Modulation of rat preadipocyte adipose conversion by androgenic status: involvement of C/EBPs transcription factors

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Abstract

Androgenic status affects rat preadipocyte adipose conversion from two deep intra-abdominal (epididymal and perirenal) fat depots differently. The aim of this study was to establish whether these site-specific alterations of adipogenesis are related to altered expressions of the transcriptional factors regulating proliferation and differentiation of preadipocytes, c-myc and CCAAT/enhancer binding proteins (C/EBPs: C/EBPα and β).

The increased proliferation of epididymal and perirenal preadipocytes from castrated rats was not linked to variations in c-myc mRNA and protein levels.

The expression of the early marker of adipogenesis, lipoprotein lipase (LPL), was decreased by androgenic deprivation in epididymal cells but remained insensitive to the androgenic status in perirenal preadipocytes. In contrast, LPL expression increased in subcutaneous preadipocytes from castrated rats, an effect which was partly corrected by testosterone treatment.

Expression of C/EBPβ was unaffected by androgenic status whatever the anatomical origin of the preadipocytes. In contrast, the mRNA and protein levels of C/EBPα were greatly decreased by androgenic deprivation in epididymal cells, an alteration which could not be corrected by *in vivo* testosterone administration.

Altogether these results demonstrated that in preadipocytes androgenic deprivation affects site-specifically the expression of LPL, an early marker of adipogenesis and of C/EBPα, a master regulator of adipogenesis. These observations contribute to an explanation of why castration induces defective adipose conversion in rat epididymal preadipocytes specifically.

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Introduction

It is well established that fat tissue development and distribution depend on sex steroid hormones. Android obesity, an important risk factor for cardiovascular diseases, is characterized by excessive accumulation of adipose tissue in abdominal and visceral regions (Bjorntorp 1991, 1996).

The presence of specific androgen receptors has been described in both precursors and mature fat cells (Xu *et al.* 1990, Dieudonné *et al.* 1998). Furthermore, these receptors are up-regulated by testosterone and their density varies with the anatomical origin of preadipocytes (De Pergola *et al.* 1990, Dieudonné *et al.* 1998) and during adipogenesis (Dieudonné *et al.* 1995, 1998). This suggests that adipose tissue might be a target for androgens.

In rats, we have previously reported that castration induces a decreased adipose tissue weight, in particular, in the epidydimal fat deposits (Lacasa *et al.* 1993). One possible explanation for these reduced adiposities could be that castration restrains the capacities of fat cell precursors to proliferate and/or to differentiate. In fact, we have observed, in rat epididymal preadipocytes but not in subcutaneous (SC) ones, that castration induces an increase in their proliferative capacity and, conversely, a decrease in their ability to differentiate (Lacasa *et al.* 1995). More recently, we have also shown that castration increases adipoconversion of perirenal preadipocytes. Furthermore, the increased proliferation of epididymal and perirenal preadipocytes is associated with high mitogen-activated protein (MAP) kinase activity (Lacasa *et al.* 1997).

Cell proliferation and differentiation processes are tightly controlled at the nuclear level by transcriptional factors which are cell specific or not. Among these factors, the product of the proto-oncogene c-myc, whose expression is activated by mitogenic signals, binds to DNA specific sequences leading to enhanced transcriptional activity controlling cellular proliferation (Amati & Lands 1994). Thus, c-myc could regulate the expression of different genes required for cell-cycle progression. On the other hand, in various cell types and tissues, c-myc expression has been shown to be regulated by sex-steroid hormones (Schuchard *et al.* 1993). For example, testosterone treatment reduces c-myc mRNA level, *in vivo*, in the

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rat ventral prostate (Quarmby *et al.* 1987) and, *in vitro*, in human prostatic cells (Wolf *et al.* 1992).

Some members of the family of transcription factors, CCAAT/enhancer binding proteins (C/EBPs) such as C/EBPα, C/EBPβ and C/EBPδ, are important regulators of adipocyte differentiation. C/EBPβ and C/EBPδ, are induced early during adipocyte differentiation in response to hormonal stimulators (Cao *et al.* 1991). In turn, these factors stimulate the expression of C/EBPα which, then, activates its own expression and that of a set of adipo-specific genes such as 422/aP2 and SCD1 which all have functional C/EBP-binding sites in their promoters (Christy *et al.* 1989). Furthermore, C/EBPα is an inhibitor of cell proliferation by inducing the expression of the growth-arrested-associated gene gadd 45 and the cyclin-dependent kinase inhibitor p21 (Constance *et al.* 1993, Timchenko *et al.* 1996).

Concerning the hormonal regulation of these transcription factors, the level of C/EBPα expression has been reported to be repressed by glucocorticoids in 3T3-L1 adipocytes (MacDouglas *et al.* 1994), but positively regulated by oestrogens in rat ovarian follicles (Piontkewitz *et al.* 1993).

Expression of C/EBPβ is rapidly induced by glucocorticoids in liver and intestine (Boudreau *et al.* 1996, Matsuno *et al.* 1996) but not in 3T3-L1 adipocytes (MacDouglas *et al.* 1994). It has been suggested that adipogenesis could be reciprocally regulated by c-myc and C/EBPα. In fact, in the 3T3-L1 preadipocyte cell line, overexpression of c-myc resulted in the inhibition of C/EBPα induction and consequently prevented adipogenesis. Conversely, overexpression of C/EBPα overcomes the inhibitory effects of Myc on adipogenesis (Freytag & Geddes 1992).

