Original Article

Suppressed Insulin Signaling and Increased Apoptosis in *Cd38*-Null Islets

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CD38 is a multifunctional enzyme capable of generating metabolites that release Ca^{2+} from intracellular stores, including nicotinic acid adenine dinucleotide phosphate (NAADP). A number of studies have led to the controversial proposal that CD38 mediates an alternate pathway for glucose-stimulated insulin release and contributes to the pathogenesis of diabetes. It has recently been shown that NAADP mediates Ca^{2+} mobilization by insulin in human pancreatic β-cells. In the present study, we report altered Ca^{2+} homeostasis and reduced responsiveness to insulin, but not glucose, in *Cd38*$^{-/-}$ β-cells. In keeping with the antiapoptotic role of insulin signaling, *Cd38*$^{-/-}$ islets were significantly more susceptible to apoptosis compared with islets isolated from littermate controls. This finding correlated with disrupted islet architecture and reduced β-cell mass in *Cd38*$^{-/-}$ mice, both in the context of a normal lab diet and a high-fat diet. Nevertheless, we did not find robust differences in glucose homeostasis in vivo or glucose signaling in vitro in *Cd38*$^{-/-}$ mice on the C57BL/6 genetic background, in contrast to previous studies by others of *Cd38* knockout mice on the ICR background. Thus, our results suggest that CD38 plays a role in novel antiapoptotic signaling pathways but does not directly control glucose signaling in pancreatic β-cells. *Diabetes* 55: 2737–2746, 2006

CD38 is part of an enzyme family with suggested roles in B-cell chronic lymphocytic leukemia, innate and adaptive immunity, smooth muscle contractility, and bone remodeling (1–8). This multifunctional enzyme is capable of generating cyclic ADP ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) from $NAD(P)^+$. Both of these metabolites mobilize Ca^{2+} from intracellular stores (9,10). Recent studies (11–14) point to a critical role for NAADP signaling in β-cell function, implicating CD38 or a related enzyme. We have demonstrated a direct role for NAADP in the initiation of insulin-stimulated Ca^{2+} signals in human β-cells (11), whereas others have implicated NAADP in MIN6 cell glucose-stimulated insulin release (12–14). We and others have found that insulin signaling in β-cells has important consequences, including the regulation of protein synthesis (15), apoptosis (J.D.J. et al., manuscript submitted), and β-cell mass (16,17). Although these findings point to a possible role for CD38 in insulin or glucose signaling in β-cells, previous studies have produced equivocal results.

Okamoto and colleagues (18–20) proposed that glucose-induced ATP generation inhibits the hydrolysis of cADPR by CD38, thereby increasing the amount of cADPR available to mobilize intracellular Ca^{2+} stores gated by the cADPR-sensitive ryanodine receptor (RyR2) complex. In support of this hypothesis, *Cd38*$^{-/-}$ mice on the outbred ICR genetic background (ICR-*Cd38*$^{-/-}$) had reduced insulin secretion from pancreatic islets and increased blood glucose levels (20), whereas mice overexpressing a *Cd38* transgene were protected from diabetes (19,20). However, subsequent experiments failed to show an acute effect of cADPR inhibitors on glucose signaling (21). Moreover, we recently showed that blocking RyR with ryanodine had no effect on glucose-stimulated insulin release from human islets (22), in apparent contrast to the situation in the MIN6 β-cell line (13). Perhaps the effects of CD38 on glucose homeostasis are indirect.

However, other evidence also points to a link between CD38 and diabetes. In one report (23), a missense mutation in CD38 was associated with diabetes in a small number of Japanese patients, although this was not seen in a European study (24). Diabetic *ob/ob* mice have dramatically reduced CD38 mRNA expression (25). Moreover, treatments that modulate CD38 activity have been shown to affect β-cell function (18,26,27). Antibodies against CD38 are markers of autoimmunity against the β-cell in both type 1 and type 2 diabetes (24,28–33), and several CD38 antibodies have agonistic properties (31–33), increasing insulin secretion acutely (32) but decreasing islet survival and function in long-term culture (33). Interestingly, we have recently shown that RyR2 is involved in β-cell apoptosis in vitro (34). Although cross-linking of CD38 with antibodies has been shown to activate prosurvival pathways in lymphocytes (2,35), to our knowledge no direct evidence that CD38 ablation leads to enhanced apoptosis has been presented.

The purpose of the present study was to further assess the role of CD38 in the regulation of β-cell function and specifically to determine whether it plays any role in apoptosis and insulin signaling in the β-cell. Our results

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Received for publication 7 November 2005 and accepted in revised form 7 July 2006.

cADPR, cyclic ADP ribose; NAADP, nicotinic acid adenine dinucleotide phosphate; RyR2, ryanodine receptor.

DOI: 10.2337/db05-1455

show that CD38 has an antiapoptotic function in pancreatic islets rather than a direct involvement in glucose signaling. We suggest possible mechanisms for the antiapoptotic effects of CD38, including a role in generating Ca^{2+} signals stimulated by insulin and the regulation of basal cytosolic Ca^{2+} levels.

RESEARCH DESIGN AND METHODS

Drugs and solutions. Reagents were from Sigma (St. Louis, MO), unless otherwise indicated. Carbachol was from Calbiochem (La Jolla, CA). Stocks were dissolved <1,000-fold. Recombinant human insulin was dissolved directly into solutions. Single-cell Ca^{2+} imaging was performed in Ringer's solution containing 5.5 mmol/l KCl, 2 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 20 mmol/l HEPES, 141 mmol/l NaCl, and 3 mmol/l glucose.

Animals and in vivo studies. The generation and characterization of *Cd38*$^{-/-}$ mice have been previously described (5). Animals used in these experiments were backcrossed for 12 generations to C57BL/6J (3), and littermate controls were used throughout. Mice were genotyped by flow cytometry of blood B-cells using phycoethrin-conjugated monoclonal anti-mouse CD38 (NIMR clone; Southern Biotechnology, Birmingham, AL) and fluorescein isothiocyanate–conjugated anti-mouse CD45R (eBiosciences, San Diego, CA) as described (5). Except where indicated otherwise, male mice were used between 3 and 6 months of age. Intraperitoneal glucose tolerance tests were performed after a 4-h fast, with tail vein blood sampled at 0, 30, 60, and 120 min after injection of dextrose (2 g/kg body wt). Insulin tolerance tests were performed by injection of insulin (0.1 units/kg body wt) after a 12-h fast. Some mice were fed a high-fat diet (TD 88137, total fat content of 6%; Harlan, Madison, WI) from weaning. Both genotypes gained significant weight to a similar extent on high-fat diets. High-fat feeding resulted in insulin resistance in both wild-type and *Cd38*$^{-/-}$ mice, as assessed by insulin tolerance test (see below). All procedures were approved by animal studies committees.

