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The metabolic cascade leading to eicosanoid precursors – desaturases, elongases, and phospholipases A_2 – is altered in Zucker fatty rats

Cécile Fèvre, Sandrine Bellenger, Anne-Sophie Pierre, Mélaine Minville, Jérôme Bellenger, Joseph Gresti, Mickaël Rialland, Michel Narce *, Christian Tessier *

INSERM UMR 866/Université de Bourgogne, Lipides Nutrition Cancer, Faculté des Sciences Gabriel, 6 Bd Gabriel, 21000 Dijon, France

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ABSTRACT

Metabolic syndrome characterized by insulin resistance and obesity is accompanied by severe lipid metabolism perturbations and chronic low-grade inflammation. However, many unresolved questions remained regarding the regulation that underlie dyslipidemia, particularly the regulation of the metabolic cascade (synthesis and release) leading to eicosanoid precursors release. This study was undertaken to investigate the regulation of desaturases/elongases and phospholipases A_2 during the establishment of metabolic syndrome. Our results showed that delta-6 desaturase as well as elongase-6 expressions were upregulated in 3-month-old Zucker fatty rats as compared to lean littermates, independently of SREBP-1c activation. We also demonstrated for the first time an increase of liver group VII phospholipase A_2 gene expression in the obese animals together with a strong specific inhibition of type IVA and VIA phospholipases A_2. These results suggest that the regulation of unsaturated fatty acids biosynthesis and signalling cascade could contribute to the development of liver lipid dysregulation related to metabolic syndrome and may be considered as new potential targets in such pathological conditions.

1. Introduction

Metabolic syndrome (MS) is a complex disease marked by the dysfunction of glucose and lipid metabolism, which is often associated with chronic low-grade inflammation. A wide variety of bioactive lipid mediators including prostaglandins, thromboxanes, leukotrienes, and lipoxins exert potent and pleiotropic effects modulating inflammatory responses. These compounds are generated via phospholipase A_2 (PLA_2) hydrolysis [1]. The PLA_2 superfamily consists of many different groups of enzymes that catalyze the hydrolysis of the sn-2 ester bond, releasing fatty acid, and particularly arachidonic acid (AA), from a variety of phospholipids. Five main types of PLA_2 have been identified, which are the secreted $sPLA_2$s, the cytosolic $cPLA_2$s, the Ca^{2+} independent $iPLA_2$s, the platelet activating factor acetyl hydrolase/oxidized lipid lipoprotein associated (Lp)-PLA_2s, and the lysosomal PLA_2s [2,3].

These enzymes mediate a variety of important physiological and pathological functions such as cellular proliferation, apoptosis, atherosclerosis [4–6]. PLA_2 isoforms play different but complementary roles in inflammatory processes [7]. Nevertheless, these enzymes have never been investigated in MS-linked inflammation. While type IIA $sPLA_2$ was the major enzyme found elevated in the systemic circulation of patients with various acute and inflammatory diseases [8], other $sPLA_2$ isoforms have been reported to be elevated locally at sites of inflammation and cell injury [9]. In addition, an increase of secreted PLA_2 level in the plasma, such as $sPLA_2$ IIA and Lp-PLA_2, is a new potential marker of inflammation [10]. Besides, type IVA $cPLA_2$ and type VIA $iPLA_2$, sources of AA [11,12], seems to be particularly implicated in inflammatory processes. More specifically, the importance of type IVA Ca^{2+}-dependent $cPLA_2$ in many different inflammatory processes has been proven through the use of null mice showing significant decreases in allergic response, damage from acute lung injury and post-ischemic brain injury [13–15]. Type VIA $iPLA_2$ has also been shown to play an important role in driving acute inflammation [16]. Consequently, the role of the different PLA_2 isoforms remains to be clearly delineated, notably in MS-linked inflammation conditions.

