In vivo expression of carbohydrate responsive element binding protein in lean and obese rats

D Letexier, O Peroni, C Pinteur, M Beylot

SUMMARY

ChREBP (Carbohydrate response element binding protein) is considered to mediate the stimulatory effect of glucose on the expression of lipogenic genes. Its activity is stimulated by glucose. Less is known on the control of its expression. This expression could be controlled by nutritional (glucose, fatty acids) and hormonal (insulin) factors. We examined the in vivo nutritional control of ChREBP expression in liver and adipose tissue of Wistar rats. Compared respectively to the fed state and to a high carbohydrate diet, ChREBP mRNA concentrations were not modified by fasting or a high fat diet in rat liver and adipose tissue. FAS and ACC1 mRNA concentrations were on the contrary decreased as expected by fasting and high fat diets and these variations of FAS and ACC1 mRNA were positively related to those of SREBP-1c mRNA and protein, but not of ChREBP mRNA. Therefore i) ChREBP expression appears poorly responsive to modifications of nutritional condition, ii) modifications of the expression of ChREBP do not seem implicated in the physiological control of lipogenesis. To investigate the possible role of ChREBP in pathological situations we measured its mRNA concentrations in the liver and adipose tissue of obese Zucker rats. ChREBP expression was increased in the liver but not the adipose tissue of obese rats compared to their lean littermates. These results support a role of ChREBP in the development of hepatic steatosis and hypertriglyceridemia but not of obesity in this experimental model.

Key-words: Lipogenesis · Obesity · Steatosis · SREBP-1c.

Address correspondence and reprint requests to:
M Beylot. INSERM U499, IFR 62, Faculté RTH LAENNEC,
University Claude Bernard-Lyon1, rue G Paradin, 69008 Lyon, France.
beylot@sante.univ-lyon1.fr

Received: May 2nd, 2005; revised: July 14th, 2005

© 2018 Elsevier Masson SAS. Tous droits réservés. - Document téléchargé le 05/10/2018 Il est interdit et illégal de diffuser ce document.
Near all tissues express the enzymes of lipogenesis, the metabolic pathway allowing the synthesis of new fatty acid molecules from non-lipid substrates, mainly carbohydrates. In mammals the two main sites of lipogenesis are liver and adipose tissue. In these tissues, lipogenesis allows in periods of high energy supply to convert carbohydrates into lipids and to store these lipids for later use in periods of low energy supply. The expression and activity of lipogenesis in these tissues are controlled by hormonal and metabolic factors. Glucagon, fatty acids and energy restriction suppress lipogenesis whereas carbohydrate intake, raised concentrations of glucose and insulin stimulate it [1-4]. Lipogenesis is controlled acutely by phosphorylation-dephosphorylation and allosteric regulation of key enzymes such as acetyl-CoA carboxylase 1 (ACAC1) [5] and on a long term basis at the level of gene transcription [3,6]. Glucose and insulin induce a coordinated stimulation of the transcription of a panel of glycolytic and lipogenic genes [2-3]. However, a full stimulation requires the simultaneous presence of high insulin and glucose concentrations whereas insulin or glucose alone induce only a moderate increase of the expression of lipogenic genes [7-8].

The stimulatory effects of insulin on the expression of the lipogenic pathway are mediated by the transcription factor SREBP-1c (sterol responsive element binding protein-1c) [9], SREBP-1c induces the expression of lipogenic genes [9-11] and its expression is stimulated by insulin [11] directly or through the transcription factor LXRa (Liver X receptor α) [12]. However SREBP-1c alone cannot fully account for the stimulation of lipogenesis by carbohydrates [8,13] and this led to the proposal that another factor is responsible for the glucose induction of lipogenic enzymes. Uyeda et al. [14-15] purified from the liver of rat and mice such a factor that they named carbohydrate response-element binding protein (ChREBP). These authors showed that glucose increases the activity of ChREBP in liver and that, once stimulated, ChREBP binds a specific carbohydrate-response element binding protein (ChREBP). These authors showed that glucose increases the activity of ChREBP in liver and that, once stimulated, ChREBP binds a specific carbohydrate-response element in the promoter of L-pyruvate kinase (L-PK) and increases the expression of L-PK [16]. They further showed that glucose stimulates ChREBP by dephosphorylation on specific amino-acids while glucagon and fatty acids inhibit it by a phosphorylation mechanism [17-19]. Initially, the role of ChREBP in the control of gene expression was clearly shown only for L-PK in liver. Evidence for a role of ChREBP in controlling the expression in liver of the genes of other lipogenic enzymes such as ACC1 and FAS has since been presented [8,20-21]. ChREBP has also been shown to control the expression of L-PK in insulin-secreting cells, extending its role outside of the liver [22]. Lastly we showed that ChREBP is also expressed in rat and human adipose tissue [23], a finding confirmed since [20,24,25] and suggesting that it could also be involved in the regulation of adipocyte lipogenesis. Therefore ChREBP appears now to be a player in the regulation of lipogenic gene expression in mediating the effects of glucose, at least in liver.

