Diet composition and lipoprotein lipase (*EC* 3.1.1.34) activity in human obesity

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1. Adipose tissue lipoprotein lipase (*EC* 3.1.1.34; AT-LPL), a rate-limiting enzyme in triglyceride storage in adipose tissue, is hormonally regulated and may be important in the maintenance of obesity.

2. In twelve obese women, AT-LPL activity was measured before weight loss, during weight loss and after 1 and 2 weeks of weight maintenance on either a high-carbohydrate or a high-protein diet.

3. When related to tissue weight, AT-LPL activity during the 2 weeks of weight maintenance was higher than the initial AT-LPL activity; there was no difference when activity was expressed per cell.

4. Changes in AT-LPL activity were not affected by diet composition. AT-LPL activity correlated with insulin levels and a change in the insulin sensitivity of AT-LPL was observed after weight loss.

Adipose tissue lipoprotein lipase (*EC* 3.1.1.34; AT-LPL) is a rate-limiting enzyme in triglyceride storage in adipose tissue (Hollenberg, 1966; Taskinen & Nikkilä, 1977). Insulin is a primary hormonal regulator (Bosello *et al.* 1984) and in non-obese individuals insulin level is correlated with AT-LPL activity (Pykälistö *et al.* 1975). AT-LPL activity is decreased in insulin-deficient diabetic patients (Pykälistö *et al.* 1975; Taskinen & Nikkilä, 1979; Taylor *et al.* 1979) and elevated in the hyperinsulinaemia associated with obesity (Guy-Grand & Bigorie, 1975; Pykälistö *et al.* 1975; Taskinen & Nikkilä, 1979; Dahms *et al.* 1981). Various characteristics of AT-LPL appear to be abnormal in the obese state (Olefsky, 1976; Kolterman *et al.* 1980; Taskinen & Nikkilä, 1981; Sadur *et al.* 1984). The decrease in AT-LPL activity in obese individuals following energy restriction is less than the decrease seen in non-obese individuals (Taskinen & Nikkilä, 1981). Likewise, the increase in AT-LPL activity following intravenous glucose infusion is smaller in obese subjects than in controls (Taskinen & Nikkilä, 1981). The response of AT-LPL activity to insulin during maintenance of euglycaemia was found to be delayed, but preserved, in obesity (Sadur *et al.* 1984).

The abnormal metabolic regulation of AT-LPL may be related to abnormalities of insulin action and binding in obesity (Olefsky, 1976; Kolterman *et al.* 1980). Reduction of the obese state is accompanied by improved insulin sensitivity and decreased insulin levels. One would, therefore, expect a decrease in AT-LPL activity following weight loss. There is, however, disagreement in the literature concerning whether or not AT-LPL activity is increased or decreased in obese individuals at a stable reduced weight. Schwartz & Brunzell (1978, 1981) have observed an increase in AT-LPL in obese individuals who are maintaining body-weight at a reduced level, and the activity decreases when the lost weight is regained. Reitman *et al.* (1982), who studied AT-LPL activity in Pima Indians before and after weight loss, did find a decrease in AT-LPL activity in individuals who were weight-stable after weight loss. Sörbis *et al.* (1981) found that a 10% weight loss had no effect on AT-LPL activity. Still another study (Rebuffe-Scrive *et al.* 1983) found that the increases in AT-

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LPL activity with refeeding after weight loss returned values only to pre-weight loss levels but not above.

There are numerous factors that could be responsible for the differing results in these studies, such as the amount of weight loss, how weight-stable the individuals were when initial measurements were taken, the percentage overweight of the study population, sex, length of time on the weight-maintenance diet, the site of the adipose tissue biopsy and the composition of the weight-maintenance diet. The aim of the present study was to determine whether the composition of the weight-maintenance diet in a weight-stable period following weight loss influences the effect of weight loss on AT-LPL activity. Variables such as serum insulin, progesterone, oestradiol, plasma lipids, fat cell size and number, and indices of body-weight were also considered.

EXPERIMENTAL

Subject characteristics

Twelve obese female subjects ranging from 120 to 202% of ideal body-weight (Metropolitan Life Insurance Height & Weight Tables, 1983) were studied. All subjects were healthy with no known endocrinological cause for their obesity and none had diabetes mellitus. Individuals were excluded if they did not have normal menstrual cycles or they ingested drugs with known effects on glucose or lipid metabolism. All had normal electrolytes, calcium, phosphorus, liver function test, electrocardiogram, complete blood counts and urine analysis. Fasting plasma glucose, serum cholesterol and triglycerides were normal in all subjects.

