

## Pancreatic $\beta$ -cell function increases in a linear dose-response manner following exercise training in adults with prediabetes

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<sup>1</sup>Department of Pathobiology, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio; <sup>2</sup>Centre of Inflammation and Metabolism, Rigshospitalet, and Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark;

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**Malin SK, Solomon TP, Blaszcak A, Finnegan S, Filion J, Kirwan JP.** Pancreatic  $\beta$ -cell function increases in a linear dose-response manner following exercise training in adults with prediabetes. *Am J Physiol Endocrinol Metab* 305: E1248–E1254, 2013. First published September 17, 2013; doi:10.1152/ajpendo.00260.2013.—Although some studies suggest that a linear dose-response relationship exists between exercise and insulin sensitivity, the exercise dose required to enhance pancreatic  $\beta$ -cell function is unknown. Thirty-five older obese adults with prediabetes underwent a progressive 12-wk supervised exercise intervention (5 days/wk for 60 min at  $\sim 85\%$  HR<sub>max</sub>). Insulin and C-peptide responses to an OGTT were used to define the first- and second-phase disposition index (DI;  $\beta$ -cell function = glucose-stimulated insulin secretion  $\times$  clamp-derived insulin sensitivity). Maximum oxygen consumption ( $\dot{V}O_{2\max}$ ) and body composition (dual-energy X-ray absorptiometry and computed tomography) were also measured before and after the intervention. Exercise dose was computed using  $\dot{V}O_2$ /heart-rate derived linear regression equations. Subjects expended  $474.5 \pm 8.8$  kcal/session ( $2,372.5 \pm 44.1$  kcal/wk) during the intervention and lost  $\sim 8\%$  body weight. Exercise increased first- and second-phase DI ( $P < 0.05$ ), and these changes in DI were linearly related to exercise dose (DI<sub>first phase</sub>:  $r = 0.54$ ,  $P < 0.001$ ; DI<sub>second phase</sub>:  $r = 0.56$ ,  $P = 0.0005$ ). Enhanced DI was also associated with increased  $\dot{V}O_{2\max}$  (DI<sub>first phase</sub>:  $r = 0.36$ ,  $P = 0.04$ ; DI<sub>second phase</sub>:  $r = 0.41$ ,  $P < 0.02$ ) but not lower body fat (DI<sub>first phase</sub>:  $r = -0.21$ ,  $P = 0.25$ ; DI<sub>second phase</sub>:  $r = -0.30$ ,  $P = 0.10$ ) after training. Low baseline DI predicted an increase in DI after the intervention (DI<sub>first phase</sub>:  $r = -0.37$ ; DI<sub>second phase</sub>:  $r = -0.41$ , each  $P < 0.04$ ). Thus, exercise training plus weight loss increased pancreatic  $\beta$ -cell function in a linear dose-response manner in adults with prediabetes. Our data suggest that higher exercise doses (i.e.,  $>2,000$  kcal/wk) are necessary to enhance  $\beta$ -cell function in adults with poor insulin secretion capacity.

aging; obesity; insulin resistance; glucose intolerance; type 2 diabetes

APPROXIMATELY 79 MILLION MEN AND WOMEN in the US are characterized as having prediabetes, of which up to 30% may develop type 2 diabetes in the next 10 years (1, 3). Although insulin sensitivity is considered a key etiological factor in the progression from prediabetes to type 2 diabetes, many insulin-resistant men and women maintain normoglycemia due to compensatory rises in insulin secretion (17). Thus, preservation of pancreatic  $\beta$ -cell function in response to glucose is fundamental to preventing type 2 diabetes, particularly in obese individuals with impaired glucose tolerance that have lost  $\sim 50$ – $70\%$  of their  $\beta$ -cell function (7). Unfortunately, the

optimal exercise prescription for enhancing pancreatic  $\beta$ -cell function is unknown.

Exercise training decreases glucose-stimulated insulin secretion in young (19), middle-aged (9, 33) and older men and women (4, 21). However, the amount of insulin needed to maintain normoglycemia is influenced by the prevailing level of insulin sensitivity. As such, the product of glucose-stimulated insulin secretion and insulin sensitivity [i.e., disposition index (DI)] may provide a more accurate view of pancreatic  $\beta$ -cell function (16). In fact, the DI is considered a better predictor of diabetes development than insulin sensitivity alone (2, 37).  $\beta$ -Cell function is viewed as a two-pool model that is characterized by the readily available pool of insulin that is released upon initial glucose stimulation (1st phase) and the synthesis of new insulin following fluctuations in postprandial glucose (2nd phase) (5). Contrasting studies suggest that moderate- (33) to high-volume (6) exercise augments first-phase pancreatic  $\beta$ -cell function. However, these studies did not characterize second-phase pancreatic function, thereby limiting our understanding of the efficacy of exercise dose on  $\beta$ -cell function across the postprandial period in men and women (6, 33). It has been suggested that, in addition to adequate first-phase pancreatic  $\beta$ -cell function, second-phase pancreatic  $\beta$ -cell function is clinically relevant for maintaining glycemic control (18). Consequently, there is a need to better understand the link between exercise dose and pancreatic  $\beta$ -cell function across the postprandial glucose time frame so that optimal lifestyle programs may be designed for type 2 diabetes prevention. Animal work suggests that exercise enhances GLUT2 transporter content (20), improves Akt signaling and glucokinase activity (10, 20, 22), and raises mitochondrial respiration in pancreatic tissue (10). Despite reports emphasizing that high doses of physical activity are needed to improve insulin sensitivity (6, 11, 24) and cardiometabolic biomarkers (e.g., total cholesterol and/or blood pressure) (13), no intervention has determined the interaction between exercise dose and  $\beta$ -cell function. Therefore, the purpose of this study was to determine the relationship between exercise dose and pancreatic  $\beta$ -cell function in men and women with prediabetes. We hypothesized that higher exercise doses would correlate with increased first- and second-phase  $\beta$ -cell function.

