CD36 deficiency increases insulin sensitivity in muscle, but induces insulin resistance in the liver in mice

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Abbreviations: 2-DG, 2-deoxy-D-[1-³H]glucose; CD36 −/−, CD36-deficient mice; CD36 +/+, wild type control littersmates; FAT, fatty acid translocase; FFA, free fatty acids; HPTLC, high performance liquid chromatography
Abstract

Objective. CD36 (fatty acid translocase) is involved in high affinity peripheral fatty acid uptake. Mice lacking CD36 exhibit increased plasma free fatty acid and triglyceride levels and decreased glucose levels. Studies in spontaneous hypertensive rats, lacking functional CD36, link CD36 to the insulin resistance syndrome. To clarify the relationship between CD36 and insulin sensitivity in more detail, we determined insulin-mediated whole body and tissue-specific glucose uptake in CD36-deficient mice.

Methods and results. Insulin-mediated whole body and tissue-specific glucose uptake was measured by D-[3H]glucose and 2-deoxy-D-[1-3H]glucose during hyperinsulinemic clamp in CD36-deficient and control mice. Whole body and muscle-specific insulin-mediated glucose uptake was significantly higher in CD36-deficient compared to control mice. In contrast, insulin completely failed to suppress endogenous glucose production in CD36-deficient mice compared to a 40% reduction in control mice. This insulin resistant state of the liver was associated with increased hepatic triglyceride content in CD36 -/- mice compared to CD36 +/+ mice (110.9 ± 12.0 and 68.9 ± 13.6 µg TG/mg protein, respectively). Moreover hepatic activation of protein kinase B by insulin, measured by Western blot, was reduced by 54%.
Conclusion. Our results show a dissociation between increased muscle and decreased liver insulin sensitivity in CD36-deficient mice.

Keywords: fatty acid transport, glucose metabolism, hepatic steatosis, hyperinsulinemic clamp.
Introduction

Increased flux of fatty acids from adipose tissue to nonadipose tissue, and increased plasma levels of free fatty acids (FFA), as a result of excessive adipose tissue mass, is a common feature of insulin resistance (1). Plasma FFA concentrations result from the balance between fatty acid release from adipose tissue and from intravascular lipolysis of triglyceride (TG)-rich lipoproteins on one hand and FFA uptake by peripheral tissues and liver on the other hand (1).

Fatty acid translocase (FAT), also known as CD36, is a receptor for several ligands, including oxidized Low-Density-Lipoproteins (LDL) and long-chain fatty acids (2-6). Abumrad et al. (2) showed that CD36 is abundant in peripheral tissues active in fatty acid metabolism, such as adipose tissue, skeletal and cardiac muscle, where it is involved in high affinity uptake of fatty acids (2,7,8). In accordance, Coburn et al. (9) showed that fatty acid uptake is considerably impaired in muscle and adipose tissue of CD36-deficient mice, resulting in increased plasma FFA concentrations (10).

There is a relationship between CD36 and glucose metabolism. For example, the spontaneous hypertensive rat (SHR), which is characterized by defects in fatty acid metabolism and insulin resistance, has mutations in the CD36 gene (11). Transgenic rescue of the deficient CD36 gene in these SHR rats improved insulin resistance as determined by an oral glucose tolerance test (12).

Remarkably, these findings were not directly corroborated in CD36 mice models.
Blood glucose levels are reduced in CD36-deficient mice (10) and increased upon muscle-specific overexpression of CD36 (13). In contrast to the SHR rat, in CD36-deficient mice whole body glucose tolerance, as determined by oral glucose tolerance tests, was diet-dependent. On a normal chow diet, CD36-deficient mice seemed more insulin sensitive since glucose tolerance as well as basal glucose uptake in cardiac and skeletal muscle were increased compared to wild type animals. Only when the diet was switched to a high fructose or high fat diet, impaired glucose tolerance could be observed in the CD36-deficient mice (14). It should be noted however that in these studies the insulin sensitivity per se in CD36-deficient mice is not confirmed in vivo by means of the hyperinsulinemic clamp analysis, which is considered as the golden standard for insulin sensitivity. This prompted us to determine whole body and tissue-specific insulin-mediated glucose uptake by hyperinsulinemic clamp in CD36-deficient versus wild type mice. We showed that whole body insulin-mediated glucose uptake was indeed increased in CD36-deficient mice. However, this increased whole body insulin sensitive glucose uptake was due to an increased insulin sensitivity in the muscle tissue, whereas the liver in these mice was insulin resistant. This dissociation can not be shown by the means of clinically most common analysis of glucose tolerance test.
Methods

