Metabolic Adaptation Follows Contractile Dysfunction in the Heart of Obese Zucker Rats Fed a High Fat "Western" Diet

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Abstract

The normal heart responds to changes in its metabolic milieu by changing relative oxidation rates of energy providing substrates. We hypothesized that this flexibility is lost when genetically obese rats are fed a high caloric, high fat 'Western' diet (WD).

Male Zucker obese (ZO) and Zucker lean (ZL) rats were fed either control or WD composed of 10 kcal% and 45 kcal% fat respectively for 7 or 28 days. Cardiac triglycerides and mRNA transcript levels were measured in situ. Substrate oxidation rates and cardiac power were measured ex vivo. Hearts from ZO rats fed WD for 7 days showed decreased cardiac power and increased cardiac triglyceride content, but no change in oleate oxidation rates or mRNA transcript levels of pyruvate dehydrogenase kinase-4 (PDK-4), uncoupling protein-3 (UCP-3), mitochondrial (MTE-1) and cytosolic thioesterase 1(CTE-1). When fed WD for 28 days, ZO rats showed no further decrease in cardiac power and no further increase in intramyocardial triglyceride levels compared to ZO rats fed the same diet for 7 days only, but did show significantly increased oleate oxidation rates and transcript levels of CTE-1, MTE-1, PDK-4 and UCP-3. In contrast, hearts from ZL rats fed WD showed increased rates of oleate oxidation and increased transcript levels of the fatty acid responsive genes investigated, and no further deterioration of contractile function.

We conclude that exposing a genetic model of obesity to the nutrient stress of WD results in an early reversible loss of metabolic flexibility of the heart which is accompanied by contractile dysfunction.

Keywords

diet; rat model of obesity; cardiac dysfunction; fatty acid metabolism

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Introduction

The normal mammalian heart prefers fatty acids as energy providing substrates. However, it is capable of changing the proportion of substrates it oxidizes in response to changes in its physiologic environment (1,2). We have proposed that prolonged exposure to an abnormal metabolic milieu in vivo results in a loss of this "metabolic flexibility", which ultimately leads to contractile dysfunction (3). A case in point is the Zucker Diabetic Fatty rat, which exhibits cardiac dilatation, reduced contractility and intramyocardial lipid accumulation (4). We have observed in the same model that administration of a PPAR-γ agonist, which restores a normal metabolic milieu, also restores cardiac function (5).

We now propose that diet-induced obesity in a genetically predisposed model of insulin resistance results in a loss of metabolic flexibility. We examined the time course of metabolic adaptation and malaptation as well as contractile function of the heart. We found that in this model, metabolic dysregulation is accompanied by an early contractile dysfunction of the heart which is subsequently compensated by mechanisms of adaptation to the altered metabolic milieu or restoration of metabolic flexibility.

Methods

Animals

Six-week-old male Zucker lean (ZL) rats and male Zucker obese (ZO) fa/fa rats were purchased from Harlan (Indianapolis, Indiana). Animals were kept in the Animal Care Center of the University of Texas Medical School at Houston under controlled conditions $(23 \pm 1° C$; 12:12-h light-dark cycle) and received standard laboratory chow and water ad libitum for 2 weeks. ZL and ZO rats were then randomly assigned to one of four feeding groups which varied both with respect to diet (control versus WD) and duration of feeding (0, 7 or 28 days). Animals were sacrificed 6 ± 1 hours after the onset of the dark phase. All protocols were approved by the Institutional Animal Care and Use Committee. Comparisons between the ZL and ZO with the same composition of diet and duration of diet (7 or 28 days) were performed to characterize the animal model. Comparisons within individual animal models exposed to different diets (e.g. ZL on control vs. WD) allowed us to characterize the effect of diet.

Control diet (CD, D12450B, Research Diet, New Brunswick, NJ) contained 10% kcal fat, 70% kcal carbohydrates, 20% kcal proteins with a caloric density of 3.85 kcal/g. High fat Western diet (WD, D12451, Research Diets, New Brunswick, NJ) had 45% kcal fat, 35% kcal carbohydrates, 20% kcal proteins with a caloric density of 4.73 kcal/g. We have used both diets previously to investigate the effects of diet-induced obesity on the heart of Wistar rats (6).

