APPL1 transgenic mice are protected from high-fat diet-induced cardiac dysfunction

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APPL1 transgenic mice are protected from high-fat diet-induced cardiac dysfunction. Am J Physiol Endocrinol Metab 305: E795–E804, 2013. First published August 6, 2013; doi:10.1152/ajpendo.00257.2013.—APPL1 (adaptor protein containing PH domain, PTB domain, and leucine zipper motif 1) has been established as an important mediator of insulin and adiponectin signaling. Here, we investigated the influence of transgenic (Tg) APPL1 overexpression in mice on high-fat diet (HFD)-induced cardiomyopathy in mice. Wild-type (WT) mice fed an HFD for 16 wk showed cardiac dysfunction, determined by echocardiography, with decreased ejection fraction, decreased fractional shortening, and increased end diastolic volume. HFD-fed APPL1 Tg mice were significantly protected from this dysfunction. Speckle tracking echocardiography to accurately assess cardiac tissue deformation strain and wall motion also indicated dysfunction in WT mice and a similar improvement in Tg vs. WT mice on HFD. APPL1 Tg mice had less HFD-induced increase in circulating nonesterified fatty acid levels and myocardial lipid accumulation. Lipidomic analysis using LC-MS-MS showed HFD significantly increased myocardial contents of distinct ceramide, sphingomyelin, and diacylglycerol (DAG) species, of which increases in C16:0 and C18:0 ceramides plus C16:0 and C18:1 DAGs were attenuated in Tg mice. A glucose tolerance test indicated less peripheral insulin resistance in response to HFD in Tg mice, which was also apparent by measuring cardiac Akt phosphorylation and cardiomyocyte glucose uptake. In summary, APPL1 Tg mice exhibit improved peripheral metabolism, reduced cardiac lipotoxicity, and improved insulin sensitivity. These cellular effects contribute to protection from HFD-induced cardiomyopathy.

cardiomyopathy; cardiac function; high-fat diet; insulin sensitivity; oxidative stress; lipotoxicity; nonesterified

Obesity and type 2 diabetes determine the extent of morbidity and mortality in heart failure and to study underlying mechanisms in animal models a high-fat diet (HFD) is often used (16). The chronic high circulating level of long-chain fatty acids which occurs in this model induces the heart to switch its metabolic substrate preference further toward use of fatty acids at the expense of glucose (46, 49). Various consequences of the ensuing lipid overload occur including accumulation of lipid intermediates such as ceramides and diacylglycerols (DAGs) and production of reactive oxygen species (ROS) (25, 43). Collectively these changes induce cardiac insulin resistance and dysfunction with chronic development of cardiomyopathy (16, 37).

Adaptor protein containing pleckstrin homology (PH) domain, phosphotyrosine binding (PTB) domain, and leucine zipper motif 1 (APPL1) was initially discovered as an Akt2 binding protein (29), and it has since been demonstrated to be an important regulator of both insulin and adiponectin signaling. Dong’s group demonstrated binding of APPL1 to adiponectin receptors and via gain- and loss-of-function experiments showed that this protein was essential in mediating adiponectin signaling and metabolic effects in skeletal muscle cells (27). Recent studies have further characterized the mechanism by which APPL1 facilitates adiponectin-stimulated AMP-activated protein kinase (AMPK) or p38 mitogen-activated protein kinase (MAPK) signaling in skeletal muscle cells (15, 47, 50). In endothelial cells, suppression of APPL1 expression decreased production of nitric oxide by adiponectin via attenuating AMPK and endothelial nitric oxide synthase (eNOS) phosphorylation (9). Overexpression and knockdown of APPL1 either in vitro or in vivo enhanced or suppressed, respectively, hepatic insulin sensitivity (8). Work by Kraegen’s group showed that APPL1 was an important mediator of metabolic effects of insulin in rat skeletal muscle (12). Furthermore, APPL1 may represent an important node for cross-talk between adiponectin and insulin signaling (12, 14, 23). Most recently, it was shown that APPL1 couples insulin-stimulated Akt activation to insulin secretion in pancreatic β-cells (10).

