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SHORT COMMUNICATION

Tadalafil increases muscle capillary recruitment and forearm glucose uptake in women with type 2 diabetes

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Abstract
Aims/hypothesis Recent evidence suggests that reduced synthesis of nitric oxide in endothelial cells, i.e. endothelial dysfunction, contributes to the impaired action of insulin in the vasculature of patients with type 2 diabetes. We investigated whether selective inhibition of phosphodiesterase-5 by tadalafil has beneficial effects on peripheral microcirculation and glucose uptake in these patients.
Methods We enrolled seven postmenopausal women with type 2 diabetes and ten age-matched healthy women as controls in a placebo-controlled study to evaluate the acute metabolic effects of tadalafil. We performed microdialysis and blood flow measurements in muscle, and sampled arterial and deep venous blood before and after a single dose of tadalafil 20 mg or placebo. Circulating glucose and insulin levels, muscle capillary recruitment as reflected by permeability surface area for glucose (PS_{glu}) and forearm glucose uptake were measured.
Results In women with type 2 diabetes, but not in the control group, tadalafil induced increases in the incremental AUC for PS_{glu} (tadalafil vs placebo 41±11 vs 4±2 ml [100 g]$^{-1}$ min^{-1}, $p<0.05$) and forearm glucose uptake (46±9 vs 8±4 μmol [100 g]$^{-1}$ min^{-1}, $p<0.05$). The variable that best predicted forearm glucose uptake was PS_{glu}, which explained 70% of its variance. However, fasting glucose and insulin concentrations were similar following treatment with placebo or tadalafil in the two groups.
Conclusions/interpretation This study suggests that tadalafil evokes positive metabolic effects in insulin-resistant women with type 2 diabetes.

Keywords Glucose-lowering drugs · Endothelial dysfunction · Insulin resistance · Microdialysis · Phosphodiesterase-5

Abbreviations

ΔAUC	AUC for the tadalafil vs placebo day
PDE-5	Phosphodiesterase-5
PS_{glu}	Permeability surface area for glucose

Introduction

Previous studies have shown strong associations between microvascular dysfunction and insulin resistance [1, 2]. Recent evidence suggests that reduced synthesis of nitric oxide in endothelial cells contributes to the impaired action of insulin in the vasculature of patients with insulin

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resistance syndrome [3]. A recent study of the pump-perfused rat hindlimb showed that stimulation of nitric oxide production by methacholine induced positive effects on muscle metabolism [4], while we have previously demonstrated beneficial effects of methacholine on capillary recruitment and forearm glucose uptake in insulin-resistant obese humans [5]. However, as methacholine cannot be used therapeutically, alternative approaches would be required to promote nitric oxide signalling. Nitric oxide promotes vasorelaxation by increasing levels of cyclic guanosine monophosphate, which is degraded by phosphodiesterase 5 (PDE-5). PDE-5 inhibition should thus result in an amplified nitric oxide signal and improved vascular function. However, little is known about the metabolic effects of PDE-5 inhibition in skeletal muscle. Here, we investigated whether the selective PDE-5 inhibitor tadalafil improves muscle capillary recruitment and forearm glucose uptake in post-menopausal women with type 2 diabetes.

Methods

Study participants This study was conducted on seven postmenopausal women with type 2 diabetes and ten age-matched healthy women as controls. Men were not included because of ethical concerns.

The type 2 diabetes women were eligible if they met the following criteria: (1) age 55 to 65 years; (2) BMI 27 to 35 kg/m^2; (3) no tobacco use; (4) HbA_{1c} 5% to 7.5% (reference values 3.9% to 5.3%); (5) no significant complications and concomitant metabolic disease as determined by medical history, physical examination and screening laboratory evaluations; and (6) no ongoing treatment with oestrogens, nitrates, beta-blockers or glucocorticoids.

The healthy controls were enrolled according to the following criteria: (1) a healthy state as determined by medical history, physical examination and screening laboratory evaluations; (2) BMI 18 to 25 kg/m^2; (3) normal glucose tolerance during a 75 g oral glucose tolerance test; (4) no current use of oestrogen or other regular medications; and (5) no tobacco use.

Clinical characteristics of the participants are shown in Table 1. All participants gave written informed consent and the study protocols were approved by the Ethics Committee at the University of Gothenburg, Sweden.

Study procedures After an overnight fast, the investigation started at 08:00 hours with participants lying supine in a room kept at 27°C. The type 2 diabetes women did not take their usual medication (except insulin) the evening before and on the morning of the study day. Catheters were inserted into a deep antecubital vein of the right forearm and into the radial artery of the left arm for sampling of deep venous and arterial blood, respectively; a catheter was also inserted into a superficial vein of the left forearm for blood sampling. Thereafter one mercury in-silastic strain gauge was placed on the upper third of the right forearm for blood flow measurements by plethysmography [5]. Muscle microdialysis for interstitial measurements of glucose was performed as previously described [6]. Calibration of the catheters was performed using urea as an internal reference.

