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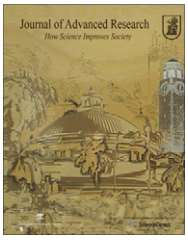

ORIGINAL ARTICLE

Effect of adrenomedullin gene delivery on insulin resistance in type 2 diabetic rats

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Abstract Type 2 diabetes mellitus is one of the common metabolic disorders that ultimately afflicts large number of individuals. Adrenomedullin (AM) is a potent vasodilator peptide; previous studies reported development of insulin resistance in aged AM deficient mice. In this study, we employed a gene delivery approach to explore its potential role in insulin resistance. Four groups were included: control, diabetic, non-diabetic injected with the AM gene and diabetic injected with the AM gene. One week following gene delivery, serum glucose, insulin, triglycerides, leptin, adiponectin and corticosterone were measured as well as the insulin resistance index (HOMA-IR). Soleus muscle glucose uptake and RT-PCR of both AM and glucose transporter-4 (GLUT 4) gene expressions were assessed. A single tail vein injection of adrenomedullin gene in type 2 diabetic rats improved skeletal muscle insulin responsiveness with significant improvement of soleus muscle glucose uptake, HOMA-IR, serum glucose, insulin and triglycerides and significant increase in muscle GLUT 4 gene expression ($P < 0.05$) compared with the non-injected diabetic rats. The beneficial effects of AM gene delivery were accompanied by a significant increase in the serum level of adiponectin (2.95 ± 0.09 versus 2.33 ± 0.17 μg/ml in the non-injected diabetic group) as well as a significant decrease in leptin and corticosterone levels (7.51 ± 0.51 and 262.88 ± 10.34 versus 10.63 ± 1.4 and 275.86 ± 11.19 ng/ml respectively in the non-injected diabetic group). The

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conclusion of the study is that AM gene delivery can improve insulin resistance and may have significant therapeutic applications in type 2 diabetes mellitus.

Introduction

Type 2 diabetes mellitus is a metabolic disease characterized by relative insulin deficiency and associated peripheral insulin resistance including skeletal muscle, liver, and adipose tissue. Multiple lines of study have shown that skeletal muscle insulin resistance is a major determinant of overall insulin resistance [1]. Improvement in whole body glucose disposal is considered to be due to the increased insulin-stimulated glucose transport in skeletal muscle mainly through increased glucose transporter-4 (GLUT 4) protein, the major glucose transporter isoform in skeletal muscle [2].

Adrenomedullin (AM) is a potent 52-amino acid vasodilator peptide originally isolated from tissue extracts of human pheochromocytoma [3]. The gene encoding adrenomedullin has been mapped and localized to a single locus of chromosome 11 and is expressed in a wide range of tissues, such as in skeletal muscle and adipose tissue [4,5] as well as in adrenal gland, kidney, heart, lung, spleen, brain, endothelial, and vascular smooth muscle cells [6]. Adrenomedullin is involved in a variety of biological activities, including vasodilatation, diuresis, and inhibition of aldosterone secretion [7].

Xing et al. [8] reported an enhanced development of insulin resistance by angiotensin II in AM deficient mice. Dobrzynski et al. [9] investigated the effect of AM gene delivery on the cardiac and renal function in streptozotocin (STZ)-induced type 2 diabetic rats and reported that it may also affect skeletal muscle, GLUT 4. These findings may imply potential beneficial effects of AM gene transfer [10].

To clarify the relationship between AM and insulin resistance, we evaluated muscle glucose uptake and GLUT 4 gene expression in addition to serum leptin, adiponectin, and corticosterone following AM gene delivery in type 2 diabetic rats.

Material and methods

Animals and experimental design

Animals were purchased from the animal care unit of Cairo Medical University; all procedures that involved animals were approved by this unit. Forty male Wistar albino rats, 12–14 weeks old, weighing 200–210 g, were each housed in a cage in a constant temperature (22–24 °C) and a light controlled room on an alternating 12:12 h light–dark cycle and had free access to food and water.

By the end of the experiment three rats died and the number was reduced to 37 rats. Animals were divided into the following groups:

- *Control group* ($n = 8$): rats receiving the standard diet.
- *Diabetic group* ($n = 10$): type 2 diabetic rats.
- *Non-diabetic-AM group* ($n = 10$): non-diabetic rats injected with the AM gene.
- *Diabetic-AM group* ($n = 9$): diabetic rats injected with the AM gene.

