Biochemical Pharmacology, Vol. 45, No. 3, pp. 579–583, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00
© 1993. Pergamon Press Ltd

EFFECTS OF RETINOIC ACID ON LIPOPROTEIN LIPASE ACTIVITY AND mRNA LEVEL *IN VITRO* AND *IN VIVO*

JASON D. OLIVER and M. PERENNA ROGERS*
Department of Biochemistry and Genetics, The Medical School, Framlington Place, The University, Newcastle upon Tyne NE2 4HH, U.K.

(*Received* 13 *July* 1992; *accepted* 13 *November* 1992)

Abstract—This study was designed to determine whether all-*trans* retinoic acid altered lipoprotein lipase (LPL) activity and mRNA levels *in vitro* and tissue LPL mRNA levels *in vivo*. Incubation of adipocytes or adipose tissue for up to 12 hr with 10^{-6} or 10^{-5} M all-*trans* retinoic acid did not decrease LPL activity. There was no change in LPL mRNA levels following 3 hr incubation of adipocytes with all-*trans* retinoic acid. Feeding all-*trans* retinoic acid for 4 days led to a significant decrease in adipose tissue LPL activity but no change in heart enzyme activity. Retinoic acid did not alter the increase in heart LPL activity observed with fasting. There were no changes in LPL mRNA levels in adipose tissue, heart or liver. Retinoic acid does not have an acute direct effect on adipose tissue LPL activity. The observed decrease in adipose tissue LPL activity *in vivo* is not due to alterations in mRNA levels and may be a secondary effect of retinoic acid.

Treatment with the vitamin A derivative retinoic acid 13-*cis* retinoate leads to increased plasma triacylglycerol levels in both humans and rats [1–4]. Triglyceride levels were found to be raised as soon as 12 hr after the start of feeding retinoic acid [1]. Both increases in triacylglycerol synthesis and decreases in triacylglycerol removal, through decreased lipoprotein lipase (LPL†) (EC 3.1.1.34) activity, have been implicated as the cause of the hyperlipidaemia [4–7]. Decreases in both skeletal muscle and adipose tissue LPL activity have been observed *in vivo* with the extent of the change depending on the animal species and form of retinoic acid under study [6, 7].

Retinoic acid receptors are members of the steroid/thyroid receptor superfamily and there is a growing list of genes the transcription of which is altered by retinoic acid. The change in transcription can occur within minutes or a few hours [8, 9] or after a longer time [10, 11]. Transglutaminase mRNA began to accumulate 15 min after treatment of macrophages with retinoic acid and reached a maximum after 9–10 hr [8]. However, the increase in Type IV collagen mRNA in F9 cells is not observed until 48 hr after the addition of retinoic acid with the peak occurring at 72–120 hr [10]. In some cases the effects of retinoic acid are transient and are maintained only in the presence of other factors [9].

There is disagreement as to whether retinoic acid has a direct or indirect effect on the enzymes involved. Thus 13-*cis* retinoate has been reported to increase triacylglycerol synthesis in rat liver post-mitochondrial supernatant but to have no effect on triacylglycerol synthesis in isolated hepatocytes [2, 5].

Retinoic acid also decreases LPL activity during the adipose conversion of 3T3 preadipocytes. While retinoic acid did not prevent the spontaneous emergence of LPL which occurs during differentiation of the preadipocytes, it did prevent the further increase produced by insulin during the terminal stage of differentiation [12].

Changes in LPL activity are brought about mainly by alteration in enzyme amount and alterations in mRNA levels have been found [13, 14]. It is possible, therefore, that retinoic acid alters LPL activity through a direct effect on transcription. On the other hand retinoic acid increases the rate of transcription of hormones such as growth hormone [11] and so the changes in LPL activity could be a secondary effect. The aim of this work was to determine whether retinoic acid has a direct acute effect on adipose tissue LPL activity and whether the levels of LPL mRNA are altered in the short or long term.

MATERIALS AND METHODS

Materials. All-*trans* retinoic acid, insulin, albumin and collagenase Type II were from the Sigma Chemical Co. (Poole, U.K.). Dexamethasone phosphate was a gift from Merck, Sharp and Dohme (Herts, U.K.). [α-^{32}P]dCTP was from Amersham (Amersham, U.K.).

