global gene expression measurements by microarray in extracted RNA, as previously described (26). Beta-blockers were initiated and up-titrated to target doses (26,27), reaching mean doses at the last EmBx and EF measurements of 75 ± 17 mg/day for 16 patients treated with carvedilol, and 169 ± 53 mg/day for 30 patients treated with metoprolol succinate. The clinical study was designed to detect differences in gene expression mediated through blockade of individual β1−, α1A− and β2-adrenergic receptors, but no systematic changes between treatment groups were detected (27) and the 3 groups, who had in common...
blockade of β1-adrenergic receptors, were combined into 1 cohort.

Eight patients had only a 3-month EmBx (1 sudden cardiac death, 1 LV assist device placement, 1 traumatic injury unrelated to the study that precluded compliance with the protocol, 5 withdrawals before 12 months), and their results were grouped with the 12-month studies in a last observation carried forward (LOCF) analysis. In these 8 subjects in the LOCF analysis, the degree of reverse remodeling was not statistically significantly different from patients with 12-month measurements (respective improvements in LVEF of 16%±5% [given as absolute percentage] vs. 22%±4%; p = 0.28). In addition to microarray, global gene expression was also measured by ribonucleic acid sequencing (RNA-Seq), in 6 “super-responders” with LVEF increases of ≥10 absolute percent compared with 6 sex- and age-matched subjects with LVEF increases of <5 absolute percent (Supplemental Appendix and Supplemental Table S1) (26,27). LVEF was measured by radionuclide single-photon emission computed tomography imaging as previously described (27,29), and a reverse remodeling responder (R) in the entire 46-patient F/NDC cohort was defined as an LOCF increase in LVEF of ≥5 absolute percent at 3 months or ≥8% at 12 months (26,27). Nonresponders (NRs) were subjects who did not meet these LVEF change criteria. EmBx and right heart catheterization were performed as previously described (26,27), and there were no procedure-related complications.

All patients signed written consent for this multicenter study conducted at the University of Colorado Anschutz Medical Campus and the University of Utah Medical Center. The study included a data and safety monitoring board and was approved by the institutional review boards at both sites.

**RNA EXTRACTION AND mRNA EXPRESSION MEASUREMENTS.** RNA extraction was performed as previously described (26,27). All individual patient mRNA measurements were from the same RNA extraction, typically involving 2 to 5 separate EmBx samples per study. Extracted RNA was stored at −80°C until use, and microarray and RNA-Seq methodologies were as previously described (26). Microarray gene expression data were normalized by log-scale robust multi-microarray analysis to yield output in fluorescence intensity on an exponential scale. RNA-Seq data transcript levels were quantified...
as fragments per kilobase of exon per million mapped reads, as previously described (26). Fold change or fold difference was calculated by log2 transformation as described (26).

**DATA ANALYSIS AND STATISTICAL TESTS.** We used both baseline comparisons between NF control subjects and patients with F/NDC plus changes from baseline in patients with F/NDC to construct an ordered classification of 4 degrees of remodeling association (Supplemental Appendix, Supplemental Table S2). For baseline studies, where only microarray data were available, alpha was set at <0.05. As described in the Supplemental Appendix (Supplemental Table S2), in serially evaluated patients with F/NDC these results were then linked to the reverse remodeling mRNA results measured by both the microarray and RNA-Seq platforms. The estimated alpha levels based on achieving \( p < 0.05 \) in more than 1 condition including directionality requirements are given in the Supplemental Appendix (Supplemental Table S2): **unequivocal evidence**, 0.0001; **evidence**, 0.002; and **possible evidence**, 0.08. The statistical significance of all gene expression data was analyzed in the same way, by nonparametric methods using Wilcoxon rank sum or signed rank tests. Correlation analysis was by Spearman rho LVEF data for which evidence of non-normal distribution was absent (26), and baseline characteristics were analyzed by Student’s \( t \)-tests or contingency table analysis.

Because several analyzed gene expression groups had small counts (NF, \( n = 4 \); RNA-Seq, \( n = 6 \) R and 6 NR; LOCF 3-month F/NDC, \( n = 5 \) Rs and 3 NRs) we used mean ± SD or, for change values, SEM as estimates of central tendency and dispersion, in both the small count groups and larger groups to which these data were compared. For microarray data (30 R and 16 NR), group size was sufficient for data to be also presented as median (interquartile range [IQR]). Thus in gene expression analyses conducted exclusively in groups where sample sizes were >6 nonparametric statistics and median (IQR) data are presented, but when smaller gene expression group sizes were analyzed or compared, nonparametric significance tests plus mean ± SD or SEM are used.

