Changes in lipoprotein lipase activities in adipose tissue, heart and skeletal muscle during continuous or interrupted feeding

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Lipoprotein lipase (LPL) activities in parametrial and interscapular adipose tissue, soleus and adductor longus muscles and hearts of female rats were measured during progressive starvation, chow re-feeding after 24 h starvation and throughout dark and light phases in rats permitted unrestricted access to chow. Adipose-tissue LPL activities declined by 50% after 6 h starvation and continued to fall as the starvation period was extended to 24 h. Skeletal-muscle LPL activities dramatically increased between 9 and 12 h of starvation. Cardiac LPL activities increased 2.5-fold within 6 h of starvation, reaching a maximum after 12 h of starvation. Adipose-tissue LPL activities increased rapidly within 2 h of re-feeding chow *ad libitum* after 24 h starvation, achieving 'fed *ad libitum*' values after 6 h. Oxidative-skeletal-muscle LPL activities also increased after 2 h of re-feeding and exceeded 'fed *ad libitum*' values throughout the 6 h

re-feeding period. Cardiac LPL activities remained up-regulated for the 6 h of re-feeding. Adipose-tissue LPL activities exceeded those of cardiac or skeletal muscle throughout both light and dark phases. The lowest adipose-tissue LPL activities were observed at 9 h into the light phase. In contrast, cardiac LPL activity declined throughout the dark phase, with a minimum at 9 h into the dark phase. No such variation was observed for skeletal-muscle LPL activities. A diurnal nadir in plasma triacylglycerol (TG) concentrations coincided with the peak in cardiac LPL activities. The results demonstrate that, during unrestricted feeding and re-feeding after prolonged starvation, changes in skeletal-muscle and adipose-tissue LPL activities are neither reciprocal nor co-ordinate. Regulation of cardiac LPL activity during the diurnal cycle may be an important aspect of both of cardiac fuel selection and whole-body TG metabolism.

INTRODUCTION

Lipoprotein lipase (LPL; EC 3.1.1.34) catalyses the hydrolysis of plasma lipoprotein triacylglycerols [in particular very-low-density lipoproteins (VLDL) and chylomicrons] to provide non-esterified fatty acids (NEFA) and 2-monoacylglycerol for tissue utilization. It is currently thought that tissue-specific variations in relative LPL activities target the degradation of triacylglycerol (TG)-rich lipoproteins to specific tissues within the body for storage or oxidation in a manner appropriate to the changing nutritional status (Robinson, 1963; Linder et al., 1976; reviewed by Robinson and Speake, 1989). Prolonged starvation is associated with a decrease in LPL activity in adipose tissue (Garfinkel et al., 1967; Nilsson-Ehle et al., 1976), but an increase in LPL activity in heart and oxidative skeletal muscle (Borensztajn and Robinson, 1970; Cryer et al., 1976; Kotlar and Borensztajn, 1977). It has been suggested that the inverse changes in LPL activities in adipose tissue and muscle evoked by prolonged starvation divert NEFA derived from circulating TG from storage in adipose tissue to oxidation in muscle (Wilson et al., 1987).

On the basis of measurements of tissue LPL activities made in either the fed state or after prolonged (24–72 h) starvation, it is generally assumed that the effects of fasting on adipose-tissue and skeletal-muscle LPL activities are reciprocal, implying tight co-ordinated regulation (see Robinson and Speake, 1989). This may not necessarily be entirely true during the nutritional transitions between the fed and fasted states. Most of the decrease in adipose-tissue LPL activity during progressive (up to 72 h) fasting appears to occur within 24 h of food withdrawal, with a maximum decrease in adipose-tissue LPL activity after 48 h of starvation (Radomski and Orme, 1971; DeGasquet and Pequignot, 1973;

Friedman et al., 1979). In contrast, other studies suggest that a maximum activity of skeletal-muscle LPL may be reached within about 12 h of food deprivation (Kotlar and Borensztajn, 1977). These results imply the possibility of the concomitant presence of relatively high activities of LPL in both adipose tissue and skeletal muscle in acute (12–24 h) starvation.