Adiponectin and insulin both mediate important effects that govern cardiac remodeling events leading to heart failure (1, 31); however, little is known regarding the role of APPL1 in mediating their cardiac effects. We have recently shown that knockdown APPL1 using siRNA attenuated adiponectin-stimulated fatty acid uptake and oxidation in primary cardiomyocytes (18) and reduced the ability of adiponectin to prevent hypoxia reoxygenation-induced oxidative stress and caspase-3 activity in cardiomyocytes (33). Suppression of APPL1 expression also attenuated adiponectin-induced nitric oxide production from endothelial cells (9). Less is known regarding possible APPL1-mediated effects of insulin on the heart and vasculature, yet recently in APPL1 knockout mice, the balance in vasoactive effects of insulin was altered by reducing vasodilation and enhancing vasoconstrictor effects (42). Furthermore, APPL1 overexpression prevented age- and obesity-induced impairments in insulin-induced vasodilation (42).
In this study, we used APPL1 transgenic (Tg) mice to examine the consequences of whole body overexpression of this protein on HFD-induced cardiac dysfunction. We focused on investigation of structural and functional changes in the heart of wild-type (WT) and APPL1 Tg mice fed HFD as well as mechanisms contributing to these changes.

**MATERIALS AND METHODS**

**Animals and echocardiography.** APPL1 transgenic (Tg) mice were generated as previously described (10, 42) and showed approximately fivefold levels of enhanced APPL1 expression in various tissues as we showed previously (see Fig. 1). All animal experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23) and were approved by the York University Animal Care Committee.

Four-week-old mice were subjected to a normal chow diet (NCD) or high-fat diet (HFD, 60% kcal from fat Research Diets) for up to 16 wk. Cardiac function and heart morphology were evaluated at multiple time points using echocardiography (Vevo 2100, VisualSonics). The animals were sedated using 3% isoflurane and maintained with positive end-expiratory pressure (PEEP). Cardiac function and heart morphology were evaluated at multiple time points using echocardiography (Vevo 2100, VisualSonics). The animals were sedated using 3% isoflurane and maintained with positive end-expiratory pressure (PEEP).

**Lipidomic analysis of lipid accumulation.** Heart sections fixed in 4% paraformaldehyde were stained with Oil red O and analyzed by bright-field microscopy as described by us before. Quantitative measurement of triglyceride (TG) content in heart tissue followed the manufacturer’s protocol for the Triglyceride Quantification Kit (BioVison). Cardiac diacylglycerols (DGs) were measured by LC-MS-MS with atmospheric pressure chemical ionization (APCI) source (6), and measured species were 16:0, 18:1, 18:0–20:4, 16:0–18:1, and 18:0–18:2 DGs. Ceramides with various acyl chains (C14:0, C16:0, C18:0, C18:1, C20:0, C24:0, C24:1) were separated by HPLC with a C18 column (XTerra C18, 3.5 μm, 2.1 × 50 mm) and ionized in positive electrospray ionization mode as described (48). Sphingolipid metabolites were monitored for multiple reaction monitoring (MRM) quantification by a bench-top tandem mass spectrometer with an electrospray ionization source.

**Insulin signaling and protein expression study using western blotting.** Mice were starved for 5–6 h, and then 2 U/kg insulin was injected via tail vein and heart tissue collected after 5 min. Samples (2 μg) were loaded onto SDS-polyacrylamide gel for Western blot analysis with antibodies specific to APPL1 [generated by us (A. Xu)], Akt, pAkt(Thr308) and β-tubulin (Cell Signaling) and pIRS(Y612) from Invitrogen. To determine oxidative stress, we used a TBARS assay kit (Cayman Chemical). For analysis of APPL1 overexpression in various tissues, WT and APPL1 Tg mice were euthanized, and various tissues were collected for Western blot analysis. Tissue homogenates were prepared in 1 ml of mammalian cell lysis buffer and clarified by centrifugation at 13,000 rpm for 15 min. Total protein (60 μg) was subjected to SDS-PAGE separation and then immunoblotted with anti-APPL1 and anti-β-tubulin antibodies as specified.

**Measurement of plasma levels of insulin, glucose, and nonesterified fatty acids and glucose tolerance test.** Serum samples were collected and prepared using a Microvette CB300Z (Sarstedt). We used the ultrasensitive insulin EIA kit (ALPCO), blood glucose meter (Braun), and LabAssay NEFA assay kit (Wako). We performed a glucose tolerance test as described by us recently (26, 41).

