Malonyl Coenzyme A and Adiposity in the Dahl Salt-Sensitive Rat: Effects of Pioglitazone

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These studies were designed to assess the effects of pioglitazone, a new oral antidiabetic agent that acts by improving insulin sensitivity, on blood pressure, plasma and tissue lipids, and insulin resistance in the Dahl salt-sensitive (Dahl-S) rat. Reaven et al had reported that male Dahl-S rats are moderately hyperinsulinemic and insulin-resistant. This was of particular interest since these rats are not obese but are hypertriglyceridemic, and on a high-salt diet they become hypertensive. In the current study, male Sprague-Dawley control and Dahl-S rats were compared when fed standard chow or high-fat, high-sucrose (HFHS) diets with or without pioglitazone (20 mg/kg body weight/d) for 3 weeks. On the standard chow diet, Dahl-S rats were hypertriglyceridemic and had high tissue levels of malonyl coenzyme A ([CoA] Dahl-S 5.0 *v* control 3.3 nmol/g in muscle, and Dahl-S 15.6 *v* control 10.7 nmol/g in liver); however, they were not hyperinsulinemic. Pioglitazone therapy decreased both malonyl CoA and plasma triglycerides toward control values, but had no effect on plasma insulin levels. On the HFHS diet, both groups became glucose-intolerant and hyperinsulinemic; however, the hyperinsulinemia was greater and more sustained in Dahl-S rats. In addition, the HFHS diet appeared to increase the mass of retroperitoneal fat in the Dahl-S but not in the control group. Treatment with pioglitazone decreased retroperitoneal fat, but as reported previously, it increased the mass of the epididymal fat pad. The results suggest that the hypertriglyceridemia of the Dahl-S rat is associated with an increase in the concentration of malonyl CoA in both liver and muscle. They also show that pioglitazone reverses both of these abnormalities independently of its effect on plasma insulin. Whether these high levels of malonyl CoA predispose the Dahl-S rat to hyperinsulinemia and possibly obesity when placed on a HFHS diet remains to be determined.

THE DAHL salt-sensitive (Dahl-S) rat has long been used as a model to study salt-induced hypertension.[1,2] Recent studies suggest that although it is not obese, it is hypertriglyceridemic and moderately hyperinsulinemic and insulin-resistant.[3] Thus, the Dahl-S rat is normal-weight, but has many of the characteristics of the insulin-resistance syndrome found in some humans with premature coronary heart disease,[4-6] essential hypertension,[4-6] and non–insulin-dependent diabetes.[6,7] Such insulin resistance in normal-weight individuals has also been found in people at risk for these disorders.[8,9]

Pioglitazone is a member of a new class of thiazolidinedione oral antidiabetic agents that act by increasing insulin sensitivity.[10] Our initial objective was to determine whether pioglitazone increases insulin sensitivity in the Dahl-S rat and, if so, whether it concurrently decreases plasma triglyceride levels and blood pressure. In addition, we wanted to assess whether an increase in insulin sensitivity is mediated through an effect on malonyl coenzyme A (CoA) and diacylglycerol (DAG) concentrations in liver and muscle. In earlier studies, we observed that pioglitazone dramatically alters the levels of these metabolites when it enhances insulin sensitivity and decreases plasma glucose and triglyceride concentrations in obese hyperinsulinemic KKA^y mice.[11] Toward these ends, male Sprague-Dawley control and Dahl-S rats were compared when fed Purina chow diets with or without pioglitazone (20 mg/kg body weight/d) for 3 weeks. In addition, a second study was performed in which rats were fed a diet high in fat and sucrose (HFHS) to create hyperinsulinemia and insulin resistance.

MATERIALS AND METHODS

Experimental Animals

Male Dahl-S (Rapp) and control Sprague-Dawley rats weighing 150 to 175 g were obtained from Harlan-Sprague-Dawley (Indianapolis, IN). Rats were randomly placed for 3 weeks on either standard rat chow or chow with pioglitazone added to obtain a drug dose of approximately 20 mg/kg body weight/d. A second group of Dahl-S and Sprague-Dawley rats weighing 60 to 85 g were placed on one of three diets for 3 weeks: (1) standard rat chow, (2) 35% fat + 22% sucrose (HFHS), and (3) HFHS + pioglitazone. The diets differed in that 35% of the total energy of the HFHS diet was derived from fat and 45% from carbohydrate, in contrast to the chow diet, which derived 15% of energy from fat and 65% from carbohydrate. Protein accounted for 20% of the total energy in both diets. Diets were obtained from Harlan Teklad (Madison, WI) and contained vitamin and mineral supplements. Pioglitazone was kindly supplied by The Upjohn Company (Kalamazoo, MI). All animals were fed ad libitum unless otherwise indicated, and were allowed free access to water. They were housed in individual cages in a temperature-controlled room (19° to 21°C) with a 12-hour light/dark cycle.