Marrino et al. (1987) found that changes in rates of hepatic TG secretion were unable to explain diurnal oscillations in plasma TG concentrations during unrestricted feeding. Instead, on the basis of comparisons of LPL activities in cardiopulmonary muscles (heart, diaphragm) and epididymal adipose tissue of male rats, measured at 2 h after entry into either the dark or light phase of a 12 h-dark/12 h-light cycle, they suggested that physiological variations in muscle LPL were primarily responsible. However, these authors did not measure LPL activities in skeletal muscle. Most studies have demonstrated not only considerably higher LPL activities in adipose tissue than in skeletal muscle, but also, marked heterogeneity between LPL activities in different skeletal-muscle types, with dramatically higher LPL activities in skeletal muscles containing high percentages of slow-twitch (oxidative) fibres than in skeletal muscles containing high percentages of fast-twitch fibres (Linder et al., 1976; Tan et al., 1977). It is clearly necessary to examine the pattern of change of LPL activities in muscle more representative of the skeletal muscle mass than the specialized rhythmically contracting heart and diaphragm. The question is also raised whether changes in skeletal-muscle or adipose-tissue LPL activities are more contributory to any changes in plasma triacylglycerolaemia observed during the fed-to-starved transition and during re-feeding after starvation.

In the present study, we define the responses of LPL activities in two white-adipose-tissue depots, one deep (parametrial) and

Abbreviations used: LPL, lipoprotein lipase (EC 3.1.1.34); FA, fatty acids; NEFA, non-esterified fatty acids; PDH, pyruvate dehydrogenase complex (EC 1.2.4.1 + EC 1.8.1.4 + EC 2.3.1.12); TG, triacylglycerol; VLDL, very-low-density lipoproteins.

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one superficial (interscapular), and in two well-characterized oxidative (postural) skeletal muscles (soleus and adductor longus) to progressive starvation (for up to 24 h) and to re-feeding rodent chow *ad libitum* for up to 6 h to rats previously starved for 24 h. Adipose-tissue and skeletal-muscle LPL activities were also measured at intervals throughout the dark (feeding) phase and the light phase in rats permitted continuous and unrestricted access to standard (high-carbohydrate/low-fat) rodent chow. The aim of the study was to assess whether reciprocal changes in LPL activities in adipose tissue and muscle might be of any significance for TG targeting under nutritional conditions of more physiological relevance than prolonged starvation. A comparison is made between the responses of heart- and skeletal-muscle LPL activities to these changes in dietary intake. Plasma TG concentrations were determined at each of the sampling points. The overall objective of the study was to obtain definitive information regarding whether, and if so to what extent, changes in LPL activities in adipose tissue and muscle are reciprocally and co-ordinately regulated, and to gain insight into the potential relative importance of adipose tissue and muscle as major sites of plasma TG clearance during continuous or interrupted feeding.

MATERIALS AND METHODS

Materials

Enzymes and coenzymes were from Boehringer Corp. (London), Lewes, Sussex, U.K. Triton WR 1339 (Tyloxapol) was obtained from Sigma. Radiolabelled triolein {glycerol tri[9,10(n)- ^3H]-oleate} was obtained from Amersham International, Amersham, Bucks., U.K. Rat chow was from Special Diets Services, Witham, Essex, U.K. Kits for measurement of plasma TG concentrations were purchased from Alpha Laboratories, Eastleigh, Hants, U.K.

Methods

Animals and diets

Female virgin albino Wistar rats (body wt. 200–250 g) supplied from Charles River were maintained on a 12 h-dark/12 h-light cycle (light from either 10:00 or 22:00 h) at an ambient temperature of $22 \pm 2^\circ\text{C}$. One group of rats was provided with standard laboratory chow diet (consisting of 52 % carbohydrate, 21 % protein, 3 % fat, 24 % non-digestible residue, all by weight) and sampled at 3 h intervals (starting at 0 h light or 0 h dark) throughout the light/dark cycle. The total daily food intake of these rats was 22.9 ± 2.5 g of chow, of which only approx. 15 % was consumed during the light period (see also Kimura et al., 1970; Bruckdorfer et al., 1974; Munday and Williamson, 1983). A second group of rats was starved for periods of up to 24 h (food withdrawal at 10:00 h; 0 h-light) and a third group was starved for 24 h and then subsequently allowed free access to standard chow for periods of up to 6 h. Some rats in each group (continuously fed, starved or starved/re-fed as indicated) were each fitted with a jugular cannula to permit the injection of Triton WR 1339 in the absence of anaesthesia, which influences peripheral fuel metabolism and hepatic TG production. Cannulation was performed under anaesthesia at 5 days before the start of the experiment. All rats were allowed access to drinking water *ad libitum*. Tissues were freeze-clamped while the rats were under surgical anaesthesia (sodium pentobarbital; 60 mg/kg body wt.; intraperitoneal injection). Tissues were rapidly excised, freeze-clamped and stored in liquid nitrogen before processing for determination of LPL activities.