**Metabolic studies in cardiomyocytes isolated from adult mice.** Adult mouse cardiomyocytes were isolated from WT or APPL1 Tg mice fed NCD or HFD for 16 wk. Glucose uptake was measured by adding 10 μl of 2-deoxyglucose (2-DG) mixture containing 195 μl ddH₂O, 22.5 μl of 0.1 M 2-DG solution, and 7.5 μl of 1 μCi/μl [3H]2-DG per well for 30 min.

**Statistics.** Data are presented as means ± SE. Comparisons of a single variable in two or more groups were analyzed by one-way ANOVA followed by Tukey’s multiple comparison tests (GraphPad Prism). Values of P < 0.05 were considered significant.

**RESULTS**

**Generation and characterization of APPL1 transgenic mice.** We generated mice expressing human APPL1 driven by the cytomegalovirus immediate early β-actin (CMV-IE-AG) promoter (10, 42). Figure 1A shows a schematic representation of the DNA fragment consisting of CMV-IE-AG promoter, human APPL1 cDNA with FLAG tag at the NH2 terminus, and the rabbit β-globin poly(A) signal. APPL1 expression was then examined in various tissues of APPL1 Tg or WT mice by Western blots analysis, as shown in Fig. 1B. Representative data shown here indicated that CMV-β-actin-driven overexpression was successful in heart, muscle, liver, kidney, brain, spleen, and white and brown adipose tissue and that Tg mice expressed on average four- to fivefold more APPL1 than WT, with only a mild increase of APPL1 expression detected in brain. This is a desirable level of mild overexpression to enable us to examine physiological consequences of APPL1 overexpression. Representative data of two lines of Tg mice are shown in Fig. 1, the former used in this study.

**Analysis of cardiac structure and function by echocardiography.** Cardiac structure and function were monitored by echocardiography after 4, 8, 12, and 16 wk of NCD or HFD feeding. Cardiac functions were similar among the four groups prior to and at 4 wk post-food treatments (data not shown). However, differences in cardiac function among the four groups were observed after 16 wk of treatment, as shown in Fig. 2. At this stage, cardiac functions including ejection fraction (EF) and fractional shortening (FS) and heart morphologies including end-diastolic volume (EDV) and end-systolic volume (ESV) were similar when the WT and Tg mice were fed NCD. How-
However, the HFD resulted in significant decrease of global cardiac functions, such as cardiac EF and FS in the WT mice compared with Tg mice (Fig. 2). Heart morphological analysis showed that there was no difference in EDV among the four groups. When the animals were fed NCD, heart ESVs of WT and Tg mice were similar. However, the Tg mice showed significantly smaller ESV than the WT mice when they were fed the HFD for 16 wk. These results indicate less dysfunction in Tg mice with Tg mice (Fig. 2). Heart morphological analysis showed that there was no difference in EDV among the four groups. When the animals were fed NCD, heart ESVs of WT and Tg mice were similar. However, the Tg mice showed significantly smaller ESV than the WT mice when they were fed the HFD for 16 wk. These results indicate less dysfunction in Tg mice compared with WT mice (Fig. 2 and Table 1). Furthermore, the measurements of blood flow velocity at ascending aorta (AoV) using PW doppler mode demonstrated that Tg mice maintained significantly better systolic function than WT mice after HFD (Fig. 2 and Table 1).

