Endothelin-1 Reduces Glucose Uptake in Human Skeletal Muscle In Vivo and In Vitro

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OBJECTIVE—Endothelin (ET)-1 is a vasoconstrictor and proinflammatory peptide that may interfere with glucose uptake. Our objective was to investigate whether exogenous ET-1 affects glucose uptake in the forearm of individuals with insulin resistance and in cultured human skeletal muscle cells.

RESEARCH DESIGN AND METHODS—Nine male subjects (aged 61 ± 3 years) with insulin resistance (M value <5.5 mg/kg/min or a homeostasis model assessment of insulin resistance index >2.5) participated in a protocol using saline infusion followed by ET-1 infusion (20 pmol/min) for 2 h into the brachial artery. Forearm blood flow (FBF), endothelium-dependent vasodilatation, and endothelium-independent vasodilatation were assessed. Molecular signaling and glucose uptake were determined in cultured skeletal muscle cells.

RESULTS—ET-1 decreased forearm glucose uptake (FGU) by 39% (P < 0.05) after the 2h infusion. ET-1 reduced basal FBF by 36% after the 2-h infusion (P < 0.05) and impaired both endothelium-dependent vasodilatation (P < 0.01) and endothelium-independent vasodilatation (P < 0.05). ETα and ETβ receptor expression was detected on cultured skeletal muscle cells. One-hour ET-1 incubation increased glucose uptake in cells from healthy control subjects but not from type 2 diabetic patients. Incubation with ET-1 for 24 h reduced glucose uptake in cells from healthy subjects. ET-1 decreased insulin-stimulated Akt phosphorylation and increased phosphorylation of insulin receptor substrate-1 serine 636.

CONCLUSIONS—ET-1 not only induces vascular dysfunction but also acutely impairs FGU in individuals with insulin resistance and in skeletal muscle cells from type 2 diabetic subjects. These findings suggest that ET-1 may contribute to the development of insulin resistance in skeletal muscle in humans. Diabetes 60:2061–2067, 2011

Endothelial dysfunction, characterized by reduced bioactivity of nitric oxide (NO) and increased activity of the vasoconstrictor and proinflammatory peptide endothelin (ET-1), is an important factor promoting the development of atherosclerosis (1). Several observations demonstrate that endothelial dysfunction is present in insulin-resistant states, including diabetes, obesity, and the metabolic syndrome (1,2). Insulin exerts important vascular actions via stimulation of NO production in the endothelium, leading to vasodilatation and increased blood flow, which in turn stimulates glucose uptake in skeletal muscle (3). These antitherogenic effects are mediated via activation of the phosphatidylinositol 3-kinase (PI3-kinase) pathway, resulting in phosphorylation of Ser-Thr kinases, such as Akt, as well as activation of endothelial NO synthase (4). Insulin resistance is associated with reduced activation of this pathway in vascular endothelial cells (5) and in skeletal muscle (6). Instead, the mitogenic-signaling pathway mediated by mitogen-activated protein (MAP) kinase (extracellular signal–related kinase [ERK] MAP) is stimulated. In endothelial cells, this change in intracellular signaling results in the stimulation of cell growth, proinflammatory effects, increased production of ET-1, and reduced bioavailability of NO (2,4). These observations indicate that endothelial dysfunction, including increased activity of ET-1, is of functional importance in insulin-resistant states.

The vascular responses to ET-1 are mediated via the two receptor subtypes, ETα and ETβ (7,8). Both types of receptors are located on vascular smooth muscle cells and mediate vasoconstriction. The ETβ receptor also is located on endothelial cells and mediates vasodilatation by stimulating the release of NO and prostacyclin (9). Recent studies suggest that ET-1 inhibits insulin-mediated glucose uptake via a plasma membrane–dependent mechanism. ET-1 impairs insulin-stimulated glucose transporter GLUT4 translocation in adipocytes (10,11) and decreases PI3-kinase activity via insulin receptor substrate (IRS)-2 Ser and Tyr phosphorylation in isolated vascular smooth muscle cells (12). Furthermore, ET-1 reduces peripheral glucose utilization (13) and insulin sensitivity in healthy volunteers (14). Selective ETα receptor blockade was shown to augment insulin-mediated glucose uptake in obese but not lean subjects (15). We have demonstrated that the dual ETα/ETβ receptor blockade acutely increases total body glucose uptake and insulin sensitivity in obese patients with insulin resistance and coronary artery disease (16). These observations suggest that endogenous ET-1 plays a role in the regulation of glucose uptake. However, it still remains unclear whether ET receptors are expressed on skeletal muscle cells and whether ET-1 affects glucose uptake in the skeletal muscle tissue of subjects with insulin resistance.

