Plasma Neuronal Growth Regulator 1 May Link Physical Activity to Reduced Risk of Type 2 Diabetes: A Proteome-Wide Study of ARIC Participants

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Habitual physical activity (PA) impacts the plasma proteome and reduces the risk of developing type 2 diabetes (T2D). Using a large-scale proteome-wide approach in Atherosclerosis Risk in Communities study participants, we aimed to identify plasma proteins associated with PA and determine which of these may be causally related to lower T2D risk. PA was associated with 92 plasma proteins in discovery ($P < 1.01 \times 10^{-5}$), and 40 remained significant in replication ($P < 5.43 \times 10^{-4}$). Eighteen of these proteins were independently associated with incident T2D ($P < 1.25 \times 10^{-3}$), including neuronal growth regulator 1 (NeGR1; hazard ratio per SD 0.85; $P = 7.5 \times 10^{-11}$). Two-sample Mendelian randomization (MR) inverse variance weighted analysis indicated that higher NeGR1 reduces T2D risk (odds ratio [OR] per SD 0.92; $P = 0.03$) and was consistent with MR-Egger, weighted median, and weighted mode sensitivity analyses. A stronger association was observed for the single *cis*-acting NeGR1 genetic variant (OR per SD 0.80; $P = 6.3 \times 10^{-5}$). Coupled with previous evidence that low circulating NeGR1 levels promote adiposity, its association with PA and potential causal role in T2D shown here suggest that NeGR1 may link PA exposure with metabolic outcomes. Further research is warranted to confirm our findings and examine the interplay of PA, NeGR1, adiposity, and metabolic health.

ARTICLE HIGHLIGHTS

- Physical activity alters tissue and plasma proteomes, which have been shown to have roles in type 2 diabetes development.
- We aimed to identify protein signatures through which physical activity may influence type 2 diabetes pathogenesis.
- Of 40 proteins associated with physical activity, 18 of these were further related to incident type 2 diabetes over an ∼24-year follow-up.
- Two-sample Mendelian randomization analysis indicated that circulating neuronal growth regulator 1 reduces risk of type 2 diabetes.
- These findings suggest that plasma neuronal growth regulator 1 may link physical activity to reduced risk of type 2 diabetes.

Physical activity (PA) is well-established for reducing risks of developing chronic diseases such as obesity and type 2 diabetes (T2D) (1). Physiologically, PA promotes insulin

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sensitivity, enhances pancreatic β-cell function, suppresses adipogenesis, reduces both oxidative burden and biomarkers of cellular senescence, and mobilizes ectopic, visceral, and subcutaneous adipose tissue stores (2–4). And yet, our understanding of the protein architecture through which PA reduces metabolic dysfunction remains incomplete.

To date, observational and intervention proteomics studies have identified circulating proteins related to or otherwise induced by PA. Creatine kinases, endothelial adhesion molecules, tumor necrosis factor, growth factors, and fatty acid-binding proteins have been found to be associated with PA (5). Aerobic PA induces differential patterns of circulating protein expression (6), indicating effects on glucose signaling, cellular stress signaling, wound healing, and apoptosis (7). Despite evidence that PA affects the plasma proteome and corresponding cardiometabolic pathways, studies have been limited in their sample sizes or numbers of interrogated proteins. Critically, which proteins may protect against the development of metabolic disease remains unclear. This study aimed to identify proteins associated with PA that may reduce T2D risk in the large community-based Atherosclerosis Risk in Communities (ARIC) study.

RESEARCH DESIGN AND METHODS

Study Population

ARIC has been described in detail previously (8). Among four U.S. communities, 15,792 male and female participants aged 45–64 were recruited. Risk factor information was obtained at the baseline (visit 1; 1987–89) and numerous follow-up examinations: visit 2 (1990–92), visit 3 (1993–95), visit 4 (1996–98), visit 5 (2011–13), visit 6 (2016–17), visit 7 (2018–19), visit 8 (2020), and visit 9 (2021–22). All participants provided written informed consent, and Institutional Review Boards at each site approved the study protocol.

