Loss of apoptosis repressor with caspase recruitment domain (ARC) worsens high fat diet-induced hyperglycemia in mice

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Abstract

Apoptosis repressor with caspase recruitment domain (ARC) is an endogenous inhibitor of cell death signaling that is expressed in insulin-producing β cells. ARC has been shown to reduce β-cell death in response to diabetogenic stimuli in vitro, but its role in maintaining glucose homeostasis in vivo has not been fully established. Here we examined whether loss of ARC in FVB background mice exacerbates high fat diet (HFD)-induced hyperglycemia in vivo over 24 weeks. Prior to commencing 24-week HFD, ARC−/− mice had lower body weight than wild type (WT) mice. This body weight difference was maintained until the end of the study and was associated with decreased epididymal and inguinal adipose tissue mass in ARC−/− mice. Non-fasting plasma glucose was not different between ARC−/− and WT mice prior to HFD feeding, and ARC−/− mice displayed a greater increase in plasma glucose over the first 4 weeks of HFD. Plasma glucose remained elevated in ARC−/− mice after 16 weeks of HFD feeding, at which time it had returned to baseline in WT mice. Following 24 weeks of HFD, non-fasting plasma glucose in ARC−/− mice returned to baseline and was not different from WT mice. At this final time point, no differences were observed between genotypes in plasma glucose or insulin under fasted conditions or following intravenous glucose administration. However, HFD-fed ARC−/− mice exhibited significantly decreased β-cell area compared to WT mice. Thus, ARC deficiency delays, but does not prevent, metabolic adaptation to HFD feeding in mice, worsening transient HFD-induced hyperglycemia.

Introduction

Apoptosis repressor with caspase recruitment domain (ARC), the protein product of the nucleolar protein 3 (Nol3) gene, is an endogenous, multifunctional inhibitor of cell death (Koseki et al. 1998, Nam et al. 2004, Davis et al. 2013, Jang et al. 2015). ARC’s N-terminal caspase recruitment domain (CARD) has been shown to interact with and inhibit numerous effectors of cell death (Geertman et al. 1996, Koseki et al. 1998, Davis et al. 2013, Jang et al. 2015). Originally, ARC was shown to interact with caspases 2 and 8 and prevent cardiac and skeletal muscle cell death (Koseki et al. 1998, Jo et al. 2004). Subsequent work demonstrated that ARC inhibits other
effectors of cell death including Bax, Bad, and p53 and that inhibition of these molecules was similarly mediated through interactions with ARC’s CARD (Gustafsson et al. 2004, Li et al. 2008, Davis et al. 2013). Moreover, ARC has been shown to diminish TNFα-induced regulated necrosis, further illustrating its multifunctional role in mitigating cell death (Kung et al. 2014). ARC has also been identified in insulin-producing islet β cells (McKimpson et al. 2013). Given its presence in β cells, which produce insulin to regulate glucose utilization, and in skeletal muscle, a primary tissue for peripheral glucose uptake and disposal, ARC is well-placed to influence whole-body glucose homeostasis.

In vitro studies have demonstrated that ARC reduces β-cell death in response to palmitate and chemically induced endoplasmic reticulum (ER) stress (McKimpson et al. 2013) as well as to endogenous islet amyloid formation (Templin et al. 2017), these being β-cell stressors associated with the pathogenesis of type 2 diabetes (T2D) (Hull et al. 2004, Özcan et al. 2004, Jurgens et al. 2011, Back & Kaufman 2012). Despite its potential to prevent β-cell loss, few studies have examined ARC’s role in maintaining whole-body glucose homeostasis in vivo. McKimpson and colleagues showed that genetic ARC deficiency did not alter islet morphology or impair glucose homeostasis in vivo in C57BL/6 mice fed with a normal chow diet (NCD) (McKimpson et al. 2017). However, loss of ARC in leptin-deficient (ob/ob) mice that are hyperphagic, obese, insulin resistant, and hyperglycemic was found to increase β-cell death, impair glucose-stimulated insulin secretion (GSIS), and exacerbate hyperglycemia (McKimpson et al. 2017). These findings were the first to show that ARC is required for β-cell survival and sufficient insulin secretion in vivo in response to a genetic model of diabetogenic stress.