The current study was therefore designed to investigate the direct effect of ET-1 on skeletal muscle glucose uptake and blood flow in insulin-resistant individuals in vivo. Furthermore, we aimed to identify ET-1 receptors as well as the effects of ET-1 on basal and insulin-stimulated glucose uptake and signaling in human skeletal muscle cells.
TABLE 1
Basal study subject characteristics (n = 9)

| Characteristics              | Value (mean ± SD) |
|------------------------------|-------------------|
| Age (years)                  | 62 ± 7            |
| BMI (kg/m²)                  | 27.8 ± 1.9        |
| M value (mg/kg/min) (n = 6)  | 4.4 ± 1.1         |
| HOMA-IR (units)              | 3.2 ± 0.2         |
| Smokers (n)                  | 1                 |
| Ex-smokers (n)               | 2                 |
| Nonsmokers (n)               | 6                 |
| Hypertension                 | 4                 |
| Prior myocardial infarction  | 1                 |
| ACE inhibitors or angiotensin receptor blockers | 2 |
| β-Blockers                   | 1                 |
| Aspirin                      | 1                 |

Fasting venous plasma insulin (pmol/L) 58.3 ± 7.8
Fasting venous plasma glucose (mmol/L) 5.1 ± 0.2
Total cholesterol (mmol/L) 3.2 ± 0.1
HDL (mmol/L) 1.2 ± 0.2
Triglyceride (mmol/L) 1.5 ± 0.3
HbA1c (%) 4.7 ± 0.1
High-sensitivity C-reactive protein (mg/L) 2.2 ± 0.5
ET-1 (pmol/L) 2.9 ± 0.2

Data are means ± SEM, unless otherwise indicated.

Endothelin-1 and Glucose Uptake

Background and Aims: Endothelin-1 (ET-1) is involved in the regulation of systemic glucose uptake in muscle. In vitro, ET receptor antagonists increase glucose uptake in cultured muscle cells. We aimed to investigate the role of ET receptors in the regulation of glucose uptake in the human forearm in vivo.

Methods: A total of 11 healthy volunteers with type 2 diabetes and 11 normal glucose tolerant subjects underwent a 2-h forearm insulin clamp study with and without ET receptor antagonists. Fasting venous plasma insulin (pmol/L) was collected repeatedly during the study protocol.

Results: Glucose uptake increased by 33% (8.7 ± 1.7 vs. 11.7 ± 2.0 μmol/min; n = 11) during the infusion of the NO donor sodium nitroprusside (SNP; 3 μmol/L) in the absence or presence of the ETA/ETB receptor antagonist bosentan (3 μmol/L). The antagonist was always added 30 min before ET-1.

Conclusions: Our results suggest that the ET system is involved in the regulation of systemic glucose uptake in muscle in vivo.
doses of acetylcholine and SNP were assessed by two-way ANOVA. Changes in FGU and arteriovenous glucose differences were assessed by one-way ANOVA for repeated measurements with the Bonferroni multiple comparison test. Differences in percentage change of phosphorylated proteins were calculated using the Wilcoxon test or the one-sample test. Differences in protein expression were compared by the Mann-Whitney U test test. A value of \( P < 0.05 \) was considered significant.

RESULTS

Study subjects. The basal characteristics of the study subjects receiving ET-1 infusion are summarized in Table 1. The subjects were overweight and had slightly elevated LDL cholesterol. Four of the subjects had hypertension, and one had a history of previous myocardial infarction.

Blood flow. Blood pressure and heart rate did not change significantly during the protocol. During the administration of ET-1, FBF was reduced by 30% \(( P < 0.01)\) at 60 min and by 36% \(( P < 0.05)\) at 120 min of infusion, compared with basal values. Infusion of ET-1 markedly inhibited acetylcholine-induced vasodilatation \(( P < 0.001)\) (Fig. 2A). In addition, the vasodilator response to SNP was slightly but significantly attenuated by ET-1 \(( P < 0.05)\) (Fig. 2B).