The mechanisms controlling the activity of ChREBP in liver have been well described [15], but the regulation of its expression is still poorly known. Recently high glucose concentrations have been shown to increase ChREBP mRNA concentrations in insulin-secreting cells [22], 3T3-L1 adipocytes [24-25] and hepatocytes [8]. In addition insulin and troglitazone stimulated ChREBP expression in 3T3-L1 adipocytes while fatty acids inhibited it [25]. However, in these in vitro experiments, an effect of glucose was observed only for very high, unphysiological, concentrations [8,22,24,25] and the effect of insulin required also very high concentrations of glucose [25]. In vivo, ChREBP mRNA concentrations were not modified by fasting nor insulin deficiency in adipose tissue [25], and increased only in rat adipose tissue [25] and mice liver [8] during the particular situation of carbohydrate refeeding after fasting. We found previously no difference in ChREBP mRNA levels in adipose tissue of human beings and rats fed a high carbohydrate (HCHO) or a high fat (HF) diet and studied in the post-absorptive state [23]. Therefore, it is presently unclear whether there is a truly physiologically relevant nutritional control of ChREBP expression. Moreover, whether modifications of ChREBP expression, in addition to modifications of its activity, could be implicated in the regulation of the expression of lipogenic genes remains also to be established. Lastly the possible role of variations of ChREBP expression in the development of pathology such as obesity, hepatic steatosis or hypertriglyceridemia has not been investigated.

In the present report we investigated the effects i) of diets with high amounts of CHO or fat (HCHO and HF diets) and ii) of fasting (compared to the fed, absorptive, state) on ChREBP expression in the adipose tissue and liver of Wistar rats. We also measured the expression level of SREBP-1c and of lipogenic genes and compared the expression of these lipogenic genes with those of SREBP-1c and ChREBP. Lastly, to determine whether ChREBP over-expression could be implicated in pathological situations such as obesity and hepatic steatosis, we measured ChREBP mRNA concentrations in the adipose tissue and liver of lean and obese Zucker rats.

**Materials and methods**

**Protocols**

Male Wistar rats (4 weeks old), male obese Zucker (ZF) rats and their lean littermates (10 weeks old) were from Charles River (L’Arbresle, France). Wistar rats were first fed during four weeks with a standard chow diet. They were then divided into two groups, one fed during six
weeks with a HCHO diet (HCHO group, n = 8) (70% CHO, 10% fat, diet D12450B of Research Diets, New Brunswick, NJ, USA) and the other fed (six weeks) with a HF diet (HF group, n = 8) (20% CHO, 60% fat with saturated and monounsaturated fatty acids, diet D12492 of Research Diets). All rats had free access to food and water. At the end of the six weeks of controlled diet, Wistar rats were studied either in the fed, absorptive, state (n = 4) or after a 48 hour fast (n = 4). Lean (n = 10) and ZF (n = 10) rats were fed one week with a standard chow diet and were studied in the fed state (n = 5) or after a 48 hour fast (n = 5). For the measurement of hepatic lipogenesis, the groups of lean and ZF rats studied in the fed state received 72 hours before sampling an intra-peritoneal injection of deuterated water (12 ml/kg in 9g/l NaCl) and ingested thereafter water enriched (3.5%) with deuterated water until the end of the protocol. In all rats, blood was sampled and perirenal adipose tissue and liver were quickly removed and snap frozen in liquid nitrogen. Samples were stored at −80°C until analysis. Samples of liver and adipose tissue were also immediately homogenized (Tris-HCl buffer 3 mM, pH 7.2, EDTA 1 mM, Sucrose 250 mM with proteases inhibitors) for the purification of nuclei and preparation of cytosolic and nuclear extracts for immunoblot analysis.

