Diet and sex hormones regulate hepatic Synaptotagmin 1 mRNA in mice

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1. ABSTRACT

The expression of Synaptotagmin 1 (Syt1) has been found to be associated with the lipid droplets in liver. Here, we studied the expression of Syt1 in Apoe-deficient mice receiving cholesterol, Western diet, squalene, and oleanolic acid. We also studied the influence of sex and impact of surgical castration. Dietary cholesterol increased hepatic Syt1 expression, an effect that was enhanced when cholesterol was combined with saturated fat present in a Western diet. This potentiation was modified by the administration of 10 mg/kg oleanolic acid or 1 g/kg squalene. Females fed chow or Western diet showed higher levels of hepatic Syt1 expression as compared to male mice on the same diet. Surgical castration of males did not modify the Syt1 expression; however, ovariectomy led to decreased levels. The data show that hepatic Syt1 expression is influenced by diet and hormonal milieu.

2. INTRODUCTION

Synaptotagmins (SYTs) belong to a family of membrane trafficking proteins characterized by
Hepatic Syt1 gene expression

Table 1. Summary of experimental conditions using Apoe-deficient mice

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Genetic background</th>
<th>Diet</th>
<th>Sex</th>
<th>Influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C57BL/6J X OLA 129</td>
<td>Commercial chow w/wo cholesterol</td>
<td>Males</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>2</td>
<td>C57BL/6J</td>
<td>Purified chow and purified Western</td>
<td>Males</td>
<td>HFD</td>
</tr>
<tr>
<td>3</td>
<td>C57BL/6J</td>
<td>Purified Western w/wo oleanolic acid</td>
<td>Males</td>
<td>Oleanolic acid</td>
</tr>
<tr>
<td>4</td>
<td>C57BL/6J</td>
<td>Purified Western w/wo squalene</td>
<td>Males</td>
<td>Squalene</td>
</tr>
<tr>
<td>5</td>
<td>C57BL/6J</td>
<td>Purified chow</td>
<td>Both sexes</td>
<td>Sex in chow diet</td>
</tr>
<tr>
<td>6</td>
<td>C57BL/6J</td>
<td>Purified Western</td>
<td>Both sexes</td>
<td>Sex in Western diet</td>
</tr>
<tr>
<td>7</td>
<td>C57BL/6J</td>
<td>Purified Western</td>
<td>Castrated and non-castrated males</td>
<td>Castration in males</td>
</tr>
<tr>
<td>8</td>
<td>C57BL/6J</td>
<td>Purified Western</td>
<td>Castrated and non-castrated females</td>
<td>Castration in females</td>
</tr>
</tbody>
</table>

HFD, high fat diet; w/wo, with or without

an N-terminal, a variable linker and two C-terminal C2-domains, C2A and C2B, designed to bind Ca\textsuperscript{2+} and phospholipids (1). SYTs are reported to participate in the docking and fusion of membrane vesicles in different cell types such as neurons, macrophages (2), pituitary cells (3), osteoblasts and osteoclasts (4), mucin-secreting airway and intestinal goblet cells (5) and muscle cells (6).

