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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 129x1/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.

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Keywords

HPA axis; hyperglycemia; anxiety-related behavior; psychogenic stress; corticosterone; background strain

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 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 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 129X1/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 (Figure 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 hr lights-on and 19:00 hr 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 d48 and 83, body composition was assessed by nuclear magnetic resonance analysis (NMR) (Echo MRI, Houston, TX).

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 minutes 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 3500×g for 15 minutes 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; 2g dextrose/kg body weight; Vedco, St. Joseph, MO) was performed in fasted mice (food removed 5 hr 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 minutes 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 (e.g., dipping head over the edge of the maze), whereas a high anxiety state is inferred when the subject avoids the open arms of the platform (Falter et al., 1992; Fernandes and File, 1996; Pellow et al., 1985; Treit et al., 1993). The EPM test was conducted under low ambient light to promote open arm exploration (Albani et al., 2015). Under these lighting conditions the automated scoring system (Clever TopScan Software; CleverSys, Reston, VA) accurately tracked behaviors that occurred in the open arms, the central square, and the portions of the closed arms that were located near the central square. However, behaviors that occurred in the far end of the closed

arms (where the dark walls further decreased the illumination) were tracked less accurately. As a result, the system provided highly reliable data relating to the number of arm entries and the time spent in each arm (as these behavioral transitions occur near the center of the maze), but less accurately recorded the distance travelled within the far ends of the closed arms. Thus, the total number of arm (open + closed) entries, instead of total distance travelled, is used as an index of locomotor activity (Walf and Frye, 2007). Head dips were manually scored by an observer blinded to genotype, with head dips being defined as incidences where the nose of the mouse extended beyond the edge of the open arm.

2.4 Forced Swim Test

On experiment d57, mice were given a one-day forced swim test (FST). Mice were placed into an inescapable opaque Plexiglas cylinder filled with water sufficiently deep to prevent standing on the bottom for 10 minutes. Resulting behavior was recorded by video camera. An observer (who was blinded to genotype) recorded the amount of time spent actively swimming (i.e., searching for an escape route) vs. immobile (i.e., doing only the minimal movements necessary to prevent drowning). Increased time spent immobile in this test is considered as a depressive-like behavior similar to behavioral despair (Pollak et al., 2010; Porsolt et al., 1979).

2.5 Statistical Analysis

All data are expressed as means $\pm$ standard error. Analyses were performed by two-way ANOVA with genotype and strain as factors with protected Fishers LSD post-hoc using GB-STAT software (Dynamic Microsystems Inc., Silver Spring, MD). When time was also a factor, a 3-way, repeated measures ANOVA was performed. Homogeneity of variance was significantly different for the plasma insulin response to restraint, so a square-root transformation was performed prior to statistical analysis of these data. Statistical significance was taken as $p < 0.05$.

3. Results

3.1 Impact of apoA-IV deficiency and mouse strain on body weight, food intake and body composition

Body weight and food intake were monitored throughout the study (Figure 2). For body weight gain over time (Figure 2A), there was a main effect of Strain ($F_{1,290} = 7.572$, $p < 0.05$; with C57 gaining less weight than 129), no main effect of Genotype and a main effect of Time ($F_{1,250} = 7.572$, $p < 0.05$; with body weight increasing over time for all groups). There were significant interactions between Strain and Genotype ($F_{1,290} = 11.46$, $p < 0.01$), Strain and Time ($F_{1,250} = 10.92$, $p < 0.0001$) and Strain X Genotype X Time ($F_{1,250} = 3.886$, $p < 0.0001$), but no interaction with Genotype and Time. Total body weight gain (Figure 2B) during the experiment (at d82, the conclusion of the experiment) showed a similar result. Weight gain was greater in 129 than C57 mice (Strain $F_{1,25} = 12.65$, $p < 0.001$) and there was no main effect of Genotype. There was a significant interaction between Strain and Genotype ($F_{1,25} = 12.91$, $p < 0.01$). Post-hoc analysis further indicated that apoA-IV deficiency increased body weight gain specifically in the 129 strain.

