

Defective Fatty Acid Uptake in the Spontaneously Hypertensive Rat Is a Primary Determinant of Altered Glucose Metabolism, Hyperinsulinemia, and Myocardial Hypertrophy*

Received for publication, January 31, 2001, and in revised form, April 20, 2001
Published, JBC Papers in Press, April 25, 2001, DOI 10.1074/jbc.M100942200

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Genetic linkage studies implicated deficiency of CD36, a membrane fatty acid (FA) transporter, in the hypertriglyceridemia and hyperinsulinemia of the spontaneously hypertensive rat (SHR). In this study we determined whether loss of CD36 function in FA uptake is a primary determinant of the SHR phenotype. *In vivo*, tissue distribution of iodinated, poorly oxidized β -methylidophenyl pentadecanoic acid (BMIPP) was examined 2 h after its intravenous injection. Fatty acid transport was also measured *in vitro* over 20 to 120 s in isolated adipocytes and cardiomyocytes obtained from SHR and from a congenic line (SHRchr4) that incorporates a piece of chromosome 4 containing wild-type CD36. SHR heart and adipose tissue exhibited defects in FA uptake and in conversion of diglycerides to triglycerides that are similar to those observed in the CD36 null mouse. However, a key difference in SHR tissues is that fatty acid oxidation is much more severely impaired than fatty acid esterification, which may underlie the 4–5-fold accumulation of free BMIPP measured in SHR muscle. Studies with isolated adipocytes and cardiomyocytes directly confirmed both the defect in FA transport and the fact that it is underestimated by BMIPP. Heart, oxidative muscle, and adipose tissue in the SHR exhibited a large increase in glucose uptake measured *in vivo* using [¹⁸F]fluorodeoxyglucose. Supplementation of the diet with short-chain fatty acids, which do not require CD36-facilitated transport, eliminated the increase in glucose uptake, the hyperinsulinemia, and the heart hypertrophy in the SHR. This indicated that lack of metabolic energy consequent to deficient FA uptake is the primary defect responsible for these abnormalities. Hypertension was not alleviated by the supplemented diet suggesting it is unrelated to fuel supply and any contribution of CD36 deficiency to this trait may be more complex to determine. It may be worth exploring whether short-chain FA supplementation can reverse some of the deleterious effects of CD36 deficiency in humans, which may include hypertrophic cardiomyopathy.

The spontaneously hypertensive rat (SHR)¹ is a widely studied rodent model of human metabolic Syndrome X, in which hypertension is associated with dyslipidemia and with insulin resistance of glucose metabolism (1). Two quantitative trait loci for defective insulin action in the SHR were identified on chromosomes 4 and 12. Quantitative trait loci for defects in glucose and fatty acid metabolism and for hypertension map to the same locus on chromosome 4 (1). Microarray screening and congenic mapping identified CD36 on rat chromosome 4 as a defective SHR gene at the peak of linkage to these quantitative trait loci (2). The SHR CD36 cDNA contains multiple sequence variants and the protein product is undetectable in SHR adipocyte plasma membrane (2).

CD36 was identified on platelets (3) as a receptor for thrombospondin, collagen (4), and oxidized lipoproteins (5). CD36 is also known as FAT for fatty acid translocase (6) since its function in long-chain FA transport was identified from its binding the reactive FA derivative sulfo-*N*-succinimidyl oleic acid, an irreversible inhibitor of FA uptake by rat adipocytes (7). Results of *in vitro* studies provided strong support for a role of CD36 in FA transport (8). Recent work with CD36 null (9, 10) and transgenic (11) mice documented that CD36 facilitates a major fraction of FA uptake by heart, oxidative skeletal muscle, and adipose tissues.

Based on the role of CD36 as a FA transporter, it was proposed that a primary genetic defect in the SHR is compromised tissue utilization of FA (2), which would contribute to the pathogenesis of insulin resistance by producing secondary alterations in basal glucose metabolism (12). Support for this interpretation was provided by the findings that both the hyperinsulinemia and insulin resistance were improved when a piece of chromosome 4 containing wild-type CD36 was integrated into the SHR genome (SHRchr4 line) (13) or with transgenic rescue (14). However, arguing against this interpretation are important phenotypic differences between the SHR and CD36 null mouse. The CD36 null mouse, in contrast to the SHR, is hypoglycemic (9) and hypoinsulinemic² while the mouse with CD36 overexpression exhibits the opposite changes (11). Also complicating the link between CD36 deficiency and the SHR phenotype are reports that some SHR lines, which apparently have normal CD36 levels, exhibit symptoms of insulin resistance (13, 15). There is no information on whether SHR tissues have loss of CD36 function. The CD36 protein

* This work was supported by National Institutes of Health Grant RO1-DK33301 (to N. A. A.), an American Heart Association Fellowship AHA0020639T (to T. H.) and Grant AHA0030345T (to A. I.), and a grant from the United States Department of Energy (to F. F. K). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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¹ The abbreviations used are: SHR, spontaneously hypertensive rat; FA, fatty acid; BMIPP, β -methylidophenyl pentadecanoic acid; 2-FDG, 2-fluorodeoxyglucose; DG, diglycerides; TG, triglycerides.