Sport and Exercise PA Assessment

For this analysis, visit 3 was the first visit at which proteomics and PA measures were both assessed and served as the baseline. The sport and exercise division of the well-validated Baecke Physical Activity Questionnaire was used to estimate physical activity (9,10).

Proteomics Measurement

Measurement of plasma proteins was performed at the SomaLogic laboratory using a modified DNA aptamer array (SomaScan version 4.0; SomaLogic, Boulder, CO), as previously described (11,12).

Covariates

Demographics were collected by questionnaires at visit 1. Current medication use, alcohol consumption, smoking status, drinking status, and alcohol intake were self-reported at multiple visits, including visit 3. BMI and waist-to-hip ratio were obtained using standard protocols at all visits. Prevalent coronary heart disease or stroke before visit 1 was based on self-report; thereafter, events were adjudicated by the ARIC Morbidity and Mortality Classification Committee. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation (mL/min/1.73 m^2), as described previously (13).

Diabetes Ascertainment

Incident T2D was determined by self-reported physician diagnosis or self-reported use of diabetes medications at visit 4 and in follow-up telephone interviews, as previously described (14).

Statistical Analysis

The visit 3 examination was conducted in 12,887 ARIC participants. Those missing proteomics data (n = 1,416), PA data (n = 96), covariate data (n = 700), or of unknown smoking status (n = 2), and non-Black or non-White individuals (n = 29) were excluded, providing a sample of 10,644 participants. Participants were randomly assigned to a discovery (n = 7,093) or replication sample (n = 3,551) (see Supplementary Fig. 1).

Multiple linear regression analysis was used to test associations between the PA exposure (per unit Baecke score) and protein outcomes (per SD) at visit 3. ANOVA determined statistical significance. Protein values outside of 6 SDs of their respective means were excluded, and adjustments were made for potential confounders measured at visit 3, including age, sex, race group, field center, education status, smoking status, alcohol drinking status, self-reported alcohol intake, BMI, waist-to-hip ratio, prevalent coronary heart disease, stroke, and T2D, use of blood pressure- and cholesterol-lowering medication, and eGFR. Bonferroni corrections were applied to the discovery sample (4,955 protein measures stipulated a significance threshold of $P < 1.01 \times 10^{-5}$) and to the replication sample (92 protein measures stipulated a significance threshold of $P < 5.43 \times 10^{-4}$).

Further analyses were performed to determine whether the 40 PA-associated proteins confirmed in the internal replication sample were related to risk of incident T2D in the entire visit 3 cohort. The final sample size of 6,039 is shown in Supplementary Fig. 1. Cox regression estimated associations between proteins identified in the primary analysis with risk of incident T2D over a median 24-year follow-up (119,633 person-years), adjusting for age, sex, field center, race group, PA, cigarette smoking status, drinking status, alcohol intake, BMI, waist-to-hip ratio, hypertension and cholesterol medication use, prevalent coronary heart disease and stroke, and eGFR at visit 3. A multiple testing threshold of $P < 1.25 \times 10^{-3}$ was applied.

Two-Sample Mendelian Randomization

Two-sample Mendelian randomization (MR) was performed to determine whether identified proteins may be causally

related to T2D by using the online MR-Base platform (15) and is described in the Supplementary Material.

Data and Resource Availability

The data that support the findings of this study are available from the ARIC Coordinating Center, but restrictions apply to the availability of these data, which were used under license for the current study and therefore are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of the ARIC Coordinating Center.

RESULTS

Discovery and replication sample characteristics were found to be balanced, as shown in Supplementary Table 1. In the discovery analysis, 92 plasma proteins were significantly associated with PA independent of multiple covariates after Bonferroni correction ($P < 1.01 \times 10^{-5}$) (Fig. 1*A*). In the replication analysis of 3,551 participants, 40 of 92 proteins remained significantly associated with PA ($P < 5.43 \times 10^{-4}$), shown in Fig. 1*B*. Estimates and CIs are shown in Supplementary Tables 2 and 3.