Animals. CD36-deficient mice (CD36 -/-) were generated by targeted homologous recombination and back-crossed 6 times to C57Bl/6. Wild type control littermates (CD36 +/-) were bred from the same cross and were therefore of identical genetic background (10). Mice used in experiments were males of 4 to 6 months of age. They were housed under standard conditions with free access to water and food (standard-rat-mouse chow diet) and experiments were performed after an overnight fast. After each experiment liver, cardiac and skeletal muscle (hind limb) and adipose tissue samples were snap frozen in liquid nitrogen and stored at -80 °C until analysis. Principles of laboratory animal care were followed and the animal ethics committee of our institute approved all animal experiments.

Plasma lipid analysis. In all experiments, tail vein blood was collected into chilled para-oxonized capillary tubes to prevent in vitro lipolysis (15). These tubes were placed on ice and immediately centrifuged at 4 °C. Plasma levels of total cholesterol, TG (without free glycerol), ketone bodies (β-hydroxybutyrate) and FFA were determined enzymatically using commercially available kits and standards ( #236691, Boehringer, Mannheim, Germany; #337-B and #310-A Sigma GPO-Trinder kit, St. Louis, MA, USA; Nefa-C kit, Wako Chemicals GmbH, Neuss, Germany).

Plasma glucose and insulin assays. Levels of plasma glucose were determined
enzymatically using a commercially available kit (#315-500, Sigma, St. Louis, MA, USA) and, during the clamp experiment whole blood glucose was measured by a Freestyle hand glucose analyzer (Disetronic, Vianen, the Netherlands). Plasma insulin was measured by radioimmunoassay, using rat insulin standards (Sensitive Rat Insulin Assay, Linco Research Inc., St Charles, Missouri, USA).

**Whole body glucose turnover studies.** Body weight matched CD36 -/- and littermate controls were anesthetized (0.5 ml/kg hypnorm; Janssen Pharmaceutical, Beerse, Belgium and 12.5 mg/kg midazolam; Roche, Mijdrecht, The Netherlands) and an infusion needle was placed in one of the tail veins. After a 2-hour infusion of D-[3 H]glucose at a rate of 0.8 µCi.kg⁻¹.min⁻¹ (Specific activity: 620 GBq/mmol, Amersham, Little Chalfont, UK) to achieve steady state levels, basal glucose parameters were determined during a 30-minute period. Thereafter a bolus of insulin (100 mU/kg, Actrapid, Novo Nordisk, Chartres, France) was administered and a hyperinsulinemic clamp was begun. Insulin was infused at a constant rate of 3.5 mU.kg⁻¹.min⁻¹ and D-[3-³H]glucose was infused at a rate of 0.8 µCi.kg⁻¹.min⁻¹. A variable infusion of 12.5% D-glucose (in PBS) was also started to maintain blood glucose at ~ 8.0 mM. Blood samples (<1 µl) were taken every 5 to 10 minutes to monitor plasma glucose levels and (if necessary) adjust the glucose pump. After reaching steady state, blood samples were taken at 20 minutes time intervals during 1-hour to determine steady state levels of [³H]glucose. An average clamp (basal and
hyperinsulinemic conditions) experiment takes 3-3.5 hours and anesthesia was maintained throughout the procedure. Total blood sample size <250 µl.

Tissue specific glucose uptake studies. To estimate the basal and insulin-stimulated glucose uptake in individual tissues 2-deoxy-D-[1-\textsuperscript{3}H]glucose (2-DG) was used in separate groups of mice. When steady state levels of glucose were reached, a bolus (2 µCi) of 2-DG (Specific activity: 344 GBq/mmol, Amersham, Little Chalfont, UK) was injected via the tail vein. Forty-five minutes later, mice were sacrificed and tissues were collected.

