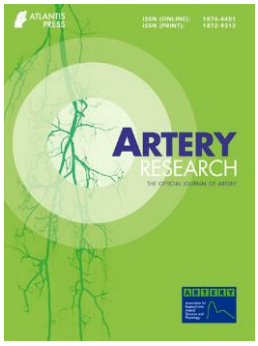

Artery Research

ISSN (Online): 1876-4401 **ISSN (Print): 1872-9312**
Journal Home Page: https://www.atlantis-press.com/journals/artres

Adiponectin negatively correlated with carotid arterial structure in the leptin-resistant Zucker diabetic fatty rat

Emmanuel Cosson, Paul Valensi, André Bado, Hubert Dabiré

To cite this article: Emmanuel Cosson, Paul Valensi, André Bado, Hubert Dabiré (2011) Adiponectin negatively correlated with carotid arterial structure in the leptin-resistant Zucker diabetic fatty rat, Artery Research 6:1, 12–20, DOI: https://doi.org/10.1016/j.artres.2011.08.001

To link to this article: https://doi.org/10.1016/j.artres.2011.08.001

Published online: 7 December 2019

Artery Research (2012) 6, 12–20

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/artres

Adiponectin negatively correlated with carotid arterial structure in the leptin-resistant Zucker diabetic fatty rat

Emmanuel Cosson [a,b,c], Paul Valensi [b,c], André Bado [d], Hubert Dabiré [a,*]

[a] INSERM U 955, Eq03 – IMRB, ENVA - Bâtiment Ferrando, 7 Avenue du Général de Gaulle, Maisons-Alfort F-94704, France
[b] Paris-Nord University, Laboratory of Nutrition, Metabolic Diseases and Cardiovascular Prevention, CRNH-IdF, Bobigny 93140, France
[c] AP-HP, Department of Endocrinology-Diabetology-Nutrition, Jean Verdier Hospital, Bondy 93140, France
[d] INSERM U773, CRB3 – Equipe 2, Paris 75890, France

Received 22 June 2011; received in revised form 29 July 2011; accepted 1 August 2011
Available online 19 August 2011

KEYWORDS
Leptin;
Adiponectin;
Luminal cross-sectional area;
Medial cross-sectional area;
Wall stress;
Zucker diabetic fatty rats

Abstract *Background:* Despite adipocytokines are implicated in arterial hemodynamic and stiffness, their effects on arterial histomorphometry remain poorly explored. The aim of the present study was to evaluate, in Zucker Diabetic Fatty (ZDF) rats, a model of type 2 diabetes with leptin resistance, carotid arterial structural changes and their determinants, with special focus on adiponectin and leptin.
Methods: Proximal aortic blood pressure (BP) was measured in conscious ZDF rats ($n = 6–8$) and their Lean controls ($n = 6–8$) at 6, 12 and 24 weeks. The contralateral carotid was harvested and fixed at the mean BP for histomorphometric quantification.
Results: Mean BP was similar in both strains and increased with age ($p < 0.001$). Medial thickness, luminal cross-sectional area (LCSA), medial cross-sectional area (MCSA) and wall stress (WS) increased with age ($p < 0.001$). LCSA and WS were higher in Lean than in ZDF rats ($p < 0.001$ for both). Leptin levels were higher in ZDF than in Lean rats ($p < 0.001$) but remained unchanged during development in ZDF rats. Adiponectin levels decreased with age in ZDF rats ($p < 0.001$) but remained unchanged in Lean rats. In all rats, adiponectin negatively correlated with medial thickness ($r = -0.50$, $p < 0.01$), LCSA ($r = -0.64$, $p < 0.001$), MCSA ($r = -0.59$, $p < 0.001$) and WS ($r = -0.43$, $p < 0.05$). These correlations were significant ($p < 0.001$) in ZDF rats considered separately ($r = -0.73$, $r = -0.87$, $r = -0.83$ and $r = -0.79$, respectively) but not in Lean rats; independently of mean BP and age after stepwise regression analyses.

Abbreviations: BP, blood pressure; Di, mean internal diameter; EP, external perimeter; h, medial thickness; IMT, intima-media thickness; IP, internal perimeter; LCSA, luminal cross-sectional area; MBP, mean BP; MCSA, medial cross sectional area; R/h, radius/medial thickness ratio; WS, wall stress; ZDF, Zucker Diabetic Fatty.

* Corresponding author. Tel.: +33 1 43 96 73 87; fax: +33 1 43 96 73 99.
E-mail addresses: hdabire@vet-alfort.fr, hubert.dabire@inserm.fr (H. Dabiré).

1872-9312/$ – see front matter © 2011 Association for Research into Arterial Structure and Physiology. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.artres.2011.08.001

Conclusion: These associations suggest a protective role for adiponectin against arterial wall thickening and wall stress. However for causal relation, further investigation is needed.
© 2011 Association for Research into Arterial Structure and Physiology. Published by Elsevier B.V. All rights reserved.

Introduction

Type 2 diabetes is associated with a high cardiovascular morbidity and mortality related to accelerated atherosclerosis. Several factors contribute to the increase of cardiovascular events, including long-term hyperglycemia, insulin resistance, dyslipidemia, hypertension, changes in clotting factors, vago-sympathetic imbalance and arterial wall structure.[1–3]

The role of adipocytokines has been extensively highlighted in the recent years.[4–7] In particular, leptin level is increased in type 2 diabetic patients, with a relative leptin resistance in this population.[4] Inversely, decreased levels of adiponectin have been shown in insulin resistance states.[2,5] Both leptin and adiponectin are likely to play a role in arterial hemodynamics.[2,4,8] Arterial stiffness and high leptin levels predict cardiovascular events.[4,7,9–12]