All deaths were induced between 12 noon and 3 PM. Rats fasted for 6 hours were anesthetized with sodium pentobarbital (6 mg/100 g body weight intraperitoneally). Soleus muscles were quickly removed, blotted, and frozen in liquid nitrogen. Epididymal fat pads were then excised, quickly weighed, and frozen, as was the caudate lobe of the liver. After this, 4 mL of blood was then removed from the abdominal aorta. The heart and other organs and the remaining liver were then removed, rinsed free of blood, blotted, and weighed. In the second (HFHS diet) study, retroperitoneal fat pads were also removed and weighed. All organs were trimmed of visible fat and connective tissue before weighing.

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Oral Glucose Tolerance Test and Blood Pressure Measurement

After 2 weeks on the diets, oral glucose tolerance tests were performed between 12 noon and 3 PM on rats from which food had been withheld for 6 hours. Glucose (1.7 g/kg body weight) was administered by gavage as a 35% glucose solution. Blood samples were taken from the tail vein before and at 15, 30, and 60 minutes after administration of the glucose load. Samples were collected in tubes containing EDTA and centrifuged, and the plasma was stored at −20°C until analyzed.

Blood pressure was measured in conscious restrained rats indirectly by tail cuff as described by Kramsch et al.[12] Reported values are the mean of five determinations.

Assays

Glucose level was measured by the hexokinase/glucose-6-phosphate dehydrogenase method,[13] and insulin was assayed by radioimmunoassay using a kit from Linco Research (St Louis, MO) and a rat insulin standard. Insulin measurements for the two studies were made in single but separate runs. Different standards were used for the two runs. We have recently learned that the standard used in the second study differed from previous standards provided by the supplier, and it yields insulin values approximately 30% lower (R. Gingrich, Linco Research, personal communication, August 1995). Plasma triglyceride (Sigma Chemical, St Louis, MO) and free fatty acids ([FFA] Wako BioProducts, Richmond, VA) were assayed with commercially available kits, and liver triglycerides were assayed by the method of Denton and Randle.[14] DAG level was measured as described by Preiss et al,[15] and malonyl CoA by the method of McGarry et al.[16]

Statistics

Values are expressed as the mean ± SEM. Statistical differences between groups were determined by ANOVA followed by the Student-Neuman-Keuls multiple comparison test or by unpaired *t* test with Bonferroni modification. It should be noted that these studies were designed to examine the effects of pioglitazone on glucose tolerance, malonyl CoA, and plasma triglycerides, and the number of rats was chosen accordingly. As a result, unexpected changes and others that are expected (eg, the decrease in plasma FFA caused by pioglitazone), although relatively large in magnitude, did not achieve statistical significance.

RESULTS

Rats on Standard Chow Diet

Plasma glucose and insulin. Dahl-S rats used in this study had normal circulating levels of insulin and glucose. Thus, when measured at 1 PM (5 hours after food removal) and during a glucose tolerance test, plasma glucose levels were nearly identical in Dahl-S and Sprague-Dawley groups. Likewise, plasma insulin levels, although slightly higher at each time point of the glucose tolerance test in Dahl-S rats, were not significantly different from those of control rats (Fig 1). Treatment with pioglitazone did not alter glucose tolerance or plasma insulin levels in either group (data not shown).

Plasma and tissue lipids. Plasma triglycerides were two-fold to threefold greater in male Dahl-S than in control Sprague-Dawley rats, as reported previously[3] (Table 1). Addition of pioglitazone to the diet resulted in a 35% decrease in plasma triglycerides in the Dahl-S group, but

