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Role of adipose and hepatic atypical protein kinase C lambda (PKCλ) in the development of obesity and glucose intolerance

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Abbreviations: PKCλ, protein kinase C-lambda; DIO, diet induced obesity; HFD, high fat diet; ip, intraperitoneal; GTT, glucose tolerance test; ITT, insulin tolerance test; PTT, pyruvate tolerance test, SREBP-1c, sterol regulatory element-binding protein-1c; FAS, fatty acid synthase; NFκB, nuclear factor kappa B

PKCλ, an atypical member of the multifunctional protein kinase C family, has been implicated in the regulation of insulin-stimulated glucose transport and of the intracellular immune response. To further elucidate the role of this cellular regulator in diet-induced obesity and insulin resistance, we generated both liver (PKC-Alb) and adipose tissue (PKC-Ap2) specific knockout mice. Body weight, fat mass, food intake, glucose homeostasis and energy expenditure were evaluated in mice maintained on either chow or high fat diet (HFD). Ablation of PKCλ from the adipose tissue resulted in mice that were indistinguishable from their wild-type littermates. However, PKC-Alb mice were resistant to diet-induced obesity (DIO). Surprisingly this DIO resistance was not associated with either a reduction in caloric intake or an increase in energy expenditure as compared with their wild-type littermates. Furthermore, these mice displayed an improvement in glucose tolerance. When maintained on chow diet, these mice were similar to wild types in respect to body weight and fat mass, yet insulin sensitivity was impaired compared with wt littermates. Taken together these data suggest that hepatic PKCλ is modulating insulin-mediated glucose turnover and response to high fat diet feeding, thus offering a deeper understanding of an important target for anti-obesity therapeutics.

Introduction

Dissecting molecular signaling pathways involved in the pathogenesis of the metabolic syndrome through the regulation of nutrient and energy homeostasis is of pivotal importance for the generation of novel strategies to prevent and treat obesity, and associated diseases such as glucose intolerance, type 2 diabetes, cancer and pulmonary diseases. Several studies over the last decade have established a close interaction between impairment of insulin signaling and dysfunction of cellular pathways in inflammation and innate immunity.[1] This convergence of metabolic and inflammatory signals is exemplified by the role of the signaling molecule protein kinase λ (PKCλ), member of the atypical subfamily of PKC isoforms (aPKC), as a critical regulator of the immune response[2] and at the same time as a crucial mediator of insulin effects on glucose transport in muscle and adipose tissue and lipid synthesis in liver.[3-5]

PKCλ, together with PKCζ, are the two highly homologous members of the aPKC subfamily. Both are serine/threonine kinases activated by phosphorylation and phosphatidylserine, but are insensitive to either calcium (Ca^{2+}) or diacylglycerol (DAG) (reviewed in ref. 6). In metabolic signaling, insulin activates aPKC through phosphatidylinositol-3 kinase (PI3K) to facilitate glucose transporter 4 (GLUT4) translocation to the plasma membrane in skeletal muscle and fat.[7] Consistent with this regulatory role, ablation of PKCλ in muscle impairs glucose uptake and this tissue-specific limitation is sufficient to induce abdominal obesity, hyperlipidemia and hepatosteatosis, all hallmarks of the metabolic syndrome.[8] Hepatic deletion of PKCλ paradoxically enhanced insulin-mediated glucose uptake in muscle and fat, but also

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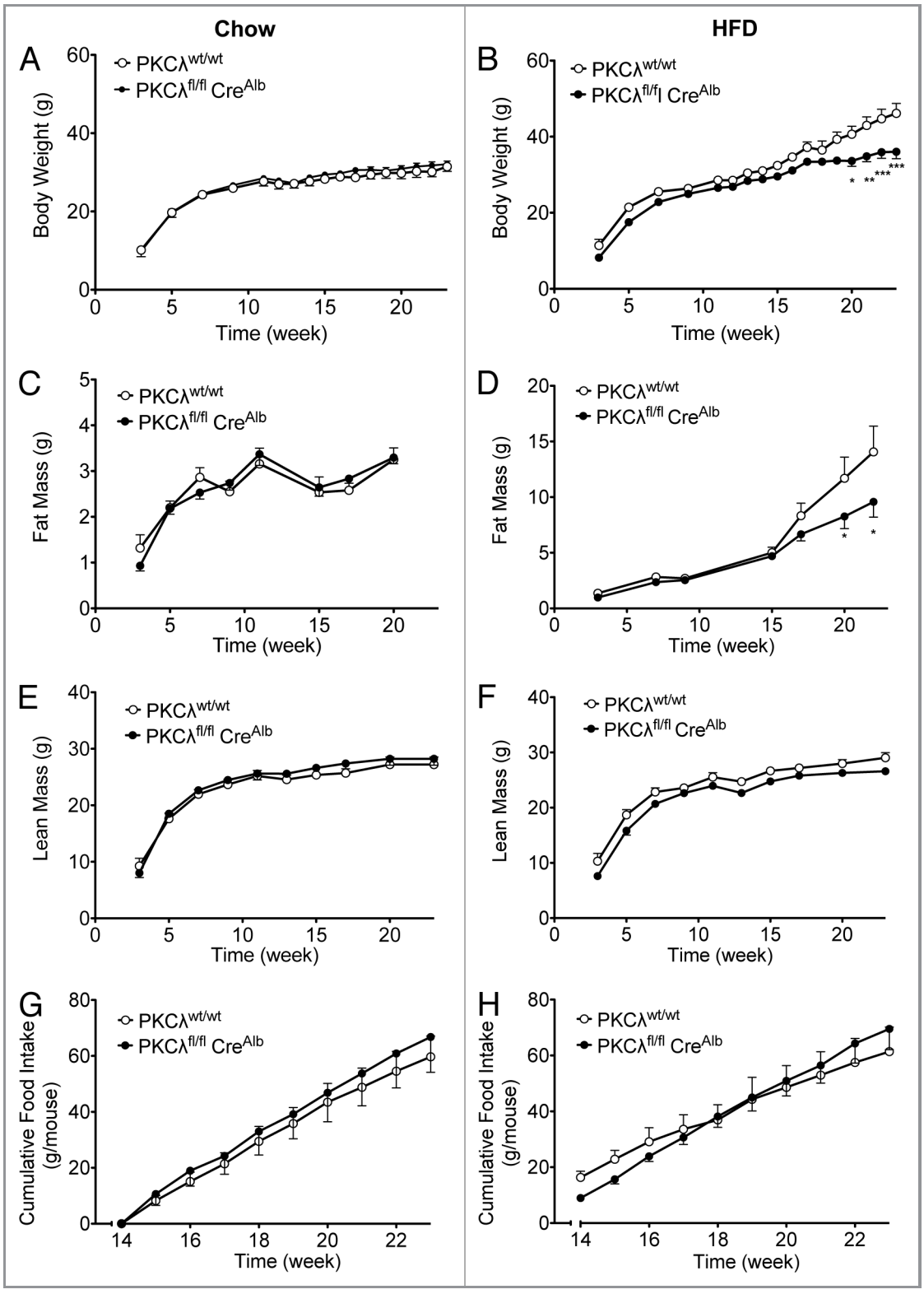

Figure 1. Body weight (A and B), fat mass (C and D) and lean mass (E and F) growth curves of chow- or HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed circles) and PKCλ$^{wt/wt}$ mice (open circles). Cumulative food intake was monitored throughout the study (G and H). All data are represented as mean ± SEM in 6–7 age-matched, male mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, as determined by 2-way ANOVA.