Mouse islet isolation by filtration. The islet isolation technique was a modification of the technique originally described by Lacy and Kostianovsky (36), with a filtration modification described by Salvalaggio et al. (37). Briefly, pancreata were distended by injecting 3 ml collagenase (0.375 mg/ml Sigma Type XI) in Hank's solution (137 mmol/l NaCl, 5.4 mmol/l KCl, 4.2 mmol/l NaH_2PO_4, 4.1 mmol/l KH_2PO_4, 10 mmol/l HEPES, 1 mmol/l $MgCl_2$, and 5 mmol/l glucose). The pancreas was removed and incubated in collagenase solution for 13 min in a 37°C water bath. The resulting disrupted pancreas was washed three times with Hank's solution with 1 mmol/l $CaCl_2$ followed by centrifugation (2,000*g*). Islets were resuspended in RPMI-1640 media (Invitrogen/Life Technologies, Carlsbad, Ca) and then poured onto a prewet 70-μm nylon mesh cell strainer (BD Biosciences, San Jose, CA). The captured islets were then rinsed into a dish with complete RPMI-1640 containing 10% fetal bovine serum and hand picked. Using this method, we are able to obtain, on average, 250 islets per mouse. These islets showed reduced apoptosis and improved insulin release compared with islets isolated using Ficoll. Improved islet engraftment has been noted by others using filtration in islet transplantation studies (37).

Ca^{2+} imaging. As described previously (11,38), islets were gently dispersed into single cells, plated on coverslips in RPMI-1640 media (containing 5.5 mmol/l glucose and 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FCS; pH 7.4 with NaOH) and maintained at 37°C, 5% CO_2, and saturated humidity. Dispersed islet cell cultures (2–4 days old) containing ~80 β-cells were imaged using Fura-4F-AM (770 nmol/l $Ca^{2+}K_d$; Molecular Probes, Eugene, OR), as described (11,38). Coverslips, placed in a narrow 32°C chamber (~300 μl) on the stage of an IX70 microscope (Olympus, Tokyo, Japan), were continuously perifused with preheated solutions to maximize control over the contents of the bath (especially ambient insulin levels). Significant Ca^{2+} responses were defined as having at least one 1-min time period with a mean $[Ca^{2+}]_c$ that was >2 SDs over the mean $[Ca^{2+}]_c$ of the 5-min pretreatment period. Ca^{2+} responses were quantified as maximal amplitude above baseline and integrated area. Unless indicated otherwise, the number of replicates reported in the figure legends and text represents the total number of islet cells in imaging experiments.

Immunofluorescence, β-cell mass, and BrdU analysis. For β-cell mass and islet architecture analysis, pancreata were fixed overnight in Bouin's solution and embedded in paraffin using standard techniques as previously described (38). For β-cell mass studies, pancreatic tissue sections were stained red with primary insulin antibody/AEC and counterstained with hematoxylin (Zymed, San Francisco, CA). Slides were imaged using a 1.25× objective on an Olympus BX41 microscope and a Nikon Coolpix 995 digital camera. Multiple images were spliced using Adobe Photoshop. Shading defects and nonred background irregularities from images were cancelled by subtracting the red channel from the green channel using MetaMorph software (Universal Imaging, Downingtown, PA). Pancreatic area and β-cell area were estimated using the intensity thresholding function of the integrated morphometry package in MetaMorph. Investigators were blinded to the source of the tissue throughout the morphometric analysis.

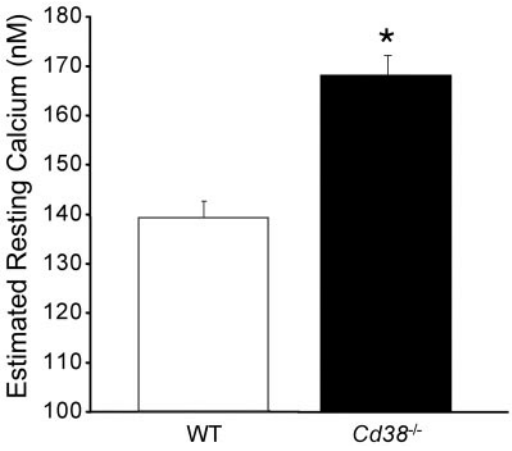

FIG. 1. *Cd38*$^{-/-}$ β-cells have elevated resting calcium. Cytosolic free Ca^{2+} concentration was measured in groups of 10–30 dispersed islet cells from wild-type (WT) or *Cd38*$^{-/-}$ mice (total cells studied were 367 for wild-type and 350 for *Cd38*$^{-/-}$). *Significant difference from wild-type controls.

BrDU labeling was performed 4 h after intravenous injection of 4-week-old mice with 5 mg/ml BrDU (200 μl volume; Sigma). After 4 h, the mice were killed and the pancreas was frozen in OCT (TissueTek) over liquid nitrogen. The pancreas was sectioned (7 μm), fixed in acetone at 4°C for 10 min, rinsed in dPBS, and then treated with 0.1% $NaBH_4$ for 15 min to quench autofluorescence. The sections were then washed in dPBS, fixed with 4 mol/l HCl for 30 min, treated with 0.1 mol/l $Na_2B_4O_7$ for 10 min, and washed in dPBS. The sections were stained with an anti-BrDU antibody coupled to Alexa-Fluor 594 (1/40 dilution; Molecular Probes) for 30 min, washed in dPBS, mounted with Prolong Antifade (Molecular Probes), and viewed on a Zeiss Axioplan 2 microscope (Oberkochen, Germany) under fluorescence using the appropriate band-pass filters. Images were captured using a 40× objective with a Zeiss Axiocam. Islet size was calculated using the Zeiss Axiovision 3.0.6 software. BrDU-positive cells were counted from a random islet area of at least ~70,000 μm^2. Experiments were repeated on three 4-week-old mice from each group.

Measurement of islet apoptosis by PCR-enhanced DNA ladder analysis. Islet apoptosis was measured using a previously described variation of a PCR-enhanced DNA-laddering protocol modified for use with small numbers of islets (34). To compare data from separate gels, band intensity was normalized to the average of the control groups.

Statistical analysis. Statistical analyses were performed using Student's unpaired *t* test or one-way ANOVA, where appropriate. Differences were considered significant when $P < 0.05$. Results are presented as means ± SE.

