ChREBP Expression in Liver, Adipose Tissue and Differentiated Preadipocytes in Human Obesity.

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ABSTRACT

ChREBP is an essential transcription factor for lipogenesis. Its physiological role in adipose tissue has been little studied and the control of its expression remains unknown in human adipocytes. We have studied ChREBP mRNA and protein expression levels in the liver and the omental (OM) and subcutaneous (SC) adipose tissues from obese and lean subjects, as well as in human differentiated preadipocytes. Liver and OM and SC adipose tissues biopsies were obtained from lean and obese patients. Human preadipocytes were isolated from the adipose tissues from obese patients and differentiated under adipogenic conditions. ChREBP expression levels were quantified by RT-PCR and Western blot analysis. We found opposite *ChREBP* regulation in the liver and adipose samples. ChREBP increased in the liver from obese compared to lean subjects, whereas the expression decreased in both adipose tissues. The mRNAs of other adipogenic markers were checked in these tissues. The pattern of *FASN* was similar to the one for *ChREBP*, *ADCY3* decreased in both adipose tissues from obese patients*, AP2* was only decreased in OM adipose tissue of obese patients and *ATG*L did not change. The levels of ChREBP mRNA and protein showed dramatic increases during the differentiation of human OM and SC preadipocytes. In conclusion, ChREBP expression has an opposite regulation in the liver and adipose tissue from obese subjects which is compatible with the increased hepatic lipogenesis and decreased adipocytic lipogenesis found in these patients. The dramatic increase of ChREBP mRNA and protein levels during preadipocytes differentiation suggests a role in adipogenesis.

Keywords: ChREBP, human liver, subcutaneous adipose tissue, omental, preadipocytes

1. INTRODUCTION

Dysregulation of the synthesis of fatty acids (lipogenesis) contributes to metabolic disease such as obesity, hepatic steatosis, diabetes and cardiovascular diseases [1]. Liver and adipose tissue are the main organs where lipogenesis takes place, glucose being the primary source of carbons for fat biosynthesis. Triglycerides (TG) are synthesized from fatty acids and glycerol; in the liver, TG can be packaged into VLDL and secreted into the circulation, stored as lipid droplets or they can enter the beta-oxidation pathway. In the adipose tissue, TG are mainly stored, thus being a major source of energy. In both tissues lipogenesis is controlled by hormonal and nutritional signals (insulin and glucose). Insulin secreted in response to elevated blood glucose levels, is well known to increase transcription of lipogenic genes mediated by the transcription factor SREBP-1 in liver [2]. However, the mechanism that activates the glucose signaling pathway by an excess of carbohydrates was not well known until the discovery of the transcription factor Carbohydrate Responsive element-binding protein (ChREBP). ChREBP activation by glucose induces the gene expression of most enzymes involved in lipogenesis [3] as well as the liver pyruvate kinase, an essential regulator of glycolysis. Other target genes of ChREBP are involved in the NADPH supply system and gluconeogenesis [4].

Despite the extensive knowledge gained in recent years regarding the role of ChREBP in the liver, its physiological role in adipose tissue has been barely studied and the control of its expression remains unknown in adipocytes. Moreover, most of the questions regarding ChREBP regulation have been always addressed in rodent models but not in human samples. To our knowledge, this is the first study involving different human tissues, such as liver and two adipose tissue depots. We have found that the regulation of ChREBP expression in differentiated preadipocytes increases in agreement

with rodent models, but there is a dysregulation in the adipose tissue of obese patients compared to lean patients, characterized by a decrease in *ChREBP* and *FASN* mRNAs, which is in contrast with the increase of the expression of these genes observed in the liver from obese versus lean patients.

2. MATERIALS AND METHODS

2.1. Subjects, tissue samples and cell cultures.

Patients were classified as obese or lean according to their body mass index (BMI), following the World Health Organization criteria, BMI $\geq$30 and <25 kg/m^2, respectively. Surgeries were performed at the Hospital Universitario La Paz (Madrid) and the Hospital Clínico Universitario S. Carlos (Madrid). Informed written consent was obtained from all the patients.

The protocols were approved by the Ethical Committees of all the institutions involved following the principles of the Declaration of Helsinki.

For expression analysis experiments in whole tissues, human adipose tissue from two locations, omental (OM) and subcutaneous (SC), were obtained from 24 obese patients undergoing bariatric surgery and from 11 lean patients who presented hiatus hernia, gastroesophageal reflux or esophageal achalasia. In addition, human liver was obtained from 9 out of the above obese patients and from 4 lean patients with esophageal achalasia.

For experiments conducted in primary cultures of OM and SC preadipocytes, human adipose tissue from both regions was obtained from 8 additional obese patients, under the above ethical considerations. Precursors cells were isolated and differentiated under defined adipogenic conditions as previously shown [5]. Differentiated preadipocytes

were harvested at 0, 3, 6 and 12 days. (mRNA, n=3 from each depot). Additionally, mature floating adipocytes were separated from the stromal-vascular cells (SVC) from adipose tissue of obese patients.

To investigate the influence of insulin on *ChREBP* mRNA expression, after a differentiation period of 10 days, cells were treated for 48 additional hours with 10% depleted newborn calf serum, and treated or not with 100 mM insulin during the last 24 h. Cells were then harvested at day 12. Depleted serum was obtained as previously described [6] and showed undetectable levels of insulin as measured by RIA.