The role of ARC in β-cell adaptation to high fat diet (HFD) feeding, a physiologically relevant metabolic stress, remains unknown. HFD feeding is a metabolic challenge that leads to weight gain, insulin resistance, and subsequent β-cell compensation to increase insulin secretion and maintain glucose homeostasis (Mosset et al. 2015, Gupta et al. 2017, Paschen et al. 2019). This challenge is commonly used to test β-cell functional capacity and glucose homeostasis when examining metabolic phenotypes in knockout mouse models (Surwit et al. 1988, Ikemoto et al. 1996, Asghar et al. 2006, Heydemann 2016, Hull et al. 2017). We hypothesized that loss of ARC would diminish β-cell compensation in response to HFD feeding, leading to impaired insulin secretion and worsened hyperglycemia in ARC-deficient mice. To address this hypothesis, we examined glycemic status, β-cell function, and β-cell area in mice with or without whole-body genetic loss of ARC that were fed with a NCD or a HFD for 24 weeks.

Materials and methods

Animals

Heterozygous ARC+/− mice on an FVB background were obtained from Richard N. Kitis (Albert Einstein College of Medicine, Bronx, NY) and maintained as ARC+/+ (WT) and ARC−/− breeding lines for this study. All mice used in this study were less than five generations post-divergence from ARC−/− mice. Mice were housed and bred in a specific pathogen-free vivarium at VA Puget Sound Health Care System (VAPSHCS) with access to food and water ad libitum. Male mice of the indicated ages were studied. All procedures were approved by the VAPSHCS Institutional Animal Care and Use Committee.

Genotyping

Polymerase chain reaction (PCR) of tail DNA using specific primers was used to determine the presence of Nol3 WT and mutant alleles. Amplification of these alleles was performed using the following primers to generate 400 bp WT products and 600 bp mutant products: forward, 5′-TTCCCTACGGTTTGGTACAGGCA-3′; reverse, 5′-TGGTACACCAAGCCGAGTGATT-3′; mutant, 5′-CCTGCCAGAGCTTGAACCCCA-3′.

Islet isolation and quantitative real-time reverse transcription PCR

Islets from 8- to 12-week-old WT and ARC−/− mice were isolated by collagenase P digestion, then recovered overnight in RPMI 1640 medium containing 10% fetal bovine serum, 1 mM sodium pyruvate, 100 μg/mL penicillin, 100 μg/mL streptomycin, and 11.1 mM glucose. The following day, islets from each genotype were lysed and total RNA was recovered using the High Pure RNA Isolation Kit (Roche). RNA was then reverse transcribed and subjected to quantitative real-time reverse transcription PCR (qRT-PCR). Loss of Nol3 mRNA in ARC−/− islets was confirmed using Taqman probes (Thermo Fisher Scientific) to quantify expression of Nol3 mRNA (Mm07299538_g1) and 18S rRNA (HS99999901_s1), with data normalized to 18S rRNA levels and expressed as fold relative to WT mice. All qRT-PCR data points represent means of triplicate determinations.
In vivo studies

NCD-fed mice consumed food containing 21% kcal from fat (LabDiet, Picolab 5058, St. Louis, MO) from weaning until analysis at 16 or 35 weeks of age. HFD-fed mice consumed food containing 60% kcal from fat (Research Diets, #D12492N, New Brunswick, NJ) for 24 weeks, starting at 10–12 weeks of age and ending at 34–36 weeks of age.

