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Adipsin is an Adipokine that Improves β Cell Function in Diabetes

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Summary

A hallmark of type 2 diabetes mellitus (T2DM) is the development of pancreatic β cell failure, resulting in insulinopenia and hyperglycemia. We show that the adipokine adipsin has a beneficial role in maintaining β cell function. Animals genetically lacking adipsin have glucose intolerance due to insulinopenia; isolated islets from these mice have reduced glucose-stimulated insulin secretion. Replenishment of adipsin to diabetic mice treated hyperglycemia by boosting insulin secretion. We identify C3a, a peptide generated by adipsin, as a potent insulin secretagogue and show that the C3a receptor is required for these beneficial effects of adipsin. C3a acts on islets by augmenting ATP levels, respiration and cytosolic free Ca^{2+}. Finally, we demonstrate that T2DM patients with β cell failure are deficient in adipsin. These findings indicate that the adipsin/C3a pathway connects adipocyte function to β cell physiology and manipulation of this molecular switch may serve as a novel therapy in T2DM.

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Introduction

The rising prevalence of obesity worldwide and its associated metabolic derangements, such as T2DM, pose an enormous public health challenge (Van Gaal et al, 2006). Diabetes is a chronic and progressive disease characterized by insulin resistance and insulinopenia resulting from β cell failure and decreases in β cell mass (Ferrannini, 2010; Kasuga, 2006; Muoio and Newgard, 2008). Despite initial success in glycemic control with oral medications, patients with T2DM typically exhibit worsening glucose homeostasis over the span of a few years (1995; Kahn et al, 2006; Matthews et al, 1998; Turner et al, 1996). The natural history of T2DM includes a continuous decline in β cell function as illustrated by longitudinal follow up studies of patients (Festa et al, 2006). This deterioration leads to the need for additional therapies as well as the cumulative development of diabetic complications.

There is a well-known association between obesity, adipose inflammation and malfunction of β cells but the molecular link remains to be established (Lumeng and Saltiel, 2011). Increasing adiposity is directly correlated with adipose inflammation and elaboration of proinflammatory cytokines such as tumor necrosis factor-a, but whether this low-grade chronic inflammation is sufficient to trigger islet dysfunction is unknown (Hotamisligil, 2006; Ouchi et al, 2011; Rosen and Spiegelman, 2006; Shoelson et al, 2006). Other factors such as amyloidosis, glucolipotoxicity, failure of β cell expansion, apoptosis and β cell dedifferentiation have also been posited (Kitamura, 2013; Leroith and Accili, 2008; Muoio and Newgard, 2008; Prentki and Nolan, 2006; Weir et al, 2009).

Adipsin was the first adipokine described (Cook et al, 1987). In fact, adipsin is one of the major proteins of adipose cells, but paradoxically decline in many animal models of obesity and diabetes (Flier et al, 1987). Adipsin was later identified to be complement factor D (Rosen et al, 1989; White et al, 1992), which catalyzes the rate-limiting step of the alternative pathway of complement activation (Xu et al, 2001). Since then, adipsin has been shown to play pivotal roles in models of ischemia reperfusion (Stahl et al, 2003) and sepsis (Dahlke et al, 2011). Functions of this molecule include both the formation of the C5-C9 membrane attack complex and the generation of a number of signaling molecules including the anaphylatoxins C3a and C5a (Ricklin et al, 2010). However, the function of adipsin in relation to energy homeostasis and systemic metabolism has been unknown.

The increasing awareness of the interplay between the immune system and adipose tissue biology focuses attention on complement biology in the pathogenesis of T2DM (Shu et al, 2012). Certain proteins of the complement pathway are preferentially expressed in the adipose tissue and some components, like adipsin, are dysregulated in models of obesity and diabetes (Choy et al, 1992; Flier et al, 1987; Zhang et al, 2007). Receptors for complement-derived peptides are widely expressed on multiple immune cell types (Ricklin et al, 2010). Studies using mice deficient in C3aR1 or employing an antagonist of the receptor have shown protection against obesity, reductions in adipose tissue inflammation, and improved insulin sensitivity (Lim et al, 2013; Mamane et al, 2009).

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We have now reassessed the role of adipsin in metabolic diseases and diabetes by performing detailed metabolic analyses of wild-type (WT) and *Adipsin*$^{-/-}$ mice (Xu et al, 2001). Mice lacking adipsin exhibit worsened glucose homeostasis when placed under the metabolic stress of diet-induced obesity. This is due primarily to an unexpected and striking requirement of adipsin for proper insulin secretion by the pancreatic β cells. C3a, downstream of adipsin catalytic action, robustly stimulates insulin secretion when coupled to hyperglycemic signals. These data establish adipsin as a major link between fat cells, obesity and β cell function.

Results

Ablation of Adipsin Leads to Decreased Adipose Tissue Inflammation but Exacerbation of Diabetes

We analyzed *Adipsin*$^{-/-}$ mice under normal conditions and with exposure to metabolic stress. On a regular diet, there was no difference in weights between the WT and adipsin-deficient groups (WT 29.0 ± 0.3g, *Adipsin*$^{-/-}$ 28.9 ± 0.3g) at 5 months of age. We also subjected WT and *Adipsin*$^{-/-}$ mice to a model of diet-induced obesity, using a high fat, high carbohydrate diet (HFD). Levels of adipsin in the blood gradually decline with increased exposure to HFD (Figures S1A and S2A). *Adipsin*$^{-/-}$ animals exhibited a mild but significant attenuation in weight gain on a HFD for 12 weeks compared to WT (WT 48.1 ± 1.1g, *Adipsin*$^{-/-}$ 46.4 ± 1.2g; $p < 0.01$). As adipsin controls the alternative complement pathway, we assessed adipose inflammation in WT and *Adipsin*$^{-/-}$ mice on a HFD. The degree of adipose inflammation was dampened in the *Adipsin*$^{-/-}$ mice compared to that of WT, with decreased expression of macrophage genes *(Cd11b, Cd11c, F4/80, Mac2)* and mast cell genes *(Mcpt4, Cma1, Cpa3)*, but not the T cell coreceptors *(Cd4* and *Cd8)* (Figure 1A). Indeed, the numbers of macrophages and crown-like structures were decreased in adipose tissues of *Adipsin*$^{-/-}$ mice as confirmed by histological analyses (Figures 1B and 1C) (Lumeng et al, 2007; Weisberg et al, 2003). Mast cells, which are putative pathogenic inflammatory cells, were similarly diminished in adipose tissues of *Adipsin*$^{-/-}$ mice, as confirmed by toluidine blue staining (Figures 1D and 1E) (Shu et al, 2012).

Obesity often positively correlates with glucose intolerance though there are notable exceptions including the aP2-deficient mice (Hotamisligil et al, 1996), and the adiponectin (Kim et al, 2007) and mitoNEET (Kusminski et al, 2012) transgenic mice. To interrogate the role of adipsin in glucose homeostasis, WT and *Adipsin*$^{-/-}$ mice were challenged with a glucose tolerance test. Under the non-diabetogenic conditions of a chow diet, *Adipsin*$^{-/-}$ mice had similar glucose tolerance compared to that of WT mice (Figure S2A). Exposure to a HFD for 8 weeks revealed similar glucose tolerance between WT and *Adipsin*$^{-/-}$ mice (Figure S2B). However, after a longer exposure of 16 weeks to a HFD, *Adipsin*$^{-/-}$ mice displayed significantly impaired glucose tolerance despite being leaner than WT mice (Figure 1F). To assess whether these changes were due to insulin resistance, we performed an insulin tolerance test on obese WT and *Adipsin*$^{-/-}$ mice on a HFD for 18 weeks. Unexpectedly, WT and *Adipsin*$^{-/-}$ mice had a similar response to exogenous insulin indicating no gross changes in insulin sensitivity (Figure 1G). Mild changes in insulin

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sensitivity cannot be ruled out. Based on these data, we considered the possibility that *Adipsin*$^{-/-}$ mice may be deficient in insulin.

Loss of Adipsin Results in Insulinopenia

To assess the possibility that adipsin regulates insulin secretion, we assayed insulin levels in WT and *Adipsin*$^{-/-}$ mice on a HFD. *Adipsin*$^{-/-}$ mice on a shorter term HFD had comparable insulin levels in response to a glucose challenge (Figure S3 A); this is consistent with the similar glucose tolerance observed between WT and *Adipsin*$^{-/-}$ mice with the shorter exposure to HFD. In contrast, *Adipsin*$^{-/-}$ mice on long term HFD had lower fasting insulin levels (Figure 2A). Importantly, insulin levels remained low upon glucose challenge, thus confirming that *Adipsin*$^{-/-}$ mice are insulinopenic (Figures 2A and S3B). These findings implicated β cell insufficiency as an obvious explanation for the hyperglycemia in *Adipsin*$^{-/-}$ mice. This may, in theory, result from either decreased β cell mass or function. To assess for a quantitative defect in β cells, we performed insulin immunohistochemistry on WT and *Adipsin*$^{-/-}$ pancreata and found no significant differences in islet morphology or β cell area (Figures 2B and 2C). To directly interrogate whether loss of adipsin impaired β cell function, we isolated islets from WT and *Adipsin*$^{-/-}$ mice that had been on a HFD. Islets from *Adipsin*$^{-/-}$ mice on a HFD exhibited diminished insulin secretion in response to glucose, recapitulating the *in vivo* phenotype (Figure 2D). The accumulation of islet-associated macrophages is sometimes correlated with deterioration in β cell function (Ehses et al, 2007; Homo-Delarche et al, 2006) . In contrast to the decreased number of adipose tissue macrophages in *Adipsin*$^{-/-}$ mice, there was no change in islet-associated macrophages between WT and *Adipsin*$^{-/-}$ mice (Figure S3C). The loss of adipsin did not affect β cell insulin secretion in response to glucose in islets from mice on a regular diet, indicating that there is not a gross developmental defect in islets from adipsin-null mice (Figure S3D). These results suggest a role for adipsin in sustaining insulin production and/or secretion in diabetes.

