RNA Editing by ADAR2 Is Metabolically Regulated in Pancreatic Islets and β-Cells

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RNA editing via the conversion of adenosine (A) to inosine (I) is catalyzed by two major families of adenosine deaminases acting on RNA (ADARs), ADAR1 and ADAR2. This genetic recoding process is known to play essential roles in the brain, due in part to changes in functional activities of edited neurotransmitter receptors and ion channels. Little is known, however, about the physiological regulation and function of A to I RNA editing in peripheral tissues and other biological processes. Here, we report that both ADAR1 and ADAR2 are expressed in the murine pancreatic islets, and ADAR2 is primarily localized in the islet endocrine cells. In contrast to ADAR1, ADAR2 transcripts in the pancreatic islets exhibit a nearly 2-fold increase in insulin-resistant mice chronically fed a high fat diet. Concurrent with this diet-induced metabolic stress, RNA editing in the islets is dramatically enhanced for the RNA transcripts encoding the ionotropic glutamate receptor subunit B. Moreover, ADAR2 protein expression is repressed in the islets under fuel deficiency condition during fasting, and this repression can be completely reversed by refeeding. We also show that, specifically in pancreatic β-cell lines, not only the expression of ADAR2 but also the glutamate receptor subunit B editing and ADAR2 self-editing are markedly augmented in response to glucose at the physiological concentration for insulin secretion stimulation. Thus, RNA editing by ADAR2 in pancreatic islets and β-cells is metabolically regulated by nutritional and energy status, suggesting that A to I RNA editing is most likely involved in the modulation of pancreatic islet and β-cell function.

RNA editing through the conversion of adenosine (A) to inosine (I) within pre-mRNAs is a genetic recoding process found in a variety of organisms, including mammals (1). The search for the RNA substrates targeted for this hydrolytic deamination modification has recently revealed abundant A to I editing sites in the human transcriptome, predominantly occurring in non-coding regions of the RNA, such as Alu repeats (2). A to I editing is catalyzed by two major families of ubiquitously expressed adenosine deaminases acting on RNA (ADARs), denoted ADAR1 and ADAR2 (1, 3). A to I RNA editing is known to affect a broad range of biological processes from pre-mRNA splicing to mRNA stability to coding capacity changes (4). Previously reported studies indicate that A to I RNA editing plays critical roles in the function and development of the central nervous system, due in a large part to the regulation via editing of the activities of neurotransmitter receptors and ion channels, including the ionotropic glutamate receptors (GluRs), G-protein-coupled serotonin-2C subtype receptor, and Kv1.1 potassium channel (5–10). Because inosine can be recognized as guanosine by the cellular protein translation apparatus, site-selective editing at crucial sites within the coding regions of RNA transcripts results in codon changes, and subsequently, altered functional behaviors of the encoded protein products. There are two major edit sites for the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) glutamate receptor subunit B (GluR-B), the Q/R and R/G sites, which have been most extensively studied. Editing at the Q/R site, which generates an arginine codon (CIG) via selective targeting of a glutamine codon (CAG) specifically by ADAR2 (11), leads to dramatic reduction in the calcium permeability of the receptor channels (12). Editing at the R/G site, targeted by both ADAR1 and ADAR2, converts an arginine (AGA) residue to glycine (IGA) and results in altered kinetic properties of the receptor channels characterized by faster recovery rates from desensitization (13). Recently reported lines of evidence also indicate that A to I RNA editing can modulate another RNA-mediated regulation pathway of gene expression, the biogenesis and maturation of microRNAs (14).

Both ADAR1 and ADAR2 exist in multiple isoforms due to alternative splicing (15, 16), and two ADAR1 enzymes have been found in mammals as a result of the presence of multiple promoters: an interferon-inducible protein enzyme of ~150 kDa (p150) and a constitutively expressed N-terminally truncated protein of about 110 kDa (p110) (17–19). Although little is known about the transcriptional regulation of ADAR2, the RNA transcripts encoding ADAR2 have been shown to undergo self-editing, which creates a 3′ splice site (AA to A) in intron 4 responsible for subsequent alternative splicing (20). This self-editing-mediated splicing event generates a 47-nucleotide insertion near the N-terminal portion and thus causes a

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1 The on-line version of this article (available at http://www.jbc.org) contains a supplemental table.