Plasma analysis. Total plasma [\textsuperscript{3}H]glucose was determined in 10 µl plasma, and in supernatants after TCA (20%) precipitation and water evaporation to eliminate [\textsuperscript{3}H]H\textsubscript{2}O. The rates of glucose oxidation were determined as previously described by Koopmans et al. (16).

Tissue analysis. Tissue samples were homogenized (~10% wet weight/vol) in PBS and samples were removed for measurement of protein content. Total TG content in homogenates was determined, after separation by high performance thin layer chromatography (HPTLC) on silica-gel-60 pre-coated plates as described previously (17,18). Quantification of the lipid amounts was performed by scanning the plates.
with a Hewlett Packard Scanjet 4c and by integration of the density areas using Tina® version 2.09 software (Raytest, Straubenhardt, Germany).

For determination of tissue 2-DG uptake, tissues were homogenized (~10%) in water, boiled, and subjected to an ion-exchange column (Dowex 1X8-100, Sigma, St. Louis, MA, USA) to separate 2-DG-6-phosphate (2-DG-P) from 2-DG, as previously described (19,20).

Calculations. Under steady-state conditions for plasma glucose concentrations, the rate of glucose disappearance equals the rate of glucose appearance. The latter was calculated as the ratio of the rate of infusion of [3-3H]glucose (dpm/min) and the steady state plasma [3H]glucose-specific activity (dpm/µmol glucose). Endogenous glucose production was calculated as the difference between the rate of glucose disappearance and the infusion rate of D-glucose. Tissue-specific glucose uptake was calculated from tissue 2-DG-P content, which was expressed as percent of 2-DG of the dosage per gram tissue, as previously described (21,22).

[3H]FFA uptake. To study in vivo the fatty acid uptake by the liver and skeletal muscle, CD36 -/- and CD36 +/- mice were anesthetized as described above.

[3H]labeled fatty acids (30 µCi [9-10(n)-3H]oleic acid) (Specific activity: 278 GBq/mmol, Amersham, Little Chalfont, UK) dissolved in 200 µl BSA solution (2 mg/ml in sterile
saline) were injected into the tail vein of the mice and after 1 minute mice were
sacrificed. Livers and skeletal muscles were collected and the amount of radioactivity
was determined. Values were corrected for entrapped blood volume in the tissues as
described by Rensen *et al.* (23) and for plasma FFA concentration.

**Glycogen content.** Five fed CD36 -/- and CD36 +/+ mice were sacrificed, skeletal
muscle and livers were collected, snap frozen in liquid nitrogen, and glycogen was
measured as previously described (24). In short, parts of these tissues were
homogenized in 1M KOH, incubated at 90 °C for 30 min, and 3M acetic acid was
added. After hydrolyzing the glycogen by aminoglucosidase (Sigma, St. Louis, MA,
USA), glucose was measured enzymatically as described above.

**Hepatic insulin signaling protein levels.** For analysis of proteins involved in the insulin
signaling pathway, we performed Western blotting and measured phosphorylated
protein kinase B (PKB-P). For this purpose 4 CD36 -/- and +/+ mice were sacrificed
10 minutes after an ip injection of insulin (50 U/kg body weight) or PBS as a control (25).
The livers were snap frozen in liquid nitrogen and parts of these tissues were
homogenized in RIPA buffer (30 mM Tris, pH 7.5, 1 mM EDTA, 150 mmol/l NaCl,
0.5% Triton X-100, 0.5% deoxycholate, 1 mmol/l sodium orthovanadate, 10 mmol/l
sodium fluoride) containing protease inhibitors (Complete, Roche, Mannheim,
Germany). Extracts were cleared by centrifugation (4°C) and protein content in the
supernatant was measured using a BCA kit (Pierce, Rockford, IL, US). Proteins (25 µg/lane) were separated by SDS-PAGE on a 8% gel and blotted on PVDF-membrane (Millipore, Bedford, MA, USA). Filters were blocked in Tris-buffered saline, containing 0.25% Tween-20 (TBST) and 5% non-fat dried milk (Protifar) (26), and incubated overnight with a phospho-Akt (Ser473) antibody (Cell signaling technology, Westburg BV, Leusden, The Netherlands). Following extensive washing in TBST, bound antibodies were detected using HRP-conjugated goat-anti-rabbit IgG (Promega, Madison, WI, USA) in a 1:5,000 dilution, followed by visualization by enhanced chemiluminescence. Blots were quantitated on a Lumimager (Roche Molecular Biochemicals, Mannheim, Germany), using LumiAnalyst software. Also total PKB expression was determined by western blot analysis using a polyclonal PKB antibody kindly provided by Dr. Boudewijn Burgering (Utrecht University, the Netherlands).