Systemic Restoration of Adipsin Augments Insulin Secretion and Improves Glucose Homeostasis

To determine if adipsin can be therapeutically relevant for the treatment of diabetes, we acutely restored adipsin expression in diabetic mice and asked if augmenting adipsin levels can improve diabetes. We have previously shown that adipsin is dramatically reduced in genetic models of obesity such as *db/db* mice (Flier et al, 1987). Importantly, these mice have no genetic defect in the *Adipsin* gene but demonstrate a potentially pathogenic loss of protein expression. Diabetic *db/db* mice were injected with adenoviral vectors expressing either control lacZ or adipsin. As expected, these vectors are taken up by the liver and cause liver secretion of adipsin into the systemic circulation. *db/db* mice injected with adipsin adenovirus robustly restored circulating adipsin levels to that above WT. In contrast, mice that received the control lacZ virus remained deficient in adipsin (Figure 3A). To determine if replenishment of adipsin in diabetic mice ameliorates hyperglycemia, glucose tolerance tests were performed. Diabetic mice that had received the adipsin adenovirus showed a significant decrease in fasting glucose levels (lacZ 182 ± 13 mg/dl, adipsin 144 ± 7 mg/dl, $p < 0.05$) and a dramatic enhancement in glucose clearance (Figure 3B). To assess if the improvements in glucose homeostasis from adipsin therapy were linked to insulin secretion,

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we assayed insulin levels. Indeed, both fasting insulin and glucose-induced insulin levels were substantially increased in the adipsin-treated *db/db* mice (Figure 3C). As insulin can suppress gluconeogenesis through either direct and/or indirect effects on the liver, we tested whether adipsin treatment resulted in decreased gluconeogenic gene expression. As expected based on insulin levels, mice treated with adipsin had significantly decreased expression of *Pepck* and *G6pc* compared to those treated with control (Figure 3D). The expression of *Crp* was reduced but many markers of inflammation such as *Il1b, Il6* and *Tnf* were unchanged between control and adipsin-treated groups (Figures S4A–4E). It is possible that adipsin or its products may have additional direct effects on the liver. There was no significant change in weight loss as a result of adenoviral infection and the fasting between the control and lacZ groups (Figure S4F). Together, our data clearly show that adipsin-directed therapy augments insulin secretion and has anti-diabetic actions.

C3a Stimulates Islet Insulin Secretion and Cytosolic Free Ca^{2+} in Response to Glucose and KCl

To assess how adipsin modulates β cell function, we examined whether adipsin and other complement factors are made locally by the islets. We found that adipsin is predominantly made by adipose tissue and not by the islets (Figure 4A). Similarly, other key components of the proximal portion of the alternative pathway, C3 and complement factor B, are both circulating factors and produced outside of the islets (Figures 4B and S5A–C). In order for adipsin to have distal/endocrine-like effects on the islets of Langerhans, it is possible that it liberates a peptide such as C3a or C5a that can act on β cells via the complement receptors. To test this idea, we first analyzed islets for expression of the C3a and C5a receptors by flow cytometry. C3aR1 was expressed in the islets but C5aR1 and C5L2 were not readily detected (Figures 4C – E). These data suggest that C3aR1 is expressed on β cells, raising the possibility that C3a may act on β cells to influence insulin secretion. C3a is detectable in the circulation at a concentration of approximately 25–50 ng/μl, though this band likely reflects both C3a and its inactivated form, C3a–desArg (Figure S5D). To determine whether C3a can induce insulin secretion, islets were subjected to a GSIS assay in the presence of C3a. Acute administration of C3a (the direct product of C3 convertase generated by adipsin) enhanced insulin secretion by 30–40% in the presence of high but not low glucose conditions (Figure 4F). C5a did not show this effect (Figure 4F). Adipsin and C3 (the precursor to C3a) failed to enhance GSIS, further confirming the specificity of C3a (Figure S5E). Over time, islets from patients with T2DM develop β cell failure, manifested by a diminished insulin secretory response to glucose (Ashcroft and Rorsman, 2012). We thus interrogated the ability of C3a to rescue β cell function in islets isolated from diabetic mice fed a HFD. C3a stimulated insulin secretion in diabetic islets, suggesting C3a is an insulin secretagogue, with potential relevance in diabetes (Figure 4G). Islets isolated from *Adipsin*$^{-/-}$ mice fed a HFD are not further stimulated by C3a (Figure S5F). This suggests that these islets may be impaired from chronic lack of C3a and/or exposure to exacerbated glucolipotoxicity in *Adipsin*$^{-/-}$ mice. In addition, adipsin or other products of adipsin action may be important in sustaining islet health.

To dissect the requirement for the C3a/C3aR1 pathway in the anti-diabetic actions of adipsin *in vivo*, we blocked C3aR1 function in *db/db* mice transduced with adipsin adenovirus. Mice

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were treated with vehicle or the C3aR1 antagonist, SB 290157 (Ames et al, 2001; Lim et al, 2013), after injection of adenovirus. The group injected with the adipsin adenovirus and also treated with the C3aR1 antagonist nearly completely reversed the improvements in glucose tolerance and insulin secretion conferred by the adipsin adenovirus alone (Figures 4H and 4I). Insulin was induced 2.1 fold in the adipsin vs. vehicle group and this was reduced by the C3aR antagonist to 1.4 fold, similar to the control groups (Figure S5G). Taken together, our data indicate that adipsin stimulates insulin secretion via actions of C3a on its receptor to improve glucose homeostasis *in vivo*.

To dissect the mechanism of C3a action on pancreatic islets, we assessed if C3a acts in the presence of KCl or the sulfonylurea tolbutamide, which is an inhibitor of the ATP-dependent K^+ channel. C3a further stimulated insulin secretion in conjunction with both KCl (Figure 5A) and tolbutamide (Figure 5B), suggesting either synergy or that its actions are downstream of the ATP-dependent potassium channel. Additionally, these data suggest that targeting the adipsin/C3a pathway can be used in combination with existing therapies for T2DM. β cell calcium (Ca^{2+}) handling is a major regulator of insulin secretion (Rorsman et al, 2012; Tarasov et al, 2012; Yang and Berggren, 2006). To determine if C3a augments the concentration of cytosolic free Ca^{2+} $[Ca^{2+}]i$ as a mechanism to increase insulin secretion, we assessed $[Ca^{2+}]_i$ in islets stimulated with C3a in response to glucose and KCl. Islets were perifused with different concentrations of glucose and KCl, in the presence or absence C3a, and then imaged to determine $[Ca^{2+}]_i$. The basal levels of $[Ca^{2+}]_i$ at 3 mM glucose were similar in control and C3a–treated islets (Figure 5C). In contrast, C3a potently augmented $[Ca^{2+}]i$ in islets in response to both glucose and KCl (Figure 5C). The response times were similar in C3a–treated and control islets. C3a robustly increased the peak $[Ca^{2+}]_i$ and area under the curve by ∼50% for glucose and nearly 100% for KCl (Figure 5D and 5E). Collectively, these results indicate that C3a enhances insulin secretion in response to multiple secretagogues at least in part by increasing $[Ca^{2+}]_i$ flux.

Glucose entry into β cells stimulates mitochondrial oxygen consumption to increase production of ATP, thereby triggering insulin release and the metabolic amplifying pathway (Maechler, 2013; Prentki et al, 2013). We assessed if C3a can augment intracellular ATP levels and increase mitochondrial oxygen consumption as a mechanism to stimulate insulin release. Treatment of islets with C3a increased intracellular ATP levels at high (20 mM) but not low (3 mM) glucose concentrations (Figure 5F). Furthermore, islets treated with C3a displayed increased oxygen consumption with glucose challenge compared to control islets (Figure 5G). The C3a–mediated increase in oxygen consumption was largely driven by ATP-coupled respiration (Figure 5H). These data suggest that C3a increases intracellular ATP levels and stimulates ATP-coupled respiration as a mechanism to enhance insulin secretion.

T2DM Patients with β Cell Failure Display Reduced Levels of Adipsin

Human patients with T2DM develop evidence of β cell dysfunction (Ferrannini, 2010; Prentki and Nolan, 2006). A subset of T2DM patients will develop significant β cell failure and require treatment with exogenous insulin. Given our data in mice that correlate adipsin expression with insulin secretion, we hypothesized that adipsin might be dysregulated in

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diabetic patients with β cell insufficiency. Adipsin expression was therefore measured in the adipose tissues of diabetic patients with or without evidence of β cell failure. Patients were segregated into two groups: T2DM or T2DM with β cell failure (T2DM-βCF) according to treatment with insulin. Patients undergoing elective abdominal surgery were matched for age, gender, BMI and fat mass (Figure S6) (Kloting et al, 2010). Importantly, the mean fasting blood glucose and hemoglobin A1c were not different between the two groups, indicating similar glycemic control (Figure S6). *Adipsin* mRNA was significantly decreased in both visceral and subcutaneous adipose tissues of T2DM-pCF compared to T2DM patients (Figure 6A). Adipokine expression generally correlates with the degree of adiposity (Deng and Scherer, 2010). We therefore tested other adipokines to rule out a general dysregulation of adipokines in patients with β cell failure, thereby assessing the specificity of the decrease in *Adipsin*. Leptin and adiponectin are extensively studied adipokines that have previously been linked to β cell function (Dunmore and Brown, 2013). We found that *Leptin* and *Adiponectin* mRNA levels were not altered between T2DM and T2DM-βCF patients (Figures 6B and 6C). These results demonstrate the specificity of the decrease in *Adipsin* in diabetic patients with β cell failure and argue against widespread alterations in adipokine gene expression.