For caloric intake over time (Figure 2C), there was no main effect of Strain or Genotype, but there was a main effect of Time ($F_{1,250} = 19.98$, $p < 0.0001$). There were also significant interactions between Strain and Time ($F_{1,250} = 3.586$, $p < 0.001$), and Strain X Genotype X Time ($F_{1,250} = 1.531$, $p < 0.0001$), but no interaction with Strain and Genotype or Genotype and Time. The average daily caloric intake over experiment days 75–82 was similar for all groups (Figure 2D). Moreover, cumulative food intake over the entire study was also similar for all groups (Figure 2E). Caloric efficiency (Figure 2F) was significantly greater in 129 mice than C57 mice (Strain $F_{1,25} = 12.71$, $p < 0.01$). There was no effect of Genotype, but there was a Strain X Genotype interaction ($F_{1,25} = 10.34$, $p < 0.01$) for caloric efficiency. Consistent with the observations above, post-hoc analysis indicated that apoA-IV deficiency increased caloric efficiency specifically in the 129 strain.

Body composition analysis on d48 revealed a main effect of Strain ($F_{1,24} = 132.1$, $p < 0.0001$), whereby C57 mice had a significantly greater proportion of lean mass (Figure 2G). There was no effect of Genotype or Strain X Genotype interaction. By extension, there was a lower proportion of fat mass in C57 mice (Figure 2H, Strain $F_{1,24} = 116.0$, $p < 0.0001$), that did not vary with apoA-IV genotype. Body composition analysis at study completion gave the same result (data not shown). Together, these data indicate that on a chow diet, apoA-IV-deficient mice gained more body weight despite equivalent caloric intake, had a higher caloric efficiency, and were overall larger (i.e., not selectively gaining fat or lean mass), specifically when on the 129 background strain.

3.2 The impact of apoA-IV deficiency and mouse strain on the plasma corticosterone response to an acute psychogenic stressor

To measure HPA axis responses to an acute restraint stress, mice were placed into ventilated plastic tubes for 30 min. The time course of the plasma corticosterone response to restraint (Figure 3A) revealed a main effect of Strain ($F_{1,87} = 68.21$, $p < 0.0001$), with 129 mice having larger corticosterone responses to restraint than C57 mice. There were also main effects of Genotype ($F_{1,87} = 7.826$, $p < 0.01$, with KO generally increasing corticosterone responses), and Time ($F_{3,75} = 164.1$, $p < 0.0001$, with post-restraint levels higher than pre-restraint), as well as a Strain X Time interaction ($F_{3,75} = 20.84$, $p < 0.0001$). There were no other interactive effects. Post-hoc analysis indicated that C57 corticosterone levels were less than 129 at 60 and 120 min for both WT and KO mice.

The plasma corticosterone AUC (Figure 3B; i.e., the area-under-the-curve of the plasma corticosterone time course, which is used as an index of the total corticosterone response) similarly showed main effects of Strain ($F_{1,25} = 51.74$, $p < 0.0001$) and Genotype ($F_{1,25} = 10.37$, $p < 0.01$), with no Strain X Genotype interaction. Post-hoc analysis indicated that C57 responses were lower than 129 regardless of genotype, and further indicated that apoA-IV deficiency increased the integrated plasma corticosterone response in the 129 strain, as opposed to the C57 strain. Collectively, these results indicate that apoA-IV deficiency increased the plasma corticosterone responses to an acute psychogenic stress, and that this effect was most prominent for the strain (129) that had the inherently larger corticosterone response.

3.3 Impact of apoA-IV deficiency and mouse strain on the hyperglycemic response to an acute psychogenic stressor

For the time course of the blood glucose response to stress (Figure 3C) there was a main effect of Strain ($F_{1,87} = 311.7$, $p < 0.0001$, with C57 having higher blood glucose levels than 129). There was a main effect of Time ($F_{3,75} = 169.8$, $p < 0.0001$, with post-restraint levels higher than pre-restraint). There were also significant interactions of Strain X Genotype ($F_{1,87} = 6.029$, $p < 0.05$), Strain X Time ($F_{3,75} = 58.96$, $p < 0.0001$) and Strain X Genotype X Time ($F_{3,75} = 3.836$, $p < 0.05$). In contrast, there was no effect of Genotype, nor a Genotype x Time interaction. Post-hoc analysis indicated that C57 mice had greater blood glucose at all time points, and also indicated that apoA-IV deficiency further increased the glycemic response specifically at 30 and 60 min after stress onset for the C57 strain. The time-dependence of this effect is further indicated by the blood glucose AUC data (Figure 3D), which revealed a main effect of Strain ($F_{1,25} = 102.9$, $p < 0.0001$), with no effect of Genotype and no Strain x Genotype interaction.