² T. Hajri, unpublished observations.

deficiency in SHR was based on reaction of adipose tissue membranes with a polyclonal antibody against rat CD36, which may have failed to recognize a mutated protein that is partially or completely functional. In addition, it is not known if CD36 has a dominant role in FA transport in rat tissues and if its loss would have similar consequences to those observed in the mouse.

We have compared uptake of FA and glucose by tissues of SHR, WKY, and the congenic SHRchr4 line (13) and tested whether diet supplementation with short-chain FA, which do not require CD36 for uptake, can improve the hyperinsulinemia and other abnormalities of the SHR.

EXPERIMENTAL PROCEDURES

Animals and Diets—Breeding pairs for SHR (NCR1BR) and WKY (NCR1BR) controls were purchased from the Charles River Company. The congenic SHR line (SHR.BN-116/Npy) harboring a segment of chromosome 4 containing CD36 (referred to here as SHRchr4) was generated by replacing the deletion variant of CD36 in the SHR with a wild-type variant from the normotensive Brown Norway rat as previously described in detail (13). Rats were housed in a facility equipped with a 12-h light cycle, were given unlimited access to water, and were fed a standard chow diet *ad libitum*.

The basal diet used was the standard chow Purina diet (number 5001) rich in carbohydrate (50% of total weight) and containing a low proportion of fat (4.5%) mostly as polyunsaturated fatty acids. The supplemented diet was prepared by adding 21.5 g of short- and medium-chain triacylglycerols (Captex 300, Arbitec Co.) to 100 g of basal diet followed by thorough mixing with a mechanical food blender. The composition of Captex 300 was 6% as caproic (C_6), 65.6% as caprylic (C_8), and 29.2% as capric (C_{10}). The final diet contained 21% fat (38% of total energy) of which 79% was short- and medium-chain fatty triacylglycerols. Rats were started on the diet at 1 week after weaning. Systolic arterial blood pressure was measured in awake rats by the indirect tail cuff method using a Physiograph (Narco Biosystem, Houston, TX) equipped with transducers and preamplifiers.

Analysis of Plasma Parameters—Rats were fasted overnight (16 h) and tail vein blood was collected into heparinized or EDTA (for FA determination) containing tubes. Plasma free fatty acids were measured using the Wako kit (Wako Chemicals, Richmond, VA). Triglycerides, cholesterol, and glucose were measured using enzymatic kits from Sigma Diagnostics. Plasma insulin was tested using a radioimmunoassay kit for rat insulin from Linco Research Inc. (St. Louis, MO).

Oral Glucose Tolerance Test—Rats, fasted overnight were given a 25% glucose solution (1.5 g/kg) orally using an intubation needle. Blood was collected for glucose determination from the tail prior to and at 10, 20, 30, 45, 90, and 120 min after the load. Glucose was measured using a Precision Q.I.D. monitoring system.

BMIPP Preparation—BMIPP was radioiodinated by the thallium-iodide exchange method and purified over a Sep-Pak RP-18 Light cartridge (Waters Corp.), as previously described (16). Specific activity of [125 I]BMIPP was in the range of 2–4 Ci/mmol and radiochemical purity, as determined by thin layer chromatography (TLC), was greater than 99%. BMIPP was dissolved in warm absolute ethanol (about 100 μ l) and added dropwise to a stirred solution of 6% FA-free bovine serum albumin at 40 °C. The solution was sterile filtered (0.22 μ m, Millipore) before injection.

Tissue Distribution of BMIPP and Fluorodeoxyglucose (2-FDG)—Each rat was injected in a lateral tail vein with 200 μ l of the radioisotope solution (14–25 μ Ci). The animals were sacrificed after 2 h and the tissues were rapidly removed, rinsed with saline, and blotted dry. Tissues were weighed and counted in a NaI Auto-Gamma counter. A sample of the injected solution was counted to determine the total injected dose.

2-[18 F]FDG in saline was injected via a lateral tail vein (about 15 μ Ci per rat) either alone or together with BMIPP. Tissues were harvested 2 h later as for BMIPP and counted for 18 F activity. When both BMIPP and 2-FDG were used in the same animal, the tissues were first counted for 2-FDG and then 24 h later for [125 I]BMIPP, once the 18 F radioactivity (half-life of 110 min) had decayed.