Synaptotagmin 1 (SYT1) is a 65-kDa integral membrane protein of synaptic vesicles and secretory granules in neuronal and neurosecretory tissues (7,8), where it serves as a Ca\textsuperscript{2+} sensor in synaptic exocytosis (9). Likewise, SYT1 has been proposed to have a role in endocytosis (10,11). In these situations, its Ca\textsuperscript{2+} binding domain plays an important role in the mechanisms of action (12). In line with the versatility of the SYT family described above, SYT1 is also expressed in other tissues. For instance, it binds to intestinal Na\textsuperscript{+}/H\textsuperscript{+} exchanger 3 and mediates cAMP- and Ca\textsuperscript{2+}-induced endocytosis in a process requiring phosphorylation of exchanger 3 in the Caco2BBE cell line (13,14). In addition, its intestinal mRNA expression has been reported to be modified by a mixture of conjugated linoleic acid isomers in rats (15). SYT1 has been localized in pancreatic acinar cells (16) and on the insulin secretory granules of pancreatic beta-cell lines, where it plays an essential role in insulin vesicle exocytosis through its Ca\textsuperscript{2+}-dependent phospholipid-binding activity (17,18). In fact, pancreatic and duodenal homeobox 1 stimulates insulin secretion in response to high glucose through the positive induction of Syt1 expression (19). It also appears to participate in the regulation of podocyte homeostasis in kidneys (20). Not surprisingly, a significant association has been reported between SYT1 polymorphisms and levels of creatinine, the most important biomarker for noninvasive assessment of kidney function (21). Using microarray analysis, with confirmation by quantitative real time PCR (RT-qPCR), our group found differential hepatic Syt1 gene expression in Apoe-deficient mice fed different conjugated linoleic acid isomers added to Western diets. Furthermore, significant associations have been observed between Syt1 expression levels and hepatic steatosis (22). Recently, we characterized the mouse hepatic Syt1 transcript as being 1807 bp in length and encoding a 421-amino acid protein with a predicted molecular mass of 47.4. kDa. However, immunoblotting of hepatic protein showed two isoforms with molecular masses that were higher than the theoretical prediction based on amino acid sequence, but lower than those found in brain. Subcellular distribution corresponded to plasma membrane, lysosomes and microsomes. Quantitative tissue distribution of Syt1 mRNA showed that the highest values corresponded to the brain, followed by liver, spleen, abdominal fat, intestine and skeletal muscle. These findings were considered to indicate an extensive role of SYT1 in different tissues and that it undergoes a posttranslational modification specific for liver function (23). On the basis of these facts, we hypothesized that Syt1 hepatic regulation might be complex. The present work was undertaken to characterize the influence of different dietary conditions and sex on Syt1 gene expression in mouse liver.

3. MATERIAL AND METHODS

3.1. Mice

Apoe-deficient mice on the C57BL/6J genetic background were obtained from Charles River (Charles River Laboratories, Barcelona, Spain). Apoe-deficient mice on the C57BL/6JxOla129 genetic background were generously provided by Dr. Nobuyo Maeda from the University of North Carolina at Chapel Hill. Blood samples were taken from the facial veins of 2-month-old mice (after four-hour fasting) to determine plasma cholesterol and to establish groups with similar initial values. All animals were housed in sterile filter-top cages in rooms maintained under a 12-h light/12-h dark cycle in the Servicio de Biomedicina y Biomateriales, University of Zaragoza (Zaragoza, Spain). All had ad libitum access to food and water, and study protocols were approved by the Ethics Committee for Animal Research of the University of Zaragoza.
Table 2. Composition of purified diets used in the different studies, based on AIN-93-recommended diets for laboratory rodents (35)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Chow (g/kg)</th>
<th>Western (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>465.7</td>
<td>371.9</td>
</tr>
<tr>
<td>Casein</td>
<td>140.0</td>
<td>111.8</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>155.0</td>
<td>123.8</td>
</tr>
<tr>
<td>Saccharose</td>
<td>100.0</td>
<td>79.9</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>40.0</td>
<td>31.9</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.0</td>
<td>39.9</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>35.0</td>
<td>27.9</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5.</td>
<td>2.0</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-</td>
<td>1.5</td>
</tr>
<tr>
<td>Palm oil</td>
<td>-</td>
<td>200</td>
</tr>
</tbody>
</table>

1 Cargill, Barcelona, Spain; 2 Lactalis Ingredients, Bourgbaré, France; 3 Azucarera Ibérica S.L., Madrid, Spain; 4 Aceites Muñoz, Toledo, Spain; 5 Vitacel R200, Rettenmaier Ibérica S.L, Barcelona, Spain; 6 AIN-93M Mineral Mix (MP Biomedicals, Lillirch, France). The salt mixture contains the following amounts (g/kg): calcium carbonate, 357; monopotassium phosphate, 250; potassium citrate monohydrate, 28; sodium chloride, 74; potassium sulphate, 46.6; magnesium oxide, 24; ferric citrate, 6.0.6; zinc carbonate, 1.6.5; manganese carbonate, 0.6.3; copper carbonate, 0.3; potassium iodate, 0.0.1; sodium selenate anhydrous, 0.0.1025; ammonium molybdate, 4H2O, 0.0.0795; sodium metasilicate, 9H2O, 1.4.5; chromium potassium sulphate, 12H2O, 0.2.75; lithium chloride, 0.0.174; boric acid, 0.0.815; sodium fluoride, 0.0.635; nickel carbonate, 0.0.318; ammonium vanadate, 0.0.066 and powdered sugar, 209.8.06. 7 AIN-93-VX Vitamin Mix (MP Biomedicals, Lillirch, France). Vitamin mixture contains the following amounts (mg/kg): nicotinic acid, 3; D-calcium pantothenate, 1.6; pyridoxine HCl, 0.7; thiamine HCl, 0.6; riboflavin, 0.6; folic acid, 0.2; D-biotin, 0.0.2; vitamin B12 (0.1% triturated in mannitol), 2.5; α-tocopherol powder (250 U/g), 30; vitamin A palmitate (250,000 U/g), 1.6; vitamin D3 (400,000 U/g), 0.2.5; phylloquinone, 0.0.75 and powdered sucrose, 959.6.55; 8 Sigma-Aldrich Quimica, Madrid, Spain and 9 Gustav Heess, Barcelona, Spain. AIN: American Institute of Nutrition.