To complement the blood glucose data, we also measured the plasma insulin response to stress. For the time course of the plasma insulin response (Figure 3E) there was a main effect of Strain ($F_{1,87} = 9.511$, $p < 0.001$, where 129 mice had overall higher plasma insulin than C57 mice) and Time ($F_{3,75} = 20.45$, $p < 0.0001$, with plasma insulin generally decreasing during restraint). There was no effect of Genotype or Strain X Genotype interaction. There were significant interactions of Strain X Time ($F_{3,75} = 3.056$, $p < 0.05$), Genotype X Time ($F_{3,75} = 5.302$, $p < 0.01$) and Strain X Genotype X Time ($F_{3,75} = 3.772$, $p < 0.05$). Post-hoc analysis indicated that at the onset of restraint, WT 129 mice had higher plasma insulin levels than WT C57 mice; the insulin levels then fell during the restraint, so that they no longer differed. ApoA-IV deficiency also reduced pre-restraint plasma insulin levels in the 129, but not the C57, strain. For the incremental plasma insulin AUC (Figure 3F) there was a significant main effect of Genotype ($F_{1,25} = 11.51$, $p < 0.01$) and a Strain X Genotype interaction ($F_{1,25} = 7.053$, $p < 0.05$), despite no main effect of Strain. Post-hoc analysis indicated that restraint stress reduced integrated plasma insulin levels to a greater extent in the 129 versus the C57 strain, and further indicated that apoA-IV deficiency blunted the hypo-insulinemic response specifically in the strain with the inherently greater insulin effect (129).

3.4 Impact of apoA-IV deficiency and mouse strain on glucose tolerance

As we observed a higher glucose response to stress in KO C57 mice compared to WT C57 mice, we then asked if this difference was due, at least in part, to impairments in glucose clearance. In order to evaluate this possibility, we administered a glucose tolerance test in which glycemic responses to an IP glucose challenge were measured in fasted mice. For the time course of the blood glucose response to an IP GTT (Figure 4A) there were main effects of Strain ($F_{1,116} = 91.49$, $p < 0.0001$, with glucose higher for C57 vs. 129 mice) and Time ($F_{4,100} = 113.9$, $p < 0.0001$) and a Strain x Time interaction ($F_{4,100} = 15.93$, $p < 0.0001$). There was not a main effect of Genotype, nor any other interactive effects. Post-hoc analysis indicated that blood glucose levels of C57 mice were greater than 129 mice at all time points (prior to and after glucose administration), and apoA-IV deficiency did not alter glucose clearance in either strain.

The blood glucose AUC in the GTT (Figure 4B) gave a similar result with a main effect of Strain ($F_{1,25} = 39.24$, $p < 0.0001$) and no main or interactive effects of Genotype. Post-hoc analysis indicated that the integrated glucose response was greater in C57 mice, and not impacted by apoA-IV deficiency. Together, these data suggest that apoA-IV deficiency did not alter glucose tolerance in either mouse strain. It should also be noted that the blood glucose levels in a few of the C57 mice reached the upper detection limit of our glucose monitors at the time points of maximal glucose excursion, therefore peak responses may be somewhat under-estimated for this strain; though this occurred in C57 mice of both genotypes, so this is unlikely to have markedly impacted interpretation of the results.

We also measured plasma insulin at baseline and at 15 min post-GTT (Figure 4C). There was a main effect of Strain ($F_{1,29} = 9.099$, $p < 0.01$) and Time ($F_{1,25} = 31.31$, $p < 0.0001$) and a Strain x Time interaction ($F_{1,25} = 9.722$, $p < 0.01$), with the 129 mice having overall greater plasma insulin, particularly at 15 min post-glucose administration. There were no main or interactive effects of Genotype for these data. Taken together with the glucose response, the GTT data suggest that the glucose and insulin responses to exogenous glucose administration varied with strain, but not apoA-IV status.