Induction of type 2 diabetes

Beginning on day 0, animals were divided into two groups (20 rats/group): one group was fed a standard rodent diet (SD: 6.5% kcal fat) and the other group was fed a high fat diet (HFD: 58% kcal fat) for a period of two weeks.

On day 14, rats on the high fat diet (HFD) were injected intraperitoneally with a single low dose of streptozotocin (STZ, 45 mg/kg i.p., in 0.01 M citrate buffer pH 4.3, Sigma, St. Louis, MO, USA) to induce type 2 diabetes mellitus. Both the low dose of STZ and the high fat diet are essential elements to induce type 2 diabetes with insulin resistance [11]. Those fed on standard diet (SD) received only the buffer solution. Subsequently all rats had free access to food and water and were continued on their respective diets till the end of the study.

On day 21, type 2 diabetes was confirmed randomly in some HFD rats by measuring fasting serum glucose and insulin.

Preparation of adrenomedullin gene

Rat AM gene was amplified by reverse transcription-polymerase chain reaction (RT-PCR) for 40 cycles and DNA was purified to prepare cloned DNA (cDNA)/lipid complex using a DNA purification kit. The purified DNA was then transfected through dilution of 0.2 μg of DNA with 0.5–5 μl of lipofectamine using the lipofectamine transfection kit (invitrogen, Carlsbad, CA, USA) then injected as 0.2 ml in the rat tail [12].

Animal treatment

On day 21, half the numbers of each of the two groups (diabetic and non-diabetic groups) were restrained manually and 0.2 ml of the prepared AM gene was administered via the tail vein over 10 s using a 3 ml needle [9]. This dose has previously been shown to result in persistent AM gene expression for up to five weeks [13]. The other half was injected only with 0.2 ml of the lipofectamine vehicle.

Blood and tissue samples

Four weeks after the beginning of the study (one week after the administration of the adrenomedullin gene), retro-orbital blood samples (2 ml each) were taken from the rats of all groups after overnight fasting (9 p.m. to 8 a.m.).

Biochemical and hormonal assays

Fasting serum glucose was measured using the oxidase–peroxidase method. Serum insulin level was analyzed using the enzyme-linked immunosorbent assay (ELISA) (Linco Research) according to the manufacturer's instructions. Total cholesterol was determined by the quantitative colorimetric determination method at 340 nm (EnzyChrom™ cholesterol assay kit: ECCH-100). Triglycerides were measured by using

the triglycerides Biovision quantification kit and its absorbance was measured spectrophotometrically at 570 nm.

Serum hormones were assessed by ELISA using the corresponding kits: adiponectin (Linco research, USA), leptin (Ray Bio research company, USA) and corticosterone (Kamiya Biomedical Company, USA).

To estimate insulin resistance, the homeostasis model assessment for insulin resistance (HOMA-IR: insulin resistance index) was used, calculated as the product of fasting insulin (in μU/ml) and fasting glucose (in mmol/l) divided by 22.5, which has been used previously in rodents [14].

Muscle collection and preparation

After collection of blood samples, the animals were anesthetized using 65 mg/kg pentobarbital sodium [15], decapitated and the soleus muscles were dissected. The soleus muscle of one side was used to assess insulin-dependent glucose uptake and the other side was used to assess adrenomedullin and glucose transporter-4 (GLUT 4) gene expression.

Competitive RT-PCR method for AM and GLUT 4 mRNAs

Total RNA was extracted from 30 mg of muscle tissue by using the single-step, acid guanidium thiocyanate, phenol–chloroform extraction (Promega, Madison, WI, USA) as described [16]. The total RNA concentration in each sample was determined from absorbance at 260 nm, and the quality of each RNA preparation was determined by 1% agarose-formaldehyde gel electrophoresis and ethidium bromide staining. The extracted RNA was reverse transcribed into cDNA using RT-PCR kit (Stratagene, USA). Moloney murine leukemia virus (MMLV); reverse transcriptase was used for synthesis of cDNA from RNA.