2.2. RNA extraction and quantitative Taqman RT-PCR

RNA of tissue samples and differentiated preadipocytes was extracted with the RNeasy lipid tissue kit (Quiagen, Madrid, Spain) following the manufacturer´s protocol. Integrity and concentration of RNA were checked with the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). cDNA from 1 µg of each RNA sample was synthesized using iScript cDNA Synthesis Kit (BioRad, Hercules, CA) according to the kit instructions. The expression levels of *ChREBP*, *FASN*, *ATGL*, *ADCY3*, *AP2* and *PPARG* mRNAs were analyzed by RT-PCR using predesigned Taqman Assays probes (Applied Biosystems, Foster City, CA) on a 7300 Real-time PCR System (Applied Biosystems, Foster City, CA). Data were referred to *IPO8* as endogenous control gene, as we have recently reported *IPO8* as an outstanding gene for normalization of gene expression studies in human adipose tissue and differentiated preadipocytes in primary cultures [7]. Liver samples were also referred to *IPO8*, after checking in some experiments that the results obtained with *IPO8* were similar to those obtained using other endogenous controls such as 18S and cyclophilin (*PPIA*)(not shown).

2.3. Western-blots

Total protein extracts were obtained from tissues and cells using standard protocols. Seventy-five µg of each sample were resolved by SDS-PAGE and transferred to nitrocellulose membranes (PROTRAN, Whatman Inc, Florham Park, NJ) in a buffer containing 25mM Tris, 200 mM glycine and 20% methanol. After blocking the membrane with 5% low-fat dried milk in Tris-buffered saline containing 0.05% Tween-20, the levels of ChREBP protein were detected using a 1:500 dilution of the specific antibody (sc-21189, Santa Cruz Biotechnology, Santa Cruz, CA), followed by a 1:7500 dilution of IRDye 800CW donkey anti-goat IgG (LI-COR Biosciences, Lincoln, NE). Beta-actin antibody (sc-47778, Santa Cruz Biotechnology, Santa Cruz, CA) was used as a loading control followed by incubation with IRDye 800 anti-mouse IgG (LI-COR, Lincoln, NE). All immunoreactive fluorescent bands were visualized on the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE) and quantified by ImageJ 1.410 (http://rsb.info.nih.gov/ij)

2.4. Statistical analysis

Results of mRNA expression among groups are shown as fold-changes which were calculated by the $2^{-\Delta\Delta Ct}$ method. Other data are means ± SEM. Statistically significant differences between two groups were determined by the Student t-test ($P< 0.05$). Due to the low sample size in some groups, significant differences were confirmed and evaluated with power analysis, using G*Power 3 software and using a minimal detectable significant power of 0.80 [8].

Correlation analysis was determined by Pearson´s correlation coefficient test (r). Data from the differentiation experiments (4 groups) were submitted to ANOVA analysis, after testing for homogeneity of variance by Bartlett´s test. Statistically significant

differences were identified by the least significant difference method.

3. RESULTS

3.1. Biochemical profile of the patients.

The biochemical parameters of the different patients included in this study are shown in Table 1. No significant differences were observed in the age of the patients among groups. As expected, BMIs from the obese groups were higher than those from their respective leans. No changes were observed in basal glucose, triglycerides or total cholesterol in plasma, between lean and obese patients used for the study of the liver. Regarding the lean and obese subjects used for the study of the adipose tissue samples, although glycemia and triglyceridemia were significantly increased ($P < 0.05$) in the obese groups compared to the lean patients, the variables were within normal ranges.

Liver biopsies from all the obese subjects showed mild (n=3) to moderate fat accumulation (steatosis) (n=6), with no inflammation signs observed. Grading of histological lesions was determined as described [9] Although fat infiltration was not studied in the hepatic samples from the lean patients, we compared alanine aminotransferase (ALT) levels in both lean and obese groups being 27.8 ± 1.9 and 44.3 ± 7.6, respectively ($p< 0.05$). Serum ALT levels are correlated to liver fat [10] and are a good predictor of hepatic steatosis [11].

3.2. ChREBP expression in liver and in omental and subcutaneous adipose tissues from lean and obese subjects.

The *ChREBP* mRNA expression was studied in the liver and the OM and SC adipose tissues from lean and obese individuals. As shown in Figure 1A, opposite regulation was found in the liver and adipose samples: the expression in the liver from obese

patients doubled that one of the leans, whereas in both adipose tissues, the expression was decreased significantly by 50% or more (in OM). In order to compare the expression level among tissues from lean individuals, *ChREBP* mRNA was referred to the liver which showed the higher levels. Although changes were not significant, the mean expression of the transcript in the SC adipose tissue from lean individuals decreased about 15% from that of the liver, whereas the one from the OM depot was even lower as this depot presented levels of *ChREBP* mRNA which were two thirds of the hepatic levels (Figure 1B). To assess the influence of the stromal vascular cells (SVC) on *ChREBP* expression, OM and SC adipose tissues from obese subjects were separated into mature floating adipocytes and SVC. ChREBP expression in SVC represented only 4 and 13% of the expression in OM and SC floating adipocytes, expressed as 100%, respectively (Figure 2).

To investigate the protein levels, tissue protein extracts were obtained and western-blots performed. Figure 3A shows a representative western-blot using liver samples from obese and lean subjects, and a positive control from human HepG2 cells. As shown, the levels of ChREBP protein were higher and clearly detected in the livers from obese individuals as compared to those from the lean, which could be hardly detected. When the levels were quantified and normalized to the beta-actin levels, ChREBP protein in the obese group increased about 3 times over the levels in the lean group (Figure 3B). The expression in the hepatic samples was further confirmed by correlation analysis: Relative *ChREBP* mRNA expression significantly correlated with ChREBP protein levels ($r= 0.84$, $p< 0.01$).

Though we tried doing western-blots with adipose tissue samples from both depots, no signal was detected even when increasing total amounts of protein up to 150 μg, suggesting that ChREBP protein levels mighy reflect the relative amounts of ChREBP

mRNA among the different tissues, liver being the tissue with the highest levels of both mRNA and protein. Non detectable bands could be also due to dilution of ChREBP protein in adipocytes with other predominant cells (SVC) whose expression is not as high as in adipocytes.

