Original Article

MOLECULAR METABOLISM

Metabolic adaptation to intermittent fasting is independent of peroxisome proliferator-activated receptor alpha

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ABSTRACT

Background: Peroxisome proliferator-activated receptor alpha (PPARA) is a major regulator of fatty acid oxidation and severe hepatic steatosis occurs during acute fasting in *Ppara*-null mice. Thus, PPARA is considered an important mediator of the fasting response; however, its role in other fasting regiments such as every-other-day fasting (EODF) has not been investigated.

Methods: Mice were pre-conditioned using either a diet containing the potent PPARA agonist Wy-14643 or an EODF regimen prior to acute fasting. *Ppara*-null mice were used to assess the contribution of PPARA activation during the metabolic response to EODF. Livers were collected for histological, biochemical, qRT-PCR, and western blot analysis.

Results: Acute fasting activated PPARA and led to steatosis, whereas EODF protected against fasting-induced hepatic steatosis without affecting PPARA signaling. In contrast, pretreatment with Wy-14,643 did activate PPARA signaling but did not ameliorate acute fasting-induced steatosis and unexpectedly promoted liver injury. *Ppara* ablation exacerbated acute fasting-induced hypoglycemia, hepatic steatosis, and liver injury in mice, whereas these detrimental effects were absent in response to EODF, which promoted PPARA-independent fatty acid metabolism and normalized serum lipids.

Conclusions: These findings indicate that PPARA activation prior to acute fasting cannot ameliorate fasting-induced hepatic steatosis, whereas EODF induced metabolic adaptations to protect against fasting-induced steatosis without altering PPARA signaling. Therefore, PPARA activation does not mediate the metabolic adaptation to fasting, at least in preventing acute fasting-induced steatosis.

Keywords PPARA; PPARalpha; Intermittent fasting; Every-other-day fasting; Steatosis; Adaptive fasting response

1. INTRODUCTION

Numerous studies have shown that intermittent fasting can prevent or delay the onset of metabolic diseases [1—3] including hepatic steatosis [4—6], while a single bout of acute fasting promotes hepatic lipid accumulation [7]. These studies indicate that the physiological response and underlying metabolic alterations associated with repeated fasting-refeeding cycles and a single acute fast are very different. In fact, acute fasting can impose systemic metabolic stress in most species [8], while fasting followed by refeeding triggers metabolic reprogramming, restores metabolic homeostasis, and is accompanied by additional alterations that protect against subsequent periods of food deprivation [8]. In this context, and according to the physiological description of stress response and adaptive response [9,10], the physiological response to the initial damage caused by a single bout of acute fasting should be considered a 'stress response', while the protective effects that result from the habitual response to repeated stress followed by a recovery period that is imposed by fasting-refeeding cycles would be an 'adaptive response'. The adaptive response that results from fasting, and in particular refeeding, regimens may consequently lead to the many beneficial metabolic effects associated with intermittent fasting.

Intermittent fasting as an intervention in human populations is somewhat impractical; however, the development of therapeutics that target fasting associated pathways are of great interest as they avoid having to impose a strict dietary regime. Given that fasting is

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characterized by the depletion of hepatic glycogen, increased lipid utilization, and elevated serum ketone bodies [8] and that peroxisome proliferator-activated receptor alpha (PPARA) is a major regulator of fatty acid oxidation (FAO) and ketogenesis, this ligand-activated nuclear receptor is considered a key mediator of the fasting response [11–13]. Indeed, *Ppara*-null or liver-specific *Ppara*-null mice show a dramatic reduction of FAO, and display severe hepatic steatosis and hypoglycemia after acute fasting [11,14–16]. These studies provide evidence of a role for PPARA during fasting but provide no evidence for its role during refeeding. Therefore, it's difficult to differentiate whether PPARA mediates a stress response to fasting, an adaptive response to fasting, or both. Moreover, current evidence on the role of pharmacological PPARA activation using a variety of agonists in steatosis is inconsistent. Some studies support the view that activation of PPARA can ameliorate steatosis in several models [17–19], some do not [20–22], and one report even suggested that PPARA can promote steatosis [23]. Collectively, current evidence indicates that although PPARA governs FAO, and loss of PPARA exacerbates acute fasting-induced steatosis and hypoglycemia in mice, the concept that PPARA is a mediator of the adaptive response to fasting remains to be elucidated. If PPARA is indeed responsible for the metabolic adaptation to fasting, it would be reasonable to expect that 1) pharmacological activation of PPARA prior to fasting would simulate adaptive response and substantially ameliorate acute fasting-induced steatosis, and 2) mice pre-conditioned by repeated fasting-refeeding cycles would activate PPARA and suppress acute fasting-induced steatosis.

Therefore, in the current study, wild-type and *Ppara*-null mice were placed on an every-other-day fasting (EODF) regimen and/or treated with the potent PPARA agonist Wy-14643 to explore the effect of PPARA on the adaptive response to fasting. The results suggest that although PPARA deficiency aggravates acute fasting-induced steatosis, EODF elicits a pronounced metabolic adaptation to prevent fasting-induced hepatic steatosis independent of PPARA.