Determination of non-esterified fatty acids in plasma

To determine non-esterified free fatty acid (NEFA) levels, spectrophotometric analysis was performed on EDTA-plasma using a commercially available kit (NEFA C, Wako Chemicals, Richmond, VA). Specimen blanks were prepared for all samples to correct for possible hemolysis.

Triglyceride extraction and quantification in heart

Tissue triglyceride concentrations were determined by digesting ~70-100mg of heart tissue in chloroform-methanol and using a triglyceride assay kit (Sigma, St. Louis, MO) as described previously (7).

Working heart perfusions

In order to investigate the time course of metabolic adaptation and maladaptation in the heart, we started our studies with hearts from animals that were six weeks old. The working heart preparation has been described previously (2). Animals were anaesthetized with chloral hydrate (60mg/100g body weight) i.p. and heparin was administered directly into the inferior vena cava. Hearts were removed rapidly and placed in ice cold Krebs-Henseleit buffer (KHB). Hearts were perfused in the working mode with recirculating KHB containing D-glucose (5 mM plus 20 μCi/L [U-14C] glucose), insulin (1ng/mL), sodium lactate (0.5 mM) and sodium oleate (0.4 mM plus 30 μCi/L [9,10-3H] oleate), which was bound to 1% BSA and dialyzed overnight. The buffer was equilibrated with $O_2:CO_2$ (95:5 % v/v). The preload was set to 15 cm H_2O, the afterload was set to 100 cm H_2O and hearts were perfused for forty minutes at 37° C. Cardiac power, glucose and oleate oxidation rates were measured at 5 minute intervals and averaged over the entire time of perfusions. At the end of the protocol, oxidation rates and cardiac power were normalized to dry weights of freeze-clamped hearts as described previously (5).

RNA extraction and quantitative RT-PCR

RNA extraction and quantitative RT-PCR was performed on non-perfused hearts as described previously (8). Primers and probes were designed across splice sites to prevent amplification of genomic DNA. The assays have been published previously (9). Data are presented as number of transcripts per nanogram of total RNA molecules.

Statistical analysis

Data are presented as mean ± standard error (SE). Statistically significant differences between groups were calculated using the analysis of variance (ANOVA), with repeated measures with post-hoc tests (Scheffe's and Dunnett's test) where appropriate.

Results

Plasma fatty acids and heart triglycerides

ZO animals fed WD showed no increase in plasma NEFA at 7 days of WD but a significant increase (70.6%) in plasma NEFA levels at 28 days compared to ZO rats fed CD for the same duration. Plasma NEFA levels were significantly increased in the ZL rats fed WD both at 7 (42.3%) and 28 days (47.2%) (Figure 1A) compared to ZL rats fed a CD for the same duration.

In the ZO rats fed WD, triglyceride content in the heart increased at both 7 and 28 days, by 165% and 153 % respectively, compared to ZO rats fed CD for the same duration Cardiac triglyceride content did not change in ZL rats fed WD or CD at 7 or 28 days. (Figure 1B). In both groups, cardiomyocytes increased in size when animals were fed WD for 28 days (Supplementary Fig 1).

Cardiac power and substrate oxidation

Cardiac power decreased by 25% in ZO rats fed WD for 7 days compared to ZO rats fed CD for 7 days. Furthermore, cardiac power significantly decreased in ZO rats fed either WD or CD for 28 days compared to ZO rats fed CD. The WD had no significant effects on cardiac power in ZL rats. Among all groups fed WD, cardiac power decreased in ZO rats compared to ZL rats for the same duration. However, ZO rats fed WD for 28 days showed no further deterioration in cardiac power compared to ZO rats fed WD for 7 days only, suggesting adaptation (Figure 2A).

We also investigated rates of oxidative substrate metabolism to study changes in ZO and ZL hearts exposed to WD. Figures 2B and C depict rates of oleate oxidation and glucose oxidation. At 7 days, both ZO rats fed WD and those fed CD had similar rates of oleate oxidation. However, 28 days of WD led to a significant increase in oleate oxidation rates (161%) in ZO rats compared to ZO rats fed CD and compared to ZO rats fed WD for only 7 days (Figure 2B). Hearts from ZL rats fed WD for 7 days also exhibited increased rates of oleate oxidation. With 28 days of WD feeding, oleate oxidation rates only trended to increase compared to ZL fed CD for the same duration.

