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Dual action of adiponectin on insulin secretion in insulin-resistant mice

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Abstract

Adiponectin is secreted by adipocytes and has been implicated as a mediator of insulin sensitivity. In this study, the acute effects of adiponectin on islets isolated from normal or diet-induced insulin resistant mice were examined. In normal islets, adiponectin (5 μg/ml) had no significant effect on insulin secretion. In contrast, in islets from mice rendered insulin resistant by high-fat feeding, adiponectin inhibited insulin secretion at 2.8 mM ($P<0.01$) but augmented insulin secretion at 16.7 mM glucose ($P<0.05$). The augmentation of glucose-stimulated insulin secretion by adiponectin was accompanied by increased glucose oxidation ($P<0.005$), but without any significant effect on palmitate oxidation or the islet ATP/ADP ratio. Furthermore, RT-PCR revealed the expression of the adiponectin receptor AdipoR1 mRNA in mouse islets, however, with no difference in the degree of expression level between the two feeding groups. The results thus uncover a potential dual role for adiponectin to modify insulin secretion in insulin resistance.

Keywords: Insulin secretion; Adiponectin; High-fat diet; Adiponectin receptors; Islets; Glucose oxidation; Insulin resistance

High-fat diet induced obesity with insulin resistance is a major risk factor for development of type 2 diabetes because it requires an islet compensation with hypersecretion of insulin for maintenance of normal glucose metabolism and in islet dysfunction, the degree of compensation is impaired leading to the disease [1–3]. Adipose tissue may be central for this development, due to its release of a number of biologically active substances, including leptin, tumor necrosis factor (TNF)-α, plasminogen-activator inhibitor type 1 (PAI-1), adipsin, resistin, and adiponectin; collectively known as adipokines [4–7]. These adipokines may be signals for or deteriorating the islet compensation in insulin resistance and therefore involved in prevention or development of type 2 diabetes. An important adipokine in this respect is adiponectin, which has been suggested to play a key role in the regulation of energy homeostasis mainly by increasing insulin sensitivity [8–10]. Furthermore, whereas levels of most adipokines are increased in obesity, adiponectin levels are decreased in human and animal models of obesity and type 2 diabetes [11,12].

Adiponectin is expressed exclusively in adipose tissue and is present in abundant concentrations in serum [13]. It is composed of an N-terminal collagenous domain and a C-terminal globular domain. In serum, adiponectin occurs in two forms, as a lower molecular weight trimer–dimer and a high molecular weight complex [14]. Adiponectin increases insulin sensitivity by increasing fatty acid oxidation in skeletal muscle, which results in decreased accumulation of triglycerides, a key factor for insulin sensitivity [15,16]. Furthermore, adiponectin suppresses hepatic glucose production [17] and may therefore be beneficial for glucose homeostasis also by this mechanism. Treatment of insulin resistant mice, obese or lipoatrophic, with physiological levels of adiponectin, consequently results in improved glucose tolerance and reduced insulin resistance [10]. The molecular mechanism of action for adiponectin is thought to

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occur via activation of the 5′-AMP-activated protein kinase (AMPK), through which adiponectin has been shown to stimulate glucose uptake in myocytes [16,18].

Accumulating data therefore clearly demonstrate a potential role of adiponectin in reducing insulin resistance in peripheral tissue. However, whether adiponectin affects β-cells is still not known. Recently, adiponectin receptors, both AdipoR1 and AdipoR2, were cloned. In mouse, AdipoR1 is expressed ubiquitously, with most abundant expression in skeletal muscle, while AdipoR2 is most abundant in liver [19]. Both receptors were identified in human and rat pancreatic β-cells, and using a clonal β-cell line (INS-1E) it was shown that adiponectin receptors were upregulated by oleate [20]. This would suggest a β-cell action of adiponectin, and accordingly, adiponectin has been suggested to counteract cytokine- and fatty acid-induced apoptosis in pancreatic β-cells (INS-1), indicating a role for the adipokine during exposure of β-cells to high levels of fatty acids [21]. However, whether adiponectin has a role in the regulation of insulin secretion is not known.