FGU. Infusion of NaCl for 30 min did not affect FGU. ET-1 infusion increased the arteriovenous glucose difference by 67% \(( P < 0.05)\) at 90 min, followed by a decrease to basal values at 120 min (Fig. 3A). Infusion of ET-1 decreased FGU from 5.8 ± 2.0 μmol/L/min × 1,000 mL at baseline (saline) to 3.4 ± 0.8 μmol/L/min × 1,000 mL at 120 min \(( P < 0.05)\) (Fig. 3B). During the protocol, arterial insulin concentrations decreased from 81.0 ± 11.0 pmol/L at baseline to 65.7 ± 7.5 pmol/L at 120 min of ET-1 infusion \(( P < 0.05)\). The arteriovenous concentration difference of insulin remained unchanged. There was no correlation between the reductions in arterial insulin concentration and FGU \(( r = 0.05, P = 0.91)\).

ET-1 effects on cultured skeletal muscle glucose uptake. Acute (1 h) exposure to ET-1 increased glucose uptake in skeletal muscle cells established from subjects with NGT (Fig. 4A and B), and this effect persisted at 2 and 6 h \(( P < 0.05)\) (Fig. 4A). At 24 h, ET-1 induced a significant reduction in glucose uptake (Fig. 4A). ET-1 did not further increase insulin-stimulated glucose uptake (Fig. 4B). In contrast, ET-1 or insulin did not increase glucose uptake in cells from subjects with type 2 diabetes (Fig. 4B).

Insulin signaling in cultured skeletal muscle cells. ET-1 decreased insulin-stimulated Akt phosphorylation in a dose-dependent manner (Fig. 5A and B). The inhibitory effect of ET-1 on insulin-induced Akt phosphorylation at both Ser\(^{473}\) \(( P < 0.001)\) (Fig. 5F) and Thr\(^{308}\) \(( P < 0.005)\) (Fig. 5D) sites was blocked by the ET receptor antagonist bosentan (Fig. 5A–D). This inhibitory effect of ET-1 exposure was evident in cells established from both subjects with NGT and subjects with type 2 diabetes (Fig. 5E and E). ET-1 increased phosphorylation of IRS-1 Ser\(^{636}\), an effect that was blocked by bosentan (Fig. 6A). Insulin increased IRS-1 Tyr\(^{612}\) phosphorylation, and ET-1 treatment did not alter this effect of insulin (Fig. 6B). ET-1 also transiently increased phosphorylation of ERK1/2, peaking at 5 min and returning to baseline by 20 min (Fig. 6C). No effect of ET-1 was noted on phosphorylation of AMPK at time points from 3 to 20 min (Fig. 6C) up to 1 h (data not shown).

DISCUSSION

Insulin resistance is associated with endothelial dysfunction and increased plasma levels of ET-1 (20). We recently demonstrated that dual ETA/ETB receptor blockade increases total-body glucose uptake and insulin sensitivity in obese patients with insulin resistance and coronary artery disease (16). This is supported by the observation that...
selective ET\textsubscript{A} receptor blockade increased glucose uptake during a hyperinsulinemic-euglycemic clamp in obese individuals (17). The current study tested the hypothesis that exogenous ET-1 reduces glucose uptake in the skeletal muscle tissue of subjects with insulin resistance. Exogenous infusion of ET-1 resulted in a significant reduction in FGU after 2 h of infusion. However, arteriovenous glucose concentrations were significantly increased during the first 90 min of ET-1 infusion and then returned to basal levels at 2 h. This change in arteriovenous glucose concentrations may be independent of the decrease in blood flow, because similar reductions in FBF were observed at 90 and 120 min of ET-1 infusion (30 and 36%, respectively). This could indicate an initial ET-1–dependent stimulation of glucose uptake into skeletal muscle, which may be obscured by the parallel reduction in blood flow, and thereby the overall FGU was unchanged.