Rates of glucose oxidation significantly decreased in hearts of ZO rats on both diets at both 7 and 28 days relative to ZL rats. The decrease in rates of glucose oxidation by hearts of ZL rats fed WD did not reach significance (Figure 2C).

Transcript levels of PPARα-regulated genes

To examine whether changes in metabolism correlate to changes in gene expression, we used real-time quantitative RT-PCR to analyze transcript levels of genes involved in fatty acid metabolism. Transcript levels of PPAR-α were the same in hearts isolated from ZL and ZO animals fed either WD or CD (Figure 3A) at all time points.

After 7 days of WD, hearts from ZO rats showed no changes in transcript levels of pyruvate dehydrogenase kinase 4 (PDK-4), uncoupling protein 3 (UCP-3), medium chain acyl-CoA dehydrogenase (MCAD), mitochondrial thioesterase-1 (MTE-1) and cytosolic thioesterase-1 (CTE-1) compared to CD. However, in ZO rats fed WD for 28 days there were strikingly increased transcript levels of CTE-1(+178%), MTE-1(+176%), PDK-4 (+200%), and UCP-3 (+276%), all $p < 0.001$ compared to ZO rats fed CD for 28 days (Figure 3B-F) and compared to ZO rats fed WD for 7 days only. ZL rats fed WD showed a significant increase in the expression of fatty acid responsive genes compared to ZL fed CD both at 7 and 28 days. Only MCAD did not change (Figure 3B-F). Transcript levels of MCAD were significantly decreased in ZO rats fed WD for 7 days compared to ZO rats fed CD for the same time (Figure 3D).

Transcript levels of genes of the lipid biosynthetic pathway

To see if changes in myocardial triglyceride content correlate to changes in gene expression, we analyzed transcript levels of glycerol-3-phosphate O-acyltransferase (GPAT), which is an important flux generating step in myocardial glycerolipid synthesis. We also investigated transcript levels of steroyl-CoA desaturase-1 (SCD-1) were investigated, which is a key enzyme in myocardial lipogenesis. Transcript levels of GPAT increased in hearts of ZO rats fed WD for both 7 and 28 days. In hearts of ZL rats fed WD, GPAT transcript levels remained at baseline at both 7 and 28 days (Figure 4A). Hearts of ZO rats showed no change in SCD-1 transcript levels in response to diet whereas transcript levels of SCD-1 were significantly decreased in hearts of ZL rats fed WD compared to those fed CD for 7 days (Figure 4B).

Transcript levels of mediators and markers of contractile function

To further elucidate changes we observed in cardiac function, we also measured transcript levels of atrial naturitic factor (ANF) and sarcoendoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a). ANF gene expression was significantly increased in the ZO rats on WD at 7 days and in ZO rats fed either diet for 28 days compared to ZO rats fed CD for 7 days only whereas ZL animals showed no change in ANF transcript levels with either CD or WD at either timepoint (Figure 4C). This data supports the functional data, as increases in ANF transcript levels mirror decreases in cardiac function. There were no changes in the gene

expression of SERCA2a in the ZO or ZL rats fed either diet at all timepoints of the study (Figure 4D).

Discussion

We investigated the effects of WD on cardiac metabolism and function in a genetically obese rat model with a missense mutation in the leptin receptor (10,11). In this model of insulin resistance and obesity, the nutrient stress of WD leads to early cardiac dysfunction, which is followed by metabolic adaptation, increased rates of long-chain fatty acid oxidation, and decreased cardiac efficiency (Supplementary Figure 2). In other words, when a nutrient stress meets a genetically predisposed animal, the heart decompensates, but a further decline in function is halted by subsequent metabolic compensatory responses, resulting in restoration of metabolic flexibility.

Alteration in substrate use and fatty acid responsive gene expression

Previous work from our laboratory has suggested that prolonged exposure to an abnormal metabolic milieu of increased fatty acids leads to a reversible loss of "metabolic flexibility" (3). For example, we have shown that administering a PPAR-γ agonist to the "metabolically inflexible" Zucker Diabetic Fatty rat improves cardiac output, reduces cardiac triglyceride content, restores suppressed glucose oxidation, and allows a tighter coupling of oxidative metabolism and contraction in Zucker diabetic fatty rats (5). We have also shown that a loss of synchrony between fatty acid delivery and oxidation rates in the "metabolically inflexible" failing human heart is associated with decreased fatty acid responsive gene expression and intramyocardial triglyceride accumulation (12). Taken together, our previous work has suggested that a chronic exposure to a high fat environment results in metabolic maladaptation due to a breakdown in the feed forward system of fatty acid oxidation, potentially resulting in loss of metabolic flexibility and lipotoxicity (13).

