Lipogenesis in arterial wall and vascular smooth muscle cells of *Psammomys obesus*: Its regulation and abnormalities in diabetes

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Abstract

Aim. – Lipogenesis is expressed in vascular smooth muscle cells (VSMCs), and such in situ lipogenesis could be providing the fatty acids for triglyceride synthesis and cholesterol esterification, and contributing to lipid accumulation in the arterial wall. This study investigated both the expression and regulation of lipogenesis in VSMCs to determine if they are modified in *Psammomys obesus* gerbils fed a high-fat diet as a model of insulin resistance and diabetes.

Methods. – Aortas were collected from diabetic and non-diabetic *P. obesus* for histological examination, measurement of lipogenic gene expression and VSMC culture.

Results. – The aortas of diabetic animals exhibited lipid deposits and foam cells as well as disorganization of elastic fibres. However, lipogenic gene expression was not modified. VSMCs in vitro from the aortas of diabetic animals had, compared with cells from non-diabetic animals, lower mRNA levels of SREBP-1c and ChREBP. An adipogenic medium stimulated moderate FAS and ACC1 expression in cells from both diabetic and non-diabetic animals, but glucose and insulin on their own had no such stimulatory action. Also, triiodothyronine (T3) had a clear stimulatory action, while angiotensin II had a moderate effect, in cells from non-diabetic *P. obesus*, but not from diabetic animals, whereas LXR agonists stimulated lipogenesis in cells from both animal groups.

Conclusion. – Lipogenesis is expressed in the arterial walls and VSMCs of *P. obesus*. However, its expression was not increased in diabetes, and did not respond to either T3 or angiotensin II. Therefore, lipogenesis in situ is unlikely to contribute to the accumulation of lipids in the arterial walls of diabetic *P. obesus* gerbils.

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Keywords: Lipogenesis; Fatty acid synthase; *Psammomys obesus*; Diabetes; Glucose; Vascular smooth muscle cells

Résumé

Lipogenèse dans la paroi artérielle et les cellules musculaires lisses vasculaires : régulation et anomalies dans le diabète.


Méthodes. – Des aortes de *Psammomys* témoins et diabétiques ont été recueillies (histologie, ARNm des gènes lipogéniques, cultures de CMLV).

Résultats. – Les aortes d’animaux diabétiques présentaient des dépôts lipidiques, des cellules spumeuses et une désorganisation des lames élastiques. Cependant l’expression des gènes lipogéniques n’était pas modifiée. *In vitro*, les CMLV de *Psammomys* diabétiques avaient des ARNm de Srebp-1c et ChREBP diminués. L’expression de FAS et ACC1 dans les cellules d’animaux témoins et diabétiques a été modérément stimulée par un milieu adipoénergique. Ni le glucose ni l’insuline seule n’ont eu d’effet. La triiodothyronine (T3) a eu une action stimulante nette et l’angiotensine une action modérée uniquement dans les cellules d’animaux témoins. Enfin des agonistes de LXR ont stimulé la lipogenèse dans les deux types de cellules.

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1. Introduction

Arterial wall foam cells, macrophages and vascular smooth muscle cells (VSMCs) incorporate labelled acetate into phospholipids and triglycerides (TG) [1], demonstrating that lipogenesis is expressed and active in these cells and tissues. More recently, Davies et al. [2] showed that human VSMCs also express lipogenic genes and that their expression, and the intracellular accumulation of TG are both increased by adipogenic differentiation medium (ADM) [3]. In addition, TO901317, a liver X receptor (LXR) agonist, can stimulate SREBP-1c and fatty acid synthase (FAS) expression, suggesting that the lipogenic action of LXR described in several tissues [4–7] is also present in VSMCs. Furthermore, Davies et al. found that FAS and SREBP-1c are both expressed in human atherosclerotic lesions, leading to the suggestion that enhanced VSMC lipogenesis may be involved in lipid accumulation and the development of atheroma [2]. Indeed, lipogenesis could be providing fatty acyl-coenzyme A for cholesterol esterification, and contributing to TG synthesis and accumulation as well. The foam cells in atheromatous plaques accumulate cholesterol, but also significant amounts of TG (8–10% of total lipids) [8,9]. Cellular TG modify the physical state of stored esterified cholesterol, and this may affect the way it is hydrolyzed and effluxed [10]. This suggests that increased TG in the arterial wall could be indicative, as in other tissues [11,12], of a general state of excess accumulation of lipid substrates with adverse effects, such as insulin resistance [13].