The reverse transcription-polymerase chain reaction specific for AM and GLUT 4 was performed as previously described [13,17]; competitive PCR reactions for each cDNA were performed by preparing a 'master mix' containing everything necessary but the primers and competitors. This master mix was allocated to the tubes containing the respective primers and then further subdivided into PCR tubes containing the respective 'competitor mix'. Each PCR tube contained a final volume of 25 μl, consisting of 0.1 μl cDNA, 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$, 200 μM dNTP (200 μM each), 5 μl competitor mix, and 1.25 U platinium thermus aquaticus (*Taq*) polymerase (Life Technologies). The competitor mix consisted of equal amounts of a specific number of the cut competitor plasmids diluted in TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 8) containing 1 ng/ml Salmon Sperm DNA (Sigma, St. Louis, MO, USA). The PCR reactions were performed in a PTC-200 PCR machine (MJ Research, Watertown, MA, USA) as follows: denaturation was performed at 95 °C, annealing at 55 °C for 1 min, and extension was performed at 72 °C for 2 min, with an additional 10-min incubation at 72 °C after completion of the last cycle.

After the PCR, the three PCR products were separated on a 2% agarose gel (BioDO, Analyser, Biometra, Germany) and stained with ethidium bromide. The gel was then photographed under ultraviolet transillumination.

The quantitative assessment of the PCR products was performed with a computerized video analysis system (Image-1/FL, Universal Imaging Corp., West Chester, PA).

A fragment of human AM was amplified by use of AM-specific oligonucleotides (forward) 5′-TTCGAGTCAAACGCTACCGC-3′ and 5′-ATCAGGCGCTCTCCACC-3′ (reverse). Glut 4 forward primer was GACATTTGGCGGAGCCTAAC and the reverse was TAACTCCAGCAGGGTGACACAG. mRNA levels were normalized by the β-actin values in the samples. The β-actin template was made from two primers: β-actin sense 5-TGTTGTCCCTGTATGCCTCT-3 and antisense 5-TAATGTCACGCACGATTTCC-3.

Glucose uptake measurement

After isolation, muscles were incubated in Krebs–Henseleit solution and gassed with carbogen (5% CO_2 and 95% O_2) as previously described [18].

The total volume of the buffer was 100 ml; 100 mg glucose was then added to this volume; insulin (soluble porcine) was added at a concentration of 250 μU per 1 ml buffer.

The pH of the freshly prepared incubation medium was adjusted at 7.4 using pH meter. Then the muscle was transferred into a sample flask with 3 ml of the incubation medium. In each set of experiments, one flask containing only 3 ml of incubation medium with no added tissues was used as control. The flasks were then placed in the metabolic shaker, under a tent of carbogen gas for 1 h at 37 °C with a shaking rate of 100 cycles/min; the continuous shaking alters the layers of incubation medium in contact with the gas phase and the muscle. After incubation, the muscle was immediately dried on filter paper and then weighed.

The glucose level was determined in 1 ml of each sample as well as in 1 ml of the control.

The insulin-stimulated glucose uptake by the muscle was calculated in mg/g of tissue/h of incubation.

$$\text{Glucose uptake} = \frac{(\text{Glucose conc. of control sample} - \text{sample glucose conc. after 1 h}) \times 3\ (\text{medium volume})}{(100 \times \text{weight of muscle in g})}.$$

Statistical methods

The results were analyzed using the SPSS computer software package version 10.0 (Chicago, IL, USA). Data were presented as mean ± SD. Data were evaluated by one-way ANOVA followed by post hoc Kruskal–Wallis and Mann–Whitney tests. Differences of $P < 0.05$ were considered significant.

Results

Effect of AM gene delivery on body weight, serum glucose, serum insulin and HOMA-IR

As revealed in Table 1, at the end of the experiment the diabetic group had significantly increased body weight, plasma

Table 1 Metabolic parameters in the studied groups at the end of the study.