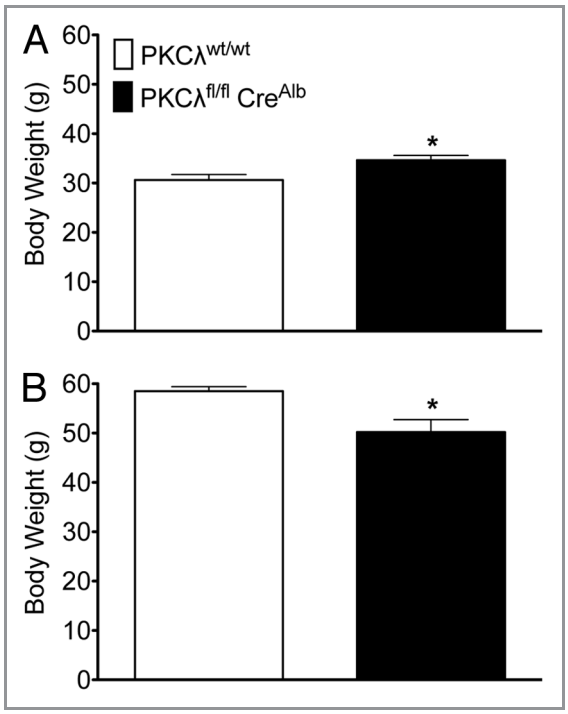

Figure 2. Body weight of 40-week-old, chow- (A) or HFD-fed (B) PKCλ$^{fl/fl}$ Cre^{Alb} (closed bars) and PKCλ$^{wt/wt}$ mice (open bars). All data are represented as mean ± SEM in 6–7 age-matched, male mice. *$p < 0.05$ as determined by Student's t-test.

prevented insulin-stimulated lipogenesis in liver,[9,10] resulting in decreased triglyceride content and expression of the sterol regulatory element-binding protein-1c (SREBP-1c) gene. In inflammatory signaling, aPKCs activate the NFκB pathway through phosphorylation of inhibitory-κBα (IκBα) and the p65 subunit of NFκB.[11,12] In addition, aPKCs regulate receptor-interacting protein and TNF receptor-associated factor 6 by binding to the scaffolding protein p62, thus modulating Toll-like receptor 4 (TLR4) and TNF-α receptor signaling cascades.[13,14] aPKC activation is further required for the LPS-induced signaling through TLR4 in macrophages[15,16] and for T-cell helper (Th2) activation, a pathway relevant in inflammatory disorders.[2]

Since aPKCs are uniquely positioned to mediate convergent intracellular immune and metabolic responses we sought to investigate whether PKCλ may also regulate cellular signaling cascades relevant for energy homeostasis in insulin-sensitive tissues such as liver and fat under high fat diet (HFD) feeding conditions. Herein we demonstrate that mice deficient for hepatic PKCλ, are resistant to diet-induced obesity and diet-induced glucose intolerance without increases in energy intake or expenditure. In contrast, we show that adipose-specific PKCλ does not play a role in diet-induced obesity and associated insulin resistance.

Results

To test the hypothesis that hepatic or adipose PKCλ modulates signaling pathways of energy homeostasis, we created hepatic and adipose tissue-specific knockouts for the PKCλ gene. This was accomplished by breeding mice expressing the Cre recombinase transgene controlled by the albumin (Alb) or the adipocyte lipid-binding protein (aP2) promotor/enhancer with mice carrying two targeted PKC-λ alleles with loxP sites (PKCλ$^{fl/fl}$) flanking an essential exon of the PKCλ gene as previously described.[2]

Hepatic-specific PKCλ deletion. *Body weight regulation and energy expenditure.* Mice deficient for hepatic PKCλ (PKCλ$^{fl/fl}$ Cre^{Alb}) were born in normal Mendelian ratios and had similar body weight at weaning as compared with their wild-type siblings (PKCλ$^{wt/wt}$; **Fig. 1A and B**). When maintained on chow diet, we did not detect differences in body weight and composition throughout the initial 23 weeks of study (**Fig. 1A, C and E**). However, when fed a high fat diet (HFD), PKCλ$^{fl/fl}$ Cre^{Alb} mice displayed resistance to diet induced obesity (DIO; **Fig. 1B and D**) as compared with their PKCλ$^{wt/wt}$ littermates. Lean mass was not different between the groups (**Fig. 1E and F**). Regardless of diet, mice from both genotypes displayed similar food intake throughout the initial 23 weeks of study (**Fig. 1G and H**). Prolonged exposure (37 weeks) of these mice to either chow or HFD resulted in a reciprocal effect on body weight. Specifically, chow-fed PKCλ$^{fl/fl}$ Cre^{Alb} mice displayed increased body weight (**Fig. 2A**) as compared with their PKCλ$^{wt/wt}$ littermates. Consistent with their earlier DIO resistance (see **Fig. 1B and D**), HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} mice displayed decreased body weight as compared with their PKCλ$^{wt/wt}$ littermates (**Fig. 2B**).

The resistance to DIO in PKCλ$^{fl/fl}$ Cre^{Alb} mice, accompanied by normal food intake (**Fig. 1H**), suggested that these mice might exhibit increased energy expenditure as compared with their PKCλ$^{wt/wt}$ littermates. To test this hypothesis, energy expenditure was monitored in 23-week-old mice on chow or HFD via indirect calorimetery over the course of one week. However, energy expenditure was not different from PKCλ$^{wt/wt}$ littermates in PKCλ$^{fl/fl}$ Cre^{Alb} mice fed either chow (**Fig. 3A and B**) or HFD (**Fig. 4A and B**). Likewise, locomotor activity and respiratory quotient were unaffected by genotype, regardless of diet (**Figs. 3 and 4C–F**).

Glucose metabolism. PKCλ$^{fl/fl}$ Cre^{Alb} mice on chow exhibited lower fasting blood glucose levels compared with their wt littermates (**Fig. 5A**) with a similar trend observed in HFD-fed mice (**Fig. 5B**). HFD feeding increased fasting insulin levels about 4-fold (from an average of 0.47 to 1.93 ng/ml) in wt mice compared with 1.9-fold (from an average of 0.49 to 0.95 ng/ml) in PKCλ$^{fl/fl}$ Cre^{Alb} mice (**Fig. 5C and D**), suggesting that that hepatic ablation of PKCλ results in enhanced insulin sensitivity. This hypothesis was supported by calculating the HOMA-Index, a widely used clinical surrogate marker for insulin sensitivity: We detected a trend toward improved HOMA-Index values in chow-fed PKCλ$^{fl/fl}$ Cre^{Alb} mice ($p = 0.0556$; **Fig. 5E**) and significantly improved HOMA-Index values in HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} mice (**Fig. 5F**), as compared with their respective wild-type controls. The regulation of glucose homeostasis by hepatic PKCλ was further examined by performing intraperitoneal glucose tolerance test (ipGTT) and insulin tolerance test (ipITT). Although blood glucose levels were similar during GTT (**Fig. 6A**, inset), the glucose lowering effect of exogenously administered insulin was reduced in PKCλ$^{fl/fl}$ Cre^{Alb} mice on chow compared with wt mice

