The lipocalin-type prostaglandin D_2 synthase knockout mouse model of insulin resistance and obesity demonstrates early hypothalamic–pituitary–adrenal axis hyperactivity

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Abstract

Obesity and diabetes are closely associated with hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis. In this study, the diet-induced obese C57BL/6 mouse was used to test the hypothesis that chronically elevated metabolic parameters associated with the development of obesity such as cholesterol and glucose can aggravate basal HPA axis activity. Because the lipocalin-type prostaglandin D_2 synthase (L-PGDS) knockout (KO) mouse is a model of accelerated insulin resistance, glucose intolerance, and obesity, it was further hypothesized that HPA activity would be greater in this model. Starting at 8 weeks of age, the L-PGDS KO and C57BL/6 mice were maintained on a low-fat or high-fat diet. After 20 or 37 weeks, fasting metabolic parameters and basal HPA axis hormones were measured and compared between genotypes. Correlation analyses were performed to identify associations between obesity-related chronic metabolic changes and changes in the basal activity of the HPA axis. Our results have identified strong positive correlations between total cholesterol, LDL-cholesterol, glucose, and HPA axis hormones that increase with age in the C57BL/6 mice. These data confirm that obesity-related elevations in cholesterol and glucose can heighten basal HPA activity. Additionally, the L-PGDS KO mice show early elevations in HPA activity with no age-related changes relative to the C57BL/6 mice.

Key Words
- lipocalin-type prostaglandin D_2 synthase
- metabolic syndrome
- hypercholesterolemia
- hyperglycemia
- hypothalamic-pituitary-adrenal axis (HPA axis)

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Introduction

Obesity and diabetes are closely associated with a hyperactive hypothalamic–pituitary–adrenal (HPA) axis. There is evidence that chronic stress can trigger obesity through the increased activity of the HPA axis. Chronic stress-induced elevations of glucocorticoids (GCs) increase expression of corticotrophin-releasing factor in the amygdale, increase the salience of compulsive activities such as the ingestion of high-fat (HF) foods, as well as increase abdominal fat depots (Dallman *et al*. 2003, Shin *et al*. 2010). Although many studies have focused on hyperactivity of the HPA axis as a cause of obesity, relatively a few have focused on the opposite relationship,

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i.e. obesity as a cause of HPA hyperactivity (Foss & Dyrstad 2011). The metabolic changes associated with obesity have the potential to aggravate the HPA axis. Elevations in glucose and cholesterol in obesity are alterations in homeostasis monitored by the hypothalamus and potentially lead to activation of the HPA axis.

The lipocalin-type prostaglandin D_2 synthase (L-PGDS) knockout (KO) mouse has been used to examine the role of L-PGDS in obesity and type 2 diabetes. This mouse model demonstrates glucose intolerance, accelerated insulin resistance, aggravated obesity in the context of increased cholesterol, and atherosclerosis (Ragolia *et al*. 2005, Tanaka *et al*. 2009). In addition, the L-PGDS KO mouse presents with hypertrophic adipocytes in visceral as well as subcutaneous stores (Ragolia *et al*. 2005, Tanaka *et al*. 2009). In human obesity, L-PGDS in cerebral spinal fluid correlates with the orexigenic peptide NPY as well as visceral adiposity and CNS markers of the HPA axis (Elias *et al*. 2011).

L-PGDS is the principle PGH_2 isomerase expressed in the CNS and was originally discovered as beta-trace. L-PGDS is produced in the choroid plexus and leptomeninges (Urade *et al*. 1993) and has been well studied in association with the regulation of sleep–wake cycle (Qu *et al*. 2006) and sensitivity to tactile pain (Eguchi *et al*. 1999). It also plays a protective, anti-inflammatory role in some neurological disorders such as Alzheimer's disease (Kanekiyo *et al*. 2007) and cerebral ischemia (Saleem *et al*. 2009). In the periphery, it is found in the vascular endothelium (Taba *et al*. 2000) as well as adipose tissue (Quinkler *et al*. 2006, Fujimori *et al*. 2007). Its role in the vasculature is thought to be a protective one, where its downstream prostaglandin (PGD_2) and its derivative 15d-PGJ_2 have anti-inflammatory properties (Sasaguri & Miwa 2004). In adipose tissue, L-PGDS and PGD_2 are linked to adipogenic differentiation (Fujimori *et al*. 2007, Fujitani *et al*. 2010).

In this study, the diet-induced obese C57BL/6 mouse was used to test the hypothesis that chronically elevated metabolic parameters associated with obesity such as cholesterol and glucose can intensify basal HPA axis activity. Because the L-PGDS KO mouse is a model of insulin resistance and obesity, it was further hypothesized that basal HPA axis activity would be greater in this model. The L-PGDS KO and C57BL/6 mice were fed a low-fat (LF) or HF diet initiated at 8 weeks of age. After 20 and 37 weeks of feeding, metabolic and HPA axis hormone measurements were compared between genotypes. Correlation analyses between metabolic parameters and ACTH and corticosterone levels were used to identify associations between obesity and HPA activity.

Materials and methods

Animals

All animal protocols were approved by the Institutions Animal Care and Use Committee and adhere to the regulations outlined by the National Institutes of Health. C57BL/6 mice were purchased from Hilltop Animal Labs, Inc. (Scottsdale, PA, USA). The L-PGDS transgenic KO C57BL/6 mice were originally obtained from the Osaka Bioscience Institute (Osaka, Japan; Eguchi *et al*. 1999) and are bred in-house. The colony is periodically tested to confirm the mutation using standard genotyping protocols. The animals were housed under local vivarium conditions and fed a standard chow diet until 8 weeks of age when the experimental diets were introduced. Animals were allowed to adapt to local conditions for at least 1 week before experimental procedures. Only male mice of both genotypes were used in the experiments.