All studies were approved by the Human Subjects Committee and were performed in the Clinical Research Center at the Harbor UCLA Medical Center after informed consent was obtained.

Experimental design

For the first 7 d, each subject consumed a liquid formula diet providing 105 kJ/kg actual body-weight and containing 50% of energy as carbohydrate, 20% as protein, 30% as fat. This was consumed on an out-patient basis and body-weight was monitored every 3 or 4 d to ensure weight maintenance. A baseline adipose tissue biopsy (biopsy 1) was obtained for AT-LPL activity assay on the morning of the 8th day. At this time blood was sampled for determination of insulin, glucose, oestradiol, progesterone, triglycerides and cholesterol. All subjects then received a liquid diet providing 3360 kJ/d, consisting of 62% of energy as carbohydrate, 22% as protein, 18% as fat (Carnation Do-It-Yourself Diet Plan^{R} mixed with 180 g low-fat milk, four times daily) and were instructed to take a daily multiple vitamin plus mineral supplement and eight 8 oz (240 g) glasses of non-energy liquids daily. This diet was consumed on an out-patient basis with bi-weekly visits to the Clinical Research Center for weight monitoring, anthropometric measurements, counselling with the dietary staff and weekly analysis of blood and urine. The length of time on the weight-loss diet varied depending on the preferences of the subjects and ranged from 1 to 5·5 months.

At the termination of the weight loss period adipose tissue was again obtained (biopsy 2) and blood was sampled as before. Following the biopsy, subjects were divided into two weight-maintenance dietary groups according to a double blind randomizing scheme: high carbohydrate (60% of energy as carbohydrate, 10% as protein, 30% as fat) or high protein (40% of energy as carbohydrate, 30% as protein, 30% as fat). Both diets were given in amounts to maintain body-weight with intakes adjusted between 80 and 105 kJ/kg actual body-weight per d. The average change in weight over the 2-week period was 0·9 kg, ranging from 0·2 to 2 kg. Adipose tissue biopsies and blood samples were repeated after 1 (biopsy 3) and 2 (biopsy 4) weeks of consuming the weight-maintenance diets.

Two additional obese women (125 and 133% of ideal body-weight (Metropolitan Life

Insurance Height & Weight Tables, 1983)) were studied to assess individual variation in AT-LPL activity over time, independent of dietary and weight changes. These individuals consumed the weight-maintenance diet used in the 1st week of the study (50% of energy as carbohydrate, 20% as protein, 30% as fat) for 2 weeks and underwent three adipose tissue biopsies on days 7, 10 and 14 of the diet.

Lipoprotein lipase activity

Adipose tissue biopsies were performed between 08.00 and 10.00 hours after an overnight fast; 300–500 mg subcutaneous adipose tissue were obtained from a small incision in the upper quadrant of the buttock about 50 mm from the midline. The area was anesthetized with lidocain (20 ml/l). Adipose tissue was cleaned of connective tissue and 100–200 mg were removed for assay of AT-LPL activity. The remaining tissue, for determination of lipid content and cell number, was kept in saline (9 g sodium chloride/l) at 37°.

A tissue homogenate (200 g/l) in ice-cold 0·25 M-sucrose–1 mM-EDTA buffer, pH 7·4, was prepared using a Kontes (Kontes Co., Vineland, N.J.) all ground glass homogenizer. Homogenates were centrifuged at 12000 ***g*** for 15 min at 4° in a Sorvall RC-5 refrigerated centrifuge. The fat-free postmitochondrial infranatant was aspirated from below the fat-cake layer and stored at −70° (Hietanen & Greenwood, 1977). AT-LPL activity was assayed using a stable radioactive substrate emulsion, according to the method of Nilsson-Ehle & Schotz (1976). Tri-[9, 10-^{3}H]oleoylglycerol (112 Ci/mmol) was obtained from New England Nuclear (Boston, MA), cold triolein from NuCheck Prep (Elysian, MN) and lecithin from Sigma (St Louis, MO). To reduce assay variability serum activator pool, the albumin and triolein substrate emulsion was the same for all assays of a given patient. All assays were run in duplicate. Incubations were carried out at 37° for 2 h in a total of 200 μl containing 100 μl of assay substrate and 100 μl infranatant or buffer or both.

