Pretranslational regulation of pyruvate dehydrogenase complex subunits in white adipose tissue during the suckling–weaning transition in the rat

Joceline MAURY,* Alan L. KERBEY,† David A. PRIESTMAN,† Mulchand S. PATEL,‡ Jean GIRARD* and Pascal FERRE§||

* Centre de Recherches sur l'Endocrinologie Moléculaire et le Développement (CNRS), Meudon-Bellevue, France, † Department of Clinical Biochemistry, John Radcliffe Hospital, Oxford, U.K., § Unité INSERM 342, Université René Descartes, Hôpital Saint-Vincent-de-Paul, 82 Ave. Denfert-Rochereau, 75014, Paris, France, and ‡ Department of Biochemistry, State University of New York, Buffalo, NY 14214–3000, U.S.A.

Total pyruvate dehydrogenase complex activity is low in white adipose tissue during the suckling period and increases markedly at weaning on to a high-carbohydrate diet. This is concomitant with an increase in the E1α, E1β and E2 subunit protein concentration and their respective mRNAs, suggesting a pretranslational control of this phenomenon. The most marked change is seen for the E1α subunit (17-fold increase in protein concentration). The changes in pyruvate dehydrogenase complex activity and subunit abundance induced by weaning on to a high-carbohydrate diet are precluded if the animals are weaned on to a high-fat diet, suggesting that the nutritional and/or related hormonal changes rather than a developmental stage are responsible for the observed adipose-tissue pyruvate dehydrogenase complex pattern.

INTRODUCTION

In the rat, the suckling–weaning transition corresponds to important nutritional and hormonal changes [1]. Milk, a high-fat low-carbohydrate diet, is progressively replaced at weaning by the adult low-fat high-carbohydrate diet. During the suckling period, plasma insulin concentrations are very low and increase after weaning on to a high-carbohydrate diet [2]. The rates of glucose utilization and lipogenesis in white adipose tissue are reduced during the suckling period and the lipogenic rate markedly increases at weaning on to a high-carbohydrate diet [1,3,4]. This increase is concomitant with pretranslational activation of the synthesis of two lipogenic enzymes, fatty acid synthase (EC 2.3.1.85) and acetyl-CoA carboxylase (EC 6.4.1.2) [5] linked to glucose absorption [6].

In white adipose tissue, the pyruvate dehydrogenase complex (PDC) is involved in the provision of carbon from glucose for lipid synthesis. PDC consists of three catalytic proteins: pyruvate dehydrogenase (subunits E1α and E1β) (EC 1.2.4.1), dihydrolipoamide acetyltransferase (subunit E2) (EC 2.3.1.12) and dihydrolipoamide dehydrogenase (subunit E3) (EC 1.8.1.4). The regulation of PDC activity is achieved by a phosphorylation/dephosphorylation cycle. Phosphorylation of serine residues in the α-subunit of the E1 component by a pyruvate dehydrogenase kinase (tightly bound to the complex) inactivates the enzyme. Dephosphorylation by a mitochondrial E1 phosphatase reactivates the complex. Pyruvate dehydrogenase kinase is affected by a number of regulatory factors, principally the concentration ratio of the metabolic pairs acetyl-CoA/CoA, NAD^+/NADH and ATP/ADP. In addition, activation of E1 phosphatase by insulin (white adipose tissue) and Ca^{2+} (muscle and liver) has been described [7].

During the suckling–weaning transition, initial but also total activity of PDC is increased in white adipose tissue [8]. The availability of polyclonal antibodies against PDC and of a human-derived cDNA probe for the various subunits has prompted us to investigate whether the PDC could also be regulated at a pretranslational level in white adipose tissue during the suckling–weaning transition in the rat.

MATERIALS AND METHODS

Chemicals

Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), benzamidine, Triton X-100, PMSF, dithiothreitol, NaF, Tween 20, and BSA were purchased from Sigma (St. Louis, MO, U.S.A.). All reagents for SDS/PAGE were from Bio-Rad (Richmond, CA, U.S.A.). Tos-Lys-CH_2Cl was from Merck (Darmstadt, Germany). Nitrocellulose membranes (BA 85) were obtained from Schleicher & Schuell (Dassel, Germany). The chemicals for enzyme assays were from Boehringer-Mannheim (Meylan, France). Non-fat dry milk was purchased from Carnation (Los Angeles, CA, U.S.A.).