At 8 weeks of age, animals were grouped according to genotype and diet and singly housed: for the C57BL/6 mice $n=20$ per group and for the L-PGDS KO mice $n=10$ per group. Animals were fed *ad libitum* with either a HF diet (5.24 kcal/g) providing 60% of the calories as fat (D12492) or a LF diet (3.85 kcal/g) with 10% of the calories as fat (D12450B) (Research Diets, Inc., New Brunswick, NJ, USA). Animals were killed by decapitation between 0900 and 1100 h after 20 or 37 weeks of feeding. Trunk blood was collected in EDTA tubes and plasma was stored at −80 °C until assay. Variations in the final n represent animals excluded due to mortality and/or no plasma collected, not enough plasma for every assay, or significant outlier.

Glucose, triglyceride, and cholesterol measurements

Whole-blood glucose measurements were made at the time of decapitation using an Accu-Chek glucose monitor (Roche) and the corresponding glucose test strips. Total cholesterol was also measured at the time of decapitation using the Cardio-Chek P.A. (Polymer Technology Systems, Inc., Indianapolis, IN, USA) and the corresponding Lipid Panel test strips, which measure cholesterol, HDL-cholesterol, and triglycerides in whole blood. Because some of the whole-blood triglyceride and HDL and measurements were out of the test-strip range, the Max Discovery kits by BIOo Scientific (Austin, TX, USA) were used to measure triglyceride, LDL, and HDL using plasma.

Plasma ACTH and corticosterone measurements

Corticosterone was measured using the DetectX corticosterone enzyme immunoassay kit (Arbor Assays) with a

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detection limit at 16.9 pg/ml. Plasma ACTH levels were determined using an enzyme immunoassay kit from Phoenix Pharmaceuticals (Burlingame, CA, USA) with a detection range of 0–25 ng/ml.

Statistical analysis

Data were analyzed using three-way fixed effects ANOVA model. In exploring the raw data, several outcome variables violated the assumptions of ANOVA, namely normality. So, we transformed the variables glucose, ACTH, and CORT using Box-Cox transformation. SAS PROC TRANSREG was used to transform the variables and made sure it satisfied the usual assumption of ANOVA. The transformed variables are then used in performing ANOVA. Genotypes, diet, and time were used as the main effects, and all possible two-way and three-way interaction terms between them were considered (Montgomery 2008). SAS PROC MIXED was used to fit the above model that was followed by Tukey–Kramer's multiple comparisons test.

Pearson correlation co-efficient was calculated to assess correlations among the outcome variables that were normally distributed. The Spearman method was used in cases where the data were not normally distributed. Multiplicity adjustments were not made as the correlation analyses were performed as an exploratory nature to examine the trend of the data. All calculations were performed using SAS 9.2 (SAS Institute, Cary, NC, USA); results were considered statistically significant when $P<0.05$. One significant outlier among the L-PGDS KO mice fed the HF diet for 37 weeks was identified using Grubb's test with a significance level of $P<0.05$. Data from this animal were excluded from all analyses.

Results

The L-PGDS KO mouse develops features of metabolic syndrome with age independent of diet

As expected, the HF diet increased body weight among both the C57BL/6 mice and the L-PGDS KO mice. Weight gain by the HF-fed L-PGDS KO mice was greater and more rapid when compared with the C57BL/6 mice. The LF-fed L-PGDS KO mice also experienced greater weight gain after 37 weeks of *ad libitum* feeding when compared with the C57BL/6 mice (Fig. 1A). ANOVA results indicate that genotype, time, and diet had significant individual effects on body weight, $P<0.0001$. The LF-fed L-PGDS KO group also developed elevated fasting total cholesterol (Fig. 1B) that can be attributed to an elevation in LDL levels (Fig. 1C). After 37 weeks of feeding, the elevations in cholesterol were not significantly different than those observed in the HF-fed L-PGDS KO mice giving rise to a significant interaction effect of genotype×diet×time ($F_{(3,105)}=3.30$, $P=0.0233$). Consistent with a metabolic syndrome, levels of HDL were lower in the LF-fed L-PGDS KO mice after 37 weeks of feeding reflected by a significant interaction effect of genotype×diet×time ($F_{(4,97)}=3.89$, $P=0.0057$) (Fig. 1D).

Fasting glucose levels were greater in all L-PGDS KO groups with the exception of the group fed the HF diet for 37 weeks (Fig. 1E). ANOVA results indicated a significant genotype effect on glucose ($F_{(1,110)}=7.68$, $P=0.0066$) as well as a significant effect of diet ($F_{(1,110)}=31.64$, $P<0.0001$).

In contrast to what was observed with cholesterol levels, fasting triglyceride levels in LF-fed L-PGDS KO mice did not increase after 37 weeks of feeding. In fact, triglyceride levels remained constant among all L-PGDS KO mice irrespective of time and diet where the C57BL/6 mice experienced elevated triglyceride with time on the LF diet as well increases in triglyceride with the HF diet. Three-way ANOVA confirmed a significant interaction effect between genotype and time ($F_{(1,108)}=7.44$, $P=0.0074$; Fig. 1F).