2 The abbreviations used are: ADAR, adenosine deaminase acting on RNA; DIO, diet-induced obesity; GluR, glutamate receptor; GluR-B, GluR subunit B; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; RT, reverse transcription; LFD, low fat diet; HFD, high fat diet; VHFD, very high fat diet.

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frameshift in the coding region that is predicted to produce a 9-kDa inactive protein due to premature termination. Furthermore, studies in mice engineered to be incapable of self-editing indicate that this self-editing event represents a negative feedback mechanism in the modulation of ADAR2 expression (21).

RNA editing by ADARs has been shown to be progressively modulated during brain development (13), and it is speculated that the extent of editing may be modulated by the “metabolic” state of the cell (4). Aberrantly high editing activity due to dysregulated ADAR enzyme in Drosophila leads to lethality at embryonic or larval stage (22). Although the precise regulatory mechanisms are poorly understood, dysfunction of RNA editing has been implicated in a number of disease states, such as epilepsy (9), chronic depression in suicide victims (23), amyotrophic lateral sclerosis (24), and malignant gliomas (25). Very little is known, however, about the physiological regulation and trophic lateral sclerosis (24), and malignant gliomas (25). Very little is known, however, about the physiological regulation and trophic lateral sclerosis (24), and malignant gliomas (25). Very little is known, however, about the physiological regulation and trophic lateral sclerosis (24), and malignant gliomas (25). Very little is known, however, about the physiological regulation and trophic lateral sclerosis (24), and malignant gliomas (25). Very little is known, however, about the physiological regulation and trophic lateral sclerosis (24), and malignant gliomas (25). Very little is known, however, about the physiological regulation and trophic lateral sclerosis (24), and malignant gliomas (25). Very little is known, however, about the physiological regulation and trophic lateral sclerosis (24), and malignant gliomas (25). Very little is known, however, about the physiological regulation and.
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For Western immunoblot analysis, whole-cell extracts were prepared by lysis with Celllytic™ M (Sigma), and the lysate was sonicated for 30 s and centrifuged for 20 min at 20,000 × g to remove the cellular debris. Tissue extracts were prepared by homogenization in the lysis buffer using a Polytron homogenizer followed by centrifugation. Proteins (~40 µg) were separated by SDS-PAGE and transferred to polyvinylidene difluoride filter membrane (Amersham Biosciences). After incubation with the desired antibodies, the blots were developed using the Amersham Biosciences ECL Plus detection system. Immunolabeled protein bands of interest were quantified using a ChemiDoc imaging system and the Quantity One software (Bio-Rad).

For double immunostaining, pancreas slices were blocked for 1 h in phosphate-buffered saline supplemented with Triton and donkey serum (0.1 M phosphate-buffered saline, 0.5% Triton X-100, and 2% normal donkey serum). The sections were then incubated for 4 h at room temperature with anti-ADAR2 antibody and antibodies against α-tubulin, insulin, glucagon, somatostatin, and pancreatic polypeptide, respectively. For negative control, the staining was done without the anti-ADAR2 antibody. Following the incubation and washing with 0.1 M phosphate-buffered saline, the sections were incubated for 2 h at room temperature with Texas Red or fluorescein isothiocyanate AffiniPure antibodies. Immunofluorescence images were obtained on a Zeiss LSM 510 META confocal microscope.

Insulin Tolerance Test, Blood Glucose, and Serum Insulin Concentrations—After an 8-h fasting, animals were injected intraperitoneally with human insulin (Eli Lilly, 0.5 milliunits/g of body weight), and blood glucose concentrations were measured at 15, 30, 45, 60, and 120 min after insulin injection using a glucometer (FreeStyle). Serum insulin concentrations were measured by radioimmunoassay kit or mouse serum adipokine LINCOplex kit (Linco Research) with the Bio-Plex system (Bio-Rad) according to the manufacturer’s instructions.

Pancreatic Islet Isolation—Pancreatic islets from mice and rats were isolated by the stationary digestion method (39). Briefly, minced pancreas were digested with Liberase enzyme solution, and the digest was fractionated by centrifugation in preformed 13, 21, 23, and 25% Ficoll gradient solutions. The islet fractions were harvested between the 13–21% gradients preformed 13, 21, 23, and 25% Ficoll gradient solutions. The islet fractions were harvested between the 13–21% gradients before preparation of the crude islet extract.

