A Cardiac Amino-Terminal GRK2 Peptide Inhibits Maladaptive Adipocyte Hypertrophy and Insulin Resistance During Diet-Induced Obesity

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HIGHLIGHTS

- Heart disease remains the leading cause of death, in part due to increasing diabetes and obesity, though the exact mechanisms linking these disorders are not fully understood.
- In a diet-induced obesity model, we found that cardiac expression of an amino-terminal peptide of GRK2, βARKnt, preserves systemic glucose tolerance and insulin sensitivity despite normal weight gain.
- βARKnt enhanced metabolic flexibility, increased energy expenditure, protected against maladaptive visceral adipocyte hypertrophy, and induced visceral fat browning.
- βARKnt further elicited cardioprotection and increased insulin-mediated AS160 signaling during metabolic stress.
- These data point to a noncanonical cardiac regulation of systemic metabolic homeostasis that may lead to new treatment modalities for metabolic syndrome.

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**SUMMARY**

Heart disease remains the leading cause of death, and mortality rates positively correlate with the presence of obesity and diabetes. Despite the correlation between cardiac and metabolic dysregulation, the mechanistic pathway(s) of interorgan crosstalk still remain undefined. This study reveals that cardiac-restricted expression of an amino-terminal peptide of GRK2 (ARKnt) preserves systemic and cardiac insulin responsiveness, and protects against adipocyte maladaptive hypertrophy in a diet-induced obesity model. These data suggest a cardiac-driven mechanism to ameliorate maladaptive cardiac remodeling and improve systemic metabolic homeostasis that may lead to new treatment modalities for cardioprotection in obesity and obesity-related metabolic syndromes. (J Am Coll Cardiol Basic Trans Science 2022;7:563-579) © 2022 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/).
acid uptake. Our group has recently found that mice overexpressing a short peptide of the amino-terminus, βARKnt, exhibit baseline hypertrophy, a normal adaptive response to transaortic constriction, and yet cardioprotection during chronic pressure overload. Interestingly, these studies uncovered a significant increase in cardiomyocyte insulin and Akt substrate of 160 kDa (AS160) signaling in the βARKnt mice, in contrast to endogenous GRK2, which is known to phosphorylate insulin receptor substrate 1 (IRS-1) and negatively regulate insulin signaling in the heart. This increase in insulin and AS160 signaling led to enhanced cardiomyocyte ATP production and spare respiratory capacity in the presence of both glucose and the fatty acid substrate palmitate. Further, these βARKnt mice exhibited a decrease in gonadal white adipose tissue (gWAT) weight, equivalent to human abdominal fat, both at baseline and 4 weeks after cardiac stress. In the present study, we subjected these cardiac-specific βARKnt transgenic (Tg) male mice to high fat diet (HFD) stress to investigate the linkage between these beneficial cardiac and metabolic effects and determine whether they might prove cardioprotective during metabolic dysfunction. Our data indicate beneficial effects on both cardiac remodeling and systemic metabolism after HFD and support the idea that the βARKnt peptide is not only cardioprotective during cardiac stress, but also demonstrates a novel means of cardioprotection and cardiac-mediated regulation of obesity and systemic metabolism during metabolic stress.

METHODS

For a detailed Methods section, see the Appendix. All animal procedures were carried out according to National Institutes of Health Guidelines on the Use of Laboratory Animals and approved by the Animal Care and Use Committees of the Lerner Research Institute. The method of euthanasia is consistent with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals. Euthanasia was accomplished by intraperitoneal injection of a single dose of ketamine/xylazine (87.5-100/10-12.5 mg/kg body weight) followed by blood collection, thoracotomy, and heart excision.

STATISTICAL ANALYSIS. All values in the text and figures are presented as mean ± SEM of independent experiments for given size N. Statistical significance was determined by 1-way analysis of variance with repeated measures (where appropriate and as indicated) and Tukey’s post hoc test for multiple pairwise comparisons, or 2-way analysis of variance with
comparable between groups, these data demonstrate that the improved glucose uptake and insulin sensitivity in the bARKnt mice is not an indirect effect of reduced obesity.

