RD-Néphrologie

INFLUENCE OF GLUCOSE METABOLISM DYSREGULATION ON EX VIVO AORTIC CALCIFICATION IN RATS

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INTRODUCTION AND AIMS: Vascular calcification is frequently observed in diabetes and metabolic syndrome and is associated with an increased risk of cardiovascular events. Insulin resistance, obesity and inflammation are key factors promoting vascular calcification. The purpose of the present work was to gain insight in the participation of glucose metabolism dysregulation on vascular calcification.

METHODS: The ability to calcify was assessed ex vivo in rat aortic rings in which the glucose metabolism is impaired. The thoracic aorta (N=45) was harvested from three insulin resistant rat models (Zucker, pre-diabetic ZDF and high fat diet) or diabetic ZDF rats. Wistar and lean fa -/+ rats served as controls. Twelve rings of each aorta were cultured for 14 days in a calcifying medium (3.8 mM phosphate) or control medium. Calcium levels (in mg/g of aorta) were determined by o-cresolphtalein complexone . Calcification (von Kossa) and fibrosis (Sirius Red) were quantified (% of the sectional stained area). Receptor of advanced glycation end products (RAGE) and carboxymethyllysine (CML) expression was assessed by immunohistochemistry on aortic rings before and after culture.

RESULTS

No calcification was observed in rings cultured with the negative control medium. Calcifying medium resulted in calcified aortas in all groups (Figure 1). Calcium content was significantly increased in Zucker and pre-diabetic ZDF insulin resistant groups (878 ± 141 and 871 ± 60 mg/g) compared to the Wistar controls (300 ± 89 mg/g). It further increased in the diabetic ZDF (1390 ± 64 mg/g) and was the highest in high fat diet rats (1659 ± 129 mg/g) (Figure 1).

	Wistar	Lean	Zucker	Pre-diabetic ZDF	Diabetic ZDF	High fat diet
Final weight (g)	277±4	299±3	652±19	334±5	323±8	640±69
Glycémia (g/L)	0.84±0.08	0.64±0.01	1.87±0.39	1.45±0.08	2.30±0.44	1.21±0.17
Insulinemia (ng/mL)	1.19±0.10	1.87±0.59	10.6±2.3	8.34±0.76	3.31±0.83	1.45±0.15
HOMA IR	1.05±0.10	1.25±0.92	20.88±0.35	12.74±0.24	8.02±0.16	1.85±0.18

Table 1. Blood parameters and rat weight

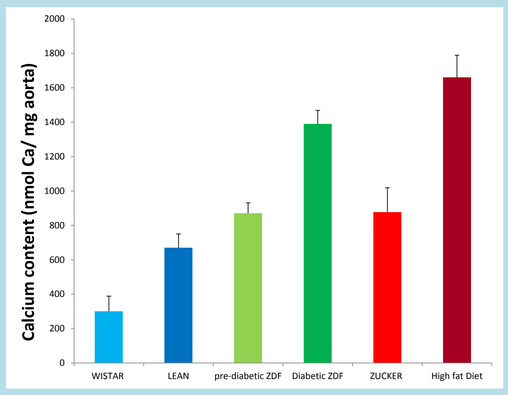

Figure 1 .Quantification of aortic calcium content in the different groups of rats by colorimetric assay.

Calcification area moderately increased in insulin-resistant Zucker and pre-diabetic ZDF groups (14 and 13%) and was the largest in high fat diet rats (23%) (Figure 2). These results are in agreement with the quantification of calcification by colorimetric assay aortic rings. For diabetic rats, aortic calcification is significantly lower (4%) to that determined by assay (Figure 2).

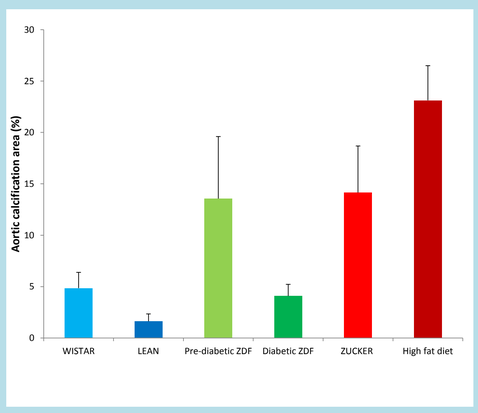

Figure 2. Quantification of calcification by von Kossa staining on rat aortic media sections of different groups.

