METABOLISM CLINICAL AND EXPERIMENTAL 65 (2016) 714–727

Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Short-term feeding at the wrong time is sufficient to desynchronize peripheral clocks and induce obesity with hyperphagia, physical inactivity and metabolic disorders in mice

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ARTICLE INFO

Article history:
Received 11 November 2015
Accepted 2 February 2016

Keywords:
Feeding rhythm
Leptin resistance
Lipogenesis
Peripheral clock

ABSTRACT

Background. The circadian clock regulates various physiological and behavioral rhythms such as feeding and locomotor activity. Feeding at unusual times of the day (inactive phase) is thought to be associated with obesity and metabolic disorders in experimental animals and in humans.

Objective. The present study aimed to determine the underlying mechanisms through which time-of-day-dependent feeding influences metabolic homeostasis.

Methods. We compared food consumption, wheel-running activity, core body temperature, hormonal and metabolic variables in blood, lipid accumulation in the liver, circadian expression of clock and metabolic genes in peripheral tissues, and body weight gain between mice fed only during the sleep phase (DF, daytime feeding) and those fed only during the active phase (NF, nighttime feeding). All mice were fed with the same high-fat high-sucrose diet throughout the experiment. To the best of our knowledge, this is the first study to examine the metabolic effects of time-imposed restricted feeding (RF) in mice with free access to a running wheel.

Results. After one week of RF, DF mice gained more weight and developed hyperphagia, higher feed efficiency and more adiposity than NF mice. The daily amount of running on the wheel was rapidly and obviously reduced by DF, which might have been the result of time-of-day-dependent hypothermia. The amount of daily food consumption and hypothalamic mRNA expression of orexigenic neuropeptide Y and agouti-related protein

Abbreviations: Actb, Actin beta; Agrp, agouti related protein; HOMA-IR, homeostasis model assessment of insulin resistance; Npy, neuropeptide Y.

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http://dx.doi.org/10.1016/j.metabol.2016.02.003

were significantly higher in DF, than in NF mice, although levels of plasma leptin that fluctuate in an RF-dependent circadian manner, were significantly higher in DF mice. These findings suggested that the DF induced leptin resistance. The circadian phases of plasma insulin and ghrelin were synchronized to RF, although the corticosterone phase was unaffected. Peak levels of plasma insulin were remarkably higher in DF mice, although HOMA-IR was identical between the two groups. Significantly more free fatty acids, triglycerides and cholesterol accumulated in the livers of DF, than NF mice, which resulted from the increased expression of lipogenic genes such as *Scd1*, *Acaca*, and *Fasn*. Temporal expression of circadian clock genes became synchronized to RF in the liver but not in skeletal muscle, suggesting that uncoupling metabolic rhythms between the liver and skeletal muscle also contribute to DF-induced adiposity.

Conclusion. Feeding at an unusual time of day (inactive phase) desynchronizes peripheral clocks and causes obesity and metabolic disorders by inducing leptin resistance, hyperphagia, physical inactivity, hepatic fat accumulation and adiposity.

1. Introduction

An internal circadian timing system entrains various physiological and behavioral rhythms such as sleep/wake cycles, body temperature, metabolism and hormone secretion to environmental cues such as light–dark cycles and temporal feeding patterns [1]. The mammalian master clock is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus [2]. The circadian oscillator in the SCN is driven by core clock genes such as *Clock*, *Arntl* (also known as [a.k.a.] *Bmal1*), *Per1*, *Per2*, *Cry1*, and *Cry2* based on transcriptional feedback loops [2]. The circadian transcription factors CLOCK and ARNTL heterodimerize and transactivate target genes such as *Per* and *Cry* by binding to E-box elements in their promoters [2]. The PER and CRY proteins heterodimerize and translocate to the nucleus where they inhibit CLOCK:ARNTL transcriptional activity [2]. Circadian oscillators called peripheral clocks are located in most peripheral organs such as the heart, lungs, liver, skeletal muscle and adipose tissues since core clock components have been identified in these tissues [2]. These peripheral clocks are synchronized to the SCN by systemic time cues that include both neuronal signals and circulating humoral factors such as glucocorticoids and insulin [1]. Components of the molecular clock regulate the expression of hundreds of metabolic output genes in peripheral tissues [1].

Feeding time is the dominant Zeitgeber for peripheral clocks in mammals. Time-imposed restricted feeding (RF) during the light phase in nocturnal rodents inverts the phase of circadian gene expression in many tissues such as the liver, heart, and kidneys, without affecting that in the SCN [3,4]. The central clock in the SCN is dispensable for entraining peripheral clocks to feeding cycles [5] and core components of the molecular clock such as *Clock* [6] or *Per* genes [7] are not essential for entraining peripheral clocks to feeding cycles. The molecular mechanisms of the RF-induced entrainment of peripheral circadian gene expression remain unknown, although humoral signals such as hormones, nutrients, body temperature and redox potential are apparently involved [3,4].

Epidemiological studies have suggested that circadian disruption caused by shift work, jet lag and sleep disorders is associated with obesity and metabolic syndrome [8,9]. Irregular eating or eating at the wrong time of day such as skipping breakfast and eating during the night might contribute to metabolic disorders induced by circadian disruption [10,11]. Studies of experimental animals have shown that RF affects body-weight, adiposity and other metabolic parameters [12–20]. Mice fed with a high-fat diet only during the sleep (light) phase over a period of six weeks gained 2.5-fold more weight than mice given the same diet during the active (dark) phase, although caloric intake and physical activity were identical between the groups [18]. Sherman et al. [12] found that mice fed with a high-fat diet for 18 weeks only during the dark phase gained 18% less body weight, decreased cholesterol by 30% and improved insulin sensitivity, compared with mice fed *ad libitum*. Hatori et al. [15] also showed that mice fed with a high-fat diet for 18 weeks only during the dark phase gained considerably less body weight than mice fed *ad libitum*, despite identical caloric intake. Importantly, RF-induced metabolic changes seemed to depend on dietary fat content [12,15]. Although many metabolic parameters are influenced by RF in experimental animals [12–19], the underlying mechanisms remain to be elucidated.

The present study aimed to quantify the early effect of RF on the behavior and physiology of mice fed with a high-fat high-sucrose diet (HFHSD), because RF seems to affect body weight gain within a few weeks [12,15,18]. Since a combination of fat and carbohydrate intake contributes to human obesity, we postulated that feeding mice with the HFHSD would mimic human diabetes and obesity more closely. We compared food consumption, wheel-running activity, hormonal and metabolic variables in blood, hepatic lipid accumulation, circadian expression of clock and metabolic genes in peripheral tissues and body weight between mice fed only during the sleep phase (DF, daytime feeding) or only during the active phase (NF, nighttime feeding). We also examined the effect of RF on behavior and physiology in mice with free access to a running wheel because metabolic status such as food consumption, body composition, hormonal secretion and metabolic gene expression remarkably differs between sedentary and exercised animals [21]. Wheel-running is a voluntary behavior in rodents [22], and the metabolic effects of experiments largely depend on such behavior [23]. To the