Fasting blood glucose and serum insulin levels (Supplemental Figure S2B) were then used to perform the homeostatic model assessment of insulin resistance (HOMA-IR) calculation (Figure 1H). Together, these blood glucose, glucose tolerance test, serum insulin, and HOMA-IR data confirm that although control mice become insulin resistant, glucose tolerance and insulin sensitivity are significantly improved in the bARKnt mice after 14 weeks of chronic HFD. Further serum analysis using mesoscale
Uplex and Rplex assays revealed dramatically elevated serum leptin levels in control mice that were significantly reduced by cardiac βARKnt expression after chronic HFD exposure (Figure 1). Recent clinical studies have revealed that insulin or leptin resistance evaluated by HOMA-IR score and serum leptin levels, respectively, were good indicators for cardiovascular disease risk. Thus, the reduction in HOMA-IR score and serum leptin levels after 14 weeks of HFD in the βARKnt mice not only indicate improved systemic metabolic performance, but also suggest protection from metabolic dysfunction–induced cardiac risk. Relevant differences were not observed for other peptide hormone regulators of metabolism such as adiponectin (Supplemental Figure S2C), glucagon like peptide-1 (GLP-1) (Supplemental Figure S2D), or glucagon (Supplemental Figure S2E). Together, these data demonstrate that despite equivalent weight gain, cardiac βARKnt expression elicits a beneficial effect on systemic insulin responsiveness and metabolic hormone signaling during HFD-induced metabolic stress. This suggests that βARKnt may induce expression of a beneficial cardiokine or other heart-mediated systemic cross-talk to metabolically active tissues.

**βARKnt MICE DEMONSTRATE METABOLIC FLEXIBILITY AND ENHANCED ENERGY EXPENDITURE FOLLOWING HFD STRESS.** To further elucidate the effect of cardiomyocyte βARKnt on systemic metabolic performance, we performed indirect calorimetry measures of energy utilization and expenditure in these NLC and Tg mice at 5 and 15 weeks after HFD. Weight gain, in addition to hyperglycemia and hyperinsulinemia in mice, drives insulin resistance and negatively alters metabolic performance, but also suggest protection from metabolic dysfunction–induced cardiac risk. Recent clinical studies have revealed that insulin or leptin resistance evaluated by HOMA-IR score and serum leptin levels, respectively, were good indicators for cardiovascular disease risk. Thus, the reduction in HOMA-IR score and serum leptin levels after 14 weeks of HFD in the βARKnt mice not only indicate improved systemic metabolic performance, but also suggest protection from metabolic dysfunction–induced cardiac risk. Relevant differences were not observed for other peptide hormone regulators of metabolism such as adiponectin (Supplemental Figure S2C), glucagon like peptide-1 (GLP-1) (Supplemental Figure S2D), or glucagon (Supplemental Figure S2E). Together, these data demonstrate that despite equivalent weight gain, cardiac βARKnt expression elicits a beneficial effect on systemic insulin responsiveness and metabolic hormone signaling during HFD-induced metabolic stress. This suggests that βARKnt may induce expression of a beneficial cardiokine or other heart-mediated systemic cross-talk to metabolically active tissues.

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**βARKnt MICE EXHIBIT REDUCED ABDOMINAL FAT WITH AN IMPROVED INFLAMMATORY AND ENERGETIC PHENOTYPE AFTER HFD STRESS.** To understand this metabolic flexibility and increased energy expenditure in the βARKnt mice, we examined the health of the gWAT. In the lean state, healthy fat is well-vascularized with minimal immune cell infiltration and releases numerous anti-inflammatory adipokines. During chronic overnutrition, adipocytes first expand in size by hypertrophy, and when they reach threshold, continue to expand in number by hyperplasia (adipogenesis). Excessive hypertrophic adipocyte enlargement leads to adipocyte death and tissue inflammation with crowning of dead adipocytes by macrophages, vascular rarefaction, proinflammatory adipokine release that generates systemic inflammatory activation, increased circulating fatty acids and lipotoxicity risk, and insulin resistance. Histological analysis of gWAT by hematoxylin and eosin staining revealed an expected increase in adipocyte size in both the NLC and TgβARKnt mice from baseline to 4 and 16 weeks of HFD, but with a visually observable decrease in adipocyte crowning in