Table 1. Echocardiography data

<table>
<thead>
<tr>
<th></th>
<th>WT-NCD</th>
<th>APPL1-NCD</th>
<th>WT-HFD</th>
<th>APPL1-HFD</th>
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<tr>
<td>EDV, µl</td>
<td>76.77 ± 2.85</td>
<td>78.69 ± 3.05</td>
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<td>ESV, µl</td>
<td>38.82 ± 2.20</td>
<td>40.19 ± 2.20</td>
<td>48.32 ± 1.15</td>
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<td>LVDD, mm</td>
<td>4.31 ± 0.08</td>
<td>4.29 ± 0.09</td>
<td>4.34 ± 0.09</td>
<td>4.30 ± 0.13</td>
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<td>LVIDS, mm</td>
<td>2.89 ± 0.07</td>
<td>2.86 ± 0.12</td>
<td>3.16 ± 0.08</td>
<td>3.11 ± 0.18</td>
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<td>LVPWD, mm</td>
<td>0.74 ± 0.03</td>
<td>0.70 ± 0.03</td>
<td>0.72 ± 0.03</td>
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<td>LVPWS, mm</td>
<td>1.08 ± 0.04</td>
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<td>1.02 ± 0.04</td>
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<td>CO, ml/min</td>
<td>17.32 ± 0.94</td>
<td>16.82 ± 0.55</td>
<td>12.4 ± 0.77</td>
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<td>EF, %</td>
<td>60.67 ± 1.34</td>
<td>63.05 ± 1.65</td>
<td>50.24 ± 0.88</td>
<td>56.27 ± 2.39</td>
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<tr>
<td>FS, %</td>
<td>32.21 ± 0.93</td>
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<td>25.35 ± 0.93</td>
<td>30.34 ± 2.09</td>
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<td>E/A ratio</td>
<td>1.73 ± 0.04</td>
<td>1.63 ± 0.03</td>
<td>1.60 ± 0.04</td>
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<td>Mean AV vel., mm/s</td>
<td>758.9 ± 27.71</td>
<td>707.9 ± 10.49</td>
<td>583.2 ± 25.28</td>
<td>733.7 ± 50.63</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>469.8 ± 11.90</td>
<td>449.0 ± 10.37</td>
<td>470.9 ± 11.18</td>
<td>466.5 ± 17.49</td>
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Values are means ± SE. WT, wild type; APPL1, APPL1 transgenic (Tg); NCD, normal chow diet; HFD, high-fat diet; EDV, end-diastolic volume; ESV, end-systolic volume; LV, left ventricular; IDD, internal diameter diastole; IDS, internal diameter systole; PWD, posterior wall thickness diastole; PWS, posterior wall thickness systole; EF, ejection fraction; FS, fractional shortening.
HFD-induced changes in circulating free fatty acids and myocardial lipid accumulation. We first examined the circulating level of free fatty acid (NEFA). Interestingly, APPL1 Tg mice showed a reduced circulating level of NEFA after HFD compared with the significantly elevated levels induced by HFD in WT mice (Fig. 4A). To examine the myocardial lipid accumulation, Oil red O staining was used to detect HFD-induced changes in myocardial neutral lipids. We detected significant increases in lipid content between heart tissue sections from WT mice fed HFD vs. NCD and to a lesser degree in Tg mice after HFD (Fig. 4B). By detecting the amount of glycerol derived from triglycerides by lipase activity, we also observed reduced HFD-induced accumulation of total triglyceride in heart homogenates from Tg compared with WT mice (Fig. 4C).

Lipidomic analysis of myocardial lipotoxicity. On the basis of these observations, we then performed lipidomic analysis to provide quantitative analysis of changes in myocardial lipid...
species. Using LC-MS-MS, we then analyzed myocardial contents of various lipid species, including ceramides and diacylglycerols (DAGs) (Fig. 5 and Table 2), two well-known lipotoxic metabolites known to induce insulin resistance (24, 40).

As expected, HFD induced a significant increase in total content of both types of lipid in the heart (Fig. 5 and Table 2). HFD-fed APPL1 Tg mice showed a significantly lower increase in the myocardial content of total ceramides than the...
HFD-fed APPL1 Tg mice did not develop this glucose intolerance on plasma glucose or insulin level after 16 wk (Fig. 6). There was no significant effect of APPL1 overexpression on systemic metabolic changes induced by 16 wk of HFD. Circulating plasma glucose and insulin levels were examined (Fig. 6), and we observed that HFD induced changes in plasma level of glucose and insulin as expected. We observed that these levels progressively increased over the diet duration up to 16 wk (data not shown). There was no significant effect of APPL1 overexpression on plasma glucose or insulin level after 16 wk (Fig. 6). Glucose tolerance tests showed that HFD-fed WT mice, as expected, exhibited a significantly impaired glucose clearance compared with NCD-fed WT or APPL1 Tg mice. Importantly, HFD-fed APPL1 Tg mice did not develop this glucose intolerance (Fig. 6).

