Original article

MOLECULAR METABOLISM

Pannexin 1 is required for full activation of insulin-stimulated glucose uptake in adipocytes

Samantha E. Adamson [1,2], Akshaya K. Meher [1], Yu-hsin Chiu [1], Joanna K. Sandilos [1], Nathaniel P. Oberholtzer [1], Natalie N. Walker [5], Stefan R. Hargett [3], Scott A. Seaman [2,3], Shayn M. Peirce-Cottler [2,3], Brant E. Isakson [2,4], Coleen A. McNamara [2,5], Susanna R. Keller [5], Thurl E. Harris [1], Douglas A. Bayliss [1], Norbert Leitinger [1,2,*]

ABSTRACT

Objective: Defective glucose uptake in adipocytes leads to impaired metabolic homeostasis and insulin resistance, hallmarks of type 2 diabetes. Extracellular ATP-derived nucleotides and nucleosides are important regulators of adipocyte function, but the pathway for controlled ATP release from adipocytes is unknown. Here, we investigated whether Pannexin 1 (Panx1) channels control ATP release from adipocytes and contribute to metabolic homeostasis.

Methods: We assessed Panx1 functionality in cultured 3T3-L1 adipocytes and in adipocytes isolated from murine white adipose tissue by measuring ATP release in response to known activators of Panx1 channels. Glucose uptake in cultured 3T3-L1 adipocytes was measured in the presence of Panx1 pharmacologic inhibitors and in adipocytes isolated from white adipose tissue from wildtype (WT) or adipocyte-specific Panx1 knockout (AdipPanx1 KO) mice generated in our laboratory. We performed *in vivo* glucose uptake studies in chow fed WT and AdipPanx1 KO mice and assessed insulin resistance in WT and AdipPanx1 KO mice fed a high fat diet for 12 weeks. Panx1 channel function was assessed in response to insulin by performing electrophysiologic recordings in a heterologous expression system. Finally, we measured Panx1 mRNA in human visceral adipose tissue samples by qRT-PCR and compared expression levels with glucose levels and HOMA-IR measurements in patients.

Results: Our data show that adipocytes express functional Pannexin 1 (Panx1) channels that can be activated to release ATP. Pharmacologic inhibition or selective genetic deletion of Panx1 from adipocytes decreased insulin-induced glucose uptake *in vitro* and *in vivo* and exacerbated diet-induced insulin resistance in mice. Further, we identify insulin as a novel activator of Panx1 channels. In obese humans Panx1 expression in adipose tissue is increased and correlates with the degree of insulin resistance.

Conclusions: We show that Panx1 channel activity regulates insulin-stimulated glucose uptake in adipocytes and thus contributes to control of metabolic homeostasis.

Keywords Pannexin 1; Adipocyte; Extracellular ATP; Glucose uptake

1. INTRODUCTION

Adipose tissue is renowned for its function in lipid storage, but it is also a key endocrine organ and metabolic dysfunction of adipocytes exacerbates insulin resistance [1]. In healthy adipose tissue, insulin stimulates glucose uptake and lipogenesis while inhibiting lipolysis, but these effects are blunted during insulin resistance [1]. Furthermore, glucose uptake in adipocytes is a major contributor to whole body insulin sensitivity [2].

The purine nucleoside adenosine accumulates extracellularly in isolated adipocyte suspensions [3–5] and is thought to be derived from degradation of extracellular ATP. Adenosine can impact adipocyte metabolic function by inhibiting lipolysis [6], increasing glucose uptake [7,8], enhancing insulin action to stimulate glucose oxidation [9], and regulating adipose tissue blood flow [10]. In addition to adenosine, extracellular nucleotides such as ATP or UTP have autocrine effects on adipocytes. They signal through the purinergic P2 receptor family, which includes ATP-gated cation channels $P2X_{1\text{-}7}$ and G-protein-coupled $P2Y_{1,2,4,6,11\text{-}14}$ receptors [11]. In brown adipose tissue, extracellular ATP caused mobilization of intracellular calcium stores, consistent with nucleotide signaling through purinergic P2 receptors [12]. Extracellular ATP also led to increased cell membrane capacitance in adipocytes [13,14], and it was suggested that ATP activates exocytosis [15]. In white adipocytes, micromolar concentrations of ATP inhibit glucose oxidation [16], but increase glycogen synthesis [17] and lipogenesis [18]. However, the effect of exogenous ATP on basal and insulin-induced glucose uptake in adipocytes is still a matter of controversy: ATP was shown in some studies to be inhibitory at millimolar

[1]Department of Pharmacology, University of Virginia, Charlottesville, VA 22908, USA [2]Cardiovascular Research Center, University of Virginia, Charlottesville, VA 22908, USA [3]Department of Biomedical Engineering, University of Virginia, Charlottesville, VA 22908, USA [4]Department of Physiology, University of Virginia, Charlottesville, VA 22908, USA [5]Department of Medicine, University of Virginia, Charlottesville, VA 22908, USA

*Corresponding author. Department of Pharmacology, University of Virginia, PO Box 800735, Charlottesville, VA 22908, USA. Tel.: +1 (434) 243 6363. E-mail: nl2q@virginia.edu (N. Leitinger).

Received June 20, 2015 • Accepted June 27, 2015 • Available online 3 July 2015

http://dx.doi.org/10.1016/j.molmet.2015.06.009

concentrations [19,20], while in other work, no effect of ATP was observed [18].

Purinergic signaling in endocrine organs has been a major research focus [reviewed by Burnstock [21]], and novel therapies based on purinergic receptors as potential drug targets for type II diabetes have been suggested [22–24]. However, there are many purinergic receptors, and modulating ATP release could represent an alternative therapeutic strategy. In this respect, it remains an open question as to how nucleosides and nucleotides are released from adipocytes in a controlled manner.

Pannexin 1 (Panx1) channels control the release of ATP and other nucleotides from many cell types [25]. For example, activation of Panx1 channels initiates paracrine signaling that controls blood vessel constriction [26,27] and clearance of dying cells [28]. Pannexins (Panx1, 2, and 3) are hexameric membrane channels that are structurally similar to connexins but do not form gap-junctions [29]. Panx1 and 3 are present in many tissues while Panx2 is mainly expressed in the brain [29]. Activation of Panx1 can proceed by various mechanisms including α1-adrenergic stimulation [30], mechano-stretch [31], and caspase-mediated cleavage of the C-terminal portion of Panx1, an irreversible process [32]. Posttranslational modification including phosphorylation and S-nitrosylation was suggested to regulate Panx1 channel function [25] and known pharmacological inhibitors of Panx1 include carbenoxolone, probenecid, mefloquine, the food dye FD&C Blue No. 1, and trovafloxacin [33–36]. Here we describe an unexpected role for Panx1 channels, controlling ATP release from adipocytes, which is required for full activation of insulin-induced glucose uptake. Since dysregulation of glucose uptake in adipocytes was shown to impact whole body insulin sensitivity [37], Panx1 may play a key role in the pathophysiology of insulin resistance.

2. METHODS

2.1. Cell-based assays

For YO-PRO® dye uptake studies, 3T3-L1 cells were confluently plated in 6 well plates, differentiated into adipocytes as described, and treated with phenylephrine for 30 min followed by staining for 10 min with YO-PRO® (1 μM) and Hoechst (1 μg/mL). ATP concentrations in supernatants were measured using Promega Cell Titer Glo reagent. Luminescence was read on a Tecan Infinite M200 plate reader. For 3T3-L1 adipocyte glucose uptake, 3T3-L1 fibroblasts were plated to confluency in 12 well plates and differentiated into adipocytes as described. Cells were pretreated with carbenoxolone or probenicid as indicated for 20 min at which point insulin was added for 15 min. Glucose uptake was measured in isolated adipocytes as described [38].