Of the 40 PA-associated proteins that internally replicated, 18 were significantly associated with risk of incident T2D ($P < 1.25 \times 10^{-3}$) over a median 24-year follow-up among 6,039 ARIC participants (n = 2,259 cases). Hazard ratios (HRs) and 95% CIs for the 10 most strongly associated proteins are shown in Fig. 2; all associations are presented in Supplementary Table 4. Of these, NeGR1 (per SD) was found to be inversely associated with T2D (HR 0.85; 95% CI 0.81–0.90; $P = 7.8 \times 10^{-11}$). A sensitivity analysis restricting to the first 10 years of follow-up was performed (n = 978 cases), and results are shown in Supplementary Table 5. Findings were consistent with those of the 24-year follow-up, although magnitudes of association were incrementally larger over the 10-year follow-up for most proteins (e.g., NeGR1 showed a stronger association with lower risk of incident T2D; HR 0.79; 95% CI 0.74–0.85; $P = 2.8 \times 10^{-10}$).

Two-sample MR analyses were conducted for the 10 proteins most strongly associated with risk of incident T2D. Results for inverse variance-weighted (IVW), MR-Egger, weighted median, weighted mode sensitivity analyses, and horizontal pleiotropy tests, as well as F statistics for genetic instruments, are shown in Table 1. Weak instrument biases were not evident based on F statistics (all $F > 10$). Results of tests for horizontal pleiotropy for all protein genetic instruments were nonsignificant.

The genetic instrument for NeGR1 comprised six protein quantitative trait loci (pQTLs) and was related to T2D based on estimates from IVW (odds ratio [OR] per SD 0.92; P = 0.03), weighted median (OR per SD 0.87; P = 0.02), and weighted mode analyses (OR per SD 0.84; P = 0.02). MR-Egger results were borderline (OR per SD 0.64; P = 0.09), likely due to the lower statistical power of the test. A single *cis*-acting pQTL for NeGR1, rs1026566, was tested and showed a stronger association with T2D than the six pQTL instrument (OR per SD 0.80; $P = 6.3 \times 10^{-5}$). The horizontal pleiotropy test for NeGR1 was null (P = 0.12).