On the basis of this indication of APPL1 Tg mice being protected from HFD-induced insulin resistance, together with the observations of cardiac dysfunction and altered myocardial lipid metabolism induced by HFD, we next directly examined cardiac insulin resistance. HFD-fed WT mice clearly showed development of insulin resistance determined by decreased levels of insulin-stimulated Akt phosphorylation on Thr308 in hearts of these mice (Fig. 7). A slight increase in basal Akt phosphorylation in hearts was observed after HFD. APPL1 Tg mice displayed better insulin sensitivity than WT after 16 wk of HFD. HFD induced insulin resistance at the level of IRS phosphorylation, which was not altered in APPL1 Tg mice. We next examined the metabolic significance of improved myocardial insulin sensitivity in APPL1 Tg mice by isolating adult primary cardiomyocytes from each group of mice and testing insulin sensitivity by treating these cells with various doses of insulin followed by analysis of glucose uptake. Although in Tg mice fed NCD compared with WT mice there was a trend of a small but not statistically significant increase in insulin sensitivity, a significantly improved insulin sensitivity (1 and 10 nM) as a result of APPL1 overexpression in mice fed HFD was observed in these glucose uptake experiments (Fig. 7). Since oxidative stress is another established consequence of myocardial lipotoxicity and associated with insulin resistance (20), we next measured the content of malondialdehyde (MDA) as a marker of lipid peroxidation. HFD elevated myocardial MDA content significantly in WT mice, whereas APPL1 overexpression was protective against HFD-induced oxidative stress (Fig. 7).

### DISCUSSION

Various animal and clinical studies have confirmed the close link among obesity, diabetes, and heart failure (2, 16, 44). Several contributing pathogenic factors have been implicated in diabetic cardiomyopathy, including changes in metabolism, lipid metabolism induced by HFD, we next directly examined cardiac insulin resistance. HFD-fed WT mice clearly showed development of insulin resistance determined by decreased levels of insulin-stimulated Akt phosphorylation on Thr308 in hearts of these mice (Fig. 7). A slight increase in basal Akt phosphorylation in hearts was observed after HFD. APPL1 Tg mice displayed better insulin sensitivity than WT after 16 wk of HFD. HFD induced insulin resistance at the level of IRS phosphorylation, which was not altered in APPL1 Tg mice. We next examined the metabolic significance of improved myocardial insulin sensitivity in APPL1 Tg mice by isolating adult primary cardiomyocytes from each group of mice and testing insulin sensitivity by treating these cells with various doses of insulin followed by analysis of glucose uptake. Although in Tg mice fed NCD compared with WT mice there was a trend of a small but not statistically significant increase in insulin sensitivity, a significantly improved insulin sensitivity (1 and 10 nM) as a result of APPL1 overexpression in mice fed HFD was observed in these glucose uptake experiments (Fig. 7). Since oxidative stress is another established consequence of myocardial lipotoxicity and associated with insulin resistance (20), we next measured the content of malondialdehyde (MDA) as a marker of lipid peroxidation. HFD elevated myocardial MDA content significantly in WT mice, whereas APPL1 overexpression was protective against HFD-induced oxidative stress (Fig. 7).

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oxidative stress, lipotoxicity, and insulin resistance (7, 25). Both adiponectin and insulin have been shown to play important roles in regulating cellular and structural remodeling events leading to heart failure (1, 31). Here, we investigated whether Tg mice with whole body overexpression of APPL1, an important component of adiponectin and insulin signaling, were more or less susceptible to cardiomyopathy induced using a model of chronic high-fat feeding. We focused on cellular metabolic alterations in the myocardium and the consequent changes in function of the heart. Since systemic effects of widespread APPL1 overexpression may also impact cardiac function, we examined whole body glucose homeostasis.

We first used echocardiography to examine cardiac function on a monthly basis upon high-fat feeding for up to 16 wk. In

Fig. 6. Analysis of HFD-induced alterations in peripheral insulin sensitivity. Changes of plasma glucose (A) and insulin (B) levels after 16 wk of each diet are shown (n = 7–10). IPGTT (C shows glucose excursions over time; D, quantitation of area under curve) indicated that HFD-induced impaired glucose tolerance in WT mice was as expected and that APPL1 overexpression conferred a protective effect (n = 5–8). Values are means ± SE. *Significance (P < 0.05) NCD vs. HFD; #significance (P < 0.05) WT vs. APPL1 Tg subjected to the same diet.