3.5 Impact of apoA-IV deficiency and mouse strain on the plasma corticosterone response to a GTT

As both mouse strain and apoA-IV status impacted the plasma corticosterone response to a restraint stressor (Figure 3A,B), we were interested in determining whether the HPA axis response to another type of psychogenic stress was similarly affected. In order to test this, we took advantage of the fact that the handling associated with an IP injection of glucose is a stressor that can activate the HPA axis, and therefore measured plasma corticosterone in the blood samples collected during the GTT. The time course of the plasma corticosterone response to GTT (Figure 4D) showed main effects of Strain ($F_{1,116} = 117.0$, $p < 0.0001$), Genotype ($F_{1,116} = 18.71$, $p < 0.001$), and Time ($F_{4,100} = 186.9$, $p < 0.0001$), with Strain X Genotype ($F_{1,116} = 11.21$, $p < 0.01$) and Strain X Time ($F_{4,100} = 17.31$, $p < 0.0001$) interactions, but no interactions between Genotype X Time and Strain X Genotype x Time. Post-hoc analysis indicated that the 129 strain had higher plasma corticosterone levels at all post-injection time points. In addition, apoA-IV deficiency increased post-injection plasma corticosterone specifically in the 129 strain.

The AUC of the plasma corticosterone response to GTT (Figure 4E) similarly revealed main effects of Strain ($F_{1,25} = 80.37$, $p < 0.0001$) and Genotype ($F_{1,25} = 9.682$, $p < 0.01$), with no Strain X Genotype interaction. Post-hoc analysis indicated that the corticosterone AUC was greater in the 129 strain, and that apoA-IV KO further increased the corticosterone AUC in the 129, but not the C57, strain. These results demonstrate that apoA-IV deficiency increased the plasma corticosterone response to GTT and that this effect was most prominent for the strain (129) that had an inherently larger corticosterone response to stress – findings that mirror those obtained with a restraint stressor (Figure 3A,B).

3.6 Impact of apoA-IV deficiency and mouse strain on non-stress plasma corticosterone and blood glucose near the peak of the circadian rhythm

Since we observed a difference in the corticosterone response to restraint and GTT stressors in the KO 129 mice compared to the WT 129 mice, we also examined potential differences in the non-stress peak circadian corticosterone level. We collected a non-stress blood sample just before the active period (lights-off) to approximate the peak circadian level. At this time, compared to C57 mice, 129 mice had greater plasma corticosterone levels (Figure 5A, Strain $F_{1,25} = 8.628$, $p < 0.001$), with no main or interactive effects of Genotype. More specifically, post-hoc analysis indicated that the KO C57 mice had lower plasma corticosterone than the KO 129 mice. In contrast, 129 (vs. C57) mice had lower blood glucose levels (Figure 5B, Strain $F_{1,25} = 94.76$, $p < 0.0001$), with no main or interactive effects of Genotype, and post-hoc analysis indicated that both WT and KO C57 mice were elevated relative to their respective 129 group. These data reveal strain differences in resting plasma corticosterone and blood glucose near the peak of the circadian rhythm that were not impacted by apoA-IV deficiency.

