

## 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- $\alpha$  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 ( $30.58 \pm 0.5$  versus  $37.88 \pm 0.7$  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.

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## 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 antidiuretics, 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- $\alpha$  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- $\alpha$  acids. Furthermore, in a double-blinded, placebo-controlled pilot

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study, diabetic subjects receiving iso- $\alpha$  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- $\alpha$  acids are not particularly stable compounds, although the reduced derivatives have been found to exhibit a greater stability [5]. Furthermore, reduced iso- $\alpha$  acids have recently shown a greater bioavailability than iso- $\alpha$  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- $\alpha$  acids [7]. One of the reduced iso- $\alpha$  acids, META060, has exhibited anti-inflammatory activity *in vitro*, mediated by the inhibition of the nuclear factor- $\kappa$ B 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 Hota-misliligil [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 (Maas-tricht, 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  $\cdot$  kg<sup>-1</sup>  $\cdot$  d<sup>-1</sup>) or rosiglitazone (1 mg  $\cdot$  kg<sup>-1</sup>  $\cdot$  d<sup>-1</sup>; 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 (rosi-glitazone 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/6J 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  $\cdot$  kg<sup>-1</sup>  $\cdot$  d<sup>-1</sup>), 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  $\cdot$  kg<sup>-1</sup>  $\cdot$  d<sup>-1</sup>), or an HFD supplemented with rosiglitazone (1 mg  $\cdot$  kg<sup>-1</sup>  $\cdot$  d<sup>-1</sup>). 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 Medizintechnik 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  $\times$  10  $\times$  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]:

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

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

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  $\mu$ g of penta-decanoic acid (C15:0) as an internal standard (Fluka, Zwijndrecht, Netherlands) was added to 15 mg of feces. Then, 200  $\mu$ L 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<sub>2</sub>CO<sub>3</sub> (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  $\times$  0.25-mm capillary gas chromatographic column (CP Sil 88, Agilent Technologies, Middelburg, 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  $\mu$ L) 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).

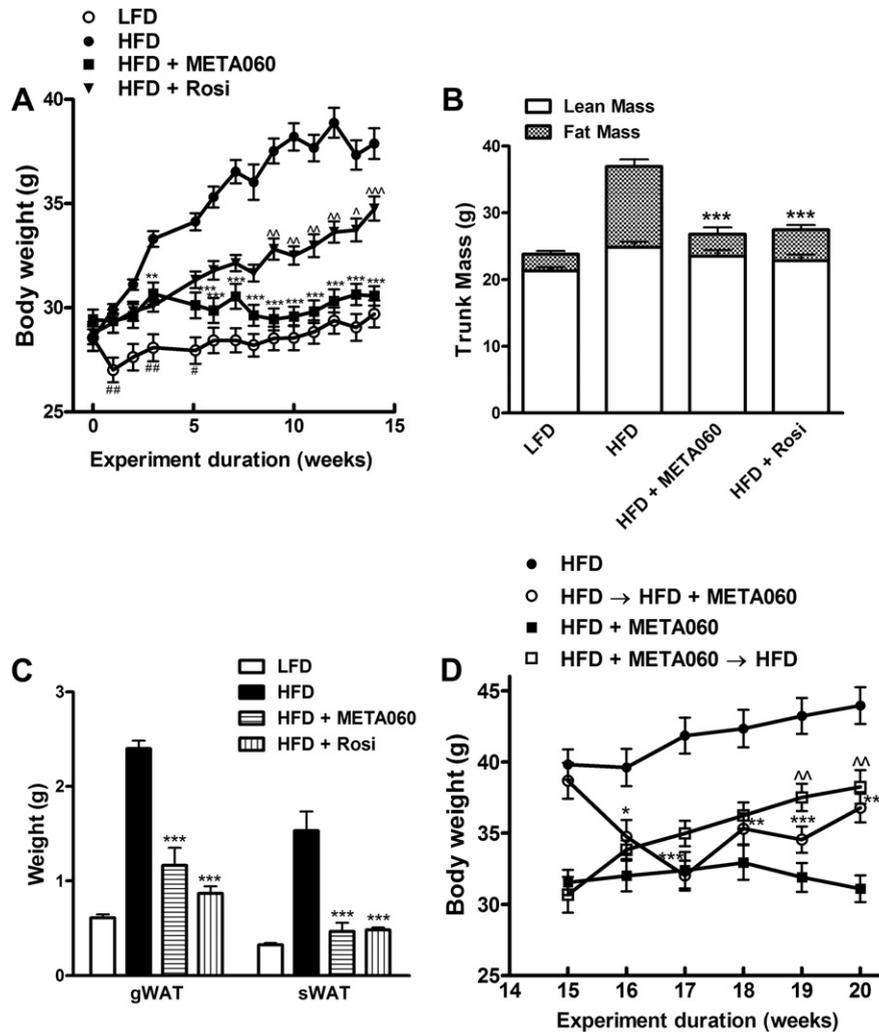
### Statistical analysis

The data are presented as mean  $\pm$  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