^{125}I-labelled Protein A, [α^{32}P]dCTP (specific radioactivity 3000 Ci/mmol) and autoradiography films were from Amersham International (Amersham, Bucks., U.K.).

Pig heart PDC was purified to homogeneity as described previously [9]. Antisera to purified pig heart and bovine kidney PDC were raised in New Zealand White rabbits.

Animals

Wistar rats bred in our laboratory were used. They were housed in a room in which the temperature was maintained at 24 °C, with light from 07.00 to 19.00 h. Studies were performed on 14–15-day-old suckling rats, 28–32-day-old rats weaned at 21 days on to a high-fat (HF) diet (percentage energy: 72% fat, 28% protein, < 1% carbohydrate) or a high-carbohydrate (HC) diet (percentage energy: 62% carbohydrate, 12% fat, 26% protein).

A group of rats was abruptly weaned at 21 days on to the HC diet and studied at 22, 23, 24 and 30 days. For this latter group

Abbreviations used: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; HC diet, high-carbohydrate diet (62% carbohydrate, 12% fat, 26% protein); HF diet, high-fat diet (72% fat, 28% protein, < 1% carbohydrate); PDC, pyruvate dehydrogenase complex; TBST, Tris-buffered saline with 0.5% Tween 20.

|| To whom correspondence and reprint requests should be addressed.

and to avoid any previous consumption of carbohydrates by the pups, the mothers were fed on an HF diet from day 15 of lactation.

Mitochondrial preparations from subcutaneous inguinal adipose tissue

Approx. 3 g of subcutaneous inguinal adipose tissue was used for each mitochondrial preparation. Mitochondria for the determination of total PDC activity were prepared as described by Denyer et al. [10]. Briefly, 3 g of adipose tissue was homogenized in buffer containing 250 mM sucrose, 5 mM Tris/HCl, pH 7.4, and 2 mM EGTA. After two 800 *g* centrifugations, the supernatants were pooled and centrifuged at 10000 *g* to pellet the mitochondrial fraction. The yield of mitochondria ($n = 8$ in each group) in mg/g wet weight was 0.21 ± 0.03, 0.16 ± 0.03, 0.17 ± 0.03, 0.2 ± 0.02 and 0.25 ± 0.01 at respectively 21, 22, 23, 24 and 30 days of age. Total PDC activity was obtained after depletion of intramitochondrial ATP using an uncoupler of oxidative phosphorylation (CCCP) and activation of PDC by the endogenous PDC phosphatase. Activation buffer contained 120 mM KCl, 20 mM Tris/HCl, pH 7.4, 5 mM potassium phosphate, 2 mM EGTA and 10 μM CCCP. Mitochondria were activated for 30 min at 30 °C.

PDC assays and protein determination

PDC activity in extracts of mitochondria was assayed spectrophotometrically by coupling to arylamine acetyltransferase (EC 2.3.1.5) and following the production of acetyl-CoA from pyruvate and CoA [11]. Assays are conducted at 30 °C in 0.75 ml of 100 mM Tris/HCl (pH 7.8)/1 mM $MgCl_2$/0.5 mM EDTA containing, in addition, 5 mM 2-mercaptoethanol, 1 mM thiamine pyrophosphate, 0.5 mM NAD^+, 0.1 mM CoA, 1 mM pyruvate, 15 μg of *p*-(*p*-aminophenylazo)benzenesulphonic acid and 50 m-units of arylamine acetyltransferase. One unit of enzyme activity is defined as the amount that converts 1 μmol of pyruvate into acetyl-CoA/min at 30 °C.

Protein concentration was determined by the method of Lowry using BSA as a standard [12].

Mitochondrial preparations for Western blotting

Subcutaneous inguinal white adipose tissue (3 g) was disrupted in sucrose buffer (0.25 M sucrose, 5 mM Tris/HCl, 2 mM EGTA, pH 7.4). After differential centrifugation, mitochondria were extracted in extraction buffer (50 mM potassium phosphate, 10 mM EGTA, 2 mM dithiothreitol, 1 mM benzamidine, 1 mM PMSF, 0.3 mM Tos-Lys-CH_2Cl, 2.5 % Triton X-100, pH 7.0) by alternate (three times) thawing (30 °C) and freezing in liquid nitrogen. They were stored at −70 °C.