Incubations. Male Porton–Wistar rats were fed *ad lib*. on standard chow pellets consisting of (by weight) 51.6% carbohydrate, 22.7% protein and 4.3% fat (the residue was non-digestible material; Special Diet Services, Witham, Essex, U.K.) and had free access to drinking water. At 7.00 a.m. the 14 hr light/10 hr dark cycle began. Epididymal adipose tissue was removed at 9.00 a.m.

Adipocytes were prepared from epididymal adipose tissue by the method of Rodbell [15]. The cells were incubated (5–7.5% final lipocrit) at 27° or 37° as indicated in Krebs–Ringer buffer (pH 7.4) containing 20 mM Hepes, 4% (w/v) defatted albumin, 5 mM glucose, 0.5% (w/v) casein hydro-

* Corresponding author. Tel. (091) 222 7443; Telex 53654 (UNINEW G); FAX (091) 222 7424.

† Abbreviations: FFA, free fatty acids; LPL, lipoprotein lipase.

lysate, 2.5 mM calcium chloride, 40 nM insulin and 0.75 μM dexamethasone phosphate [14]. Heparin (5 IU/mL) was present when LPL activity was measured. All-*trans* retinoic acid was prepared as a 10^{-3} M stock solution in ethanol and stored at −20° in the dark. Equivalent amounts of ethanol were added to control incubations. Half epididymal fat pads were incubated for up to 5 hr in the above medium except that the buffer was Krebs–Henseleit bicarbonate and the glucose and defatted albumin were 10 mM and 2% (w/v), respectively. For long-term incubations of adipose tissue, 1/6 fat pads were incubated for 8 or 12 hr in Krebs–Henseleit bicarbonate medium as above but in the absence of heparin in order to help to maintain enzyme activity within the tissue. The incubations were at 32° in a 95% oxygen:5% carbon dioxide atmosphere [14].

In vivo *experiments*. Groups of three rats were fed either all-*trans* retinoic acid-treated pellets or control pellets for 4 nights. All-*trans* retinoic acid was dissolved in acetone and applied to the dry pellets to give 100 μg retinoic acid/g dry diet and 40 mL acetone/100 g pellets. The control pellets were treated with 40 mL acetone/100 g pellets. After the 4 nights feeding the rats were either killed at 9.00 a.m. or the food was removed and the rats killed at 4.00 p.m. One fat pad and half a heart from each rat were taken for LPL assay and the other fat pad, half a heart and sample of liver were taken for RNA isolation.

LPL assays. Adipocyte suspensions were homogenized with an equal volume of 10 mM barbital buffer (pH 7.4) containing 4 mg/mL Triton X-100, 0.4 mg/mL SDS and 5 IU/mL heparin [16]. After 10 min on ice the homogenates were centrifuged at 1800 *g* for 10 min and 0.5 mL of the infranate assayed for LPL activity.

At the end of the incubations adipose tissue was removed from the medium and 0.5 mL of the medium assayed for LPL activity immediately. The tissue was homogenized in 5 mM barbital buffer (pH 7.4) containing 2 mg/mL Triton X-100 and 0.2 mg/mL SDS.

The homogenates were kept on ice for 10 min (or stored at −20° overnight) and then centrifuged as above and the infranate assayed for LPL activity. Whole fat pads from the *in vivo* experiments were homogenized in 2% defatted albumin and acetone–ether powders were made [17]. The powders were homogenized in 5 mM barbital buffer (pH 7.4) containing 2.5 IU heparin/mL and assayed for LPL activity.

LPL was assayed by the release of free fatty acid (FFA) from an Intralipid substrate in the presence of serum as previously described [14]. LPL activity is expressed as μmol FFA released/hr/g wet weight of tissue or for fat cell activity, /g wet weight of fat pad equivalent.

RNA extraction. Tissue was homogenized in ice-cold 3 M lithium chloride, 6 M urea, 50 mM sodium acetate and 0.05% (w/v) SDS immediately following incubation or removal from the rat. The total tissue RNA was then extracted by the method of Auffray and Rougeon [18]. Following the removal of 2 mL of medium, adipocyte RNA was extracted from the remaining 1 mL suspension by the method of Chomczynski and Sacchi [19]. The electrophoresis and northern blotting of total RNA (1.5–5.0 μg) and the labelling of cDNA was as described previously [14]. The blots were washed as described [20] and subjected to autoradiography by using preflashed hyperfilm-MP (Amersham) with intensifying screens at −70°. The autoradiographs were scanned by a Chromoscan densitometer (Joyce–Loebl). The LPL cDNA was removed by washing at 65° in 0.005 M Tris–HCl, 0.02 M Na_2EDTA and 0.1 × Denhardt's solution (pH 8.0) for 2 hr. The blots were then reprobed using an α-tubulin cDNA probe. The results are expressed as a ratio of LPL mRNA to α-tubulin mRNA. Retinoic acid did not alter α-tubulin mRNA levels. Linearity of response was observed with increasing concentrations of total RNA. The LPL cDNA probe was a gift from M. C. Schotz, Wadsworth Medical Centre, Los Angeles, CA, U.S.A. and the α-tubulin probe was a gift from P. Middleton, University of Newcastle upon Tyne, U.K.