RNA Isolation and RT-PCR—Total RNA from islets was isolated with a mirVana™ miRNA isolation kit (Ambion). Total RNA from mouse tissues and cultured β-cells was isolated with TRIzol reagent (Invitrogen) followed by digestion with RNase-free DNase I (Roche Diagnostics) to eliminate possible DNA contamination. First-strand cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase and random hexamer primers (Invitrogen). Real-time quantitative PCR was performed using the SYBR Green PCR system (Bio-Rad), with actin serving as an internal control for normalization. Specific oligonucleotide primers for target gene sequences were listed in supplemental Table S1.

Analysis of RNA Editing—For quantitative analysis of the Q/R site editing of GluR-B, PCR products targeting the pre-mRNA were amplified using a 5′-digoxigenin-labeled primer and digested by Tau1 (Fermentas), which can cleave the DNA fragments derived only from the edited RNA. After analysis by 1.5% agarose gel electrophoresis, the digoxigenin-labeled DNA fragments were transferred onto a nylon membrane (Amersham Biosciences) and visualized by a digoxigenin luminescent detection kit (Roche Applied Science). Editing efficiency was quantified using the Quantity One software (Bio-Rad). For assessment of the GluR-B R/G site editing, gel-purified PCR products were either directly sequenced to analyze the A/G electropherograms at the editing site or subcloned into pGEM®-T Easy Vector (Promega) for subsequent sequencing of individual clones.

Statistical Analysis—Student’s t test was performed for two groups, and one-way analysis of variance was performed for more than two groups, with p values of <0.05 considered statistically significant.

RESULTS

Up-regulation of ADAR2, but Not ADAR1, Expression Levels in Pancreatic Islets of Obese Mice Fed High Fat Diet—To investigate the possible physiological function of A to I RNA editing in metabolic homeostasis, we first examined the tissue expression patterns of ADAR1 and ADAR2 genes in the diet-induced obesity (DIO) mouse model. The mice were fed for 16 weeks on one of the three experimental diets with 10% (LFD), 45% (HFD), and 60% fat (VHFD) in calories. In comparison with those on LFD, mice fed HFD and VHFD exhibited more dramatic body weight increases (Fig. 1A) with significant differences starting to be observed at 4 and 2 weeks, respectively. This could largely be attributed to the increases in body fat (data not shown). An insulin tolerance test showed that chronic feeding of HFD or VHFD resulted in apparently lowered insulin sensitivity, i.e. impaired hypoglycemic response to insulin, when compared with feeding of LFD (Fig. 1B). Concomitantly with the onset of severe insulin resistance in mice fed VHFD, overt hyperinsulinemia occurred progressively after 8 weeks (data not shown), and significantly higher blood insulin levels were observed in mice fed VHFD than those fed LFD at 16 weeks (8.66 ± 0.58 versus 0.84 ± 0.14 ng/ml, n = 17, p < 0.01) (Fig. 1C). These data indicate that in high fat diet-induced obesity, systemic insulin resistance developed in mice. Under these conditions, the pancreatic β-cells were presumably under metabolic stress, primarily because of the compensatory actions to meet the demand for excessive insulin production and secretion to maintain normal blood glucose level.

In the DIO mice chronically fed HFD for 16 weeks, we then analyzed, by real-time quantitative reverse transcription-PCR (RT-PCR), the levels and tissue distribution patterns of ADAR1 and ADAR2 mRNA. As expected, ADAR2 was most abundantly expressed in the brain (Fig. 1D). Interestingly, the pancreatic islets of mice fed HFD or VHFD showed a nearly 2-fold increase in the level of ADAR2 transcripts when compared with the control mice fed LFD (Fig. 1D, p < 0.05). On the other hand, no significant alterations in the ADAR2 mRNA level were evident in the other tissues examined, including brain, lung, liver,
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FIGURE 1. Expression and tissue distribution profile of ADAR1 and ADAR2 in diet-induced obesity mice.
A–C, high fat diet-induced obesity led to impaired insulin sensitivity and hyperinsulinemia. C57BL/6N male mice were fed with LFD, HFD, or VHFD after weaning at 4 weeks of age. Body weight (A) was measured during the feeding process. Insulin tolerance test (B) and measurement of blood insulin levels (C) were performed for mice at 16 weeks on the diets. Data are shown as mean ± S.E. (n = 17 per group). *, p < 0.05; **, p < 0.01. D and E, ADAR1 and ADAR2 transcriptional levels in different tissues of mice in response to chronic HFD feeding for 16 weeks. Relative mRNA levels of ADARs were assessed by real-time RT-PCR with actin mRNA utilized as an internal control for normalization. Expression in islets was analyzed for mice fed all three diets. Values are shown as mean ± S.E. (n ≥ 3 for non-islet tissues; n = 3 pools of islets, each pool from 4–6 mice), *, p < 0.05.