### METHODS

**Subjects.** Thirty-five older ( $66.8 \pm 0.8$  yr) obese (BMI =  $35.1 \pm 0.7$  kg/m<sup>2</sup>) men ( $n = 16$ ) and women ( $n = 19$ ) who were previously involved in obesity-related lifestyle modification studies were included in this study (31). Subjects were recruited via advertisements

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in the Cleveland, OH, area. All subjects underwent health screenings that included a resting and exercise stress test with 12-lead electrocardiogram, medical history, and physical examination as well as blood and urine chemistry analysis. Subjects were excluded if they smoked, were physically active ( $>60$  min/wk), were weight unstable ( $>2$  kg within the previous 6 mo), had known chronic disease (i.e., renal, liver, heart, etc.), and/or were taking medications known to affect glucose homeostasis. All subjects provided written signed and verbal informed consent, and the Cleveland Clinic Institutional Review Board approved the study.

**Metabolic control period.** Subjects underwent a 3-day inpatient stay in our Clinical Research Unit prior to metabolic assessments. Subjects were provided weight maintenance meals [resting metabolic rate  $\times 1.2$ ;  $\sim 55\%$  carbohydrate (CHO), 30% fat, and 15% protein] and refrained from strenuous activity before testing. Respiratory gases ( $\dot{V}O_2$  and  $\dot{V}CO_2$ ) were analyzed by indirect calorimetry for determination of resting metabolic rate and substrate oxidation ( $V_{max}$  Encore, Viasys, Yorba Linda, CA) before and after training, as described previously (35).

**Body composition and cardiometabolic risk factors.** Weight was assessed on a digital platform with minimal clothing, and height was recorded on a stadiometer. Computerized tomography (Siemens Medical Solutions, Malvern, PA) was used to determine total abdominal fat and visceral adipose tissue (31). Dual X-ray absorptiometry (Lunar Prodigy, Madison, WI) was used to quantify total body fat and fat-free mass. Blood pressure was obtained after a 10-min rest period, and fasting blood triglyceride and cholesterol levels were obtained. Maximal oxygen consumption ( $\dot{V}O_{2max}$ ) was determined (Jaeger Oxycon Pro; Viasys) during a treadmill exercise test according to standard criteria (35), and maximal heart rate ( $HR_{max}$ ) was used to prescribe exercise intensity.

**Exercise training.** Subjects participated in a supervised aerobic exercise program 5 days/wk at 60–65% of  $HR_{max}$  for the first 4 wk. Thereafter, the exercise intensity was increased and maintained at 80–85%  $HR_{max}$ . Subjects exercised for 50–60 min, with a 10-min warmup and cooldown.  $\dot{V}O_{2max}$  was repeated at weeks 4 and 8 to ensure that the appropriate exercise intensity was maintained throughout training. Subjects also met weekly with a dietitian to assess proper nutrient intake (i.e.,  $\sim 55\%$  CHO, 30% fat, and 15% protein). Food records were averaged over a 3-day period before and during the last week of the intervention to determine total caloric and macronutrient intake.

**Exercise dose.** Linear regression equations of  $\dot{V}O_2$  as a function of HR were performed at four distinct time points using week 0, 4, 8, and 12  $HR_{max}$  tests. These regression equations allowed us to compute the average  $\dot{V}O_2$  in liters per minute for each training session based on the average HR obtained at that particular session. The average oxygen uptake was then converted into energy units (1 liter of  $O_2 = 5$  kcal) to obtain the energy expended per minute for each session (27). This energy expenditure value was summed for each exercise session performed over the 12-wk program and then averaged, and the product of average kilocalories per minute and the duration of the exercise bout enabled us to calculate the kilocalories expended per session (i.e., exercise dose). These were then summed to obtain the caloric expenditure per week (i.e., exercise volume). To account for changes in energy expenditure as a function of improved cardiovascular fitness, we applied week 0, 4, 8, and 12  $\dot{V}O_{2max}$  regression equations to weeks 1–3, 4–7, 8–10, and 11–12, respectively. This approach allowed us to account for individual differences in response to exercise. The exercise dose difference between individuals is thus due to their initial fitness and training-induced improvements in fitness.

**Insulin sensitivity.** Subjects underwent a 120-min euglycemic hyperinsulinemic clamp after an overnight fast. A primed, constant infusion ( $40$  mU $\cdot$ m $^{-2}$  $\cdot$ min $^{-1}$ ) of insulin was administered via catheters placed in an antecubital vein. Glucose (20%) was infused at a variable rate to maintain plasma glucose at 90 mg/dl (34). Insulin sensitivity

was defined as the average glucose infusion rate during the final 30 min of the clamp divided by ambient insulin concentrations.

**Pancreatic  $\beta$ -cell function.** After an overnight fast, a 75-g oral glucose tolerance test (OGTT) was performed. Blood samples were obtained from an antecubital vein at 0, 30, 60, 90, and 120 min for the determination of glucose, insulin, and C-peptide concentrations. Area under the curve during the OGTT was calculated using the trapezoidal method. First- and second-phase glucose-stimulated insulin secretion (GSIS) was calculated by dividing plasma insulin by glucose area under the curve during the first 30 and last 60 min of the OGTT. The first- and second-phase DI was used to characterize pancreatic  $\beta$ -cell function and calculated as GSIS  $\times$  insulin sensitivity. C-peptide was also used in place of insulin to more accurately reflect prehepatic insulin secretion. Because of limited sample volume and available sample with aprotinin, C-peptide analysis was performed in 23 subjects. Hepatic extraction was also estimated as insulin divided by C-peptide area under the curve during the OGTT (15).

**Biochemical analysis.** All samples were centrifuged at 1,000 rpm for 10 min at 4°C and stored at  $-80^\circ\text{C}$  until analysis. Plasma glucose was collected in a lithium-heparin vacutainer, and samples were measured using a glucose oxidase assay (YSI 2300 STAT Plus; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and C-peptide were collected in vacutainers containing EDTA and the protease inhibitor aprotinin, and samples were analyzed using a radioimmunoassay (Millipore, Billerica, MA). Plasma triglycerides and cholesterol were analyzed using enzymatic methods with an automated platform (Roche Modular Diagnostics, Indianapolis, IN).

**Statistical analysis.** Data were analyzed using the statistical program R (Leopard build 64-bit; The R Foundation, Vienna, Austria). Pre- and postintervention data were compared using paired two-tailed *t*-tests. As part of secondary analysis to generalize our findings, we report male and female responses to exercise training. Differences (post – pre) between men and women after training were compared using unpaired two-tailed *t*-tests. Bivariate linear regression analysis was used to determine associations between study outcomes. Insulin sensitivity and GSIS were also used as covariates using multivariate regression analysis to adjust for their influence on the association between exercise dose and DI. Significance was accepted as  $P < 0.05$ , and data are reported as means  $\pm$  SE.