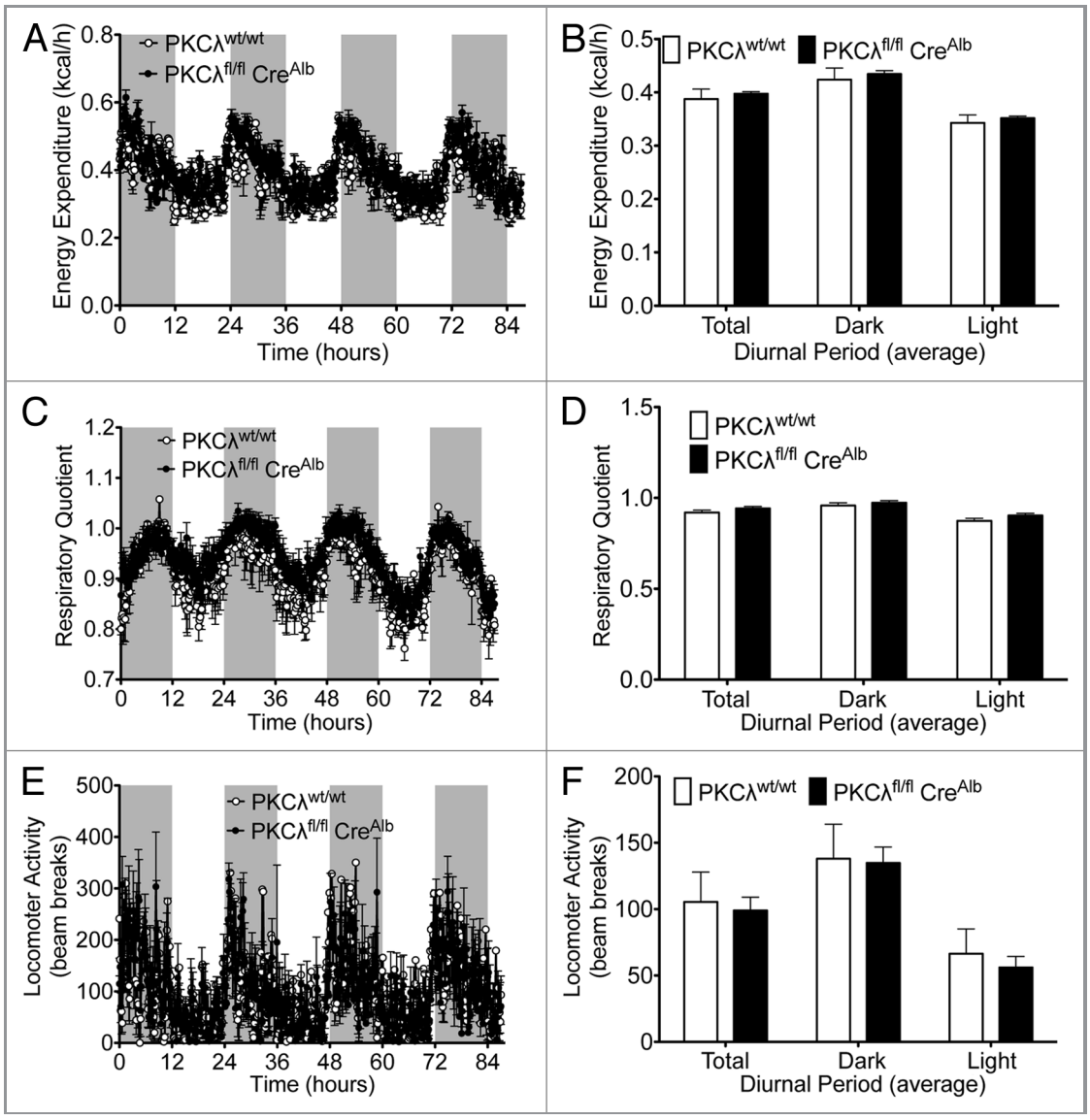

Figure 3. Dynamic and average energy expenditure (A and B), respiratory quotient (C and D) and locomotor activity (E and F) in chow-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed circles/bars) and PKCλ$^{wt/wt}$ (open circles/bars) mice as measured by indirect calorimetry. All data are represented as mean ± SEM in 6–7 age-matched, male mice. No differences were determined by 2-way ANOVA.

(**Fig. 6C and E**), indicating that contrary to our hypothesis, insulin-mediated glucose transport was impaired. However, PKCλ$^{fl/fl}$ Cre^{Alb} mice on HFD exhibited an improved response to ipGTT compared with their PKCλ$^{wt/wt}$ littermates. Specifically, the glucose excursion 60 min after glucose challenge (**Fig. 6B**) and the area under the curve for the duration of the test (**Fig. 6B**, inset) were reduced in PKCλ$^{fl/fl}$ Cre^{Alb} mice. Analysis of ipITT in HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} mice and their PKCλ$^{wt/wt}$ littermates revealed no difference in glucose clearance (**Fig. 6D and F**), suggesting that hepatic glucose output may be different in the absence of PKCλ. Since we also observed similar glucose lowering effects of exogenously administered insulin in chow and HFD fed PKCλ$^{fl/fl}$ Cre^{Alb} mice (**Fig. 6C and D**), we concluded that ablation of hepatic PKCλ makes mice resistant to the effect of HFD on insulin-mediated glucose uptake.

We next conducted pyruvate tolerance tests (ipPTT) to elucidate the contribution of hepatic glucose output to the divergent ipGTT and fasting glucose levels observed in these mice. Chow-fed PKCλ$^{fl/fl}$ Cre^{Alb} mice displayed similar glucose excursions (**Fig. 7A**) and increase of glucose levels of ipPTT (**Fig. 7C**) as compared with their PKCλ$^{wt/wt}$ littermates. Under HFD feeding conditions, relative glucose appearance revealed similar glucose excursions (**Fig. 7B**) but, an enhanced hepatic glucose output in PKCλ$^{fl/fl}$ Cre^{Alb} mice (**Fig. 7D**) during the first 30 min, suggesting a higher gluconeogenic potential in the absence of PKCλ. We concluded that the observed differences in fasting glucose levels can neither be explained by increased insulin-mediated glucose uptake nor by reduced hepatic gluconeogenic potential. Taken together our results draw a complicated picture of PKCλ's various roles in glucose homeostasis, indicating