We then performed correlation analysis of the *ChREBP* mRNA levels in OM and SC adipose depots as well as in liver with the biochemical parameters of the patients included in the study (BMI, glucose, cholesterol and triglycerides). Only the expression levels in the OM location, the most affected one with higher difference between groups, presented a significant correlation with the BMI of the patients (Figure 4).

3.3. FASN, AP2 and *ATGL* mRNA expression in liver and in OM and SC adipose tissues from lean and obese subjects.

As an opposite regulation of *ChREBP* mRNA expression was found in liver and adipose tissues, other markers involved in adipogenesis were checked in these tissues. Thus, the *FASN* mRNA, a marker of lipogenesis, was checked in the three tissues. As shown in Figure 5A, the pattern was very similar to the one for *ChREBP*: obesity doubled hepatic *FASN* mRNA levels, while decreased them to one third in both OM and SC adipose tissues.

The transcripts of AP2, a key mediator of intracelular transport of fatty acids, were also determined and shown in Figure 5B. Although no changes were observed in liver and SC adipose tissue from lean and obese subjects, a significant decrease was seen in OM adipose tissue from obese patients.

When mRNA levels of ATGL, a main lipase involved in lipolysis, were measured, no changes were observed in any of the tissues between lean and obese patients (Figure 5C). Adenylyl cyclases (ADCY) are enzymes involved inthe first steps of lipolysis.

ADCY3 mRNA levels were measured and are shown in Figure 5D. While the hepatic levels did not change with obesity, the expression in both OM and SC adipose depots were decreased in the obese group.

3.4. ChREBP expression during differentiation of preadipocytes.

To further investigate the role of ChREBP in the adipocyte development in humans, we isolated precursors cells from both OM and SC adipose depots from obese patients. These primary cultures of preadipocytes were differentiated to adipocytes in the presence of 100 nM insulin and harvested at different days (0, 3, 6 and 12 days). Adipocyte differentiation was checked by visual inspection of the accumulated lipids in the cells and by analyzing the expression of well known markers of adipocytic differentiation such as *PPARG* and *AP2* , which increased as expected through adipogenesis (Figure 6, panels A and B). The levels of expression of *ChREBP* mRNA showed dramatic increases at all days tested in both OM and SC locations. The pattern was similar in both depots, as the relative fold-change increased at day 3 and reached the maximal levels at days 6 - 12 of differentiation (Figure 6, panel C). Protein extracts were isolated from the differentiated preadipocytes and the levels of CHREBP protein were measured by Western blots showing increases at all days when compared to day 0 of differentiation (Figure 6, panel D). When the differentiation of the cells was performed in the absence of insulin during the last 2 days, a dramatic decrease was observed in *ChREBP*, *AP2* and *PPARG* mRNA expression (not shown), confirming the positive role of insulin on their expression in human adipocytes.

4. DISCUSSION

ChREBP is a transcription factor with a critical role in the induction of glycolytic and lipogenic genes by glucose, by means of its binding to the ChREs present in the promoters of those target genes which are present, among other tissues, in both liver and WAT. Though there are studies regarding the ChREBP physiological function and activity, very few have been focused in the transcriptional regulation of the gene. Moreover, most of them have been performed in mammals others than humans [12-14] and studied almost exclusively the liver. Inactivation of ChREBP causes a complex phenotype which can not be atributed only to changes in the liver, as this protein is expressed in other important tissues. To our knowledge this is the first study describing ChREBP regulation on human liver in vivo and human differentiated preadipocytes from samples of obese subjects.

Regarding adipocytes *in vitro*, *ChREBP* mRNA expression increased dramatically during the differentiation process of human preadipocytes in culture. Our findings using human preadipocytes are in agreement with the increased *ChREBP* mRNA, obtained during differentiation of murine 3T3 L1 preadipocytes [15]. The CHREBP protein levels were also increased in our samples. As other important transcription factors which control adipocyte differentiation and are regulated by insulin and nutrients, ChREBP is also regulated by insulin in 3T3L1 preadipocytes [15]. Our results show that during human preadipocytes differentiation, the *ChREBP* transcripts were clearly down-regulated in the absence of insulin during the last 48 hr. Such changes suggest that the regulation of *ChREBP* mRNA in human differentiated preadipocytes is highly sensitive to the presence of insulin, similarly to the results obtained in other models such as 3T3L1 which are responsive to insulin stimulation [15].

The protein and mRNA ChREBP levels tended to be higher in liver than in adipose tissues of lean individuals, in agreement with previous results in rats [15, 16]. In fact,

267 the protein levels were only detected in the liver but not in the adipose tissues.
268 However, ChREBP protein was clearly detectable in differentiated preadipocytes in
269 contrast to the whole tissue suggesting a "dilution" effect. In this line, the ChREBP
270 mRNA in SVC cells represented only about 5 and 10% of the one present in mature
271 adipocytes, from OM and SC depots, respectively, suggesting that ChREBP expression
272 is mainly due to adipocytes.

273 When comparing lean and obese patients, opposite regulation was found in liver and
274 adipose tissue. While hepatic ChREBP expression increased in obesity, a decrease was
275 observed in both adipose tissues, being the OM location more affected. This result
276 confirms the study by Letexier and coworkers [17]

277 As stated above, a possible limitation of this kind of studies is the inherent complexity
278 of the tissues studied in terms of the differences in regulation among cell-types present.
279 In this regard, adipocytes can represent 70% of the total adipose mass, but only 25% of
280 the cellular population. The other 75% of the cells of the adipose tissue are fibroblasts,
281 macrophagues, blood cells, endothelial cells and preadipocytes [18]; although SVC
282 present low expression of ChREBP mRNA compared to mature adipocytes, we can not
283 exclude this limitation, as we could only measure the transcripts in samples from obese
284 patients but not in those of lean individuals. In contrast to adipocytes, the hepatocytes
285 are the predominant cells (60% of the total number) of the liver and perform the
286 majority of its functions [19]. Between SC and OM adipose tissues, there are also
287 morphological and functional differences and, in addition, specifically OM fat (and
288 visceral fat, in general) is clearly associated to increased risks to develop obesity-
289 associated disorders such as metabolic syndrome and insulin resistance. Likely the
290 reduction in the expression of ChREBP in adipose tissue in obesity reflects down-
291 regulation of the pathway leading to lipogenesis to prevent further fat accumulation and