It may thus be suggested that modified expression of c-myc and consequently of C/EBPα could explain the defective adipogenesis observed in epididymal cells after castration. In order to test this hypothesis, we have presently compared the effects of androgenic status on the expression of c-myc and C/EBPα (mRNA and protein), as well as on early events of adipogenesis in rat preadipocytes from two intra-abdominal (epididymal and perirenal) and one SC (femoral) localizations.

Materials and Methods

Materials

Fetal bovine serum (FBS) was obtained from Gibco-BRL (Grand Island, NY, USA). Phenol red-free Dulbecco's modified Eagle's medium (DMEM) and DMEM–Ham's F12 (50:50 mix) were obtained from Sigma Chemical Co. (St Louis, MO, USA). The antisera and synthetic peptides specific for C/EBPα (SC61), C/EBPβ (SC130) and GADD153/CEBP Homologous Protein (CHOP) (SC793) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antiserum specific for c-myc (OP30) was from Oncogene Science (Cambridge, MA, USA). Western blotting protocols and random sequence hexanucleotide primers DNA labelling (Megaprime kit) were from Amersham International plc (Amersham, Bucks, UK). The PCR purification kit (QUIAquick) was obtained from Quiagen (Santa Clarina, CA, USA). Restriction enzymes (Bam HI and Sal I) were purchased from Promega (Madison, WI, USA). Testosterone RIA tests were provided by Biomérieux (Marcy l'Etoile, France). All other chemicals were of reagent grade.

Animals

Procedures with experimental animals were authorized and followed the guidelines of the Ministry of Agriculture (France) (authorization 006614). Male Sprague–Dawley rats (125–150 g) were castrated under pentobarbital anaesthesia (40 mg/kg i.p.) and treated as previously described (Lacasa *et al.* 1993, 1995). Briefly, 5 days after the operation, half of the castrated rats received one s.c. injection of testosterone propionate (0·5 mg/100 g body weight) every other day for 10 days (cast+T) while the other half (cast) and the sham-operated rats (sham) received the vehicle (propyleneglycol) only. One day after the last injection, rats were killed by decapitation. Serum testosterone levels were measured as described in Lacasa *et al.* (1993) and were on the day of death: $1{\cdot}5 \pm 0{\cdot}2$, less than 0·1 and $13{\cdot}5 \pm 3{\cdot}7$ nmol/l in sham, cast and cast+T rats. The characteristics of SC and epididymal adipose tissues of the different experimental groups were given in Lacasa *et al.* (1993). Perirenal adipose tissue weights were: $3{\cdot}05 \pm 0{\cdot}28$, $2{\cdot}90 \pm 0{\cdot}30$ and $2{\cdot}8 \pm 0{\cdot}28$ g for sham, cast and cast+T rats.

Cell culture

Cell preparation and culture were performed as described in Deslex *et al.* (1987). Briefly, preadipocytes obtained from the stroma-vascular fraction of adipose tissue by collagenase digestion were plated at a density of $1–2 \times 10^4$ cells/cm^2 in 8% FBS–DMEM. After 12 h, cultures were washed and fed with 8% FBS–DMEM. Medium was changed every other day. At confluence (3 days post-plating), cells were allowed to differentiate in DMEM–Ham's F12 containing 5 μg/ml insulin, 10 μg/ml transferrin and 200 pM tri-iodothyronine (ITT medium) and in the absence of serum as described in Deslex *et al.* (1987). Whatever the anatomical origin, at least 80% of the control cells in culture were fully differentiated at days 8–10 post-confluence.

c-myc expression was examined in proliferating pre-adipocytes cultured for 1–2 days post-plating in 8% FBS–DMEM and then serum-deprived for 18 h. Early differentiated preadipocytes (EDP) (2–3 days in ITT medium) were used for lipoprotein lipase (LPL) and

C/EBPβ expression studies while C/EBPα expression was investigated in late-differentiated preadipocytes (LDP) (6–8 days in ITT medium).

Preparation of cellular extracts

Preadipocyte fraction was prepared as follows. Proliferating or differentiated preadipocytes were scraped and sonicated in cold buffer containing 50 mM Tris (pH 8·0), 120 mM NaCl, 1 mM EDTA, 1% NP40, 0·1% SDS, 0·57 mM PMSF, 0·5 mM sodium deoxycholate, 1 mM orthovanadate, 30 mM β-glycerophosphate, 25 μg/ml aprotinin and 20 μg/ml leupeptin. After centrifugation at 100 000 ***g*** for 15 min at 4 °C, the resulting supernatant was denaturated with Laemmli buffer (v:v) and stored at − 20 °C.

Western blot analysis

Equal amounts of protein (20–75 μg) and prestained molecular weight markers were subjected to SDS-PAGE (12·5%-15% acrylamide). Proteins were transferred to polyvinylidenedifluoride membranes. The filters were subsequently stained to verify equal protein loading and transfer. After blocking by Tris-buffered saline (TBS) containing 0·1% Tween 20 (TBS-T) and 2·5% gelatin for 2 h, filters were incubated overnight with the primary antibody diluted in TBS-T/2·5% gelatin (0·5 μg/ml). Membranes were washed and incubated with the secondary antiserum coupled to peroxidase (1:5000 dilution in TBS-T) for 1 h and extensively washed with TBS-T. Filters were next incubated with the enhanced chemiluminescence detection solution and then exposed to X-ray films. Reprobing of the membranes gave identical results. Specificity of the immunoreactive proteins was verified by loss of sample immunoreactivity when incubated with the antiserum neutralized with the corresponding specific peptide. Signals were quantified by densitometry. Control experiments with various amounts of protein (10–75 μg) were performed to ensure that the densitometric signal intensity was proportional to the amount of protein loaded.