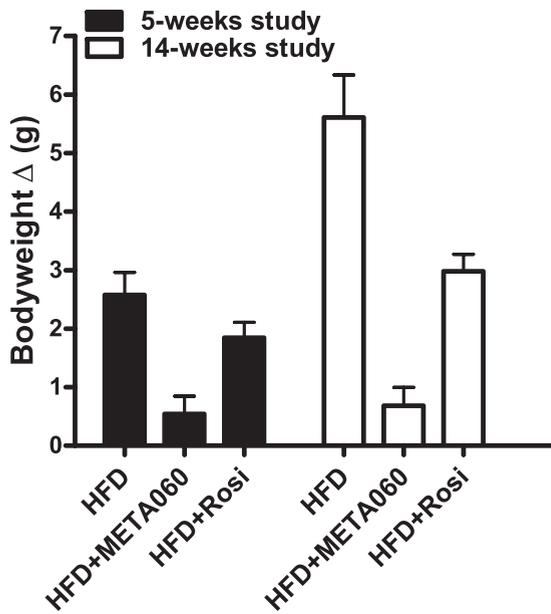


**Fig. 1.** META060 prevents HFD-induced obesity. (A) Mice were fed an LFD, an HFD, or an HFD supplemented with META060 100 mg · kg<sup>-1</sup> · d<sup>-1</sup> or Rosi 1 mg · kg<sup>-1</sup> · d<sup>-1</sup> for 14 wk. Mouse body weights were recorded every week (*n* = 12 per group). \*\* *P* < 0.01, \*\*\* *P* < 0.001 HFD versus HFD + META060; ^ *P* < 0.05, ^^ *P* < 0.01, ^^<sup>^</sup> *P* < 0.001 HFD + META060 versus HFD + Rosi; # *P* < 0.05, ## *P* < 0.01 HFD + META060 versus LFD. (B) After a 4-h fast, the lean body mass and fat mass were determined by dual-energy x-ray absorptiometry (*n* = 6 per group for HFD and META060, *n* = 12 per group for Rosi). \*\*\* *P* < 0.001 HFD versus HFD + META060 and HFD versus HFD + Rosi. (C) After the mice were sacrificed, masses of gWAT and sWAT were determined (*n* = 6 per group for HFD and META060, *n* = 12 per group for Rosi). \*\*\* *P* < 0.001 HFD versus HFD + META060 and HFD versus HFD + Rosi. (D) At 14 wk, half the mice in the HFD group were shifted to HFD + META060 group, and half the mice in the HFD + META060 group were shifted to the HFD-only group. Body weights were recorded weekly (*n* = 6 per group). \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001 HFD versus HFD → HFD + META060; ^^ *P* < 0.01 HFD + META060 versus HFD + META060 → HFD. Data are presented as mean ± SE. gWAT, gonadal white adipose tissue; HFD, high-fat diet; LFD, low-fat diet; Rosi, rosiglitazone; sWAT, subcutaneous white adipose tissue.

with META060 100 mg · kg<sup>-1</sup> · d<sup>-1</sup> or rosiglitazone 1 mg · kg<sup>-1</sup> · d<sup>-1</sup> for 14 wk. Previous studies in a mouse model of collagen-induced arthritis have reported an effect of META060 with 50 mg · kg<sup>-1</sup> · d<sup>-1</sup> for decreasing cartilage degradation and bone erosion, and doses up to 250 mg · kg<sup>-1</sup> · d<sup>-1</sup> were well tolerated [8]. Therefore, a dose of 100 mg · kg<sup>-1</sup> · d<sup>-1</sup> was selected for the present study investigating the effect of META060 on body weight and metabolism in HFD-fed mice. Rosiglitazone is an antidiabetic agent from the thiazolidinedione class of drugs. Its mechanism of action is well known, involving the activation of peroxisome proliferator-activated receptor-γ, and studies in HFD-fed mice have reported a decrease of insulin levels with a dose of rosiglitazone 1 mg · kg<sup>-1</sup> · d<sup>-1</sup> [14]. The mice receiving the HFD supplemented with META060 maintained similar body weights as those on the LFD over 14 wk and body weights were significantly lower in the HFD-fed mice at week 3 and every subsequent time point up to week 14 (Fig. 1A).

