Myocardial hypertrophy and enhanced left ventricular contractility in Zucker diabetic fatty rats

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Abstract

Heart failure is known to be a complication of insulin-dependent (IDDM) and noninsulin-dependent diabetes mellitus (NIDDM) even in the absence of coronary heart disease or hypertension. The mechanisms leading to diabetic cardiomyopathy are unknown. The aim of the study was to characterize structural and functional alterations in hyperinsulinemic Zucker diabetic fatty (ZDF) rats treated with or without insulin. Diabetic animals showed a twofold increase in cardiomyocyte volume with increased left ventricular ANP but not BNP mRNA levels in spite of a reduced plasma renin activity (PRA) 2 months after onset of diabetes compared to nondiabetic littermates. These changes were associated with an increase in left ventricular performance as assessed by echocardiography. Insulin treatment led to a significant increase in body weight (BW), total heart weight, myocardial protein content, and left ventricular mass (LVM). Perivascular fibrosis and laminin thickness were significantly augmented in diabetic rat myocardium irrespective of insulin treatment, whereas interstitial collagen I and fibronectin were similarly found in diabetic and control myocardium. Initial stages of diabetic cardiomyopathy in hyperinsulinemic rats are characterized by cardiomyocyte hypertrophy and enhanced cardiac contractility. It is suggested that hyperinsulinemia may be involved in cardiac hypertrophy. © 2004 Elsevier Inc. All rights reserved.

Keywords: Hyperinsulinemia; Diabetes mellitus type II; ANP; Hypertrophy; Obesity

1. Introduction

The metabolic syndrome, a concurrence of disturbed glucose and insulin metabolism, overweight, abdominal fat distribution, dyslipidemia, and hypertension [1], plays a pivotal role in public health. A concomitant presentation of all components of the syndrome is rare; therefore, in the view of most experts, three out of the four main findings are sufficient for defining the syndrome. The components of the metabolic syndrome are major risk factors for type II diabetes mellitus and heart disease [2], accompanied by high cardiovascular morbidity and mortality [3].

Interestingly, as documented by the Framingham study, the incidence of congestive heart failure in diabetic patients is markedly increased (2.5–5 fold for men and women) independently of age, arterial hypertension, and coronary artery disease [4]. However, little is known about cardiac changes in the metabolic syndrome that could explain the development of heart failure. Most of the previous experimental studies on cardiac alterations in diabetes were performed in animals with alloxan- or streptozotocin-induced insulin-deficient diabetes [5], imitating the insulin-dependent diabetes mellitus (IDDM). Pathological findings in these animals include destruction of myocytes by apoptosis [6], as well as interstitial and perivascular fibrosis [7]. A study in our group showed myocardial hypertrophy [8] in a streptozotocin rat model. Only a limited number of studies have looked at experimental models of the metabolic syndrome. Most of the studies have been made on the Otsuka Long–Evans Tokushima fatty (OLETF) rats, a model with rather mild adiposity and hyperglycemia developing significant hyperglycemia after 30 weeks of age [9]. To study more precisely the cardiovascular changes in the metabolic syndrome, we chose the Zucker diabetic fatty rat (ZDF/Drt-fa) that was obtained by inbred strains of obese Zucker fatty rats with high glucose level. This model is characterized by early onset of marked hyperglycemia (after 9–12 weeks of age), hyperphagia,
adiposity, hyperinsulinemia, and hyperlipidemia, thereby resembling clinical features of the metabolic syndrome [10]. As shown by genetic studies, ZDF (fa/fa) rats bear a mutation in the obese gene (ob) product leptin receptor gene [11]. Leptin mainly coordinates energy metabolism and expenditure, thereby regulating body fat composition [12]. In this model, the state of hyperinsulinemia deteriorates with time to an IDDM state with lack of insulin comparable to the course in humans. Although insulin treatment is widely used as a therapeutic option for patients with poorly controlled noninsulin-dependent diabetes mellitus (NIDDM), its influence on morphological and functional changes of the heart is largely unknown [13]. Data from the Framingham Study led to the assumption that long-term treatment of diabetic patients with insulin may increase the risk for developing congestive heart failure [4]. In-vitro studies clearly demonstrated that insulin has trophic effects leading to proliferation of a variety of cells including fibroblasts [14]. It is therefore intriguing to speculate that insulin treatment may contribute to the process of cardiac remodeling.