3.7 Impact of apoA-IV deficiency and mouse strain on anxiety-related behavior in the elevated plus-maze test

Given the well-established links between HPA axis activity and stress-related behaviors (Packard et al., 2016), we also assessed the impact of strain and apoA-IV deficiency on stress-associated behaviors. Anxiety-related behavior was tested in the EPM, and the amount of time spent in the two open arms, the two closed arms, and total entries to the 4 arms, was recorded. Open arm time (Figure 6A) had a Strain x Genotype interaction ($F_{1,25} = 7.067$, $p < 0.05$), with no main effects of Strain or Genotype, indicating that apoA-IV deficiency decreased open arm time specifically in 129 mice. Time spent in the closed arms (Figure 6B) indicated a main effect of Strain ($F_{1,25} = 7.081$, $p < 0.05$) and a Strain x Genotype interaction ($F_{1,25} = 4.871$, $p < 0.05$), with no main effect of Genotype. Post-hoc analysis revealed that the 129 strain spent less time in the closed arms, and that apoA-IV deficiency increased closed arm time specifically in 129 mice. The time spent engaging in head-dipping behavior while on the EPM (Figure 6C) had a main effect of Strain ($F_{1,25} = 7.092$, $p < 0.05$) and a Genotype x Strain interaction ($F_{1,25} = 11.46$, $p < 0.01$), with no main effect of Genotype, such that 129 spent more time head-dipping, and apoA-IV deficiency reduced head-dipping time specifically in the 129 strain. There were no differences in the total number of arm entries (Figure 6D) suggesting that the general activity of each group was similar. Collectively, the EPM data suggest that the 129 strain generally exhibits less anxiety-related behaviors, as they spent more time in exploratory head-dipping behavior and less time in the closed arms. Moreover, apoA-IV deficiency increased anxiety-related behaviors, including reduced open arm time, greater closed arm time, and less time spent head-dipping, specifically in the 129 strain.

3.8 Impact of apoA-IV deficiency and mouse strain on depressive-like behavior in the forced swim test

We assessed the impact of strain and apoA-IV deficiency on depression-like behavior in the FST (Figure 7). During the FST, the percent time spent swimming (Figure 7A), immobile

(Figure 7B), and climbing (Figure 7C) were not impacted by either strain or genotype. These results suggest that neither strain nor apoA-IV status impact depression-like behavior.

4. Discussion

These results demonstrate for the first time that apoA-IV regulates physiological responses to stress, and furthermore indicate that this regulation depends upon genetic background. Metabolically, ApoA-IV-deficient mice gain greater body weight despite equivalent caloric intake when on the 129 (vs. C57) background strain. ApoA-IV deficiency also increases the HPA axis response to two different types of stress (restraint and glucose injection), and promotes anxiety-related behaviors in the EPM, primarily in the 129 strain. In contrast, apoA-IV deficiency elevates stress-induced hyperglycemia specifically in the C57 strain at 30 and 60 min after stress onset, without significantly altering IP glucose tolerance in either strain. This suggests that the apoA-IV effect on post-stress hyperglycemia in C57 mice is not secondary to impaired glucose clearance. Rather the rapid increases in blood glucose that occur during stress are primarily mediated by the adrenal medullary release of epinephrine, under the control of the sympathetic nervous system (Bialik et al., 1989; Tank and Lee Wong, 2015). By extension, apoA-IV deficiency may elevate stress-induced sympathetic drive to the adrenal medulla, resulting in greater post-stress blood glucose levels that are primarily observed early after stress onset. The present data also emphasize the importance of comparing HPA axis, autonomic and behavioral stress responses concurrently, as assessment of any of these alone would have failed to identify a role for apoA-IV in one of the strains, underscoring the fact that the stress response is multifaceted and is not fully reflected by any one measure alone (Ulrich-Lai and Herman, 2009).

4.1 Impact of mouse strain on physiological stress responses

There was a significant effect of mouse strain on physiological stress responses in our study. In response to stress, C57 mice had lower plasma corticosterone responses and greater hyperglycemic responses compared to 129 mice. More specifically, the data indicate that 129 mice may utilize HPA axis activation as their primary response to stress, with little-to-no accompanying sympathomedullary activation. In contrast, C57 mice may utilize a large sympathomedullary activation, coupled with a more modest HPA axis activation, in order to respond to stress. Such strain-dependent differences in the extent of post-stress HPA axis activation have been previously shown in mice (Anisman et al., 2001; Cryan and Holmes, 2005; Miller et al., 2010; Moloney et al., 2015), though the C57 and 129 strains have not previously been compared. Moreover, while strain-dependent differences in autonomic nervous system drive to the cardiovascular system have been reported (Shusterman et al., 2002), to our knowledge this is the first report of strain-dependent differences in stress-induced hyperglycemia. Together these findings suggest that the magnitude of physiological stress responses can vary markedly among strains, indicating that these are driven to a large extent by an individual's genetics.

