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Exogenous administration of spermine improves glucose utilization and decreases bodyweight in mice

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ABSTRACT

Polyamines are highly charged low molecular weight aliphatic polycations and are ubiquitously present in all living cells. In addition to their previously reported role in cell proliferation and cancer, recent studies support their role in energy homeostasis and glucose metabolism. In the present study we have evaluated a polyamine—spermine for its effect on glycemic, lipid and body weight parameters. High fat diet induced obese mice (6 week old male C57B6/J mice fed on high fat diet for 22 weeks) were dosed with spermine intraperitoneally at two different doses (5 mg/kg and 10 mg/kg body weight) for 4 weeks and its effect on body weight, glycemic and lipid parameters was monitored. We found that at a dose of 10 mg/kg bodyweight, spermine treatment resulted in a 24% reduction in the body weight and 18% reduction in the fasting glucose compared to untreated controls. Besides, spermine treated mice exhibited improved glucose utilization associated with improved fat oxidation and loss of white adipose mass. Our study is promising in the direction of exploring the spermine and their analogs for treatment of metabolic syndrome.

1. Introduction

The polyamines, putrescine, spermidine and spermine, exist in all living cells (Mandal et al., 2013) and due to cationic nature, they can interact with negatively charged macro-molecules such as DNA, RNA, proteins and phospholipids (Igarashi and Kashiwagi, 2000). They are known to influence cellular functions like cell proliferation, differentiation and cell death (Janne et al., 2004; Thomas and Thomas, 2001, 2003). Several studies conducted in the past have pointed out the role of exogenously supplied spermine in cancer progression (Glikman et al., 1990; Thomas and Thomas, 1994), animal growth (Smith, 1990), food intake (Zasloff et al., 2001), insulin sensitivity and energy expenditure (Lockwood and East, 1974; Lockwood et al., 1971).

Effect of spermine on cancer is well known and the spermine analog bis(ethyl)norspermine (BENSpm) has been characterized for anti-tumor activity and this molecule has undergone Phase I and II clinical trials (Bernacki et al., 1995; Wolff et al., 2003).

The potential for exogenous dietary polyamines to contribute to growth and health has been addressed in the literature (Sousadias and Smith, 1995). Studies indicate that supplementation of amino acid diets with 0.2% putrescine can promote whole-body growth of chicks (Smith, 1990) and this finding supports the notion that putrescine may be an essential nutrient for the chick (Anon, 1992). Besides, supplementation of putrescine and ethylamine in calves and neonatal pigs is shown to enhance enterocyte proliferation and to reduce the adverse effects of soy protein (Grant et al., 1989, 1990). A naturally occurring spermine metabolite of cholesterol named as MSI-1436 isolated from the dogfish shark, *Squalus acanthias*, has been shown to exhibit appetite suppressant activity in rodents (Zasloff et al., 2001). These studies indicate that polyamines and their analogs can influence various aspects of food absorption, intake and animal growth.

In addition to their effect on food consumption and growth, polyamines are also known to influence glucose metabolism. Studies conducted by Lockwodd et al. indicate that spermidine and spermine can stimulate the conversion of glucose to carbon

Abbreviations: ACC, Acetyl CoA carboxylase; AMP, Adenosine monophosphate; AMPK, 5'AMP activated protein kinase; AUC, Area under the curve; CPT, Carnitine palmitoyl transferase; FFA, Free fatty acids; HFD, High fat diet; PDK, Pyruvate dehydrogenase kinase; SSAT, Spermidine/spermine *N*1-acetyltransferase; PK, Pharmacokinetics; TG, Triglycerides

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dioxide and inhibit lipolysis in isolated rat adipose cells (Lockwood et al., 1971). Evidence has also been presented suggesting that polyamines can facilitate glucose transport and inhibit lipolysis by suppressing cyclic AMP levels (Clo et al., 1979). Recent studies have shown that the enhanced polyamine flux (due to SSAT expression) can lead to reduced whole body WAT (white adipose tissue) mass with a reduction in acetyl-CoA and malonyl-CoA in WAT in mice (Jell et al., 2007; Pirinen et al., 2007). Supporting the role of polyamines in energy metabolism further, spermidine and spermine are shown to increase the stability of insulin mRNA and stimulate proinsulin biosynthesis in isolated rat adipocytes (Lockwood and East, 1974). Although these studies imply the role of polyamines in glucose and fat metabolism, the potential of polyamines as agents controlling the glucose and lipid metabolism has not been evaluated in animal models and validation of the polyamine metabolic pathway as a target for diabetes or metabolic syndrome is still not fully explored. In the current study, we have validated the potential of exogenously administered spermine to control glucose and lipid metabolism in diet induced obese mice. Our studies indicate that spermine treatment in mice results in reduced body weight, improved glucose utilization and increased fatty acid oxidation. The study holds promise that polyamines and their analogs optimized for pharmacokinetic properties could be a potential anti-diabetic agents.

