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Coexisting role of fasting or feeding and dietary lipids in the control of gene expression of enzymes involved in the synthesis of saturated, monounsaturated and polyunsaturated fatty acids

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ABSTRACT

In the liver, maintaining lipid homeostasis is regulated by physiological and exogenous factors. These lipids are synthesized by Fasn, elongases and desaturases. Interactions in an organism among these factors are quite complex and, to date, relatively little is known about them. The aim of this study was to evaluate the coexisting role of physiological (insulin, fasting and feeding) and exogenous (dietary lipids) factors in the control of gene expression of Fasn, elongases and desaturases via Srebf-1c in liver from rats. Gene expression of encoding enzymes for fatty acid synthesis and fatty acid composition was evaluated in liver from rats in fasting and feeding (at 30, 60, 90 and 120 min after feeding) when food intake (adequate or high-lipid diet) was synchronized to a restricted period of 7 h. Fasn, Scd and Fads2 were induced during 120 min after initial feeding in both dietary groups. This induction may be activated in part by insulin via Srebf-1c. Also, we showed for the first time that Elovl7 may be regulated by insulin and dietary lipids. The failure to synthesize saturated and monounsaturated fatty acids is consistent with a downregulation of Fasn and Scd, respectively, by dietary lipids. A higher content of LC-PUFAs was observed due to a high expression of Elovl2 and Elovl5, although Fads2 was suppressed by dietary lipids. Therefore, elongases may have a mechanism that is Srebf-1c-independent. This study suggests that a high-lipid diet triggers, during 120 min after initial feeding, a tight coordination among *de novo* lipogenesis, elongation, and desaturation and may not always be regulated by Srebf-1c. Finally, upregulation by feeding (insulin) of Fasn, Scd, Fads2 and Srebf-1c is insufficient to compensate for the inhibitory effect of dietary lipids.

1. Introduction

Dietary fat is an important macronutrient for growth and development of all organisms. It provides substrates for energy metabolism and acts as a structural component of membranes. Dietary fat regulates gene expression and signaling molecules and thus performs key biological functions such as regulation of lipid metabolism, cell division and inflammation (Jump et al., 2005). Some fatty acids are essential for life and are required for fetal and postnatal growth and neuronal development and cognition (Helland et al., 2003). Therefore, it is necessary to maintain the homeostasis of fatty acid content.

The liver plays a central role in whole-body lipid metabolism where fatty acid elongation and desaturation are two key metabolic routes for the synthesis of saturated, monounsaturated, and long-chain polyunsaturated fatty acids (LC-PUFAs). In the hepatic cell, saturated fatty acids either synthesized *de novo* in the cytosol by fatty acid synthase (Fasn) or derived from the diet can be further desaturated by stearoyl-CoA desaturase (Scd) to produce monounsaturated and/or elongated fatty acids by elongases Elovl1, Elovl6 and Elovl7 into long-chain fatty acids (16C, 18C, 20C and 22C) by specific membrane-bound enzymes localized in the endoplasmic reticulum (ER). LC-PUFAs such as arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are synthesized from their dietary precursor essential PUFA linoleic acid (LA) and α-linolenic acid (ALA) by fatty acid desaturases (Fads) 1 and 2 and elongases Elovl2 and Elovl5 (Guillou et al., 2010). Enzymes involved in the fatty acid synthesis are expressed in nearly all human tissues with the greatest activities found in the liver. Significant levels of expression of these in hepatic tissue are also indicative of their central roles in maintaining overall lipid homeostasis (Cho et al., 1999a,b). Expression and activity of those enzymes changes in concert as a

Abbreviations: LC-PUFAs, long-chain polyunsaturated fatty acids; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; ALA, α-linolenic acid; Fads1, fatty acid desaturase 1; Fads2, fatty acid desaturase 2; Elovl, elongation of very long chain fatty acids; Srebf-1c, sterol-regulatory element binding transcription factor 1c; SREs, sterol regulatory elements; Fasn, fatty acid synthase; ALD, adequate lipid diet; HLD, high lipid diet; DEPC, diethylpyrocarbonate; Chrebp, carbohydrate-responsive element-binding protein.

