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Basic nutritional investigation

META060 protects against diet-induced obesity and insulin resistance in a high-fat–diet fed mouse

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ABSTRACT

Objective: We investigated whether a reduced iso-α acid derived from an extract of *Humulus lupulus L.*, META060, had an effect on weight gain, body composition, and metabolism in a high-fat–diet (HFD) fed mouse model.
Methods: Weight gain was monitored for up to 20 wk in mice receiving a low-fat diet, an HFD, or an HFD supplemented with META060 or rosiglitazone. Body composition was determined using dual-energy x-ray absorptiometric analysis. Indirect calorimetric measurements were performed to investigate the energy balance in the mice, and oral glucose tolerance tests were administered to examine the effect of META060 on the glycemic response.
Results: The HFD-fed mice administered META060 for 14 wk had a significantly lower mean weight than HFD-fed mice (30.58 ± 0.5 versus 37.88 ± 0.7 g, $P < 0.05$). Indirect calorimetric measurements showed an increased metabolic flexibility in mice supplemented with META060. In addition, glucose tolerance was improved, comparable to the effects of rosiglitazone treatment.
Conclusions: META060 has potential therapeutic value for managing obesity and insulin resistance, and further research into the mechanism of action is warranted.

Introduction

The management of obesity has become a primary goal for health care practitioners in response to the rising epidemic of obesity-related chronic diseases, including type 2 diabetes mellitus and cardiovascular disease. Pharmaceutical approaches that alter appetite, metabolism, or fat absorption include antidepressants, central nervous system stimulants, or peripherally acting antiobesity drugs, and all have been associated with adverse effects (reviewed by Kaplan [1]). Many people seek natural therapies as an alternative to pharmaceuticals for weight management. Yerba mate, yohimbe, aloe, pyruvate, St. John's wort, dandelion, and herbal diuretics have been used for weight loss, although significant clinical studies supporting their efficacy are lacking (reviewed by Pittler et al. [2]).

Iso-α acids derived from the hop plant (*Humulus lupulus L.*) have been found to decrease plasma triacylglycerol and free fatty acid (FA) levels in mice [3,4]. C57BL/6N mice fed a high-fat diet (HFD) exhibited improved glucose tolerance after 14 d and decreased insulin resistance after 10 d of administration of iso-α acids. Furthermore, in a double-blinded, placebo-controlled pilot

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study, diabetic subjects receiving iso-α acids for 8 wk had an average 10.1% decrease in blood glucose levels and a 6.4% decrease in glycated hemoglobin levels [4].

Iso-α acids are not particularly stable compounds, although the reduced derivatives have been found to exhibit a greater stability [5]. Furthermore, reduced iso-α acids have recently shown a greater bioavailability than iso-α acids in humans [6].

Previous work in our laboratory to screen various botanical extracts for lipogenic activity has resulted in the identification of a family of reduced iso-α acids [7]. One of the reduced iso-α acids, META060, has exhibited anti-inflammatory activity in vitro, mediated by the inhibition of the nuclear factor-κB pathways [8,9]. Several reports have suggested a link between obesity-induced inflammation and related metabolic disorders such as insulin resistance (reviewed by Hummasti and Hotamisligil [10] and Olefsky and Glass [11]). The objectives of the present study were to determine the effects of META060 compared with rosiglitazone, a commonly used drug in the treatment of type 2 diabetes mellitus, on body weight, energy metabolism, glucose tolerance, and insulin sensitivity in HFD-induced obese mice.

Materials and methods

Animals and dietary intervention

Wild-type C57Bl/6J male mice were purchased from Charles River (Maastricht, The Netherlands). The mice were housed under standard conditions with access to water and food ad libitum. For the 14-wk dietary intervention, the study was started when the animals were 19 wk of age. The mice were fed a low-fat diet (LFD; 10% energy derived from lard fat; D12450, Research Diet Services, Wijk bij Duurstede, The Netherlands), with a caloric content of 3.85 kcal/g, an HFD (45% energy derived from lard fat; D12451, Research Diet Services), with a caloric content of 4.73 kcal/g, or an HFD supplemented with META060 (100 mg · kg^{-1} · d^{-1}) or rosiglitazone (1 mg · kg^{-1} · d^{-1}; SmithKline Beecham Farma, Rijswijk, The Netherlands). META060 was supplied by Hopsteiner, (St Paul, MN, USA) and standards were purchased from ASBC (New York, NY, USA). The chemical composition of META060 has been described previously [12]. META060 or rosiglitazone was added to the self-made HFD. Briefly, rosiglitazone tablets (rosiglitazone maleate; Avandia, SmithKline Beecham Farma) or META060 powder were crushed in a mortar with a pestle. Subsequently, the powder was mixed with the 45% lard HFD powder diet (45% energy derived from lard fat; D12451, Research Diet Services). For the HFD plus META060, 1.875 g of META060 per kilogram of HFD powder was used. For rosiglitazone, 12 mg of powder was added to 1 kg of HFD powder. The pellets were made by adding 2% agar (Sigma, Zwijndrecht, Netherlands), freeze-dried to remove water, and stored at −20°C. A fixed dosage was used throughout the dietary intervention. Based on previously assessed food intake data, we knew that C57Bl/J6 mice on an HFD plus META060 (D12451, Research Diet Services) eat approximately 2.5 g of diet per day. Each treatment group in the 14-wk intervention included 12 mice, and mice were weighed weekly. The food intake was monitored weekly by weighing the food in the cages manually. After 14 wk, animals in the LFD or HFD plus rosiglitazone groups were sacrificed, after 4 h of fasting. Mice from the HFD group were randomly divided into 2 groups: six were shifted to the HFD plus META060 (100 mg · kg^{-1} · d^{-1}), and the remaining six mice continued receiving the HFD for 6 wk. Likewise, mice supplemented with HFD plus META060 were divided into two groups: six were shifted to the HFD, and the remaining six mice continued receiving the HFD plus META060.