Tissue collection, histology, and quantitative microscopy

Mice were euthanized, and pancreas, liver, epididymal adipose, inguinal adipose, brown adipose, gastrocnemius muscle, and vastus medialis muscle tissue were excised, and mass was recorded. After excision, pancreas sections were fixed in 4% (wt/vol) phosphate-buffered paraformaldehyde, processed, and embedded in paraffin. To visualize β cells, 4-µm pancreas sections were cut and stained with mouse monoclonal anti-insulin antibody (1:2018, 1:2000, Sigma-Aldrich) followed by goat anti-mouse Alexa Fluor 488 (#A-11001, Life Technologies, 1:200). To visualize α cells, sections were incubated with rabbit monoclonal anti-glucagon antibody (#Ab92517, Abcam; 1:100) followed by goat anti-rabbit Alexa Fluor 546 (#A-11010, Life Technologies, 1:200). Sections were mounted with polyvinyl alcohol containing Hoechst 33258 to visualize nuclei. Islet area, β-cell area, and α-cell area were imaged with a fully automated NiE microscope (Nikon) equipped with NIS Elements High Content software and automated JOBS-based acquisition and analysis algorithms (NIS Elements, Nikon). Islet images were captured at 20× magnification, then insulin- and glucagon-positive area was quantified using automated analysis software (NIS Elements, Nikon). Binary layers for insulin and glucagon were used in post-processing steps to calculate total islet area, then insulin- and glucagon-positive areas were calculated on a per islet basis. All islets visible on each section were quantified, with an average of 34 ± 2 islets analyzed per replicate per condition. Ki67 and cleaved caspase 3 staining and analysis was performed by automated immunohistochemistry through the University of Washington Diabetes Research Center’s Cellular and Molecular Imaging Core using antibodies specific to Ki67 (clone D3BS, Cell Signaling, #12202) and cleaved caspase 3 (clone D3E9, Cell Signaling, #9579). Antibody complexes were visualized using 3,3’-diaminobenzidine, and slides were counterstained with hematoxylin. Slides were scanned with a 20× objective using a NanoZoomer Digital Pathology System (Hamamatsu City, Japan), and digital images were imported into Visiopharm software (Hoersholm, Denmark) for analysis. The Visiopharm software was trained to label positive staining for Ki67, cleaved caspase 3, and background counterstain (hematoxylin) using a project-specific configuration. Observers were blinded to genotype of the pancreas section for all quantitative microscopy.

Intravenous glucose tolerance tests

Mice were fasted for 16 h, then anesthetized with intraperitoneal sodium pentobarbital for the duration of the procedure. Under anesthesia, a catheter was placed in the carotid artery, and glucose was administered at a dose of 1 g/kg body weight, after which the catheter was flushed with heparinized saline. Blood was sampled 5 min before (fasting sample) and 2, 5, 10, 20, 30, and 45 min after glucose administration for measurement of plasma glucose and insulin. Following the 10-, 20-, and 30-min blood draws, red blood cells were resuspended in heparinized saline and reinfused via carotid artery to prevent hypovolemic/anemic shock. First- and second-phase insulin secretion were calculated as the ratio of incremental area under the curve (iAUC) of insulin over iAUC of glucose for 0–5 min and for 5–45 min, respectively.

Insulin tolerance tests

Mice were fasted for 4 h and a baseline blood sample was taken via tail vein. Insulin was injected intraperitoneally at a dose of 1 U/kg body weight, and plasma glucose was measured at 0, 15, 30, and 60 min after insulin administration using a handheld glucometer (Accu-Chek, Roche) on blood samples obtained via tail tip.

Glucose and insulin assays

Plasma was separated from blood by centrifugation and stored at −30°C for subsequent analysis. Plasma glucose concentrations were determined using a plate-based colorimetric assay utilizing the glucose oxidase method, and plasma insulin concentrations were determined using an ultrasensitive mouse insulin ELISA assay (#80-INSMSU-E10, ALPCO, Salem, NH, USA). Each measure represents the mean of triplicate determinations.