## RESULTS

**Subject and exercise characteristics.** Prior to the intervention, men and women were on average obese, dyslipidemic, prehypertensive, and glucose intolerant (Table 1). Subjects reduced total food intake ( $1,868.2 \pm 59.0$  vs.  $1,676.7 \pm 61.3$  kcal/day,  $P < 0.02$ ), which was attributable mainly to lower carbohydrate ( $254.9 \pm 7.4$  vs.  $234.9 \pm 9.2$  g/day,  $P < 0.01$ ) and fat ( $61.0 \pm 2.2$  vs.  $52.7 \pm 2.7$  g/day,  $P < 0.02$ ), since protein did not change statistically ( $76.7 \pm 2.6$  vs.  $71.7 \pm 2.8$  g/day,  $P = 0.06$ ). Subjects expended  $\sim 474.5 \pm 8.8$  kcal/session (men =  $481.6 \pm 16.2$  vs. women =  $468.4 \pm 9.0$ ,  $P = 0.48$ ) during the intervention. The average exercise intensity was  $7.9 \pm 0.1$  kcal/min (men =  $8.0 \pm 0.3$  vs. women =  $7.8 \pm 0.2$ ;  $P = 0.48$ ), with  $2,372.5 \pm 44.1$  kcal expended over the 5-day training week (men =  $2,408.3 \pm 81.1$  vs. women =  $2,342.3 \pm 45.1$ ,  $P = 0.48$ ).

**Responses to exercise.** Exercise training reduced total body fat and weight by  $\sim 6$ – $8$  kg ( $P < 0.001$ ; Table 1) and increased cardiovascular fitness by 12% in men and women ( $P < 0.0001$ ). There was also an increase in basal fat oxidation ( $0.77 \pm 0.08$  vs.  $0.99 \pm 0.09$  mg $\cdot$ kg fat-free mass $^{-1}$  $\cdot$ min $^{-1}$ ,  $P = 0.02$ ) despite no change in resting metabolic rate after the intervention ( $1,548 \pm 52.7$  vs.  $1,549.6 \pm 44.1$  kcal/day,  $P = 0.96$ ). There was no

Table 1. Metabolic characteristics before and after exercise training

Variable	Group Baseline (n = 35)	Group, $\Delta$	Male Baseline (n = 16)	Males, $\Delta$	Female Baseline (n = 19)	Females, $\Delta$
Age, yr	66.8 $\pm$ 0.8		65.0 $\pm$ 1.9		65.9 $\pm$ 0.9	
Body weight, kg	99.0 $\pm$ 2.4	-8.1 $\pm$ 0.7**	96.3 $\pm$ 3.4	-7.9 $\pm$ 0.7**	93.7 $\pm$ 3.0	-5.9 $\pm$ 0.6***
BMI, kg/m <sup>2</sup>	35.1 $\pm$ 0.7	-2.8 $\pm$ 0.2**	34.2 $\pm$ 1.1	-2.7 $\pm$ 0.2**	35.8 $\pm$ 1.1	-2.3 $\pm$ 0.2***
Body fat, %	43.0 $\pm$ 1.3	-3.8 $\pm$ 0.6**	42.0 $\pm$ 1.7	-3.6 $\pm$ 0.6**	48.2 $\pm$ 1.4 <sup>^</sup>	-2.1 $\pm$ 0.5***
Fat mass, kg	42.5 $\pm$ 1.6	-6.7 $\pm$ 0.7**	41.5 $\pm$ 1.9	-6.3 $\pm$ 0.7**	45.2 $\pm$ 2.3	-4.7 $\pm$ 0.7***
FFM, kg	56.4 $\pm$ 2.0	-1.2 $\pm$ 0.4**	54.8 $\pm$ 2.5	-1.2 $\pm$ 0.4*	48.1 $\pm$ 1.9 <sup>^</sup>	-1.2 $\pm$ 0.4*
Total Abdominal fat, cm <sup>3</sup>	612.6 $\pm$ 22.7	-109.7 $\pm$ 12.4**	596.8 $\pm$ 27.1	-105.7 $\pm$ 12.2	646.2 $\pm$ 27.5	-97.1 $\pm$ 19.2
Visceral fat, cm <sup>3</sup>	151.4 $\pm$ 14.0	-30.6 $\pm$ 6.5**	147.0 $\pm$ 13.7	-29.3 $\pm$ 6.2**	132.8 $\pm$ 3.9	-20.9 $\pm$ 3.9**
$\dot{V}O_{2max}$ , ml/min	2,080.8 $\pm$ 85.9	263.6 $\pm$ 44.5**	2,319.2 $\pm$ 99.9	255.7 $\pm$ 42.5**	1,761.9 $\pm$ 70.9 <sup>^</sup>	209.9 $\pm$ 48.5**
$\dot{V}O_{2max}$ , ml·kg <sup>-1</sup> ·min <sup>-1</sup>	37.8 $\pm$ 1.6	5.0 $\pm$ 1.0**	34.5 $\pm$ 1.9	5.4 $\pm$ 0.8**	34.8 $\pm$ 2.7	5.3 $\pm$ 1.4**
Total cholesterol, mg/dl	204.7 $\pm$ 4.8	-25.0 $\pm$ 3.8**	204.6 $\pm$ 6.7	-36.3 $\pm$ 5.3**	204.8 $\pm$ 7.2	-15.0 $\pm$ 4.4***
Triglycerides, mg/dl	160.6 $\pm$ 12.0	-43.7 $\pm$ 9.4**	166.8 $\pm$ 18.1	-62.7 $\pm$ 15.3**	155.1 $\pm$ 16.3	-26.8 $\pm$ 10.4**
HDL cholesterol, mg/dl	46.3 $\pm$ 2.5	-1.4 $\pm$ 0.8	43.0 $\pm$ 2.9	0.8 $\pm$ 1.8	50.3 $\pm$ 4.1	-5.6 $\pm$ 0.8***
Systolic BP, mmHg	133.0 $\pm$ 3.2	-13.1 $\pm$ 2.5**	134.8 $\pm$ 4.8	-14.7 $\pm$ 3.6**	131.0 $\pm$ 4.9	-14.4 $\pm$ 11.4**
Diastolic BP, mmHg	79.3 $\pm$ 2.1	-6.5 $\pm$ 1.9**	79.6 $\pm$ 3.1	-6.7 $\pm$ 2.7*	78.9 $\pm$ 2.8	-6.9 $\pm$ 2.8*

Data are reported as means  $\pm$  SE. BMI, body mass index; FFM, fat-free mass; BP, blood pressure.  $\Delta$  = change between post- and pretraining. Clamp-derived insulin sensitivity was defined as GIR/I. To convert total cholesterol and HDL mg/dl to mM, divide by 38.67. To convert triglyceride mg/dl to mM, divide by 88.57. \* $P$  < 0.05 and \*\* $P$  < 0.01 compared with baseline; <sup>^</sup> $P$  < 0.05 compared with males.

difference between men and women in fat oxidation or resting metabolic rate following the intervention (data not shown).