2.2. Mice

$Panx1^{fl/fl}Adipoq^{Cre}$ mice were generated through appropriate breeding between the $Adipoq^{cre}$ mice [39] with $Panx1^{fl/fl}$ mice [36]. Littermate controls were used for all experiments. All animal studies were approved by the Animal Care and Use Committee at the University of Virginia. Mice were fed a high fat diet containing 60% cal from fat and 0.2% cholesterol (Bioserv) or normal chow (Teklad). Fat and lean masses were measured by EchoMRI™-100H Body Composition Analyzer. Glucose and insulin tolerance tests were performed in accordance with recommendations published by Ayala et al. [40]. For glucose tolerance test, mice were fasted for 6 h and then injected with 1 g/kg glucose i.p. and blood glucose levels were measured from tail blood by glucometer (OneTouch Ultra) over 2 h. For insulin tolerance test, mice were fasted 6 h and then injected with 0.75 U/kg insulin i.p. and blood glucose levels were measured from tail blood by glucometer (OneTouch Ultra) over 1 h. *In vivo* glucose uptake studies were performed as described [41]. In brief, mice were fasted 6 h followed by intraperitoneal injection of 2 g/kg glucose containing 10 μCi [^{3}H] 2-deoxy-D-glucose. Gastrocnemius muscle and perigonadal adipose tissue were collected 2 h post injection and snap frozen. 2-deoxyglucose uptake in tissues was determined by passing tissue homogenates over poly-prep chromatography columns with AG1-X8 resin (Bio-rad) and then calculating the difference in radioactive counts between total homogenate and column eluent, normalizing to specific activity of glucose as determined by serum samples processed with perchloric acid.

2.3. Electrophysiology

Patch clamping of 3T3-L1 adipocytes with active caspase 3 was performed as described previously [32]. Whole-cell recordings were made at room temperature using Axopatch 200B amplifier (Molecular Devices) with a bath solution composed of 140 mM NaCl, 3 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES and 10 mM glucose (pH 7.3). Borosilicate glass patch pipettes (3–5 MΩ) were filled with an internal solution containing 30 mM tetraethylammonium chloride, 100 mM $CsMeSO_4$, 4 mM NaCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 10 mM HEPES, 10 mM EGTA, 3 mM ATP-Mg, and 0.3 mM GTP-Tris (pH 7.3). Ramp voltage commands were applied by using pCLAMP software and Digidata1322A digitizer (Molecular Devices). HEK293T cells were transiently transfected using Lipofectamine2000 (Invitrogen), and underwent serum depletion for 2–4 h before patch recording in order to reduce basal insulin receptor signaling. Basal Panx1 current was recorded, and then insulin (180 nM) was applied to the bath solution, followed by CBX (50 μM). Note that no CBX-sensitive current was observed in HEK293T cells without heterologously expressing Panx1 [32]. Constructs used in HEK293T heterologous system include mouse Panx1 wildtype construct [42,43], human Panx1(TEV) construct [32], and an EGFP-tagged human insulin receptor construct (Addgene) [44].

2.4. Human adipose tissue samples

Omental adipose tissue samples were obtained from patients undergoing bariatric surgery. All protocols and procedures were approved by the Institutional Review Board at the University of Virginia (IRB HSR #14180). HOMA-IR was calculated using the formula: HOMA-IR = fasting insulin × fasting glucose/405 [45].

2.5. Statistical analysis

Statistical analyses were performed with Graph Pad Prism (GraphPad, San Diego, CA). Student's t-test or ANOVA with post hoc comparison tests were used as appropriate. F test was performed in Prism to determine if variances were similar among groups.

3. RESULTS

3.1. Pannexin 1 channels are expressed and functional in adipocytes

The functional role of Pannexin 1 (Panx1) in adipose tissue has not been reported. To examine whether adipocytes express Panx1, we used immunohistochemistry. Panx1 protein expression was clearly observed on membranes of adipocytes (arrows) in adipose tissue from wild-type C57Bl6 mice, while the staining was absent in adipose tissue from *Panx1 knockout (KO)* mice (Figure S1A). To explore the functionality of Panx1 channels in adipocytes we performed experiments with cultured 3T3-L1 adipocytes and primary adipocytes isolated from wild-type or *Panx1 KO* mice, using known activators of Panx1 channel

function [28,30,32]. We found that Panx1 expression in 3T3-L1 adipocytes is induced by insulin (Figure S1B), which is in line with reports that cAMP response elements play a role in transcriptional regulation of Panx1 [46]. First indications for a functional role of Panx1 in adipocytes came from experiments where treatment of 3T3-L1 adipocytes with the α-adrenergic receptor agonist phenylephrine (PE) caused a dose-dependent increase in the uptake of YO-PRO®, a green-fluorescent dye that can enter cells via open Panx1 channels [28,47] (Figure 1A). Furthermore, PE treatment induced the release of ATP from 3T3-L1 adipocytes into the media (Figure 1B). PE-induced ATP release was abrogated by a Panx1 intracellular loop peptide (IL2) corresponding to a region of the intracellular loop portion of the Panx1 channel (K191—K200) (Figure 1B **inset**) that disrupts α-adrenergic-dependent activation of Panx1 channels [48]. In contrast, a C-terminal peptide (CT2) corresponding to the C-terminal region of Panx1 had no effect (Figure 1B). To further examine whether PE-induced ATP release from adipocytes was dependent on Panx1 channel function, we treated adipocytes isolated from wildtype or global Panx1 knockout mice with PE, which resulted in significant ATP release into the media only when Panx1 was present (Figure 1C). These results show that Panx1 is not only present on adipocytes but also that channel function can be activated to release ATP through the known mechanism of alpha-adrenergic stimulation.

Another important mechanism of Panx1 channel opening is cleavage of the C-terminal tail of the channel by activated caspase-3 (CASP3), particularly relevant for Panx1-dependent ATP release from apoptotic cells [28,32]. To test if this mechanism can induce Panx1 opening in adipocytes, we applied activated CASP3 to cultured 3T3-L1 adipocytes through the patch pipette, which induced a current with Panx1-like voltage-dependent properties that was blocked by the Panx1 inhibitor carbenoxolone (CBX) [32] (Figure 1D). These results suggested that Panx1 channels may be activated during apoptosis of adipocytes.

To further investigate caspase-dependent activation of adipocyte Panx1 during apoptosis, we exposed 3T3-L1 adipocytes to UV irradiation. Induction of apoptosis was confirmed by the formation of apoptotic bodies (Figure S2A) and by radiation dose-dependent uptake of YO-PRO® and propidium iodide, dyes known to enter cells via Panx1 [47] (Figure S2B and C). Further support for caspase-dependent opening of Panx1 channels induced by UV irradiation of adipocytes was demonstrated by a significant increase in ATP release into the supernatant that was blocked by treatment with the Panx1 inhibitor carbenoxolone (CBX) (Figure S2D and Figure 1E) and the pan-caspase inhibitor zVAD (Figure 1E). Together, these results show that opening of Panx1 channels can be induced in adipocytes either by α-adrenergic stimulation or via caspase-mediated C-terminal cleavage during apoptosis.