However the relationships between arterial structure and adipocytokines are poorly known. Matsuda *et al*. have demonstrated that adiponectin-deficient mice showed severe neointimal thickening in mechanically injured arteries, whereas adenovirus-mediated supplement of adiponectin attenuated neointimal proliferation.[13] In humans, adiponectin but not leptin levels have been shown to be negatively associated with intima-media thickness (IMT) in middle-aged healthy white subjects, in type 2 diabetes and in 64 year-old women whatever their glycemic status.[14–16] In another report, the negative association between IMT and adiponectin observed in men disappeared after adjustment for HbA1c and insulin resistance index.[17] Störk *et al*. have recently shown in post-menopausal non-diabetic women that low levels of adiponectin were associated with adverse changes in morphology and function of central arteries over a 12-month period, independently of other cardiovascular risk factors[18] No association was observed for leptin,[18] and some authors suggest to consider leptin/adiponectin ratio as a better atherosclerotic marker.[19] Furthermore, low adiponectin levels have been associated with increased plaque volume, lipid-rich plaque and pathological intimal thickening.[20,21]

To our knowledge the putative association between adipocytokines and arterial wall structure has never been studied in animals. Zucker Diabetic Fatty (ZDF) rats develop with time obesity, insulin resistance, diabetes and dyslipidemia, with controversial data regarding arterial blood pressure (BP).[22] In addition, ZDF rats are leptin-resistant because of homozygous mutation of the leptin receptor and are therefore of interest to explore the role of adipocytokines, especially adiponectin, in arterial structural changes.

The aim of the present study was then to evaluate, during development in ZDF rats, carotid wall structural changes, and to explore their determinants, with a special focus on adiponectin and leptin.

Methods

Animals

Male ZDF rats (Gmi-*fa*/*fa*, $n = 22$) and their age-matched male controls (Lean (*?/fa)*; $n = 20$) were obtained from Charles River France (L'Arbresle, France) at 5 or 6 weeks of age and were acclimatized for at least one week before the experiments. The animals were maintained at 22–24 °C with light on from 0600 to 1800. They were fed A04 (UAR) with tap water *ad libitum*. The study was performed at the 6th, 12th, and 24th week of age, after an overnight fasting. The protocol was approved by the Animal Ethic Committee of Institut National de la Santé et de la Recherche Médicale, Paris, France. The investigation conforms 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).

Blood pressure recording

The technique for BP measurement in rats has been recently described in details.[23] Briefly, the rats were anaesthetized with pentobarbital sodium (60 mg/kg, ip). A polyethylene catheter, filled with heparinised 0.9% NaCl (50 U/ml), was inserted into the descending aorta, through the right common carotid artery, to measure proximal (central) aortic BP. The catheter was tunneled subcutaneously under the skin of the back to exit between the scapulae and was plugged with a short piece of stainless steel wire. The rats were allowed to recover during 24 h in individual cages. Then, in unanesthetised unrestrained rats, the catheter was connected to a signal processor (MacLab 8, AD Instruments, Oxfordshire, UK) via a pressure transducer (BP-T, EMKA Technologies, Paris, France). Aortic BP signals were recorded during at least 1 h on-line at a sampling rate of 1000 points/sec (Chart version 5.2, AD Instruments) and stored on a microcomputer (PowerMac 4400/200, Apple). Further, a stationary 60 s recording, selected at the end of the recording was analyzed beat-to-beat by means of Chart version 5.2 software. This software automatically detected the minimal value of proximal BP (diastolic BP), maximal value of proximal BP (systolic BP) and calculated mean BP (MBP) and heart rate (HR).

Biochemical assays

Blood samples were taken just after BP recording and before the rats were euthanized. Plasma glucose was analyzed using the Infinity glucose test (Thermotrace, Melbourne, Australia). Plasma insulin concentration was assayed with an ELISA kit (ELIT) obtained from Eurobio (Les Ulis, France). Serum triglycerides and total cholesterol

were determined using the IL Test (Instrumentation Laboratory, Milano, Italy) and serum free fatty acids were measured by spectrometric method using the Wako NEFA C Test (Wako Chemicals, Neuss, Germany). The index of insulin resistance was appreciated by the homeostatic model assessment (HOMA). Serum leptin and total adiponectin concentrations were assessed with mouse leptin and adiponectin RIA kits, respectively (Linco Research CAT # HZDP-61HK, St. Charles, MO, USA).

Carotid wall histomorphometry

The left carotid artery was carefully excised. A 1-cm long sample was perfused *in vitro* with 10% formol containing phosphate-buffered saline at its individual peripheral MBP to provide the tissue fixation closest to the physiological *in situ* state of the vessel. To that purpose, the carotid segment was mounted in a specially designed organ chamber and connected to a perfusion line both containing 10% formol phosphate-buffered saline. The carotid segment was submitted constantly to the MBP of the animal for 20 min. The sample was secondarily dehydrated and then embedded in paraffin. Two sections (6 μm thickness) were cut and stained with orceine for double-blind measurement of carotid external (EP) and internal (IP) perimeters, and medial thickness (h, the distance between the external and the internal elastic laminae was measured 4 times and the average was calculated; μm). All the measurements (Fig. 1), including luminal cross-sectional area ($LCSA = IP^2/4\pi$), were performed with a computer-directed colour analysis (Quant'image software, Talence, France). Mean internal diameter ($Di = IP/\pi$), medial cross-sectional area ($MCSA = (EP^2/4\pi)$-LCSA), wall stress ($WS = MBP \times Di/2h$; Lamé's equation) were also calculated. The radius/medial thickness ratio (R/h with $R = Di/2$) was calculated. Elastin content was quantified according to the analysis of optic density.