Analysis of BMIPP Lipid Incorporation—Lipids were extracted from frozen tissue according to Folch *et al.* (17). Aliquots were chromatographed next to known standards on aluminum-backed silica plates (Analtech, Inc.). Petroleum ether:diethyl ether:glacial acetic acid (70:30:1, v/v/v) was used to resolve polar lipids, diglycerides, fatty acids, and triglycerides to determine the BMIPP distribution in each lipid

class as a percentage of total counts on the plate.

Measurement of CD36 Protein Levels—CD36 expression was measured by flow cytometry and Western blotting. Flow cytometry was performed on platelets prepared by centrifuging platelet-rich plasma at 1800 \times g for 10 min. A 10- μ l aliquot of washed platelets (about 3×10^7 cells) suspended in 0.02 M phosphate-buffered saline with 9 mM EDTA and 0.1% bovine serum albumin (PEB) was incubated with a 1:100 dilution of anti-CD36 antibody and then with 1:150 fluorescein isothiocyanate-labeled secondary antibody. The cells were washed, resuspended in PEB, and assayed by flow cytometry (FACScan, Becton Dickinson). CD42 polyclonal antibody was used as a platelet marker. Data were analyzed as the percentage of gated cells expressing CD36.

For Western blots, adipose tissue was homogenized in 1 ml of ice-cold TES buffer (20 mM Tris, 1 mM EDTA, 250 mM sucrose). The homogenate was centrifuged (16,000 \times g) to yield a pellet (P1), which was layered on 38% sucrose and centrifuged at 86,000 \times g to pellet out mitochondria and nuclei (P2). The band at the interphase was harvested, diluted (1:7) in TES buffer, and centrifuged at 350,000 \times g to yield a total microsomal pellet (P3). Samples from both P1 and P3 (20–50 μ g of protein) were subjected to electrophoresis according to Laemmli (18) followed by transfer to a nylon-supported nitrocellulose membrane. Briefly, the membrane was incubated with anti-CD36 antibody (2 h, 1:1000 dilution), washed, then incubated (1 h, 1:10,000) with secondary antibody labeled with horseradish peroxidase. Detection of immune complex was according to the directions of the ECL kit (Amersham Pharmacia Biotech).

Preparation of Adipocytes and Cardiomyocytes—Adipocytes were isolated from the epididymal fat of male rats after tissue digestion with collagenase type I (1 mg/ml, Worthington Biochem). Suspensions were kept in a lipocrit of 30% and assayed as described earlier (19) and in the legend of Fig. 4. Cardiomyocytes were isolated from perfused hearts as described by Luiken *et al.* (20). Suspensions were kept at about 25% cell density and were assayed within 2 h after isolation using the same assay conditions as for adipocytes.

RESULTS

FA Uptake Is Reduced in Heart and Adipose Tissues of SHR—To evaluate whether the SHR phenotype involves loss of CD36 function in FA uptake, we compared biodistribution of the slowly oxidized FA analog [125 I]BMIPP in WKY controls and SHR. As documented previously in studies on both humans and laboratory animals (10, 21), BMIPP, a 3-methyl branched (15C) FA analogue, is rapidly extracted from blood-like native FA (21, 22). It equilibrates within 2–3 min and is incorporated into cellular lipids at rates almost identical to those of native FA (22–24). The stable iodination of BMIPP coupled with its prolonged tissue retention allow sensitive comparisons of tissue capacities for FA uptake *in vivo*. The CD36 null mouse exhibits a 60–80% defect in BMIPP uptake by oxidative skeletal muscle, heart, and adipose tissue and the magnitude of the defect is similar when data from BMIPP are compared with those with the rapidly oxidized FA analog, 15-(*p*-iodophenyl)pentadecanoic acid, or with native palmitate (10).

The biodistribution data for BMIPP in SHR and WKY showed that of the several tissues examined, which included blood, liver, intestine, lung, hip muscle, heart, and adipose tissue, impaired BMIPP uptake in SHR was observed only in heart (25%) and fat tissue (60%) (Fig. 1).

In CD36 null mice, tissues where FA uptake was deficient exhibited a defect in converting labeled diglycerides (DG) to triglycerides (TG) with a severalfold increase in the ratio of DG to TG (10). As shown in Fig. 2, such a block in DG to TG conversion was also observed in SHR heart. Table I, which documents BMIPP lipid distribution for different tissues, shows that the defect in intracellular processing of BMIPP is observed in oxidative (diaphragm) but not in glycolytic (hip) muscles. It is also measured in the kidney and in adipose tissue but not in the liver. Another point documented by Table I is that the defect in intracellular incorporation of BMIPP is observed early during growth since the rats used in these experiments were aged 9–10 weeks, as opposed to 14–16 weeks in the experiment shown in Fig. 2.