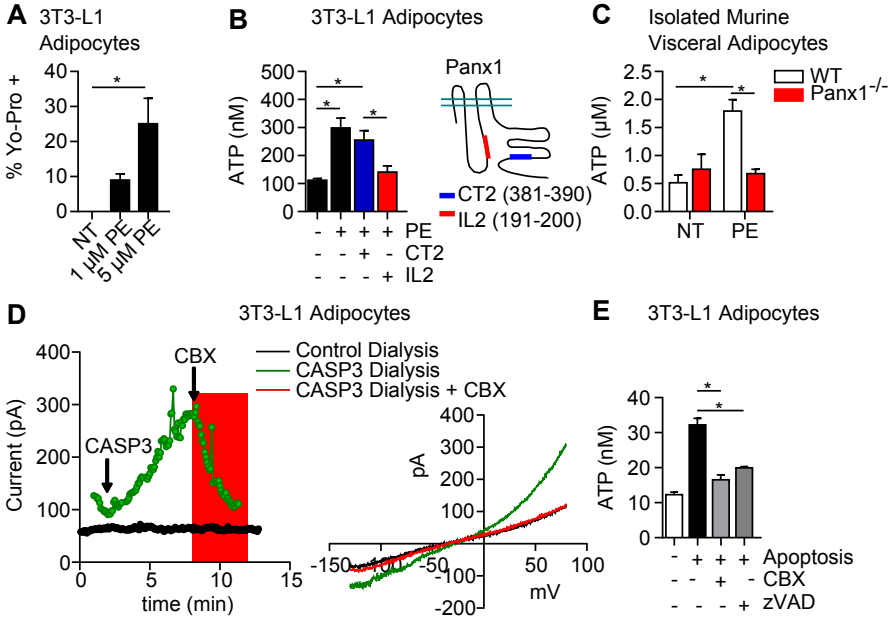

Figure 1: Pannexin 1 channel function in adipocytes is regulated by alpha-adrenergic stimulation or via caspase-mediated C-terminal cleavage during apoptosis. (**A**) 3T3-L1 adipocytes were treated with indicated concentrations of phenylephrine (PE) for 30 min and then stained with 1 μM YO-PRO® and 1 μg/mL Hoechst for 10 min. Experiment was performed in triplicate. Total cells were quantitated by counting Hoechst-positive cells. Cells positive for YO-PRO® indicate cells in which Panx1 channels have been activated and opened, allowing dye to enter. Data are expressed as mean ± s.e.m. *p = 0.0087 by Student's t-test. (**B**) 3T3-L1 adipocytes were treated with 5 μM PE with or without pretreatment with a Panx1 intracellular loop peptide (IL2) or a C-terminal peptide (CT2). The IL2 peptide corresponds to a region of the Panx1 intracellular loop (aa 191—200) while the CT2 peptide is from a region of the C-terminal tail corresponding to aa 381—390 (inset) [48]. ATP release into the media was measured using cell-titer glo assay (Promega). Experiment was performed in triplicate. Data are expressed as mean ± s.e.m. *p < 0.05 by Student's t-test. (**C**) Adipocytes were isolated from perigonadal adipose tissue of *WT* or *Panx1 KO* mice, and ATP release was measured upon stimulation with phenylephrine (PE, 5 μM, 15 min) or no treatment (NT). Experiment was performed in triplicate. Data are expressed as mean ± s.e.m. *p < 0.04 by 2-way ANOVA with Sidak's multiple comparison test. (**D**) Whole cell patch clamping of 3T3-L1 adipocytes reveals a carbenoxolone-sensitive current when active caspase-3 is present in the pipette indicating that adipocyte Panx1 is activated by the caspase-cleavage mechanism. Arrows indicate time at which active caspase 3 (CASP3) or carbenoxolone (CBX) was added. Control dialysis shown in black. Current—voltage relationship (IV) curves are shown at right. (**E**) 3T3-L1 adipocytes were exposed to 400 mJ/cm^3 UV irradiation (Stratalinker) to induce apoptosis and incubated with or without 400 μM carbenoxolone (CBX) or zVAD, a pan-caspase inhibitor (50 μM) for 1 h. ATP was measured in the supernatant by cell-titer glo assay (Promega). n = 8. Data are expressed as mean ± s.e.m. *p < 0.0001 by ANOVA with Tukey's multiple comparison test.

3.2. Full activation of insulin-stimulated glucose uptake in adipocytes requires ATP release by Panx1 channels

To explore the role of Panx1 in adipocytes *in vivo*, we crossed *Panx1*$^{F/F}$ mice with Adiponectin-Cre (*Adipq*$^{Cre+}$) mice [39] to generate an adipocyte-specific Panx1 KO mouse (*AdipPanx1KO*). Excision of exon 3 of the Panx1 gene in *Panx1*$^{F/F}$*Adipq*$^{Cre+}$ (*AdipPanx1KO*) mice was observed in adipose tissues including visceral, subcutaneous, and brown adipose tissue but not in liver, lung, pancreas, spleen, or brain (Figure S3A). Remaining *Panx1* expression in visceral, subcutaneous, and brown adipose tissue from *AdipPanx1KO* mice is derived from resident immune cells and other stromal and vascular cells that are present in whole adipose tissue preparations. Whole mount staining of visceral adipose tissue pieces from *WT* and *AdipPanx1KO* mice with an antibody specific for Panx1 [42] and subsequent confocal microscopy demonstrated distinctive punctate Panx1 staining in adipocytes of WT mice, which was undetectable in adipocytes from *AdipPanx1KO* mice (Figure S3B). Panx1 deficiency in adipocytes did not affect cell size, since analysis using a BioSorter® demonstrated that adipocytes isolated from gonadal adipose tissue of *WT* and *AdipPanx1KO* mice showed identical cell size distribution (Figure S3C).

To examine a role for Panx1 in glucose uptake in adipocytes, we treated cultured or primary adipocytes with insulin for 15 min, and basal and insulin-stimulated glucose uptake was measured in the presence and absence of two distinct pharmacological Panx1 inhibitors. Glucose uptake into cultured 3T3-L1 adipocytes was significantly increased after treatment with insulin; notably, pretreatment with carbenoxolone (CBX) or probenecid (Prob) resulted in significantly blunted insulin-stimulated glucose uptake (Figure 2A). In addition, insulin-stimulated glucose uptake was significantly impaired in adipocytes isolated from *AdipPanx1KO* mice compared to adipocytes isolated from *WT* mice (Figure 2B). Since pharmacological as well as genetic inhibition of Panx1 in adipocytes resulted in blunted insulin-stimulated glucose uptake, we hypothesized that Panx1-mediated ATP release was responsible for the effect. Indeed, the addition of exogenous ATP rescued the compromised insulin-stimulated glucose uptake in *Panx1*-deficient adipocytes (Figure 2B).

To test whether a lack of adipocyte Panx1 would inhibit glucose uptake *in vivo*, lean WT and *AdipPanx1KO* mice were given a bolus of glucose containing a trace amount of radioactive [^{3}H] 2-deoxy-D-glucose such that organs harvested at the end of the study could be assessed for glucose uptake. While the rate of glucose uptake was similar in gastrocnemius muscle from WT and *AdipPanx1KO* mice, the absence of Panx1 in adipocytes resulted in a 20% decreased rate of glucose uptake in perigonadal white adipose tissue (Figure 2C).

These results demonstrate that Panx1-dependent ATP release is required for insulin-stimulated glucose uptake in adipocytes, and they suggest a potentially novel mechanism of Panx1 channel activation by insulin.

3.3. Insulin induces Panx1 channel activation and ATP release

To examine whether insulin activates Panx1 channels, we first assessed Panx1-dependent ATP release from primary adipocytes isolated from *WT* mice. Treatment with insulin for 30 min caused a significant increase in extracellular ATP, which was blocked by addition of the Panx1 inhibitor probenecid (Prob) (Figure 3A). Next, we tested the ability of insulin to activate Panx1 channels using HEK293 cells co-transfected with expression plasmids for the human insulin receptor (hIR) and murine *Panx1*. Insulin treatment evoked a current with Panx1-like voltage-dependent properties that was blocked by the Panx1 inhibitor carbenoxolone (CBX) (Figure 3B). Insulin-stimulated Panx1 currents were also seen in HEK293 cells co-transfected with expression plasmids for the human insulin receptor (hIR) and human Panx1 with a mutated caspase cleavage site [32] (Figure 3C). These data identify insulin as a novel mediator of Panx1 channel activation and show that insulin-induced activation of Panx1 is independent of caspase-mediated cleavage.

