Increased Hepatic CD36 Expression Contributes to Dyslipidemia Associated With Diet-Induced Obesity

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OBJECTIVE—The etiology of type 2 diabetes often involves diet-induced obesity (DIO), which is associated with elevated plasma fatty acids and lipoprotein associated triglycerides. Since aberrant hepatic fatty acid uptake may contribute to this, we investigated whether increased expression of a fatty acid transport protein (CD36) in the liver during DIO contributes to the dyslipidemia that precedes development of type 2 diabetes.

RESEARCH DESIGN AND METHODS—We determined the effect DIO has on hepatic CD36 protein expression and the functional consequence of this in terms of hepatic triglyceride storage and secretion. In addition, in vivo adenoviral gene delivery of CD36 to the livers of lean mice was performed to determine if increased hepatic CD36 protein was sufficient to alter hepatic fatty acid uptake and triglyceride storage and secretion.

RESULTS—During DIO, CD36 protein levels in the liver are significantly elevated, and these elevated levels correlate with increased hepatic triglyceride storage and secretion. These alterations in liver lipid storage and secretion were also observed upon forced expression of hepatic CD36 in the absence of DIO and were accompanied with a marked rise in hepatic fatty acid uptake in vivo, demonstrating that increased CD36 expression is sufficient to recapitulate the aberrant liver lipid handling observed in DIO.

CONCLUSIONS—Increased expression of hepatic CD36 protein in response to DIO is sufficient to exacerbate hepatic triglyceride storage and secretion. As these CD36-mediated effects contribute to the dyslipidemia that often precedes the development of type 2 diabetes, increased hepatic CD36 expression likely plays a causative role in the pathogenesis of type 2 diabetes. ***Diabetes*** **56:2863–2871, 2007**

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ALT, alanine aminotransferase; BMIPP, 15-p-[^{123}I]iodophenyl-3-(R,S)-methyl pentadecanoic acid; DIO, diet-induced obesity; NEFA, nonesterified fatty acid; SPECT, single photon emission computed tomography; VLDL, very-low-density lipoprotein.

Type 2 diabetes is currently a global epidemic, and the number of individuals affected by this disease continues to rise (1). Much of this increase in the number of type 2 diabetic individuals is correlated with the growing number of overweight or obese individuals (1). Although type 2 diabetes has been correlated with obesity, the disease is multifactorial and includes genetic factors, environmental factors, and a shift toward a more sedentary lifestyle (1). Ultimately, however, type 2 diabetes develops as a direct result of insulin resistance (particularly in skeletal muscle and liver [2,3]) and the eventual decrease in β-cell insulin secretory capacity (3). Although the exact cause of insulin resistance in skeletal muscle and liver is not known, one possible mechanism is increased fatty acid uptake in both of these organs (2–4). Indeed, elevated levels of plasma fatty acids that are induced by diets rich in fatty acids and the resulting skeletal muscle insulin resistance both contribute to hepatic insulin resistance (5), increased hepatic glucose output (6), hepatic steatosis (4), and increased hepatic triglyceride secretion in VLDLs (7). Such dramatic fatty acid–induced aberrant liver function further contributes to the dyslipidemia associated with the eventual development of type 2 diabetes (8). While exposure of the liver to elevated levels of plasma fatty acids may have a number of deleterious effects on liver function, we investigated whether these profound changes in liver function are a result of molecular changes within the hepatocyte, which alter hepatic fatty acid uptake.

While previous reports have indicated that a deletion variant of *Cd36* may be a quantitative trait loci for insulin resistance in the spontaneously hypertensive rat (SHR) (9,10), emerging evidence has suggested that increased CD36 expression actually contributes to insulin resistance in humans with type 2 diabetes (11,12) and that inhibition of CD36 at the level of the pancreatic β-cell and the skeletal muscle may prevent cellular lipotoxicity and subsequent peripheral insulin resistance (12,13). Although CD36 is not believed to play a significant role in fatty acid uptake in the liver (14–17), postprandial uptake of fatty acid by the liver is increased in type 2 diabetic patients, indicating the existence of a regulatory mechanism controlling hepatic fatty acid uptake (18). In addition, mouse models of high-fat diet–induced steatohepatitis and genetic models of obesity have shown that CD36 transcript levels increase in the liver (19–21), suggesting that CD36 protein levels may also be increased. However, whether this translates into increased CD36 protein expression and the effects this may have on hepatic fatty acid transport have not been explored.

