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Original article

Influence of dark phase restricted high fat feeding on myocardial adaptation in mice

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ABSTRACT

Prolonged high fat feeding is associated with myocardial contractile dysfunction in rodents. However, epidemiological data do not necessarily support the concept that fat-enriched diets adversely affect cardiac function in humans. When fed in an *ad libitum* manner, laboratory rodents consume chow throughout the day. In contrast, humans typically consume food only during the awake phase. Discrepancies between rodent and human feeding behaviors led us to hypothesize that the time of day at which dietary lipids are consumed significantly influences myocardial adaptation. In order to better mimic feeding behavior in humans, mice were fed (either a control or high fat diet) only during the 12-hour dark phase (*i.e.*, no food was provided during the light phase). We report that compared to dark phase restricted control diet fed mice, mice fed a high fat diet during the dark phase exhibit: 1) essentially normal body weight gain and energy balance; 2) increased fatty acid oxidation at whole body, as well as skeletal and cardiac muscle (in the presence of insulin and/or at high workloads) levels; 3) induction of fatty acid responsive genes, including genes promoting triglyceride turnover in the heart; 4) no evidence of cardiac hypertrophy; and 5) persistence/improvement of myocardial contractile function, as assessed *ex vivo*. These data are consistent with the hypothesis that ingestion of dietary fat only during the more active/awake period allows adequate metabolic adaptation, thereby preserving myocardial contractile function. This article is part of a Special Issue entitled "Focus on cardiac metabolism".

1. Introduction

Common environmental factors within Western society, such as excess caloric intake, physical inactivity, and sleep deprivation are strongly associated with the development of modern day cardiometabolic diseases, including obesity and type 2 diabetes mellitus, as well as cardiovascular disease (CVD) [1,2]. In terms of nutritional influences, significant research efforts have focused on the quantity and/or quality (*i.e.*, nutritional content) of calories ingested as a means of interrogating the effects of, and potential mechanisms responsible for, diet-induced alterations in heart function. Regarding macronutrients, the influence of dietary lipids/fat on myocardial function has received much attention in both human- and animal-based research, resulting in substantial controversy. It is therefore not clear whether high or low fat diets are cardioprotective or cardiotoxic in humans [3–7]. In rodents, prolonged Western/high fat feeding has often been reported to modestly depress baseline myocardial contractile function [8–12]. These effects appear to be diet composition- and duration-dependent, as well as influenced by the species and strain of the rodent. For example, feeding C57/Bl6 mice a high fat diet for up to 12 weeks does not appear to adversely affect myocardial contractile function, despite development of marked obesity [13]. Continuation of high fat feeding for 16 weeks or longer, however, has been shown by multiple laboratories to cause myocardial contractile dysfunction in several mouse strains [8,9]. With regard to macronutrient composition, feeding rats a diet composed of 45% fat (more akin to a Western diet) attenuates myocardial contractile function to a greater extent than a 60% fat diet (more akin to a high fat/low carbohydrate Atkins diet) [11]. Similarly, we have recently reported that feeding mice a 45% high fat diet for 16 weeks in an *ad libitum* fashion depresses cardiac function [8]. These differences likely reflect distinct adaptation at multiple levels, including systemic and cardiac-specific.

The response of the myocardium to high fat diets is complicated further by the co-existence of additional disease/stress states. For instance, elevated fatty acid availability due to chronic high fat feeding [14] or acute increases *ex vivo* [15], reduces ischemia/reperfusion tolerance in rodent models, potentially due to alterations in myocardial metabolism

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(*e.g.*, an uncoupling of glycolysis from glucose oxidation, causing H^+ accumulation). In contrast, high fat feeding has been shown to improve myocardial contractile function during pressure overload-induced hypertrophy and permanent LAD occlusion-induced heart failure (potentially due to re-activation of fatty acid oxidation and reversal of an energy deficient state) [16–18].

The use of genetically altered mouse models has been essential for elucidation of novel mechanisms involved in the pathogenesis of human disease, including diet-induced myocardial dysfunction. It is, however, important to note that differences between rodent and human feeding behaviors exist. The most common experimental approach in rodent nutritional studies typically involves continual access to a single specialized diet. Under such conditions, the rodent consumes the diet throughout the 24-hour period in a contiguous manner, although a time-of-day dependent oscillation exists with respect to the quantity of food consumed (approximately one third of daily calories during the light (less active/sleep) period and two thirds during the dark (more active/awake) period, for wild-type mice) [19]. In contrast, humans typically consume meals with often distinct caloric quantity and quality, at discrete times of the waking hours. The potential importance of these differences in feeding behavior between humans and laboratory rodents has recently been highlighted by several studies reporting that the time-of-day at which a high fat diet is consumed influences multiple cardiometabolic syndrome parameters (including adiposity and glucose tolerance) [20–22]. However, to date the impact on myocardial function is unknown.

The purpose of the present study was to investigate the adaptation of the heart to high fat feeding, when restricted only to the dark phase. The effects of dark phase restricted high fat feeding on both extra-cardiac (*e.g.*, whole body energy balance, humoral factors, skeletal muscle metabolism) and myocardial adaptation (*e.g.*, metabolism, gene expression, contractile function) were assessed. In marked contrast to our recently reported observations for *ad libitum* high fat fed mice [8], we report that restricting high fat feeding to the dark phase results in adequate adaptation at whole body and myocardial levels, which is associated with preservation of myocardial contractile function.