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Tg|ARKnt mice demonstrate metabolic flexibility in utilizing fat and increased heat production (energy expenditure) following HFD stress. Mice were fed ad libitum and subjected to indirect calorimetry analysis to assess light and darkness cycle of (A) Respiratory exchange ratio (RER) in NLC and Tg|ARKnt mice after 5 weeks of HFD. Values represent measurements of 3 consecutive days (n = 4-6 per group). Quantitative measurements of RER in these mice at (B) 30°C, (C) room temperature (22°C), and (D) 4°C. (E) Heat production in NLC and Tg|ARKnt mice after 5 weeks of HFD. Values represent measurements of 3 consecutive days (n = 4-6 per group). Quantitative measurements of heat production in these mice at (F) 30°C, (G) room temperature (22°C), and (H) 4°C. (I) RER in NLC and Tg|ARKnt mice after 15 weeks of HFD. Values represent measurements of 3 consecutive days (n = 4-6 per group). Quantitative measurements of RER in these mice at (J) 30°C, (K) room temperature (22°C), and (L) 4°C. (M) Heat production in NLC and Tg|ARKnt mice after 15 weeks of HFD. Values represent measurements of 3 consecutive days (n = 4-6 per group). Quantitative measurements of heat production in these mice at (N) 30°C, (O) room temperature (22°C), and (P) 4°C. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 by 1-way ANOVA with repeated measures and Tukey post hoc test relative to corresponding NLC light phase at 5 or 15 weeks. †P < 0.05; ††P < 0.01; †††P < 0.001; ††††P < 0.0001 by 1-way ANOVA with repeated measures and Tukey post hoc test relative to corresponding NLC dark phase at 5 or 15 weeks post-HFD. Abbreviations as in Figure 1.
the βARKnt tissues compared with control (Figure 3A). Interestingly, and consistent with our pressure overload study, gWAT weight was significantly reduced in the βARKnt mice at 4 and 16 weeks after HFD compared with control mice (Figure 3B). Further, there was a coordinate decrease in average adipocyte size in these mice at 16 weeks compared with NLC (Figure 3C), despite no overall difference in
cell number between groups (Figure 3D). Reverse transcription polymerase chain reaction analysis of adipokine mRNA expression further revealed a significant decrease in proinflammatory tumor necrosis factor alpha (TNFα) (Figure 3E) and increase in anti-inflammatory transforming growth factor beta (TGFβ) (Figure 3F) in Tg mice, though only at 4 weeks after HFD. Interestingly, we also observed a significant increase in serum IL-10 in both groups (Supplemental Figure S5A), though more robust in NLC, and coordinate with our mRNA results, a significant decrease in circulating TNFα levels in the βARKnt mice compared with control mice at 16 weeks of HFD (Supplemental Figure S5B). Together, these data demonstrate maladaptive adipose tissue remodeling and dysfunction in control mice that is abrogated in mice with cardiomyocyte βARKnt expression during acute and chronic HFD stress.

Under normal conditions, white fat acts mainly as a lipid storage depot. However, under certain conditions, it can undergo beiging to adopt a more brown adipose tissue–like phenotype. During beiging, white adipocytes increase mitochondrial content and the energy expenditure marker mitochondrial uncoupling protein 1 (UCP-1) expression to utilize fatty acids for heat production, or thermogenesis. To determine whether the βARKnt gWAT might be undergoing beiging, we performed immunohistochemical staining for UCP-1 protein expression in Tg and NLC gWAT at baseline, and 4 and 16 weeks after HFD feeding (Figure 3G). Coordinate reverse transcription polymerase chain reaction analysis revealed a significant increase in UCP-1 mRNA at baseline and 16 weeks, with a trend at 4 weeks, and this translated to a trend at baseline and 4 weeks, and significant increase in UCP-1 protein expression in the βARKnt, but not NLC, gWAT at 16 weeks of HFD (Figure 3I). These data suggest an increase in gWAT beiging and thermogenic activity in mice with cardiomyocyte βARKnt expression, consistent with the increased energy expenditure observed during our indirect calorimetry measures. Together, these data demonstrate that despite equivalent weight gain during chronic HFD stress, and characteristic maladaptive remodeling of the gWAT in NLC mice, βARKnt mice exhibit reduced abdominal fat weight and adipocyte size, and increased markers of anti-inflammation and browning, suggesting a significantly improved gWAT phenotype following HFD stress.