292 weight gain. Curiously, in our study only the *ChREBP* mRNA expression in the OM
293 adipose tissue, but not the one in liver or SC fat, was negatively correlated to the
294 increasing BMI of the patients. This is in contrast to literature which shows that visceral
295 fat pads were reduced in the *ChREBP* deficient mice [12] and in a similar way,
296 ChREBP inhibition in ob/ob mice reduced considerably the weight of white adipose
297 tissue [14]. This lack of correlation might be due to differences among species. Recent
298 reports of species- specificity have been published for another gene, PNPLA3, which is
299 associated to fatty liver in humans but not in mice [20]. It has been shown that a high
300 BMI is associated with liver steatosis and that the severity of the steatosis decreases
301 after weight loss [21] and moreover, visceral fat rather than SC fat is a good predictor
302 for the presence of fatty liver [22] due to its contribution through the portal vein. At this
303 respect, several studies in humans and rodents have suggested that ChREBP expression
304 is associated with the excesive accumulation of triglycerides observed in hepatic
305 steatosis [14] which can be caused by an augmented lipogenesis. In fact, all liver
306 samples from the obese subjects in our study show mild to moderate fat infiltration.
307 In murine hepatocytes it has been shown that ChREBP binds to the ChRE of lipogenic
308 genes promoters like FASN and ACC [23]. As expected in our study, the opposite
309 regulation observed in liver and adipose tissue of the ChREBP mRNA was paralleled
310 by a similar pattern in the lipogenic enzyme FASN. In lean healthy humans, adipose
311 tissue lipogenesis, although active, is less responsive than hepatic lipogenesis to acute or
312 prolonged carbohydrate overfeeding [24]. Similar results have been shown in human
313 obesity: there is an enhanced hepatic lipogenesis but no evidence for an increased
314 lipogenic capacity of SC adipose tissue in obese subjects [25]. Our study confirms those
315 results and further shows that this occurs not only in adipose tissue from SC origin but
316 also from visceral localization. Although we do not know the exact mechanism of this

317 opposite regulation, it could be due to a different tissue specific regulation. It has been
318 recently described that O-linked β-N-acetylglucosamine (O-GlcNAcylation) is a novel
319 mechanism increasing ChREBP activity in the liver [26], but there are no reports on
320 adipose tissue. Alternatively, the regulation of the lipogenic enzymes promoters by
321 ChREBP could be different in liver and adipose tissue. That is the case for SREBP-1c, a
322 well established transcription factor which regulates FASN in hepatocytes. Despite the
323 activation process of SREBP-1c is intact in adipocytes, it does not bind to the functional
324 SRE/E-box site on the FASN promoter in adipocytes [27]. This opposite regulation
325 might have arisen as a consequence of the adipose tissue expansion limit, which once
326 reached, will reduce the capacity of the adipose tissue to store energy efficiently, and
327 lipids begin to accumulate in other tissues such as the liver, contributing to lipotoxicity
328 and associated pathologies (insulin resistance, diabetes or fatty liver) [28]. In a recent
329 study with adipose tissues from obese subjects we found a generalized decrease in the
330 expression of enzymes involved in lipogenesis, as well as in lipolysis, [29], both likely
331 due to a defense mechanism. Regarding lipolysis, the mRNA expression of two
332 enzymes, ADCY3 and ATGL, were assayed in the liver and adipose tissues samples of
333 our present study. No changes were observed in the tissue expression levels of ATGL
334 between lean and obese subjects, however, ADCY3 expression decreased in adipose
335 tissue of OM and SC origin, while no differences were seen in the liver. These data are
336 in agreement with previous results, as *ATGL* mRNA levels were found to be unaffected
337 in obesity [30] [31] and four isoforms of ADCY, ADCY3 included, were decreased in
338 adipose tissue of obese subjects [29]. Adipocytes release fatty acids into the circulation
339 when nutrients are needed but a state of obesity would not be a situation in which
340 energy expenditure is required. Thus, lipolysis in these tissues of obese patients could
341 be inhibited to avoid a higher excess of free fatty acids. Among fatty acids,

polyunsaturated fatty acids (PUFA) are potent inhibitors of lipogenesis by downregulating *ChREBP* gene expression [32]. In situations in which lipolysis is high, there is a selective mobilization of polyunsaturated fatty acids (PUFA) to circulation [33], if lipolysis were inhibited in some way, mobilization of PUFA would be also decreased resulting in accumulation inside the adipocytes. This might be happening in the adipose tissue of the obese patients of our study, and this putative increase in adipose tissue PUFA could be suppressing lipogenic enzymes by inhibiting ChREBP expression. In addition, a decrease of AP2 could reduce NEFA release, in that case in absence of AP2, lipolysis would decrease and the NEFA (and concomitantly PUFA) content inside the adipocyte could increase up to 3-fold [34] .

On the other hand, hepatic PUFA are clearly decreased in the liver of obese patients with steatosis [35], and this decrease might account for the higher levels of hepatic *ChREBP* mRNA, as the inhibition by PUFA could be abolished.

In conclusion, the present study shows for the first time data on the regulation of ChREBP expression in human differentiated preadipocytes and an opposite regulation of its expression in the liver and adipose tissue from obese subjects. These results are compatible with a limited lipogenic/adipogenic capacity in adipocytes which might lead to spillover of lipids excess to other tissues such as the liver with an increased lipogenesis contributing to lipotoxicity and comorbilities found in obese patients.

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FIGURE LEGENDS.