Statistical analyses

Normality of data distribution in each data set was tested using the D’Agostino-Pearson normality test. Normally distributed data were analyzed using two-tailed Student’s t-tests, while non-normally distributed data were analyzed using non-parametric tests.
using Mann–Whitney U tests. Longitudinal data were analyzed using repeated measures two-way ANOVA, with significant results followed by Fisher’s least significant difference post hoc analysis of specific time points. No outliers were excluded from the data sets presented. Statistical analyses were conducted with GraphPad Prism 8 software (GraphPad). Data are presented as mean ± standard error, with a value of $P < 0.05$ considered significant.

Results

**ARC$^{-/-}$ mice fed with an NCD do not display alterations in islet, β-cell, or α-cell area at 16 or 35 weeks of age**

We first examined WT and ARC$^{-/-}$ mice fed with an NCD. At 16 weeks of age, we observed significantly reduced body weight in ARC$^{-/-}$ mice (Fig. 1A), but no difference in average islet area (Fig. 1B), β-cell area (Fig. 1C), or α-cell area (Fig. 1D) was found between WT and ARC$^{-/-}$ mice. At 35 weeks of age, we found a trend toward reduced body weight in ARC$^{-/-}$ mice (Fig. 2A, $P=0.09$) and again observed no difference in average islet area (Fig. 2B), β-cell area (Fig. 2C), or α-cell area (Fig. 2D) between genotypes. However, WT mice displayed a significant increase in islet size from 16 to 35 weeks (from 14,259 ± 1786 µm$^2$ to 25,328 ± 3547 µm$^2$, $P = 0.04$), whereas islet size in ARC$^{-/-}$ did not differ over this period (13,626 ± 1137 µm$^2$ vs 18,304 ± 2842 µm$^2$, $P = 0.22$). Loss of ARC mRNA expression (Nol3) was confirmed in ARC$^{-/-}$ isolated islets (Fig. 1E). Representative images of islets from 16- (Fig. 1F) and 35- (Fig. 2E) week-old WT and ARC$^{-/-}$ mice are displayed.

**ARC$^{-/-}$ mice fed with an NCD display normal glucose homeostasis and insulin secretion at 35 weeks of age**

Thirty-five-week-old ARC$^{-/-}$ mice exhibited no difference in fasting plasma glucose (Fig. 3A) or fasting plasma insulin (Fig. 3B) compared to WT mice. Following intravenous glucose administration, neither plasma glucose (Fig. 3C) nor insulin (Fig. 3D) differed in ARC$^{-/-}$ compared to WT mice. First-phase and second-phase insulin secretion were not different between 35-week-old WT and ARC$^{-/-}$ mice, as measured by IAUC of insulin over IAUC of glucose from 0 to 5 (Fig. 3E) or 5 to 45 (Fig. 3F) min, respectively.

**ARC$^{-/-}$ mice exhibit worsened HFD-induced hyperglycemia**

Next, a 24-week HFD feeding study of WT and ARC$^{-/-}$ mice ending at 35 weeks of age was started. When analyzed over the full 24-week HFD feeding period, ARC$^{-/-}$ mice exhibited higher plasma glucose levels compared to WT mice (Fig. 4A, $P < 0.05$). Non-fasting plasma glucose was not different between groups prior to HFD feeding (Fig. 4A). From baseline, ARC$^{-/-}$ mice displayed a greater increase in plasma glucose after 4 weeks of HFD feeding compared to WT mice (55.8 ± 20.5 mg/dL vs 2.2 ± 9.4 mg/dL, $P = 0.03$, Fig. 4B). Plasma glucose increased to equivalent absolute levels in ARC$^{-/-}$ and WT mice after 8 weeks of HFD feeding. After 16 weeks of HFD feeding, ARC$^{-/-}$ mice displayed significantly higher absolute plasma glucose (244.0 ± 8.5 mg/dL vs 178.0 ± 6.4 mg/dL, $P < 0.001$, Fig. 4A) and change in plasma glucose (62.62 ± 13.99 mg/dL vs −22.60 ± 8.04 mg/dL, $P < 0.001$, Fig. 4B) compared to WT mice. After 24 weeks of HFD, the observed difference in plasma glucose was
no longer present (Fig. 4A and B), although ARC−/− mice exhibited a trend for increased change in plasma glucose at this time (18.0 ± 12.9 vs –8.4 ± 6.1 mg/dL, P = 0.08, Fig. 4B).