L-PGDS KO mice experience early elevations in HPA axis activity relative to C57BL/6 mice but do not experience age-associated changes in ACTH levels

The C57BL/6 mice experience the expected age-associated change in HPA axis activity, i.e. with both the LF and the HF diet, their fasting ACTH levels increased significantly from 20 to 37 weeks of feeding (Fig. 2A). With the LF diet, corticosterone levels do not increase with age as would be expected, reflecting the increase in ACTH. When fed a HF diet, the C57BL/6 mice experience elevations in corticosterone compared with their LF-fed counterparts (Fig. 2B). In the L-PGDS KO mice, ACTH levels were elevated at 20 weeks relative to the C57BL/6 mice but did not increase with age leading to a significant interaction effect of genotype and time ($F_{(1,108)}=32.00$, $P<0.0001$; Fig. 2A). Among the L-PGDS KO mice, corticosterone levels were elevated compared with the C57BL/6 mice as well as with HF feeding; however, there was no change with time on either diet. Three-way ANOVA revealed a significant effect of genotype ($F_{(1,106)}=9.78$, $P=0.0023$) as well as a significant interaction effect of diet and time ($F_{(1,106)}=5.67$, $P=0.0191$) (Fig. 2B).

Because leptin plays a known regulatory role in the HPA axis (Malendowicz *et al.* 2007), leptin levels were

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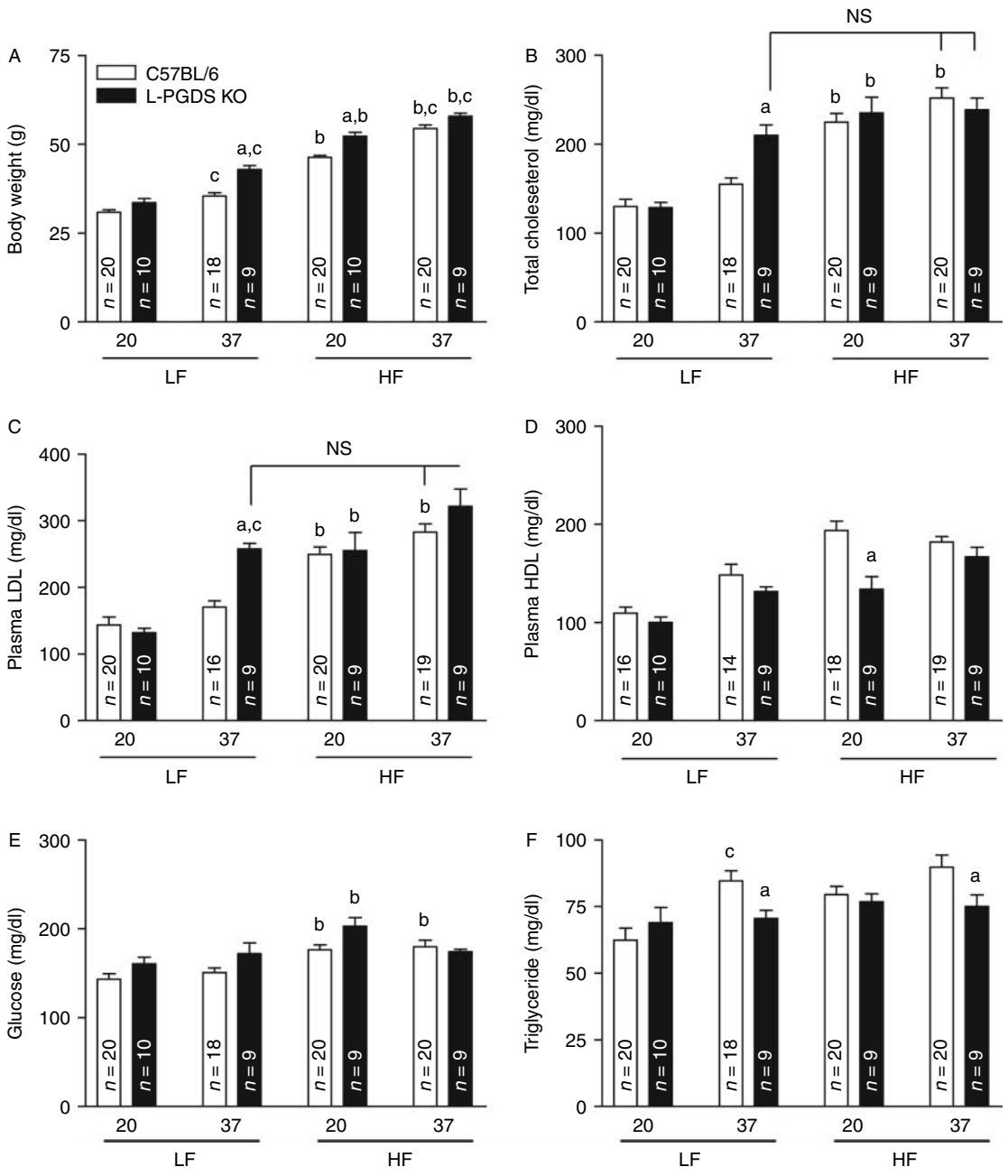

Figure 1

Metabolic parameters in C57BL/6 mice and L-PGDS KO mice maintained on LF and HF diets for 20 and 37 weeks. (A) Body weight, (B) Total cholesterol, (C) Plasma LDL, (D) Plasma HDL, (E) Glucose and (F) Triglyceride were measured. Data are presented as mean ± S.E.M. and *n* is indicated for each group within or above the bar. Comparisons between individual groups were made using the Tukey–Kramer adjustment after significant single or interaction effects found through three-way ANOVA. [a]Significantly different from C57BL/6 counterpart, [b]significantly different from LF fed of same genotype, [c]significantly different from 20 weeks of same genotype and diet.

measured and compared between genotypes. As expected and consistent with increases in body weight and metabolic indices, leptin levels increased significantly with HF feeding among both genotypes (Fig. 2C). Also consistent with metabolic indices, leptin levels among LF-fed L-PGDS KO mice were significantly elevated after 37 weeks when compared with their C57BL/6 counterparts. No significant differences in leptin between