epidydimal fat, and muscle of the DIO mice (Fig. 1D, p > 0.05). Among all the tissues examined, ADAR1 displayed prominent expression level in the pancreatic islets, with its relative abundance almost comparable with that detected in the brain. In contrast to ADAR2, ADAR1 transcript level showed little change in any tissue analyzed in the DIO mice (Fig. 1E, p > 0.05). Therefore, expression level of ADAR2 is up-regulated selectively in the pancreatic islets in the metabolically stressed DIO mice, indicating that ADAR2 and ADAR2-mediated RNA editing may be regulated in a manner that is unique to pancreatic islets.

Cellular Localization of ADAR2 in Mouse Pancreatic Islets—We next examined the cellular localization of ADAR2 enzyme in mouse pancreatic islets by immunofluorescent histochesistry staining. Co-staining with dual antibodies against ADAR2 and tubulin demonstrated robust positive ADAR2 signals primarily in the islets, consistent with the tissue profile results for ADAR2 expression (Fig. 1D), whereas ADAR2 protein was nearly undetectable in the non-islet areas harboring other cell types, e.g. the pancreatic acinar cells (Fig. 2A). Whether the pancreatic islets were from the DIO or lean control mice, similar expression patterns of ADAR2 were observed in all four major endocrine cell types of the islets, as shown by co-localized signals from the double immunostaining using anti-ADAR2 antibody together with antibodies against insulin (β-cells; Fig. 2B), glucagon (α-cells; Fig. 2C), somatostatin (δ-cells; Fig. 2D), and pancreatic polypeptide (F- or pp-cells; Fig. 2E), respectively. Immunohistochemistry analysis was unable to reveal apparent differences in the relative abundance of ADAR2 protein in different endocrine cells. Nonetheless, the observation that ADAR2 appears to be expressed in an islet-specific fashion suggests a likely role for ADAR2 in the endocrine function of the pancreatic islets.

Enhanced GluR-B RNA Editing In Pancreatic Islets under HFD-induced Metabolic Stress—To determine whether the enhanced expression level of ADAR2 in pancreatic islets leads to enhanced editing activity, we attempted to examine the site-selective A to I editing of the three best characterized coding RNA substrates, the RNA transcripts encoding GluR-B, serotonin 2C receptor, and Kv1.1 potassium channel. Although the serotonin 2C receptor mRNA and Kv1.1 potassium channel mRNA were undetectable, the GluR-B RNA was considerably expressed in the pancreatic islets when analyzed by RT-PCR. We measured the editing efficiency of the two known editing sites targeted by ADAR2 of the GluR-B RNA, the Q/R and R/G sites, using a Taq restriction digestion-based editing assay or by direct sequencing electropherogram analysis using the RT-PCR products. The editing at the Q/R site of the mature GluR-B mRNA was nearly 100% in the islets (data not shown) in both the DIO mice and the lean control mice, supporting the notion that edited GluR-B pre-mRNA might be spliced preferentially (5). Then we performed the analysis of the Q/R site editing at the pre-mRNA level. The Q/R site editing of the GluR-B pre-mRNA exhibited an increase by ~2-fold in the islets from the obese mice fed HFD or VHFD when compared with that from the lean control mice fed LFD (45.9 or 47.9 versus 28.5%, n = 17, p < 0.01) (Fig. 3, A and B). Moreover, direct sequencing of the RT-PCR products demonstrated that editing at the R/G site of GluR-B mRNA also increased considerably in the DIO mice (Fig. 3C). Subsequent sequence analysis of 108 individual GluR-B cDNA subclones (54 clones each, respectively, for the LFD-fed control mice and for the VHFD-fed DIO mice) also indicated an elevation of R/G site editing in the islets of the obese mice (38.9 versus 29.6%) (Fig. 3D). Curiously, most of the clones obtained encoded the "flip" form of the GluR-B subunit (97 out of 108). On the other hand, examination in the brain of the DIO mice of the GluR-B RNA showed no significant alteration in editing at the R/G site (data not shown). These results are consistent with our finding that ADAR2 expression was markedly elevated in the islets of the DIO mice, indicating that the ADAR2-mediated RNA editing is responsive to the HFD-induced metabolic stress that is exerted specifically on the islets.