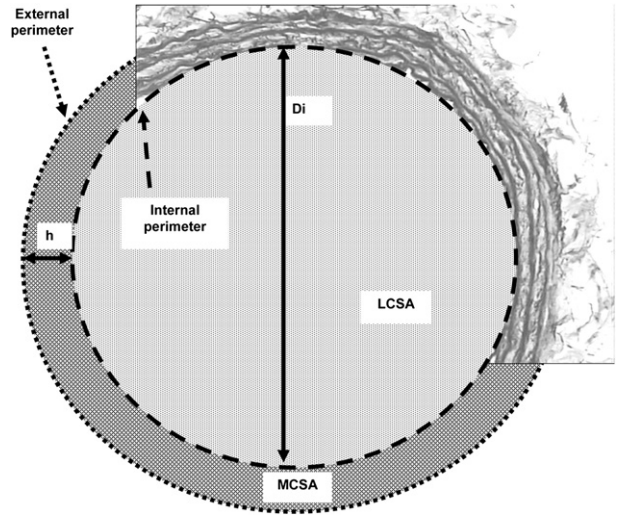

Figure 1 Schematic representation of a section of carotid artery for the determination of histomorphometric parameters: h, medial thickness; Di, mean internal diameter; LCSA, luminal cross-sectional area; MCSA, medial cross-sectional area.

Statistical analyses

Results are given as means ± SEM. Two ways ANOVA followed by a Fischer Protected Least Significant Difference (PLSD) tests for multiple comparisons were used to assess the significance of the results. To evaluate the major determinants of medial thickness, MCSA, LCSA and wall stress, robust stepwise regression analysis was used. Variables entered in the model were body weight, age, MBP (except for wall stress) and adipocytokines (adiponectin for ZDF rats, leptin for Lean rats). The statistical analysis was performed with NCSS 6.0 package software (Hintze JL, Kaysville, Utah, USA). A p value < 0.05 was considered as statistically significant.

Results

Body weight and biochemical parameters

Table 1 shows the changes in body weight and biochemical parameters with age in the two strains. ZDF rats were overweight compared with Lean control rats at wks 6 and 12, but underweight at wk 24. They were diabetic at wk 12 and 24 but not at wk 6, with hyperinsulinemia at 6 and 12 wks but not at wk 24. Plasma lipid levels were higher in ZDF rats as compared with Lean rats at every age. The index of insulin resistance, HOMA, was higher in ZDF rats compared to Lean rats, whatever the age, but was significantly reduced at wk 24 of age as compared to wk 12 of age. Adiponectin levels significantly and progressively decreased with age in the ZDF rats, whereas it did not change with age in the Lean control rats. The significant interaction shows that in ZDF rats, adiponectin was significantly higher at wk 6, but lower at wk 12 and 24 as compared to their controls. Leptin levels were higher in ZDF rats than in Lean rats at wks 6 and 12. Leptin/adiponectin ratio was higher at every age in ZDF rats than in Lean rats.

Blood pressure, carotid histomorphometric parameters and wall stress

Table 2 and Fig. 2 show the changes in systolic BP and carotid arterial structure with ageing in each strain. Systolic BP was similar in ZDF and Lean rats at wks 6, 12 and 24, with an increase between wks 6 and 12, and stability between wks 12 and 24. The same profile was observed for diastolic BP and MBP (results not shown). Wall stress was lower in ZDF rats and increased with age, especially in Lean rats. R/h ratio was lower in ZDF than in Lean rats (strain effect <0.001) whereas elastin content was similar and stable with ageing in both strains (Table 2). Medial thickness was higher in ZDF rats than in Lean rats only at wk 12, and increased significantly ($p < 0.001$) with age in both strains. LCSA was significantly lower in ZDF rats than in Lean rats (strain effect <0.001) at wks 6 and 12. MCSA was similar in the 2 strains and significantly increased with age in both strains (age effect <0.001) (Fig. 2).

Table 1 Weight and biochemical characteristics of ZDF rats and their Lean controls.

	Strain	Age (weeks)			*p* value (ANOVA)		
		6	12	24	Strain	Age	Interaction
Number of rats	ZDF	7	7	8			
	Lean	7	7	6			
Body weight (g)	ZDF	140 ± 6	326 ± 4[b]	357 ± 8[b]	0.165	<0.001	<0.001
	Lean	120 ± 4[a]	290 ± 6[a,b]	392 ± 8[a,b]			
Plasma glucose level (mmol/l)	ZDF	7.79 ± 0.19	21.7 ± 1.5[b]	30.1 ± 1.7[b]	<0.001	<0.001	<0.001
	Lean	7.02 ± 0.32	7.64 ± 0.18[a]	9.05 ± 0.35[a,b]			
Plasma insulin level (pmol/l)	ZDF	337 ± 38	672 ± 102[b]	287 ± 45	<0.001	<0.001	<0.001
	Lean	42±5[a]	130 ± 18[a,b]	243 ± 15[b]			
Total cholesterol (mmol/l)	ZDF	2.60 ± 0.10	3.24 ± 0.17[b]	5.07 ± 0.35[b]	<0.001	<0.001	<0.001
	Lean	1.91 ± 0.10[a]	1.83 ± 0.05[a]	2.56 ± 0.07[a,b]			
Triglycerides (mmol/l)	ZDF	0.47 ± 0.08	2.58 ± 0.56[b]	0.83 ± 0.18[b]	<0.001	<0.001	<0.001
	Lean	0.27 ± 0.04[a]	0.29 ± 0.05[a]	0.27 ± 0.03[a]			
Free fatty acids (mmol/l)	ZDF	1.36 ± 0.16	3.13 ± 0.32[b]	1.92 ± 0.18[b]	<0.001	<0.001	<0.001
	Lean	0.84 ± 0.08[a]	0.95 ± 0.06[a]	0.97 ± 0.06[a]			
HOMA	ZDF	16.29 ± 8.19	89.19 ± 8.19[b]	55.10 ± 7.66[b]	<0.001	<0.001	<0.001
	Lean	1.81 ± 8.19[a]	5.72 ± 7.22[a,b]	13.47 ± 8.84[a,b]			
Leptin (ng/ml)	ZDF	10.20 ± 1.22	9.66 ± 0.34	8.89 ± 0.93	<0.001	<0.01	<0.001
	Lean	1.31 ± 0.18[a]	2.91 ± 0.41[a]	7.67 ± 0.84			
Adiponectin (μg/ml)	ZDF	18.71 ± 1.43	6.00 ± 0.62[b]	3.21 ± 0.45[b]	0.095	<0.001	<0.001
	Lean	8.24 ± 0.96[a]	8.40 ± 1.26[a]	7.15 ± 0.85[a]			
Leptin/adiponectin ratio	ZDF	0.57 ± 0.09	1.76 ± 0.26[b]	2.93 ± 0.35[b]	<0.001	<0.001	<0.001
	Lean	0.17 ± 0.02[a]	0.37 ± 0.06[a,b]	1.17 ± 0.19[a,b]			