RESULTS

Role of Cd38 in β-cell Ca^{2+} homeostasis and insulin signaling. The regulation of intracellular Ca^{2+} is a major function of CD38. First, we examined Ca^{2+} homeostasis in unstimulated cells. Analysis of the resting Ca^{2+} concentration from all experiments indicated that *Cd38*$^{-/-}$ islet cells had higher basal cytosolic Ca^{2+} compared with wild-type cells (Fig. 1). This finding suggests that CD38, directly or indirectly, regulates the set point for cytosolic Ca^{2+} in β-cells. Next, we examined the role of CD38 in the mobilization of Ca^{2+} by extracellular signals. The possibility that CD38 played a role in the responsiveness of islet cells to insulin was examined using a wide range of insulin concentrations, from 2 pmol/l to 2 μmol/l, since the concentration dependence of autocrine insulin signaling in primary β-cells is bell shaped (11,15). The majority of dispersed islet cells responded to at least one concentration of insulin. Notably, there were 50% fewer responses to insulin in cells from *Cd38*$^{-/-}$ mice (Fig. 2). Among cells

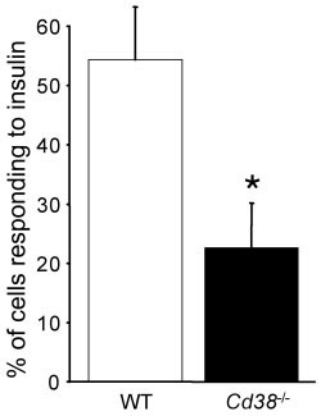

FIG. 2. *Cd38*$^{-/-}$ β-cells are less responsive to insulin. Quantification of the percentage of cells exhibiting a significant (2 SDs above baseline) Ca^{2+} response to any concentration of insulin in multiple independent experiments illuminates a signaling defect in *Cd38*$^{-/-}$ β-cells. *Significant difference from wild-type (WT) control. See Fig. 3 for example traces and number of cells studied.

responding to insulin, wild-type cells appeared to be more sensitive since they showed maximal responses at 0.2 nmol/l insulin, while *Cd38*$^{-/-}$ cells showed maximal responses to 2 nmol/l insulin (Fig. 3*A* and *B*). The amplitude of the Ca^{2+} signal induced by insulin was lower in *Cd38*$^{-/-}$ cells, although this was not statistically significant (Fig. 3*C*). The observation that insulin signaling was not completely abolished in *Cd38*$^{-/-}$ β-cells suggests that other members of the CD38 family may play a compensatory role. Ca^{2+} signals in response to 5 μmol/l carbachol and 100 μmol/l ATP, both IP_3-generating agonists, did not appear to be reduced (not shown), suggesting that CD38 plays a specific role in insulin responsiveness. Together, these results indicate that CD38 controls the responsiveness of single islet cells to insulin.

Role of Cd38 in islet apoptosis and β-cell mass. Insulin is a potent islet survival factor (39,40) (J.D.J. et al., manuscript submitted). As such, we directly tested the hypothesis that *Cd38*$^{-/-}$ islets, with their reduced sensitivity to insulin, would exhibit increased apoptosis. The effects of *Cd38* deficiency on apoptosis induced by a number of treatments were examined using a semiquantitative PCR-enhanced DNA-laddering assay (38). These experiments showed that *Cd38*$^{-/-}$ islets are significantly more susceptible to apoptosis at physiological glucose concentrations, in the presence of the free fatty acid palmitate or in the absence of serum (Fig. 4*A* and *B*). Interestingly, *Cd38*$^{-/-}$ islets were not more susceptible to endoplasmic reticulum stress induced by thapsigargin or cell death induced by ryanodine. This suggests that apoptosis via disruption of *Cd38* and intracellular Ca^{2+} stores, potentially downstream of NAADP (11), were not additive. Acutely isolated *Cd38*$^{-/-}$ islets did not exhibit changes in the phosphorylation status of Akt or glycogen synthase-kinase 3 (Fig. 4*C*), suggesting the possible involvement of other antiapoptotic pathways. Interestingly, CD38 mRNA in isolated wild-type islets was upregulated in serum-free conditions (Fig. 4*D*).

Increased islet apoptosis can result in decreased β-cell mass and disrupted islet architecture (41). *Cd38*$^{-/-}$ mice displayed a small but significant decrease in β-cell mass under both normal conditions and under conditions of high-fat feeding (Fig. 5*A*). No difference was seen in pancreatic weight or whole-body weight. The decreased β-cell mass was paralleled by a disruption in islet architecture (Fig. 5*B*). Whereas in the normal islet, glucagon-containing α-cells are distributed exclusively in the islet mantle, in *Cd38*$^{-/-}$ islets, glucagon staining was distributed more diffusely between the insulin-secreting β-cells in the islet core, as seen in several mouse models of increased islet apoptosis (38,42). No difference in β-cell uptake of BrdU was observed in *Cd38*$^{-/-}$ islets either when measuring the total number of proliferating cells per islet (Fig. 5*C* and *D*) or when measuring the total number of proliferating cells per unit area in the islet (data not shown). Since β-cell mass reflects the balance between β-cell apoptosis and proliferation (38,43,44), these data suggest that the decrease in β-cell mass seen in *Cd38*$^{-/-}$ mice is due to increased apoptosis rather than reduced proliferation.

Role of Cd38 in glucose homeostasis in vitro and in vivo. Previous reports (19,20) of a critical role for CD38 in calcium signaling induced by glucose prompted us to examine glucose signaling in our *Cd38*$^{-/-}$ mice. An extensive examination of >350 β-cells from C57BL/6 *Cd38*$^{-/-}$ mice and their littermate controls showed no significant difference in the Ca^{2+} responses to 10 mmol/l glucose (Fig. 6*A–C*). Similar results were seen with 6 mmol/l glucose (n = 13 for wild type, n = 13 for *Cd38*$^{-/-}$; not shown) and 20 mmol/l glucose (n = 12 for wild type, n = 15 for *Cd38*$^{-/-}$; not shown). Glucose signaling in wild-type control islet cells was also not reduced by nicotinamide (Fig. 6*D*), which has been shown to inhibit CD38 enzyme activity (45), but was strongly dependent on the influx of extracellular calcium through voltage-gated Ca^{2+} channels in primary β-cells (Fig. 6*E*), as established by others (46).