After 14 wk, the META060-supplemented mice weighed 19% less than the HFD-fed control mice (30.58 ± 0.5 versus 37.88 ± 0.7 g, *P* < 0.05) and were comparable in weight to mice fed the LFD for 14 wk (29.71 ± 0.7 g). The mice supplemented with rosiglitazone did not gain as much weight as those without supplementation, although they gained significantly more weight than the HFD/META060- or LFD-fed mice (Fig. 1A). During the 14-wk dietary intervention, no differences in food intake were observed.

To determine whether the decreased weight gain in the META060-supplemented mice reflected a lower fat accumulation compared with the HFD-only fed mice, the body composition of these mice was determined by DEXA analysis. The total body fat of the mice supplemented with META060 was significantly lower than that of the HFD-fed mice (3.29 ± 1.0 versus 12.12 ± 1.1 g, *P* < 0.001; Fig. 1B). At the end of the experiment, the organs were dissected out and weighed. The META060 supplementation decreased gonadal (1.17 ± 0.2 versus 2.40 ± 0.09 g,



**Fig. 2.** Comparison of 5-wk body weight increases in the 5-wk and 14-wk studies. The mice were fed an HFD, an HFD supplemented with META060, or an HFD supplemented with Rosi in two independent studies of different durations (5 or 14 wk). In the two studies, the mice were weighed at 5 wk. The mean body weight difference from baseline  $\pm$  SE is represented for each group ( $n = 9$ –12 per group).  $\Delta$ , difference; HFD, high-fat diet; Rosi, rosiglitazone.

$P < 0.001$ ) and subcutaneous ( $0.47 \pm 0.09$  versus  $1.53 \pm 0.2$  g,  $P < 0.001$ ) white adipose tissue masses in the HFD-fed mice compared with no-supplement controls (Fig. 1C).

At 15 wk, half the mice in the HFD group were shifted to the HFD/META060 group, and half the mice in the HFD/META060 group were shifted to the HFD-only group. Body weight was monitored weekly for 5 wk in these four treatment groups. Although the animals maintained on the HFD for the entirety of the experiment continued to gain weight, those shifted to the HFD/META060 group lost a significant amount of weight during weeks 16 and 17, after which they began to gain weight again (Fig. 1D). A concomitant decrease in food intake was observed in the first 2 wk after switching diets, followed by a rebound to even higher levels than the food intake in mice in the HFD/META060 group that did not switch diets (data not shown), perhaps a reflection of an adjustment to the palatability differences between the distinct diets.

#### META060 increased RER and metabolic flexibility in mice fed an HFD

To investigate how META060 protects against HFD-induced obesity, an independent 5-wk study was initiated with three treatment groups: HFD, HFD supplemented with META060 ( $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ), or HFD supplemented with rosiglitazone ( $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ). In the first 5 wk of the 14-wk intervention study, the average weight gained in the HFD group was  $5.61 \pm 0.7$  g, whereas the mice supplemented with META060 gained  $0.68 \pm 0.3$  g. In the 5-wk study, the average weight gained in the HFD group was  $2.58 \pm 0.4$  g, and mice supplemented with META060 gained  $0.54 \pm 0.9$  g (Fig. 2). Despite differences in the absolute weight gained, which was likely due to the difference in age of the mice at the start of each study, the META060 supplementation reproducibly decreased the relative HFD-induced body weight gain in the two experiments.

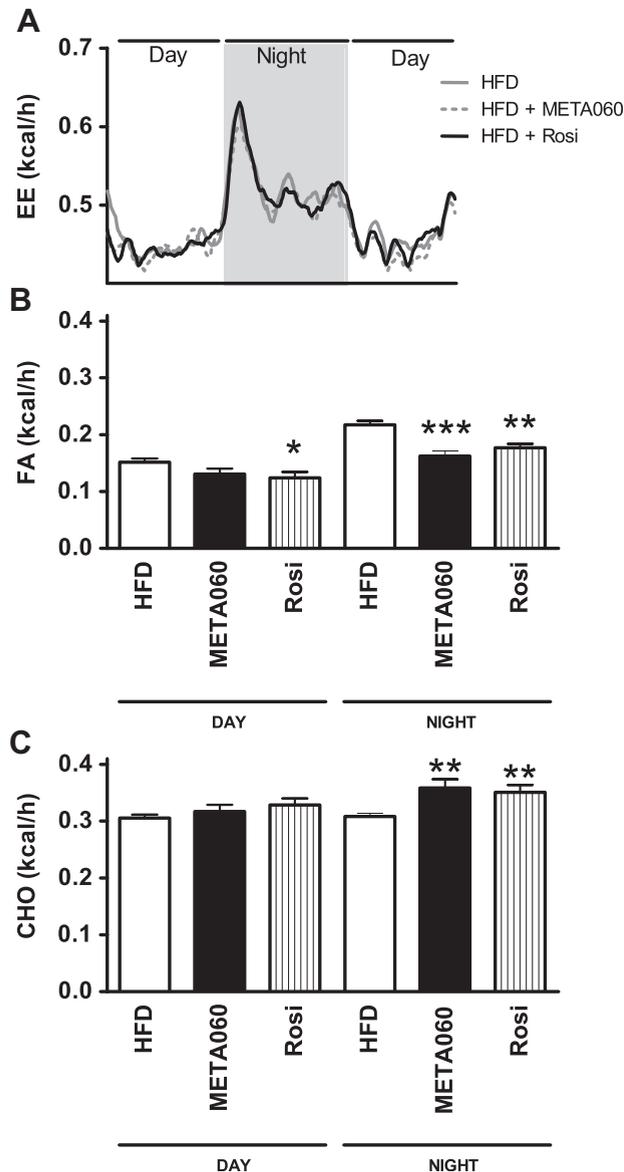
Whole-body substrate use was examined for approximately 36 h during week 4 of the dietary intervention. Four weeks of dietary intervention was chosen because, at this time point in the 14-wk study, body weight was still increasing and a new set point had not yet been reached.

