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Caloric restriction improves glucose homeostasis, yet increases cardiometabolic risk in caveolin-1-deficient mice

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ABSTRACT

Background and Purpose: The plasma membrane protein caveolin-1 (CAV-1) has been shown to be involved in modulating glucose homeostasis and the actions of the renin-angiotensin-aldosterone system (RAAS). Caloric restriction (CR) is widely accepted as an effective therapeutic approach to improve insulin sensitivity and reduce the severity of diabetes. Recent data indicate that polymorphisms of the CAV-1 gene are strongly associated with insulin resistance, hypertension and metabolic abnormalities in non-obese individuals. Therefore, we sought to determine whether CR improves the metabolic and cardiovascular (CV) risk factors in the lean CAV-1 KO mice.
Materials/Methods: Twelve- to fourteen-week-old CAV-1 knockout (KO) and genetically matched wild-type (WT) male mice were randomized by genotype to one of two dietary regimens: *ad libitum* (*ad lib*) food intake or 40% CR for 4 weeks. Three weeks following the onset of dietary restriction, all groups were assessed for insulin sensitivity. At the end of the study, all groups were assessed for fasting glucose, insulin, HOMA-IR, lipids, corticosterone levels and blood pressure (BP). Aldosterone secretion was determined from acutely isolated Zona Glomerulosa cells.
Results: We confirmed that the CAV-1 KO mice on the *ad lib* diet display a phenotype consistent with the cardiometabolic syndrome, as shown by higher systolic BP (SBP), plasma glucose, HOMA-IR and aldosterone levels despite lower body weight compared with WT mice on the *ad lib* diet. CAV-1 KO mice maintained their body weight on the *ad lib* diet, but had substantially greater weight loss with CR, as compared to caloric restricted WT mice. CR-mediated changes in weight were associated with dramatic improvements in glucose and insulin tolerance in both genotypes. These responses to CR, however, were more robust in CAV-1KO *vs.* WT mice and were accompanied by reductions in plasma glucose, insulin and HOMA-IR in CAV-1KO but not WT mice. Surprisingly, in the CAV-1 KO, but not in WT mice, CR was associated with increased SBP and aldosterone levels, suggesting that in CAV-1 KO mice CR induced an increase in some CV risk factors.
Conclusions: CR improved the metabolic phenotype in CAV-1 KO mice by increasing insulin sensitivity; nevertheless, this intervention also increased CV risk by inappropriate adaptive responses in the RAAS and BP.

1. Introduction

Caloric restriction (CR) is widely recognized as a fundamental component of the prevention and treatment of diabetes, insulin resistance and the metabolic syndrome [1] especially in the obese population [2–4]. CR improves HOMA-IR, hepatic and adipocyte insulin signaling, and blood pressure (BP) [2,4–6]. Several predisposing factors for cardiometabolic disturbances - driven by genetics and environment - have been explored extensively in the last decade [7–9].

Insulin resistance, diabetes and the metabolic syndrome also occur in non-obese individuals, who constitute up to 20–60% of patients with type 2 diabetes in many populations (*e.g.* in northern European [10] and Asian countries [11]) where obesity is not "epidemic". This "metabolically unhealthy non-obese (MUN)" phenotype can present with similar metabolic abnormalities as seen in obese individuals. For example, studies in individuals with type 2 diabetes (T2DM) have demonstrated that MUN individuals have the same cardiovascular (CV) risks as do obese patients [9,10,12,13]. However, the factors underlying MUN are less clear than for those who are obese.

Our group has shown that polymorphisms of the caveolin 1 (CAV-1) gene were strongly associated with insulin resistance in hypertensive

Abbreviations: CAV-1, caveolin-1; HOMA, homeostasis model assessment; Ip-GTT, intraperitoneal glucose tolerance test; IP-ITT, intraperitoneal insulin tolerance test; IR, insulin resistance; ad lib, ad libitum; CR, caloric restricted group; BP, blood pressure; WT, wild type.

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individuals and that CAV-1 KO mice also display abnormal metabolic trends [8], suggesting that the CAV-1 KO mouse model may be translationally relevant to humans who carry polymorphic variants of the CAV-1 gene. Evidence demonstrates co-aggregation and co-heritability between insulin resistance and hypertension, suggesting a close-linked genetic susceptibility of the two conditions [14]. Together with obesity and/or dyslipidemia, these pro-atherogenic risk factors are often classified as the metabolic syndrome (MetS). Recently, we also found that MetS risk is modified by an interaction between the CAV-1 genotype and body mass index (BMI), whereby the minor allele carrier status strongly predicted MetS and diabetes in non-obese but not in obese individuals and was associated *in silico* with decreased CAV-1 expression [15]. Interestingly, the CAV-1 KO mouse is also lean.

The CAV-1 protein is a key component of caveolae, important microdomains on the surface of the plasma membrane of most cells. Highly abundant in adipose and vascular tissues, CAV-1 has been shown to play an intricate role in cholesterol transport/efflux and in the regulation of signaling pathways critical for glucose and BP homeostasis [8,16–20].

Effects of CR on insulin resistant individuals with a lean phenotype are largely unknown. Therefore, understanding the CR-mediated changes in the CAV-1 KO mouse model might reveal important information for the clinical use of CR in non-obese diabetic and/or insulin-resistant humans carrying CAV-1 gene polymorphisms. We hypothesized that CR would improve all of the components of the metabolic syndrome in the lean, CAV-1 KO mouse.

