Modulation of glucose uptake in adipose tissue by nitric oxide-generating compounds

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There is increasing evidence that endogenous nitric oxide (NO) influences adipogenesis, lipolysis and insulin-stimulated glucose uptake. We investigated the effect of NO released from S-nitrosoglutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP) on basal and insulin-stimulated glucose uptake in adipocytes of normoglycaemic and streptozotocin (STZ)-induced diabetic rats. GSNO and SNAP at 0.2, 0.5, and 1 mM brought about a concentration-dependent increase in basal and insulin-stimulated 2-deoxyglucose uptake in adipocytes of normoglycaemic and STZ-induced diabetic rats. SNAP at 1.0 mM significantly elevated basal 2-deoxyglucose uptake ($115.8 \pm 10.4\%$) compared with GSNO at the same concentration ($116.1 \pm 9.4\%$; $P < 0.05$) in STZ-induced diabetic rats. Conversely, SNAP at concentrations of 10 mM and 20 mM significantly decreased basal 2-deoxyglucose uptake by $50.0 \pm 4.5\%$ and $61.5 \pm 7.2\%$ respectively in adipocytes of STZ-induced diabetic rats ($P < 0.05$). GSNO at concentrations of 10 mM and 20 mM also significantly decreased basal 2-deoxyglucose uptake by $50.8 \pm 6.4\%$ and $55.2 \pm 7.8\%$ respectively in adipocytes of STZ-induced diabetic rats ($P < 0.05$). These observations indicate that NO released from GSNO and SNAP at 1 mM or less stimulates basal and insulin-stimulated glucose uptake, and at concentrations of 10 mM and 20 mM inhibits basal glucose uptake. The additive effect of GSNO or SNAP, and insulin observed in this study could be due to different mechanisms and warrants further investigation.
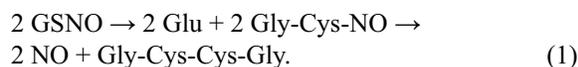
1. Introduction

S-nitrosothiols (RS-N=O or RSNOs) are an important class of nitric oxide (NO)-donor drugs. S-nitrosoglutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP) are two RSNOs which are commonly used sources of NO in the biomedical field (Williams 1985). GSNO possesses potent anti-platelet aggregatory activity and has a half-life of 2.7 h (Matthew and Kerr 1993). The decomposition of GSNO *in vivo* depends upon the extracellular milieu where metal ion-dependent breakdown and *trans*-nitrosation reactions are

possible. The enzyme γ-glutamyl transpeptidase enhances the decomposition of GSNO forming S-nitrosocysteinylglycine and glutamate (Singh *et al* 1996). S-nitrosocysteinylglycine (Gly-Cys-NO) is more susceptible to transition metal ion-dependent decomposition than GSNO with its subsequent breakdown to release NO (equation 1; Gordge *et al* 1998). The therapeutic potential of SNAP is limited by the rapid and unpredictable nature of its decomposition due to the catalytic effect of trace Cu^+ ions formed by the reduction of Cu^{2+} by thiols or ascorbate (Askew *et al* 1995). The half-life of SNAP is 1.15 h (Matthew and Kerr 1993) and

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decomposition of RSNOs like SNAP involves the homolytic cleavage of the S-NO bond with the release of NO and the formation of an alkyl thiyl radical followed by the combination of two thiyl radicals to give the corresponding disulphide (RS-SR; Al-Sa'doni and Ferro 2000).