R (R Foundation, Vienna, Austria), GraphPad Prism (GraphPad Software, La Jolla, California) and XLSTAT/Excel (Microsoft, Redmond, Washington) were the statistical software packages used.

The mRNA expression of genes associated with remodeling is presented. See Figure 2 for further description. Abbreviations as in Figures 1 and 2.
**TABLE 2** Change From Baseline Data, Microarray, and RNA-Seq

| Gene   | Microarray | R | p Value | NR | R vs. NR | RNA-Seq | R | p Value | NR | R vs. NR | Level of Evidence Remodeling Association* |
|--------|------------|---|---------|----|---------|----------|---|---------|----|---------|------------------------------------------|
| RAS    | ACE2       | 0.675 | <0.0001 | 1.144 | 0.60 | 0.591 | 0.0006 | 0.402 | 0.031 | 1.216 | 0.69 | 0.331 | 0.004 | U.E |
|        | ACE        | 1.040 | 0.65 | 1.02 | 0.67 | 1.016 | 0.95 | 1.065 | 0.69 | 1.087 | 0.44 | 0.980 | 0.44 | N.E |
|        | AGT        | 1.049 | 0.32 | 1.078 | 0.30 | 0.97 | 0.76 | 0.924 | 0.44 | 1.049 | 1.00 | 0.973 | 0.18 | N.E |
|        | AGTR1      | 1.479 | 0.003 | 1.253 | 0.13 | 1.181 | 0.44 | 1.814 | 0.031 | 1.404 | 0.031 | 1.293 | 0.18 | P.E |
|        | AGTR2      | 0.964 | 0.21 | 1.181 | 0.23 | 0.816 | 0.060 | 0.900 | 0.31 | 1.703 | 0.44 | 0.528 | 0.24 | N.E |
| Proteases | TMPRSS2 | 1.000 | 0.84 | 1.000 | 0.63 | 1.000 | 0.62 | Transcript not detected | NE |
|         | TMPRSS11D | 0.943 | 0.044 | 1.017 | 0.99 | 0.928 | 0.23 | 0.977 | 1.00 | 2.149 | 0.59 | 0.452 | 0.84 | N.E |
|         | CTSL1      | 1.180 | 0.006 | 1.085 | 0.013 | 0.918 | 0.92 | 1.000 | 0.16 | 0.999 | 0.69 | 1.101 | 0.18 | N.E |
|         | ADAM17     | 1.018 | 0.56 | 1.025 | 0.67 | 0.993 | 0.92 | 0.865 | 0.094 | 1.042 | 0.44 | 0.830 | 0.65 | N.E |
|         | FURIN      | 1.043 | 0.82 | 0.952 | 0.22 | 1.10 | 0.55 | 0.912 | 0.31 | 9.938 | 0.31 | 0.972 | 0.48 | N.E |
| remodeling | NPPB     | 0.522 | <0.0001 | 1.783 | 0.38 | 0.295 | 0.0015 | 0.078 | 0.031 | 1.404 | 0.84 | 0.055 | 0.009 | U.E |
|         | MYH6       | 1.171 | 0.052 | 0.821 | 0.002 | 1.426 | 0.0002 | 2.426 | 0.062 | 0.885 | 0.56 | 2.742 | 0.015 | E |
|         | ATP2A1     | 1.050 | 0.077 | 0.989 | 0.60 | 1.062 | 0.056 | 1.388 | 0.031 | 1.005 | 1.00 | 1.044 | 0.31 | P.E |
|         | PLN        | 1.108 | 0.001 | 0.986 | 0.43 | 1.123 | 0.0006 | 1.281 | 0.22 | 1.034 | 0.44 | 1.239 | 0.24 | E |
|         | ADRB1      | 1.335 | 0.001 | 1.124 | 0.53 | 1.187 | 0.16 | 1.596 | 0.031 | 1.084 | 0.84 | 1.473 | 0.041 | E |
| Reference | GAPDH     | 1.026 | 0.99 | 0.983 | 0.30 | 1.044 | 0.69 | 0.994 | 0.84 | 1.071 | 0.44 | 0.928 | 0.48 | N.E |

*FC values are calculated from mean values as described in the methods; values <1.0 indicate a decrease in expression and >1.0 an increase. The p values are calculated by Wilcoxon rank sum of messenger ribonucleic acid readouts as described in the methods. GAPDH baseline NF and F/NDC were, respectively, 13.45 ± 0.08 and 13.40 ± 0.13 fluorescence units; p = 0.69. *Level of evidence of remodeling association is as described in the Supplemental Appendix and Supplemental Table S2. E = evidence; FC = log; fold change; NE = no evidence; PE = possible evidence; RAS = renin-angiotensin system; RNA-Seq = ribonucleic acid sequencing; UE = unequivocal evidence; other abbreviations as in Table 1.