Data are mean ± SEM.
HOMA, homeostatic model assessment.
[a] $p < 0.05$ versus ZDF at the same age.
[b] $p < 0.05$ versus week 6 for the same strain.

Correlations between arterial structural parameters and adipocytokines

Table 3 shows the univariate correlations in the overall series of rats and in ZDF rats and Lean rats considered separately. Medial thickness correlated positively with age, body weight and MBP (except in Lean rats taken separately). Similar correlations were observed between LCSA or MCSA and age, body weight and MBP. Wall stress correlated with body weight and age (except in Lean rats considered separately) (Table 3).

Adiponectin significantly and negatively correlated to medial thickness, LCSA, MCSA and wall stress in the overall population and in particular in ZDF rats. There were no correlations in Lean rats considered separately (Table 3). In contrast, leptin significantly and positively correlated to medial thickness, LCSA and MCSA, only in Lean rats considered separately. No correlation was observed

Table 2 Blood pressure, carotid histomorphometry and wall stress in ZDF rats and their Lean controls.

	Strain	Age (weeks)			*p* value (ANOVA)		
		6	12	24	Strain	Age	Interaction
Number of rats	ZDF	7	7	8			
	Lean	7	7	6			
Systolic blood pressure (mmHg)	ZDF	121 ± 3	131 ± 5[b]	136 ± 3[b]	0.069	<0.001	0.916
	Lean	125 ± 4	138 ± 3[b]	140 ± 3[b]			
	Lean	69.5 ± 2.5	84.5 ± 2.5[b]	102.2 ± 2.5[b]			
Wall stress (mmHg)	ZDF	750 ± 27	882 ± 21[b]	926 ± 41[b]	<0.001	<0.001	0.168
	Lean	834 ± 49	1082 ± 28[a,b]	993 ± 40[b]			
Radius/medial thickness ratio	ZDF	7.66 ± 0.31	7.69 ± 0.22	7.95 ± 0.29	<0.001	0.158	0.089
	Lean	8.20 ± 0.20	9.16 ± 0.17[a]	8.37 ± 0.34			
Elastin content (%)	ZDF	23 ± 1	24 ± 2	21 ± 3	0.837	0.177	0.716
	Lean	21 ± 3	25 ± 1	21 ± 1			

Data are mean ± SEM.
[a] $p < 0.05$ versus ZDF at the same age.
[b] $p < 0.05$ versus week 6 for the same strain.

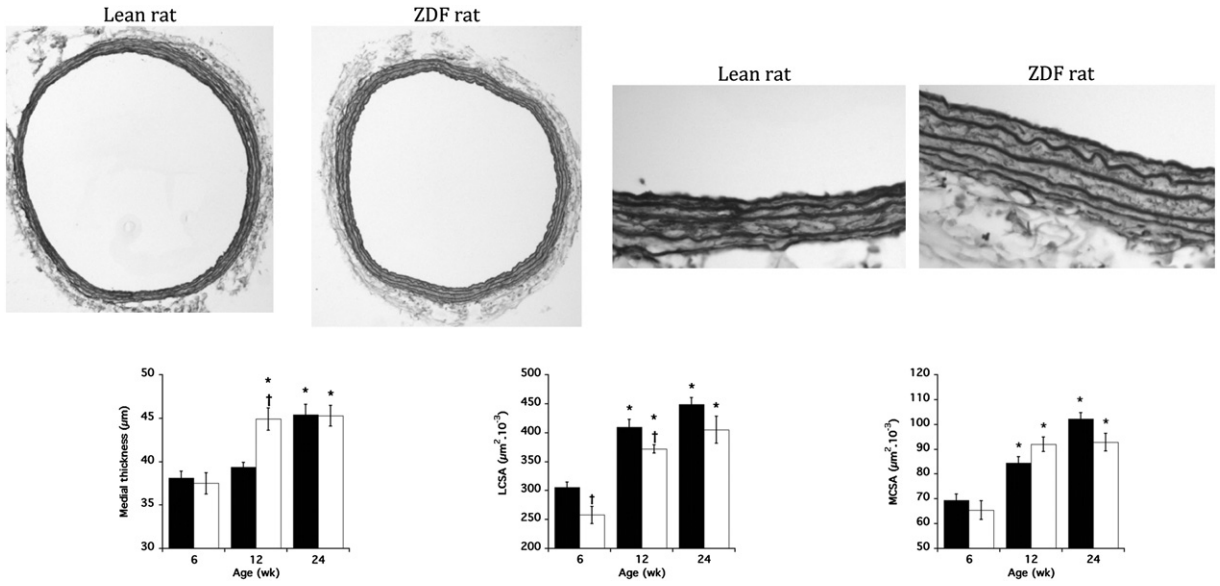

Figure 2 Representative histological images of carotid arteries of a Lean and a ZDF rat at 12 weeks old (orceine staining; x10 and x40) showing significant decrease in luminal cross-sectional area (LCSA) and increase in medial thickness in ZDF rats. The changes of the mean values of medial thickness, LCSA and media cross-sectional area (MCSA) are presented at the bottom. Each bar is the mean ± SEM of 6–8 rats. Full bars, Lean rats; open bars, ZDF rats. $*p < 0.001$ compared to 6 weeks for the same strain; $\dagger p < 0.05$ compared to Lean rats at the same age.