SDS/PAGE

Equal amounts of mitochondrial protein (100 μg) were fractionated by one-dimensional SDS/PAGE (10 % gel) [13] and electrophoretically transferred to nitrocellulose membranes. Purified pig heart PDC was used as a control. Apparent M_r values were calculated with reference to the mobility of the following proteins: phosphorylase *b* (M_r 97400), BSA (M_r 69000), ovalbumin (M_r 46000), carbonic anhydrase (M_r 30000).

Immunoblotting

Membranes were blocked with 3 % (w/v) BSA in TBST for 3 h at room temperature, and for another 3 h at room temperature with 2 % (w/v) non-fat dry milk in TBST. Then they were incubated with PDC antiserum diluted 1:5000 in TBST/milk overnight at 4 °C, washed twice in TBST at room temperature and allowed to react with 0.5 μCi/ml ^{125}I-Protein A in TBST/milk for 2 h at room temperature. The filters were again washed three times (10 min each time) in TBST, exposed to Hyperfilm MP and quantified by scanning densitometry.

Whatever the PDC antiserum (pig heart or bovine), PDC from rat white adipose tissue showed four bands with apparent M_r values of 68000, 55000, 46000 and 39000 (results not shown). These apparent M_r values are quite similar to the M_r of the rat heart PDC components previously reported [14]. When antiserum raised to pig heart PDC was used, E1α and E2 subunits reacted strongly but the signals of E1β and E3 were lower than the signals of the other two subunits and required longer exposure of the autoradiograms.

There was no cross-reactivity of PDC E1α, PDC E1β and PDE E2 antibodies with the E1 and E2 subunits of the other two α-oxoacid dehydrogenase complexes [15–17].

Quantification of mRNA coding for PDC subunits

Total RNA was isolated as previously described [18]. For Northern-blot analysis, 40 μg RNA samples were treated as described [5]. The integrity and relative amounts of RNA were assessed by Methylene Blue staining of the RNA on blots. cDNA probe of the human PDC E1α subunit was kindly provided by B. H. Robinson [19]. Human E1β, E2 and E3 cDNA probes were as reported previously [20–22].

The probes were labelled with ^{32}P using the Megaprime labelling system kit (Amersham International).

Hybridizations were performed as described previously [5] except that prehybridization lasted 24 h. In order to verify that each lane was loaded with the same amount of total RNA, each membrane was probed with an excess amount of a [γ-^{32}P] oligonucleotide specific for the 18S RNA [23]. This oligonucleotide was labelled at its 5′ end with [γ-^{32}P]ATP by T4 polynucleotide kinase. An RNA ladder (Boehringer-Mannheim) was used as the size marker.

There was no cross-reactivity of PDC E1α, PDC E1β and PDC E2 cDNA probes with the E1 and E2 subunit mRNAs of the other two α-oxoacid dehydrogenase complexes [15–17].

Statistical analysis

Results are expressed as means ± S.E.M. Statistical analysis was performed by Student's *t* test for unpaired data.

RESULTS

PDC activity in white adipose tissue of suckling and weaned rats

We measured total PDC activity in freshly extracted mitochondria of white adipose tissue from 15-day-old suckling rats and 30-day-old rats weaned at 19 days on to the HC diet. Total PDC activity was 45 ± 4 m-units/mg of mitochondrial protein in suckling rats ($n = 10$), and this increased 4-fold after weaning on to the HC diet in 30-day-old rats [180 ± 18 m-units/mg of mitochondrial protein ($n = 10$; $P < 0.001$)]. Similar results have been obtained on whole adipose tissue ([8] and results not shown), suggesting that the differences observed are not linked to any artifact in mitochondrial preparations.

E1α and E1β subunits

Suckling rats were kept with their mother until 21 days of age, and then abruptly weaned on to the HC diet. Protein and mRNA