Figure 1. ChREBP mRNA in liver, omental and subcutaneous adipose tissue from lean and obese subjects. A) ChREBP mRNA is normalized to IPO8. Each tissue is compared to its respective lean group. B) Groups are expressed vs lean liver considered as 1. Data are means± SEM. * $p<0.05$ *vs* the respective lean subjects, # $p<0.05$ *vs* lean liver. The number of observations appears in brackets and is the same for both panels.

Figure 2. ChREBP mRNA in floating adipocytes (Ad) and stromal vascular cells (SVC) from obese patients. SVC from omental (OM) and subcutaneous (SC) adipose tissue were compared to their respective floating mature adipocytes. Data are mean ± SEM, * p< 0.05, n=2/group.

Figure 3. ChREBP mRNA and protein expression in livers from lean and obese subjects. A) Western-blot of ChREBP protein as compared to ß-actin in liver extracts. A positive control of HepG2 cells extract (Hep) is included. B) Quantification of ChREBP levels compared to ß-actin protein expressed as mean ± SEM, * p< 0.05, n=4 and n=8 for lean and obese patients, respectively.

Figure 4. Correlation analysis between BMI and ChREBP mRNA expression in omental adipose tissue. Correlation was determined by Pearson´s correlation coefficient test (r), n=35 (11 lean and 24 obese).

Figure 5. *FASN*, *AP2*, *ATGL* and *ADCY3* mRNA in liver and adipose tissues from lean/ obese subjects. *FASN* (A), *AP2* (B), *ATGL* (C) and *ADCY3* (D) mRNA expression are normalized to IPO8. Results are mean ± SEM, * p< 0.05 vs its respective lean group. n appears in brackets.

Figure 6. *PPARG*, *AP2* and *ChREBP* mRNA and ChREBP protein in omental and subcutaneous differentiated preadipocytes. Cells were harvested at different days of differentiation (0, 3, 6 and 12 days). Results are mean ± SEM from duplicates of three

independent experiments from each depot and are normalized to IPO8, * p< 0.05 vs its respective 0 day, # p< 0.05 vs its respective day3. A representative Western-blot is shown with ChREBP levels as compared to ß-actin.

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Table 1

Table 1. Biochemical parameters from the obese and lean subjects included in the study.

	LEAN LIVER	OBESE LIVER	LEAN ADIPOSE	OBESE ADIPOSE	OBESE CULTURES	Range
Sex (M/F)	2/2	1/8	4/7	5/19	3/5	
Age (years)	44.2±6.4	42.9±3.3	47.6±3.6	44.0±2.3	41.1±5.5	
BMI (kg/m2)	23.6±0.8	47.8 ±2.2 [a]	23.0±0.5	49.8± 1.5[b]	47.9±2.1 [b]	
Glucose (mg/dl)	89.5±5.4	106.1±12.8	86.3±2.6	99.8± 5.9[b]	115.6±8.0 [b]	60-115
Cholesterol (mg/dl)	186.3±10.5	172.9±12.5	193.8±6.6	186±.28.9	195.6±12.0	120-240
Triglycerides (mg/dl)	69.3±7.1	81.9±4.8	65.9±5.6	99.3± 9.9[b]	152.4 ±17.0[b]	50-175

Results are means ± SEM. [a] and [b] represent a statistical significant difference ($p<0.05$) versus the respective lean group, comparing livers ([a]) and adipose tissues ([b]), respectively.

Figure 1

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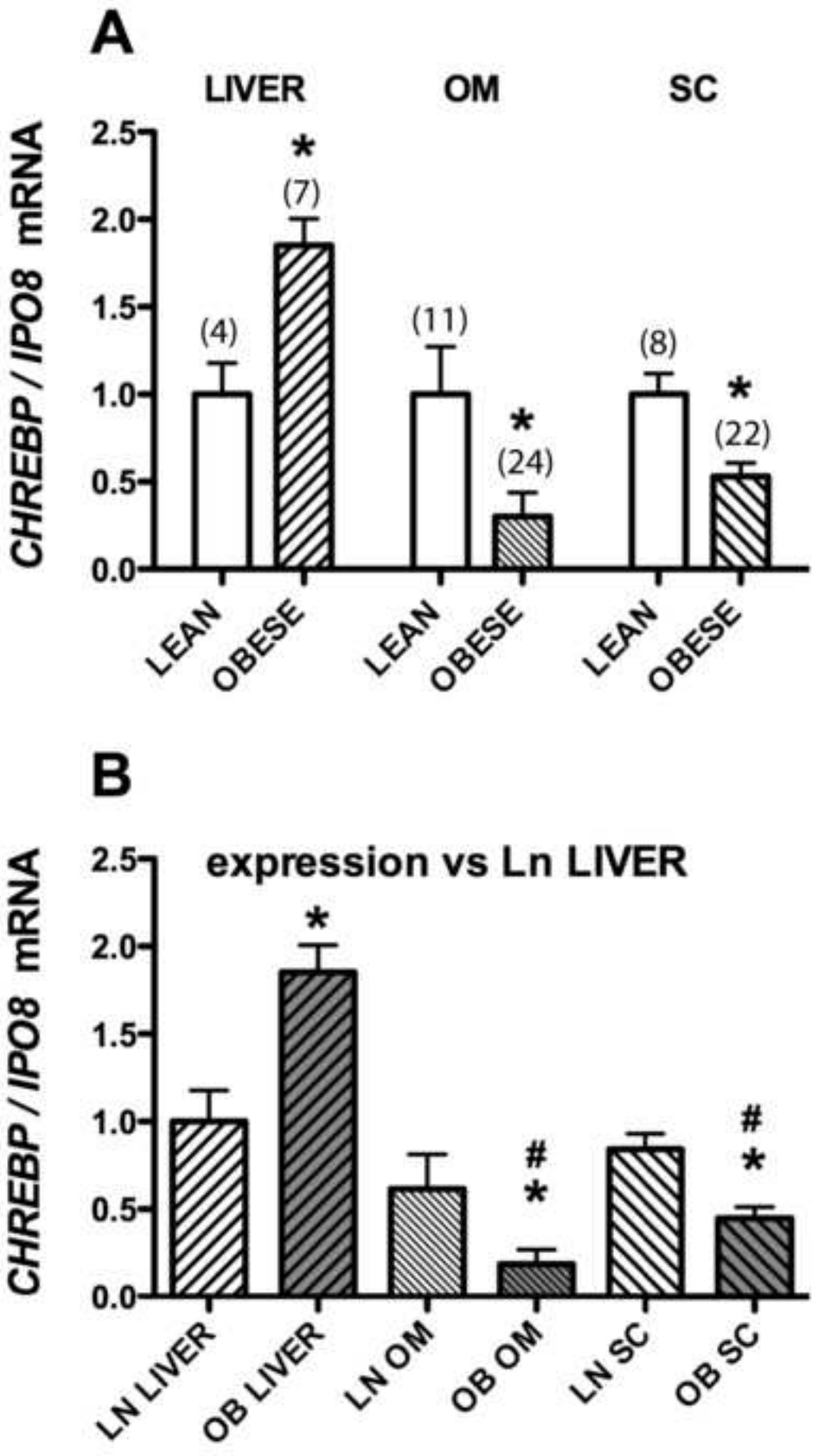