Measurement of LPL activities

LPL activities were measured by a modification of the technique of Nilsson-Ehle and Ekman (1977), in which tissue samples are dried to a powder with acetone/ether and then resolubilized in a buffer (pH 8.1) containing 1 M ethylene glycol (see Cruz and Williamson, 1992). Extracts were then used in an assay containing [^3H]triolein as substrate and fasted-rat serum as a source of apolipoprotein C-II (necessary for the activation of LPL; Korn, 1955). ^3H -labelled fatty acids, released during incubation for 1 h at 37°C , were extracted and determined by the method of Nilsson-Ehle and Schotz (1976). LPL activity is expressed as nmol of fatty acid released/min per mg of acetone/ether-dried powder.

Treatment with WR 1339

An estimate of the rate of entry of TG into the circulation was obtained by a modification of the method of Otway and Robinson (1967) using the detergent Triton WR 1339, which prevents the removal of TG from the plasma (Scanu, 1965). Triton WR 1339 [1.0 ml of a 10 % (w/v) solution in 0.9 % NaCl] was injected via the indwelling cannula of conscious unrestrained rats. Blood (0.4 ml) was collected via the same indwelling cannula after 1.0 h, 1.5 h and 2 h. The increase in total plasma TG was calculated from the rate of increase in plasma TG concentration, assuming a plasma volume of 4 % of the body weight (Otway and Robinson, 1967). The results are expressed as μmol of TG entering the circulation/h per 200 g body wt.

Measurement of plasma TG concentration

TG concentrations were measured in plasma of mixed blood obtained from the chest cavity at the time of killing, by using kits.

Statistical analysis

Data were analysed and statistical significances of differences were assessed by Student's unpaired *t* test. There were 6 to 8 animals in each group unless otherwise specified.

RESULTS AND DISCUSSION

LPL activities in adipose tissue and skeletal muscle

LPL activities were measured in four white-adipose-tissue depots and in four individual skeletal muscles differing in their fibre compositions (Table 1). Irrespective of the nutritional status of the donor rats (fed *ad libitum* or 24 h-fasted), LPL activities in the parametrial and perirenal adipose-tissue depots were similar, and somewhat higher than those in the more superficial (interscapular and subcutaneous) adipose-tissue depots. Starvation (24 h) was associated with a significant decline in LPL activities in all four adipose-tissue depots (Table 1). In confirmation of previous studies, LPL activities in the fed state in skeletal muscles (soleus and adductor longus) containing high percentages of slow-twitch fibres were at least 4-fold higher than in those (extensor digitorum longus, tibialis anterior and gastrocnemius) containing high percentages of fast-twitch fibres (Table 1). The distinction between slow-twitch and fast-twitch skeletal muscles in terms of their relative LPL activities were even more pronounced after 24 h starvation: LPL activities in the former, but not the latter, muscle types were significantly increased by 1.8–2.1-fold after 24 h starvation (Table 1). On the basis of the results shown in Table 1, parametrial and interscapular adipose tissue (the superficial depot with the higher LPL activity in both

Table 1 LPL activities in a range of adipose-tissue depots and individual skeletal muscles of rats fed *ad libitum* and 24 h-starved rats

Experimental details are given in the Materials and methods section. The results are means $\pm$ S.E.M., with the numbers of rats shown in parentheses. Values that are statistically different by Student's *t* test from those of the corresponding rats fed *ad libitum* are shown by **P* < 0.001.