Statistical analysis. The Mann-Whitney nonparametric test for 2 independent samples was used to define differences between CD36 -/- and CD36 +/+ mice. The criterion for significance was set at P<0.05. All data are presented as mean ± SD.
Results

Body weight, food intake and plasma lipid concentrations. CD36 -/- mice weighed significantly less than age matched CD36 +/+ mice and food intake of CD36 -/- mice was also decreased as compared to CD36 +/+ mice (Table I). There were no significant differences in cholesterol concentrations (Table I). In accordance with previously published studies (9), plasma concentrations of FFA and TG were significantly increased in CD36 -/- mice compared to CD36 +/+ mice (Table I)(10).

CD36 deficiency increases insulin sensitivity. To investigate the supposed improved glucose tolerance in CD36 -/- mice (14) (and Table I), we measured insulin sensitivity of whole body glucose metabolism by hyperinsulinemic clamp. To avoid possible confounding effects of the lower body weight in CD36 mice we took body weight matched CD36 -/- and wild type littermates (21.0 ± 2.0 and 21.4 ± 2.5 g, respectively) for the clamp analysis. After an overnight fast (basal), to exclude effects of food intake, [3H]glucose turnover measurements showed that CD36 -/- mice tended to have a higher whole body glucose uptake as compared to CD36 +/+ mice, but no significant difference was found (Fig. 1A). Basal glucose oxidation was significantly increased in CD36 -/- mice compared to wild type littermates (Fig. 1B). Interestingly, muscle glycogen content was not significantly changed in CD36 -/- mice compared to littermate controls (2.6 ± 0.9 and 2.1 ± 0.4 mg glycogen/g tissue, respectively), despite the increased muscle glucose uptake. For hyperinsulinemic
conditions, glucose levels were clamped at 8 mmol/l, mimicking normal postprandial glucose levels in mice. The insulin levels during the clamp studies were approximately 10-fold higher (387.5 ± 156.0 pmol/l in CD36 +/+ vs. 340.2 ± 141.8 pmol/l in CD36 -/- mice) than those measured under fasted conditions in CD36 +/+ mice (Table I). Under these conditions, CD36 -/- mice showed a significant increase in whole body glucose uptake compared to CD36 +/+ mice (Fig. 1A). The increment of insulin-mediated over basal whole body glucose uptake was also significantly increased in CD36 -/- mice compared to wild type controls (58 ± 9 and 40 ± 8 µmol/kg.min, respectively, p<0.05). Insulin-mediated glucose oxidation did not show a significant increase in CD36 -/- mice compared to littermate controls (Fig. 1B).

CD36 deficiency enhances insulin sensitivity in skeletal muscle, but leads to hepatic insulin resistance. Glucose uptake in skeletal (hind limb) muscle and adipose tissue was measured under basal and hyperinsulinemic conditions 45 minutes after a bolus of 2-DG was administered (Fig. 2). In skeletal muscle, a significant increase in glucose uptake in CD36 -/- mice was observed both under basal as well as under hyperinsulinemic conditions as compared to CD36 +/+ mice (Fig. 2). In adipose tissue, there were no differences in glucose uptake between the two genotypes (results not shown). CD36 -/- mice show comparable endogenous glucose production as compared to CD36 +/+ mice under basal conditions (Fig. 3A). Interestingly however, for hyperinsulinemic conditions, endogenous glucose
production was significantly higher in CD36 −/− mice as compared to CD36 +/+ mice (Fig. 3A). In accordance with our previous studies in wild type mice (27), insulin inhibited endogenous glucose production by approximately 40% in CD36 +/+ mice (Fig. 3B). In contrast, however, endogenous glucose production is not inhibited by insulin in CD36 −/− mice (Fig. 3B), indicating that these mice have liver-specific insulin resistance.