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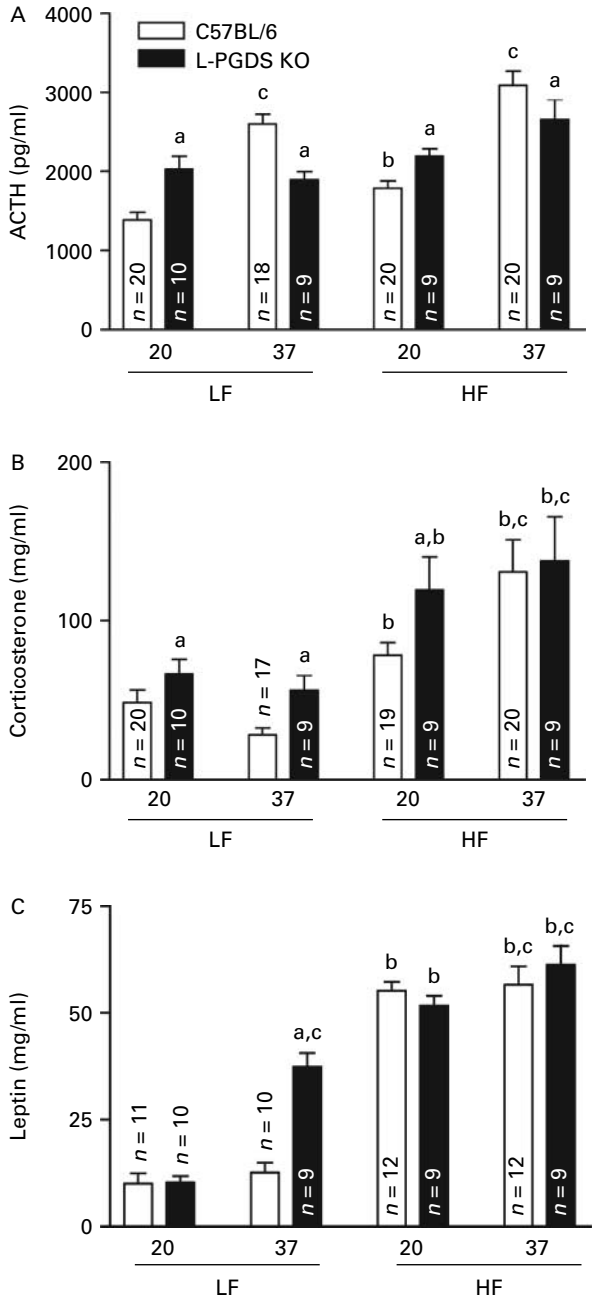

Figure 2
HPA axis hormone and leptin levels in C57BL/6 and L-PGDS KO mice maintained on LF and HF diets for 20 and 37 weeks. (A) ACTH, (B) Corticosterone, and (C) Leptin were measured. Data are presented as mean ± S.E.M. and *n* is indicated for each group within or above the bar. Comparisons between individual groups were made using the Tukey–Kramer adjustment after significant single or interaction effects found through three-way ANOVA. [a]Significantly different from C57BL/6 counterpart, [b]significantly different from LF fed of same genotype, [c]significantly different from 20 weeks of same genotype and diet.

genotypes were found at 20 weeks under a LF diet or with HF feeding consistent with previous reports (Fig. 2C; Ragolia *et al*. 2005).

Correlation analyses of metabolic parameters and HPA axis hormones

In order to reveal relationships between elevated metabolic parameters associated with obesity and HPA axis activity, we performed correlation analyses. These analyses were performed by combining LF- and HF-fed groups of the same genotype and comparisons were made with regard to age, i.e. 20 weeks of feeding (28 weeks of age) and 37 weeks of feeding (45 weeks of age). First, to identify relationships between metabolic parameters and the peripheral branch of the HPA axis, correlations with corticosterone were made. Due to the strong *in vivo* relationship between corticosterone and glucose, the strong positive correlations between these two parameters observed in the C57BL/6 mice were expected. These correlations trended toward positive in the L-PGDS KO mice but were not significant (Fig. 3A and B). In addition, we identified significant correlations between corticosterone and total cholesterol among the C57BL/6 and L-PGD KO mice and this positive correlation strengthened with age (Fig. 3C and D). In line with the correlation results of corticosterone and total cholesterol, significant positive correlations between corticosterone and LDL and corticosterone and HDL were found among the C57BL/6 mice. Among L-PGDS KO mice, positive correlations between corticosterone and LDL and HDL also strengthened with age (Fig. 3E, F, G and H).

To identify relationships between metabolic parameters and the central branch of the HPA axis, correlations were made with ACTH. If elevated metabolic parameters associated with obesity aggravate the HPA axis then it was expected that positive correlations with ACTH would exist. As with corticosterone in the C57BL/6 mice, ACTH positively correlated with glucose and with total cholesterol, with the strength of the correlations increasing with time (Fig. 4A, B, C and D). Correlations of ACTH and LDL followed a similar trend, although at 45 weeks they did not reach significance (Fig. 4E and F). This may have been due to a few data points that approached the point of significant outlier. By contrast, no significant correlations were found between ACTH and HDL levels in these mice (Fig. 4G and H). Among L-PGDS KO mice, significant positive correlations between ACTH and all metabolic parameters were found at 20 weeks but not after 37 weeks of feeding (Fig. 4A, B, C, D, E, F, G and H).