3.2. Experimental designs

A summary of all experiments undertaken is shown in Table 1.

3.2.1. Effect of dietary cholesterol on Syt1 expression in male Apoe-deficient C57BL/6J mice fed a commercial chow diet

Mice were assigned randomly to one of the following experimental groups: a) a control group (n=7) fed a standard chow diet (B & K Universal Ltd, Humberside, UK) and b) a cholesterol group (n=7) fed a diet supplemented with 0.1% (w/w) cholesterol. The nutritional intervention lasted 10 weeks (24).

3.2.2. Effect of a Western diet on Syt1 expression in male Apoe-deficient C57BL/6J mice

Two study groups were established: a) one (n=13) receiving a purified chow diet and b) the other (n=9) receiving a purified Western diet containing 0.1% cholesterol and 20% palm oil (Gustav Heess, S.L., Barcelona, Spain) (Table 2) (25).

3.2.3. Effect of dietary oleanolic acid on Syt1 expression in male Apoe-deficient C57BL/6J mice fed a Western diet

Two study groups were established: a) one (n=8) receiving a purified Western diet and b) the other (n=9) receiving the same diet but supplemented with 0.0.1% oleanolic acid (Extrasynthese, Genay, France), equivalent to a dose of 10 mg/kg mouse, assuming a daily intake of 3 g per mouse (26).

3.2.4. Effect of dietary squalene on Syt1 expression in male Apoe-deficient C57BL/6J mice fed a Western diet

Two study groups were established: a) a control group (n=9) receiving a purified Western diet and b) a treatment group (n=10) receiving the same diet but containing 1% squalene (Sigma, Madrid, Spain), equivalent to a dose of 1 g/kg mouse, assuming a daily intake of 3 g per mouse (27).

3.2.5. Effect of sex on Syt1 expression in Apoe-deficient C57BL/6J mice fed a chow diet

Two study groups were established: a) male mice (n=13) and b) female mice (n=13), both of which received a purified chow diet (Table 2) for 11 weeks.

3.2.6. Effect of sex on Syt1 expression in Apoe-deficient C57BL/6J mice fed a Western diet

Two study groups were established: a) male mice (n=9) and b) female mice (n=10), both of which received a purified Western diet for 11 weeks.

3.2.7. Effect of surgical castration on Syt1 expression in male Apoe-deficient C57BL/6J mice fed a Western diet

Two study groups of males were established: a) a control group (n=9) that received a purified Western diet and b) a group of orchietomized mice (n=9) that received the same diet, both for 11 weeks.

3.2.8. Effect of surgical castration on Syt1 expression in female Apoe-deficient C57BL/6J mice fed a Western diet

Two study groups of females were established: a) a control group (n=9) that received a purified Western diet and b) a treatment group (n=10) receiving the same diet but containing 1% squalene (Sigma, Madrid, Spain), equivalent to a dose of 1 g/kg mouse, assuming a daily intake of 3 g per mouse (27).
Hepatic Syt1 gene expression

diet and b) a group of ovariectomized mice (n=9) that received the same diet, both for 11 weeks.