**ARC−/− mice display reduced body weight but equivalent weight gain in response to HFD**

At 10–12 weeks of age before HFD feeding began, ARC−/− mice had lower body weight than WT mice (26.2 ± 0.6 g vs 30.6 ± 0.8 g, P < 0.001, Fig. 4C), and this difference in body weight remained until the end of the 24-week HFD study (40.9 ± 1.7 g vs 46.8 ± 1.6 g, P = 0.02, Fig. 4C). However, the degree of body weight gain due to HFD was not different between WT and ARC−/− mice (Fig. 4D). Vastus medialis muscle, epididymal adipose and inguinal adipose tissue mass were decreased in ARC−/− compared to WT mice (Fig. 4E and F), but no differences in pancreas, liver, gastrocnemius muscle or brown adipose tissue mass were observed between genotypes (Fig. 4E and F).

**ARC−/− mice fed with a HFD display reduced β-cell area but no differences in α-cell area, islet area, proliferation or apoptosis compared to WT mice after 24 weeks of HFD**

Following 24 weeks of HFD feeding, ARC−/− mice had decreased β-cell area compared to WT mice (72.69 ± 1.83% vs 77.32 ± 1.27% insulin/islet area, P = 0.04, Fig. 5A). No difference in α-cell area existed at this time point (3.33 ± 0.73% vs 2.94 ± 0.44% glucagon/islet area, P = 0.64, Fig. 5B), but a trend toward reduced islet area was observed in ARC−/− mice (28,111.5 ± 4355.33 µm² vs 39,292.7 ± 4021.7 µm², P = 0.07, Fig. 5C). Ki67 expression tended to be increased in ARC−/− pancreas sections (P = 0.06, Fig. 5D), while cleaved caspase 3 expression was unaltered (P = 0.27, Fig. 5E) following HFD feeding.

**ARC−/− mice display unaltered glucose homeostasis, insulin secretion, and insulin sensitivity after 24 weeks of HFD**

At the end of the HFD feeding period, ARC−/− mice exhibited no difference in fasting plasma glucose (Fig. 6A, 127.5 ± 12.0 mg/dL vs 117.3 ± 6.9 mg/dL, P = 0.49) or fasting plasma insulin (Fig. 6B, 181.48 ± 33.65 pM vs 221.57 ± 63.39 pM, P = 0.57) compared to WT mice. Upon challenge with intravenous glucose, neither plasma glucose (Fig. 6C) nor insulin (Fig. 6D) differed in ARC−/− compared to WT mice. The incremental area under the curve (iAUC)of insulin over iAUC of glucose was not significantly different from 0 to 5 min (Fig. 6E, 2.90 ± 0.35 vs 4.13 ± 0.86, P = 0.19) or from 5 to 45 min (Fig. 6F, 1.96 ± 0.40 vs 2.76 ± 0.80, P = 0.37). Furthermore, no difference in insulin sensitivity was observed between WT and ARC−/− mice after the HFD feeding period as measured by intraperitoneal insulin tolerance test (Fig. 6G and H).
In contrast to this earlier study, when we challenged ARC−/− mice with HFD for 24 weeks exhibited accelerated and prolonged hyperglycemia along with lower body weight throughout this interval. By the end of the HFD feeding period, however, hyperglycemia had resolved in ARC−/− mice. Thus, our data indicate that ARC deficiency delays, but does not prevent, metabolic adaptation to HFD feeding in mice, worsening transient HFD-induced hyperglycemia.