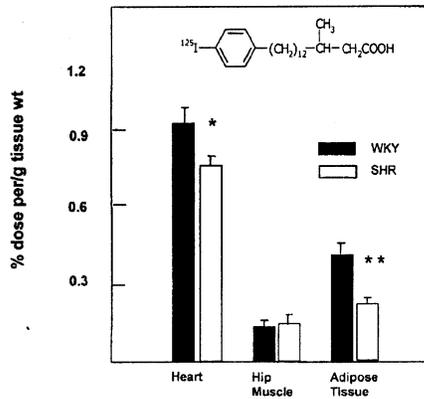


FIG. 1. BMIPP biodistribution in WKY and SHR rats. Rats were injected with 62 μCi of [^{125}I]BMIPP. Tissues were removed 2 h after injection. Uptake is expressed as percent of injected dose per gram tissue. Means are shown \pm S.E. ■, WKY ($n = 6$); □, SHR ($n = 6$). **, $p < 0.01$; ***, $p < 0.001$. Inset, structure of BMIPP.

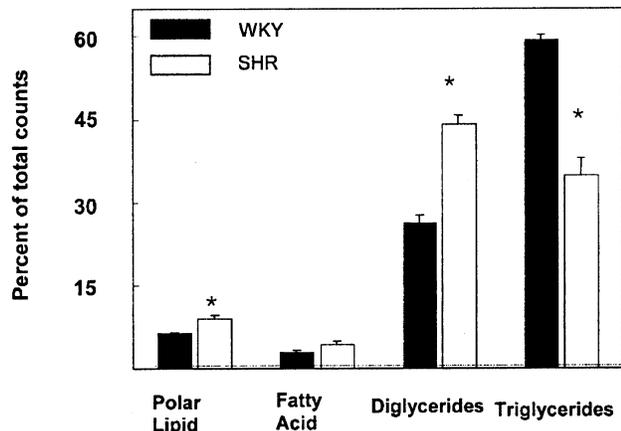


FIG. 2. Incorporation of BMIPP in heart lipid. Tissue samples were extracted for lipids and BMIPP incorporation was evaluated by TLC on Silica Gel G. Means \pm S.E. represent the percent of total counts recovered in each lipid fraction. Polar lipids include phospholipids and monoacylglycerides. ■, WKY ($n = 6$); □, SHR ($n = 6$). **, $p < 0.01$; ***, $p < 0.001$.

A significant finding in the oxidative tissues of the SHR is shown in Table II. Despite the defect in BMIPP uptake present in heart and diaphragm, there was a severalfold increase in accumulation of free BMIPP, a finding that was not observed in the CD36 null mouse.

The defective FA utilization present in some SHR tissues was paralleled by a compensatory increase in glucose uptake, as shown in Fig. 3. A large increase in uptake of the glucose tracer 2- ^{18}F FDG was observed in heart and diaphragm. Smaller but still significant increases were measured in intercostal and hip muscles and in adipose tissue. These data are in line with our findings with the CD36 null mouse.³

Since the ability to detect metabolic defects in the SHR may depend on the type of wild-type strain used for comparison, we examined BMIPP and 2-FDG uptake in tissues from SHR and a congenic line (SHRchr4) that incorporates a piece of chromosome 4 containing wild-type CD36. We also compared FA uptake by adipocytes isolated from the two lines. Panel A of Fig. 4 shows CD36 expression by adipose tissue from congenic SHRchr4, WKY, and SHR using a polyclonal anti-rat CD36 antibody. No detectable CD36 protein was observed in SHR adipose tissue (panel A) or platelets (not shown), while expres-

TABLE I
Incorporation of BMIPP into cellular lipids

Tissue samples from an experiment similar to that shown in Fig. 3 were Folch-extracted and the lipids evaluated by TLC. Values reflect the percent of total counts recovered in each lipid fraction. Polar lipids include phospholipids and monoacylglycerol-3-phosphates. Means, shown \pm S.E., are from one experiment typical of two others.

	Diglycerides	Triglycerides	Polar lipids
	% of total radioactivity		
WKY ($n = 6$)			
Liver	48 \pm 1	26.7 \pm 0.6	23 \pm 1
Kidney	49 \pm 1	23 \pm 2	25 \pm 1
Adipose	41 \pm 3	45 \pm 3	11 \pm 5
Hip muscle	61 \pm 4	11 \pm 1	22 \pm 2
Diaphragm	51 \pm 3	31 \pm 2	17 \pm 4
Heart	42 \pm 2	45 \pm 3	12.3 \pm 0.9
SHR ($n = 6$)			
Liver	45 \pm 2	28 \pm 1	25 \pm 1
Kidney	56.6 \pm 0.6 ^a	18 \pm 1 ^b	23.1 \pm 0.7
Adipose	53 \pm 3 ^b	36 \pm 3 ^b	8 \pm 3
Hip muscle	67 \pm 2	13 \pm 1	17 \pm 2
Diaphragm	60.8 \pm 0.7 ^b	20.5 \pm 0.7 ^a	14.1 \pm 0.2
Heart	48.7 \pm 0.9 ^b	29 \pm 1 ^a	16.7 \pm 0.3 ^b

^a $p < 0.005$.