3.3. RNA isolation

At sacrifice, the livers were immediately removed and frozen in liquid nitrogen. RNA was isolated from each liver using Tri-reagent (AMBION, TX, USA). DNA contaminants were removed by TURBO DNase treatment using the DNA removal kit from AMBION. RNA was quantified by absorbance at A

The integrity of the 28 S and 18 S ribosomal RNAs was verified by agarose gel electrophoresis.

3.4. Quantification of mRNA

The potential changes in Syt1 mRNA expression were determined by RT-qPCR analysis of individual samples using equal amounts of DNA-free RNA from each sample taken from each animal. First-strand cDNA synthesis was performed using the First Strand synthesis kit (Thermo Scientific, Madrid, Spain). RT-qPCR reactions were carried out using the Sybr Green PCR Master Mix (Applied Biosystems, Foster City, CA). The primers were designed using Primer Express® (Applied Biosystems) and checked by BLAST analysis (NCBI) to verify gene specificity, as well as to ensure amplification of cDNA but not genomic DNA (28). RT-qPCR reactions were performed on a Step One Real Time PCR System (Applied Biosystems) following the standard procedure. The relative amount of all mRNAs was calculated using the comparative 2\(^{-}\Delta\Delta\text{Ct}\) method and normalized to the reference Cyclophilin B mRNA expression.

3.5. Hepatic lipid analysis

Tissues (10 mg) were homogenized in 1 mL of PBS. An aliquot was saved to determine protein concentration by the BioRad dye binding assay (BioRad, Madrid, Spain). The total lipids were extracted from one volume of the tissue homogenates according to Folch's method (29) using a chloroform-methanol (2:1, v/v) solvent system, evaporated under N\(_2\) stream and dissolved in 100 \(\mu\)L of isopropanol. Cholesterol and triglycerides were measured by colorimetric assay with Infinity kits (Thermo Scientific).

3.6. Liver histology analysis

Aliquots of liver, stored in neutral formaldehyde, were embedded in paraffin. Sections (4 \(\mu\)m) were stained with hematoxylin and eosin and observed under a Nikon microscope. Hepatic fat content was evaluated by quantifying the extent of fat droplets in each liver section with hematoxylin and eosin and observed under a Nikon microscope. Hepatic fat content was evaluated by quantifying the extent of fat droplets in each liver section with Adobe Photoshop 7.0. and expressed as percentage of total liver section (27).

3.7. Statistical analysis

Data are expressed as means ± standard deviation of the values. The data were analyzed using the Statistical Package for Social Sciences (SPSS) program (SPSS, Chicago, IL, USA) or Instat 3.0.2 software for Windows (GraphPad, S. Diego, CA, USA). For parametric distributions, a Student’s t test was employed. When the variables did not show normal distribution (according to the Shapiro-Wilk test), or failed to show homology of variance, data were analyzed with the Mann-Whitney U test. Correlations between variables were sought using the Spearman correlation test. The statistical significance was set at p < 0.05.

4. RESULTS

4.1. Nutritional regulation of hepatic Syt1 expression in Apoe-deficient mice

For the initial characterization of the dietary regulation of this gene expression in mice, historical hepatic RNA samples from males that had consumed a chow diet with and without cholesterol (24) were analyzed for Syt1 expression. Cholesterol supplementation increased the liver surface area occupied by lipid droplets, as well as hepatic cholesterol and triglyceride contents (Figure 1A, B and C). As shown in Figure 1D, the presence of dietary cholesterol induced a modest increase in the hepatic expression of this gene. Hepatic cholesterol content was associated with hepatic Syt1 expression (Figure 1E).

A second study focused on the combination of cholesterol and a high-fat diet (HFD) containing palm oil as source of long-chain saturated fatty acids, provided as a purified Western diet to male C57BL/6J mice (Figure 2). The consumption of a Western diet significantly increased the liver surface area occupied by lipid droplets (Figure 2A, B and C), as well as hepatic cholesterol and triglyceride contents. In this experimental setting, there was a significant elevation of hepatic Syt1 expression (Figure 2D).