**OGTT and pancreatic  $\beta$ -cell function.** Exercise training enhanced clamp-derived insulin sensitivity ( $P$  < 0.0001; Table 2), and reduced plasma glucose in response to the OGTT (Fig. 1). Although exercise decreased circulating insulin after exercise training (Fig. 2), C-peptides showed only subtle reductions, particularly during the first 30 min of the OGTT (Fig. 3). Overall, there were no sex differences in response to exercise training for blood glucose, insulin, or C-peptide (Table 2).

Hepatic extraction was higher in response to the OGTT after the intervention (1st phase: 0.07  $\pm$  0.01 vs. 0.09  $\pm$  0.01 ng·ml<sup>-1</sup>· $\mu$ U<sup>-1</sup>·ml<sup>-1</sup>, 30 min; 2nd phase: 0.08  $\pm$  0.01 vs. 0.11  $\pm$  0.01 ng·ml<sup>-1</sup>· $\mu$ U<sup>-1</sup>·ml<sup>-1</sup>, 60 min;  $P$  < 0.05) for combined groups. There were no differences in hepatic extraction between men and women (data not shown). Nevertheless, in general, exercise decreased GSIS (Table 2). When GSIS was corrected for changes in ambient insulin sensitivity, first- and second-phase DI (i.e.,  $\beta$ -cell function) were elevated after training ( $P$  < 0.05; Table 2).

Table 2. Glucose homeostasis before and after exercise training

Variable	Group Baseline	Group, $\Delta$	Male Baseline	Males, $\Delta$	Female Baseline	Females, $\Delta$
Glucose AUC <sub>0-30</sub>	-327.5 $\pm$ 76.5	-327 $\pm$ 76.5**	3,759.8 $\pm$ 70.7	-231.1 $\pm$ 101.1*	4,294.1 $\pm$ 94.8 <sup>^</sup>	-408.7 $\pm$ 111.4*
Glucose AUC <sub>60-120</sub>	10,659.9 $\pm$ 402.6	-766.4 $\pm$ 310.3**	9,380.7 $\pm$ 475.6	-301.3 $\pm$ 393.6	11,737.1 $\pm$ 514.0 <sup>^</sup>	-1,158.1 $\pm$ 455.7*
Glucose AUC <sub>0-120</sub>	20,036.5 $\pm$ 591.8	-1,535.9 $\pm$ 446.0**	17,854.3 $\pm$ 651.6	-668.2 $\pm$ 556.2	21,874.1 $\pm$ 714.4 <sup>^</sup>	-2,266.5 $\pm$ 640.5*
Insulin AUC <sub>0-30</sub>	1,642.5 $\pm$ 210.9	-614.6 $\pm$ 0.6**	1,735.8 $\pm$ 384.5	-726.5 $\pm$ 232.7 <sup>^^</sup>	1,564.0 $\pm$ 224.4	-520.4 $\pm$ 183.5*
Insulin AUC <sub>60-120</sub>	8,008.4 $\pm$ 1,186.0	-3,324.8 $\pm$ 905.2**	7,184.5 $\pm$ 1,225.2	-2,620.0 $\pm$ 737.0 <sup>^^</sup>	8,702.3 $\pm$ 1,944.3	-3,918.4 $\pm$ 1,533.0* <sup>^</sup>
Insulin AUC <sub>0-120</sub>	13,018.3 $\pm$ 1,720.0	-5,315.0 $\pm$ 1,201.3**	1,226.0 $\pm$ 1,993.7	-4,726.9 $\pm$ 1,024.3 <sup>^^</sup>	13,685.4 $\pm$ 2,729.6	-5,810.2 $\pm$ 2,062.8*
C-peptide AUC <sub>0-30</sub>	117.5 $\pm$ 16.6	-25.0 $\pm$ 10.4**	112.4 $\pm$ 20.5	-26.4 $\pm$ 14.8	122.5 $\pm$ 27.0	-23.7 $\pm$ 15.7
C-peptide AUC <sub>60-120</sub>	538.5 $\pm$ 72.6	-60.5 $\pm$ 53.4	515.1 $\pm$ 68.0	-52.0 $\pm$ 65.2	561.9 $\pm$ 131.9	-69.1 $\pm$ 87.9
C-peptide AUC <sub>0-120</sub>	1,078.03 $\pm$ 72.6	-127.0 $\pm$ 97.5	1,032.4 $\pm$ 136.8	-132.4 $\pm$ 123.2	1,123.5 $\pm$ 252.6	-121.6 $\pm$ 157.4
First-phase						
INS <sub>0-30</sub> /GLC <sub>0-30</sub>	0.41 $\pm$ 0.05	-0.14 $\pm$ 0.04**	0.46 $\pm$ 0.10	-0.18 $\pm$ 0.06*	0.37 $\pm$ 0.05	-0.10 $\pm$ 0.05*
Second-phase						
INS <sub>60-120</sub> /GLC <sub>60-120</sub>	0.77 $\pm$ 0.11	-0.30 $\pm$ 0.08**	0.77 $\pm$ 0.13	-0.28 $\pm$ 0.09*	0.78 $\pm$ 0.18	-0.30 $\pm$ 0.13*
First-phase C-peptide <sub>0-30</sub> /GLC <sub>0-30</sub>	0.030 $\pm$ 0.004	0.03 $\pm$ 0.04	0.03 $\pm$ 0.01	-1.58 $\pm$ 0.08	0.03 $\pm$ 0.01	-1.64 $\pm$ 0.10
Second-phase						
C-peptide <sub>60-120</sub> /GLC <sub>60-120</sub>	0.056 $\pm$ 0.007	-0.006 $\pm$ 0.005	0.06 $\pm$ 0.01	-0.007 $\pm$ 0.006	0.05 $\pm$ 0.01	-0.003 $\pm$ 0.007
First-phase DI (INS)	0.008 $\pm$ 0.001	0.002 $\pm$ 0.001**	0.008 $\pm$ 0.001	0.002 $\pm$ 0.001*	0.008 $\pm$ 0.001	0.001 $\pm$ 0.001
Second-phase DI (INS)	0.015 $\pm$ 0.002	0.003 $\pm$ 0.001**	0.014 $\pm$ 0.001	0.003 $\pm$ 0.001*	0.013 $\pm$ 0.002	0.002 $\pm$ 0.002
First-phase DI (C-peptide)	0.001 $\pm$ 0.000	0.0004 $\pm$ 0.0001**	0.001 $\pm$ 0.001	0.0004 $\pm$ 0.0001*	0.002 $\pm$ 0.001	0.0002 $\pm$ 0.0001 <sup>†</sup>
Second-phase DI (C-peptide)	0.001 $\pm$ 0.000	0.001 $\pm$ 0.000**	0.001 $\pm$ 0.001	0.002 $\pm$ 0.001**	0.001 $\pm$ 0.001	0.002 $\pm$ 0.003* <sup>‡</sup>
Insulin sensitivity	0.026 $\pm$ 0.002	0.019 $\pm$ 0.002**	0.025 $\pm$ 0.004	0.025 $\pm$ 0.004**	0.026 $\pm$ 0.003	0.014 $\pm$ 0.002***