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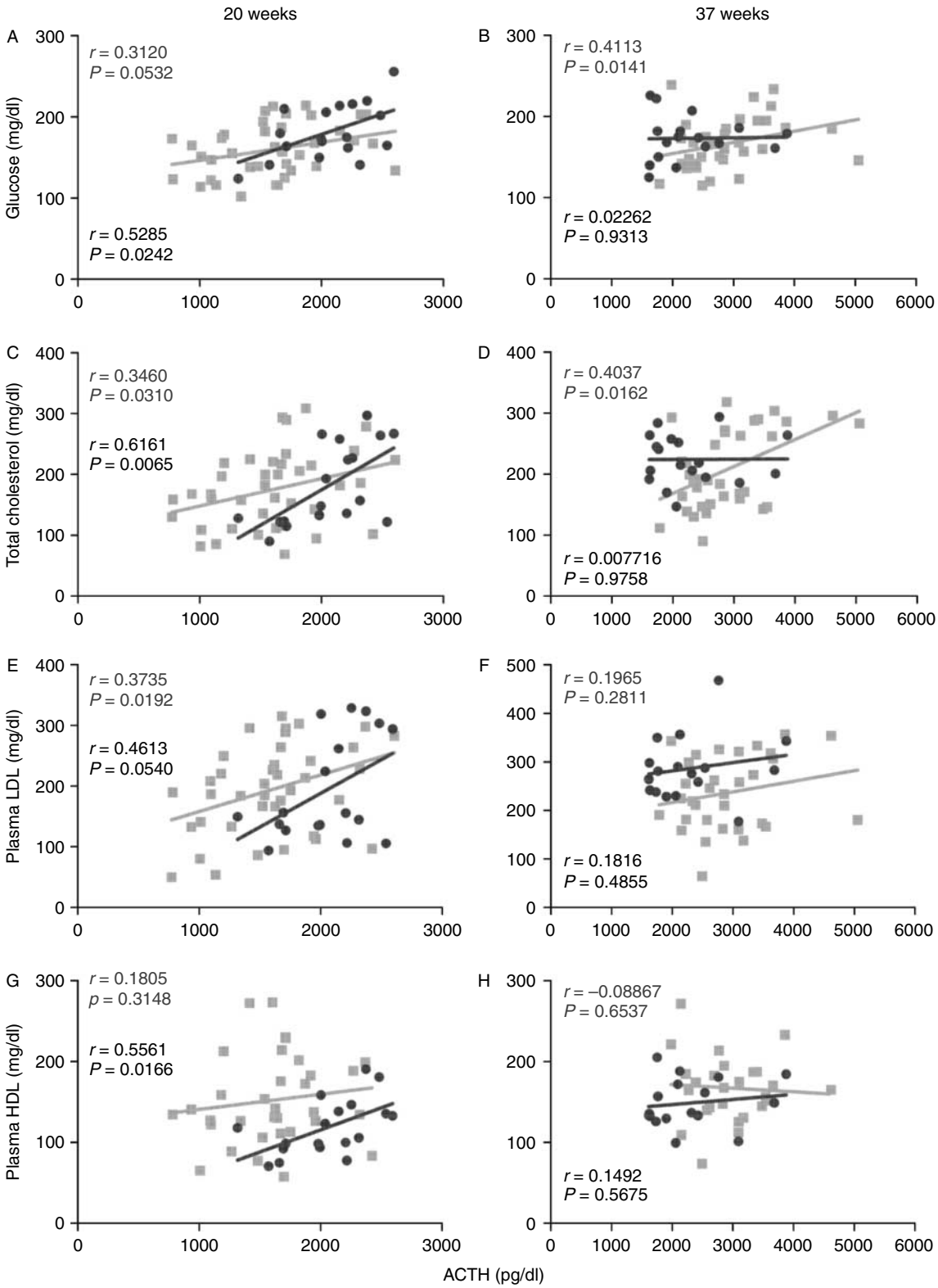

Figure 3

Correlation analyses of glucose (A & B), total cholesterol (C & D), and LDL (E & F) and HDL (G & H) with ACTH in C57BL/6 mice and L-PGDS KO mice. Analyses were performed combining LF- and HF-fed measurements after 20 weeks of feeding (28 weeks of age) and after 37 weeks of feeding (45 weeks of age). Gray squares – C57BL/6, black circles – L-PGDS KO.