Two experiments were set up to investigate whether the increased Syt1 expression induced by HFD was modulated by other dietary components. In the first (Figure 3), male mice receiving an oleanolic acid-supplemented Western diet were compared to those receiving the unsupplemented HFD. Oleanolic acid administration induced a significant reduction of hepatic Syt1 expression (Figure 3D), with no significant changes in hepatic cholesterol or triglyceride contents. However, the liver surface area occupied by lipid droplets was found to be significantly increased (Figure 3C) and negatively associated with hepatic Syt1 expression (Figure 3E). In the second experiment (Figure 4), the effect of a squalene-enriched Western diet was explored, again in males. The addition of this compound increased the hepatic Syt1 expression while it reduced hepatic cholesterol and triglyceride contents. No significant changes were noted in relation to the percentage of liver surface occupied by lipid droplets and there were no significant associations between hepatic Syt1 expression and these liver parameters.
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Overall, these experiments underline the exquisite nutritional regulatory role of hepatic Syt1 expression at the transcriptional level in Apoe-deficient mice, where cholesterol and saturated fat increase its levels, and the latter can be modulated by minor dietary components such as oleanolic acid or squalene.

4.2. Female hormones are involved in hepatic Syt1 expression in Apoe-deficient mice

To explore the influence of sex, two different experiments were carried out in Apoe-deficient mice. In one, hepatic Syt1 expression was analyzed in males and females being fed a purified chow diet. As shown in Figure 5, females had higher levels of gene expression and significantly higher hepatic cholesterol content than males, whereas no differences in triglyceride content were observed. There was a significant positive association between hepatic Syt1 values and hepatic cholesterol concentrations. In view of the effects of HFD described above, the second experiment was designed to assess the influence of sex in mice fed a purified Western diet. As shown in Figure 6, this experimental approach revealed significant differences between sexes in terms of hepatic Syt1 expression. However, no significant difference was observed in the percentage of liver surface occupied by lipid droplets, and the levels of hepatic cholesterol and triglycerides were significantly lower in females than in males. A significant inverse association was also found between hepatic Syt1 values and hepatic triglyceride levels. These data indicate that, irrespective of diet, sex plays an important role in hepatic Syt1 expression.

To analyze whether sex differences were due to hormonal changes, mice of both sexes underwent surgical castration and were fed a purified Western
diet. As shown in Figure 7, no significant change in Syt1 expression was observed in orchiectomized males; nor was there any significant change in hepatic cholesterol or in hepatic triglycerides. However, there was a significant increase in the liver surface occupied by lipid droplets in orchiectomized males. No significant association was found between Syt1 expression and lipid parameters (data not shown). In contrast, ovariectomized females (Figure 8) showed a significant decrease in Syt1 expression compared to control females. Ovariectomy resulted in significant increases in hepatic cholesterol and triglycerides, and in the surface area occupied by lipid droplets. A significant inverse association was also found between hepatic Syt1 values and those of hepatic triglycerides (Figure 8E). These results indicate that the release of ovarian hormones is necessary to induce the increased hepatic Syt1 expression observed in females.

5. DISCUSSION

This experimental approach was designed to evaluate putative hepatic Syt1 transcriptional changes induced by dietary components and sex. We found that, in Apoe-deficient mice, dietary cholesterol produced an increase that was potentiated by its combination with saturated fat. The latter effect was seen to be modulated by minor dietary components such as oleanolic acid or squalene, administered at pharmacological doses. Likewise, our study revealed a sex-related regulatory influence irrespective of the diet provided, with female sex acting as a positive regulator. In an attempt to establish the role of sex hormones, both males and females were subjected to castration. This experiment revealed that ovarian hormones are crucial for the increased hepatic Syt1 expression observed in females.
In a previous study using extreme dietary conditions with conjugated linoleic acid isomers and Apoe-deficient C57BL/6JxOla129 mice on a mixed genetic background, we identified hepatic Syt1 as an important gene whose expression is susceptible to modulation in response to diet or dramatic changes in hepatic fat content (22). Recently, our group characterized mouse hepatic Syt1 mRNA in detail, finding an important difference in length compared to the brain transcript (23). The present work explored the influence of several dietary components, especially cholesterol and saturated fat, on its hepatic expression. This would be in agreement with the results found in mice receiving a n-3 polyunsaturated fatty acid-depleted diet and in those deficient in stearoyl-CoA desaturase 1 (Table 3), in which saturated fatty acids tend to accumulate in liver. However, the discrepancy between the findings in mice overexpressing lipin 1-beta and those lacking medium-chain acyl-coenzyme A dehydrogenase (Table 3) may suggest that the nature of fatty acids is important. In this regard, the differences in the results observed in mice receiving different conjugated linoleic acid isomers (22) reinforce this notion. Overall, nutritional components are critical modulators of hepatic Syt1 expression.