Data are reported as means  $\pm$  SE. AUC, area under the curve; INS, insulin; GLC, glucose; DI, disposition index. First- and second-phase glucose-stimulated insulin secretion (GSIS) calculations were derived from a 75-g oral glucose tolerance test. GSIS derived from AUC insulin [ $\mu$ U·ml<sup>-1</sup>·mg<sup>-1</sup>·dl, 30 (or 60) min] and C-peptide [ng·ml<sup>-1</sup>·mg<sup>-1</sup>·dl, 30 (or 60) min] blood measurements. Insulin sensitivity was defined as GIR/I from the euglycemic hyperinsulinemic clamp (mg·kg<sup>-1</sup>·min<sup>-1</sup> per  $\mu$ U/ml). To convert glucose mg/dl to mM, divide by 18. To convert insulin  $\mu$ U/ml to pM, multiply by 6. To convert C-peptide ng/ml to nM, multiply by 0.333.  $\beta$ -Cell function was determined using GSIS and C-peptide secretion multiplied by clamp-derived insulin sensitivity (i.e., DI). <sup>†</sup> $P$  = 0.07, \* $P$  < 0.05, and \*\* $P$  < 0.01 compared with baseline <sup>‡</sup>( $P$  = 0.07); <sup>^</sup> $P$  < 0.05 and <sup>^^</sup> $P$  < 0.01 compared with males.

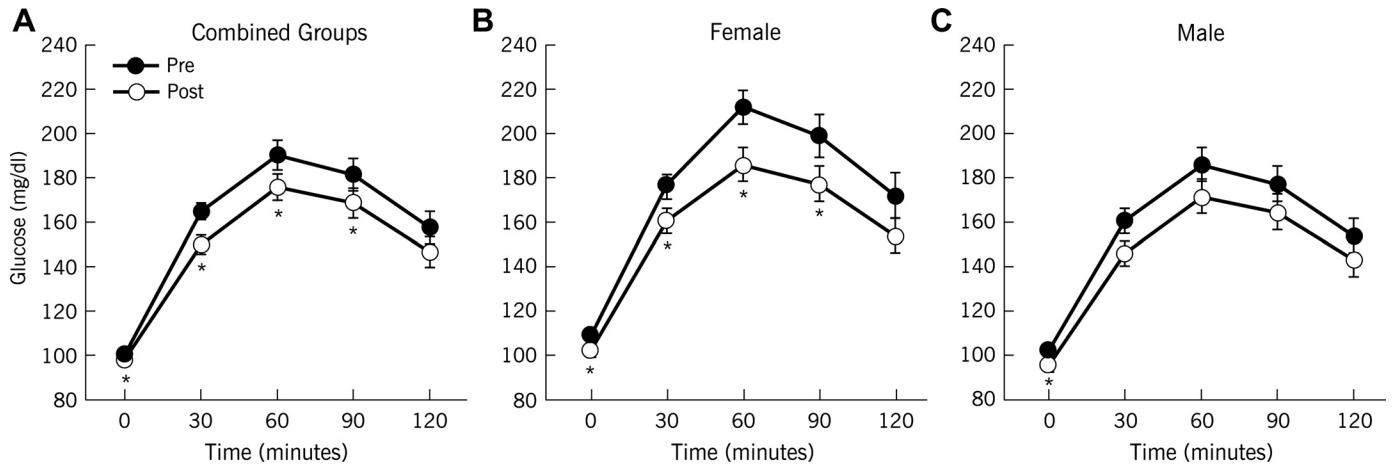


Fig. 1. Effect of exercise training with weight loss on plasma glucose responses. Data are means  $\pm$  SE. Pre, pretraining; post, posttraining. \* $P < 0.05$  compared with pretraining.

**Correlational analysis.** First- and second-phase DI were both linearly associated with exercise dose (Fig. 4). The insulin- and C-peptide-derived calculations of DI were correlated at baseline (DI<sub>first phase</sub>:  $r = 0.68$ ,  $P < 0.001$ ; DI<sub>second phase</sub>:  $r = 0.61$ ,  $P < 0.001$ , respectively) and after the intervention (DI<sub>first phase</sub>:  $r = 0.78$ ,  $P < 0.00001$ ; DI<sub>second phase</sub>:  $r = 0.68$ ,  $P = 0.0006$ , respectively). Thus, all remaining correlations were analyzed using the insulin-derived DI to minimize type 1 and type 2 statistical errors. Baseline DI was inversely correlated with enhanced DI after exercise (DI<sub>first phase</sub>:  $r = -0.37$ ,  $P = 0.04$ ; DI<sub>second phase</sub>:  $r = -0.41$ ,  $P = 0.02$ ). Increased DI was also positively correlated with increased  $\dot{V}O_{2\max}$  after training (DI<sub>first phase</sub>:  $r = 0.36$ ,  $P = 0.04$ ; DI<sub>second phase</sub>:  $r = 0.41$ ,  $P = 0.02$ ). However, the change in DI did not correlate with reductions in body fat (DI<sub>first phase</sub>:  $r = -0.21$ ,  $P = 0.25$ ; DI<sub>second phase</sub>:  $r = -0.30$ ,  $P = 0.10$ ) or visceral adiposity (DI<sub>first phase</sub>:  $r = 0.06$ ,  $P = 0.73$ ; DI<sub>second phase</sub>:  $r = 0.12$ ,  $P = 0.50$ ) after the intervention. Lower fasting glucose after training was associated with elevated first-phase DI ( $r = -0.39$ ,  $P = 0.02$ ) and increased fat oxidation ( $r = 0.41$ ,  $P = 0.01$ ) but not insulin sensitivity ( $r = -0.16$ ,  $P = 0.33$ ) or second-phase DI ( $r = -0.17$ ,  $P = 0.33$ ) after training. Multiple linear regression analysis for first-phase DI indicated that insulin