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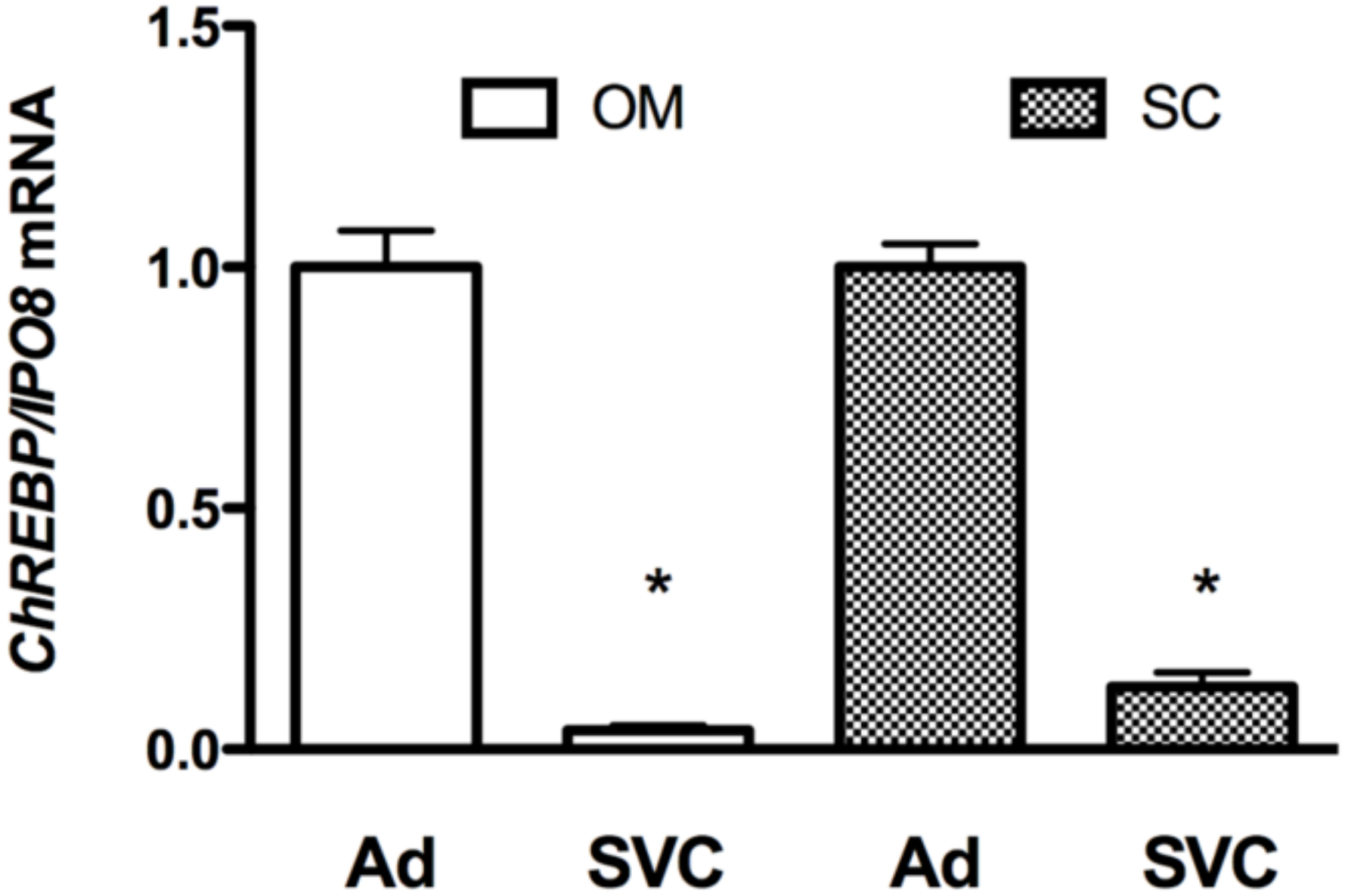