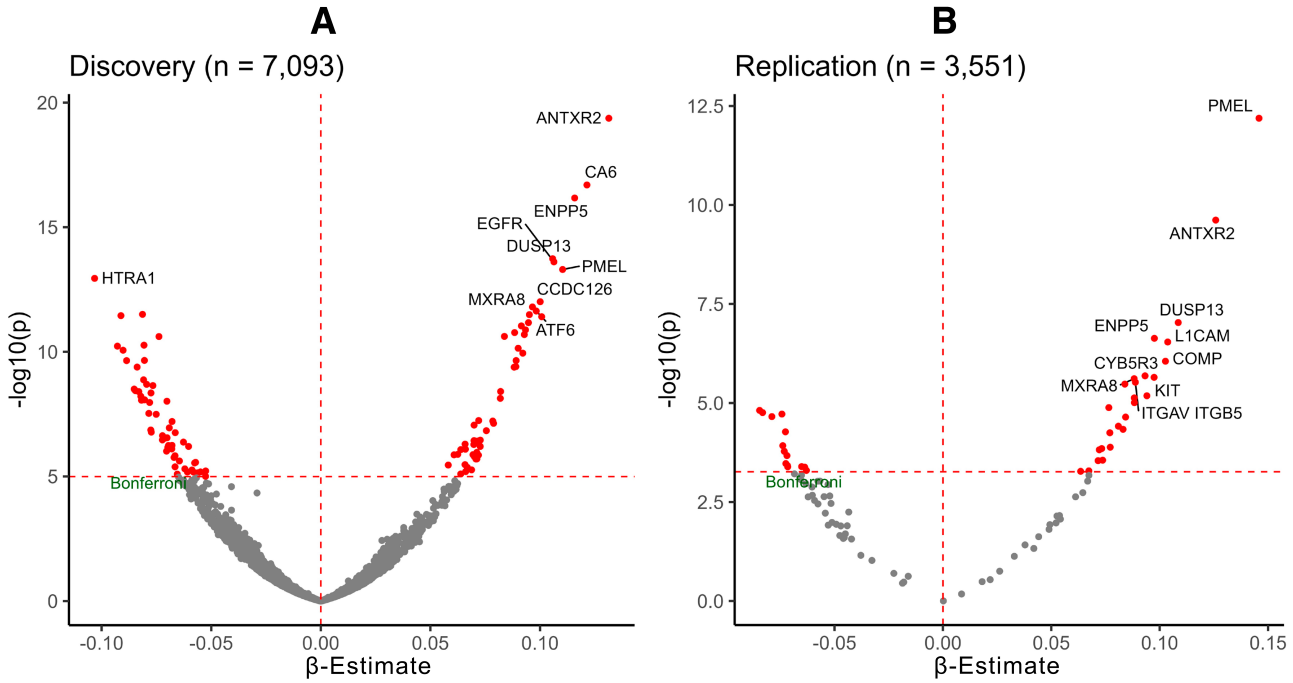

Figure 1—Volcano plots showing cross-sectional analysis of physical activity and plasma proteins in discovery (*A*) and internal replication (*B*) samples.

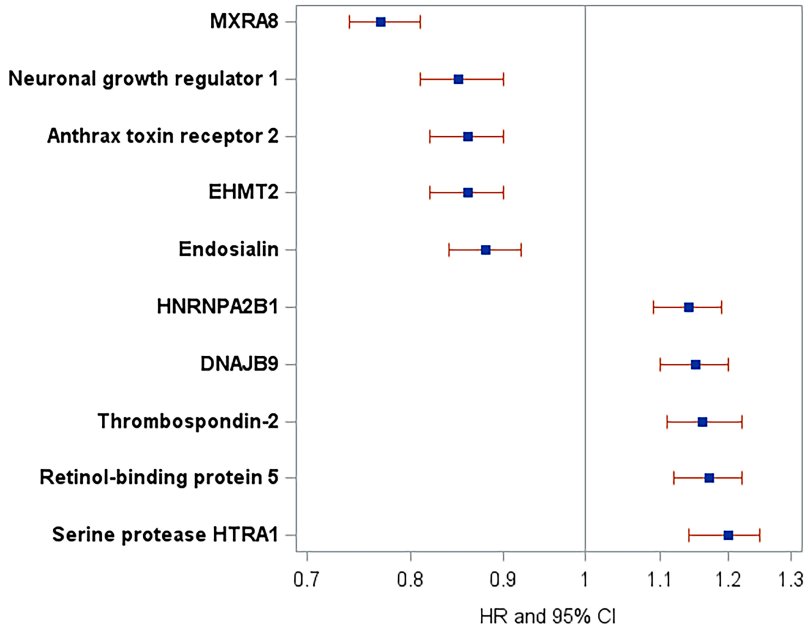

Figure 2—Estimated HRs and 95% CIs for the top 10 plasma proteins (per SD) associated with incident T2D in 6,039 ARIC participants (n = 2,259 for cases). Results for all 40 PA-associated plasma proteins ($P < 5.4 \times 10$) are shown in Supplementary Table 4. DNAJB9, DnaJ homolog subfamily B member; EHMT2, histone-lysine *N*-methyltransferase; HNRNPA2B1, heterogeneous nuclear ribonucleoproteins A2/B1; MXRA8, matrix remodeling-associated protein 8.

To further examine NeGR1, participant characteristics are shown across NeGR1 quintiles in Supplementary Table 6. Significantly lower proportions of Black participants, current smokers, and individuals taking blood pressure and cholesterol medication were evident across successive quintiles of plasma NeGR1 concentrations. Compared with participants in the referent, those in the top quintile showed considerably lower mean BMI (-3 kg/m^2) and fasting glucose (-15 mg/dL) as well as a 54% lower proportion of individuals with prevalent T2D.