It was therefore our aim to assess changes in myocardial morphology, extracellular matrix proteins, and their functional consequences in rats with metabolic syndrome alone and in combination with insulin treatment.

2. Methods

2.1. Experimental design

Male ZDF rats (BW 106–158 g; n = 13) and male Zucker lean rats (BW 85–118 g; n = 7) were obtained at an age of 5 weeks from Genetic Models (Indianapolis, IN). Animals were maintained on RMH-B rat chow from Hope Farms (Woerden, The Netherlands) with water ad libitum. All animals were individually housed in a 12:12-h dark/light cycle controlled room. The protocols had been approved by the local committee on animal research and conform with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996). At the age of 13 weeks, 1 week after the development of hyperglycemia, the animals were divided into three groups: (1) Zucker lean rats (control group; n = 7), (2) ZDF rats without insulin treatment (n = 6), and (3) ZDF rats treated with insulin (n = 6). An insulin (Actrapid HM U500, Novo Nordisk, Mainz, Germany) dose of 25.0 U/kg/day in the first 2 weeks and 75.0 U/kg/day in the following 4 weeks was required to normalize serum glucose levels in diabetic rats treated with subcutaneously implanted Alzet osmotic minipumps (Model 2ML2 and 2ML4, Charles River Wiga, Sulzfeld, Germany). Pumps were exchanged after 2 weeks and insulin dose was adapted as indicated above. BW and blood glucose level was determined every week (Accu-Chek Plus Roche, Mannheim, Germany). At the age of 18 weeks, systolic blood pressure and heart rate were measured by indirect tail-cuff method as previously described [15] using an automated cuff inflator-pulse detection system (W+W electronic AG, BP recorder no. 8005, Basel, Switzerland). After 6 weeks of insulin treatment, at the age of 19 weeks, echocardiography was performed. The next day, animals were sacrificed, the heart was removed, rinsed in PBS, and frozen in liquid nitrogen for further biochemical and histological analysis.

2.2. Echocardiography

Echocardiography was performed at the end of the study for quantification of left ventricular diameter, wall thickness, and performance by an experienced sonographer who was blinded to treatment. Animals were slightly anesthetized (methohexital sodium 30 mg/kg ip) and two-dimensional (short-axis) images and motion-mode (M-mode) images of the left ventricle were obtained from a parasternal view using a Hewlett Packard Sonos 5500 device with a 12.5 MHz transducer. Inner diastolic (IDD), systolic diameter (IDS), diastolic (IVSD), systolic interventricular septum thickness (IVSS), diastolic (LVPWD), and systolic left ventricular posterior wall thickness (LVPWS) were measured by the leading-edge method from at least three consecutive cardiac cycles on the M-mode tracing at a precision of 0.1 mm as proposed by the American Society for Echocardiography [16] (recording speed 100 mm/s). Diastolic and systolic left ventricular volumes were approximated according to Pawlush et al. [17] as IDD3 and IDS3, respectively, and were used to estimate ejection fraction (EF in %) as [1 – (IDS3/IDD3)] × 100. Fractional shortening (FS in %) was derived as (IDD – IDS)/IDD × 100. Relative left ventricular wall thickness (RLVWT) as index of left ventricular remodeling was calculated as (IVSD + LVPWD)/IDD. Left ventricular mass (LVM) was calculated according to a modified formula of Devereux and Reicheck [18] as LVM = 1.04 × [(IDD + 2 × LVPWD)2 – IDD2 – 13.6]. M-mode tracings were coded and analysis of the echocardiographic data was blinded for the study group.