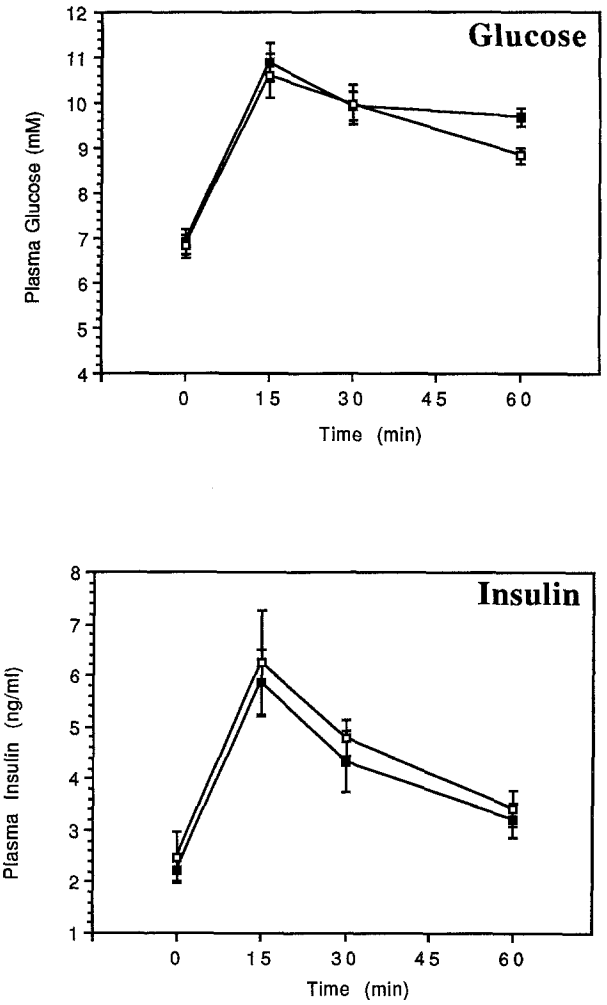


Fig 1. Plasma glucose and insulin response to an oral glucose load (1.7 g/kg body weight). Results are the mean ± SEM for 8 rats per group. (■) Control rats; (□) Dahl-S rats.

was without effect in control rats. Liver triglycerides were 7.4 ± 0.9 (n = 5) and 8.2 ± 0.6 μmol/g (n = 7), respectively, in control and Dahl-S rats on a chow diet, and 5.5 ± 0.7 (n = 7) and 5.9 ± 0.6 (n = 6) when these rats were placed on pioglitazone. None of the differences were statistically significant.

Table 1. Characteristics of Control and Dahl-S Rats Fed Chow or Chow + Pioglitazone

	Control	Dahl-S	Control + Pz	Dahl-S + Pz
Plasma triglycerides (mg/dL)	47 ± 5	111 ± 12*	47 ± 4	73 ± 9†‡
Body weight (g)	287 ± 9	290 ± 8	293 ± 6	292 ± 8
Heart weight (g)	0.97 ± 0.03	0.99 ± 0.03	1.08 ± 0.02	1.03 ± 0.06
Epididymal fat weight (g)	0.89 ± 0.09	0.87 ± 0.06	1.44 ± 0.06*	1.14 ± 0.11§

NOTE. Results are the mean ± SEM for 8 rats per group.
*$P < .001$ *v* chow-fed control rats.
†$P < .001$ *v* chow + pioglitazone-fed control rats.
‡$P < .001$ *v* chow-fed Dahl-S rats.
§$P < .05$ *v* chow-fed control rats.

The concentration of malonyl CoA was 50% greater in both the soleus muscle and liver of Dahl-S rats than in control rats (Fig 2). Treatment with pioglitazone decreased malonyl CoA levels by 30% to 50% in Dahl-S rats, but was without effect in the controls. DAG mass was similar in the soleus muscle of the two groups (129 ± 18 pmol/mg wet weight [control] *v* 152 ± 22 [Dahl]) and was not altered by pioglitazone (134 ± 15 pmol/mg wet weight [control] *v* 145 ± 26 [Dahl]). Similar findings were observed in liver (193 ± 11 pmol/mg wet weight [control] *v* 211 ± 22 [Dahl-S]; 216 ± 27 [control + pioglitazone] *v* 233 ± 19 [Dahl-S + pioglitazone]).

Blood pressure. After 2 weeks of study (1 week before death), blood pressures were 129 ± 4 (n = 8) mm Hg and 120 ± 9 (n = 8) mm Hg in control rats on standard diets and diets containing pioglitazone, respectively. Comparable blood pressures for the Dahl-S group were 138 ± 7 and 130 ± 5. None of the differences were statistically significant.