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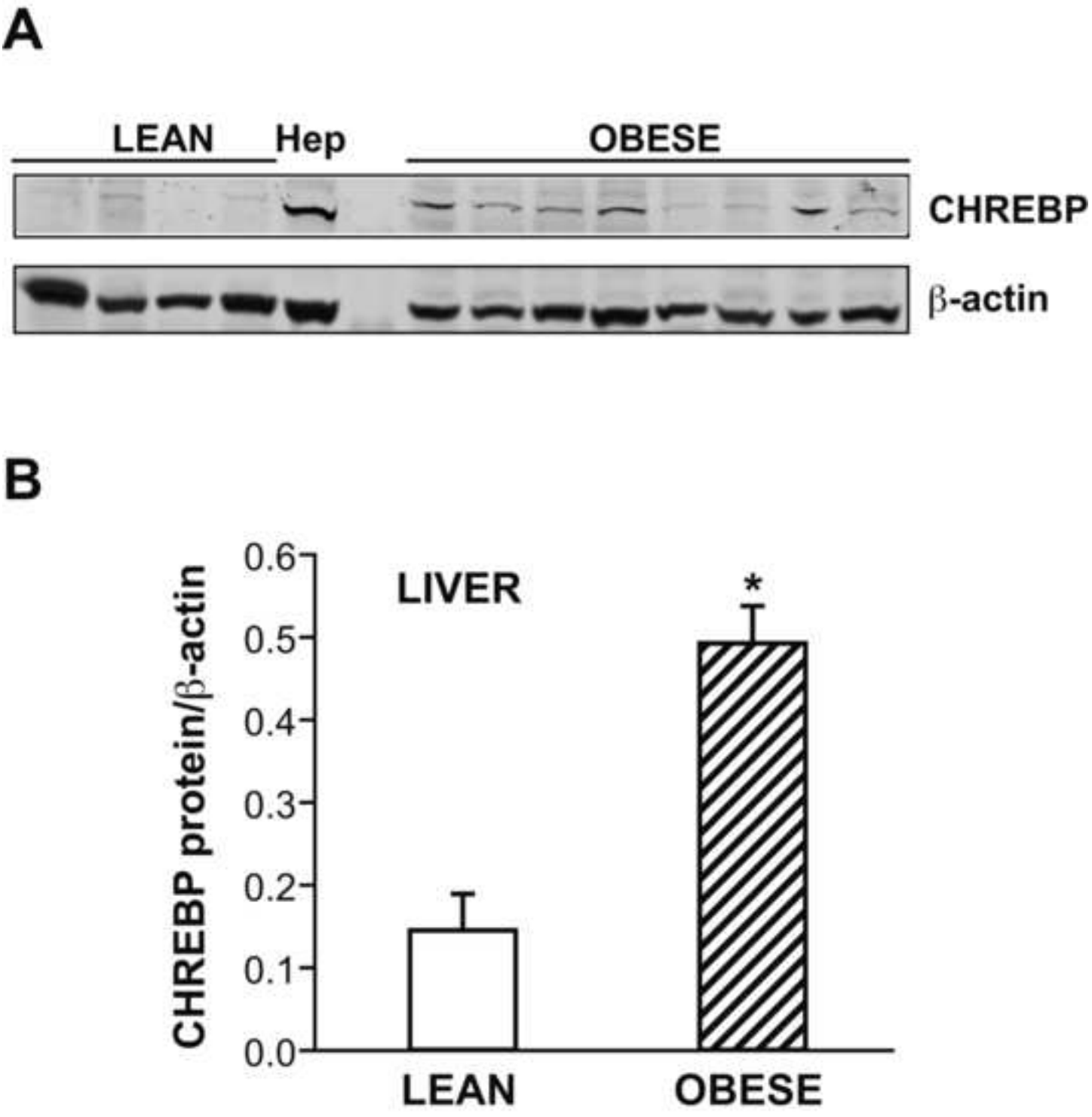

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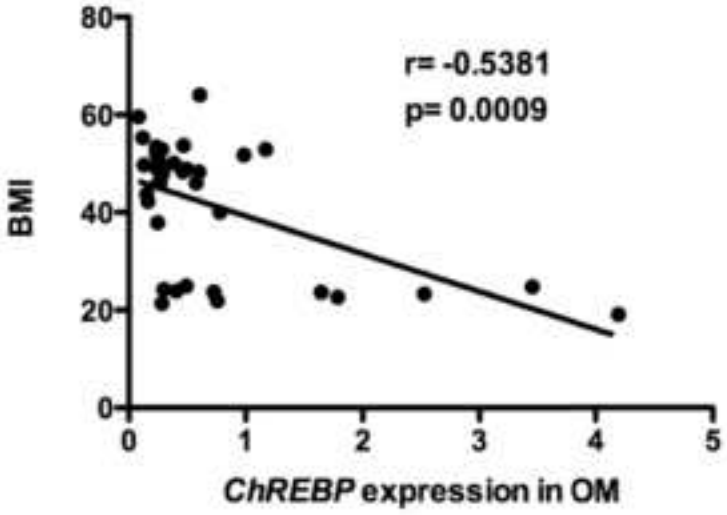