Northern blot analysis

Total RNAs were extracted following the acid–guanidinium–isothiocyanate protocol as previously described (Chomczynski & Sacchi 1987). RNA samples (20–40 μg) were separated on denaturing gels containing 1% agarose, 12·5% formaldehyde. The electrophoresed RNAs were capillary transferred onto a Hybond N^+ membrane in 20 × SSC and cross-linked to this membrane (1 h at 80 °C). Prehybridizations were carried out for 3 h at 68 °C in Church solution (0·5 M sodium phosphate, 1 mM EDTA and 7% SDS). Hybridizations with ^{32}P-cDNA probes labelled by random priming (2–5 10^8 d.p.m./μg) were performed overnight at 68 °C. The hybridized membranes were then washed twice in 2 × SSC, 0·1% SDS at room temperature for 15 min and once in 0·5 × SSC, 0·1% SDS at 65 °C for 5 min before being exposed to an X-ray film at − 80 °C. The band intensities were quantified by densitometry. Membranes were next dehybridized in boiling 0·5% SDS and then hybridized with probe specific for the ribosomal acidic protein PO chosen as an internal control because its expression is sex-steroid independent (La Borda 1991).

To verify the integrity of the loaded RNA, parallel gels were run and stained with ethidium bromide to visualize 28S and 18S ribosomal RNAs.

The CMV-NF-IL6 vector was obtained by inserting a 1 kb Sal I fragment containing the NF-IL6 coding region into one Sal I site of CMVp eucaryotic vector (Akira *et al.* 1990). A 1·3 kb Not I fragment containing the C/EBPα coding region was inserted into one Not I site of CMVp eucaryotic vector. The resulting plasmid was named hCMV-C/EBPα (Timchenko *et al.* 1995). The probes were specific for rat c-myc and ribosomal acidic protein PO as described in Nishimura *et al.* (1992) and La Borda (1991) respectively. The oligonucleotide primer pairs specific for rat LPL were: sense 5′-GCGGATTTC GTAGATGTC-3′/antisense 5′-TGCCTTGCTGGGG TTTTC-3′. These probes were prepared by RT-PCR methods using rat proliferating preadipocyte RNA (c-myc, ribosomal acidic protein PO) and rat adipocyte RNA (LPL). The PCR products were purified with a PCR purification kit.

Other determinations

Protein concentrations were measured following the dye-binding procedure (Bradford 1976) using bovine serum albumin as standard. All results are expressed as means ± S.E.M. from at least three individual experiments. Comparisons between groups were made using analysis of variance with Bonferroni *P* values.

Results

As previously reported (Lacasa *et al.* 1994, 1995, 1997) and summarized in Table 1, androgenic status affects adipose conversion of preadipocytes differently according to their anatomical origin. To obtain more information on these effects at a molecular level, we studied the expression of some transcription factors involved in preadipocyte proliferation and differentiation.

Androgenic status and c-myc expression

Serum-stimulated expression of c-myc was first studied in proliferating preadipocytes from different fat depots.

In preliminary experiments using cells deprived of serum for 18 h, we found that c-myc mRNA and protein

Table 1 Influence of castration on adipoconversion of preadipocytes from different anatomical localizations in the rat

	Proliferative capacity (cell number)	**Differentiation capacity** (GPDH activity)
Anatomical origin of fat depots		
Femoral SC	Unchanged	Unchanged
Epididymal	Increased	Decreased
Perirenal	Increased	Increased

expressions were maximally induced after 1-h and 3-h serum exposure respectively.

It should be noted that from several experiments, no reproducible differences in c-myc expression (mRNA and protein) could be observed between SC and deep intra-abdominal preadipocytes from control rats (data not shown).

As shown in Figs 1 and 2, the steady-state levels of c-myc mRNA and protein were both decreased by castration in epididymal preadipocytes. However, *in vivo* treatment by testosterone failed to reverse these effects.

These experiments indicate that the increased proliferation of deep intra-abdominal preadipocytes induced by castration is not linked to variations in c-myc expression.

Androgenic status and early events of adipogenesis

Androgenic status differently influences the adipoconversion process in deep intra-abdominal preadipocytes as assessed by morphological observations and glycerol-3-phosphate dehydrogenase (GPDH) activity, a late marker of differentiation (Lacasa *et al.* 1995, 1997). To examine whether the early steps of adipogenesis are also affected by androgenic status, the expression of one marker of these events, LPL (Ailhaud *et al.* 1992) was measured in EDP from the different fat depots. When normalized to ribosomal protein, LPL expression was found to be identical in preadipocytes from sham rats whatever their anatomical origin. However, as shown in Fig. 3, LPL expression was almost completely abolished by castration in epididymal EDP, a defect which could not be restored to control values by *in vivo* testosterone treatment. Surprisingly, LPL expression was markedly enhanced (+250%) by castration in SC EDP, an effect which was completely prevented by testosterone replacement. In contrast, however, perirenal EDP were found to be insensitive to the androgenic status in terms of LPL expression.