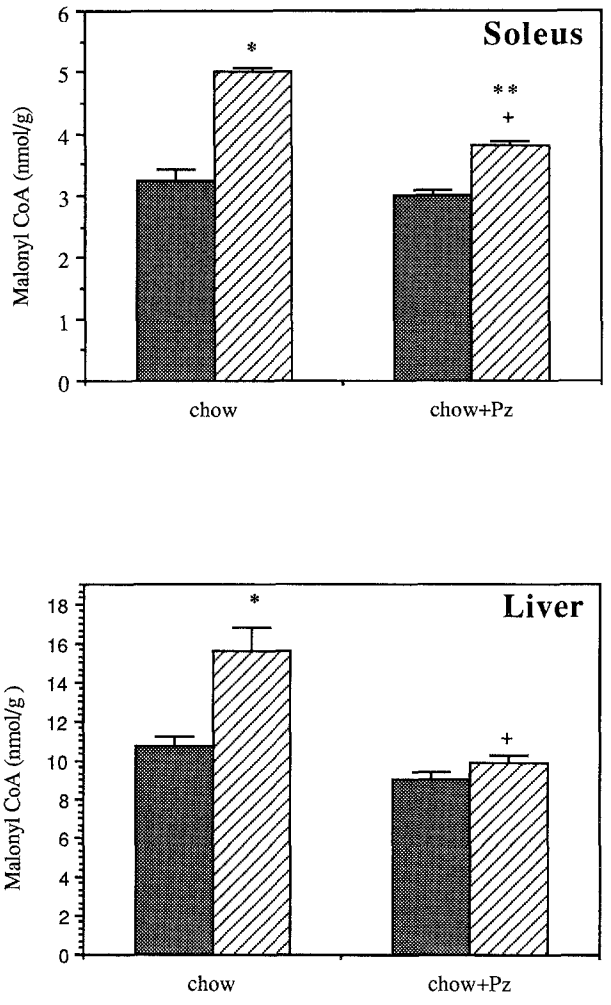


Fig 2. Effects of pioglitazone on malonyl CoA concentrations in soleus muscle and liver of control (■) and Dahl-S (▨) rats. Results are the mean ± SEM for 6 to 8 rats per group. Significantly different (*P* < .001) from: *chow-fed control rats, +chow-fed Dahl-S rats, **chow + pioglitazone-fed control rats.

Organ and body weights. Treatment with pioglitazone had no effect on body weight (Table 1). In rats on the standard chow diet, pioglitazone caused a nearly 50% increase in the mass of the epididymal fat pad in Sprague-Dawley control rats (Table 1). Interestingly, it also increased epididymal fat pad mass in the Dahl-S group; however, the increase was only half as large. The weights of the liver and soleus muscle were not affected by pioglitazone in either group (data not shown). Heart weight tended to be increased in pioglitazone-treated rats (Table 1); however, the differences were not statistically significant.

Rats on a HFHS Diet

Plasma glucose and insulin. To determine whether hyperinsulinemia and glucose intolerance would develop more readily in Dahl-S rats than in Sprague-Dawley control rats when they were nutritionally stressed, the two groups (initial weight, ~50 g) were fed a diet containing 35% of calories as fat (*v* 10% in standard chow) and 22% as sucrose for 3 weeks. Nagy et al[17] have previously reported that rats fed such a diet for 5 weeks become obese, hyperinsulinemic, and glucose-intolerant.

Both control and Dahl-S rats showed some impairment of glucose tolerance (Fig 3) and an increased plasma insulin response (Fig 4) after 3 weeks on the HFHS diet. Plasma insulin levels were greater in Dahl-S rats than in control rats at each time point of the glucose tolerance test; however, only at the 60-minute time point was the difference statistically significant. Pioglitazone had no effect on either glucose tolerance or plasma insulin levels in Dahl-S rats on this diet. In the control group, for reasons unknown, it increased plasma insulin levels at the 60-minute time point of the glucose tolerance test. The low insulin levels in this study are in part attributable to the use of an insulin standard different from that used in the earlier study.

Plasma and tissue lipids. As in the earlier study in which older rats were used, plasma triglyceride concentration tended to be higher in Dahl-S rats than in Sprague-Dawley control rats, although the difference was not statistically significant (Table 2). Ingestion of the HFHS diet had no effect on plasma triglyceride levels in either group. Pioglitazone tended to decrease plasma triglycerides and FFA levels in all groups studied (Table 2).

Malonyl CoA levels in soleus muscle were increased in the younger Dahl-S rat compared with control rats (Fig 5) on the standard chow diet, in agreement with findings in older animals (Fig 2). Ingestion of the HFHS diet had no effect on the concentration of malonyl CoA in control rats. It significantly diminished the level in Dahl-S rats; however, malonyl CoA levels in these rats were still higher than in the control group. DAG concentrations in liver and soleus muscle were similar in control and Dahl-S rats on the chow diet, and were not altered by either the HFHS diet or pioglitazone (data not shown).