$$2 \text{ GSNO} \rightarrow 2 \text{ Glu} + 2 \text{ Gly-Cys-NO} \rightarrow$$
$$2 \text{ NO} + \text{Gly-Cys-Cys-Gly}. \quad (1)$$

$$2 \text{ RSNO} \rightarrow 2 \text{ NO} + \text{RS-SR}. \quad (2)$$

Adipose tissue is the largest reservoir of lipids in the body. Nitric oxide (NO) is a key signal molecule that is found in practically all tissues including adipose tissue (Moncada and Higgs 1993). Endothelial and inducible NO synthase (eNOS and iNOS) have been demonstrated in white adipose tissue of rat (Ribiere *et al* 1996) indicating that this tissue is a potential source of NO production. Nitric oxide is involved in adipose tissue biology by influenc-ing adipogenesis and insulin-stimulated glucose uptake. *In vitro*, exogenous NO inhibits cell proliferation and stimulates the expression of two adipogenic marker genes, peroxisome proliferator-activated receptor *γ* and uncoupling protein-1, in rat brown pre-adipocytes (Nisoli *et al* 1998). *In vivo*, NOS blockade reduces insulin-mediated glucose uptake in both brown and white adipose tissues (Roy *et al* 1998).

In spite of the involvement of NO in adipocyte metabolic signalling and the presence of isoforms of NOS in adipocytes, the role of NO in adipose tissue is still ambiguous. Recently, Tanaka *et al* (2003) reported that NO released from sodium nitroprusside (SNP) stimulates glucose transport through insulin-independent glucose transporter-4 (GLUT-4) translocation in 3T3-L1 adipocytes. However, the effects of other NO donors on glucose uptake in adipose tissues have not been extensively studied. We hypothesized that increased NO generated from its donors may alter basal and/or insulin-stimulated glucose uptake in adipose tissues of both normoglycaemic and diabetic rats. To test this hypothesis, we investigated the effect of GSNO and SNAP on basal and insulin-stimulated glucose uptake in isolated adipocytes.

2. Materials and methods

2.1 *Animals–Experimental design*

Sixteen Sprague-Dawley rats (8 males and 8 females) were obtained from the Preclinical Animal House of the Department of Basic Medical Sciences, University of the West Indies. The animals were housed in an animal room and fed standard laboratory diet and water *ad libitum*. All procedures were approved by and conducted in accordance with the guidelines of the University of the West Indies

Animal Care and Use Committee. All experiments were conducted when the rats were 10–12 weeks of age after an overnight period of food restriction of 12 h.

The rats were divided into two groups, experimental (4 males and 4 females) and control (4 males and 4 females). Diabetes was induced in the experimental group of Sprague-Dawley rats as previously described (Masiello *et al* 1998). Briefly, nicotinamide (Sigma Chemical Co., St. Louis, MO, USA; 180 mg/kg body weight) dissolved in 0.2 ml of saline was administered intraperitoneally 15 min before an intravenous administration of streptozotocin (STZ) (Sigma; 65 mg/kg body weight), also dissolved in 0.2 ml of saline. The normoglycaemic (control) group received 0.2 ml of saline. Blood from the tail vein was tested for glucose concentration once a week for 4 weeks using a glucometer (Miles Inc. Diagnostics Division, Indiana, USA). At the end of 4 weeks, the rats were weighed and an oral glucose tolerance test administered, using 1.75 mg of glucose/kg of body weight. Diabetic (determined based on values from WHO; World Health Organization 1999) and control rats were euthanized using diethyl ether in accordance with the University of West Indies ethical committee standards.

GSNO was prepared by reacting reduced glutathione with sodium nitrite under acidic conditions at 0°C, followed by the addition of cold acetone (Hart 1985). The resulting precipitate was filtered off, washed, and dried under laboratory conditions to give the light-pink solid GSNO, with similar ultraviolet and visible absorption maxima and elemental composition as reported (Hart 1985). SNAP was synthesized as previously described (Field 1978).

2.2 *Adipocytes isolation and glucose uptake studies*

Glucose transport in isolated adipocytes was measured by the use of radiolabelled 2-deoxyglucose (2-deoxy-D-[1,2-^{3}H(N)]glucose) as described previously (Shepherd *et al* 1993). Subcutaneous fat pads were excised from male and female rats, weighed, and digested by collagenase (1 mg/ml) at 37°C for 30 min with constant shaking (100 rpm). Digestion was performed using glucose- and insulin-free oxygenated Krebs-Ringer bicarbonate (KRB) buffer supplemented with 30 mM N-(2-hydroxyethyl)-piperazine-N′-ethanesulphonic acid (HEPES), 2.5% bovine serum albumin (BSA) [Radioimmunoassay (RIA) grade] and 200 nM adenosine. After digestion, isolated cells were separated from connective tissue by filtration through a fine nylon mesh, washed four times, and re-suspended in the same buffer.