2. MATERIALS & METHODS

2.1. Mice

All procedures involving mice were performed under protocols approval by the National Cancer Institute Animal Care and Use Committee. Six-week-old male C57BL/6N mice were purchased from Charles River lab. Six-to eight-week-old wild-type (WT) or *Ppara*-null (*Ppara*$^{-/-}$) mice on the C57BL/6N background were described previously [24,25]. Mice were housed in a temperature- and light-controlled vivarium with free access to water and standard rodent chow (NIH-07, Envigo, Huntingdon, United Kingdom), a standard control grain diet (F3028, Bio-Serve, Flemington, NJ), or a standard control grain diet containing 0.1% Wy-14643 (Bio-Serv, Flemington, NJ). Male 2- to 4-month-old mice were used in all experiments unless otherwise indicated.

2.2. EODF and Wy-14643 treatment

Mice were randomly divided into control (CON) and intermittent fasting groups. All mice were fed with standard chow diet and co-housed (2 mice per cage) for two weeks prior to study initiation to allow for acclimation to the animal facility. The CON group was allowed unrestricted, *ad libitum* access to a chow diet, while the intermittent fasting group was fed on alternating 24 h periods of free access to food followed by fasting for 20 cycles, referred to as every-other-day fasting (EODF). After the pre-conditioned intermittent fasting period, the EODF mice were divided in half; one set was killed at the end of a 24 h feeding period, and the other set was killed at the end of a 24 h fasting period. Control mice were also split into two groups with half of the animals killed at the end of a 24 h feeding period and the remaining half killed after a 24 h fasting period to match the fasted EODF group. In the *Ppara*-null versus WT mouse study, mice were fasted 16 h due to the pronounced steatosis and liver damage observed in fasted *Ppara*-null mice in response to prolonged fasting. For Wy-14643 studies, the mice were allowed unrestricted access to a standard control grain diet for two weeks to allow for acclimation to the grain diet, and then randomly separated into two groups and placed on either a diet containing 0.1% Wy-14643 (100 mg/kg/day) or a matching grain control diet for two days. Half of the mice were then killed in the fed state, and the other half were killed after fasting.

2.3. Respiratory exchange ratio assay

Respiratory exchange ratios (RER) were measured by indirect calorimetry in mice after 14 cycles of EODF treatment using a 12-chamber Environment Controlled CLAMS (Columbus Instruments, Columbus, OH) with one mouse/chamber as previously described [26]. After a 48 h chamber acclimatization, the mice were monitored for 24 h during the fed state followed by 20 h during the fasting state. During testing, water was provided *ad libitum*. Locomotor activity was recorded at the same time using an infrared beam interruption system.

2.4. Serum glucose and fasting blood glucose assay

For assaying serum glucose, blood samples were collected into tubes containing no anticoagulant from retro-orbital sinus per a standard procedure (Parasuraman et al., 2010) and then were sequentially clotted for 30 min on ice, centrifuged at 2,000 g for 15 min at 4 °C, pipetted off the top yellow serum layer, and measured using a Glucometer (Bayer, Pittsburgh, PA). Fasting blood glucose was detected using a Glucometer (Bayer, Pittsburgh, PA) by tail bleeds in mice after fasting for 16 h.

2.5. Serum biochemical assays

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed in a 96-well microplate using a commercial ALT or AST assay kit (Catachem, Bridgeport, CT), and monitored at 340 nm for 5 min with a microplate reader (BioAssay Systems, Harvard, CA). Serum ketone bodies and non-esterified fatty acid (NEFA) were assayed using Wako Clinical Diagnostics kits (Wako USA, Richmond, VA). Serum insulin levels were tested with an ultra-sensitive mouse insulin ELISA kit (90080, Crystal Chem, Downers Grove, IL).

2.6. Liver lipid and glucose analysis

For analysis of liver lipid and glucose content, 20 mg of frozen liver was homogenized in 400 μl of 50 mM Tris + 5% Triton-X 100, then samples were heated to 80–100 °C and cooled to room temperature (repeated twice). After centrifugation, the supernatants were diluted 1–5 fold, lipids were quantified using Wako Clinical Diagnostics kits (Wako USA, Richmond, VA), and glucose concentrations assayed using a Glucometer (Bayer, Pittsburgh, PA).

2.7. Serum acylcarnitines profiling

Serum acylcarnitines were determined as previously described [27]. In brief, 5 μl serum was diluted with 45 μl acetonitrile containing 0.1 μM D3-palmitoylcarnitine and centrifuged at 16,000 g for 15 min at 4 °C. The supernatant was removed, dried under vacuum, re-dissolved in HPLC-grade water containing 2% acetonitrile, and assayed by LC-MS. All MS data were analyzed by Waters MassLynx software.