Although we did not directly compare the LFD group during the 5-wk study, we knew from published and experimental data that 5 wk of HFD feeding in mice results in an unaltered total energy expenditure (kilocalories per hour) but in changes RER, fat (FA), and CHO oxidation. Daytime RER was  $0.84 \pm 0.04$  versus  $0.94 \pm 0.04$ , and night-time RER was  $0.84 \pm 0.03$  versus  $0.93 \pm 0.04$  for the HFD versus LFD group, respectively ( $P < 0.05$ ). The daytime FA oxidation was  $0.17 \pm 0.05$  versus  $0.07 \pm 0.04$ , and the night-time FA oxidation was  $0.19 \pm 0.05$  versus  $0.08 \pm 0.06$  for the HFD versus LFD group, respectively ( $P < 0.05$ ). The META060 or rosiglitazone intervention started from day 0, when the animals were switched from chow to the HFD, as described in MATERIALS AND METHODS.

The nocturnal and diurnal data were analyzed separately to distinguish between periods of low (diurnal) and high (nocturnal) physical activity. The total energy expenditure was similar across all dietary intervention groups (Fig. 3A). For food intake, no significant differences were observed between the groups (data not shown). Despite similarities in total energy expenditure and food intake, the HFD-fed mice supplemented with META060 or rosiglitazone exhibited a significantly lower mean nocturnal FA oxidation rate than the HFD-only group ( $0.16 \pm 0.01$  and  $0.18 \pm 0.01$  versus  $0.22 \pm 0.01$  kcal/h,  $P < 0.001$  and  $P < 0.01$ , respectively), and rosiglitazone had a lower mean diurnal FA oxidation rate compared with the control group ( $0.12 \pm 0.01$  versus  $0.15 \pm 0.01$  kcal/h,  $P < 0.05$ ; Fig. 3B). In addition, the nocturnal CHO oxidation levels were increased in the HFD-fed mice that received META060 or rosiglitazone compared with controls ( $0.36 \pm 0.02$  and  $0.35 \pm 0.01$  versus  $0.31 \pm 0.01$  kcal/h,  $P < 0.01$ ; Fig. 3C). This increased CHO-to-fat oxidation ratio was reflected in the RER. META060 and rosiglitazone significantly increased the RER in HFD-fed mice during the diurnal period compared with the HFD-only-fed mice ( $0.88 \pm 0.00$  and  $0.89 \pm 0.01$  versus  $0.86 \pm 0.01$ ,  $P < 0.05$  and  $P < 0.01$ , respectively) and during the nocturnal period ( $0.87 \pm 0.01$  and  $0.86 \pm 0.00$  versus  $0.83 \pm 0.01$ ,  $P < 0.001$ , respectively; Fig. 4A, B). To test the ability of the animals to adjust the fuel oxidation to fuel availability (metabolic flexibility), the animals were fasted overnight; subsequently, the food was replaced and the RER was monitored. The META060- and rosiglitazone-supplemented mice had a significantly higher RER when the food was replaced compared with the HFD-only-treated mice ( $0.94 \pm 0.00$  and  $0.94 \pm 0.00$  versus  $0.92 \pm 0.00$ ,  $P < 0.001$ ), indicating greater metabolic flexibility in the META060- or rosiglitazone-fed animals (Fig. 4C). Physical activity measurements did not show differences in either treatment group compared with the HFD-only-fed mice (data not shown).