We found that ARC−/− mice on a FVB background fed with an NCD displayed reduced body weight but unaltered β-cell area and glucose homeostasis compared to their WT counterparts. In contrast, ARC−/− mice challenged with HFD for 24 weeks exhibited accelerated and prolonged hyperglycemia along with lower body weight throughout this interval. By the end of the HFD feeding period, however, hyperglycemia had resolved in ARC−/− mice. Thus, our data indicate that ARC deficiency delays, but does not prevent, metabolic adaptation to HFD feeding in mice, worsening transient HFD-induced hyperglycemia.

While this study is the first to examine how loss of ARC impacts metabolic adaptation to HFD feeding, McKimpson and colleagues previously challenged ARC−/− mice using the ob/ob model of leptin deficiency, marked obesity, insulin resistance, and hyperglycemia (McKimpson et al. 2017). In both our study and theirs, unchallenged ARC−/− mice were found to exhibit normal glucose metabolism and islet morphology. However, when ob/ob and ARC−/− mice were crossed, the resulting mice (ob/ob;ARC−/−) demonstrated significantly increased non-fasting glucose, reduced β-cell area, glucose intolerance, impaired GSIS, and reduced insulin sensitivity compared to ob/ob mice alone (McKimpson et al. 2017). In contrast to this earlier study, when we challenged ARC−/− mice with the milder and more physiologically relevant metabolic stress of HFD feeding, we observed a less severe metabolic phenotype. Similar to ob/ob;ARC−/− mice, our ARC−/− mice exhibited significantly increased non-fasting glucose and reduced terminal β-cell area compared to their WT counterparts. However, HFD-induced hyperglycemia resolved in both WT and ARC−/− mice by the end of the 24-week HFD feeding period, and at this time, ARC−/− mice exhibited unaltered glucose tolerance, GSIS, and insulin sensitivity. Thus, the differing metabolic challenges applied to ARC−/− mice in these studies likely resulted in different phenotypic outcomes, with ARC−/− mice exhibiting a delayed but intact ability to adapt metabolically to HFD feeding. Together, these studies indicate that ARC deficiency alone does not cause β-cell loss or hyperglycemia in vivo, but loss of ARC exacerbates hyperglycemia in response to metabolic challenges such as HFD feeding and genetically-induced obesity and insulin resistance.

We found that ARC−/− mice exhibited significantly reduced body weight compared to WT mice both at baseline and over 24 weeks of HFD feeding. We also observed that β-cell area was reduced in ARC−/− mice at the end of HFD feeding, a time when plasma glucose, glucose tolerance, and GSIS did not differ from WT mice. Additionally, our

Discussion

ARC plays an important role in regulating cell death signaling (Koseki et al. 1998, Neuss et al. 2001) in multiple cell types, including insulin-producing islet β cells (McKimpson et al. 2013, 2017, Templin et al. 2017). Given that β-cell loss can contribute to the development of insulin secretory dysfunction and hyperglycemia (Butler et al. 2003, Rahier et al. 2008), ARC has attracted attention as a molecule that could be targeted to maintain β-cell survival and function to prevent diabetes. In this study, a mouse model of whole-body genetic ARC deficiency (ARC−/−) was used to examine, for the first time, whether loss of ARC impairs metabolic adaptation to the physiologically relevant stress of HFD feeding and, if so, whether β-cell