2. Materials and methods

2.1. Animals

C57B6J male mice were housed at 22 ± 3 °C, with a relative humidity of 50–70% on a 12 h light and 12 h dark cycle with artificial fluorescent tubes. Mice aged 6 weeks were fed on high fat diet (D12492 60% kcal fat from lard from Research Diets, US) for 22 weeks. Body weight, fasting glucose, glucose tolerance and serum TG were measured in these animals after 22 weeks and mice were randomized into 3 groups based on these parameters (G2, G3 and G4, $n=8$ in each group). These parameters were not significantly different between these groups. Spermine was dissolved in phosphate buffered saline and the mice in groups G3 and G4 were dosed twice daily with 5 mg/kg and 10 mg/kg doses of spermine respectively for 4 weeks through an intra-peritoneal route. Mice in group G2 were administered with phosphate buffered saline through an intra-peritoneal route and they served as untreated controls. Age matched animals ($n=8$) that were fed on a normal chow diet were used as lean control (designated as group G1). Mice in different groups were continued on the respective diets (*i.e.* G2, G3 and G4 mice on high fat diet and G1 mice on chow diet) during the treatment period. All animal experiments were conducted according to ethical guidelines set by the institutional animal ethics committee.

2.2. Oral glucose tolerance test

Mice were fasted for 6 h and initial (time 0 min) glucose level was measured using an Accu-check glucometer from the blood collected from the tail tip cut. After this, glucose was administered to mice through oral gavauze at 2 g/kg body weight and blood glucose levels were measured after 15, 30, 60, 90 and 120 min.

2.3. Estimation of triglyceride

100 mg of liver samples was collected in 1 ml of phosphate buffered saline (pH 7.4) and lysed using a tissue lyser (25 Hz for 5 min). The liver triglyceride was extracted according to Folch's method of lipid extraction with some modifications (Folch et al., 1957). Briefly, 0.3 ml of 10% liver homogenate was extracted with 1.5 ml of chloroform:methanol (2:1) and the organic layer was separated and dried in a vacuum dryer, then residue was re-suspended in absolute isopropyl alcohol and triglyceride levels were estimated by using a DiaSys Diagnostic Systems GmbHkit as per the manufacturer's instructions.

2.4. Real time quantitative PCR

Total RNA was extracted from the liver using Tri-reagent (Sigma, St. Louis, MO, USA), followed by chloroform extraction and isopropyl alcohol precipitation. cDNA was synthesized by reverse transcription (ABI, Foster City, CA, USA). The cDNA was amplified using MESA Green PCR Master Mix (Eurogenetic, Belgium). Quantification was done by a ddCt method and beta actin gene was used as endogenous control. Each sample was run in duplicate and the data are represented as Mean ± S.E.M. Primers used in this study are as follows:

LIPE forward 5'-AGACACCAGCCAACGGATAC-3'
LIPIE Reverse 5'GCGGTTAGAAGCCACATAGC 3';
CPT1a forward 5'-AAC ATC GTG AGT GGC GTC CTC TTT-3'
CPT1a reverse 5'ATT CTG GTG CTG CGG CTC ATT T-3'
CPT1b forward 5'-CGG GAC AAA GGC AAG TTC TG-3'
CPT1b reverse 5'-GGT ACA GGA ACG CAC AGT CTC A-3'
PDK4 forward 5'-TGT GGT CCC TAC AAT GGC TCA A-3'
PDK4 reverse 5'-AGC ATC CGA GTA GAA ATG CGG T-3'
PPARd forward 5'-TCCCAGGGTCAGAAGCTAGA-3'
PPARd reverse 5'-CCAGTCTGGATGCTGCTACA-3'
ATGL forward 5'-TCATGTGACACAGCGAGTGAG-3'
ATGL reverse 5'-AAAGAGGAGCCAAGCACAAA-3'

3. Results

3.1. Animal groups used in the study

C57B6/J mice were fed on high fat diet as explained in Section 2 and were divided into 3 groups—G2, G3 and G4. Fasting glucose levels, serum triglyceride levels and body weight parameters in these mice (G2, G3 and G4) were significantly different at the beginning of the study when compared to group G1 mice (chow diet control mice) as shown in Table 1. The mice were monitored routinely for any behavioral changes, signs of toxicity and lethality. The spermine dose used in the study did not result in any lethality and upon pathological examinations none of the mice showed any detectable pathological abnormalities.

3.2. Effect of spermine on body weight and feed consumption

As shown in Fig. 1, within 2 weeks after treatment animals exhibited decrease in body weight. By the 2nd week of treatment with spermine mice in group G3 showed a 6% decrease in the body weight compared to untreated mice (group G2). By the 4th week of treatment mice treated with 5 mg/kg (group G3) and 10 mg/kg (group G4) doses of spermine showed 4% and 24% decrease in the body weight. Epididymal fat pad is one of the largest visceral fat depots in mice and is an index of the obesity in diet induced obese mice (Casteilla et al., 2008). Hence at the end of 4 weeks treatment animals were sacrificed and weight of epididymal fat was measured. As indicated in Fig. 2A, mice in groups G3 and G4 exhibited significant decrease in the weight of their epididymal fat mass. In group G2, where the mice were fed with high fat diet for 22 weeks without any treatment, there was about 4 fold increase in the total epididymal fat mass. With spermine treatment for 4 weeks at 5 mg/kg (group G3) there was a 22% decrease in the