Regarding MS, it is now accepted that inflammation associated with visceral obesity induces insulin resistance, which is sufficient to promote the progression of cardiovascular diseases. This is accompanied by severe liver lipid metabolism perturbations leading to liver steatosis [17]. However, many contradictory and unresolved questions remain regarding the molecular and cellular mechanisms that underlie this hepatic lipid dysregulation. Nevertheless, higher liver

Abbreviations: MS, metabolic syndrome; AA, arachidonic acid; UFA, unsaturated fatty acid; SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acid; ZF, Zucker fatty; ZL, Zucker lean; PUFA, poly-unsaturated fatty acid

* Corresponding authors. Narce is to be contacted at Tel.: +33 3 80 39 63 17. Tessier, Tel.: + 33 3 80 39 63 23.
E-mail addresses: michel.narce@u-bourgogne.fr (M. Narce), christian.tessier@u-bourgogne.fr (C. Tessier).

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expression of elongases (Elovl-5 and -6) and desaturases (delta-5, -6, and SCD-1) have been shown in ob/ob mice, compared with their lean littermates [18]. In contrast, resistance to high-fat-diet-induced hepatosteatosis and obesity has been associated with an increased capacity to convert *n*–6 unsaturated fatty acids (UFA) into AA via higher expressions of microsomal Elovl-5 and delta-5, -6 desaturases [19].

Altogether, these data underlie that the regulation of the different steps leading to the synthesis and release of eicosanoid precursors could play an important part in the modulation of MS-associated inflammation. We still do not know how liver UFA biosynthetic and signalling enzymes (desaturases, elongases, and phospholipases A_2) are regulated during the establishment of MS. To better understand these aspects, we investigated here the liver UFA biosynthesis and signalling pathways (desaturases, elongases, and phospholipases A_2) in 3-month-old Zucker rats. Indeed, it is important to understand cellular mechanisms leading to liver steatosis in order to prevent and to develop novel therapeutic strategies targeting for liver insulin resistance during MS.

2. Materials and methods

2.1. Animals, organs, and blood parameters measurements

Animal experimental procedures were approved by the Ethic Committee on Experimental Animals of the University of Burgundy, Dijon, France. Ten-week-old male Zucker fatty (*fa/fa*, ZF) and Zucker lean (*fa/+*, ZL) rats (Charles River Laboratories, L'Arbresle, France) were housed under controlled temperature (25 ± 1 °C) and humidity (60 ± 5%) with a 12-h day/12-h night cycle. Animals were fed standard laboratory chow (commercial standard pellets; Safe, U.A.R. A04, Villemoisson-sur-Orge, France) containing 4% lipids and were all sacrificed at 14 weeks of age.

For oral glucose tolerance test (OGTT) at 12 weeks of age, rats received, after a 12-h overnight fast, an oral glucose injection (2 g glucose/kg body weight). Glycemia were measured using a One Touch II glucometer (Lifescan, Johnson and Johnson, Milpitas, CA, USA) before gavage, and after 15, 30, 60, and 120 min.

For plasma triglycerides and cholesterol measurements, rats were fasted 12-h overnight and blood samples were collected on 10% EDTA under isofluorane anesthesia. Plasma were isolated by centrifugation (20 min at 200*g*) and then stored at −80 °C until use. Plasmatic triglycerides and cholesterol were measured according to the manufacturer's instructions, using a colorimetric kit (Diasys, Condom, France). Before insulin measurement, plasma were centrifuged to remove fat and insulin was measured using an ELISA kit (Linco Research, St. Charles, MO, USA).

At 14 weeks of age, non-anesthetized, fed rats were sacrificed and plasma were prepared as previously described. Livers and pancreas were isolated and stored at −80 °C until use. For immunofluorescence studies, pancreas were incubated for 24 h in a 30% sucrose solution. They were then embedded in a matrix (Shandon M-1 embedding matrix, Thermo Fisher Scientific, Cergy-Pontoise, France) and rapidly frozen at −20 °C until use. Pancreas were then cut with a cryostat (Leica CM 3050 S, Leica Camera AG, Solms, Germany) into 10-μm thin cross-sections, stuck on microscope Superfrost slices (Microm, Francheville, France) and stored at −80 °C.

2.2. Secreted PLA_2 activity

$sPLA_2$ activity was carried out directly on plasma collected at sacrifice, using a $sPLA_2$ Assay Kit (SPI-bio, Montigny-le-Bretonneux, France) according to the manufacturer's instructions. Enzyme activity measurement is based on the hydrolysis of 1,2-dithio analog of diheptanoyl phosphatidylcholine by $sPLA_2$. Thiols released in solution by PLA_2 activities present in homogenates combined with DNTB added to the reaction medium to form a colored compound, detected at 414 nm.

