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Improved metabolic status and insulin sensitivity in obese fatty (*fa*/*fa*) Zucker rats and Zucker Diabetic Fatty (ZDF) rats treated with the thiazolidinedione, MCC-555

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1 We examined the effect of chronic (21 days) oral treatment with the thiazolidinedione, MCC-555 ((±)-5-[{6-(2-fluorbenzyl)-oxy-2-naphy}methyl]-2,4-thiazolidinedione) on metabolic status and insulin sensitivity in obese (*fa*/*fa*) Zucker rats and Zucker Diabetic Fatty (ZDF) rats which display an impaired glucose tolerance (IGT) or overt diabetic symptoms, respectively.

2 MCC-555 treatment to obese Zucker rats (10 and 30 mg kg^{-1}) and diabetic ZDF rats (10 mg kg^{-1}) reduced non-esterified fatty acid concentrations in both rat strains and reduced plasma glucose and triglyceride concentrations in the obese Zucker rats. Liver glycogen concentrations were significantly increased by chronic MCC-555 treatment in both obese Zucker rats (30 mg kg^{-1} day^{-1}) and diabetic ZDF rats (10 mg kg^{-1} day^{-1}), as compared with vehicle-treated lean and obese rats and there was a significant increase in hepatic glycogen synthase activity in MCC-555-treated diabetic ZDF rats as compared to vehicle-treated controls.

3 During a euglycaemic hyperinsulinaemic clamp, MCC-555-treated obese Zucker rats and diabetic ZDF rats required significantly higher glucose infusion rates to maintain stable glucose concentrations (2.01 ± 0.19 mg min^{-1} and 6.42 ± 1.03 mg min^{-1}, respectively) than vehicle-treated obese controls (0.71 ± 0.17 mg min^{-1} and 2.09 ± 0.71 mg min^{-1}; $P < 0.05$), demonstrating improved insulin sensitivity in both Zucker and ZDF rats. MCC-555 treatment also enhanced insulin-induced suppression of hepatic glucose production in ZDF rats as measured using infusions of [6-^{3}H]-glucose under clamp conditions.

4 In conclusion, we have demonstrated that MCC-555 improves metabolic status and insulin sensitivity in obese Zucker and diabetic ZDF rats. MCC-555 may prove a useful compound for alleviating the metabolic disturbances and IGT associated with insulin resistance in man.

Keywords: MCC-555; thiazolidinedione; metabolism; obese (*fa*/*fa*) Zucker rats; diabetes

Introduction

Non-insulin dependent diabetes mellitus (NIDDM) is an increasingly common and important cause of morbidity and mortality throughout the world. Hyperglycaemia in NIDDM is due to variable combinations of a relative decrease in insulin secretion and by insulin insensitivity in peripheral tissues, notably the liver and skeletal muscle (DeFronzo, 1988). NIDDM and the pre-diabetic state of impaired glucose tolerance (IGT) have been estimated to affect at least 3–5% of the population world-wide, and 10–20% of subjects over 60 years of age (Harris, 1996). Current treatment of NIDDM patients hinges on attempts to correct insulin deficiency with insulin secretagogues (especially sulphonylureas) or insulin itself. The biguanides, notably metformin, reduce hyperglycaemia and improve some aspects of the insulin insensitivity associated with NIDDM (Williams, 1994).

The thiazolidinediones are a novel class of compounds that enhance insulin action and are currently under evaluation as antidiabetic drugs (Suter *et al*., 1992; Nolan *et al*., 1994; Kumar *et al*., 1996). Their action is thought to be primarily on adipose tissue where they act as agonists at the peroxisome proliferator activator receptor-gamma subtype (PPAR-gamma) nuclear receptor (Lehmann *et al*., 1996; Wilson *et al*., 1996). This alters the transcription of multiple genes leading to reduced lipolysis, increased triglyceride accumulation and decreased plasma non-esterified free fatty acid concentrations (Forman *et al*., 1995). The reduction in plasma non-esterified fatty acid concentrations leads to increased glucose utilization in muscle (Randle *et al*., 1964), reduced hepatic gluconeogenesis and improved suppression of hepatic glucose output by insulin (Bowen *et al*., 1991; Oakes *et al*., 1994).

Several thiazolidinediones are undergoing clinical trials and have been shown to be beneficial in both NIDDM (decreasing blood glucose and plasma insulin concentrations) and in impaired glucose tolerance (Suter *et al*., 1992; Nolan *et al*., 1994) suggesting that thiazolidinediones, such as troglitazone, may be useful in preventing NIDDM.