3.4. Absence of Panx1 in adipocytes exacerbates diet-induced insulin resistance without affecting energy expenditure or adipose tissue inflammation

Impaired glucose uptake in adipose tissue was shown to adversely affect high fat diet-induced insulin resistance [2,37]. To test whether the 20% reduction in glucose uptake rate that we observed in the adipose tissue of *AdipPanx1KO* mice (Figure 2C) would have an effect on the development of insulin resistance, we fed *WT* and *AdipPanx1KO* mice a diabetogenic high fat diet. The absence of Panx1 from adipocytes slightly, but significantly, exacerbated measures of insulin resistance, including glucose and insulin tolerance after 12 weeks of high fat diet feeding (Figure 4A and B). However, no difference in weight gain or adiposity was observed between the groups (Figure 4C and D). Serum free fatty acid levels were also similar between high fat diet-fed *WT* and *AdipPanx1KO* mice (Figure 4E), and high fat feeding increased serum insulin levels in both *WT* and *AdipPanx1KO* mice to the same extent (Figure 4F).

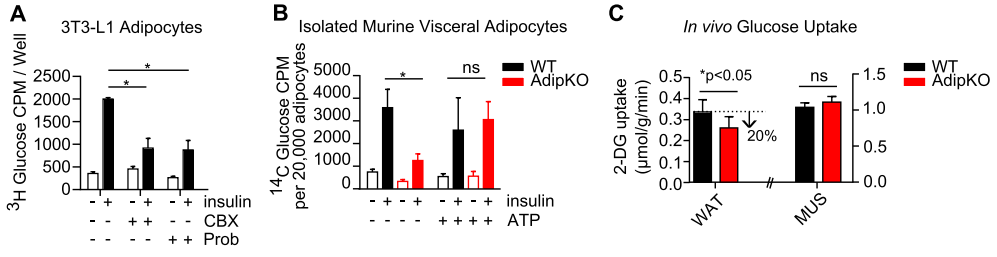

Figure 2: Full activation of insulin-stimulated glucose uptake in adipocytes requires ATP release by Panx1 channels. (**A**) Blockade of Pannexin-1 channels with carbenoxolone (100 μM, CBX) or probenecid (1 mM, Prob) significantly decreases insulin-stimulated ^{3}H-glucose uptake in 3T3L1-adipocytes. Data are expressed as mean ± s.e.m. *$p < 0.001$ by 2 way ANOVA with Tukey's multiple comparisons test. (**B**) Insulin-stimulated glucose uptake is significantly decreased in adipocytes isolated from perigonadal adipose tissue of adipocyte-specific Pannexin-1 null mice. Addition of exogenous ATP (50 μM) restores insulin-stimulated ^{14}C-glucose uptake in adipocytes isolated from *Panx1* null mice. Data are expressed as mean ± s.e.m. *$p < 0.003$ by 1 way ANOVA with Tukey's multiple comparisons test. (**C**) *In vivo* [^{3}H] 2-deoxy-D-glucose uptake was assessed in perigonadal white adipose tissue (WAT) and gastrocnemius muscle (MUS) in age-matched, male, chow fed WT and *AdipPanxKO* littermates ($n = 6$). Data are presented as mean ± s.e.m. *$p < 0.05$ by paired t-test. Dotted line indicates the 20% decrease in glucose uptake in WAT in *AdipPanxKO* mice compared to WT.

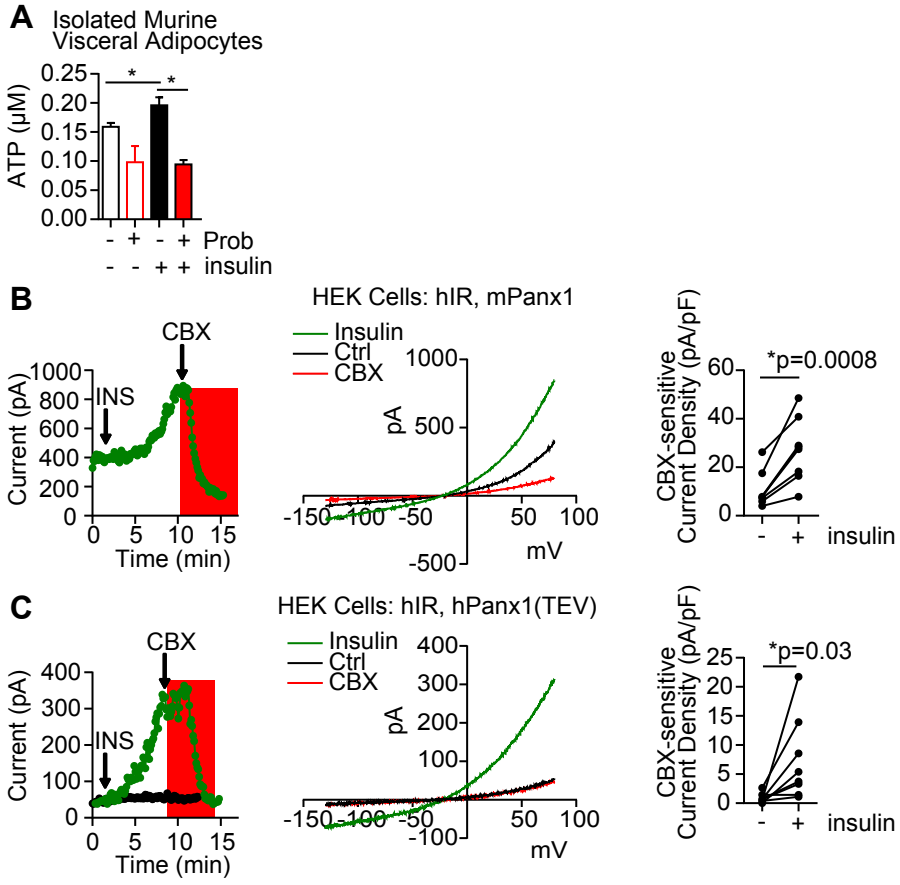

Figure 3: Insulin induces Panx1 channel activation and ATP release. (**A**) Adipocytes isolated from perigonadal adipose tissue of WT mice release ATP upon insulin stimulation, which can be blocked by treatment with the Panx1 inhibitor probenecid. Data are expressed as mean ± s.e.m. *p < 0.05 by Student's t-test. (**B**) Whole cell patch clamp of HEK cells transfected with human insulin receptor and mouse Panx1 reveals a Panx1 dependent current upon treatment with insulin that is abolished by addition of the Panx1 inhibitor carbenoxolone. Current–voltage relationship curve is shown in middle. Insulin treatment significantly increases CBX-sensitive current density (n = 8 cells). (**C**) Whole cell patch clamp of HEK cells transfected with human insulin receptor and human Panx1 in which the C-terminal caspase cleavage site has been replaced with a TEV protease cleavage site reveals a Panx1 dependent and CBX-sensitive current upon treatment with insulin. Current–voltage relationship curve is shown in middle. Insulin treatment significantly increases CBX-sensitive current density (n = 8 cells).

Next, we investigated whether differences in insulin resistance in *AdipPanx1KO* mice could be a result of alterations in whole body energy expenditure or inflammation in the adipose tissue. Chow-fed *WT* and *AdipPanx1KO* mice had similar metabolic rates and mobility (Figure 4G–J) while *AdipPanx1KO* mice on a high fat diet revealed small but non-significant decreases in light and dark cycle metabolic rates, compared to WT mice on high fat diet (Figure 4G–I). However, we did not observe differences in food intake (not shown), or overall mobility (Figure 4J). Analysis of the stromal vascular fractions of perigonadal adipose tissue from high fat-fed *WT* and *AdipPanx1KO* mice by flow cytometry revealed no difference in absolute numbers of $CD45^+/CD11b^+$ cells, which include macrophages. There was also no difference in the relative abundance of phenotypically polarized macrophage subsets M1 ($CD11b^+/CD11c^+$) and M2 ($CD11b^+/CD206^+$) (Figure S4A). Moreover, analyses of mRNA levels of several pro and anti-inflammatory cytokines in the stromal vascular fraction of perigonadal adipose tissue from high fat fed *WT* and *AdipPanx1KO* mice, including *tnfα*, *il1β*, *il18*, *ifnγ*, and *mcp1* as well as *il4*, *arg1*, *mgl1*, *il10*, and *ym1*, revealed no significant difference (Figure S4B), except for *mgl1* mRNA levels, which were significantly lower in SVF from *AdipPanx1KO* mice.