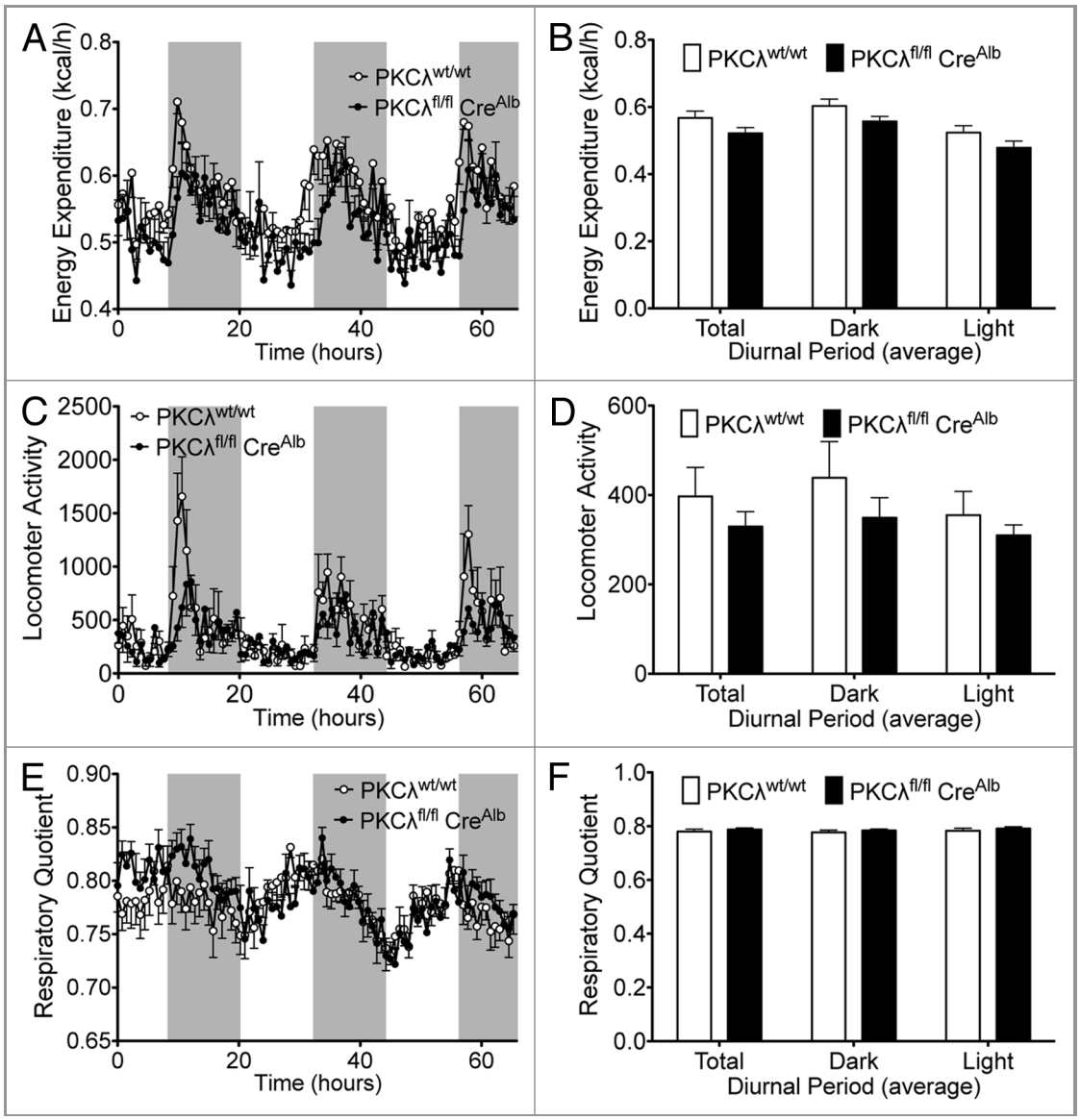

Figure 4. Dynamic and average energy expenditure (A and B), respiratory quotient (C and D) and locomotor activity (E and F) in HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed circles/bars) and PKCλ$^{wt/wt}$ (open circles/bars) mice as measured by indirect calorimetry. All data are represented as mean ± SEM in 6–7 age-matched, male mice. No differences were determined by 2-way ANOVA.

that ablation of hepatic PKCλ protects against DIO-induced hyperglycemia, but at the same time it impairs insulin-mediated glucose uptake under lean, chow feeding conditions.

Lipid metabolism. Prior reports on the hepatic action of PKCλ suggest that this pathway regulates hepatic lipogenesis.[9,10] To gain insight into lipid metabolism in our model, we measured cholesterol and triglycerides at the termination of the study (week 40). Plasma cholesterol levels were similar in PKCλ$^{fl/fl}$ Cre^{Alb} and their PKCλ$^{wt/wt}$ littermates, regardless of diet (**Fig. 8A**). Furthermore, we found that plasma triglycerides were significantly elevated in chow-fed PKCλ$^{fl/fl}$ Cre^{Alb} mice as compared with their PKCλ$^{wt/wt}$ littermates (**Fig. 8B**). However, while HFD exposure in PKCλ$^{wt/wt}$ mice elevated plasma triglycerides as compared with chow-fed PKCλ$^{wt/wt}$ mice, we observed no further increase in HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} mice (**Fig. 8B**). These effects on plasma triglycerides were not associated with liver injury/dysfunction as determined by Alanine transaminase (**Fig. 8C**). Together these findings suggest that in our model, ablation of hepatic PKCλ modulates triglyceride metabolism in chow fed animals, an effect that is not evident under high fat feeding conditions.

Adipose-specific PKCλ deletion. As in the hepatic specific ablation, mice deficient for adipose PKCλ (PKCλ$^{fl/fl}$ Cre^{aP2}) were born in normal Mendelian ratios and had similar body and fat mass at weaning as compared with their wild-type siblings (PKCλ$^{wt/wt}$; **Fig. 9A and B**). When maintained on chow diet, these mice showed similar body composition compared with wt littermates throughout the initial 20 weeks of study (**Fig. 9B and C**). At week 20, all mice were monitored for energy expenditure differences and

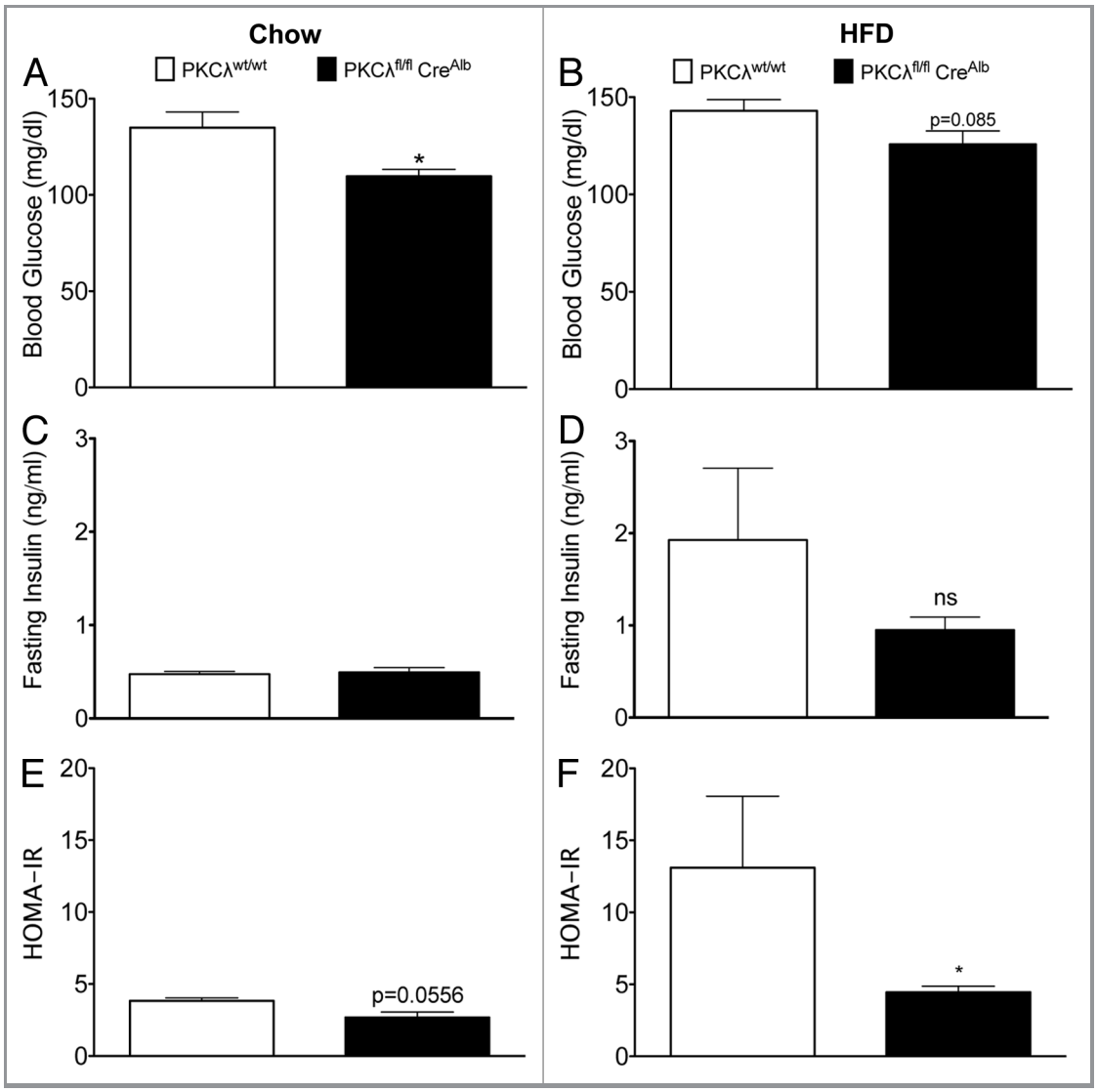

Figure 5. Blood glucose levels (A and B) of chow- and HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed bars) and PKCλ$^{wt/wt}$ (open bars) mice following 6 h fast. Plasma insulin (C and D) in HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed bars) and PKCλ$^{wt/wt}$ (open bars) mice following 6 h fast. HOMA-INDEX (e and f) in HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed bars) and PKCλ$^{wt/wt}$ (open bars) mice. All data are represented as mean ± SEM in 6–7 age-matched, male mice at week 25. *$p < 0.05$ as determined by one-way ANOVA.