Figure 3
Thirty-five-week-old ARC−/− mice fed with a normal chow diet (NCD) display unaltered fasting and glucose-stimulated plasma glucose and insulin levels compared to wild type (WT) mice. (A) Fasting plasma glucose and (B) fasting plasma insulin were not different between 35-week-old WT (open squares) and ARC−/− (closed circles) mice fed with an NCD. Intravenous glucose- (1 g/kg body weight) stimulated (C) plasma glucose and (D) plasma insulin were not different between genotypes. In response to intravenous glucose stimulation, the change in insulin as a function of the change in glucose (IAUC insulin/IAUC glucose) was not different between WT (open squares) and ARC−/− mice (closed circles) (E) from 0 to 5 or (F) from 5 to 45 min. n = 6–7/genotype.
data show that cleaved caspase 3, a marker of apoptosis, was not increased in ARC−/− pancreas sections at this time point, while Ki67, a marker of proliferation, tended to be increased. Thus, we do not believe the reduced β-cell area observed in ARC−/− mice in our study is pathological but rather the result of lower body weight and reduced β-cell secretory demand elicited from HFD feeding.

The body weight phenotype identified in ARC−/− mice in this study was not observed in the McKimpson and colleagues study of of ARC−/− mice (McKimpson et al. 2017). This difference between studies could have arisen due to the different genetic backgrounds used in these studies, with this study examining mice on an FVB background and the previous work using mice on a C57Bl/6 (B6) background. While B6 mice are prone to obesity, insulin resistance, and glucose intolerance, FVB mice (also known as 129) are relatively resistant to these phenotypes (Almind & Kahn 2004, Champy et al. 2008, Tschöp et al. 2011). This resistance to obesity in FVB compared to B6 mice is related to higher metabolic rate, while caloric intake on a HFD is similar between backgrounds (Almind & Kahn 2004). Therefore, it is possible that effects of ARC deficiency on metabolic rate or energy expenditure were more pronounced in our study using FVB mice and that these effects underly the body weight phenotype we observed. Although we did not collect food intake data in this study, McKimpson and colleagues previously found that food intake was increased by ~30% in ARC−/− mice compared to mice with intact ARC expression on an ob/ob background (McKimpson et al. 2017), suggesting reduced food intake is not likely to explain the lower body weight observed in ARC−/− mice in our study.

Several different HFD experimental designs have been used to induce metabolic dysfunction in mice, with these designs eliciting varying degrees of insulin resistance, hyperglycemia, and insufficient insulin secretion (Winzell & Ahrén 2004, Mosser et al. 2015). Over a period of months, metabolic adaptation to the diet takes place so that
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This adaptation includes expansion of β-cell mass and function to increase insulin secretion and restore plasma glucose levels to normal (Ahrén et al. 2010, Gupta et al. 2017). We observed that HFD-induced hyperglycemia was accelerated and prolonged in ARC−/− mice and that resolution of this hyperglycemia occurred after 24 weeks of HFD feeding vs after 16 weeks in WT mice. ARC−/− mice also displayed decreased β-cell area after 24 weeks of HFD compared to WT mice. Although we did not measure plasma insulin after 16 weeks of HFD, it appears plausible that loss of ARC in the β-cell impaired expansion of β-cell mass and function in response to HFD and contributed to the worsened hyperglycemia observed in ARC−/− mice at this time point. However, at completion of HFD feeding, ARC−/− mice exhibited unaltered GSIS, and when compared to NCD-fed mice of the same age, HFD increased maximal intravenous GSIS by 39% in WT mice vs 35% in ARC−/− mice. Given the similar stimulation of insulin responses by HFD between genotypes, our data do not suggest failed β-cell compensation in ARC−/− mice at the end of the study.

One link between ARC’s mechanism of action and β-cell adaptation to HFD is found in ER homeostasis. As demand for β-cell insulin production increases, cells undergo an adaptive response to facilitate increased insulin biosynthesis in part by expanding ER size (Marchetti et al. 2007), and this process has been shown to occur in association with obesity and β-cell compensation (Orikurede et al. 2013). When this adaptive response is inadequate to meet insulin demand, ER stress signaling initiates cell death pathways, and ARC is involved in this process (McKimpson et al. 2013, 2017). Previous in vitro studies showed that ARC overexpression reduces β-cell death and ARC knockdown increases β-cell death in response to thapsigargin-induced ER stress (McKimpson et al. 2013). Although we have not assessed ER stress in ARC−/− islets in this study, we believe ARC’s role in maintaining β-cell ER homeostasis could have contributed to the transient worsening of HFD-induced hyperglycemia observed in ARC−/− mice.