Moderate reductions in adipose production of adipsin may potentially be compensated by proportional increases in adipose tissue mass. Since adipsin is a secreted factor derived from adipose tissues, we asked if the reductions in *Adipsin* mRNA seen in T2DM-βCF patients translated to lower circulating adipsin. To this end, we tested serum adipsin levels in patients with T2DM and T2DM-βCF. Circulating adipsin levels were significantly decreased in T2DM-βCF compared to T2DM patients (Figure 6D). The decline in adipsin was not a consequence of an overall reduction in adipokines as circulating leptin and adiponectin were unaltered between T2DM and T2DM-βCF patients (Figures 6E and 6F). Earlier studies had shown that human subjects with mild to moderate obesity did not have reduced levels of circulating adipsin (Napolitano et al, 1994; Pomeroy et al, 1997). Although there is a reduction in adipsin production per unit of adipose tissue, the expansion of fat mass in obesity may compensate to keep circulating adipsin levels high. Taken together, this suggests that adipose production of adipsin and systemic levels of adipsin may initially be higher in metabolic syndrome and early stages of diabetes as a compensatory mechanism but then decline with adipose dysfunction. These T2DM patients may then develop β cell failure. Our data provide evidence for adipsin as a key molecular link to the development of β cell failure in T2DM.

Discussion

Obesity has long been correlated with insulin resistance. In addition, obesity, at least in its early stages, often provokes compensatory hyperinsulinemia. These clinical observations have raised the possibility that adipose cells themselves might send important signals concerning their status to the pancreatic islets. There have been conflicting reports on whether the adipokine adiponectin can directly boost insulin secretion (Okamoto et al, 2008; Staiger et al, 2005; Winzell et al, 2004). Recently, a small molecule adiponectin receptor agonist has been reported and found to improve insulin sensitivity but had no effects on insulin secretion (Okada-Iwabu et al, 2013). The present study identifies adipsin, one of the

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most abundant and specifically expressed adipose proteins, as a circulating factor linking fat cells and obesity to β cell function. Just as incretins provide cues from the gastrointestinal system, adipsin appears to impart signals along the adipose to pancreatic islet axis. In addition, we find that adipsin is selectively decreased in T2DM patients with β cell failure. It will be important to determine the factors that suppress *Adipsin* expression *in vivo* in rodents and humans. Our prior work suggests that there are transcription factors or cofactors that bind to the *Adipsin* gene specifically in obesity (Platt et al, 1994). Understanding the factors that inhibit *Adipsin* expression can help us to develop strategies to reverse this repression.

Replenishment of adipsin for a few days to diabetic *db/db* mice with moderately severe diabetes was able to have significant anti-diabetic effects. And as adipsin is an abundant circulating factor normally found in the bloodstream, this suggests that adipsin may be an excellent therapy for diabetes with a wide therapeutic window. As is the case with many new potential therapies, validation of adipsin in additional animal models of diabetes with β cell failure will be needed prior to potential clinical testing. The data here suggest that adipsin acts, at least in part, via C3a to potentiate insulin secretion. Adipsin/factor D cleaves factor B only when in complex with C3b, catalyzing the formation of the C3 convertase (C3bBb), which can act on C3 to liberate C3a. C3a potentiates insulin secretion only when glucose levels are elevated. At low glucose conditions, C3a does not induce β cells to release insulin. This makes C3a an ideal drug that has a built-in negative feedback system protecting against hypoglycemia. Furthermore, C3a is rapidly inactivated by serum carboxypeptidases to the inert C3a–desArg. Thus, it is highly likely that while adipsin is secreted mainly or exclusively by fat and reaches islets via the circulation, it meets the C3bB pro-convertase complex in the vicinity of the β cells. The islets may be a privileged docking site of the pro-convertase to allow adipsin to amplify generation of C3a locally before its conversion to the inert C3a–desArg. Future research focusing on the regulation of C3a production at the level of the islets is needed. It is also possible that C3aR1 may have other diabetes modifying functions in other metabolic tissues since it is fairly widely expressed. Additionally, strategies aimed at antagonizing the carboxypeptidase(s), responsible for inactivation of C3a specifically near the islets, draw a potential parallel with incretins and inhibition of dipeptidyl peptidase-4. This approach may potentially be limited by the anaphylatoxin activity of C3a, translating to a narrower therapeutic window.

C3aR1 is a G protein-coupled receptor that is pertussis toxin sensitive; in other systems it has been shown to enhance $[Ca^{2+}]_i$ flux and activate MAPK/ERK, Rho and NF-kB signaling pathways. C3a may work by a number of these different mechanisms on pancreatic islets. Isolated islets perifused with glucose or KCl in the presence of C3a show 50–100% increases in $[Ca^{2+}]_i$. These effects may be from modulation of the SERCA pump to affect Ca^{2+} stores that, when released, would increase peak $[Ca^{2+}]_i$. In addition, C3a drives islet mitochondrial respiration, potentially as a result of increases in ATP and/or in intramitochondrial Ca^{2+} and their effects on many enzymes of the Krebs cycle (Tarasov et al, 2012). There may be other C3a signaling pathways that do not directly affect Ca^{2+} feeding into the metabolic amplifying pathway of nutrient-induced insulin secretion (Henquin, 2011). C3aR1 is expressed in a number of metabolic tissues and may have other functions aside from its effects on the pancreatic islets.

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It is widely accepted that full length C3a is the active molecule on C3aR1, whereas C3a–desArg is the inactive form. There have been some data suggesting that C3a–desArg stimulates lipogenesis but these have been disputed (Baldo et al, 1993; Wetsel et al, 1999). Importantly, our study used active C3a at relatively low doses, consistent with a *bona fide* C3a–based mechanism. However, it is unclear whether the less active C3a–desArg could affect the β cell functions shown here when present at supraphysiologic doses (Ahren et al, 2003). Future studies dissecting the mechanisms of C3a/C3aR1 signaling on β cells will help to determine the conserved and specialized signaling pathways utilized by β cells.

Here we show that the actions of adipsin in the augmentation of insulin secretion are at least partly through C3aR1. The *Adipsin*$^{-/-}$ and *C3aR1*$^{-/-}$ mice show some similarities on a HFD. Both mice are leaner and have decreased adipose tissue inflammation compared to WT (Mamane et al, 2009). At first glance, there seems to be discrepancies with the adipsin-null mice showing exacerbated glucose intolerance and the *C3aR1*$^{-/-}$ mice with improved glucose tolerance. In Mamane et al, glucose tolerance of the *C3aR1*$^{-/-}$ mice was examined at 8 weeks on a HFD. Similarly, at 8 weeks of HFD, we observed a small non-significant trend towards improved glucose tolerance in the *Adipsin*$^{-/-}$ mice (Figure S2B). It is only after a longer exposure to HFD, which presumably allows β cell failure to develop, that the insulinopenia phenotype is unmasked. Ablation of adipsin would disrupt generation of the C3 convertase but still permits low levels of C3a generated from spontaneous hydrolysis of C3, the so-called "C3 tickover." Another possibility is that adipsin may have other effects that do not depend on C3aR1.

The present study is the first report of altered adipsin levels in a subset of patients with T2DM. Measurement of adipsin levels may also prove to be valuable from a diagnostic standpoint to identify those patients at high risk of developing β cell failure and accelerated diabetes. T2DM patients may be risk stratified based on their adipsin level and those with the lowest levels may warrant closer follow up even when other glycemic indices such as the hemoglobin A1c are well-controlled. This group may benefit from earlier introduction of insulin therapies. Alternatively, there may be a group of patients with high levels of adipsin who may be protected from T2DM such as the "metabolically healthy but obese" (Klöting et al, 2010; Primeau et al, 2011). We chose insulin therapy as a surrogate for β cell insufficiency but not all patients being treated with insulin may have *bona fide* β cell failure. Thus the level of adipsin in T2DM patients with β cell failure may be even lower. It is also possible that exogenous insulin therapy may repress *Adipsin* but not the other adipokines *(Adiponectin* and *Leptin)* tested in our study. Future prospective studies using adipsin to predict the development of T2DM in nondiabetic patients or progression of diabetes in T2DM patients are needed to answer these questions. Thus, we present a novel adipose to pancreas pathway with potential diagnostic and therapeutic utility in metabolic diseases.

Experimental Procedures

Mice and treatments

WT and *Adipsin*$^{-/-}$ mice were backcrossed 7–10 generations to C57BL/6 background. Controls from same backcrossed generations were used for experiments. Mice were fed a regular chow diet or a 60% kCal HFD (Research Diets). *db/db* mice were purchased from

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Jackson laboratories. For adenoviral expression studies, mice were injected i.v. with lacZ or adipsin-expressing adenoviral vectors (Life Technologies) and assayed 5 days later. For C3aR1 antagonist experiments, SB 290157 (Millipore) dosed at 30 mg/kg or vehicle (10% ethanol) was administered by i.p. injections b.i.d. from days 3–5 after adenovirus. Mice were fasted overnight for glucose tolerance tests and given an i.p. injection of D-glucose (1 g/kg body weight for HFD and *db/db* mice; 2 g/kg for regular diet mice and for insulin induction). For insulin tolerance tests, mice were fasted for 5 hours and given an i.p. injection of insulin (0.75 units/kg body weight). Blood samples were taken from the tail vein and measured with a glucometer. The mice were housed in a facility accredited by the American Association for Laboratory Animal Care. The Institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Center approved studies using animals.