Type 2 diabetes and insulin resistance are risk factors for atherosclerosis characterized by high concentrations of insulin and/or glucose. Lipogenesis is stimulated by insulin and glucose in tissues such as liver [3,14]. Such stimulation, if present in the arterial walls, could result in increased tissue lipid accumulation, further aggravating resistance to insulin and possibly contributing to accelerated atherosclerosis. However, contrary to what is observed in liver [15,16], lipogenesis expression is not increased in the adipose tissue [17] and skeletal muscle of insulin-resistant and type 2 diabetic patients, and resists the action of insulin [18]. Furthermore, genetic models of insulin resistance and type 2 diabetes (Zucker obese and diabetic rats) reveal that the expression of lipogenesis in the arterial wall and VSMCs is not increased and is resistant to insulin [19]. These results suggest that the previously described insulin resistance in the arterial walls of Zucker rats [13] and of VSMCs from diabetic patients [20] also involves the lipogenic pathway. To clarify the issue, the present study aims to determine the level of expression of lipogenesis, and its regulation in the arterial wall and VSMCs of another diet-induced model of insulin resistance and diabetes—namely, Psammomys obesus—when receiving a high-fat diet.

2. Material and methods

2.1. Studies in vivo

P. obesus gerbils, also known as the ‘fat sand rat’, were captured in the Algerian Sahara, and housed in individual cages in an animal facility (University of Algiers) under controlled temperature and lighting conditions, with free access to food and water. During a 2-week acclimation period, the animals were fed the halophilic plants, rich in water and mineral salts, that they normally eat in the desert [21–23]. Thereafter, they were separated into two groups. One group (n = 5) was fed natural plants from the same halophilic family, but growing along the edge of sea (Salicornia; composition per 100 g: water 80.8 g; mineral salts 6.9 g; lipids 0.4 g; proteins 3 g; carbohydrates 8.4 g; and 45–50 kcal/100 g). The other group (n = 5) received a high-fat diet, comprising the salicorne plants plus the daily addition of one-quarter of cooked egg yolk (composition per 100 g: water 40–46 g; proteins 13.5–17.5 g; carbohydrates 0.2 g; lipids 30–31 g; cholesterol 1.2–1.3 g; and 370–400 kcal/100 g). Both groups of gerbils were followed for 6 months, with measurements of their body weight, plasma glucose, insulin, total cholesterol and TG in a fed state. After 6 months, the animals were sacrificed (fed state) for plasma collection, tissue sampling (aortas and liver) and VSMC culture. The thoracic aorta was removed, flushed with cold isotonic saline and cleaned of any perivascular adipose tissue. One part was flash-frozen in liquid nitrogen before storage at −80 °C until analysis, another part was used in the histological analysis (Masson’s trichrome stain) and the remaining part used for VSMC culture. Liver samples were flash-frozen with liquid nitrogen and stored at −80 °C until analysis, with one part reserved for histology (Masson’s trichrome stain).

2.2. Studies in vitro on VSMCs

Explants were obtained from the thoracic aortas [24] of the control (non-diabetic) and diabetic gerbils. After removing the adventitia (collagenase), small fragments were placed in DMEM (5 mM glucose with 15% fetal calf serum) and maintained at 37°C in an air/CO2 (95%/5%) atmosphere until confluence. The VSMCs were then trypsinized and subcultured. For the experiments, 10⁶ VSMCs/mL/well were seeded into six well plates in the usual medium for at least 2 days. At 24 h before starting the experiments, the culture medium was replaced by a basal medium with no fetal calf serum. On the first experimental day, cells were collected from two wells for baseline values (D0), while the culture medium was replaced in the other four wells with test substances added at appropriate concentra-
tions. All experiments were done with cells at passage 3–5 or earlier.

2.3. Effects of glucose and adipogenic differentiation medium

Cells were cultured for either 3 or 7 days [2] in DMEM with either basal (5 mM) or high (25 mM) glucose concentrations and with or without ADM (ADM; final concentrations in culture medium: insulin 1.2 μM; dexamethasone 100 nM; triiodothyronine (T3) 1 nM; and 3-isobutyl 1-methyl-xanthine 0.25 mM) [2]. Cells remained viable throughout the experiments. The effects of insulin (for 3 days and 7 days) and of T3 (for 3 days) on their own (same final concentrations) were also tested. In addition, as the arterial wall and VSMCs express components of the renin–angiotensin system [20], and angiotensin II (AngII) stimulates FAS expression and lipogenesis in adipose cells and liver [25,26], the effects of AngII (100 nM, for 3 days) were also assessed.