βARKnt MICE EXHIBIT A MORE PRESERVED BROWN FAT MORPHOLOGY AFTER HFD STRESS. In contrast to the lipid storage function of white fat, BAT is saturated with mitochondria dedicated to producing heat through UCP-1–dependent uncoupled respiration of metabolites, thereby significantly modulating energy utilization and expenditure. Because the βARKnt mice exhibited metabolic flexibility under different temperature conditions and enhanced heat production after HFD stress, we investigated whether βARKnt mice would demonstrate improved morphology and increased BAT UCP-1 expression. To determine whether the morphology of the subscapular BAT depots would be improved in the βARKnt mice compared with control mice, we performed histological analysis of BAT by hematoxylin and eosin staining in Tg and NLC mice at baseline, and 4 and 16 weeks after HFD stress (Figure 4A). These data revealed a robust increase in lipid droplet storage within the BAT of NLC mice at 4 and 16 weeks after HFD, that was markedly restrained in the βARKnt mice. Although overall BAT weight was no different between these groups (Figure 4B), average cell area was significantly increased only in control BAT at 16 weeks (Figure 4C), with a coordinate reduction in cell count per unit area in the NLC, compared with βARKnt BAT (Figure 4D). No change in UCP-1 mRNA expression, or difference in anti- and proinflammatory markers, were observed over the time course of chronic HFD stress in these groups (Figure 4E, Supplemental Figure S6). Together, these data demonstrate that, in contrast to the significant lipid accumulation in NLC mice after chronic HFD, BAT morphology is improved in mice with cardiomyocyte βARKnt expression. Interestingly, mRNA data indicate no overall change in tissue health or function between groups, suggesting that the increase energy expenditure observed in the βARKnt mice during indirect calorimetry was not due to enhanced BAT activity, and must be due to other metabolically active tissues. Further studies will be needed to confirm that there is in fact no difference in BAT-mediated thermogenesis in the βARKnt mice during chronic HFD stress.

βARKnt MICE EXHIBIT ENHANCED CARDIAC FUNCTION AND INSULIN-MEDIATED AS160 SIGNALING AFTER HFD STRESS. In response to diet-induced obesity, the heart initially undergoes adaptive remodeling, but cannot maintain this adaptive state and transitions into maladaptive remodeling, including pathological hypertrophy, fibrosis, and cardiac insulin resistance. To interrogate whether the enhanced systemic glucose tolerance and insulin sensitivity, and improved abdominal fat phenotype would correlate with preserved cardiovascular function, we performed echocardiographic analysis in these NLC and
TgβARKnt mice at baseline, and 4 and 14 weeks after HFD stress. In parallel to the role of cardiomyocyte βARKnt in regulating systemic metabolism and energy expenditure, we observed enhanced cardiovascular performance. Echocardiography revealed a significant increase in fractional shortening in βARKnt mice at 4 and 14 weeks after HFD, despite no overall change in control mice (Figure 5A). Further, cardiac output was significantly increased in βARKnt compared with a trending decrease in NLC mice from 4 to 14 weeks after HFD (Figure 5B). Numerous other measures of cardiovascular structure and function were also enhanced in the Tg mice compared with control mice (Table 1), some of which have been previously shown to be affected by cardiomyocyte βARKnt expression irrespective of metabolic stress.23 Interestingly, although left ventricular mass was significantly increased in βARKnt, but not NLC mice, at 4 weeks, this measure was equivalently elevated in both groups by 14 weeks (Figure 5C). This was accompanied by a comparable increase in heart weight (Figure 5D, Supplemental Figure S7A) and lung weight (Figure 5E, Supplemental Figure S7B) in both groups after 16 weeks of chronic HFD stress. These data are accompanied by equivalent increases in both tibia length (Supplemental Figure S7C) and body weight (Supplemental Figure S7D) at 4 and 16 weeks of HFD feeding as expected for aging-related growth and HFD-induced weight gain, respectively.

To determine whether βARKnt expression was able to reduce maladaptive left ventricular remodeling after chronic HFD stress, we performed Masson Trichrome staining of 4-chamber paraffin sections taken at the level of the aortic outflow tract. These images revealed a proportional increase in cardiac size in the NLC and TgβARKnt hearts from 4 to 16 weeks after HFD (Figure 5F). Quantification of ventricular myocyte and fibrotic area revealed no differences after acute HFD stress, but a significant decrease in myocyte area (Figure 5G) and increase in fibrotic area (Figure 5H) in the βARKnt mice at after chronic metabolic stress. Although these differences are mild, an increase in fibrosis is generally considered at odds
with an increase in cardiac output and stroke volume, and will require future investigation. Of note, the increase in fibrotic area (Figure 5H) and left ventricular posterior wall thickness during systole (Table 1), and decrease in end-systolic volume (Table 1) in the βARKnt mice, indicates left ventricular hypertrophy and is consistent with our previous results during cardiac stress. 23