High triglyceride levels in the liver correlate with hepatic insulin resistance in CD36 −/− mice. In search for a possible explanation for hepatic insulin resistance in CD36 −/− mice, we analyzed [3H]fatty acid uptake and TG content in liver and muscle tissue from CD36 −/− and CD36 +/+ mice after an overnight fast (Fig. 4). CD36 −/− mice have significantly increased fatty acid uptake in the liver (Fig. 4A). In addition, hepatic TG content was significantly higher in CD36 −/− mice compared to CD36 +/+ mice (Fig. 4B). CD36 −/− mice also have increased plasma ketone bodies (Table I), which is indicative of increased hepatic ²-oxidation. We observed a 25% decrease in muscle FA uptake in CD36 −/− mice (Fig. 4C). Interestingly, no difference in muscle TG content was observed between the two genotypes (Figure 4D).

Hepatic insulin resistance is associated with defects in insulin signaling. In order to obtain a molecular basis for the hepatic insulin resistance, we analyzed protein kinase B S473 phosphorylation, a marker of the activity of the insulin signal
transduction pathway in response to intraperitoneal insulin injection. As shown in Figure 5, insulin-induced phosphorylation of protein kinase B was significantly reduced in CD36 \(^{-/-}\) mice, whereas total PKB expression was unaltered.
Discussion

Increased fatty acid flux from adipose tissue to nonadipose tissue is fundamental to metabolic changes that are characteristic of the insulin resistance syndrome (1). The present study shows that in the absence of CD36 insulin sensitivity in muscle is increased. However, in this mouse model, more fatty acids are directed to the liver, leading to hepatic insulin resistance.

After an overnight fast (basal state) plasma glucose levels were lower in CD36 -/- mice, whereas no differences were found in insulin levels (Table I). However after a 4 hour fast, we found significant lower plasma insulin levels in CD36 -/- mice as compared to CD36 +/- mice (49.4 ± 16.3 and 72.8 ± 23.2 pmol/l, respectively, p<0.05). In accordance, Hajri et al. (14) found decreased plasma insulin levels in CD36 -/- mice as compared to controls. They also showed that CD36 -/- mice cleared an intraperitoneal glucose bolus more efficiently then their wild type littermates. Although we did not observe significant differences in basal whole body glucose uptake between fasted CD36 -/- and fasted wild type mice, we did find an increased glucose uptake specifically in skeletal muscle tissue under these basal conditions. These observations confirm the increased glucose tolerance as performed by Hajri et al. (14). Furthermore, basal glucose oxidation was significantly higher in CD36 -/- mice showing indeed high glucose utilization in these mice. These data suggest that the increased glucose uptake and oxidation exceed the endogenous glucose production in CD36 -/- mice leading to the observed lower fasting plasma glucose
concentrations in these mice. Muscle glycogen content was similar between the two genotypes. These data seem to further confirm the notion that CD36 -/- mice use the increased basal, and insulin-mediated glucose uptake as well, directly for oxidation/energy production, rather than storage as glycogen.