The isolated adipocytes (300 μl/1.5 ml eppendorf tube) were incubated with or without various concentrations (0.2 mM, 0.5 mM, 1.0 mM, 10 mM and 20 mM) of SNAP or GSNO for 15 min at 30°C in oxygenated KRB buffer,

followed by treatment with or without insulin (100 nM) for 30 min. Adipocytes were then incubated for 30 min at 30°C in KRB buffer containing 8 mM 2-deoxy-D-[1,2-³H(N)]glucose (2.25 μCi/ml); 32 mM [¹⁴C] mannitol (0.3 μCi/ml); and 1% BSA. Glucose uptake was stopped by spinning the suspension through dinonyl phthalate oil. Cells were lysed in 1 ml of lysis buffer containing 0.1% Triton X-100 for 45 min. Aliquots of the cell lysates were used for liquid scintillation counting in a Beckman LS6000 scintillation counter programmed for dual-channel counting.

2.3 *Statistical analysis*

All results shown in the figures are expressed as means ± SEM. Analysis of the data was done using the Sigma Plot and Sigma Statistics software packages (Jandel Scientific). To evaluate the effects of SNAP and GSNO on basal and insulin-stimulated glucose uptake in adipocytes, values of each group were compared by either a paired Student's *t* test or two-way analysis of variance (ANOVA) followed by the Benferroni multiple comparison test (Clark and Yang 1986). *P* values less than 0.05 were considered to be significant in all cases.

3. **Results**

3.1 *Blood glucose and plasma insulin*

Table 1 gives a summary of the body weights, blood glucose and plasma insulin concentrations of the rats used in the study. The body weights of the control rats before treatment with saline was 170.82 ± 15.83 g while that after treatment was 251.51 ± 21.00 g. Conversely, the body weights of the rats before treatment with STZ and nicotinamide was 238.79 ± 10.03 g while that after treatment was 234.74 ± 7.34 g. There was a significant difference in the body weights of the rats before treatment with either saline or STZ and nicotinamide in both groups. However, there was no difference in the body weights of the rats before and after treatment with saline in the control group, or before and after treatment with STZ and nicotinamide in the experimental group. Fasting plasma insulin concentration in STZ-induced diabetic rats was not significantly different from that in control rats. However, the postprandial plasma insulin concentration in STZ-induced diabetic rats was approximately half that of the control rats (*P* < 0.05).

An abnormal glucose tolerance curve was obtained for the STZ-induced diabetic rats compared to the normoglycaemic rats (figure 1). After administration of the oral glucose load, the blood glucose concentration increased to a maximum

**Table 1.** Summary of the characteristics of the rats used in the study.

	Controls	Diabetic
Body Weights (g)		
Before treatment with nicotinamide and streptozotocin in saline, or saline only	170.82 ± 15.83	238.79 ± 10.03
After treatment with nicotinamide and streptozotocin in saline, or saline only	251.51 ± 21.00	234.74 ± 7.34
Plasma insulin concentration (μIU/ml)		
Postprandial	249.00 ± 7.56	119.83 ± 0.62*
Fasting	120.75 ± 7.07	112.25 ± 4.50

Results are expressed as means ± SEM of eight or more separate experiments with eight rats in each group. Statistical significant difference between the values in the STZ-induced diabetic group and the control group is shown as *P* < 0.05. Rats in the control group were administered with saline while rats in the experimental group were treated with STZ and nicotinamide.

of 19.00 ± 0.90 mmol/l in the STZ-induced diabetic rats compared to 6.74 ± 0.46 mmol/l in the normoglycaemic rats at 1.0 h. In addition the increase in blood glucose concentration is faster in the STZ-induced diabetic rats as at 0.5 h there is already an increase of about 80% of the maximum which is reached at about 1.0 h, while in the controls it is only about 40% of the maximum which is reached much later (1.5 h). After 2 h, the blood glucose approached fasting levels in the controls, but remained persistently high in the STZ-induced diabetic rats (*P* < 0.05).