Moreover, our data further suggest that the impact of strain on stress responses may not be to increase (or decrease) all stress responses uniformly, but rather varies the extent to which HPA axis vs. sympathetic (glucose) responses to stress are utilized. In other words, different

strains of mice may respond to stress with their own unique coping strategy, such that 129 have large HPA axis responses coupled with limited sympathomedullary activation, whereas C57 have large sympathomedullary responses coupled with more modest HPA axis activation. Prior work in outbred Wistar rats and an originally feral strain of rats, Wild Type Groningen, (both genetically diverse populations) identified two subtypes of stress coping responses among various individuals; one with a higher HPA axis response (corticosterone) and another with a higher sympathetic (noradrenaline) response (De Boer et al., 2017; de Boer et al., 1990; Koolhaas et al., 2010; 1999). It is intriguing to speculate that the current results utilizing the inbred 129 and C57 mouse lines illustrate an analogous concept, with the 129 similar to those rats with an HPA axis-dominant stress response and the C57 similar to those rats with a sympathomedullary-dominant stress response. Thus, the current results support the idea that there are individual differences in the relative extent (or ratio) of HPA axis vs. sympathetic responses to stress that can depend on genetic factors. In turn, an individual's coping strategy might predispose them to certain pathophysiological conditions. For example, during chronic stress, repeated and/or prolonged HPA axis activation can promote visceral adiposity and immunosuppression, suggesting that those with an HPA axis-dominant response may be preferentially predisposed for these stress-related disorders (De Boer et al., 2017; de Boer et al., 1990; Koolhaas et al., 2010; 1999). In contrast, repeated or prolonged sympathetic activation during chronic stress can promote hypertension, atherosclerosis and autoimmune disease, suggesting that those with a sympathomedullary-dominant response may be preferentially predisposed for these stress-related disorders (De Boer et al., 2017; de Boer et al., 1990; Koolhaas et al., 2010; 1999). It will be important for future work to focus on understanding the short- and long-term physiological impact of acute and chronic stress among individuals that rely primarily on HPA axis vs. sympathetic responses.

4.2 Impact of apoA-IV on physiological stress responses

The data also suggest that the lack of apoA-IV significantly increased responses to stress in a strain-dependent manner, increasing post-stress corticosterone in the 129 strain, and increasing stress-induced hyperglycemia in the C57 strain. In other words, endogenous apoA-IV inhibits HPA axis stress reactivity or inhibits sympathomedullary-mediated hyperglycemia differentially depending on the background strain. Potentially, exogenous administration of apoA-IV-based therapeutics may be used to limit HPA axis and/or sympathetic responses to stress, which could in turn reduce the development of stress-related psychiatric and cardiometabolic diseases. Our results suggest that the impact and efficacy of apoA-IV-based therapeutics will likely vary with an individual's genetics.

In addition, the present work suggests that apoA-IV deficiency does not alter glucose clearance in an IP GTT in either the 129 or C57 strains. This result is in contrast to prior work from our group demonstrating that a lack of apoA-IV in the C57 strain impairs glucose tolerance in an IP GTT, without altering insulin sensitivity in an insulin sensitivity test (ITT) (Wang et al., 2012) [Note that prior work has not previously addressed the impact of apoA-IV on glucose homeostasis in the 129 strain.] As the primary focus of the present study was stress, the experimental design and group sizes ($n = 7-8/\text{group}$) were powered for stress-related measures. In contrast, since the focus of our prior paper was specifically glucose

homeostasis (Wang et al., 2012), it was designed and powered specifically for glucose tolerance testing ($n = 17/\text{group}$). Thus, the observation that apoA-IV deficiency significantly impairs glucose tolerance in the earlier study (with larger group sizes), while tending towards a non-significant increase in the present study is not surprising. Importantly though, apoA-IV deficiency significantly altered multiple stress-related outcomes in the present experiment, supporting the *a priori* power analyses that indicated group sizes of $n = 7\text{--}8$ for stress-related end points. Related to this idea, while the present work did not identify an effect of apoA-IV deficiency on glucose tolerance in the 129 strain, it will be important for subsequent work to re-address this question using an experimental design that is focused on, and optimized for, this specific question.