In this study, we therefore investigated the acute effect of adiponectin on mouse islets. We demonstrate that the adiponectin receptor AdipoR1 is abundantly expressed on mouse islets and that whereas insulin secretion in normal islets was not affected by adiponectin, insulin resistance uncovered a dual action of the adipokine on insulin secretion. This suggests that adiponectin may have a potential role in modulating insulin secretion in insulin resistance.

Materials and methods

Animals. Female C57BL/6J mice, weighing ~18 g, were purchased from Taconic (Skensved, Denmark). The animals were maintained in a temperature-controlled room (22 °C) on a 12 h light–dark cycle. The study was approved by the Local Animal Ethics Committee, Lund, Sweden. One week after arrival, the mice were started on the different diets. Mice were divided into two groups, fed either a low-fat (11% fat by energy) or a high-fat diet (58% fat by energy, Research Diets, New Brunswick, NJ), and kept on the different diets for 8–12 weeks. Food intake and body weight were measured once a week, and blood samples were taken regularly from the intraorbital, retrobulbar plexus from nonfasted, anesthetized mice to measure basal levels of blood glucose, insulin, and adiponectin.

Islet isolation and insulin secretion. For insulin secretion studies, islets were isolated from the pancreas by collagenase digestion and hand-picked under microscope. Batches of islets were preincubated in Hepes balanced salt solution containing 125 mM NaCl, 5.9 mM KCl, 1.28 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM Hepes (pH 7.4), 3.3 mM glucose, and 0.1% fatty acid free bovine albumin (Boehringer–Mannheim, GmbH, Germany) for 60 min. Thereafter, islets in groups of three were incubated in 200 μl of the Hepes buffer with varying concentrations of glucose with or without addition of 5 μg/ml adiponectin (full-length recombinant mouse adiponectin, BioCat GmbH, Heidelberg, Germany) and incubated for 60 min at 37 °C. After incubation, aliquots of 25 μl in duplicates were collected and stored at −20 °C until analysis of insulin.

RT-PCR and real time PCR analysis of islet mRNA for identification of adiponectin receptors. Total mRNA was extracted from isolated islets, skeletal muscle (gastrocnemius), and liver from high-fat and low-fat fed mice and 500 ng was reverse transcribed using random primer and Superscript II reverse transcriptase (Invitrogen). For amplification of Adipo-R1 and R2 primers (Adipo-R1, forward (5′-CTTCTACT GCTCCCCACAGC-3′) and reverse (5′-TCCCAGGAACACTCCTG CTC); Adipo-R2, forward (5′-CCACACAACACAAGAATCCG-3′) and reverse (5′-CCCTTCTTCTTGGGAGAATGG-3′)) were selected to eliminate non-specific amplification of genomic DNA. The PCR conditions were 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 1 min for 34 cycles. The RT-PCR product was confirmed by sequencing. As an internal standard, cDNA for the housekeeping gene hypoxanthine phosphoribosyltransferase (HPRT) (137 bp) was amplified using specific sense (5′-CAGTCCCAGCGTCGTGATTA-3′) and antisense (5′-AGCAAGTCTTTCAGTCCTGTC-3′) primers. PCR products were separated by electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining with UV light using a Gel Doc 1000 Documentation System (Bio-Rad Laboratories, Richmond, CA).

Real time reverse-transcription polymerase chain reaction analyses were performed in a fluorescent temperature cycler (LightCycler, Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. Five hundred nanograms of total RNA from islets was used for each RT reaction. The 20-μl amplification mixture contained 1.2 μl RT reaction products plus 3 mM $MgCl_2$, 0.5 μM of each primer, and 1× LightCycler DNA Master SYBR Green I mix (Roche Molecular Biochemicals). The PCR cycling conditions included an initial denaturation at 96 °C for 20 s followed by 40 cycles at 96 °C for 2 s; 60 °C for 15 s; and 72 °C for 15 s.