2.3. Tissue preparation

Rats were killed by decapitation. Peripheral blood was collected for determination of HbA1c, insulin levels, and plasma renin activity (PRA). Hearts were excised, rinsed with saline, and blotted dry. The whole heart weight was determined. Four chamber slices were cut for immunohistochemical studies, prepared with tissue tek (Sekura, Finetek Europe, Netherlands), frozen in liquid nitrogen, and stored at −80°C until analyzed. The remaining heart was dissected free from the atra, cut into right and left ventricular tissue, and stored at −80°C.

2.4. Northern blot analysis

Northern blot analysis was performed as previously described [19]. Briefly, total RNA was extracted from left
ventricles using Trizol reagent (Canadian Life Technologies, Burlington, ON, Canada) according to the manufacturer's instructions. 20 μg were separated in 1% agarose gels under denaturing conditions. RNA was transferred onto nylon membranes (Gene Screen Plus, NEN, Dreieich, Germany) and UV cross-linked. Hybridization, washes, and autoradiography were performed. Different exposures of all autoradiograms were obtained to ensure that laser scanning (Personal Densitometer no. 50301, Molecular Dynamics) could be performed within the linear range of densitometry. cDNA probes for atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were amplified by PCR with specific primer pairs (ANP: sense primer 5'-AGGGCTTCTTCCTCTGCTG-3'; antisense primer 5'-GGATCTTTTGCGATCTGCTC-3'; BNP: sense 5'-TCACTTCTGCAGCATGGATC-3'; antisense primer 5'-TGAGCCAGGAGGTC-3') and labeled with α32-P dCTP (specific activity 3000 Ci/mmol, Amersham, Dreieich, Germany). To equalize for loading differences, all densitometric values were normalized to the housekeeping gene GAPDH.

2.5. Measurement of total protein

Measurement of total protein was performed in tissue homogenates from left ventricular myocardium in homogenization buffer (5 mM Tris–HCl pH 7.4, 300 mM sucrose, 0.1 mM EDTA, and 0.01 mM PMSF). Protein concentration was determined in duplicate according to method of Bradford.

2.6. Measurement of HbA1c, insulin, and C-peptide

HbA1c serum levels were determined by liquid chromatography (HPLC Bio-Rad Diamat MDL 723, Hercules, CA). Immunoreactive insulin (IRI) was measured by radioimmunassay with a polyclonal antibovine insulin antisem from Sigma-Aldrich (Taufkirchen, Germany) and a kit from Linco Research (St. Charles, MO). C-peptide was determined by a rat C-peptide RIA kit (Linco Research). For each assay, 100 μl of rat serum was used. All experiments were performed at least in duplicate.

2.7. Morphometry, tissue histochemistry, and quantitative image analysis

Morphometry and automatic image analyses were performed for quantification of structural changes in the left ventricular free wall using a computer-assisted image analysis system device (Olympus Optical, Hamburg, Germany) as previously described [20]. Briefly, frozen specimens were sectioned (5 μm), fixed with acetone at −20°C for 10 min, and stained with hematoxylin/eosin. Elastin staining was done with orcein according to a standard protocol [21]. Adjacent sections were selected for immunohistochemistry using the following antibodies: collagen I 1:200, Southern Biotechnology, Birmingham, AL, 1:1000; fibronectin 1:20, Chemicon International, Temecula, USA; and laminin, prediluted, Sigma, Deisenhofen, Germany. DAB was used as substrate for HRP-conjugated secondary antibodies. Finally, sections were dehydrated and embedded with entellan (Merck, Darmstadt, Germany). Sections were visualized by light microscopy using an oil immersion objective with a calibrated magnification of ×400 or ×200. Visual fields had 757 × 506 square pixels, with a resolution of