We conclude from these data that the greater propensity to develop insulin resistance in high fat-fed *AdipPanx1KO* mice is neither due to alterations in energy expenditure nor to adipose tissue inflammation, but can be attributed to diminished glucose uptake in adipocytes as a result of defective Panx1-mediated ATP release.

3.5. Pannexin 1 expression in human adipose tissue is associated with obesity and insulin resistance

Neither expression levels nor potential functional roles of Panx1 in adipose tissue have been reported in the context of human pathology. A previous study compared global gene expression in subcutaneous adipose tissue between lean and obese Pima Indians using gene arrays [49]. Data extracted from the data sets deposited at NCBI (GDS1498[ACCN]) demonstrate that Panx1 expression in both males and females was significantly increased in obese compared to lean subjects (Figure 5A). Thus, we examined Panx1 expression in visceral adipose tissue obtained from 23 morbidly obese patients during bariatric surgery. We found that relative Panx1 mRNA expression levels significantly correlated with fasting blood glucose levels, and this correlation was especially strong in Caucasian females (Figure 5B). Subjects in the tertile with the highest relative Panx1

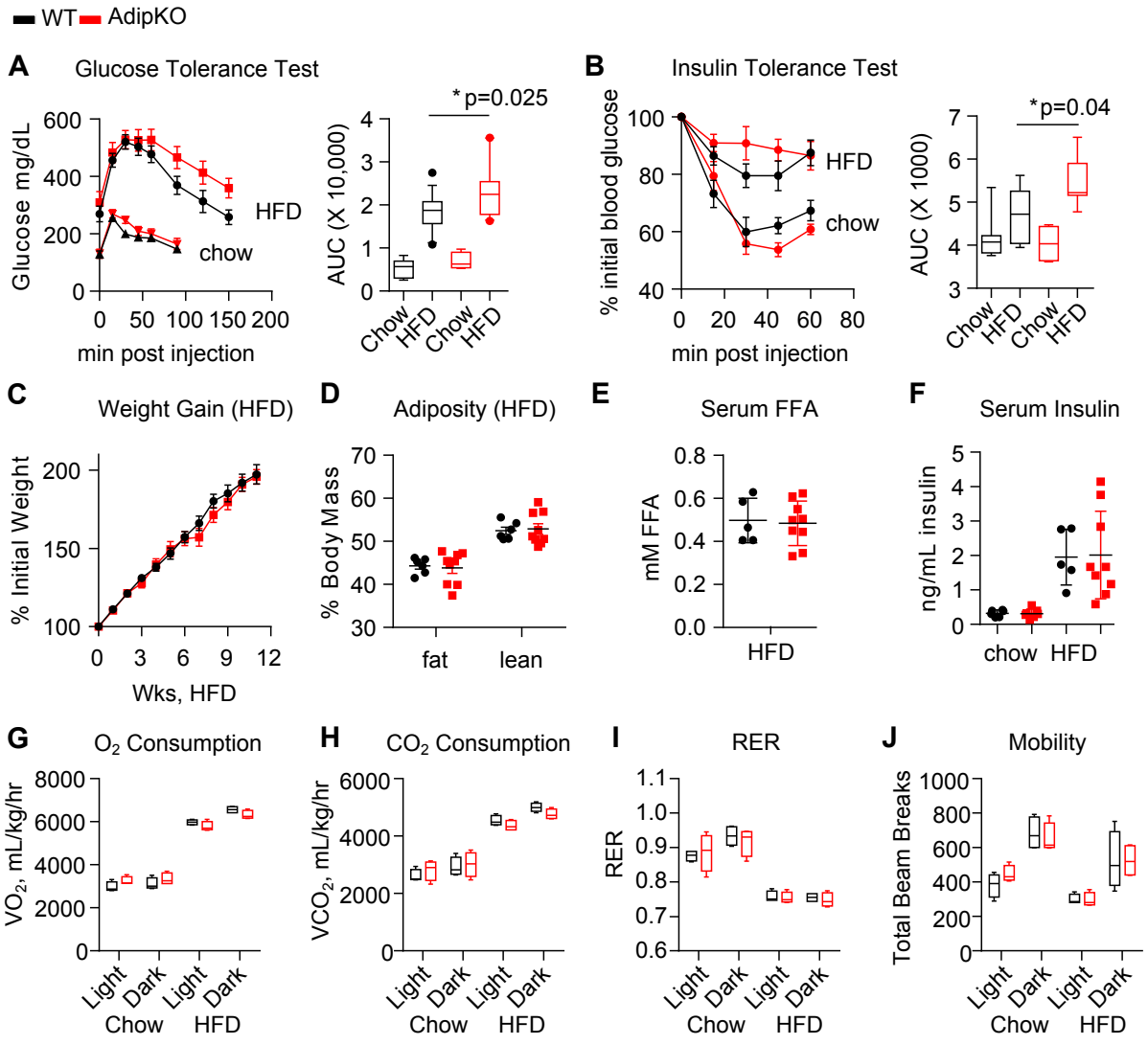

Figure 4: Weight gain, energy expenditure, serum free fatty acids and insulin are not different between high fat fed WT and *AdipPanx1KO* mice. (**A**) Intraperitoneal glucose tolerance test was performed on age-matched, male, WT and *AdipPanxKO* littermates that had been fed chow or high fat diet (60% fat) for 12 weeks. Mice were injected i.p. with 1 g/kg glucose, and blood glucose was measured in tail vein blood via glucometer (One Touch Ultra). Data are presented as mean ± s.e.m and representative of 3 independent experiments (WT HFD n = 6, *AdipPanxKO* HFD n = 9, WT chow n = 7, *AdipPanxKO* chow n = 6). Combined area under the curve (AUC) analysis of glucose tolerance tests reveals that *AdipPanxKO* mice are significantly more glucose intolerant after high fat feeding compared to WT mice (WT HFD n = 18, *AdipPanxKO* HFD n = 14, WT chow n = 7, *AdipPanxKO* chow n = 4); *p = 0.025 by 2-tailed Student's t-test. Box plots represent the 10th to 90th percentile. Three mice in the high fat diet group (WT n = 1, *AdipPanxKO* n = 2) did not respond to diet as evidenced by AUC not being different from chow groups and thus were excluded from the analysis. One data point (WT n = 1) was greater than 2 standard deviations from the mean and thus was excluded. (**B**) Intraperitoneal insulin tolerance tests (0.75 U/kg) was performed on age-matched, male, WT and *AdipPanxKO* littermates that had been fed chow or high fat diet (60% fat) for 12 weeks. n = 6–7 mice per group. Data are expressed as mean + s.e.m. Box plots represent the 10th to 90th percentile. *p < 0.05 by Student's t-test. (**C**,**D**) Male WT and *AdipPanx1KO* littermates were fed a high fat diet (60% fat) for 12 weeks. Mice on a high fat diet were weighed weekly. Adiposity (%fat) and lean mass (%lean) were assessed by echo MRI. Data are representative of 3 independent experiments, n = 6 WT, n = 9 *AdipPanx1KO*. Data are expressed as mean ± s.e.m. (**E**) Serum free fatty acids (FFA) were measured after 12 weeks of high fat feeding in WT (n = 5) and *AdipPanxKO* (n = 9) mice using the colorimetric FFA kit from Wako. Data are expressed as mean ± s.d. (**F**) Serum insulin in WT and *AdipPanxKO* mice that were either fed chow diet or HFD for 12 weeks were measured after a 5 h fast. (n = 6 WT chow, n = 7 *AdipPanxKO* chow, n = 5 WT high fat diet, n = 9 *AdipPanxKO* high fat diet). Data are expressed as mean ± s.d. (**G**–**I**) High fat diet-fed (12 weeks) and chow-fed WT and *AdipPanx1KO* mice (n = 4 per group) were placed in metabolic cages for 72 h. Average VO_2 consumption, VCO_2, and respiratory exchange ratio (RER) by animal are shown for light and dark periods. The initial 4 h of readings were not part of average light values as this was time for mice to acclimate. Box plots represent the 10th to 90th percentile. (**J**) Locomotion was recorded as X- and Y-axis beam breaks during light and dark cycle. Total beam breaks were not different between WT and *AdipPanx1KO* mice indicating no overall difference in locomotion. Box plots represent the 10th to 90th percentile.

mRNA levels also had significantly higher HOMA-IR scores (a measure of insulin resistance) (Figure 5C). These data suggest that Panx1 may also play a role in adipocyte metabolism in humans, in a way that increased Panx1 expression may help counterbalance decreasing insulin sensitivity.

