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Research report

Prolonged metformin treatment leads to reduced transcription of Nrf2 and neurotrophic factors without cognitive impairment in older C57BL/6J mice

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HIGHLIGHTS

- A mouse model was used to determine the effect of high fat diet and metformin treatment on cognition, brain neurotrophic factor expression and Nrf2 expression.
- Transcripts of three major neurotrophic factors were decreased in brains of metformin treated mice.
- Brain protein and mRNA levels of the antioxidant regulatory factor Nrf2 were decreased by metformin treatment.
- Long-term high dose metformin use may create disadvantageous biochemical conditions in the brain.

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ABSTRACT

Long-term use of anti-diabetic agents has become commonplace as rates of obesity, metabolic syndrome and diabetes continue to escalate. Metformin, a commonly used anti-diabetic drug, has been shown to have many beneficial effects outside of its therapeutic regulation of glucose metabolism and insulin sensitivity. Studies on metformin's effects on the central nervous system are limited and predominantly consist of in vitro studies and a few in vivo studies with short-term treatment in relatively young animals; some provide support for metformin as a neuroprotective agent while others show evidence that metformin may be deleterious to neuronal survival. In this study, we examined the effect of long-term metformin treatment on brain neurotrophins and cognition in aged male C57Bl/6 mice. Mice were fed control (C), high-fat (HF) or a high-fat diet supplemented with metformin (HFM) for 6 months. Metformin decreased body fat composition and attenuated declines in motor function induced by a HF diet. Performance in the Morris water maze test of hippocampal based memory function, showed that metformin prevented impairment of spatial reference memory associated with the HF diet. Quantitative RT-PCR on brain homogenates revealed decreased transcription of BDNF, NGF and NTF3; however protein levels were not altered. Metformin treatment also decreased expression of the antioxidant pathway regulator, Nrf2. The decrease in transcription of neurotrophic factors and Nrf2 with chronic metformin intake, cautions of the possibility that extended metformin use may alter brain biochemistry in a manner that creates a vulnerable brain environment and warrants further investigation.

Abbreviations: HF, high fat; HFM, high fat plus metformin; BDNF, brain derived neurotrophic factor; NGF, nerve growth factor; NT3, neurotrophin 3; Nrf2, nuclear factor erythroid 2-related factor.

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1. Introduction

The incidence of type-2 diabetes mellitus (T2DM) continues to emerge in epidemic proportions throughout the developed world. This disease is ranked fourth in leading causes of global death by disease [1]. The NIH reports that about 8.3% of the United States is affected by diabetes; and for those aged over 65 years, this rate increases to 26.9% [2,3]. Given that obesity and physical inactivity are the primary factors that lead to the development of T2DM, the vast majority of cases could be controlled or reversed through lifestyle changes, such as increased exercise and a modification of dietary habits. Still, most type-2 diabetics require treatment with prescribed medication for prolonged periods of time [3], often several years. Consequently, chronic use of anti-diabetic agents is increasingly prevalent within general populations.

Several effective anti-diabetic agents have been developed and are currently in use. These agents provide benefits such as increased insulin sensitivity through up-regulation of glucose transporters, a suppressed rate of intestinal glucose absorption by inhibition of α-glucosidases, and increased insulin secretion from beta cells [4]. These treatments are important for regulating blood glucose and insulin levels, thereby preventing the consequential damage to peripheral organs and nerves.

Aside from the injurious effects on peripheral systems, T2DM also has deleterious effects on brain function. Several studies have shown impaired cognition and memory performance in patients with T2DM [5–7]. Others have shown an increased risk for the development of dementia and specifically, Alzheimer's disease (AD) [8–10]. Additionally, studies suggest that insulin resistance in the brain is a major contributing factor to the symptoms of AD, ultimately affecting synaptic plasticity. Conversely, effective glycemic control is associated with cognitive improvement [11]. With this correlation between cognition and T2DM, it would be important to be aware of any potential risks and or benefits of commonly used diabetes treatments on brain health. Interestingly, one post-mortem study, which examined the association between diabetes treatments and AD neuropathology, found that only in combination with insulin treatment are anti-diabetic drugs associated with decreased AD neuropathology [12]. Therefore, there is growing interest in evaluating the impact of anti-diabetic agents on brain chemistry and function.