Nutritional status ...	LPL activities (nmol of FA released/min per mg of acetone-dried tissue)	
	Fed <i>ad libitum</i>	Starved (24 h)
Adipose tissue		
Parametrial	4.61 $\pm$ 0.20 (8)	1.18 $\pm$ 0.28 (6)*
Interscapular	3.27 $\pm$ 0.27 (9)	0.64 $\pm$ 0.11 (7)*
Perirenal	4.04 $\pm$ 0.45 (8)	0.99 $\pm$ 0.16 (8)*
Subcutaneous	1.82 $\pm$ 0.24 (9)	0.22 $\pm$ 0.06 (8)*
Skeletal muscles		
Soleus	0.38 $\pm$ 0.05 (8)	0.80 $\pm$ 0.08 (7)*
Adductor longus	0.39 $\pm$ 0.05 (8)	0.70 $\pm$ 0.02 (8)*
Tibialis anterior	0.08 $\pm$ 0.02 (8)	0.07 $\pm$ 0.01 (9)
Extensor digitorum longus	0.05 $\pm$ 0.02 (8)	0.09 $\pm$ 0.02 (7)
Gastrocnemius	0.06 $\pm$ 0.02 (8)	0.08 $\pm$ 0.02 (8)

the fed and starved state) were chosen as representative adipose-tissue depots. Soleus and adductor longus muscles were selected for more detailed study on the basis of their relatively high LPL activities in the fed state and because they responded to 24 h starvation with the greatest absolute increases in total LPL activities.

Adipose-tissue and skeletal-muscle LPL activities during progressive starvation and re-feeding after 24 h starvation

Significant (*P* < 0.05) decreases in LPL activities were observed in parametrial and interscapular adipose tissue within 6 h of the onset of starvation [to 63 % and 50 % of the respective initial (0 h light) values; Figure 1]. Further significant (*P* < 0.01) decreases in adipose-tissue LPL activities occurred as the starvation period was extended to 24 h, by which time LPL activities were only 26 % (parametrial) and 20 % (interscapular) of each corresponding initial value (Figure 1). Re-feeding chow *ad libitum* after 24 h starvation led to a dramatic enhancement of LPL activities in parametrial (148 %; *P* < 0.05) and interscapular (194 %; *P* < 0.001) adipose-tissue depots within 2 h (Figure 1). Adipose-tissue LPL activities further increased as the re-feeding period was extended beyond 2 h and were restored to initial (0 h light) values after 6 h of re-feeding (Figure 1). A striking correlation was observed between the pattern of changes in adipose-tissue LPL activities (the present work, Figure 1) and plasma insulin concentrations (Holness and Sugden, 1991; Holness et al., 1991), shown as line traces in Figure 1.

Skeletal-muscle LPL activities remained at 90–95 % of the initial (0 h light, continuously fed) value over the first 9 h of starvation (Figure 1b). Major (approx. 2-fold) and rapid increases in LPL activities of both skeletal muscles were observed from 9 to 12 h of starvation (Figure 1), subsequent to the initial starvation-induced decreases in adipose tissue LPL activities and plasma insulin concentrations (see Figure 1). Extending the period of starvation from 12 h to 24 h did not elicit any further changes in skeletal-muscle LPL activities (Figure 1b). Contrary to any predictions that might be based on the concept of reciprocal control of LPL activities in adipose tissue and skeletal

Figure 1 LPL activities in adipose tissue and skeletal muscle during progressive starvation and re-feeding after 24 h starvation

LPL activities (mean $\pm$ S.E.M.) were measured in parametrial ($\blacktriangle$) and interscapular ($\bullet$) adipose tissue (a) and soleus ($\blacktriangle$) and adductor longus ($\bullet$) skeletal muscles (b). Tissue LPL activities were measured in acetone-dried extracts of freeze-clamped tissues at 3 h intervals during progressive (up to 24 h) starvation and at 2 h intervals during chow re-feeding *ad libitum* after 24 h starvation. The 0 h re-fed value refers to LPL activities measured after 24 h starvation. Statistically significant effects of starvation were observed in both adipose-tissue depots at 6 h (*P* < 0.05 in each case), 9 h (*P* < 0.01 in each case) and 24 h (*P* < 0.001 in each case) and in both skeletal muscles at 12 h (*P* < 0.01 in each case) and 24 h (*P* < 0.01 in each case). Statistically significant effects of re-feeding after 24 h starvation were observed in both parametrial and interscapular adipose tissue at 2 h (*P* < 0.05 and *P* < 0.01 respectively), 4 h (*P* < 0.05 and *P* < 0.001 respectively) and 6 h (*P* < 0.001 in each case). Statistically significant effects of re-feeding were observed in both soleus and adductor longus at 2 h (*P* < 0.05 and *P* < 0.01 respectively) and also in adductor longus at 4 h (*P* < 0.01) and 6 h (*P* < 0.001).