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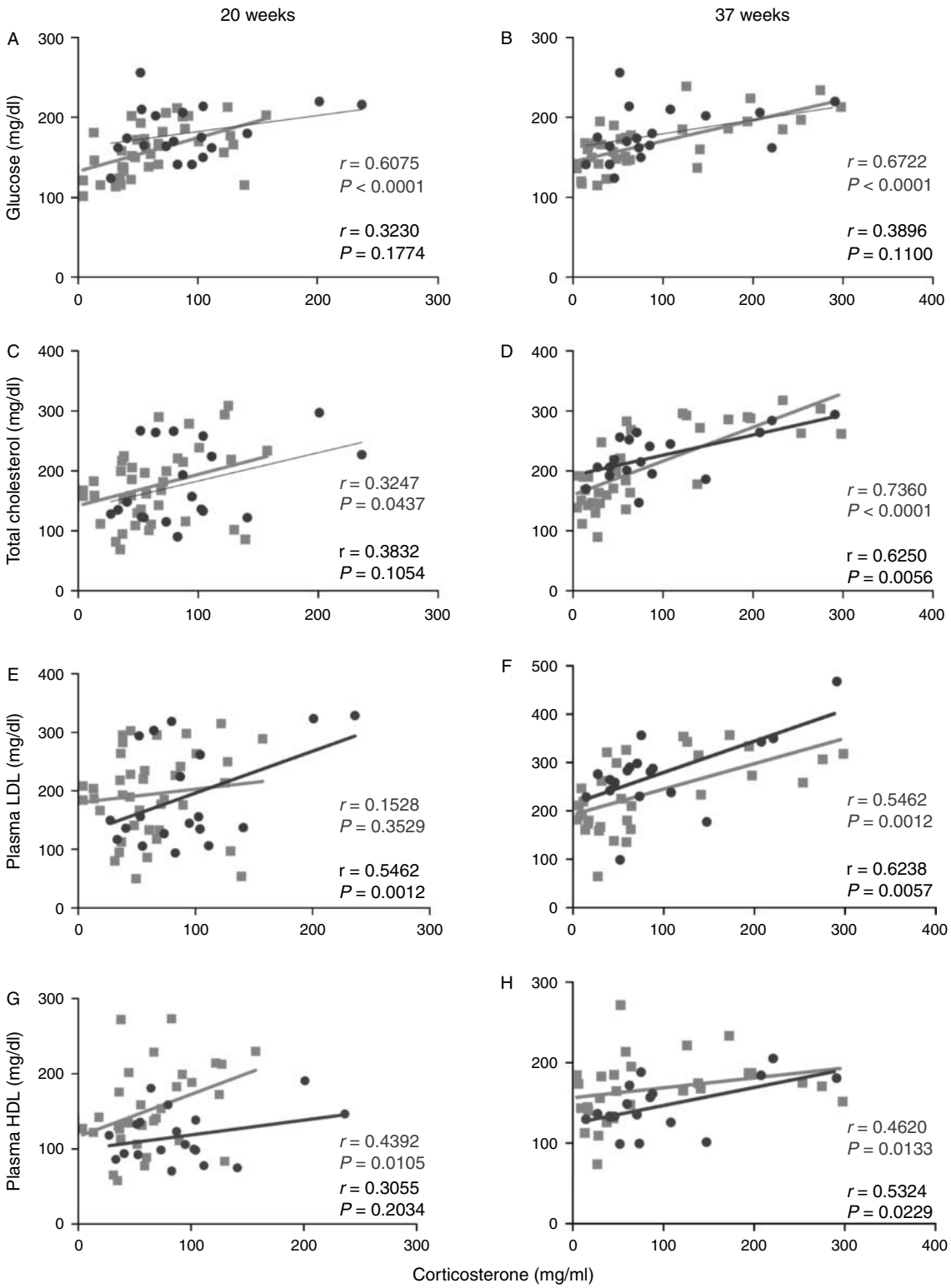

Figure 4

Correlation analyses of glucose (A & B), total cholesterol (C & D), and LDL (E & F) and HDL (G & H) with corticosterone in C57BL/6 mice and L-PGDS KO mice. Analyses were performed combining LF- and HF-fed measurements after 20 weeks of feeding (28 weeks of age) and after 37 weeks of feeding (45 weeks of age). Gray squares – C57BL/6, black circles – L-PGDS KO.

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Correlation analysis of ACTH, corticosterone, and leptin

It is generally accepted that ACTH and corticosterone correlate. Our analysis showed a significant correlation only after 37 weeks of feeding among the C57BL/6 mice and no correlation of HPA axis hormones among L-PGDS KO mice (Fig. 5A and B).

Correlation analyses between leptin and HPA axis hormones were also performed due to leptin's known regulatory role in this axis. Strong positive correlations were found between leptin and both ACTH and corticosterone among both genotypes with the correlations increasing in significance with length of feeding time (Fig. 5C, D, E and F).

Discussion

The L-PGDS KO mouse maintained on a HF diet is a model of increased cholesterol in the context of obesity (Tanaka *et al*. 2009). In our study, the L-PGDS KO mice also developed some characteristic features of metabolic syndrome independent of diet. Metabolic syndrome is defined as having three or more disorders relating to metabolism, i.e. obesity, high blood pressure, elevated fasting glucose, and elevated lipids with a decreased HDL. KO mice maintained on the LF diet from 8 weeks until 45 weeks of age experienced increased body weight as well as an elevated total cholesterol that could be attributed to increased LDL and decreased HDL cholesterol when compared with C57BL/6 mice on the same diet. The elevations in total cholesterol and LDL cholesterol are not significantly different from those observed in the C57BL/6 mice or L-PDGDS KO mice fed a HF diet for the same length of time (Fig. 1B and C). Consistent with the increase in body weight gain and metabolic parameters, the LF-fed L-PGDS KO mice also experienced significantly increased leptin levels after 37 weeks.

C57BL/6 mice fed a HF diet are a well-established model of metabolic syndrome and, consistent with this, they experienced an elevation in fasting triglyceride levels when fed a HF diet. Of note is the fact that this increase did not persist after 37 weeks of feeding due to an age-associated increase in fasting triglyceride among mice maintained on a LF diet. These data are consistent with the reports of a shift in fatty acid metabolism toward lipogenesis with aging in mice (Kuhla *et al*. 2011). It was expected that in L-PGDS KO mice, triglyceride levels in response to HF diet would mirror trends observed with cholesterol measurements as others found a trend toward higher fasting triglyceride levels in L-PGDS KO mice after 12 weeks of HF feeding (Tanaka *et al*. 2009). However, no significant difference in fasting triglyceride was found in L-PGDS KO mice with 20 weeks of HF feeding. Moreover, unlike the control mice, fasting triglyceride levels did not increase with age and after 37 weeks of feeding were lower than those observed in the control animals under both LF and HF diets giving rise to a significant interaction effect of genotype×time (Fig. 1F). Glucose levels in HF-fed L-PGDS KO mice were also not elevated above LF-fed mice after 37 weeks of feeding. Lower triglyceride levels as well as lower glucose levels in the L-PGDS KO mice could be indicative of increased conversion of glucose to triglyceride along with increased triglyceride storage in adipocytes. Adipocytes found in fat depots of L-PGDS KO mice are significantly larger than those of wild-type mice (Ragolia *et al*. 2005, Tanaka *et al*. 2009), and L-PGDS and PGD_2 expression positively correlate with adipogenic differentiation (Fujimori *et al*. 2007, Fujitani *et al*. 2010). Adipogenesis in developing L-PGDS KO mice is impaired, and therefore they have significantly lower numbers of adipocytes. The increased lipid storage in individual cells may be to compensate for decreased numbers of adipocytes.