#### META060 had no effect on fecal FA composition and concentration

Because META060 decreased fat oxidation, we investigated whether fat absorption was impaired in the META060-supplemented mice. The fecal FA composition and concentration were determined in samples collected during metabolic cage experiments (data not shown). No difference was found in total fecal weight. Furthermore, the quantitative gas chromatographic analysis showed equal fecal FA composition and fecal FA content in all treatment groups. Together with the

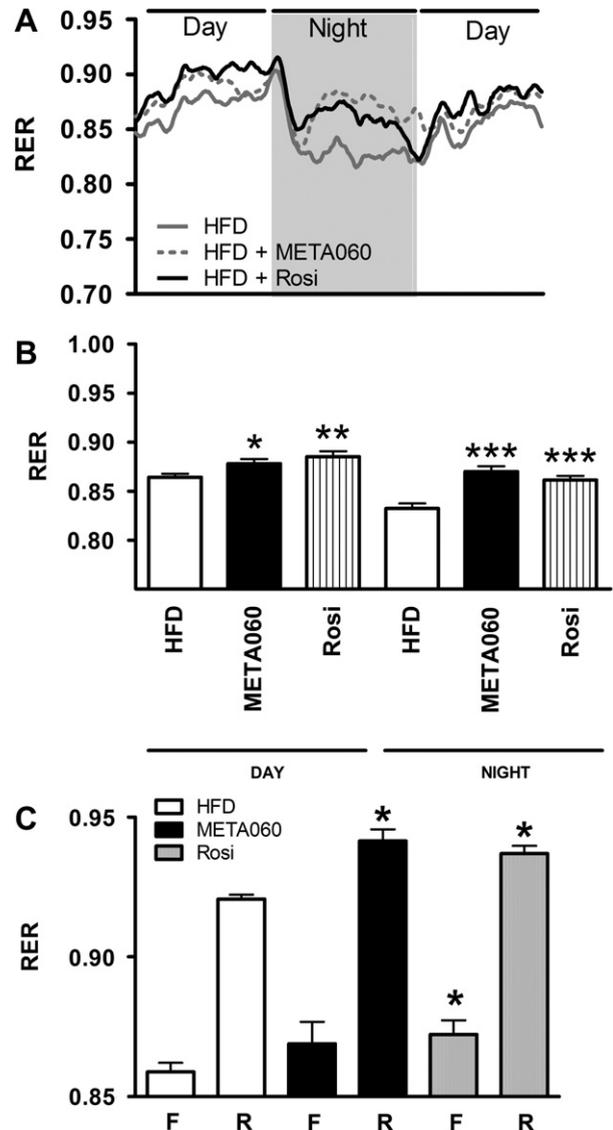


**Fig. 3.** METAO60 affects substrate use. During week 4 of the dietary intervention, oxygen consumption and carbon dioxide production were recorded for 36 h in HFD-fed mice without supplementation or supplemented with METAO60 100 mg · kg<sup>-1</sup> · d<sup>-1</sup> or Rosi 1 mg · kg<sup>-1</sup> · d<sup>-1</sup>. The night period is represented by the shaded area on the graph. (A) EE was calculated as described in MATERIALS AND METHODS. (B) FA and (C) CHO oxidations for the day or night phase were calculated for the HFD-fed mice without supplementation or in those supplemented with METAO60 or Rosi. Data are presented as mean ± SE (*n* = 8 per group). \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001. CHO, carbohydrate; EE, energy expenditure; FA, fatty acid; HFD, high-fat diet; Rosi, rosiglitazone.

equivalent food intake, this implies a similar intestinal absorption of lipids.