Adipose tissue cell size and number

Tissue for determination of lipid content was blotted dry and weighed. Duplicate pieces of 50–100 mg were extracted with chloroform: methanol (2:1, v/v), according to the method of Folch *et al.* (1957), and the lipid content determined gravimetrically. Duplicate tissue samples (30–50 mg) for determination of cell number were fixed in osmium tetroxide (20 g/l in 0·05 M-collidine–hydrochloric acid buffer, pH 7·4) and counted electronically in a Coulter Counter as described by Hirsch & Gallian (1968).

Fat cell size (μg triglyceride/cell) was calculated by dividing triglyceride/g tissue by the number of cells/g.

Statistical analysis

Results were analysed using repeated measures analysis of variance, treating each subject as her own control throughout the four periods and treating the diet as a grouping factor. Comparisons between treatment periods were analyzed by suitable linear contrasts. Potential confounders (i.e. insulin, progesterone, oestradiol, plasma lipids, fat cell size and indices of body-weight) were examined as non-constant covariables within the repeated measures analysis. Synergism was considered to be present when there was a significant interaction term between diet and main effects or contrasts. Groups were compared by unpaired Student's *t* test and correlations performed with linear least squares regression. All analyses were carried out with suitable BMDP routines (Dixon, 1983).

RESULTS

The characteristics of the subjects in the two dietary groups are shown in Table 1. There were no differences in serum cholesterol, triglycerides, glucose and insulin between the two groups (values not shown).

Table 1. *Characteristics of obese female volunteers*

(Mean values and standard deviations for six subjects. At the end of weight-loss period patients were randomly assigned to a weight-maintenance diet either high in protein (30% of energy) or high in carbohydrate (60% of energy), but of equal energy density. Unpaired *t* tests showed no significant differences of any comparison between the two groups)

	High protein		High carbohydrate	
Wt-maintenance diet	Mean	SD	Mean	SD
Age (years)	35·0	7	31·0	7
Initial wt (kg)	87·6	19·4	105·7	24·8
Initial percentage ideal body-wt	142·4	14·5	168·3	28·2
Change in body-wt (kg)	−10·5	3·2	−14·4	9·4
Period on diet (months)	3·3	1·7	3·5	1·6
Age at onset of obesity (years)	15·8	6·3	13·5	8·3

Table 2. *Body-weight, adipose tissue cell size and adipose tissue lipoprotein lipase (EC 3.1.1.34; AT-LPL) activity before and at the end of weight loss in twelve obese female volunteers*

(Mean values and standard deviation)

Biopsy no....	Before wt loss 1		During wt loss 2	
	Mean	SD	Mean	SD
Body-wt (kg)	96·7***	23·3	84·0	22·5
Ideal body-wt (%)	155·3***	25·3	135·0	26·2
Cell size (μg triglyceride/cell)	0·50**	0·07	0·42	0·08
AT-LPL activity (nmol FFA/g tissue per min)	3·87	2·20	3·42	2·10
AT-LPL activity (nmol FFA/10^6 cells per min)	2·61	1·44	1·95	1·05

FFA, free fatty acids.
Mean values were significantly different from those for biopsy 2. ** $P < 0.005$, *** $P < 0.001$.

Baseline values (biopsy 1) for cell size and AT-LPL activity, as well as values after weight loss (biopsy 2) while still consuming the hypoenergetic diet, are shown in Table 2. These initial values from both groups have been combined because treatments did not differ until randomization to a weight-maintenance diet in the last 2 weeks of the study. The mean weight loss for the twelve subjects was 12·4 kg with a range of 6 to 30 kg. When initial AT-LPL activity per 10^6 cells was correlated with percent ideal body-weight, ten of the twelve subjects had values on the regression line described by Schwartz & Brunzell (1981), with a correlation coefficient of 0·74 ($P < 0.025$) (Fig. 1). The remaining two patients had extremely high initial AT-LPL values and were omitted from the correlation calculation but are shown in Fig. 1 and will be discussed later.

AT-LPL activity did not significantly differ at $P < 0.05$ from biopsy 1 to biopsy 2 (Table 2). The change in AT-LPL activity per 10^6 cells was not significantly related to the amount of weight lost, percentage ideal body-weight, initial body-weight or time on the weight-loss diet. There was a significant decrease in cell size (Table 2) with weight loss ($P < 0.005$) and, not surprisingly, this decrease was correlated with the weight loss (kg) (r 0·73, $P < 0.01$).