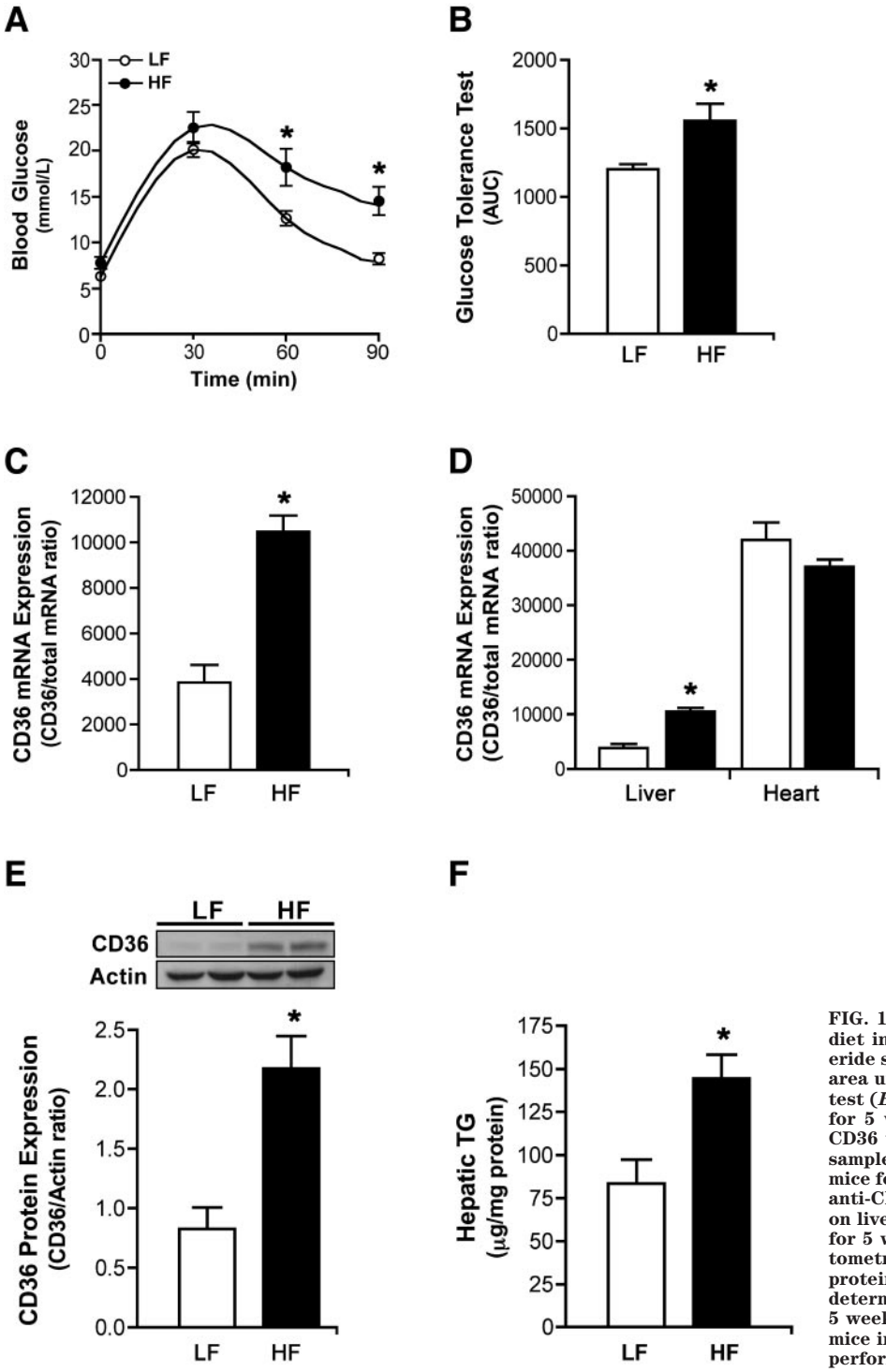

FIG. 1. Increased weight gain resulting from an HF diet increases CD36 expression and hepatic triglyceride storage in mice. Glucose tolerance test (*A*) and area under the curve (AUC) of the glucose tolerance test (*B*) of mice fed an HF diet or a standard LF diet for 5 weeks. Quantitative RT-PCR measurement of CD36 transcript levels in liver (*C* and *D*) and heart samples (*D*) were performed on RNA extracted from mice fed an LF or HF diet. Immunoblot analysis using anti-CD36 and anti-actin antibodies were performed on liver extracts (*E*) from mice fed an LF or HF diet for 5 weeks. Immunoblots were quantified by densitometry and normalized against actin as a control for protein loading (*E*). Triglyceride (TG) levels were determined in livers of mice fed an LF or HF diet for 5 weeks (*F*). Values are the means ± SE of $n = 4–5$ mice in each group. *$P < 0.05$ indicates comparisons performed between LF- and HF-fed mice.

RESULTS AND DISCUSSION

To investigate if diet-induced obesity (DIO), a risk factor for the development of type 2 diabetes, can regulate hepatic CD36 expression, C57BL6 mice were fed a standard low-fat chow diet (LF; 10% kcal from lard) or a high-fat diet (HF; 60% kcal from lard) for a 5-week period. In accordance with the well-documented model of HF feeding (22), mice fed a HF diet rapidly gained weight, culminating in a significant increase in body weight in mice fed a HF diet compared with mice fed a LF diet (10.2 ± 0.9 vs. 1.8 ± 0.3 g, respectively, $P < 0.05$). While fasting plasma glucose levels did not differ in mice fed either diet (LF 6.4 ± 0.3 vs. HF 7.8 ± 0.6 mmol/l, P = NS), mice fed a HF diet for 5 weeks did display delayed glucose clearance as determined by a glucose tolerance test (Fig. 1*A* and *B*). Although hepatic glucose output and insulin measurements were not performed on mice after 5 weeks of LF or HF diets, glucose and insulin tolerance tests indicated that early stages of insulin resistance did not develop in mice until ~10 weeks of HF diet feeding (data not shown), indicating that at 5 weeks of HF diet mice are glucose intolerant. Consistent with dietary fatty acid consumption, fasting plasma nonesterified fatty acid (NEFA) levels were significantly increased in mice fed an HF diet

compared with mice fed an LF diet (2.0 ± 0.08 vs. 1.7 ± 0.07 mmol/l, respectively, $P < 0.05$). In addition, CD36 mRNA levels were significantly increased in livers from mice fed an HF diet compared with mice fed an LF diet (Fig. 1*C*), and when compared with an organ that expresses high levels of CD36, such as heart, hepatic CD36 mRNA levels increased from ~10% of what is present in the heart in the LF group to ~28% of what is present in the heart in the HF group (Fig. 1*D*). Furthermore, CD36 protein expression (Fig. 1*E*) was significantly increased in livers from mice fed an HF diet compared with mice fed an LF diet, indicating that DIO, or possibly elevated plasma NEFA levels, was sufficient to induce hepatic CD36 protein expression. It has previously been shown that CD36 transcript levels are increased in the livers of mice fed an HF diet and that these transcriptional changes occur via a peroxisome proliferator–activated receptor γ–mediated mechanism (19,20). Congruent with this, our findings demonstrate that increased CD36 transcript levels translate into elevated CD36 protein levels and increased CD36 localized to the plasma membrane (data not shown). This finding is consistent with the high level of plasma membrane–localized CD36 found in heart and skeletal muscle (for review, see ref. 23). Associated with the 2.6-fold increase in CD36 protein expression was a 1.7-fold increase in hepatic triglyceride storage (Fig. 1*F*), suggesting a potential role for aberrant CD36 expression in enhanced liver fatty acid uptake during DIO.