These data on reduced plasma insulin levels and increased muscle glucose uptake are indicative for increased insulin sensitivity of the CD36 -/- mice compared to controls. Hajri et al. (14) showed by in vitro studies that the insulin-mediated glucose uptake in isolated muscle tissue of CD36 -/- mice is increased. By using hyperinsulinemic clamp analysis we showed in vivo for the first time that in CD36 -/- mice insulin-mediated whole body and skeletal muscle-specific glucose uptake was increased when compared to their controls. Hence, CD36 -/- mice indeed exhibit increased insulin sensitivity. Also in other studies, decreased fatty acid uptake in skeletal muscle has been linked to increased insulin sensitivity, with respect to glucose uptake (19,28). Thus, despite elevated plasma FFA and TG levels, CD36 deficiency significantly improved insulin sensitivity in peripheral tissues (this study and (14)). Also in mice overexpressing human apoC1 strongly elevated plasma FFA and TG levels were found in combination with increased insulin sensitivity (29). Hence, increased plasma FFA and TG levels per se do not necessarily result in peripheral insulin resistance. On the contrary, increased plasma fatty acid levels, as a consequence of decreased uptake of fatty acids in the peripheral tissues lead to increased muscle insulin sensitivity.
We could not find significant differences in muscle TG content between CD36 -/- and CD36 +/- mice, whereas Hajri et al. (14) found a decreased muscle TG content in CD36 -/- mice. We can not explain these differences, but they may be due to differences in dietary conditions (6.5% vs 3.5% fat in our chow diet) and or fasting conditions. A positive relationship between muscle TG content and insulin resistance is observed in mice (30) and in patients with type 2 diabetes (29,31,32). Our own data from previous studies (27) did not show a direct positive correlation between muscle TG accumulation and insulin resistance. Thus the reduced FA uptake per se and/or reduced intracellular metabolites other than TG (acyl CoAs and DAGs) determine muscle insulin sensitivity in CD36 -/- mice.

CD36 -/- weighed significantly less than age matched CD36 +/- control mice, suggesting that the reduced flux of fatty acids to the peripheral tissues and/or reduced food intake in CD36 -/- mice leads to lower body weight. In CD36 -/- mice we observed a significant deceased uptake of [3H]fatty acids in adipose tissue (results not shown) while no differences were observed in 2-deoxyglucose uptake by adipose tissue as compared to CD36 +/- mice (data not shown). This is in line with previous studies (33-37). In mice lacking the Very-Low-Density-Lipoprotein Receptor (VLDLr), we and others have seen a decreased whole body FFA uptake and decreased body weight: these mice are protected from obesity and insulin resistance when feeding them a high fat diet (33,34). ApoC1 strongly inhibits lipoprotein binding to the VLDLr and overexpression of apoC1 also leads to a decreased adipose tissue FFA
uptake with decreased body weight and improved insulin sensitivity (35,36). Also in the absence of lipoprotein lipase (LPL) in adipose tissue, mice on an ob/ob background showed lower body weights (37). All these models together indicate that a disturbance in the delivery of fatty acids to the peripheral tissues leads to a decreased body weight, mainly reflected in less adipose tissue. To exclude the possible confounding effect of reduced body weights in CD36 -/- mice we used body weight matched littermates for the clamp analysis. Forced caloric restriction leads to body weight loss and reduced body fat content, but increased not only muscle but also liver insulin sensitivity (38-41). We can not exclude that a changed body composition in CD36 -/- mice compared to littermate controls effects muscle insulin sensitivity. But this possible body composition change can not, to our opinion, explain the liver-specific insulin resistance in CD36 -/-.

The enhanced muscle insulin sensitivity of CD36 -/- mice contrasts with the data obtained in spontaneous hypertensive rats, in which the absence of CD36 induces insulin resistance due to a suggested defective fatty acid uptake (11,12). Transgenic expression of CD36 in SHR led to improved glucose tolerance and higher incorporation of glucose into muscle glycogen compared to SHR rats (12). The authors, however, did not document in vivo insulin-mediated whole body and muscle-specific glucose uptake using hyperinsulinemic clamp experiments (12). As an explanation for the discrepancy between the data obtained in mice and rats, Hajri et al. (14) suggest that in the SHR there is evidence that muscle fatty acid uptake exceeds oxidative capacity,
despite CD36 deficiency. It is likely, however, that differences in genetic backgrounds between rats and mice determine the discrepant effects of CD36 on insulin sensitivity. Moreover, differences are found between different SHR strains. Although Gotoda et al. (42) detected in their SHR strain no CD36 deficiency, the same phenotype was found as in the SHR described in the study by Aitman et al. (11).