Fig. 7. Analysis of HFD-induced alterations in myocardial insulin sensitivity and oxidative stress. A and B: myocardial insulin signaling in heart homogenates was examined by Western blot analysis. HFD decreased insulin (2 U/kg, 5 min) -stimulated p-Akt (T308) level in WT mice. Heart tissues from APPL1 Tg mice exhibited higher insulin-stimulated p-Akt compared with WT. Analysis of p-IRS (Y612) indicated that HFD induced insulin resistance at this level, which was not altered in APPL1 TG mice. Quantification of p-Akt level shows insulin-stimulated fold increase over PBS-injected mouse hearts (n = 3–4). Values are means ± SE. *Significance (P < 0.05) NCD vs. HFD; #significance (P < 0.05) WT vs. APPL1 Tg subjected to the same diet.

C: insulin-sensitizing effect of APPL1 upon exposure to HFD was also demonstrated by in vitro glucose uptake experiments in primary cardiomyocytes isolated from each group of animals (n = 3–5). Data are presented as fold over basal level of glucose uptake from each experimental group. *Significance (P < 0.05) between basal and insulin-stimulated level; #statistical difference between WT and Tg mice fed HFD. D: myocardial oxidative stress levels determined by measuring MDA content (n = 4–5). Values are means ± SE. *Significance (P < 0.05) NCD vs. HFD; #significance (P < 0.05) WT vs. APPL1 Tg subjected to the same diet.
agreement with previous literature (16, 25), we observed that cardiomyopathy in mice induced in HFD is a progressive event, and significant cardiac dysfunction manifested at 16 wk. At that time, analysis of ejection fraction, fractional shortening, and end systolic volume indicated cardiac dysfunction in WT mice fed HFD. However, HFD did not change the cardiac function significantly, because APPL1 Tg mice demonstrated significantly less cardiac dysfunction compared with WT mice. These data suggested that moderate overexpression of this protein confers cardioprotective effects. Additionally, we studied cardiac global and regional functions using speckle tracking echocardiography, which provides accurate analysis of tissue deformation strain and motion during cardiac cycles (5). In clinical studies, it has been shown that subtle changes in strain of myocardial tissue motion are associated with cardiomyopathy and cardiovascular risk factors even with the normal cardiac structure and functional parameters (13, 30). This analysis allowed us to access myocardial tissue deformations, which included myocardial shortening in the longitudinal axis and thickening in the radial axis during systole, as measures of tissue strain and strain rate (SR). Our data collectively indicated that HFD impaired both systolic and diastolic function, especially in the radial axis (reduced strain and strain rate), with dysynchronous endocardial motion of the heart. However, moderate APPL1 overexpression alleviated these effects. Thus, echocardiography analyses clearly showed that significantly less HFD-induced cardiac dysfunction occurred in APPL1 Tg mice.

After observing the beneficial effect of APPL1 overexpression on cardiac function, we investigated the cellular mechanisms that might be responsible. Elevated circulating NEFA have been shown to lead to elevated myocardial uptake of these species (16), and their subsequent metabolism influences the development of cardiomyopathy. The HFD model of obesity and diabetes is known to induce lipid accumulation in various peripheral tissues, including the heart, and myocardial lipotoxicity is known to be a major player contributing to subsequent cardiac dysfunction (16, 25, 43, 51). Our data, derived from the commonly used approach of Oil red O staining of heart tissue sections as well as the more quantitative analysis of total TG content in heart tissue, clearly showed a significant increase in myocardial lipid accumulation in hearts subjected to HFD. A much lower extent of lipid accumulation was observed in APPL1 Tg mice. Furthermore, we used the LC-MS-MS method to analyze myocardial contents of ceramides and DAGs, two lipid metabolites known to induce insulin resistance (24, 40). Ceramides, DAGs, and TG were increased by HFD feeding, and hearts from APPL1 Tg mice showed decreased levels of these lipid metabolites compared with the hearts from WT mice. Cardiac ceramide levels correlate with lipotoxic cardiac dysfunction in various animal models such as those with cardiomyocyte-specific transgenes for PPARα, PPARγ, and acyl-CoA synthase (11, 19, 39). In addition, the fact that pharmacological inhibition of ceramide biosynthesis improved cardiac dysfunction in lipotoxic heart clearly indicates the involvement of ceramide in cardiac metabolism (34). In particular, we observed that reduced C18:0 ceramide in HFD-fed APPL1 Tg hearts correlated with improved cardiac function. Although cardiac DAG is associated with β-adrenergic response and cardiac function via PKC activation (17), we did not find any statistically significant change in HFD-fed APPL1 hearts. These results indicated that altered ceramide levels, rather than DAG, are implicated in improved cardiac function in HFD-fed APPL1 Tg mice hearts. The indication that sphingomyelin levels in HFD-fed APPL1 Tg hearts were elevated compared with WT controls indicates that fatty acids were directed toward sphingomyelin synthesis and resulted in reduced ceramide and sphingomyelin synthesis. This suggests that the regulation of ceramide/sphingomyelin balance by adiponectin or insulin deserves further study (21).