During the study protocol, we observed a reduction in arterial insulin levels by 19%. The underlying cause for this change is unknown, but this could contribute to the reduction in glucose uptake. The lack of constant insulin levels during the experiment is a limitation of the current study. We did not include constant intra-arterial insulin infusion into the study protocol because this is known to increase blood flow in an NO-dependent manner and to stimulate glucose uptake in skeletal muscle (21). We recently demonstrated that infusion of ET-1 in combination with insulin did not affect FGU in the forearm of patients with insulin resistance (22). The reduction in arterial insulin concentrations did not result in a decrease of arteriovenous glucose concentrations below the basal level observed during the initial 30 min of saline infusion. This observation suggests that the moderate fall in insulin levels did not significantly affect local FGU, per se. Furthermore, in the current study, there was no correlation between the reduction in insulin levels and the ~40% reduction noted in FGU. Thus, the reduction in FGU may be related to other factors, such as the direct effects of ET-1 on insulin signaling and blood flow. From the present data, it cannot be determined whether the effect of ET-1 on FGU in vivo is the result of a direct effect on skeletal muscle cells or secondary to the reduction in blood flow.

To gain additional insight into the complex nature of these changes in vivo, we examined the effect of ET-1 in cultured human skeletal muscle, giving us an opportunity to determine any direct and flow-independent effects of ET-1 on glucose uptake. In skeletal muscle cells from healthy subjects with NGT, acute ET-1 exposure (up to a 6-h exposure) increased glucose uptake in agreement with previous observations in rodent muscle (23). At 24 h, however, ET-1 significantly reduced glucose uptake. This implies that prolonged exposure to ET-1 reduces glucose uptake, which is in agreement with the present in vivo findings and our previous observations that the ET receptor blockade increased basal glucose uptake in insulin-resistant individuals in vivo (22) when sustained increased levels of ET-1 have been noted. Twenty-four-hour ET-1 exposure reduced both basal and insulin-stimulated glucose uptake in cultured muscle cells while maintaining an insulin response, suggesting that at this time point there is an overall reduction in basal glucose transport (22). Of interest, ET-1 did not affect glucose uptake in cells cultured from individuals with type 2 diabetes, indicating a quantitative difference regarding the effect of ET-1 on glucose metabolism in insulin-sensitive and insulin-resistant states. We observed that both ET\textsubscript{A} and ET\textsubscript{B} receptors are expressed in skeletal muscle cells, suggesting a direct effect of ET-1 that could be mediated by both subtypes of receptor.

We also aimed to dissect molecular signaling events underlying the ET-1 effects on glucose uptake. ET-1 decreased insulin-stimulated Akt phosphorylation in a dose-dependent manner. This finding is supported by previous observations in rat skeletal muscle and vascular smooth muscle cells, suggesting that ET-1 inhibits activation of the PI3-kinase pathway and insulin-stimulated Akt phosphorylation (12,23). In primary human skeletal muscle cells, Akt Ser\textsuperscript{473} phosphorylation primarily is a reflection of phosphorylation on Akt2, whereas Thr\textsuperscript{308} represents both Akt1 and Akt2 (24). Because ET-1 exerts similar effects on both Akt phosphorylation sites, this implies that the effect of ET-1 to reduce insulin-stimulated Akt phosphorylation is evident for both Akt1/Akt2, with Akt2 making the most significant contribution. ET-1 increased in IRS-1 Ser\textsuperscript{636} phosphorylation, which could be prevented by the ET receptor antagonist bosentan. Phosphorylation of IRS-1 on Ser\textsuperscript{636} is a negative signal, which acts as a negative-feedback control mechanism decreasing IRS-1 tyrosine phosphorylation after prolonged insulin exposure (25). Insulin also induces phosphorylation of IRS-1 at several different tyrosine sites (25), with Tyr\textsuperscript{612} being important for subsequent activation of PI3-kinase and activation of

FIG. 4. A: Glucose uptake in skeletal muscle cells at the basal state and after 1-, 2-, 6-, and 24-h treatment with 10 nmol/L ET-1. *P < 0.05; **P < 0.01. Data are means ± SEM (n = 3–5). B: Basal and insulin-stimulated glucose uptake in cells established from subjects with NGT (n = 11) and subjects with type 2 diabetes (T2DM) (n = 11) under basal conditions and after a 1-h ET-1 treatment. *P < 0.05 by Student t test. Data are means ± SEM.
Akt. However, ET-1 treatment had no effect on insulin-induced IRS-1 Tyr612 phosphorylation, suggesting that the ET-1–mediated impairment noted on Akt phosphorylation and glucose transport is independent of PI3-kinase activation. This is in agreement with results in adipocytes, where ET-induced impairment in glucose transport has been shown to be mediated via PI3-kinase–independent mechanisms (11,26). Furthermore, in skeletal muscle cells, a reduction in glucose uptake was only evident after longer ET-1 exposure (24 h) in cells derived from subjects with NGT. Thus, although reduced Akt phosphorylation may contribute to the observed reduction in glucose uptake, it is likely that deregulation of additional pathways are required for ET-induced impairment in skeletal muscle glucose uptake.