In this study, there is a disconnect between cardiac triglycerides and fatty acid oxidation. With 7 days of WD feeding, ZO rats display an increase in cardiac triglyceride content by 165% but no increase in oleate oxidation and no corresponding changes in transcript levels of fatty acid responsive genes investigated (except MCAD, which decreased) compared to ZO rats on CD for the same time (Figure 1B). Though contrary to data from ZDF and failing human hearts, this observation is in line with our previously published study investigating the effects of acute fasting in this model, which also noted an increase in myocardial triglyceride levels and a decrease in cardiac power without corresponding increases in fatty acid responsive genes or oleate oxidation rates (13).

In addition, we observed decreased transcript levels of MCAD in ZO rats fed WD for 7 days compared to ZO rats on CD for the same time. Decreased MCAD transcript levels are associated with a reduction in MCAD activity and a reduction in rates of fatty acid oxidation in canine models of pacing-induced cardiac failure (14,15). Accordingly, the decreased cardiac power noted in ZO rats at 7 days on WD may reflect a delay in the ability to increase myocardial mitochondrial fatty acid oxidation and may be responsible for the cardiac dysfunction seen at that time (Supplementary Figure 3). In contrast, ZL rats fed WD for 7 days increase oleate oxidation rates and transcript levels of fatty acid responsive genes and maintain cardiac power. This suggests that obese rats have lost their capacity to adapt to a WD and show no change in gene expression or exogenous substrate oxidation at 7 days. It is reasonable to assume that this inability of the ZO hearts to increase oleate oxidation rates and to adapt to WD after 7 days may be the cause for a mismatch between fatty acid uptake and oxidation in the cardiomyocytes, resulting in the massive myocardial triglyceride levels seen in our model (13). After 28 days of WD, ZO rats display a long-term metabolic adaptation and show no further decrease in cardiac power. Exogenous oleate oxidation rates

increase, which is associated with increased transcript levels of fatty acid responsive genes and there is no further increase in intramyocardial triglyceride levels. Taken together, ZO rats exposed to WD show contractile dysfunction at 7 days and a later adaptation to the abnormal metabolic milieu by 28 days, which prevents a further decrease in cardiac power.

Lipotoxicity

In addition to reduced fatty acid oxidation levels, increased *de novo* lipogenesis also likely contributes to the rapid accumulation of intramyocardial lipid in ZO rat hearts by 7 days. Increased expression of lipogenic enzymes such a GPAT, an enzyme of fatty acid esterification, at both 7 and 28 days also likely contributes to the rapid accumulation of triglyceride in cardiac tissue of these rat hearts during these timepoints. ZO rats on both diets were also unable to decrease expression levels of stearoyl Co-A desaturase (SCD-1), which regulates fatty acid synthesis, unlike ZL rats which displayed a significant decrease in SCD-1 gene expression with exposure to WD both at 7 days. These observations suggest that ZO rats are unable to downregulate fatty acid synthesis to the same extent as the ZL rats, which may be due to the effects of defective leptin signaling on SCD-1 and AMP-activated protein kinase (AMPK) activity in our model (16,17).

Although the exact mechanism remains elusive, the phenomenon of cardiac "lipotoxicity" is characterized by the accumulation of ceremide, diacyl glycerol, and triglycerides (18). Cardiac overexpression of fatty acyl-CoA synthetase (FACS) or human lipoprotein lipase has also been shown to result in lipid accumulation, cardiac hypertrophy and gradual development of contractile dysfunction (19). An interesting new aspect of the metabolic changes in lipotoxicity is the possible role of AMPK.