Antibodies and reagents

Recombinant C3a and C5a proteins (R&D) were used at a concentration of 100 nM. Adipsin (R&D) and C3 (Calbiochem) proteins were used at 1 μg/ml. Anti-adipsin antibody (Santa Cruz) was used for Western blot analysis. Antibodies to C3aR1 (Santa Cruz), C5aR1 (Biolegend) and C5L2 (Hycult) were used for flow cytometry. RNA isolation and reverse transcription were performed with Trizol (Life Technologies), RNeasy (Qiagen) and cDNA synthesis (Agilent) kits. qPCR reactions were done with SYBR green on an ABI 7900HT (Life Technologies).

Islet studies

Islets were isolated from pancreata with collagenase digestion as previously described (Danial et al, 2008). For static incubation (batch release) assays, we handpicked islets and placed them into basal Krebs buffer containing 3 mM glucose followed by transfer into Krebs solution containing the indicated concentrations of glucose or secretagogues. After 45 min incubation at 37°C, islets were pelleted and supernatants were collected. We solubilized the pellet to assess intracellular insulin content. Insulin was measured by ELISA (Crystal Chem).

Real-time measurements of mitochondrial oxygen consumption rate (OCR) were performed using the XF24 extracellular flux analyzer instrument and the AKOS algorithm (Seahorse). Islets were rinsed with sodium bicarbonate-free DMEM supplemented with 0.5% BSA and 3 mM glucose and 60 islets were distributed per well. After baseline measurements, substrates or inhibitors of interests were injected. In each experiment, the injection order was as follows: glucose (20 mM), oligomycin (5 μM), and rotenone and antimycin A (5 μM). The respiratory rate of each islet sample was measured at 37°C and analyzed.

For measurements of cytoplasmic free Ca^{2+} concentration, islets were loaded with 2 μM Fura-2/AM (Molecular Probes) for 60 min in the presence or absence of 100 nM C3a in a buffer containing (in mM) 125 NaCl, 5.9 KCl, 1.28 $CaCl_2$, 1.2 $MgCl_2$, 25 HEPES, and 3 glucose and 0.1% BSA (pH 7.4). After loading, a single islet was transferred to an open perifusion chamber maintained at 37°C and $[Ca^{2+}]_i$ was measured as the 340/380 nm fluorescence ratio using a Spex Fluorolog spectrophotometer coupled to a Zeiss Axiovert 35 M microscope with a Zeiss Fluar 40×/1.30 oil objective (Carl Zeiss). Concentrations of 20

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mM glucose and 50 mM KCl (in the presence of 3 mM glucose) were used for islet stimulation.

For measurements of intracellular ATP, batches of 50 islets were incubated in Krebs buffer with 3 mM glucose for 1 hour and then transferred to Krebs buffer containing 3 or 20 mM glucose with C3a. ATP levels were quantified by the CellTiter-Glo kit (Promega) with ATP standards. For flow cytometry, islets were dispersed into a single cell suspension with trypsin/EDTA treatment. After standard staining with primary and secondary antibodies, the cells were analyzed on a FACSCanto II (BD Biosciences).

Light microscopy and histological analyses

For histological studies of islets, pancreata were dissected, fixed in 10% formalin, and laid flat for paraffin embedding. Paraffin sections with the largest tissue surface area were used. For insulin immunohistochemistry, we used an anti-insulin antibody (Dako) with biotinylated anti-guinea pig antibody. HRP-conjugated Avidin-biotin complex reagent was used following the manufacturer's protocol (Vector). Signals were developed using DAB. For islet counting and β cell area determination, images covering the entire section were obtained. Islet and total pancreas cross-sectional areas were analyzed with Aperio Imagescope. For insulin and Mac-2 immunofluorescence staining, slides were blocked, then incubated with anti-insulin (Dako) and anti-Mac-2 (Cedarlane) antibodies. Slides were washed in PBS and incubated with fluorescently labeled anti-guinea pig and anti-rat antibodies (Life Technologies). 40–60 islets from each pancreas were scored for islet-associated macrophages. Fluorescence images were captured on a Nikon Ti inverted microscope equipped with a cooled CCD camera (Orca R2, Hamamatsu), 20x PlanApo VC NA0.75 objective, and Lumencor Sola LED light source using NIS-Elements software.

For immunohistochemistry analysis of crown-like structures (CLS), serial sections were incubated with anti-MAC-2 (Cedarlane) primary antibody. Biotinylated and HRP-conjugated secondary antibodies were from Vector. Histochemical reactions were performed using Vectastain ABC Kit (Vector) and Sigma Fast 3,3′-diaminobenzidine (Sigma) as the substrate. Sections were counterstained with hematoxylin. Toluidine blue staining was used to enumerate mast cells in purple (metachromatic staining) with a blue background (orthochromatic staining) (Enerbäck et al, 1986). CLS and mast cell density were obtained by counting the total number of CLS or mast cells in each section compared with the total number of adipocytes and expressed as number/10,000 adipocytes.

Patient studies

We investigated *Adipsin, Leptin*, and *Adiponectin* mRNA expression in paired omental and subcutaneous adipose tissue samples obtained from 187 extensively characterized Caucasian obese men (n=65) and women (n=130) who underwent open abdominal surgery for elective cholecystectomy, sleeve gastrectomy, Roux-en-Y bypass surgery as described previously (Bluher et al, 2009). With oral glucose tolerance tests, we identified individuals with T2DM (n=90) or normal glucose tolerance (NGT) (n=97). Among the T2DM cohort, 42 obese individuals (BMI = 34.3 ± 4.9 kg/m^2) were matched for age, gender, BMI and body fat mass, then patients were grouped according to treatments with insulin alone or metformin

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(2g BID). The phenotypic characteristics of the two study groups are given in supplementary figure S6. All study protocols have been approved by the Ethics committee of the University of Leipzig. All participants gave written informed consent before taking part in the study.

Plasma insulin was measured with an enzyme immunometric assay for the IMMULITE automated analyzer (Diagnostic Products). Adiponectin and leptin serum concentrations were measured as previously described (Kloting et al, 2010). Serum adipsin was measured by an ELISA (Aviscera). Human *Adipsin, Leptin* and *Adiponectin* mRNA expression were measured by quantitative TaqMan assay, and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems). *Adiponectin* and *Leptin* expression were measured as described previously (Kloting et al, 2010). Human *Adipsin, Leptin* and *Adiponectin* mRNA expression were calculated relative to the mRNA expression of *Hypoxanthine phosphoribosyltransferase 1 (HPRT1)*, all determined by premixed assays on demand (Applied Biosystems). The following statistical tests were used: paired student's test, chi quadrate test, and Pearson's simple correlation. Statistical analysis was performed using SPSS version 12.0.

Statistical analyses

Unless otherwise stated, data are presented as mean ± s.e.m. Statistical analysis was carried out using Student's t-test when comparing two groups and ANOVA when comparing multiple groups. Differences were considered significant at $P < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. Diabetes. 1995; 44:1249–1258. [PubMed: 7589820]

Ahren B, Havel PJ, Pacini G, Cianflone K. Acylation stimulating protein stimulates insulin secretion. International journal of obesity and related metabolic disorders. journal of the International Association for the Study of Obesity. 2003; 27:1037–1043.

Ames RS, Lee D, Foley JJ, Jurewicz AJ, Tornetta MA, Bautsch W, Settmacher B, Klos A, Erhard KF, Cousins RD, et al. Identification of a selective nonpeptide antagonist of the anaphylatoxin C3a

receptor that demonstrates antiinflammatory activity in animal models. J Immunol. 2001; 166:6341–6348. [PubMed: 11342658]

Ashcroft FM, Rorsman P. Diabetes mellitus and the beta cell the last ten years. Cell. 2012; 148:1160–1171. [PubMed: 22424227]

Baldo A, Sniderman AD, St-Luce S, Avramoglu RK, Maslowska M, Hoang B, Monge JC, Bell A, Mulay S, Cianflone K. The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. The Journal of clinical investigation. 1993; 92:1543–1547. [PubMed: 8376604]

Bluher M, Bashan N, Shai I, Harman-Boehm I, Tarnovscki T, Avinaoch E, Stumvoll M, Dietrich A, Kloting N, Rudich A. Activated Ask1-MKK4-p38MAPK/JNK stress signaling pathway in human omental fat tissue may link macrophage infiltration to whole-body Insulin sensitivity. The Journal of clinical endocrinology and metabolism. 2009; 94:2507–2515. [PubMed: 19351724]

Choy LN, Rosen BS, Spiegelman BM. Adipsin and an endogenous pathway of complement from adipose cells. The Journal of biological chemistry. 1992; 267:12736–12741. [PubMed: 1618777]

Cook KS, Min HY, Johnson D, Chaplinsky RJ, Flier JS, Hunt CR, Spiegelman BM. Adipsin a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. Science. 1987; 237:402–405. [PubMed: 3299705]

Dahlke K, Wrann CD, Sommerfeld O, Sossdorf M, Recknagel P, Sachse S, Winter SW, Klos A, Stahl GL, Ma YX, et al. Distinct different contributions of the alternative and classical complement activation pathway for the innate host response during sepsis. J Immunol. 2011; 186:3066–3075. [PubMed: 21263075]

Danial NN, Walensky LD, Zhang CY, Choi CS, Fisher JK, Molina AJ, Datta SR, Pitter KL, Bird GH, Wikstrom JD, et al. Dual role of proapoptotic BAD in insulin secretion and beta cell survival. Nature medicine. 2008; 14:144–153.