<p>| Table 1 |
| Biometric data at 19 weeks |</p>
<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Heart weight (mg)</th>
<th>Heart rate (l/min)</th>
<th>C-peptide (pmol/l)</th>
<th>Serum glucose (mg/dl)</th>
<th>HbA1c (% of control)</th>
<th>Systolic blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=7)</td>
<td>342 ± 11</td>
<td>1372 ± 33</td>
<td>484 ± 4</td>
<td>593 ± 98</td>
<td>96 ± 7</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>ZDF (n=7)</td>
<td>380 ± 8*</td>
<td>1341 ± 46</td>
<td>416 ± 13*</td>
<td>913 ± 65*</td>
<td>477 ± 26*</td>
<td>301 ± 12*</td>
</tr>
<tr>
<td>ZDF + Ins (n=6)</td>
<td>466 ± 26*</td>
<td>1530 ± 53*</td>
<td>421 ± 18*</td>
<td>639 ± 162*</td>
<td>251 ± 73*</td>
<td>179 ± 19*</td>
</tr>
</tbody>
</table>

Ins = insulin; ZDF = Zucker diabetic fatty rat.
* t Test p < .05 versus control.
* ANOVA/multiple range test statistically significant different versus control.
* t Test p < .05 versus ZDF.
* ANOVA/multiple range test statistically significant different versus ZDF.
0.2 µm/pixel (area = 0.016 mm²). Automatic analysis used an 8-bit color system that translates colors to 256 Gy values for automatic border detection. Slices were accepted for quantitative analysis (1) if cross sections of cardiomyocytes with centrally located nucleus were present and (2) if their cellular membranes were intact. Cardiomyocyte width (CMW) was assessed by marking their borders on laminin slices [22], and cardiomyocyte volumes were assessed by determining cardiomyocyte length (CML) on laminin slices and by calculating CMW/2 × π × CML. The degree of perivascular fibrosis was determined by measurement of the thickness of the collagen I area surrounding the vessels. The thickness of the laminin layers that surrounded cardiomyocytes was measured. The areas positive for fibronectin and collagen I were determined by quantitative image analysis. Area fractions were calculated as the sum of all positive areas related to the area of the entire visual field × 100. Twenty randomly selected visual fields were analyzed to calculate the average of the respective volume fractions. For each parameter, 50 visual fields were analyzed to calculate averages (variance <2%). All measurements were performed by two blinded investigators.

2.8. Statistical analysis

Statistical analysis was performed using SPSS 7.5 (SPSS, USA). Results are expressed as mean ± SEM. Comparisons between multiple groups were assessed by one-way analysis of variance, Students t test, and ANOVA with multiple range test. The strength of the relationship between two variables was assessed by calculation of the product–moment correlation coefficient r. Statistical significance was accepted at p < .05.

3. Results

3.1. Biometric data

As shown in Fig. 1, BW in ZDF rats steadily increased over time compared to nondiabetic lean animals. Treatment with insulin led to a further increase in BW 3 weeks after the beginning of treatment. Absolute heart weight was significantly higher in the treatment group, but not in nontreated control rats compared to age-matched control rats (Table 1). As expected, treatment with insulin decreased blood glucose level towards normoglycemic values in diabetic animals. The diabetic index given as serum glucose level per unit plasma insulin was about fivefold higher in the ZDF group (Fig. 2). Diabetic animals had a 3.0-fold increased level of glycosylated HbA1c when compared to control animals. This was significantly reduced by insulin treatment (1.8-fold increase; Table 1). Plasma C-peptide levels were markedly elevated in diabetic ZDF rats indicating severe hyperinsulinemia. C-peptide levels appeared slightly lower in animals

![Image](https://example.com/image1.png)

Fig. 2. Diabetic index. Diabetic index given as serum glucose level per unit plasma insulin in nondiabetic control rats (Ctr) and diabetic animals without (ZDF) and with insulin treatment (ZDF + Ins). *p < .05 versus Ctr; #p < .05 versus ZDF.