The oligonucleotide specific primers for rat AdipoR1, AdipoR2, and HPRT are described above. After PCR, a C_T value was obtained using the software provided by the manufacturer. Relative quantification of the PCR products was then based upon value differences using the comparative C_T method. AdipoR1 and R2 mRNA levels were normalized with respect to HPRT level in each sample.

Insulin, glucose, and adiponectin measurements. Insulin was determined radioimmunochemically using a guinea pig anti-rat insulin antibody, ^{125}I-labelled human insulin as tracer, and rat insulin as standard (Linco Res., St. Charles, MO). Free and bound radioactivity were separated by use of an anti-IgG (goat anti-guinea pig) antibody. The sensitivity of the assay is 17 pmol/L and the coefficiency of variation is less than 3% at both low and high levels. Glucose was measured using the glucose oxidase method. Plasma adiponectin was measured using a radioimmunoassay kit with a multispecies rabbit anti-adiponectin antiserum and ^{125}I-labelled murine adiponectin as tracer (Linco Res.). Recombinant mouse adiponectin was used as standard. Free and bound radioactivity were separated by use of an anti-IgG (goat anti-guinea pig) antibody. The sensitivity of the assay is 0.8 ng/ml and the coefficiency of variation is less than 8.2% at both low and high levels.

Fuel oxidation. Palmitate and glucose oxidation were measured in islets isolated from low-fat or high-fat fed mice [22,23]. Batches of 30 islets in quadruplicates were incubated in a reaction mixture consisting of 0.5 mM palmitic acid complexed to 1% fatty acid free bovine serum albumin, with 0.5 μCi [1-^{14}C]palmitic acid (NEN, Boston, MA, specific activity 55 mCi/mmol) as tracer, 0.8 μM L-carnitine, and glucose, at final concentrations of 2.8 or 16.7 mM with and without addition of 5 μg/ml adiponectin. For glucose oxidation, islets were incubated with 0.1 or 0.7 μCi [^{14}C]glucose (NEN, Boston, MA, specific activity 310 mCi/mmol) and 2.8 or 16.7 mM glucose, respectively, with and without 5 μg/ml adiponectin. The reaction was terminated after 2 h and the amount of released $^{14}CO_2$, trapped with benzetonium hydroxide, was determined by scintillation counting.

ATP/ADP ratio measurement. The ATP/ADP ratio was measured in overnight cultured islets under similar conditions as above. In brief, for determination of ATP/ADP ratio in islets, batches of 10 islets were incubated in 125 mM NaCl, 5.9 mM KCl, 1.28 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM Hepes (pH 7.4), and 0.1% fatty acid free bovine albumin, containing 2.8 or 16.7 mM glucose with and without 5 μg/ml

adiponectin at 37 °C for 5 min. The reaction was stopped by addition of TCA and nucleotides were extracted. The ATP concentration was measured using a luciferase-based assay (ATP monitoring kit, Thermo Labsystems, Finland). ADP was comeasured after converting the ATP irreversible to AMP with ATP sulfurylase [24].

Statistical analysis. Data are presented as means ± SEM. Multiple comparisons between the different groups were performed by one-way ANOVA and Tukey's post hoc test to calculate statistical difference between the groups. Significant statistical difference was considered at $P < 0.05$.