^b $p < 0.05$.

TABLE II

Incorporation of BMIPP into cellular free FA in myocardium from WKY and SHR as compared to that in myocardium from wild type and CD36 null mice

Rat data are from the experiments shown in Table I while the mouse data are from (10). Means are shown \pm S.E. Rat data are from one experiment ($n = 6$) typical of two others. Mouse data are from one experiment ($n = 3-5$) typical of two more. Values for mouse diaphragm were not determined.

	WKY	SHR	Wild type	CD36 -/-
	% of total BMIPP in lipids counts			
Heart	1.1 \pm 0.5	5.1 \pm 0.7 ^a	3.1 \pm 0.5	1.9 \pm 0.3
Diaphragm	1.5 \pm 0.3	4.6 \pm 0.3 ^a		

^a $p < 0.005$.

sion on both adipose tissue (panel A) and platelets (not shown) could be observed in the SHRchr4 line.

Panel B shows that CD36 expression reversed the defects in uptake of FA and glucose in the SHR. Uptake of 2-FDG by SHRchr4 heart and adipose tissue was decreased by 400 and 46%, respectively, which supported an improvement in FA utilization. In line with this, initial rates of FA uptake by cardiomyocytes (panel C) and adipocytes (panel D) obtained from SHRchr4 were increased 2-3-fold above levels measured in cells from the parent SHR line, documenting reversal of the defect in FA transport with CD36 expression.

We next tested whether the defect in FA uptake constitutes a primary cause of the hyperinsulinemia and insulin resistance observed in the SHR. We examined whether supplying SHR tissues with the FA they can utilize would reverse the defects caused by lack of CD36. The saturable component of FA uptake, presumably facilitated by CD36, does not recognize short-chain FA (25). Also in line with this is the finding that pure CD36 does not bind short-chain FA.

SHR and WKY rats were started on the diet supplemented with short- and medium-chain FA at about 5 weeks of age and maintained on it for a period of 3 months. As shown in Table III, there were no significant differences in body weights between WKY and SHR at the end of the 3-month diet treatment. However, a significant decrease in SHR heart weight and in the heart to body weight ratio was observed on the FA-supplemented diet. The effect was small and not significant in WKY. The decrease in heart weight occurred without a change in blood pressure, which remained significantly higher in the

³ T. Hajri and N. Abumrad, unpublished observations.

FIG. 3. $2\text{-}^{18}\text{F}$]FDG tissue distribution in WKY and SHR rats. Rats were injected with $17\ \mu\text{Ci}$ of $2\text{-}^{18}\text{F}$]FDG. Tissues were removed 2 h after injection. Uptake is expressed as percent of injected dose per gram tissue. Means are shown \pm S.E. ■, WKY ($n = 6$); □, SHR ($n = 6$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

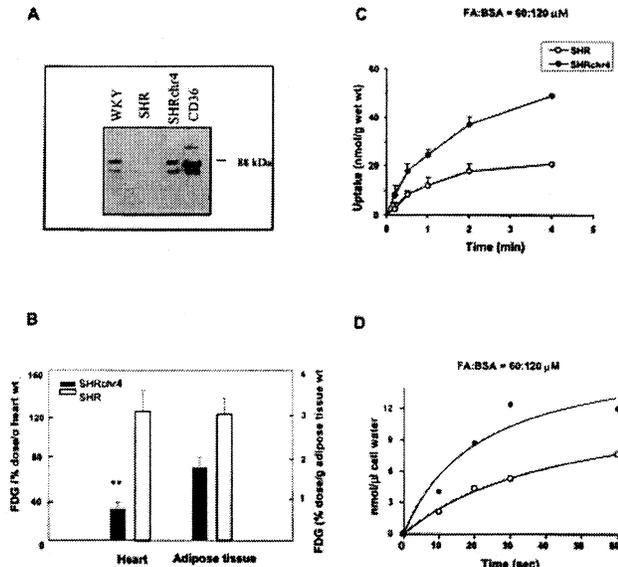
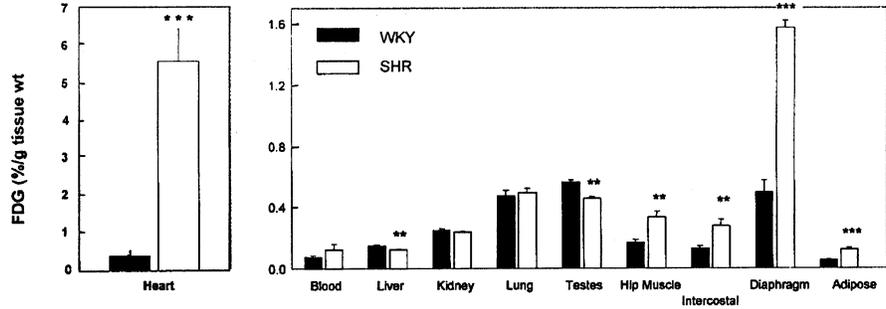