For the 5-wk dietary intervention, 12-wk-old mice were fed an HFD, an HFD supplemented with META060 (100 mg · kg^{-1} · d^{-1}), or an HFD supplemented with rosiglitazone (1 mg · kg^{-1} · d^{-1}). Each dietary group consisted of nine animals. Body weight was measured weekly during the dietary intervention. All experiments were approved by the animal ethics committee of the Leiden University Medical Center.

Dual-energy x-ray absorptiometry

Animals were subjected to dual-energy x-ray absorptiometric (DEXA) analysis after 4 h of fasting. The animals were weighed and sedated by a single intraperitoneal injection of a mixture of acepromazine (6.25 mg/kg; Neurotranq, Alfasan International BV, Weesp, The Netherlands), midazolam (6.25 mg/kg; Dormicum, Roche Diagnostics, Mijdrecht, The Netherlands), and fentanyl (0.31 mg/kg; Janssen Pharmaceuticals, Tilburg, The Netherlands). The sedated animals were scanned in toto using a small-animal DEXA scanner (pDEXA, Norland Stratec Medizintechinik GmbH, Birkenfeld, Germany) and the data were analyzed by the software supplied by the manufacturer. Fat mass and lean body mass were determined.

Indirect calorimetry

Groups of eight mice were subjected to individual indirect calorimetric measurements for a period of 4 consecutive days using a Comprehensive Laboratory Animal Monitoring System (Columbus Instruments, Columbus, OH, USA). The cages were made of clear Plexiglas (30 × 10 × 9 cm, length by depth by height). Before the start of the experiment, the animals were acclimated to the cages and the single housing for a period of 24 h. The experimental analysis started at 09:00 h and continued for 36 h. In the next 36 h of monitoring, the animals were fasted overnight, and then food was replaced to assess the metabolic flexibility. The analyzed parameters included real-time food and water intakes, meal size, frequency, and duration. Oxygen consumption (V_{O_2}) and carbon dioxide production rates (V_{CO_2}) were measured at intervals of 7 min. The respiratory exchange ratio (RER), a measurement for the metabolic substrate choice, was calculated as the ratio of V_{CO_2} to V_{O_2}. CHO and fat (FA) oxidation rates were calculated using the following formulas [13]:

$$\text{CHO} = \left(\left[4.585 \times V_{CO_2}\right] - \left[3.226 \times V_{O_2}\right]\right) \times 4/1000$$

$$\text{FA} = \left(\left[1.695 \times V_{O_2}\right] - \left[1.701 \times V_{CO_2}\right]\right) \times 9/1000$$

The total energy expenditure was calculated from the sum of CHO and FA oxidation. The activity was monitored as two-dimensional infrared beam breaks.

Fecal FA composition and concentration

Feces were collected over 4 d during week 4 of the 5-wk dietary intervention. Feces were weighed, freeze-dried, and ground, and fecal FAs were subsequently derivatized by methyl esterification. Therefore, 2 mL of methanol/hexane (4:1 v/v) containing 80 μg of penta-decanoic acid (C15:0) as an internal standard (Fluka, Zwijndrecht, Netherlands) was added to 15 mg of feces. Then, 200 μL of acetyl chloride (Merck, Darmstadt, Germany) was added, and the samples were incubated at 95°C. After subsequent cooling to 4°C, 5 mL of 6% K_2CO_3 (Sigma) was added and the samples were centrifuged (10 min, 4000 rpm, 4°C). The upper hexane layer was isolated and used for gas chromatographic analysis of FA methyl esters. The FA methyl esters were separated on a 50-m × 0.25-mm capillary gas chromatographic column (CP Sil 88, Agilent Technologies, Middelbrug, Netherlands) in a 3800 gas chromatograph (Varian, Agilent Technologies, Middelburg, Netherlands) equipped with a flame ionization detector. The injector and flame ionization detector were kept at 270°C. The column temperature was programmed from 170°C to 210°C. The FA methyl esters were introduced by split injection (split ratio 20:1). The quantification was based on the ratio of the area of the individual FA to the internal standard.

Oral glucose tolerance test

Glucose and insulin levels were determined after overnight fasting during week 5 of the 5-wk dietary intervention and after 14 wk of the 14-wk dietary intervention. Blood was obtained by tail bleeding, and the glucose and insulin concentrations were determined. Subsequently, the mice received an intragastric load of D-glucose (2 g/kg) provided as a 20% solution in phosphate buffered saline. Additional blood samples (30 μL) were collected by tail bleeding at 5, 15, 30, 60, 90, and 120 min after glucose loading for measurements of plasma insulin and glucose concentrations. The glucose concentration was determined with a glucose analyzer (Accu-Check, Sensor Comfort, Roche Diagnostics GmbH, Germany) and the insulin concentration was determined by an immunoassay (Chrystal Chem, Inc., Drovers Grove, IL, USA).

Statistical analysis

The data are presented as mean ± standard error. Statistical differences were calculated using the unpaired *t* test (SPSS 17, SPSS, Inc., Chicago, IL, USA) or two-way analysis of variance with the Bonferroni post hoc test (GraphPad Prism, San Diego, CA, USA). $P < 0.05$ was regarded as statistically significant.

Results

Supplementation with META060 for 14 wk prevented HFD-induced obesity

To determine the effect of META060 on HFD-induced obesity, the mice were fed an LFD, an HFD, or an HFD supplemented