Deng Y, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome. Annals of the New York Academy of Sciences. 2010; 1212:E1–E19. [PubMed: 21276002]

Dunmore SJ, Brown JE. The role of adipokines in beta-cell failure of type 2 diabetes. The Journal of endocrinology. 2013; 216:T37–T45. [PubMed: 22991412]

Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, Gueripel X, Ellingsgaard H, Schneider MK, Biollaz G, et al. Increased number of islet-associated macrophages in type 2 diabetes. Diabetes. 2007; 56:2356–2370. [PubMed: 17579207]

Enerbäck, L.; Miller, HRP.; Maryhofer, G. Methods for the identification and characterization of mast cells by light microscopy. In: Befus, D.; Bienenstock, J.; Denburg, JA., editors. In Mast cell differentiation and heterogeneity. New York: Raven Press; 1986. p. 167-181.

Ferrannini E. The stunned beta cell: a brief history. Cell metabolism. 2010; 11:349–352. [PubMed: 20444416]

Festa A, Williams K, D'Agostino R Jr, Wagenknecht LE, Haffner SM. The natural course of beta-cell function in nondiabetic and diabetic individuals: the Insulin Resistance Atherosclerosis Study. Diabetes. 2006; 55:1114–1120. [PubMed: 16567536]

Flier JS, Cook KS, Usher P, Spiegelman BM. Severely impaired adipsin expression in genetic and acquired obesity. Science. 1987; 237:405–408. [PubMed: 3299706]

Henquin JC. The dual control of insulin secretion by glucose involves triggering and amplifying pathways in beta-cells. Diabetes research and clinical practice. 2011; 93(Suppl 1):S27–S31. [PubMed: 21864748]

Homo-Delarche F, Calderari S, Irminger JC, Gangnerau MN, Coulaud J, Rickenbach K, Dolz M, Halban P, Portha B, Serradas P. Islet inflammation and fibrosis in a spontaneous model of type 2 diabetes, the GK rat. Diabetes. 2006; 55:1625–1633. [PubMed: 16731824]

Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006; 444:860–867. [PubMed: 17167474]

Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science. 1996; 274:1377–1379. [PubMed: 8910278]

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Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. The New England journal of medicine. 2006; 355:2427–2443. [PubMed: 17145742]

Kasuga M. Insulin resistance and pancreatic beta cell failure. The Journal of clinical investigation. 2006; 116:1756–1760. [PubMed: 16823472]

Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, Schraw T, Durand JL, Li H, Li G, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. The Journal of clinical investigation. 2007; 117:2621–2637. [PubMed: 17717599]

Kitamura T. The role of FOXO1 in beta-cell failure and type 2 diabetes mellitus. Nature reviews Endocrinology. 2013; 9:615–623.

Kloting N, Fasshauer M, Dietrich A, Kovacs P, Schon MR, Kern M, Stumvoll M, Bluher M. Insulin-sensitive obesity. American journal of physiology Endocrinology and metabolism. 2010; 299:E506–E515. [PubMed: 20570822]

Klöting N, Fasshauer M, Dietrich A, Kovacs P, Schön MR, Kern M, Stumvoll M, Bluher M. Insulin sensitive obesity. American Journal of Physiology - Endocrinology and Metabolism. 2010

Kusminski CM, Holland WL, Sun K, Park J, Spurgin SB, Lin Y, Askew GR, Simcox JA, McClain DA, Li C, et al. MitoNEET-driven alterations in adipocyte mitochondrial activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity. Nature medicine. 2012; 18:1539–1549.

Leroith D, Accili D. Mechanisms of disease using genetically altered mice to study concepts of type 2 diabetes. Nature clinical practice Endocrinology & metabolism. 2008; 4:164–172.

Lim J, Iyer A, Suen JY, Seow V, Reid RC, Brown L, Fairlie DP. C5aR and C3aR antagonists each inhibit diet-induced obesity, metabolic dysfunction, and adipocyte and macrophage signaling. FASEB journal official publication of the Federation of American Societies for Experimental Biology. 2013; 27:822–831. [PubMed: 23118029]

Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of clinical investigation. 2007; 117:175–184. [PubMed: 17200717]

Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. The Journal of clinical investigation. 2011; 121:2111–2117. [PubMed: 21633179]

Maechler P. Mitochondrial function and insulin secretion. Molecular and cellular endocrinology. 2013; 379:12–18. [PubMed: 23792187]

Mamane Y, Chung Chan C, Lavallee G, Morin N, Xu LJ, Huang J, Gordon R, Thomas W, Lamb J, Schadt EE, et al. The C3a anaphylatoxin receptor is a key mediator of insulin resistance and functions by modulating adipose tissue macrophage infiltration and activation. Diabetes. 2009; 58:2006–2017. [PubMed: 19581423]

Matthews DR, Cull CA, Stratton IM, Holman RR, Turner RC. UKPDS 26: Sulphonylurea failure in non-insulin-dependent diabetic patients over six years. UK Prospective Diabetes Study (UKPDS) Group. Diabetic medicine: a journal of the British Diabetic Association. 1998; 15:297–303. [PubMed: 9585394]

Muoio DM, Newgard CB. Mechanisms of disease:molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. Nature reviews Molecular cell biology. 2008; 9:193–205.

Napolitano A, Lowell BB, Damm D, Leibel RL, Ravussin E, Jimerson DC, Lesem MD, Van Dyke DC, Daly PA, Chatis P, et al. Concentrations of adipsin in blood and rates of adipsin secretion by adipose tissue in humans with normal, elevated and diminished adipose tissue mass. International journal of obesity and related metabolic disorders journal of the International Association for the Study of Obesity. 1994; 18:213–218.

Okada-Iwabu M, Yamauchi T, Iwabu M, Honma T, Hamagami K, Matsuda K, Yamaguchi M, Tanabe H, Kimura-Someya T, Shirouzu M, et al. A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. Nature. 2013; 503:493–499. [PubMed: 24172895]

Okamoto M, Ohara-Imaizumi M, Kubota N, Hashimoto S, Eto K, Kanno T, Kubota T, Wakui M, Nagai R, Noda M, et al. Adiponectin induces insulin secretion in vitro and in vivo at a low glucose concentration. Diabetologia. 2008; 51:827–835. [PubMed: 18369586]

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Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nature reviews Immunology. 2011; 11:85–97.

Platt KA, Claffey KP, Wilkison WO, Spiegelman BM, Ross SR. Independent regulation of adipose tissue-specificity and obesity response of the adipsin promoter in transgenic mice. The Journal of biological chemistry. 1994; 269:28558–28562. [PubMed: 7961801]

Pomeroy C, Mitchell J, Eckert E, Raymond N, Crosby R, Dalmasso AP. Effect of body weight and caloric restriction on serum complement proteins, including Factor D/adipsin: studies in anorexia nervosa and obesity. Clinical and experimental immunology. 1997; 108:507–515. [PubMed: 9182900]

Prentki M, Matschinsky FM, Madiraju SR. Metabolic signaling in fuel-induced insulin secretion. Cell metabolism. 2013; 18:162–185. [PubMed: 23791483]

Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. The Journal of clinical investigation. 2006; 116:1802–1812. [PubMed: 16823478]

Primeau V, Coderre L, Karelis AD, Brochu M, Lavoie ME, Messier V, Sladek R, Rabasa-Lhoret R. Characterizing the profile of obese patients who are metabolically healthy. International journal of obesity (2005). 2011; 35:971–981. [PubMed: 20975726]

Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement a key system for immune surveillance and homeostasis. Nature immunology. 2010; 11:785–797. [PubMed: 20720586]

Rorsman P, Braun M, Zhang Q. Regulation of calcium in pancreatic alpha- and beta-cells in health and disease. Cell calcium. 2012; 51:300–308. [PubMed: 22177710]

Rosen BS, Cook KS, Yaglom J, Groves DL, Volanakis JE, Damm D, White T, Spiegelman BM. Adipsin and complement factor D activity: an immune-related defect in obesity. Science. 1989; 244:1483–1487. [PubMed: 2734615]

Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature. 2006; 444:847–853. [PubMed: 17167472]

Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. The Journal of clinical investigation. 2006; 116:1793–1801. [PubMed: 16823477]

Shu CJ, Benoist C, Mathis D. The immune system's involvement in obesity-driven type 2 diabetes. Seminars in immunology. 2012; 24:436–442. [PubMed: 23333525]

Stahl GL, Xu Y, Hao L, Miller M, Buras JA, Fung M, Zhao H. Role for the alternative complement pathway in ischemia/reperfusion injury. The American journal of pathology. 2003; 162:449–455. [PubMed: 12547703]

Staiger K, Stefan N, Staiger H, Brendel MD, Brandhorst D, Bretzel RG, Machicao F, Kellerer M, Stumvoll M, Fritsche A, et al. Adiponectin is functionally active in human islets but does not affect insulin secretory function or beta-cell lipoapoptosis. The Journal of clinical endocrinology and metabolism. 2005; 90:6707–6713. [PubMed: 16204361]

Tarasov AI, Griffiths EJ, Rutter GA. Regulation of ATP production by mitochondrial Ca(2+). Cell calcium. 2012; 52:28–35. [PubMed: 22502861]

Turner R, Cull C, Holman R. United Kingdom Prospective Diabetes Study 17 a 9-year update of a randomized, controlled trial on the effect of improved metabolic control on complications in non-insulin-dependent diabetes mellitus. Annals of internal medicine. 1996; 124:136–145. [PubMed: 8554206]

Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature. 2006; 444:875–880. [PubMed: 17167476]

Weir GC, Marselli L, Marchetti P, Katsuta H, Jung MH, Bonner-Weir S. Towards better understanding of the contributions of overwork and glucotoxicity to the beta-cell inadequacy of type 2 diabetes. Diabetes, obesity & metabolism. 2009; 11(Suppl 4):82–90.