Statistical analysis. All data expressed as mean ± SD were evaluated statistically by Student's *t*-test.

RESULTS AND DISCUSSION

Adipocyte LPL activity and mRNA levels

In order to determine whether retinoic acid has a

Table 1. Effect of retinoic acid on LPL mRNA levels and LPL activity in isolated adipocytes

	Retinoic acid concentration (M)			
	Control	10^{-7}	10^{-6}	10^{-5}
LPL/α-tubulin mRNA				
27°*	100.0 ± 11	111 ± 13	100 ± 9	102 ± 11
LPL activity (μmol FFA/hr per g)				
27°†	18.0 ± 1.9		16.3 ± 2.6	
37°*	15.8 ± 1.6		16.3 ± 4.1	16.1 ± 1.4

The mRNA results are expressed as the values of LPL/α-tubulin mRNA as a percentage of the control values.

All results are expressed as means ± SD.

* N = 4; † N = 6.

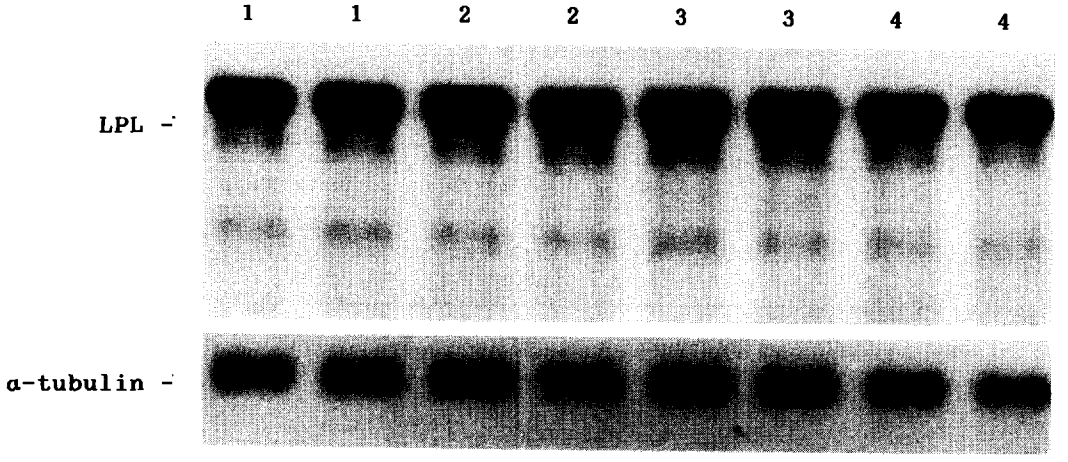

Fig. 1. Northern blot analysis of LPL and α-tubulin mRNA levels in isolated adipocytes. Adipocytes were incubated for 3 hr at 27° in the presence or absence of retinoic acid. Duplicate 1.5 μg of total adipocyte RNA was subjected to electrophoresis and northern blotting as described in Materials and Methods. Key: (1) control incubations; (2) 10^{-7} M retinoic acid; (3) 10^{-6} M retinoic acid; (4) 10^{-5} M retinoic acid. The densitometric results from these autoradiographs are given in Table 1.

direct effect on adipocyte LPL activity rat adipocytes were incubated in the presence and absence of retinoic acid at concentrations up to 10^{-5} M for up to 5 hr.

The first series of experiments, in which LPL mRNA levels were also measured, were carried out at 27°. No significant changes occurred in LPL mRNA levels in adipocytes incubated in the presence of 10^{-7}–10^{-5} M retinoic acid as compared to control incubations (Table 1, Fig. 1). There was also no change in LPL activity with 10^{-6} M retinoic acid.