4. DISCUSSION

The contribution of extracellular nucleotide signaling to the control of metabolic homeostasis in adipocytes has been known for some time; however, the mechanisms of nucleotide release from adipocytes are

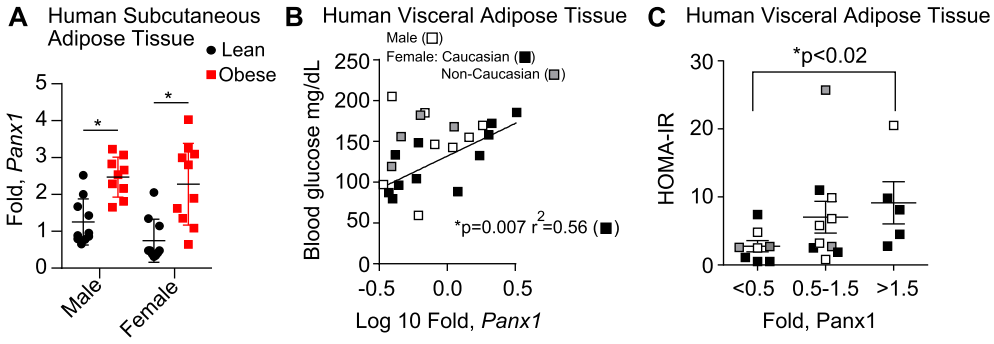

Figure 5: Pannexin 1 expression in human adipose tissue is associated with obesity and insulin resistance. (**A**) Data from NCBI gene array (GDS1498[ACCN]) [49] were analyzed for Panx1 expression in subcutaneous adipose tissue from lean male (n = 10), lean female (n = 10), and obese male (n = 9) and obese female (n = 10) human subjects. Data are normalized to lean samples, each point represents one human subject, error bars indicate s.d. *p < 0.0001 by 2 way ANOVA with Sidak's multiple comparison test. (**B**) Omental fat samples were obtained from human subjects prior to bariatric surgery and analyzed for Panx1 mRNA levels normalized to 18S mRNA. The average of all samples was set to 1 and log 10 values of Panx1 mRNA were plotted against the blood glucose levels of patients at time of surgery, revealing a positive and significant correlation ($r^2 = 0.181$ and p = 0.04 by linear regression). The correlation of Panx1 levels with blood glucose levels was particularly pronounced in Caucasian females ($r^2 = 0.56$, n = 11, black line). Each point represents one human subject, female subjects are shown as gray (non-Caucasian) and black (Caucasian) squares, male subjects as white squares. (**C**) Samples from **B** were grouped into tertiles based on low (<0.5 fold, n = 8), medium (0.5–1.5 fold, n = 10), and high Panx1 expression (>1.5 fold, n = 5) and plotted against HOMA-IR, a clinical measure of insulin resistance. *p < 0.02 by Student's t-test. Each point represents one human subject; error bars represent s.e.m.

not known. Our data establish Panx1 channels as mediators of controlled extracellular nucleotide release from adipocytes. Furthermore, we demonstrate that Panx1-dependent ATP release is required for full activation of insulin-induced glucose uptake in adipocytes and we identify insulin as a novel mediator of Panx1 channel activation.

Although pharmacologic Panx1 inhibitors such as carbenoxolone and probenicid are known to have off-target effects, the use of multiple inhibitors as well as genetic deletion of Panx1 in our study collectively support a role for Panx1 channels in ATP release from adipocytes. Together with highly specific electrophysiological channel recordings, YO-PRO® dye has been shown to enter apoptotic cells in a Panx1-dependent manner [28], adding further evidence for Panx1 channel function in adipocytes.

The most widely studied molecule that is released by Panx1 channels is ATP; however, Panx1 channels have a pore size upon activation that can accommodate molecules of up to 1 kDa in size [29]. So it is conceivable that other molecules that could impact metabolic signaling in adipocytes in an autocrine fashion are also released via Panx1. Our finding that addition of exogenous ATP to Panx1-deficient adipocytes rescues inhibited insulin-induced glucose uptake strongly suggests a role for ATP or its metabolites in this context. Extracellular ATP is rapidly metabolized and we calculated an approximately 6 min half-life of exogenous ATP added to 3T3-L1 adipocytes (data not shown). The degradation of ATP by ectonucleotidases including CD39 and CD73, which are expressed on adipocytes [50], provides ligands for a variety of purinergic receptors and adenosine, which was shown to enhance glucose uptake and block lipolysis [7,8]. Further studies are needed to identify the autocrine purinergic signaling pathways that are activated subsequently to Panx1 channel opening in adipocytes.

Our finding that insulin stimulates opening of Panx1 channels is intriguing and points to a novel mechanism by which the function of this channel is regulated. We demonstrate that insulin activates channel opening in a caspase-independent manner, pointing to a transient, reversible mechanism of activation. Utilizing an HEK293 heterologous expression system enabled us to explore insulin-mediated Panx1 channel activation by obtaining electrophysiologic data about Panx1 channel function. Feasibility of this approach is supported by previous reports that HEK293 cells express endogenous proteins involved in the insulin signaling pathway, including Irs1 [51], Akt [52,53], and mTOR [54]. Previous studies have identified putative phosphorylation sites on the Panx1 channel that may contribute to its function, suggesting that insulin-induced kinases may regulate channel opening. However, further studies are needed to elucidate this new mechanism of Panx1 channel activation.

Our data demonstrate that genetic deletion of Panx1 in adipocytes leads to exacerbation of diet-induced metabolic dysfunction in mice, which manifests in increased insulin resistance. Although we see no overt changes in lean, chow-fed AdipPanx1KO mice in the glucose tolerance test, we did observe decreased glucose uptake specifically in white adipose tissue. Upon high fat diet challenge, a deficit in glucose uptake in adipocytes may begin to manifest in whole body glucose tolerance over time [2]. Neither inflammation nor energy expenditure were significantly affected by adipocyte-selective deletion of Panx1 in mice. Inflammation and macrophage infiltration into adipose tissue are associated with the development of insulin resistance [55,56]. In general, extracellular ATP is considered a pro-inflammatory mediator and ATP that is released from various cell types attracts macrophages [28,57]. Our initial hypothesis was that, during obesity, Panx1-mediated ATP release from adipocytes would initiate inflammatory cross talk between dying adipocytes and adipose tissue macrophages [58]; however, we found no differences in the abundance or inflammatory state of adipose tisue macrophages when Panx1 was absent from adipocytes. In fact, inflammatory cytokines that are produced in adipose tissue during obesity may override signals mediated by extracellular nucleotides [47]. In this context, it has been shown that the ATP-gated cation channel P2X7, which is implicated in mediating inflammasome activation, is dispensable for diet-induced inflammation and insulin resistance [59]. Together, we conclude that the impairment in glucose uptake is an underlying cause to exacerbate insulin resistance in response to high fat diet in adipocyte-specific Panx1 deficient mice.

Our observation of an association of Panx1 expression with obesity and insulin resistance in humans further supports a role for Panx1 in metabolic homeostasis. Upregulation of Panx1 expression in response to obesity and insulin resistance may reflect a protective mechanism by which decreased insulin sensitivity is counteracted by an increased ability of the tissue to release bioactive nucleotides. However, further studies, particularly in human subjects, are required to definitively support this hypothesis.