We have examined the effects of the thiazolidinedione, MCC-555 ((±)-5-[{6-(2-fluorbenzyl)-oxy-2-naphy}methyl]-2,4-thiazolidinedione) on plasma insulin, glucose and non-esterified fatty acid concentrations and insulin sensitivity of obese (*fa*/*fa*) Zucker rats and Zucker Diabetic Fatty (ZDF) rats. MCC-555 has been demonstrated to reduce the hyperglycaemia and hyperinsulinaemia in the diabetic KKA^y mouse model to levels measured in non-diabetic mice, with a greater potency that either pioglitazone or troglitazone (Kadowaki, personal communication) and lacks haematological and cardiac side effects (Ishii *et al*., 1996). Both obese Zucker rats and diabetic ZDF rats develop morbid obesity through the *fa* mutation in the extracellular domain of the leptin receptor, which interferes with leptin's centrally-mediated appetite-suppressing and thermogenic actions, tending to severe insulin resistance (Clark & Palmer, 1982;

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Peterson, 1994; Sturgis *et al*., 1995; Sreenan *et al*., 1996). Unlike the ZDF rats, which are fully diabetic, obese Zucker rats represent an important animal model for the study of impaired glucose tolerance (IGT) since they display a mild hyperglycaemia, pronounced hyperinsulinaemia and a marked reduction in insulin sensitivity (Terrettaz & Jeanrenaud, 1983). ZDF rats, unlike obese Zucker rats, have a β-cell defect which progresses to a state of insulin deficiency analogous to human NIDDM, characterized by marked hyperglycaemia and overt diabetic symptoms of polyuria and polydipsia and ultimately to weight loss (Peterson, 1994).

The aims of the study were to determine whether MCC-555 can improve insulin sensitivity in both obese Zucker rats and diabetic ZDF rats and also attenuate the key metabolic disorders associated with the diabetic state in ZDF rats.

Methods

Animal procedures

Male lean (204–236 g) and obese (341–403 g) Zucker rats were obtained from Harlan Olac, Bicester, U.K. Ten week old male lean (276–331 g; Gmi-*fal*$^+$) and diabetic (319–372 g; *fa*/*fa*) ZDF rats were purchased from Genetic Models Inc. (Indianapolis, IN, U.S.A). Animals were housed individually in wire-bottomed cages for a period of 1 week before treatment and maintained on a 12 h dark/light cycle (lights on 07.00) with free access to standard lab chow (CRM, Biosure, Cambridge, U.K.) and tap water. Groups of eight obese Zucker rats and eight diabetic ZDF rats were dosed with vehicle (0.5% sodium carboxymethylcellulose in water 5 ml/kg^{-1}/day^{-1}) or MCC-555 (Mitsubishi Chemical Company, Yokohama, Japan) suspension in vehicle (10 or 30 mg kg^{-1}/day^{-1}; 5 ml kg^{-1}) by gavage for 21 days. Groups of eight lean Zucker or eight non-diabetic ZDF rats received vehicle alone for 21 days. Body weight, food and water consumption were recorded daily. Twenty-four hours after the last dose of vehicle or MCC-555, fed rats were killed using CO_2 inhalation and blood removed *via* cardiac puncture into cold heparinized tubes. After a brief centrifugation at 4°C, plasma was frozen in aliquots for the later determination of glucose, non-esterified fatty acid, triglyceride and insulin concentrations. Approximately 1 g of liver was dissected, snap-frozen in liquid nitrogen and stored at −40°C for later measurements of hepatic glycogen concentrations and glycogen synthase activity. The carcasses of a group of vehicle-treated lean and vehicle and MCC-555-treated obese Zucker rats were homogenized using a food processor and frozen for later estimations of the body lipid content.

All animal care, monitoring and procedures were carried out in accordance with strict guidelines issued by the U.K. Home Office.