Fibrosis localized in the same place as the calcium deposits (Figure3).

Figure 3. Sirius red staining of aorta sections. The distribution of collagen is similar to the staining of calcification by von Kossa.

RAGE and CML expression - before culture - was low and diffuse in control rat aortas. It was more marked in the adventitia of Zucker rat aortas. Both expressions were intense in the adventitia and intima of prediabetic and with a stronger intensity, in diabetic ZDF rat aortas (Figure 4).

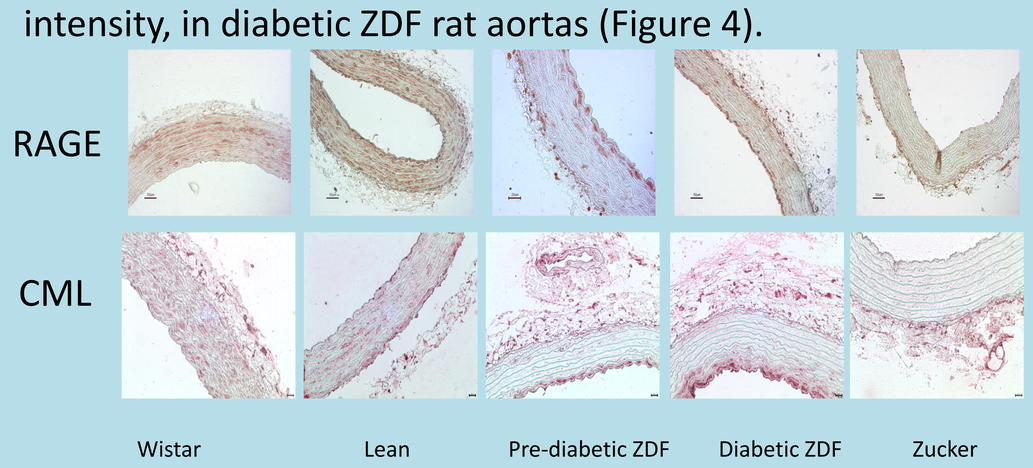

Figure 4. Representative images of immunostaining by RAGE and CML of aorta sections before culture .

After 14 days of culture, RAGE expression was more intense and diffuse in aortic rings isolated from high fat diet rats than in the others groups (Figure 5).

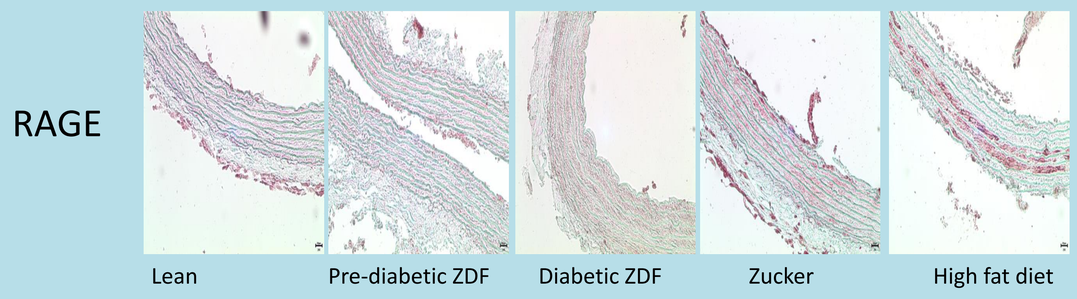

Figure 4. Representative images of immunostaining by RAGE of aorta sections after culture in calcifying medium.

CONCLUSIONS: Glucose metabolism dysfunction exacerbated ex vivo vascular calcification of thoracic aorta in rat and the different calcifying patterns suggest the participation of different mechanisms depending on the model studied. The alteration in carbohydrate metabolism, especially hyperglycaemia induces tissue damage and could stimulate the activation of inflammatory response by the advanced glycation end products (AGEs). In our study, insulin resistant rats, Zucker and pre-diabetic ZDF, have a similar calcification profile with strong calcification of the media. In contrast, calcification is rather adventitial in diabetic ZDF rats while in high fat diet rats calcification is distributed on the two tunics, media and adventitia. Expression of RAGE and CML in aortas before culture are not correlated with aortic calcification in the different groups. Yet, both RAGE expression and calcification are diffuse and intense in cultured aortic rings isolated from high fat diet rats. This suggests that the advanced glycation end products (AGEs) and their RAGE receptors have a role in the vascular calcification mainly in rats fed high fat diet.

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