To investigate whether insulin-mediated AS160 signaling is similarly enhanced in βARKnt-expressing cardiomyocytes as was observed in our previous pressure-overload study, we assessed insulin signaling via Western blot in ventricular lysates from NLC and TgβARKnt mice at 4 and 16 weeks after HFD stress. These studies revealed a significant increase in insulin receptor (InsR) protein expression levels in βARKnt compared with NLC mice at 4 weeks, with only a trend at 16 weeks (Figure 6A, Supplemental Figures S8A and S9D). This led to a significant increase in downstream levels of phosphorylated Akt at 4 weeks (Figure 6B, Supplemental Figures S8B and S9A), with no difference in total Akt expression (Figure 6C, Supplemental Figures S8C and S9B). There was a similar increase in phosphorylated AS160 in the βARKnt, but not NLC, mice at 4 weeks, with a further significant increase at 16 weeks (Figure 6D, Supplemental Figure S8D), but no difference was observed in total AS160 protein levels (Figure 6E, Supplemental Figures S8E and S9B). This translated to a trend toward an increase at 4 weeks and significant increase at 16 weeks after HFD stress in downstream serine 2448 phosphorylation of mechanistic target of rapamycin protein kinase (mTOR), suggesting enhanced mTORC1 signaling in the βARKnt lysates (Figure 6F, Supplemental Figure S8F) that was accompanied by a trend toward increased total mTOR (Figure 6G, Supplemental Figures S8G and S9C). These studies also revealed a coordinate increase in total glucose transporter 4 (GLUT4) protein expression in the βARKnt mice compared with control mice after chronic HFD stress (Figure 6H, Supplemental Figure S8H). Together, these data suggest enhanced cardiac insulin signaling and glucose metabolism in the βARKnt mice during metabolic stress that may underlie the observed increase in cardiovascular function.}

**NEITHER ENHANCED gWAT ACTIVATION NOR NATRIURETIC PEPTIDE ACTIVITY EXPLAINS THE IMPROVED gWAT PHENOTYPE AND SYSTEMIC METABOLISM OBSERVED IN THE βARKnt MICE.** To
elucidate the underlying mechanism(s) by which cardiomyocyte-specific βARKnt expression enhances gWAT phenotype during metabolic stress, we further interrogated gWAT activation. Catecholamines act as prominent regulators of gWAT function, wherein by binding to β3-adrenergic receptors (β3ARs) they stimulate cAMP production and enhanced protein kinase A (PKA) activity, resulting in lipolysis and free fatty acid release.32,36 Therefore, we performed immunohistochemical staining for β3AR protein expression in the gWAT of NLC and TgβARKnt mice at baseline, and 4 and 16 weeks after HFD (Figure 7A). Quantification of these data revealed no significant difference in receptor expression between groups throughout the time course of the study (Figure 7B). Additionally, serum epinephrine levels showed no significant difference, and serum norepinephrine levels were equivalently enhanced over time in the βARKnt and NLC mice in response to HFD stress (Figures 7C and 7D). Recent insights have shown that β3ARs and natriuretic peptides (NPs) share a common pathway for activation of gWAT and gWAT browning.36,37 PKA and PKG, which are stimulated by 3ARs and NPs, respectively, share a closely related motif for substrate phosphorylation of mTOR to enhance mTORC1-mediated adipocyte browning.35,36

The atrial (ANP) and B-type (BNP) endocrine-like NPs are normally produced by atrial myocytes; however, their expression in ventricular myocytes is significantly enhanced in response to cardiovascular and cardiometabolic stress. NP stimulation of NP receptor A (NPRA) activates adipocyte browning through mTORC1 signaling,35,36 whereas NP receptor C (NPRC) acts as a clearance receptor involved in NP degradation.35,40 Therefore, the ratio of NPRA:NPRC determines NP signaling capacity in abdominal fat. ANP (Figure 7E) and NPRC (Figure 7G) mRNA levels were comparable between groups; however, βARKnt mice exhibited increased NPRA gene expression after 4 weeks of HFD stress (Figure 7F). This resulted in a significant increase in the NPRA:NPRC ratio at 4 weeks (Figure 7H). Although these data suggest that gWAT in βARKnt mice may have enhanced NP responsiveness at 4 weeks, we did not observe a significant increase in UCP-1 protein expression until 16 weeks of HFD. Together, these data demonstrate that neither an increase in β3AR nor NP activation is sufficient to explain the enhanced gWAT UCP-1 and thermogenic activity observed in the βARKnt mice in this study. This highlights the need for additional gWAT signaling studies and suggests there may be a noncanonical heart-to-fat pathway in these mice.