Figure 5

Thirty-five-week-old ARC−/− mice fed with a HFD for 24 weeks display reduced β-cell area but no differences in α-cell area, islet area, proliferation or apoptosis compared to wild type (WT) mice. Following the HFD feeding study period, (A) β-cell area (insulin/islet area) was reduced in ARC−/− (closed circles) vs WT, open squares) mice. (B) α-cell area (glucagon/islet area), (C) average islet area, (D) Ki67 area, and (E) cleaved caspase 3 area were not significantly different between WT (open squares) and ARC−/− (closed circles) mice. n = 12–15 genotype. *p < 0.05.
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Has been reported in skeletal muscle (Koseki et al. 1998, Davis et al. 2013). One would hypothesize that decreased adipose tissue mass in ARC−/− mice would have resulted in improved insulin sensitivity and glucose tolerance, but we did not observe such improvements in this study. Although studies have shown that ARC is expressed in white and brown adipose tissue depots (Rosell et al. 2014, Liu et al. 2015, Min et al. 2019), relatively little is known about the role of ARC in adipose tissue. Thus, it is possible that loss of ARC in adipose tissue impacts glucose homeostasis. Additional studies utilizing models of tissue-specific ARC deficiency are needed to understand ARC’s role in regulating metabolism in insulin-sensitive peripheral tissues.

In summary, we demonstrated that unchallenged whole-body ARC−/− mice on an FVB background exhibit reduced body weight but no alterations in islet morphology, glucose tolerance, or GSIS compared to WT mice in vivo. In contrast, when challenged with 24 weeks of HFD feeding, ARC−/− mice displayed decreased body weight but exhibited accelerated and prolonged hyperglycemia compared to WT mice. By the end of the study, ARC−/− mice had undergone metabolic adaptation to HFD feeding similar to WT mice, with no alterations in plasma glucose or plasma insulin observed in either the fasted or glucose-stimulated state, but with decreased β-cell area. Our study shows that ARC promotes early metabolic adaptation to HFD feeding and as such is a molecule that could be targeted to maintain glucose homeostasis in the setting of T2D.

Figure 6
Thirty-five–week-old ARC−/− mice fed with a high fat diet (HFD) for 24 weeks display unaltered fasting and glucose-stimulated plasma glucose and insulin concentrations, and unaltered insulin sensitivity compared to wild type (WT) mice. Following 24 weeks of HFD feeding, (A) fasting plasma glucose and (B) fasting plasma insulin were not different between WT (open squares) and ARC−/− (closed circles) mice. Intravenous glucose (1 g/kg body weight)-stimulated (C) plasma glucose and (D) plasma insulin were not different between genotypes. In response to intravenous glucose stimulation, the change in insulin as a function of the change in glucose (iAUC insulin/iAUC glucose) was not different between WT (open squares) and ARC−/− (closed circles) mice (E) from 0 to 5 or (F) from 5 to 45 min. (G) Insulin administered at a dose of 1 U/kg body weight reduced absolute plasma glucose levels to similar degrees in WT (open squares) and ARC−/− (closed circles) mice. (A–F) n = 9–10/genotype, (G–H) n = 12–13/genotype.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
A T T participated in study design, performed research, analyzed and interpreted data, and wrote the manuscript. C W S, M F H and N E participated in study design, performed research, analyzed and interpreted data, and revised/approved the manuscript. R N K, R L H, S Z and S E K participated in study design, analyzed and interpreted data, and revised/approved the manuscript. A T T and S E K are responsible for the integrity of the work as a whole.
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