## RESULTS

**BASELINE CHARACTERISTICS.** Baseline characteristics for the 46 subjects with F/NDC and 4 NF control subjects are given in Table 1. At baseline, the F/NDC and NF characteristics were similar, except for measurements and biomarkers of ventricular dysfunction and remodeling. In patients with F/NDC, LVEF was more reduced than RVEF (Figure 1A), but both were p < 0.05 versus NF. Right heart catheterization data trended abnormal in F/NDC but no measurement was p < 0.05 versus NF. BNP was elevated in patients with F/NDC, LVEF was more reduced than RVEF (Figure 1A), but both were p < 0.05 versus NF. These data describe a relatively young (mean age 45.6 ± 13.2 years), well-compensated HFpEF population with moderate LV dysfunction and remodeling (LVEF: 27.2 ± 9.0%, LV end-diastolic volume: 232 ± 93 ml). The baseline characteristics of the R and NR groups are also given in Supplemental Table S3. A markedly greater duration of heart failure in NRs versus Rs (55.7 ± 67.0 months vs. 7.0 ± 10 months, respectively; p = 0.011) was the only difference.

The protocol mandated angiotensin converting enzyme (ACE) inhibitor background therapy and diuretic agents as needed. Spironolactone was administered as tolerated, and an angiotensin receptor blocker (ARB) could be substituted in patients who are ACE inhibitor-intolerant. Table 1 gives the ACE inhibitor and ARB doses in enalapril and losartan equivalents (39), at both baseline and the average dose during the 12-month study. All 4 of the NF control subjects were on ACE inhibitors for suspected and ultimately unproved heart muscle disease, and 44 of the 46 patients were on an ACE inhibitor at baseline. There is no difference in ACE inhibitor dose (in mg) between patients with F/NDC and NF control patients, and no difference between reverse remodeling Rs and NRs in average dose of ACE inhibitors or ARBs.

**REVERSE REMODELING AS MEASURED BY LVEF AND RVEF OVER 3 OR 12 MONTHS.** Figure 1B plots baseline, LOCF (8 patients at 3 months and 38 at 12 months), and the LOCF – baseline change for both LVEF and RVEF, by R and NR groups in the F/NDC cohort. For Rs versus NRs, there is a marked increase...
in LVEF (by 21.2 ± 1.8 [SEM absolute percent] vs. 0.9 ± 0.8%; p < 0.0001) and a lesser, nonsignificant increase in RVEF (respectively, 9.4 ± 2.1% vs. 4.9 ± 3.1%; p = 0.24). Remodeling data for the selected super-Responder subcohort and control subjects are in the Supplemental Appendix (Supplemental Table S1), and follow the same pattern, except that the LVEF change from baseline was larger than in the entire cohort (30.7 ± 4.2% vs. 21.2 ± 1.8%; p = 0.035).

**BASELINE CHANGES IN mRNA EXPRESSION IN PATIENTS WITH NDC VERSUS NF CONTROL SUBJECTS.** ACE2, other RASs, and proteases. Figure 2 contains F/NDC versus NF baseline mRNA abundance data, for ACE2, 2 associated RAS genes (AGT and ACE), and 5 proteases that have been implicated in CoV-2 or CoV-2-cell membrane priming and fusion (12–14,31,32). Compared with NF control subjects, ACE2 is substantially up-regulated in F/NDC, by 1.97-fold (p = 0.008). ACE expression is unchanged from control, as is angiotensinogen. The angiotensin II type 1 and type 2 receptors (AGTR1, AGTR2) are plotted in Figure 3 and are not different between NF and F/NDC. None of the proteases shown in Figure 2 exhibit differences in mRNA expression in F/NDC versus NF, and only 1 (down-regulation of cathepsin L-like 3) of 11 additional proteases (Supplemental Appendix and Supplemental Table S3) exhibited any change in the F/NDC group.

**Remodeling-associated genes.** At baseline, NPPB, PLN, and ATP2A2 exhibited changes similar to those in previously reported reverse transcription polymerase chain reaction (PCR) data (27). NPPB was up-regulated by 8.8-fold in F/NDC, and its expression versus that of ACE2 was significantly related (Spearman rho = 0.73; p < 0.0001). Unlike in previous studies using reverse transcriptase PCR (26,27,33,34), MYH6 was unchanged between NF and F/NDC but was markedly up-regulated on reverse remodeling (Table 2) consistent with previously reported data (26,27,34).