DISCUSSION

Among a cohort of >10,000 participants, habitual PA was associated with 92 proteins in a cross-sectional discovery analysis. In the internal replication, 40 associations remained significant, and 18 were subsequently shown to be related to T2D risk over median 24-year follow-up. Two-sample MR analysis identified higher plasma NeGR1 as a causal protective factor against T2D pathogenesis.

NeGR1 May Link PA and Adiposity, T2D Risk

It is well established that PA reduces risks of obesity and T2D and that obesity increases risk of T2D. Our findings may provide a proteomic signature linking these phenomena when considered with previous evidence. Specifically, experimental studies have demonstrated that genetic deficiency in NeGR1 promotes greater white adipose tissue stores and higher plasma glucose and insulin levels (16). By contrast, adenoviral-mediated suppression of NeGR1 increases body weight in rodents independent of food intake (17). Genome-wide association studies have further identified variants in *NEGR1* associated with BMI and obesity (18,19), suggesting that the *NEGR1* gene is one of many genetic determinants of adiposity. In the context of this study, the higher levels of NeGR1 associated with PA shown here may negatively influence BMI and, consequently, risk of T2D (Fig. 3*A*). And yet, greater adiposity has also been shown to reduce the secretion of NeGR1, which then contributes to disruptions in metabolic homeostasis (20). If true in humans, then greater adiposity is also an upstream regulator and suppressor of NeGR1 (Fig. 3*B*).

Our findings are consistent with a combination of Figs. 3*A* and *B*, represented in Fig. 3*C*. Plasma NeGR1 was inversely associated with incident T2D independent of BMI and waist-to-hip ratio (HR per SD 0.85; $P = 3.3 \times 10^{-11}$) (Fig. 1). Removing BMI and waist-to-hip ratio from the regression model resulted in a 40% increase in the magnitude of this association (HR per SD 0.79; $P = 1.4 \times 10^{-22}$). Taken together, NeGR1 may serve as a link between PA and BMI and BMI with T2D (Fig. 3*C*).

Our two-sample MR analysis showed that genetically higher NeGR1 levels (per SD) correspond to an 8% to 16% lower risk of T2D (Table 1), corresponding to the IVW (P = 0.03) and weighted mode estimates (P = 0.02), respectively. In addition, the single *cis*-acting pQTL for NeGR1 (rs1026566) showed a stronger association and may represent a more accurate effect size of NeGR1 on T2D, since the pQTL is located on the *NEGR1* gene and is therefore less susceptible to horizontal pleiotropy (21). Given the previous evidence that

Table 1—Two-sample MR analyses of PA-related plasma proteins most significantly associated with T2D in secondary analysis

			IVW or Wald		MR-Egger		Weighted median	
Protein	pQTL (*n*)	*F* statistic	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
NEGR1	6	540.7	0.92 (0.86, 0.99)	3.4E−02	0.64 (0.43, 0.94)	8.8E−02	0.87 (0.78, 0.98)	1.7E−02
NEGR1 (*cis*)	1	250.1	0.80 (0.72, 0.89)	6.3E−05	—	—	—	—
MXRA8	8	456.6	1.15 (1.06, 1.25)	9.5E−04	0.36 (0.12, 1.07)	1.2E−01	0.95 (0.84, 1.07)	4.2E−01
ANTXR2	12	1,118.2	1.10 (1.04, 1.16)	5.5E−04	0.98 (0.63, 1.54)	9.4E−01	1.08 (1.00, 1.17)	4.1E−02
HTRA1	12	307.7	1.44 (1.30, 1.59)	6.3E−12	0.49 (0.03, 8.00)	6.2E−01	1.14 (0.96, 1.37)	1.4E−01
EHMT2	8	1,013.1	1.03 (0.97, 1.09)	3.4E−01	1.07 (0.95, 1.20)	3.2E−01	1.03 (0.99, 1.07)	9.8E−02
CD248	9	440.1	0.94 (0.86, 1.03)	1.7E−01	1.06 (0.56, 1.99)	8.7E−01	0.96 (0.84, 1.09)	5.0E−01
THBS2	4	107.6	0.98 (0.78, 1.22)	8.6E−01	1.12 (0.25, 4.93)	9.0E−01	0.93 (0.75, 1.17)	5.4E−01
RBP5	3	288.9	1.07 (0.94, 1.23)	3.2E−01	0.55 (0.21, 1.45)	4.4E−01	1.00 (0.85, 1.16)	9.5E−01
HNRNPA2B1	3	159.7	1.06 (0.89, 1.27)	5.2E−01	0.58 (0.15, 2.20)	5.7E−01	1.06 (0.87, 1.30)	5.7E−01
DNAJB9	2	49.8	1.11 (0.79, 1.55)	5.5E−01	—	—	—	—