We assessed the role of CD38 in glucose signaling in vivo by comparing wild-type and *Cd38*$^{-/-}$ mice on the C57BL/6 genetic background. Random blood glucose was not different between wild-type and *Cd38*$^{-/-}$ mice, irrespective of sex or diet (Fig. 7*A*). Glucose tolerance was not significantly different in *Cd38*$^{-/-}$ mice, although there was a trend toward higher 30-min glucose values in female *Cd38*$^{-/-}$ mice on both diets (Fig. 7*B–E*). Insulin tolerance tests were also normal in *Cd38*$^{-/-}$ mice on both diets (Fig. 7*F* and *G*). Significant insulin resistance was observed in the high-fat diet mice. Together, these results strongly suggest that CD38 is not required for glucose signaling.

DISCUSSION

In the present study, we used islets from *Cd38* knockout mice to address the functional role of CD38 in the endocrine pancreas. Our study has produced two major findings. First, *Cd38* deficiency leads to defects in insulin signaling, islet survival, and β-cell mass. Second, CD38 is not required for glucose signaling and is dispensible for normal glucose homeostasis, even under the stress of a high-fat diet.

In a previous study, we demonstrated that insulin mobilizes Ca^{2+} from multiple compartments in β-cells, including a novel class of intracellular Ca^{2+} stores sensitive to the *Cd38*-derived metabolite NAADP (11). Our findings now provide further evidence that a *Cd38*/NAADP pathway is present in pancreatic islet cells and confirm that this system plays a role in insulin signaling. Our results show that *Cd38* was required for optimum sensitivity of islet cells to insulin, rather than being absolutely necessary. Where it has been examined in other studies, Ca^{2+} signals in response to endogenous agonists are only par-

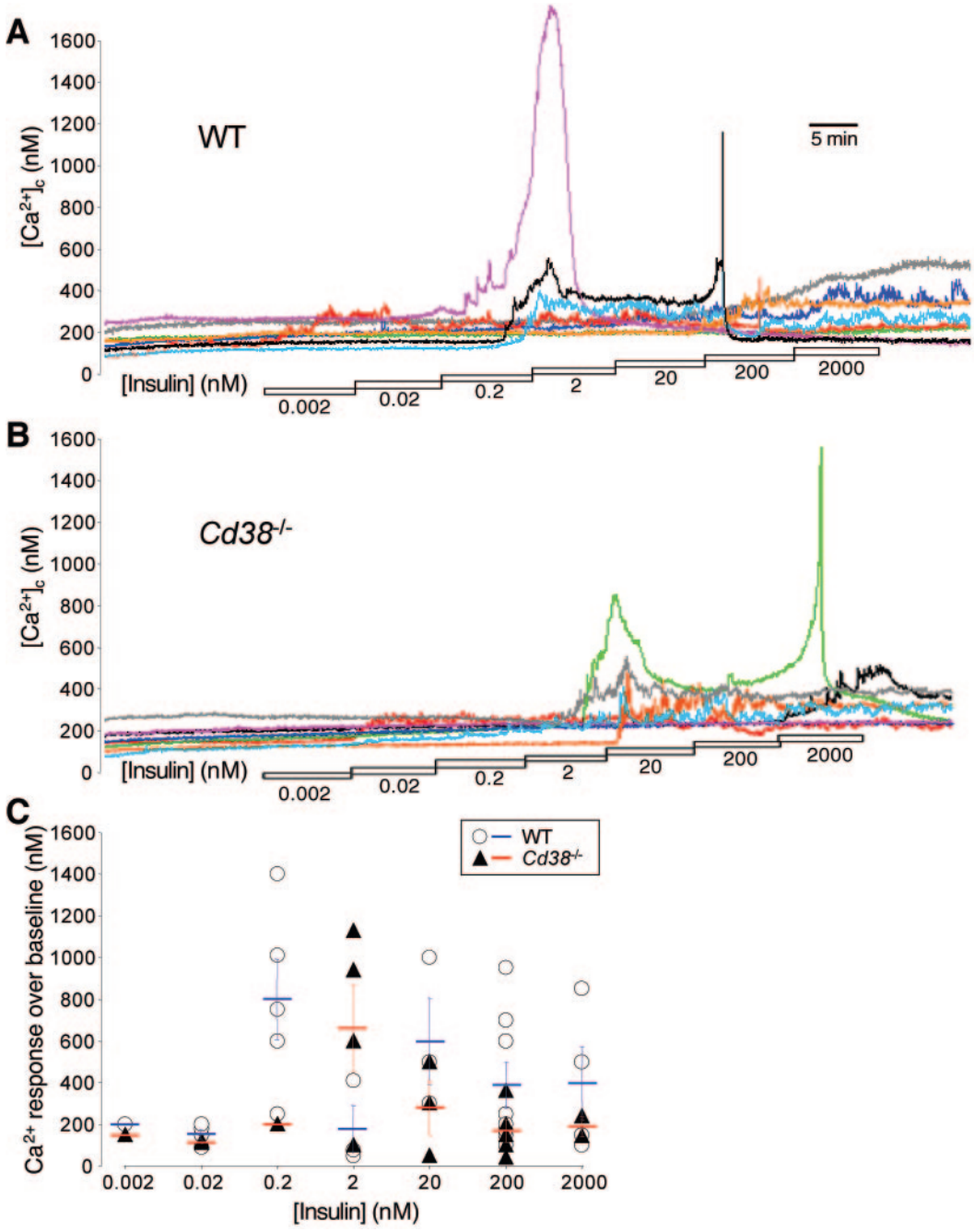

FIG. 3. Reduced sensitivity and amplitude of insulin-stimulated Ca^{2+} signals in *Cd38*$^{-/-}$ β-cells. *A* and *B*: Example traces of multiple cells in a representative experiment wherein dispersed islets cells were exposed to stepwise increases in insulin. The total number of cells studied was 271 for wild-type (WT) and 250 for *Cd38*$^{-/-}$. *C*: Ca^{2+} response amplitudes and the insulin concentration at which the responses were initiated from a representative experiment. Cells from *Cd38*$^{-/-}$ islets responded less frequently to 0.2 nmol/l insulin, reflecting an apparent decrease in sensitivity. The mean Ca^{2+} response amplitude (cross bars ± SE) is given for each concentration of insulin.

tially inhibited in *Cd38*$^{-/-}$ cells from various tissues (3,47,48), suggesting the possible compensation by other members of the CD38 family (e.g., CD157) or other enzymes capable of generating cADPR or NAADP. Since calcium signals generated by insulin were completely dependent on NAADP (11), our current results suggest that additional NAADP-generating enzymes may be involved in insulin signaling. The involvement of CD38 in β-cell insulin signaling is further evidence of a previously undescribed arm of the insulin signaling pathway. The present study also demonstrates that this pathway plays an important role in controlling the susceptibility to apoptosis. Indeed, an increasing body of evidence points to a critical role for the insulin signaling pathway in the survival of β-cells. Insulin exerts antiapoptotic effects on isolated islets (39,40) (J.D.J. et al., manuscript submitted), and mice lacking β-cell insulin receptors or IRS2 display reduced β-cell mass (16,17). In accordance with this, we found that β-cell mass was reduced and islet architecture disrupted in *Cd38*$^{-/-}$ mice. The selective increase in apoptosis seen at physiological glucose levels, but not in the presence of thapsigargin, is reminiscent of the phenotype of mice haploinsufficient for *pdx-1*, a gene linked to human diabetes (38) and involved in β-cell insulin signaling (J.D.J., K.S.P., unpublished observations).