These observations indicate that castration alters site-specifically an early marker of adipogenesis such as LPL whose expression is either increased in SC, or decreased in epididymal or unchanged in perirenal EDP.

Androgenic status and the C/EBPs transcriptional factors

C/EBPβ which is expressed early during the course of adipogenesis activates the expression of C/EBPα which is then positively autoregulated (MacDouglas & Lane 1995).

Therefore, to gain more information about the molecular basis underlying the effects of castration on adipogenesis

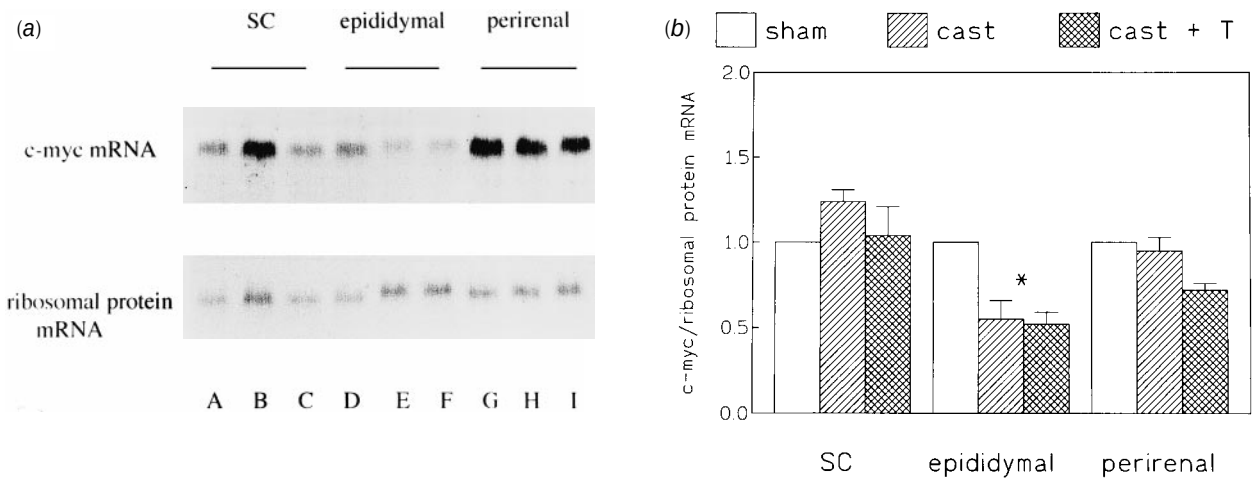

Figure 1 Influence of androgenic status on c-myc mRNA expression in rat proliferating preadipocytes from femoral SC, epididymal and perirenal fat depots. Total RNAs of proliferating preadipocytes from femoral SC (A, B and C), epididymal (D, E and F) and perirenal (G, H and I) fat depots from sham (A, D and G), cast (B, E and H) and cast+T (C, F and I) rats were hybridized with rat c-myc probe. (*a*) Representative Northern blot of c-myc mRNA. (*b*) Densitometric analysis of c-myc Northern blots. The data are means ± S.E.M. of five separate experiments. (*b*) This figure shows variations in c-myc mRNA/ribosomal protein mRNA percentage ratios. 100% is assigned to each type of preadipocyte from sham rats (control) for c-myc mRNA and ribosomal protein mRNA. Then, for each experimental group, the respective c-myc mRNA and ribosomal protein mRNA were expressed relative to the corresponding controls. Next, c-myc mRNA/ribosomal protein mRNA percentage ratios were calculated. *P<0·05, cast and cast T vs sham.