As LPL is more stable at 27° than at higher temperatures [2], it was possible that any effect of retinoic acid might be more evident at 37°. Therefore, further experiments were carried out at this temperature. However, again there was no change in LPL activity on incubation with either 10^{-6} or 10^{-5} M retinoic acid (Table 1). This observation extended to the LPL activity measured after 5 hr incubation which was 14.3 ± 1.1 and 14.4 ± 1.9 μmol FFA/hr/g adipose tissue equivalent in the presence of 10^{-6} and 10^{-5} M retinoic acid, respectively, compared to 15.2 ± 1.6 μmol FFA/hr/g adipose tissue equivalent in the absence of retinoic acid (N = 4).

Adipose tissue LPL activity

The site of action of LPL is outside the adipocyte at the capillary endothelium. Ligands may alter LPL activity within the adipocyte or after its secretion from the cell. Therefore, to determine whether retinoic acid alters LPL activity in adipose tissue, which includes extracellular enzyme, enzyme activity was measured in both tissue and medium following incubation. Table 2 shows the results of one such experiment where 10^{-6} M retinoic acid did not alter LPL activity in the tissue nor that released into the medium even after 5 hr of incubation. The mean difference between the activity in the paired retinoic acid and control incubations was 3.7 ± 2.7 for the tissue and 3.0 ± 2.4 for the media (all time points). The above results were confirmed in two further experiments. Increasing the concentration of retinoic acid in the incubations to 10^{-5} M did not alter the result as the LPL activity in the tissue incubated for 5 hr was 31.7 ± 5 μmol FFA/hr/g adipose tissue in the presence of retinoic acid and 31.9 ± 8.5 (N = 5) in its absence. The medium LPL activity was 21.0 ± 11.0 μmol FFA/hr/g adipose tissue in the presence of 10^{-5} M retinoic acid and 19.0 ± 11.4 (N = 5) in its absence.

A further experiment investigated the longer term effect of retinoic acid on adipose tissue LPL activity *in vitro*. After incubation of adipose tissue pieces for 8 hr at 32° the LPL activity was 25.7 ± 8.7 μmol FFA/hr/g and 23.5 ± 7.4 μmol FFA/hr/g when incubated in the presence of 10^{-6} and 10^{-5} M retinoic acid, respectively, compared to 23.9 ± 9.4 μmol FFA/hr/g in the absence of retinoic acid (N = 5). The enzyme activity following incubation for 12 hr was 18.0 ± 5.1 μmol FFA/hr/g and 21.2 ± 5.4 μmol FFA/hr/g when incubated in the presence of 10^{-6} and 10^{-5} M retinoic acid, respectively, compared to 19.8 ± 6.9 μmol FFA/hr/g in the control incubations (N = 5).

The findings that incubation with retinoic acid for up to 12 hr does not decrease LPL activity show that retinoic acid does not have an acute direct effect on this enzyme in isolated adipocytes or whole adipose tissue. The finding that retinoic acid prevented the rise in LPL activity produced by insulin during the terminal stage of differentiation of 3T3 preadipocytes could be explained as a specific effect of retinoic acid on the differentiation process or as different behaviour of established cell lines and isolated cells as has been previously found with tumour necrosis factor (see Ref. 14) and insulin [21]. While this work was in progress Antras *et al.* [22] reported no effect

Table 2. Effect of retinoic acid on LPL activity in rat adipose tissue incubated *in vitro*

Time of incubation (hr)	Lipoprotein lipase activity (μmol FFA/hr/g)			
	Control		Retinoic acid-treated	
	Tissue	Medium	Tissue	Medium
3	40.5 ± 8.0	22.2 ± 8.9	42.8 ± 2.8	22.7 ± 8.6
4	45.1 ± 6.2	31.4 ± 9.4	44.4 ± 5.2	28.4 ± 13.0
5	38.4 ± 4.8	33.0 ± 11.5	41.9 ± 12.8	31.9 ± 12.8

Rat epididymal fat pads were cut in half and one half incubated in the presence of 10^{-6} M retinoic acid and the other half in its absence at 32°. At the end of the incubations, LPL activity was measured in the tissue and medium.

All values are means ± SD. N = 3 for each time point.

Table 3. Effect of retinoic acid on LPL activity and mRNA levels in adipose tissue and heart *in vivo*

Tissue and treatment	LPL activity (μmol FFA/hr/g)	LPL/α-tubulin mRNA
Adipose tissue		
Control	74.4 ± 31.5	100 ± 15
Retinoic acid	40.3 ± 17.0*	100 ± 11
Heart (fed)		
Control	37.0 ± 9.5	100 ± 10
Retinoic acid	37.7 ± 8.0	93 ± 14
Heart (fasted)		
Control	191.0 ± 29.4	100 ± 16
Retinoic acid	170.0 ± 39.0	113 ± 21

Rats were treated as indicated in the text. LPL mRNA values are expressed relative to α-tubulin mRNA and are given as a percentage of control values.