Epididymal and retroperitoneal fat pads and body weights. The mass of the epididymal fat pad relative to total body weight was the same in control and Dahl-S rats and was not altered by the HFHS or chow diet. In contrast, the HFHS diet caused a 26% increase in the mass of the retroperito-

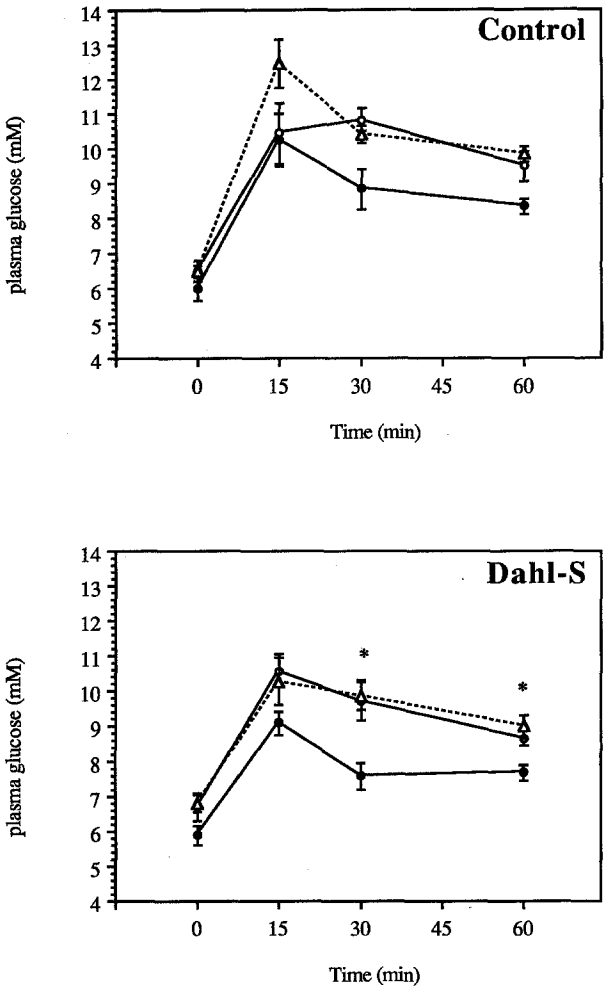


Fig 3. Effects of HFHS diet on plasma glucose response to an oral glucose load (1.7 g/kg body weight) in control and Dahl-S rats. Results are the mean ± SEM for 8 rats per group. (●) Chow diet; (○) HFHS diet; (△) HFHS + pioglitazone. *Significantly different ($P < .01$) from chow-fed Dahl-S rats.

neal fat pad in Dahl-S but not in control rats (Fig 6). A larger mass of retroperitoneal fat (47%) was also seen in Dahl-S compared with control rats on this diet when they were treated with pioglitazone. Equally noteworthy, whereas pioglitazone increased the mass of the epididymal fat pad, it decreased the mass of the retroperitoneal fat pad, suggesting a differential effect on the two tissues. Total body weight was greater in rats on the HFHS diet versus the standard diet, with the increases possibly being larger in the Dahl-S groups (Table 2). Pioglitazone did not appear to have an independent effect on body weight or on the mass of the liver or soleus, although it tended to increase the weight of the heart relative to total body weight in Dahl-S rats fed the HFHS diet (Table 2). More rats need to be studied to determine whether this effect is significant.

DISCUSSION

The results were surprising in several respects. One of these was that inbred Dahl-S rats fed a standard chow diet were neither overtly hyperinsulinemic nor insulin-resistant. This contrasts with an earlier study by Reaven et al[3] in which moderate hyperinsulinemia (39 ± 2 *v* 27 ± 2 μU/mL in control rats at 1 PM) and a 20% decrease in insulin stimulation of glucose transport into adipocytes were found in Dahl-S rats. Differences of this magnitude were possibly missed in the present study, due to the small number of rats used. In most respects, the two studies were similar. Rats weighing 200 and 290 g were evaluated in this study, and 250-g rats by Reaven et al.[3] In both instances, insulin measurements were performed after 5 hours of food deprivation at approximately 1 PM. The two studies differed in that inbred Dahl-S rats were used here, and outbred rats in the earlier study.