We hypothesized that the acute ET-1–mediated increase in glucose uptake noted in cultured skeletal muscle would be a result of a transient stress response because ET-1 alone did not increase Akt phosphorylation and thus is unlikely to use an insulin-like signaling cascade. However, ET-1 exposure of skeletal muscle to ET-1 did not increase phosphorylation of AMPK, and the molecular signal mediating the increased glucose uptake remains to be determined. Acute exposure of skeletal muscle cells to ET-1 increased...
phosphorylation of ERK MAP kinase, which is known to be associated with cell proliferation and migration as well as excessive formation of reactive oxygen species (27).

In the current study, we show that ET-1 impairs both endothelium-dependent and endothelium-independent vasodilatation in subjects with insulin resistance. This result extends our previous findings that exogenous ET-1 markedly reduces endothelium-dependent vasodilatation in healthy subjects (18). This effect of ET-1 is independent of its vasoconstrictor effects (18). Furthermore, ET receptor blockade acutely improves endothelium-dependent vasodilatation in subjects with insulin resistance (28) and atherosclerosis (18,29). Altogether, these observations suggest that elevated levels of ET-1, in addition to alterations in glucose uptake, contribute to endothelial dysfunction in subjects with insulin resistance.

FIG. 6. A: Basal and insulin-stimulated expression of phosphorylated IRS-1 at the Ser<sup>636</sup> site, under control conditions and after a 1-h exposure to ET-1 in the absence and presence of the ET receptor antagonist bosentan (3 μmol/L). *P < 0.05 by Mann-Whitney U test. Data are means ± SEM (n = 10). B: Basal and insulin-stimulated expression of phosphorylated IRS-1 at the Tyr<sup>612</sup> site, under control conditions and after a 1-h exposure to ET-1 in the absence and presence of the ET receptor antagonist bosentan (3 μmol/L). Data are means ± SEM (n = 8). C: Phosphorylation of ERK1/2 and AMPK under control conditions and 3, 5, 10, and 20 min of ET-1 (10 nmol/L) treatment. Total expression of actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control.

FIG. 7. Western blot analysis of ETA (A) and ETB (B) receptor expression in cells established from subjects with NGT (n = 8) and subjects with type 2 diabetes (T2DM) (n = 8). Data are means ± SEM.
In conclusion, the current study demonstrates that prolonged exposure to ET-1 impairs glucose uptake both in vivo and in cultured skeletal muscle cells. In cultured human muscle established from healthy subjects, ET-1 induces a biphasic response, with an initial stimulation, followed by a reduction, in glucose uptake. ET-1 does not elicit an increase in glucose uptake in muscle cells established from type 2 diabetic subjects. Furthermore, ET-1 interferes with insulin signaling in cultured skeletal muscle cells, reducing Akt phosphorylation and increasing phosphorylation of IRS-1 Ser636. These observations indicate that ET-1 signaling may exacerbate metabolic dysregulation in insulin resistance.

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A.S. and F.S. wrote the manuscript, researched the data, and contributed to the discussion. D.E.D.-G. researched the data. F.B., T.G., J.P., and A.K. contributed to the discussion and reviewed and edited the manuscript. E.R. researched the data and contributed to the discussion.