Table 1 Clinical characteristics of women participants

	Control	Type 2 diabetes	*p* value
n	10	7	
Age (years)	60±5	61±6	NS
BMI (kg/m^2)	22.7±1.0	29.5±3.4	0.0006
Waist circumference (cm)	82±5	100±12	0.002
Systolic BP (mmHg)	118±10	140±11	0.003
Diastolic BP (mmHg)	78±7	87±5	0.028
HbA_{1c} (%)[a]	4.4±0.1	5.5±0.8	0.0008
Plasma glucose (mmol/l)	4.7±0.3	7.0±1.2	0.0006
Serum insulin (pmol/l)	30±8	90±36	0.0006

Data presented as mean ± SD

[a] Reference value 3.9–5.3%

Study protocol The study participants received either placebo or 20 mg tadalafil orally on one occasion and the opposite treatment after an interval of 4 to 6 weeks. From 1 h before tadalafil or placebo until 4 h after treatment measurements were taken every 15 min (muscle dialysates) or 30 min (blood flow and blood sampling). Baseline was defined as the mean of the measurements taken in the 1 h before administration of tadalafil or placebo. Samples collected were immediately stored at −20°C until analysed.

Calculations We used substrate balance studies and the equations of Fick and Renkin to estimate muscle glucose uptake and capillary recruitment in response to tadalafil in the fasting state [6, 7]. Permeability surface area for glucose (PS_{glu}) was calculated as described previously [7].

Analytical methods Metabolite concentrations in dialysates and plasma fractions were determined with a colorimetric (glucose) and an ultraviolet (urea) method on a microdialysis analyser (CMA 600; CMA Microdialysis, Stockholm, Sweden). Serum insulin concentrations were measured by an ultrasensitive insulin ELISA (Mercodia, Uppsala, Sweden) with a detection limit of 0.4 pmol/l and intra-and interassay coefficients of variation of 5.3% and 2.7%, respectively.

Statistical analysis Comparisons within and between the groups were performed using two-way ANOVA for repeated measures. AUC and incremental AUC were calculated by the trapezoidal integration method. The correlation between pairs of variables was assessed by a simple linear regression analysis, while multivariate relationships were analysed using a general linear model. Data are shown as mean ± SEM. A two-sided p value of $p<0.05$ was considered statistically significant. Statistical analyses were carried out using software from StatView (Abacus Concepts, Berkeley, CA, USA) and SAS Institute (Cary, NC, USA).

Results

Compared with placebo, tadalafil did not affect the incremental AUC for either PS_{glu} or forearm glucose uptake in the control group, but did promote increases in both of these measurements in the type 2 diabetic women (Fig. 1a–d). Tadalafil did not affect the incremental AUC for forearm blood flow in any of the study groups (Fig. 1e–f).

The difference in AUC for the tadalafil vs placebo day (ΔAUC) for PS_{glu} correlated with ΔAUC for forearm glucose uptake in the control group (r^2 0.74, $p<0.01$) and in the type 2 diabetic women (r^2 0.89, $p<0.01$).

Multiple linear regression analysis showed that ΔAUC PS_{glu} was an independent predictor of forearm glucose uptake and accounted for approximately 70% of its variance (Electronic supplementary material [ESM] Table 1). Other confounders measured did not substantially modify the prediction by ΔAUC PS_{glu} alone and only accounted for additional 5% of variance (ESM Table 1).

No significant differences in serum insulin and plasma glucose levels were shown at baseline, and at 2 and 4 h following treatment with placebo or tadalafil in both study groups (data not shown).