sensitivity (estimate =  $1.6 \times 10^{-1}$ ; SE =  $4.6 \times 10^{-2}$ ;  $t$ -value = 3.5,  $P = 0.001$ ), GSIS (1st phase: estimate =  $9.4 \times 10^{-3}$ , SE =  $2.6 \times 10^{-3}$ ,  $t$ -value = 3.5,  $P = 0.001$ ), and exercise dose (estimate =  $2.9 \times 10^{-5}$ , SE =  $1.3 \times 10^{-5}$ ,  $t$ -value = 2.2,  $P = 0.03$ ) were all significant predictors of  $\beta$ -cell function. For second-phase DI, the results indicated that insulin sensitivity (estimate =  $2.0 \times 10^{-1}$ , SE =  $7.7 \times 10^{-2}$ ,  $t$ -value = 2.6,  $P = 0.01$ ) and GSIS (2nd phase: estimate =  $1.2 \times 10^{-2}$ , SE =  $3.3 \times 10^{-3}$ ,  $t$ -value = 3.6,  $P = 0.001$ ) were significant predictors of  $\beta$ -cell function, whereas exercise dose trended toward significance (estimate =  $3.6 \times 10^{-5}$ , SE =  $2.2 \times 10^{-5}$ ,  $t$ -value = 1.6,  $P = 0.10$ ).

## DISCUSSION

The major finding from this study is that exercise training with modest energy reduction increased both first- and second-phase pancreatic  $\beta$ -cell function, calculated using both plasma insulin and C-peptide, in a linear dose-response manner in men and women with prediabetes. Our data are consistent with Davis et al. (6), who reported that high-dose compared with low-dose exercise enhanced first-phase  $\beta$ -cell function in overweight children. However, Slentz et al. (33) reported increased

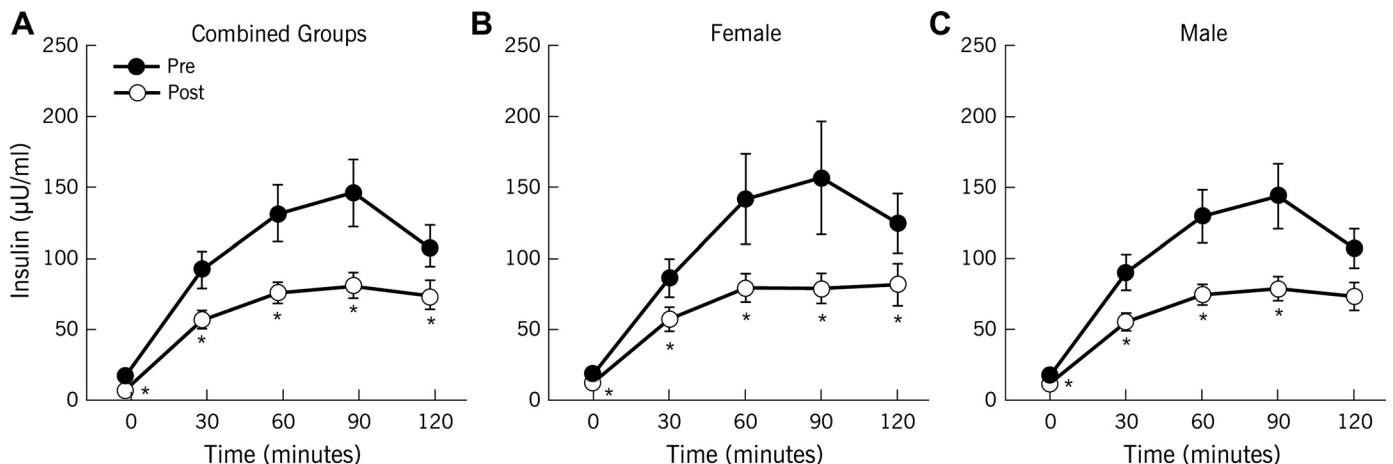


Fig. 2. Effect of exercise training with weight loss on plasma insulin responses. Data are means  $\pm$  SE. \* $P < 0.05$  compared with pretraining.