To confirm that elevated CD36 expression can increase hepatic fatty acid uptake, primary cultures of mouse hepatocytes were infected with a recombinant adenovirus harboring mouse CD36 cDNA (Ad.CD36) or control adenovirus expressing no transgene (Ad.Null). Immunoblot analysis revealed that levels of total cellular CD36 (Fig. 2*A*) and plasma membrane–associated CD36 (Fig. 2*B*) were significantly increased in Ad.CD36-infected hepatocytes compared with Ad.Null-infected hepatocytes, demonstrating that Ad.CD36-transduced cells had increased levels of properly localized CD36. Consistent with the role of CD36 as a fatty acid transport protein in other tissues such as skeletal muscle (24) and adipose tissue (14), fatty acid uptake into Ad.CD36-infected hepatocytes was significantly elevated compared with Ad.Null-infected hepatocytes as measured by the fluorescent fatty acid analog BODIPY c16 (Fig. 2*C*). In addition, Ad.Null- and Ad.CD36-infected hepatocytes were incubated in the presence of [^{3}H]palmitate for 2 h, which was the time necessary to detect changes in ^{3}H-TG levels. These primary mouse hepatocytes were used to determine ^{3}H-TG cellular incorporation, ^{3}H-TG secretion, and ^{3}H-palmitate oxidation. Furthermore, separate sets of hepatocytes were collected 16 h after adenoviral infection to determine the levels of triglyceride before the onset of the [^{3}H]palmitate experiments. Before treating with [^{3}H]palmitate, baseline triglyceride levels in the two groups of cells were similar (Ad.Null 29.2 ± 0.7 vs. Ad.CD36 28.5 ± 1.6 μg/mg protein, P = NS), indicating that alterations in baseline triglyceride did not influence label distribution. After the 2-h incubation period, palmitate oxidation was not significantly different between the two groups of cells (Ad.Null 45.8 ± 1.2 vs. Ad.CD36 49.0 ± 2.8 nmol/mg protein, P = NS), and therefore, an equal fraction of the [^{3}H]palmitate was lost to oxidation in the Ad.Null- and Ad.CD36-infected cells. In contrast, there was a significant increase in ^{3}H-TG cellular incorporation (Fig. 2*D*) and ^{3}H-TG secretion (Fig. 2*E*) in Ad.CD36-infected hepatocytes compared with Ad.Null-infected hepatocytes (Fig. 2*D* and *E*). However, when the amount of ^{3}H-TG secreted (Fig. 2*E*) was normalized to the amount of ^{3}H-palmitate incorporated into triglyceride (Fig. 2*D*), there was no significant difference between Ad.Null- and Ad.CD36-infected hepatocytes (Fig. 2*F*). This finding suggests that increased triglyceride secretion was directly related to CD36-mediated fatty acid uptake as opposed to CD36 being involved in channeling intracellular fatty acids to oxidation or secretory pathways and/or directly modifying the processes involved in hepatic triglyceride secretion.

To assess if increased CD36 expression alone is sufficient to produce the liver-specific effects observed with DIO, lean mice maintained on an LF diet were administered a single systemic dose of either Ad.Null or Ad.CD36 via tail vein injection. Seven days after adenoviral administration, the levels of hepatic CD36 mRNA (Fig. 3*A*) and protein (Fig. 3*B*) expression were significantly increased in Ad.CD36-injected mice to approximately the same levels as observed with the HF-feeding model (Fig. 1*C* and *E*). There were no significant differences in body weight (Ad.Null 21.3 ± 0.3 vs. Ad.CD36 21.2 ± 0.4 g, P = NS) or liver weight–to–body weight ratio (Ad.Null 4.5 ± 0.1 vs. Ad.CD36 4.8 ± 0.1 g, P = NS) nor were there any significant changes in fasting plasma glucose levels (Ad.Null 4.6 ± 0.4 vs. Ad.CD36 5.3 ± 0.3 mmol/l, P = NS) or tolerance to a glucose load (data not shown) between Ad.Null- and Ad.CD36-injected mice. Although previous reports have suggested that adenoviral administration can cause liver damage, as evidenced by plasma levels of alanine aminotransferase (ALT) higher than 200 units/l (25), mean plasma ALT levels in Ad.Null- or Ad.CD36-injected mice were not different and ranged from 47–123 units/l (data not shown), indicating an absence of liver damage. Moreover, and in agreement with low dietary fatty acid intake, NEFA levels were unaltered in the two groups of mice (Ad.Null 1.2 ± 0.04 vs. Ad.CD36 1.3 ± 0.08 mmol/l, P = NS) and were lower than mice fed an HF diet. In addition, single photon emission computed tomography (SPECT) imaging and direct in vivo fatty acid uptake measurements in Ad.Null- and Ad.CD36-expressing mice using the radio-labeled fatty acid analog 15-p-[^{123}I]iodophenyl-3-(R,S)-methyl pentadecanoic acid (BMIPP) indicate that hepatic fatty acid uptake was significantly elevated in Ad.CD36-expressing mice compared with Ad.Null-expressing mice (Fig. 3*C*; sagittal and axial views [*left*] and quantified scintigraphs [*right*]). Consistent with these data, direct measurements of ^{123}I incorporation into livers extracted from mice 1 h postinjection of BMIPP also demonstrated a significant increase in fatty acid uptake in Ad.CD36-expressing mice compared with Ad.Null-expressing mice (Fig. 3*D*). However, there was no difference in fatty acid uptake in other tissues that express high levels of CD36 such as heart (Fig. 3*E*), skeletal muscle (Fig. 3*F*), and adipose tissue (Fig. 3*G*), suggesting that peripheral organs/tissues are not changed with respect to fatty acid clearance. Nonetheless, since the kinetics of fatty acid uptake/clearance is very different from tissue uptake/clearance of triglycerides (26), it remains to be determined whether VLDL-triglyceride production or clearance is the major cause of the increased triglyceride accumulation in the livers of Ad.CD36-expressing mice.