muscle by insulin, LPL activities in soleus and adductor longus increased, rather than decreased, from 24 h-starved values in response to re-feeding after 24 h starvation (Figure 1). The enhancement of skeletal-muscle LPL activities observed after re-feeding [increases in LPL activities of 31 % (*P* < 0.05) in soleus and 40 % (*P* < 0.01) in adductor longus at 2 h after re-feeding] was less marked than that observed in adipose tissue. Skeletal-muscle LPL activities continued to exceed initial (0 h light, continuously fed) values throughout the 6 h re-feeding period [by 150–176 % in soleus (*P* < 0.001) and by 108–151 % in adductor longus (*P* < 0.001)] (Figure 1). Extending the period of re-feeding to 24 h elicited significant suppression of skeletal-muscle LPL activities to values similar to those observed in rats fed *ad libitum* (91 % and 78 % of fed values in soleus and adductor longus respectively).

Kiens et al. (1989) examined the changes in skeletal-muscle LPL activities during euglycaemic/hyperinsulinaemic clamp in

Figure 2 LPL activities in adipose tissue and skeletal muscle during continuous and unrestricted feeding during the light and dark phases

LPL activities (means $\pm$ S.E.M.) were measured in parametrial ($\blacktriangle$) and interscapular ($\bullet$) adipose tissue (a) and soleus ($\blacktriangle$) and adductor longus ($\bullet$) muscles (b). Tissue LPL activities were measured at 3 h intervals during the light and dark phases in rats permitted access to chow *ad libitum*. Both light and dark phases were of 12 h duration, starting at 10:00 h. Values statistically significantly different from the 0 h-light value were observed in interscapular adipose tissue at 3 h light ($P < 0.05$) and in both parametrial and interscapular adipose tissue at 6 h light ($P < 0.05$ for both depots) and 9 h light ($P < 0.05$ and $P < 0.001$ respectively).

man after an overnight fast. They observed that an acute increase in insulin concentrations during the clamp was associated with a decline in skeletal-muscle (vastus lateralis) LPL activity. This decline correlated closely with an insulin-induced increase in leg glucose uptake. These authors therefore proposed that changes in skeletal-muscle LPL activity were secondary to an insulin-dependent increase in skeletal-muscle glucose metabolism. Using recent measurements *in vivo* of rates of glucose uptake/phosphorylation by skeletal muscles of conscious unrestrained rats (Sugden et al., 1990; Holness and Sugden, 1991), we found no obvious inverse correlation between LPL activities and rates of glucose uptake/phosphorylation in either soleus or adductor longus during re-feeding after 24 h starvation. However, the increase in LPL activities observed in oxidative skeletal muscles after 12 h of starvation (the present work) parallels significant decreases in rates of glucose uptake/phosphorylation (Holness and Sugden, 1990b) and also in the capacity for pyruvate oxidation (Holness et al., 1989) in both muscles. It is thus plausible that increased skeletal-muscle LPL activities during starvation may be secondary to decreased glucose utilization. Conversely, increased local production of FA via LPL for skeletal-muscle oxidation may permit suppression of glucose utilization via the provision of a preferred substrate. Since plasma NEFA and ketone-body concentrations are relatively low after 12 h of starvation (Holness et al., 1989), these lipid

substrates are unlikely to make major contributions to skeletal-muscle energy supply over the period from 0 to 12 h starvation.

Adipose-tissue and skeletal-muscle LPL activities during continuous and unrestricted feeding during the light and dark phases