In low energy states, the enzyme AMPK plays a distinct role in the regulation of malonyl CoA levels and increases both glucose uptake and fatty acid oxidation in the heart in response to decreased intracellular ATP/AMP ratio (20). The induction of fatty acid oxidation by leptin has already been shown to be mediated by AMPK in skeletal muscle (21) and in isolated cardiomyocytes (22). AMPK activation is also associated with increased β-oxidation, reduction in fatty acid CoA, palmitoyl-CoA and ceremide in oxidative skeletal muscle from ob/ob mice (17,23). In this study, ZO rats show no change in p-AMPK or p-ACC levels after 7 days of WD but show significant increases in p-AMPK and p-ACC after 28 days of WD (Supplementary Figure 4B and 4C). AMPK activation may be responsible for increased oleate oxidation rates and preservation of glucose oxidation rates seen at 28 days in the ZO rats fed a WD. Additionally, AMPK activation may serve to increase the cells capacity to utilize both glucose and oleate to produce ATP in the face of impaired insulin signaling and explain the maintenance of contractile function in ZO hearts fed WD for 28 days.

Insulin resistance

Insulin suppresses myocardial fatty acid oxidation. As such, increased reliance on fatty acids could be coupled to insulin resistance. Mice with heart-specific overexpression of PPAR-α develop a cardiomyopathy with increased sensitivity to ischemic insult, increased rates of lipid oxidation and reduced glucose metabolism, all of which are characteristic features of the diabetic heart (24). Here we report that ZO rats show a significant decrease in AKT phosphorylation at 28 days irrespective of diet (Supplementary Figure 4A). The data indicate that the development of insulin resistance with age in the ZO rats is associated with cardiac dysfunction after 28 days on both diets. Whether insulin resistance is a marker or a mediator of contractile dysfunction (or altered fatty acid oxidation) remains unclear.

Limitations

The effects of a WD on cardiac function and metabolism in a genetic model of obesity have never been investigated using a longitudinal approach. The present study has determined substrate utilization in cardiac muscle in the ZO rat and provided a framework for explaining cardiac dysfunction after short and long term feeding of WD in animals that are genetically predisposed to growing obese. While we continue to investigate the causality, in this study we display a striking association between early contractile dysfunction and delayed metabolic adaptation in the ZO hearts faced with the nutrient stress of a WD when compared to the hearts of ZL rats, which exhibit normal leptin signaling. The exact mechanism by which aberrant leptin signaling leads to a loss of metabolic flexibility is still unknown. Studies utilizing the same isolated working rat heart perfusion apparatus have suggested that stimulation of cardiac fatty acid oxidation by leptin is mediated by p38 MAPK, which is activated by AMPK (25,26). However, further studies are required to identify the targets of p30 MAPK which mediate this effect. Manipulating fatty acid oxidation in a controlled manner may be able to address these important questions.