**Integrins.** Table 3 contains NF versus F/NDC baseline data for 10 integrins previously reported to bind to ACE2 (18,19), facilitate viral internalization (17), or be associated with LV remodeling (35) or cardiac myocyte injury protection (36). Only 1, the laminin binding integrin ITGA7, has not been reported to be involved in pathogenic virus cell internalization. Five integrins in Table 3 bind to the arginine-glycine-aspartic acid (RGD) motif, recently identified in the CoV-2 spike protein binding domain (16) and used by multiple viruses for binding to integrins. Of the ACE2 binding integrins, ITGA5 expression was 1.28-fold higher in patients with F/NDC compared with NF control subjects (p = 0.039), whereas ITGB1 and ITGA2 were not different. ITGA5 has also been associated with virus internalization (17) and LV remodeling where it decreases with LV assist device treatment (35). Of the 6 non-Ace2 binding integrins listed in Table 3 that have been associated with virus internalization, 4 reached a p value of <0.05 for differences between patients with F/NDC and NF control subjects, 2 up-regulated (ITGB3 and ITGA4) in patients with F/NDC and 2 down-regulated (ITGB6 and ITGA6). For the 3 genes previously associated with remodeling other than ITGA5, 2 (ITGA6 and ITGB6) were down-regulated in patients with F/NDC consistent with the decreased expression of ITGA6 in human LVs with dilated cardiomyopathies (35), or the up-regulation previously reported with LV assist device treatment (35). The laminin binding integrin ITGA7 was up-regulated in patients with F/NDC.

**CHANGES IN mRNA EXPRESSION ON REVERSE REMODELING.** ACE2, other RASs, and proteases. Changes in R or NR fold changes based on mean values are given in Table 2, whereas median (IQR) values for microarray data are presented in Supplemental Tables S3 and S4. In microarray measurements, the near 2-fold up-regulated ACE2 at baseline was down-regulated as LV remodeling improved, declining to 0.675-fold (an approximate 1.5-fold decrease; p < 0.0001). Three other RAS genes (ACE, AGT, and AGTR2) and only 1 of the proteases shown in Table 2 exhibited changed expression on reverse remodeling. The protease that was down-regulated in patients with F/NDC (cathepsin L-like gene 3) was not changed with reverse remodeling (fold change from baseline: 1.03; p = 0.62) (Supplemental Table S3). Although in the R versus NR comparison, PRSS1 was down-regulated on reverse remodeling (fold change: 0.90; p = 0.042) (Supplemental Table S3) the change was related to an up-regulation in the NR group rather than a change in the R group. AGTR1 met criteria for possible evidence (p < 0.08) for an association with remodeling, with R and not NR up-regulation on microarray measurements. RNA-Seq data exhibited a decrease in ACE2 expression on reverse remodeling (p = 0.004), with a greater fold change of 0.40-fold (approximate 2.5-fold decline) than in microarray data. Thus, ACE2 met criteria for unequivocal evidence (p < 0.0001) of a remodeling association. RNA-Seq data for all other RAS and protease genes (Table 2, Supplemental Table S4) were similar to microarray measurements.
Remodeling-associated genes. As expected, these genes generally changed their expression in directions opposite to their baseline levels (Table 2 and Supplemental Table S4). NPPB, which encodes a precursor of a counter-regulatory peptide considered the premier biomarker of pathologically remodeled ventricular myocardium in tissue (27,34) or plasma (37), exhibited expression behavior very similar to ACE2 including an unequivocal evidence rating for remodeling association. MYH6, PLN, and ADRB1 all had remodeling associated ratings of evidence (p < 0.002), whereas ATP2A2 rated possible evidence. Integrins. Reverse remodeling changes in integrins are given in Table 2 and Supplemental Table S4. For the p < 0.05 baseline-changed genes, ITGB3, ITGB6, and ITGA5 had at least 1 platform showing significant change on reverse remodeling and thus qualified for evidence of a remodeling association (Table 3). ITGA4 and ITGA6 expression did not significantly change on reverse remodeling, and their baseline changes therefore qualified as possible evidence. For ITGA7, both microarray and RNA-Seq data met criteria for dynamic down-regulation of the baseline up-regulated expression and a rating of unequivocal evidence of a remodeling association, with a pattern identical to that of ACE2 and NPPB. Thus, of the 10 integrins examined, 5 had at least some evidence of an association with remodeling.