Values are means ± SD of six animals.

* Significantly different from the respective control ($P < 0.05$).

of 10^{-5} M all-*trans* retinoic acid on LPL mRNA levels in 3T3-F442A adipocytes although there was a decrease in adipsin mRNA levels. Direct effects have been observed in other cells. Incubation of murine bone marrow macrophages with retinoic acid decreased the secretion of LPL but the maximum decrease was not observed until 6 days although a 46% decrease occurred by 16 hr [23].

Effect of retinoic acid treatment in vivo

Retinoic acid treatment has previously been observed to alter LPL activity [6, 7]. To determine whether retinoic acid *in vivo* alters LPL mRNA levels rats were fed all-*trans* retinoic acid. The weight of the rats fed retinoic acid increased by a factor of 1.16 ± 0.03 while the weight of the controls increased by a factor of 1.19 ± 0.03. The amount of food eaten by the rats was similar for both groups with the retinoic acid-treated rats eating 19.4 g/night/rat and the control rats eating 20.8 g/night/rat.

Retinoic acid treatment had no effect on heart LPL activity or mRNA levels (Table 3). However, there was a significant fall in adipose tissue LPL activity in the retinoic acid-treated rats to 54% of the control values (Table 3). No corresponding effect on adipose tissue LPL mRNA levels was observed (Table 3). Similar values were obtained when the LPL mRNA levels were normalized with β-actin. These findings show that the change in adipose tissue LPL activity is not the result of decrease mRNA levels.

LPL activity is higher in the heart in the fasted state than the fed state and so an effect of retinoic acid might be more evident during fasting. Therefore, LPL activity and mRNA levels were determined in the hearts taken from rats killed at 4.00 p.m. following 4 nights of retinoic acid treatment. The results in Table 3 show that all-*trans* retinoic acid did not cause a significant change in either heart LPL activity or mRNA levels.

The results presented here confirm that one cause of the elevated plasma triacylglycerol levels observed in humans and rats treated with vitamin A or retinoic acid derivatives [1–4] could be the decrease in LPL activity in adipose tissue. However, no effect on LPL activity was observed when adipose tissue was incubated for up to 12 hr with all-*trans* retinoic acid. These findings suggest that the decrease in enzyme activity observed *in vivo* may be a secondary effect of retinoic acid.

No signal was detected for LPL mRNA in total liver RNA extracted from either retinoic acid-treated or control rats. LPL mRNA is not normally detected in adult liver [24, 25]. However, Enerback *et al.* [26] found that a single dose of tumour necrosis factor, which like retinoic acid decreases LPL activity during the adipose conversion of 3T3 preadipocytes, produced a marked increase in LPL mRNA in guinea-pig liver. As tumour necrosis factor also decreases the levels of LPL mRNA [27] it is of interest that retinoic acid does not induce the appearance of LPL mRNA in rat liver.

Acknowledgement—J.D.O. holds a studentship from the Science and Engineering Research Council.