Despite normal levels of NEFA in Ad.Null- and Ad.CD36-injected mice, hepatic CD36 expression was still able to significantly increase hepatic triglyceride levels (Fig. 4*A*),

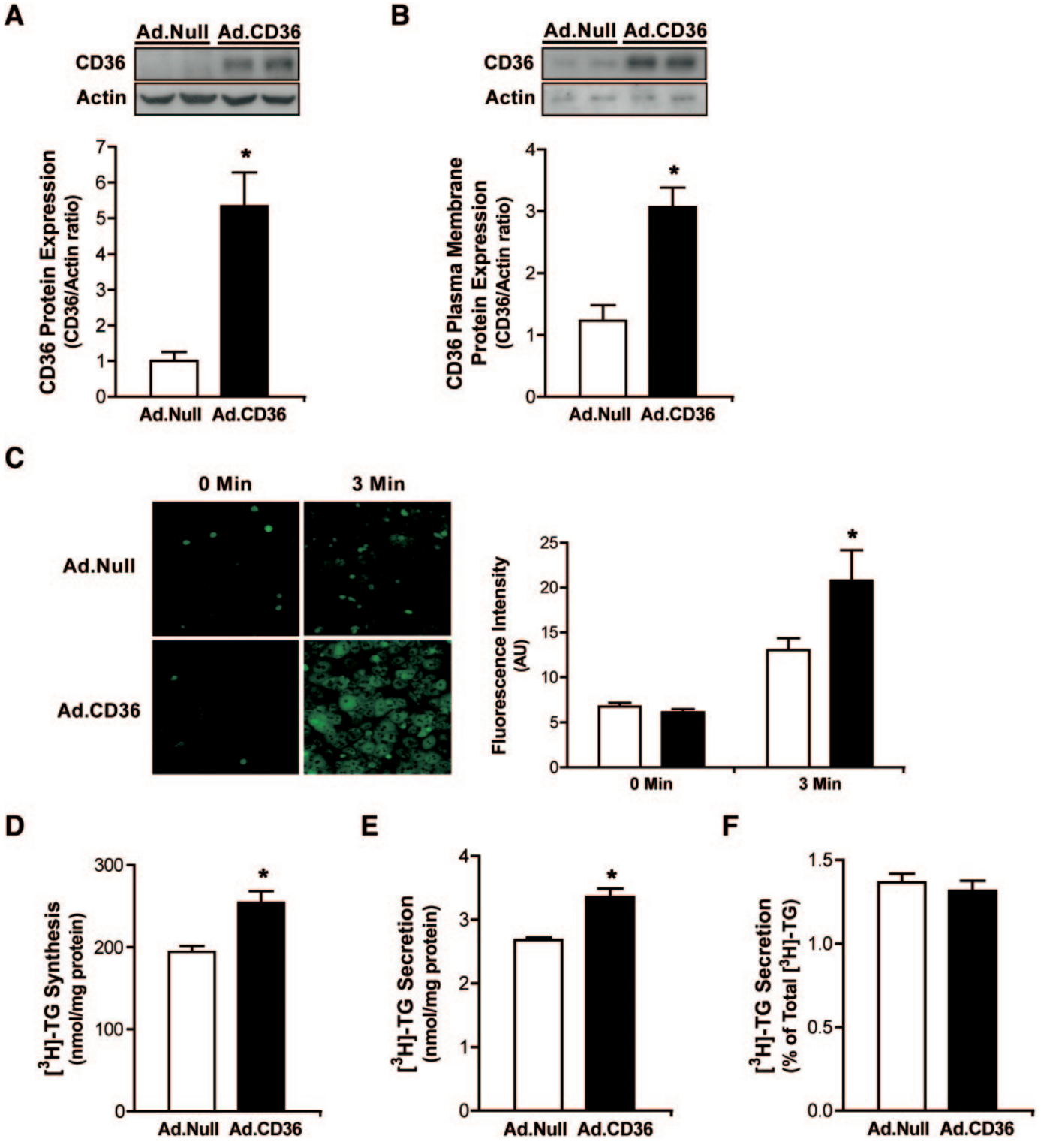

FIG. 2. Effects of increased CD36 expression on fatty acid uptake and triglyceride secretion in freshly isolated primary cultures of mouse hepatocytes. Extracts from mouse hepatocytes transduced with an empty adenovirus (Ad.Null) or with an adenovirus expressing CD36 (Ad.CD36) were subjected to immunoblot analysis using anti-CD36 antibody and anti-actin antibodies (*A*). Plasma membranes isolated from Ad.Null- and Ad.CD36-transduced hepatocytes were also assessed using the same antibodies (*B*). *C*: Fatty acid transport into Ad.Null- (□) or Ad.CD36- (■) transduced hepatocytes was determined by the uptake of the fatty acid analog BODIPY C16 (*left panel*, representative fluorescence image; *right panel*, quantified fluorescence intensity expressed as arbitrary units [AU]) and by determining the amount of ^{3}H-palmitate incorporated into intracellular triglyceride (TG) (*D*). ^{3}H-palmitate secreted as triglyceride was also measured in Ad.Null- or Ad.CD36-transduced hepatocytes (*E*) and normalized to the amount of ^{3}H-palmitate incorporated into triglyceride (*F*). Values are the means ± SE of $n = 3–4$ independent hepatocyte isolations. *$P < 0.05$ indicates comparisons performed within Ad.Null- or Ad.CD36-infected hepatocytes. (Please see http://dx.doi.org/10.2337/db07-0907 for a high-quality digital representation of this figure.)