LPL activities during the dark and light phases in tissues of rats permitted free access to standard rodent diet are shown in Figure 2. The rats sampled in the dark phase were maintained on a reverse light/dark cycle (dark from 10:00 h) to permit direct comparison with rats sampled during the light phase (light from 10:00 h). The patterns of changes in LPL activities occurring throughout the light and dark phases were qualitatively similar in parametrial and interscapular white adipose tissue (Figure 2). In support of previous findings (Marrino et al., 1987), adipose-tissue LPL activities measured at 3 h into the light phase were not significantly different from those measured at 3 h into the dark phases. However, significant (up to 3-fold) changes in adipose-tissue LPL activities were detected during the light and dark phases in the present experiments where the frequency of sampling times was increased. LPL activities in both adipose-tissue depots declined significantly (by 33% and 27% respectively; $P < 0.05$ in both cases) within 6 h of the onset of the light phase, and in each case a minimum was observed after 9 h into the light phase. Adipose-tissue LPL activities were restored over the last 3 h of the light phase. The decline in adipose-tissue LPL activities during the first 9 h of the light phase (Figure 2) closely parallels the fall in plasma insulin concentrations known to occur over this period (Holness et al., 1992; shown as line traces in Figure 2). Similarly, the high adipose-tissue LPL activities observed during the last 9 h of the dark (feeding) period are concomitant with high insulin concentrations (Figure 2). However, adipose-tissue LPL activities are relatively high at the onset and during the first 3 h of the dark phase, times at which insulin concentrations remain relatively low (Figure 2). These findings imply that factor(s) other than insulin may direct plasma TG towards storage in adipose tissue via modulation of adipose-tissue LPL activity. Interestingly, LPL activities for each adipose-tissue depot during the first 9 h of the light phase in rats permitted unrestricted access to chow did not differ significantly from those observed over the corresponding period of starvation (compare Figures 1a and 2a). In contrast, the restoration of adipose-tissue LPL activities during the last 3 h of the light phase is clearly dependent on continued access to food, as it is not observed in rats denied access to chow.

There was no evidence for any dramatic or sustained change in skeletal-muscle LPL activities in response to light or darkness (Figure 2b). It is clear from the asynchronous changes in adipose-tissue and skeletal-muscle LPL activities observed during continuous and unrestricted feeding and also during progressive starvation and re-feeding after 24 h starvation that LPL activities in these individual tissues are likely to be subject to independent regulation in responses to changes in carbohydrate and insulin status.

Cardiac LPL activities during starvation, re-feeding and continuous and unrestricted feeding during the light and dark phases

Previous studies have reported a significant increase in cardiac LPL activity after 8 h of fasting (Borensztajn et al., 1970). In the present experiments, a 2.5-fold increase in cardiac LPL activity was observed after 6 h of starvation (Figure 3). A further significant ($P < 0.05$) increase in cardiac LPL activity was

Figure 3 Cardiac LPL activities and plasma TG concentrations during progressive starvation and re-feeding after 24 h starvation

Cardiac LPL activities ($\blacktriangle$) and plasma TG concentrations ($\triangle$) were measured at 6 h intervals during progressive (up to 24 h) starvation and at 2 h intervals during chow re-feeding *ad libitum* after 24 h starvation. The 0 h re-fed value refers to cardiac LPL activity and plasma TG concentrations measured after 24 h starvation. Results are means $\pm$ S.E.M. Statistically significant effects of starvation on plasma TG concentrations were observed at 12 h ($P < 0.05$) and on cardiac LPL activity at 6 h ($P < 0.05$), 12 h ($P < 0.001$) and 24 h ($P < 0.05$). Statistically significant effects of re-feeding after 24 h starvation on cardiac LPL activities were observed at 2 h ($P < 0.05$), 4 h ($P < 0.01$) and 6 h ($P < 0.05$). Statistically significant effects of re-feeding on plasma TG concentrations were observed at 2 h ($P < 0.05$), 4 h ($P < 0.01$) and 6 h ($P < 0.01$).

observed between 6 and 12 h of fasting, such that cardiac LPL activities after 12 h of starvation were 4.3-fold higher than the initial (fed) value (Figure 3). Thus up-regulation of cardiac LPL activity is one of the earliest responses to food withdrawal, occurring concomitantly with down-regulation of adipose-tissue LPL (Figure 1), depletion of hepatic glycogen (Holness and Sugden, 1989) and inactivation of hepatic and peripheral lipogenesis (Holness and Sugden, 1990a).

Inactivation of cardiac PDH by phosphorylation is also an early response to food withdrawal, an approx. 70% decline being observed within 6 h of the onset of starvation (Holness and Sugden, 1989). This decline is attenuated when mitochondrial FA oxidation is inhibited (Holness and Sugden, 1989), even though there is no major increase in the circulating supply of NEFA or ketone bodies (Holness and Sugden, 1990b) or TG (Figure 3). The present results, demonstrating a very rapid response of cardiac LPL within 6 h of the onset of starvation, emphasize the potential for local cardiac production of FA from circulating TG and raise the possibility that FA derived from circulating TG (rather than from adipose-tissue TG) is used as oxidative substrate by the heart during this period.