Measured parameters	Control group	Diabetic group	Non-diabetic-AM group	Diabetic-AM group
Body weight (g)	200.63 ± 9.42	257 ± 9.2*	217 ± 10.05*	252.67 ± 16.85[3]
Serum glucose (mmol/l)	3.88 ± 0.9	10.31 ± 2.2*	3.28 ± 0.77	8.45 ± 1.12+,#
Serum insulin (μU/ml)	10.89 ± 1.08	31.92 ± 6.8*	12.09 ± 0.85	22.43 ± 4.32+,#
HOMA-IR	1.84 ± 0.3	15 ± 5.73*	1.76 ± 0.43	8.45 ± 2.08+,#

* Significantly compared to control group.
+ Significantly compared to diabetic group.
# Significantly compared to non-diabetic-AM group.

glucose and insulin levels as well as the HOMA-IR compared to the control group ($P < 0.05$). The non-diabetic-AM group showed a significant increase in the body weight ($P < 0.05$) compared to the control group but without significant differences in serum glucose and insulin levels as well as in the HOMA-IR ($P > 0.05$).

The diabetic-AM group had a highly significantly increased body weight, serum glucose and insulin and HOMA-IR compared to the non-diabetic-AM group ($P < 0.001$). Compared to the diabetic group, the diabetic-AM group showed no statistical significance in body weight ($P > 0.05$), while serum glucose and insulin and HOMA-IR were significantly improved ($P < 0.001$), denoting the beneficial effect of AM gene delivery on insulin resistance.

Effect of AM gene delivery on serum triglycerides and cholesterol

As shown in Table 2, the diabetic group had a highly significantly elevated serum cholesterol ($P < 0.001$) without a significant increase in its serum triglycerides compared to the control group. There were no significant differences in both serum TG and cholesterol between the non-diabetic-AM and the control group ($P > 0.05$), and the diabetic-AM group still had a significantly elevated serum level of cholesterol compared to the non-diabetic-AM group ($P < 0.05$).

AM gene delivery improved serum TG and cholesterol of the diabetic-AM group compared to the diabetic group ($P < 0.05$), highlighting another beneficial effect of AM in type 2 diabetes.

Effect of AM gene delivery on serum corticosterone, leptin and adiponectin

As observed in Table 3, at the end of the study the diabetic and the diabetic-AM groups showed a significant increase of serum corticosterone and leptin levels with a significant decrease in serum adiponectin ($P < 0.05$) compared to the control and non-diabetic-AM groups respectively. No statistical significance was demonstrated in these parameters between the non-diabetic-AM group and the control group; the diabetic-AM group showed a significant decrease in serum corticosterone and leptin and a significant increase in serum adiponectin ($P < 0.05$) compared to the diabetic group, which may contribute to the improvement of the insulin resistance state following AM gene delivery.

Effect of AM gene delivery on muscle glucose uptake and GLUT 4 expression

Fig. 1A and B reveal that the diabetic group muscle adrenomedullin expression compared to the control group was not

Table 2 Serum triglycerides and cholesterol in the studied groups at the end of the study.

Measured parameters	Control group	Diabetic group	Non-diabetic-AM group	Diabetic-AM group
Serum triglycerides (mg/dl)	63.68 ± 4.87	70.2 ± 7.2	58.6 ± 6.35	61.58 ± 4.07+
Serum cholesterol (mg/dl)	129.5 ± 8.53	159.47 ± 16.9*	126.83 ± 5.01	144.8 ± 9.75+,#

* Significantly compared to control group.
+ Significantly compared to diabetic group.
# Significantly compared to non-diabetic-AM group.

Table 3 Serum corticosterone, leptin and adiponectin in the studied groups at the end of the study.

Measured parameters	Control group	Diabetic group	Non-diabetic-AM group	Diabetic-AM group
Serum corticosterone (ng/ml)	250.37 ± 9.28	275.86 ± 11.19*	240.05 ± 7.52	262.88 ± 10.34+,#
Serum leptin (ng/ml)	3.49 ± 0.56	10.63 ± 1.4*	4.22 ± 0.5	7.51 ± 0.57+,#
Serum adiponectin (μg/ml)	3.76 ± 0.23	2.33 ± 0.17*	3.91 ± 0.22	2.95 ± 0.09+,#

* Significantly compared to control group.
+ Significantly compared to diabetic group.
# Significantly compared to non-diabetic-AM group.