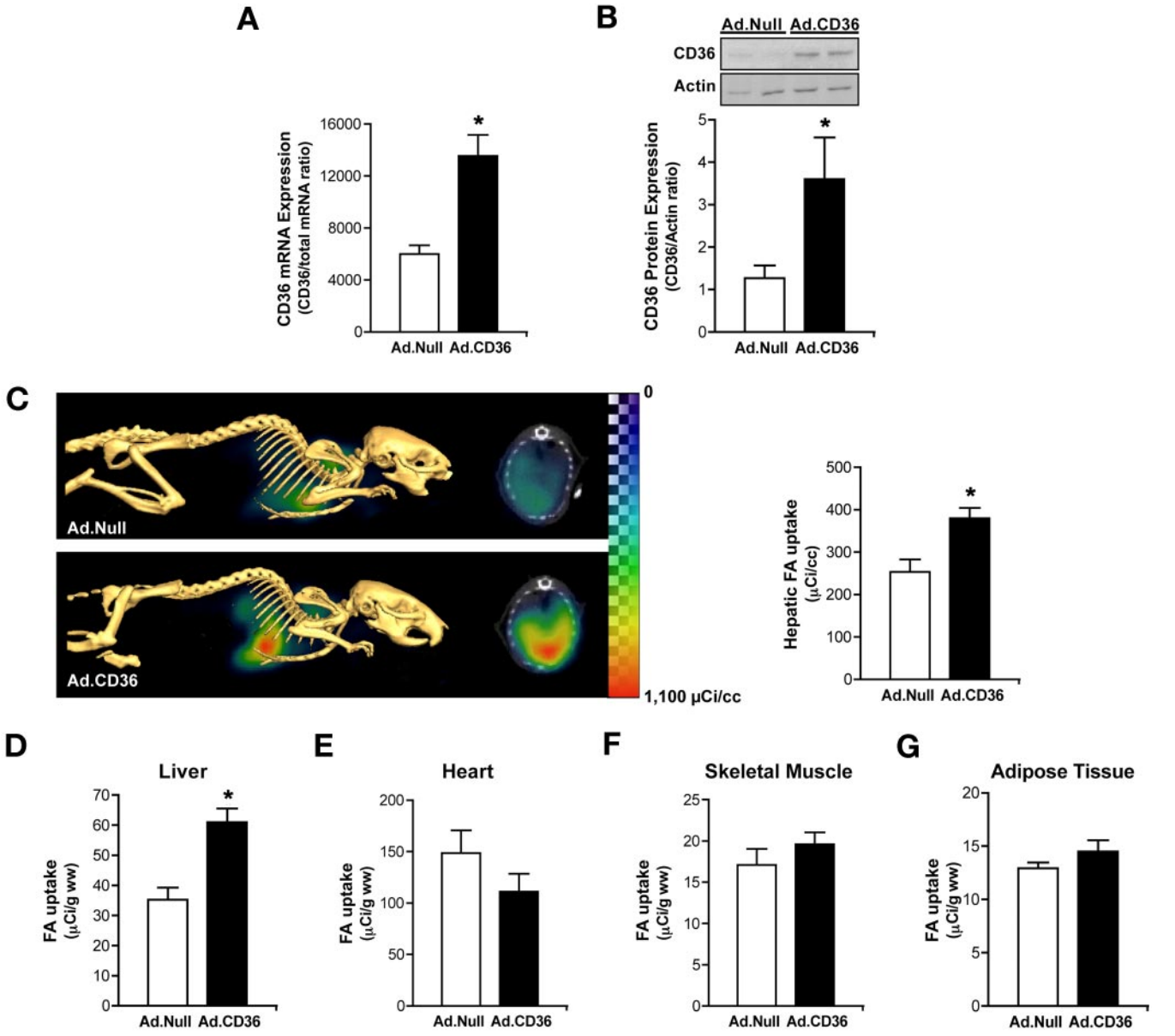

FIG. 3. Forced expression of CD36 in the liver via adenoviral gene delivery increases hepatic CD36 protein levels and hepatic fatty acid uptake in mice fed a standard chow diet. Seven days following systemic injection of Ad.Null or Ad.CD36 into mice, liver homogenates were subjected to quantitative RT-PCR (*A*) and immunoblot analysis using anti-CD36 antibody and anti-actin antibodies (*B*). Immunoblots were quantified by densitometry and normalized against actin as a control for protein loading (*B*). A separate group of adenovirus-treated mice were used to determine in vivo hepatic fatty acid (FA) uptake using BMIPP. Representative SPECT/computed tomography dual-modality images of mice from Ad.Null- and Ad.CD36-injected mice are shown in the sagittal view of *C* (*left* side of images) and images of the livers from these mice in the axial views (*right* side of images). Quantification of hepatic scintigraphy is shown in µCi/cc (*C*, graph). Liver (*D*), heart (*E*), skeletal muscle (*F*), and adipose tissue (*G*) collected from mice that were killed 1 h post-BMIPP injection were quantified for the level of ^{123}I incorporation using a γ counter and expressed as µCi/g wet wt (g ww). Values are the means ± SE of $n = 4–5$ mice in each group. *$P < 0.05$ indicates comparisons performed between Ad.Null- and Ad.CD36-infected mice. (Please see http://dx.doi.org/10.2337/db07-0907 for a high-quality digital representation of this figure.)

which is consistent with the elevated in vivo fatty acid uptake measurements in these mice (Fig. 3*C*). In addition, plasma triglyceride levels (Fig. 4*B*) were increased accordingly in Ad.CD36-injected mice, which is consistent with our observations that triglyceride secretion is related to CD36-mediated fatty acid uptake in the isolated mouse hepatocytes (Fig. 2*E* and *F*). Although we cannot rule out the possibility that altered VLDL-triglyceride clearance contributed to the elevated levels of triglyceride in the plasma of mice on an HF diet (27–29), this is unlikely in the Ad.CD36-injected mice given the liver-specific increase in CD36 in these mice.

