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Metformin improves putative longevity effectors in peripheral mononuclear cells from subjects with prediabetes. A randomized controlled trial

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Abstract *Background and aims:* Prediabetes increases cardiovascular risk and is associated with excess mortality. In preclinical models, metformin has been shown to exert anti-ageing effects. In this study, we sought to assess whether metformin modulates putative effector longevity programs in prediabetic subjects.

Methods and results: In a randomized, single-blind, placebo-controlled trial, 38 prediabetic subjects received metformin (1500 mg/day) or placebo for 2 months. At baseline and after treatment, we collected anthropometric and metabolic parameters. Gene and protein levels of *SIRT1*, *mTOR*, *p53*, *p66Shc*, SIRT1 activity, AMPK activation, telomere length, and *SIRT1* promoter chromatin accessibility were determined in peripheral blood mononuclear cells (PBMCs). Plasma N-glycans, non-invasive surrogate markers of ageing, were also analysed.

Compared to baseline, metformin significantly improved metabolic parameters and insulin sensitivity, increased SIRT1 gene/protein expression and *SIRT1* promoter chromatin accessibility, elevated *mTOR* gene expression with concomitant reduction in p70S6K phosphorylation in subjects' PBMCs, and modified the plasma N-glycan profile. Compared to placebo, metformin increased SIRT1 protein expression and reduced p70S6K phosphorylation (a proxy of mTOR activity). Plasma N-glycans were also favourably modified by metformin compared to placebo.

Conclusion: In individuals with prediabetes, metformin ameliorated effector pathways that have been shown to regulate longevity in animal models.

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Introduction

Diabetes is characterized by a reduced life expectancy, especially in the presence of obesity and insulin resistance [1]. Vice versa, lower plasma glucose concentrations and better glucose tolerance are associated with longer life expectancy [2]. Metformin has been shown to effectively prevent progression of prediabetes to overt diabetes [3]. In

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addition to its anti-hyperglycaemic actions, metformin blunts inflammation, improves cell survival [4,5] and exerts anticancer effects by suppressing the mammalian target of rapamycin (mTOR)/S6K1 axis [6]. AMP-activated dependent kinase (AMPK) is one putative target of metformin action and favourably affects cellular resistance to stress, inflammation, and ageing [7–9]. Furthermore, metformin improves lifespan in animal models through an anti-ageing pathway driven by mTOR [10,11]. Metformin has also been shown to protect endothelial cells from hyperglycaemic damage by directly stimulating the expression/activity of Sirtuin-1 (SIRT1), a deacetylase involved in metabolism [12] and longevity [13], and by modulating SIRT1 downstream targets FoxO1 and p53/p21 [14]. Indeed, SIRT1, AMPK and mTOR form a network that connects cellular metabolism and longevity programmes [15]. While this network has been demonstrated in animal models, the effect of metformin on longevity-related pathways has not yet been clarified in humans.

Protein glycosylation, i.e. the enzymatic addition of sugars to proteins, plays an emerging role as ageing modulator by modifying protein functions [16]. Glycans contribute to the folding and conformational stability of many proteins, mediate host–pathogen interactions and innate immunity, and serve as ligands that mediate cell trafficking, adhesion, and signalling [16]. Many studies have substantiated the importance of changes in glycan structures during development and disease [16,17], but it is unknown whether this pathway is affected by metformin.

This study was designed to test the hypothesis that metformin affects putative longevity-promoting pathways in peripheral blood mononuclear cells (PBMCs) and plasma N-glycans of prediabetic individuals.

Methods

Study population

The study was approved by the Ethical Committee of the University Hospital of Padova and carried out in accordance with International Ethical Guidelines and the principles of the Declaration of Helsinki. Written informed consent was obtained from all subjects. Patients attending the outpatient clinic of the Division of Metabolic Diseases of the University of Padova Medical Centre who had IFG (fasting plasma glucose 100–125 mg/dl [5.5–6.9 mmol/l] or IGT (2-h plasma glucose 140–199 mg/dl [7.7–11.1 mmol/l]) were recruited. Exclusion criteria were: diagnosis of diabetes, acute disease or infection, chronic inflammatory disease, pregnancy or lactation.

Randomization and intervention

Thirty-eight prediabetic patients were recruited in a randomized, single (patient) blind, placebo-controlled trial, from January 2011 to December 2012. Subjects were assigned to metformin 500 mg tris in die (n = 19) or placebo (n = 19), based on a balanced randomly-generated sequence available to the investigators. Metformin was purchased from Merck-Serono. One film-coated tablet contained 500 mg metformin hydrochloride corresponding to 390 mg metformin base (tablet core: povidone K30; magnesium stearate; film-coating hypromellose). In both groups, diet and life-style advices were given as standard care offered routinely for outpatients.

The following data were collected at baseline and at the end of the two month treatment period: weight, height, BMI, waist circumference, blood pressure, heart rate, prevalence of hypertension, and information on other medications. A blood sample was drawn in fasting conditions to isolate peripheral blood mononuclear cells (PBMCs), determine serum lipid profile and analyse N-glycans. In PBMCs, gene and protein expression of *SIRT1*, *mTOR*, *p53*, *p66Shc*, SIRT1 activity, AMPK activation, telomere length, and chromatin accessibility of the *SIRT1* promoter were determined (see the online appendix). All subjects underwent a standard 75 g oral glucose tolerance test followed by frequent blood sampling for glucose, insulin, and C-peptide determination. The insulin sensitivity index (Si) was calculated, as described elsewhere [12].

Statistical analysis

Data are expressed as mean ± standard error or as percentage, where appropriate. The Kolmogorov–Smirnov test was used to test the normal distribution of the variables of interest. Comparison of continuous data between the 2 groups (metformin versus placebo) was performed using the 2-tailed unpaired Student's T test for normal variables and the Mann–Whitney test for non-normal variables. The Chi-square test was used for categorical data. The 2-tailed paired Student's t test for normal variables or the Wilcoxon rank test for non-normal variables were used to compare paired datasets. Correlations were checked using Pearson's r or Spearman's rho coefficients for normal and non-normal data, respectively. The study primary endpoint was the change in SIRT1 protein content in PBMCs at the end of the study period compared to baseline. Secondary endpoints were changes in protein and gene expression of other longevity pathway effectors and metabolic parameters. The study was powered to detect a 0.2 arbitrary units (AUs) difference between the 2 groups in SIRT1 protein expression, with the baseline dataset at 1.0 AUs (sigma = 0.22; alpha = 0.05; 1-beta = 0.80). Statistical significance was accepted at $p < 0.05$.

Results

Effects of metformin on metabolic parameters

All intention-to-treat analyses are based on the 19 patients per group who were enrolled, randomized, and completed the study. The drug was well tolerated and there was no drop-out. Clinical characteristics of the subjects at baseline and at study end are outlined in Table 1. There were no significant differences between the 2 groups in terms of