2.4. Effects of liver X receptor and pregnane X receptor agonists

TO901317 [an LXR and pregnane X receptor (PXR) agonist] was from Calbiochem (Merck KGaA, Darmstadt, Germany), and the LXR agonists (GW3965 and paxillin) and PXR agonist (5-pregnen-3β-ol-20-one-16α-carbonitrile; PCN) were from Sigma-Aldrich. Compounds were dissolved in ethanol (10–30 mM stock solution), and used at final concentrations of 10 μM (TO901317 and paxillin) and 30 μM (PCN). VSMCs were cultured without (control) and with one of the agonists for 3 days. Ethanol was added to the control cultures at the same final concentration as in the test cultures.

In some experiments, the activity of the lipogenic pathway was determined by measuring the incorporation of deuterium from deuterated water into the palmitate of cellular TG, as described elsewhere [27].

2.5. Determinations

Metabolites were measured by enzymatic methods [28], and insulin by Elisa (Crystal Chem, Downers Grove, IL, USA). For VSMC and liver TG concentrations, lipids were extracted by chloroform/methanol (1:2). After drying (nitrogen), the extracted lipids were dissolved in propanol for determination of TG concentrations [29]. Measurements of deuterium enrichment in the palmitate of VSMC, TG and calculation of the contribution of lipogenesis to the cellular TG pool were performed as described elsewhere [15,27,28,30,31].

Total RNA, purified according to the TRIzol® protocol (Invitrogen, Cergy-Pontoise, France) and the addition of DNase treatment, was reverse-transcribed using SuperScript II (Invitrogen) and random hexamers. Real-time polymerase chain reaction (PCR) was performed with the MyQ thermal cycler and iQ SYBR Green Supermix (Bio-Rad, Marnes-la-Coquette, France). The results are expressed as the target over 18S RNA concentration ratio (ng/μg). Primer sequences are shown in the supplemental table (Table S1, available with this article online). As there are no published sequences for the selected target mRNAs in P. obesus, primer pairs designed to measure these targets in humans, rats or mice were tested, and only those amplifying one cDNA fragment of a size comparable to that amplified in human, rat or mouse samples were selected. Sequences were determined in several amplicons to compare them with sequences in rats, humans and mice (see Table S1 online). Also tested were primers for other mRNAs [fatty acid translocase (FAT), very low-density lipoprotein (VLDL) receptors, lipoprotein lipase (LPL)]. Although these primers were not used in the present study, their sequences and the sequence, when obtained, of the amplicon are presented in the supplemental data (see Table S1 online).

2.6. Statistical analysis

Results are expressed as means ± SEM. Comparisons were performed using the two-tailed t test for unpaired values (comparisons of two groups), or one-way analysis of variance (Anova), followed by the Dunnett procedure to locate the differences (more than two groups), P < 0.05 was considered a significant difference. Calculations were performed using GraphPad Prism 4.02 software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Hormone and metabolite values

The high-fat diet induced only a trend for higher body weight from month 1 (119 ± 6 g vs 107 ± 12 g) to 5 (132 ± 9 g vs 119 ± 16 g) of the study, and mostly increased plasma TG and cholesterol over the 6-month study (Fig. 1). Diabetes was also induced as early as the end of month 1 of the diet. Hyperglycaemia developed despite a large increase in plasma insulin—from 70 ± 15 pM initially to 794 ± 72 pM at month 2 (P < 0.01) and 921 ± 205 pM at month 4 (P < 0.01), indicating insulin resistance. Insulin returned to near-baseline values (70 ± 15 pM) at month 6, thus revealing the evolution towards relative insulin deficiency.

3.2. Histology of liver and aorta

Diabetic P. obesus gerbils had hepatic steatosis at 6 months (liver TG content: 26.75 ± 2.66 mg/g vs 6.73 ± 2.60 mg/g; P < 0.01; Figs. S1 and S2, available with this article online). Their aortas also showed major alterations, with disorganized and ruptured elastic laminae, lipid deposits, foam cells and increased collagen deposition. However, arterial TG content could not be determined in these aortas due to the limited amount of tissue. Thus, it remains uncertain whether lipid deposition resulted from the accumulation of cholesterol alone or together with TG.
3.3. Liver and arterial wall expression of genes involved in de novo lipogenesis

Levels of mRNA were measured for the key enzymes [acetyl-coenzyme A carboxylase (ACC1), FAS] for de novo lipogenesis (DNL), for synthesis of monounsaturated fatty acids (SCD-1) and for the transcription factors controlling their expression (SREBP-1c and ChREBP) (Fig. 2). FAS mRNA concentrations were decreased in livers from diabetic animals. Decreases in ACC1, SCD-1 (0.78 ± 0.08 ng/μg vs 2.39 ± 1.32 ng/μg of 18S RNA) and SREBP-1c mRNAs were not significant most likely because of the small number of animals investigated, and were probably related to the inhibitory action of the high-fat diet. In aortas, in contrast, no decrease was observed in diabetic animals, and there was even a trend towards higher values of ACC1, SCD-1 (1.68 ± 0.44 ng/μg vs 0.48 ± 0.31 ng/μg) and ChREBP mRNA levels.