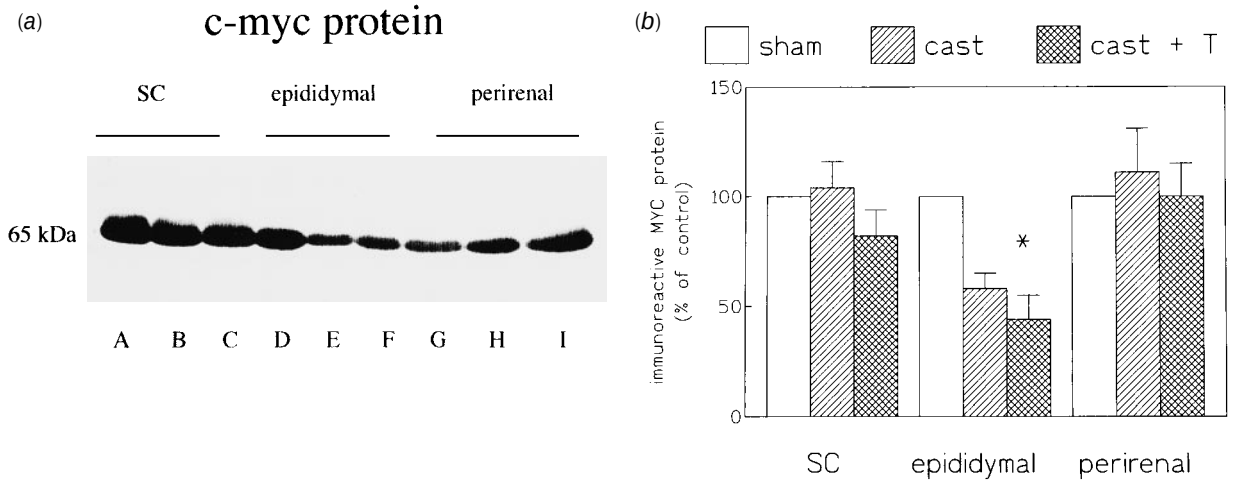

Figure 2 Influence of androgenic status on c-myc protein expression in rat proliferating preadipocytes from femoral SC, epididymal and perirenal fat depots. Cellular extracts of proliferating preadipocytes from femoral SC (A, B and C), epididymal (D, E and F) and perirenal (G, H and I) fat depots from sham (A, D and G), cast (B, E and H) and cast+T (C, F and I) rats were probed with rat c-myc antiserum. (*a*) Representative Western blot of c-myc protein. (*b*) Densitometric analysis of c-myc Western blots. The data are means ± S.E.M. of five separate experiments and are expressed as percentage of control values (100% is assigned to each type of preadipocyte from sham rats). **P*<0·05, cast and cast T vs sham.

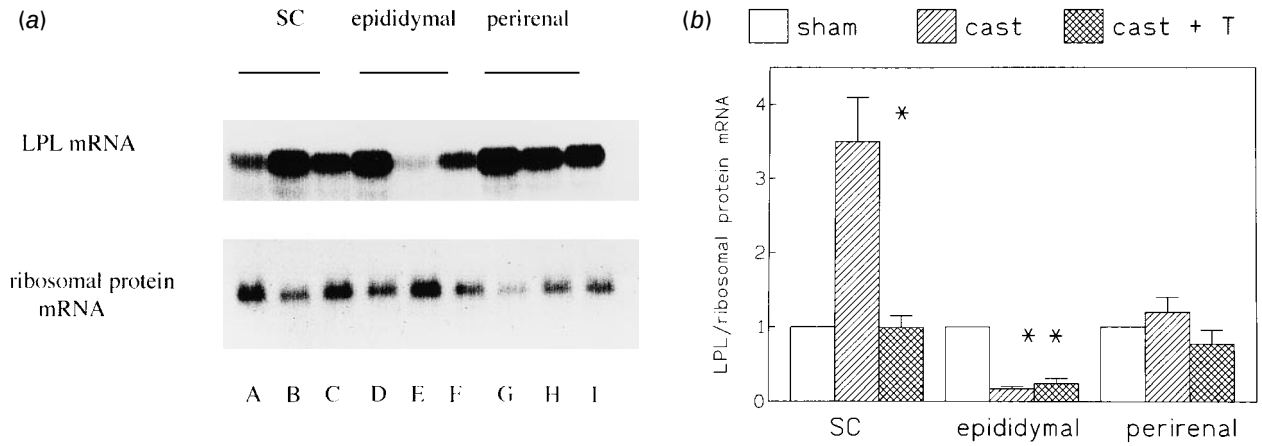

Figure 3 Influence of androgenic status on LPL mRNA expression in rat differentiated preadipocytes from femoral SC, epididymal and perirenal fat depots. Total RNAs of differentiated preadipocytes from femoral SC (A, B and C), epididymal (D, E and F) and perirenal (G, H and I) fat depots from sham (A, D and G), cast (B, E and H) and cast+T (C, F and I) rats were hybridized with rat LPL probe. (*a*) Representative Northern blot of LPL mRNA. (*b*) Densitometric analysis of LPL Northern blots. The data are means ± S.E.M. of six separate experiments. (*b*) The figure shows variations in LPL mRNA relative to ribosomal protein mRNA. 100% is assigned to each type of preadipocyte from sham rats (control) for LPL mRNA and ribosomal protein mRNA. Then, for each experimental group, the respective LPL mRNA and ribosomal protein mRNA were expressed relative to the corresponding controls. Next, LPL mRNA/ribosomal protein mRNA percentage ratios were calculated. **P*<0·05, cast vs cast T and sham; ***P*<0·05, cast and cast T vs sham.

described above, expressions of these transcriptional factors (mRNA and protein) were next studied. C/EBPβ mRNA and protein expressions were found to be unaffected by the androgenic status whatever the anatomical origin of EDP (Fig. 4).

A single C/EBPα mRNA has been shown to give rise to two major alternative translation products, p42 C/EPBα and p30 C/EBPα (Ossipow *et al.* 1993). C/EBPα mRNA level was greatly decreased in epididymal LDP from castrated rats (Fig. 5). Similarly, the levels