3.2 *Effect of GSNO on glucose uptake*

Figures 2 and 3 show that GSNO induced a concentration-dependent increase in basal 2-deoxyglucose uptake in adipocytes of normoglycaemic and STZ-induced diabetic rats respectively. GSNO significantly elevated 2-deoxyglucose uptake above the basal level at 0.5 mM (44.8 ± 5.7%) and 1.0 mM (55.2 ± 7.1%) in normoglycaemic rats (*P* < 0.05; figure 2). The elevation of 2-deoxyglucose uptake above basal level was greater in adipocytes of STZ-induced diabetic rats (figure 3) with significant effect at 0.5 mM (97.4 ± 7.3%) and 1.0 mM (116.1 ± 9.4%; *P* < 0.05). GSNO at 1.0 mM increased insulin-stimulated 2-deoxyglucose uptake above that of insulin only by 62.2 ± 5.0 % in normoglycaemic rats (*P* < 0.05). Incubation of
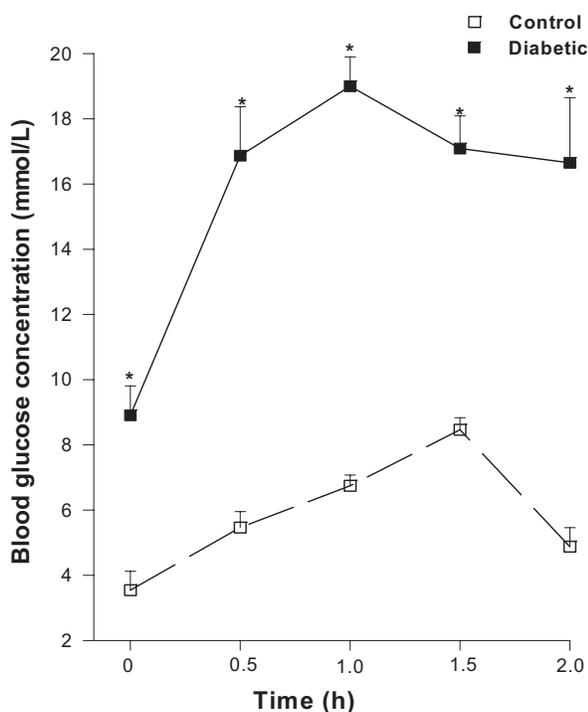
Figure 1. Oral glucose tolerance curve showing the effect of STZ on blood glucose concentrations in rats treated with 65 mg/kg of STZ and 180 mg/kg of nicotinamide, and controls administered with saline.

Values are expressed as Mean ± SEM of eight values from separate experiments. Statistical significance differences between values of the STZ-induced diabetic group and the control group is shown by *$P < 0.05$.

adipocytes of normoglycaemic rats with 10 and 20 mM of GSNO resulted in reduction of 2-deoxyglucose uptake by 50.8 ± 6.4% and 55.2 ± 7.8% respectively compared with basal uptake in the absence of GSNO ($P < 0.05$; figure 2). Similar results of 50.0 ± 6.5% (10 mM of GSNO) and 61.50 ± 7.4% (20 mM of GSNO) were observed in adipocytes of STZ-induced diabetic rats ($P < 0.05$; figure 3).

Figure 2 shows that GSNO (1 mM) and insulin (100 nM) elevated 2-deoxyglucose uptake (1.35 ± 0.15 pmol/mg/min; $P < 0.05$ and 1.05 ± 0.58 pmol/mg/min respectively) compared to that in the absence of either agent (0.87 ± 0.12 pmol/mg/min) in adipocytes of normoglycaemic rats (figure 2). When used in combination at these concentrations with one another, there was an additive effect on the stimulation of 2-deoxyglucose uptake (1.70 ± 0.37 pmol/mg/min; $P < 0.05$).