4.3 Impact of mouse strain and apoA-IV on stress-related behaviors

Previous research has demonstrated that there are important strain-dependent differences in both anxiety-related and depression-like behaviors; however, to our knowledge this is the first study to directly compare these two particular strains (C57BL6J and 129X1/SvJ). The present results indicate that the C57 (C57BL6J) show more anxiety-related behaviors than 129 (129X1/SvJ) in the EPM test, with no differences between the two strains in depression-like behavior in the FST. Prior work has compared other sub-strains of the C57 and 129 families to each other using multiple tests of stress-related behaviors and obtained varying results, suggesting that stress-related behavioral end points are strongly influenced by genetic background (Contet et al., 2001; Miller et al., 2010; Moloney et al., 2015). Importantly, apoA-IV-deficiency increased behavioral anxiety exclusively in the 129 strain, suggesting that endogenous apoA-IV normally reduces anxiety in a manner dependent upon an individual's genetics. Intriguingly, apoA-IV contributes to the regulation of both the HPA axis and behavioral anxiety primarily in the 129 strain, supporting prior observations that anxiety-related behaviors are strongly-linked with HPA axis function (Packard et al., 2016). Notably, depression-like behavior in the FST was not impacted by either strain or apoA-IV status, indicating that endogenous apoA-IV regulates specific types of stress-related behavior.

4.4 Potential mechanisms for apoA-IV effects on stress-related end points

Given that apoA-IV is expressed in both brain and peripheral metabolic organs (Apfelbaum et al., 1987; Liu et al., 2001) and that these mice have a global apoA-IV deficiency, it is unknown whether apoA-IV-mediated stress reduction occurs via central and/or peripheral mechanisms. In support of the idea that apoA-IV can act in brain to influence stress regulation, apoA-IV is expressed in brain regions related to metabolic function and stress responses (Shen et al., 2008), and central administration of exogenous apoA-IV impacts food intake (Gotoh et al., 2006). Also, apoA-IV dysfunction has also been linked to neurodegenerative (Császár et al., 1997) and cognitive (Corbett et al., 2007) diseases in people, suggesting that apoA-IV can modulate neuronal function. Together this suggests that apoA-IV may act directly in brain to regulate HPA axis activation and/or glucocorticoid negative feedback, as well as the autonomic response to stress. Alternatively, multiple roles have been described for apoA-IV peripherally, including protecting against atherosclerosis, protecting lipoproteins from oxidation, having anti-inflammatory properties, reversing cholesterol transport, absorbing intestinal lipids, slowing gastric emptying, promoting

insulin 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). As these metabolic systems can also indirectly alter stress responses (Ulrich-Lai and Ryan, 2014), peripheral apoA-IV effects may be critical to apoA-IV's regulation of stress. In the future it will be important to determine the extent to which apoA-IV affects HPA axis, hyperglycemic and anxiety-related stress responses via central versus peripheral mechanisms, as well as whether these mechanisms are strain-dependent.

4.5 Conclusion

In summary, while stress generally activates both the HPA axis and sympathomedullary systems, the relative amount of HPA axis vs. sympathomedullary activation varies between mouse strains, with 129 mice exhibiting an HPA axis-dominant response and C57 mice exhibiting a sympathomedullary-dominant response. Furthermore, ApoA-IV deficiency selectively increased the predominant stress response for each strain. Taken together, these data indicate that apoA-IV is an important factor that reduces stress reactivity in a manner that depends on genetic background. The results therefore support that idea that apoA-IV is a potential mechanism linking co-morbid stress-related disorders, such as cardiovascular disease, obesity/diabetes and psychiatric disorders. This work also has important implications for the development and optimization of apoA-IV-based therapeutics, indicating that their efficacy may vary with genetic background.

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Highlights

- ApoA-IV deficiency increases the HPA response to stress primarily in 129x1/SvJ mice
- ApoA-IV deficiency increases anxiety-related behavior exclusively in the 129 strain
- ApoA-IV deficiency increases post-stress hyperglycemia only in the C57Bl/6J strain
- ApoA-IV deficiency does not alter depression-like behavior in either mouse strain
- Endogenous ApoA-IV attenuates acute stress reactivity in a strain-dependent manner

Figure 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 days 48 and 83, and behavioral testing in the elevated plus-maze and forced swim tests on d47 and d57, respectively.