Conventional dogma holds that hypertriglyceridemia occurs in insulin-resistant rodents and humans because hepatic lipid synthesis remains sensitive to insulin at the same time hepatic and peripheral carbohydrate metabolism are insulin-resistant.[4,6] In the apparent absence of hyperinsulin-

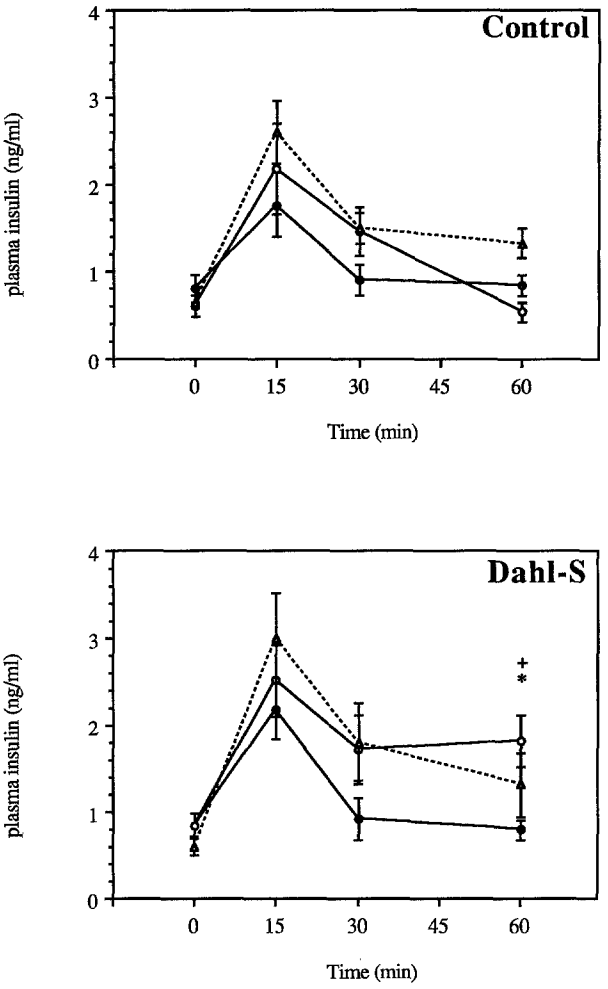


Fig 4. Effects of HFHS diet on plasma insulin response to an oral glucose load (1.7 g/kg body weight) in control and Dahl-S rats. Results are the mean ± SEM for 8 rats per group. (●) Chow diet; (○) HFHS diet; (△) HFHS + pioglitazone. Significantly different ($P < .05$) from: +chow-fed Dahl-S rats, *HFHS-fed control rats.

Table 2. Characteristics of Control and Dahl-S Rats Fed Chow, HFHS, or HFHS + Pioglitazone

	Control	Dahl-S	Control + HFHS	Dahl-S + HFHS	Control + HFHS + Pz	Dahl-S + HFHS + Pz
Body weight (g)	188 ± 7	175 ± 7	200 ± 8	199 ± 10	206 ± 9	206 ± 10
Heart weight (% body weight)	0.47 ± 0.02	0.46 ± 0.01	0.43 ± 0.02	0.43 ± 0.02	0.49 ± 0.02	0.49 ± 0.01
Plasma TG (mg/dL)	75 ± 9	159 ± 52	70 ± 4	110 ± 19	48 ± 3*	64 ± 4
Plasma FFA (mmol/L)	0.39 ± 0.06	0.53 ± 0.12	0.37 ± 0.03	0.44 ± 0.04	0.20 ± 0.03	0.34 ± 0.05

NOTE. Results are the mean ± SEM for 5 to 10 rats per group.

Abbreviations: Pz, pioglitazone; TG, triglycerides.

*Significantly different from HFHS-fed control rats ($P < .05$) by unpaired t test with Bonferroni modification.

emia in the Dahl-S rat, one has to attribute its hypertriglyceridemia to hepatic overproduction or peripheral underutilization of very–low-density lipoprotein triglyceride due to other causes. Thus, the finding that malonyl CoA levels in liver are increased in the Dahl-S rat is of special interest. Malonyl CoA is an inhibitor of carnitine palmitoyl transferase I, the enzyme that catalyzes the transfer of long-chain fatty acyl CoA (LCFA CoA) into the mitochondria, where they are oxidized. When the concentration of malonyl CoA is increased, LCFA CoA transport into the mitochondria diminishes and more LCFA CoA is available in the cytosol for glycerolipid synthesis.[18] In liver, this leads to an increase in the production of triglyceride, and secondarily of very–low-density lipoproteins. The observation that treatment with pioglitazone, which decreases plasma triglycerides (Table 1), also decreases hepatic malonyl CoA levels (Fig 2) is consistent with this notion. Less clear are why malonyl CoA levels are increased in the first place in the liver and muscle of the Dahl-S rat and how pioglitazone acts to decrease them. The possibilities of a primary abnormality in acetyl CoA carboxylase (ACC), the enzyme that catalyzes malonyl CoA formation, or of insulin resistance and hyperinsulinemia not detected by the glucose tolerance test should be evaluated. Also to be determined is why ingestion of the HFHS diet diminished malonyl CoA levels in muscle of the Dahl-S rat. Hypothetically, it could relate to inhibition of ACC. Fatty acyl CoA, the concentration of which might be increased after the HFHS diet,[19] activates an adenosine monophosphate kinase that inhibits the 265-kD ACC in liver. It remains to be determined whether such increases in LCFA CoA have a similar effect in muscle. The concentration of malonyl CoA in liver is regulated principally by a 265-kD ACC and in muscle by a 280-kD isozyme that appears to be different structurally from its hepatic counterpart.[20,21] Possibly, these enzymes have a common regulatory control that is altered in the Dahl-S rat and is corrected by pioglitazone.