In this study, the intake of oleanolic acid, a pentacyclic triterpene, decreased Syt1 expression, as did dietary supplementation with Boswellia serrata, an extract rich in particular derivatives of boswellic acid, also a pentacyclic triterpene-based compound (30). However, squalene, a linear triterpene, showed the opposite effect. These data indicate that the chemical structure of triterpenes may be crucial in the final outcome of this gene expression. Due to the anti-inflammatory properties of oleanolic and boswellic acids, its decreasing action would be in agreement with absence of c-Jun N-terminal

Figure 3. Effect of dietary oleanolic acid on hepatic steatosis and Syt1 expression in Apoe-deficient mice fed a purified Western diet. Representative liver micrographs (x400 magnification) from Apoe-deficient mice consuming Western (control) (A) and oleanolic acid-enriched (B) diets. Morphometric evaluation of surface of hepatocyte occupied by fat, cholesterol (Chol) and triglyceride (TG) contents (C). Analysis of hepatic Syt1 expression determined by RT-qPCR normalized to Cyclophilin B (D). Data are expressed as mean ± SD for each group. Statistical analyses were done according to the Mann-Whitney U test. *P < 0.0.5 vs control. Relationship between percentage of hepatic surface occupied by fat and Syt1 gene expression (E). Open squares correspond to controls and black squares to oleanolic acid-fed mice. Correlations were calculated according to Spearman’s test.
Figure 4. Effect of dietary squalene on hepatic steatosis and Syt1 expression in Apoe-deficient mice fed a purified Western diet. Representative liver micrographs (x400 magnification) from Apoe-deficient mice consuming Western (control) (A) and squalene-enriched (B) diets. Morphometric evaluation of surface of hepatocyte occupied by fat, cholesterol (Chol) and triglyceride (TG) contents (C). Analysis of hepatic Syt1 expression determined by RT-qPCR normalized to Cyclophilin B (D). Data are expressed as mean ± SD for each group. Statistical analyses were done according to the Mann-Whitney U test. *P < 0.05 vs control.

Table 3. Changes in hepatic Syt1 expression according to the Genome Expressed Omnibus data bank

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Type of change</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid receptor coactivator-2-deficient female mice</td>
<td>Decreased</td>
<td>GDS4785</td>
</tr>
<tr>
<td>SIRT1-deficient mice</td>
<td>Decreased</td>
<td>GDS3666</td>
</tr>
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<td>c-Jun N-terminal kinase 1-deficient livers of diet-induced obese mice</td>
<td>Decreased</td>
<td>GDS3001</td>
</tr>
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<td>Dietary supplement of Boswellia serrata</td>
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<td>GDS1227</td>
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<tr>
<td>NADPH-cytochrome P450 reductase-deficient mice</td>
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<td>GSE2362</td>
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<td>Medium-chain acyl-coenzyme A dehydrogenase-deficient mice</td>
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<td>GDS4548</td>
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<tr>
<td>Concanaavalin A-induced fulminant hepatitis model</td>
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<td>GDS3752</td>
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<tr>
<td>SIRT4-deficient mice</td>
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<td>GDS4823</td>
</tr>
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<td>Senescent activated hepatic stellate cells</td>
<td>Increased</td>
<td>GDS3492</td>
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<td>Stearoyl-CoA desaturase 1-deficient mice</td>
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<tr>
<td>Kupffer cell depletion in high-fat-diet-induced steatosis</td>
<td>Increased</td>
<td>GDS4166</td>
</tr>
<tr>
<td>Hepatitis B virus (HBV)-associated acute liver failure in humans</td>
<td>Increased</td>
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<tr>
<td>Glycosylphosphatidylinositol-specific phospholipase D overexpression effect in hepatoma cells</td>
<td>No change</td>
<td>GDS2049</td>
</tr>
</tbody>
</table>

kinase 1. This kinase is activated by various stimuli, including UV light, interleukin-1, tumor necrosis factor-α (TNF-α), and CD28 co-stimulation (31), some of which also play a role in hepatitis (32). For this reason, an
Increased expression was found in hepatitis B virus and in Kupffer cell depletion (Table 3), whereas, fulminant hepatitis induced a decrease. These results also suggest that the regulation of this gene may be influenced by several inflammatory compounds.