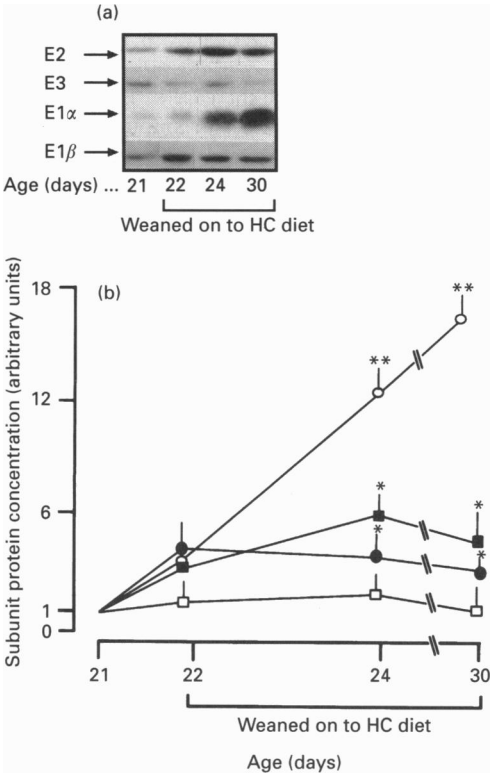

Figure 1 Western-blot analysis of PDC subunit proteins in adipose-tissue mitochondria during the suckling–weaning transition in rats

Suckling rats (21 days old) were abruptly weaned on to the HC diet and studied up to 30 days of age. At each stage, mitochondrial extracts (100 μg) from adipose tissue were fractionated on a one-dimensional SDS/10% polyacrylamide gel, transferred to nitrocellulose and probed with a 1:5000 dilution of polyclonal antibody against bovine PDC. The membranes were then labelled with ^{125}I-protein A and autoradiographed. (**a**) Representative Western blot. (**b**) Protein concentrations of the various subunits were estimated by scanning densitometry from their respective autoradiograms. Results are expressed as fold increase over the 21-day value. They are means ± S.E.M. of four determinations in each group. Each determination corresponds to adipose tissue pooled from 10–12 rats. ○, E1α subunit; ●, E1β subunit; ■, E2 subunit; □, E3 subunit. Difference from value for 21-day-old suckling rats statistically significant: * $P < 0.05$; ** $P < 0.001$.

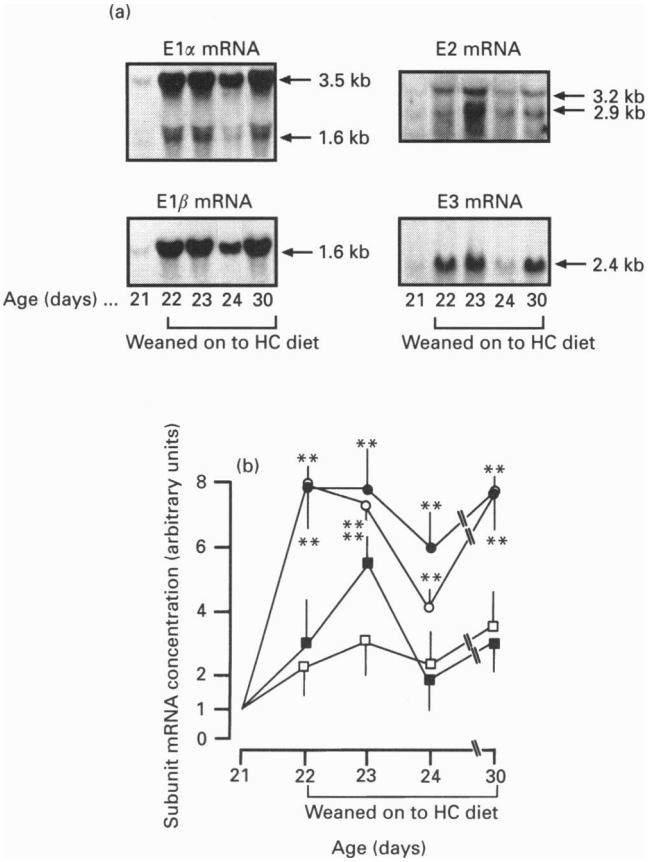

Figure 2 Northern-blot analysis of PDC subunit mRNA in adipose tissue during the suckling–weaning transition in rats

Suckling rats (21 days old) were abruptly weaned on to the HC diet and studied up to 30 days of age. At each stage, total RNA was extracted from white adipose tissue and 40 μg of RNA was treated as described in the Materials and methods section. Blots were hybridized with the various human PDC subunit cDNAs and the 18S RNA oligonucleotide. (**a**) Representative Northern blots. (**b**) mRNA concentrations of the various subunits were estimated by scanning densitometry from their respective autoradiograms. Results, corrected using the 18S RNA abundance, are expressed as fold increase over the 21-day value. They are means ± S.E.M. of four determinations in each group. Each determination corresponds to adipose tissue pooled from 10–12 rats. ○, E1α subunit; ●, E1β subunit; ■, E2 subunit; □, E3 subunit. Difference from value for 21-day-old suckling rats statistically significant: ** $P < 0.001$.

concentrations of the PDC subunits were followed for 9 days. The E1α subunit protein was hardly detectable in suckling rats, but increased 12-fold after 3 days of weaning and 17-fold after 9 days (Figure 1). In order to analyse whether these changes in E1α protein concentration were linked to a pretranslational event, we hybridized total RNA from adipose tissue with a human E1α cDNA probe.