Euglycaemic hyperinsulinaemic clamp studies

Twenty four hours after the last dose of vehicle or MCC-555, rats were anaesthetized with pentobarbitone (30 mg kg^{-1} body weight) and the right jugular vein was catheterized. A priming intraperitoneal injection of [6-^{3}H]glucose (4 μCi of 32.0 Ci mmol^{-1}, Amersham International, Little Chalfont, Bucks, U.K.) was given before the infusion of [6-^{3}H]glucose at 0.2 μCi min^{-1} for 45 min. A stable blood glucose specific radioactivity was reached after 30 min and used to calculate basal glucose turnover. Hyperinsulinaemia was achieved by infusing a mixture of soluble human insulin (Humulin-S, Eli Lilly and Company Ltd, Basingstoke, Hants, U.K.; 14 mu · min^{-1} in obese Zucker and 2 mu min^{-1} in lean Zucker, diabetic ZDF and non-diabetic ZDF rats) dissolved in isotonic saline with 1% bovine serum albumin (Sigma Chemical Co., Poole, Dorset, U.K.) and [6-^{3}H] glucose (0.2 μCi min^{-1}) at 20 μl min^{-1} for 1 h. After 1 min, 5% glucose in isotonic saline was co-infused at an initial rate of 1 mg min^{-1} through the jugular vein. Blood glucose concentrations were measured every 5 min from the tail vein using an Exactech electrochemical meter (Medisense, Abingdon, Oxon, U.K.) and the glucose infusion rate adjusted to maintain stable blood glucose levels of 4.2 mmol l^{-1} in lean Zucker and non-diabetic ZDF rats, 5.2 mmol l^{-1} in obese Zucker rats and 20 mmol l^{-1} in diabetic ZDF rats. Glucose appearance and uptake were calculated from blood specific activity as described by Terrettaz & Jeanrenaud (1983). Blood samples were collected at 0, 30 and 90 min of clamping into cold heparinized tubes, immediately centrifuged and kept on ice. Fifty microlitres of plasma were deproteinized with 25 μl 83 mM $ZnSO_4$ and 25 μl 87 mM $Ba(OH)_2$, centrifuged (14,000 r.p.m.) for 1 min and 50 μl of the supernatant was evaporated to remove ^3H_2O in order to determine blood glucose specific activity. Radioactivity was measured by scintillation spectrometry using 3 ml scintillation cocktail (Cocktail T. BDH, Poole, Dorset, U.K.) and an LKB counter (counting efficiency = 45%). Total unlabelled glucose appearance was calculated from plasma glucose concentrations and glucose specific activity in blood, taking into account the difference in glucose space in lean and obese rats (Katz *et al*., 1974; glucose space = 25% of body weight).

Biochemical measurements

Plasma insulin concentrations were measured using a commercially available radioimmunoassay kit (Pharmacia/Upjohn Diagnostics U.K., Lewes, East Sussex). Plasma glucose, non-esterified fatty acid and triglyceride concentrations were measured using standard kits from Boehringer Mannheim (Milton Keynes, Bucks., U.K.) and Sigma Chemical Company, (Poole, Dorset, U.K.).

Whole-body fat mass was measured using a modified method of Folch *et al*. (1956). Briefly, the frozen homogenized carcass was weighed, freeze-dried and homogenized in a food processor. A small amount of the homogenized tissue (1.5 g) was added to a mixture of chloroform/methanol (2 : 1) and homogenized further using a polytron to dissolve the fat. The extract was filtered through fat-free Whatman filter paper and mixed with 0.05% (w/v) $CaCl_2$ (0.2 vols). When the mixture had separated into two phases, the upper phase was removed and the interphase washed three times with chloroform/methanol/$CaCl_2$ (2 : 1 : 0.02) taking care not to disturb the lower layer. The lower organic phase containing the dissolved lipids was placed in preweighed glass scintillation vials and the solvent evaporated off. The vials containing the remaining lipids were reweighed. The percentage body fat mass was calculated from the dry carcass mass.

Liver glycogen content was measured using the method of (Lowry, 1972) and hepatic glycogen synthase activity was measured using [^{14}C]-UDP-glucose (292 mCi mmol^{-1}, Amersham International plc, Little Chalfont, Bucks, U.K.) according to the method of Thomas *et al*. (1968). Liver glycogen concentrations are expressed as μmol glucose incorporated into glycogen per g tissue wet weight, the glycogen synthase activity as μmol min^{-1} g wet weight^{-1}.

Statistical analyses

Differences between MCC-555-treated rats and vehicle-treated rats were analysed using one- or two-way analysis of variance (ANOVA) followed by Bonferroni modified *t*-tests for multiple comparisons.

Results

Zucker rats

As expected, vehicle-treated obese rats ate more than lean rats (Figure 1). Daily food consumption of MCC-555-treated obese Zucker rats did not differ significantly from vehicle-treated obese rats at either dose throughout. However, the cumulative food consumption of obese rats treated with 30 mg kg^{-1}, but not with 10 mg kg^{-1} (not shown), MCC-555 was significantly greater than vehicle-treated obese rats during the last 10 days of the study. Both groups of obese rats were demonstrated significantly hyperphagic, as compared to vehicle-treated lean rats. There was no change in the daily water consumption of MCC-555-treated obese Zucker rats as compared to controls (Table 1). Daily administration with 30 mg kg^{-1} MCC-555 did not significantly alter the final body weights of obese Zucker rats, as compared to vehicle-treated obese controls, but both groups of obese rats remained significantly heavier than vehicle-treated lean animals (Table 1) and there was no effect on body weight following daily administration of 10 mg kg^{-1} MCC-555 (not shown). The percentage body fat was 4 fold larger in untreated obese Zucker rats than in lean rats, but was not significantly modified by MCC-555 treatment (Table 1).