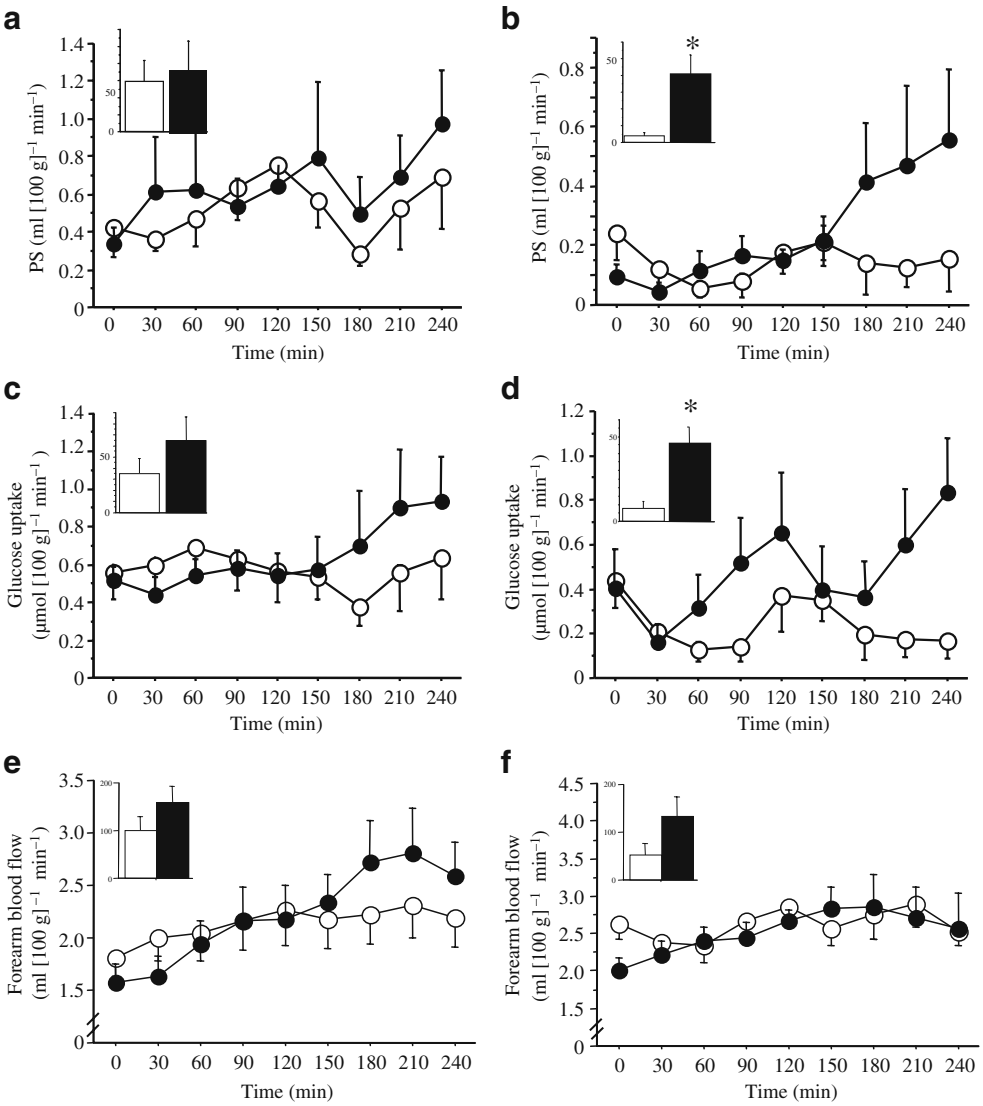

Fig. 1 Incremental AUC for PS_{glu} (**a**, **b**), forearm glucose uptake (**c**, **d**) and forearm blood flow (**e**, **f**) in healthy controls (**a**, **c**, **e**) and patients with type 2 diabetes (**b**, **d**, **f**) following placebo (white circles/bars) or tadalafil 20 mg (black circles/bars). All negative incremental AUC values were set to zero, leaving means above zero in bar graph insets. *$p<0.05$ compared with placebo

Discussion

Our evaluation of the potential of PDE-5 inhibition to improve peripheral microcirculation and metabolism in women with type 2 diabetes showed that tadalafil increased capillary recruitment and muscle glucose uptake in the forearm of insulin-resistant humans. Our study is the first to suggest that PDE-5 inhibition may be a novel approach to improve glucose uptake in patients with type 2 diabetes.

Our current findings are in agreement with our previous study, which showed increased capillary surface area and glucose uptake in insulin-resistant obese patients following methacholine infusion [5]. Interestingly, a recent study in high-fat fed mice showed that chronic treatment with the PDE-5 inhibitor sildenafil resulted in increased muscle glucose uptake, but without activation of downstream insulin signalling in the muscle extracts [8]. The authors thus speculated that glucose uptake was facilitated by improved capillary recruitment or by increased cyclic guanosine monophosphate levels in skeletal muscle cells [8]. This could plausibly explain our results, indicating that a component of muscle glucose uptake is independent of PS_{glu}. In contrast to our findings, a recent study in women with the metabolic syndrome showed an effect of tadalafil on beta cell function, whereas insulin resistance as measured by the minimal model was unchanged [9]. In the absence of this test in the present study, one should consider that positive effects of tadalafil on metabolism may be mediated by effects on insulin secretion, insulin sensitivity or both.

Our observation of a strong correlation between ΔAUC PS_{glu} and ΔAUC forearm glucose uptake in both study groups is consistent with previous studies showing that a microvascular response is critical for glucose uptake in skeletal muscle [4, 10]. Thus, it is possible that increased nutritive blood flow may enhance glucose delivery to insulin-resistant muscle in the postabsorptive state, too. Our multiple regression analysis showed that forearm blood flow did not substantially affect the variance of forearm glucose uptake. The results are in agreement with the concept that muscle glucose uptake is more dependent on capillary recruitment than on forearm blood flow in type 2 diabetes patients [6, 10].

The participants experienced some tadalafil-related side effects such as myalgia, dyspepsia and nausea, but hypotension was not observed. Chronic treatment with low-dose tadalafil is not associated with tachyphylaxis or serious side effects [11], indicating that it is a safe drug to use.

In conclusion, this study implies that tadalafil has acute positive effects on capillary recruitment and glucose uptake in insulin-resistant skeletal muscle of type 2 diabetic women. As we investigated the response to tadalafil in the fasting state, further studies will be required to delineate whether PDE-5 inhibition has beneficial effects on the vasculature and beta cell function after a meal, and on insulin sensitivity assessed by glucose clamp in chronic studies.

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Duality of interest M. Sjöstrand is employed by AstraZeneca Research and Development, Sweden. All other authors declare that there is no duality of interest associated with this manuscript.

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