![Image](https://example.com/image2.png)

Fig. 3. Cardiomyocyte volume. To visualize, cell borders cross sections from left ventricular tissue from 19-week-old nondiabetic control rats (Ctr) and diabetic animals without (ZDF) and with insulin treatment (ZDF + Ins) were stained with an antibody against laminin. Specimens were accepted for quantitative analysis if cross sections of cardiomyocytes with intact cell membranes and centrally located nucleus were present. *p < .05 versus Ctr.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>LVM (mg)</th>
<th>Ejection fraction (%)</th>
<th>Fractional shortening (%)</th>
<th>RLVWT</th>
<th>IDD (mm)</th>
<th>IDS (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 7)</td>
<td>820 ± 57</td>
<td>85 ± 2</td>
<td>47 ± 2</td>
<td>0.46 ± 0.02</td>
<td>7.6 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>ZDF (n = 7)</td>
<td>941 ± 68</td>
<td>90 ± 1*</td>
<td>53 ± 2*</td>
<td>0.46 ± 0.03</td>
<td>7.7 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>ZDF ± Ins (n = 6)</td>
<td>1012 ± 71*</td>
<td>91 ± 1*</td>
<td>55 ± 2*</td>
<td>0.54 ± 0.03*</td>
<td>7.3 ± 0.3</td>
<td>2.0 ± 0.2</td>
</tr>
</tbody>
</table>

IDD, inner diastolic diameter of the left ventricle; IDS, inner systolic diameter of the left ventricle; Ins, Insulin; LVM, left ventricular mass; RLVWT, relative left ventricular wall thickness; ZDF, Zucker Diabetic Fatty rat.

* t Test p < .05 versus control.

ANOVA/multiple range test statistically significant different versus control.
receiving insulin treatment (Table 1). Interestingly, heart rate was significantly lower in all diabetic animals and blood pressure was the same in the nontreated ZDF group and even reduced in the insulin-treated ZDF group (Table 1).

3.2. Echocardiography

To evaluate cardiac performance in vivo, echocardiography was performed at the end of the study. The inner diastolic diameter of the left ventricle (IDD) showed no significant difference in the three groups, indicating the absence of a cardiac dilatation (Table 2). The thickness of the posterior wall of the left ventricle (LVPWD), however, showed a moderate but significant increase (+17%) in the insulin-treated diabetic group, but not in nontreated animals, compared to the control group (data not shown). Furthermore, the RLVWT and the LVM significantly increase only in insulin-treated ZDF rats, whereas the ejection fraction and the fraction shortening as indices of the left ventricular systolic function significantly increased in all diabetic animals compared to nondiabetic littermates (Table 2). However, because of significant increase in BW, there was no difference in LVM index between the different groups.

3.3. Morphometry and cardiac extracellular matrix proteins

As shown in Figs. 3 and 4, there was a marked enlargement of the cardiomyocyte cell volume from diabetic animals. This was mainly due to an increase in diameter, whereas cardiomyocyte length remained unchanged in all groups (Ctr: 107 ± 2.4 μm; ZDF: 108 ± 5.8 μm; ZDF + Ins: 106 ± 1.8 μm). Interestingly, cardiomyocyte diameter correlated significantly with HbA1c and serum glucose levels (Pearson’s r = .65 and .84, respectively). Since changes in extracellular matrix proteins have been made responsible for functional alterations in the diseased heart, myocardial collagen I, fibronectin, and laminin were determined by quantitative image analysis. Pericellular laminin thickness revealed a significant increase of about 40% in both diabetic groups in comparison to the control group, whereas there was no difference in fibronectin, interstitial collagen I (Fig. 5), or elastin content (data not shown). However, marked perivascular fibrosis with collagen I was observed in left ventricular tissue from diabetic rats compared to aged-matched control rats. Conversely, there was no difference in myocardial interstitial fibrosis between all groups (Fig. 5). Again, perivascular collagen I and laminin thickness significantly correlated with HbA1c values for the individual data from all groups (Pearson’s r = .66 and .60, respectively; p < .05).