One finding of this study that proved striking was the increased hepatic Syt1 expression in female mice and its suppression following ovariectomy. Interestingly, female mice lacking steroid receptor coactivator-2 also showed decreased hepatic expression of this gene (Table 3). Steroid receptor coactivator-2 promotes the transcriptional activation of estrogen receptor in some tissues (33). These results indicate the influence of estradiol or other female sex hormones on a positive regulation of this gene.

In our initial description of hepatic Syt1 gene expression (22), we found significant associations with hepatic steatosis in Apoe-deficient mice fed different conjugated linoleic acid isomers, in Cbs-deficient mice and in olive oil-fed Apoe-deficient mice. In the present study, this association was observed only when oleanolic acid was administered (Figure 3). Interestingly, in three experiments, Syt1 expression was associated with hepatic triglycerides (Figures 6 and 8) or cholesterol (Figures 1, 2 and 5). There are two major differences between the earlier study and the present report: genetic background and diet composition. The present study was conducted using C57BL/6J mice, whereas the previous one involved Ola129xC57BL/6J hybrids. These strains have been shown to differ considerably in terms of hepatic fat content (34), a circumstance that may have influenced the outcome. The second important difference is the use of an American Institute of Nutrition (AIN)-93 purified diet (35) in the present study, rather than the commercial chow employed in the earlier one. We were obliged to make this change because of the high variability among
Hepatic Syt1 gene expression

Figure 6. Effect of sex on hepatic steatosis and Syt1 expression in Apoe-deficient mice fed a purified Western diet. Representative liver micrographs (x400 magnification) from male (A) and female (B) Apoe-deficient mice consuming Western diets. Morphometric evaluation of surface of hepatocyte occupied by fat, cholesterol (Chol) and triglyceride (TG) contents (C). Analysis of hepatic Syt1 expression was determined by RT-qPCR normalized to Cyclophilin B (D). Data are expressed as mean ± SD for each group. Statistical analyses were done according to the Mann-Whitney U test. *P < 0.0.5 vs male. Relationship between percentage of hepatic triglyceride content and Syt1 gene expression (E). Open squares correspond to males and black squares to females. Correlations were calculated according to Spearman’s test.

Figure 7. Effect of orchiectomy on hepatic steatosis and Syt1 expression in male Apoe-deficient mice fed a purified Western diet. Representative liver micrographs (x400 magnification) from sham (control) (A) and surgically castrated (B) male Apoe-deficient mice consuming Western diets. Morphometric evaluation of surface of hepatocyte occupied by fat, cholesterol (Chol) and triglyceride (TG) contents (C). Analysis of hepatic Syt1 expression determined by RT-qPCR normalized to Cyclophilin B (D). Data are expressed as mean ± SD for each group. Statistical analyses were done according to the Mann-Whitney U test. *P < 0.0.5 vs control.
Hepatic Syt1 gene expression

Control mice and their atherosclerotic lesions observed in our lab over the years when using commercial chows and the impossibility of obtaining the same batch from one year to another. Undoubtedly, dietary components are an important source of variation (36) and, in this respect, this study, performed under well-defined conditions of mouse strain and purified diets, maximizes the control of this variability.

In conclusion, the present report identifies three major factors in hepatic Syt1 regulation defined by dietary components, inflammation and sex. Regarding diet, cholesterol and the nature of fatty acids are involved. Anti-inflammatory modulators such as oleanolic acids seem to play a role as well. Finally, the detection of higher levels of hepatic gene expression found in females, but not observed in ovariectomized mice, suggests that female sex hormones are involved in hepatic Syt1 expression.

6. ACKNOWLEDGMENTS

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Abbreviations: PCR, Polymerase Chain Reaction, RT, reverse transcriptase; qRT, Quantitative Real Time; Tg, Triglycerides

Key Words: Synaptotagmin 1, Cholesterol, Apolipoprotein E Deficient Mice, Squalene, High-Fat Diet, Syt1, sex

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