After 21 days of MCC-555 treatment, plasma triglyceride and non-esterified fatty acid concentrations were significantly reduced in obese Zucker rats receiving 30 mg kg^{-1} MCC-555, as compared with vehicle-treated rats, but plasma triglyceride concentrations were not reduced to levels observed in lean rats (Table 1). The modest hyperglycaemia of obese Zucker rats was partially normalized by 30 mg kg^{-1} MCC-555 (Table 1) and 10 mg kg^{-1} MCC-555 treatment but their hyperinsulinaemia was unchanged (Table 1). Liver glycogen concentrations, but not glycogen synthase activity, were significantly increased in MCC-555-treated rats (30 mg kg^{-1}), as compared with both vehicle treated obese and lean rats (Table 1).

Infusion of insulin to both lean and obese Zucker rats during euglycaemic hyperinsulinaemic clamping conditions elevated plasma insulin concentrations in all groups (Table 3). During the last minute of the clamp, significantly higher glucose infusion rates were required to maintain euglycaemia in vehicle treated lean and MCC-555-treated obese rats, as compared with vehicle-treated obese rats (Table 3). Basal hepatic glucose production was similar in all three groups of Zucker rats (Figure 2), but hyperinsulinaemia during clamping completely inhibited hepatic glucose production in vehicle-treated lean rats with no significant change in vehicle-treated obese rats. However, chronic MCC-555 treatment failed to significantly reduce hepatic glucose production in obese

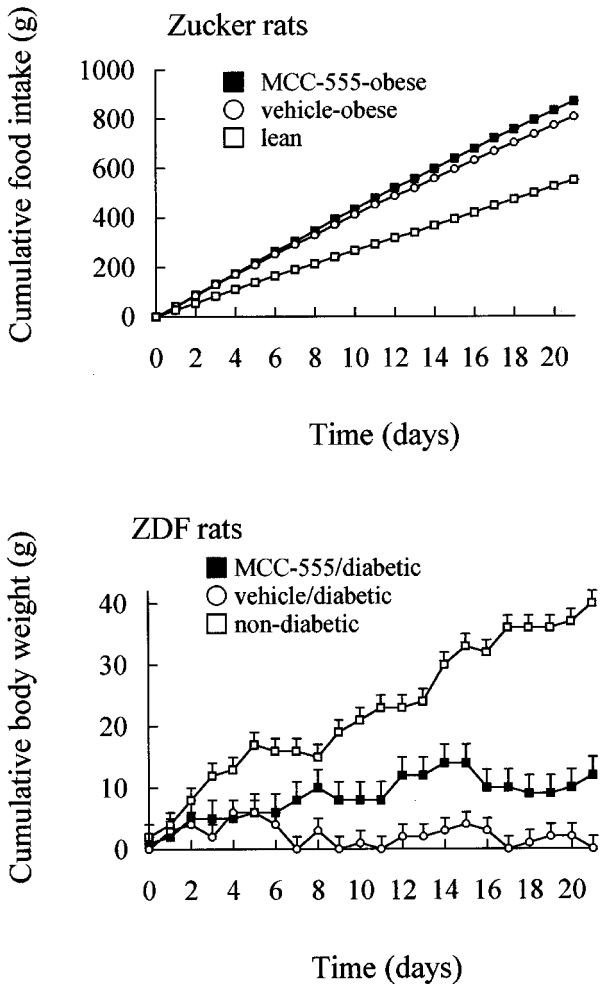

Figure 1 Cumulative food consumption in MCC-555- and vehicle-treated obese Zucker rats and in lean Zucker rats. Also cumulative body weight in MCC-555- and vehicle-treated diabetic ZDF rats and vehicle-treated non-diabetic rats. Data shown as mean ± s.e.mean for groups of eight rats/group.