FIG. 4. CD36 expression, 2-FDG tissue uptake, and FA transport by isolated adipocytes and myocytes from SH and congenic SHchr4 rats. **A**, expression of CD36 protein in adipose tissue from SHR, WKY, and SHRchr4. Protein levels were detected using a polyclonal antibody against purified denatured rat adipose CD36. Purified recombinant CD36 protein from a baculovirus expression system was used as a positive control on Western blots. Data are typical of at least three experiments. **B**, $2\text{-}^{18}\text{F}$]FDG biodistribution in WKY and SHR rats. Rats were injected with $17\ \mu\text{Ci}$ of $2\text{-}^{18}\text{F}$]FDG. Heart and adipose tissues were removed 2 h after injection. Uptake is expressed as percent of injected dose per gram tissue. Data are typical of two experiments with $n = 4$ per group per experiment. Means are shown \pm S.E. ■, WKY ($n = 4$); □, SHRchr4 ($n = 4$). **C**, FA uptake in isolated cardiomyocytes from SHR and SHRchr4. Cardiomyocytes (25% cell density) were prepared as described in Luiken *et al.* (20) and assayed for transport of palmitate ($60\ \mu\text{M}$) complexed to bovine serum albumin at a ratio of 0.5. Data are typical of three experiments. **D**, FA uptake in isolated adipocytes from SHR and SHRchr4. Adipocytes were isolated and assayed for transport of palmitate ($60\ \mu\text{M}$, FA:bovine serum albumin (BSA) = 0.5) as previously described (19). Data are representative of three experiments ($n = 2$ rats per group per experiment).

SHR and was largely unaffected by the diet.

Table IV shows that blood glucose levels were slightly increased by the FA diet in both rat groups but were not significantly different between the groups on either diet. In contrast, insulin levels, which were about 2.4 times higher in SHR were normalized by the FA-supplemented diet while no effect was observed on blood insulin in the WKY. The FA diet normalized the insulin/glucose ratio in SHR, which dropped from 1.4 to 0.4. Short-chain FA supplementation significantly decreased blood-free FA and slightly increased blood TG in both groups.

To determine whether the lower insulin levels and insulin/glucose ratios in SHR fed the experimental diet reflected improved insulin responsiveness, glucose tolerance was examined

in the two groups comparing basal and FA-supplemented diets (Fig. 5). SHR and WKY fed the FA diet cleared glucose at similar rates, which mainly reflected a slower clearance in the WKY with little change in the SHR. The short-chain FA diet had a significant effect in sparing glucose utilization by SHR tissues. As shown in Fig. 6, $2\text{-}^{18}\text{F}$]FDG uptake by SHR hearts, which was greatly increased on the basal diet, was decreased to levels indistinguishable from those measured in WKY hearts.

DISCUSSION

The present study documents that the SHR exhibits loss of CD36 function in FA uptake in fat and oxidative muscle, tissues that normally express high CD36 levels (6). An associated defect in conversion of DG to TG is observed and may reflect an important role of CD36 in directing the FA to specific metabolic sites. Of note is the observation of a significant defect in BMIPP lipid incorporation in the kidney (Table I), which is a tissue with low CD36 levels, suggesting an important role of CD36 in the kidney cells where most TG accumulation occurs.

SHR tissues deficient in FA uptake exhibit a large increase in basal glucose uptake, which is eliminated by provision of short-chain FA that do not require CD36 for transport (Fig. 6 and Table IV). The short-chain FA-supplemented diet also eliminated the hyperinsulinemia of the SHR and lowered the fasting insulin/glucose ratio down to the level present in wild-type mice. This indicates that short-chain FA may be beneficial when hyperinsulinemia and insulin resistance reflect defects in FA uptake. In line with this, a diet supplemented with short-(octanoic) and medium-chain FA decreased insulin levels and improved carbohydrate intolerance in a patient with lipodystrophy from lipoproteinlipase deficiency (26). In addition to the hyperinsulinemia, the FA-supplemented diet also alleviated SHR heart hypertrophy, which links the symptom to defective FA metabolism since it is reversed by provision of energy from FA oxidation (Table III). In contrast to myocardial hypertrophy, hypertension was not improved indicating that it is independent of FA-generated energy. Although CD36 deficiency could be contributing to the hypertension, the link is complex and may reflect multiple influences as previously suggested (14). It may be worth exploring whether short-chain FA supplementation may reverse some of the deleterious effects of CD36 deficiency in humans, which may include hypertrophic cardiomyopathy (27).