### 3.3 *Effect of SNAP on glucose uptake*

Figures 4 and 5 show that SNAP induced a concen-tration-dependent elevation of basal 2-deoxyglucose uptake

in adipocytes of normoglycaemic and STZ-induced dia-betes rats respectively. SNAP significantly elevated 2-deoxyglucose above the basal level at 1.0 mM (60.5 ± 6.3%) in normoglycaemic rats ($P < 0.05$; figure 4). The elevation of 2-deoxyglucose uptake by SNAP over basal levels was more pronounced in adipocytes of STZ-induced diabetic rats with values of 97.4 ± 8.4% and 115.8 ± 10.4% at 0.5 mM and 1.0 mM respectively ($P < 0.05$; figure 5). SNAP at 1.0 mM significantly increased insulin-stimulated 2-deoxyglucose uptake by 42.9 ± 5.6% in normoglycaemic rats, and by 233.0 ± 14.6% in STZ-induced diabetic rats ($P < 0.05$). SNAP at 20 mM significantly decreased basal 2-deoxyglucose uptake by 54.7 ± 9.1% ($P < 0.05$) in adipocytes of normoglycaemic rats (figure 4). The reduction in basal 2-deoxyglucose was more pronounced in adipocytes of STZ-induced diabetic rats with values of 50.0 ± 4.5% (10 mM of SNAP) and 61.5 ± 7.2% (20 mM of SNAP) ($P < 0.05$; figure 5).

Figure 4 shows that SNAP (1 mM) and insulin (100 nM) elevated 2-deoxyglucose uptake (1.38 ± 0.18 pmol/mg/min; 1.16 ± 0.15 pmol/mg/min respectively) compared to that in the absence of either agent (0.86 ± 0.20 pmol/mg/min) in adipocytes of normoglycaemic rats. When used in combination at these concentrations with one another, there was an additive effect on the stimulation of 2-deoxyglucose uptake (1.79 ± 0.17 pmol/mg/min; $P < 0.05$). Similarly results of 0.98 ± 0.17 pmol/mg/min (1 mM of SNAP; $P < 0.05$), 0.53 ± 0.08 pmol/mg/min (100 nM of insulin) and 0.42 ± 0.07 pmol/mg/min (in the absence of either agent) was observed in adipocytes of STZ-induced diabetic rats (figure 5). The 2-deoxyglucose uptake in adipocytes treated with 1 mM of SNAP and 100 nM of insulin was 1.77 ± 0.20 pmol/mg/min ($P < 0.05$).

