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# Apolipoprotein A-IV constrains HPA and behavioral stress responsivity in a strain-dependent manner

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ABSTRACT

There is a critical gap in our knowledge of the mechanisms that govern interactions between daily life experiences (e.g., stress) and metabolic diseases, despite evidence that stress can have profound effects on cardiometabolic health. Apolipoprotein A-IV (apoA-IV) is a protein found in chylomicrons (lipoprotein particles that transport lipids throughout the body) where it participates in lipid handling and the regulation of peripheral metabolism. Moreover, apoA-IV is expressed in brain regions that regulate energy balance including the arcuate nucleus. Given that both peripheral and central metabolic processes are important modulators of hypothalamic-pituitary-adrenocortical (HPA) axis activity, the present work tests the hypothesis that apoA-IV activity affects stress responses. As emerging data suggests that apoA-IV actions can vary with background strain, we also explore the strain-dependence of apoA-IV stress regulation. These studies assess HPA axis, metabolic (hyperglycemia), and anxiety-related behavioral responses to psychogenic stress in control (wildtype) and apoA-IV-deficient (KO) mice on either the C57Bl/6J (C57) or 129 × 1/SvJ (129) background strain. The results indicate that apoA-IV KO increases post-stress corticosterone and anxiety-related behavior specifically in the 129 strain, and increases stress-induced hyperglycemia exclusively in the C57 strain. These data support the hypothesis that apoA-IV is a novel factor that limits stress reactivity in a manner that depends on genetic background. An improved understanding of the complex relationship among lipid homeostasis, stress sensitivity, and genetics is needed to optimize the development of personalized treatments for stress- and metabolism-related diseases.

## 1. Introduction

The majority of Americans are overweight or obese, increasing their risk for a number of serious disorders including diabetes, cardiovascular diseases, cancer and liver disease; yet much remains unknown about the intrinsic and extrinsic factors regulating body weight (Ogden et al., 2014). Stress is a prevalent extrinsic factor that can have profound effects on physiology and behavior. For example, stress exposure evokes physiological responses that include activation of the hypothalamic-pituitary-adrenocortical (HPA) axis to elevate circulating glucocorticoids (cortisol in humans and corticosterone in mice), and activation of the sympathetic nervous system (Ulrich-Lai and Herman, 2009). The sympathetic nervous system then promotes rapid (seconds to minutes) effects, such as increased heart rate, blood pressure, and blood glucose, while the HPA axis promotes slower (minutes to hours) but more sustained effects on the metabolic, cardiovascular and immune systems (Ulrich-Lai and Herman, 2009; Ulrich-Lai and Ryan, 2014). Importantly, heightened stress responses have been linked to metabolic disease. For instance, elevated cortisol levels are associated with obesity (Jackson et al., 2017), and type 2 diabetes is associated with increased neuroendocrine responses to stress, as well as a higher incidence of stress-related disorders including depression (Hackett et al., 2014; Siddiqui et al., 2015; Tabák et al., 2014). Although stress is implicated in metabolic diseases, the underlying mechanisms are not clear (Ulrich-Lai and Ryan, 2014). Discovery of novel elements that modify the magnitude of physiological and behavioral responses to stress could help to identify individuals at risk of developing such diseases. These factors may also be therapeutic targets to prevent disease or improve outcomes in these populations.

Diverse cardiometabolic functions have been identified for apolipoprotein A-IV (apoA-IV), including contributing to satiation, protecting against atherosclerosis, protecting lipoproteins from oxidation, having anti-inflammatory properties, reversing cholesterol transport, absorbing intestinal lipids, slowing gastric emptying, promoting insulin

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secretion, and reducing hepatic gluconeogenesis (Duverger et al., 1996; Kohan et al., 2012; Li et al., 2015; Vowinkel et al., 2004; Wang et al., 2012; Weinstock et al., 1997). Given this diversity of functions, apoA-IV has been recognized as a potential therapeutic target, as well as a critical factor meriting investigation as a key regulator of metabolic processes (Kohan et al., 2015; Wang et al., 2015). ApoA-IV is primarily produced by the intestine (constituting as much as 3% of the protein made by enterocytes) and is also made by hepatocytes (in rodents) and in brain (Rodriguez et al., 1997; Shen et al., 2008; Tso et al., 2004). In the intestine, apoA-IV is packaged into chylomicrons in response to fat ingestion. As chylomicron triglycerides are metabolized in blood, apoA-IV is released and largely circulates freely in plasma, however some apoA-IV incorporates into high-density lipoproteins (HDL) particles (Kohan et al., 2015; Wang et al., 2015). Although apoA-IV does not cross the blood-brain barrier, it communicates with the brain via the vagus nerve (Lo et al., 2012; Shen et al., 2008). ApoA-IV is also expressed within brain regions that regulate metabolism, including the paraventricular, ventromedial, and arcuate hypothalamic nuclei, and the nucleus of the solitary tract (Liu et al., 2004; Shen et al., 2008). Further, apoA-IV acts in brain to influence energy balance. Specifically, apoA-IV interacts synergistically with melanocortins and leptin in the hypothalamus to reduce food intake (Gotoh et al., 2006; Shen et al., 2007). One mechanism mediating this effect is the inhibition of orexigenic neurons and activation of anorexigenic neurons in the arcuate nucleus (Yan et al., 2016).

