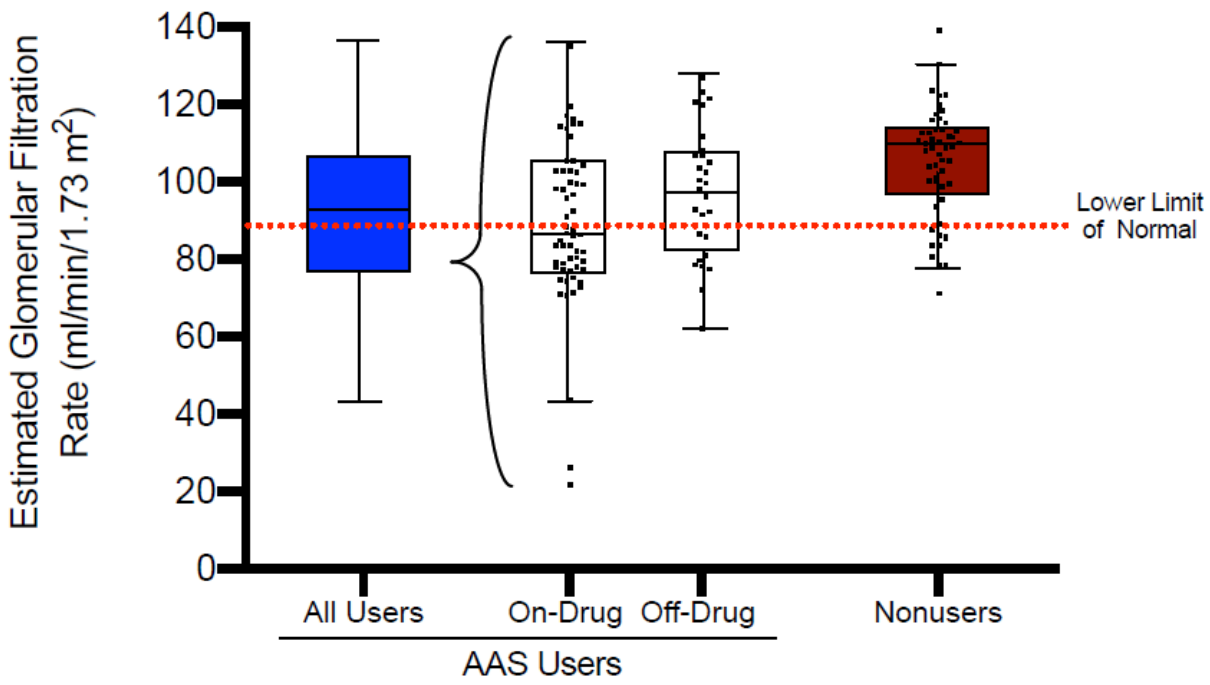

Figure S1. **Estimated glomerular filtration rate in supraphysiologic-dose anabolic-androgenic steroid users and nonusers.** Boxplots for anabolic-androgenic steroid (AAS) users (N=85), shown as an entire group (**Left**), and shown with accompanying scatterplot as subgroups of individuals who were on-drug (N=57) and off-drug (N=28) at the time of evaluation (**Middle**)**;** and a group of nonusers (N=52) (**Right**).