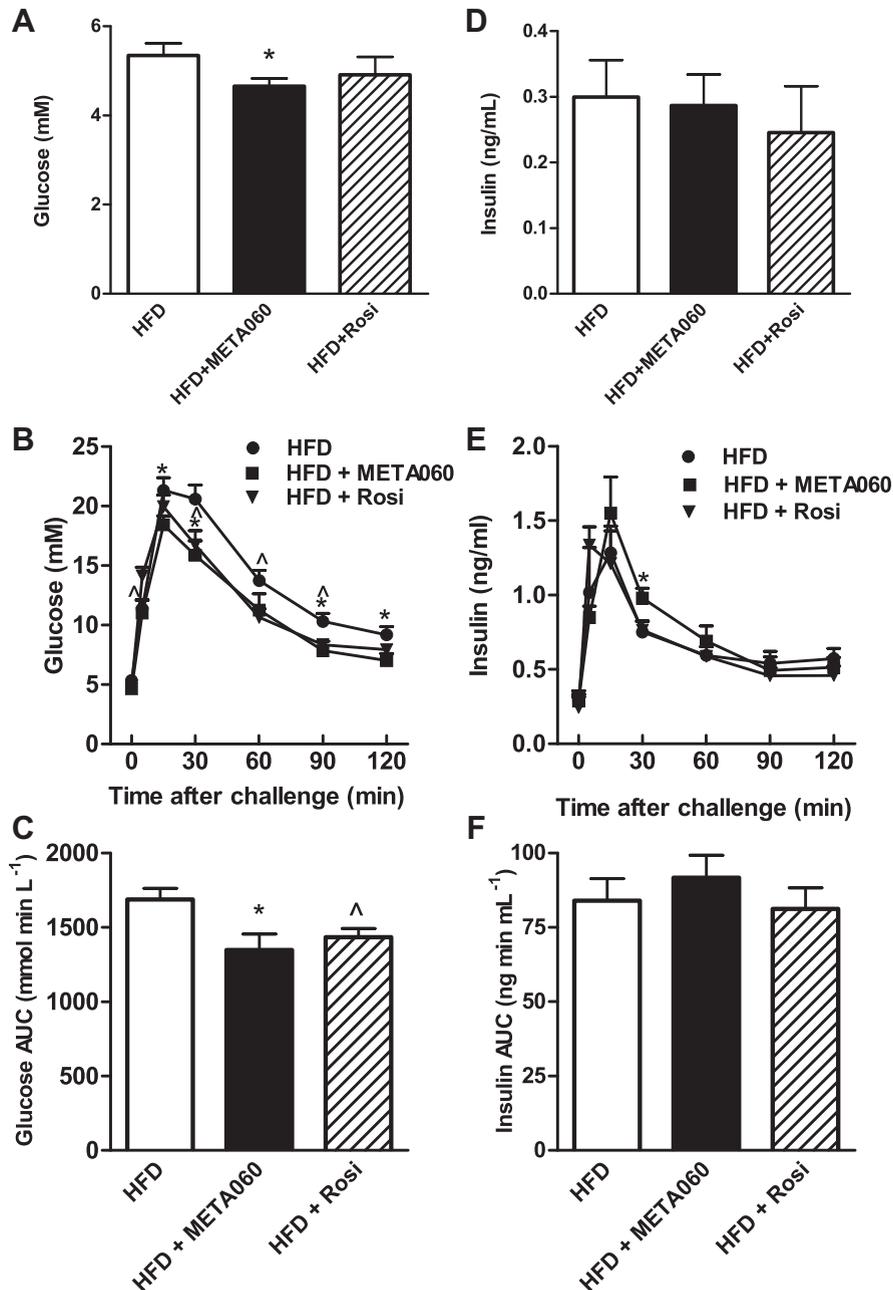
#### METAO60 improved glucose tolerance in HFD-fed mice

Because an increased CHO-to-fat oxidation ratio and an increased metabolic flexibility suggest protection against HFD-induced insulin resistance, oral glucose tolerance tests were performed during week 5 of the dietary intervention. After an overnight fast, blood glucose concentrations were lower in METAO60-supplemented mice compared with HFD-only-treated mice (4.66 ± 0.2 versus 5.34 ± 0.3, *P* < 0.05; Fig. 5A), whereas



**Fig. 4.** METAO60 increases RER and metabolic flexibility. (A) The RER was monitored for 36 h in HFD-fed mice without supplementation or supplemented with METAO60 100 mg · kg<sup>-1</sup> · d<sup>-1</sup> or Rosi 1 mg · kg<sup>-1</sup> · d<sup>-1</sup>. (B) The RER for the day or night phase was calculated for each treatment group (*n* = 8 per group). \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001. (C) The HFD-fed mice with or without supplementation with METAO60 or Rosi for 4 wk were fasted overnight and then feeding was resumed. The RER was calculated after fasting and after refeeding (*n* = 8 per group). \* *P* < 0.05. Data are presented as mean ± SE. F, after fasting; HFD, high-fat diet; R, after refeeding; RER, respiratory exchange rate; Rosi, rosiglitazone.

fasting insulin levels were not significantly different among treatment groups (Fig. 5D). After the glucose challenge, plasma glucose and insulin levels were determined at intervals up to 120 min, and areas under the curve (AUCs) were calculated. Glucose concentrations were significantly decreased for mice supplemented with METAO60 compared with HFD-fed mice at 15, 30, 90, and 120 min after the glucose challenge, and the mean AUC was 20% lower than in HFD-fed mice (*P* < 0.05; Fig. 5B, C). Rosiglitazone also significantly decreased the plasma glucose levels at 5, 30, 60, and 90 min after the glucose challenge, and the mean AUC was 15% lower than in HFD-fed mice (*P* < 0.05). These observations show that METAO60 and rosiglitazone improved glucose tolerance in mice fed an HFD for 5 wk. This may be due to an increased insulin sensitivity in response to an oral glucose



**Fig. 5.** META060 improves glucose tolerance. The mice were fed an HFD or an HFD supplemented with META060  $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  or Rosi  $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ . During week 5 of the dietary intervention and after an overnight fast, the blood samples were collected, and (A) glucose and (D) insulin concentrations were determined. Oral glucose tolerance tests were performed in fasted mice. (B) Glucose and (E) insulin concentrations were recorded at time points up to 120 min and AUCs for (C) glucose and (F) insulin were calculated. Values are presented as mean  $\pm$  SE ( $n = 7\text{--}9$  per group). \*  $P < 0.05$  HFD versus HFD + META060,  $P < 0.05$  HFD versus HFD + Rosi. AUC, area under the curve; HFD, high-fat diet; Rosi, rosiglitazone.