The AT-LPL activity per g adipose tissue for all four biopsies is shown in Fig. 2. Repeated measures analysis revealed a significant increase in AT-LPL activity per g tissue during the weight-maintenance period (biopsies 3 and 4), compared with both the initial value (biopsy

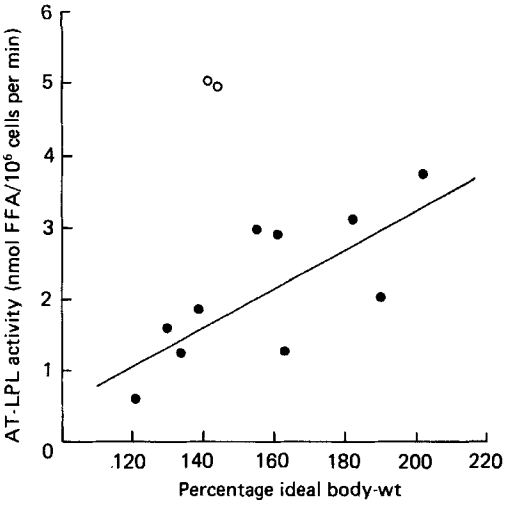

Fig. 1. Relation between adipose tissue lipoprotein lipase (*EC* 3.1.1.34; AT-LPL) activity (nmol free fatty acids (FFA)/10^6 cells per min) and percentage ideal body-weight for ten weight-stable obese female volunteers (●) (r 0·74, $P < 0·025$). (○), Two individuals with very high AT-LPL activity who were not included in the calculation of the regression equation ($y = 0·027 \times -2·19$).

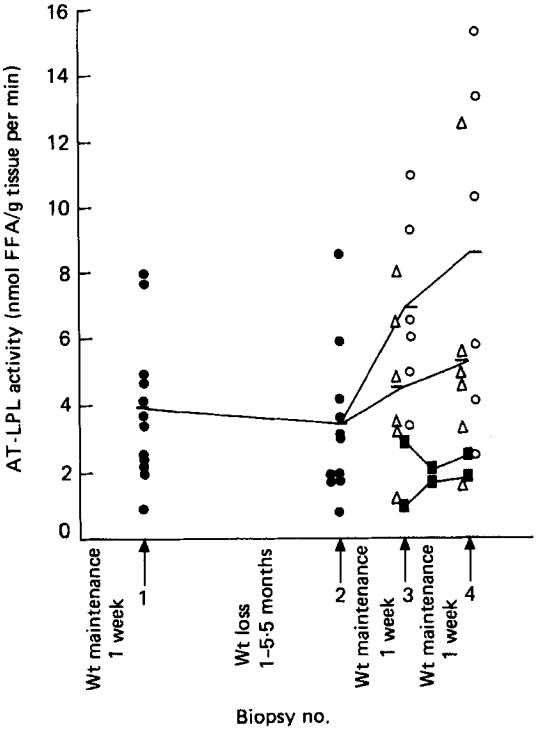

Fig. 2. Adipose tissue lipoprotein lipase (*EC* 3.1.1.34; AT-LPL) activity in obese subjects before (biopsy 1) and at the end of weight loss (biopsy 2) and during subsequent weight maintenance (biopsies 3 and 4). (●) Individuals before and at the end of weight loss; (△), individuals consuming the high-protein weight-maintenance diet; (○), those consuming the high-carbohydrate weight-maintenance diet; (−), group means aligned with appropriate symbols; (■), AT-LPL activity in two obese individuals who were weight-stable with no previous weight loss.