Table 1 Effects of chronic MCC-555 treatment (30 mg kg^{-1} day^{-1}) on body weight, food intake, water consumption in obese Zucker rats, compared with vehicle-treated obese and lean controls

	MCC-555-treated obese rats (n = 8)	*Vehicle-treated obese rats (n = 8)*	*Vehicle-treated lean rats (n = 8)*
Initial body weight (g)	393 ± 9**	389 ± 10**	260 ± 10
Final body weight	493 ± 12**	467 ± 9**	306 ± 10
Water consumption at day 20 of dosing (ml)	22.8 ± 1.5	21.5 ± 0.7	19.5 ± 2.7
Percentage body fat	19.7 ± 1.2**	22.1 ± 1.2**	5.2 ± 0.4
Plasma triglycerides (mmol l^{-1})	3.9 ± 0.2**†	5.2 ± 0.3**	1.1 ± 0.1
Plasma fatty acids (mmol l^{-1})	0.11 ± 0.02*†	0.32 ± 0.05	0.30 ± 0.04
Plasma glucose (mmol l^{-1}	9.5 ± 1.0†	13.3 ± 1.1**	7.5 ± 0.5
Plasma insulin (μu ml^{-1})	145 ± 6**	136 ± 8**	18 ± 2
Glycogen synthase D activity (μmol min^{-1} g $tissue^{-1}$)	3.1 ± 0.1*	3.2 ± 0.2	2.4 ± 0.2
Liver glycogen (μmol glucose g $tissue^{-1}$)	322 ± 28*†	238 ± 26	222 ± 13

Data shown as mean ± s.e.m. *$P < 0.05$, **$P < 0.01$ versus vehicle-treated lean rats, †$P < 0.05$ versus vehicle-treated obese rats.

Zucker rats under hyperinsulinaemic conditions. At baseline there was no significant difference in the whole-body glucose uptake between MCC-555-treated obese rats and vehicle-treated lean and obese rats (Figure 2). During clamping conditions, however, whole-body glucose uptake increased by 100% in vehicle-treated lean rats and by 58% in MCC-555-treated obese rats. In contrast, whole-body glucose uptake was increased by only 11% in vehicle-treated obese rats (Figure 2).

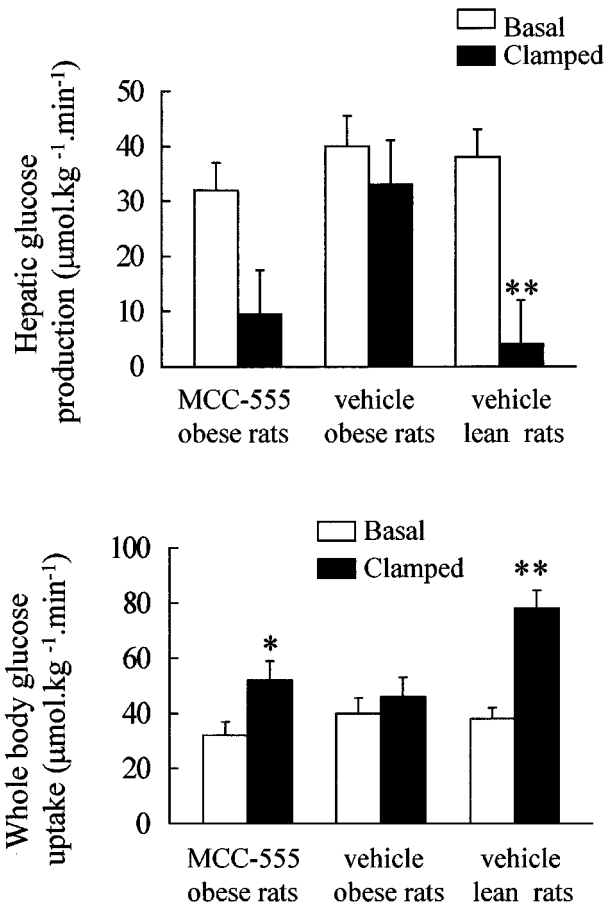

Figure 2 Hepatic glucose production and whole body glucose uptake in MCC-555-treated and vehicle-treated lean and obese Zucker rats under basal and hyperinsulinaemic conditions. Data shown as mean±s.e.mean for groups of eight rats. $*P<0.05$, $**P<0.01$ as compared to basal hepatic glucose output or whole body glucose uptake.

Diabetic ZDF rats

There was no significant increase in body weight gain of vehicle-treated diabetic ZDF rats during the experimental period whereas vehicle-treated lean ZDF rats demonstrated a significant increase in body weight (Figure 1, Table 2). MCC-555-treated diabetic rats demonstrated a small increase in body weight over the experimental period that was significantly different from vehicle-treated controls by day 21 (Figure 1, Table 2). Daily and the cumulative food consumption of vehicle- and MCC-555-treated diabetic ZDF rats were not significantly different from one another, but both groups ate significantly more than non-diabetic, lean controls (Table 2). The marked polydipsia of diabetic rats was not significantly attenuated by MCC-555 treatment nor were there any significant alterations in plasma glucose or triglyceride concentrations (Table 2). However, plasma insulin concentrations were significantly reduced in MCC-555-treated diabetic ZDF rats prior to clamping (Table 3) and there was also a trend for lower insulin concentrations in another group of MCC-555-treated diabetic ZDF rats, as compared to vehicle-treated diabetic ZDF rats (Table 2).