Metformin is a highly prescribed anti-hyperglycemic drug. It is often a first line oral treatment for T2DM, and one of only two oral anti-diabetics listed in the 16th World Health Organization Model List of Essential Medicines and is currently being investigated, in clinical trials, as a potential treatment for AD. Metformin is known to elicit many of its effects through 5' adenosine monophosphate-activated protein kinase (AMPK) activation which in turn decreases gluconeogenesis in the liver and improves insulin binding to receptors in liver and muscle thus increasing insulin sensitivity and glucose uptake [13]. AMPK activation by metformin has been shown to be secondary to its inhibition of mitochondrial respiratory chain complex 1 [14]. Metformin has also been shown to decrease intestinal absorption of glucose [15,16]. Studies on metformin's effects in the central nervous system are limited and predominantly consist of in vitro studies, some of which show support for metformin as a neuroprotective agent [17], increasing hippocampal neurogenesis and enhancing cognitive function [18] however, other studies have shown evidence that metformin may increase risk of developing Alzheimer's disease [19] and be deleterious to neuronal survival acting through its AMPK activating mechanism [20]. Most of these studies utilize a paradigm of short-term metformin treatment.

In this study, we used a mouse model to assess effects of a high-fat (HF) diet and long-term treatment with metformin on brain neurotrophins and cognitive function.

2. Materials and methods

2.1. Animals and diets

Forty eight, twelve month-old male C57Bl/6 mice were obtained from the breeding colony at the National Institute on Aging (NIA). Mice were randomly assigned to one of three dietary groups: standard/control (C), high fat (HF), and high fat plus metformin (HFM) ($n = 16$/group). Diets included the AIN-93 G standard diet (C), AIN-93 G modified high fat diet to provide 60% of calories from fat by the addition of hydrogenated coconut oil (HF), and the AIN-93 G modified high fat diet with the addition of 1.0% metformin by weight (HFM). Mice were maintained on the diets for 6 months. During the first month all animals were fed ad libitum (AL). Food intake and body weight were measured on a biweekly basis. To ensure that effects seen in the metformin treated group did not result from differences in caloric intake, after the initial four weeks, HF-fed mice were fed daily an amount of HF diet that was equivalent to the average daily caloric intake of mice fed on the HFM diet. After 4 months on respective diets, mice were tested for learning and memory performance, motor coordination, body composition and glucose tolerance according to procedures described below.

2.2. Housing

Animal housing conditions consisted of a 12-h light/dark cycle with temperatures between 22 and 24 °C (according to animal protocols and NIH guidelines). Mice were single housed in cages which contained a voluntary free-spinning running wheel. Running wheels were connected to a computerized automated monitoring system which kept record of the number of wheel rotations for each individual cage (Columbus Instruments, Columbus, OH).

2.3. Rotarod motor performance

Motor coordination performance was tested using an automated, motorized rotarod treadmill for mice (Med. Associates Inc., St. Albans, VT). The rotating drum (3 cm in diameter) is divided into test zones, by round divider plates, allowing for 5 mice to be tested at one time. Mice were habituated to the rotarod one day prior to testing by first being placed on the non-rotating drum for 10 s immediately followed by a 120 s period with the drum rotating at a constant 4.0 rpm. During testing, mice were placed on the rotating drum which was set to gradually accelerate from 4 to 40 rpm over a 300 s interval. Mice were forced to move at increasing speeds to avoid the 16.5 cm fall to the platform. Each mouse received 3 trials, with a 30-min inter-trial interval. Latencies before falling were measured and averaged across the 3 trials as the dependent variable.

2.4. Morris water maze

The Morris water maze test was used to measure hippocampal based spatial memory and learning function. The water maze apparatus consisted of a white circular plastic tank (100 cm diameter and 70 cm high) which was filled with water (24 ± 1 °C) made opaque by the addition of white DryTemp® paint powder (Palmer Paint Products Inc.,Troy, MI, USA). Visual cues of objects varying in geometric shapes and shades were affixed to a clear, plastic cylinder surrounding the interior wall of the pool and extending approximately 30 cm above the pool surface. A clear, circular (10 cm diameter) escape platform was submerged a few millimeters below the water surface. Mice were trained 4 trials/day, for 5 consecutive days. Each acquisition trial was started by placing the mouse in the water facing the wall of the tank. The location of entry of the mouse was changed for every trial such that mice entered the maze from each