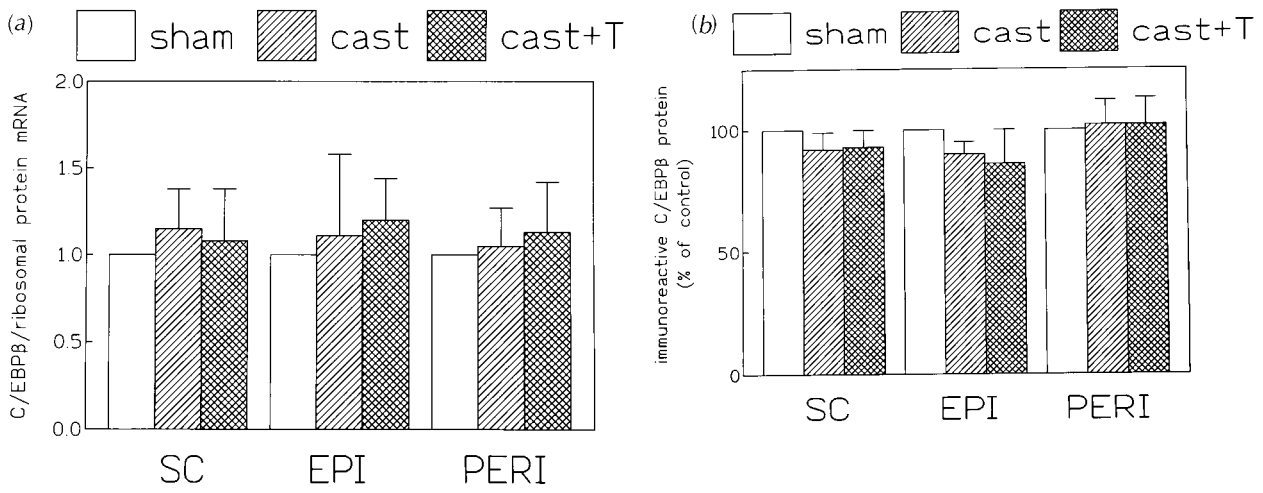

Figure 4 Influence of androgenic status on C/EBPβ mRNA and protein expression in rat differentiated preadipocytes from SC, epididymal and perirenal fat depots. (*a*) Densitometric analysis of C/EBPβ Northern blots. Total RNA of differentiated preadipocytes from femoral SC, epididymal (EPI) and perirenal (PERI) fat depots from sham, cast and cast+T rats were hybridized with C/EBPβ probe. The data are means ± S.E.M. of five separate experiments. The graph shows C/EBPβ mRNA/ribosomal protein mRNA percentage ratios. 100% is assigned to each type of preadipocyte from sham rats (control) for C/EBPβ mRNA and ribosomal protein mRNA. Then, for each experimental group, the respective C/EBPβ mRNA and ribosomal protein mRNA were expressed relative to the corresponding controls. Next, C/EBPβ mRNA/ribosomal protein mRNA percentage ratios were calculated. (*b*) Densitometric analysis of C/EBPβ Western blots. Cellular extracts of differentiated preadipocytes from femoral SC, epididymal and perirenal fat depots from sham, cast and cast+T rats were probed with C/EBPβ polyclonal antiserum. The data are means ± S.E.M. of five separate experiments and are expressed as percentage of control values (100% is assigned to each type of preadipocyte from sham rats).

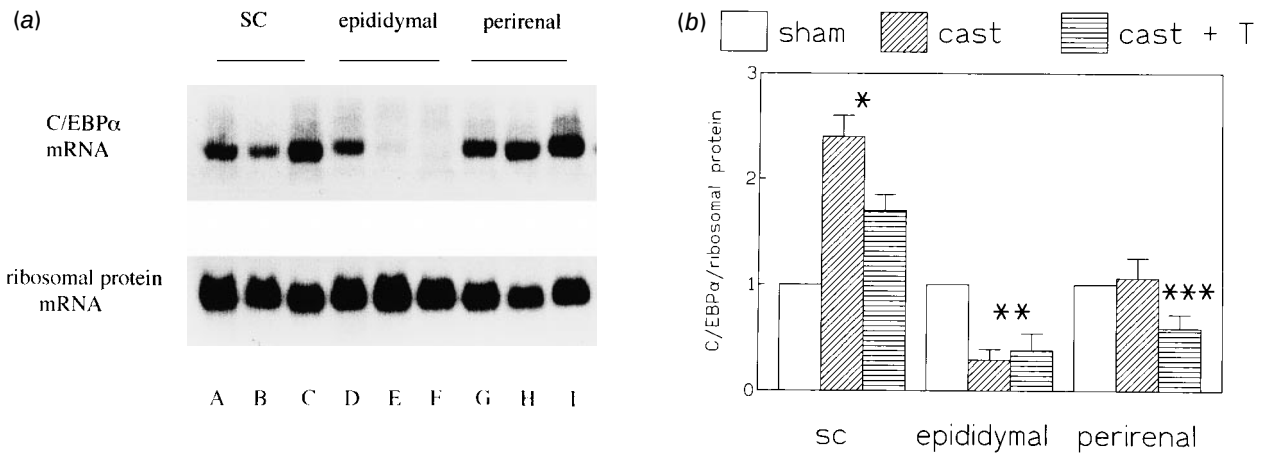

Figure 5 Influence of androgenic status on C/EBPα mRNA expression in rat differentiated preadipocytes from femoral SC, epididymal and perirenal fat depots. Total RNAs of differentiated preadipocytes from femoral SC (A, B and C), epididymal (D, E and F) and perirenal (G, H and I) fat depots from sham (A, D and G), cast (B, E and H) and cast+T (C, F and I) rats were hybridized with C/EBPα probe. (*a*) Representative Northern blot of C/EBPα mRNA. (*b*) Densitometric analysis of C/EBPα Northern blots. The data are means ± S.E.M. of five separate experiments. (*b*) This figure shows variations in C/EBPα mRNA relative to ribosomal protein mRNA. 100% is assigned to each type of preadipocyte from sham rats (control) for C/EBPα mRNA and ribosomal protein mRNA. Then, for each experimental group, the respective C/EBPα mRNA and ribosomal protein mRNA were expressed relative to the corresponding controls. Next, C/EBPα mRNA/ribosomal protein mRNA percentage ratios were calculated. **P*<0·05, cast vs sham and cast T; ***P*<0·05, cast and cast T vs sham; ****P*<0·05, cast T vs sham and cast.