Plasma non-esterified fatty acid concentrations were, however, significantly reduced in MCC-555-treated versus vehicle-treated diabetic ZDF rats (Table 2). There was a significant increase in the gonadal and perirenal fat pad mass, liver glycogen content and hepatic glycogen synthase activity of MCC-555-treated diabetic rats as compared to vehicle-treated diabetic and lean controls (Table 2).

Infusion of insulin to both diabetic and lean ZDF rats during clamping conditions elevated plasma insulin concentrations in all groups (Table 3). Steady-state glucose infusion rates required to maintain euglycaemia were significantly different between MCC-555- and vehicle-treated diabetic rats (Table 3). However, glucose infusion rates for MCC-555-treated diabetic rats remained less than those required for non-diabetic vehicle-treated controls (Table 3).

Basal hepatic glucose production was significantly greater in diabetic ZDF rats, with or without MCC-555 treatment, when compared with lean rats (Figure 3). During clamp conditions there was a significant inhibition in hepatic glucose production in both vehicle-treated lean rats and diabetic rats treated with MCC-555 (Figure 3). However, vehicle-treated diabetic rats did not exhibit significant suppression of glucose production during clamping. Whole-body glucose uptake was significantly increased in diabetic ZDF rats treated with MCC-555 as compared with vehicle-treated diabetic controls, but did not

Table 2 Effects of chronic MCC-555 treatment on food and water consumption and plasma chemistry of diabetic ZDF rats as compared to vehicle-treated diabetic and lean (non-diabetic) controls

	MCC-555-treated obese diabetic rats (n=10)	*Vehicle-treated diabetic rats (n=8)*	*Vehicle-treated lean rats (n=9)*
Cumulative body weight gain (g) over 21 days	9±3**†	0±4**	40±4
Food consumption (g day^{-1}) at day 20 of treatment	46.2±3.5**	42.6±3.3**	23.4±1.5
Water consumption (ml day^{-1}) at day 20 of treatment	149±17**	160±16**	29±4
Gonadal fat mass (g)	7.0±0.3**†	5.9±0.2**	2.1±0.1
Perirenal fat mass (g)	8.5±0.5**†	6.3±0.4**	1.9±0.1
Plasma triglycerides (mmol l^{-1})	4.4±0.4**	5.0±0.6**	1.4±0.1
Plasma fatty acids (mmol l^{-1})	0.18±0.003*†	0.26±0.04*	0.07±0.01
Plasma glucose (mmol l^{-1})	38.9±3.0**	43.6±2.3**	14.1±0.7
Plasma insulin (μu ml^{-1})	29.5±3.4	37.5±6.8**	26.0±1.8
Glycogen synthase activity (μmol min^{-1} g $tissue^{-1}$)	1.1±0.1*†	0.8±0.1	0.8±0.2
Liver glycogen (μmol glucose g $tissue^{-1}$)	300±30*†	230±20	220±10

Data shown as mean±s.e.mean for groups of eight to ten rats. $*P<0.05$, $**P<0.01$ as compared to vehicle-treated lean rats, $\dagger P<0.05$ as compared to vehicle-treated diabetic rats.

Table 3 Insulin concentrations and steady-state glucose infusion rates before and during euglycaemic hyperinsulinaemic clamping in Zucker rats and ZDF rats following vehicle or MCC-555 treatment

	Zucker rats		
	MCC-555-treated obese rats (n=8)	*Vehicle-treated obese rats* (n=8)	*Vehicle-treated lean rats* (n=8)
Initial insulin concentrations (μu ml^{-1})	73.3±6.2**	73.7±58.9**	16.5±2.2
Insulin concentrations during clamping (μu ml^{-1})	233.3±42.4	208.6±21.0	141.9±35.2
Final glucose infusion rates (mg min^{-1})	2.01±0.19†	0.71±0.17**	2.56±0.15
	ZDF rats		
	MCC-555-treated obese rats (n=8)	*Vehicle-treated obese rats* (n=7)	*Vehicle-treated lean rats* (n=10)
Initial insulin concentrations (μu ml^{-1})	10.0±1.7†	35.9±7.4	31.9±5.9
Insulin concentrations during clamping (μu ml^{-1})	733.6±61.3	639.4±71.1	795.0±45.7
Final glucose infusion rates (mg min^{-1})	6.42±1.03**†	2.09±0.71**	9.25±0.28

Data shown as mean±s.e.mean for groups of eight rats. **$P<0.01$ versus lean rats, †$P<0.05$ versus vehicle-treated obese rats.