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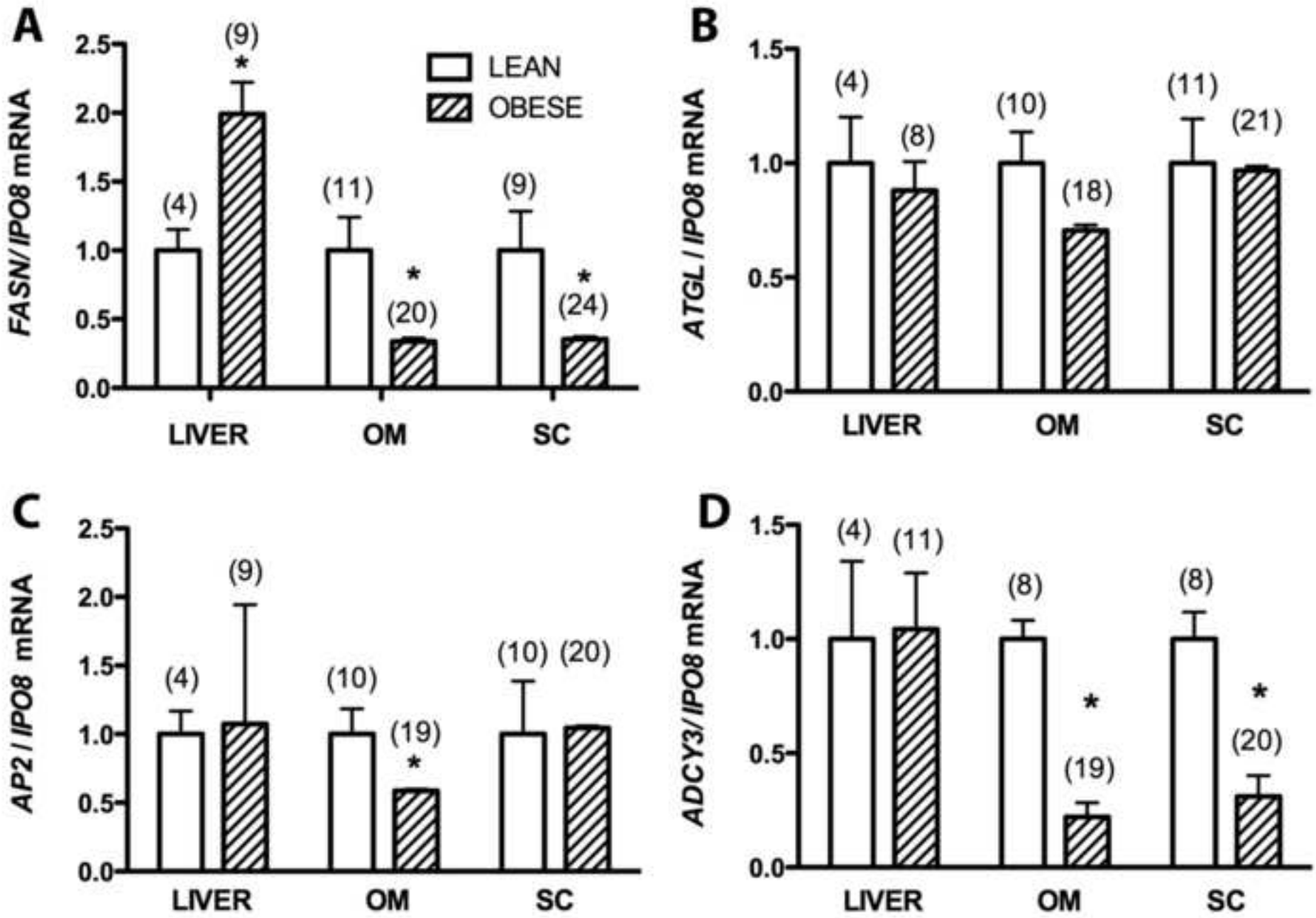
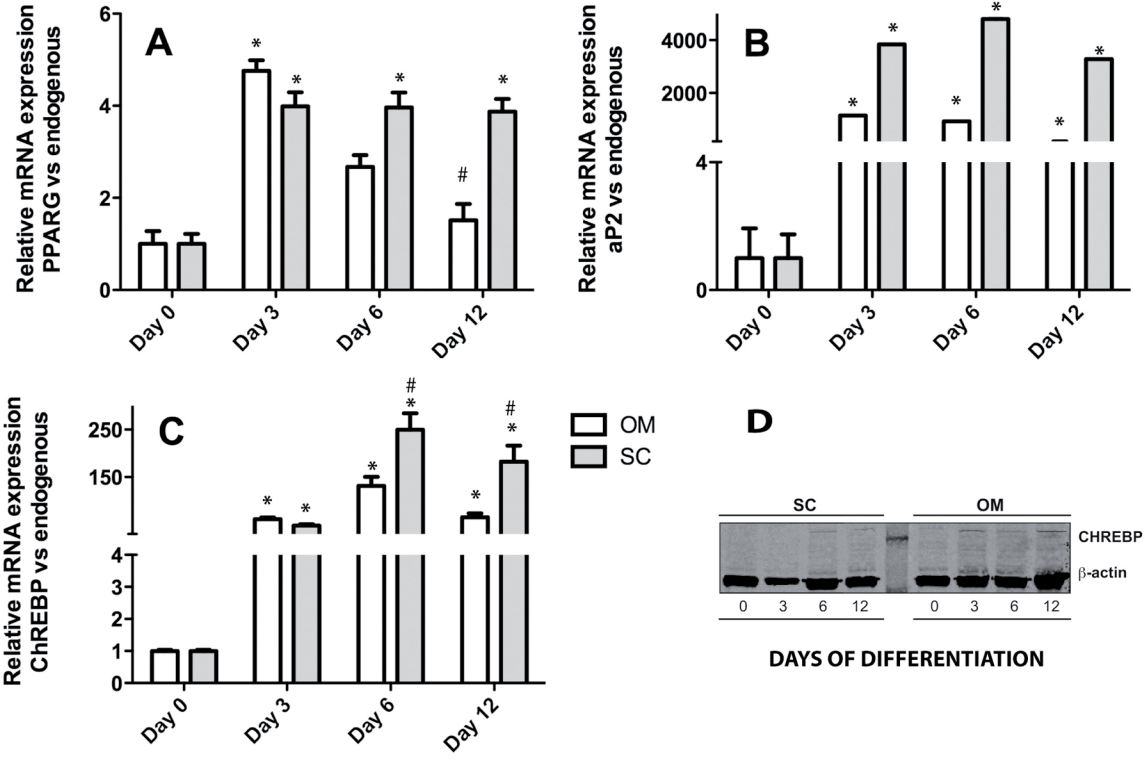


Figure 6


A
Relative mRNA expression PPARG vs endogenous
Day 0
Day 3
Day 6
Day 12
B
Relative mRNA expression aP2 vs endogenous
C
Relative mRNA expression ChREBP vs endogenous
OM
SC
D
SC
OM
CHREBP
β-actin
0 3 6 12
0 3 6 12
DAYS OF DIFFERENTIATION