2. Materials and methods

2.1. Animals

Male wild-type mice (on FVB/N background) were housed under temperature-, humidity-, and light-controlled conditions either at the Children's Nutrition Research Center (Baylor College of Medicine) or at the University of Alabama at Birmingham. A strict 12-hour light/12-hour dark cycle regime was enforced (lights on at 6 AM; zeitgeber time [ZT] 0). Mice received food and water *ad libitum*, unless otherwise specified. Mice were housed in standard micro-isolator cages, prior to initiation of feeding protocols (during which time mice were housed either in wire-bottom or CLAMS (Comprehensive Laboratory Animal Monitoring System) cages to prevent consumption of bedding or feces). All animal experiments were approved by the respective Institutional Animal Care and Use Committees.

2.2. Rodent diets and feeding studies

A high fat diet (45% calories from fat, Research Diets, New Brunswick, NJ; catalog number D12451) and a control diet (10% calories from fat, Research Diets, New Brunswick, NJ; catalog number D12450B) were utilized for this study; these same diets were utilized in our recently published *ad libitum* feeding studies [8]. Diets were matched for protein content, and fat and carbohydrate were derived from the same source for each diet. Mice were randomly assigned to one of four feeding groups: 1) *ad libitum* control diet; 2) *ad libitum* high fat; 3) *d*ark *p*hase restricted *c*ontrol *di*et (DPCD); and 4) *d*ark *p*hase restricted *h*igh *f*at (DPHF). For the latter two groups, mice were fasted during the 12-hour light phase (which represents the less active period for these nocturnal animals). When housed within wire bottom cages (for terminal studies, such as humoral factors, skeletal and cardiac muscle incubations/perfusions, and gene expression), feeding regimes were enforced manually (*i.e.*, addition/removal of food from the cage on a daily basis). When housed within the CLAMS, feeding regimes were enforced in a computer-controlled automated fashion by the CLAMS. In order to control for the potential stress associated with opening and closing of the CLAMS feeders, *ad libitum* fed mice were exposed to this same intervention twice daily (at ZT0 and ZT12, for only 1 min). Feeding regimes were initiated when mice were 12 weeks of age, and were enforced for either 12 weeks or 16 weeks (depending on the endpoint measurements).

2.3. Non-invasive mouse monitoring

Twenty-four hour patterns of food intake, energy expenditure (indirect calorimetry), and physical activity were measured using a CLAMS (Columbus Instruments Inc., Columbus, OH). This instrument also enforced the feeding regimes in an automated, computer-controlled manner. Body weight was monitored in mice at weekly intervals.

2.4. Humoral factor measurement

Plasma glucose, non-esterified fatty acid, triglyceride, cholesterol, glycerol, insulin, adiponectin, and leptin concentrations were measured using commercially available kits (Thermo Scientific, Waltham, MA; Wako Diagnostics, Richmond, VA; Crystal Chem Inc., Downers Grove, IL; Thermo Scientific, Waltham, MA).

2.5. Ex vivo assessment of skeletal muscle metabolism

Soleus muscle metabolism was assessed *ex vivo* using the isolated intact muscle preparation, essentially as described previously [23]. Briefly, muscles were rapidly excised from mice, tied at resting tension onto stainless steel clips, and pre-incubated in standard Krebs–Henseleit buffer supplemented with 8 mM glucose, 0.4 mM oleate conjugated to 3% BSA (fraction V, fatty acid-free; dialyzed), and 1 mUnit/ml insulin. Following a 45 minute pre-incubation period, muscles were transferred to incubation media (Krebs–Henseleit buffer supplemented with 8 mM glucose and 0.4 mM oleate conjugated to 3% BSA, as well as tracer amounts of [U-^{14}C]-glucose (0.5 mCi/l) and [9,10-^{3}H]-oleate (0.75 mCi/l), in either the absence or presence of a maximal concentration of insulin (1 mUnit/ml)). During both the pre-incubation and incubation periods, agitation, temperature (37 °C), and gassing (95% oxygen, 5% carbon dioxide) were maintained. Following a 60 minute incubation period, muscles were briefly blotted dry, and rapidly frozen in liquid nitrogen. Rates of oleate and glucose oxidation were determined as described previously [23].

2.6. Ex vivo assessment of heart metabolism and contractile function

Myocardial contractile function and metabolism were determined *ex vivo* through isolated working mouse heart perfusions, as described previously [8,24,25]. All hearts were perfused in the working mode in a non-recirculating manner with a preload of 12.5 mm Hg and an afterload of either 50 mm Hg (baseline) or 80 mm Hg (high). Standard Krebs–Henseleit buffer was supplemented with 8 mM glucose, 0.4 mM oleate conjugated to 3% BSA (fraction V, fatty acid-free; dialyzed), 0.05 mM L-carnitine, and 0.13 mM glycerol. Radiolabeled tracers ([U-^{14}C]-glucose (0.12 mCi/l) and [9,10-^{3}H]-oleate (0.067 mCi/l)) were utilized to monitor oxidative substrate metabolism. For the initial 30 min, hearts were perfused under baseline conditions (*i.e.*, 50 mm Hg afterload, no insulin). An insulin challenge was next performed; maximal insulin responsiveness was assessed for 30 min (*i.e.*, 1 mUnit/ml insulin). Finally, a workload challenge was performed; responsiveness