Table 3 Univariate correlations with carotid wall histomorphometric parameters.

	All rats		ZDF rats		Lean rats	
	r	*p* value	r	*p* value	r	*p* value
Medial thickness						
Age	0.68	< 0.001	0.59	< 0.01	0.83	< 0.001
Body weight	0.73	< 0.001	0.78	< 0.001	0.73	< 0.001
Mean blood pressure	0.59	< 0.001	0.86	< 0.001	0.39	0.235
Adiponectin	−0.50	<0.01	−0.73	<0.001	−0.13	0.611
Leptin	0.35	<0.05	−0.15	0.553	0.73	<0.001
Leptin/Adiponectin ratio	0.56	<0.001	0.56	<0.05	0.58	<0.01
Luminal cross-sectional area						
Age	0.74	< 0.001	0.75	< 0.001	0.82	< 0.001
Body weight	0.83	< 0.001	0.87	< 0.001	0.91	< 0.001
Mean blood pressure	0.69	< 0.001	0.69	< 0.001	0.70	< 0.001
Adiponectin	−0.64	<0.001	−0.87	<0.001	−0.14	0.599
Leptin	−0.01	0.964	−0.35	0.160	0.75	<0.001
Leptin/Adiponectin ratio	0.33	<0.05	0.57	<0.05	0.75	<0.001
Media cross-sectional area						
Age	0.77	< 0.001	0.90	< 0.001	0.64	< 0.01
Body weight	0.88	< 0.001	0.77	< 0.001	0.91	< 0.001
Mean blood pressure	0.68	< 0.001	0.86	< 0.001	0.60	< 0.001
Adiponectin	−0.59	<0.001	−0.83	<0.001	−0.15	0.551
Leptin	0.22	0.215	−0.21	0.414	0.78	<0.001
Leptin/Adiponectin ratio	0.49	<0.01	0.57	<0.05	0.76	<0.001
Wall stress						
Age	0.43	< 0.01	0.65	< 0.01	0.38	0.113
Body weight	0.57	< 0.001	0.78	< 0.001	0.58	< 0.01
Adiponectin	−0.43	<0.05	−0.79	<0.001	0.12	0.658
Leptin	−0.18	0.319	−0.34	0.185	0.38	0.130
Leptin/Adiponectin ratio	0.12	0.496	0.48	<0.05	0.40	0.113

between wall stress and leptin (Table 3). The leptin/adiponectin ratio significantly and positively correlated to medial thickness, LCSA and MCSA, but not with wall stress, except in ZDF rats considered separately (Table 3).

Table 4 shows the results of stepwise regression analyses for the determinants of arterial structural parameters in ZDF and in Lean rats. In ZDF rats, MBP and body weight were the only determinants of medial thickness and MCSA, respectively whereas adiponectin was the only independent determinant of LCSA and wall stress. In Lean rats, age and body weight were significant determinants of medial thickness and, LCSA and MCSA, respectively. The two variables were independently associated to wall stress, explaining 34% and 17% of its variance, respectively. Adjustment to other cardiovascular risk factors as total cholesterol and HOMA did not change these results in both ZDF and Lean rats (not shown).

Discussion

The present study evaluated vascular wall structure and wall stress in ZDF rats and investigated the correlations with adipocytokines in this model of type 2 diabetes characterized by a leptin resistance. In contrast to their Lean controls, leptin levels were high but did not increase with ageing along with fat mass. Conversely, adiponectin levels decreased with ageing in ZDF rats, but did not change in Lean rats. Carotid histomorphometric analysis showed that LCSA was lower in ZDF rats compared to Lean rats and that medial thickness tended to increase earlier in ZDF rats than in Lean rats. Lower LCSA together with similar medial thickness may suggest that the intima was larger in ZDF rats than in Lean rats. Furthermore, the lower R/h ratio observed in ZDF rats than in Lean rats suggested a eutrophic internal remodeling in the formers. Simultaneously, BP was similar in both strains at every age as we previously reported.[23] As a result, wall stress was lower in ZDF rats than in Lean rats. Finally, the major finding of our study was that adiponectin negatively correlated with medial thickness, LCSA, MCSA and wall stress. These correlations were especially observed in ZDF rats, independently of body weight, age and MBP. Conversely, independent positive correlations between leptin and LCSA and between leptin and MCSA were observed in Lean rats.

Compared to Lean rats, ZDF rats revealed to be heavier from 7 to 23 wks old. They had similar body weight as Lean rats between 20 and 31 wks old and became lighter than Lean rats between 28 and 36 wks old.[24,25] Likewise, Szöcs et al. have recently shown that body weight gain in ZDF rats was linear and high from 7 to 12.5 weeks (0–150 g) but became very less between 15.5 and 18 weeks olds (15 and 5 g).[26] Our results are therefore in accordance with the literature as our ZDF rats were heavier than Lean rats at 6 and 12 wks old but lighter than Lean rats at 24 wks old.

Body weight may play an important role in arterial structural parameters. Indeed, in obese subjects and type 2 diabetic patients, IMT is higher than in controls and is associated to body weight.[15] In the present experiments, the lower LCSA together with similar medial thickness suggests that the intima was larger in ZDF rats than in Lean rats. As in humans, in Lean rats, body weight was associated to LCSA, MCSA and wall stress, independently of MBP, age or leptin. By contrast, in ZDF rat, body weight was associated only to MCSA, whereas adiponectin is the major determinant of LCSA and wall stress. These results suggest that in type 2 diabetes, adiponectin may play a major role in arterial structural changes whereas body weight, but not leptin may be the major determinant of arterial structure in obesity.