Conclusions

We conclude that exposing a genetic model of obesity to WD results in an early reversible loss of metabolic flexibility of the heart which is accompanied by contractile dysfunction. The exact mechanisms for this delayed metabolic await elucidation. Nonetheless, it is tempting to speculate that the genetic make up of an organism predisposes an organ like the heart to an abnormal metabolic stress including lipotoxicity, insulin resistance, and aberrant leptin signaling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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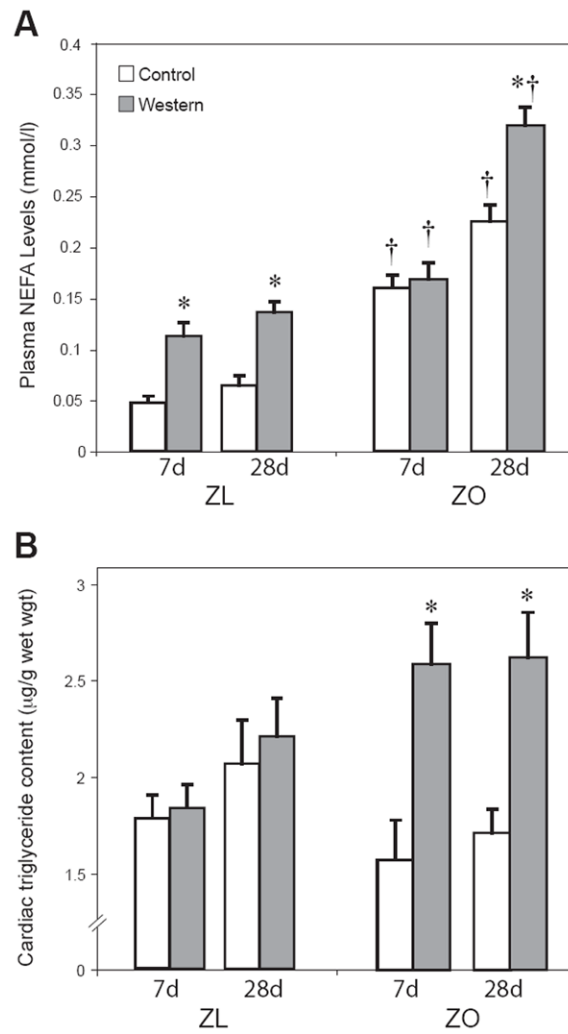
Figure 1.
Plasma NEFA (A), and cardiac triglyceride content (B). Groups: ZL = Zucker lean; ZO = Zucker obese. WD = high fast diet; CD = control diet. Data are means ± SE for 8 independent observations. * p < 0.01 for WD vs. CD; † p < 0.05 for ZL vs. ZO.
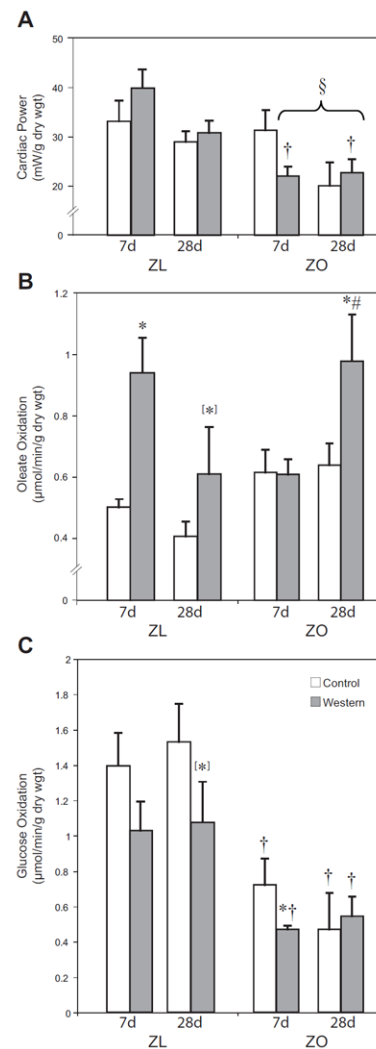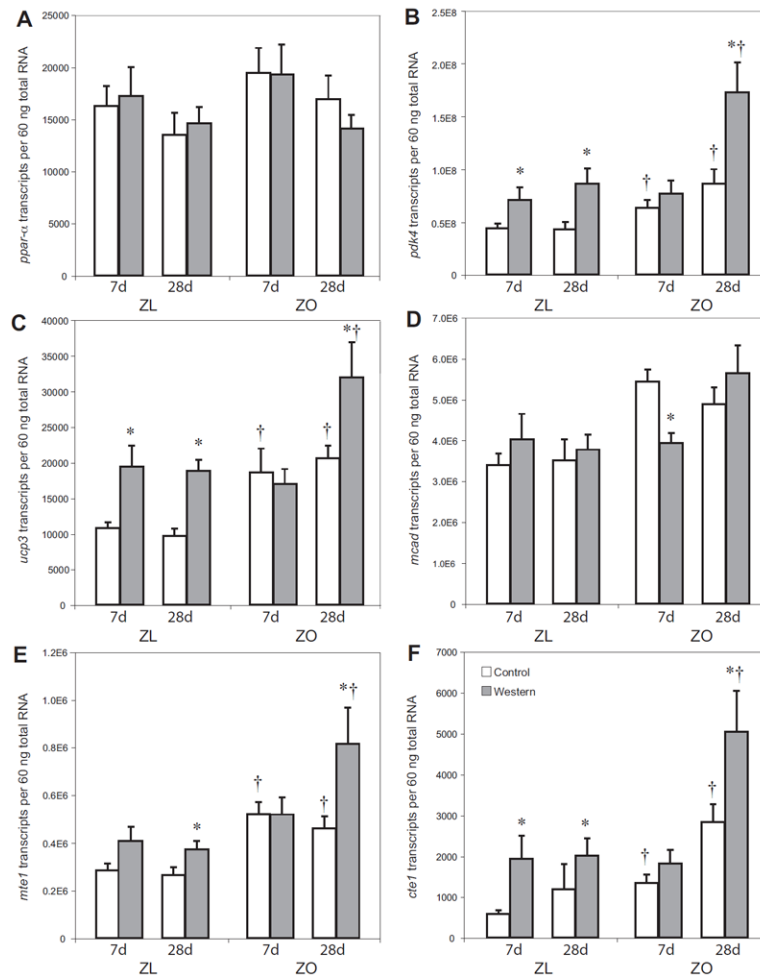
Figure 2.
Cardiac power (A), oleate oxidation (B) and glucose oxidation (C) of perfused hearts from lean and obese Zucker rats. Groups are the same as in Figure 1. Data are means ± SE. * p < 0.05 for WD vs. CD; † p < 0.05 for ZL vs. ZO; [*] p < 0.1 for WD vs. CD; § p < 0.05 for ZO fed CD for 7 days vs. ZO fed WD for 7 days, ZO fed WD for 28 days and ZO fed CD for 28 days.