Results and discussion

Adiponectin levels in C57BL/6J mice

Feeding C57BL/6J mice a high-fat diet is a well-characterized model resulting in insulin resistance, defective islet compensation, and impaired glucose tolerance [3,25,26]. Basal circulating glucose is elevated by approximately 1 mM, which is apparent already after one week on the diet and remains elevated after one year on the diet. Basal insulin is however progressively increased over time. In this study, we had mice on high-fat diet for 8–12 weeks, which increased glucose and insulin levels as expected from previous results (Table 1). In this study, we also examined the circulating adiponectin levels in high-fat and low-fat fed mice. It is known from several other models of insulin resistance that adiponectin levels correlate negatively with insulin resistance and therefore are reduced in obesity [11,12]. Since treatment of obese mice with adiponectin has resulted in decreased body weight and reduced insulin resistance, this reduction in adiponectin levels would be of pathophysiological relevance [10,27,28]. However, here we found that adiponectin levels were not significantly different between the normal and the insulin resistant, high-fat fed mice, being 12.8 ± 0.5 and 12.3 ± 0.4 μg/ml in normal and insulin resistant mice, respectively, after two months of high-fat feeding. This suggests that different models of insulin resistance and obesity differ in this respect, perhaps depending on various degrees of insulin resistance. For example, the more severely insulin resistant ob/ob mice are associated with reduced adiponectin levels [11]. Another possibility is differences in the different forms of circulating adiponectin. Adiponectin forms multiple species of different molecular weights, and it circulates mainly as a hexamer of relatively low molecular weight (LMW) and a large high molecular weight (HMW) multimeric structure and it has been suggested that the biological activity of adiponectin is dependent on alteration of the oligomeric distribution [29]. In db/db mice the total adiponectin levels are slightly reduced compared to wild type littermates, but db/db mice had a far lower percentage of circulating HMW adiponectin. It was suggested that it is not the absolute levels of adiponectin but rather the amount of HMW complex that is important for adiponectin bioactivity, and thus the amount of HMW adiponectin complex would be a more relevant indicator of insulin sensitivity [29]. It is not known if a difference in the circulating oligomers of adiponectin in high-fat fed C57BL/6J mice would explain the unexpected finding that adiponectin levels are not reduced in this model of insulin resistance.

Expression of adiponectin receptors

In mouse, AdipoR1 is abundantly expressed in several tissues including heart, kidney, liver, lung, skeletal muscle, and spleen, while AdipoR2 is predominantly expressed in liver [19]. Previous studies have shown that the two different adiponectin receptors are expressed in rat and human islets [20]. Using a clonal β-cell line (INS-1E) it was also shown that 24 h of incubation of the cells with 0.5 mM palmitate or oleate resulted in a slight upregulation of both adiponectin receptor subtypes. This led us to examine the expression of the adiponectin receptors in islet from low-fat and high-fat fed mice, respectively. After two months on high-fat or normal diet, the expression of AdipoR1 and AdipoR2 mRNA was examined in isolated islets by RT-PCR and real time PCR. It was found that mouse islets expressed AdipoR1 abundantly while AdipoR2 was not detected (Figs. 1A and B). This result suggests that in isolated mouse islets, only AdipoR1 could mediate adiponectin actions. AdipoR1 expression was, however, not significantly altered by high-fat feeding as assessed by real time PCR (Fig. 1C). Our present findings thus show that AdipoR1 is expressed also in mouse islets. AdipoR1 was earlier found to have high affinity for the globular form of adiponectin, mediating increased 5′-AMP-activated kinase, PPAR-α activity, and fatty acid

Table 1
Basal levels of circulating glucose, insulin, and adiponectin in non-fasting female mice fed high-fat ($n = 30$) or low-fat diet ($n = 30$) at the start of the experiment and after two months on the different diets

	Glucose (mM)		Insulin (pmol/L)		Adiponectin (μg/ml)	
	LF-diet	HF-diet	LF-diet	HF-diet	LF-diet	HF-diet
Start	7.8 ± 0.2	7.8 ± 0.2	126 ± 14	125 ± 20	9.8 ± 0.3	10.1 ± 0.4
2 Months	7.3 ± 0.2	8.8 ± 0.2**	80 ± 8	140 ± 18*	12.8 ± 0.5	12.3 ± 0.4

* $P < 0.05$.
** $P < 0.01$.