Adipose tissue is not only a simple energy pool, but also secretes metabolic hormones, enzymes, anti-fibrinolytic proteins, and inflammatory cytokines.[6,27] In contrast to adiponectin, other adipocytokines, including leptin, are markedly up regulated in obesity. Despite higher concentrations, the inhibitory effect on food intake of leptin is not enhanced, suggesting a leptin resistance.[2,4] The model of ZDF rats is therefore interesting since these rats genetically resist to leptin.[22] As a result, we showed, as others, that leptin levels were high in this model and did not increase in parallel with adipose tissue growth.[28] In agreement with the leptin resistance, leptin did not correlate in ZDF rats with histomorphometric parameters. In contrast, positive correlations were observed in Lean rats between leptin levels and medial thickness, LCSA and MCSA. The correlation with LCSA and MCSA disappeared in the stepwise regression analysis when body weight was introduced as an independent variable, suggesting that in Lean rats, body weight influences more arterial structure than leptin.

In humans no correlation was found between leptin and IMT in post-menopausal women, in type 2 diabetic patients or in controls.[15,18] Furthermore, leptin-deficient *ob*/*ob* mice develop only small lesion after arterial injury even if kept on atherogenic diet despite severe obesity and proatherogenic metabolic profile. Administration of leptin enhances lesion formation in wild type and *ob*/*ob* mice, despite reducing body weight and plasma lipids and improving insulin sensitivity.[29] These experiments and others have suggested that leptin promotes remodeling and neointimal growth; with a protective effect due to leptin resistance[30] and that adipocytokines may play a crucial role, which is expected to be emphasized in case of leptin resistance.

One of the most interesting features of adiponectin is that its adipose tissue expression and plasma concentration are reduced in overweight and obese subjects.[31] In the present study, adiponectin decreased accordingly with age in ZDF rats as previously reported.[32] However, despite a higher body weight than, and similar plasma glucose level as Lean rats, a high level of plasma adiponectin was observed in ZDF rats at 6 wks old. This seems unexpected. Nonetheless, Szöcs et al. have shown a negative association between plasma glucose and plasma adiponectin in ZDF rats and have suggested that the impact of metabolic deterioration on circulating adiponectin is by far superior to established direct influences of body weight.[26] Therefore, the changes in biochemical parameters observed in the present experiments are in agreement with those reported by others.[24,26]

In the present study, in ZDF rats, adiponectin correlated with MCSA, LCSA and wall stress, independently of age and BP. In humans, a negative correlation was also observed between adiponectin and IMT in post-menopausal women whatever their glucose status,[16,18] in middle-aged healthy

Table 4 Stepwise regression analysis of the determinants of arterial structural parameters in ZDF rats and their Lean controls.

Variable	ZDF rats					Lean rats				
	In	Standard coefficient	R^2 increment	t value	*p* value	In	Standard coefficient	R^2 increment	t value	*p* value
Medial thickness										
Mean blood pressure	Yes	0.867	0.752	6.737	<0.001	No		0.023	1.083	0.297
Body weight	No		0.017	1.008	0.330	No		0.009	0.679	0.500
Adiponectin	No		0.013	0.879	0.394					
Leptin						No		0.014	0.836	0.417
Age	No		0.007	0.628	0.540	Yes	0.840	0.706	6.002	<0.001
	Adjusted global $R^2 = 0.75$					Adjusted global $R^2 = 0.71$				
Luminal cross-sectional area										
Mean blood pressure	No		0.009	0.718	0.485	No		0.005	0.687	0.503
Body weight	No		0.010	0.776	0.450	Yes	0.915	0.838	8.803	<0.001
Adiponectin	Yes	−0.871	0.759	−0.868	<0.001					
Leptin						No		0.000	0.102	0.920
Age	No		0.007	0.670	0.514	No		0.004	0.604	0.556
	Adjusted global $R^2 = 0.76$					Adjusted global $R^2 = 0.84$				
Media cross-sectional area										
Mean blood pressure	No		0.014	0.919	0.374	No		0.002	0.478	0.640
Body weight	Yes	0.865	0.749	6.684	<0.001	Yes	0.928	0.862	9.667	<0.001
Adiponectin	No		0.000	0.025	0.981					
Leptin						No		0.000	0.127	0.901
Age	No		0.001	0.211	0.836	No		0.019	1.475	0.162
	Adjusted global $R^2 = 0.75$					Adjusted global $R^2 = 0.86$				
Wall stress										
Body weight	No		0.007	0.496	0.628	Yes	1.732	0.339	3.095	<0.01
Adiponectin	Yes	−0.785	0.616	−4.904	<0.001					
Leptin						No		0.005	0.371	0.717
Age	No		0.005	0.415	0.684	Yes	−1.224	0.169	−2.187	<0.05
	Adjusted global $R^2 = 0.62$					Adjusted global $R^2 = 0.50$				

In, entering or removal of variable after stepwise regression; Yes, variable entering in stepwise regression model; No, variable is removed after stepwise regression. Adiponectin was used only in ZDF and leptin only in Lean rats.

white subjects,[14] in patients with coronary arterial disease,[33] in men[17] and in people with[34] or without diabetes.[15] Such an association does not mean a causal relationship. Therefore, animal studies might further highlight the potential role of adiponectin on vessel wall structure as shown in adiponectin-deficient mice and in rabbits.[13,35] In the latter, local transfer of adiponectin gene in aorta adventitia or intima reduced the development of atherosclerotic plaques.