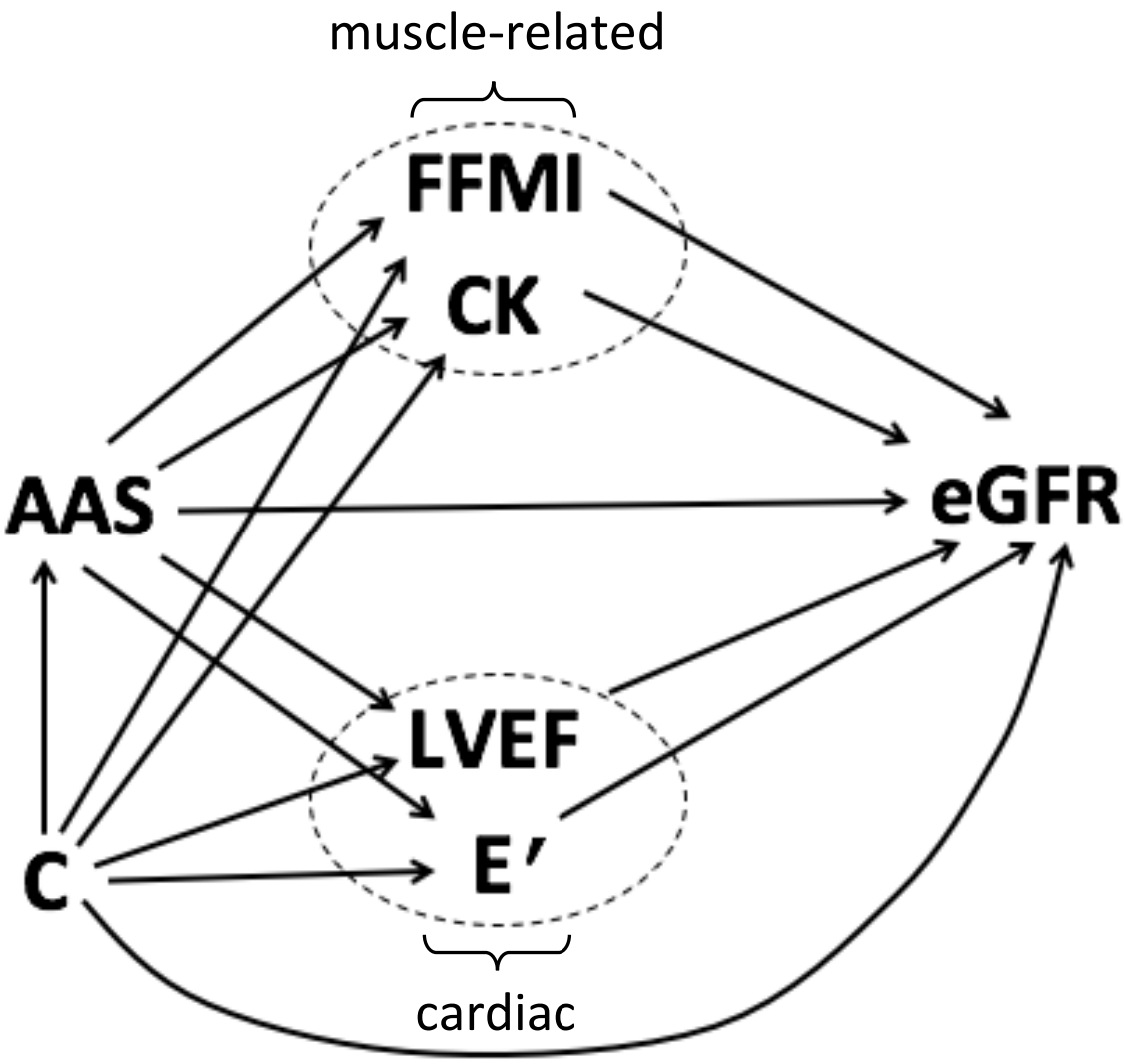

Figure S2. **Directed acyclic graph (DAG) showing causal paths related to candidate mediators of the effect of anabolic-androgenic steroids ("AAS") on estimated glomerular filtration rate ("eGFR").** The two sets of candidate mediators are muscle-related variables (fat-free mass index ("FFMI") and creatine phosphokinase ("CK")) and cardiac variables (left ventricular ejection fraction ("LVEF") and early left ventricular relaxation velocity (E')). "C" represents a set of confounding variables, some of which have been controlled for in the analysis (see text), and some of which represent hypothetical unknown variables that cause residual confounding. Note that, for simplicity, this DAG does not consider effects of eGFR on candidate mediators, nor does it consider dependencies among the candidate mediators (see text for a discussion of these possible effects).