then exposed to HFD. We found that PKCλ$^{fl/fl}$ Cre^{aP2} mice developed diet-induced obesity at similar rates than wt littermates, suggesting that ablation of PKCλ in adipose tissue does not modulate body composition (40 weeks; **Fig. 9A**). Despite the similar body and fat mass, food intake over the course of this study showed a trend to be decreased in PKCλ$^{fl/fl}$ Cre^{aP2} mice as compared with their PKCλ$^{wt/wt}$ littermates (**Fig. 9D**). Surprisingly, but consistent with our observations in PKCλ$^{fl/fl}$ Cre^{Alb} mice, energy expenditure in PKCλ$^{fl/fl}$ Cre^{aP2} mice was not different from PKCλ$^{wt/wt}$ littermates (**Fig. 10A and B**). Likewise, respiratory quotient (**Fig. 10C and D**) and locomotor activity (**Fig. 10E and F**) were unaffected by genotype. Glucose metabolism analysis at week 20 revealed no differences in fasting glucose levels (**Fig. 9F**) and similar response to glucose tolerance test in chow-fed PKCλ$^{fl/fl}$ Cre^{aP2} mice and their PKCλ$^{wt/wt}$ littermates (**Fig. 9E**, inset), indicating that adipose tissue specific deficiency of PKCλ has no effect on whole body glucose metabolism.

Discussion

We herein report for the first time to our knowledge that mice deficient in hepatic PKCλ are protected against diet-induced obesity and hyperglycemia. Since this resistance was not a result of reduced energy intake or increased expenditure, we propose that the observed difference in body weight may be due to a small difference in energy balance that is below the detection limit of the generally insensitive 24 h indirect calorimetry, but nevertheless relevant for chronic body composition development, as previously described.[17] Consistent with reduced adiposity, glucose tolerance was improved in HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} mice

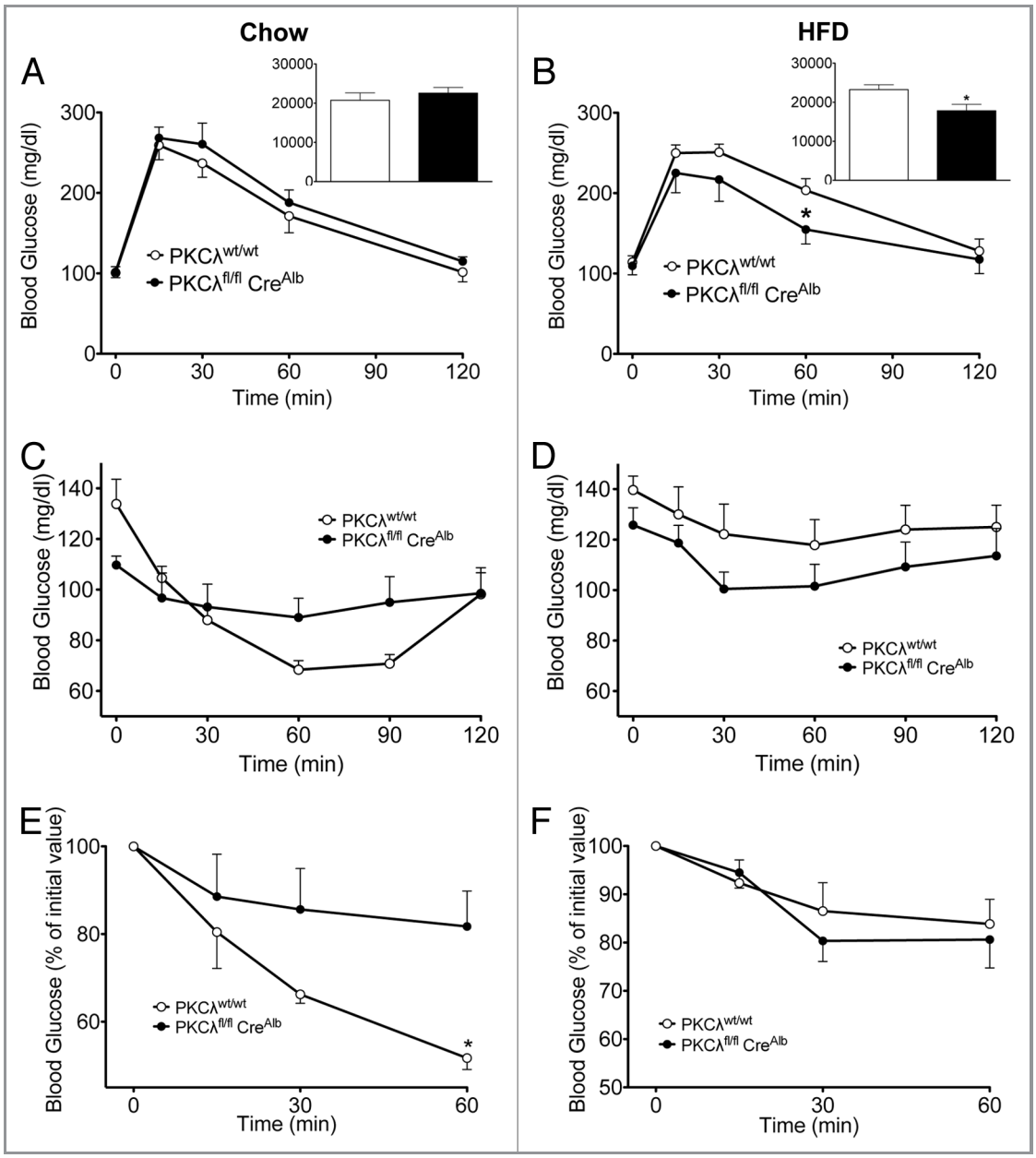

Figure 6. Glucose tolerance as assessed by glucose excursion (A and B) and area under the curve analyses (inset of A and B) of chow- and HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed circles) and PKCλ$^{wt/wt}$ (open circles) mice following a 2 g/kg dose of ip glucose at week 20. Insulin tolerance as assessed by glucose excursion (C and D) and glucose clearance [as % of initial blood glucose (E and F)] of chow- and HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed circles) and PKCλ$^{wt/wt}$ (open circles) mice following a 0.75 U/kg dose of ip insulin at week 25. All studies were conducted after a 6 h fast and all data are represented as mean ± SEM in 6–7 age-matched, male mice. *$p < 0.05$ as determined by 2-way ANOVA or Student's t-test.

compared with their wt littermates, pointing to a beneficial effect on diet induced hyperglycemia and glucose intolerance by silencing PKCλ in liver. Our findings are confirmed by emerging data utilizing small-molecule inhibitors of hepatic PKCλ in rodents.[18]

Lower fasting glucose and insulin levels reflected significantly improved HOMA-IR index values, a well-established surrogate measure of insulin action,[32,33] suggesting that HFD-induced insulin resistance is reduced in the absence of hepatic PKCλ. Furthermore, HFD-induced fasting hyperinsulinemia was enhanced in wt mice compared with PKCλ$^{fl/fl}$ Cre^{Alb} mice indicating a possible impaired early-phase insulin secretion during glucose challenge in wt mice.[20] To our surprise, results obtained with ipGTT and ipPTT showed that the observed improvement in glucose metabolism is not due to enhanced insulin-mediated glucose transport or hepatic gluconeogenesis. We therefore speculate that hepatic deficiency of PKCλ may reduce glycogenolysis and consequently lowering fasting glucose levels.[21] Furthermore, we observed that lean and obese PKCλ$^{fl/fl}$ Cre^{Alb} mice exhibited similar glucose disappearance rates during ipITT