	Weighted mode		Horizontal pleiotropy test		
	OR (95% CI)	*P* value	Intercept	SE	*P* value
NEGR1	0.84 (0.76, 0.93)	1.8E−02	0.034	0.017	1.2E−01
NEGR1 (*cis*)	—	—	—	—	—
MXRA8	0.93 (0.83, 1.03)	2.1E−01	0.087	0.041	7.6E−02
HTRA1	1.18 (0.95, 1.47)	1.7E−01	0.052	0.068	4.6E−01
EHMT2	1.03 (1.00, 1.07)	1.2E−01	−0.008	0.007	2.6E−01
CD248	1.02 (0.81, 1.28)	9.0E−01	−0.008	0.021	7.2E−01
THBS2	0.86 (0.61, 1.21)	4.4E−01	−0.007	0.042	8.8E−01
RBP5	0.96 (0.82, 1.12)	6.8E−01	0.046	0.033	4.0E−01
HNRNPA2B1	1.00 (0.79, 1.26)	9.9E−01	0.034	0.038	5.4E−01
DNAJB9	—	—	—	—	—

genetic variants of *NEGR1* are related to BMI, we independently examined associations of BMI with both our main genetic instrument and the *cis*-acting pQTL (Supplementary Table 7). The significant associations with BMI denote the presence of vertical or horizontal pleiotropy. Whereas the former would not affect the validity of the MR results, the latter is a potential confounder. This potential for confounding must be balanced with the nonsignificant horizontal pleiotropy test for the main genetic instrument ($P = 0.12$) (Table 1) and the possibility that BMI serves as a partial mediator between NeGR1 and T2D.

Strengths and Limitations

This study used an aptamer array in a large cohort study, allowing for the interrogation of thousands of proteins and a second stage of confirmatory replication. Statistically conservative Bonferroni corrections for multiple comparisons were applied in the primary and secondary analyses. This typically reduces type I errors but likely inflated type II errors. The two-sample MR analysis was performed using the large deCODE Health Study cohort and a meta-analysis of three large genome-wide association studies, which provided precise estimates of pQTL-protein and pQTL-outcome associations, respectively. In terms of limitations, self-reported PA is known for measurement error (22). In addition, some aptamer measurements deviate from those of mass spectrometry or immunobased assays (23,24); however, weakly correlated aptamer measures would be expected to bias associations toward the null (25). Further validation of aptamer measurements is warranted.

Conclusions

Of the dozens of PA-associated circulating proteins identified and confirmed in this study, 18 were related to incident T2D, and two-sample MR showed that NeGR1 may be a protective factor against T2D. When considered with experimental and genetic evidence, NeGR1 levels may link PA with both BMI and T2D risk. Additional research is warranted to confirm our findings and further examine the mechanistic underpinnings of PA, NeGR1, adiposity, and metabolic health.