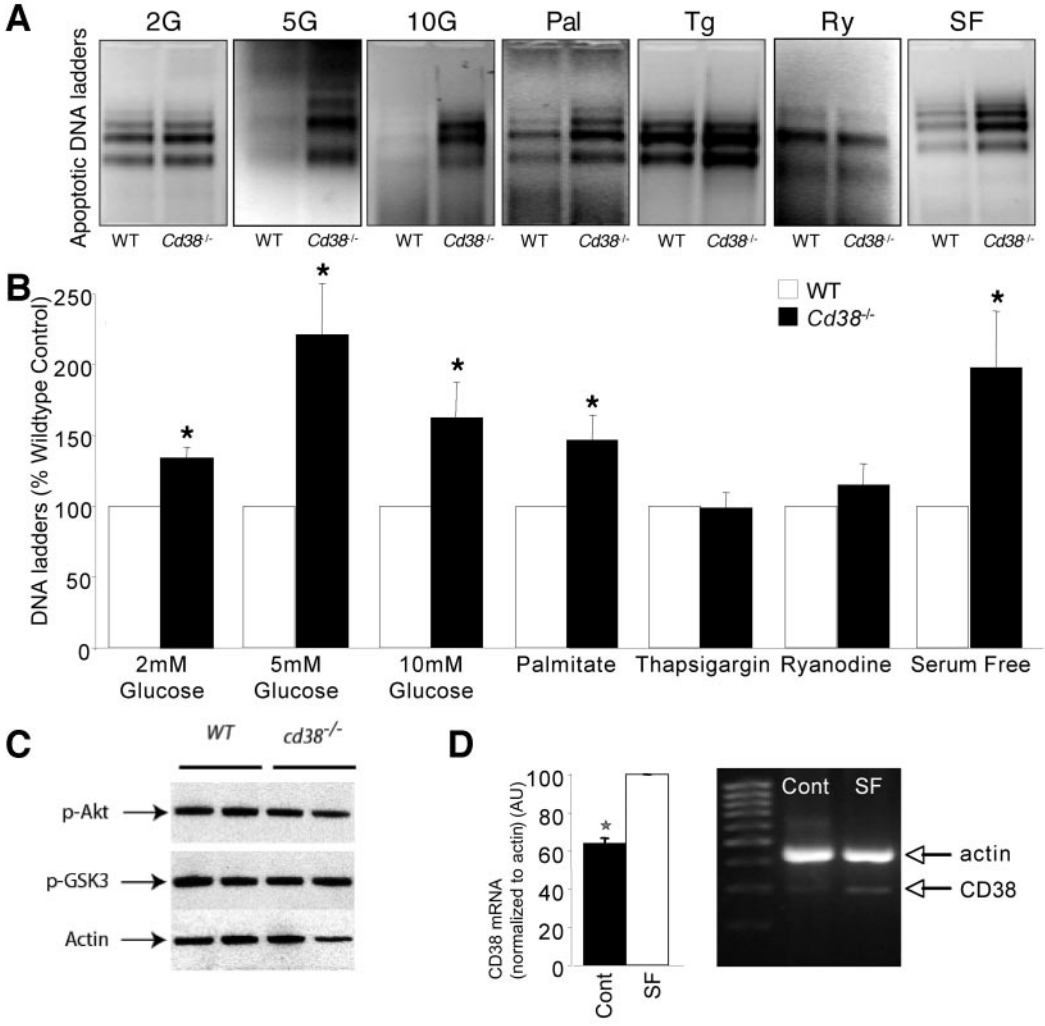

FIG. 4. *Cd38*$^{-/-}$ islets have increased sensitivity to apoptosis. *A*: PCR-enhanced DNA ladder analysis of apoptosis in groups of 10 isolated wild-type (WT) or *Cd38*$^{-/-}$ islets cultured for 5 days in media containing 2 mmol/l glucose (2G; $n = 6$), 5 mmol/l glucose (5G; $n = 8$), or 10 mmol/l glucose (10G; $n = 8$) as indicated. Apoptosis was also assessed in islets cultured at 10 mmol/l glucose (10G) for 3 days with the addition of 250 μmol/l palmitate, 1 μmol/l thapsigargin, or 50 μmol/l ryanodine ($n = 8$). Apoptosis was also compared in islets cultured for 7 days in serum-free conditions ($n = 8$). *B*: Results from the experiments in *A* are quantified as percentage of DNA laddering in wild-type controls for each condition. *Significant difference compared with islets from wild-type littermate controls under the same treatment conditions. *C*: Representative Western blot of phosphorylated Akt, phosphorylated glycogen synthase kinase-3 (*p*-GSK3), and actin (as a loading control) ($n = 4$) indicate that classical insulin signaling targets are not altered in *Cd38*$^{-/-}$ islets. *D*: RT-PCR demonstrates CD38 mRNA is increased in serum-free (SF) conditions compared with control (Cont) (10% fetal bovine serum) ($n = 3$).

CD38 may also prevent β-cell apoptosis through the cADPR-mediated activation of RyR2, which may explain why the apoptotic effects of *Cd38* knockout and ryanodine were not additive. We have shown that Ca^{2+} release from RyR2 inhibits the expression of calpain-10, a diabetes susceptibility gene and proapoptotic protease in β-cells (34,49). Thus, it is possible that *Cd38* and calpain-10 comprise part of a genetic network influencing diabetes risk in humans. Given the link between obesity and type 2 diabetes, this novel β-cell apoptosis pathway may represent an important intersection between acquired risk factors, such as high circulating lipids, and genetic predisposition. *Cd38* gene expression and RyR activity are both suppressed in *ob/ob* mice, a genetic animal model of obesity and diabetes (25). *Cd38*$^{-/-}$ islets were also more susceptible to apoptosis induced by palmitate in vitro. Although the fat feeding resulted in a threefold increase in β-cells mass, the effects of CD38 deficiency were not proportionally larger with the high-fat diet. This finding, combined with out BrdU staining, suggests that CD38 is not involved in β-cell proliferation but instead mediates the general maintenance of β-cell mass.