**DISCUSSION**

Herein, we investigated whether the enhanced cardiomyocyte AS160 signaling and reduced gWAT weight observed in our βARKnt line would translate to cardioprotection and/or improved systemic metabolism in a mouse model of HFD-induced metabolic dysfunction. Cardiac-specific TgβARKnt mice exhibited equivalent weight gain over time on HFD, yet enhanced systemic glucose tolerance and insulin sensitivity, with a coordinate increase in metabolic flexibility and energy expenditure. Interrogation of the white adipose tissue phenotype revealed reduced gWAT weight and adipocyte size, and increased markers of anti-inflammation and browning both before and after HFD stress that was not due to classical activation of the gWAT through β3AR or natriuretic peptides. This was accompanied by an improved brown adipose tissue morphology. More importantly, the βARKnt mice exhibited enhanced cardiovascular function, despite no improvement in tissue remodeling, which may be again due to enhanced

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**TABLE 1 Measures of Echocardiographic Parameters From NLC and Tg(βARKnt) Mice at Baseline, and 4 and 14 Weeks After HFD**

| Parameter | Baseline | 4 Weeks | 14 Weeks |
|-----------|----------|---------|----------|
| HR (beats/min) | 453.90 ± 10.88 | 493.40 ± 7.41 | 491.30 ± 16.43 |
| TG | 438.90 ± 12.48 | 482.20 ± 11.99 | 449.40 ± 10.00 |
| EF (%) | 52.42 ± 1.29 | 52.33 ± 1.99 | 51.87 ± 2.16 |
| LVSP (mmHg) | 0.96 ± 0.03 | 1.01 ± 0.03 | 1.06 ± 0.04 |
| LVPWd (mm) | 0.65 ± 0.02 | 0.70 ± 0.02 | 0.88 ± 0.05 |
| LVtd (mm) | 2.88 ± 0.07 | 2.85 ± 0.08 | 2.72 ± 0.10 |
| ESV, mL | 3.91 ± 0.06 | 3.87 ± 0.06 | 3.68 ± 0.08 |
| EDV, mL | 25.11 ± 1.57 | 18.16 ± 1.26 | 17.65 ± 1.31 |
| SV, mL | 34.80 ± 0.95 | 33.88 ± 1.37 | 29.39 ± 1.09 |

**Values are mean ± SEM. n = 11–12 per group.**

- **P** < 0.05
- **P** < 0.01
- **P** < 0.001
- **P** < 0.0001 by 2-way analysis of variance (ANOVA) with repeated measures and Tukey post hoc test relative to corresponding baseline. **P** < 0.05 by 2-way ANOVA with repeated measures and Bonferroni post hoc test relative to corresponding NLC at baseline, 4 or 14 weeks post-HFD.

EDV = end-diastolic volume; EF = ejection fraction; ESV = end-systolic volume; HR = heart rate; LVd = left ventricular internal diameter during diastole; LVtd = left ventricular internal diameter during systole; LVPWd = left ventricular posterior wall thickness during diastole; LVPWD = left ventricular posterior wall thickness during systole; SV = stroke volume.
cardiovascular insulin-mediated AS160 signaling in these hearts.

The canonical role of GRK2 in regulating normal heart function through G-protein coupled receptor (GPCR) desensitization has been well established; however, in addition to this classical activity, GRK2 has been shown to display noncanonical regulatory functions in numerous cell signaling routes including mitochondrial function and apoptosis, and insulin signaling.8,15 Furthermore, GRK2 has been demonstrated to affect cardiac and systemic metabolic activity, though the beneficial versus detrimental effects are time and tissue dependent.27,41 Although overexpression of GRK2 displays resistance to HFD-induced obesity and enhanced GLUT4 levels in gWAT, cardiac-restricted expression of βARKct negatively alters systemic metabolism and enhances the obesogenic phenotype.27 Recently, our lab demonstrated that cardiac expression of βARKnt after pressure overload positively modulates cardiac metabolism and reduces gWAT size, in addition to its cardioprotective effects.23 Thus, whereas increased GRK2 is detrimental for the heart and fine for the metabolic system, βARKnt is beneficial for both, protecting the heart and reducing only the abdominal fat pad.

Insulin-sensitive tissues including adipose tissues, liver, skeletal muscle, and the heart play a crucial role in regulating systemic metabolism.42,43 Over the past decade, great progress has been made in interrogating the complexity of these tissues and their interactive roles in metabolism.42 These studies have revealed that obesity-driven insulin resistance in these tissues is the primary cause for the development of systemic dysregulation and T2D.42,43 During insulin resistance, normally insulin-sensitive tissues lose their response, leading to impaired glucose uptake and resulting in elevated blood glucose levels that stimulate pancreatic β cell secretion of more insulin in a detrimental feed-forward cycle.29,44,45 There are several
hypotheses regarding the mechanistic pathways responsible for insulin resistance during obesity, including adipocyte dysfunction, inflammation, hyperinsulinemia, endoplasmic reticulum stress, and mitochondrial dysfunction.44 Although there is as of yet no unifying mechanism for insulin resistance, these mechanistic pathways are typically associated with obesity.