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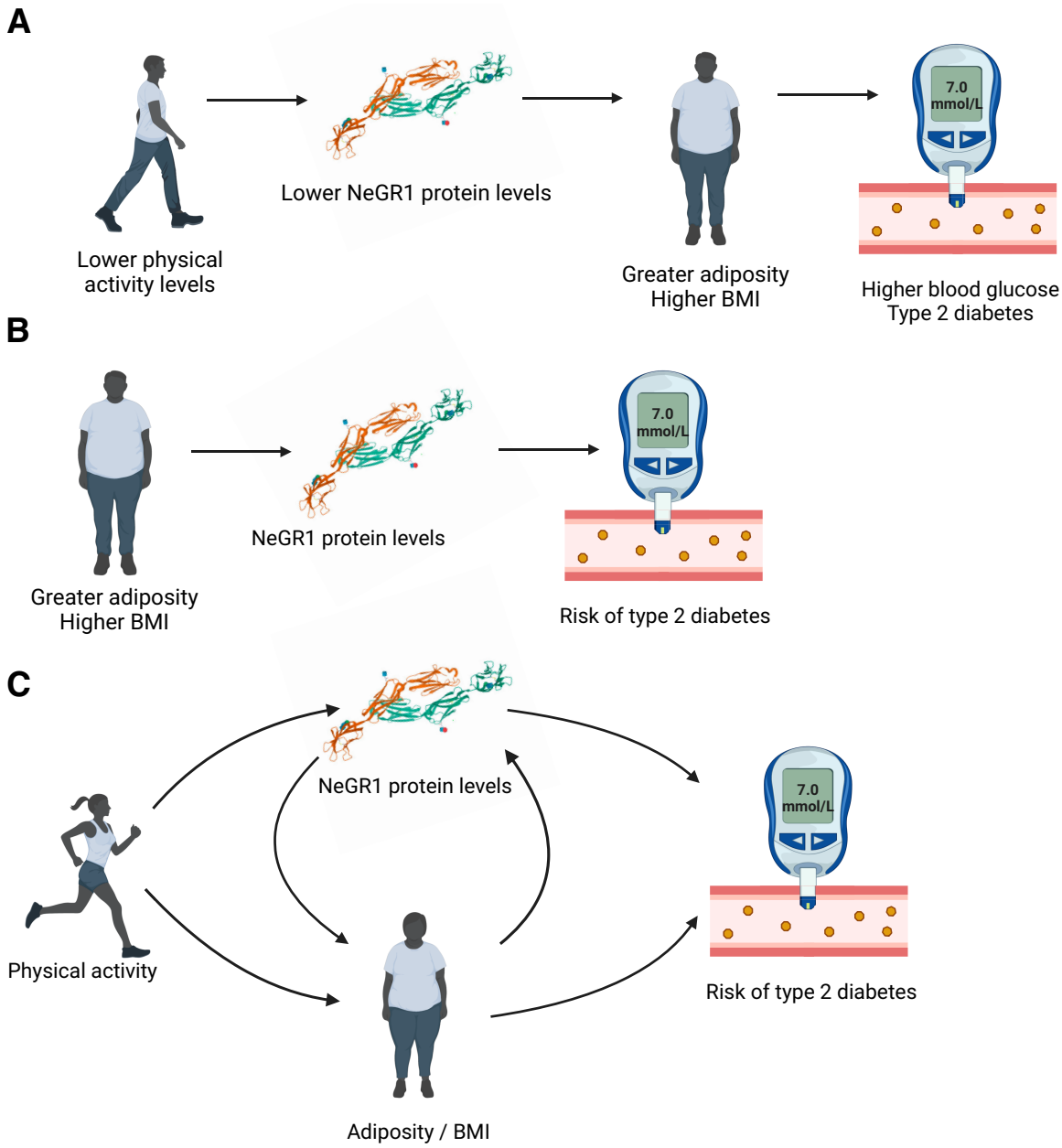

Figure 3—*A*: Lower PA levels are associated with lower NeGR1; low NeGR1 has been shown to promote adiposity, which promotes T2D. *B*: Greater adiposity has been shown to reduce NeGR1 secretion, which promotes risk of T2D. *C*: Physical activity is independently related to both NeGR1 levels and adiposity, and these appear to influence one another; NeGR1 and adiposity are both independently and causally related to type 2 diabetes. Created with BioRender.com.

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manuscript. J.S.P., W.T., M.R.R., R.T.D., P.L.L., W.G., K.P.G., P.P., E.D.M., and M.A.P. critically revised manuscript content and made important intellectual contributions. All authors approved the final version for publication. M.A.P. is the guarantor of this work, and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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