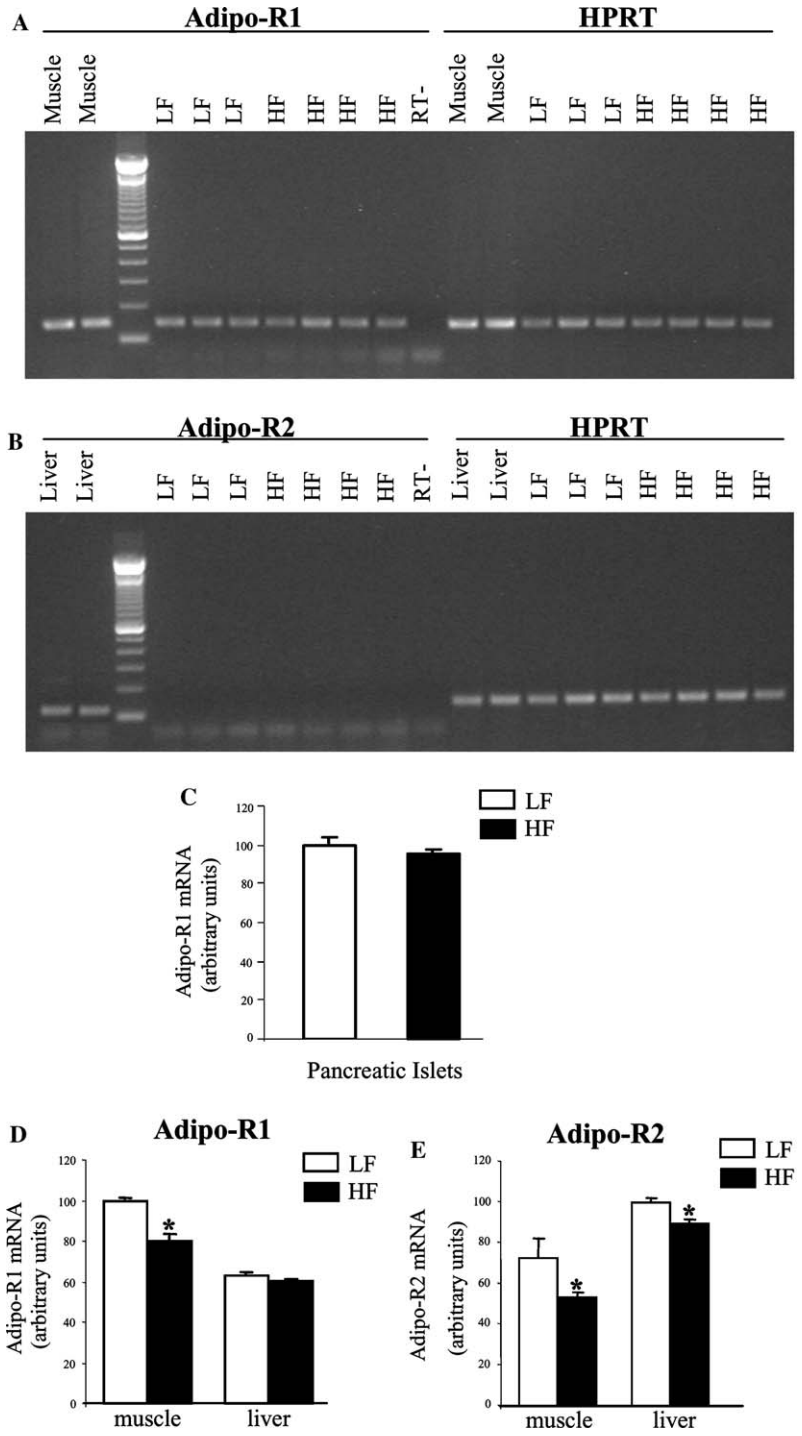

Fig. 1. Expression of adiponectin receptors AdipoR1 and AdipoR2 in mouse islets, muscle, and liver. RT-PCR of (A) AdipoR1 and (B) AdipoR2 in islets from low-fat (LF) or high-fat (HF) fed mice. Total RNA was extracted from 300 to 400 islets in each sample, which contained pooled islets from three different mice. The housekeeping gene HPRT (hypoxanthine phosphoribosyltransferase) was used as internal standard. Skeletal muscle and liver were used as control tissues for the expression of AdipoR1 and AdipoR2, respectively. (C) Real time PCR quantification of AdipoR1 mRNA expression in mouse islets from low-fat (LF) or high-fat (HF) fed mice. AdipoR1 and R2 mRNA levels were normalized with respect to HPRT level in each sample. The data are means ± SEM from four different experiments where islets were isolated and pooled from three mice in each feeding group. $^*P < 0.05$. Real time PCR quantification of (D) AdipoR1 and (E) AdipoR2 expression in skeletal muscle (gastrocnemius) and liver from high-fat and low-fat fed mice. The data are means ± SEM from one experiment where tissues were isolated from six different mice in each feeding group. $^*P < 0.05$.

oxidation in myocytes [15,16,18,19]. The failure to observe any AdipoR2 expression in mouse islets is probably due to lack of or extremely low expression of this receptor type, because our technique readily showed expression of AdipoR2 in other mouse tissues, such as liver and skeletal muscle.

It has been demonstrated that in mice fasted 48 h, AdipoR1 and AdipoR2 were increased in both liver and muscle [30], while both receptors were decreased in several tissues from ob/ob mice. Interestingly, in this study, the expression of AdipoR1 and AdipoR2 in skeletal muscle was decreased in high-fat fed mice compared to low-fat fed mice (Fig. 1D), confirming the notion that adiponectin receptor expression appears to be inversely regulated by insulin [30]. The expression of AdipoR2 in the liver was also reduced by high-fat feeding, while expression of AdipoR1 was unchanged in the liver by the diet (Fig. 1E). However, in the high-fat diet model, the mRNA expression of AdipoR1 in isolated islets