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. The Journal of clinical investigation. 2003; 112:1796–1808. [PubMed: 14679176]

Wetsel RA, Kildsgaard J, Zsigmond E, Liao W, Chan L. Genetic deficiency of acylation stimulating protein (ASP(C3ades-Arg)) does not cause hyperapobetalipoproteinemia in mice. The Journal of biological chemistry. 1999; 274:19429–19433. [PubMed: 10383458]

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White RT, Damm D, Hancock N, Rosen BS, Lowell BB, Usher P, Flier JS, Spiegelman BM. Human adipsin is identical to complement factor D and is expressed at high levels in adipose tissue. The Journal of biological chemistry. 1992; 267:9210–9213. [PubMed: 1374388]

Winzell MS, Nogueiras R, Dieguez C, Ahren B. Dual action of adiponectin on insulin secretion in insulin-resistant mice. Biochemical and biophysical research communications. 2004; 321:154–160. [PubMed: 15358228]

Xu Y, Ma M, Ippolito GC, Schroeder HW Jr, Carroll MC, Volanakis JE. Complement activation in factor D-deficient mice. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98:14577–14582. [PubMed: 11724962]

Yang SN, Berggren PO. The role of voltage-gated calcium channels in pancreatic beta-cell physiology and pathophysiology. Endocrine reviews. 2006; 27:621–676. [PubMed: 16868246]

Zhang J, Wright W, Bernlohr DA, Cushman SW, Chen X. Alterations of the classic pathway of complement in adipose tissue of obesity and insulin resistance. American journal of physiology Endocrinology and metabolism. 2007; 292:E1433–E1440. [PubMed: 17244723]

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Highlights

- Adipsin-null mice develop exacerbated diabetes due to insulinopenia
- Replenishment of adipsin augments insulin secretion in vivo
- C3a, a peptide downstream of adipsin, enhances glucose-stimulated insulin secretion
- Type II diabetic patients with β cell failure are deficient in adipsin

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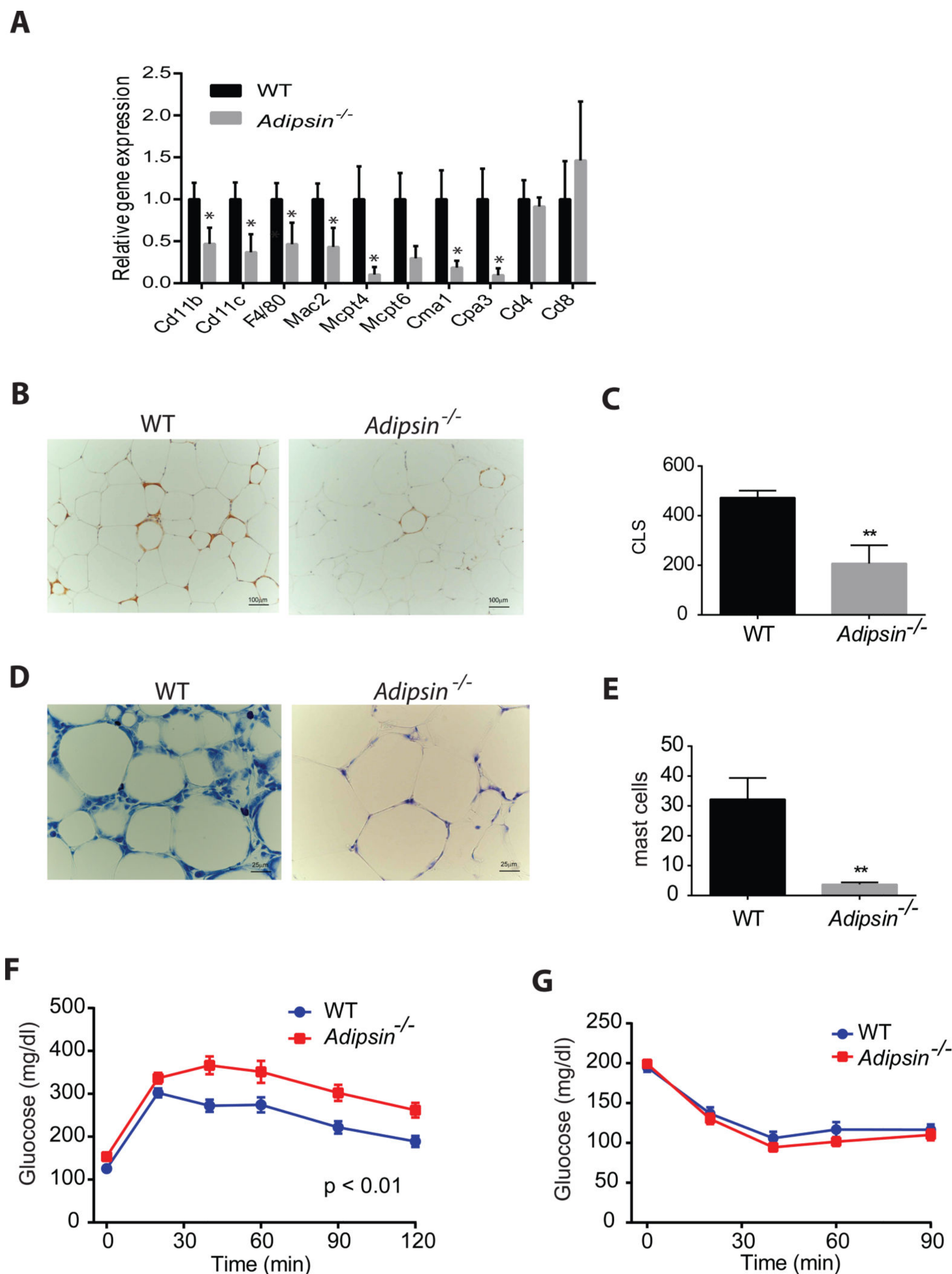

Figure 1. Adipsin Regulates Adipose Tissue Inflammation and Protects Against Diabetes

(**A**) WT and *Adipsin*$^{-/-}$ mice were fed a high fat diet (HFD) for 4 months starting at 1 month of age and visceral adipose tissues were assayed for immune infiltration by qPCR. (**B** and **C**) WT and *Adipsin*$^{-/-}$ adipose tissues were stained for crown-like structures (CLS). Representative pictures are shown (**B**) and numbers of CLS (**C**) were quantitated. Scale bar, 100 μm. (**D** and **E**) WT and *Adipsin*$^{-/-}$ adipose tissues were stained with toluidine blue for mast cells. Representative pictures are shown (**D**) and numbers of mast cells (**E**) were quantitated. Scale bar, 25 μm. (**F**) Glucose tolerance test (GTT) was performed on WT and

Adipsin$^{-/-}$ mice fed a HFD for 16 weeks with measurement of blood glucose concentrations. (**G**) Insulin tolerance test (ITT) was performed on WT and *Adipsin*$^{-/-}$ mice fed a HFD for 18 weeks with measurement of blood glucose concentrations. For GTT and ITT experiments, n = 12–16 per genotype. *$P < 0.05$, **$P < 0.01$. See also Figures S1 and S2.

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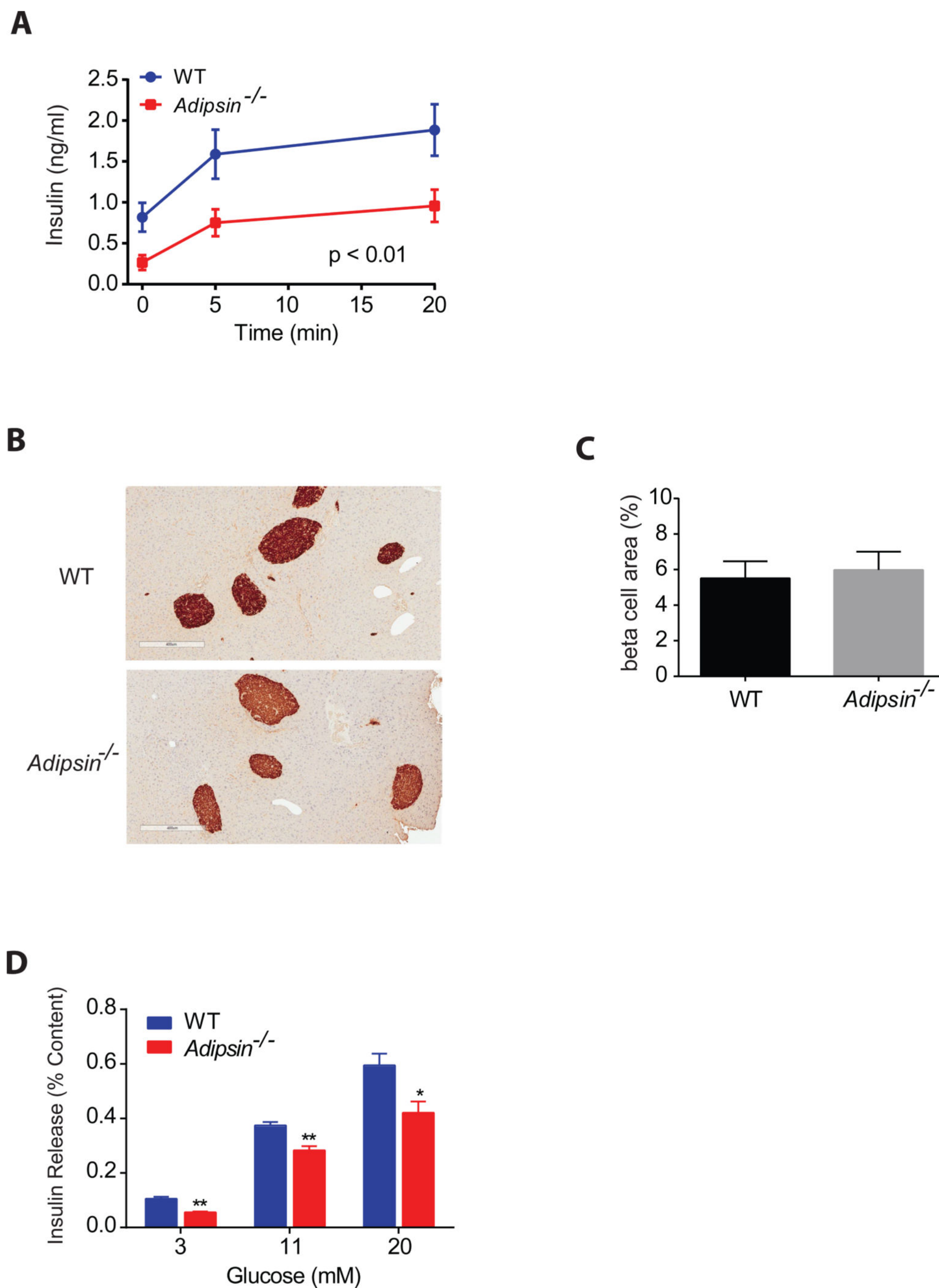