Item S1. Details of the 2017 study of cardiac function that furnished data for the present study of kidney function

Study design

The data presented in the present study of kidney function were obtained in the course of a recent observational study of the cardiovascular toxicity associated with long-term use of supraphysiologic doses of anabolic-androgenic steroids (AAS).[1] Details of this study ("the cardiac study") are presented in the original paper[1] and are summarized here.

The cardiac study used a cross-sectional cohort design – a method that we have presented in detail in formal terms in a prior statistical publication.[2] The cross-sectional cohort design begins by defining a dynamic cohort of individuals from a given source population, who in theory could have been enumerated in the past and followed to the present (the "conceptual cohort"). However, rather than sampling from this theoretical conceptual cohort, one samples from individuals currently available (the "study cohort"). Using this design, estimates of effects derived from the study cohort are valid with respect to the conceptual cohort, with requirements for validity similar to those of other retrospective designs (e.g., case-control and retrospective cohort studies). Applying this approach, we sampled from a source population of male weightlifters in local gymnasiums and then compared exposed (AAS-using) and nonexposed (non-AAS-using) men. We chose this source population because the great majority of AAS users are male[3] and lift weights regularly.[4, 5] We did not recruit from athletic events because, contrary to some popular beliefs,[6] most AAS users are not competitive athletes and instead use AAS primarily to enhance personal appearance.[5, 7, 8]

Recruitment and evaluation of participants

We employed recruitment methods that have been used successfully over a series of studies of AAS users in the last 20 years, and which are detailed in several of our previous papers.[1, 9, 10] In the specific case of the cardiac study, we posted advertisements in

approximately 15 gymnasiums in the vicinity of Boston, Massachusetts, inviting men age 34-55 who could bench press at least 275 pounds for at least one repetition, now or in the past, to participate in a medical and psychological research study. Men were offered $550 for participating in the study, which required a three-hour screening visit followed by a five-hour cardiac and general medical evaluation.

The bench-press requirement in the study advertisement was designed simply to obtain a group of muscular men, likely to contain a substantial portion of AAS users. However, the study's focus on AAS was not disclosed in the advertisement, in order to minimize selection bias that might occur if respondents knew in advance the exposure variable of interest. Similarly, when advertisement respondents were screened by telephone, they were invited to participate without inquiring about their AAS use. In the course of the study we received about 100 telephone inquiries from men who met the age and bench-press criteria and who were invited for a screening interview. Men who participated in the study referred about 100 other potential candidates who in turn were invited for a screening interview if appropriate.

It should be acknowledged that in a study such as this, which depended upon recruiting illicit drug users and non-users from the field, there is a greater risk of selection bias than would be encountered in a typical medical study involving, say, sequentially evaluated patients, or a specific well-defined cohort. For example, weightlifters who chose to respond to our advertisement, or who were referred by prior study participants, might arguably have been more motivated to participate if they were financially strained and eager to earn the $550 study compensation, or if perhaps if they had experienced medical or psychiatric symptoms of some type that had stimulated their interest in participating in a medical study. Conversely, individuals who had used AAS in the past, but subsequently discontinued AAS and ceased to lift weights, may have been underrepresented because they were less likely to have seen our advertisement in a local gymnasium.

At the screening interview, all participants first provided written informed consent for the study as approved by the McLean Hospital Institutional Review Board. They then were assessed for demographic features; athletic history; height, weight, and body fat; psychiatric and medical history; history of AAS use, including specific drugs, doses, and durations of use; history of use of other performance-enhancing drugs and of classical drugs of abuse; urine testing for AAS; and testing of hair samples for classical drugs of abuse. Qualifying men were then referred for a second study visit at Massachusetts General Hospital, where they received an evaluation focusing primarily on cardiac measures, including physical examination, electrocardiography, echocardiography, Holter monitoring, and computed tomography coronary angiography. At the cardiac evaluation, men also provided blood for standard chemistries, hematology, cystatin C, and endocrine measures. The values for creatinine and cystatin C, obtained from these blood samples, represented the primary data used in the present analysis of kidney function. It should be noted that since the original study was focused on cardiac function, we unfortunately did not obtain more detailed measures of kidney function such as albuminuria, proteinuria, or other serum or urine biomarkers of kidney injury.