CD38 deficiency leads to an increase in the basal cytosolic Ca^{2+} concentration in β-cells. Prolonged Ca^{2+} elevation could promote apoptosis in pancreatic β-cells, as it would mimic chronic hyperstimulation with glucose (34,38,50–52). We speculate that the chronic increase in basal Ca^{2+} seen in *Cd38*$^{-/-}$ β-cells may by related to indirect long-term effects of the RyR2 pathway on gene expression. In preliminary microarray studies, blocking RyR reduced the expression of a number of hyperpolarizing K^+ channels (J.D.J, K.S.P, unpublished observations). A decrease in K^+ channel density would be expected to increase membrane excitability and Ca^{2+} influx. Another possible explanation for this increased Ca^{2+} involves the ability of insulin to decrease membrane excitability by opening ATP-sensitive K^+ channels in β-cells (53).

Our study suggests that islet apoptosis is a primary alteration in *Cd38*$^{-/-}$ mice since it can be observed in the absence of changes in glucose signaling. This idea is

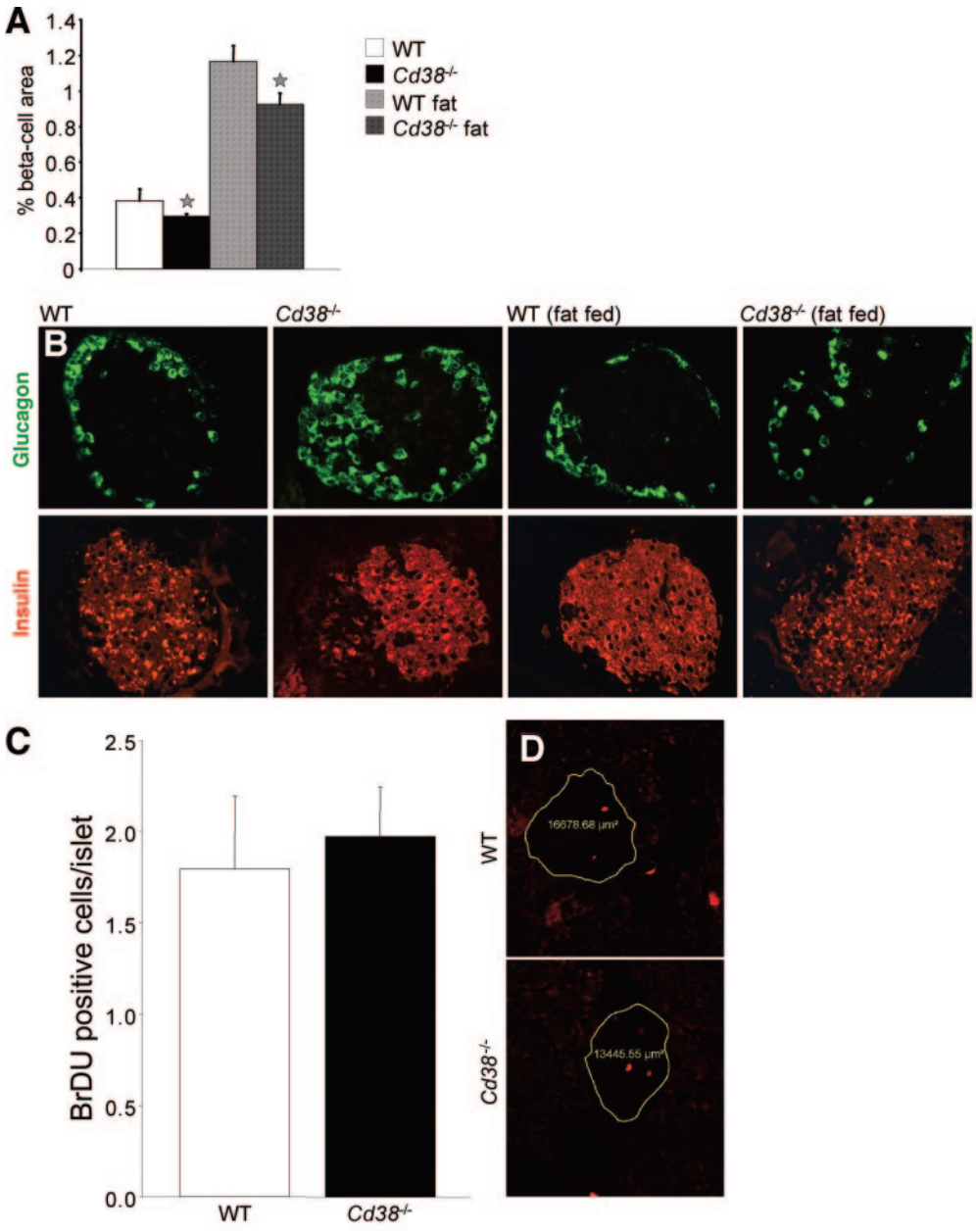

FIG. 5. Reduced β-cell mass and disrupted islet architecture in *Cd38*$^{-/-}$ mice. *A*: Differences in β-cell mass, estimated by quantifying the area of insulin-positive β-cells as a percentage of total pancreas area in each pancreatic tissue section, were examined in wild-type (WT) or *Cd38*$^{-/-}$ mice fed either a normal lab diet or high-fat diet as indicated (wild-type, $n = 4$; *Cd38*$^{-/-}$, $n = 4$). *B*: Glucagon immunofluorescence (green) and insulin immunofluorescence (red) of pancreatic islets from wild-type or *Cd38*$^{-/-}$ mice fed either a normal lab diet or high-fat feed as indicated. *C*: Fluorescent BrDU labeling on sections from wild-type and *Cd38*$^{-/-}$ mice were quantified as described in RESEARCH DESIGN AND METHODS ($n = 3$ mice for each group, and a minimum of 5–11 islets were examined for each mouse). *D*: Examples of BrDU-labeled islets.

consistent with studies in other cell types. For example, CD38-derived cyclic ADP-ribose has been shown to improve the survival and engraftment of hematopoietic stem cells (54). In T-cells, inactivation of CD38 by ADP-ribosylation results in increased apoptosis (55), whereas stimulation of CD38 with cross-linking antibodies leads to activation of the Akt and Erk prosurvival pathways (35). The possibility that Akt is downstream of CD38 is interesting, since Akt has been implicated in insulin signaling and glucose homeostasis (56,57). Although we did not observe changes in the phosphorylation status of the total pool of Akt in *Cd38*$^{-/-}$ islets, it remains possible that the localization or activity of Akt in a subpopulation of CD38-positive β-cells could mediate the effects of altered insulin signaling.