WAT is a complex organ that not only acts as a reservoir for energy storage, but also senses energy demands and releases adipokines to modulate other metabolic tissues.43 In a high-energy state, WAT secretes adipokines such as leptin and adiponectin to regulate food intake, increase energy expenditure, and enhance insulin sensitivity and glucose metabolism.46 Interestingly, recent studies have shown that sustained BAT thermogenic activation leads to WAT beiging, and these brown-like adipocytes eventually produce heat via UCP-1 to improve systemic metabolism energy balance.47 Further, this increase in gWAT UCP-1 content in mice is strongly linked to protection against diet induced obesity and systemic dysfunction.33,48 Although some investigators suggest that WAT beiging may induce additional improvements in energy expenditure and metabolite utilization through mechanisms independent of UCP-1, the actual relevance of WAT beiging to systemic metabolism remains unclear. Interestingly, the βARKnt peptide elicited improved WAT activity via increased beiging and thermogenesis with no indication of altered BAT function in response to obesity. These findings reveal that cardiac βARKnt beneficially
induces browning in gWAT, rather than BAT, to protect against HFD stress and systemic dysfunction. The mechanism by which cardiac βARKnt increases gWAT, not BAT, UCP-1 will require further investigation. These findings suggest that although the effects of obesity on insulin-sensitive tissues is well-established, a unique link between the heart and adipose tissues via cardiometabolic modulation and/or cardiokine secretion may provide new insights for adipose and systemic dysfunction.

One caveat of this study is that we did not directly measure insulin responsiveness or metabolite uptake in WAT, BAT, liver, or skeletal muscle, so we cannot exclude the regulatory role of these tissues in the improved systemic metabolism. In ob/ob mice and isolated hearts of HFD mice, obesity does not impair mitochondrial fat metabolism but rather impairs insulin-stimulated glucose oxidation in a manner similar to diabetes, resulting in glucose intolerance and insulin resistance.49,50 Future studies could directly measure changes in glucose and fatty acid uptake into these tissues following fasting and a bolus injection of [3H]2-deoxyglucose or 2-bromopalmitate, with or without insulin stimulation, followed by tissue recovery counting.51,52 Comparing the heart to other metabolically active tissues would allow us to determine whether it is just the cardiac βARKnt enhancing cardiovascular metabolism or if the heart is somehow signaling to increase insulin sensitivity and metabolite uptake in the systemic tissues as well. Our previous study demonstrated that cardiomyocytes did not undergo a switch to glucose utilization, as is often seen during cardiovascular disease and considered detrimental. Rather, they had enhanced fatty acid and glucose utilization that contributed to the increased ATP production and spare respiratory capacity.23 Thus, these future studies would also be important to fully understand our indirect calorimetry results, particularly whether the increase in observed RER was due only to a decrease in lipid use or could be due to increased glucose utilization, or a combination effect.

Our previous study identified a novel interaction of βARKnt with endogenous AS160 in the heart, and enhanced cardiac insulin and AS160 signaling. AS160 is expressed in multiple insulin-responsive tissues, though predominantly WAT, skeletal muscle, and the heart.53 In fact, AS160 was first discovered in adipocytes, where improper Akt-dependent activation led to defective glucose uptake.54 Patients carrying a premature stop mutation in 1 allele of AS160 (R362X), resulting in lower levels of AS160 protein and a dominant-negative truncated variant, exhibit severe insulin resistance during puberty and post-prandial hyperinsulinemia, due to impaired insulin-stimulated GLUT4 translocation.55 Impaired insulin-induced phosphorylation of AS160 has also been demonstrated in type 2 diabetic patient muscle, with phosphorylation and insulin sensitivity improved by endurance training.56 Knockout models of AS160 have also demonstrated systemic insulin resistance without diabetes, wherein insulin resistance, reduced GLUT4 levels, and glucose uptake were greatest in the skeletal muscle and WAT.57 In other studies, specific ablation of AS160 in skeletal muscle, adipocytes, hepatocytes, and neurons lead to tissue-specific cross-talks causing whole-body insulin resistance.57,58 Unfortunately, due to limitations, these mouse models cannot evaluate the cardiometabolic influence of AS160 on insulin-responsive physiological changes in glucose uptake or the metabolic flexibility and efficiency of myocytes. Further, little information is known about how disruption in AS160 signaling alters cardiac insulin resistance and metabolic function. Nonetheless, the studies point to the role of AS160 as a central node regulating glucose uptake and systemic metabolic homeostasis. The present study revealed an increase in insulin-mediated AS160 signaling in the βARKnt hearts during acute and chronic HFD-induced metabolic stress, similar to what we previously observed during cardiac stress.23 Importantly, though, because βARKnt is only expressed in the hearts of these mice, the observed changes in systemic tissue function and insulin responsiveness are not due to a direct effect of βARKnt/AS160 in those tissues but through some other means of systemic signaling. Future studies will be needed to elucidate whether AS160 plays a causative role in the beneficial effects of βARKnt on systemic metabolism and the underlying mechanism of these effects.