**DISCUSSION**

**IN PATIENTS WITH MILD-MODERATE F/NDC, MYOCARDIAL ACE2 GENE EXPRESSION DYNAMICALLY REGULATES WITH LV REMODELING INDEPENDENT OF RAS INHIBITOR TREATMENT AND MAY EXPLAIN HEIGHTENED COVID-19 CLINICAL RISKS.** Figure 4 illustrates the positioning and role of ACE2 within the renin-angiotensin-aldosterone system. In the human LV, this zinc-dependent carboxypeptidase catalyzes the conversion of the octapeptide angiotensin II to the counter-regulatory heptapeptide angiotensin-(1-7) with high activity and efficiency (21). ACE2 exerts a very important function in the heart, by reducing levels of angiotensin II as well as by generating a counter-regulatory peptide that activates MAS1 receptor pathway signaling through Gαi and multiple downstream events including reducing extracellular signal related kinase 1/2 MAP kinase activation (38,39). We found that in a setting where RAS inhibitors were not a potentially confounding variable ACE2 mRNA expression was substantially up-regulated in intact failing/remodeled ventricular myocardium, confirming and extending previous work in explanted human hearts (20,22,23). From its up-regulated expression in F/NDC at baseline, with reverse remodeling ACE2 then down-regulated toward control values in measurements by both mRNA
platforms to yield an *unequivocal evidence* rating for remodeling association corresponding to a p value of <0.0001. In the current study, the source of starting material was interventricular septum that reflects molecular changes in either the RV (33) or LV (27), and the reverse remodeling changes were mostly confined to the LV. In addition, the mRNA expression of the 42% ACE2 homologous (40) enzyme ACE was not altered at baseline or on reverse remodeling. Angiotensinogen was similarly unchanged in failing/remodeled ventricular myocardium. The behavior of ACE2 gene expression was analogous and substantially related (baseline values Spearman rho of 0.73; p < 0.0001) to that of NPPB, another counter-regulatory gene whose remodeling association rating was *unequivocal evidence*.

The up-regulation of ACE2 in remodeled intact LV myocardium from subjects with only mild-moderate HFpEF indicates that this regulatory change is not confined to end-stage disease in patients who have been treated with RAS inhibitors (20,22,23), and suggests that increased expression of ACE2 in remodeled LVs could be the reason why patients infected with CoV-2 underlying heart muscle disorders are at heightened clinical risk (2,3).

**PROTEASES THAT PARTICIPATE IN CoV-2-CELLULAR MEMBRANE PRIMING AND FUSION DO NOT EXHIBIT REGULATED expression IN F/NDC.** We also evaluated the expression of 5 proteases that participate in fusion of viral and cell membranes (12-14,31,32) when triggered by SARS coronavirus binding to ACE2 (41,42). Expression of CTSL1 (13), TMPRSS11D (13,14), ADAM17 (31), and FURIN (13,32) were detected by both microarray and RNA-Seq platforms. In contrast, TMPRSS2 (12-14), recently implicated in CoV-2-membrane fusion in model systems (12), was low abundance as measured by microarray and could not be detected by RNA-Seq. None of these proteases and only 1 (cathepsin L-like 3) of an additional panel of 11 others exhibited differences between NF control subjects and patients with F/NDC, and none changed expression in Rs with reverse remodeling. However, this does not exclude the possibility that protease protein abundance or enzyme activity may have changed with remodeling.

**INTEGRINS, PARTICULARLY ITGA5, ARE REGULATED WITH REMODELING AND ARE CANDIDATES FOR CoV-2 BINDING AND CELL ENTRY.** We considered that integrins could be a potential participant in CoV-2 cell entry and found *evidence* or *unequivocal evidence* of remodeling-associated up-regulation in 5 of the 10 investigated. ITGA5, rated *evidence* of an association with remodeling, and its protein product can bind to ACE2 (19) plus a motif in CoV-2 (16) and thus is a candidate for involvement in CoV-2 cell binding and internalization. The only integrin that achieved *unequivocal evidence* of remodeling association was the laminin binding cardiac and skeletal muscle integrin ITGA7, whose gene product has not been reported to bind to ACE2 or pathogenic viruses and which, like ACE2 and BNP, can be viewed in a counter-regulatory context (36). Five of the 10 integrins evaluated bound to an RGD motif, common in pathogenic viruses as a means of cell surface binding and virus internalization, and recently identified in CoV-2 (16). Of these, only ITGA5 and ITGB3 were up-regulated, meaning if CoV-2 did utilize RGD-integrin binding for internalization, these 2 monomers might predispose to greater cell entry. However, these integrins do not dimerize with each other (43), and the ITGA5-ITGB1 or ITGAV-ITGB3 protein product heterodimers α5β1 or αvβ3, both commonly used by pathogenic viruses for cell entry, would need to be formed. If RGD binding is disregarded (17), the other up-regulated integrin (rated as *possible evidence*) was ITGAA4, which also does not dimerize with ITGB3 (43). Alternatively, up-regulated integrins could affect virus replication in cardiac myocytes via interaction with integrin linked kinase (44), a pseudokinase adaptor molecule known to bind to ITGB1 and ITGB3 gene products (45).