Our finding of normoglycemia in *Cd38*$^{-/-}$ mice is in contrast to the model proposed by Okamoto et al. (18) based in part on studies of transgenic mice overexpressing *Cd38* and *Cd38*$^{-/-}$ mice on the ICR genetic background (19,20). We and others did not observe a significant defect in glucose tolerance in *Cd38*$^{-/-}$ mice on the C57BL/6 genetic background (58). It is possible that genetics could account for the differences in whole-body glucose metabolism. However, the previous studies (19,20) also reported a robust impairment in insulin release in response to 20–30 mmol/l glucose in vitro and reduced Ca^{2+} response to glucose in islets isolated from ICR-Cd38$^{-/-}$ mice. Comparing our findings with the results of Okamoto and colleagues (20) illustrates the potentially critical role of genetic background in the susceptibility to diabetes in

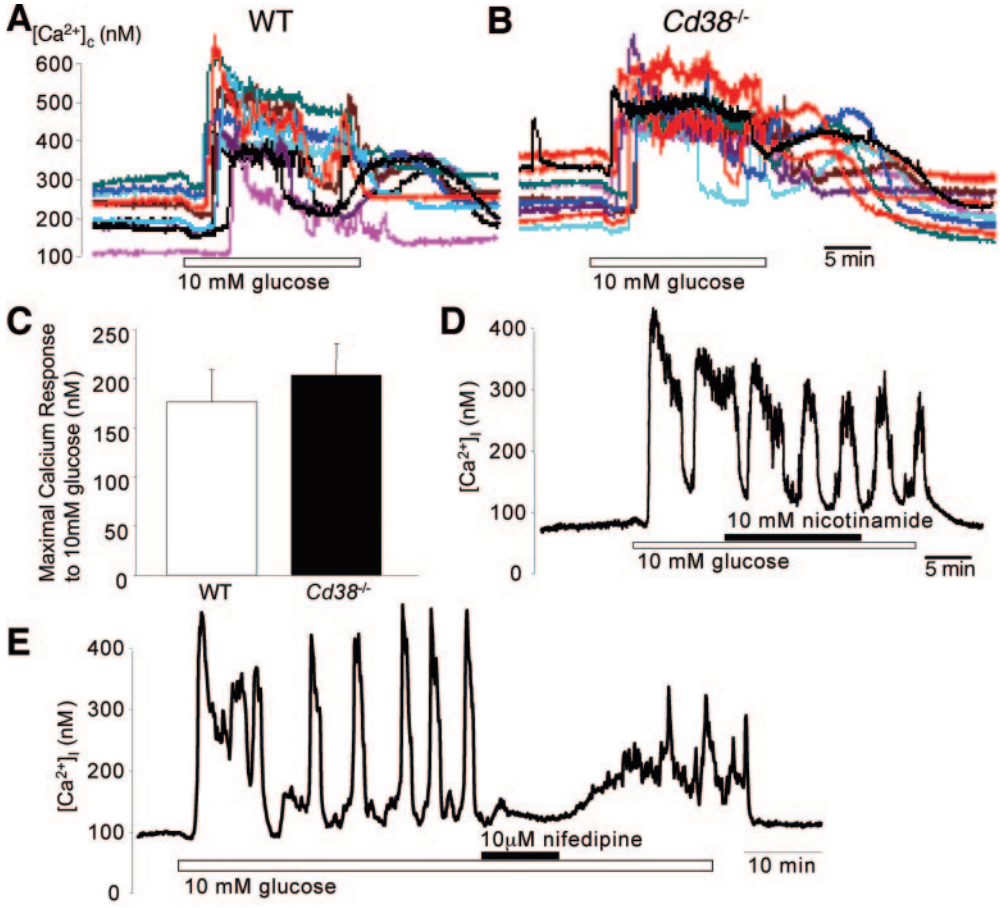

FIG. 6. *Cd38* is not involved in glucose signaling in single β-cells. *A* and *B*: Example of multiple wild-type (WT) and *Cd38*$^{-/-}$ β-cells responding simultaneously to a stepwise increase from 3 to 10 mmol/l glucose. *C*: Quantification of baseline-substracted Ca^{2+} responses to 10 mmol/l glucose (wild-type, $n = 368$; *Cd38*$^{-/-}$, $n = 350$). Glucose-stimulated Ca^{2+} signals were also not different when quantified using the area under the curve (data not shown). *D*: Ca^{2+} signals in response to 10 mmol/l glucose are not altered by acute exposure to 10 mmol/l nicotinamide, a CD38 antagonist, in β-cells from wild-type C57BL/6J mice ($n = 28$). *E*: Glucose-stimulated Ca^{2+} signals are abolished by 10 μmol/l nifedipine in β-cells from wild-type C57BL/6J mice ($n = 10$).

Cd38 knockout mice. Indeed, the *CD38* mutation seen in Japanese patients (23) was not observed in a European cohort (24). We await reports from large-scale human genetic studies to clarify the role of variation in the *CD38* gene in type 1 and type 2 diabetes.

In our study of β-cells from *Cd38*$^{-/-}$ mice on the C57BL/6 genetic background, a direct role for CD38 in glucose signaling was not found. It would be surprising if the fundamental mechanisms of glucose signaling differed between mouse strains. One possibility would be that islet apoptosis was more severe in the ICR-*Cd38*$^{-/-}$ mice and that this led to a secondary defect in glucose signaling. Since an analysis of islet apoptosis has not been reported for the ICR-*Cd38*$^{-/-}$ mice, it is difficult to know the exact reason for these discordant findings. At least, our data suggest that the model proposed by Okamoto et al. (18) cannot be generally applied to β-cell function in mice from different genetic backgrounds. Importantly, our previous studies (21,22) of human islet cells, as well as studies on rodent islets, argue against a major role for cADPR or RyR in glucose-stimulated insulin release. Although the CD38/RyR2 pathway may be an accessory pathway modulating insulin release, especially in cell lines, the bulk of the evidence supports a model whereby localized Ca^{2+} influx through voltage-gated Ca^{2+} channels subsequent to the closure of ATP-sensitive K^+ channels is the primary trigger of glucose-stimulated insulin release (46,59–62).