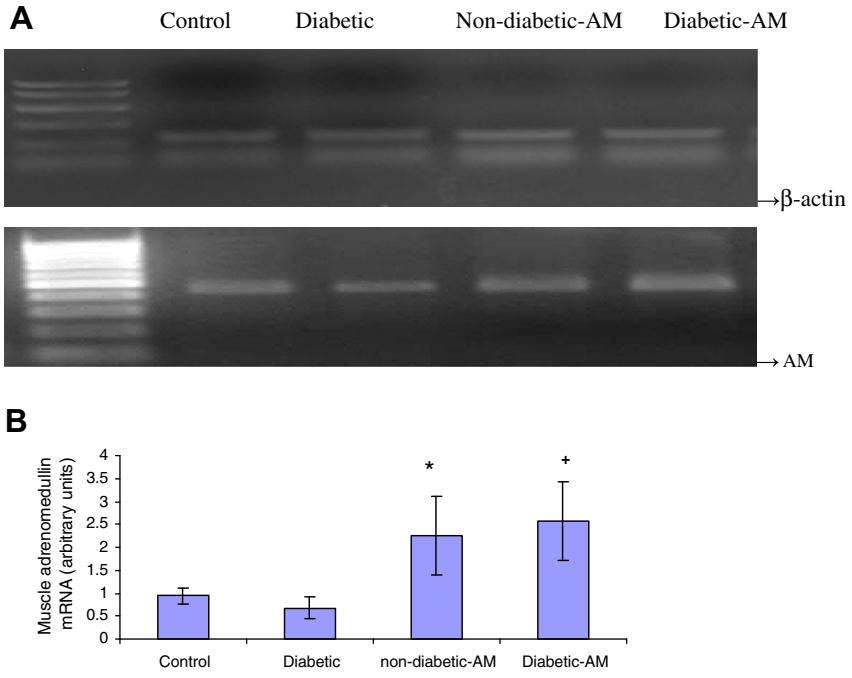

Fig. 1 Soleus muscle adrenomedullin in the different groups. PCR products of adrenomedullin mRNA compared to β-actin (A) and adrenomedullin muscle level in arbitary units (B) in the different studied groups. *Significantly compared to control group. +Significantly compared to diabetic group.

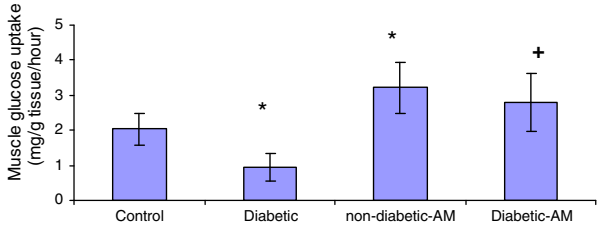

Fig. 2 Soleus muscle glucose uptake in the different studied groups. *Significant compared to control group. +Significantly compared to diabetic group.

significantly different ($P > 0.05$) although its muscle glucose uptake (Fig. 2) and GLUT 4 expression (Fig. 3A and B) were significantly decreased ($P < 0.05$).

The diabetic-AM group showed no statistically significant difference in either muscle adrenomedullin expression or glucose uptake (Figs. 1 and 2) ($P > 0.05$), while it still showed a significant decrease in GLUT 4 expression compared to the non-diabetic-AM group ($P < 0.001$) (Fig. 3).

Meanwhile AM gene delivery significantly increased muscle adrenomedullin expression, glucose uptake and GLUT 4 expression ($P < 0.01$) in the non-diabetic-AM and diabetic-AM groups compared to the control and the diabetic groups respectively.

Discussion

Insulin resistance has received great attention because of its public health importance. Many studies have tried and continue to try to improve it. In this study, AM gene delivery in non-diabetic-AM and in diabetic-AM rats was capable of improving soleus muscle insulin-stimulated glucose uptake and GLUT 4 expression compared to their controls not receiving AM gene delivery. In addition it improved the measures of insulin resistance (plasma glucose, insulin and cholesterol as well as HOMA-IR) in the diabetic group. These findings indicate a promising effect of AM gene delivery in insulin resistance.

Similarly, Dobrzynski et al. [9] observed an increased skeletal muscle membrane-bound GLUT 4, which has improved glucose utilization of AM-treated STZ-diabetic rats. They attributed this effect to AM interaction via the Akt pathway. Furthermore, insulin-stimulated glucose uptake into the isolated skeletal muscle was significantly attenuated in aged AM deficient ($AM^{+/-}$) mice [19].