Of 165 men evaluated in screening interviews, 25 did not progress to the subsequent cardiac evaluation. Of these, 10 qualified for the cardiac evaluation but withdrew from the study before the cardiac evaluation could be performed (9 of whom were AAS users and 1 a nonuser). Twelve additional men were excluded because they reported less than 2 years of total lifetime AAS use, which we defined as the threshold duration of use for inclusion in this study of long-term AAS use; and 3 men were excluded because they showed findings on drug testing that contradicted their self-reports, or displayed a degree of muscularity (as calculated by their fat-free mass index[11]) suggesting that they had surreptitiously used AAS even though they denied doing so on interview. Thus, the final sample consisted of 86 men reporting at least two years of cumulative lifetime supraphysiologic-dose AAS exposure and 54 equally experienced male weightlifters reporting no AAS exposure. Among the AAS users, 58 were currently taking AAS at

the time of evaluation and 28 had formerly used AAS but were not currently doing so. These 28 past users had last ingested AAS a median (interquartile range) of 15 (5, 70) months prior to evaluation. Of this total sample of 140 men, 3 (1 current AAS user and 2 nonusers) lacked a cystatin C measurement, leaving a final sample of 57 current users, 28 past users, and 52 nonusers for analysis in the present paper.

Works cited:

1. Baggish AL, Weiner RB, Kanayama G, Hudson JI, Lu MT, Hoffmann U, Pope HG, Jr. Cardiovascular Toxicity of Illicit Anabolic-Androgenic Steroid Use. *Circulation.* 2017;135(21):1991-2002.

2. Hudson JI, Pope HG, Jr., Glynn RJ. The cross-sectional cohort study: an underutilized design. *Epidemiology.* 2005;16(3):355-359.

3. Pope HG, Kanayama G, Athey A, Ryan E, Hudson JI, Baggish A. The lifetime prevalence of anabolic-androgenic steroid use and dependence in Americans: current best estimates. *American Journal on Addictions.* 2014;23:371-377.

4. Bahrke MS, Yesalis CE, Kopstein AN, Stephens JA. Risk factors associated with anabolic-androgenic steroid use among adolescents. *Sports Med.* 2000;29(6):397-405.

5. Pope HG, Jr., Wood RI, Rogol A, Nyberg F, Bowers L, Bhasin S. Adverse health consequences of performance-enhancing drugs: an Endocrine Society scientific statement. *Endocr Rev.* 2014;35(3):341-375.

6. Kanayama G, Pope HG. Misconceptions about anabolic-androgenic steroid abuse. *Psychiatric Annals.* 2012;42(10):371-375.

7. Ip EJ, Barnett MJ, Tenerowicz MJ, Perry PJ. The Anabolic 500 survey: characteristics of male users versus nonusers of anabolic-androgenic steroids for strength training. *Pharmacotherapy.* 2011;31(8):757-766.

8. Parkinson AB, Evans NA. Anabolic androgenic steroids: a survey of 500 users. *Med Sci Sports Exerc.* 2006;38(4):644-651.

9. Kanayama G, Pope HG, Cohane G, Hudson JI. Risk factors for anabolic-androgenic steroid use among weightlifters: a case-control study. *Drug Alcohol Depend.* 2003;71(1):77-86.

10. Pope H, Kanayama G, Hudson J. Risk factors for illicit anabolic-androgenic steroid use in male weightlifters: A cross-sectional cohort study. *Biological Psychiatry* 2012;71:254-261.

11. Kouri EM, Pope HG, Jr., Katz DL, Oliva P. Fat-free mass index in users and nonusers of anabolic-androgenic steroids. *Clin J Sport Med.* 1995;5(4):223-228.