As expected, the C57BL/6 mice experienced an increase in basal ACTH with age as well as a concomitant relative hypocorticism; corticosterone levels did not increase to mirror the increase in ACTH levels. These results are consistent with previous experiments examining age-related changes in HPA activity of male C57BL/6 mice and have been explained as a consequence of a relative hyposensitivity of the adrenal with aging in mice (Dalm *et al*. 2005). By contrast, the L-PGDS KO mice did not experience age-associated changes in basal HPA axis hormones; ACTH did not increase with age under either diet. These data suggest an association of L-PGDS and/or its PGD_2 product and stress axis activity with aging. CNS levels of L-PGDS increase with aging in humans (Miwa *et al*. 2008) and in animal models (Chen *et al*. 2009). L-PGDS levels also correlate with basal CRH (Elias *et al*. 2011). Thus, there is the possibility that lack of increased L-PGDS levels with age results in decreased CRH production and therefore ACTH production and release. Further study is needed to test this hypothesis.

Although the L-PGDS KO mice did not experience HPA axis hyperactivity with age, their ACTH and corticosterone levels were increased relative to the C57BL/6 mice at 28 weeks of age after 20 weeks of feeding on either diet (Fig. 2). This is not surprising and many factors could be contributing to the early changes in the stress axis of these animals. Both increased production of

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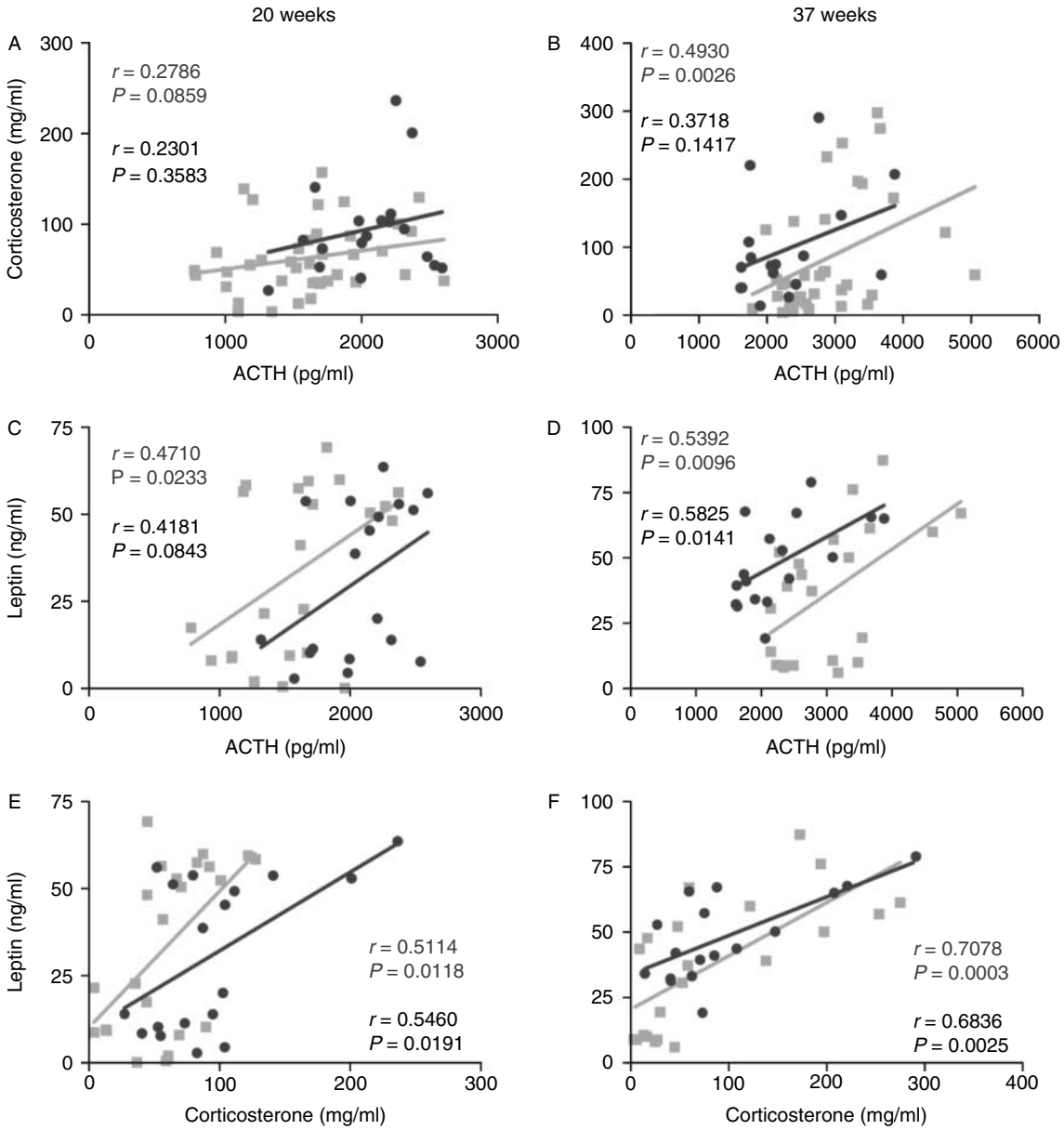

Figure 5

Correlation analyses of leptin and the HPA axis hormones in C57BL/6 mice and L-PGDS KO mice. (A & B) ACTH vs corticosterone, (C & D) ACTH vs leptin, (E & F) corticosterone vs leptin. Analyses were performed combining LF- and HF-fed measurements after 20 weeks of feeding (28 weeks of age) and after 37 weeks of feeding (45 weeks of age). Gray squares – C57BL/6, black circles – L-PGDS KO.