2. Materials and Methods

2.1. Animals and CR Procedure

Details for the animals used in this study are provided in the Supplementary Data. The experimental design is indicated in Fig. 1. Day 0 was defined as the day when animals of both genotypes were randomized to *ad lib* or CR. Animals were housed in individual cages on day-5, with *ad lib* access to both water and chow. After acclimation, twenty-four hour urine was collected on day-3 and BP was measured on day−2. Food was provided twice/d and food intake of individual animals was measured daily to establish basal food intake. On day 0 mice from each genotype were randomized into two subgroups: *ad lib* and 40% CR (*i.e.* 60% of *ad lib* intake), for a total of four genotype-diet groups ($n = 12$/group): WT-*ad lib*, CAV-1 KO-*ad lib* (CAV-*ad lib*), WT-40% CR (WT-CR) and CAV-1 KO-40% CR (CAV-CR). CR mice were given a diet corresponding to approximately 60% of the amount of food consumed by mice of the same genotype fed an *ad lib* diet. Body weight and food intake were measured periodically at 4 pm. Mice remained on *ad lib* or CR diets for 4 wk. On day 26, mice were transferred to individual metabolic cages for 24-h urine collection, and the assessment of food and water intake. The next day, BP was measured. At completion of the experiment, mice were euthanized under deep anesthesia with isoflurane, the abdominal and thoracic cavities were opened, blood samples were collected from the abdominal aorta in BD microtainer tubes (EDTA) and the kidneys and adrenal glands were rapidly harvested. Kidney samples were snap frozen and stored at −80 °C until processed for transcript assessment. Adrenal glands were immediately processed for acute stimulation studies. Blood samples were separated and plasma kept at −80 °C until assayed.

2.2. Insulin Measurements and HOMA-IR Calculation

Plasma samples collected at day 28 were stored at −80 °C until assayed. Measurements were made by enzyme-immunolinked assay, using the Mouse Ultrasensitive Insulin kit (Alpco Diagnostics, Salem, NH), as previously described [8]. Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated to assess changes in insulin resistance (fasting insulin (mU/L) × fasting glucose (mg/dL)/405) [21].

2.3. Fasting Glucose and Glycemic Response to Intraperitoneal Glucose Tolerance Test (GTT)

Glucose metabolism was assessed on day 0 and day 21 of the experiment. Mice were fasted for 15–18 h (overnight), and the next morning baseline body weight (BW) was recorded. Mice were challenged with intraperitoneal (ip) glucose (1.5 mg/g BW) consistent with our previous studies in rodents [18]. At 0, 15, 30, 60, 90 and 120 min, blood was sampled from the tail for glucose determinations with a glucometer (AlphaTrak 2, Abbott, IL) At the end of the GTT, mice were returned to cages and allowed access to food and water. Areas under the curves (AUC) were calculated by using the trapezoid method [22].

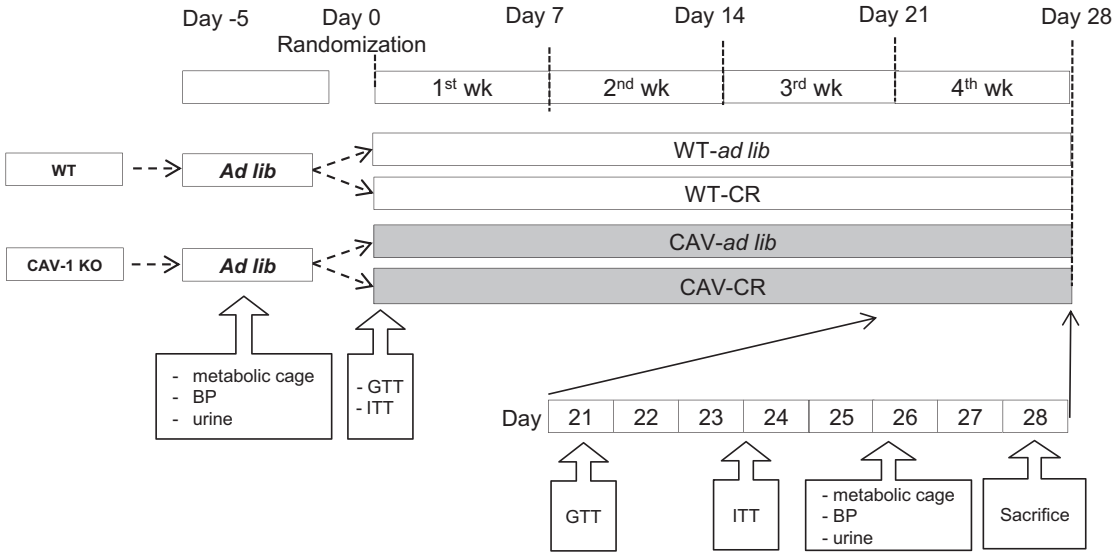

Fig. 1. Experimental design. On day-5 mice of both genotypes were acclimatized in an individual cage with free access to *ad lib* diet and water. On day 0, WT and CAV-1 KO mice were randomized into *ad lib* (control for each genotype) or CR groups for 4 weeks. The timing for performance of the GTT, ITT, BP measurement, urine collection and sacrifice are indicated.