3.4. Expression of genes involved in de novo lipogenesis in vascular smooth muscle cells cultures

Concentrations of mRNA were measured under basal conditions (D0; DMEM with 5 mM glucose) and after 3 and 7 days (D3, D7) of culture in the presence of high glucose concentrations (25 mM) and/or adipogenic medium (ADM) and compared. In the initial state (D0), mRNA concentrations of ChREBP and SREBP-1c were lower in VSMCs of diabetic P. obesus [1.26 ± 1.08 × 10^{-5} ng/μg vs 6.64 ± 1.30 × 10^{-5} ng/μg of 18S RNA (P < 0.05) and 1.85 × 10^{-6} ± 0.9 × 10^{-6} ng/μg vs 1.35 × 10^{-4} ± 3.71 × 10^{-5} ng/μg of 18S RNA (P < 0.01), respectively], but with no associated decrease in the mRNAs of FAS and ACC1 (data not shown). Tests were carried out to determine whether or not lipogenic gene expression was regulated by metabolic (glucose) or hormonal (insulin, T3, AngII) factors, and whether or not there were any differences in such regulation between the cells of the control and diabetic animals. In fact, in both, ADM induced an increase in ACC1 mRNA (D3 and D7), and a transient increase in FAS mRNA (D3) (Fig. 3), with no significant modifications of either ChREBP or SREBP-1c. SCD-1 was also stimulated on D3 and D7 (0.023 ± 0.002 ng/μg vs 0.010 ± 0.001 ng/μg and 0.089 ± 0.006 ng/μg vs 0.095 ± 0.001 ng/μg of 18S RNA, respectively; P < 0.01 for both) in cells from the controls, but only on D7 in cells from the diabetics (0.042 ± 0.009 ng/μg vs 0.0016 ± 0.005 ng/μg; P < 0.01). However, cellular TG content was not modified (data not shown), nor was the contribution of lipogenesis to this TG pool (controls: from 1.77 ± 0.15% to 1.67 ± 0.31%; diabetics: 1.98 ± 0.10% to 2.01 ± 0.51%). Surprisingly, the high glucose concentration (25 mM) had no stimulatory effect except for an increase in ChREBP in the VSMCs from the diabetic gerbils.

In addition, on testing the effects of insulin and T3 on their own, insulin induced only a small increase on D7 in FAS and ACC1 mRNA levels in cells from diabetic animals (data not shown), and had no effects on lipogenesis (data not shown). In contrast, T3 induced, in the cells of control animals, a large increase in the lipogenic mRNAs measured, but had only a weak action on SREBP-1c in the cells of diabetic animals (Fig. S3; online), while AngII increased SREBP-1c expression only in the cells of the controls.

TO901317 can stimulate the expression of lipogenic genes in human VSMCs [2]. However, it is both an LXR and...
PXR agonist [32]. PXR agonists stimulate lipogenesis in liver [27] and PXR is expressed at least at the mRNA level in VSMCs (data not shown). Comparing its effects with those of paxillin and GW3965 (LXR agonists) and of PCN (PXR agonist), TO901317 was found to increase the expression of FAS and ACC1 in cells from both control and diabetic animals (Fig. S2; online), but had no significant effects on SREBP-1c and ChREBP (data not shown). This stimulation of lipogenesis expression was associated in the controls with increases in cellular TG content (from 25.3 ± 4.0 μg/10^6 to 48.6 ± 2.2 μg/10^6 cells; *P < 0.01) and with the contribution of DNL to this TG pool [from 1.68 ± 0.14% to 2.54 ± 0.19%; *P < 0.01, an increase of 0.42 ± 0.05 μg/10^6 to 1.24 ± 0.12 μg/10^6 cells (*P < 0.001) of TG synthesized with fatty acids from DNL].

4. Discussion

Despite its limited availability, *P. obesus* gerbils are a classic model of insulin resistance and diabetes [33]. Whether receiving a standard laboratory or high-fat diet, these animals develop insulin resistance and hyperglycaemia in spite of raised insulin levels, which can then progress to type 2 diabetes with relative insulin deficiency [21,33], as in the present study. With diabetes, these animals become hyperlipidaemic, which can lead to vascular complications with macroangiopathy [34], resembling an early stage of human atheroma. In the present study, such histological abnormalities of the arterial wall were indeed observed in diabetic animals, along with foam cells, lipid deposits and disorganization of elastic fibres.