The livers of CD36 -/- mice are severely insulin resistant with regard to the suppression by insulin of endogenous glucose production. Under normal conditions hepatic expression of CD36 is very low (2) and plasma membrane FA-binding protein (FABPpm) is the main hepatic fatty acid transporter (43). Consequently, in CD36-deficient mice, increased plasma FFA levels lead to an increased flux of fatty acids towards the liver (9). This increased flux of fatty acids towards the liver of CD36 -/- mice results in increased β-oxidation reflected in increased plasma levels of ketone bodies and in excess storage of TG in the liver. The increased hepatic TG content and enhanced beta-oxidation might seem contradictive, but to our understanding the increased FA-flux towards the liver (+300%) in CD36 -/- mice exceeds the increased beta-oxidation capacity (~+50%). When FA uptake exceeds utilization the fatty acids are stored as TG in CD36 -/- mice. Increased hepatic TG content correlates with impaired suppression of endogenous glucose production by insulin (40,44). Furthermore, epidemiological evidence has been presented that liver fat accumulation is associated with high circulating plasma FFA levels and hepatic insulin resistance in humans (45,46). Accordingly, the phosphorylation of PKB in the liver by
insulin in CD36 -/- mice is in line with hepatic insulin resistance. PKB, also called Akt, is an important downstream target of the insulin signaling pathway, regulating hepatic glucose metabolism (47). Increased plasma FFA concentrations and increased hepatic FA uptake observed in these CD36 -/- mice may lead to an increase in hepatic intracellular fatty acid derivatives such as fatty acyl CoA, diacylglycerol and ceramides. These metabolites may interfere, directly or indirectly, with the insulin signaling cascade leading to downstream effects in the insulin signaling pathway, and in that way may contribute to insulin resistance in the liver of these CD36 -/- mice (28).

The liver-specific insulin resistance found in these CD36 -/- mice could in fact be the basis of the diet-induced impaired glucose tolerance shown by Hajri et al. (14). One could argue that the higher basal-hyperinsulinemic difference in glucose concentration in CD36 -/- mice compared to wild type controls (4.9 mM and 2.8 mM, respectively) induced higher endogenous insulin secretion in the CD36 deficient mice leading to higher portal insulin supply to the liver. To our opinion this would mean that even higher portal insulin levels in these CD36 -/- mice do not lead to suppression of the endogenous glucose production by the liver. Supporting our conclusion that the livers of CD36-/- mice are insulin resistant.

In conclusion, our data indicate that impaired peripheral fatty acid uptake in CD36-deficient mice leads to increased plasma FFA levels without inducing insulin resistance in muscle. The increased plasma FFA levels on its turn lead to an increase in fatty acid flux towards the liver which results in increased hepatic β-
oxidation and TG storage and consequently liver-specific insulin resistance. These observations show that tissue fatty acid uptake determines actual tissue insulin sensitivity and not plasma FFA concentrations per se. Using hyperinsulinemic clamp analysis we were able to show a dissociation of whole body and muscle-specific insulin sensitivity on one hand and liver-specific insulin resistance on the other hand.

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References


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Figure legends

Figure 1
Whole body glucose uptake (A) and glucose oxidation (B) in overnight fasted body weight matched CD36 -/- and CD36 +/- mice under basal and hyperinsulinemic clamp conditions.

**Basal**: After a 2-hour infusion of D-[3H]glucose to achieve steady state levels, basal glucose parameters were measured over a 30-minute period.

**Hyperinsulinemic**: After the basal period, insulin and D-[3-3H]glucose were administered and the hyperinsulinemic clamp was started as described in the methods section. After reaching steady state (equal glucose concentrations), blood samples were taken at 20 minutes time intervals during 1 hour to determine steady state levels of [3H]glucose.

Values represent the mean ± SD of 6 mice per group. *P<0.05, indicating the difference between CD36 -/- and CD36 +/- mice, using nonparametric Mann-Whitney tests.