Intriguingly, evidence suggests that apoA-IV may also regulate stress responses. For example, peripheral metabolic status can modulate HPA axis and sympathetic nervous system activity (Ulrich-Lai and Ryan, 2014). Moreover, the specific hypothalamic and brainstem regions that express apoA-IV are critical sites for stress regulation (Packard et al., 2016; Ulrich-Lai and Herman, 2009; Ulrich-Lai and Ryan, 2014). ApoA-IV is therefore well-positioned to modulate multiple types of physiological and emotional/behavioral responses to stressor exposure. Thus, in the present work we use apoA-IV-deficient mice to test the hypothesis that apoA-IV regulates neuroendocrine (HPA axis), metabolic (glycemic) and behavioral (anxiety-related and depression-like) responses to acute psychogenic stress. Unpublished preliminary evidence suggests that the ability of apoA-IV to influence metabolic regulation varies among mouse strains (personal communication from Chih-Wei Ko) thus it follows that the stress effects of apoA-IV may also be strain-dependent. The present work therefore tests the impact of apoA-IV-deficiency in two common background strains of mice, C57BL6J (C57) and 129 × 1/SvJ (129), in order to test the secondary hypothesis that the role of apoA-IV in stress regulation varies with genetic background.

## 2. Materials and methods

### 2.1. Animals

ApoA-IV-deficient mice (KO) were originally obtained from J.L. Breslow (The Rockefeller University, New York, NY) and deficiency was verified by northern and western blot (Weinstock et al., 1997). Adult (> 8 weeks old at experiment onset on d0 (Fig. 1)) male KO and wild type (WT) controls were bred in-house on either the C57 or 129 background strain (at least 6 generations backcrossed) and genotyped by PCR in order to yield 4 groups: WT C57, KO C57, WT 129 and KO 129 (n = 7–8 per group). Mice were individually housed in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) −accredited facility on a 12 h light/dark cycle (07:00 h lights-on and 19:00 h lights-off) with *ad libitum* access to rodent chow (Teklad LM-485; Envigo, Madison, WI) and water. All experiments were approved by the University of Cincinnati Institutional Animal Care and Use Committee (IACUC) and were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Body weight and food intake were monitored throughout the study. On experiment day (d) 48 and d83, body composition was assessed by nuclear magnetic resonance analysis (NMR) (Echo MRI, Houston, TX).

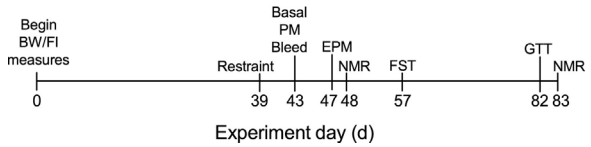


**Fig. 1.** Time line of the experimental design: Body weight and food intake measurements began on d0. Mice received restraint stress testing on d39, measurement of afternoon, basal plasma corticosterone and blood glucose on d43, assessment of body composition by NMR on d48 and d83, and behavioral testing in the elevated plus-maze and forced swim tests on d47 and d57, respectively.

### 2.2. Blood collection and analysis

On the morning of experiment d39 (near the nadir of the circadian rhythm), non-fasted mice were given an acute 30-min restraint stress by placing them in a well-ventilated plastic tube. Mice were tested in a non-fasted state since fasting itself can confound measurements of HPA axis responsivity (Makimura et al., 2003). Serial blood samples were collected from the tip of the tail at 0, 30, 60 and 120 min following the initiation of restraint. Care was taken to quickly obtain the 0-min blood sample within 2–3 min of first touching the animal's cage in order to ensure measurement of pre-stress corticosterone levels (see below) (Vahl et al., 2005). One drop of blood was used to measure glucose (Precision Xtra monitors and strips, Abbott, Abbott Park, IL) and the remainder was collected into EDTA-coated tubes and placed onto ice. Blood samples were then centrifuged at 3500xg for 15 min at 4 °C, and plasma was collected and stored at −80 °C until measurement of corticosterone by radioimmunoassay (MP Biomedicals, Orangeburg, NY) or insulin by enzyme-linked immunosorbent assay (EMD Millipore, Billerica, MA).

On experiment d43, non-stress blood was again collected from non-fasted mice in the late afternoon (near the peak of the circadian rhythm). Blood glucose and plasma corticosterone were assessed as described above. On experiment d82, an intraperitoneal (IP) glucose tolerance test (GTT; 2 g dextrose/kg body weight; Vedco, St. Joseph, MO) was performed in fasted mice (food removed 5 h prior). Serial blood samples were collected just prior to (i.e., at 0 min) and at 15, 30, 60 and 120 min following glucose administration for blood glucose and plasma corticosterone measurement as described above. Because the blood collection schedule for the GTT contains 5 sampling time points, and we needed to keep to the total blood collection volume below the threshold of a hemorrhagic stress response, we collected additional blood for the measurement of plasma insulin just prior to glucose administration (i.e., at 0 min) and at the approximate peak of the insulin response (i.e., at 15 min) (Bielohuby et al., 2013; Chambers et al., 2011; Wang et al., 2012).

### 2.3. Elevated plus-maze

On experiment d47, mice were given an elevated plus-maze (EPM) test for behavioral anxiety. The EPM test is based on the observation that rodents display an innate avoidance of heights and/or exposed spaces (Pellow et al., 1985). The mouse was placed near the center of an elevated platform that is shaped like a "+", in which two non-adjacent arms of the "+" have walls (enclosed arms), and the other two arms do not (open arms). The mouse was allowed to move freely through the apparatus for 5 min and the resulting behavior was recorded by a video camera mounted directly above the apparatus. A low anxiety state is inferred when the subject spends increased time in the open arms of the platform, and/or engages in ethological indices of exploratory behavior