Item S2. Exploratory analyses of possible mediators of the effect of anabolic-androgenic steroids (AAS) on estimated glomerular filtration rate (eGFR)

Rationale

In an attempt to explain the association between AAS exposure and decreased eGFR, as presented in the main text of this paper, it seems plausible that this association might be caused, in part, by at least two types of mediators (i.e., effects of AAS that could in turn influence eGFR). First, muscle breakdown stimulated by weight-lifting, especially in highly muscular AAS users, may cause kidney injury through repeated episodes of rhabdomyolysis and pigment nephropathy.[1] Second, cardiovascular dysfunction (which we demonstrated among current AAS users in our prior cardiovascular study[2]) could lead to depressed eGFR through mechanisms including reduced renal perfusion secondary to impaired forward flow and/or elevated central venous pressures or neurohormonal derangement.[3-5]

Methods

We conducted exploratory analyses of variables that might plausibly be mediators of the effect of AAS on eGFR. The first set comprised "muscle-related" variables, including body muscle mass (as assessed by fat-free mass index[6]) and creatine phosphokinase levels, which could reflect recent muscle breakdown from weightlifting.[7] The second set comprised cardiac variables, obtained in the prior cardiac study, including left ventricular ejection fraction and early left ventricular relaxation velocity.

For orientation, we have diagrammed our hypotheses related to potential mediators using a directed acyclic graph (DAG), which is a form of causal modeling that has proven useful in many epidemiologic applications.[8-11] Figure S2 provides a DAG that diagrams the plausible causal relationships between the variables of interest, highlighting the potential mediating role of muscle-related and cardiac variables.

For this DAG to be valid (that is, for it to represent accurately the statistical independencies and dependencies among the variables), it is necessary for it to satisfy three

major assumptions. The first, which applies to any mediation analysis, is that there is no confounding, either in the form of a) exposure-outcome confounding; b) exposure-mediator confounding; or c) mediator-outcome confounding (including any variable that is both an effect of exposure and a mediator-outcome confounder). These three forms of confounding are represented in the DAG. Note that technically one also needs to consider another form of mediator-outcome confounding, which is any variable that is both an effect of exposure and a mediator-outcome confounder; however, for clarity of exposition, we have not represented this form of confounding in the DAG. We have controlled for all of the above types of confounding by using the same set of variables that we used to control for exposure-outcome confounding in the main analysis. Although we have no reason from the scientific literature to suspect that there are any specific variables, other than those that we used in the main exposure-outcome analyses, that would markedly influence the mediation analyses, residual confounding always remains a threat to validity. The expected effect of most forms of residual confounding in the mediation analysis in the specific context of this application would be similar to those for the exposure-outcome association, namely to bias the results away from the null.

The second, which pertains to our choice of modeling variables in the DAG, and which also is required for our analytic method (described below), is that a variable presumed to be a mediator cannot be a cause of the candidate mediator under evaluation. In our case, this means that cardiac variables are not causes of muscle-related variables, and vice versa.

The third, which relates to our hypotheses in this case, is that there is no effect of changes in eGFR on the muscle-related or cardiac variables. This assumption likely holds for the muscle-related variables. However, it may be violated, to a greater or lesser degree, for the cardiac variables. This issue is considered in detail in the discussion below. The expected effect of any influence of eGFR on cardiac function would be to bias the estimate of the indirect effect away from the null.

Note that it is further assumed that there is no causal relationship among variables within either of the two sets of candidate mediator variables (muscle-related and cardiac) that would affect the estimates of indirect effects derived from the models below to non-negligible extent. This assumption seems reasonable, particularly given that the estimates derived for indirect effects themselves must be considered rather crude approximations.

To calculate estimates of the indirect effect related to a given mediator, we first note that the total effect of an exposure on an outcome, in the context of a mediator with which the exposure might interact, can be decomposed into four components, as described by VanderWeele (2014)[12]:

1) that due to neither mediation nor interaction, termed the *controlled direct effect*;

2) that due to just interaction but not mediation, termed the *reference interaction*;

3) that due to both mediation and interaction, termed the *mediated interaction*; and

4) that due to just mediation but not interaction, termed the *pure indirect effect*.