Figure 2
Glucose uptake (as measured by 2-deoxy-[3H]D-glucose uptake) in skeletal muscle in overnight fasted CD36 -/- and +/- mice under basal and hyperinsulinemic clamp...
conditions. Values represent the mean ± SD of 5 mice per group. *P<0.05, indicating the difference between CD36 −/− and CD36 +/+ mice, using nonparametric Mann-Whitney tests.
Figure 3

Endogenous glucose production (A) and inhibition of endogenous glucose production by insulin (B) in CD36 -/- and CD36 +/+ mice.

Endogenous glucose production was measured under basal and euglycemic-hyperinsulinemic clamp conditions as described in the methods section. The difference in endogenous glucose production under basal and insulin inhibiting conditions (3A) was calculated as the % inhibition of endogenous glucose production by insulin.

Values represent the mean ± SD of 6 mice per group. * P<0.05, indicating the difference between CD36 -/- and CD36 +/+ mice, using nonparametric Mann-Whitney tests.

Figure 4

Liver (A and B) and skeletal muscle (C and D) [3H]free fatty acid (FFA) uptake (A and C) and triglyceride (TG) content (B and D) in CD36 +/+ and -/- mice.

After an overnight fast, CD36 +/+ and CD36 -/- mice were given a [3H]FFA bolus and 1 minute after the injection mice were sacrificed and the amount of radioactivity in liver (A) and skeletal muscle (C) tissue was determined as described in the methods section.

Triglycerides were determined in liver (B) and skeletal muscle (D) homogenates from CD36 -/- and CD36 +/+ mice after separation by HPTLC as indicated in the methods.
Values represent the mean ± SD of 3 (A and C) or 6 (B and D) mice per group.

*P < 0.05, indicating the difference between CD36 -/- and CD36 +/+ mice, using nonparametric Mann-Whitney tests.

**Figure 5**

Western Blot analysis of hepatic insulin receptor beta chain and phosphorylated PKB in CD36 -/- and +/+ mice after an ip injection of insulin. Intensity determination of the Western Blots expressed relative intensities corrected for total PKB. Values represent mean ± SD for n = 4 animals per group. *P < 0.05, indicating the difference between CD36 -/- and CD36 +/+ mice, using nonparametric Mann-Whitney tests.
Table I

**Body weight, food intake and plasma lipid, glucose and insulin concentrations in CD36 -/- and CD36 +/- mice.**

<table>
<thead>
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<th>Genotype</th>
<th>Body weight (g)</th>
<th>Food intake (g/24h)</th>
<th>Plasma levels</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>TC (mmol/l)</td>
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<tr>
<td>CD36 +/-</td>
<td>29.7 ± 3.1</td>
<td>4.6 ± 0.7</td>
<td>1.55 ± 0.05</td>
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<td>CD36 -/-</td>
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Plasma cholesterol (TC), triglyceride (TG), free fatty acids (FFA), ketone body (KB), glucose and insulin levels were measured after overnight fasting as described in the methods section.

**Abbreviations:** 2-DG, 2-deoxy-D-[1-3H]glucose; CD36 -/-, CD36-deficient mice; CD36 +/-, wild type control littermates; FAT, fatty acid translocase; FFA, free fatty acids; HPTLC, high performance thin layer chromatography; TG, triglyceride; SHR, spontaneous hypertensive rat; VLDLr, very-low-density-lipoprotein receptor.
Values represent the mean ± SD of 6 mice per group. *P<0.05, indicating the difference between CD36 -/- and CD36 +/+ mice, using nonparametric Mann-Whitney tests.
Figure 1

A

Glucose uptake (μmol.min⁻¹.kg BW⁻¹)

<table>
<thead>
<tr>
<th>Condition</th>
<th>CD36 +/+</th>
<th>CD36 -/-</th>
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<td>Basal</td>
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<td>Hyperinsulinemia</td>
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</table>

B

Glucose oxidation (μmol.min⁻¹.kg BW⁻¹)

<table>
<thead>
<tr>
<th>Condition</th>
<th>CD36 +/+</th>
<th>CD36 -/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Hyperinsulinemia</td>
<td>80</td>
<td>120</td>
</tr>
</tbody>
</table>
Figure 2

% glucose uptake /g tissue

Skeletal muscle

CD36

Basal

Hyperinsulinemia

*
Figure 3
Figure 4
Figure 5