The magnitude of the myocardial FA defect is underestimated in the SHR by BMIPP as compared with the CD36 null mouse (25% in SHR versus about 60–80% in CD36 null mice (10)). We believe this is most likely a result of rat versus mouse differences in cellular esterification of incoming FA, which is the process measured by BMIPP. TG levels are severalfold lower in the rat as compared with the mouse heart and they turnover less actively (28). As a result, FA incorporation into TG in the rat myocardium is affected much less than in the mouse by a drop in FA supply consequent to deficient membrane transport. FA oxidation in SHR heart (not measured by

TABLE III

Body and heart weights and mean systolic blood pressure of rats fed chow without (basal) or with short-chain FA supplementation

Rats were maintained on the diet beginning at week 5 and for a period of 3 months. Values of the same row sharing the same superscript are significantly different with $p < 0.01$. $n = 5$ for the basal diet and 6 for the +FA diet.

	Basal		+ Short-chain FA	
	SHR	WKY	SHR	WKY
BW (g)	265 ± 34	293 ± 34	277 ± 30	280 ± 34
Heart W (g)	2.04 ± 0.26 ^a	1.68 ± 0.22	1.65 ± 0.11 ^a	1.5 ± 0.02
H/B (%)	0.72 ± 0.02 ^b	0.57 ± 0.02	0.62 ± 0.03 ^b	0.55 ± 0.02
SBP	198 ± 7.5 ^c	128 ± 6 ^c	190 ± 4.1 ^d	116 ± 14.3 ^d

TABLE IV

Plasma parameters of rats fed chow without (basal) or with short-chain FA supplementation

Rats were kept on the diet for a period of 3 months. Blood samples were obtained from the tail vein or from the vena cava before the rats were killed. Measurements were obtained at 6 and 12 weeks with similar results and only the 12 weeks data are shown. Values of the same row sharing the same superscript are significantly different with $p < 0.05$. $n = 5$ for the basal diet and 6 for the +FA diet.

	Basal		+ Short-chain FA	
	SHR	WKY	SHR	WKY
BW (g)	265 ± 34	293 ± 34	277 ± 30	280 ± 34
Glucose (mg/dl)	63 ± 2 ^a	70 ± 4 ^b	98.3 ± 7 ^a	95 ± 7 ^b
Insulin (ng/dl)	87.5 ± 10.9 ^{a,b}	36.9 ± 6.6 ^a	42.0 ± 9.2 ^b	32.3 ± 7.4
Ins/Gluc	1.39 ± 0.1 ^{a,b}	0.53 ± 0.09 ^a	0.43 ± 0.09 ^b	0.34 ± 0.09
FFA (nmol/dl)	190 ± 16 ^a	165 ± 11 ^b	115 ± 11 ^a	124 ± 8 ^b
TG (mg/dl)	49 ± 7	36 ± 3	66 ± 2 ^b	54 ± 3 ^b
Cholesterol (mg/dl)	75 ± 6 ^a	123 ± 3 ^a	70 ± 9 ^b	118 ± 10 ^b

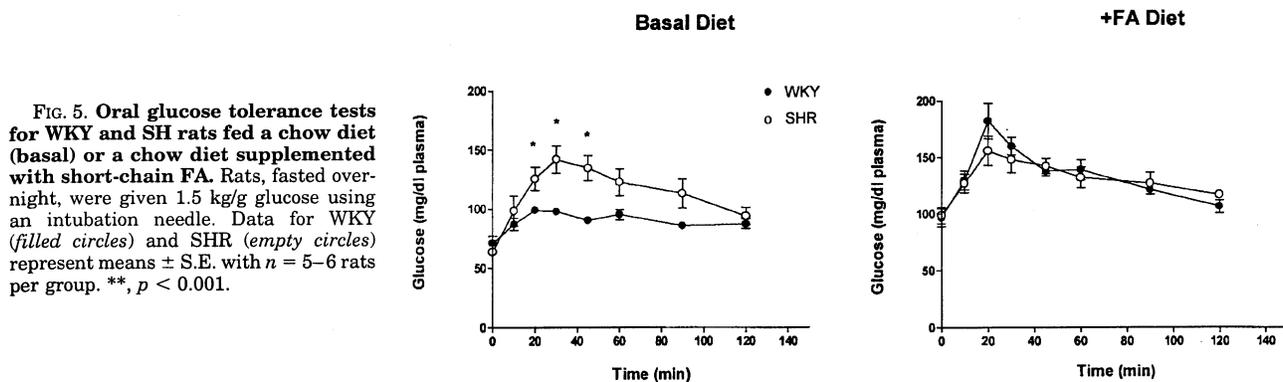


FIG. 5. Oral glucose tolerance tests for WKY and SH rats fed a chow diet (basal) or a chow diet supplemented with short-chain FA. Rats, fasted overnight, were given 1.5 kg/g glucose using an intubation needle. Data for WKY (filled circles) and SHR (empty circles) represent means ± S.E. with $n = 5-6$ rats per group. **, $p < 0.001$.