The present study aimed to determine the level of expression of lipogenesis in the arterial wall and VSMCs of diabetic and non-diabetic *P. obesus*, and whether or not its regulation was modified in diabetes. Lipogenesis has been investigated in the liver and adipose tissue of these animals, and previous studies found an increase in hepatic lipogenesis in diabetic *P. obesus* [35,36]. However, this was observed in animals fed a high-carbohydrate diet, and presenting with high glucose and insulin levels. In the present study, we investigated desert gerbils receiving a high-fat diet and in a state of diabetes with low insulin concentrations. The reduced expression of lipogenic genes found in the liver can probably be explained by the inhibitory action of the high-fat diet and the relative insulin deficiency, and the observed hepatic steatosis was probably mostly due to the accumulation of dietary fat. There was also suppression of SREBP-1c and ChREBP expression in VSMCs of diabetic *P. obesus*, although these decreases were not apparent in their aortas, most likely because VSMCs are only one cellular component of the arterial wall and the values measured in aortas are a combination of the values of various cells. In general, lipogenesis was not overexpressed in the aortas and VSMCs of the diabetic *P. obesus* gerbils in our study, and was probably suppressed instead. These results are consistent with those seen in Zucker rats [19].
Fig. 3. Lipogenic mRNA concentrations in cultured vascular smooth muscle cells from control and diabetic *Psammomys obesus* gerbils after 3 and 7 days of culture under basal conditions (glucose 5 mM, white columns), in the presence of raised glucose concentrations (25 mM, black columns) and in the presence of adipogenic differentiation medium (ADM), without (hatched columns) and with (dotted columns) raised glucose concentrations. *P<0.05, **P<0.01, ***P<0.001 vs basal conditions.
On investigating the regulation of lipogenic genes in VSMCs, one surprising result was the lack of stimulatory effect of high glucose concentrations, except for ChREBP in cells from diabetic animals. In fact, high glucose levels had a somewhat inhibitory action in contrast to the moderate stimulatory action of ADM on FAS and ACC1 expression. However, ascertaining whether or not this is specific to VSMCs of P. obesus or a general characteristic of the species requires further studies of isolated hepatocytes and adipocytes. The moderate effect of ADM may have been due to the action of insulin, T3 or dexamethasone alone, or due to an additive or synergistic action of the hormones in combination. Insulin alone had no significant effect on cells from either diabetic or non-diabetic animals, and such resistance could be a general characteristic of the species, as there are data in vivo showing that even normoglycaemic and normoinsulinaemic P. obesus have a lowered response of glucose metabolism to exogenous insulin [33]. In cells from non-diabetic animals, T3 stimulated mostly the expression of lipogenic genes, including ChREBP, showing that the stimulatory action of thyroid hormones on ChREBP expression, as described in liver [37], is also present in other tissues. However, these effects of T3 on lipogenic genes were lost in the VSMCs from diabetic animals. The moderate action of AngII seen in VSMCs from the controls was also lost in cells from diabetic animals. Such differences in the response to T3 and AngII were previously found in VSMCs from Zucker rats and their controls [19]. However, the lack of action of AngII in VSMCs from diabetic animals is surprising, as the expression of its type 1 receptor (AT1R) is increased in VSMCs from human diabetics [20]. As for the loss of action of T3 in cells from diabetic P. obesus, recent data show a decrease in the expression of the thyroid hormone receptor TRα1 in the adipose tissue of obese or type 2 diabetic patients [38]. If present in VSMCs, such a decrease could contribute to the loss of T3 effect.

The present study confirms the stimulatory effect of TO901317 on the expression of lipogenic genes in VSMCs [2,19], an effect that was present in cells from diabetic animals and reproduced only in part (FAS only) by other LXR agonists (paxillin and GW3965). The latter compounds are considered to be selective LXR agonists, whereas TO901317 is both an LXR and PXR agonist. Previously, PCN, a PXR agonist, was found to have no effect on the expression of lipogenic genes in rat VSMCs [19]. However, in the present experiments, PCN also reproduced in part the effects of TO901317, thereby suggesting that the effects of this dual LXR and PXR agonist in this animal species result in the stimulation of both receptors.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary material (Figs. S1–S3, Fig. S1–S3 and Table S1) related to this article can be found at http://www.sciencedirect.com, doi:10.1016/j.diabet.2010.01.003.

References