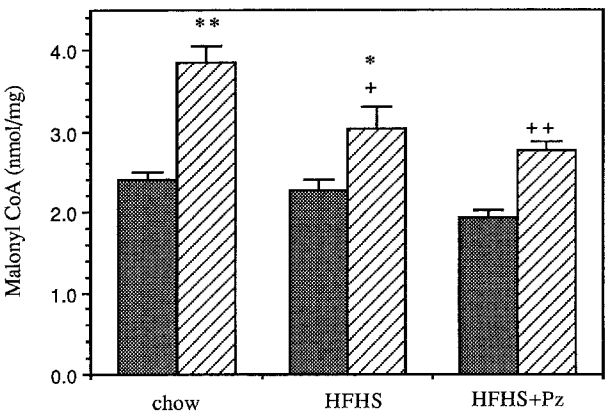
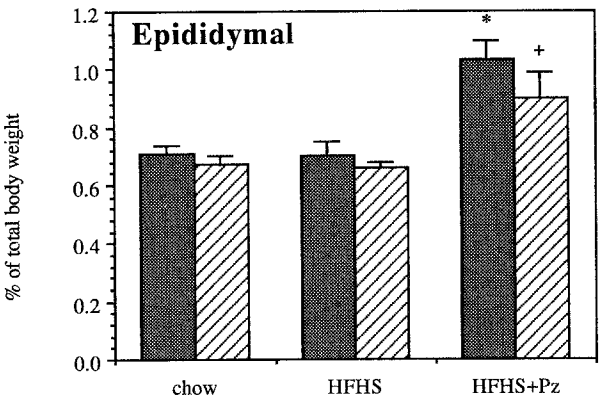


Fig 5. Effects of HFHS diet and HFHS + pioglitazone diet on soleus muscle malonyl CoA concentration in control and Dahl-S rats. Results are the mean ± SEM for 8 to 10 rats per group. (■) Control rats; (▨) Dahl-S rats. Significantly different from: **chow-fed control rats ($P < .001$), *chow-fed Dahl-S rats ($P < 0.01$), +HFHS-fed control rats ($P < .05$), ++HFHS + pioglitazone-fed control rats ($P < .01$).



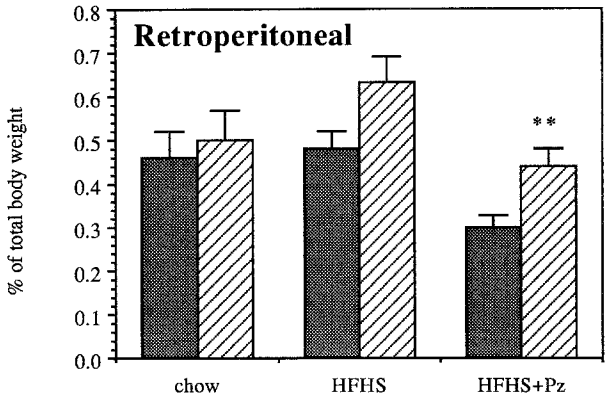


Fig 6. Effects of HFHS diet and HFHS + pioglitazone diet on fat pad weight of control and Dahl-S rats. Results are the mean ± SEM for 7 to 10 rats per group and are expressed relative to body weight, due to the modest difference in weight of the different groups (Table 2). (■) Control rats; (▨) Dahl-S rats. Significantly different from: *HFHS-fed control rats ($P < .001$), +HFHS-fed Dahl-S rats ($P < .05$), **HFHS + pioglitazone-fed control rats ($P < .05$).