In agreement with the hyperlipidemia observed in rodent models of DIO (30,31) and in humans (32), the elevated plasma triglyceride in Ad.CD36-injected mice appears to be primarily contained within the VLDL fraction (Fig. 4*C*). Hepatic cholesterol (Fig. 4*D*) and plasma cholesterol levels (Fig. 4*E*) were not significantly different in Ad.Null- and Ad.CD36-injected mice, and the amount of cholesterol in the HDL fraction was almost identical (Fig. 4*F*). Furthermore, plasma cholesteryl ester (Fig. 4*G*) levels did not differ between Ad.Null- and Ad.CD36-injected mice; however, increased expression of hepatic CD36 was able to promote cholesteryl ester formation within the

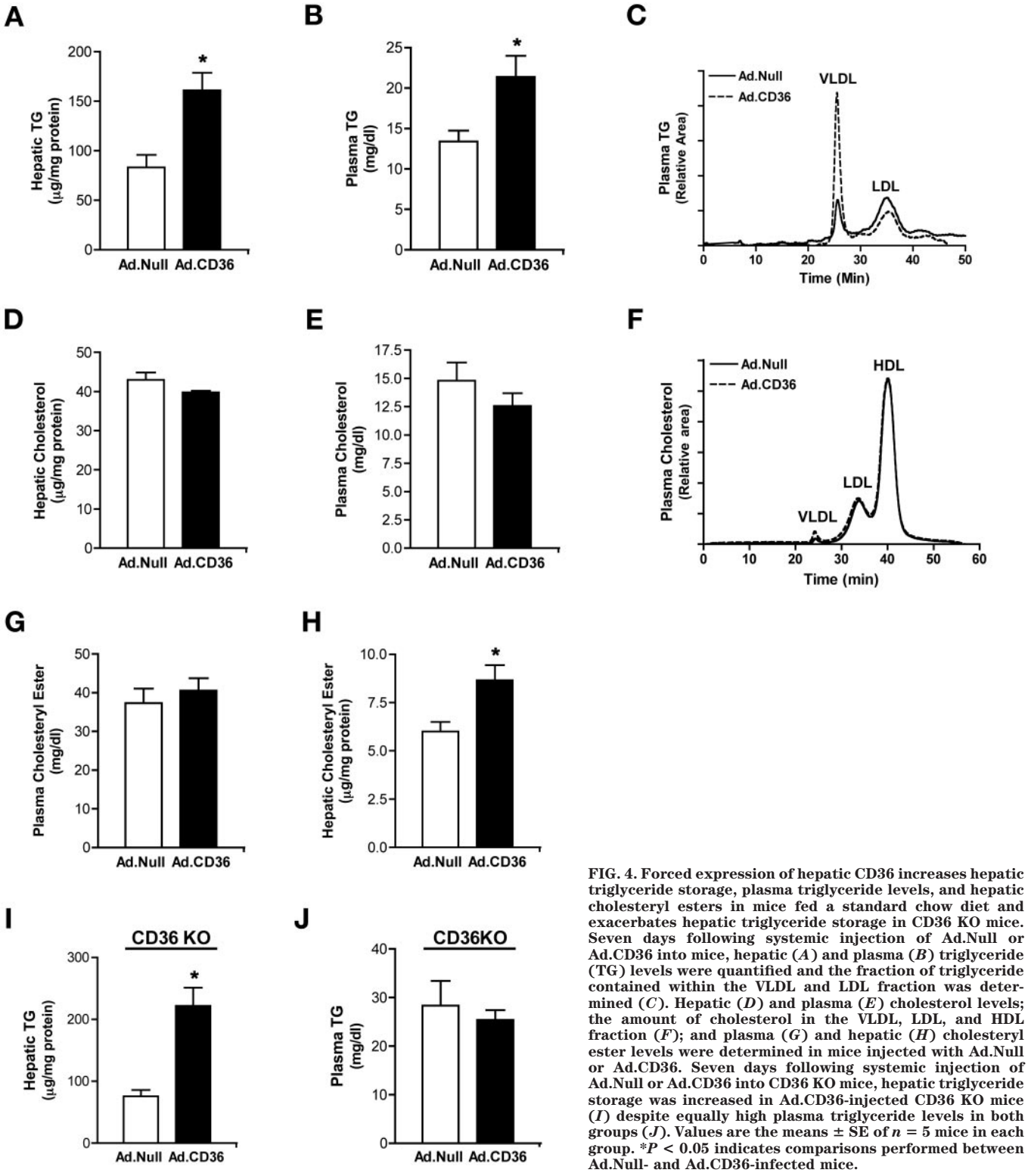

FIG. 4. Forced expression of hepatic CD36 increases hepatic triglyceride storage, plasma triglyceride levels, and hepatic cholesteryl esters in mice fed a standard chow diet and exacerbates hepatic triglyceride storage in CD36 KO mice. Seven days following systemic injection of Ad.Null or Ad.CD36 into mice, hepatic (*A*) and plasma (*B*) triglyceride (TG) levels were quantified and the fraction of triglyceride contained within the VLDL and LDL fraction was determined (*C*). Hepatic (*D*) and plasma (*E*) cholesterol levels; the amount of cholesterol in the VLDL, LDL, and HDL fraction (*F*); and plasma (*G*) and hepatic (*H*) cholesteryl ester levels were determined in mice injected with Ad.Null or Ad.CD36. Seven days following systemic injection of Ad.Null or Ad.CD36 into CD36 KO mice, hepatic triglyceride storage was increased in Ad.CD36-injected CD36 KO mice (*I*) despite equally high plasma triglyceride levels in both groups (*J*). Values are the means ± SE of $n = 5$ mice in each group. *$P < 0.05$ indicates comparisons performed between Ad.Null- and Ad.CD36-infected mice.

liver (Fig. 4*H*), thereby emphasizing the secondary effects of CD36-mediated fatty acid uptake on cholesteryl ester formation and VLDL secretion in the mouse liver. Finally, consistent with CD36 having a functional role in hepatic fatty acid uptake resulting in increased hepatic triglyceride storage, Ad.CD36-injected CD36 knockout (KO) mice had significantly elevated levels of hepatic triglyceride compared with Ad.Null-injected mice (Fig. 4*I*), despite equally high plasma triglyceride levels in both groups (Fig. 4*J*). These data also show that increased CD36 expression in the liver can further increase fatty acid uptake and subsequent hepatic triglyceride storage even when plasma fatty acid and triglyceride levels already significantly favor maximal hepatic fatty acid uptake. These data also confirm that our method of expressing CD36 produces a protein that is functional in terms of fatty acid transport. Interestingly, plasma triglyceride levels did not increase in CD36 KO mice following Ad.CD36 injection, suggesting

that alterations in plasma triglyceride clearance may be more important in the control of plasma triglyceride levels than hepatic VLDL-triglyceride production in the CD36 KO mouse, as previously reported (28,33). However, since plasma triglyceride levels are already extremely high in the CD36 KO mouse (28,33), the contribution of hepatic VLDL-triglyceride production in the CD36 KO mice infected with Ad.CD36 may be more difficult to detect than the contribution of hepatic VLDL-triglyceride production to plasma triglyceride levels in control mice.

CONCLUSIONS