Figure 2. Adipsin Regulates Insulin Secretion *in vivo* and *in vitro*

(**A**) WT and *Adipsin*$^{-/-}$ mice were fed a HFD diet for 4 months starting at 1 month of age and challenged with i.p. glucose injections, and plasma insulin levels were assayed. N = 8–12 mice per genotype. (**B** and **C**) Pancreata from WT and *Adipsin*$^{-/-}$ mice fed a HFD diet for 4 months were collected and insulin immunohistochemistry staining was performed. Representative pictures are shown (**B**) and β cell area was quantitated (**C**). Scale bar, 400 μm. (**D**) Glucose-stimulated insulin secretion assay on islets from WT and *Adipsin*$^{-/-}$ mice fed a HFD diet. $*P < 0.05$, $**P < 0.01$. See also Figure S3.

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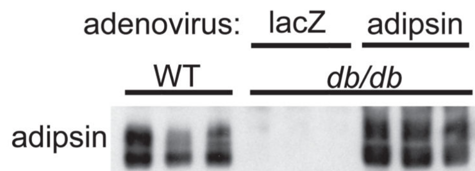
A



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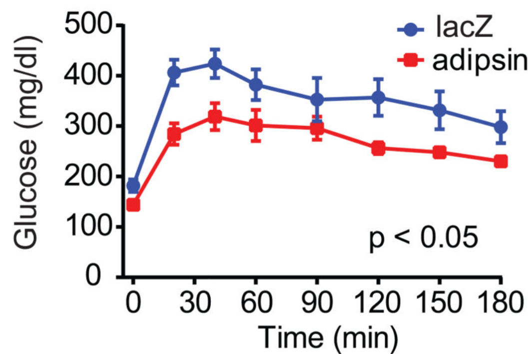

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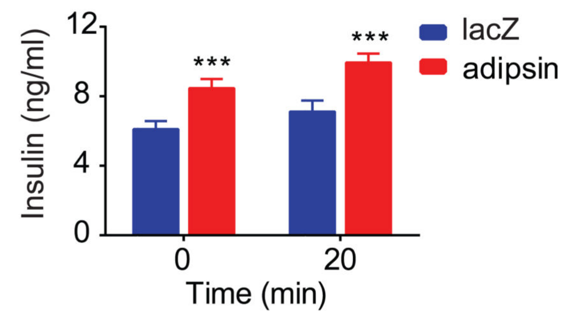

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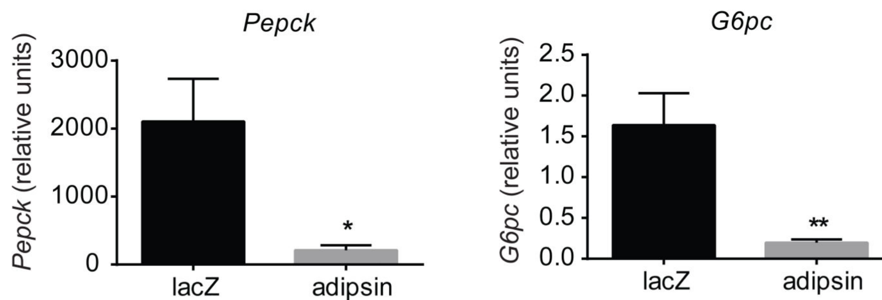

Figure 3. Restoration of Adipsin Improves Insulin Secretion and Glucose Homeostasis

(**A**) Diabetic *db/db* mice (3 month old) were treated i.v. with 2×10^9 IFU of control lacZ or adipsin adenovirus vectors and 5 days later serum adipsin was assessed by Western blot. (**B** and **C**) Control lacZ- and adipsin-transduced mice were challenged by an i.p. GTT with measurements of blood glucose (**B**) and plasma insulin (**C**). N = 6 mice per group. (**D**) Gluconeogenesis was determined by hepatic *Pepck* and *G6pc* gene expression. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. See also Figure S4.

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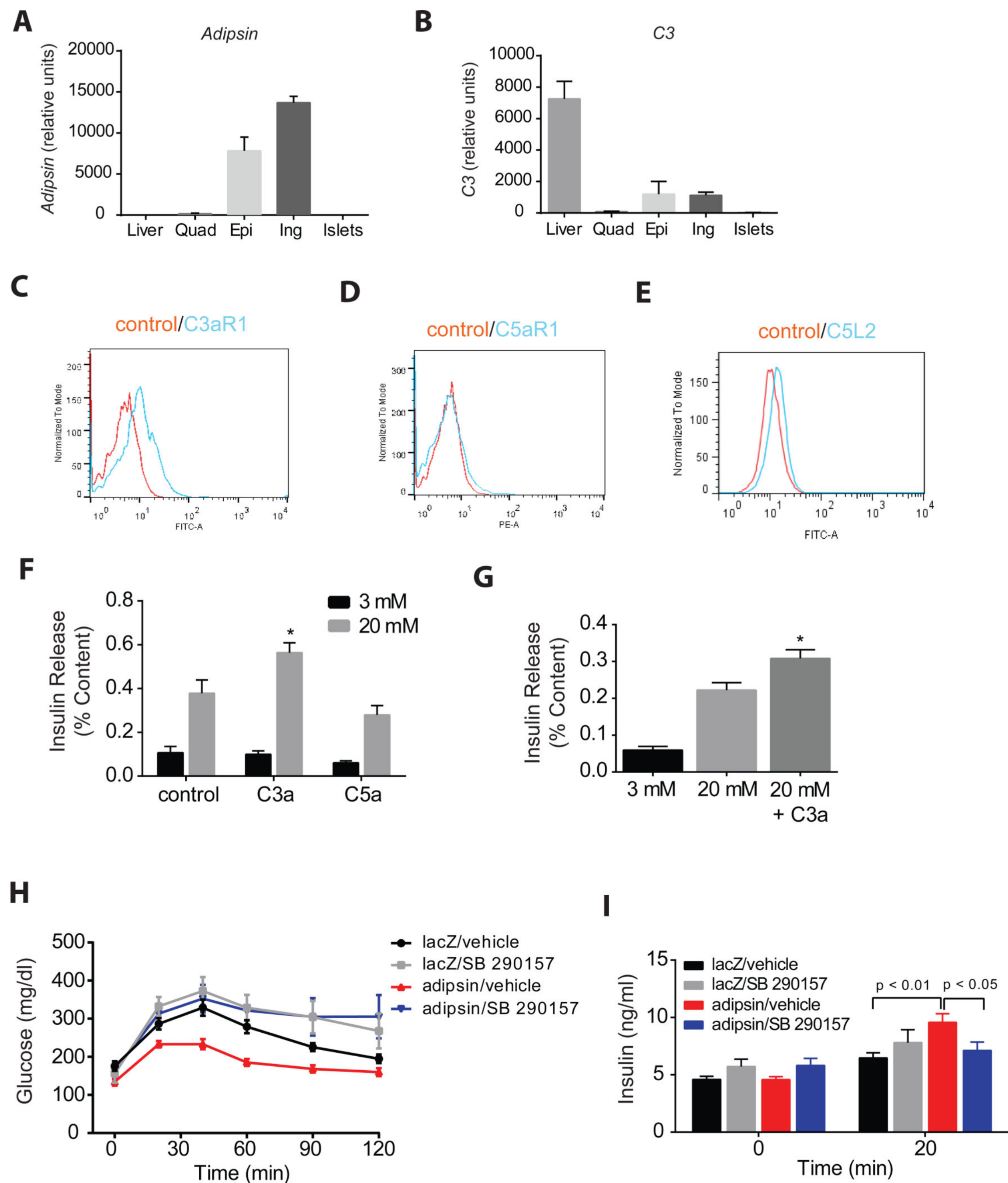

Figure 4. C3a Stimulates Pancreatic β Cells to Secrete Insulin

(**A** and **B**) Relative expression of *Adipsin* (**A**) and *C3* (**B**) in liver, quadriceps muscle (quad), islets, epididymal (epi) and inguinal (ing) fat were quantitated by qPCR. (**C** to **E**) Flow cytometry was performed on islet cells with antibodies to receptors C3aR1 (**C**), C5aR1 (**D**) and C5L2 (**E**). (**F** and **G**) Islets from WT mice on a chow diet (**F**) or HFD (**G**) were subjected to a GSIS assay with recombinant C3a or C5a (100 nM) at the indicated concentrations of glucose. (**H** and **I**) *db/db* mice (3 month old) transduced with lacZ or adipsin adenovirus were treated with vehicle or C3aR antagonist (SB 290157) and subjected

to GTT with measurements of blood glucose (**H**) and plasma insulin (**I**). Data were pooled from 2 experiments. Statistics for GTT assays between groups: lacZ/vehicle vs. adipsin/vehicle, $p = 0.05$; adipsin/vehicle vs. adipsin/SB 290157, $p < 0.01$; lacZ/SB 290157 vs. adipsin/vehicle, $p < 0.01$. N = 12–15 mice per group. *$P < 0.05$. See also Figure S5.