Several other lipids examined in this study warrant mention. Plasma FFA levels were not increased in the Dahl-S rat. Earlier reports[22] suggested that pioglitazone diminishes plasma FFA in control and insulin-resistant rodents. It tended to do the same in this study; however, the decreases were not statistically significant, possibly because of the small number of rats studied. The absence of an abnormality in DAG content in muscle and liver of the Dahl-S rat is also noteworthy. High concentrations of DAG have been observed in liver and muscle of insulin-resistant KKA^y mice[11] and in muscle in other insulin-resistant states.[23,24] In KKA^y mice, pioglitazone paradoxically increased total DAG content at the same time it diminished insulin resistance. As suggested elsewhere,[11] there appear to be at least two biochemically distinct pools of DAG in muscle: one derived from phospholipid hydrolysis and the other synthesized de novo. These pools may respond differently to pioglitazone in these and non–insulin-resistant mice. The absence of such changes in the Dahl-S rat is in keeping with the apparent lack of insulin resistance in these animals.

HFHS diets cause hyperinsulinemia, insulin resistance, and obesity when fed to Sprague-Dawley rats for 5 weeks[17] and C57BL/J6 mice for 16 weeks.[25] Diets high in fat alone produce a similar constellation of events in rats and mice, with the effects substantially greater in some strains (eg, Osborne-Mendel rat[26,27] and C57BL mice[25]) than in others. The HFHS diet used here produced moderate hyperinsulinemia and glucose intolerance in both control and Dahl-S rats, with the hyperinsulinemia more sustained in the latter (Fig 4). It did not significantly increase epididymal fat mass relative to total body weight in either group after 3 weeks (Fig 6), possibly reflecting their relatively short time on the diet. In contrast, it appeared to increase retroperitoneal fat in Dahl-S rats. Whether this increase in retroperitoneal fat reflects its greater sensitivity than epididymal fat to the hyperinsulinemia caused by the HFHS diet or to other factors remains to be established.

The possible relation of the high concentration of malonyl CoA in liver and muscle of the Dahl-S rat to its propensity to obesity merits comment. It has been suggested that in many instances, obesity is a disorder of fat partitioning.[28,29] According to this hypothesis, certain individuals (and experimental animals) become more obese when placed on a high-fat diet because of an inability to oxidize dietary fat.[29,30] In keeping with this notion, a high respiratory quotient and other indicators of a low rate of fatty acid oxidation (eg, low ketone body levels) have been observed in non-obese humans[8,31] and experimental animals[28] predisposed to obesity. Also, an impaired ability to oxidize dietary fat[32] has been found in formerly obese women as compared with lean women. Malonyl CoA, and the enzyme that catalyzes its formation, ACC, have been shown to be components of a fuel-sensing mechanism that responds to changes in fuel supply and energy expenditure in muscle[33] and other cells that oxidize fatty acids.[34,35] Thus, high concentrations of malonyl CoA are found in these tissues when glucose is present in excess, whereas malonyl CoA levels decrease when they are glucose-deprived. A dysregulation of such a mechanism leading to a sustained increase in the concentration of malonyl CoA in muscle and liver[33] could explain why oxidation of ingested fat is impaired in individuals predisposed to obesity. If this hypothesis is correct, high levels of malonyl CoA will be found in muscle and other tissues of such individuals. Also, therapies aimed at decreasing malonyl CoA (eg, exercise or caloric restriction) should be especially efficacious in preventing obesity in these subjects. This hypothetical schema applies to humans predisposed to obesity who are not yet obese. Once they are obese, fatty acid oxidation appears to be increased, as judged by the finding that their respiratory quotient decreases to values similar to or less than those of non-obese subjects.[8,31] We believe this results from an increase in the cytosolic concentration of LCFA CoA due to high plasma FFA levels and an increase in intracellular triglyceride stores and turnover. Such increases in LCFA CoA could enhance fat oxidation both by inhibiting ACC[21,36] and thereby decreasing the concentration of malonyl CoA and by diminishing malonyl CoA inhibition of CPT 1.[36] However, direct proof of this is needed.

An unexpected finding, which needs to be studied in other models, was that pioglitazone treatment decreased retroperitoneal fat pad mass in rats fed a HFHS diet at the same time it increased the mass of the epididymal fat pad. To our knowledge, this is the first report that an agent can differentially affect the mass and presumably the metabolism of these two adipose tissue depots. Whether defined genetic and/or metabolic effects of pioglitazone on the fat cell (eg, its ability to diminish tumor necrosis factor mRNA[37]) are also different in these two depots remains to be determined. If so, this could have bearing on the ability of the thiazolidinediones to enhance insulin sensitivity in insulin-resistant subjects.[38,39] Also of note is the finding that thiazolidinediones have been shown to interact with peroxisome proliferator-activated receptor γ.[40] Activation of this family of genes induces enzymes involved in fatty acid oxidation. Such a mechanism of action could explain the effect of pioglitazone in this study.

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