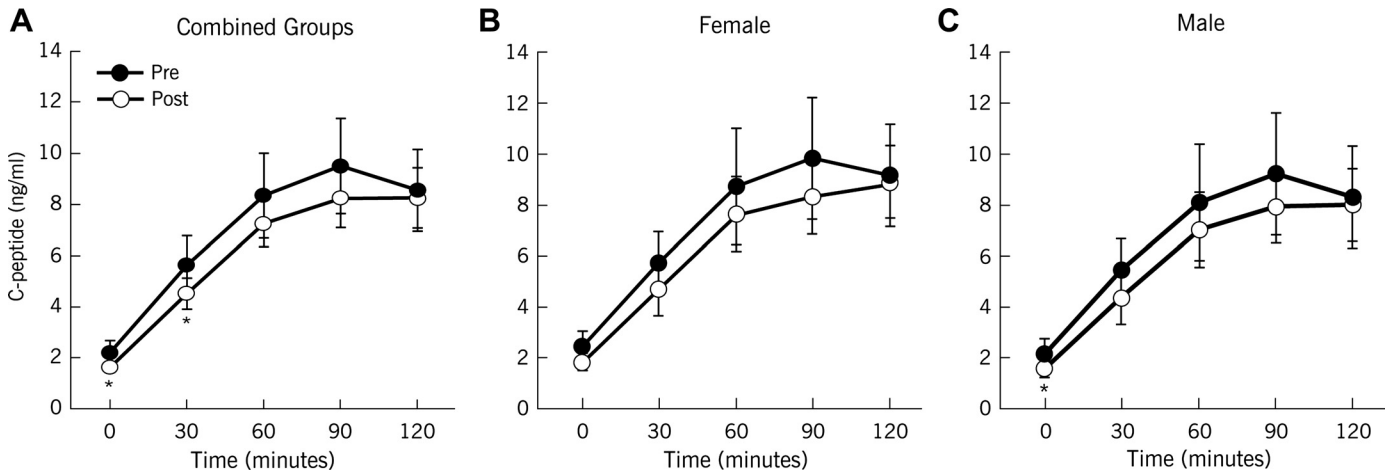


Fig. 3. Effect of exercise training with weight loss on plasma C-peptide responses. Data are means  $\pm$  SE. Note that C-peptide analysis included 23 of the 35 subjects. \* $P < 0.05$  compared with pretraining.

first-phase  $\beta$ -cell function after moderate compared with high-volume exercise in middle-aged, overweight, normoglycemic adults. The inconsistency in the literature regarding exercise dose and pancreatic function may be related to methodology. Our data and the report by Davis et al. (6) used the OGTT to characterize  $\beta$ -cell function, whereas the study by Slentz et al. (33) used the intravenous glucose tolerance test. Pancreatic

function derived from intravenous glucose tolerance test is dependent on  $\beta$ -cell glucose responsiveness and the readily available release of insulin, whereas the OGTT additionally characterizes the processing and synthesis of new insulin as well as evoking incretin and neural-mediated effects on the pancreas (19). Thus, these different methodological approaches are likely to have contributed to the somewhat different quan-

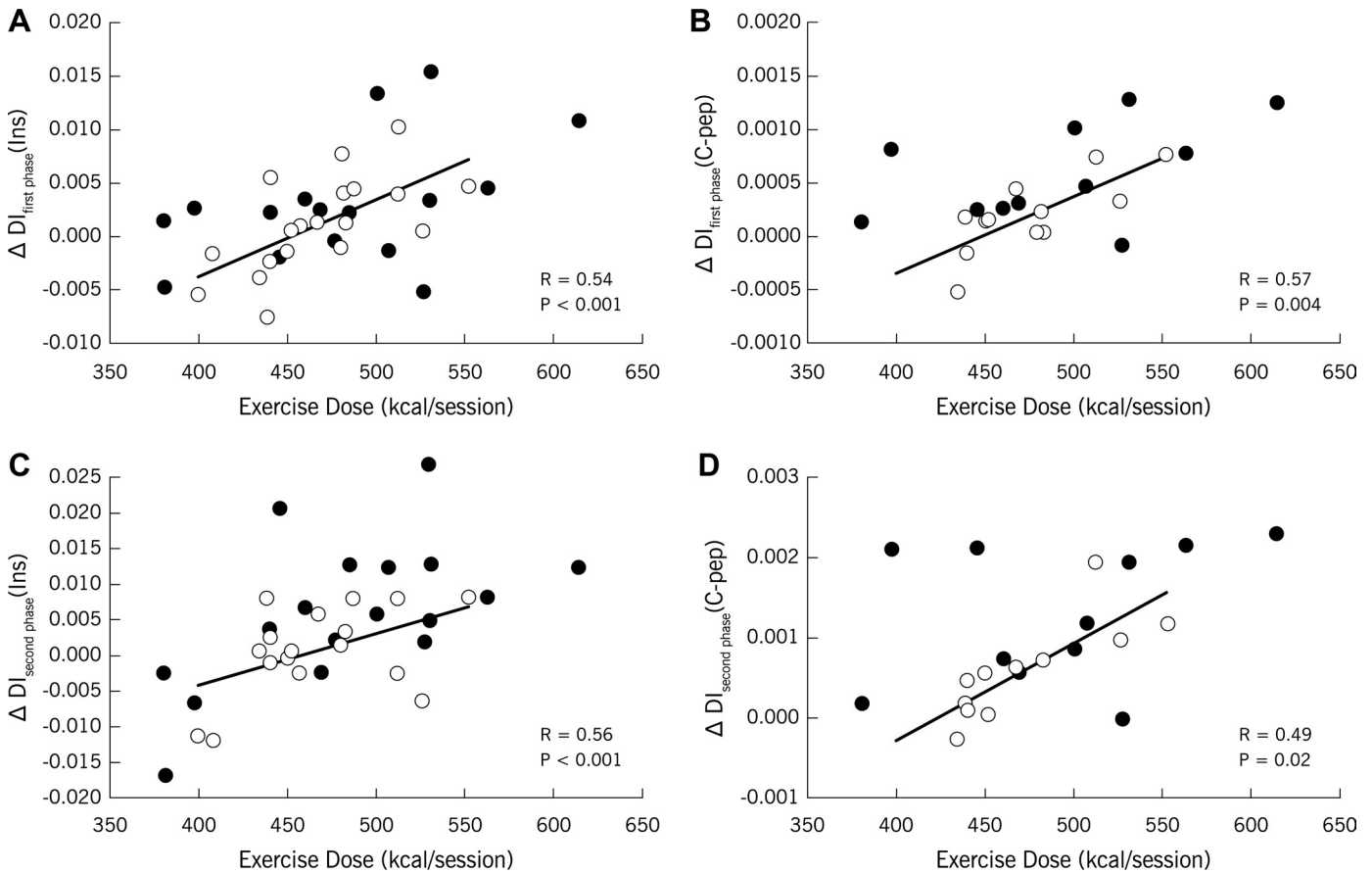


Fig. 4. Correlations between the change ( $\Delta$ ) in 1st- and 2nd-phase disposition index (DI; or  $\beta$ -cell function) derived from insulin (A and C) and C-peptide (B and D) with exercise energy expenditure (kcal/session). Average kcal/min during each exercise session was averaged over the 12-wk intervention and was used to calculate the kcal expended/session (i.e., exercise dose).  $\circ$ , Women;  $\bullet$ , men.

titative insulin responses and hence, contrasting conclusions regarding the effectiveness of lifestyle modification programs, including exercise, on  $\beta$ -cell function.