In summary, we show here for the first time that Panx1 on adipocytes can be activated to release ATP by known mechanisms including α-adrenergic stimulation and caspase-mediated cleavage, and we also identify insulin as a novel mediator of Panx1 activation. We demonstrate that ATP released by Panx1 is required for insulin-stimulated glucose uptake in adipocytes and that mice with genetic deletion of Panx1 in adipocytes experience exacerbated insulin resistance when fed a high fat diet. Moreover, we discovered an association between insulin resistance and Panx1 channel expression in adipose tissue from obese humans. Together, we identify Panx1 as a key regulator of nucleotide release in adipose tissue implying an unexpected role for this channel in controlling metabolic homeostasis.

AUTHOR CONTRIBUTIONS

S.E.A. designed and performed the experiments, analyzed the data, and co-wrote the manuscript. A.K.M., Y.C., J.K.S., and S.R.H. performed experiments and analyzed data. N.N.W. and S.A.S. performed experiments and provided technical expertise. C.M. provided human adipose samples and expertise. S.P., B.I., S.K., T.E.H., and D.A.B. provided expertise and contributed to the discussion. N.L. designed and supervised the study, analyzed the data, and co-wrote the manuscript.

ACKNOWLEDGMENTS

We are grateful to Kodi S. Ravichandran (UVa) and Philipp Scherer (UT Southwestern) for providing Panx1 floxed mice and adipoQ-cre mice, respectively, and to Joanne Lannigan for continuous support in the Flow Cytometry Core Facility at UVa. Anti-Panx1 antibody was kindly provided by Silvia Penuela (University of Western Ontario) and human adipose tissue samples by Peter T. Hallowell (UVa). This study was supported by grants from the National Institutes of Health NIH-P01HL120840 (to NL, DAB, BEI), NIH-R01DK101946 (to TEH), and a shared equipment grant to the UVa FACS core facility (S10RR025460-01). SEA and AKM were supported by AHA pre- and postdoctoral fellowships, respectively. SEA was also supported by an NIH training grant (HL007284).

CONFLICT OF INTEREST

The authors declare no competing financial interests.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2015.06.009.

REFERENCES

[1] Samuel, V.T., Shulman, G.I., 2012. Mechanisms for insulin resistance: common threads and missing links. Cell 148:852—871.

[2] Shepherd, P.R., Kahn, B.B., 1999. Glucose transporters and insulin action — implications for insulin resistance and diabetes mellitus. The New England Journal of Medicine 341:248—257.

[3] Kather, H., 1988. Purine accumulation in human fat cell suspensions. Evidence that human adipocytes release inosine and hypoxanthine rather than adenosine. The Journal of Biological Chemistry 263:8803—8809.

[4] Turpin, B.P., Duckworth, W.C., Solomon, S.S., 1977. Perifusion of isolated rat adipose cells. Modulation of lipolysis by adenosine. The Journal of Clinical Investigation 60:442—448.

[5] Schwabe, U., Ebert, R., Erbler, H.C., 1973. Adenosine release from isolated fat cells and its significance for the effects of hormones on cyclic 3′,5′-AMP levels and lipolysis. Naunyn-Schmiedeberg's Archives of Pharmacology 276:133—148.

[6] Dhalla, A.K., Chisholm, J.W., Reaven, G.M., Belardinelli, L., 2009. A1 adenosine receptor: role in diabetes and obesity. Handbook of Experimental Pharmacology, 271—295.

[7] Martin, S.E., Bockman, E.L., 1986. Adenosine regulates blood flow and glucose uptake in adipose tissue of dogs. The American Journal of Physiology 250:H1127—H1135.

[8] Green, A., 1983. Catecholamines inhibit insulin-stimulated glucose transport in adipocytes, in the presence of adenosine deaminase. FEBS Letters 152:261—264.

[9] Schwabe, U., Schonhofer, P.S., Ebert, R., 1974. Facilitation by adenosine of the action of insulin on the accumulation of adenosine 3′:5′-monophosphate, lipolysis, and glucose oxidation in isolated fat cells. European Journal of Biochemistry/FEBS 46:537—545.

[10] Martin, S.E., Lenhard, S.D., Schmarkey, L.S., Offenbacher, S., Odle, B.M., 1993. Adenosine regulates coronary blood flow during increased work and decreased supply. The American Journal of Physiology 264:H1438—H1446.

[11] Junger, W.G., 2011. Immune cell regulation by autocrine purinergic signalling. Nature Reviews Immunology 11:201—212.

[12] Lee, S.C., Pappone, P.A., 1997. Effects of P2 purinergic receptor stimulation in brown adipocytes. The American Journal of Physiology 273:C679—C686.

[13] Chowdhury, H.H., Grilc, S., Zorec, R., 2005. Correlated ATP-induced changes in membrane area and membrane conductance in single rat adipocytes. Annals of the New York Academy of Sciences 1048:281—286.

[14] Lee, S.C., Pappone, P.A., 1997. Membrane responses to extracellular ATP in rat isolated white adipocytes. Pflugers Archiv: European Journal of Physiology 434:422—428.

[15] Lee, S.C., Pappone, P.A., 1999. ATP can stimulate exocytosis in rat brown adipocytes without apparent increases in cytosolic Ca2+ or G protein activation. Biophysical Journal 76:2297—2306.

[16] Chang, K.J., Cuatrecasas, P., 1974. Adenosine triphosphate-dependent inhibition of insulin-stimulated glucose transport in fat cells. Possible role of membrane phosphorylation. The Journal of Biological Chemistry 249:3170—3180.

[17] Tamura, S., Dubler, R.E., Larner, J., 1983. Stimulation of maximal intracellular insulin action on glycogen synthase by preincubation of adipocytes with adenosine 5′-triphosphate. The Journal of Biological Chemistry 258:719—724.

[18] Schodel, J., Weise, I., Klinger, R., Schmidt, M., 2004. Stimulation of lipogenesis in rat adipocytes by ATP, a ligand for P2-receptors. Biochemical and Biophysical Research Communications 321:767—773.

[19] Hashimoto, N., Robinson, F.W., Shibata, Y., Flanagan, J.E., Kono, T., 1987. Diversity in the effects of extracellular ATP and adenosine on the cellular processing and physiologic actions of insulin in rat adipocytes. The Journal of Biological Chemistry 262:15026—15032.

[20] Yu, Z., Jin, T., 2010. Extracellular high dosages of adenosine triphosphate induce inflammatory response and insulin resistance in rat adipocytes. Biochemical and Biophysical Research Communications 402:455—460.

[21] Burnstock, G., 2014. Purinergic signalling in endocrine organs. Purinergic Signalling 10:189—231.

[22] Yelovitch, S., Barr, H.M., Camden, J., Weisman, G.A., Shai, E., Varon, D., Fischer, B., et al., 2012. Identification of a promising drug candidate for the treatment of type 2 diabetes based on a P2Y(1) receptor agonist. Journal of Medicinal Chemistry 55:7623—7635.

[23] Pacheco, P.A., Ferreira, L.G., Alves, L.A., Faria, R.X., 2013. Modulation of P2 receptors on pancreatic beta-cells by agonists and antagonists: a molecular target for type 2 diabetes treatment. Current Diabetes Reviews 9:228—236.

[24] Piwkowska, A., Rogacka, D., Jankowski, M., Angielski, S., 2013. Metformin reduces NAD(P)H oxidase activity in mouse cultured podocytes through purinergic dependent mechanism by increasing extracellular ATP concentration. Acta Biochimica Polonica 60:607—612.

[25] Sandilos, J.K., Bayliss, D.A., 2012. Physiological mechanisms for the modulation of pannexin 1 channel activity. The Journal of Physiology 590:6257—6266.