4. Discussion

The study reveals that SNAP and GSNO at concentrations of 1 mM or lower stimulate basal 2-deoxyglucose uptake in adipocytes obtained from normoglycaemic and STZ-induced diabetic rats, and this effect appears to be additive to the stimulation produced by insulin in adipocytes from both types of rats. Higher concentrations GSNO (10 mM, 20 mM) and SNAP (10 mM and 20 mM) induced significant inhibition of basal glucose uptake in normoglycaemic and STZ-induced diabetic rats and to a lesser extent insulin-stimulated glucose uptake. If we interpret the actions of SNAP and GSNO in terms of the NO released on account of their application, as is plausible, these observations indicate dual, concentration-dependent, effects of exogenous NO on glucose uptake in rat adipocytes. Similar effects of NO have been observed in other tissues as in the *β*-cells of the pancreas where endogenous NO plays a role as a physiological mediator and is also involved in pathophysiological processes (Spinas 1999).
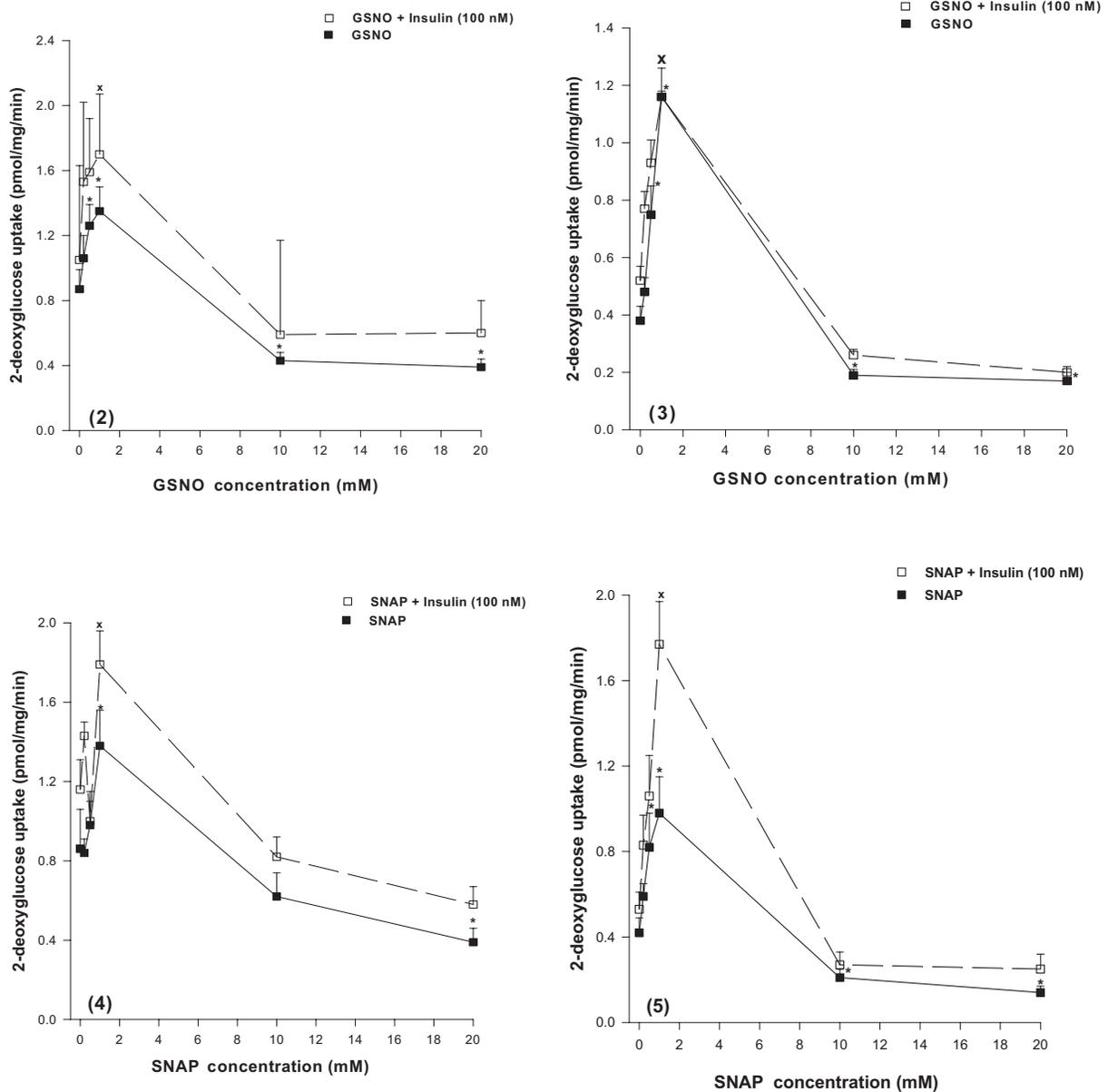
Figures 2–5. Effects of GSNO (**2**, **3**) and SNAP (**4**, **5**) on basal and insulin-stimulated glucose uptake in adipocytes of normoglycaemic rats (**2**, **3**) and STZ-induced diabetic rats (**4**, **5**).