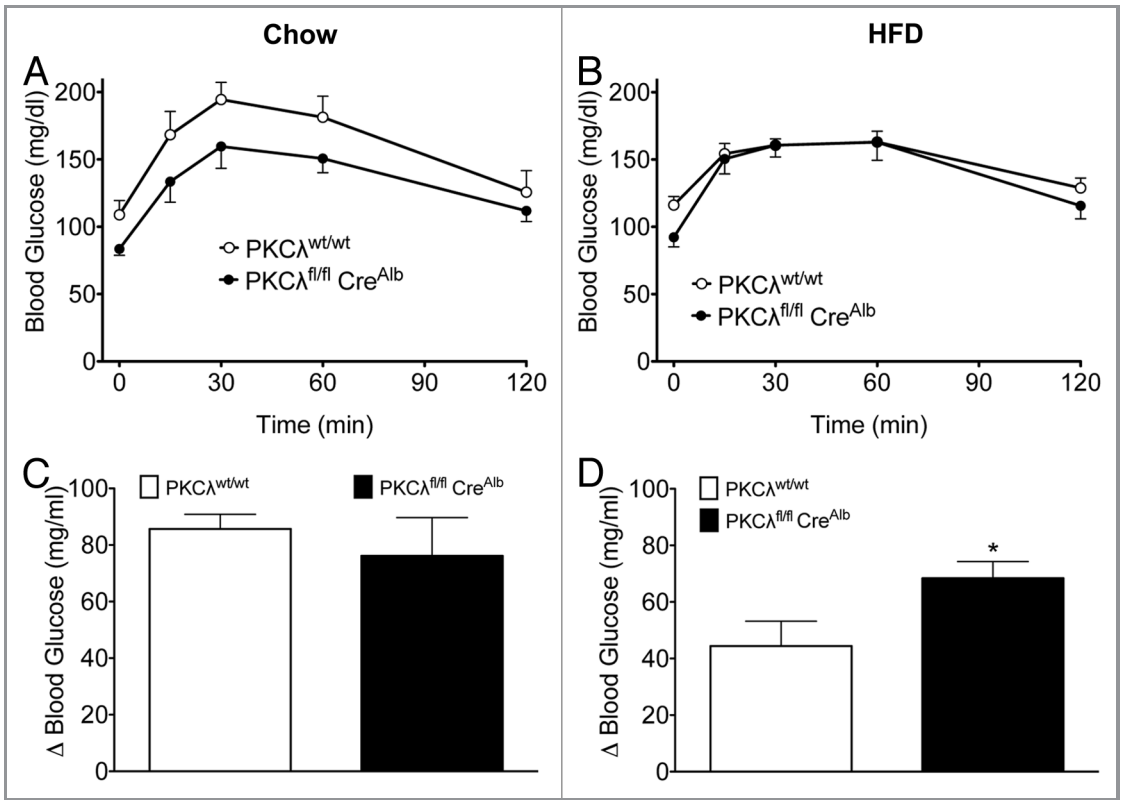

Figure 7. Pyruvate tolerance as assessed by glucose excursion (A and B) and glucose appearance during the first 30 min following ip pyruvate challenge (2 g/kg) (C and D) of chow- and HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed circles) and PKCλ$^{wt/wt}$ (open circles) mice following a 2 g/kg dose of ip pyruvate at week 26. All data are represented as mean ± SEM in 6–7 age-matched, male mice. *$p < 0.05$ as determined by Student's t-test.

in contrast to wt mice in which insulin-mediated glucose uptake was markedly blunted by HFD feeding. This lack of diet-induced worsening of insulin action indicates that alterations in hepatic nutrient metabolism due to the absence of PKCλ translates into impaired insulin-mediated glucose transport that is no longer responsive to the effect of HFD. In light of these observations we hypothesize that while insulin-mediated glucose transport is principally impaired, glucose-stimulated insulin secretion may be preserved in PKCλ$^{fl/fl}$ Cre^{Alb} mice under HFD feeding conditions.

Our finding that fasting triglyceride levels are increased in lean PKCλ$^{fl/fl}$ Cre^{Alb} mice to similar levels detected in obese wt and PKCλ$^{fl/fl}$ Cre^{Alb} mice suggests that at least lipid metabolism may be modulated by the deficiency of PKCλ in liver. Although at this time we do not fully understand the underlying mechanisms, there is growing evidence suggesting that alterations of fatty acids/triglyceride metabolism are an important determinant of insulin sensitivity.[22] In addition, as outlined in our introduction, PKCλ modulates inflammatory responses by activating the NFκB pathway[11,12] and modulating Toll-like receptor 4 (TLR4) and TNF-α receptor signaling cascades.[13,14] Since it is well established that these pathways are implicated in the development of diet-induced insulin resistance and dyslipidemia,[23] it seems plausible that ablation of PKCλ in the liver may diminish HFD-induced inflammation and protect against diet-induced obesity, dyslipidemia and glucose intolerance through inhibition of its inflammatory signaling function. We thus propose that PKCλ ablation in liver may disrupt insulin and/or inflammatory signaling cascades similar to the hepatic insulin resistance observed in patients with hyperglycemia and dyslipidemia.[19] The resulting increase in de novo lipogenesis and VLDL secretion[19] may thus be responsible for the deterioration of the observed insulin-mediated glucose uptake in PKCλ$^{fl/fl}$ Cre^{Alb} mice.

While our investigation confirms certain findings reported previously on the ablation of hepatic PKCλ in mice, we also made observations that contradict prior investigations on some fronts: First, similar to Matsumoto et al., we found that chow fed PKCλ$^{fl/fl}$ Cre^{Alb} mice exhibited a body composition and response to GTT similar to their wild-type controls.[9] However, in the same report the authors describe a paradoxically beneficial effect of hepatic PKCλ ablation on insulin sensitivity in chow-fed mice. In contrast, our studies suggest that insulin-mediated glucose transport is impaired in the absence of hepatic PKCλ. Our data are consistent with the hypothesis that PKCλ is important for proper insulin signaling and are in line with the results obtained in muscle specific deletion of PKCλ.[8] We thus speculate that the contradicting findings by Matsumoto may be a consequence of changes in the genetic background, based on emerging evidence that even slight differences have profound effects on an observed phenotype.[24]

In our model, fasting plasma cholesterol levels are unaffected by PKCλ ablation, regardless of diet. However, fasting plasma triglyceride levels are significantly elevated in chow-fed PKCλ$^{fl/fl}$ Cre^{Alb} mice. Though we did not directly measure genes important

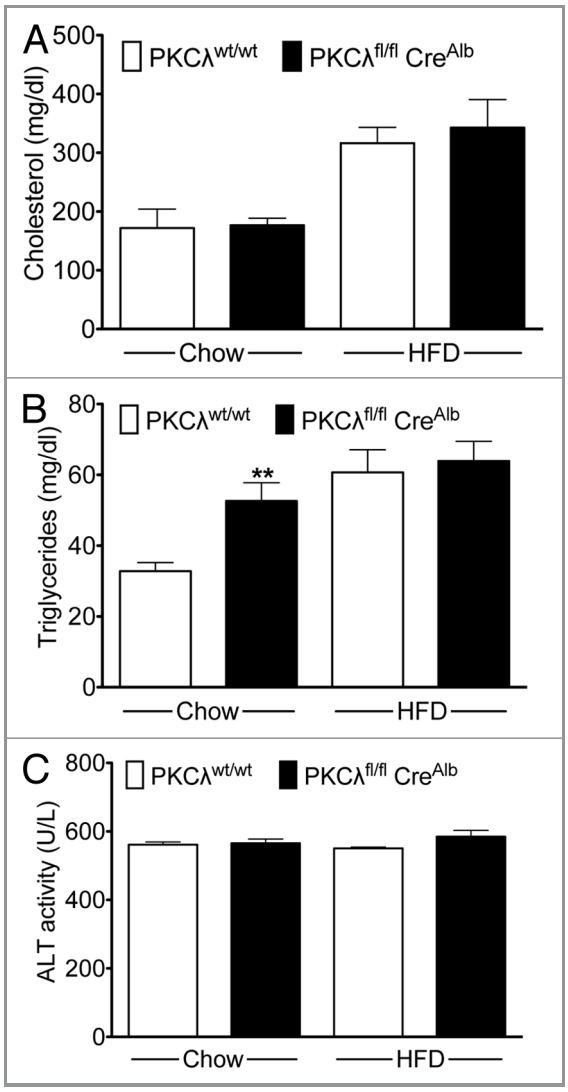

Figure 8. Fasting cholesterol (A), triglyceride (B) and alanine transaminase concentrations of chow- and HFD-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed bars) and PKCλ$^{wt/wt}$ (open bars) mice at sacrifice. All data are represented as mean ± SEM in 6–7 age-matched, male mice. **p < 0.01 as determined by 1-way ANOVA.