The role played by adiponectin may be multifactorial. First, adiponectin has been shown to exhibit antiproliferative properties. This effect may be exerted through inhibition of growth factors.[36,37] Adiponectin has been reported to inhibit angiogenesis, endothelial cell proliferation and migration and to be tightly related to endothelial function.[38–41] When the vascular endothelium is injured, adiponectin accumulates in the subintimal space of the arterial wall through its interaction with collagen in the vascular intima.[42] Adiponectin inhibits tumor necrosis factor (TNF) α-induced monocyte adhesion and expression of endothelial-leucocyte adhesion molecule 1 (E-selectin), vascular cell adhesion molecule-1 (VCAM-1)[43] and intracellular adhesion molecule-1 (ICAM-1) on the endothelium.[44] Recently it has been shown that adiponectin exerts an NO-dependent vasodilatation in resistance arteries of Lean rats but not ZDF rats, and this may contribute to endothelium dysfunction in ZDF rats, leading to the changes observed in arterial structure.[45] Adiponectin also suppresses lipid accumulation in monocyte-derived macrophages through the suppression of macrophage scavenger receptor expression.[13] Finally, adiponectin may also play a beneficial role through a decrease in arterial stiffness,[46] as suggested by a negative correlation between these parameters in patients with hypertension,[9] metabolic syndrome[47] and in Black asymptomatic young adults.[48] Furthermore, the changes in arterial stiffness under glitazones or metformin correlate negatively with changes in plasma adiponectin levels.[10]

In conclusion, the different associations observed between arterial structure and adipocytokines in ZDF rats, a model of insulin resistance, although not providing a direct causal relationship, strongly suggest that adiponectin may play a protective role against arterial wall thickening and wall stress and that the balance between leptin and adiponectin may be crucial in obesity-related arterial structural changes. The data further suggest that modifying adiponectin levels may be beneficial against cardio-metabolic disease.[3] In this respect, further experiments remained to be performed to draw a clear-cut conclusion.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

We thank Mrs Monique Herissé for assistance in the investigations.

References

1. Safar ME. Systolic blood pressure, pulse pressure and arterial stiffness as cardiovascular risk factors. *Curr Opin Nephrol Hypertens* 2001;**10**:257–61.
2. Valensi P, Chanu B, Cosson E. Obesity, metabolic syndrome, diabetes and arterial hypertension. *Immun Endoc Metab Agents Med Chem* 2006;**6**:407–23.
3. Szmitko PE, Teoh H, Stewart DJ, Verma S. Adiponectin and cardiovascular disease: state of the art? *Am J Physiol Heart Circ Physiol* 2007;**292**:H1655–63.
4. Beltowski J. Leptin and atherosclerosis. *Atherosclerosis* 2006; **189**:47–60.
5. Okamoto Y, Kihara S, Funahashi T, Matsuzawa Y, Libby P. Adiponectin: a key adipocytokine in metabolic syndrome. *Clin Sci* 2006;**110**:267–78.
6. Fantuzzi G, Mazzone T. Adipose tissue and atherosclerosis: exploring the connection. *Arterioscler Thromb Vasc Biol* 2007; **27**:996–1003.
7. Antoniades C, Antonopoulos AS, Tousoulis D, Stefanadis C. Adiponectin: from obesity to cardiovascular disease. *Obes Rev* 2009;**10**(3):269–79.
8. Ohashi K, Kihara S, Ouchi N, Kumada M, Fujita K, Hiuge A, et al. Adiponectin replenishment ameliorates obesity-related hypertension. *Hypertension* 2006;**47**:1108–16.
9. Mahmud A, Feely J. Adiponectin and arterial stiffness. *Am J Hypertens* 2005;**18**:1543–8.
10. Araki T, Emoto M, Teramura M, Yokoyama H, Mori K, Hatsuda S, et al. Effect of adiponectin on carotid arterial stiffness in type 2 diabetic patients treated with pioglitazone and metformin. *Metab Clin Exp* 2006;**55**:996–1001.
11. Snijder MB, Flyvbjerg A, Stehouwer CDA, Frystyk J, Henry RMA, Seidell JC, et al. Relationship of adiposity with arterial stiffness as mediated by adiponectin in older men and women: the Hoorn Study. *Eur J Endocrinol* 2009;**160**:387–95.
12. Windham BG, Griswold ME, Farasat SM, Ling SM, Carlson O, Egan JM, et al. Influence of leptin, adiponectin, and resistin on the association between abdominal adiposity and arterial stiffness. *Am J Hypertens* 2010;**23**:501–7.
13. Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, et al. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem* 2002;**277**:37487–91.
14. Iglseder B, Mackevics V, Stadlmayer A, Tasch G, Ladurner G, Paulweber B. Plasma adiponectin levels and sonographic phenotypes of subclinical carotid artery atherosclerosis: data from the SAPHIR Study. *Stroke* 2005;**36**:2577–82.
15. Dullaart RPF, de Vries R, van Tol A, Sluiter WJ. Lower plasma adiponectin is a marker of increased intima-media thickness associated with type 2 diabetes mellitus and with male gender. *Eur J Endocrinol* 2007;**156**:387–94.
16. Behre CJ, Brohall G, Hulthe J, Wikstrand J, Fagerberg B. Are serum adiponectin concentrations in a population sample of 64-year-old Caucasian women with varying glucose tolerance associated with ultrasound-assessed atherosclerosis? *J Intern Med* 2006;**260**:238–44.
17. Nilsson PM, Engström G, Hedblad B, Frystyk J, Persson MM, Berglund G, et al. Plasma adiponectin levels in relation to carotid intima media thickness and markers of insulin resistance. *Arterioscler Thromb Vasc Biol* 2006;**26**:2758–62.
18. Störk S, Bots ML, Angerer P, von Schacky C, Grobbee DE, Angermann CE, et al. Low levels of adiponectin predict worsening of arterial morphology and function. *Atherosclerosis* 2007;**194**:e147–53.
19. Kotani K, Sakane N, Saiga K, Kurozawa Y. Leptin: adiponectin ratio as an atherosclerotic index in patients with type 2 diabetes: relationship of the index to carotid intima-media thickness. *Diabetologia* 2005;**48**:2684–6.