Values are expressed as Mean ± SEM of eight values from separate experiments. Statistical significance differences are shown between values of 2-deoxyglucose uptake at different concentrations of GSNO (**2**, **3**) and SNAP (**4**, **5**) compared with basal level (*$P < 0.05$), and between different concentrations of GSNO (**2**, **3**) and SNAP (**4**, **5**) and insulin compared with insulin only ($^{x}P < 0.05$).

SNAP and GSNO at 1 mM or less appear to exert their effects via the mediation of NO in the stimulation of basal 2-deoxyglucose uptake in normoglycaemic and STZ-induced diabetic rats. The study showed that SNAP (1 mM) or GSNO (1 mM) when used alone significantly elevated 2-deoxyglucose uptake ($P < 0.05$) while insulin (100 nM) elevated 2-deoxyglucose uptake but not at a significant level. When adipocytes from normoglycaemic rats were treated with GSNO (1 mM) and insulin (100 nM) in normoglycaemic rats, and those from normoglycaemic and STZ-induced diabetes rats treated with SNAP (1 mM) and insulin (100 nM) there was an additive effect on the stimulation of 2-deoxyglucose uptake at a significant level as seen in figures 2, 4 and 5. This supports the hypothesis

that separate signalling pathways regulate basal and insulin-signalling glucose uptake. The mechanism of NO stimulation of glucose uptake may involve an insulin-independent pathway and the involvement of GLUT-4 translocation (Tanaka *et al* 2003). Gyanylate cyclase have been found to be involved in the insulin-independent pathway as experiments using the guanylate cyclase inhibitor LY83583 resulted in significant reduction of SNP-stimulated 2-deoxyglucose uptake (Tanaka *et al* 2003). The NO-guanylate cyclase pathway partially contributes to AMP-activated protein kinase (AMPK) in heart muscle (Li *et al* 2004). The catalytic alpha subunit (α1) of AMPK is expressed in 3T3-L1 adipocytes. There is evidence that NO-stimulated glucose uptake is associated with the activation of α1 AMPK in skeletal muscle (Higaki *et al* 2001) and this mechanism may occur in adipocytes. In the same study by Tanaka and colleagues, NO from SNP stimulated basal glucose uptake in 3T3-L1 adipocytes and this effect appears to be additive to the stimulation produced by insulin. Insulin was found to induce GLUT-4 translocation and thus glucose uptake through phosphorylation of IRS-1 or Akt in 3T3-L1 adipocytes. However, NO did not induce phosphorylation of IRS-1 and Akt during the stimulation of glucose uptake (Tanaka *et al* 2003), suggesting that the insulin receptor/Akt pathway is not involved in NO function. The results of our studies are in consonance with that of Tanaka and colleagues. However, further investigation is required to determine the exact mechanism.

It was observed that SNAP and GSNO at 10 mM and 20 mM significantly inhibited basal glucose uptake in STZ-induced diabetic rats and normoglycaemic rats. The detailed mechanism of NO-induced inhibition of glucose uptake mechanism is poorly understood. We suggest that the observed decrease in basal glucose uptake by NO released from these donors in both STZ- induced diabetic and normoglycaemic rats could be due to the cellular toxicity of NO at higher concentrations (Karnieli *et al* 1986) and its direct inhibition of the intrinsic or functional activity of GLUT-4 (Garvey *et al* 1991), which negatively alters the recruitment of the GLUT-4 from an intracellular pool to plasma membrane (Bao *et al* 1995). Furthermore, inhibition of basal glucose uptake by NO from SNAP at 10 mM and 20 mM was greater in adipocytes from STZ-induced diabetic rats compared to normoglycaemic rats. In STZ-induced diabetic rats cellular GLUT-4 gene expression is reduced and impaired glucose uptake is due to decrease in GLUT-4 protein and mRNA content (Ducluzeau et al 2002). The toxic effect of the higher concentration of NO released from SNAP may cause the down-regulation of GLUT-4 and a greater reduction in the cell surface content of GLUT-4 in adipocytes (Karnieli et al 1986) from STZ-induced diabetic rats compared with normoglycaemic rats. This may result in alterations in the trafficking of the GLUT-4 vesicles or

in the exposure or activation of GLUT-4 (Kahn *et al* 1991). Increasing NO released from GSNO and SNAP can become problematic in diabetic rats as NO potentiates the formation of peroxynitrite which has enormous potential to increase the oxidative modification of proteins by oxidizing different amino acids such as cysteine, tryptophan, methionine and tyrosine (Wedgwood *et al* 2001).

