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Dietary supplementation with evodiamine prevents obesity and improves insulin resistance in ageing mice

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ABSTRACT

Evodiamine is a major alkaloid extracted from the fruit of *Evodia fructus*, which reduces diet-induced obesity in young animals. We investigated the effects of long-term dietary supplementation with evodiamine on obesity, insulin resistance and longevity in normal ageing mice. Twelve-month-old C57BL/6J male mice were fed with standard chow with or without 1 or 10 mg evodiamine per kg food and maintained until death. Supplementation with low dose evodiamine prevented body weight gain and improved glucose tolerance in the mice, in which increased AMPK phosphorylation and down-regulation of mTOR signalling responsible for regulating energy metabolism were detected in white adipose tissue. However, evodiamine supplementation did not increase lifespan, and the high dose evodiamine caused excessive reduction in body weight, which could have side effects in aged animals. Thus, evodiamine at a low dose (1 mg/kg food) prevents obesity and insulin resistance even when ingestion begins in middle age.

1. Introduction

Excess caloric intake, in contrast to caloric restriction, induces obesity and type-2 diabetes mellitus, accelerating the progression of cardiovascular diseases and cancers, and reducing longevity. Indeed, obesity, characterized by excessive fat deposition mainly in white adipose tissue (WAT) but also leading to fat accumulation in the liver, skeletal muscle and pancreas, is a serious health risk in industrialized societies (Kolonin, Saha, Chan, Pasqualini, & Arap, 2004). Ageing is an important factor that increases susceptibility to obesity

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Abbreviation: WAT, white adipose tissue; BAT, brown adipose tissue; IPGTT, intraperitoneal glucose tolerance test; mTOR, serine/threonine protein kinase mammalian target of rapamycin; S6K, ribosomal S6 protein kinase; AMPK, AMP-activated protein kinase; UCP, uncoupling protein

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because the basal metabolic rate and activity generally decline with age, although there are individual variations in the degree of their attenuation. Indeed, the prevalence of overweight and obesity increases with age until 50–60 years in humans (Kopelman, 2000). Since obesity develops as the result of energy imbalance when energy intake exceeds energy expenditure, interventions to reduce caloric intake through hormonal regulation and/or to increase energy expenditure by increasing thermogenic function such as uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) would be reasonable ways to prevent or cure obesity (Bray & Tartaglia, 2000; Guerra, Koza, Yamashita, Walsh, & Kozak, 1998). Although UCP1 is not essential for longevity under conditions of standard diet and normal housing temperature, deficiency increases susceptibility to obesity with age in combination with a high-fat (HF) diet (Kontani et al., 2005). Recent findings that functional BAT, despite its reduction with age, exists even in adult humans have accelerated basic and clinical studies on the stimulation of BAT formation and activity as a potential therapeutic target against age-related diseases (Mattson, 2010; Yoneshiro et al., 2011); however, an alternative strategy independent of UCP1 thermogenesis is needed for BAT-negative individuals.

Previous studies involving calorie restriction have revealed several putative molecular bases underlying the regulation of age-related diseases and longevity. In particular, recent advances towards understanding the molecular mechanisms of longevity have been made by the study of signalling pathways such as insulin/insulin-like growth factor I (IGF-1) and serine/threonine protein kinase mammalian target of rapamycin (mTOR) in multiple model organisms (Kennedy, Steffen, & Kaeberlein, 2007; Kenyon, 2010; Zoncu, Efeyan, & Sabatini, 2011). Insulin signalling is intimately linked to the nutrient-responsive mTOR signalling pathway via activation of Akt (White, 2002; Zoncu et al., 2011). The activation of mTOR phosphorylates its downstream protein ribosomal S6 protein kinase (S6K), which participates in several processes including protein synthesis and proliferation (Shima et al., 1998; Zoncu et al., 2011). mTOR activation is also negatively regulated by AMP-activated protein kinase (AMPK), which regulates various cellular processes including glucose and lipid metabolism (Hardie, 2007; Kahn, Alquier, Carling, & Hardie, 2005). Hyperactivation of mTOR by nutrients, Akt, or hyperinsulinaemia results in serine phosphorylation of insulin receptor substrate 1 (IRS1) by S6K, which is involved in insulin resistance (Draznin, 2006). On the other hand, mice deficient in S6K are protected against age- and diet-induced obesity while exhibiting enhanced insulin sensitivity owing to the loss of the negative feedback loop from S6K to IRS1 (Selman et al., 2009; Um et al., 2004). Rapamycin is a macrolide antibiotic and is used as an anti-tumour drug because it prevents cell growth and proliferation by inhibiting mTOR activation (Zoncu et al., 2011). Harrison et al. (2009) in a large-scale mouse study reported the striking results that dietary supplementation with rapamycin extends lifespan in mice even when initiated at 600 days of age. This impact of rapamycin intervention on lifespan extension emphasizes the importance of the mTOR signalling pathway in preventing age-related diseases and regulating longevity.