3.4. Renin activity, protein expression, and myocardial ANP expression

Since the renin–angiotensin–aldosterone system (RAAS) plays a pivotal role in the development of hypertrophy in various forms of myocardial disease, we determined its plasma activity in diabetic and nondiabetic animals. Surprisingly,
we found a reduction of approximately 50% in renin activity in diabetic individuals, independently of an additional treatment with insulin (Fig. 6). ANP and BNP are important markers for cardiac hypertrophy [23] and heart failure [24]. ANP mRNA was twofold increased in left ventricular myocardium of diabetic animals, whereas BNP mRNA was unchanged (Fig. 7). mRNA levels for ANP significantly correlated with HbA1c for the individual data from all groups (Pearson’s $r = .7; p < .05$). Furthermore, total protein content of the left ventricular tissue was 7–13% higher in the diabetic group (in mg/g wet weight, Ctr: 144 $\pm$ 4; ZDF: 154 $\pm$ 3*; ZDF + Ins: 162 $\pm$ 4*; *$p < .05$ vs. Ctr).

**Fig. 5.** Morphometric analysis of ECM proteins. Specific phenotypic pattern of myocardial extracellular matrix proteins in nondiabetic control rats (Ctr) and diabetic animals without (ZDF) and with insulin treatment (ZDF + Ins). Values are given in mean ± S.E.M. *$p < .05$ versus Ctr.

**Fig. 6.** Renin plasma activity. Activity of plasma renin in 19-week-old nondiabetic control rats (Ctr) and diabetic animals without (ZDF) and with insulin treatment (ZDF + Ins). Values are given in mean ± S.E.M. *$p < .05$ versus Ctr.

**Fig. 7.** Myocardial expression of natriuretic peptides. Northern blot analysis of left ventricular mRNA levels for ANP and BNP in nondiabetic control rats (Ctr) and diabetic animals without (ZDF) and with insulin treatment (ZDF + Ins). Each mRNA value was corrected for GAPDH mRNA value and given as mean ± S.E.M. *$p < .05$ versus Ctr.
4. Discussion

As demonstrated in the present study, cardiac changes in the metabolic syndrome of the ZDF rat are characterized by a marked hypertrophy of single cardiomyocytes, an increase in myocardial protein content and up-regulation of ANP expression without a significant increase in total heart weight. This is not due to an increase of hemodynamic load as caused by alterations in blood pressure or activation of the RAAS. At this stage, there is a concentric remodeling of the heart with enhanced left ventricular function as assessed by echocardiography. Additional treatment with insulin led to a significant increase in BW, total heart weight, RLVWT, and LVM, respectively. However, there were no changes in single myocyte volume, extracellular matrix proteins or myocardial expression of natriuretic peptides.

Previous clinical data show that myocardial dysfunction frequently occurs in diabetic patients with metabolic syndrome even in the absence of significant macro- or microvascular disease, indicating the existence of a primary cardiomyopathy. The mechanisms, however, responsible for cardiomyopathy in these patients remain unclear.

The general process by which the ventricular myocardium experiences changes in structure and function is often referred to as myocardial remodeling [25]. The central feature of this process is an increase in ventricular mass and on the cellular level hypertrophy of individual cardiomyocytes. A number of factors now have been identified as potential causes of cardiomyocyte hypertrophy, including mechanical stress [26], the sympathetic nervous system [27], the RAAS [26], growth factors, and inflammatory cytokines [28].