IL1β and sleep disturbances are potential contributors to increased HPA axis activity in L-PGDS KO mice. Lack of L-PGDS is associated with increased production of IL1β in L-PGDS KO mice (Tanaka *et al*. 2009). Increased production of this inflammatory cytokine is in turn associated with increased stimulation of the HPA axis (Hsieh *et al*. 2010, Sasayama *et al*. 2011). PGD_2 produced from L-PGDS also plays a significant somnogenic role in physiological sleep in rodents and humans (Hayaishi 2002, Jordan *et al*. 2004, Qu *et al*. 2006). L-PGDS deficiency in the KO animals would result in lower PGD_2 levels and disturbances in sleep. Sleep disturbances can have a significant impact on HPA axis activity resulting in significant elevations in basal HPA hormone levels (Balbo *et al*. 2010).

Chronic stress and subsequent elevated GC result in the release of glucose from hepatic stores and the redistribution of fat to abdominal depots. Therefore, stress and the associated basal HPA hyperactivity have been

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implicated as a trigger in the development of obesity and diabetes (Dallman *et al*. 2004). Our data confirm the strong *in vivo* relationship between glucose and corticosterone and also support the hypothesis that elevated metabolic indices can intensify HPA axis activity. ACTH levels correlated with glucose and total cholesterol levels, and these correlations also strengthened with time, confirming HPA axis hyperactivity. Therefore, the HPA axis can be aggravated by a HF diet as well as participate in the pathological progression of type 2 diabetes.

The mechanism behind HF diet activation of the HPA axis needs to be explored. It has been established that uncontrolled or poorly controlled diabetes is associated with an upregulated HPA axis (Chan *et al*. 2001). This increased activity is reportedly related to insulin deficiency as opposed to hyperglycemia; basal ACTH and corticosterone in streptozotocin (STZ)-treated rats are normalized with insulin but not phloridzin (Chan *et al*. 2005). Hypothalamic insulin resistance occurs with HF feeding and is attributed to the accumulation of lipid in this region (Posey *et al*. 2009). Therefore, our data are in line with these data suggesting that diet-induced obesity creates an insulin-deficient environment in the brain resulting in a hyperactive HPA axis.

The positive correlation between ACTH and total cholesterol levels in C57BL/6 mice after 20 and 37 weeks of feeding, as well as in the L-PGDS KO mice after 20 weeks of feeding, could possibly be explained by stimulation of the liver-X receptor-α in the pituitary by oxysterol. A high cholesterol diet or the administration of the synthetic liver X receptor agonist increases *POMC* mRNA in the pituitary and also increases pituitary production of ACTH resulting in elevated plasma ACTH (Matsumoto *et al*. 2009).

In addition, the impact of HF feeding on ACTH levels must be considered. Glucagon-like peptide-1 (GLP1), released in response to food intake, can increase the activity of the HPA axis in rodents and humans (Gil-Lozano *et al*. 2010). Therefore, bouts of hyperphagia due to the more palatable food among HF-fed animals may contribute to elevated circulating ACTH levels. However, GLP1-induced increases are transient and ACTH levels return to baseline within 20 min of injection (Gil-Lozano *et al*. 2010).

The correlation analyses also indicate a positive relationship between elevated total cholesterol and corticosterone. Others have concluded that in addition to *de novo* synthesis of cholesterol and catabolism of intracellular stored cholesterol, cholesterol derived from plasma lipoproteins is a significant source of the cholesterol needed in adrenal steroidogenesis (Morris & Chaikoff 1959, Hoekstra *et al*. 2010). Mice fed probucol to deplete plasma lipoproteins experience impaired adrenal steroidogenesis (Hoekstra *et al*. 2010). These data along with the strong correlation between corticosterone and total cholesterol in our study point to the possibility that an increase in lipoprotein would increase adrenal basal steroidogenesis and secretion.

It is generally expected that ACTH and corticosterone correlate, especially in the presence of an environmental stressor due to the fact that ACTH stimulates adrenal corticosterone release. Here, however, we found a significant correlation only after 37 weeks of feeding in the C57BL/6 mice and no correlation among the L-PGDS KO mice. Additionally, leptin, which plays a regulatory role in the HPA axis in mice, strongly correlated with both ACTH and corticosterone among both genotypes. Centrally, leptin increases ACTH secretion while peripherally it suppresses ACTH-stimulated steroid production (Malendowicz *et al*. 2007). However, leptin can also raise hepatic 11β-hydroxysteroid dehydrogenase (11β-HSD) type I activity in mice (Liu *et al*. 2003). Therefore, our data suggest that in mice, obesity-related increases in leptin disrupts the feedback between central and peripheral branches of the HPA axis by increasing ACTH secretion and inhibiting ACTH-stimulated adrenal corticosterone release. However, it can increase corticosterone through sources other than ACTH stimulation. This would explain the absence of the correlation between ACTH and corticosterone, the increases in corticosterone with HF feeding, and the significant correlations of leptin with both ACTH and corticosterone.

Overall, our results demonstrate that changes in metabolic indices associated with obesity promote increased activity in the HPA axis. The L-PGDS KO mouse displays features of the metabolic syndrome in the absence of a HF diet as well as with HF feeding and this correlates with HPA axis hyperactivity at 28 weeks of age. However, unlike the C57BL/6 model that displays age-related increases in HPA activity, the L-PGDS KO mice appear resistant to changes in HPA activity with age and long-term HF feeding indicating that these events are dependent on L-PGDS expression.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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