of p42 and the p30 doublet isoforms resulting from post-translational modification (MacDouglas & Lane 1995) were diminished by castration (Fig. 6). *In vivo* testosterone treatment failed to correct these defects (Figs 5 and 6). In contrast, in perirenal LDP, C/EBPα mRNA level was unaffected by castration but decreased (− 40%, *P*<0·05)

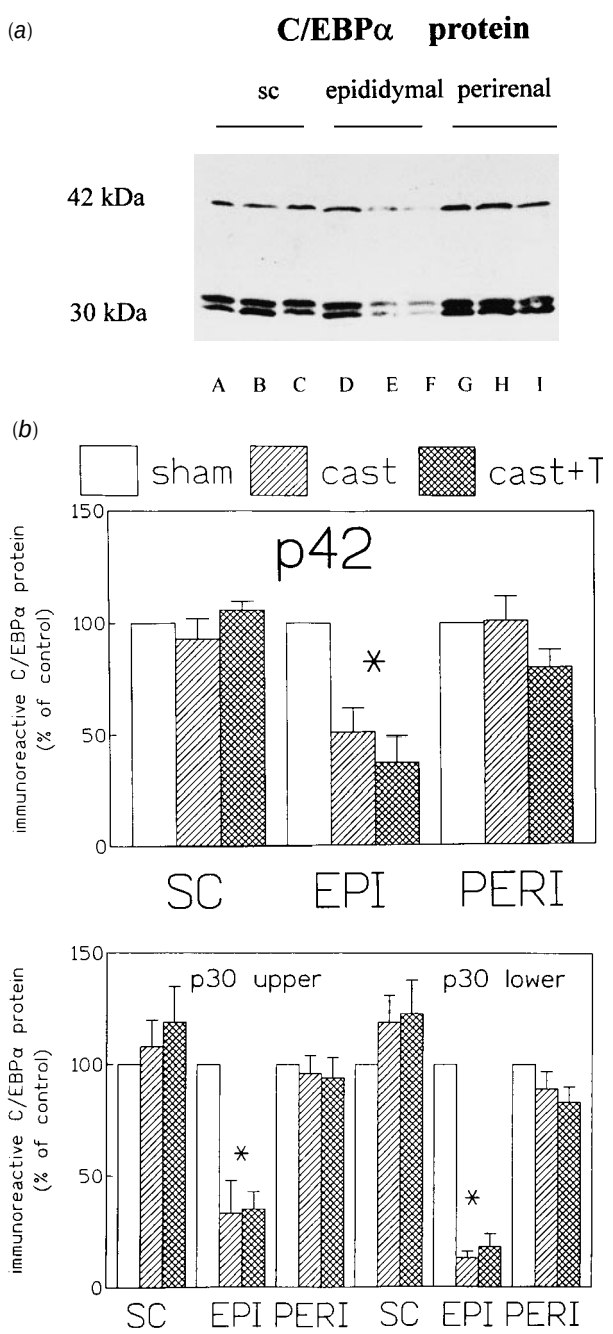

Figure 6 Influence of androgenic status on C/EBPα protein expression in rat differentiated preadipocytes from femoral SC, epididymal and perirenal fat depots. Cellular extracts of differentiated preadipocytes from femoral SC (A, B and C), epididymal (D, E and F) and perirenal (G, H and I) fat depots from sham (A, D and G), cast (B, E and H) and cast+T (C, F and I) rats were probed with C/EBPα polyclonal antiserum. (*a*) Representative Western blot of C/EBPα protein. (*b*) Densitometric analysis of C/EBPα Western blots. The data are means ± S.E.M. of five separate experiments and are expressed as percentage of control values (100% is assigned to each type of preadipocyte from sham rats). *P<0·05, cast and cast T vs sham.

by testosterone treatment. Finally, in SC LDP, castration induced a significant increase in C/EBPα mRNA but not in C/EBPα protein expression, an effect which was partly prevented by testosterone replacement.

Taken together these data strongly suggest that the defective adipogenesis observed in epididymal LDP after castration is linked to a decreased expression of the transcriptional factor C/EBPα but not of its upstream activator C/EBPβ.

Discussion

We have previously reported that the adipogenesis of deep intra-abdominal preadipocytes studied *in vitro* is differently affected by the androgenic status depending on the anatomical origin of the precursor cells. In fact, we found that the differentiation process was either enhanced or completely inhibited by castration in perirenal and epididymal preadipocytes respectively (Lacasa *et al.* 1995, 1997).

In contrast, both epididymal and perirenal preadipocytes elicited increased proliferation capacities after castration (Lacasa *et al.* 1995, 1997). The latter finding led us to hypothesize that the androgenic status may alter c-myc induction in response to serum mitogenic factors in both perirenal and epididymal preadipocytes. As shown in the present study, however, this hypothesis can be ruled out since the steady-state levels of c-myc mRNA and protein were found to be unchanged in perirenal and even decreased in epididymal preadipocytes after castration. This negative observation suggests that, like in Swiss 3T3 fibroblasts (Mehmet *et al.* 1997), only low levels of Myc are sufficient to reach full mitogenic response of rat preadipocytes, e.g. that above a given level, c-myc protein would not further increase the preadipocyte proliferation rate.