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result of nutritional, hormonal, developmental, and tissue-specific factors (Jump et al., 2008; Pauloin et al., 2010; Rodriguez-Cruz et al., 2006, 2011; Tang et al., 2003). For example, a high fat diet significantly lowers the hepatic abundance of Elovl5, Scd, Fads 1 and 2 mRNA, and this is paralleled by a comparable reduction in enzyme activity (Jump et al., 2008; Qin et al., 2009; Rodriguez-Cruz et al., 2006, 2011).

Insulin regulates hepatic *de novo* lipogenesis, at least in part, by controlling the master regulator sterol regulatory element binding transcription factor 1c (Srebf-1c) (Matsuzaka et al., 2002; Qin et al., 2009; Wang et al., 2006). It is well known that, in liver, elongases (Elovl2, Elovl5, and Elovl6), desaturases (Fads1, and Fads2) and Fasn expression is positively regulated by Srebf-1c. It has been suggested that the tight maintenance of cellular levels of fatty acids (saturated, monounsaturated and polyunsaturated) is mainly controlled by Srebf-1c activity, mediated by sterol regulatory elements (SREs) present in the regulatory region of these target genes (Kumadaki et al., 2008; Matsuzaka et al., 2002; Nara et al., 2002; Teran-Garcia et al., 2007). Although it is well known that, in mammals, hepatic elongases and desaturases work in concert to synthesize fatty acids, very little is known about the regulation of these fatty acid elongases (Jump et al., 2008).

Importantly, PUFAs are key regulators of Srebf-1c activation and, in consequence, they control fatty acid synthesis in the liver (Qin et al., 2009). In this context, enhanced levels of endogenously synthesized or diets high in LC-PUFAs are negative regulators of lipogenesis that inhibit Srebf-1c gene transcription and induce mRNA Srebf-1c instability (Tang et al., 2003), resulting in a downregulation of both de *novo* lipogenesis and LC-PUFAs synthesis through this well-designed negative feedback system (Rodriguez-Cruz et al., 2006). However, it is necessary to consider that, at the same time, maintaining overall lipid homeostasis is regulated by physiological factors (circadian rhythms, age, fasting, hormones, feeding and genetic background) and exogenous factors (dietary lipids) (Guillou et al., 2010). Interactions during a physiological stage in an organism among various factors such as fasting, feeding, dietary lipids and hormones like insulin, are obviously quite complex. To date, relatively little is known about these factors.

The purpose of the present study was to extend those previous observations by evaluating, at the same time and in an organism, the role of several physiological factors (insulin, fasting and feeding) and exogenous factors (dietary lipids) in the control of expression of enzymes involved in fatty acid synthesis to maintain lipid homeostasis in the liver. In addition, we explored the possible mechanism of regulation of these genes involved in saturated, monounsaturated and polyunsaturated fatty acid synthesis by Srebf-1c.

2. Material and methods

2.1. Materials

Fatty acid methyl ester standards for chromatographic analysis were obtained from PolySciences (Niles, IL). All other chemicals and solvents were reagent molecular grade from usual commercial sources.

2.2. Experimental animals and tissue collection

Thirty male Sprague Dawley weanling rats were obtained from the Animal Care Facility of the Centro Médico Nacional Siglo XXI of the Mexican Institute of Social Security (IMSS) in México City. Animals were housed (a maximum of five rats per cage) at 22 ± 2 °C with a 12-h light/dark cycle and had free access to water. To synchronize food intake, rats were trained to consume the experimental diets during a restricted period of 7 h (0800–1500 h) for 15 consecutive days. Rats were randomly divided into two groups and assigned to an adequate lipid diet (ALD; 5% lipids) or a high lipid diet (HLD) (10% lipids). Dietary composition was previously reported elsewhere (Rodriguez-Cruz et al., 2006, 2011) and is listed in Table 1. Lipids were obtained from corn oil, whose fatty acid composition was 12.1% C16:0, 2.2% C18:0, 30.7% C18:1, 1% C18:3n-3, and 54% C18:2n-6. Rats were euthanized according to the Mexican Official Norm (NOM-062-ZOO-1999). On day 15, rats were decapitated after diethyl ether anesthesia at 0, 30, 60, 90 and 120 min after they began to ingest their meal. Blood samples were immediately collected and serum was separated and frozen at −20 °C to quantify insulin by radioimmunoassay (Human Insulin RIA Kit, Linco Research, St. Charles, MO, USA) and glucose by glucose oxidase (Glucose, Trinder GOD-PAP, SPINREACT, Mexico City) methods. The liver was subsequently removed and harvested, rapidly frozen in liquid nitrogen, and stored at −70 °C for subsequent RNA isolation and total lipid extraction. The protocol was approved by the Animal Care Ethics Committee of the IMSS, México.