for lipogenic programming, this observation was unexpected based on previous reports.[9,10] One reason for these non-consistent results is the fact that Matsumoto et al. determined lipid parameters during a randomly fed state whereas we fasted the mice for 6 h to shed light into hepatic triglyceride metabolism. Our results seem to confirm a growing body of evidence that PKCλ may have divergent metabolic functions depending upon nutritional status as postulated by Farese et al. in a recent review.[5]

In addition to its hepatic role, PKCλ is also known to participate in insulin-stimulated glucose uptake in adipocytes.[25-27] Activation of the atypical PKCs by insulin appears to occur downstream of PI3K, as the relative activity of this kinase is subject to wortmanin inhibition.[28] The regulatory importance of this interaction is evidenced by the impaired glucose transport observed in studies utilizing pharmacological or dominant-negative inhibition of atypical PKC signaling.[29,30] However, PKCλ ablation from all tissues is embryonic lethal, so these earlier studies were limited to cell-based assays. Our current strategy of targeted PKCλ deletion in adipose tissue allowed for the assessment of the effects of this tissue specific kinase on energy and glucose homeostasis in the whole organism. In light of the prior evidence surrounding PKCλ in adipocytes and its role in insulin-stimulated glucose transport, we expected these mice to exhibit impaired glucose tolerance. As such, our observation that mice lacking adipose PKCλ were of similar glucose tolerance to their WT littermates was unexpected, but not unique. In a recent review Farese et al. pointed to unpublished results where their lean adipocyte-specific PKCλ knockout mice have mild glucose intolerance but, unlike lean muscle-specific PKCλ-knockout mice, do not develop obesity or hyperlipidemia, perhaps reflecting a failure to develop increases in either serum insulin levels or hepatic SREBP-1c expression.[5] Based on our and their findings that adipocyte PKCλ deficient mice have a relatively normal response to glucose challenge suggests that other tissues (skeletal muscle and liver) are able to compensate for the presumed lack of uptake into adipose tissue. This hypothesis is supported by the observation that adipose tissue is responsible for less than 5% of the postprandial glucose load.[31] In view of our findings in mice lacking hepatic PKCλ, and prior studies in mice deficient for skeletal muscle PKCλ[8], these data suggest that adipose PKCλ plays a subtle role, if any, in whole body glucose homeostasis. Furthermore, for the first time to our knowledge we have determined that adipose PKCλ does not impact energy homeostasis and the development of diet-induced obesity

In summary, this report suggests that hepatic PKCλ is an important regulator of glucose and energy homeostasis. Specifically these data implicate hepatic PKCλ in the development of diet induced obesity and impaired insulin action. Furthermore, this report details the relative contributions of hepatic PKCλ in contrast to PKCλ in adipose tissue. Importantly, our data confirm and build upon previous observations and suggest that inhibition of hepatic PKCλ may be an important target in the treatment of obesity.

Materials and Methods

Animals. Mice carrying the atypical protein kinase C PKCλ$^{fl/fl}$ allele[2] have been described previously. Alb- and Ap2-Cre mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). Mice from all lines were bred in our facilities and maintained on a C57/B6 background. The PKCλ$^{fl/fl}$ mice were crossed with Alb-Cre or Ap2-Cre mice to get PKCλ$^{fl/fl}$ Cre^{Alb} or PKCλ$^{fl/fl}$ Cre^{aP2} genotypes. DNA was extracted from tail snips and PCR was used to establish genotypes. All animals were housed on a 12:12 h light-dark cycle at 22°C. Only male mice were used for the studies and had free access to water and were fed ad libitum with either a regular chow diet (LM-485; Teklad, 5.6% fat) or a high fat diet (D12331; Research Diets Inc., 58% kcal fat) after weaning. All studies were approved by, and performed according to, the guidelines of the Institutional Animal Care and Use Committee of the University of Cincinnati.