was unchanged (Fig. 1C). Thus, these results indicate that there is a blunted response to adiponectin in high-fat fed mice due to downregulation of adiponectin receptors in both liver and skeletal muscle, but not in islets. In the high-fat fed mice, leptin levels are elevated [31]. It is possible that the elevated levels of leptin together with adiponectin serve to modulate insulin secretion during high-fat feeding.

Adiponectin modulates insulin secretion

To determine whether adiponectin has any acute effects on insulin secretion, freshly isolated islets were incubated at 2.8 or 16.7 mM glucose with and without addition of 5 μg/ml adiponectin for 1 h. In islets from normal mice, adiponectin had no significant effect on insulin secretion (Fig. 2). In contrast, in islets from insulin resistant mice, adiponectin inhibited insulin secretion at 2.8 mM (164 ± 27 versus 75 ± 11 pmol/L, $P = 0.007$) whereas the adipokine augmented insulin secretion at 16.7 mM glucose (1900 ± 380 versus 1040 ± 120 pmol/L, $P = 0.031$). Hence, the maximal glucose-stimulated insulin secretion (GSIS) was augmented by approximately 80% by adiponectin. Islets from insulin resistant mice had elevated basal and GSIS compared to normal islets, which is interpreted to be due to the islet compensation that occurs with insulin resistance. The increased effect of adiponectin on GSIS in insulin resistance may therefore be a mechanism behind the islet compensation. Therefore, adiponectin may be a safe mechanism preventing deterioration both of insulin sensitivity and islet dysfunction in insulin resistance. Since the expression of AdipoR1 did not differ between the two groups, the altered regulation of insulin secretion by adiponectin in high-fat fed mice may be related to altered adiponectin signal transduction in insulin resistant β-cells. The inhibition of basal but augmentation of stimulated insulin secretion by adiponectin suggests a dual mechanism in islets in insulin resistance. This is similar to previous reports on effects of leptin on insulin secretion, where it was suggested that leptin in a dual manner modulates both basal and glucose-stimulated insulin secretion in isolated ob/ob islets [32].