**CARDIAC MYOCYTE CELL ENTRY OF CoV-2, YET TO BE ESTABLISHED, IS POTENTIALLY POSSIBLE BASED ON BINDING AND INTERNALIZATION MECHANISMS BEING PRESENT IN HUMAN VENTRICULAR MYOCARDIUM.** Despite substantial evidence that myocardial involvement is common and potentially devastating in COVID-19, identification of CoV-2 virus in cardiac myocytes has not been reported. Cardiac findings on autopsy of patients who had COVID-19 are limited to a single case of severe pulmonary involvement in which on tissue examination no myocardial pathologic findings were observed (46), and 2 pulmonary death cases in which postmortem myocardial needle biopsy findings were deemed likely to be secondary to pre-existing underlying conditions (47). One of the 2 needle biopsy subjects had a negative tissue block CoV-2 PCR (47). There is thus far only a single case report of an EmBx in a COVID-19 PCR-proven case, in a patient in cardiogenic shock (48). This patient had electron microscopy-imaged coronavirus in ventricular myocardial interstitial cells but not in cardiac...
myocytes, despite evidence of myofibril lysis (48). However, based on remodeling associated up-regulation in ACE2 and the demonstration that all other myocardial cell entry constituents exist in human ventricular myocardium, it would be surprising if CoV-2 cannot bind to, enter, replicate, and damage human cardiac myocytes. Myocytes contribute approximately 70% of tissue volume in the human LV (49), and ACE2 is definitely cardiac myocyte-expressed according to cell marker findings (20), single-cell RNA data (50), and the degree of enzyme activity in vivo (21) and in explanted hearts (22). Thus cardiac myocytes appear to be a vulnerable target for CoV-2, and this needs to be addressed by further histopathologic investigation in hearts exhibiting CoV-2-associated myocardial dysfunction.

**ACE2 AS A POTENTIAL THERAPEUTIC TARGET IN CoV-2 INFECTION.** As has been noted by others (51), ACE2 and its receptor for the spike protein binding domain are an attractive therapeutic target for treating or preventing CoV-2 infections. ACE2 small molecule inhibitors have been developed (52) and dramatically lower ACE2 activity in failing human LV preparations, at nanomolar concentrations (22). However, ACE2 is an important counter-regulatory enzyme in the heart, responsible for converting angiotensin II to angiotensin-(1-7) that is anti-proliferative, anti-fibrotic, and a vasorelaxant. Based on gene ablation, ACE2 is considered an “essential regulator of heart function” (53). It follows that any ACE2 inhibitor would need to block CoV-2 binding without decreasing enzyme activity, which may be possible through antibody inhibition (11) or the use of decoy receptors (54). Another possibility would be to deploy an ACE2 activator such as diminazene (55) if an ACE2 CoV-2 receptor inhibitor diminishes ACE2 activity. However, if any of these approaches are taken, it would be important to rule out other mechanisms of CoV-2 cell entry that would be uninhibited, such as virus-integrin binding.

**ACKNOWLEDGMENTS** The authors thank Rachel Rosenberg, MS, MBA, and Laura Hofstatter for manuscript proofing and handling.

**ADDRESS FOR CORRESPONDENCE:** Dr. Michael R. Bristow, Division of Cardiology, University of Colorado, Denver/Anschutz Medical Campus, B-139 Research 2, 12700 East 19th Avenue, Aurora, Colorado 80045. E-mail: michael.bristow@cuanschutz.edu.
COMPETENCY IN MEDICAL KNOWLEDGE: ACE2, a counter-regulatory enzyme robustly expressed in human LV and RV and the major pathway for breaking down angiotensin II into the antihypertrophic, antibfotic, and vasorelaxant peptide angiotensin-(1-7), has been hijacked by severe acute respiratory syndrome coronaviruses as the binding site for initiation of cell entry. ACE2 is up-regulated in eccentrically remodeled/failing LVs, and then decreases expression with reverse remodeling. This regulatory behavior was independent of RAS inhibitors as doses of ACE inhibitors or ARBs were not different in patients with reverse remodeling compared with in those without reverse remodeling. ACE2’s remodelling expression behavior was identical to NPPB, another counter-regulatory gene that leads to generation of the antiproliferative vasodilator B-type natriuretic peptide. Up-regulated ACE2 should be viewed as beneficial to a remodeled, failing heart, although it may increase the risk of CoV-2 cell invasion and cytopathology. It stands that any therapeutic approach involving ACE2 should focus on inhibiting CoV-2 binding, while maintaining enzyme activity.

TRANSLATIONAL OUTLOOK: The data presented are another example of reverse translation, in this case where an enzyme that was originally characterized and shown to be up-regulated in failing, pathologically remodeled human hearts was discovered to be the cell transducer for a worldwide pandemic. The precise biologic mechanisms involved in severe acute respiratory syndrome coronavirus cell entry and damage are now being investigated at the basic science level, and therapeutic strategies involving ACE2 inhibition or intervention at downstream events may result in forward translation back to the clinical setting.