The human E1α cDNA probe detected in the rat two mRNA species at 3.5 and 1.6 kb, as in the human [19,24,25] (Figure 2). In the rat, however, the 3.5 kb species is more abundant, the converse situation to the human. The E1α subunit mRNA was barely detectable before weaning (Figure 2), but increased markedly (8-fold) to reach a maximal concentration 24 h after weaning on to the HC diet.

In the PDC, E1α is associated with E1β to form an $\alpha_2\beta_2$ tetramer. We therefore followed the protein and mRNA concentration of the E1β subunit. The E1β protein concentration increased 4-fold 1 day after weaning and then plateaued (Figure 1).

As described in the human heart [20], the human E1β cDNA probe detected in the rat a major mRNA at 1.6 kb. During the suckling–weaning transition, E1β subunit mRNA concentration followed a pattern similar to that of E1α (Figure 2).

E2 and E3 subunits

We then analysed the other subunit components of the PDC, namely E2 and E3. E2 subunit protein concentration increased 6-fold 3 days after weaning and then plateaued (Figure 1). The human E2 cDNA probe detected in the rat two major mRNAs at 3.2 and 2.9 kb as described previously in the human [26] (Figure 2). E2 subunit mRNA concentration increased 5-fold 2 days after weaning on to the HC diet and then decreased (Figure 2).

E3 subunit concentration did not increase significantly throughout the weaning period (Figure 1). As described previously in rats [22], a single 2.4 kb mRNA was detected by the human E3 cDNA probe (Figure 2). E3 subunit mRNA concentration increased steadily after weaning on to the HC diet but did not peak to the same extent as the other subunits (Figure 2).

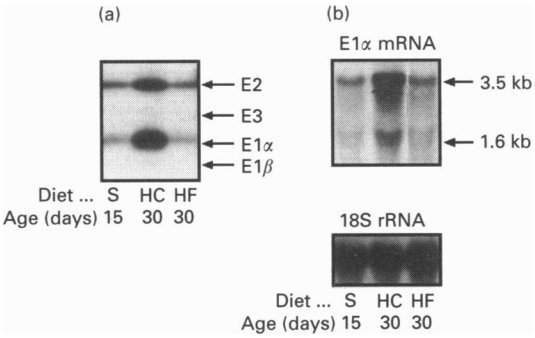

Figure 3 Effects of the HF weaning diet on PDC E1α and E2 subunit protein and E1α mRNA concentrations

Fifteen-day-old suckling rats (S) were weaned on to either the HC or HF diet and studied at 30 days of age for adipose-tissue protein content of PDC subunits using the pig heart antiserum (**a**) and for the E1α mRNA and 18S rRNA concentrations (**b**). The blots are representative of three independent experiments.

In summary, the E1α subunit shows the largest increase in concentration after weaning, followed by E2 and E1β, whereas E3 is not increased. The changes are concomitant with variations in their respective mRNA abundance.

Association of changes in E1α and E2 subunits with changes in diet

In order to test whether the marked change observed for the E1α subunit was linked to a developmental stage *per se* or to the nutritional changes, rats were weaned at 21 days on to an HC or the HF diet. Total PDC activity did not increase when rats were weaned on to the HF diet (24±3 m-units mg of mitochondrial protein in 30-day-old rats compared with 45±4 in suckling rats). The increase in E1α and E2 subunit protein and E1α mRNA concentrations at weaning were abolished when rats were weaned on to the HF diet (Figure 3).

DISCUSSION

It is now well established that PDC activity is modulated on a short-term basis by serine phosphorylation/dephosphorylation of its E1α subunit [7,27,28]. Reversible phosphorylation of PDC is considered to be the major mechanism of regulation [7]. The proportion of PDC in the active form is under metabolic and hormonal control in different tissues including white adipose tissue [7,27,28].

However, a number of studies have shown that total PDC activity may also be regulated in white adipose [8,29–32]. In these studies, changes in total PDC activity were linked to long-term modifications of the nutritional (high-fat diet, suckling period), physiological (lactation) or pathological (obesity, insulin-resistance) status.