20. Marso SP, Mehta SK, Frutkin A, House JA, McCrary JR, Kulkarni KR. Low adiponectin levels are associated with atherogenic dyslipidemia and lipid-rich plaque in nondiabetic coronary arteries. *Diabetes Care* 2008;**31**:989—94.
21. Gustafsson S, Lind L, Söderberg S, Ingelsson E. Associations of circulating adiponectin with measures of vascular function and morphology. *J Clin Endocrinol Metab* 2010;**95**:2927—34.
22. Russell JC, Proctor SD. Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. *Cardiovasc Pathol* 2006;**15**: 318—30.
23. Cosson E, Valensi P, Laude D, Mesangeau D, Dabire H. Arterial stiffness and the autonomic nervous system during the development of Zucker diabetic fatty rats. *Diabetes Metab* 2009;**35**: 364—70.
24. Hoshi S, Shu Y, Yoshida F, Inagaki T, Sonoda J, Watanabe T, et al. Podocyte injury promotes progressive nephropathy in zucker diabetic fatty rats. *Lab Invest* 2002;**82**:25—35.
25. Mizuno M, Sada T, Kato M, Koike H. Renoprotective effects of blockade of angiotensin II AT1 receptors in an animal model of type 2 diabetes. *Hypertens Res* 2002;**25**:271—8.
26. Szöcs Z, Brunmair B, Stadlbauer K, Nowotny P, Bauer L, Luger A, et al. Age-dependent development of metabolic derangement and effects of intervention with pioglitazone in Zucker diabetic fatty rats. *J Pharmacol Exp Ther* 2008;**326**: 323—9.
27. Matsuzawa Y, Funahashi T, Nakamura T. Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. *Ann N Y Acad Sci* 1999; **892**:146—54.
28. Janssen SW, Martens GJ, Sweep CG, Ross HA, Hermus AR. Zucker diabetic fatty rats plasma leptin levels are correlated with plasma insulin levels rather than with body weight. *Horm Metab Res* 1999;**31**:610—5.
29. Schäfer K, Halle M, Goeschen C, Dellas C, Pynn M, Loskutoff DJ, et al. Leptin promotes vascular remodeling and neointimal growth in mice. *Arterioscler Thromb Vasc Biol* 2004;**24**:112—7.
30. Bodary PF, Shen Y, Ohman M, Bahrou KL, Vargas FB, Cudney SS, et al. Leptin regulates neointima formation after arterial injury through mechanisms independent of blood pressure and the leptin receptor/STAT3 signaling pathways involved in energy balance. *Arterioscler Thromb Vasc Biol* 2007;**27**:70—6.
31. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257**:79—83.
32. Altomonte J, Harbaran S, Richter A, Dong H. Fat depot-specific expression of adiponectin is impaired in Zucker fatty rats. *Metab Clin Exp* 2003;**52**:958—63.
33. Kojima S, Funahashi T, Maruyoshi H, Honda O, Sugiyama S, Kawano H, et al. Levels of the adipocyte-derived plasma protein, adiponectin, have a close relationship with atheroma. *Thromb Res* 2005;**115**:483—90.
34. Karakitsos D, De Groot E, Patrianakos AP, Parthenakis F, Boletis J, Karabinis A, et al. Adiponectin and cardiovascular remodeling in end-stage renal disease and co-morbid diabetes mellitus. *Am J Nephrol* 2006;**26**:340—7.
35. Li C-J, Sun H-W, Zhu F-L, Chen L, Rong Y-Y, Zhang Y, et al. Local adiponectin treatment reduces atherosclerotic plaque size in rabbits. *J Endocrinol* 2007;**193**:137—45.
36. Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, et al. Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem* 2003;**24**(278):2461—8.
37. Wang Y, Lam KSL, Xu JY, Lu G, Xu LY, Cooper GJS, et al. Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner. *J Biol Chem* 2005;**280**:18341—7.
38. Brakenhielm E, Veitonmäki N, Cao R, Kihara S, Matsuzawa Y, Zhivotovsky B, et al. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. *Proc Natl Acad Sci U.S.A* 2004;**101**:2476—81.
39. Fernandez-Real J-M, Castro A, Vázquez G, Casamitjana R, López-Bermejo A, Peñarroja G, et al. Adiponectin is associated with vascular function independent of insulin sensitivity. *Diabetes Care* 2004;**27**:739—45.
40. Takano H, Kodama Y, Kitta Y, Nakamura T, Obata J-ei, Mende A, et al. Transcardiac adiponectin gradient is independently related to endothelial vasomotor function in large and resistance coronary arteries in humans. *Am J Physiol Heart Circ Physiol* 2006;**291**. H2H264641—6.
41. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, et al. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation* 2002;**105**:2893—8.
42. Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, et al. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. *Horm Metab Res* 2000;**32**:47—50.
43. Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, et al. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2002;**106**:2767—70.
44. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999;**100**:2473—6.
45. Schmid PM, Resch M, Steege A, Fredersdorf-Hahn S, Stoelcker B, Birner C, et al. Globular and Full-Length adiponectin Induce NO-Dependent Vasodilation in resistance arteries of zucker Lean but not zucker diabetic fatty rats. *Am J Hypertens* 2011;**24**:270—7.
46. Tsioufis C, Dimitriadis K, Selima M, Thomopoulos C, Mihas C, Skiadas I, et al. Low-grade inflammation and hypoadiponectinaemia have an additive detrimental effect on aortic stiffness in essential hypertensive patients. *Eur Heart J* 2007;**28**:1162—9.
47. Kim SG, Ryu OH, Kim HY, Lee KW, Seo JA, Kim NH, et al. Effect of rosiglitazone on plasma adiponectin levels and arterial stiffness in subjects with prediabetes or non-diabetic metabolic syndrome. *Eur J Endocrinol* 2006;**154**:433—40.
48. Nguyen QM, Srinivasan SR, Xu J-H, Chen W, Berenson GS. Racial (black-white) divergence in the association between adiponectin and arterial stiffness in asymptomatic young adults: the Bogalusa heart study. *Am J Hypertens* 2008;**21**:553—7.