Figure 3.
Expression of PPAR-α and fatty acid response gene-regulated genes in lean and obese Zucker rat hearts. Transcripts for PPAR-α (A), PDK-4 (B), UCP-3 (C), MCAD (D). MTE-1 (E) and CTE-1 (F). Groups are the same as in Figure 1. Data are means ± SE for seven to eight independent observations. * $p < 0.05$ for WD vs. CD; † $p < 0.05$ for ZL vs. ZO.
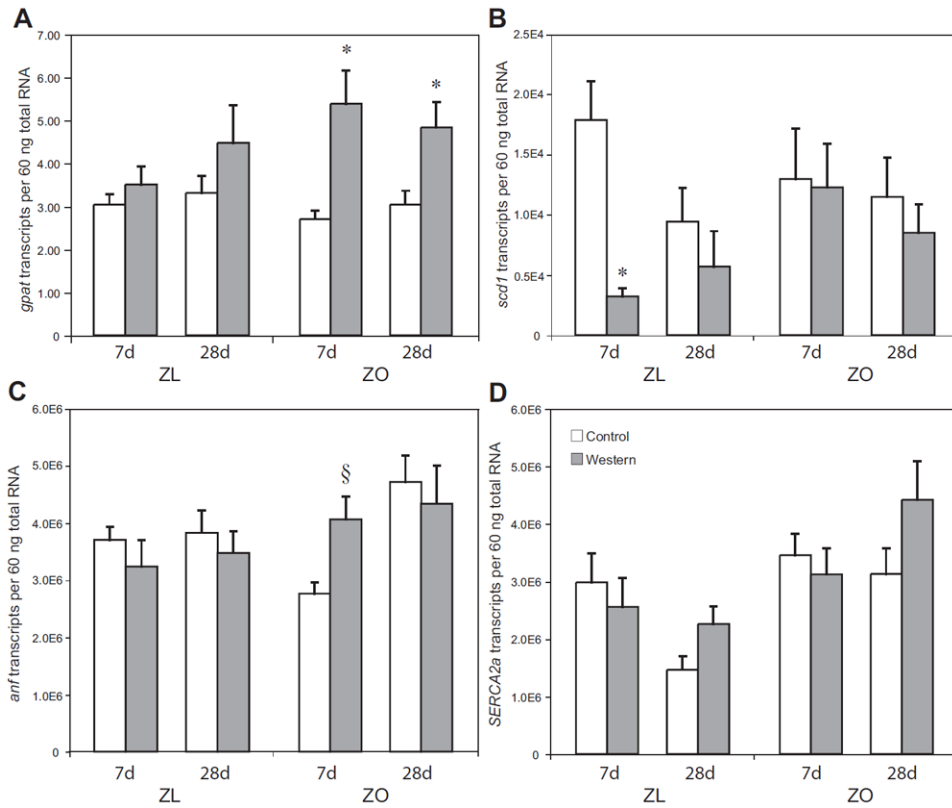
Figure 4.
Expression of enzymes involved in fat synthesis and potential modulators of contractile function in lean and obese Zucker rat hearts. Groups are the same as in Figure 1. Transcripts coding for GPAT (A), SCD-1 (B), ANF (C), and SERCA2a (D). Data are means ± SE for seven to eight independent observations. * p < 0.05 for WD vs. CD; † p < 0.05 for ZL vs. ZO; § p < 0.05 for ZO fed CD for 7 days vs. ZO fed WD for 7 days, ZO fed WD for 28 days and ZO fed CD for 28 days.