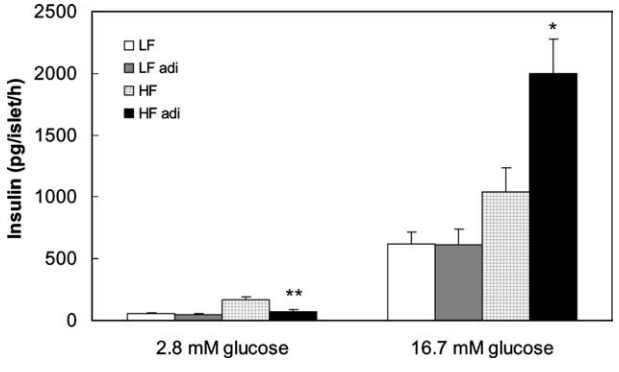

Fig. 2. Insulin secretion from freshly isolated islets incubated for 1 h at 2.8 or 16.7 mM glucose with and without addition of 5 μg/ml full-length mouse adiponectin. Islets were isolated from low-fat (LF) or high-fat (HF) fed mice. The results are expressed as means ± SEM of three independent experiments with $n = 5$ for each incubation condition. *$P < 0.05$; **$P < 0.01$.

Effect of adiponectin on islet metabolism

To examine potential molecular mechanisms underlying the effect of adiponectin on insulin secretion, studies on fuel oxidation and ATP/ADP ratio were undertaken in isolated islets. Adiponectin has been found to stimulate fatty acid oxidation in myocytes [15,18], and fat oxidation may reflect modulation of a lipid signal of potential importance for insulin secretion. Therefore, the effect of adiponectin on palmitate oxidation was tested on freshly isolated mouse islets from normal and insulin resistant mice. Islets were incubated with [^{14}C]palmitate at 2.8 or 16.7 mM glucose with and without 5 μg/ml adiponectin for 2 h and the released $^{14}CO_2$ was measured. We could not observe any significant effect with adiponectin on palmitate oxidation, neither in normal nor in islets from insulin resistant mice (Fig. 3A). If anything, there was a slight decrease in palmitate oxidation at low glucose with adiponectin in insulin resistant islets. These results are in agreement with a recent study, where both full-length and globular adiponectin were unable to increase palmitate oxidation in rat islets [20]. To investigate if adiponectin instead could affect β-cell glucose metabolism, glucose oxidation was measured. The experiment was performed as for palmitate oxidation with the exception that islets were incubated with [U-^{14}C]glucose instead of [^{14}C]palmitate for 2 h and the released $^{14}CO_2$ was measured. Adiponectin had no effect on glucose oxidation at low glucose (2.8 mM) concentration. However, at high glucose (16.7 mM), glucose oxidation was significantly increased with adiponectin in islets from insulin resistant mice (Fig. 3B). The increased oxidation of glucose could thus explain the observed potentiation in GSIS with adiponectin in islets from insulin resistant mice. There were, however, no differences observed in the ATP/ADP ratio after