Regulation of ADAR2 by Energy Deficiency Status in Pancreatic Islets—To study the possibility that ADAR2 expression in the islets is regulated not only by excessive dietary fat intake but also by energy deficiency status, we analyzed the ADAR2 pro-
tein levels in the islets of rats subjected to fasting and refeeding. In male rats 8 weeks of age, fasting for 24 h caused a dramatic decrease in the serum levels of glucose (from 123.3 ± 5.48 to 93.5 ± 7.18 mg/dl, n = 6, p < 0.05) and insulin (from 5.51 ± 0.82 to 0.73 ± 0.23 ng/ml, n = 6, p < 0.01), both of which were reversed by refeeding for 24 h after fasting (124.83 ± 6.47 mg/dl for glucose and 4.48 ± 0.16 ng/ml for insulin) (Fig. 4, A and B). ImmunobLOTS of the islet extracts revealed that fasting induced a marked decrease (by ~70%) in ADAR2 protein expression level in the pancreatic islets (Fig. 4C) in comparison with the control rats fed ad libitum. In contrast, no apparent difference was observed in ADAR2 protein expression level in the cerebrum and liver (Fig. 3C) of control rats fed ad libitum. Moreover, the repression by fasting of ADAR2 protein expression was completely reversed by refeeding (Fig. 3C). These findings indicate that ADAR2 expression in the pancreatic islets responds to the energy status in vivo and that RNA editing by ADAR2 in the islets may function in a metabolically coordinated manner in relation to the nutrient and hormonal milieu, e.g. the blood glucose level.

Regulation by Glucose of ADAR2 Expression and ADAR2-mediated Editing in Pancreatic β-cells—Given the fact that glucose serves as the most potent physiological stimulus of pancreatic islet and β-cell function, we investigated the effects of glucose, at the physiological concentration for insulin secretion stimulation, on ADAR2 expression and RNA editing in the pancreatic β-cell lines. When the rat pancreatic β-cell line INS-1 cells precultured in 2.8 mM glucose were cultured in the presence of 16.7 mM glucose, the ADAR2 mRNA level, as analyzed by real-time RT-PCR, was stimulated by up to 10-fold at 3–12 h (Fig. 5A). Moreover, glucose induced a slight but noticeable reduction (~20%) in the ADAR1 mRNA level (Fig. 5B). It is worth noting that the glucose-stimulated elevation in the ADAR2 mRNA level displayed a similar time-dependent trend as that of insulin (Fig. 5C), a well studied glucose-inducible gene in the β-cells (40). However, the decline in the insulin transcript level after the maximal stimulation appeared to be greater than that observed for ADAR2, reaching to a point at 24 h lower than the initial basal level. This likely resulted from the transcriptional
repression of insulin biosynthesis arising from chronic exposure to the elevated concentration of glucose (40). Consistent with the increase of ADAR2 transcripts, the ADAR2 protein level was also considerably elevated, with approximately 2–3-fold increases observed after 6 h of stimulation, whereas ADAR1 protein level showed little changes (Fig. 5D). To confirm that the observed glucose up-regulation of ADAR2 expression is specific to pancreatic β-cells, we analyzed the effect of glucose stimulation in rat pheochromocytoma PC-12 cells and rat glioma C6 cells. No significant induction by high concentration of glucose was detected for the ADAR2 protein expression in PC-12 (Fig. 5E) or C6 cells (data not shown).

In parallel to the up-regulated ADAR2 expression, glucose stimulation resulted in progressively augmented Q/R site editing (41). β-cells are known to possess two signaling pathways for glucose sensing, the K<sub>ATP</sub> channel-dependent (42) and -independent (43) pathways. The former involves a number of ion channels responsible for the depolarization of the cell membrane, whereas the latter acts via G protein-coupled receptors. Thus, similar to neuronal cells sensing neurotransmitters, pancreatic cells possess the characteristic receptors for metabolites and hormones and ion channels that modulate the insulin secretion pathways. However, the precise biochemical mechanisms involved in the regulation of pancreatic β-cell function are not completely understood. In this study, we have observed strong coordinate regulation of ADAR2-mediated RNA editing and altered insulin secretion demand. In DIO mice with manifest insulin resistance, insulin secretion stimulated by glucose or other insulin secretagogues in pancreatic β-cells involves a sequential cascade of regulated cellular processes, including closure (inactivation) of ATP-dependent K<sup>+</sup> (K<sub>ATP</sub>) channels, membrane depolarization, activation of voltage-dependent Ca<sup>2+</sup> channels, and a rise in cytosolic calcium concentration (41).