Elevated levels of plasma fatty acids induced by diets rich in fatty acids contribute to hepatic insulin resistance (5), increased hepatic glucose output (6), hepatic steatosis (4,34), and increased hepatic triglyceride secretion in VLDL (7). Such dramatic fatty acid–induced aberrant liver function further contributes to the dyslipidemia associated with the eventual development of type 2 diabetes (8,35). While exposure of the liver to elevated levels of plasma fatty acids may have a number of deleterious effects on liver function, we show that increased hepatic CD36 protein expression is a regulatory mechanism that, in addition to what has been shown for FABPpm (36) and FATP5 (37,38), also exists in the mouse liver to control fatty acid uptake. In addition, we provide evidence that increased hepatic CD36 protein expression in response to diets rich in fatty acids and/or obesity (Fig. 1*E*) contributes to aberrant liver fatty acid uptake and subsequent dyslipidemia (Fig. 1*F*). These findings are entirely consistent with those of Degrace et al. (39) where hepatic CD36 expression has been shown to increase in a mouse model of human hypercholesterolemia. Moreover, we show that elevations in hepatic CD36 protein expression in lean mice directly affect hepatic fatty acid uptake, triglyceride storage, and VLDL-triglyceride secretion and therefore are sufficient to recapitulate the hepatic and plasma fatty acid phenotype observed in DIO. Whether other genes involved in fatty acid handling or lipoprotein assembly are altered in the liver secondary to increased CD36 expression is currently unknown. Nevertheless, these data show that increased expression of CD36 in the liver occurs in response to diets rich in fatty acids and that this increases hepatic fatty acid uptake and exacerbates hepatic triglyceride storage and secretion similar to what has been shown with FATP5 (40). While this increase in CD36 expression is likely an early adaptive process that may remove excess plasma fatty acids to protect extrahepatic organs from insulin resistance, we propose that increased expression of CD36 in the liver eventually becomes maladaptive by promoting prolonged and more severe hepatic fatty acid uptake and triglyceride storage and by exacerbating hepatic triglyceride secretion.

While the extensive changes that occur at the cellular level in the liver, as a result of DIO and insulin resistance, makes identifying the effects of a single protein extremely difficult, we have successfully identified CD36 as one specific protein that is abnormally expressed in the liver during the development of DIO; this protein, by itself, is sufficient to cause dyslipidemia and may play a causative role in the pathogenesis of type 2 diabetes. Based on our findings, we propose that specific inhibition of hepatic CD36 fatty acid transport activity may prove to be a novel strategy to normalize hepatic fatty acid uptake and triglyceride secretion in individuals consuming an HF diet and thus may be useful in the prevention of insulin resistance and the eventual development of type 2 diabetes and related diseases.

RESEARCH DESIGN AND METHODS

Male C57BL6 mice, aged 10–12 weeks (Charles River Laboratories), and CD36 KO male mice (41) were used in this study. All experiments involving mice were performed with the approval of the University of Alberta Animal Policy and Welfare Committee. In some instances, mice were randomly divided into an LF-diet group (D12450B; Research Diets) and an HF-diet group (D12492; Research Diets) and given free access to water and food unless otherwise stated.

Glucose tolerance tests. Following a 5-h fast, mice were injected intraperitoneally with a 50% glucose solution (2 g/kg). Glucose from blood collected from the tail before and following glucose injection (at 30, 60, and 90 min) was measured using an ACCU-CHEK Advantage glucometer (Roche Diagnostics).