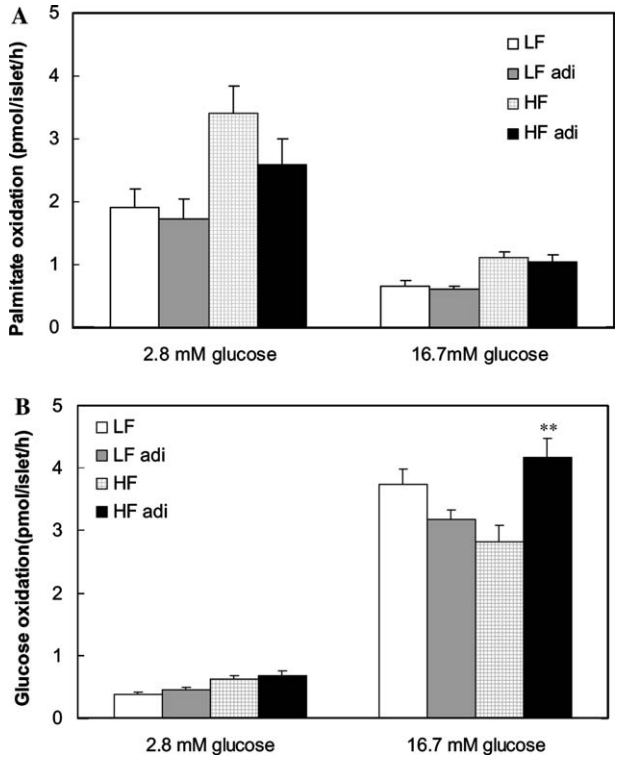

Fig. 3. Fuel oxidation in freshly isolated islets. Batches of 30 islets were incubated for 2 h in 2.8 or 16.7 mM glucose, with and without 5 μg/ml adiponectin together with either [^{14}C]glucose or [^{14}C]palmitate. Islets were isolated from low-fat (LF) or high-fat (HF) fed mice. (A) Palmitate oxidation and (B) glucose oxidation were expressed as means ± SEM of three independent experiments where each condition was run in quadruplicate. $^{**}P < 0.01$.

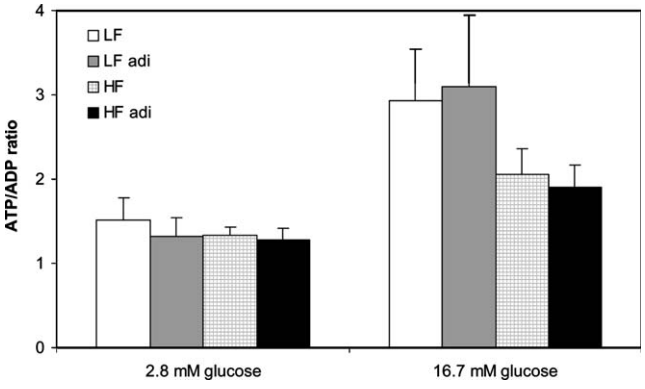

Fig. 4. ATP/ADP ratio in overnight cultured islets. Batches of 10 islets were incubated at 2.8 or 16.7 mM glucose with and without 5 μg/ml adiponectin for 5 min. Islets were isolated from low-fat (LF) or high-fat (HF) fed mice. The reaction was stopped by addition of TCA and nucleotides were extracted. The ATP levels were measured using a luciferase-based assay. The results are from two different experiments where each condition was run in triplicate.

treatment with adiponectin (Fig. 4). This finding is at present difficult to reconcile with a model of stimulated glucose metabolism by adiponectin. Therefore, further studies are required for understanding the molecular mechanism of adiponectin-induced stimulation of GSIS.

To conclude, we found that adiponectin exhibits a glucose-dependent dual effect on insulin secretion in islets from insulin-resistant (high-fat fed) mice in that the adipokine decreases basal insulin secretion but potentiates GSIS. This effect may be secondary to fuel partitioning with, if anything, a slightly reduced fatty acid oxidation but in particular increased glucose oxidation. In contrast, in normal islets adiponectin does not appear to have any acute effects on insulin secretion. The results thus uncover a potential role for adiponectin to modify insulin secretion in insulin resistance. This supports a role of adiponectin as a potential signal involved in islet compensation to insulin resistance. In particular, the augmentation of GSIS in insulin resistance may help the islet to sufficiently compensate the reduced insulin action. Since adiponectin is known to increase insulin sensitivity [11,12], this adipokine therefore seems to be of importance in preventing deterioration of glucose homeostasis in insulin resistance by both increasing insulin sensitivity and augmenting glucose-stimulated insulin secretion.

Acknowledgments

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