[26] Billaud, M., Sandilos, J.K., Isakson, B.E., 2012. Pannexin 1 in the regulation of vascular tone. Trends in Cardiovascular Medicine 22:68—72.
[27] Sridharan, M., Adderley, S.P., Bowles, E.A., Egan, T.M., Stephenson, A.H., Ellsworth, M.L., et al., 2010. Pannexin 1 is the conduit for low oxygen tension-induced ATP release from human erythrocytes. American Journal of Physiology. Heart and Circulatory Physiology 299:H1146—H1152.
[28] Chekeni, F.B., Elliott, M.R., Sandilos, J.K., Walk, S.F., Kinchen, J.M., Lazarowski, E.R., et al., 2010. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature 467:863—867.
[29] Penuela, S., Gehi, R., Laird, D.W., 2013. The biochemistry and function of pannexin channels. Biochimica et Biophysica Acta 1828:15—22.
[30] Billaud, M., Lohman, A.W., Straub, A.C., Looft-Wilson, R., Johnstone, S.R., Araj, C.A., et al., 2011. Pannexin1 regulates alpha1-adrenergic receptor-mediated vasoconstriction. Circulation Research 109:80—85.
[31] Kienitz, M.C., Bender, K., Dermietzel, R., Pott, L., Zoidl, G., 2011. Pannexin 1 constitutes the large conductance cation channel of cardiac myocytes. The Journal of Biological Chemistry 286:290—298.
[32] Sandilos, J.K., Chiu, Y.H., Chekeni, F.B., Armstrong, A.J., Walk, S.F., Ravichandran, K.S., et al., 2012. Pannexin 1, an ATP release channel, is activated by caspase cleavage of its pore-associated C-terminal autoinhibitory region. The Journal of Biological Chemistry 287:11303—11311.
[33] Dahl, G., Qiu, F., Wang, J., 2013. The bizarre pharmacology of the ATP release channel pannexin1. Neuropharmacology 75:583—593.
[34] Silverman, W., Locovei, S., Dahl, G., 2008. Probenecid, a gout remedy, inhibits pannexin 1 channels. American Journal of Physiology. Cell Physiology 295: C761—C767.
[35] Wang, J., Jackson, D.G., Dahl, G., 2013. The food dye FD&C Blue No. 1 is a selective inhibitor of the ATP release channel Panx1. The Journal of General Physiology 141:649—656.
[36] Poon, I.K., Chiu, Y.H., Armstrong, A.J., Kinchen, J.M., Juncadella, I.J., Bayliss, D.A., et al., 2014. Unexpected link between an antibiotic, pannexin channels and apoptosis. Nature 507:329—334.
[37] Shepherd, P.R., Gnudi, L., Tozzo, E., Yang, H., Leach, F., Kahn, B.B., 1993. Adipose cell hyperplasia and enhanced glucose disposal in transgenic mice overexpressing GLUT4 selectively in adipose tissue. The Journal of Biological Chemistry 268:22243—22246.
[38] Mullins, G.R., Wang, L., Raje, V., Sherwood, S.G., Grande, R.C., Boroda, S., et al., 2014. Catecholamine-induced lipolysis causes mTOR complex dissociation and inhibits glucose uptake in adipocytes. Proceedings of the National Academy of Sciences of the United States of America 111:17450—17455.
[39] Wang, Z.V., Deng, Y., Wang, Q.A., Sun, K., Scherer, P.E., 2010. Identification and characterization of a promoter cassette conferring adipocyte-specific gene expression. Endocrinology 151:2933—2939.
[40] Ayala, J.E., Samuel, V.T., Morton, G.J., Obici, S., Croniger, C.M., Shulman, G.I., et al., 2010. Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. Disease Models & Mechanisms 3:525—534.
[41] Lansey, M.N., Walker, N.N., Hargett, S.R., Stevens, J.R., Keller, S.R., 2012. Deletion of Rab GAP AS160 modifies glucose uptake and GLUT4 translocation in primary skeletal muscles and adipocytes and impairs glucose homeostasis. American Journal of Physiology. Endocrinology and Metabolism 303:E1273—E1286.
[42] Lohman, A.W., Billaud, M., Straub, A.C., Johnstone, S.R., Best, A.K., Lee, M., et al., 2012. Expression of pannexin isoforms in the systemic murine arterial network. Journal of Vascular Research 49:405—416.
[43] Lohman, A.W., Weaver, J.L., Billaud, M., Sandilos, J.K., Griffiths, R., Straub, A.C., et al., 2012. S-nitrosylation inhibits pannexin 1 channel function. The Journal of Biological Chemistry 287:39602—39612.
[44] Ramos, R.R., Swanson, A.J., Bass, J., 2007. Calreticulin and Hsp90 stabilize the human insulin receptor and promote its mobility in the endoplasmic reticulum. Proceedings of the National Academy of Sciences of the United States of America 104:10470—10475.
[45] Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C., 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412—419.
[46] Dufresne, J., Cyr, D.G., 2014 Dec. Regulation of the pannexin-1 promoter in the rat epididymis. Biology of Reproduction 91(6):143.
[47] Qu, Y., Misaghi, S., Newton, K., Gilmour, L.L., Louie, S., Cupp, J.E., et al., 2011. Pannexin-1 is required for ATP release during apoptosis but not for inflammasome activation. Journal of Immunology 186:6553—6561.
[48] Billaud, M., Choi, Y., Lohman, A.W., Parpaite, T., Butcher, J.T., Mutchler, S.M., et al., 2015. A molecular signature in the pannexin 1 intracellular loop confers channel activation by the α1 adrenoreceptor in smooth muscle cells. Science Signalling 8(364):ra17.
[49] Lee, Y.H., Nair, S., Rousseau, E., Allison, D.B., Page, G.P., Tataranni, P.A., et al., 2005. Microarray profiling of isolated abdominal subcutaneous adipocytes from obese vs non-obese Pima Indians: increased expression of inflammation-related genes. Diabetologia 48:1776—1783.
[50] Muller, G., Wied, S., Walz, N., Jung, C., 2008. Translocation of glycosylphosphatidylinositol-anchored proteins from plasma membrane microdomains to lipid droplets in rat adipocytes is induced by palmitate, H_2O_2, and the sulfonylurea drug glimepiride. Molecular Pharmacology 73:1513—1529.
[51] Wharton, J., Meshulam, T., Vallega, G., Pilch, P., 2005 Apr 8. Dissociation of insulin receptor expression and signaling from caveolin-1 expression. The Journal of Biological Chemistry 280(14):13483—13486.
[52] Amoroso, F., Falzoni, S., Adinolfi, E., Ferrari, D., Di Virgilio, F., 2012 Aug 16. The P2X7 receptor is a key modulator of aerobic glycolysis. Cell Death & Disease 3:e370.
[53] Zaslavsky, A., Li, S., Xu, Y., 2005 Jul 18. Sphingosine-1-phosphate induces a PDGFR-dependent cell detachment via inhibiting beta1 integrin in HEK293 cells. FEBS Letters 579(18):3899—3906.
[54] Ekim, B., Magnuson, B., Acosta-Jaquez, H.A., Keller, J.A., Feener, E.P., Fingar, D.C., 2011 Jul. mTOR kinase domain phosphorylation promotes mTORC1 signaling, cell growth, and cell cycle progression. Molecular and Cellular Biology 31(14):2787—2801.
[55] Odegaard, J.I., Chawla, A., 2008. Mechanisms of macrophage activation in obesity-induced insulin resistance. Nature Clinical Practice. Endocrinology & Metabolism 4:619—626.
[56] Sun, S., Ji, Y., Kersten, S., Qi, L., 2012. Mechanisms of inflammatory responses in obese adipose tissue. Annual Review of Nutrition 32:261—286.
[57] Elliott, M.R., Chekeni, F.B., Trampont, P.C., Lazarowski, E.R., Kadl, A., Walk, S.F., et al., 2009. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature 461:282—286.
[58] Adamson, S.E., Leitinger, N., 2014. The role of pannexin1 in the induction and resolution of inflammation. FEBS Letters 588:1416—1422.
[59] Sun, S., Xia, S., Ji, Y., Kersten, S., Qi, L., 2012. The ATP-P2X7 signaling axis is dispensable for obesity-associated inflammasome activation in adipose tissue. Diabetes 61:1471—1478.