Primary culture of mouse hepatocytes. Primary hepatocytes were isolated by collagenase perfusion and plated as previously described (42). Cultures were transduced with Ad.CD36 or Ad.Null 2–4 h after plating at a titer of 10 plaque-forming units/cell. Sixteen hours postinfection, hepatocytes were lysed and equal amounts of protein were subjected to SDS-PAGE and immunoblot analysis. In some instances, cell lysates were homogenized in a Potter-Elvejhem glass homogenizer, frozen in liquid nitrogen, and the plasma membrane was isolated as previously described (43,44) before SDS-PAGE and immunoblot analysis.

Fatty acid uptake experiments. Stock solutions of 4,4-difluoro-5,7-dimethyl-4-bora-3α,4α-diaza-*s*-indacene-3-hexadecanoic acid (BODIPY-C16; Molecular Probes) were prepared in DMSO (Sigma). Hepatocytes were cultured on collagen-coated microscope coverslips, and at 16 h postinfection, the cells were serum starved for 3 h and rinsed with 1 × PBS. Hepatocytes were incubated for 3 min in 1 × PBS supplemented with BODIPY-C16 to a final concentration of 100 nmol/l, rinsed three times with ice-cold 1 × PBS, and fixed in ice-cold 3.7% paraformaldehyde for 15 min. Coverslips were washed and mounted on microscope slides using the Prolong Antifade kit from Molecular Probes. The fluorescence intensity of 90–100 cells was quantified using Image J (NIH) software and expressed as arbitrary units. In vivo hepatic fatty acid uptake was determined in Ad.Null- and Ad.CD36-expressing mice by dual-modality imaging using a combined SPECT/computed tomography imaging system (Gamma Medica). Mice were injected via the tail vein with 1 mCi of the radio-labeled fatty acid analog BMIPP (Molecular Insight Pharmaceuticals), and image acquisition started 5 min thereafter. Hepatic scintigraphy was completed 20 min after BMIPP injection and quantified using Amira software (Gamma Medica). After 1 h, mice were killed, tissues were removed, and the level of incorporated ^{123}I was determined directly using a γ counter.

^{3}H-palmitate oxidation and incorporation into cellular and secreted lipids. Sixteen hours after adenoviral infection, hepatocytes were incubated for 2 h with 0.05 mmol/l [^{3}H]palmitate (5 μCi/ml) bound to BSA in a 3:1 molar ratio. Following treatment, an aliquot of medium was collected and utilized to determine palmitate oxidation essentially as previously described (45). In separate cellular isolations and treatments, the medium was collected and cells scraped and sonicated in PBS (pH 7.4). Lipids from the medium and the cell lysates were extracted and separated by thin-layer chromatography as described (46). Additional sets of hepatocytes were collected 16 h after adenoviral infection to determine the levels of triglyceride before the onset of the [^{3}H]palmitate experiments.

Injection of recombinant adenoviruses. A single dose of adenovirus (2.85×10^{8} plaque-forming units) harboring no transgene (Ad.Null) or mouse CD36 (Ad.CD36; a gift from F. de Beer, Lexington, KY) was injected into male C57BL6 mice via the tail vein. Six days postinjection, mice were fasted (16 h) and anesthetized with metofane. A large blood sample was drawn via cardiac puncture and livers dissected immediately, frozen in liquid nitrogen, and stored at −80°C until further analysis was done. Plasma ALT levels were measured by the INFINITY ALT kit (ThermoTrace).

Determination of liver and plasma FFA, triglycerides, cholesterol, and cholesteryl esters. Crushed liver tissues from mice following a 16-h fast were homogenized, the phospholipids were digested with phospholipase C (2 h, 30°C), lipids extracted, and the amount of triglyceride, cholesterol, and cholesteryl ester determined by gas-liquid chromatography as previously described (47). Plasma from mice following a 16-h fast was collected in the presence of EDTA and immediately stored on ice to inhibit lipase activity without the use of chemical inhibition. An aliquot of plasma was collected and separated into lipoprotein fractions using high-performance liquid chromatography with an Amersham Bioscience Superose column attached to a Beckman Systems Gold or Nouveau Gold apparatus. The remainder of the plasma samples were immediately frozen and stored at −80°C. In-line assays for total

triglyceride and cholesterol were performed as described (47), and plasma NEFA levels were measured using the NEFA C kit (Wako Chemicals).

Liver membrane preparation. Liver tissues were prepared in ice-cold sucrose homogenation buffer as previously described (48). In brief, tissues were homogenized on ice in a 20-s burst and the homogenate centrifuged at 1,000*g* for 20 min at 4°C to remove nuclei and cell debris. The supernatant was centrifuged at 100,000*g* for 30 min at 4°C, and the subsequent pellet containing the final membrane fraction was suspended in ice-cold homogenation buffer.

Immunoblot analysis. Equal amounts of protein were subjected to 10% SDS-PAGE, transferred to nitrocellulose, immunoblotted with affinity-purified rabbit polyclonal antibody to CD36 and goat polyclonal anti-actin (I-19; Santa Cruz Biotechnology), and visualized using the Amersham Pharmacia enhanced chemiluminescence Western blotting detection system.

Quantitative RT-PCR analysis. RNA extraction and the cyclophilin and CD36-specific quantitative RT-PCR assay of samples was performed using previously described methods (49,50). Gene expression data are represented as mRNA molecules per nanogram total RNA, which was not different when expressed as a ratio to the cyclophilin mRNA (data not shown).

Statistical analysis. Data are expressed as means ± SE. Comparisons between groups were performed using the unpaired Student's two-tailed *t* test or ANOVA with a Bonferroni post hoc test of pairwise comparisions where appropriate. A probability value of <0.05 is considered significant.

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