Evodiamine is a major alkaloidal compound extracted from the fruit of *Evodia fructus* (*Evodia rutaecarpa* Benth., Rutaceae), which has been used for many years as a traditional Chinese herbal medicine for the treatment of pain, vomiting, and pyrexia. Studies have demonstrated that evodiamine exhibits anti-nociceptive, anti-obesity, vasodilatory, anti-tumour and anti-inflammatory effects (Chiou, Chou, Shum, & Chen, 1992; Heo, Yun, Yi, Noh, & Park, 2009; Kobayashi et al., 2001; Takada, Kobayashi, & Aggarwal, 2005; Wang et al., 2006). We have also demonstrated that evodiamine improves diet-induced obesity and glucose tolerance in a UCP1-independent manner in UCP1-knockout mice, which were given an HF diet supplemented with 300 mg evodiamine/kg food for two months (Wang et al., 2008; Wang, Wang, & Yamashita, 2009). Supplementation of evodiamine (300 mg/kg food, approximately 30 mg/kg body weight per day) inhibited body weight gain by 52% in the control mice in the UCP1-knockout mice fed with HF diet. Our studies showed that evodiamine increased phosphorylation of extracellular signal-regulated kinase (ERK) and reduced the expression of peroxisome proliferator-activated receptor-γ in preadipocytes, strongly inhibiting their differentiation into mature adipocytes. Bak et al. (2010) reported that daily injection of evodiamine (10 mg evodiamine/kg body weight) for 2 weeks improved insulin resistance and the undesirable effect of rosiglitazone on body weight gain in db/db mice. More recently, we reported that daily injection of evodiamine (3 mg evodiamine/kg body weight) for one week improved obesity and insulin resistance in obese/diabetic KK-Ay mice via inhibition of insulin-stimulated mTOR–S6K activation and stimulation of AMPK phosphorylation in mature adipocytes (Wang et al., 2013). Thus, evodiamine shows great potential for the prevention of metabolic diseases including obesity and diabetes (Yamashita, 2014). However, the effects of long-term supplementation of low dose evodiamine on age-related pathophysiology and longevity are not clear. In the present study we carried out longitudinal experiments to clarify the effects of evodiamine at low dosages (1 and 10 mg evodiamine/kg food, equivalent to approximately 0.1 and 1 mg/kg body weight per day, respectively) on obesity, insulin resistance and lifespan in normal ageing mice.

2. Materials and methods

2.1. *Experimental animals*

Twelve-month-old male C57BL/6J mice were provided by the ageing farm at the National Institute for Longevity Sciences. Mice which did not show overt signs of diseases were used in the diet study. The mice were maintained under artificial lighting for 12 h per day and provided with standard chow (11.6% kcal from fat; Diet No. CE-2, CLEA Japan, Inc.) and tap water *ad libitum* in our animal facility at 23 °C. Mice were divided randomly into three groups and were fed with the standard chow without or with 1 or 10 mg evodiamine (Kishida Chemical, Osaka, Japan) per kg food (referred to as EL or EH, respectively, 10 mice per group). Evodiamine dose of supplementation was estimated on the assumption that the 12-month-old male mouse weighing 35 g consumes approximately 3.5 g of standard chow per day; that is, 10 and 1 mg evodiamine/kg food supply, 1 and 0.1 mg evodiamine/kg body weight per day, respectively. One mg evodiamine per kg food was the lowest concentration that could be prepared in a compound diet. These mice were housed in groups of 3 and 4 and maintained until