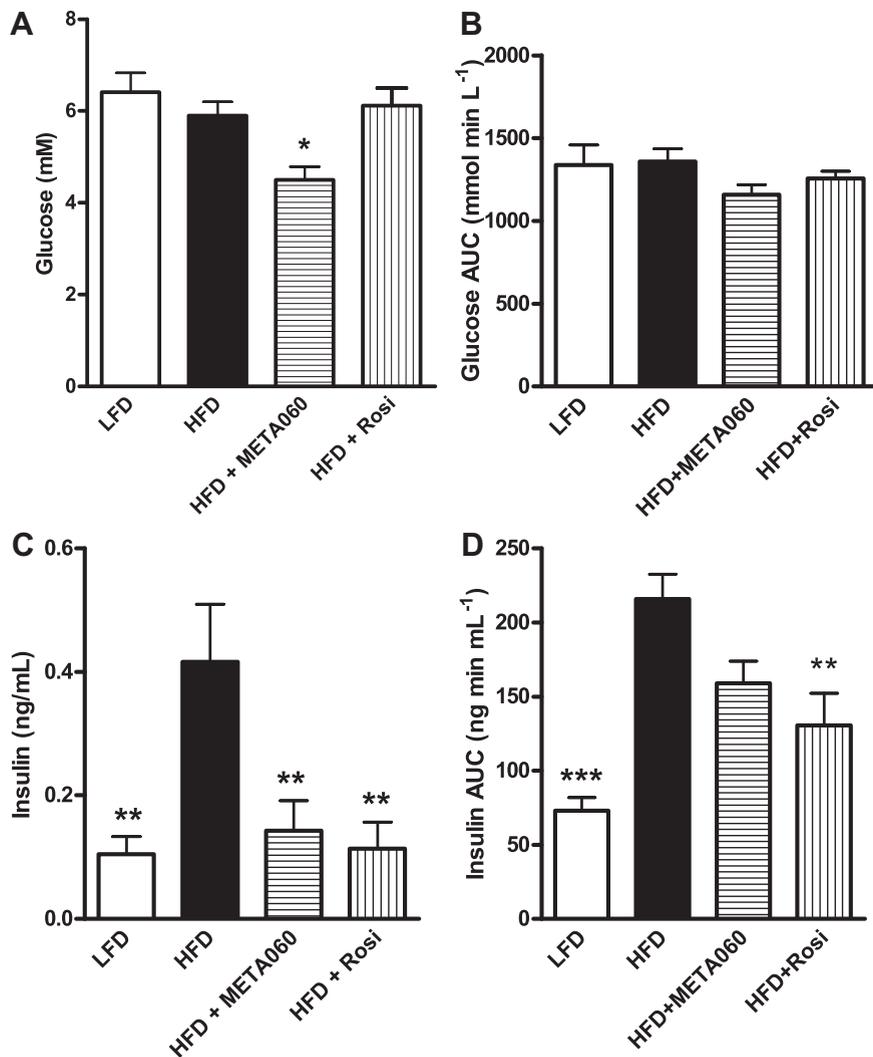
load because the time course and AUC for plasma insulin levels were comparable in all groups (Fig. 5E, F).

After 14 wk of the dietary intervention, the fasting blood glucose concentration in the META060-supplemented mice was significantly lower than in the HFD-fed mice ( $4.5 \pm 0.3$  versus  $5.9 \pm 0.3$  mmol/L,  $P < 0.05$ ; Fig. 6A). Moreover, the fasting insulin concentration was significantly decreased in the META060-supplemented mice compared with the HFD-fed mice ( $0.14 \pm 0.05$  versus  $0.42 \pm 0.09$  ng/mL,  $P < 0.001$ ; Fig. 6C). This implies that after long-term META060 supplementation, insulin sensitivity in HFD-fed mice was increased. Oral glucose tolerance tests were performed in mice and the blood glucose and insulin

concentrations were recorded at several time points up to 120 min after the challenge. The AUC for glucose was similar among all groups (Fig. 6B). However, the AUC for insulin was increased in the HFD group, and only rosiglitazone supplementation had a statistically significant effect on decreasing the insulin response compared with the HFD group (40%,  $P < 0.05$ ; Fig. 6D).