The elevation of glucose uptake in adipocytes that were stimulated by insulin was greater in adipocytes of normoglycaemic rats than in STZ-induced diabetic rats. The insulin responsive GLUT-4 predominates in insulin target tissues (fat and muscle) and mediates the bulk of insulin-stimulated glucose transport activity. The lower glucose uptake observed in the STZ-induced diabetic rats could be due to cellular abnormality in adipocytes which is intrinsic to the glucose transport effector system (Ducluzeau *et al* 2001). The decreased response to insulin may have resulted from defect in the trafficking and translocation of GLUT-4 to the plasma membrane (Rothman *et al* 1992). Investigators have found that cellular depletion of GLUT-4 is found in adipocytes of individuals suffering from diseases characterized by insulin resistance such as obesity and non-type II diabetes mellitus (Garvey *et al* 1989). In addition, pre-translational events specifically suppress gene expression of the GLUT-4 glucose transporter isoform in adipocytes, and this process appears to be a key mechanism of cellular resistance in type II diabetes mellitus and obesity (Garvey *et al* 1991). Insulin receptor kinase activity, the expression and insulin-stimulated phosphorylation of IRS-1 are reduced in adipose tissues of diabetic subjects. Events downstream of IRS-1 are also impacted, as insulin stimulation of phosphoinositol-3-kinase (PI3K) activity and phosphorylation of protein kinase B/Akt are impaired (Freidenberg *et al* 1988).

The concentrations of GSNO and SNAP used in this study are similar to the concentrations of SNP used in other studies that have assessed the role of NO in basal and insulin-stimulated glucose uptake (Balon and Nadler 1994; Young and Leighton 1998). The stimulatory effects of the NO donors appear to be varied and dependent on the cell or tissue type examined. In this study we found that the effect of NO on glucose uptake in adipocytes was similar to its effect in skeletal muscle (McGrowder *et al* 2003) except there was a lower rate of glucose uptake in adipocytes.

Studies done to evaluate the rate of NO decomposition of RSNO showed that SNAP released NO at a faster rate than GSNO, with SNAP having a shorter half-life than GSNO (Matthew and Kerr 1993; Askew *et al* 1995). A direct determination of the stable oxidative products of NO, nitrate (NO_3^-) and nitrite (NO_2^-) have been used by McGrowder and colleagues to assess NO production by SNAP and GSNO *in vivo* (McGrowder *et al* 1999, 2001). We suggest that

based on the reaction time of this experiment approximately half of SNAP would have been decomposed compared to about one quarter of GSNO *in vitro*. However, this requires further investigation. One of the limitations of the study is the significant difference in the body weights of the rats in the control and experimental group. Ideally the investigators wanted both groups to have the same weights. However, this was not possible due to challenges experienced (at the time the experiments were conducted) with obtaining rats of the appropriate weights from the animal house. The authors believe that this did not compromise the objective, which was to compare the body weights of the rats before and after treatment with either STZ and nicotinamide, or saline in the experimental and control groups respectively.

In summary, SNAP and GSNO at concentrations of 1 mM or lower stimulate basal 2-deoxyglucose uptake in adipocytes from normoglycaemic and STZ-induced diabetic rats, and GSNO and SNAP at 10 mM and 20 mM brought about a significant inhibition of basal and insulin-stimulated glucose uptake. Both effects are likely due to the NO released from SNAP and GSNO. The stimulating effect of NO and insulin is additive. This could be due to different pathways of action, a possibility that warrants further investigation. The effect of exogenous NO on glucose uptake gives a clearer understanding of its involvement in metabolic regulation with potential physiological and pathophysiological implications.

Acknowledgements

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