With regard to the early events of adipogenesis, our results clearly show that androgen deprivation substantially abolishes the expression of the early marker LPL in epididymal preadipocytes but not in perirenal cells. In the early steps of the differentiation process, LPL expression has been shown to be regulated, at least in part, through the cAMP signalling pathway (Couturier *et al.* 1998) while, later on, LPL is induced by transactivators such as peroxysome proliferator-activated receptors γ (PPARγs) and members of the C/EBP family (Spiegelman & Flier 1996). We have previously observed that castration specifically blunted the cAMP production by confluent and differentiated preadipocytes (D Lacasa & B Agli, unpublished results). Thus this alteration could be responsible for the decreased LPL expression in these cells.

However, as shown in the present study, castration increases and *in vivo* testosterone treatment decreases LPL expression without altering the adipogenic capacities of SC cells (Lacasa *et al.* 1995). These findings are consistent with our previous observation showing that the lipogenic

activity (^{3}H glucose incorporation into triacylglycerol) was either enhanced in rat SC adipocytes or decreased in epididymal cells by castration (Lacasa *et al.* 1993). On the whole, these results indicate that androgen deprivation influences, in two opposite ways, the lipogenic pathway of SC and epididymal adipocytes mainly through a positive or negative regulation of LPL expression.

The mechanisms whereby castration site-specifically influences the lipogenic pathway remain unsettled. In any case, these site-related opposite effects cannot be related to differences in androgen receptor (AR) densities since AR numbers are lower in SC than in deep intra-abdominal preadipocytes (De Pergola *et al.* 1990, Dieudonné *et al.* 1998) and AR are down-regulated by castration whatever the anatomical origin of the cells (Dieudonné *et al.* 1998).

Expression of C/EBPβ appears unaffected by the androgenic status, suggesting that modified expression of this transcriptional factor is not involved in the defective adipogenesis of epididymal preadipocytes.

A different situation was observed concerning the expression of C/EBPα, another member of this transcriptional factor family.

As shown in the present study, the steady-state levels of C/EBPα mRNA and protein are dramatically decreased in epididymal preadipocytes after castration. Under our experimental conditions, *in vivo* testosterone administration was unable to restore to normal C/EBPα expression. Since CHOP, the product of a growth-arrested-associated gene (gadd 153) belonging to the C/EBP family, is a negative modulator of C/EBPα, β and ∂ (MacDouglas & Lane 1995), we have also examined the expression of CHOP in epididymal preadipocytes. Our preliminary results indicate, however, an apparent insensitivity of CHOP expression to castration (data not shown). The present study also indicates that the expression of C/EBPβ, another C/EBPα modulator, is unaltered by the androgenic status in epididymal cells. It is worthy of note that these cells exhibit a reduced number of lipid droplets due to defective lipogenic capacities (Lacasa *et al.* 1995). Thus, fatty acid (FA) availability is probably reduced in these cells and consequently the transcriptional activity of PPARγ because FAs are physiological activators of this transcription factor (Spiegelman & Flier 1996). Since a positive regulatory loop has been postulated between PPARγ and C/EBPα (Brun *et al.* 1997), reduced PPARγ transcriptional activity could well contribute to explain why the differentiation process in epididymal cells cannot proceed and why various adipocyte genes including C/EBPα are not induced after castration.

C/EBPα has a strong antiproliferating role by inducing the growth-arrested-associated gene, gadd 45, and the cyclin-dependent kinase inhibitor p21 (Constance *et al.* 1993, Timchenko *et al.* 1996). Thus, the reduced expression of C/EBPα observed in epididymal preadipocytes after castration could contribute to an explanation of the increased proliferation capacities of these cells but not of the perirenal preadipocytes where C/EBPα expression is not modified by castration. Further experiments will be needed to establish whether inductions of p21 and gadd 45 by C/EBPα are differently affected by castration in epididymal and perirenal preadipocytes.

Expression of C/EBPα which is sufficient to trigger adipogenesis is positively autoregulated to maintain the fully differentiated state of the cells (MacDouglas & Lane 1995). Because of all the properties of C/EBPα, the low level of C/EBPα expression found in epididymal preadipocytes after castration could provide a convenient explanation not only for the increased proliferative capacity, but also for the defective adipogenesis characterizing these cells. However, the mechanisms underlying the site-specific differences in C/EBPα modulation by androgen deprivation in preadipocytes remain to be determined.

Recent experiments from our laboratory indicate that *in vivo* testosterone administration up-regulates AR number in epididymal preadipocytes from castrated rats (Dieudonné MN, unpublished results). However, as shown here, this treatment failed to correct some of the alterations induced by castration (i.e. LPL and C/EBPα expressions) in these cells. This indicates that these alterations cannot fully account for androgen and/or AR deficiencies. These observations rather suggest that lack of some products of the testis other than testosterone (steroidal and non-steroidal testicular factors) could play a significant role in these effects of castration.

In conclusion, this study provides strong evidence that the mechanism whereby androgenic status specifically depresses the adipose conversion of epididymal preadipocytes, involves, at least in part, a reduced expression of C/EBPα, a master regulator of adipogenesis.

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