Measurements of mRNA levels and SREBP-1c protein amounts

Total RNA was extracted from liver and adipose tissue samples using the RNeasy total RNA kit (Qiagen, Courtaboeuf, France) with the addition of treatment with DNAase. Concentrations and purity were verified by measuring optical density at 260 and 280 nm. Their integrity was checked by agarose gel electrophoresis. mRNA concentrations of ACC1, FAS and SREBP-1c were measured by RT-competitive PCR as previously described [23,26,27]. Other mRNAs levels were determined by semi-quantitative RT-PCR using beta-actin as internal standard. Primers used are shown in table I. The protocols used for these RT-PCR has been published in detail previously [23]. The amounts of the precursor (in cytosolic extracts) and mature forms (in nuclear extracts) of SREBP-1c protein were quantified by immunoblots as previously described [23].

Metabolites and hormone concentrations

Plasma glucose, triglycerides (TG), non-esterified fatty acids (NEFA) and total cholesterol were measured by enzymatic method [28,29] and insulin by ELISA (Cristal Chem Inc, Downers Grove, Il, USA) respectively.

Hepatic lipogenesis

Measurements of the deuterium enrichments in plasma water and in the palmitate of plasma TG and the calculation of the contribution of hepatic lipogenesis to this pool of plasma TG were performed as previously described [27,30,31].

Statistics

Results are shown as mean and sem. Comparisons between the different groups of Wistar rats were performed by one way analysis of variance followed by the Newman-Keuls test to locate the significant differences. Comparisons between lean and obese Zucker rats were made by two tailed Student’s t test.

**Results**

**Wistar rats**

The body weight gain of the rats fed the HF or HCHO diet and their final weight were comparable. Plasma glucose, cholesterol, TG and insulin concentrations were decreased (P < 0.05) by fasting in both groups of rats, without differences between the HF and HCHO groups (table II). Fasting increased plasma NEFA in all

---

**Table I**

<table>
<thead>
<tr>
<th>Primers used for RT-PCR.</th>
<th>Sense (5’ - 3’)</th>
<th>Antisense (5’ - 3’)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-actin</td>
<td>GAGGAGGCCCAGAGCAAGAGA</td>
<td>GGGTGTTGAGGCTCTGAAGACA</td>
<td>225</td>
</tr>
<tr>
<td>INSIG1</td>
<td>GCAGAAATGTGGAGACACTG</td>
<td>GAGCATGATCTTCCACACCT</td>
<td>463</td>
</tr>
<tr>
<td>INSIG 2</td>
<td>ATGAAACCATGCGCAGAAGGAGGAG</td>
<td>CAAAATGCCCACCAGTGCC</td>
<td>456</td>
</tr>
<tr>
<td>CReBP</td>
<td>CTGTGTTCTCACCAGAAGTGGAA</td>
<td>CACGGTGAGAAGGAGTGGAGCA</td>
<td>702</td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>GGCGAGCCATGATTGCAC</td>
<td>CTCTCTCTATACAGGCCC</td>
<td>311</td>
</tr>
<tr>
<td>FAS</td>
<td>GTGGGCTGCTGACGGGCAAG</td>
<td>TGGGGCTGAGGCTGGGGCA</td>
<td>514</td>
</tr>
<tr>
<td>ACC1</td>
<td>GTTGCACAAAAAGATTGCAC</td>
<td>CGACATTACATGCTCCGAC</td>
<td>504</td>
</tr>
</tbody>
</table>
rats. However rats fed the HF diet had in the fed state higher plasma NEFA concentrations (P < 0.05) than the HCHO group. This difference disappeared after the 48 hour fast (Table II).

Despite these modifications in metabolic (glucose, fatty acids) and hormonal factors (insulin) proposed as playing a role in the regulation of ChREBP expression [8,22,24-25], mRNA concentrations of ChREBP in liver and adipose tissue were not modified by fasting in the HF nor in the HCHO groups (fig. 1). They were also not different between these two groups of rats, either in the fed or in the fasted state. Contrasting with this lack of variation in ChREBP mRNA concentrations, liver and adipose tissue mRNA levels of FAS and ACC1 were largely decreased (P < 0.01 or 0.001) by fasting in the HCHO group (fig. 2). Feeding a HF diet also decreased, in rats studied in the fed state, the concentrations of FAS and ACC1 mRNA in liver (P < 0.001) and adipose tissue (P < 0.01) (fig. 2). Fasting also induced a decrease of FAS and ACC1 mRNA levels in the liver of HF rats (P < 0.05, fig. 2). In adipose tissue of HF rats, only FAS mRNA levels were decreased (P < 0.05) by fasting.