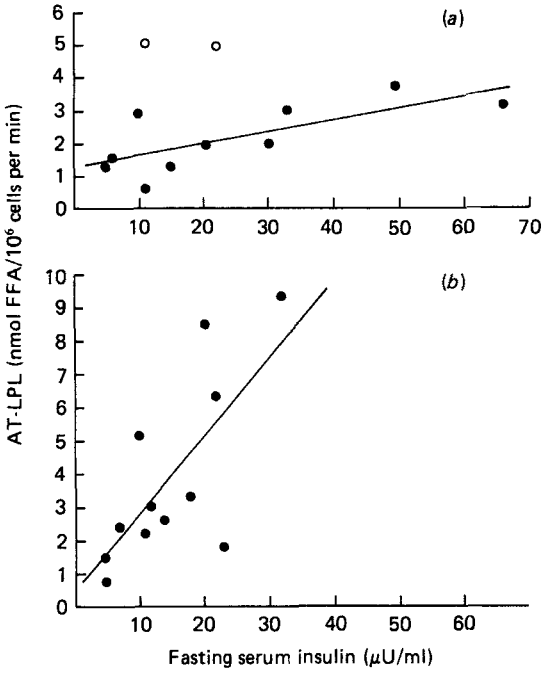

Fig. 3. Relation between adipose tissue lipoprotein lipase (*EC* 3.1.1.34; AT-LPL) activity (nmol free fatty acids (FFA)/10^6 cells per min) and serum insulin concentration (*a*) before weight loss (biopsy 1) and (*b*) after weight loss followed by 2 weeks of weight maintenance (biopsy 4). (○), Two individuals with high initial AT-LPL activity who were excluded from the calculation of the regression equation ($y = 0{\cdot}036\,x + 1{\cdot}24$, r 0·73; $P < 0{\cdot}01$). All twelve individuals after weight loss are included in the regression equation ($y = 0{\cdot}234\,x + 0{\cdot}53$, r 0·71; $P < 0{\cdot}01$).

1) ($P < 0{\cdot}026$) and the activity while still on the hypoenergetic diet (biopsy 2) ($P < 0{\cdot}004$). There was no significant interaction with diet. At biopsy 3 the means (and SD) for the high-protein and high-carbohydrate diets were 4·47 (2·45) and 6·86 (2·81), respectively; at biopsy 4 these values were 5·34 (3·78) and 8·57 (5·22) respectively. When results were expressed per 10^6 cells, AT-LPL activity during the weight maintenance period (biopsies 3 and 4) was significantly greater than activity after weight loss (biopsy 2) ($P < 0{\cdot}007$), but not greater than the initial baseline measurement (biopsy 1). Values from the two control patients who were biopsied three times without weight loss are included in Fig. 2 to illustrate that the changes seen in the experimental subjects were not due to normal variation in AT-LPL activity or to the effect of repeated biopsies.

Other potential covariants of AT-LPL activity which were examined included serum insulin, progesterone, oestradiol and indices of body-weight. There was no statistical interaction between these variables and diet. Therefore, they had no effect on the response of AT-LPL activity to diet.

Fig. 3 illustrates the correlations between AT-LPL activity and plasma insulin concentration before weight loss (biopsy 1) and following weight loss and 2 weeks of weight maintenance (biopsy 4). There was a significant decrease in fasting serum insulin concentration after weight loss when the initial value (biopsy 1) is compared with those at biopsies, 2, 3 and 4 ($P < 0{\cdot}05$). Since there was no significant difference in fasting serum insulin between the high-carbohydrate and high-protein groups at any of the four time points, values have been combined in Fig. 3. The correlation between baseline AT-LPL

activity and serum insulin (Fig. 3(*a*)) is significant only if the two individuals with high initial AT-LPL activity are omitted (r 0·73, $P < 0·025$). The slope of this line is 0·036. At a stable lower weight (biopsy 4) (Fig 3(*b*)) the correlation between AT-LPL activity and serum insulin was r 0·71 ($P < 0·01$) and the slope increased to 0·23. This line includes the two individuals who were 'outliers'. This could suggest a change in the sensitivity of AT-LPL activity to insulin with weight loss.

There were no differences in cholesterol and triglyceride levels between groups. Insulin, triglycerides and glucose remained constant during the weight-maintenance period and were not different between groups.

DISCUSSION

There is considerable disagreement in the literature concerning the changes in AT-LPL activity in weight-stable obese individuals following weight loss (Schwartz & Brunzell, 1981; Sörbis *et al.* 1981; Rebuffe-Scrive *et al.* 1983). In the present study at weight maintenance, AT-LPL activity per 10^6 cells (biopsies 3 and 4) did not significantly increase above the pre-weight-loss baseline value (biopsy 1). This agrees most closely with the results of Sörbis *et al.* (1981) and fails to support the fourfold increase reported by Schwartz & Brunzell (1981). When the values are expressed as AT-LPL activity per g tissue (Fig. 2), there is a significant increase during weight maintenance compared with the pre-weight-loss value. AT-LPL activity per 10^6 cells reflects total body AT-LPL activity since fat cell number changes little with weight loss, whereas the weight (g) of fat decreases proportionately with weight loss. As a result most investigators report their values as AT-LPL activity per 10^6 cells (Schwartz & Brunzell, 1981; Rebuffe-Scrive *et al.* 1983); however, AT-LPL activity per g reflects the concentration of enzyme in the adipose tissue. It is unclear how an increase in AT-LPL activity per g tissue affects the body's capacity to store fat and, therefore, regain weight.