Proteins quantity was measured using the bicinchoninic acid method (BCA kit, Sigma, Lyon, France).

2.3. Pancreas immunofluorescence

Pancreatic slices were dried 2 h at 37 °C, fixed in a solution containing 75% methanol, 20% formaldehyde, 5% acetic acid, and rehydrated in PBS 10 mM pH 7.4. Cross-sections were permeabilized and saturated with TBS 10 mM, 10% BSA, 0.1% Triton X100, 0.2% Tween 20 at room temperature. Slices were then incubated overnight at +4 °C in a wet room with anti-insulin antibody (Jackson ImmunoResearch, West Grove, PA, USA) at a 1:200 final dilution in TBS 10 mM, 4% BSA, 0.01% Triton X100, 0.02% Tween 20. Control slices were treated with TBS-BSA-Triton-Tween alone. Slices were then exposed for 3 h at room temperature to a rhodamine–antirabbit IgG (1:200 dilution) (Jackson ImmunoResearch). Nuclei were stained with a Hoechst 33342 solution (1:100 dilution) for 5 min. The coverslips were mounted in FluoPrep medium (BioMérieux, Craponne, France), and images were acquired using an inverted fluorescence microscope Axiovert 200 M (Zeiss, Oberkochen, Germany) with appropriate filters and a cooled charged-coupled device camera controlled with AxioVision software (Zeiss).

2.4. Liver microsomes preparation

At sacrifice, livers were quickly removed and rinsed with 0.15 M NaCl and weighed. 3.5 g of each liver were cut into thin slices and homogenized at 4 °C in a Potter-Elvehjem homogenizer with 18 mL of a solution containing 0.05 M phosphate buffer pH 7.4 and 0.25 M saccharose. Liver microsomes were then prepared as previously described [20]. Microsomal protein concentration was determined by the method of Layne [21], using bovine serum albumin as standard.

2.5. SCD-1, delta-6 n–3, and delta-6 n–6 desaturase activities

SCD-1, delta-6 *n*–3, and delta-6 *n*–6 desaturase activities were estimated in liver microsomes, using 50, 40, and 40 nmol of [1-^{14}C] stearic, [1-^{14}C] alpha-linolenic or [1-^{14}C] linoleic acids, respectively, as substrates. 2.5 mg of microsomal proteins were incubated at 37 °C for 20 min in a shaken glass with a total volume of 1.1 mL incubation medium containing 72 mM phosphate buffer pH 7.4, 4.8 mM $MgCl_2$, 0.5 mM coenzyme A, 3.6 mM ATP, and 1.2 mM NAPDH. Incubations were stopped by adding 7.5 mL of chloroforme/methanol (1:1, v/v) [22]. Total lipids were extracted from incubation mixtures, according to Narce et al. [23]. Briefly, 2 mL chloroform, HCl 0.05 M, and NaCl 35% were added to an aliquot (4 mL) of the incubation mixtures; total lipids were extracted after centrifugation. Then saponification and transmethylation were carried out using methanol NaOH 0.5 M and 14% boron trifluoride in methanol, respectively, for 20 min at 80 °C, according to Slover and Lanza [24]. The distribution of radioactivity between each substrate and the corresponding products was determined by reverse-phase high-performance liquid chromatography as described by Bellenger et al. [25] using a Waters Set (510 HPLC pump and 410 differential refractometer; Millipore, Molsheim, Germany) equipped with two monolithic columns [26]. Analyses were carried out isocratically using pure acetonitrile or acetonitrile/water (93:7, v/v) for SCD-1 and D6D, respectively, as a mobile phase, at a flow rate of 1 mL/min. The fatty acid methyl ester mixtures were dissolved in pure acetonitrile before injection. ^{14}C radioactivity was directly measured by liquid scintillation with a Packard Tri-Carb model 1900 TR (Packard, Meriden, CT, USA) liquid scintillation analyzer. The fatty acid methyl esters were identified according to their retention times by comparison with standards: mixture reference standard 1B (Nu Check Prep, Elysian, MN, USA) for SCD-1