APPL1 overexpression enhanced peripheral insulin sensitivity, including in the heart. We demonstrated that, whereas hearts of WT mice on HFD for 16 wk exhibited insulin resistance, the APPL1 Tg mice hearts had a lesser extent of insulin resistance. Our data suggest that this could have occurred as a result of several mechanisms, including the lack of lipotoxic-induced, in particular ceramide-induced, insulin resistance and also reduced oxidative stress (11, 19, 20, 34, 39). Therefore, we examined the effect of APPL1 overexpression on HFD-induced cellular oxidative stress. The close relationship between diabetes and increased oxidative stress in various tissues is well established. In particular, elevated ROS levels have been reported to cause a number of adverse outcomes in the heart, such as mitochondriopathy, myocyte hypertrophy, and myocardial fibrosis, which may lead to heart failure (3). As expected, HFD elevated the level of lipid peroxidation, a measure of cellular damage induced by oxidative stress; however, APPL1 Tg mice maintained a normal level of oxidative stress under HFD conditions. Furthermore, we also found that APPL1 Tg mice exhibited higher content of glutathione (GSH), an antioxidant molecule, compared with WT when challenged by HFD (data not shown).

Simply overexpressing APPL1 is also likely to directly improve insulin sensitivity (8, 12, 36, 42); however, in our study we did not find that APPL1 Tg mice on NCD had improved insulin sensitivity. We also observed improved insulin sensitivity and metabolism in peripheral tissues. Both WT and APPL1 Tg mice showed similar body weight gains with a small but significant increase in plasma glucose levels upon HFD challenge. The relatively normal level of plasma glucose maintained even after the chronic exposure to HFD might be accounted for by the significant elevation of plasma insulin level. This observation is consistent with numerous animal studies demonstrating the relationship between HFD-induced insulin resistance and elevated insulin secretion with β-cell volume expansion (4, 45). Glucose tolerance tests showed that HFD results in impaired glucose tolerance in WT mice and that APPL1 Tg subjected to HFD had comparable glucose tolerance to NCD-fed mice. This observation could be expected based on related literature (8, 12, 36, 42) demonstrating insulin sensitizing actions of APPL1 in liver and skeletal muscle. Indeed, APPL1 levels were significantly decreased in endothelium of Zucker diabetic fatty rats (38), and a recent study suggested that chronic exercise in mice increased hepatic APPL1 expression, which, at least in part, accounted for improved insulin sensitivity (28). However, in a human study, there was higher APPL1 expression in type 2 diabetic muscle, and weight loss in these individuals was associated with reduced skeletal muscle APPL1 level (22). The limitations of mouse models that constitutively overexpress or lack a specific protein include the fact that potential compensatory effects may ensue, and this must be borne in mind when interpreting data from this and other studies. Future
studies ideally will examine the direct consequences of inducible cardiac-specific APPL1 overexpression.

In summary, here, we found that APPL1 Tg mice are protected from HFD-induced cardiac dysfunction. This is underpinned by contributory mechanisms including reduced circulating NEFA, reduced lipid accumulation, in particular ceramide, reduced oxidative stress, and improved insulin sensitivity. The overall observations support recent suggestions that targeting lipotoxicity may be at least as effective as glycemic control as a therapeutic approach for diabetic cardiomyopathy (25) and that modulation of APPL1 function may be one way to do so.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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