Figure 2. Body weight, food intake and body composition

The time course of body weight gain throughout the experiment (A), and the total body weight gain at the conclusion of the experiment (d82; body weight measured just before the pre-GTT fast) (B), indicate that apoA-IV deficiency (KO) increased body weight gain relative to wild-type (WT) mice when on the 129 background, but not when on the C57 background. The time course of the average daily caloric intake throughout the experiment (C), the average daily caloric intake on experiment d75–82 (D), and the cumulative caloric intake over the entire experiment (E) indicate that food intake was similar among the groups. However, caloric efficiency (F) is significantly greater for KO 129 mice. NMR analysis of body composition on d48 shows that C57 mice have greater percent lean mass (G) and less percent fat mass (H) compared to 129 mice, with no effect of apoA-IV deficiency. Symbols denote significant differences on post-hoc analysis: * $p < 0.05$ vs respective WT group, # $p < 0.05$ vs respective 129 group. (n = 7–8/group)

Figure 3. HPA axis and glycemic responses to acute restraint stress

The plasma corticosterone response (A) to an acute (30-min) restraint stress is less in C57 mice compared to 129, and is greater in KO mice compared to WT (note that while the ANOVA indicate a main effect of Genotype, no individual group differences were identified between the two genotypes on the post-hoc analysis). The incremental corticosterone AUC (B) is greater for the 129 strain, and is also increased by apoA-IV deficiency, with post-hoc analysis indicating that the apoA-IV KO effect was most prominent for the 129 strain. The time course (C) and AUC (D) of the plasma glucose response to restraint stress indicate that C57 (vs. 129) mice have higher stress-induced hyperglycemia, and that apoA-IV deficiency increases the glucose response specifically in C57 mice at 30 and 60 min after stress onset (such that the apoA-IV KO effect is not observed when the AUC of the entire time course is analyzed). The time course (E) and AUC (F) of the plasma insulin response to restraint stress indicate that 129 (vs. C57) mice have greater reductions in plasma insulin following restraint, and that apoA-IV deficiency blunts this effect. Symbols denote significant differences on post-hoc analysis: * $p < 0.05$ vs respective WT group, # $p < 0.05$ vs respective 129 group. (n = 7–8/group)

Figure 4. Glycemic and HPA axis responses to a glucose tolerance test (GTT)

The time course (A) and AUC (B) of the plasma glucose response to an intraperitoneal administration of glucose (2 g dextrose/kg body weight) is greater in the C57 mouse strain, and not affected by apoA-IV deficiency in either strain. Plasma insulin levels (C) from the GTT are greater for 129 mice. The time course (D) and AUC (E) of the plasma corticosterone response to the GTT are greater in the 129 strain, and apoA-IV deficiency enhances the plasma corticosterone response primarily in the 129 strain. Symbols denote significant differences on post-hoc analysis: * $p < 0.05$ vs respective WT, # $p < 0.05$ vs respective 129. (n = 7–8/group)

Figure 5. Basal plasma corticosterone and blood glucose in the afternoon

Afternoon non-stress plasma corticosterone (i.e., near the peak of the circadian rhythm) is lower in C57 (vs. 129) mice and not affected by apoA-IV deficiency (A). Afternoon non-stress blood glucose is higher in C57 (vs. 129) mice and also not impacted by apoA-IV deficiency (B). Symbols denote significant differences on post-hoc analysis: # $p < 0.05$ vs respective 129 group. (n = 7–8/group)

Figure 6. Behavior in the elevated plus-maze

Time mice spent in the open arms (A), closed arms (B), dipping their head over the edge (C) and total arm entries (D), of the elevated plus-maze (EPM). C57 mice spend greater time in the closed arms and less time dipping their head over the edge compared to 129 mice. ApoA-IV deficiency reduces open arm time, increases closed arms time, and decreases time spent head-dipping, specifically in the 129 strain. There were no differences in total arm entries. Symbols denote significant differences on post-hoc analysis: * $p < 0.05$ vs respective WT, # $p < 0.05$ vs respective 129. (n = 7–8/group)

Figure 7. Behavior in the forced swim test

Percent time spent swimming (A), immobile (B) and climbing (C) in the forced swim test was unaffected by either strain or apoA-IV status. (n = 7–8/group)