REFERENCES

1. Guan WJ, Ni ZY, Liang WH, et al., for the China Medical Treatment Expert Group for COVID-19. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708-20.
2. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054-62.
3. Shi S, Qin M, Shen B, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol 2020;5:802-10.
4. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med 2020;46:846-8. erratum: Intensive Care Med 2020;46:1294-1297.
5. Inciardi RM, Lupi L, Zaccone G, et al. Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). JAMA Cardiol 2020;5:1-6.
6. Hu H, Ma F, Wei X, Fang Y. Coronavirus fulminating myocarditis saved with glucocorticoid and the cardiovascular system. Nat Rev Cardiol 2020;17:259-60.
7. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. Nat Rev Cardiol 2020;17:259-60.
8. Lippi G, Lavie CJ, Sanchis-Gomar F. Cardiac troponin I levels in patients with coronavirus disease 2019 (COVID-19): evidence from a meta-analysis. PloS Cardiovas Dis 2020;6:3-90.
9. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the per fus conformation. Science 2020;367:1260-3.
10. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 Spike glycoprotein. Cell 2020;181:281-92.e6.
11. Hoffmann M, Klein-Webhe H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271-80.e8.
12. Belouzard S, Chu VC, Whittaker GR. Activation of the SARS coronavirus spike protein by sequential proteolytic cleavage at two distinct sites. Proc Natl Acad Sci U S A 2009;106:5871-6.
13. Bertram S, Glowacka I, Mueller MA, et al. Cleavage and activation of the severe acute respiratory syndrome coronavirus spike protein by human airway trypsin-like protease. J Virol 2011;85:13363-72.
14. Hagemeijer MC, Rottier PJM, de Haan CAM. Biogenesis and dynamics of the coronavirus replicative structures. Viruses 2012;4:3245-69.
15. Sigrist CJA, Bridge A, Le Mercier P. A potential role for integrins in host cell entry by SARS-CoV-2. Antiviral Res 2020;177:104759.
16. Hussein HA, Walker LR, Abdel-Raouf UM, Desouky SA, Montasser AK, Akula SM. Beyond RGD: virus interactions with integrins. Arch Virol 2015;160:2669-81.
17. Lin Q, Keller RS, Weaver B, Zisman LS. Interaction of ACE2 and integrin beta1 in failing human heart. Biochim Biophys Acta 2004;1689:175-8.
18. Clarke NE, Fisher MJ, Porter KE, Lambert DW, Turner AJ. Angiotensin converting enzyme (ACE) and ACE2 bind integrins and ACE2 regulates integrin signalling. PLoS One 2012;7:e34747.
19. Shen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. Cardiovasc Res 2020;116:1097-100.
20. Zisman LS, Meixell GE, Bristow MR, Canver CC. Angiotensin-(1-7) formation in the intact human heart: in vivo dependence on angiotensin II as substrate. Circulation 2003;108:1679-81.
21. Zisman LS, Keller RS, Weaver B, et al. Increased angiotensin-(1-7)-forming activity in failing human heart: evidence for upregulation of the angiotensin-converting enzyme homologue ACE2. Circulation 2003;108:1707-12.
22. Goulter AB, Goddard MJ, Allen JC, Clark KL. ACE2 gene expression is up-regulated in the human failing heart. BMC Med 2004;2:19.
23. Ferrario CM, Jessup J, Chappell MC, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation 2005;111:2605-10.
24. Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM.
Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. Hypertension 2004;43:970-6.

26. Toni LS, Carroll IA, Jones KL, et al. Sequential analysis of myocardial gene expression with phenotypic change: use of cross-platform concordance to strengthen biologic relevance. PLoS One 2019;14:e0221519. erratum: PLoS One 2019;14:e0224389.

27. Kao DP, Lowes BD, Gilbert EM, et al. Therapeutic molecular phenotype of beta-blocker-associated reverse-remodeling in nonischemic dilated cardiomyopathy. Circ Cardiovasc Genet 2015;8:270-83.

28. Leary PJ, Kromnal RA, Blumene DA, et al. Histamine H2 receptor polymorphisms, myocardial transcripts, and heart failure (from the Multi-Ethnic Study of Atherosclerosis and Beta-Blocker Effect on Remodeling and Gene Expression Trial). Am J Cardiol 2018;121:256-61.

29. Sucharov CC, Kao DP, Port JD, et al. Myocardial microRNAs associated with reverse remodeling in human heart failure. JCI Insight 2017;2:e89169.