REFERENCES

1. Bönh F, Pernow J. The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. Cardiovasc Res 2007;76:8–18
2. Jansson PA. Endothelial dysfunction in insulin resistance and type 2 diabetes. J Intern Med 2007;262:173–183
3. Vincent MA, Montagnani M, Quon MJ. Molecular and physiologic actions of insulin related to production of nitric oxide in vascular endothelium. Curr Diab Rep 2003;3:279–288
4. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. Endocr Rev 2007;28:463–491
5. Zeng G, Nyström FH, Ravichandran LV, et al. Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. Circulation 2000;101:1539–1545
6. Krook A, Björnholm M, Galuska D, et al. Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. Diabetes 2000;49:284–292
7. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. Nature 1999;348:730–732
8. Sakurai T, Yanagisawa M, Takaku Y, et al. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature 1990;348:732–735
9. de Nucci G, Thomas R, D’Orleans-Juste P, et al. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostanoids and endothelium-derived relaxing factor. Proc Natl Acad Sci USA 1988;85:9797–9800
10. strawbridge AB, Elmdorfd JS. Phosphatidylinositol 4,5-bisphosphate reverses endothelin-1-induced insulin resistance via an actin-dependent mechanism. Diabetes 2005;54:1608–1705
11. Arai H, Zhou QL, Chatterjee A, et al. Endothelin-1 modulates insulin signaling through phosphatidylinositol 3-kinase pathway in vascular smooth muscle cells. Diabetes 1999;48:1120–1130
12. Alhborg G, Weitzberg E, Lundberg JM. Endothelin-1 infusion reduces splanchic glucose production in humans. J Appl Physiol 1994;77:121–126
13. Ottoisson-Seeberger A, Lundberg JM, Alvestrand A, Alhborg G, Exoginores endothelin-1 causes peripheral insulin resistance in healthy humans. Acta Physiol Scand 1997;161:211–220
14. Leof A, Vaishnava P, Baron AD, Mather KJ. Endothelin limits insulin action in obese/insulin-resistant humans. Diabetes 2007;56:728–734
15. Alhborg G, Shemyakin A, Bönh F, Gonon A, Pernow J. Dual endothelin receptor blockade acutely improves insulin sensitivity in obese patients with insulin resistance and coronary artery disease. Diabetes Care 2007;30:591–596
16. Rabinowitz D, Zierler KL. Forearm metabolism in obesity and its response to intra-arterial insulin: evidence for adaptive hyperinsulinaemia. Lancet 1961;2:690–692
17. Bönh F, Alhborg G, Pernow J. Endothelin-1 inhibits endothelin-dependent vasodilatation in the human forearm: reversal by ETA receptor blockade in patients with atherosclerosis. Clin Sci (Lond) 2005;109:321–327
18. Al-Khalili L, Chibalin AV, Kannisto K, et al. Insulin action in cultured human skeletal muscle cells during differentiation: assessment of cell surface GLUT4 and GLUT1 content. Cell Mol Life Sci 2003;60:991–998
19. Wheatcroft SB, Williams II, Shah AM, Kearney MT. Pathophysiological implications of insulin resistance on vascular endothelial function. Diabet Med 2003;20:255–268
20. Vincent MA, Clerk LH, Lindner JR, et al. Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake in vivo. Diabetes 2004;53:1418–1423
21. Shemyakin A, Salehzadeh F, Bönh F, et al. Regulation of glucose uptake by endothelin-1 in human skeletal muscle in vivo and in vitro. J Clin Endocrinol Metab 2010;95:2359–2366
22. Wilkes JJ, Hevera A, Olefsky J. Chronic endothelin-1 treatment leads to insulin resistance in vivo. Diabetes 2003;52:1804–1809
23. Bouzakri K, Zachrisson A, Al-Khalili L, et al. siRNA-based gene silencing reveals specialized roles of IRS-1/Akt2 and IRS-2/Akt1 in glucose and lipid metabolism in human skeletal muscle. Cell Metab 2006;4:389–96
24. Gual P, Le Marchand-Brustel Y, Tanti J-F. Positive and negative regulation of IRS1/Akt signal in insulin resistance and type 2 diabetes. J Biomed Biotechnol 2006;2006:2006
25. Elmendorf JS. Signals that regulate GLUT4 translocation. J Membr Biol 2010;236:250–261
26. Avogaro A, de Kreutzenberg SV, Fadini GP. Oxidative stress and vascular disease in diabetes: is the dichotomization of insulin signaling still valid? Free Radic Biol Med 2008;44:1209–1215
27. Shemyakin A, Bönh F, Wagner H, Efendic S, Bävenholm P, Pernow J. Enhanced endothelin-dependent vasodilatation by dual endothelin receptor blockade in individuals with insulin resistance. J Cardiovasc Pharmacol 2006;47:385–390
28. Bönh F, Beltran E, Pernow J. Endothelin receptor blockade improves endothelial function in atherosclerotic patients on angiotensin converting enzyme inhibition. J Intern Med 2005;257:263–271