FAS and ACC1 mRNA concentrations appear therefore totally unrelated with those of ChREBP, either in adipose tissue or in liver. The nutritionally induced modifications of FAS and ACC1 mRNA levels on the contrary appear parallel to those of SREBP-1c mRNA in adipose tissue and liver (fig. 1) and to those of the amount of the precursor and mature forms of SREBP-1c protein in liver (fig. 3). In adipose tissue, only the precursor form of SREBP-1 protein could be quantified whereas the variation of the amount of this precursor form of SREBP-1 (fig. 3) were parallel to those of SREBP-1c, ACC1 and FAS mRNA concentrations. ACC1 and FAS mRNA concentrations were positively related to those of SREBP-1c mRNA in adipose tissue (FAS: r = 0.716 P < 0.001; ACC1: r = 0.988 P < 0.001) and in liver (FAS: r = 0.989 P < 0.001; ACC1: r = 0.893 P < 0.001). ACC1 and FAS mRNA levels were also positively related with the amounts of the precursor and mature forms of SREBP1 protein (P < 0.01 for both) in liver and with the amount of the precursor form in adipose tissue (P < 0.05).

We also measured the mRNA concentrations of INSIG-1 and INSIG-2, encoding for two proteins intervening in the control of lipogenesis by blocking the cleavage of the precursor form of SREBP [24,32-35]. These concentrations in adipose tissue were not different between the HCHO and HF groups and were also not modified by fasting (data not shown). In liver these mRNA concentrations were not different between the HCHO and HF groups but were modified by fasting: in both groups INSIG-1 mRNA levels were decreased by the 48 hour fast (HCHO group: from 0.79 ± 0.08 to 0.27 ± 0.11; HF group: 0.76 ± 0.10 to 0.19 ± 0.03, P < 0.01) while INSIG-2 mRNA concentrations increased on the contrary (HCHO group: from 0.39 ± 0.10 to 0.94 ± 0.17; HF group: 0.52 ± 0.09 to 0.92 ± 0.13, P < 0.05).

**Lean and Zucker rats**

Obese Zucker rats as expected had higher concentrations of plasma TG than their lean controls (2.22 ± 0.27 vs 0.56 ± 0.11 mM P < 0.01 in the fed state, 1.98 ± 0.32 vs 0.38 ± 0.03 mM P < 0.05 in the fasted state). Plasma cholesterol concentrations were also higher in obese rats (4.40 ± 0.19 vs 2.26 ± 0.10 mM P < 0.001 in the fed state and 3.30 ± 0.15 vs 1.48 ± 0.06 mM in the fasted state, P < 0.01). The fractional contribution of hepatic lipogenesis to the plasma TG pool, measured in the fed state, was also higher in the obese Zucker group (74.8 % ± 34.7 % 0.06 % P < 0.01). The mRNA concentrations of ChREBP, expressed relative to D-actin, were higher in the liver of obese rats (fed state: 3.35 ± 0.59 vs 0.34 ± 0.08 p < 0.001; fasted state: 1.06 ± 0.21 vs 1.04 ± 0.03 P < 0.05) (fig. 4). In addition these mRNA concentrations were decreased during fasting in the obese group (P < 0.01) but not in the lean group. Contrary to what was observed in liver, ChREBP mRNA levels were comparable in the adipose tissue of obese and lean rats (fed state: 0.11 ± 0.02 vs 0.13 ± 0.02; fasted state: 0.10 ± 0.03 vs 0.10 ± 0.01) without any differentiations.
ces between the fed and fasted state. Lastly, as appreciated by mRNA concentrations, ChREBP in obese rats was much less expressed in adipose tissue than in the liver ($P < 0.05$).

**Discussion**

We investigated in the present report whether ChREBP expression is controlled by nutritional factors in vivo and if such a control could, in addition to the acute modifications of ChREBP intra-cellular location and activity [15], be implicated in the physiological regulation of lipogenesis. Both fasting and a HF diet induced in rats, compared respectively to the fed state and to a HCHO diet, the expected changes in the expression of FAS, ACC1 and SREBP-1c with dramatic decreases of their mRNA concentrations, and of SREBP-1 protein, in liver and less marked decreases in adipose tissue. The modifications of FAS and ACC1 mRNA levels were parallel with those of SREBP-1c mRNA and protein both in liver and adipose tissue. ChREBP mRNA concentrations were on the contrary unaffected by fasting or a HF diet compared respectively to the fed state and the HCHO diet. This lack of change in

![Figure 1](image-url)