In the present work, we show that total PDC activity is low when rats are fed milk, a low-carbohydrate, high-fat diet, and increases at weaning on to the adult high-carbohydrate diet. This is concomitant with a marked increase in E1α and a lower one in E1β subunit protein (17- and 4-fold respectively). As the functional unit of E1 is an $\alpha_2\beta_2$ tetramer, a similar increase in the E1α and E1β subunits could have been expected. It should be pointed out, however, that we cannot compare the respective amounts of each subunit in terms of protein concentration but only their respective fold increase.

E2 subunit is also increased at weaning, but to a much lower extent. Although we cannot conclude from the present study what the roles of E1α, E1β and E2 in the increase in total PDC activity at weaning are, the large increase in E1α concentration observed could suggest that the cellular amount of E1α is rate-limiting for PDC formation. It is noteworthy that in the pancreatic β-cell, a decrease in total activity of PDC and branched-chain oxoacid dehydrogenase is also concomitant with a dramatic decrease in their respective E1α subunit protein and mRNA concentrations [33,34]. The E1α subunit is the rate-limiting step for overall PDC reaction [7] and is the site of short-term regulation by phosphorylation/dephosphorylation.

The fact that the E3 subunit does not follow a similar pattern to the other subunits fits with the fact that it is common to several dehydrogenases.

As described for other lipogenic enzymes modified during the suckling–weaning transition (fatty acid synthase and acetyl-CoA carboxylase) [5], the increase in E1α, E1β and E2 subunit protein concentrations is concomitant with changes in the concentrations of their respective mRNAs. This suggests a major control at a step involving transcription and/or stability of mRNA, although some translational or post-translational mechanisms could also be involved, as, for instance, E1α and E1β mRNA increase to the same extent whereas the increase in protein is much lower for the latter. It must also be underlined that the method of expressing the results (per mitochondrial protein) does not reveal any change that might occur in the number of mitochondria per cell. This might explain some of the discrepancies observed between the pattern of protein and mRNA concentrations.

The transition from a high-fat low-carbohydrate diet to a high-carbohydrate low-fat diet is probably responsible for the changes observed in the expression of specific PDC subunits. Indeed, (i) these changes occur extremely rapidly after abrupt weaning on to the HC diet, and (ii) they are precluded by weaning on to the HF diet which prevents both the increased carbohydrate availability and the enhanced insulin concentration [35] seen after weaning on to the HC diet. Hyperglycaemia and/or hyperinsulinaemia could be involved in the stimulation of PDC subunit gene expression after weaning on to the HC diet. Indeed, the expression of other lipogenic enzymes (fatty acid synthase and acetyl-CoA carboxylase) is increased by glucose and insulin in cultured adipose tissue of suckling rats [6], and PDC E1α mRNA concentration is up-regulated by increasing glucose concentrations in cultured pancreatic islets of adult rats [33].

In conclusion, we have shown for the first time that the expression of specific PDC subunit genes and particularly the E1α gene can be modulated, probably through changes in nutrition. The E1α gene could belong to the rapidly expanding family of genes that are regulated by specific nutrients, although a role for insulin cannot be ruled out at this stage [36,37]. The recent characterization of its promoter [38] should allow us to investigate what factors and DNA elements are involved in this control.

REFERENCES

1 Issad, T., Coupé, C., Pastor-Anglada, M., Ferré, P. and Girard, J. (1988) Biochem. J. **251**, 685–690
2 Girard, J. R., Ferré, P., Kervran, A., Pégorier, J. P. and Assan, R. (1977) in Glucagon: Its Role in Physiology and Clinical Medicine (Foa, P. P., Bajaj, J. S. and Foa, N. L., eds.), pp. 563–581, Springer-Verlag, New York
3 Gandemer, G., Pascal, G. and Durand, G. (1982) Int. J. Biochem. **74**, 797–804
4 Tsujikawa, M. and Kimura, S. (1980) J. Nutr. Sci. Vitaminol **26**, 367–374
5 Coupé, C., Perdereau, D., Ferré, P., Hitier, Y., Narkewicz, M. and Girard, J. (1990) Am. J. Physiol. **258**, E126–E133
6 Foufelle, F., Gouhot, B., Pégorier, J. P., Perdereau, P., Girard, J. and Ferré, P. (1992) J. Biol. Chem. **267**, 20543–20547