30. Peri-Okońsky PA, Mi X, Khairon Y, et al. Target doses of heart failure medical therapy and blood pressure: insights from the CHAMP-HF registry. J Am Coll Cardiol HF 2019;7:350-8.

31. Haga S, Yamamoto N, Nakai-Murakami C, et al. Modulation of TFN-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TFN-alpha production and facilitates viral entry. Proc Natl Acad Sci U S A 2008;105:8083-8.

32. Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus-2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res 2020;176:104742.

33. Lowes BD, Minobe WA, Abraham WT, et al. Changes in gene expression in the intact human heart: down-regulation of alpha-myosin heavy chain in hypertrophied, failing ventricular myocardium. J Clin Invest 1997;100:2315-24.

34. Lowes BD, Gilbert EM, Abraham WT, et al. Myocardial gene expression in dilated cardiomyopathy treated with beta-blocking agents. New Engl J Med 2002;346:1357-65.

35. Dullens HFJ, Schipper MEJ, van Kuijk J, et al. Integrin expression during reverse remodeling in the myocardium of heart failure patients. Cardiovasc Pathol 2012;21:291-8.

36. Okada H, Lai NC, Kawaraguchi Y, et al. Integrins protect cardiomyocytes from ischemia/reperfusion injury. J Clin Invest 2013;123:4394-408.

37. Martin FL, Chen HH, Catalotti A, Burnett JC Jr. B-type natriuretic peptide: beyond a diagnostic. Heart Fail Clin 2008;4:449-54.

38. Santos RAS, Simoes e Silva AC, Maric C, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci U S A 2003;100:8258-63.

39. Tallant EA, Ferrario CM, Gallagher PE. Angiotensin-(1-7) inhibits growth of cardiac myocytes through activation of the mas receptor. Am J Physiol Heart Circ Physiol 2005;289:H1560-6.

40. Donoghue M, Hsieh F, Baronas E, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res 2000;87:E1-9.

41. Heald-Sargent T, Gallagher T. Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence. Viruses 2012;4:557-80.

42. Belouard S, Millet JK, Licitra BN, Whitaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses 2012;4:1011-33.

43. Takada Y, Ye X, Simon S. The integrins. Genome Biol 2007;8:215.

44. Esfandiarei M, Suarez A, Amaral A, Si X, Racke KE, El Ghouzzi V. ACE2-induced TNF-alpha production and facilitation of viral entry by the SARS-CoV-2 spike glycoprotein. Elife 2020;9:e557.

45. Legate KR, Fässler R. Mechanisms that regulate integrin-mediated cell adhesion and migration. J Cell Sci 2009;122:187-98.

46. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 2020;8:420-2.

47. Tian S, Xiong Y, Liu H, Niu L, Guo J, Liao M, Xiao SY. Pathological study of the 2019 novel coronavirus disease (COVID-19) through post-mortem core biopsies. Mod Pathol 2020;33:1007-14.

48. Tavazzi G, Pellegrini C, Maurelli M, et al. Myocardial localization of coronavirus in COVID-19 cardiogenic shock. Eur J Heart Fail 2020;22:911-5.

49. Tang Y, Nyengaard JR, Andersen JB, Baandrup U, Gurdersen HJ. The application of stereological methods for estimating structural parameters in the human heart. Anat Rec 2009;292:1630-47.

50. Guo J, Xiangxiang W, Li Q, et al. Single-cell RNA analysis on ACE2 expression provides insight into SARS-CoV-2 blood entry and heart injury. medRxiv preprint April 4, 2020. Available at: https://doi.org/10.1101/2020.03.31.20047621. Accessed April 21, 2020.

51. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med 2020;46:586-90.

52. Dales NA, Gould AE, Brown JA, et al. Substrate-based design of the first class of angiotensin-converting enzyme-related carboxypeptidase (ACE2) inhibitors. J Am Chem Soc 2002;124:11852-3.

53. Crackower MA, Sarao R, Oudit GY, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 2002;417:822-8.

54. Hofmann H, Geier M, Marzi A, et al. Susceptibility to SARS coronavirus 5 protein-driven infection correlates with expression of angiotensin converting enzyme 2 and infection can be blocked by soluble receptor. Biochem Biophys Res Commun 2004;319:1216-21.

55. Qaradakhi T, Gadaneck LK, McSweeney KR, et al. The potential actions of angiotensin-converting enzyme II (ACE2) activator diminazene aceturate (DIZE) in various diseases. Clin Exp Pharmacol Physiol 2020;47:751-8.

**KEY WORDS** angiotensin converting enzyme 2, coronavirus disease 2019, integrins, proteases, ventricular remodeling

**APPENDIX** For supplemental explanations and tables, please see the online version of this paper.

Go to http://www.acc.org/jacc-journals-cme to take the CME/MOC/ECME quiz for this article.