2.3. RNA isolation, cDNA synthesis and quantitative real-time (qRT)-PCR

Frozen tissue was pulverized and total RNA was isolated from the liver (0.1–0.2 g) using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA concentration was determined by absorbance at 260 nm and diluted to 1 μg/μl in RNAse-free water, and its integrity was evaluated using electrophoresis with 1% agarose and ethidium bromide staining (1.25 ng/μl, Sigma, Mexico City). First-strand cDNA from tissue was synthesized from 2 μg of total RNA with the MMLV reverse transcriptase. All reagents used were from Invitrogen. Before qRT-PCR analysis, cDNA quality was tested by final-point PCR amplifying the reference β-actin gene.

Relative mRNA levels of target genes and reference transcript β-actin were determined by qRT-PCR using cDNA preparation for liver. Synthesized cDNA was mixed with LightCycler Fast Start DNA SYBR Green I (Roche, Indianapolis, IN) and with various sets of gene-specific forward and reverse primers (Elovl1, Elovl2, Elovl5, Elovl6, Elovl7, Fads1, Fads2, Scd and Srebf-1c) and then subjected to RT-PCR quantification using the Light Cycler 3.5 detection System (Roche). qRT-PCR was performed in specially designed LightCycler Capillaries in a total volume of 20 μl containing 2 μl of the cDNA sample, 40 pmol of each primer, and 4 μl of Master^{PLUS} SYBER Green. For each reaction, the polymerase was activated by preincubation at 95 °C for 10 min.

Table 1
Composition of experimental diets.

Ingredient	ALD (g/kg diet)	HLD
Casein	222	222
Glucose	307.5	272.5
Corn starch	307.5	272.5
Corn oil[a]	50	100
Vitamin mix[b]	10	10
Mineral mix[c]	40	60
Cellulose	63	63
Energy (kJ/g)	15.82	16.53

ALD, adequate lipid diet; HLD, high lipid diet.

[a] Fatty acid composition is 12.1% palmitic acid (C16:0), 2.2% stearic acid (C18:0), 30.7% oleic acid (C18:1), 1% linolenic acid (C18:3n-3), and 54% linoleic acid (C18:2n-6).

[b] Vitamin mixture contained (per kg): *p*-aminobenzoic acid, 11.01 g; ascorbic acid, 101.66 g; biotin, 0.044 g; cyanocobalamin, 2.97 g; calcium pantothenate, 6.61 g; choline dihydrogen citrate, 349.69 g; folic acid, 0.20 g; inositol, 11.01 g; menadione, 4.95 g; niacin, 9.91 g; pyridoxine HCl, 2.20 g; riboflavin, 2.20 g; thiamin HCl, 2.20 g; dry retinyl palmitate, 3.96 g; dry ergocalciferol, 0.44 g; dry DL-α-tocopherol acetate, 24.23 g; corn starch, 466.67 g.

[c] Mineral mixture contained (per kilogram): ammonium molybdate 0.025 g; calcium carbonate 292.9 g; calcium phosphate 4.3 g; cupric sulfate 1.56 g; ferric citrate 6.23 g; magnesium sulfate 99.8 g; manganese sulfate 1.21 g; potassium iodide 0.005 g; potassium phosphate 343.1 g; sodium chloride 250.6 g; sodium selenite 0.015 g; zinc chloride 0.2 g.