7 Randle, P. J., Kerbey, A. L. and Espinal, J. (1988) Diabetes Metab. Rev. **4**, 623–638
8 Issad, T., Ferré, P., Pastor-Anglada, M., Baudon, M. A. and Girard, J. (1989) Biochem. J. **264**, 217–222
9 Kerbey, A. L., Radcliffe, P. M., Randle, P. J. and Sugden, P. H. (1979) Biochem. J. **181**, 427–433
10 Denyer, G. S., Lam, D., Cooney, G. J. and Caterson, I. D. (1989) FEBS Lett. **250**, 464–468
11 Kerbey, A. L., Randle, P. J., Cooper, R. H., Whitehouse, S., Pask, H. T. and Denton, R. M. (1976) Biochem. J. **154**, 327–348
12 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
13 Laemmli, U. K. (1970) Nature (London) **227**, 680–685
14 Matuda, S., Shirahama, T., Saheki, T., Miura, S. and Mori, M. (1983) Biochim. Biophys. Acta **741**, 86–93
15 Wexler, I. D., Kerr, D. S., Ho, L. et al. (1988) Proc. Natl. Acad. Sci. U.S.A., **85**, 7336–7340
16 Fisher, C. W., Chuang, J. L., Griffin, T. A., Lau, K. S., Cox, R. P. and Chuang, D. T. (1989) J. Biol. Chem. **264**, 3448–3453
17 Nakano, K., Matuda, S., Yamanaka, T. et al. (1991) J. Biol. Chem. **266**, 19013–19017
18 Leturque, A., Postic, C., Ferré, P. and Girard, J. (1991) Am. J. Physiol. **260**, E588–E593
19 DeMeirleir, L., MacKay, N., Lam Hon Wah, A. M. and Robinson, B. H. (1988) J. Biol. Chem. **263**, 1991–1995
20 Ho, L., Javed, A. A., Pepin, T. J. et al. (1988) Biochem. Biophys. Res. Commun. **150**, 904–908
21 Thekkumkara, T. J., Jesse, B. W., Ho, L. et al. (1987) Biochem. Biophys. Res. Commun. **145**, 903–907
22 Pons, G., Raefsky-Estrin, C., Carothers, D. J. et al. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**,, 1422–1426
23 Chan, Y. L., Gutell, R., Noller, H. F. and Wool, I. G. (1984) J. Biol. Chem. **259**, 224–230
24 Dahl, H. H. M., Hunt, S. M., Hutchinson, W. M. and Brown, G. K. (1987) J. Biol. Chem. **262**, 7398–7403
25 Wexler, I. D., Kerr, D. S., Ho, L. et al. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 7336–7340
26 Coppel, R. L., McNeilage, L. J., Surh, C. D. et al. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 7317–7321
27 Denton, R., Randle, P., Bridges, B. et al. (1975) Mol. Cell. Biochem. **9**, 27–53
28 Wieland, O. H. (1983) Rev. Physiol. Biochem. Pharmacol. **96**, 124–170
29 Stansbie, D., Denton, R. M., Bridges, B. J., Pask, H. T. and Randle, P. J. (1976) Biochem. J. **154**, 225–236
30 Lombardo, Y. and Menahan, L. (1978) Life Sci. **22**, 1033–1042
31 Kilgour, E. and Vernon, R. G. (1987) Biochem. J. **243**, 69–74
32 Macaulay, S. L. and Larkins, R. G. (1988) Metabolism **37**, 958–965
33 MacDonald, M. J., Kaysen, J. H., Moran, S. M. and Pomije, C. E. (1991) J. Biol. Chem. **266**, 22392–22397
34 MacDonald, M. J., McKenzie, D. I., Kaysen, J. H. et al. (1991) J. Biol. Chem. **266**, 1335–1340
35 Girard, J. R., Ferré, P., Pégorier, J.-P. and Duée, P. H. (1992) Physiol. Rev. **72**, 507–562
36 Girard, J. R., Perdereau, D., Foufelle, F., Prip-Buus, K. and Ferré, P. (1994) FASEB J. **8**, 36–42
37 Vaulont, S. and Kahn, A. (1994) FASEB J. **8**, 28–35
38 Chang, M., Naik, S., Johanning, G. L., Ho, L. and Patel, M. S. (1993) Biochemistry **32**, 4263–4269

Received 21 March 1995/12 June 1995; accepted 15 June 1995