High fasting and postprandial glucose concentrations are linked to the deterioration of first- and second-phase  $\beta$ -cell function, respectively (18). Consequently, improving both first- and second-phase  $\beta$ -cell function may be necessary to optimally manage hyperglycemia. Exercise increases  $\beta$ -cell function in adults with type 2 diabetes, whereas it reduces compensatory insulin secretion in normal glucose-tolerant individuals (8, 34). However, there are limited data examining the impact of exercise on pancreatic  $\beta$ -cell function in adults with prediabetes (4). We observed a significant inverse association between low-baseline  $\beta$ -cell function and a rise in  $\beta$ -cell function after our intervention. These data are consistent with the view that individuals with poor insulin secretion at the start of a lifestyle intervention are likely to respond by increasing  $\beta$ -cell function. Circulating insulin is influenced by whole body insulin sensitivity and the capacity to secrete insulin. Thus, it is not possible to properly evaluate GSIS from circulating insulin during an OGTT without taking insulin sensitivity into account. The DI was developed (2, 5, 16) as the product of both GSIS and insulin sensitivity and serves as an index of  $\beta$ -cell function that reflects the integrated capacity for whole body glucose disposal. In our study, although insulin sensitivity likely contributed to improvements in  $\beta$ -cell function, neither insulin sensitivity nor GSIS alone correlated with reductions in circulating glucose levels. Thus, the improvement in DI may be a more clinically relevant determinant of glycemic control when exercise is used as an intervention (2, 37). Moreover, the improvement in  $\beta$ -cell function is specific to the postprandial period, as there were clear reductions in fasting insulin and C-peptide concentrations (Fig. 2 and 3). Taken together, our findings suggest that some exercise is better than none for glycemic control, and increasing doses of exercise appear to be important for enhancing  $\beta$ -cell function in adults with poor insulin secretion capacity (11).

The relationship between exercise dose and pancreatic function may be linked to obesity (17, 28). It is reasonable to expect that individuals expending more kilocalories during exercise would have greater reductions in body fat, which in turn, would lead to lower blood lipids and/or inflammatory markers that impair  $\beta$ -cell function (36, 38). However, we did not observe a direct association between reductions in body fat and  $\beta$ -cell function, suggesting that the improvement in  $\beta$ -cell function after exercise and a modest reduction in calorie intake may be independent of changes in fat mass. Alternatively, higher exercise doses were linked to increased  $\dot{V}O_{2\max}$ , and this enhanced fitness was directly associated with  $\beta$ -cell function. We did not design this study to determine the exact mechanism by which  $\dot{V}O_{2\max}$  contributes to higher  $\beta$ -cell function, but previous work in rodents suggests that exercise enhances GLUT2 transporter content (20), improves Akt signaling and glucokinase activity (10, 20, 22), and raises mitochondrial respiration (10). Consistent with improved mitochondrial capacity, we did observe a significant increase in fat oxidation after our intervention. Thus, we speculate that the greater energy expenditure from fat after exercise reduces lipotoxicity in the pancreas and improves glucose regulation (28, 30, 32). It is worth noting, however, that we detected a significant correlation between improvements in first-phase  $\beta$ -cell function and

reductions in fasting hyperglycemia. This association is consistent with work highlighting that glucotoxicity is a factor impairing insulin secretion (32). Thus, it is possible that improvements in glucolipotoxicity contributed to improvements in  $\beta$ -cell function. Finally, we recognize that insulin-resistant muscle may secrete myokines that affect  $\beta$ -cell mass and/or secretion (12, 26). As a result, it remains possible that exercise training altered the release of an unknown myokine and improved the cross-talk between skeletal muscle and the pancreas to elevate  $\beta$ -cell function (14). Whether other organs such as the liver or adipose tissue secrete hormones that modulate pancreatic function awaits further investigation.

Hepatic extraction is a potential factor influencing circulating insulin (15, 23, 29). In our study, exercise reduced postprandial plasma insulin to a larger extent than C-peptide concentrations. This difference is likely related to hepatic extraction and suggests that our insulin-derived  $\beta$ -cell function calculations may underestimate insulin secretion. However, plasma insulin was significantly associated with C-peptide-derived calculations of  $\beta$ -cell function. Thus, our findings suggest that plasma insulin provides a reasonable estimate of  $\beta$ -cell function. Another factor to consider is that the number of kilocalories expended during exercise in this study was within a narrow range (i.e., ~400–600 kcal/session). Thus, it is possible that expending more or less kilocalories (e.g., 900 or 200 kcal/session) during each exercise session could yield different results (24). Although subjects were counseled to maintain nutritional intake throughout the study, we observed less food intake during the intervention. Therefore, caloric restriction in addition to energy expenditure may have contributed to improvements in  $\beta$ -cell function. However, we did not detect any relationship between changes in body fat and  $\beta$ -cell function, and this suggests that weight loss via energy deficit is unlikely to be the primary mechanism leading to improved  $\beta$ -cell function after lifestyle modification. Taken together, our data suggest that future work is needed to determine the most time-efficient exercise strategy to maximize energy expenditure, as this appears to be an important factor for improving  $\beta$ -cell function.

In conclusion, our findings suggest that exercise interventions expending >2,000 kcal/wk increase pancreatic  $\beta$ -cell function in a linear dose-response manner in men and women. This observation is clinically relevant since current health guidelines recommend that men and women must expend between 1,000 and 2,000 kcal/wk to receive cardiometabolic benefit. Because glucose-intolerant adults appear to have blunted responses to exercise interventions (25), it would seem appropriate to tailor exercise intensity, duration, and mode to maximize energy expenditure for optimal glucoregulatory effects.

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#### DISCLOSURES

The authors report no conflicts of interest, financial or otherwise.

#### AUTHOR CONTRIBUTIONS

S.K.M. and J.P.K. contributed to the conception and design of the research; S.K.M., T.P.S., A.B., S.F., J.F., and J.P.K. performed the experiments; S.K.M.,

T.P.S., A.B., and S.F. analyzed the data; S.K.M., T.P.S., A.B., S.F., and J.P.K. interpreted the results of the experiments; S.K.M. prepared the figures; S.K.M. drafted the manuscript; S.K.M., T.P.S., A.B., S.F., J.F., and J.P.K. edited and revised the manuscript; S.K.M., T.P.S., A.B., S.F., J.F., and J.P.K. approved the final version of the manuscript.

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