In the L6 skeletal muscle cell line, adrenomedullin can bind to CGRP receptors, activating adenylate cyclase and cAMP-dependent protein kinases [20]. Nishimatsu et al. [21] reported that AM is capable of directly activating Akt via phosphatidylinositol 3-kinase (PI-3 kinase) in rat aorta. Phosphorylated Akt can also stimulate cellular glucose usage and stimulate GLUT 4 translocation to the membrane in skeletal muscle [22]. These known Akt activities could help explain the observed beneficial effects of AM gene delivery in the STZ-induced diabetic rats in this study.

Previous studies indicated that skeletal muscle-specific inhibition of insulin signaling is adequate to cause insulin resistance [23]. Insulin increases skeletal muscle glucose transport through translocation of the GLUT 4 isoform of the glucose transporter from intracellular sites to the cell surface [24]. There is a good correlation between the GLUT 4 content of a muscle and maximally stimulated glucose uptake [25].

In contrast, Garvey et al. [26] found that the muscle GLUT 4 glucose transporter level was normal in type 2 diabetes, and stated that the insulin resistance is due to impaired

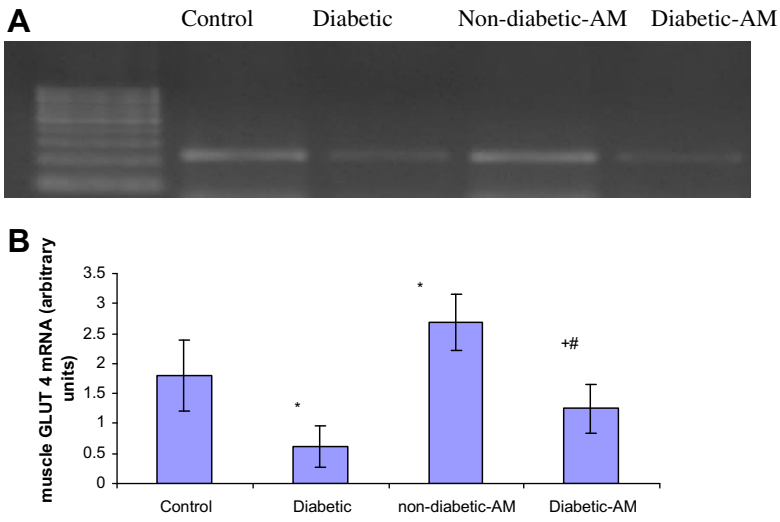

Fig. 3 Soleus muscle GLUT 4 the different groups. PCR products of GLUT 4 mRNA (A) and its muscle level in different groups in arbitary units (B). *Significantly compared to control group. +Significantly compared to diabetic group. #Significantly compared to non-diabetic-AM group.

translocation and trafficking of intracellular GLUT 4 with consequent accumulation of GLUT 4 in a dense membrane compartment from which insulin is unable to recruit GLUT 4 to the cell surface.

Multiple lines of study have shown diabetic patients to have increased oxidative stress. Moreover, *in vitro* study has demonstrated reactive oxygen species (ROS) to impair insulin internalization in endothelial cells [27], block insulin receptor substrate (IRS) phosphorylation, and impair PI-3 kinase activity in hepatocytes [28], and reduce the translocation of GLUT 4 in adipocytes [29].

The AM-ROS axis may play a role in the pathophysiology of insulin resistance. AM has been shown to be up-regulated by ROS [30]. It was also reported that a deficiency of AM induces a higher oxidative stress by stimulating ROS production, but not by impairing the scavenging system that is regulated by AM and that AM not only inhibited ROS production but also had better effect on glucose homeostasis compared to superoxide dismutase mimetic [31]. Since it has been suggested that the production of activated oxygen species has a major role in the development of STZ-induced diabetes [32], the antioxidant effect of AM could be another plausible mechanism by which AM could improve insulin resistance, although ROS were not measured in this work.