Although we did not note significant defects in glucose homeostasis in the *Cd38*$^{-/-}$ mice under the conditions tested, it remains possible that additional β-cell stress could provoke β-cell failure and diabetes, especially in female *Cd38*$^{-/-}$ mice, which showed a trend toward glucose intolerance in our study. One potential example is type 1 diabetes, where the increased islet apoptosis demonstrated in the present study may have significant ramifications during autoimmune attack. In this respect, it is notable that diabetes occurs predominantly in female NOD mice (63) and that CD38 expression is controlled by estrogen in other tissues (64). Interestingly, cytokines responsible for β-cell apoptosis in type 1 diabetes, namely tumor necrosis factor α, interleukin-1β, and interferon γ, have all been shown to alter CD38 expression in other cells types (65,66). While this report was in revision, Leiter et al. (58) reported that CD38 deficiency significantly accelerated diabetes in NOD mice. While the CD38$^{-/-}$:NOD study focused largely on the roles of CD38 in the immune system, their results also pointed to effects in nonhematopoietic cells (58). In summary, the results of the present study clarify the molecular role of CD38 in autocrine insulin signaling, Ca^{2+} homeostais, and β-cell apoptosis, while arguing against a primary role in glucose-dependent Ca^{2+} signaling.

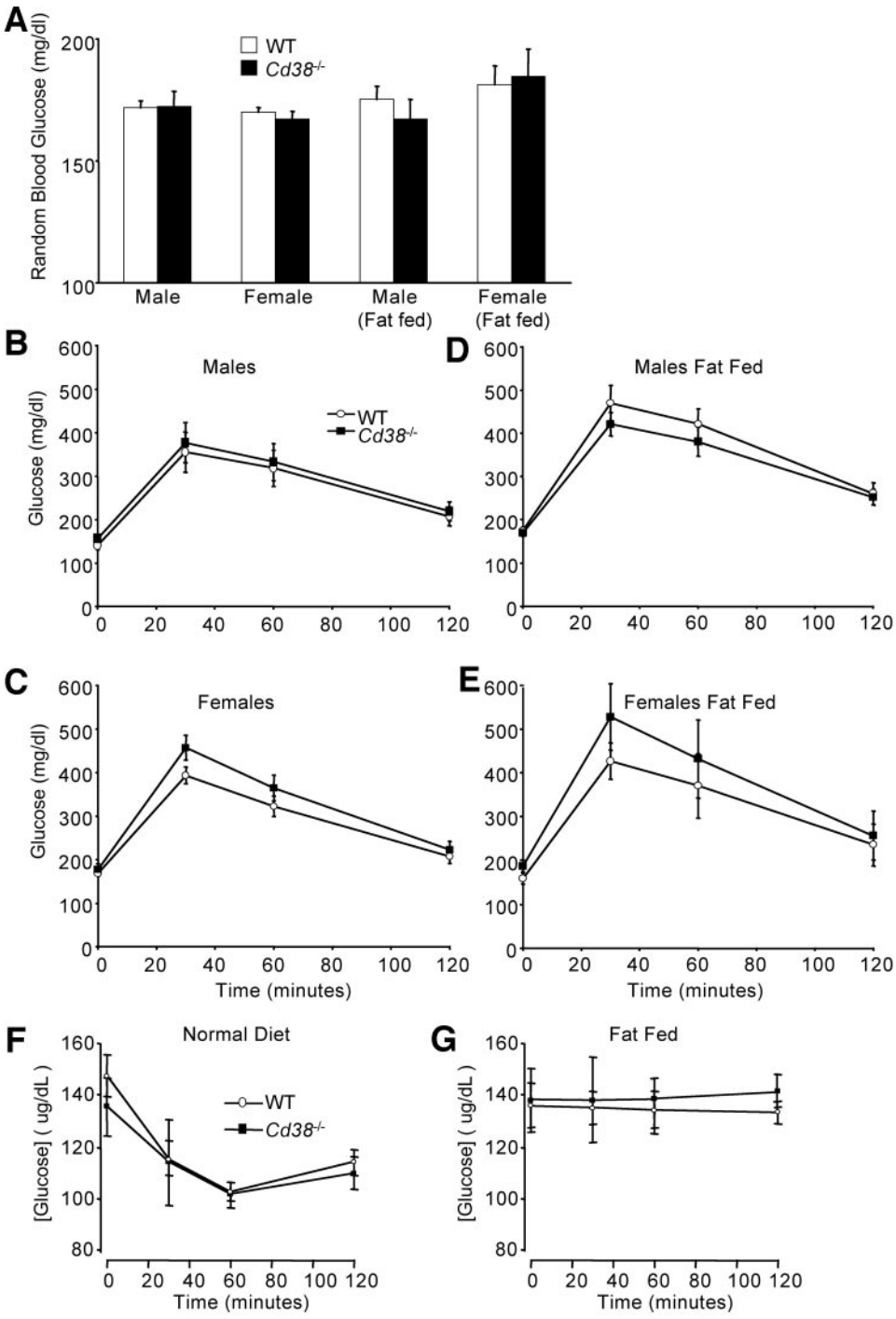

FIG. 7. Effects of CD38 knockout on in vivo glucose homeostasis. *A*: Random nonfasting blood glucose was measured in male and female mice fed a normal diet or a high-fat diet. *B–E*: Intraperitoneal glucose tolerance test on equal numbers of wild-type (WT) and *Cd38*$^{-/-}$ mice of both sexes and diets are shown (males $n = 19$, females $n = 9$, male fat fed $n = 6$, female fat fed $n = 4$). No significant differences in areas under the curve were seen. Similar results were seen at 12 weeks (after 8 weeks of high-fat diet) and at 6 months (after 5 months of high-fat diet). Insulin tolerance tests on male *Cd38*$^{-/-}$ mice and their wild-type littermate controls under non–high-fat–fed conditions (*F*) ($n = 6$) or after a high-fat diet (*G*) (wild-type, $n = 8$; *Cd38*$^{-/-}$, $n = 4$) show normal insulin sensitivity.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health (NIH) Grant DK-31842 (to K.S.P.), the Kovler Foundation, NIH Grant AI-057996 (to F.E.L.), and the Trudeau Institute (T.D.R.). J.D.J. was supported by scholarships and operating grants from the Canadian Institutes of Health Research (MOP-69012), the Michael Smith Foundation for Health Research (CI-SCH-01403-1), and the Juvenile Diabetes Research Foundation (2-2005-926).

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