slowly oxidized BMIPP) is significantly impaired as can be documented from FA transport measurements (20–120 s) in myocytes and from the large increase in FDG uptake. A previous report (29) noted a 60–80% lower rate of palmitate oxidation by perfused hearts from SHR as compared with those from Harlan Sprague-Dawley rats.

Based on the above, a key characteristic of the SHR is that CD36 deficiency in this model impairs FA oxidation proportionately much more than FA esterification possibly via changes in concentration of regulatory intermediates such as citrate and malonyl-CoA (30, 31). This likely underlies the 3–5-fold accumulation of free BMIPP in SHR muscle (Table II). Alternatively, free BMIPP accumulation may reflect CD36-independent abnormalities in FA activation, intracellular transport, mobilization or oxidation, which could lead to dilution of BMIPP. Presence of an additional defect in FA metabolism would be consistent with the report that the SHRSP line, which has CD36, exhibits insulin resistance (32) but it is not supported by direct data.

The free FA accumulation observed in SHR muscle is likely to play a role in the insulin resistance or hyperinsulinemia that are observed in this model (13) possibly by producing alterations in insulin signaling (33). Another potential contributor is the high rate of basal glucose uptake, which would increase insulin requirements. Overexpression of glut 1, which leads to high basal glucose transport, is associated with insulin resist-

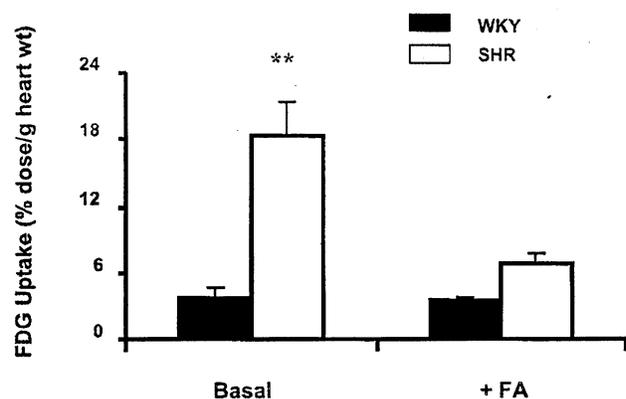


FIG. 6. Effect of a diet rich in short-chain FA on glucose utilization by hearts of WKY and SH rats. Rats, fasted overnight, were injected with 15 μ Ci of 2-[¹⁸F]FDG in a lateral tail vein. Tissues were harvested 2 h later. Counts are shown as percent of the dose injected divided by the weight of the tissue and represent means of five observations. **, $p < 0.01$.

ance (34). In line with this, provision of SHR tissues with short-chain FA and the consequent sparing of glucose utilization abolish the hyperinsulinemia. Although the drop in blood FA that was associated with short-chain supplementation may

have played a role by limiting insulin secretion (35), this is not supported by the fact that FA decreased in both WKY and SHR while the decrease in insulin levels was only observed in SHR. In addition, the insulin response to a glucose load in SHR versus WKY fed the FA diet was slightly higher at 30 min and identical at 60 min (data not shown). The lack of a change in the glucose tolerance curve and the lower insulin/glucose ratio present in SHR fed the FA-supplemented diet both support an improvement in insulin responsiveness.

In summary, CD36 is important for FA uptake by adipose and muscle tissues of the rat and its deficiency in the SHR impairs FA oxidation to a larger extent than it impairs FA esterification. The disproportionate inhibition of FA oxidation coupled with the high rate of glucose utilization result in hyperinsulinemia and insulin resistance in the SHR and both abnormalities can be reversed by providing energy from FA oxidation.

Acknowledgments—We thank the PET scanning facility at North Shore University Hospital in Manhasset and Ralph Mataracchieri in particular for preparation and donation of the fluorodeoxyglucose, Dr. J. Gao for help with the heart perfusion/myocyte isolation experiments, A. L. Beets and Dr. S. J. Kennel for help with the BMIPP studies, Dr. A. Birzgalis and L. C. Moore for blood pressure measurements, and the Abitec Corporation (Jamesville, Wisconsin) for the generous gift of Captex 300.

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