The first two components sum to the *direct effect*, and the second two components sum to the *indirect effect*. When there is no mediator-by-exposure interaction, then components 2 and 3 are absent. Although alternative terminology has been used in the mediation literature for some of these components, the decomposition of effects as used here is unambiguous from the mathematical representation presented below.[12-14]

As shown in the main text of this paper (Table 1), AAS users and nonusers differed markedly on muscle-related variables (body muscle mass and creatine phosphokinase), and also on cardiovascular variables (left ventricular ejection fraction and early left ventricular relaxation velocity). Given that the association between AAS use and eGFR differed by AAS subgroup, as reported above, we estimated indirect effects of both the muscle-related and cardiovascular variables separately for current users and past users.

To estimate these effects, we fitted models using "seemingly unrelated regression" (Stata command "sureg"). We fitted two (or three, in the case of considering two mediators

within the same set in the same analysis) regression models simultaneously, as described by VanderWeele and Vansteelandt (2013): 1) model(s) with the mediator(s) as the outcome, and user subgroup status (current or past) as the predictor; and 2) models with eGFR as the outcome, and mediator(s), user subgroup, and mediator-by-user subgroup interaction (if necessary). Both models included the same set of covariates using to control for confounding in the analysis of the exposure-outcome association presented in the main text.

The models can be written as:

(1a) $E [M_{(1)} | USER, COV] = \beta_{01} + \beta_{11}$ USER + $\boldsymbol{\beta_{21}}$ COV;

(1b) $E [M_{(2)} | USER, COV] = \beta_{02} + \beta_{12}$ USER + $\boldsymbol{\beta_{22}}$ COV;

(2) $E [eGFR | USER, M_{(1)}, M_{(2)}, COV] = \theta_0 + \theta_1$ USER + $\theta_2 M_{(1))} + \theta_3 M_{(2)} + \theta_4$ USER * $M_{(1)}$ + θ_5 USER * $M_{(2)}$ + $\boldsymbol{\theta_6}$ COV;

where $M_{(1)}$ are $M_{(2)}$ are two mediators within a given set (with model 1b being used when considering two mediators, and being dropped when considering only one mediator); USER is either current user or past user, depending on which sub-group of AAS users is being considered; and COV is the vector of covariates specified in the methods of the main text that represent potential confounders. Note that the interaction terms θ_4 USER * $M_{(1)} + \theta_5$ USER * $M_{(2)}$ are included in the initial model fitting, but are dropped if they fail to achieve statistical significance at alpha = 0.05, 2-tailed.

Following VanderWeele and Vansteelandt (2013), the indirect effect is calculated from the simultaneous fitting of the above models as follows:

(a) for final models without interaction terms: $\beta_{01} \theta_2 + \beta_{11} \theta_3$; and

(b) for final models with interaction terms: $\beta_{01} \theta_2 + \beta_{11} \theta_3 + \beta_{01} \theta_4 + \beta_{11} \theta_5$.

We calculated the indirect effects described above using the estimated regression coefficients from these jointly fit models for the four mediators, both individually and paired as "muscle-related" and "cardiac" sets. We estimated the mean indirect effect, along with 95% confidence intervals and nominal *P* values; we set the threshold for acceptance of an indirect

effect as worthy of consideration at a nominal *P* value of < 0.01. All models were fitted models using Stata 14.2 software (StataCorp, College Station, TX).

Before fitting final models, we performed regression diagnostics and found that the association between eGFR and cardiac variables was heavily influenced by two observations. Therefore, for mediation analyses, we rank-transformed these two variables. We also performed a sensitivity analysis excluding these two observations.