## Discussion

In the present study, we investigated the effects of META060 on HFD-induced obesity and insulin resistance. Supplementation with META060 decreased the weight gain in the HFD-fed mice.



**Fig. 6.** Long-term META060 supplementation decreases plasma glucose and insulin concentrations. Fasting (A) plasma glucose and (C) insulin concentrations were determined in mice fed an LFD, an HFD, or an HFD supplemented with META060  $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  for 14 wk as described in MATERIALS AND METHODS. Oral glucose tolerance tests were performed in fasted mice, and blood concentrations of glucose and insulin were determined at time points up to 120 min. The AUCs are presented for (B) glucose and (D) insulin. Values are presented as mean  $\pm$  SE ( $n = 5\text{--}6$  per group). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . AUC, area under the curve; HFD, high-fat diet; LFD, low-fat diet; Rosi, rosiglitazone.

This effect was significant after 3 wk and was sustained for up to 20 wk. Furthermore, when the META060 feeding was terminated, the mice began to gain weight rapidly. META060 inhibited the fat accumulation in HFD-fed mice as evidenced by a decrease in adipose tissue mass in mice supplemented with META060 compared with the HFD-fed control mice. In addition, META060 improved glucose tolerance after 5 wk of supplementation. Moreover, long-term META060 supplementation in HFD-fed mice clearly decreased the fasting blood glucose and insulin levels. These data suggest that META060 improves glucose homeostasis similarly to rosiglitazone and prevents HFD-induced obesity and insulin resistance.

Rosiglitazone, an antidiabetic drug from the class of thiazolidinediones, increases insulin sensitivity through its action on the peroxisome proliferator-activated receptor- $\gamma$  and has exhibited anti-inflammatory activity through a mechanism involving nuclear factor- $\kappa$ B [15,16]. Although the mechanistic target(s) of META060 has not been identified, previous studies have indicated that META060 has potent inhibitory effects on several kinases regulating the nuclear factor- $\kappa$ B pathway, including glycogen synthase kinase-3 and phosphatidylinositol-3 kinase [12]. In this

study, META060 showed effects on insulin sensitivity similar to that of rosiglitazone, prompting us to speculate whether the improvement of glucose tolerance in META060-treated mice is mediated through a peroxisome proliferator-activated receptor- $\gamma$ -dependent mechanism. However, rosiglitazone was not as effective at preventing weight gain in HFD-fed mice as was META060, suggesting an alternative or additional mechanism of action for META060.

Results from the metabolic experiments indicated that supplementation with META060 increased the RER, metabolic flexibility, and the CHO-to-fat oxidation ratio in HFD-fed mice. These observations are congruent with the increased insulin sensitivity and improved CHO handling induced by META060. Differences in metabolism and weight also were observed when the fat intake or absorption was not consistent across treatment groups. However, the metabolic experiments also indicated that META060 did not affect total energy expenditure, food intake, or FA secretion into the feces and thus do not explain the decrease in weight gain of META060-supplemented mice. Therefore, metabolic measurements may not be sufficient to resolve a mechanism for the global effects of META060 on the mouse metabolism.

The mice used in the 5-wk study were slightly younger than those in the longer-term experiment, and age may have a potential impact on physical activity, food intake, energy expenditure, or other metabolic processes. Although mice of different ages may have distinct metabolic characteristics contributing to the results we observed, the effects of META060 on weight gain and glucose homeostasis were consistent in the short-term and long-term experiments.

Results from *in vitro* studies in a human cecal cell line have shown that META060 increases Glucagon-like-peptide-1 (GLP-1) secretion (data not shown). Because GLP-1 is an insulin-sensitizing hormone, this *in vitro* effect of META060 is consistent with the *in vivo* effects on glucose homeostasis. The activation of GPR120, a G-protein-coupled receptor that regulates GLP-1 secretion [17–19], may function as a mechanistic target for META060-dependent GLP-1 secretion, although further studies will be required to investigate this possibility.

Future studies will focus on elucidating the mechanism of action underlying the effects of META060 on preventing weight gain in HFD-fed mice and investigating whether META060 is effective in decreasing weight in obese animals. META060 decreases fasting plasma glucose and insulin concentrations, and further research into its activity on insulin signaling and hepatocyte metabolism is needed. The present data suggest that META060 may have therapeutic value as an antidiabetic or antiobesity agent, and future investigations will evaluate its potential clinical use.

## Conclusion

META060 supplementation significantly decreased the amount of weight gained in mice on an HFD. Indirect calorimetric measurements showed an increased metabolic flexibility in mice, and the mice exhibited an improved glucose tolerance comparable to the effects of rosiglitazone treatment. We conclude that META060 has potential therapeutic value for managing obesity and insulin resistance.

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