The peak cardiac LPL activity was observed after 12 h of starvation (Figure 3), with a decline in cardiac LPL activity between 12 and 24 h of starvation (Figure 3). However, cardiac LPL activities after 24 h of starvation were still 1.8-fold higher than those observed in the continuously fed *ad libitum* state (Figure 3). Borensztajn et al. (1970) also observed a rapid rise in cardiac LPL activity, succeeded by a decline as the starvation

Figure 4 Cardiac LPL activities and plasma TG concentrations during continuous and unrestricted feeding during the light and dark phases

Cardiac LPL activities ($\blacktriangle$) and plasma TG concentrations ($\triangle$) were measured at 3 h intervals during the light and dark phases in rats permitted access to chow *ad libitum*. Both light and dark phases were of 12 h duration, starting at 10:00 h. Results are means $\pm$ S.E.M. Cardiac LPL activities statistically significantly different from the 0 h-light value were observed at 6 h dark ($P < 0.05$) and at 9 h dark ($P < 0.05$). Plasma TG concentrations statistically significantly different from the 0 h-light value were observed at 9 h light ($P < 0.05$).

period was extended. Interestingly, the decline in cardiac LPL from 12 to 24 h of starvation coincides with a significant rise in circulating plasma NEFA concentrations (Holness et al., 1989) which may provide an alternative supply of energy substrate for the heart. Re-feeding for up to 6 h after 24 h of starvation failed to elicit any significant change in cardiac LPL activities. Thus, as in skeletal muscle, cardiac LPL activities remained significantly higher (approx. 1.7-fold; $P < 0.05$) than 'fed *ad libitum*' values for a considerable period after access to food was restored after prior starvation.

Previous studies (Marrino et al., 1987) reported that LPL activities in heart and diaphragm at 2 h into the dark phase were 1.7-fold and 2.3-fold higher respectively compared with activities measured at 2 h into the light phase in rats allowed unrestricted access to chow. Significant differences between cardiac LPL activities measured between 0 and 3 h into the light phase compared with activities measured between 0 and 3 h into the dark phase were not observed in the present experiments (Figure 4). However, there was considerable variation in cardiac LPL activities during the diurnal cycle, with a substantial decline in cardiac LPL activity after 6 h of darkness compared with the 0 h dark value (Figure 4). The lowest cardiac LPL activity during the light and dark cycle was observed after 9 h of darkness, and this activity was only 34% of the 0 h-dark value and only approx. 50% of that observed during most of the light phase (Figure 4).

In contrast with the rapid inactivation of cardiac pyruvate dehydrogenase (PDH), skeletal-muscle PDH inactivation is a relatively late event in the overall response to starvation, with significant inactivation occurring only after 12–15 h of starvation (Holness et al., 1989). It is therefore of interest that the response of skeletal-muscle LPL activity to starvation, like that of PDH, is more refractory than that of heart and, furthermore, heart (but not skeletal-muscle) LPL activity shows considerable diurnal

variation. Taken together, the results suggest either that the factor(s) initiating the response of muscle energy metabolism to starvation differ between heart and skeletal muscle or that the sensitivity of cardiac LPL to common regulatory factor(s) is greater than that of skeletal-muscle LPL. The decline in cardiac (but not skeletal-muscle) LPL activity during the dark phase is consistent with increased sensitivity of cardiac LPL activity to changes in dietary intake compared with skeletal muscle. A common feature of both cardiac and skeletal muscle is, however, the retention of relatively high LPL activities during the initial (0–6 h) phase of re-feeding after prolonged starvation.

Plasma TG concentrations during starvation, re-feeding and unrestricted feeding

Studies *in vitro* (Mangiapane and Brindley, 1986; Barlett and Gibbons, 1988) suggest suppression of hepatic VLDL production by insulin, the concentrations of which decline during starvation and increase during re-feeding (see Figure 1). The only significant change in triacylglycerolaemia observed during starvation in the present experiments was an approx. 50 % decline ($P < 0.05$) after 12 h (Figure 3): this decline was coincident with the initial increase in skeletal-muscle LPL activities (Figure 1) and the highest observed activity of cardiac LPL (Figure 3). A more sustained change in triacylglycerolaemia was observed during chow re-feeding after 24 h starvation, where plasma TG concentrations were consistently and significantly (59–80 %) lower than in the continuously fed *ad libitum* (0 h light) state (Figure 3). Plasma TG concentrations declined to 53 % of the initial (starved) value within 2 h of re-feeding and remained significantly ($P < 0.05$) lower than the starved value after 4 h of re-feeding (Figure 3).