As demonstrated in our study, rats with NIDDM show a significant cardiac hypertrophy. The ZDF rat is characterized by peripheral insulin resistance with initial hyperinsulinemia and obesity [29] resulting from a mutant leptin receptor [11]. Several studies have underlined the importance of insulin as a growth factor in a variety of cells [30]. Insulin promotes its known effects through a heterotetrameric transmembrane protein tyrosine kinase receptor (insulin receptor), which also can be detected at the surface of cardiomyocytes [31]. Recent in vitro data suggest that insulin, by activating its receptor, is capable to induce the JAK-2/STAT1 pathway [32] in the heart, which is known to be involved in growth promoting pathways stimulated by different peptides [33]. Indirect evidence supporting the notion that insulin per se favors cardiac hypertrophy comes from transgenic models. Disruption of the insulin-sensitive glucose transporter GLUT4 in mice resulted in hyperinsulinemia associated with decreased longevity and cardiac hypertrophy [34]. Further in vitro experiments by Flier et al. [35] showed that fibroblast DNA synthesis and amino acid transport in response to physiological and supraphysiologic concentrations of insulin can be competitively inhibited by pretreatment with a monoclonal antibody directed specifically against the insulin-like growth factor 1 (IGF-1) receptor. Similarly, T-lymphocytes treated with high insulin concentrations showed a significant increase in proliferation. Again, this effect was blunted by preincubation with an antibody against the IGF-1 receptor [36]. These findings suggest that at supraphysiologic concentrations, the growth promoting effects of insulin are mediated in part through the IGF-1 receptor. Corresponding experiments with cardiomyocytes have not yet been reported. However, the hypothesis that hyperinsulinemia is involved in cardiac hypertrophy is underlined by our findings that additional treatment with insulin resulted in an increase in heart weight, total protein content, and relative wall thickness.

As demonstrated by Phillips et al., the phenotype in ZDF rats is a consequence of a mutation in the leptin receptor gene [11]. It is suggested that leptin plays a pivotal role in obesity. It is mainly secreted by the adipose tissue increasing with obesity and acts by stimulating the sympathetic nervous system and reduces appetite [12]. Albeit the leptin receptor (Ob-R) can be detected on cardiomyocytes and act through the Janus kinase (Jak), the signal transducers and activators of transcription (STAT), or mitogen-activated protein (MAP) kinase pathway, the exact functional role of hyperleptinemia in cardiac hypertrophy has not been determined yet [37]. However, since ZDF rats bear a missense mutation in the leptin receptor, hyperleptinemia is unlikely to be involved in cardiac hypertrophy as seen in this study.

At the age of 19 weeks, an early phase of diabetes, we observed an improved function of the heart as assessed by echocardiography. The mechanism behind the improved systolic function in diabetic animals may be complex and cannot be answered with our experiments. One explanation for this phenomenon may be the combination of a reduced afterload, as shown by the significant decrease in the renin-angiotensin activity and the increase in myocardial contractile mass. In this setting, insulin induced changes in myocardial gene expression (e.g., up-regulation of contractile proteins) potentially playing a role in the improved function of the heart. Interestingly, these findings are in clear contrast to experiments of Zhou et al. [38], who showed a significant reduction of the contractile function (fractional shortening) determined by echocardiography in 20-week-old obese ZDF rats. Albeit the experimental setting was comparable to ours, the difference can be due to the cardiodepressant effects of the used anesthetic regime with pentobarbital sodium. Furthermore, the control group in the aforementioned study was rather small. Ren et al. [39] studied the contraction and relengthening profile of isolated ventricular myocytes from 14-week-old ZDF rats. They found a significant reduction in peak shortening and a prolonged relengthening duration, indicating systolic and diastolic dysfunction. Whether this contradiction is due to the nondiabetic strain of ZDF rats used in the aforementioned study is not presently known.