REFERENCES

1. Gerber LE and Erdman JW, Comparative effects of

all-*trans* and 13-*cis* retinoic acid administration on serum and liver lipids in rat. *J Nutr* **110**: 343–351, 1980.
2. McMaster J, Rogers MP, Sherratt HSA and Shuster S, Effects of isotretinoin on lipid metabolism in the rat. *Arch Dermatol Res* **281**: 116–118, 1989.
3. Lyons F, Laker MF, Marsden JR, Manuel R and Shuster S, Effect of oral 13-*cis*-retinoic acid on serum lipids. *Br J Dermatol* **107**: 591–595, 1982.
4. Bershad S, Rubinstein A, Paterniti JR, Le N, Poliak SC, Heller B, Ginsberg HN, Fleischayer R and Brown WV, Changes in plasma lipids and lipoproteins during isotretinoin therapy for acne. *N Engl J Med* **313**: 981–985, 1985.
5. Erdman JW, Elliott JG and Lachance PA, The effect of three forms of Vitamin A upon *in vitro* lipogenesis from three cholesterol precursors. *Nut Reports Int* **16**: 47–57, 1977.
6. Vahlquist C, Lithell H, Michaelsson G, Selinus I, Vahlquist A and Vessby B, Plasma fat elimination, tissue lipoprotein lipase activity and plasma fatty acid composition during sequential treatment with etretinoate and isotretinoin. *Acta Derm Venereol* (Stockh) **67**: 139–144, 1987.
7. Gerber LE and Erdman JW, Hyperlipidemia in rats fed retinoic acid. *Lipids* **16**: 496–501, 1981.
8. Chiocca EA, Davies PJA and Stein JP, The molecular basis of retinoic acid action. *J Biol Chem* **263**: 11584–11589, 1988.
9. Rodan SB, Wesolowski G, Hilton DJ, Nicola NA and Rodan GA, Leukaemia inhibitory factor binds with high affinity to preosteoblastic RCT-1 cells and potentiates the retinoic acid induction of alkaline phosphatase. *Endocrinology* **127**: 1602–1608, 1990.
10. Hellaakoski T, Pajunen L, Kivirikko KI and Pihlajaniemi T, Increases in mRNA concentrations of the α and β subunits of prolyl 4-hydroxylase accompany increased gene expression of Type IV collagen during differentiation of mouse F9 cells. *J Biol Chem* **265**: 11413–11416, 1990.
11. Morita S, Matsuo K, Leng S, Yamashita S, Izumi M and Nagataki S, Stimulatory effect of retinoic acid on growth hormone expression: the interaction of retinoic acid and tri-iodothyronine in rat pituitary cells. *J Endocrinol* **125**: 251–256, 1990.
12. Pairault J, Quignard-Boulange A, Dugail I and Lasnier F, Differential effects of retinoic acid upon early and late events in adipose conversion of 3T3 preadipocytes. *Exp Cell Res* **177**: 27–36, 1988.
13. Ong JM, Kirchgessner T, Schotz MC and Kern PA, Insulin increases the synthetic rate and messenger RNA level of lipoprotein lipase in isolated rat adipocytes. *J Biol Chem* **263**: 12933–12938, 1988.
14. Mackay AG, Oliver JD and Rogers MP, Regulation of lipoprotein lipase activity and mRNA content in rat epididymal adipose tissue *in vitro* by recombinant tumour necrosis factor. *Biochem J* **269**: 123–126, 1990.
15. Rodbell M, Metabolism of isolated fat cells. *J Biol Chem* **239**: 680–685, 1964.
16. Semb H and Olivecrona T, Nutritional regulation of lipoprotein lipase in guinea-pig tissues. *Biochim Biophys Acta* **878**: 330–337, 1986.
17. Ashby P, Tolson AM and Robinson DS, The heterogeneity of the lipoprotein lipase of rat epididymal adipose tissue. *Biochem J* **171**: 305–311, 1978.
18. Auffray C and Rougeon F, Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. *Eur J Biochem* **107**: 303–314, 1980.
19. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium thyocyanate-phenol-chloroform extraction. *Anal Biochem* **162**: 156–159, 1987.
20. *Membrane Transfer and Detection Methods*, Amersham International plc, U.K. 198521.
21. Semenkovich CF, Wims M, Noc L, Etienne J and Chan L, Insulin regulation of lipoprotein lipase activity in 3T3-L1 adipocytes is mediated at post-transcriptional and posttranslational levels. *J Biol Chem* **264**: 9030–9038, 1989.
22. Antras J, Lasnier F and Pairault J, Adipsin gene expression in 3T3-F442A adipocytes is post-transcriptionally down-regulated by retinoic acid. *J Biol Chem* **266**: 1157–1161, 1991.
23. Goldman R, Control of lipoprotein lipase secretion by macrophages: effect of macrophage differentiation agents. *J Leukoc Biol* **47**: 79–86, 1990.
24. Mackay AG and Rogers MP, Lipoprotein lipase mRNA in rat tissues. *Biochem Soc Trans* **17**: 177, 1989.
25. Wion KL, Kirchgessner TD, Lusis AJ, Schotz MC and Lawn RM, Human lipoprotein lipase complementary DNA sequence. *Science* **235**: 1638–1641, 1987.
26. Enerback S, Semb H, Tavernier J, Bjursell G and Olivecrona T, Molecular cloning and sequence analysis of cDNA encoding lipoprotein lipase of guinea-pig. *Gene* **64**: 97–106, 1988.
27. Cornelius P, Enerback S, Bjursell G, Olivecrona T and Pekala PH, Regulation of lipoprotein lipase mRNA content in 3T3-L1 cells by tumour necrosis factor. *Biochem J* **249**: 765–769, 1988.