In the current study, serum leptin and corticosterone were significantly increased in the STZ-diabetic group compared to the control group while serum adiponectin was significantly decreased. Previous studies reported that diabetes is associated with elevated plasma levels of glucocorticoids. Contributors include: a hyperactive hypothalamic–pituitary–adrenal axis with increasing signaling by hypothalamic CRH and increased adrenal glucocorticoid production [33] and abnormalities in negative feedback regulation by cortisol at the pituitary level due to some metabolic disorders [34]. Increased body weight, glucocorticoids and insulin as well as the high fat diet increase leptin secretion [35] and can explain our finding in the diabetic group. Moreover, the reduction of serum adiponectin, the most abundant adipose-secreted protein, may be related to atrophy of adipocytes and/or other diseases that might be induced by diabetes mellitus [36].

Iemura Inaba et al. [37] suggested that AM may improve glucose intolerance via improving glucose incorporation in adipose tissue. Fukai et al. [5] demonstrated AM expression in adipocytes of obese rats and speculated that the upregulation of AM may contribute to adipokine dysregulation and development of metabolic syndrome; however, these authors did not investigate such a hypothesis.

Interestingly, AM gene delivery in the current study was accompanied by a significant increase in the serum adiponectin level and a decrease in both corticosterone and leptin levels in the diabetic-AM group compared with the non-injected diabetic group, with concomitant improvement in the insulin resistance state.

It is well known that glucocorticoid excess contributes to muscle insulin resistance and reduces glucose uptake by decreasing GLUT 4 translocation to the cell surface [38] and adiponectin can decrease insulin resistance by enhancing fatty acid oxidation and glucose disposal in muscle and liver through the AMP kinase [39]. In addition, adiponectin potentiates the effects of leptin on glucose and lipid oxidation [40] and has an anti-inflammatory effect [41]. Thus reduction of corticosterone and increase in adiponectin can contribute to amelioration of the insulin resistance state seen in the diabetic-AM group.

The effects of AM on adrenal glucocorticoid release are doubtful and probably mediated by the increase in adrenal blood flow rate and the inhibition of ACTH release by pituitary corticotropes [14]. The decrease in both serum insulin and corticosterone in the diabetic-AM group can explain the decrease in leptin level. Also to our knowledge AM has no direct stimulatory effect on adiponectin secretion but may have an indirect effect through decreasing corticosterone, which is known to inhibit adiponectin secretion [42]. Moreover, adiponectin levels increase when insulin sensitivity improves [43].

In the present study, the type 2 diabetic group showed a non-significant difference in soleus muscle adrenomedullin

gene expression although it showed other parameters of insulin resistance together with reduced insulin stimulated muscle glucose uptake and muscle GLUT 4 content compared to the control group.

The role of hyperglycemia in upregulating adrenomedullin is controversial. *In vitro* data suggested that hyperglycemia might increase vascular adrenomedullin expression [44]. However, this notion could not be substantiated *in vivo* [45], and other studies found an increase in circulating AM in patients with type 2 diabetes and attributed this to acute hyperinsulinemia [46] and increased oxidative stress [47].

In this study the AM gene was delivered to diabetic rats. However, many physiological conditions such as exercise [48], moving from low to high altitude [49] and pregnancy in both rats [50] and women [51] can increase plasma adrenomedullin. However, it is difficult to describe the exact mechanisms that regulate adrenomedullin synthesis and secretion *in vivo*.

With respect to the effect of adrenomedullin on insulin secretion, conflicting results have been found. Specifically, Mulder et al. [52] first reported the stimulatory effects of adrenomedullin on insulin secretion from isolated rat islets, while Martinez et al. [53] clearly demonstrated the inhibitory role of adrenomedullin on insulin secretion *in vitro* that might play a role in the homeostasis of pancreatic islets. The vasodilatory effect of adrenomedullin may also have some influence on insulin secretion by elevating the pancreatic perfusion rate, but this remains to be proven. In this study serum insulin in the diabetic group receiving AM was significantly decreased but it still remains to be proven whether this is attributable to improved insulin resistance or to a direct effect of AM on the pancreas.

Conclusions

In this study, we demonstrated that AM may be a potentially useful peptide to counteract the deleterious effects of a diabetic state through its upregulating effect on skeletal muscle GLUT 4, reduction in serum corticosterone and improvement of serum adiponectin and this may stimulate future investigation into the potential therapeutic use of AM in insulin resistance.

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