Although a central function of A to I RNA editing in the pancreatic islets during fasting and refeeding. Rats were divided into three groups: fed ad libitum (Ad lib); 24 h of fasting; and 24 h of fasting followed by 24 h of refeeding. Plasma glucose (A) and insulin (B) levels were measured (mean ± S.E., n = 6 each group). *, p < 0.05; **, p < 0.01. C, immunoblots showing ADAR2 and β-actin expression from extracts of pooled tissues (n = 3 per group): the pancreatic islets, cerebrum, and liver. The blots represent two independent experiments, and the graph below indicates the change in -fold over the expression level in mice fed ad libitum after normalization to β-actin as internal loading control. *, p < 0.05.

FIGURE 4. Repression and derepression of ADAR2 protein expression in pancreatic islets during fasting and refeeding. Rats were divided into three groups: fed ad libitum (Ad lib); 24 h of fasting; and 24 h of fasting followed by 24 h of refeeding. Plasma glucose (A) and insulin (B) levels were measured (mean ± S.E., n = 6 each group). *, p < 0.05; **, p < 0.01. C, immunoblots showing ADAR2 and β-actin expression from extracts of pooled tissues (n = 3 per group): the pancreatic islets, cerebrum, and liver. The blots represent two independent experiments, and the graph below indicates the change in -fold over the expression level in mice fed ad libitum after normalization to β-actin as internal loading control. *, p < 0.05.

**DISCUSSION**

Although a central function of A to I RNA editing in the central nervous system in the mammals and the fruit fly (5–13, 37) is well established, the physiological regulation and functional roles of ADAR-mediated RNA editing in tissues other than the brain have been nebulous. In this study, we report our novel findings that 1) ADAR1 and ADAR2 are not only prominently expressed in the murine pancreatic islets, but importantly, that ADAR2 expression is also selectively regulated by the systemic energy metabolism status in an islet-specific fashion; 2) ADAR2-mediated GluR-B RNA editing is also regulated in islets in response to dietary fat-induced metabolic stress characterized by hyperinsulinemia; 3) glucose, the most potent stimulus for insulin secretion of the pancreatic β-cells, acts as an enhancer specifically in the β-cells for ADAR2 expression and ADAR2 editing activity. Our results suggest that, in addition to the central nervous system, A to I RNA editing by ADAR2 in pancreatic islets and β-cells may play an important role in energy metabolism that has yet to be elucidated.

Pancreatic β-cells are a key component in glucose homeostasis, sensing glucose by changes in metabolic flux and responding by synthesis and secretion of insulin (27). Insulin secretion stimulated by glucose or other insulin secretagogues in pancreatic β-cells involves a sequential cascade of regulated cellular processes, including closure (inactivation) of ATP-dependent K<sup>+</sup> (K<sub>ATP</sub>) channels, membrane depolarization, activation of voltage-dependent Ca<sup>2+</sup> channels, and a rise in cytosolic calcium concentration (41). β-cells are known to possess two signaling pathways for glucose sensing, the K<sub>ATP</sub> channel-dependent (42) and -independent (43) pathways. The former involves a number of ion channels responsible for the depolarization of the cell membrane, whereas the latter acts via G protein-coupled receptors. Thus, similar to neuronal cells sensing neurotransmitters, pancreatic cells possess the characteristic receptors for metabolites and hormones and ion channels that modulate the insulin secretion pathways. However, the precise biochemical mechanisms involved in the regulation of pancreatic β-cell function are not completely understood. In this study, we have observed strong coordinate regulation of ADAR2-mediated RNA editing and altered insulin secretion demand. In DIO mice with manifest insulin resistance, insulin secretion is chronically enhanced to compensate for the higher demand for insulin production to control blood glucose level, resulting in hyperinsulinemia. Under these stress conditions, caused by chronic intake of surplus dietary fat, ADAR2-medi-
ADAR2 transcripts increased in the β-cells upon glucose stimulation (~5-fold at 24 h; Fig. 5A), the self-editing of ADAR2 transcripts was augmented simultaneously (~3-fold at 24 h; Fig. 7C), consistent with the dynamic elevation of ADAR2 protein level (~3-fold at 24 h; Fig. 5D). However, as a negative mechanistic regulator of ADAR2 protein translation (21), the enhanced self-editing efficiency might have served as a "brake" to prevent further increases in ADAR2 protein level that presumably accumulated during the glucose stimulation. Yet other factors that may affect the translation efficiency or protein stability of ADAR2 cannot be excluded.