**Figure 1**
Concentrations of ChREBP and SREBP-1c mRNA in the liver (panel A) and adipose tissue (panel B) of rats receiving a HCHO or HF diet and studied in the fed state or after a 48 h fast. * $P < 0.05$, *** $P < 0.001$ vs fed rats of the same group, $§ P < 0.05$ vs the fed group of HCHO rats; § $p < 0.05$ vs the starved HCHO group.
ChREBP mRNA levels was observed despite modifications in plasma concentrations of glucose, insulin and NEFA that should have, according to in vitro studies [8,22,24,25], induced an increased ChREBP expression in the fed state and in the HCHO group. We investigated the effect of a HF diet containing saturated and monounsaturated fatty acids since such fatty acids clearly decreased adipocytes ChREBP expression in vitro [25]. This effect was not observed in vivo during the present study. Therefore, although it is clear that it can be modified in particular situations such as high CHO refeeding after fasting [8,25], ChREBP expression appears, compared to SREBP-1c expression, poorly responsive to nutritional factors in vivo. Of course, these results should not be interpreted as showing that ChREBP plays no role in the physiological nutritional control of lipogenesis but that it intervenes mainly by the modifications of its intra-cellular localization and of its activity previously described [15,17,18] and poorly through increase or decrease of its expression.

To obtain insights on the possible role of ChREBP in pathological situations we measured its mRNA concentration in the liver and adipose tissue of lean and obese Zucker rats. ZF rats had as expected increased hepatic lipogenic rates and high plasma TG levels. Our finding of

![Figure 2](attachment:image.png)

**Figure 2**
Concentrations of FAS and ACC1 mRNA in the liver (panel A) and adipose tissue (panel B) of rats receiving a high carbohydrate (HCHO) or high fat (HF) diet. Rats were studied in the fed state or after a 48 h fast (starved). * P < 0.05, ** P < 0.01, *** P < 0.001 vs fed rats of the same group; $$ P < 0.01, $$$ P < 0.001 vs the fed group of HCHO rats; § P < 0.05 vs the starved HCHO group.
increased ChREBP mRNA concentrations in liver supports a role for ChREBP in stimulating, in addition to SREBP-1c [36,37], the expression and activity of liver lipogenic pathway in this model of hepatic steatosis and hypertriglyceridemia. Whether ChREBP is overexpressed in the liver of other experimental models, and in human steatosis, will be important to determine in order to strengthen or not this possibility. ChREBP mRNA concentrations were on the contrary not modified in the adipose tissue of obese rats. This result and our previous finding of low expression levels of ChREBP mRNA in the adipose tissue of human subjects with long standing obesity [23] do not support a role for adipose ChREBP in the development or maintenance of excess fat stores in adipocytes. This is consistent with previous reports showing that FAS, ACC1 and SREBP-1c mRNA concentrations are not increased, but rather decreased in the adipose tissue of human subjects [27] and experimental models of obesity [36,38]. However these observations were done in human subjects and in animals with established, long standing obesity and could be an adaptative response aimed at limiting a further expansion of fat storage in adipose tissue. The situation  

Figure 3
Amounts of the precursor (liver and adipose tissue) and mature (liver) forms of SREBP-1c proteins (OD arbitrary units/µg of proteins) in the liver and adipose tissue of rats fed a HCHO or HF diet. Rats were studied in the fed state or after a 48 h fast (starved). Panel A: data in liver (precursor form p: upper part, mature form p: lower part). Panel B: upper part, precursor form in adipose tissue; lower part representative immunoblot of Srebp-1c in liver.

© 2018 Elsevier Masson SAS. Tous droits réservés. - Document téléchargé le 05/10/2018 Il est interdit et illégal de diffuser ce document.
could be different during the initial, dynamic phase of the constitution of obesity, as suggested by animal studies [39]. This will be an important point to solve during future studies.

In conclusion, our results show that ChREBP expression in liver and adipose tissue is poorly responsive to changes in nutritional conditions and therefore that modifications of ChREBP expression plays little role in the nutritional control of lipogenesis. However an increased expression of ChREBP could be implicated in the development of pathological situations such as hepatic steatosis and hypertriglyceridemia.

Acknowledgments – This work was supported in part by a grant from the AFERO (Association Française d’Etude et Recherche sur l’Obésité) and from the ministère délégué à la Recherche (appel d’offre 2003 : plates-formes d’explorations fonctionnelles thématisées). O Peroni was supported by a grant from the Fondation pour la Recherche Médicale.

References

37. Shimomura I, Bashmakov Y, JD H. Increased levels of nuclear SREBP-1c is associated with fatty livers in two mouse models of diabetes mellitus. J Biol Chem 1999;274:30028-32