Table of Findings: Indirect Effects of Long-Term Supraphysiologic-Dose AAS Use on eGFR Mediated by Muscle and Cardiac Variables

Variable	Indirect Effect of Group (Mediated by Variable) on eGFR, mL/min/1.73m^2			
	Current AAS Users		Past AAS Users	
	Estimate (95% CI)	*P* Value*	Estimate (95% CI)	*P* Value*
Muscle Set				
Fat-free mass index	2.5 (-2.0, 7.0)	0.28	4.9 (0.2, 9.6)	0.04
Creatine phosphokinase (CK)	-2.1 (-.6.2, 2.1)	0.32	1.4 (-0.8, 3.6)	0.22
Total Muscle	-0.4 (-5.2, 6.0)	0.88	6.0 (1.0, 11.0)	0.02
Cardiac Set				
Left ventricular ejection fraction	-11.2 (-17.3, -5.8)	<0.001 †	0.3 (-2.0, 2.6)	0.80
Early left ventricular relaxation velocity (E')	-6.2 (-10.8, -1.6)	<0.009 †		
Total cardiac	-12.5 (-21.4, -4.9)	<0.001 †	0.1 (-2.4, 2.6)	0.95

AAS = anabolic-androgenic steroid; eGFR = estimated glomerular filtration rate.
* Nominal *P* value
† Exceeds threshold for acceptance (nominal *P* value <0.01)

We found that the two muscle variables, analyzed separately and as a set, showed virtually no indirect effects among the current users. Among the past users, we found a modest indirect effect, but this effect failed to meet the criterion for acceptance, and furthermore was opposite to the direction predicted (i.e., greater muscle mass and muscle breakdown was associated with increased eGFR, rather than reduced eGFR).

However, the two cardiac variables, analyzed individually and as a set, yielded high estimates for indirect effects among current users. In particular, the estimated mean indirect effect of this set was a decrease of 12.5 mL/min/1.73m^2 in eGFR, which represented 74% of the total effect of AAS use on eGFR. By contrast, among past users, the cardiac variables, whether analyzed individually and as a set, yielded very low estimates which failed to meet the criterion for acceptance. Note that the separate estimates of indirect effects provided for the individual cardiac variables should not be considered as independent from one another, in that these variables were highly correlated with each other, and their association with AAS use and with eGFR is likely due to common causal pathways.

Finally, in the sensitivity analysis regarding the two influential observations in the analysis of the cardiac set, when these two influential observations (which are also those with the lowest eGFR) are excluded, the estimates for the indirect effects of left ventricular ejection fraction, early left ventricular relaxation velocity, and the combined cardiac set are reduced by 30-33%, but all remain statistically significant.

Other Considerations

Our mediation analyses found that greater muscle breakdown, as reflected by body muscle mass and creatine phosphokinase (analyzed separately and in combination), yielded very low and statistically nonsignificant estimates for indirect effects on eGFR. By contrast, cardiac function, as reflected by left ventricular ejection fraction and early left ventricular relaxation velocity (also when analyzed separately and in combination), yielded high, statistically significant estimates for indirect effects on eGFR among the current AAS users, but not among the past AAS users, where the estimates were low and statistically nonsignificant.

Although subject to important limitations, discussed below, these findings would suggest that muscle breakdown has very little mediating effect, and hence accounts for very little of the effect of AAS on eGFR. By contrast, the findings would suggest that cardiac function might perhaps account for a substantial portion of the effect of AAS on eGFR among the current users, though not among the past users. If true, this interpretation would mean that current AAS use produces an indirect, cardiac-mediated effect on kidney function (i.e., current use leads to depressed cardiac function, which in turn leads to reduced kidney function), as well as a direct effect on the kidney, given the persistent decrease in eGFR even after subtracting any indirect cardiac effect. One might speculate that renal hypoperfusion stemming from reductions in cardiac output and/or elevations in central venous pressure, coupled with dysregulated neurohormonal "cardiorenal" interaction, may in part underlie our observations.

However, these findings and interpretations must be considered cautiously in light of several possible threats to their validity. Among these threats, as discussed in the main text of this paper, are selection bias; information bias in the form of classification and measurement error; and confounding. In addition, there are threats specific to the mediation analysis. Perhaps the greatest of these additional threats is the possibility of reverse causation – namely, that kidney function might be influencing the proposed mediator variable, rather than the mediator variable influencing kidney function. Such reverse causation would seem unlikely to be the case

for the muscle-related variables, since it seems implausible that kidney function would markedly affect creatine phosphokinase levels or body muscle mass. However, reverse causation is a more serious consideration with regard to the cardiac variables. Given the complex interplay between kidney function and heart function, in which abnormalities in either organ can lead to dysfunction in the other,[15, 16] there might be a substantial effect of kidney function on cardiac function – and if so, this would bias upward the estimated indirect effect of AAS on eGFR mediated by cardiac function. Thus, our estimates likely represent an *upper bound* for the indirect effects of cardiac function on eGFR and a *lower bound* for the direct effects of current AAS use on eGFR.