Whether values are expressed per 10^6 cells or per g tissue they still represent detectable tissue AT-LPL activity and do not account for the distribution of enzyme between the active extracellular form and the mostly undetectable intracellular form. Fasting (24 h) in rats has been shown to reduce the extracellular form of the enzyme as well as the intracellular pool (Lasunción & Herrera, 1983). In the present study, all adipose tissue biopsies were performed after an overnight fast. However, it is possible that the long-term dietary manipulations could also alter the distribution of the enzyme. AT-LPL activity would need to be assessed differently to answer this question.

Other variables which could complicate comparisons between studies include the period of time on the low-energy diet, the amount of weight lost, the percentage ideal body-weight and the rate of weight loss. None of these variables were found to be covariants in the present study population. The role of AT-LPL in the obese state may also vary depending on whether the obesity is primarily due to adipocyte hypertrophy or hyperplasia. In the present study the early age of onset of obesity and a relatively small fat cell size compared with other findings in the literature (Schwartz & Brunzell, 1981; Rebuffe-Scrive *et al.* 1983) suggest primarily hyperplastic obesity. Gluteal adipose tissue was used in the present study and there is work which indicates that the metabolism of the fat cell may vary depending on the site of biopsy (Lithell & Boberg, 1978; Bosello *et al.* 1984) and the fat distribution of the individual (Kissebah *et al.* 1982).

Differences in individuals within a study population may also explain contradictory results reported in the literature. A specific set point for fat mass or fat cell size has been postulated (Sims *et al.* 1973; Bray & Campfield, 1975; Brunzell & Greenwood, 1983). According to this theory, deviation above or below the set point will stimulate counter-regulatory mechanisms to come into play and drive the individual back to the set point.

Schwartz & Brunzell (1981) have hypothesized that if AT-LPL activity fluctuates to maintain fat cell size or mass at a predetermined set point, then the baseline AT-LPL activity depends on where the subject lies in relation to their set point. If an individual were losing or gaining weight or were above or below the set point initially, AT-LPL activity, as well as any changes resulting from weight loss, will be distorted. This provides a possible explanation for the two subjects who did not fit the regression line of AT-LPL *v.* percentage ideal body-weight (Fig. 1). In these two subjects initial AT-LPL was increased in relation to percentage ideal body-weight. This suggests that these individuals may have been below their set point at the start of the study.

In the present study the effect of the carbohydrate and protein contents of the weight-maintenance diet on AT-LPL activity in weight-stable individuals after weight loss was specifically examined. Diet composition failed to have an effect on AT-LPL activity (Fig. 2); therefore differences in diet composition are unlikely to account for the differences seen between studies.

While the present study does not explain the discrepant results in the literature, the changing relation between insulin and AT-LPL activity with a change in weight (Fig. 3) may point to an important effector in the regulation of AT-LPL activity. Initial AT-LPL activity correlated positively with insulin in ten of the twelve subjects. Reports in the literature vary as to whether insulin relates to AT-LPL activity in obese individuals (Reitman *et al.* 1982; Bosello *et al.* 1984). However, after weight loss and stabilization (biopsy 4) the slope of the line changed dramatically and the two individuals who did not lie on the line now do. It is possible that these two individuals did not have the same decreased AT-LPL sensitivity to insulin that the others had, but it seems more likely, as noted earlier, that they were starting the study at a weight below their set point. With weight loss the AT-LPL activity became relatively more sensitive to regulation by insulin and activity was increased despite a decrease in serum insulin. This finding agrees with other reports that there is a suppressed AT-LPL response to insulin in obesity (Sadur *et al.* 1984). In obese individuals the decreased sensitivity of AT-LPL to insulin (Sadur *et al.* 1984) may prevent further increases in cell size above the set point despite hyperinsulinaemia. Therefore, AT-LPL activity may change to regulate fat cell size to the preset level, whether this level is obese or normal. The factors determining this preset level and signals affecting AT-LPL activity have yet to be determined.

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