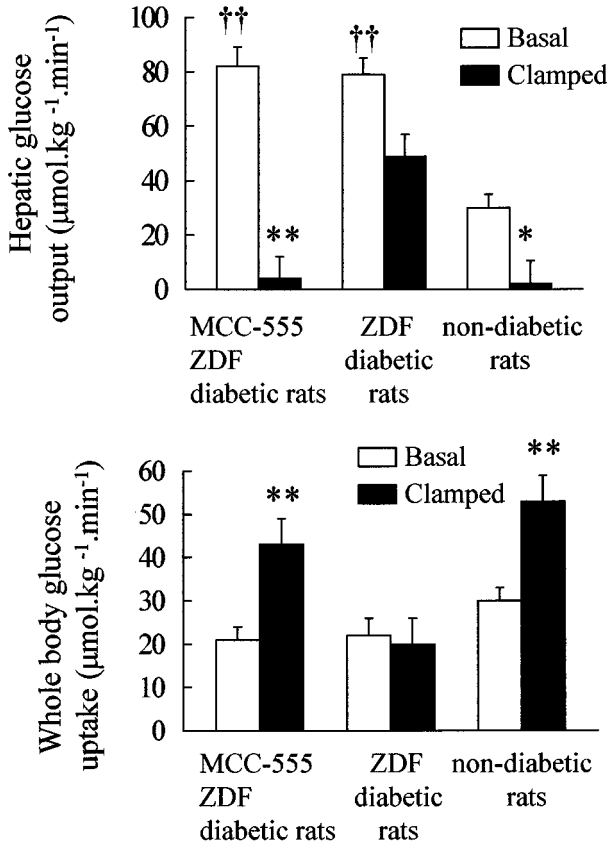

Figure 3 Hepatic glucose production and the whole body glucose uptake of vehicle-treated non-diabetic and diabetic ZDF rats and diabetic ZDF rats treated with MCC-555 under basal and euglycaemic hyperinsulinaemic conditions. Data shown as mean± s.e.mean for groups of seven to ten rats. *$P<0.05$, **$P<0.01$ as compared to basal hepatic glucose output or whole body glucose. ††$P<0.01$ versus non-diabetic ZDF rats.

quite reach levels measured in vehicle-treated lean non-diabetic controls (Figure 3).

Discussion

Reduced sensitivity to various biological effects of insulin is a fundamental abnormality of noninsulin dependent diabetes mellitus (NIDDM) in animals and humans (Terrettaz & Jeanrenaud, 1983) and drugs that significantly improve insulin sensitivity are likely to be potentially useful in the treatment of the disease. Here, we examined the effects of chronic MCC-555 treatment on the metabolic status of obese Zucker rats, which display morbid obesity associated with insulin insensitivity and impaired glucose tolerance and on ZDF rats which are overtly diabetic.

Chronic treatment of obese Zucker and diabetic ZDF rats with MCC-555 significantly increased insulin sensitivity, as determined by the increase in glucose infusion rates to maintain euglycaemia under hyperinsulinaemic conditions. The significant reduction in plasma non-esterified fatty acid concentrations following MCC-555-treatment to both obese Zucker and diabetic ZDF rats is consistent with studies of other thiazolidinediones, such as troglitazone and BRL 49653 (rosiglitazone) (Oakes *et al*., 1994; Sreenan *et al*., 1996) and suggests that MCC-555 acts as a potent PPAR-gamma agonist thereby increasing gene expression coding for enzymes that regulate lipolysis in adipose tissue (Lehmann *et al*., 1995). The reduction in non-esterified fatty acid concentrations have been suggested to play a key role in improving insulin sensitivity in skeletal muscle and adipose tissue *via* alterations in metabolism acting through the glucose-fatty acid cycle (Randle *et al*., 1964; Oakes *et al*., 1994). However, we cannot exclude the possibility that MCC-555 may improve insulin sensitivity through the blockade of protein kinase C translocation from the cytoplasmic to particulate fractions, which has been demonstrated with other thiazolidinediones such as BRL 49653 (Schmitz-Peiffer *et al*., 1997) and troglitazone (Bähr *et al*., 1996) and has been suggested to participate in improved insulin-sensitive glucose transport (Schmitz-Peiffer *et al*., 1997).