Finally, there are several other assumptions required for our proposed DAG to be valid, as enumerated in the Methods section above.

In summary, the findings from the mediation analysis, though intriguing, must be interpreted cautiously in light of the limitations above. Future longitudinal studies attempting to address these limitations will be required to assess their validity.

Works Cited

1. Sakthirajan R, Dhanapriya J, Varghese A, Saravanakumar K, Dineshkumar T, Balasubramaniyan T, Gopalakrishnan N, Abraham Kurien A. Clinical profile and outcome of pigment-induced nephropathy. *Clin Kidney J.* 2018;11(3):348-352.

2. Baggish AL, Weiner RB, Kanayama G, Hudson JI, Lu MT, Hoffmann U, Pope HG, Jr. Cardiovascular Toxicity of Illicit Anabolic-Androgenic Steroid Use. *Circulation.* 2017;135(21):1991-2002.

3. Dries DL, Exner DV, Domanski MJ, Greenberg B, Stevenson LW. The prognostic implications of renal insufficiency in asymptomatic and symptomatic patients with left ventricular systolic dysfunction. *J Am Coll Cardiol.* 2000;35(3):681-689.

4. Hillege HL, Girbes AR, de Kam PJ, Boomsma F, de Zeeuw D, Charlesworth A, Hampton JR, van Veldhuisen DJ. Renal function, neurohormonal activation, and survival in patients with chronic heart failure. *Circulation.* 2000;102(2):203-210.

5. Mahon NG, Blackstone EH, Francis GS, Starling RC, 3rd, Young JB, Lauer MS. The prognostic value of estimated creatinine clearance alongside functional capacity in ambulatory patients with chronic congestive heart failure. *J Am Coll Cardiol.* 2002;40(6):1106-1113.

6. Kouri EM, Pope HG, Jr., Katz DL, Oliva P. Fat-free mass index in users and nonusers of anabolic-androgenic steroids. *Clin J Sport Med.* 1995;5(4):223-228.

7. Bosch X, Poch E, Grau JM. Rhabdomyolysis and acute kidney injury. *N Engl J Med.* 2009;361(1):62-72.
8. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology.* 1999;10(1):37-48.
9. Hernán MA, Robins JM. *Causal inference: What If*. Boca Raton, Florida (available online at https://www.hsph.harvard.edu/miguel-hernan/causal-inference-book/) Chapman & Hall/CRC 2020.
10. Kanayama G, Pope HG, Hudson JI. Associations of anabolic-androgenic steroid use with other behavioral disorders: an analysis using directed acyclic graphs. *Psychol Med.* 2018;48(15):2601-2608.
11. Robins JM, Hernan MA, Brumback B. Marginal structural models and causal inference in epidemiology. *Epidemiology.* 2000;11(5):550-560.
12. VanderWeele TJ. A unification of mediation and interaction: a 4-way decomposition. *Epidemiology.* 2014;25(5):749-761.
13. VanderWeele TJ. Mediation Analysis: A Practitioner's Guide. *Annu Rev Public Health.* 2016;37:17-32.
14. VanderWeele TJ, Vansteelandt S. Mediation Analysis with Multiple Mediators. *Epidemiol Methods.* 2013;2(1):95-115.
15. Ronco C, Bellasi A, Di Lullo L. Cardiorenal Syndrome: An Overview. *Adv Chronic Kidney Dis.* 2018;25(5):382-390.
16. Bock JS, Gottlieb SS. Cardiorenal syndrome: new perspectives. *Circulation.* 2010;121(23):2592-2600.