Although the mechanism by which adipose tissue dysfunction contributes to cardiac pathology has been thoroughly investigated,43 whether the heart can reciprocally modulate adipose tissue or other metabolically active organs remains unclear. In response to cardiac or metabolic stress, and depending upon disease stage and etiology, disrupted metabolic processes lead the heart to secret proteins into the circulation, called cardiokines.59 These proteins can act as autocrine (self), paracrine (adjacent cell), or endocrine (peripheral blood) factors to induce cellular responses, with endocrine actions of particular interest in seeking to understand when and how
the heart initiates communication with peripheral organs. Although our preliminary data suggest that the reduced gWAT weight and being observed in the βARKnt mice could not be explained by enhanced β2AR, circulating catecholamines, or NP stimulation, future studies would be needed to exclude these processes and fully elucidate the mechanism by which cardiac βARKnt beneficially influences the gWAT, or other metabolically active tissue, phenotype. This will include elucidating whether cardiac βARKnt stimulates secretion of cardiokine(s) that directly act on the adipose tissue or liver. Although our data suggest cardiac-driven regulation of systemic metabolism, it may instead be an indirect effect of significant alterations in cardiomyocyte metabolism, or metabolite usage. This would still represent an as yet unknown level of interorgan sensing, or whole-body metabolite sensing, that would be worthy of investigation.

CONCLUSIONS

In summary, the present study demonstrates that cardiac-restricted expression of βARKnt beneficially modulates abdominal fat function and systemic metabolism through improvements in insulin sensitivity and energy utilization. These findings also provide further support that cardiac βARKnt ameliorates pathological left ventricular remodeling through direct modulation of insulin signaling pathways within cardiomyocytes and translates them into beneficial effects on systemic metabolism. The growing incidence of obesity and metabolic syndrome emphasizes the need for therapeutic interventions that improve myocardial function while simultaneously reducing abdominal fat. Therefore, a better understanding of the molecular alterations responsible for the improved adipose tissue phenotype in the βARKnt mice may identify therapeutic targets to enhance the function of adipose tissue and systemic metabolism. Further, these studies will provide insight into cardiac regulation of systemic insulin responsiveness and may lead to new treatment modalities for cardioprotection in obesity and obesity-related metabolic syndromes.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Heart disease remains the leading cause of death, and cardiovascular mortality rates positively correlate with the presence of diabetes and metabolic syndrome. Independent of sex, age, and developmental status of the country, the worldwide prevalence of obesity and severe obesity (body mass index ≥35 kg/m²) continues to progress with adverse effects on cardiovascular risk factors including plasma glucose and lipids, arterial blood pressure, inflammation, and cardiorespiratory fitness. Despite the correlation between cardiac and metabolic dysfunction, the exact mechanistic links are not fully understood. This study reveals that cardiac-restricted expression of a peptide of the GRK2 amino-terminus (βARKnt) preserves systemic insulin responsiveness and reduces maladaptive adipose tissue remodeling despite diet-induced obesity. These data suggest that altering cardiac function may induce a clinically relevant improvement in both cardiac and systemic responses to obesity and metabolic disease.

TRANSLATIONAL OUTLOOK: In the midst of a growing recognition of the effect of adipose tissue dysfunction and adipokine imbalance on cardiovascular disease in the obese population, a less-well understood mechanism is that of the effect of cardiac metabolism and function on whole-body metabolic homeostasis. This study demonstrates that cardiac-restricted expression of a GRK2 amino-terminal peptide (βARKnt) preserves insulin sensitivity and glucose tolerance in a model of diet-induced obesity. Moreover, cardiac βARKnt expression protects against maladaptive adipocyte hypertrophy and adipose tissue dysfunction. Elucidating the mechanism whereby cardiac βARKnt improves both cardiac and systemic responses to obesity will provide insight into cardiac regulation of systemic metabolic homeostasis and lead to new treatment modalities for metabolic syndrome.
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**APPENDIX** For an expanded Methods section and supplemental figures, please see the online version of this paper.