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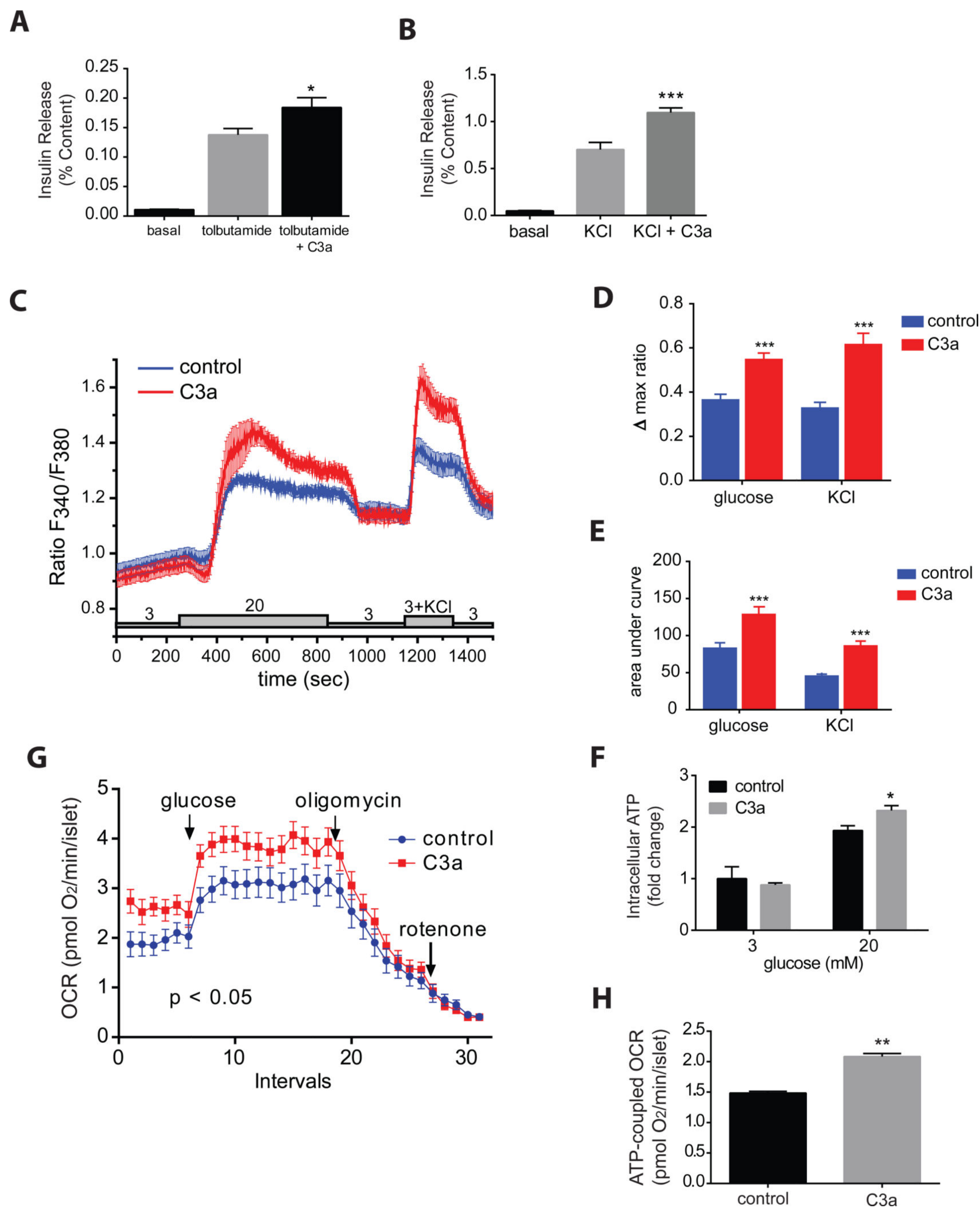

Figure 5. C3a Stimulates Cytoplasmic Free Ca^{2+} ($[Ca^{2+}]_i$) and Oxygen Consumption in Islets

(**A** and **B**) Islets were treated with C3a (100 nM) in conjunction with 30 mM KCl (**A**) or 0.25 mM tolbutamide (**B**) and assayed for insulin secretion. (**C–E**) $[Ca^{2+}]_i$ was measured in islets treated with control or C3a and stimulated with 20 mM glucose, washed with 3 mM glucose, stimulated with KCl and then washed with 3 mM glucose. The peak $[Ca^{2+}]_i$ (**D**) and $[Ca^{2+}]_i$ area under the curve (**E**) are quantified. (**F**) Intracellular ATP levels were determined after treatment of islets with C3a at 3 or 20 mM glucose. Data were pooled from 3 experiments. (**G** and **H**) Oxygen consumption rates (OCR) were measured in islets treated

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with C3a and the following treatments: 20 mM glucose, oligomycin, rotenone and antimycin A. Statistical analysis was performed on traces prior to oligomycin. Data were pooled from 3 experiments. (**H**) ATP-coupled respiration of islets is quantified. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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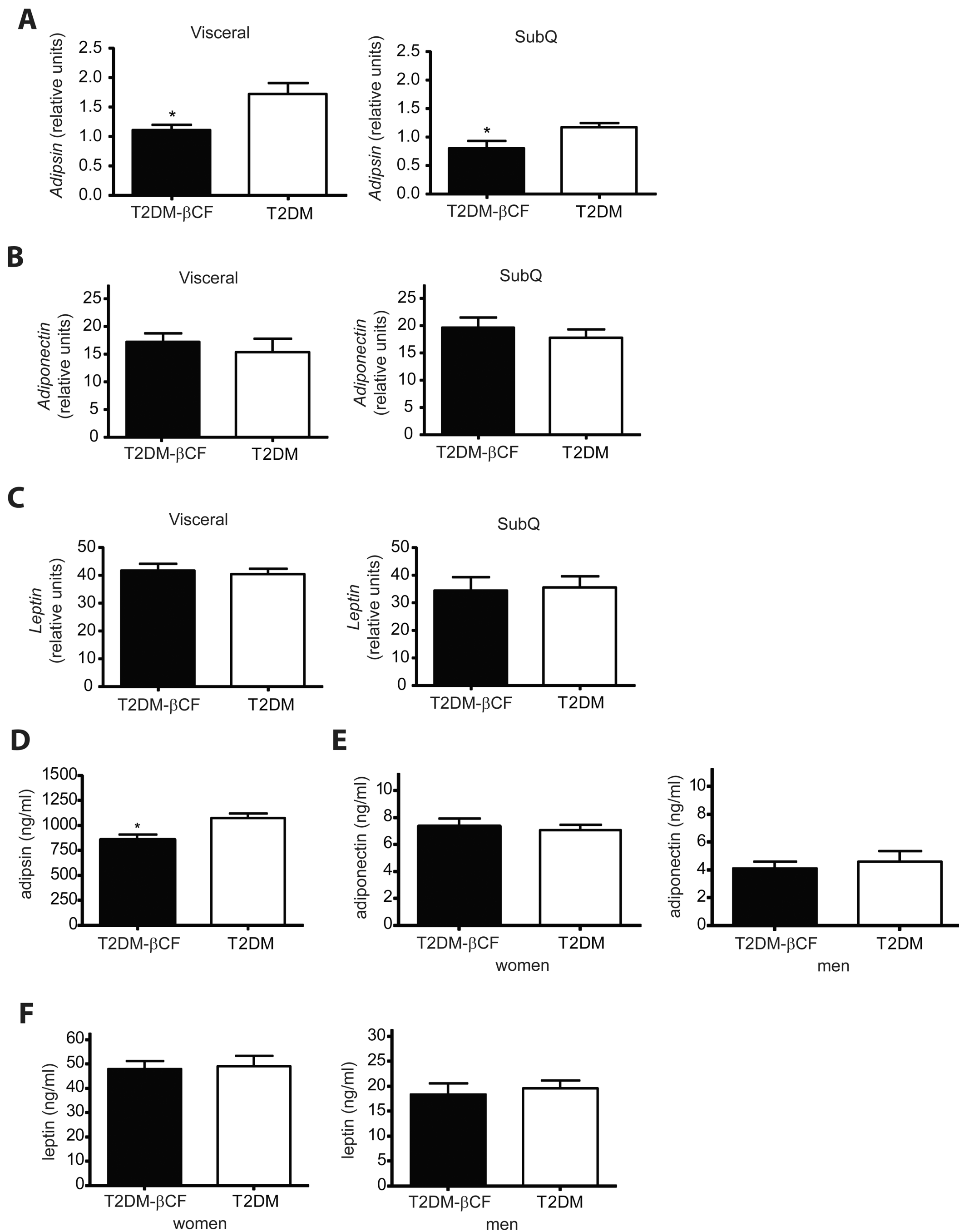

Figure 6. Adipsin is Decreased in Patients with Type II Diabetes Mellitus (T2DM) with β Cell Failure

(**A** to **F**) T2DM patients were classified according to those on oral metformin therapy (T2DM) or T2DM patients with β cell failure (T2DM-βCF) for those on insulin therapy. (**A** to **C**) Visceral and subcutaneous (SubQ) adipose tissue samples from T2DM and T2DM-βCF patients were analyzed for *Adipsin* (**A**), *Adiponectin* (**B**) and *Leptin* (**C**) mRNA levels. (**D** to **F**) Circulating adipsin (**D**), adiponectin (**E**) and leptin (**F**) levels were measured from

blood samples of T2DM and T2DM-βCF patients. Adiponectin and leptin are plotted according to male and female. *$P < 0.05$. See also Figure S6.

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