Rates of TG entry into the circulation measured at 2 h after re-feeding standard (52 % carbohydrate/3 % fat) rodent chow after 24 h starvation (which estimates hepatic VLDL secretion plus TG derived from dietary fat) were not significantly higher than those measured after 24 h starvation, which estimates hepatic VLDL secretion (entry rates of 20.09 ± 3.12 and 22.39 ± 4.1 μmol of TG/h per 200 g body wt. respectively; $P > 0.05$). These results suggest that the increased rate of entry of TG from the gastrointestinal tract during re-feeding is balanced by suppression of hepatic TG secretion and are consistent with the concept that dietary lipid input and/or a post-prandial rise in insulin leads to suppression of hepatic TG output. They also indicate that a decreased rate of entry of TG into the circulation after re-feeding cannot account for the observed decrease in triacylglycerolaemia (Figure 3). Although in the present experiments whole-tissue (rather than 'functional') LPL activity was measured, it is most likely that the sustained use of circulating TG by muscle (as well as increased use of TG by adipose tissue) after re-feeding may be a significant factor underlying the observed decline in triacylglycerolaemia. The sustained use of circulating TG may also underlie the latency in the ability of heart and oxidative skeletal muscle to oxidize carbohydrate, assessed by measurement of the expressed (active) form of PDH (Sugden and Holness, 1989).

At only one time point during the light and dark phases of unrestricted feeding were plasma TG concentrations significantly different from the initial (0 h light) values (a 49 % decline in TG concentrations at 9 h light; $P < 0.01$ versus 0 h light; Figure 3). We found no statistically significant difference between rates of entry of TG into the circulation during the period of decline in TG during the light phase and the corresponding period during the dark phase (entry rates of 14.84 ± 0.74 and 14.80 ± 1.23 μmol of TG/h per 200 g body wt. respectively). However, strikingly, the sampling time at which the lowest plasma TG concentrations

were observed (9 h light) coincided with the peak of cardiac LPL activity (see Figure 4) and is clearly not explicable in terms of increased LPL activities in either adipose tissue (in which LPL activities had declined) or skeletal muscle, in which LPL activities were unchanged (see Figure 2). There is therefore a strong possibility that the heart makes a greater contribution to whole-body TG clearance during unrestricted feeding than might be experienced on the basis of its contribution to body mass. This may reflect the high rates of blood flow to this organ.

Concluding remarks

Adipose-tissue LPL activities during unrestricted feeding during the light and dark phases were consistently (by at least 2.6-fold and up to 10.7-fold) higher than the highest skeletal-muscle LPL activities observed. It is therefore likely that adipose tissue, rather than skeletal muscle, makes the major contribution to whole-body TG clearance when changes in food intake are appetite-led and access to food is unrestricted. There are concomitant relatively high activities of LPL in both adipose tissue and oxidative muscle at 12 h after food withdrawal, but the partitioning of available TG between the two tissue types is not known with certainty. However, the two situations where LPL activities in adipose tissue and skeletal muscle are comparable and therefore where skeletal muscle might potentially make a more significant contribution to TG clearance is after prolonged (> 12 h) starvation and during the first few hours of re-feeding after a starvation period exceeding 12 h. In the former situation, the dietary input of TG is absent (necessitating the maintenance of the body TG pool by synthesis *de novo*), but concentrations of alternative lipid fuels (NEFA and ketone bodies) are relatively high; in the latter situation, circulating NEFA and ketone-body concentrations are low, but there is a dietary supply of TG. Since plasma TG concentrations are lower during re-feeding than after starvation, it is tempting to speculate that utilization of TG by skeletal muscle is even greater during re-feeding after prolonged starvation than during starvation itself. In this connection, it is of interest that high concentrations of FA can potentially inhibit LPL activity, both by displacement of LPL from endothelial binding sites and also by end-product inhibition and suppression of LPL activation by apolipoprotein CII (Olivecrona and Bengtsson, 1984; Saxena et al., 1989; Saxena and Goldberg, 1990). It could be envisaged that re-feeding after prolonged starvation, with the associated decline in circulating NEFA (and ketone body) supply removes a restraint on the potentially high skeletal-muscle LPL activities.

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