The fact that ADAR2 expression was found in all four major types of endocrine cells in the pancreatic islets (Fig. 2) indicates that the function of A to I RNA editing is perhaps not limited to the β-cells. It remains to be seen whether ADAR2-mediated RNA editing is under similar metabolic regulation in other endocrine cells, such as the α-cells. From the known ADAR editing substrates found in the central nervous system, it is tempting to speculate that islet-specific ion channels and G protein-coupled receptors (10, 37) likely exist as editing substrates for ADARs, and modifications by editing may influence the islet and β-cell function, e.g. exerting a finer level of tuning in the control of insulin secretion pathway(s) involved in sensing nutrients, metabolites, and hormonal stimuli. It is intriguing that some of the newly identified potential A to I editing RNA substrates in Drosophila indeed encode, aside from voltage- and ligand-gated ion channels, several components of the synaptic release machinery, which may serve similar prominent roles in the regulated release of secretory vesicles from the islet endocrine cells (37). In this regard, our findings suggest that A to I RNA editing represents a likely regulatory mechanism underlying the molecular pathways of the pancreatic islets and β-cells in sensing the metabolic energy status of the body.

Glutamate has been reported to regulate insulin secretion from β-cells through acting on AMPA glutamate receptors (36). Glutamate is known to be co-secreted with glucagon, and a paracrine-like glutamate signaling pathway has also been suggested to mediate cell-cell communications as a novel regulatory mechanism for islet function (44). However, it remains to be determined whether editing by ADARs of glutamate receptor channels in the islets has any effect on the glutamate signaling for stimulation of insulin secretion. Also, further investigations will be necessary to understand the mechanisms
underlying the energy regulation of ADAR2 in pancreatic islets and β-cells. Although the observed regulation by glucose of ADAR2 expression and editing in the β-cell lines is consistent with the findings in vivo in the two types of animal models under energy stress, glucose may not be the sole modulator of editing by ADAR2 in the islets and β-cells. Obesity is known to be associated with chronic activation of cellular stress signaling and inflammatory pathways (45–47) that are thought to be responsible for the systemic insulin resistance, and the fasting-refeeding process triggers dramatic changes not only in the serum glucose level but in a great number of other factors such as insulin, leptin, and free fatty acids (48). Whether ADAR2-mediated RNA editing in the islets and β-cells is also modulated by other metabolic molecules, cellular signaling mediators, or hormonal regulators remains to be elucidated. A unique property of β-cells is that insulin secretion is stimulated by glucose, and insulin possesses an important autocrine function by exerting profound effects on β-cells (49). However, insulin appears unable to affect ADAR2 expression or ADAR2-mediated RNA editing in β-cell lines. The ADAR1 gene is known to be regulated by interferon (19), and here, our results now demonstrate that the ADAR2 gene and ADAR2-mediated RNA editing events are physiologically regulated by nutrient status in the peripheral tissue of crucial function in the homeostatic energy control. Interestingly, it has been recently reported that forebrain ischemia in adult rats selectively expresses expression of ADAR2 enzyme, and expression of a constitutively active cAMP-responsive element-binding protein is able to induce expression of endogenous ADAR2 in the neurons analyzed (50). It is possible that similar signaling components may be employed for the regulation of A to I editing machinery in the pancreatic islet endocrine cells.

RNA-based mechanisms for gene regulation play important roles in various cellular pathways. MicroRNA molecules, which have been shown to serve as a novel type of RNA substrates for A to I editing (14), are reported to regulate the insulin secretion process in β-cells (51). It is unclear whether these RNA-mediated gene regulation pathways are implicated in the dysfunction of pancreatic β-cells and metabolic disorders. Further studies of the function of ADAR enzymes in pancreatic islets and β-cells will lead us to better understanding of not only the fundamental physiology of A to I RNA editing but the basic biology of pancreatic islets and β-cells.

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