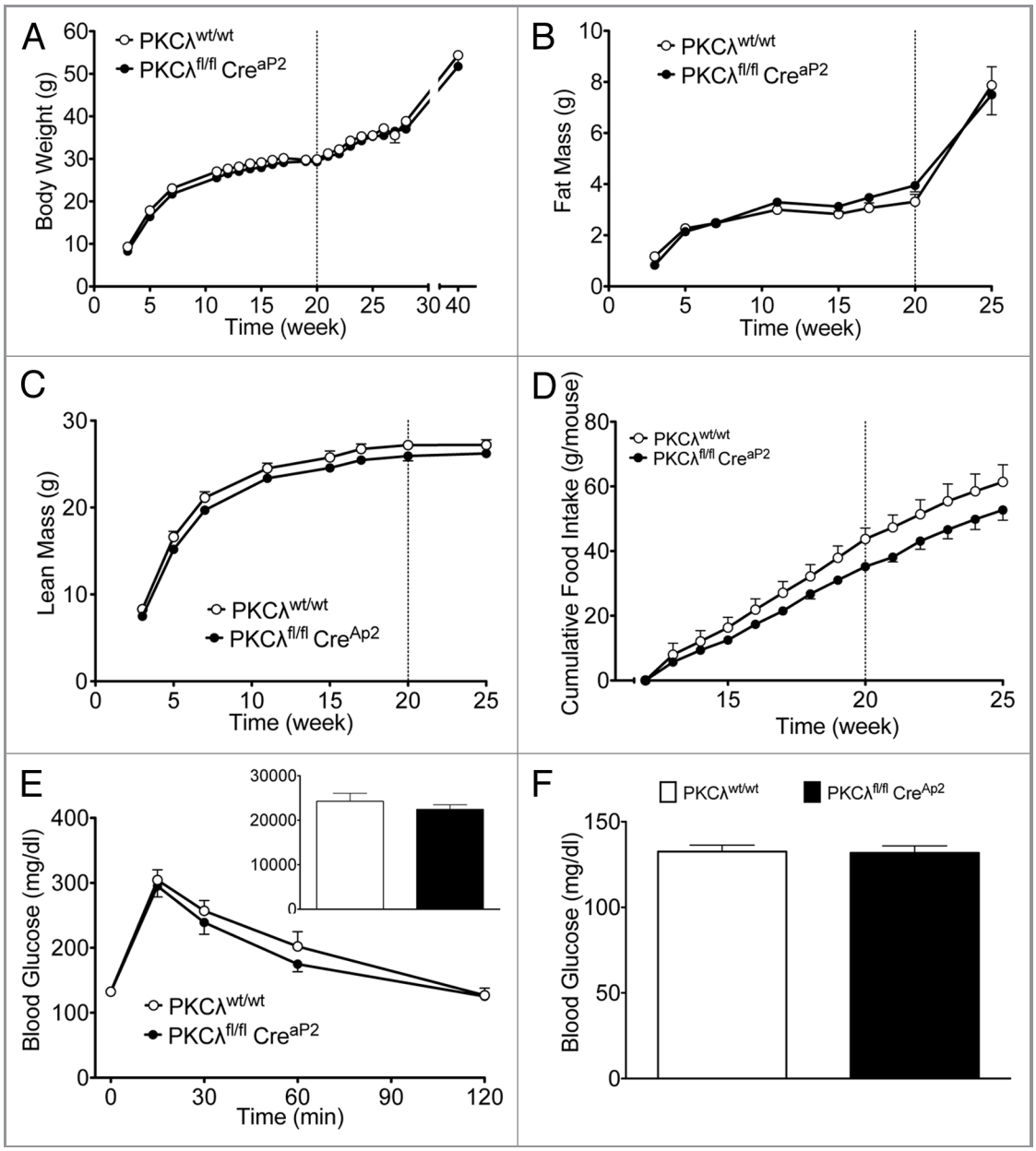

Figure 9. Body weight (A), fat mass (B), lean mass (C) and cumulative food intake (D) in PKCλ$^{fl/fl}$ Cre^{aP2} (closed circles) and PKCλ$^{wt/wt}$ mice (open circles). Glucose tolerance as assessed by glucose excursion (E) and area under the curve analyses (inset of E) of 20-week-old, chow-fed PKCλ$^{fl/fl}$ Cre^{Alb} (closed circles) and PKCλ$^{wt/wt}$ (open circles) mice following a 2 g/kg dose of ip glucose at week 20. All data are represented as mean ± SEM in 6–7 age-matched, male mice. No differences were determined by 2-way ANOVA or Student's t-test.

Body composition and plasma analysis. Whole body composition (fat and lean mass) was measured using NMR technology (EchoMRI). Individual samples for plasma triglyceride and cholesterol levels were measured by enzymatic assay kits (TR22421 and TR13421; Thermo Electron) in mice that have been fasted for 6 h.

Insulin, pyruvate and glucose tolerance test. For measurements of insulin, pyruvate, and glucose tolerance, mice were fasted for 6 h before the intraperitoneal challenge. Glucose tolerance tests were conducted with 25% D-glucose (G8270; Sigma) in 0.9% saline, such that the final dose was 2.0 g glucose/kg body weight in all animals. Insulin tolerance tests were conducted with 100 U/ml human insulin (Lilly Humolog) in 0.9% saline, such that the final dose was 0.75 U/kg. Pyruvate tolerance tests were conducted with sodium pyruvate (P2256; Sigma) in 0.9% saline, such that the final dose was 2 g/kg. Glucose levels (mg/dl) were measured from tail blood using a handheld glucometer (Freestyle lite) before (0 min) and at 15, 30, 60 and 120 min after injection. Glucose tolerance was assessed by area under the curve analysis, insulin tolerance by glucose clearance over the initial 60 min of the insulin challenge, and pyruvate tolerance by glucose appearance over the initial 30 min

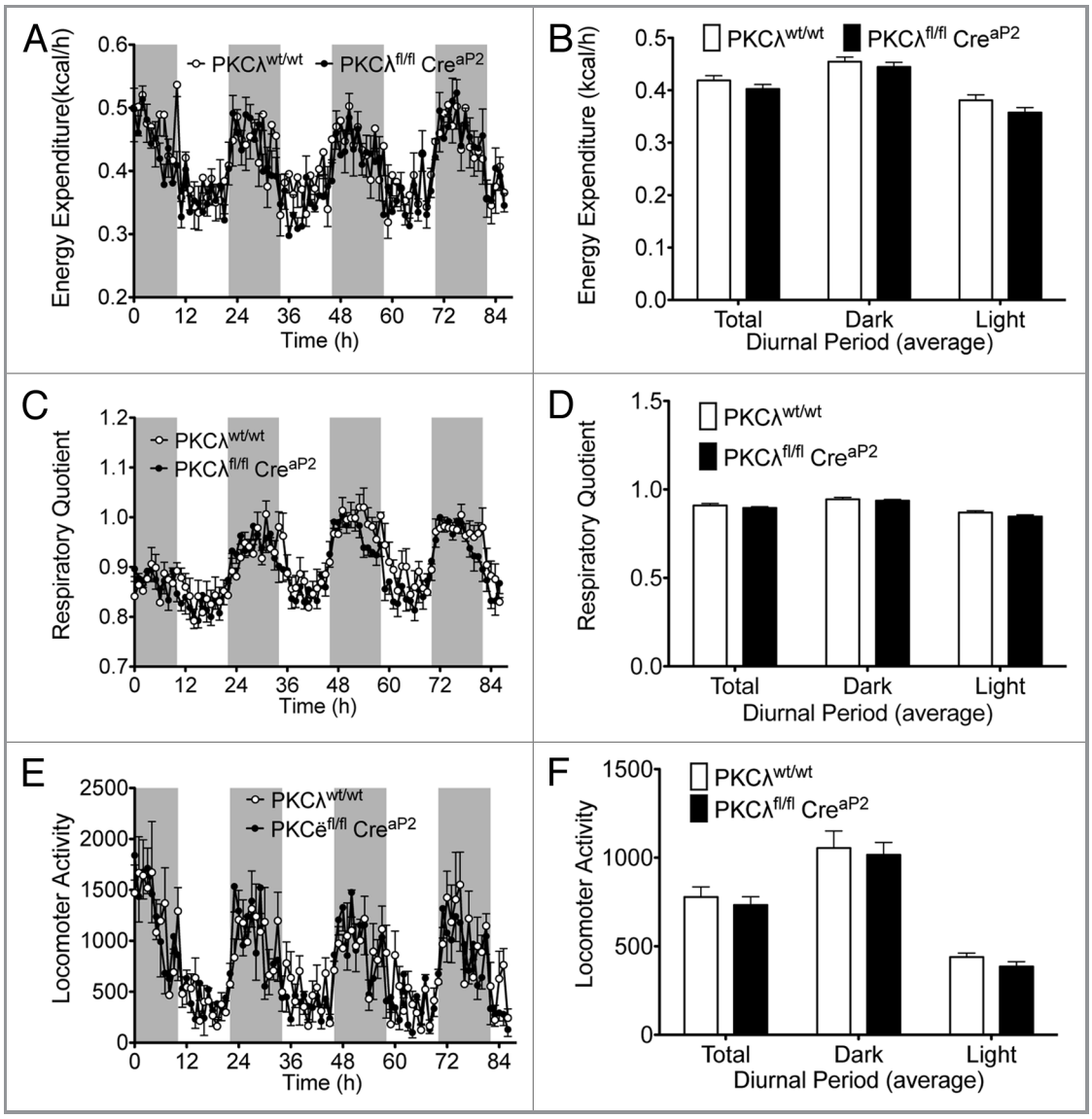

Figure 10. Dynamic and average energy expenditure (A and B), respiratory quotient (C and D) and locomotor activitiy (E and F) in 20-week-old, chow-fed PKCλ$^{fl/fl}$ Cre^{aP2} (closed circles) and PKCλ$^{wt/wt}$ (open circles) mice as measured by indirect calorimetery. All data are represented as mean ± SEM in 6–7 age-matched, male mice. No differences were determined by 2-way ANOVA.

of the challenge. Plasma insulin levels were measured with the Ultra Sensitive Rat Insulin ELISA kit (Crystal Chem) using rat insulin as the standard. HOMA-INDEX has been calculated as described previously.[32,33]

Energy expenditure and locomotor activity. Energy intake and expenditure, as well as home-cage activity, were assessed using a combined indirect calorimetry system (TSE Systems). O_2 consumption and CO_2 production were measured every 60 min for a total of 8 d (including 24 h of adaptation) to determine the respiratory quotient and energy expenditure. Food intake was determined continuously via scales located within the sealed-cage environment. Home-cage locomotor activity was determined using a multidimensional infrared light-beam system with beams scanning the bottom and top levels of the cage, and activity being expressed as beam breaks.

Statistical analysis. All statistical analysis was performed with Prism 5.0 (GraphPad Software). Statistical differences between groups were determined by Student's t-test or two-way anova, as appropriate. A p value of less than 0.05 was considered significant.

Disclosure of Potential Conflicts of Interest

M.H.T. is a consultant for Roche Pharmaceuticals and receives Roche research funds.

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