The consequence of MCC-555-induced reductions in plasma non-esterified fatty acid concentrations, and the improvement in insulin sensitivity was to reduce hepatic glucose production and increase glucose uptake into peripheral tissues. An increase in glucose entry into insulin-sensitive tissues was also observed by the significant increase in hepatic glycogen concentrations in both Zucker and ZDF rats under hyperinsulinaemic conditions and increased fat pad mass in diabetic ZDF rats. The finding that glycogen concentrations in MCC-555-treated obese Zucker and diabetic ZDF rats were significantly greater than those of lean Zucker or non-diabetic rats suggests that the thiazolidinedione significantly increased the sensitivity of hepatic insulin-responsive elements above those of lean or non-diabetic animals resulting in enhanced glucose entry into the liver. Furthermore, the attenuation in hepatic glucose production following infusion of the insulin in

MCC-555-treated rats, as compared to vehicle-treated non-diabetic controls, is further evidence for increased insulin sensitivity in diabetic ZDF rats.

The improvements to insulin sensitivity resulted in an improved metabolic status in both obese Zucker and diabetic ZDF rats demonstrated by the modest increase in body weight gain in MCC-555-treated ZDF diabetic rats, without affecting food consumption. Despite the marked hyperphagia in diabetic ZDF rats, they do not continue to gain weight after 15–20 weeks of age due to heavy losses of energy as glycosuria and catabolism resulting in lean ZDF rats exceeding them in bodyweight. Hyperglycaemia is typical of mature ZDF rats as a result of plasma insulin reductions from high values observed in young ZDF rats to those observed in young and older lean rats (Peterson 1994). This indicates that insulin deficiency has become the critical factor in causing metabolic decompensation, and that the syndrome has progressed beyond the phase where insulin insensitivity is the limiting factor and where improvements in insulin action would be most beneficial. The body weight gain in diabetic ZDF rats following MCC-555 treatment is probably the result of increased glucose entry into adipose tissue and the conversion to triglycerides leading to increased fat mass. Increased body weight gain has been observed following 10 days of pioglitazone treatment to diabetic ZDF rats, but as there was no increase in fat deposition, the increased weight was attributed to water retention (deSouza *et al*., 1995). The physiological mechanism resulting in the slight increase in food intake during MCC-555 treatment to obese Zucker rats is currently unknown, but has been also observed with other thiazolidinediones, such as BRL 49653 (rosiglitazone) (Eldershaw *et al*., 1995; Wang *et al*., 1997), suggesting that this phenomenon is a general property of these compounds.

We could not find evidence for changes in plasma glucose concentrations in MCC-555-treated diabetic ZDF rats although there was a small, but significant reduction in plasma glucose concentration in obese Zucker rats. Some thiazolidinediones have previously been reported to reduce plasma glucose and insulin concentrations in the diabetic ZDF rat and obese Zucker rat (Sturgis *et al*., 1995; deSouza *et al*., 1995; Sreenan *et al*., 1996) but the effect may depend on age and severity of hyperglycaemia and the dose of thiazolidinedione used in the study, possible insulin secretagogue actions or effects on insulin clearance. Recently Smith *et al*. (1997) have also failed to observe a change in plasma insulin or glucose following treatment to diabetic ZDF rats with the potent thiazolidinedione, BRL 49653 (rosiglitazone), in older rats where the diabetic syndrome has been fully established. As we used older ZDF diabetic rats, which were 18 weeks of age at the end of the study by which the β-cell deficiency, hyperglycaemia and relative insulin deficiency had been fully established, it is probably difficult to improve β-cell function at this late stage in the development of the model. Sturgis and co-workers (1995) demonstrated that pioglitazone administered to 7-week old pre-diabetic ZDF rats prevented the hyperglycaemia that usually develops at 8–10 weeks of age. However, pioglitazone administration at 11 weeks of age had no effect on plasma glucose or insulin concentrations in either obese Zucker or diabetic ZDF rats (Sturgis *et al*., 1995; Hirshman *et al*., 1995) supporting the contention that age and severity of diabetic symptoms determine the effects of thiazolidinediones on plasma insulin and glucose concentrations. Possibly other factors, such as thiazolidinedione-induced changes in insulin clearance and/or insulin secretagogue actions (Masuda *et al*., 1995) may account for the lack of change in plasma insulin concentrations in MCC-555-treated obese Zucker rats despite increased insulin sensitivity.

In conclusion, we have demonstrated that chronic treatment of obese Zucker and diabetic ZDF rats with the thiazolidinedione, MCC-555, significantly improves insulin sensitivity and metabolic status of these rats. The overall effects of MCC-555 in overtly diabetic ZDF rats was to partially reverse the catabolic metabolic consequences of the diabetic state. In view of this improved responsiveness to insulin, MCC-555 may prove a promising candidate for the treatment of NIDDM.

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