A second main finding was a significant reduction in PRA, which was not modified by additional treatment with
insulin. Decreased release of renin has been reported in normotensive diabetic patients [40]. Hypertension, nephropathy, and autonomic dysfunction have been proposed as the potential causative factors of this abnormality. However, decreased renin activity has been also described in normotensive diabetic patients without overt nephropathy and autonomic dysfunction, suggesting that other unidentified mechanisms may be involved in renin release. In rats with streptozotocin, induced diabetes with marked hyperglycemia plasma renin is progressively decreased [41]. In the same experiment, the hematocrit is decreased after 8 weeks of diabetes. It was suggested that hyperglycemia causes a decrease in PRA secondary to volume expansion. Supporting this hypothesis, we found a significant inverse correlation between serum glucose levels and PRA.

Increased myocardial mRNA expression of the natriuretic peptides ANP and BNP has been used as an indirect marker for cardiac hypertrophy and failure. The differences in expression of ANP and BNP in the present study suggest that in the diabetic state, BNP gene expression is regulated differently from that of ANP. Differential expression of BNP and ANP has been shown in other models of experimental heart failure. Langenickel et al. [42] showed that induction of a small aortocaval shunt in rats resulted in an approximately 50% increase in total heart weight due to chronic volume overload, without clinical signs of overt heart failure. In these experiments, cardiac hypertrophy was accompanied by a significant increase in myocardial ANP but not BNP expression, whereas animals with large shunts and clinical signs of heart failure also showed an increase in BNP expression. This observation led to the hypothesis that myocardial ANP expression is less dependent on the severity of heart failure and is already induced in the compensated state, when hypertrophy occurs, but left ventricular end-diastolic pressure is still normal. Furthermore, the molecular regulation of ANP and BNP differs. The regulatory regions of the proximal promoter of both genes share only 50% sequence homology [43], suggesting a distinct gene expression in the diabetic state. Since one of the main action of natriuretic peptides is natriuresis and diuresis, it is conceivable that due to the hyperglycemia-induced volume expansion (also not measured in our study), the increase in ANP may be an apparent attempt to overcome the water retention, thereby maintaining the physiological volume homeostasis. This is underlined by the finding in this study that myocardial ANP expression strongly correlates to HbA1c values.

Of note is the observation that ZDF rats display a significant perivascular fibrosis, as seen in hearts from diabetic patients. Furthermore, pericellular laminin deposition was markedly enhanced in diabetic animals. Both findings were also found in OLETF rats [9] and were not influenced by insulin treatment. Albeit the exact mechanism for this phenomena is unclear, TGF-β1, a growth factor that is involved in tissue fibrosis by stimulating extracellular matrix proteins, might be a potential candidate. A previous report on another rat model for NIDDM (Otsuka Lang–Evans Tokushima fatty rats) showed an up-regulation of cardiac TGF-β1 in the prediabetic state (14 weeks), which remained increased until the animals became insulin dependent (54 weeks). These rats developed significant perivascular fibrosis without fibrosis of the interstitium at the age of 30 weeks [9]. This led to the supposition that TGF-β may influence myocardial remodeling in diabetic cardiomyopathy. The analysis of the functional importance of TGF-β in diabetic cardiomyopathy warrants further investigation.

Summarizing the aforementioned data, the myocardial changes in this animal model of the metabolic syndrome with diabetes mellitus are characterized by the development of compensated concentric hypertrophy of the heart, with a decrease in the serum activity of renin and a significant increase in perivascular fibrosis without increase of interstitial collagen content. Additional treatment with insulin seems to augment the hypertrophic response of the heart.

It is intriguing to speculate that on one hand, the myocardial substrate delivery to the diabetic heart is impaired, and on other hand, energy expenditure is increased due to cardiac hypertrophy, thereby finally becoming maladaptive and leading to the energy-depleted state with the clinical feature of heart failure.

5. Summary

The manuscript describes for the first time the functional and morphological changes of the heart in early NIDDM of ZDF rats. The main finding is that at this stage, hyperinsulinemia is associated with marked myocardial hypertrophy and enhanced contractility, independent of alterations in hemodynamic load as caused by changes in blood pressure or activation of the RAAS.

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